Cue-potentiated feeding in rats

Michael David Kendig

School of Psychology

Faculty of Science

The University of Sydney

2017

A thesis submitted to fulfil requirements for the degree of Doctor of Philosophy.

Abstract

Cue-potentiated feeding (CPF) describes the stimulation of food consumption by cues that have become associated with food. Determining under what conditions CPF occurs is important for understanding whether exposure to food cues contributes to overeating. A history presented in **Chapter 1** describes how the study of CPF developed from incidental findings in early experiments to Weingarten's (1983) influential paper, through to contemporary models that focus primarily on the neural circuits underlying CPF. There have been fewer attempts to characterise the broader nature of the effect, particularly in relation to whether CPF is 'specific' to the paired food. This formed the general focus of the present thesis. **Chapter 2** outlines three experiments using a training procedure in which laboratory rats received intermixed exposures to a 'Plus' context containing palatable food and to a 'Minus' context containing no food. CPF was found to be specific to the training food even when testing a palatable and familiar alternative. However, contexts paired with a variety of foods enhanced consumption of other foods never eaten in that environment. Experiments in **Chapter 3** explored individual differences in CPF and found that the effect did not correlate with consumption of palatable food at baseline or during training. Results also suggested that consumption of palatable food in training was not matched by an equivalent reduction in home-cage chow intake. **Chapter 4** reports a series of experiments in which methodological changes hypothesised to enhance the CPF effect reversed the predicted pattern of consumption. These results are discussed with reference to theories of incentive contrast. The effects of diet-induced obesity on CPF were explored in **Chapter 5.** The present results are integrated with existing literature and directions for future research are outlined in **Chapter 6**, which discusses CPF with reference to specificity and variety; individual differences; and the sensitivity of the effect to procedural parameters.

Acknowledgements

I am deeply grateful to my supervisors, Laura Corbit and Bob Boakes, for their invaluable support and guidance. Thank you both for encouraging me to think independently but critically; to develop my own ideas, and for reigning them in when necessary. Thank you also to Kieron Rooney, whose encouragement and advice has been a huge source of support. The passion the three of you share for research is contagious and I am fortunate to have had such inspiring mentors.

I am grateful for insightful feedback on this work from members of the learning labs at the University of Sydney and the University of New South Wales. I would especially like to thank Ben Colagiuri, Nathan Holmes, Kelly Clemens, and Vincent Laurent for their advice on research and on more general aspects of academic life.

The support and humour of many fantastic friends around the lab has made the ups and downs of this journey far more bearable. In particular, thank you to Sarah Martire, Jess Lee, Ash Morse, Dominic Tran, Llew Mills, Bec Kuhnert, and Kirill Fayn for your friendship. To Manuel Menouhos and Alison Salo, thank you for your humour in the animal labs and for so cheerily obliging my many husbandry requests over the years. Several technical crises were averted thanks to the technical wizardry of Nenad Petkovski and Philip Leung; thank you both.

To Mum and Dad, and my sisters Caralee and Kathy, thank you for your unwavering support and encouragement. Seeing you was always a happy period of solace in between lab stints. Finally, thank you to my confidante and best friend, Justine Fam. Pei, you have been a rock of support and I am so grateful for your patience and assistance. Thank you for your unending love; for helping me stay focused; for making life so much fun; and for putting up with Blue Monday on repeat more than any spouse should be reasonably expected to tolerate.

Statement of originality

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis has not been submitted for any degree or other purposes. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Michael D. Kendig

Care and use of animals

All experimental procedures were carried out in accordance with the recommendations of the *Australian code for the care and use of animals for scientific purposes* (8th Edition, 2013), and were approved by the Animal Ethics Committee at the University of Sydney (Protocol 589). All efforts were made to minimise animal suffering.

Publications associated with this thesis

Kendig, M. D., Boakes, R. A., & Corbit, L. H. (accepted) Variety overcomes the specificity of cue-potentiated feeding in rats. *Journal of Experimental Psychology: Animal Learning and Cognition.*

- This manuscript reports the experiments presented in Chapter 2 of this thesis.

Kendig, M. D., Cheung, M. K. A., Raymond, J. S., & Corbit, L. H. (2016). Contexts paired with junk food impair goal-directed behaviour in rats: implications for decision making in obesogenic environments. *Frontiers in Behavioral Neuroscience, 10,* 216.

- This paper formed a significant component of the candidate's research program. It is distinct from the study of cue-potentiated feeding reported in the main body of this thesis but is relevant for aspects of its interpretation and is therefore included as Appendix G.

Conference presentations associated with this thesis

Kendig, M. D., Boakes, R. A., & Corbit, L. H. "Variety and cue-potentiated feeding." Paper presented at the Australian Learning Group Mid-Year Conference, held July 12-14th, 2017 in Katoomba, New, Australia.

Kendig, M. D., Boakes, R. A., & Corbit, L. H. "The role of variety and contrast in the magnitude and selectivity of cue-potentiated feeding." Paper presented at the 2015 meeting of the Inter-University Neuroscience and Mental Health Conference, held September 24-25th at University of New South Wales, Australia.

Kendig, M. D., Boakes, R. A., & Corbit, L. H. "The number and type of palatable foods associated with a context affect the selectivity of cue-potentiated feeding." Paper presented at the 2015 meeting of the Society for the Study of Ingestive Behavior, held July 7-11th in Denver, USA.

Kendig, M. D., Boakes, R. A., & Corbit, L. H. "The effects of motivational state on cue-potentiated feeding." **Poster** presented at the joint meeting of the Australian Learning Group and the International Society for Comparative Psychology, held July 14-16th, 2016, in Sydney, Australia.

List of Figures

Chapter 5

List of Tables

Chapter 4

Table of Contents

Chapter 1: Introduction

"We wish to suggest, then, that ingestion of food is determined more by the external

situation than by the actual interoceptive stimulation."

(H. F. Harlow, 1932, p. 219)

1.1. Scope

Few would deny that choices about what, when, and how much we eat are influenced by the environment around us. As the opening quote attests, the general finding that eating could be stimulated by external factors was known early in experimental psychology's history – and surely in conventional wisdom for some time prior. However, systematic study of how this occurred began only relatively recently. This introduction will provide a history of research on the modulation of feeding behaviour by external stimuli, beginning with examples from studies of social facilitation, drive theory, and resistance to satiation spanning the middle half of the twentieth century. What is notable about these early studies is that feeding was not the primary interest *per se*, but rather a behaviour that could be manipulated and measured relatively easily. Enhanced feeding induced by external stimuli was even a hindrance in some designs.

By contrast, there were only intermittent attempts to characterise the effects of *conditioned* external cues on feeding until the 1980s. The most influential of these was a study by Weingarten (1983) that is often referenced as the seminal demonstration of cue-potentiated feeding. Weingarten's model was significant both for its experimental precision and because it was framed as a challenge to the prevailing belief that energy intake was controlled by homeostatic mechanisms. The ways in which Weingarten and others influenced the development of theories of energy balance will be discussed in the context of the

emergence of obesity as a global public health challenge. Next, contemporary animal models of cue-potentiated feeding will be reviewed. The introduction will conclude by outlining the research questions addressed by the present thesis.

It is also important to clarify what is outside the scope of the introduction. Since the experiments in this thesis are conducted on laboratory rats, the introduction will focus on research in non-human animals and not in people, except to summarise key findings that demonstrate the progression of the field. Nor will it cover the large literatures on the modulation of feeding by acute or chronic stress, or neuroimaging studies on responses to palatable food.

1.2. Socially facilitated feeding

Social facilitation describes instances where the presence of a conspecific performing a particular behaviour initiates or increases the performance of that behaviour in another animal (Clayton, 1978). Though the conditions under which social facilitation occurs and the behaviours that are affected vary widely, a substantial proportion of the literature has studied feeding. While comprehensive reviews are provided by Clayton (1978) and Guerin (1993, 2010) the purpose of the present overview is simply to describe the many circumstances in which the presence of conspecifics invigorates or prolongs feeding. Of particular relevance is how some authors interpreted social facilitation in terms of learning and conditioning.

Ecological and observational studies across a range of species have demonstrated that animals will eat a novel food more readily if they observe a conspecific eating it nearby (Dally, Clayton, & Emery, 2008). Sometimes, enhanced feeding occurs even in the presence of animals from different species (Rubenstein, Barnett, Ridgely, & Klopfer, 1977). In rat colonies, young animals learn which foods are safe to consume and which are to be avoided

by observing the behaviour of adults (Galef & Laland, 2005), and feeding behaviour is often highly synchronised and regularly initiated by 'pioneer' rats procuring food (Barnett & Spencer, 1951). Livestock animals such as pigs and cows typically increase their feeding rate in group housing (Keeling & Hurnik, 1995; Phillips, 2004), but often compensate by making fewer or shorter visits to feeders (Nielsen, Lawrence, & Whittemore, 1995; Harb et al., 1985)[1].

1.2.1. Coaction methods

Such comparisons between individually- and group-housed animals were classified by Clayton (1978) as *coaction* methods and were also applied in laboratory settings. Harlow[2] (1932) found greater food intake when rats were housed in groups (pairs or triplets) than individually, but that feeding was unaffected if one rat in a pair was restrained in a small cage. Similarly, studies in juvenile chicks suggested that enhanced feeding in pairs emerged only when complete social contact was allowed and not when chicks were separated by a wire mesh or plexiglass barrier (Tolman, 1964; Tolman & Wilson, 1965). While this suggested that social facilitation relied on active competition for a single food source, a host of subsequent studies demonstrated enhanced feeding in animals separated by barriers (Hoyenga & Aeschleman, 1969) and in other experimental preparations that removed competition (Harlow & Yudin, 1933; Dally et al., 2008; Strobel, 1972).

---

[1] This is suggested to reflect the tendency of herd animals to synchronise feeding, which may be adaptive by enhancing foraging efficiency and reducing the risk of predation (Clayton, 1978; Nielsen, 1999).
[2] This appears to be one of only two studies by Harlow on feeding, before shifting focus to the attachment work for which he is renowned.

Interpreting these results can be complicated by the use of individual housing as a baseline. Thus, a difference assumed to reflect stimulated feeding in groups may equally be driven by suppressed consumption in isolation (Clayton, 1978), which itself may be moderated by whether or not, and for how long animals are raised in groups or alone (Guerin, 1993). The presence of other animals may also alleviate stress in experiments where tests are conducted in unfamiliar environments (Harlow & Yudin, 1933). Few experiments addressed these possibilities systematically. Coaction approaches were often unable to measure consumption by individual animals (body weight change was often used as a surrogate measure) and to identify which specific aspect of feeding was augmented (e.g. latency to eat versus rate of feeding).

1.2.2. Resumption methods

The second method of studying social facilitation tested whether animals that had just eaten to satiety could be stimulated to recommence eating by the introduction of a hungry companion. This *resumption* method, though less common (Clayton, 1978), is more comparable to methods used to study cue-potentiated feeding today. Two experiments in the 1920s showed that satiated hens were prompted to recommence eating upon the introduction of a second, hungry hen (Fischel, 1927; Bayer, 1929). These papers apparently inspired Harlow's (1932) study in rats, which began with a failed attempt of the resumption method. Other studies had greater success, however. Ross and Ross (1949b) found that placing a hungry puppy with its satiated littermates stimulated them to eat up to 200% of the amount just consumed, while another study showed that satiated dogs recommenced lever-pressing for food when another dog or the experimenter entered the room (James, 1954). A more recent experiment in monkeys found that providing fresh food to a pair of animals stimulated

consumption of stale feed by observers in surrounding cages (Galloway, Addessi, Fragazy, & Visalberghi, 2005).

These effects, however, are often modulated by social hierarchies. Bayer (1929) noted that dominant hens would reliably attack their submissive partners in an attempt to prevent them eating. This effect of '*Futterneid*' – food envy – was not evident in submissive hens, which nonetheless began eating upon the introduction of their dominant peer. Moreover, the direction of social hierarchy effects varies between and even within species. One study found that only submissive puppies exhibited social facilitation (James, 1953), while another reported the effect in all puppies within a litter (James & Cannon, 1955). Dominant pigs exhibited a greater increase in feeding upon the introduction of a hungry pen-mate (Hsia & Wood-Gush, 1984), whereas submissive cattle showed stronger social facilitation following a shift from individual to group feeding sessions (Harb et al., 1985). What may explain this variability is that group feeding sessions might establish social hierarchies rather than measure pre-existing ones; indeed, this was the explicit aim of group feeding sessions in some studies (Harlow & Yudin, 1933).

1.2.3. *The role of learning*

Researchers debated the extent to which socially facilitated feeding was innate or learned. Harlow (1932) and Tolman (1964) argued that social facilitation was independent of learning, since their data indicated that enhanced feeding in groups was evident in animals' first group session and remained stable thereafter. However, Harlow and Yudin (1933) suggested that the introduction of another animal served as a conditioned, excitatory stimulus that facilitated feeding. James and Gilbert (1955) found that puppies raised in isolation only exhibited social facilitation after repeated group feeding sessions. These authors hypothesised

that repeated group feeding sessions established companion animals as secondary reinforcers, or discriminative stimuli associated with food, that elicited feeding behaviour (James, 1954; James & Gilbert, 1955). Tolman (1964) explored the idea that social facilitation could be traced to specific behaviours, and demonstrated that tapping a pencil on a desk or on his birds' beaks stimulated feeding to a similar extent to the presence of another animal. A study in ducklings found that feeding was elicited by presenting a wooden pole which ducklings were previously trained to peck to gain access to an imprinted stimulus (Hoffman, Stratton, & Newby, 1969).

1.2.4. Interim summary 1

It is clear that the presence of conspecifics often enhances feeding over the long-term (in coaction studies) and can trigger eating in the short-term despite satiety (in resumption studies). These effects vary dramatically across species and methods. An obvious point is that group settings are generally arousing for animals, and may energise additional appetitive behaviours as well as feeding itself (Keeling & Hurnik, 1996). On the other hand, other behaviours that preclude feeding could also be enhanced in social settings (e.g. play in young rats; Harlow, 1932, Experiment 3). Finally, it is difficult to isolate the specific component of the companion animal's behaviour that enhances eating in these studies (though see Tolman, 1964). All of this underscores the importance of understanding the behavioural repertoire of the animal under study. To quote Guerin (1993):

"*Background psychological and ethological studies are needed to assess any effects of any proposed mechanism* [for social facilitation]. *You must 'know your animal' first, whether this is a human or an armadillo.*" (Guerin, 1993, p. 127.)

Nonetheless, the complexity of social facilitation effects in non-human animals does not undermine their relevance to understanding human eating behaviour. Indeed, individuals' meal size, duration and even food choices can be affected profoundly by social facilitation and other social processes such as modelling (De Castro, 1997; Stroebele & De Castro, 2004). Together, this demonstrates that in addition to other forms of conditioned cues that are the focus of the present thesis, social factors are an important class of external influences on feeding.

1.3. Studies of drive theory and resistance to satiation

1.3.1. Drive theory

Throughout the twentieth century experimental psychology adopted more systematic methods and stricter experimental control in an attempt to study behaviour in a more scientific fashion. A highly influential theory of behaviour that was emblematic of these aims was outlined by Clarke Hull in his seminal work *Principles of Behavior* in 1943. Hull's theory centred on the concept of drive, a theoretical construct thought to motivate animals' behaviour in response to their biological needs. For example, depriving an animal of food was thought to increase its hunger drive, which motivated food-seeking and consumption. An animal's food consumption could then be used to infer the strength of its hunger drive (Bolles, 1967). The concept of drive, which was also applied to other biological needs such as thirst and sexual behaviour, was widely accepted, but was defined in varied and largely descriptive terms until Hull refined it within a model that generated testable predictions (Bolles, 1967). These were most often explored using feeding behaviour.

An important question within this framework was the extent to which drive states, such as hunger, could be *conditioned*, *acquired*, or *externalised* to cues. This was tested by

pairing environmental stimuli with a drive state and measuring behaviour in that environment. One such study exposed two groups of rats to a striped box for 30-min/day after either 1-h or 22-h food deprivation (Calvin, Bicknell, & Sperling, 1953a). This was intended to pair the box with low and high hunger drive, respectively. In feeding tests in the box (held after 12-h deprivation) the 'high-drive' group ate significantly more. The authors concluded that the motivating properties of the hunger drive became associated with the box, eliciting greater consumption at test (Calvin et al., 1953a).

This study is noteworthy for two reasons. First, it is one of the few experiments that actually found evidence for conditioned drives. By contrast, most tests of conditioned hunger did not find support for this concept, including two studies that failed to replicate the findings of Calvin et al. within a few years of its publication (Siegel & MacDonnell, 1954; Scarborough & Goodson, 1957). One exception, albeit from a more recent literature with different aims, was the finding that rats ate significantly more in an environment previously associated with a long period of food deprivation than in one where food was available (Roitman, van Dijk, Thiele, & Bernstein, 2001). At the time, however, most authors concluded there was very weak evidence for the existence of conditioned drives (Bindra, 1978; Bolles, 1967; Morgan, 1979). Weingarten (1985) noted that there was no more reason to expect an environment paired with the absence of food to elicit eating than to elicit any other behaviour that had also been unavailable (e.g. copulation!). However, Bolles (1967) made the important distinction that the seeming inability to pair external stimuli with hunger was not to say they were unable to affect eating.

The second reason for presenting Calvin et al. (1953a) as an example of conditioned hunger is that these researchers conducted a parallel study in which the same striped box contained food. In this case, the intention was to establish the box as a secondary reinforcer, through its association with the primary reinforcer of food, rather than with the hunger drive

(Calvin, Bicknell & Sperling, 1953b). Two groups of rats were fed a food ration for 30-min per day, either in the box or in the home cage prior to exposure to the empty box 1-h later, while a third group always remained in home cages. The dependent variable was chow consumption in the box in four tests held over two days. The authors reasoned that if the secondary reinforcer – the box – functioned like a primary reinforcer, it should reduce the primary drive with which it was associated – hunger – and reduce eating. The results, however, showed that the group previously fed in the box ate more than the rats fed in the home cage. Thus, what appeared to be conditioned to the box for the group fed within it was not a reduction in hunger drive, but the act of eating.

1.3.2. *Resistance to satiation*

A fundamental prediction of drive theories was that increasing or decreasing the strength of a drive should produce corresponding changes in the behaviours under its control. Researchers most often tested this hypothesis by studying whether an instrumental response for food learned while hungry would persist when the motivation for food was removed. This was often achieved using satiation procedures in which animals could eat freely prior to a test of instrumental responding. This acute pre-feeding was often in addition to extended periods of *ad-libitum* feeding in the home-cage to allow rats' body weight to return to pre-deprivation levels (Capaldi & Myers, 1978). 'Resistance to satiation' described instances where instrumental responding persisted despite these various satiation manipulations (Morgan, 1974).

It was crucial in these designs to verify that the animal was truly satiated; otherwise, continued instrumental performance might simply reflect residual hunger (Morgan, 1979). A recurring problem, however, was that satiation – at least when defined by the cessation of

eating – was notoriously difficult to achieve[3]. This was because animals would often continue to eat food upon placement in the test environment. Morgan (1979, p. 190) observed that:

"…*in practice it is often very difficult to eliminate consummatory responding in a situation where the animal has learned to eat; and then matters become much more complicated.*"

Unfortunately, because such studies viewed food consumption only as a means to satiation, consumption in the test phase was rarely reported, let alone analysed (Morgan, 1974).

A series of studies by Elizabeth Capaldi and colleagues addressed this issue directly by measuring consumption in satiated tests. These studies trained hungry rats to locate food pellets in a goal-box at the end of a straight alley, where running speed was the dependent variable during training and the index of resistance to satiation at test (e.g. Capaldi & Myers, 1978). After training, rats were returned to *ad-libitum* feeding until baseline body weight was restored. During tests, rats were pre-fed the reward pellets in the home-cage for 15-min prior to placement in the maze, where pellets awaited in the goal-box. Despite unrestricted home-cage chow and pre-feeding of the pellets, rats continued to traverse the alley and ate the goal-box pellets on a majority of test trials. Capaldi and Myers (1978) concluded that the persistence of running versus that of eating were largely unrelated, based on the observation that the groups that continued to run during test did not necessarily eat, and *vice versa*.

A later study employed a similar method in which training consisted of reinforced alley running trials, as described above, or direct placement into the goal-box with pellets inside (Capaldi, Davidson, & Myers, 1981). Two other groups were not pre-exposed to the

---

[3] Miller (1955) was perhaps most pessimistic about inferring anything from food consumption. He suggested it was informative only in long term studies of body weight regulation and energy balance, but too variable to accurately reflect hunger over the short-term.

alley or pellets. On test days rats were provided with 50 reward pellets immediately prior to placement in the alley. Although all groups ate pellets in pre-feeding, goal-box pellets were only eaten by the groups that had previously eaten pellets in the apparatus, and never by other groups. Later experiments in this paper showed that consumption in the goal-box was highly persistent: satiated rats ate 4 pellets in the goal-box on each of four daily trials over at least 10 test days. However, 'satiation' consisted of unrestricted home-cage chow access but no pre-feeding of pellets[4].

Capaldi and colleagues explained their general finding – the continuation of running and eating despite multiple levels of satiation – by emphasising an associative process. Specifically, the presentation of pellets during pre-feeding and placement in the running alley were considered cues that elicited the consummatory response by 'force of habit' (Capaldi et al., 1978). A second process hypothesised to drive resistance to satiation was the rewarding effect of eating pellets while satiated (Capaldi et al., 1981). While these data certainly suggest that associative factors prolonged feeding, no studies appear to have ruled out the possibility that consumption would continue in *any* environment, such as other alleys never paired with pellets (Capaldi et al., 1981). Such tests were not necessary for the experimenters' purposes but limit the extent to which results can be confirmed as conditioned effects. Nonetheless, it is noteworthy that feeding after satiation persisted so reliably over multiple days.

1.3.3. Interim summary 2

The research discussed so far has covered experiments in which feeding was enhanced by various external social or environmental cues. Despite widely different aims and

---

[4] Another study by Capaldi and Myers (1979) compared resistance to satiation in rats that, after alley training under food deprivation and subsequent re-feeding, were satiated either with the reward pellets, chow mash, or chow alone. Consumption of pellets in tests declined over repeated tests in the pellet-satiated but not the chow- or mash-satiated groups.

methods, these studies are consistent in their use of feeding as a tool: measuring food intake was ideal for logistical reasons, but understanding the control of feeding was not the primary aim. Thus, the ubiquity of feeding behaviour across species made it ideal for studies of social facilitation. The relative ease of measuring and manipulating feeding made it a popular choice for testing predictions of drive theory and identifying the properties of resistance to satiation. Although associative explanations were often proposed to explain the outcomes, experiments often lacked the necessary control conditions. Learning and conditioning processes are difficult to isolate in the complex social interactions that characterise social facilitation studies, whereas the continuation of feeding in tests of resistance to satiation was rarely compared with feeding in other environments. Therefore, these literatures are best viewed as weak evidence for feeding driven by conditioned environmental cues.

1.4. Cues in their own right

1.4.1. Early studies

The idea that environments or stimuli associated with food could acquire the ability to stimulate feeding was acknowledged as early as a 1937 textbook of psychology, which explained how:

"*The baby who has acquired the habit of eating when offered fruit or candy, even in absence of hunger, will rapidly develop interest in the fruit or candy store, in doing this or that to earn the tidbits, in pennies that will procure them, in uncles who furnish the pennies.*" (Dashiell, 1937, p. 121.)

This quote is emblematic of the broad, anecdotal way in which external cues were acknowledged. It appears that because examples were relatable and easy to generate, what

comprised an 'external' cue was not defined rigorously but instead encompassed anything

other than internal hunger signals (Herman & Polivy, 2008).

Within the animal literature, until the late 1960s there were only sporadic attempts

(e.g. Drew, 1937) to test how initially neutral stimuli paired with food could affect feeding

behaviour. Unlike the research reviewed so far, the stated intention of these studies was to

study the control of feeding by learned cues. As for any field of study in its infancy,

terminology was varied, with food-paired cues and contexts alternatively referred to as

feeding-related stimuli (Valle, 1968), signals for feeding (Zamble, 1973), external events or

situational factors (Grant & Milgram, 1973), and eventually conditioned cues by Weingarten

(1983).

An early study by Drew (1937) reported an extensive set of tests on what external

factors could stimulate satiated rats to eat. The paper was impressive for its originality,

though no numerical data or statistical analyses were reported. The more unusual stimuli

tested were turning a tap on and off, making the rat tug on a piece of food held by the

experimenter, and allowing the rat to venture from its home-cage to retrieve pieces of food

situated on adjacent tables of various shapes. The only manipulation that reliably induced

feeding was placement in an environment where feeding had previously occurred. Drew

concluded that this likely constituted an ongoing habit[5] learned in rats' prior training in that

environment.

Three decades later Valle (1968) maintained rats on a restricted feeding regimen

wherein they were fed lab chow in a distinct context (white box) for 1-h/day for 15 days. Two

hours after the 15th training session, rats received a 10-min feeding test of pellet consumption

---

[5] The term 'habit' is defined today as a behaviour insensitive to changes in the value of its outcome and one driven by a simple stimulus-response mechanism (Balleine & Dickinson, 1998a). Drew's use of the term to describe persistent eating elicited by the environment is generally consistent with this definition.

in the home cage. During this interval rats either remained in the home cage or were re-exposed to the context for 15 or 60 min immediately prior to the test. The latter two groups ate significantly more pellets than rats that remained in the home-cage. Of interest, this effect was weaker in groups that had received additional handling at unpredictable times during training, suggesting that rats used handling as a food-predictive stimulus. A similar study exposed rats to a distinctive chamber containing food pellets for 30-min/day for 6 days (Grant & Milgram, 1973). Rats either had unrestricted access to pellets in the home-cage or were fed only in the context and for 30 min afterwards in the home cage. After 3 days of unrestricted food in the home cage, tests of pellet consumption in the context found that rats ate significantly more if they were previously exposed hungry rather than satiated (Grant & Milgram, 1973). However, these rats weighed significantly less than the satiated group at test, suggesting energy depletion may have contributed to their greater intake.

While Valle (1968) and Grant and Milgram (1973) used contexts paired with food, Zamble (1973) kept rats in their home-cages at all times and used a discrete cue procedure. Each day, animals were given a 20g food ration for 30 min at unpredictable times. For some rats, feeding was always preceded by turning off the light in the colony room for 15 min, while for other rats the offset of the light and feeding times were unpaired. Over 25 days, rats that received 'signalled' feeding sessions came to eat significantly more of their food ration and lost significantly less body weight than those given unpaired feeding. Experiment 2 compared forward and backward pairings of an auditory cue with feeding sessions separated by a highly variable interval (4-44 hours). Once again, the cue enhanced food intake only when it preceded food availability, leading to differences in weight change despite identical access to food in both groups[6].

---

[6] The experiment was terminated after 30 days due to dangerously low intake and high body weight loss in the group given backward pairings.

A study by Lovibond (1980) appears to be the first to demonstrate cue-potentiated feeding in a within-subjects design. During training, rats learned to associate two contexts with food and two with no food. Rats were exposed to one context in each pair while hungry, and to the other after minimal food deprivation. At test, rats ate significantly more in the two food-paired contexts, which also increased their general activity and rates of lever pressing. Lovibond (1980) suggested that the food-paired contexts aroused a single central appetitive system directed toward procuring food. His second experiment was based on the results of Zamble (1973) described above. During training rats were exposed to a cue (tone or light, counterbalanced) that predicted the subsequent presentation of food, while a second cue was not paired with food. In a series of consumption tests, the food-paired cue enhanced eating by 11% relative to the unpaired cue and by 13% relative to when no cue was presented. The modest size of potentiated feeding despite statistical significance is something many researchers have subsequently confirmed.

The first study of the pharmacological control of feeding driven by external cues was by Schallert, Pendergrass and Farrar (1982). For three weeks rats were fed twice per day using a distinct routine. At feeding times, the colony room light was switched off, a light adjacent to each cage turned on, and the experimenter offered pellets to the animal[7]. Tests assessed the effects of the satiety peptide cholecystokinin (CCK) on feeding in response to this sequence of food cues. Minimally deprived rats injected with saline began eating within half a minute, on average, and ate almost 5g of food. CCK significantly increased the latency to eat and reduced the amount eaten and time spent eating. It also inhibited food intake in rats not given conditioning. However, this study lacked a demonstration that the effects of the cues were associative, and could not have been produced by any arousing stimulus. The

---

[7] After repeated training sessions rats "…*reared up with their heads out of the cage and seized the first 10 g pellet from a forceps*" (p. 83).

authors argued that, under saline, rats given conditioning in Experiment 1 ate more than rats given random exposures to the cues in Experiment 2 while on free chow. However, this comparison was across experiments and confounded deprivation state with conditioning.

1.4.2. *Weingarten's model*

If not the first, then certainly the most influential, demonstration of cue-potentiated feeding was published in *Science* by Harvey Weingarten in 1983. Though his most renowned work on feeding occurred while in a faculty position within the Department of Psychology at McMaster University in Canada, Weingarten's graduate study at Yale under the supervision of Terry Powley was primarily physiological. He published on aspects of the cephalic phase response, particularly gastric acid secretion and its measurement (Weingarten & Powley, 1980a) and effects of hypothalamic lesions on its production (Weingarten & Powley, 1980b). Notably, one study reported a conditioned increase of gastric acid secretion in an environment paired with food (Weingarten & Powley, 1981), demonstrating an interest in applying principles of learning to understand the stimulus control of physiological processes.

Weingarten's seminal paper in 1983 reported the results of two experiments, each using 7 rats. Animals were housed individually and all training and testing occurred in the home cage. The training phase in Experiment 1 lasted 11 days and involved feeding rats six meals each day that were always preceded by a 270-s CS+ consisting of a light and buzzer compound. The liquid meal was rats' only food source and was delivered into a food-cup during the last 30-s of the cue. In the midpoint of the interval separating each meal (on average, 3.5 h) a pure tone was played to establish this cue as a CS-. Across training, latency to approach the food-cup after the onset of the CS+ fell rapidly to below 5-s. During the subsequent 21-day test phase rats had unrestricted access to the liquid diet from a bottle

within the cage. Once a day, Weingarten tested whether presentation of the CS+ and CS- cues elicited approach to the food-cup and consumption. Despite free access to food, rats continued to reliably approach the food-cup within 5-s of CS+ onset. The time spent exploring the food-cup during CS+ presentations and eating after its offset was comparable to the training phase. Presentations of the CS- had no such effects.

Weingarten's second experiment examined the contribution of cue-potentiated feeding to overall energy intake. After an identical training phase, rats were again allowed unrestricted access to the liquid diet from a bottle inserted into the cage. On some days, a single presentation of the CS+ was followed by delivery of a 15-ml meal to the food-cup. Total consumption was compared between these days and those on which no cue was played. Presenting the CS+ stimulated consumption that comprised 20% of rats' total daily energy intake. However, rats compensated for this cued meal by consuming less from the bottle on these days, such that total energy intake did not differ between days that contained a CS+ presentation and those that did not.

Two aspects of Weingarten's methods were particularly novel. The first was that stimulatory effects of the CS+ on consumption were demonstrated under conditions in which rats had simultaneous, unrestricted access to the same food from another source (the bottle). This was in contrast to studies in which consumption in the presence of food-paired cues or contexts was tested *after* a discrete satiation phase. Thus, testing in the home-cage removed the need for context shifts and the necessary handling that had been shown to contribute to conditioning effects (Valle, 1968). The second novel aspect of the paper was its analysis of the extent to which intake triggered by the CS+ contributed to total energy intake. For the first time, the effects of food cues were not evaluated solely in terms of a single test or against a given control condition, but within the longer-term context of that animal's energy balance. Weingarten concluded by suggesting the possibility that although his animals were

able to successfully compensate for cued meals, in other populations "…*persistent responding to conditioned cues would result in positive energy balance and obesity.*" (Weingarten, 1983, p. 432).

Full details of Weingarten's method and results appear to have been constrained by the condition of brevity that came with a publication in *Science*. The following year, however, Weingarten published a set of four experiments that further characterised his effect (Weingarten, 1984a). Each of these studies used an identical training procedure to Weingarten (1983) but varied aspects of the test phase, when the same liquid diet was available *ad-libitum*. An important detail clarified in the methods section is that the six meals provided during training provided 70% of rats' intake under *ad-libitum* conditions. This indicated that learning about the CS+ and CS- occurred in a state of hunger.

Experiment 1 in Weingarten (1984a) was actually the same experiment as that reported in his 1983 paper, but with far more data[8]. The main addition to the previously reported results was a demonstration that the latency to approach the food cup and feeding time in response to the CS+ did not differ significantly between the last day of conditioning and the last day of testing. This suggested that the magnitude of cue-induced feeding was robust over 21 days of testing, despite the free availability of food and the fact that CS- tests were also reinforced (rats often ate this meal, but well after the CS- played). Subsequent experiments showed that meals elicited by the CS+ were comparable in size to spontaneous meals from the *ad-lib* bottle (Experiment 2), even when the food cup contained stale milk from the previous day (Experiment 3). Experiment 4 replicated the earlier result that rats compensated for cue-elicited meals, regardless of whether the CS+ was played once or five

---

[8] This is suggested by the fact that mean starting body weight and the *t* statistics reported for anticipatory food-cup activity and feeding time are identical in Weingarten (1983) and Weingarten (1984a).

times per day. In the latter case meals triggered by the CS+ comprised 50% of daily energy

intake; however, not every cue presentation prompted intake (Weingarten, 1984a).

It was no surprise that Weingarten's first attempt to understand the mechanisms of the

effect tested the role of cephalic phase responses. Weingarten suggested that exposure to

conditioned cues elicited physiological responses in peripheral tissue, such as the secretion of

gastric acid and insulin. In turn, these were detected by the brain, which subsequently

initiated food-seeking behaviour. Weingarten's schematic of this mechanism is depicted in

Figure 1.1.
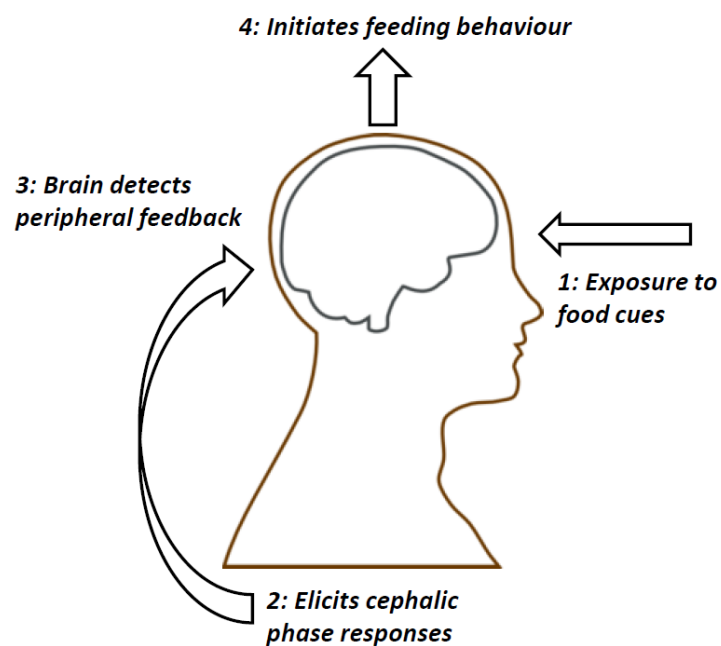
Figure 1.1. Weingarten's hypothesised mechanism for cephalic phase responses as the

critical physiological mediator of cue-potentiated feeding. Adapted from Weingarten

(1984b).

To test this, Weingarten (1984b) took advantage of the fact that cephalic phase responses are mediated primarily by cholinergic receptors. Therefore, after conditioning of the kind described above, rats received feeding tests preceded by an injection either of a cholinergic antagonist, atropine methyl nitrate, or saline. Contrary to his hypothesis, the latency to eat and amount eaten in response to the CS+ were no different under these conditions, indicating that feeding driven by conditioned cues did not depend on cholinergic aspects of the cephalic phase response. This null effect was unrelated to the efficacy of the dose of antagonist, which Weingarten showed was effective in inhibiting gastric acid secretion promoted by exogenous insulin. By contrast, pre-treating rats with CCK reduced meal size elicited by the cue and the time spent eating, but not anticipatory food-cup behaviour or the latency to eat (Weingarten, 1984b). Except for the latter measure, these results were consistent with the effects of CCK reported by Schallert et al. (1982) and with later studies of CCK's effects on instrumental responding (Balleine, Davies, & Dickinson, 1995).

Weingarten and Martin (1989) conducted additional tests of the mechanisms of cue-potentiated feeding. This paper explored the dissociation between the anticipatory and consummatory aspects of the effect; namely, food-cup behaviour in the presence of the cue (latency to approach and time spent exploring) and meal size. Conditioning an aversion to the liquid milk diet using injections of lithium chloride had no effect on food-cup activity during the presence of the cue, but reduced cue-induced meal size. This result was taken to indicate that presenting the cue did not evoke a specific representation of the paired food, but rather energised a general appetitive state geared toward food-seeking that ceased when rats tasted the now aversive-food (Weingarten & Martin, 1989, Experiment 1).

Experiments 2-4 explored whether cue-potentiated feeding was affected by a dopamine antagonist, α-flupentixol, the opioid antagonist naloxone, and an acute pre-meal of

the liquid diet delivered via oral gavage. Treatment with α-flupentixol blunted anticipatory food-cup activity but had no effect on meal size; conversely, naloxone reduced meal size but was without effect on food-cup activity. In each case, effects appeared to be dose-dependent. These results are consistent with many subsequent reports that place dopamine primarily as mediating incentive motivation processes and opiates as mediating the hedonic processing of rewards (Berridge, Robinson, & Aldridge, 2009). The final experiment demonstrated that both anticipatory and consummatory behaviour were reduced when rats were given 8 or 12ml of the liquid diet by gavage, indicating that satiety more completely suppressed behaviour than either pharmacological treatment. It is worth noting, though, that rats consumed 5.5ml even after the 12ml pre-meal.

1.4.3. Interim summary 3

In four studies spanning 6 years, Weingarten had established a well-controlled model of cue-potentiated feeding and identified several of its key behavioural properties. When considering its substantial influence, it is surprising that Weingarten's experimental work on cue-potentiated feeding appeared to end in 1989. His publication history thereafter suggests a greater focus on research involving people, including a demonstration of eating after satiation in undergraduate students (Cornell, Rodin, & Weingarten, 1989) and broadening to topics such as food cravings (e.g. Weingarten & Elston, 1990; 1991) and sweet taste responsivity (Looy & Weingarten, 1992; Looy, Callaghan, & Weingarten, 1992) with fewer animal studies (e.g. Warwick & Weingarten, 1995). Weingarten's research directly inspired at least one study of cue-potentiated feeding by Birch, McPhee, Sullivan, and Johnson (1989), who found that contexts paired with food enhanced consumption in preschool children. In rats, however, there would be little further published research on cue-potentiated feeding until the late 1990s.

1.5. Contribution to theories of food intake

At this point it is timely to consider these results within their broader context. The demonstrations of cue-potentiated feeding described so far were one of several types of findings that challenged the prevailing homeostatic theories of food intake and weight regulation that dominated much of the 20th century. These homeostatic, 'set-point' or 'depletion' models centred on the notion that physiological mechanisms triggered food intake in the short-term and regulated body weight over the longer term. For example, Mayer (1955) made the very influential proposal that eating was initiated by changes in glucose utilisation (this he termed the *glucostatic* theory), and that body fat stores mediated body weight over the longer term (a more tentatively suggested *lipostatic* hypothesis) (Mayer, 1955). Some years later, Le Magnen (1981) proposed that an acute drop in plasma glucose triggered the initiation of a meal, and that periodic shifts between the storing and burning of body fat stores explained rats' tendency to feed mostly during the dark phase[9]. Homeostatic models often proposed that the control of feeding was mediated by the hypothalamus. Such proposals followed demonstrations of hyperphagia and obesity produced by lesions to the ventromedial sub-region (Kennedy, 1950), or by electrical or chemical stimulation of the *lateral* hypothalamus (e.g. Epstein, 1960; Grossman, 1960), contrasted with the complete inanition produced by lesions to this region (Anand & Brobeck, 1952).

The main criticism of purely homeostatic models was that they failed to account for the many, varied circumstances in which rats could be prompted to eat (Kanarek, 1981; Toates, 1981). Moreover, others reported that manipulating levels of endogenous glucose failed to induce meals under certain conditions (Friedman, 1981; David, 1981; Woods & Ramsay, 2000) and that predictions that were supported in animals maintained under severe

---

[9] This 'dual-periodicity' account stimulated a great deal of interest, as evidenced by the 23 commentaries that it generated from eminent feeding researchers from all disciplines of science (see Le Magnen, 1981).

food deprivation did not hold in animals fed *ad-libitum* (Weingarten, 1985). Together, this suggested that homeostatic mechanisms were one of several interacting determinants of feeding behaviour, and directed attention towards external factors.

The first and most influential theory of internal and external cues was developed by Stanley Schacter in the 1960s. Schacter proposed that obesity was characterised by a reliance on external cues to initiate eating, resulting from an inability to sense internal signals of hunger and satiety. Schacter and his students devised a range of experimental situations in which normal-weight participants reduced their food intake (often of crackers and under the guise of taste tests) after various manipulations, such as pre-feeding with sandwiches, the threat of an upcoming unavoidable shock and being led to believe dinnertime had arrived by manipulating a clock in the test room (Schacter, 1968). In each case, consumption by obese participants was either unaffected by the external cue or, in the latter clock study, *increased*. Schacter reported similar findings across a range of observational, situational and experimental studies (for review, see Schacter, 1971).

As with homeostatic theories, however, Schacter's theory was criticised for being overly simplistic: internal and external control over feeding was not a dichotomy, and sensitivity to one form of cue over the other was not determined only by weight (Rodin, 1981). For example, Meyers and Stunkard (1980) reported that normal-weight, overweight and obese individuals were equally susceptible to the influence of external cues on dessert choices in an observational setting at a hospital cafeteria. Several of Schacter's students proposed modifications to his original hypothesis that described ways in which internal and external cues could interact, and distinguishing between types of external cues (see Herman & Polivy, 2008, for review).

Theories of feeding developed outside the Schacter laboratory argued that feeding was best explained by interactions between internal and external cues (e.g. Toates, 1981). The appeal to learning and conditioning processes was often based on processes such as flavour-nutrient learning, conditioned satiety and sensory-specific satiety (Bellisle, 1979; Booth, 1981; Rolls et al., 1981) and did not consider feeding elicited by conditioned cues. Indeed, the study of cue-potentiated feeding was only just beginning. Weingarten's own 'two-factor' theory proposed that two separate hunger systems had independent and interactive effects on feeding. The first, *internal* hunger was induced by energy depletion and was characterised by its slow onset and non-specificity; the second, *external*, hunger was produced by exposure to conditioned cues which rapidly provoked a desire for a specific food (Weingarten, 1985). This distinction resembles more recent descriptions of homeostatic versus hedonic feeding systems (e.g. Saper, Chou, & Elmquist, 2002) and distinct 'metabolic' versus 'cognitive' brain systems thought to underlie them (Berthoud, 2007, 2012).

Interest in external cues was heightened by the emergence in the late 20th century of obesity and metabolic disease as major public health challenges. Many suggested that such a rapid increase must relate more to environmental than genetic causes. The term 'obesogenic environment' was coined in 1999 to describe the myriad ways in which technological, social and environmental features of modern societies serve to promote energy intake and reduce energy expenditure (Swinburn et al., 1999). Among the most noteworthy features of so-called obesogenic environments is their ready access to foods that are highly palatable, calorically dense, and signalled by salient and ubiquitous cues (Hetherington, 2007). It follows that overeating – and long-term weight gain – is more likely in societies saturated with cues and environments signalling food, and in which far less energy need be expended to obtain it. This is an appealing argument in evolutionary terms, as outlined by Berthoud (2007):

"These forebrain systems evolved to engage powerful emotions for guaranteed supply and ingestion of beneficial foods from a sparse and often hostile environment. They are now simply overwhelmed with an abundance of food and food cues that is no longer interrupted by frequent famines." (Berthoud, 2007, p. 486.)

Modern theories express this basic idea in various ways that ascribe slightly different roles for external cues. In their 'boundary model', Herman and Polivy (1984, 2005) proposed that external cues were the primary determinants of food intake, aside from instances of complete satiation or significant hunger (i.e., the boundaries). A similar theory suggested that environmental cues only influence body weight within a 'settling zone' determined by biology (Levitsky, 2005). Other accounts propose that responding to food cues established through Pavlovian conditioning is an adaptive process that helps organisms prepare for meals (Pavlov, 1927; Woods & Ramsay, 2000). De Castro (1996, 2010) has argued that environmental cues are the primary determinant of food intake, and that physiological mechanisms exert subtle effects that are evident only over the longer term. Davidson and colleagues suggest that food cues act within a vicious cycle of obesity and cognitive decline mediated by the hippocampus. In their model, food cues prompt consumption of palatable but ultimately unhealthy foods that promote obesity and impair hippocampal function; in turn, individuals are less able to inhibit responding to food cues and overeat further (Davidson, Kanoski, Walls, & Jarrard, 2005; Davidson, Sample, & Swithers, 2014; see also Parent, 2016). In sum, it is clear that most contemporary theories of food intake and obesity assign an important role for external cues.

1.6. Current cue-potentiated feeding paradigms

Most research on cue-potentiated feeding over the past two decades has come from the laboratories of Peter Holland and Gorica Petrovich. Their paradigm was initially developed as part of a broader project in collaboration with Michela Gallagher assessing the role of the amygdala in various learning tasks (Holland & Petrovich, 2005). To avoid repetition, the training and test procedures that are common to most studies by these researchers will be described first; herein, these are collectively referred to as the Holland-Petrovich method. The Holland-Petrovich method has been used most fruitfully to identify the neurocircuitry underlying cue-potentiated feeding. Various experiments have also tested the importance of procedural variables and behavioural manipulations. Greater attention will be given to this latter class of variables, since these form the focus of the present thesis. Studies from other groups will be integrated where relevant.

1.6.1.   The Holland-Petrovich method

The Holland-Petrovich method resembles Weingarten's procedure in that rats are trained hungry and tested satiated. During training rats are typically housed individually and maintained at 85% of free-feeding weights. Unlike Weingarten's studies, daily training sessions occur in conditioning chambers and not in the home-cage. Pavlovian training typically begins with two days in which an auditory cue (most often a tone or white noise) is established as a CS+ by delivering two 45-mg pellets at the end of a 10-s stimulus presentation. Subsequently, rats undergo 10-11 days of discrimination training in which CS+ presentations are intermixed with non-reinforced presentations of a CS- cue (white noise or tone). Some studies employ between-subjects designs in which a single cue is rewarded for a *Paired* group, whereas an *Unpaired* group receive equivalent, non-contingent presentations of the cue and pellets. The emergence of conditioning over training is indexed by the increase

in anticipatory magazine entries during the CS+, relative to the CS- or to rats given unpaired training.

After training rats receive unrestricted access to chow in the home cage for 7-14 days. This allows body weight to return to pre-training levels and reduces the extent to which rats are hungry at test. Test days often begin with *ad-libitum* pre-feeding of the test food, pellets, before the cue/s are tested. This additional satiation is implemented because the pellets are palatable and readily eaten by non-deprived rats; thus, reducing otherwise high consumption should yield clearer effects. For the pre-feeding treatment rats are placed in the conditioning chambers with an ample supply of pellets (e.g. 50) in the food-cup. After some time, typically 5-10 min, rats and any remaining pellets are removed. In some studies, this pre-feeding procedure is repeated a second time; in others, rats have already been pre-fed in home cages. Finally, rats are returned to the chamber where a large number of pellets are now available for a 5-10 min test containing ten 10-s presentations of the cue.

Cue-potentiated feeding is demonstrated by higher consumption in the CS+ test relative to the CS- test in within-subjects designs or relative to rats given unpaired training in between-subjects designs. Because of the multiple preceding pre-feeding sessions, consumption in the cued test is often small in absolute terms, though statistically robust. Thus, in contrast to Weingarten's model, in which the CS+ triggered spontaneous meals in free-feeding rats, the Holland-Petrovich method provides stricter control over food intake immediately prior to the test to show that cues prolong or extend consumption despite ample pre-feeding. This is reflected in terminology: whereas Weingarten referred to meals elicited or *induced* by cues, the effect is often described as cue-*potentiated* feeding by Holland and Petrovich. This latter term will be used herein for consistency and is abbreviated as CPF.

Holland, Petrovich and Gallagher (2002) used this method to compare CPF between rats with lesions to the basolateral (BLA) or central nuclei (CeA) of the amygdala. BLA-lesioned rats failed to exhibit CPF, despite comparable training performance and pre-feeding intakes to the CeA- and sham-surgery groups, which each ate more in the presence of the CS+. However, BLA-lesioned rats exhibited the shortest latency to enter the food cup during the CS+ test. Notably, the magnitude of CPF was similar regardless of whether the pellets were presented in the food-cup (i.e. the trained location) or in a bowl on the other side of the chamber, suggesting that the effect was not driven by conditioned approach behaviour.

Holland and Gallagher (2003) replicated this result in a study that also tested instrumental responding for the pellet reward signalled by the cues – i.e., Pavlovian-to-instrumental transfer (PIT). After Pavlovian training in which cues were presented for 2-min (rather than the standard 10 s), BLA-lesioned rats showed no elevation in feeding to the CS+, unlike CeA- and sham-lesioned rats. By contrast, the effects of the lesions were reversed during PIT tests, where instrumental responding during the CS+ was elevated for BLA- and sham-lesioned but impaired in CeA-lesioned rats[10]. Experiment 2 replicated this double dissociation when 10-s, rather than 2-min, stimuli presentations were used in Pavlovian training and PIT testing. Several other studies confirmed that CPF is impaired by BLA lesions (Holland, Hatfield, & Gallagher, 2001[11]; Galarce, McDannald, & Holland, 2010) and is unaffected by lesions to the CeA (Holland & Hsu, 2014).

---

[10] Similar effects of BLA and CeA lesions have been found on tasks measuring fear-induced *inhibition* of feeding, as measured by the consumption of food in the presence of a tone previously paired with shock (Petrovich & Lougee, 2011; Petrovich, Ross, Mody, Holland, & Gallagher, 2009).

[11] In that study, the cue that ultimately served as the CS+ was originally only weakly associated with food reward within a more complex serial conditioning procedure designed to assess attentional processing. It was then re-trained as a first-order CS+ and subsequent tests showed a robust CPF effect in sham-operated rats that was blunted in those with BLA lesions.

Petrovich, Setlow, Holland, and Gallagher (2002) demonstrated that the role of the BLA in CPF depended on its connections with the lateral hypothalamus (LHA). Rats received lesions to the BLA and LHA either in opposing hemispheres or in the same hemisphere. Because LHA-BLA connections are mostly ipsilateral, this abolished functional connectivity for the former group but retained it in one hemisphere for the latter group, while equating total tissue damage (Petrovich et al., 2002). Rats with ipsilateral or sham lesions showed significantly greater intake in the presence of the CS+, whereas those with contralateral lesions ate similarly low amounts in CS+ and CS- tests. However, groups did not differ in a second-order conditioning task where a new (visual) cue was followed by presentations of the original (auditory) CS+. Therefore, BLA-LHA disconnection affected the ability of the CS+ to promote feeding but not to drive new learning (Petrovich et al., 2002).

Subsequent studies characterised the role of cortical regions in CPF. Petrovich, Holland, and Gallagher (2005) injected a retrograde tracer (FluoroGold) into the lateral hypothalamus on the day after standard Pavlovian training, allowing for projections to this area to be identified. Thirteen days later, eating in the presence of the CS+ and CS- was tested in two 5-min tests held on the same day, separated by 25-min and with order counterbalanced (CS- or CS+ test first). Rats were culled immediately after the second test. This arrangement ensured that the first and second cue tests were aligned with the maximum mRNA induction of two immediate-early genes, H1a and Arc, respectively. Rats ate significantly more in the CS+ than CS- test, despite their temporal proximity. Within the basolateral/basomedial amygdala and the orbitomedial frontal cortex, a significantly higher percentage of the total FluoroGold-positive neurons were H1a- or Arc-positive[12] during the

---

[12] FluroGold-positive neurons that were positive for both IEGs were recorded as 'nonselective', since this indicated the projection neuron was not specific to the CS+ or CS- test.

CS+ than in the CS- test. These results indicated that projections from these frontal regions to the LHA were activated during the potentiated feeding driven by the CS+.

Two other studies clarified the role of the frontal cortex in CPF. The first by McDannald, Saddoris, Gallagher and Holland (2005) showed that CPF was intact in rats given bilateral lesions of the lateral orbitofrontal cortex (OFC). However, OFC-lesioned rats were poorer in a differential outcome-expectancy task that tested lever pressing for distinct outcomes during various discriminative stimuli. Poorer performance by OFC-lesioned rats on this task suggested that CPF did not require the retrieval of specific outcome representations (McDannald, Saddoris, et al., 2005), a result with implications for the specificity of cue-potentiated feeding. Whereas McDannald, Saddoris and colleagues targeted the lateral OFC, another study found that lesions to the ventromedial prefrontal cortex that incorporated the *medial* OFC abolished CPF when a context (not cue) was paired with food (Petrovich, Ross, Holland, & Gallagher, 2007a).

More recent research has explored the peptide and neurotransmitter systems that mediate CPF. Unsurprisingly, a host of peptides involved in the regulation of feeding have been shown to influence the control of feeding by learned cues. The peptide orexin (also referred to as hypocretin) is produced within the lateral hypothalamus and appears important for the expression of CPF, as indicated by a study in which systemic injections of an orexin antagonist blocked the effect in rats (Cole, Mayer, & Petrovich, 2015). Another study using the Holland-Petrovich method adapted for the home-cage found that presentations of a conditioned food cue induced neuronal activation in orexin neurons within the lateral hypothalamus (Petrovich, Hobin, & Reppucci, 2012). The same study found no effects on LHA melanin-concentrating hormone (MCH) neurons, a result which contrasts two other studies reporting that deletion of the MCH-1 receptor blocked CPF in mice (Johnson, 2011; Sherwood, Holland, Adamantidis, & Johnson, 2015).

Several studies have assessed how CPF is moderated by ghrelin, an orexigenic peptide secreted from the stomach. One study using a method adapted from Weingarten's (1983) original protocol found that ghrelin microinjections to the ventral hippocampus enhanced the number of meals induced by a food-paired CS+ relative to a CS- paired with no food (Kanoski, Fortin, Ricks, & Grill, 2013). Of interest, CPF was not observed following vehicle injections, suggesting that the cue exerted only weak effects on feeding under baseline conditions. Walker, Ibia and Zigman (2012) found that oral administration of a ghrelin antagonist blocked CPF in mice. This was due to increased consumption during the CS- rather than reduced consumption during the CS+, a finding which is somewhat difficult to reconcile with ghrelin's function as an orexigenic peptide. A final study showed that peripheral administration of a ghrelin antagonist had no effect on CPF as measured in consumption, but delayed the onset of feeding in response to the CS+ relative to vehicle-treated rats (Dailey, Moran, Holland, & Johnson, 2016).

1.6.3. *Interim summary 4*

Lesion and neuroanatomical tracing studies have revealed that the lateral hypothalamus, basolateral and basomedial nuclei of the amygdala, and ventromedial prefrontal cortex are key structures that contribute to the expression of CPF. Study of the effects of feeding peptides on CPF have produced somewhat more mixed results, with this variability perhaps due to whether pharmacological interventions are region-specific or global (e.g. knockout models). In addition, the manipulation of feeding peptides appears to exert more subtle influences on the distribution of meal patterns as well as (or instead of) changes to absolute consumption. However, CPF is clearly sensitive both to manipulations of hormones generated in the CNS (e.g. orexin) as well as those produced by peripheral organs (e.g. ghrelin).

The specificity of CPF

In modern environments people receive broad exposure to a multitude of cues and environments associated with various foods. The extent to which these cues drive overeating depends, in part, on whether their effects are specific or general. That is, do cues prompt consumption only of the food they have previously signalled or of a wider range of foods? Studies in humans suggest that the effects of food cues may partly generalise to other foods, provided these are sufficiently similar. Two studies showed that after exposure to an olfactory pizza cue, participants reported greater desires to eat and larger anticipated portion sizes of not only pizza, but a range of other savoury foods (Ferriday & Brunstrom, 2008, 2011). Importantly, however, Ferriday and Brunstrom (2011) found that the desire to eat sweet foods was unaffected. This divide between sweet and savoury foods is consistent with earlier work: Cornell, Rodin and Weingarten (1989) found that eating a small bite of pizza or ice cream prompted greater consumption only of that food when both were subsequently available to consume *ad-libitum*[13]. Similarly, a study of restrained eaters found that the smell of pizza or cookies selectively enhanced consumption only of that food (Federoff, Polivy, & Herman, 2003).

Most research in rats has found that CPF is *selective* or *specific* to the training food. Few early experiments tested this issue, often because the paired food in training was also rats' maintenance diet (e.g. Zamble, 1973; Weingarten, 1983). More recently, two studies by Petrovich and colleagues indicated that CPF was specific to the training food (Petrovich et al., 2007a; Petrovich, Ross, Gallagher, & Holland, 2007b). The method used in these studies differed in several important respects from the standard Holland-Petrovich procedure. First, rather than discrete auditory cues, the conditioning chamber itself served as the conditioned

---

[13] However, the effect was far stronger when pizza was the primed food than ice cream. Cornell and colleagues suggested that pizza may have constituted a more salient cue (seemingly due to the smell).

stimulus. Training consisted of six 10-min exposures to the context, which contained pellets for Paired groups and no food for Unpaired groups. Second, the 7g of reward pellets (approximately 150) presented in the context for Paired groups far exceeded the total amount earned in discrete cue studies. Third, a milder deprivation schedule was used wherein rats received unrestricted chow in the home cage for 24-h after each training session. Food was removed the following day so that rats were then hungry for training the following day. Finally, the interval between the end of training and tests was shorter (2-3 days) than the standard 7-14 days, presumably because there was no weight loss from which to recover.

Petrovich et al. (2007a) held four CPF tests on consecutive days. No pre-feeding was conducted prior to placement in the context for the 10-min test. The first and fourth tests measured consumption of the pellets used in training and found significantly greater consumption in rats given Paired training. Critically, the second test measured consumption of a novel pellet formula, while the third test presented an alternative but familiar food, home-cage chow. No CPF effect was found in either of these two tests; instead, consumption was minimal in both groups. This suggested that CPF was specific to the food paired with the context during training (Petrovich et al., 2007a).

The second demonstration of specificity by Petrovich et al. (2007b) also used chow as the alternative, familiar food. Rats received three CPF tests that measured pellets, then chow, then pellets once again (Petrovich et al., 2007b). Here, however, the context test lasted 20-min and rats were removed so that fresh food could be inserted after 10-min. Once again, consumption was higher in Paired than Unpaired rats when the test food was pellets, but consumption was at floor when chow was tested. Removing rats after the halfway point of tests also allowed for examination of the time-course of CPF. Of interest, the difference between Paired and Unpaired groups was more pronounced in the second half of the test,

with comparably high intakes of pellets in the first 10-min (Petrovich et al., 2007b). This would appear to support the rationale for pre-feeding used in other studies.

In contrast to the above results are those from a context conditioning study by Boggiano, Dorsey, Thomas, and Murdaugh (2009). In the first phase of this experiment rats received seven 24-h exposures to a distinct 'Cookie cage' containing Oreos, chow, and water, distributed over 22 days in which rats otherwise had unrestricted chow and water in the home cage. Tests found that chow consumption in the Cookie cage over 24-h was greater than in the home cage, and that providing a morsel of Oreo (2g) enhanced this effect. Subsequently, the same rats were re-trained in a better-controlled design where seven 4-h exposures to the Cookie cage were intermixed with seven 4-h exposures to a distinctly marked Chow cage over 14 days. In tests, rats ate significantly more chow in the Cookie than in the Chow cage after 4 and 24-h. Providing a 2g morsel of Oreo reduced consumption of chow in the Chow cage, but not in the Cookie cage. Boggiano and colleagues hypothesised that placement in the Cookie cage blunted rats' satiety responses, fostering greater consumption of chow in this environment.

A caveat for interpreting these results as evidence against the specificity of CPF is that chow was provided in the Cookie and Chow cages in training[14]. Their results are also inconsistent with two studies from the Petrovich lab that found CPF to be specific when pellets and chow were available concurrently during tests of similar duration. These studies employed between-subjects designs and administered all training and testing in the home-cage. During training, home cages were brought into the conditioning room and 10 x 10-s presentations of a cue were made over 5-min, either paired or unpaired with pellets (the latter

---

[14] It seems likely that at least some chow was consumed during the 4 to 24-hr conditioning sessions (although these data were not reported) and, consequently, that the Cookie cage was also associated with chow, if only weakly. Therefore, it is difficult to conclude whether the overeating of chow constitutes a non-specific CPF effect.

group received pellets after a delay). In the first study, pellet intake stimulated by the cue was greater in rats given Paired training, with minimal chow intake by both groups over the 75-min test (Petrovich et al., 2012). Similarly, the second study found that exposure to the cue enhanced pellet but not chow consumption over a 4-h test, but only when rats were tested satiated and not food-deprived, when consumption was high in both groups (Reppucci & Petrovich, 2012). Experiment 2 in this paper showed that the CPF effect persisted for the first two of four tests over a 2-week period; increased intake by the Unpaired group nullified the effect on latter tests.

Whereas these studies showed a clear failure for CPF to transfer to chow, the absence of the effect in such tests is typically because consumption is minimal in both groups or conditions. This may suggest that the potential to detect CPF effects is constrained by the low palatability of chow relative to the reward pellets paired with the cue. Stronger evidence for the specificity of CPF comes from a series of experiments from the Holland laboratory that have paired two auditory cues with sucrose and maltodextrin solutions. Two key procedural differences in this work are that (1) during training the liquid rewards are presented intermittently during longer-duration CS presentations (typically 2-min), rather than at the offset of the CS+ as described previously; and (2) CPF tests compare the *rate* of consumption of one of the rewards in the presence of its predictive CS, the CS paired with the alternate reward, and with no cue. Studies using this method have shown that consumption of each reward is enhanced only by its predictive CS and not by the CS that predicts the alternative reward (Galarce, Crombag, & Holland, 2007; Delamater & Holland, 2008; Galarce & Holland, 2009; Holland, 2014; Holland & Hsu, 2014).

The type of specificity found in these experiments is often also shown in instrumental responding. Thus, if animals are trained to perform two instrumental responses for the two rewards, presentation of the Pavlovian cues increases responding only of the action earning

the same reward; i.e., specific Pavlovian-to-instrumental transfer (PIT) (e.g. Corbit &

Balleine, 2005). However, inhibitory associations between each cue and the *absence* of the

other reward also contribute to the expression of specific PIT (Laurent & Balleine, 2015).

Additionally, presenting a cue paired with an outcome not used as a reward in instrumental

training can produce a general elevation in responding (Corbit & Balleine, 2005). These

results indicate that the associative history of a food can influence the circumstances in which

animals will work for and consume it in the presence of other cues. In the case of CPF, it

remains to be seen how food cues affect consumption of alternative foods that are without

associations with other cues, and which are of similar palatability and familiarity to the paired

food. Indeed, evidence from studies in humans suggests that the effects of food cue exposure

can generalise to alternatives.

1.7. Summary and outline of the present thesis

Three broad research questions shaped the experiments reported in this thesis. First, to

what extent is CPF specific to the training food, and under what conditions might this

specificity be overcome? Second, are all animals equally susceptible to overeating triggered

by food cues, and can vulnerability to CPF be predicted on an individual or a group level?

Third, how do motivational manipulations and procedural parameters chosen for training and

test affect the expression of CPF?

These aims are addressed in various ways across the following experimental chapters.

Chapter 2 reports three experiments that establish a protocol for studying CPF in which

contexts are paired with palatable foods, and which test the effects of variety on CPF.

Experiments in **Chapter 3** explore whether individual differences in eating behaviour can

predict susceptibility to CPF. **Chapter 4** reports a series of experiments in which

methodological changes that seemed likely to enhance the CPF effect failed to do so. These results are discussed with reference to incentive contrast. **Chapter 5** presents an experiment assessing the effects of diet-induced obesity on CPF. The present results are integrated with existing research and directions for future research are outlined in **Chapter 6**.

Chapter 2: Variety and CPF

Note: a manuscript reporting the three experiments in this chapter has been accepted for

publication in the Journal of Experiment Psychology: Animal Learning and Cognition.

2.0. Introduction

The experiments in Chapter 2 studied the specificity of CPF using a protocol in which contexts were trained as food-paired stimuli. Experiments applied within-subjects designs in which a 'Plus' context was paired with palatable food and a 'Minus' context was paired with no food or chow. As outlined in Chapter 1, evidence that CPF is specific to the training food comes from two forms of results. First, several studies have demonstrated that food-paired cues or contexts do not augment intake of chow or novel pellet varieties (Petrovich et al., 2007a, 2007b, 2012). Second, experiments involving two cues paired with separate liquid rewards show that consumption of each reward is enhanced or sustained selectively by its predictive cue (Galarce et al., 2007; Delamater & Holland, 2008).

A general observation that inspired the present experiments was that modern food environments contain a wide range of palatable foods that are signalled by salient cues (e.g. signs and advertisements) and which are often available alongside one another in distinct environments. Therefore, exposure to a food cue rarely involves access only to that food; to the contrary, it would seem we are just as often exposed to multiple food cues with multiple foods available. This prompted two research questions that focused on the specificity of CPF. The first was whether CPF would be specific when testing an alternative food that was palatable and familiar. The second was how a context paired with a variety of foods would affect the size and specificity of CPF.

Experiment 2.1 examined whether the use of contexts as food-paired stimuli provided suitable conditions to observe CPF. Thus, after context conditioning, an initial CPF test measured consumption of the training food in the contexts. The specificity of CPF was then addressed in a second test that measured consumption of an alternative palatable food. The effects of variety were explored in Experiment 2.2, which focused on the potential for CPF to 'transfer' to an alternative food. Experiment 2.3 replicated and extended the results from Experiment 2.2 with a minor change in procedure.

2.1. Experiment 2.1: CPF using contexts

Experiment 2.1 trained food-deprived rats to associate one environment with consumption of a palatable food, and another environment with no food. In keeping with most past CPF research, rats were then given unrestricted access to chow in home cages prior to tests. The measure of CPF was whether, after pre-feeding the training food, consumption would be potentiated in the Plus context relative to the Minus context. To address the specificity of this effect, a second set of tests then measured consumption of a palatable and familiar alternative food.

2.1.1. Method

Subjects

Sixteen female adult Long-Evans rats were purchased from the University of Adelaide. We chose female animals because evidence in humans indicates that reactivity to food cues is more strongly associated with binge eating and body weight in females than in males (Sobik, Hutchison, & Craighead, 2005). Nonetheless, the use of female animals is rare in animal CPF experiments and will be considered in Chapter 6 (General discussion). Mean

body weight was 301g (range: 246 – 411g) at the beginning of the experiment. They were housed 4 per cage within a temperature- and humidity-controlled colony room maintained on a 12:12 reverse dark:light cycle (lights off at 0900h). For one week prior to experimental procedures animals were handled regularly and a restricted feeding schedule (10-13g/rat/day) was introduced. The maintenance diet was laboratory chow (*"Rat and Mouse Cubes"*; 14.23 kJ/g; Specialty Feeds®; see Appendix A).

Apparatus

Training and testing was conducted in 12 identical operant chambers (Med Associates; East Fairfield, VT) housed within sound- and light-attenuating shells. A 3 W, 24 V houselight mounted on the top-centre of the wall opposite the levers and magazine provided illumination. Visual, tactile and olfactory dimensions of these chambers were manipulated to provide two distinct contexts, as shown in Appendix A. One context had striped walls, a rough sandpaper floor and was scented with 10% rosewater solution, while the second had spotted walls, smooth plastic floor and was scented with 10% vanilla essence solution (Queen, Australia). Wall decorations were black and white laminated paper fitted around the exterior of the chamber. Odour solutions were pipetted onto a folded paper towel and placed in the bedding tray below the floors on which the rats were placed. These two environments were assigned as Plus and Minus contexts in a counterbalanced fashion (*n* = 8 each configuration).

The palatable food paired with the Plus context during training was Froot Loops® (FL) (Kellogg's®; 16.33 kJ/g), a sweet cereal eaten readily by rats (e.g. Ahn & Phillips, 2012). The alternative palatable food was Banana bread® (BB) (Coles®, 13.5 kJ/g). A full list of foods used throughout this thesis is available in Appendix A.

Procedure

Training

Over the 12 days of training rats received six 30-min exposures each to the Plus and Minus contexts in an intermixed, semi-random order, such that rats received no more than two consecutive exposures to the same environment (order: MPPMMPMPPMMP). Context conditioning sessions were held between 1500 and 1700 hrs each day. In each Plus session, 16 FL (~4 g) were provided in a Petri dish centred against the side wall of the chamber. No food was available in the Minus context. During training rats were fed a daily chow ration (~12 g/rat) at least 30-min after the end of each session. After the twelfth training session rats were returned to free food for six days. The recovery of body weight was monitored during this time. On two of these days rats were familiarised for 20-min to the individual cages used for pre-feeding. These were plastic tubs (40 x 25 x 20 cm) with wire tops, and were located in a room adjacent to where training occurred.

Test

The first CPF test compared consumption of FL in the Plus and Minus contexts. The two test days were separated by 2 days and their order was counterbalanced. Each test began with 20-min access to a dish of 25-30 FL in individual feeding cages. Rats were then immediately transferred to the Plus or Minus context for a 30-min test, with a new dish of FL available. Consumption was measured after 10, 20, and 30 min by quickly removing and weighing the dish of FL. This typically took no more than 15-s per chamber; rats were not touched and remained in the context. After the second FL test, rats remained in their home cages for 2 days with free chow and water. In the afternoon of the second day rats were pre-exposed to the alternative food, BB, while being weighed. Each rat was fed 1g individually and all rats took and readily ate this piece within 1-min.

42

The second CPF test began the following day and compared BB consumption in the Plus and Minus contexts. The procedure was identical to Test 1 except that a single day of rest separated the two tests. Test order was counterbalanced such that rats were tested in the reverse configuration to that used in Test 1. A procedural error was that on the second test day the 8 rats due to be tested in their Minus context were tested in the Plus context a second time. These rats were tested in the Minus context after a day of home-cage rest. Data from the second Plus test were used for these rats because pre-feeding intakes on this test were more comparable to those prior to the Minus context test.

2.1.2. Results

Body weight

Food restriction during training reduced rats to an average of $93.6 \pm 0.6\%$ [SEM] of their free-feeding weights. Weight increased for all rats during the six days of re-feeding, although rats remained marginally lighter than their prior *ad-libitum* weights at the time of both tests (Test 1: $97.0 \pm .9\%$ and Test 2: $97.8 \pm .9\%$ of free-feeding weights).

Training

Figure 2.1.1 shows that FL consumption increased and then plateaued over the six Plus context sessions. In practice, almost all rats ate all the available FL by the end of training. This observation was supported by significant linear ($F(1, 15) = 39.15, p < .001$) and quadratic trends ($F(1, 15) = 7.49, p = .015$) in a repeated-measures ANOVA.

Experiment 2.1: Training
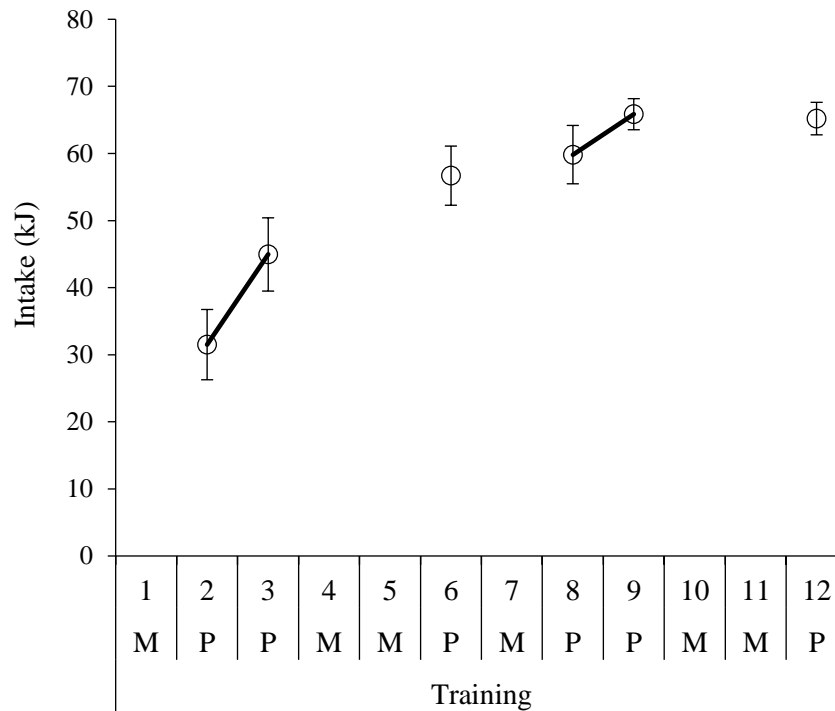
Figure 2.1.1. Training consumption in Experiment 2.1. Rats were provided with a dish of approximately 4g (~65 kJ) of FL in each Plus context training session (P). On other days, rats were exposed to a Minus context (M) where no food was available. Consumption of FL was nearly complete by the 6th Plus session.

Tests

Consumption during pre-feeding and in the contexts is shown in Figure 2.1.2. In each figure, consumption is collapsed across the two context tests and shows total intake after 30-min. Pre-feeding consumption prior to the Plus versus Minus tests was analysed in repeated-measures ANOVAs. Total intake in the 30-min context test was analysed using 2 x (2) mixed-ANOVAs (test order x [context]).

Consumption in the training food test is displayed in Figure 2.1.2.A. During pre-feeding rats ate $2.01 \pm .2$ g (32.7 kJ) prior to the Minus test and $2.49 \pm .3$ g (40.6 kJ) prior to the Plus test; this difference was statistically significant ($F(1, 15) = 6.11, p = .026$). This unexpected result appears to be a chance effect, since conditions prior to these two pre-

44

feeding periods were identical and test order was counterbalanced. Analysis of consumption in the test phase found a significant main effect of context ($F(1, 14) = 4.63$, $p = .049$), indicating higher consumption in the Plus than Minus context, despite higher intake in the pre-feeding period. The main effect of test order and the test order x context interaction were not significant (both $F < 1$). Further analyses indicated that although consumption was higher in the Plus context in each 10-min bin, the difference was not statistically significant in any bin individually (all $p > .17$; data not shown).

Consumption in the test of the alternative food is shown in Figure 2.1.2.B. Pre-feeding consumption prior to the Minus ($3.12 \pm .33$ g) and Plus tests ($3.11 \pm .30$ g) did not differ significantly ($F < 1$). Analysis of consumption in the test phase found no main effect of context ($F < 1$), no main effect of test order ($F(1, 14) = 3.88$, $p = .069$) and no context x test order interaction ($F < 1$). There was no effect of context within any of the three 10-min bins (all $p > .12$; data not shown).

Finally, data from the two tests were collapsed and analysed in a (2) x (2) within-subjects ANOVA, with context (Plus or Minus) and test type (training or alternative food) as factors. Consumption in kJ was used and test order was omitted given this factor did not interact in prior analyses, as described above. This analysis found a significant main effect of context was significant ($F(1, 15) = 6.85$, $p = .019$) but no main effect of test type ($F(1, 15) = 1.12$, $p = .31$) and no test type x context interaction ($F < 1$).

A) Training food **B) Alternative food**
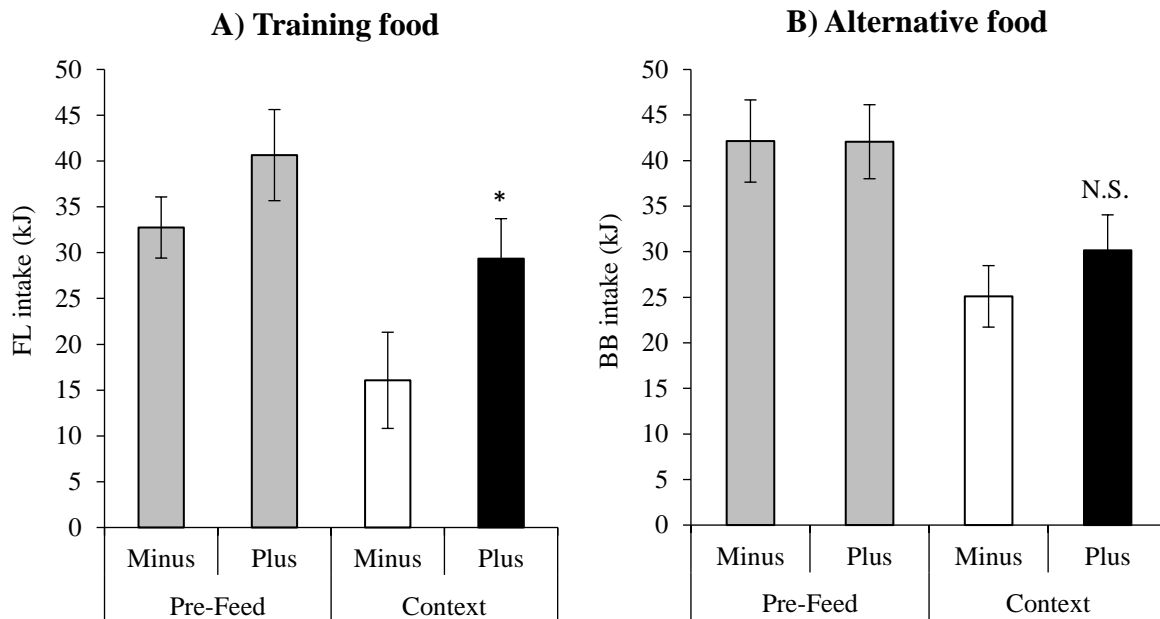
Figure 2.1.2. Experiment 2.1 test results. Panel A: Rats ate significantly more of the training food in the Plus than in the Minus context, despite higher consumption in pre-feeding prior to this test (*$p < .05$ for difference between Plus and Minus in both Pre-feed and Context phases). Panel B: Consumption of an alternative palatable food, Banana Bread, did not differ between contexts.

2.1.3. Discussion

Experiment 2.1 tested the specificity of CPF following training in which mildly hungry rats learned to associate one context with consumption of a palatable food, FL, and another with no food. Tests compared FL consumption in these 'Plus' and 'Minus' contexts after 6 days of free-feeding in home cages. In addition to free access to chow in the home cage, immediately prior to tests rats were satiated on the test food. The key demonstration of CPF was that rats ate almost twice as much when subsequently placed in the Plus context than when placed in the Minus context (see Fig. 2.1.2.A), despite eating somewhat more during the pre-feeding period prior to it.

Results of Test 2 indicated that consumption of an alternative palatable food, which was pre-exposed prior to testing, did not differ significantly between Plus and Minus

contexts. Caution should be taken when using this as evidence for the specificity of CPF because the interaction term from a combined analysis of the two tests was not statistically significant. Nonetheless, the absence of a CPF effect on this test is consistent with previous literature where food-paired cues or contexts selectively promote further consumption only of the food they signal during training (Petrovich et al., 2007a, 2007b, 2012; Delamater & Holland, 2008). Although past studies have demonstrated that CPF is robust over multiple tests without re-training (Reppucci & Petrovich, 2012; Petrovich et al., 2007a, 2007b), in the present experiment the order of the training food and alternative food tests was not counterbalanced. As such, the possibility that repeat testing contributed to the non-significant results in Test 2 cannot be dismissed entirely. For example, presenting food in the Minus context during Test 1 violated its past association with no food, perhaps explaining higher consumption in the Minus context in Test 2 than Test 1 (compare Fig. 2.1.2., panels A and B). Therefore, Experiment 2.2 focused on testing an alternative food in a design that also assessed the effects of variety on CPF.

2.2. Experiment 2.2: The effects of variety on CPF

Training in past CPF experiments has involved pairing each cue with a single food. Modern societies, however, are typified not only by an abundance of food cues, but also by enormous variety in food products and flavours. There would appear at least as many opportunities for food cues and environments to become associated with multiple foods as single ones. Shopping centre food courts, buffets, movie theatres, sporting events, and our own kitchens are but a few examples of 'contexts' that, now more than ever, may be associated with multiple foods. In the case of discrete cues, fast food signs for different brands – e.g. the archetypal 'golden arches' – could feasibly be associated with multiple foods, since many fast food chains feature ever-broadening menu choices. Even food cues

that are more proximal to eating itself might share common elements with several foods, such as the sound of a chip packet opening or of food sizzling on a barbecue. Perhaps the only sure form of 'specific' cue for any given food is the smell, sight, or taste of that food itself.

Providing a variety of foods either successively (i.e., one food after another) or simultaneously (i.e., multiple foods together) increases consumption in both people (e.g. Rolls et al., 1981) and in rats (e.g. Sclafani & Springer, 1976; Treit, Spetch, & Deutsch, 1983; Rolls, Van Duijvenvoorde, & Rowe, 1983; Warwick & Schiffman, 1991). Indeed, variety is so reliably obesogenic that animal models frequently employ 'cafeteria', 'western' or 'supermarket' diets, consisting of varied exposure to multiple palatable foods, to induce weight gain with no interest in variety *per se*. Additionally, instrumental responding can be enhanced when it is rewarded by a variety of outcomes, as shown in rats (Bouton, Todd, Miles, León, & Epstein, 2013; Thrailkill, Epstein, & Bouton, 2014), children (Temple, Giacomelli, Roemmich, & Epstein, 2008), and adults (Myers Ernst & Epstein, 2002).

To our knowledge, no studies have examined the interactions between food cues and exposure to variety on measures of feeding. In particular, it is currently unknown whether the size and specificity of CPF effects is altered by variety. That is, will an environment paired with multiple palatable foods enhance consumption more than one paired with only a single palatable food? In addition, will exposure to variety foster feeding on alternative foods? To this end, Experiment 2.2 compared CPF between groups for which the Plus context was paired with either one or three palatable foods, or with chow. All groups were also exposed to a Minus context containing no food, as in Experiment 2.1. To focus on the specificity of CPF under these conditions, we tested consumption of an alternative food that was pre-exposed prior to training but which was never previously presented in either training context.

The training procedure in Experiment 2.2 incorporated three changes as part of a less restrictive design. First, rats were not food-deprived during training. This allowed for CPF tests to be conducted more proximally to training. Second, we did not restrict the amount of palatable food available in Plus context training sessions. In CPF studies using discrete cues, rats typically receive very few rewards in daily training sessions whereas studies using contexts have tended to provide a larger but capped amount (e.g. 7g pellets; Petrovich et al., 2007a, 2007b) or unlimited food (Boggiano et al., 2009). We were interested in the extent to which consumption would continue to increase across training and whether the CPF effect might be larger following greater training consumption. The third change was that no pre-feeding was conducted prior to CPF tests. This precluded the possibility that variability or group differences in pre-feeding might obscure CPF or complicate the interpretation of effects, and allowed a direct assessment of the contexts' ability to promote consumption of an alternative food.

2.2.1. Method

Subjects

Thirty-one female Sprague-Dawley rats were used. At the beginning of the experiment mean body weight was 295g (range: 264 – 332g). Animals were previously used in unrelated conditioning experiments where discrete cues signalled the delivery of reward pellets. Neither the pellets nor the cues were used in the present experiments. Housing conditions were as described for Experiment 2.1, except that the colony room was maintained on a 12:12h light:dark cycle (lights on 0900 – 2100 hrs) and rats were tested during the light cycle.

Foods

Chow and water were available *ad-libitum* throughout all experimental procedures. The palatable foods (PF) paired with the Plus context were selected to provide a variety of tastes and textures. Thus, we used Oreo cookies® (Nabisco, USA, 20.33 kJ/g), Banana Bread® (Coles, Australia, 13.50 kJ/g), and Burger Rings® (herein 'Rings'; Smiths, Australia, 21.93 kJ/g) (see Appendix A). These foods were not pre-exposed prior to training. The test food for all rats was Froot Loops (FL), which was familiarised prior to training but never paired with the Plus context.

Design

The key manipulation was in terms of the number and type of foods that were paired with the Plus context. At the start of the experiment rats were randomly assigned to *Chow* (*n* = 10), *Single* (*n* = 11) and *Variety* (*n* = 10) groups. For rats in the *Single* group the Plus context was always paired with the same PF, such that four rats received Oreos, three received Burger Rings, and four received Banana Bread. For the *Variety* group the Plus context was paired with all 3 PF, with a single PF presented in each Plus session. Variety was provided successively rather than simultaneously because the latter scenario would likely increase intake relative to the *Single* group (Rolls et al., 1983; Treit et al., 1983). The order in which the 3 PF were presented over days for the *Variety* group was varied, such that subsets received "ABC", "BCA", and CAB" orders. Thus, on every Plus training day, all 3 PF were provided to similar numbers of rats in *Single* and *Variety* groups. We considered this important in order to more accurately compare training consumption between groups over days. The *Chow* group underwent identical training and test procedures but received standard chow in the Plus context. This assessed the extent to which CPF might be affected by nonspecific effects of exposure to PF.

Procedure

Froot Loop familiarisation

Several days prior to training, rats were familiarised to the test food, FL. Thirty FL were scattered in each home cage, and the experimenter confirmed each rat sampled them. On the next day, inspection of the cage bedding confirmed all were eaten.

Training

The preparation of the Plus and Minus contexts was as described in Experiment 2.1. Daily training sessions were 30-min in length and were held between 1400 and 1600 hrs. For 20 days rats received intermixed exposures to the Plus and Minus contexts (10 sessions in each context, order: MMPMPMPPMPMMPMMPPMPP). Training was longer than in Experiment 2.1 in order to increase exposure to the 3 foods for the *Variety* group. After the last training session rats were given a single day of rest in the home cage prior to tests.

Test

The CPF test compared consumption of FL in the Plus and Minus contexts, within-subjects. Each test was 30 min in length and no pre-feeding was conducted. Intake was not measured after 10 and 20 min since these data were not illuminating in Experiment 2.1. Test order was counterbalanced and the two test days were separated by a single day of rest in the home-cage.

2.2.2. Results

Training

Since the training foods differed in energy density, consumption in grams was converted to kJ and analysed in a 3 x (10) mixed-ANOVA (group x [session]). Consumption in Plus sessions during training is shown in Figure 2.2.1. *Single* and *Variety* groups steadily increased their intake of PF, while chow consumption in the *Chow* group remained low. These observations were confirmed by a significant linear trend over sessions ($F(1, 28) = 75.99$, $p < .001$), a significant group x session linear interaction trend ($F(2, 28) = 21.17$, $p < .001$), and a significant main effect of group ($F(2, 28) = 25.64$, $p < .001$). Post-hoc pairwise comparisons (Tukey HSD correction) showed that the *Chow* group consumed fewer kJ than both *Single* and *Variety* groups (both $p < .001$) but gave no indication that the latter two groups differed ($p = .93$). A separate analysis comparing *Single* and *Variety* groups failed to find any difference in their rates of increase in consumption ($F < 1$).

Experiment 2.2: Training
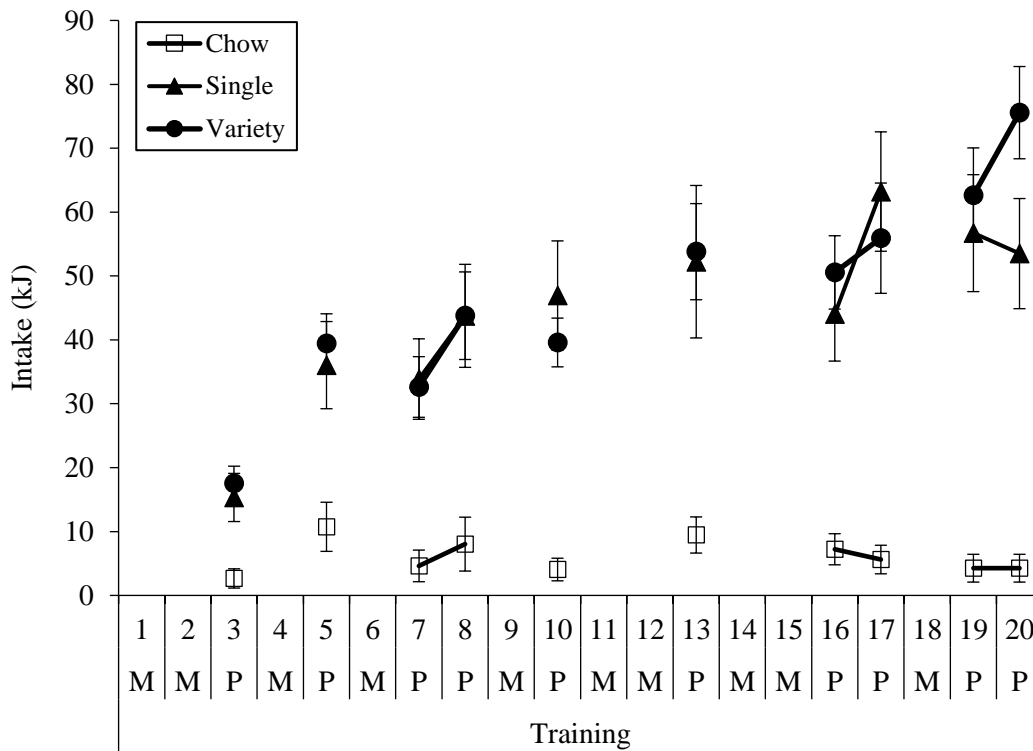
Figure 2.2.1. Training consumption in Experiment 2.2. *Single* and *Variety* groups steadily increased their consumption of PF during Plus training sessions (P) at a similar rate. *Chow* rats ate minimal amounts of lab chow. Intermixed with these Plus sessions were sessions in the Minus context (M) where no food was available.

Test

Compiled test data are shown in Figure 2.2.2 and were analysed in a 3 x 2 x (2) mixed-ANOVA (group x test order x [context]). This analysis found a significant main effect of context ($F(1, 25) = 5.04$, $p = .034$) and a significant group x context interaction ($F(2, 28) = 4.41$, $p = .023$). There was no main effect of group ($F(2, 25) = 1.75$, $p = .19$) or of test order ($F < 1$). The group x test order interaction was significant ($F(2, 25) = 3.75$, $p = .038$) but not the context x test order or group x context x test order interactions ($F(2, 25) = 3.76$, $p = .064$ and $F(2, 25) = 2.53$, $p = .10$, respectively). The context main effect and group x context interaction indicated that consumption was greater in the Plus than in the Minus context, but

53

that this effect differed between groups. Analyses of simple effects indicated that the

difference between consumption in the Plus and Minus contexts was not significant for the

Chow and *Single* groups (both $F < 1$), but that the *Variety* group ate significantly more in the

Plus than in the Minus context ($F(1, 9) = 11.80$, $p = .007$).
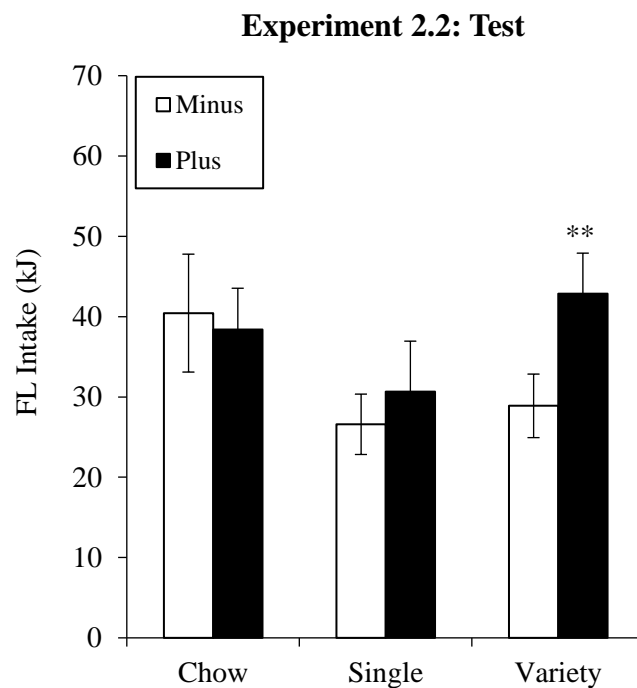
Experiment 2.2: Test

Figure 2.2.2. Experiment 2.2 test results. After pairing a Plus context with 1 (*Single*) or 3 (*Variety*) PF, or with chow (*Chow*), only the *Variety* group overate FL in this context relative to a Minus context paired with no food. **p < .01 refers to the effect of context in the *Variety* group.

2.2.3. Discussion

The key result from this experiment was that only the *Variety* group exhibited CPF

when an alternative food was tested, by eating significantly more FL in the Plus than in the

Minus context. This effect was not explained by differences in training consumption, since

the *Single* and *Variety* groups ate substantial and similar amounts of PF during training (see

Fig. 2.2.1). Furthermore, all groups were given equivalent pre-exposure to the test food prior to test. Therefore, the effect in the *Variety* group appears to relate to the provision of variety in this environment and not simply a history of eating more. Of note, the Plus context did not enhance consumption for the *Single* group, which received the same palatable food in every Plus training session. This group was effectively a replication of Experiment 2.1, where all rats were trained with a single food, and where no CPF effect was found on an alternative food. Therefore, the results are consistent with past CPF studies that have found the effect to be specific. The present result extends previous research by indicating that CPF does not transfer to an alternative food even when this food is familiar and palatable.

Finally, there was no CPF effect in the *Chow* group, which received laboratory chow in Plus sessions. With free access to chow in the home cage, *Chow* rats ate minimally during Plus sessions, far less than intake of palatable food by *Single* and *Variety* groups. At test, however, the *Chow* group ate ample amounts of Froot Loops in both contexts, with their consumption at least as high as other groups'. This may reflect positive contrast, such that the availability of a more palatable food at test was highly salient and drove high consumption in both contexts (whereas *Single* and *Variety* groups were accustomed to such highly palatable foods).

2.3. Experiment 2.3: Replication with chow in the Minus context

The first aim of Experiment 2.3 was to replicate the effect of variety on CPF observed in Experiment 2.2. A second aim was to test whether this 'variety effect' would transfer to a completely novel palatable food. If the variety manipulation facilitated a CPF effect on a familiar but not a novel palatable food, this would indicate that prior experience of consuming the test food is necessary for the effect. By contrast, observing CPF in the *Variety*

group on a novel food might suggest the effect of the manipulation involved, in part, preventing or blunting neophobia toward novel foods. To explore these possibilities, Experiment 2.3 first tested for CPF using a familiar food, Froot Loops, and then, after brief re-training of the contexts, tested consumption of a novel food in a second set of tests.

A minor procedural change was implemented after noting that in Experiment 2.2, the provision of unrestricted chow in home cages meant that Minus context training sessions were the only periods when rats could not eat. Although the training sessions were relatively brief, it nonetheless seemed possible that the Minus context may have become a signal for the absence of food. This possibility is important because past research indicates that cues or contexts paired with the absence of food can promote feeding if food is subsequently made available in their presence (Roitman et al., 2001; Galarce & Holland, 2009; Holland, 2014; Holland & Hsu, 2014). Consequently, we hypothesised that the absence of food in the Minus context during training may have enhanced consumption at test to a greater extent than would a more neutral environment.

A counter-argument to this possibility is the observation that the *Chow* group ate similarly in their (chow-paired) Plus and (empty) Minus contexts at test. However, this group only occasionally ate very small amounts of chow in training sessions, suggesting the distinction between the Plus and Minus contexts was minimal. By contrast, the absence of food in the Minus context during training was likely more salient for *Single* and *Variety* groups, which came to eat substantial amounts of food in the Plus context. In addition, placement in the Minus context followed a sequence of cues – transporting cages from the colony to the test room and necessary handling – that also preceded Plus sessions. This possibility was easily addressed by providing chow in the Minus context during training. Therefore, Experiment 2.3 sought to replicate and extend the results of Experiment 2.2 under

conditions where there was now no possibility that the Minus context was associated with the absence of food.

2.3.1. Method

Subjects

Forty adult female Sprague-Dawley rats were used. The experimental history of the rats and housing conditions were as described in Experiment 2.2 except that the colony room was maintained on a reverse cycle dark:light schedule (lights off 0900 – 2100 hrs) and rats were tested between 0930 and 1230 hrs each day. At the beginning of the experiment mean body weight was 301g (range: 273 – 331g).

Foods

The same palatable foods were used as in Experiment 2.2, except for Burger Rings. These were replaced with another savoury food, Sausage Roll® (Coles®, 11.1 kJ/g), after choice preference tests indicated that this food was more equally matched with Oreos and Banana Bread (see Experiment 3.2). As in Experiment 2.2, Test 1 measured consumption of FL. The novel palatable food presented in Test 2 was Mini Jam Roll® (Coles®, 11.95 kJ/g).

Design

At the beginning of the experiment rats were randomly assigned to *Chow* ($n = 12$), *Single* ($n = 14$), and *Variety* ($n = 14$) groups. The preparation of the Plus and Minus contexts was as described in Experiment 2.2.

Procedure

Froot Loop familiarisation

FL were pre-exposed in the home cage as described in Experiment 2.2.

Training

Twenty total training sessions were administered (10 exposures each to Plus and Minus contexts) in the following order: MPPMPMMPPMMPMMPPMPMP. As described, the main methodological change was that chow (15g or 4-5 pellets) was provided in a Petri dish in each Minus context session for all groups. Therefore, for the *Chow* group both "Plus" and "Minus" contexts contained chow. Of the 14 rats in the *Single* group, 5 received Oreos, 5 received Banana Bread and 4 received Sausage Roll during Plus sessions. The *Variety* group received access to all three foods, one per Plus session, with different sequences of exposure used for subsets of rats.

Tests

Test 1 measured FL consumption in the Plus and Minus contexts. After 18 days of training, rats remained in their home cages for one day before two CPF tests that were separated by a single day of rest. Each test lasted 30-min and no pre-feeding was conducted. The order in which contexts were tested was counterbalanced. After the second test day rats were left in home cages for a week of rest, and then received a Minus and Plus re-training session on consecutive days. For the Plus re-training session, rats in the *Variety* group received their second most-consumed food during training. Then, Test 2 measured consumption of the novel food, Jam Roll. The test procedure was identical to Test 1 except that the order in which contexts were tested was reversed (rats tested M->P in Test 1 were now tested P->M and *vice versa*).

2.3.2. Results

Training

Intakes in kilojoules are displayed in Figure 2.3.1 and were analysed in a 3 x (2) x (10) mixed-ANOVA (group x [context] x [session]). *Single* and *Variety* groups steadily increased their consumption of palatable foods in the Plus context and ate little chow in the Minus context. The *Chow* group ate similarly low amounts of chow in both contexts. These observations were supported statistically by significant linear interaction trends between session and context ($F(1, 37) = 62.84$, $p < .001$) and session, context, and group ($F(2, 37) = 20.78$, $p < .001$). Averaged over sessions, there were significant main effects of context ($F(1, 37) = 250.41$, $p < .001$), group ($F(2, 37) = 45.30$, $p < .001$) and a significant interaction between context and group ($F(2, 37) = 65.24$, $p < .001$). A separate analysis comparing Plus context consumption between *Single* and *Variety* groups found no significant difference in the increase in consumption across sessions (linear interaction trend: $F(1, 26) = 1.36$, $p = .26$; quadratic interaction trend: $F < 1$), and no main effect of group ($F < 1$).

Experiment 2.3: Training
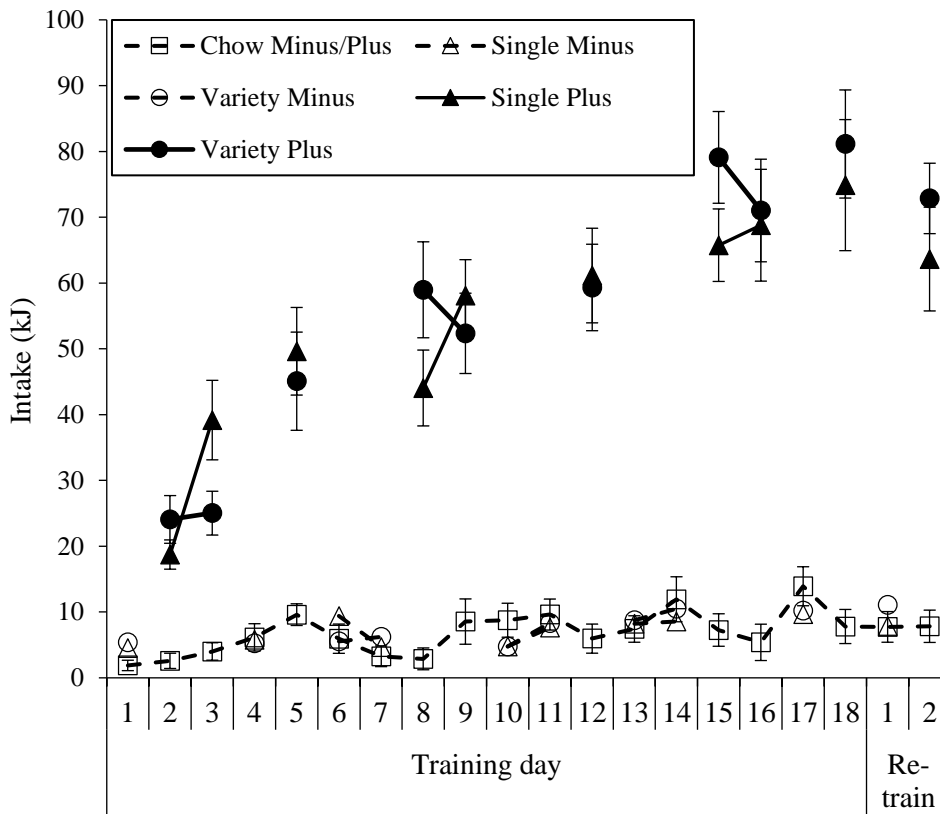
Figure 2.3.1. Training consumption in Experiment 2.3. Chow consumption in the Minus context is shown in unfilled symbols, while filled symbols show palatable food intake for the *Single* and *Variety* groups. Only one data series is used for the *Chow* group since both 'Plus' and 'Minus' contexts contained chow.

Tests

Consumption in Test 1 (FL) and Test 2 (novel food) is shown in Figure 2.3.2.A and 2.3.2.B, respectively. These data suggested that a CPF effect was exhibited only by the *Variety* group in both tests. To compare the strength of CPF between groups and across tests, compiled data were first analysed in a 3 x 2 x (2) x (2), (group x order x [test] x [context], mixed-ANOVA. This analysis revealed a significant interaction between context and group ($F(2, 34) = 5.57$, $p = .008$) and significant 3-way interactions between test, context and order ($F(1, 34) = 21.88$, $p < .001$) and between context, order and group ($F(2, 34) = 3.94$, $p = .029$). A main effect of test ($F(1, 34) = 40.89$, $p < .001$), suggested lower consumption in the novel

food than familiar food test; however, this appeared to be an artefact of energy density, since intakes in grams did not differ between tests ($F < 1$). No other main or interaction effects were significant (largest $F(1, 34) = 2.85$, $p > .10$).

To clarify the nature of the interaction between context and group, separate 2 x 2 x (2) ANOVAs assessed the effects of test (familiar or novel food), context, and order for each group. The main effect of context was significant for the *Variety* group ($F(1, 12) = 26.30$, $p < .001$, but not for the *Single* or *Chow* groups (both $F < 1.51$). Unexpectedly, the 3-way interaction between test, context, and order was significant for each group ($F(1, 10) = 6.45$, $p = .029$; $F(1, 12) = 6.49$, $p = .026$; $F(1, 12) = 9.61$, $p = .009$, for *Chow, Single* and *Variety* groups respectively), while the context x order interaction was significant for the *Chow* group ($F(1, 10) = 6.23$, $p = .032$) and *Variety* group ($F(1, 12) = 6.71$, $p = .024$). The main effect of test was significant within each group (reflecting the lower energy density of the novel food: $F(1, 10) = 23.69$, $p = .001$; $F(1, 12) = 10.39$, $p = .007$; $F(1, 12) = 8.81$, $p = .012$ for *Chow, Single* and *Variety* groups, respectively). No other main or interaction effects were significant.

The interactions involving test order were somewhat difficult to interpret from the above analysis, given that this factor identified rats that were tested Minus-Plus for Test 1 and Plus-Minus for Test 2 from those that were tested Plus-Minus for Test 1 and Minus-Plus for Test 2. The key result was that the context main effect was only significant for the *Variety* group. Therefore, the final analysis consisted of separate (2) x 2 ANOVAs ([context] x test order) for the *Variety* group on Test 1 and Test 2. At Test 1 the main effect of context was significant ($F(1, 12) = 5.78$, $p = .033$) with no context x order interaction and no main effect of order (both $F < 1$). At Test 2 the main effect of context was significant ($F(1, 12) = 13.61$, $p = .003$) as well as the context x order interaction ($F(1, 12) = 31.86$, $p < .001$) with no main effect of order ($F(1, 12) = 1.27$, $p = .28$). Inspection of the data indicated that the context x

order interaction reflected the tendency for consumption to increase over repeat tests. (This was also the case for order interactions within the *Single* and *Chow* groups.) Importantly, the Plus context significantly enhanced intake only for the *Variety* group despite the influence of test order.
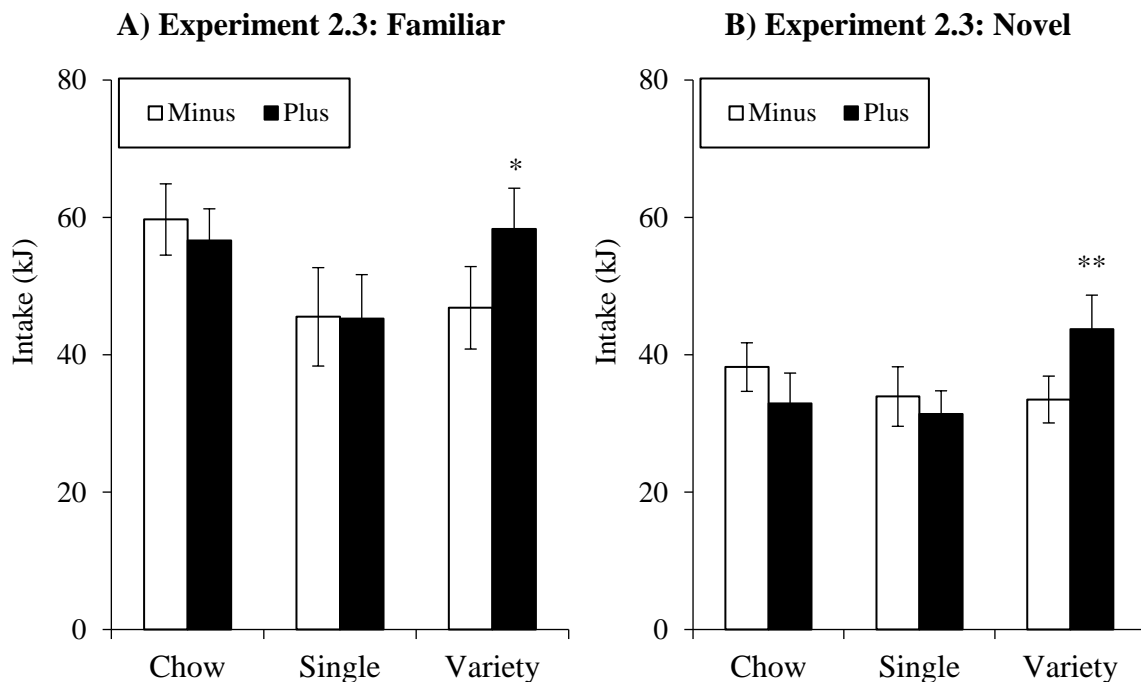
Figure 2.3.2. Experiment 2.3 test results. In both tests, only the *Variety* group ate significantly more of a familiar food (Panel A) and a novel food (Panel B) in the Plus context. *$p < .05$; **$p < .01$ for the effect of context within the *Variety* group.

2.3.3. Discussion

Experiment 2.3 aimed to replicate the effect of variety on CPF when chow was provided in Minus context training sessions. This change was made in light of research demonstrating that cues signalling the absence of food can stimulate intake when food is available. We hypothesised that providing chow in the Minus context during training might reduce consumption in this context during CPF tests. Results did not support this prediction:

although rats ate appreciable but small quantities of chow during Minus context training sessions, consumption in the Minus context in both tests remained substantial. The relatively brief duration of training sessions and free access to chow in the home cage were likely to make the absence of chow less salient than in previous studies using longer training sessions (Roitman et al., 2001) or utilising food deprivation (Galarce & Holland, 2009).

The results of CPF tests replicated those from Experiment 2.2. When testing a familiar food never previously exposed in the contexts, the Plus context enhanced consumption only in the *Variety* group. Consumption was unaffected by context for the *Single* group, despite a similar history of consuming significant amounts of palatable food in the Plus context. There was no opportunity to observe CPF in the *Chow* group, since the two training contexts both contained chow and differed only arbitrarily in their decoration. This group ate substantial amounts of FL in both contexts in Test 1, mirroring the result from Experiment 2.2 and reinforcing the idea that positive contrast contributed to this effect.

Notably, only the *Variety* group ate significantly more of a novel food in the Plus context in Test 2. This effect emerged despite an interaction with test order that reflected the tendency for consumption to increase across repeat tests. These results could suggest that the variety manipulation functioned by increasing acceptance of, or reducing neophobia towards, novel foods. However, the fact all groups readily ate the novel food suggests that effects of neophobia, if any, were confined to the early portion of the test. An alternative possibility is that exposure to variety produced a different form of learning within the context, perhaps encoding a more general association with eating rather than with the specific features of the foods used in training.

2.4. General discussion

The experiments in Chapter 2 tested CPF using a context conditioning protocol wherein animals were given access to palatable food within a distinct environment. To test CPF we compared consumption of palatable food in this 'Plus' context with another distinct 'Minus' context paired with no food. The primary aim was to examine the extent to which CPF could extend to foods other than those paired with predictive stimuli during training. The first key result is that, for contexts paired with a single food, CPF was specific even when the alternative food was familiar and highly palatable. The second and more novel finding is that this specificity was overcome when the Plus context was paired with a variety of foods.

Results from the training food test in Experiment 2.1 indicated that our general procedure, in terms of the use of contexts as food-paired stimuli and the palatable foods used, provided appropriate conditions to observe CPF. No CPF effect was found when an alternative palatable food was tested in Experiment 2.1, nor in the *Single* group in Experiments 2.2 and 2.3. The Plus context was associated with consumption of a highly palatable food for these rats, but did not potentiate consumption of familiar or novel alternative foods. The absence of CPF was not due to neophobia: rats ate ample amounts in tests but not in a way that was modulated by their environment. Thus, data from Experiment 2.1 and the *Single* groups in Experiments 2.2 and 2.3 are consistent with past research showing that CPF is specific when cues are paired with a single food (Petrovich et al., 2007a, 2007b, 2012; Delamater & Holland, 2008; Galarce et al., 2007).

The novel result is that this specificity was overcome when the Plus context was associated with multiple foods. The current study is the first, to our knowledge, to confine exposure to variety to a distinct context and to test the ability of this context to potentiate

feeding. The most likely process underlying this 'variety effect' is generalisation: the range of tastes and textures provided by the three foods encoded a broader representation of food reward to the context. This allowed the association formed with the Plus context to generalise to the test scenario, facilitating consumption of alternative foods for the *Variety* group. By contrast, the *Single* group's exposure to the same food throughout training may have conditioned a stronger specific association with the context that rendered the presentation of an alternative food at test more surprising.

In the present experiments, variety was spaced such that one food was provided per Plus session. This was in order to avoid a difference in consumption that likely would have emerged had the *Variety* group received access to three foods simultaneously, as shown in much past work (Rolls et al., 1981, 1983; Raynor & Epstein, 2001). Consequently, *Single* and *Variety* groups did not differ significantly in the rate of increase or in average consumption during training. This appears to rule out the possibility that the CPF effect in the *Variety* group was explained by differences in conditioned satiety with the Plus context (Booth, 1972) or other factors arising from one group simply eating more in training. Other data indicates that variety can promote overeating by weakening associations between flavours and their post-ingestive consequences (Martin, 2016; Hardman, Ferriday, Kyle, Rogers, & Brunstrom, 2015). However, compromised flavour-nutrient learning would not appear to explain the present data, since rats were exposed to FL prior to the variety manipulation. Additionally, rats were not food-deprived for training, suggesting that consumption of the palatable foods in the Plus context was driven more by their palatability than by a need to relieve hunger.

The lack of an effect of variety on training intake is interesting to compare with a rat study in which instrumental responding was higher when it was rewarded by both sucrose and grain pellets than only with sucrose pellets (Thrailkill et al., 2014). Of interest, this 'variety effect' emerged in rats trained on random-interval (RI)-3 or RI-6 schedules, but not

when RI-12 and RI-24 schedules were used. An earlier study from this group reported that no variety effect was found when variety was given between, rather than within, sessions (Bouton et al., 2013, Experiment 2). These findings are consistent with the present results, where variety was provided between sessions separated by 24-72 hours, and produced comparable consumption relative to animals receiving the same food. They also suggest that providing simultaneous variety might produce larger effects, although as described, the likely increase in training intake might necessitate additional control groups.

Finally, consumption in CPF tests may have been influenced by incentive contrast between the training and test foods (e.g. Flaherty & Largen, 1975). This is most strongly suggested by comparing the *Chow* and *Single* groups in Experiments 2.2 and 2.3. Though neither group exhibited CPF, intake by the *Chow* group appeared higher overall than by the *Single* group. This would appear to be explained by the simple fact that FL were more palatable than the chow provided to the *Chow* group (positive contrast) and less palatable for the *Single* group (negative contrast). For the *Variety* group, Froot Loops were unexpected, but with prior exposure to multiple foods in this environment, negative contrast was reduced and the generally excitatory properties of the context drove further consumption. This idea is explored further in coming chapters.

Chapter 3: The role of individual differences in CPF

3.0. Introduction

The general aim of the experiments in Chapter 3 was to continue to explore properties of the 'variety effect' described in Chapter 2. Thus, under what conditions do contexts paired with palatable food enhance consumption of various other foods? To this end, the same general method was retained: training consisted of intermixed exposures to a *Plus* context containing palatable food or chow and to a *Minus* context containing no food, prior to CPF tests measuring consumption in each environment. The first two experiments in Chapter 3 tested CPF when the test food was chow (Experiment 3.1) and when preferences for the training and test foods were identified for each rat (Experiment 3.2). The third experiment used a larger sample of animals and focused on the transfer of an alternative and familiar palatable food, Froot Loops (Experiment 3.3).

Experiments also sought to identify whether individual differences in eating behaviour would correlate with the strength of the CPF effect. This aim was generated by the observation that despite the significant group differences reported in Chapter 2, CPF was often variable within each group. Animal models of diet and obesity have demonstrated the importance of individual differences on a range of measures. For example, while *obesity-prone* animals rapidly gain weight when fed a high-fat, high-sugar diet, their *obesity-resistant* counterparts weigh no more than controls (Levin, Dunn-Meynell, Balkan, & Keesey, 1997). This phenotype extends beyond body weight: obesity-prone rats have been shown to exhibit greater anxiety and higher craving for food when their high-fat, high-sugar diet is removed (Pickering, Alsio, Hulting, & Schioth, 2009). Additionally, rats' approach behaviour toward a sucrose-paired cue *prior* to an obesogenic diet can predict body weight gain (i.e., differentiate prone and resistant rats; Robinson et al., 2015). Similarly, studies of sign- versus goal-

tracking use individual differences to classify rats as the former or the latter, according to whether the presentation of a food cue elicits approach toward the location of food (goal-trackers) or toward the cue itself (sign-trackers) (Boakes, 1977; Flagel, Watson, Akil, & Robinson, 2008).

In what appears to be the only study to examine individual differences in the study of CPF, Boggiano et al. (2009) reported that 'binge-prone' and 'binge-resistant' animals showed similar degrees of overeating in a palatable food-paired environment. This classification was based on animals' consumption of Oreo cookies in the home-cage prior to training. The present experiments adopted a similar approach by measuring baseline palatable food consumption prior to context conditioning. To do this we exploited the fact that our current protocol already involved familiarising rats to the test food, FL. While in Chapter 2 this familiarisation was done only once in the home-cage, experiments in Chapter 3 added individual consumption tests to provide an index of *baseline FL intake*, which was then compared with the CPF effect.

The intuitively most likely outcome was that rats with a high baseline intake of FL would exhibit a larger CPF effect. Alternatively, the propensity for these rats to eat more might work against a CPF effect if their consumption was higher in both Plus and Minus contexts at test. A second variable where a relationship with CPF was examined was the amount of food consumed in the Plus context during training. Some variability in this measure was observed in Chapter 2, and we reasoned that consuming larger amounts of food in the Plus context might encode a stronger association with eating, and elicit greater consumption at test. Alternatively, greater consumption might reduce attention toward elements of the context to weaken conditioning.

Therefore, experiments in Chapter 3 examined correlations between baseline FL intake, training consumption, and the CPF effect. Several features of the data analyses are important to note. First, in order to perform these correlations, CPF was expressed as the proportion of total intake at test eaten in the Plus context, i.e. Proportion = Plus / (Plus + Minus), such that values above 0.5 indicated greater consumption in the Plus context. Expressing CPF as a proportion rather than absolute intake in the Plus context ensured correlations would reflect the strength of CPF rather than the simple tendency for some rats to eat more than others. Second, nonparametric Spearman's correlations were used for all analyses, since baseline FL consumption was often not normally distributed (as described below) and because we did not necessarily hypothesise linear correlations. Third, a correlational approach was chosen because it seemed to better reflect the spread of the data. For example, initial analyses explored categorical methods that identified sub-groups of rats based on baseline FL consumption; however, these appeared arbitrary when tertiles (or other equal-n divisions) were formed. When more rigorous methods of categorisation were used (e.g. k-means clustering), the resulting groups were often too unequal in terms of group size or variance to be compared statistically.

3.1. Experiment 3.1: The variety effect does not transfer to chow

The primary aim of this experiment was to test whether the effect of the variety manipulation described in Chapter 2 would transfer to a familiar but less palatable food, chow. Thus, after context conditioning for *Chow*, *Single*, and *Variety* groups, the first CPF test measured chow consumption in the Plus and Minus contexts. A second CPF test then measured consumption of a palatable alternative food, Froot Loops (FL). The second aim was to assess whether the CPF effect on this latter test could be predicted by baseline intake of

FL, measured prior to training. To this end, rats were familiarised to FL first in the home-cage (like Chapter 2), and then in an individual 30-min consumption test.

3.1.1. Method

Subjects

Twenty-four female adult Hooded Wistar rats were used. At the start of the experiment mean body weight was 262g (range 234 – 287g). As in Experiments 2.2 and 2.3, rats had previously been used in a Pavlovian conditioning experiment in which discrete cues predicted delivery of a food pellet to a magazine. Neither the pellets nor the cues were used in the present experiment. Rats were group-housed ($n = 6$/cage) in a temperature- and humidity-controlled room with free access to chow and water throughout all procedures. The colony room was maintained on a standard 12:12 light:dark cycle (lights on at 0900h); training and testing occurred between 0900 and 1200 hrs each day.

Apparatus

The preparation of the contexts was as described in Chapter 2. Baseline FL consumption was measured in the pre-feeding chambers described in Experiment 2.1, which were located in a separate room to where training took place.

Procedure

Baseline FL intake

On Day 1 rats were familiarised to FL in home cages. FL (~32/cage) were scattered in the cage bedding and observation confirmed they were sampled by each rat within a 15-min period. On Day 2 a 30-min consumption test was held in individual feeding cages where 25 FL were available in a Petri dish. Consumption in this session was taken as the measure of

baseline FL intake. Subsequently, rats were assigned to *Chow, Single*, or *Variety* groups

(each $n = 8$) that were matched on this measure and on body weight.

Training

The 18-day training phase began 6 days later, and consisted of nine 30-min exposures

each to the Plus and Minus contexts, intermixed such that rats were not in the same context

on more than two consecutive days. All rats were trained with the same sequence of Plus and

Minus sessions (order MPPMPMPMMPMPMMPPMP). Rats were trained in three 8-rat

squads, each containing rats from the three groups. In the Plus context, the *Chow* group

received standard chow and the *Single* group received either Oreos ($n = 3$), Banana Bread ($n

= 3$), or Burger Rings ($n = 2$), as described in Experiment 2.2. The *Variety* group ($n = 8$) was

exposed to all three of these foods in a cycled, counterbalanced order.

CPF Tests

After the 18th training session rats remained in home cages for four days with free

access to chow and water. Next, Test 1 measured chow consumption in the Plus and Minus

contexts on consecutive days. The order in which the contexts were tested was

counterbalanced within each group. In each 30-min test, 20g of chow was presented in a glass

Petri dish. Two days after Test 1, rats received six re-training sessions (3 Plus and 3 Minus

sessions; order: MMPPMP). After a day of rest in the home-cage, Test 2 assessed

consumption of FL. Once again, test order was counterbalanced and tests were held on

consecutive days. On these tests, 25 FL were available in a Petri dish.

3.1.2. Results

One rat in the *Chow* group developed abdominal cysts during training and was euthanised. Its data were excluded from analyses.

Baseline FL intake

As shown in Figure 3.1.1, during the 30-min consumption test rats ate on average 2.20 ± .26g [SEM]; or 7 to 8 Froot Loops. There was no evidence that any one colour/flavour (Green, Yellow, Orange, Purple or Red) was systematically preferred or avoided. The Shapiro-Wilk test for normality was marginally significant ($W = .92$, $p = .073$).
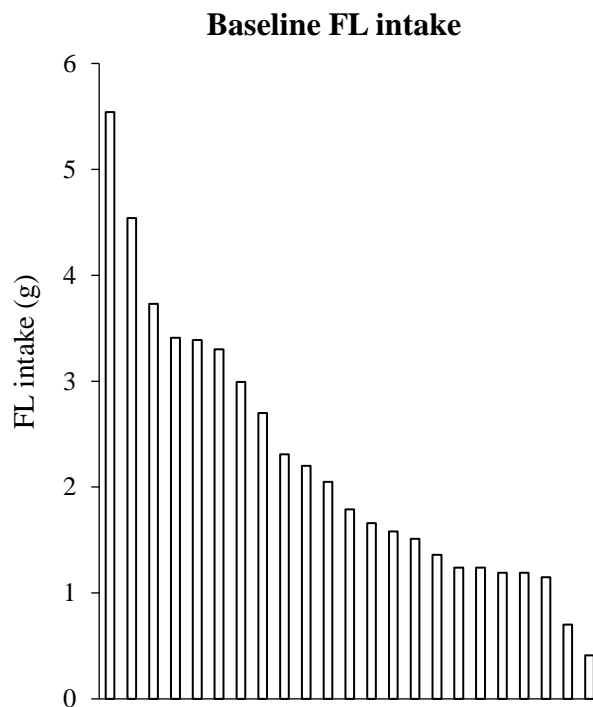
Baseline FL intake

Figure 3.1.1. Baseline FL intake in Experiment 3.1. Consumption by individual animals in a 30-min test.

Food intake during training is shown in Figure 3.1.2. Since foods differed in energy density, consumption in grams was converted to kJ for presentation and analyses. A 3 x (9) (group x [session]) mixed-ANOVA showed a significant increase in consumption over sessions (linear trend: $F(1, 20) = 48.30$, $p < .001$) that differed significantly between groups (group x session linear interaction trend: $F(2, 20) = 12.33$, $p < .001$). This reflected the increase in palatable food consumption by *Single* and *Variety* groups and the consistently low chow intakes in the *Chow* group. On average, the *Chow* group ate significantly less than the *Single* and *Variety* groups (Tukey post-hoc, both $p < .001$), which did not differ from each other ($p = .59$). A separate analysis of the two latter groups indicated that the rate of increase over sessions did not differ ($F < 1$).

Consumption during re-training was analysed in a 3 x (3) (group x [session]) mixed-ANOVA. This analysis found no significant trend over sessions and no group x session interaction trends (all $F < 1$), but a significant main effect of group ($F(2, 20) = 24.10$, $p < .001$). As in initial training, the *Chow* group ate significantly less than both *Single* and *Variety* groups (both $p < .001$; Tukey post-hoc) with no significant difference between the latter groups ($p = .99$).

Experiment 3.1: Training consumption
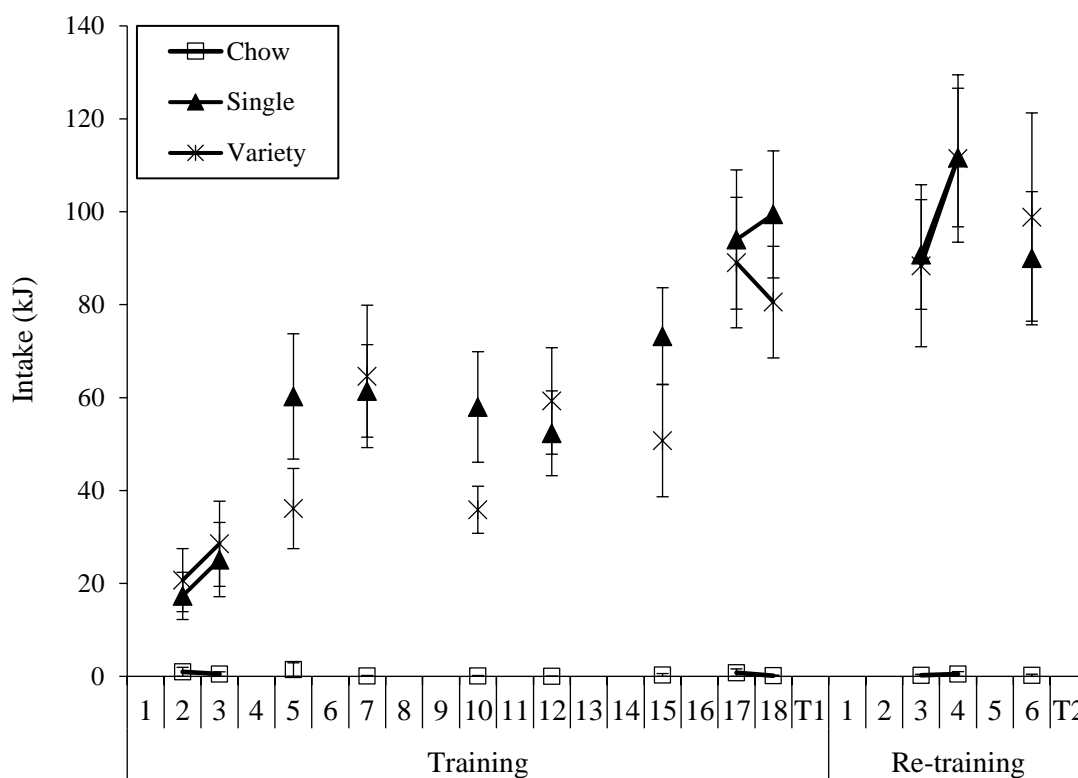
Figure 3.1.2. Training consumption in Experiment 3.1. In Plus sessions, *Single* and *Variety* groups steadily increased their intake of palatable foods at a comparable rate, while the *Chow* group ate minimal amounts of chow. T1 = Test 1 (chow); T2 = Test 2 (FL).

Test 1: Chow

Compiled data from Test 1 are shown in Figure 3.1.3.A. Statistical analysis of these data was precluded by a floor effect: most rats (16/23) ate nothing in either context. The other seven rats (2 *Chow*, 2 *Single*, 3 *Variety*) ate an average of 0.45g in the Plus test and 0.61g in the Minus test.

Test 2: FL

Compiled data from the second CPF test, when FL were available, are displayed in Figure 3.1.3.B and were analysed in a 3 x 2 x (2) mixed-ANOVA (group x test order x [context]). This analysis found no significant main effect of context, no context x group interaction, and no main effect of group (all $F < 1$). However, there was a significant context x test order interaction ($F(1, 17) = 33.66$, $p < .001$). The main effect of test order and the group x test order interaction were marginally significant ($F(1, 17) = 4.15$, $p = .057$ and $F(2, 17) = 3.08$, $p = .072$, respectively). The main effect of test order suggested that overall, rats tested Plus-> Minus ate more than rats tested Minus->Plus. Inspection of the data indicated that the interactions involving test order were driven by the tendency for rats to eat more on their second test, regardless of which context was tested. To confirm this, separate 3 x [2] ANOVAs were run for the two test order cohorts. These analyses found that while rats tested Minus->Plus ate significantly more in their second (Plus) test (context main effect: $F(1, 9) = 11.69$, $p = .008$), rats tested Plus->Minus also ate significantly more on their second test in the *Minus* context ($F(1, 8) = 56.47$, $p < .001$).
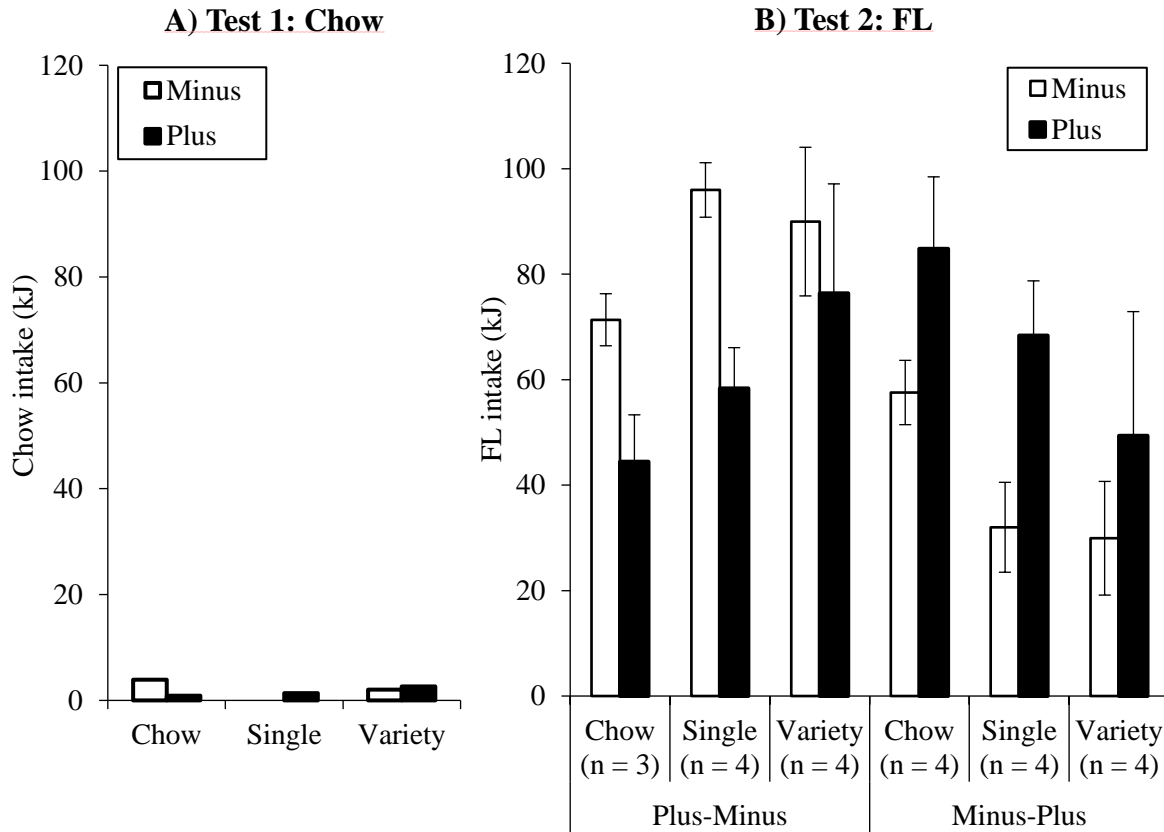
Figure 3.1.3. Experiment 3.1 test results. Panel A: consumption in both contexts was minimal when chow was the test food. Panel B: no overall CPF effect and no group differences were found when a palatable alternative food, FL, was tested (see text for details).

Correlations with CPF

As described above, Spearman's nonparametric correlations assessed the relationships between baseline FL intake, training consumption, and the CPF proportion measure. The correlations of the former two variables with CPF are displayed in Figure 3.1.4. Overall, no correlations between either baseline FL intake and CPF (Panel A: $r_s = .17$, $n = 23$, $p = .44$), between training intake and CPF (Panel B: $r_s = -0.02$, $n = 23$, $p = .94$) or between baseline FL intake and training consumption ($r_s = -0.06$, $n = 23$, $p = .79$) were detected. These relationships were then explored within each of the three groups. The only significant result from these analyses was a positive correlation between baseline FL and training intakes

within the *Chow* group ($r_s = 1$, $n = 7$, $p < .001$), indicating that each rat's rank for training

intake corresponded perfectly to that for baseline FL intake. Otherwise, no significant

correlations were found for the *Single* or *Variety* groups (largest $r_s = .52$, $n = 8$, $p = .18$).
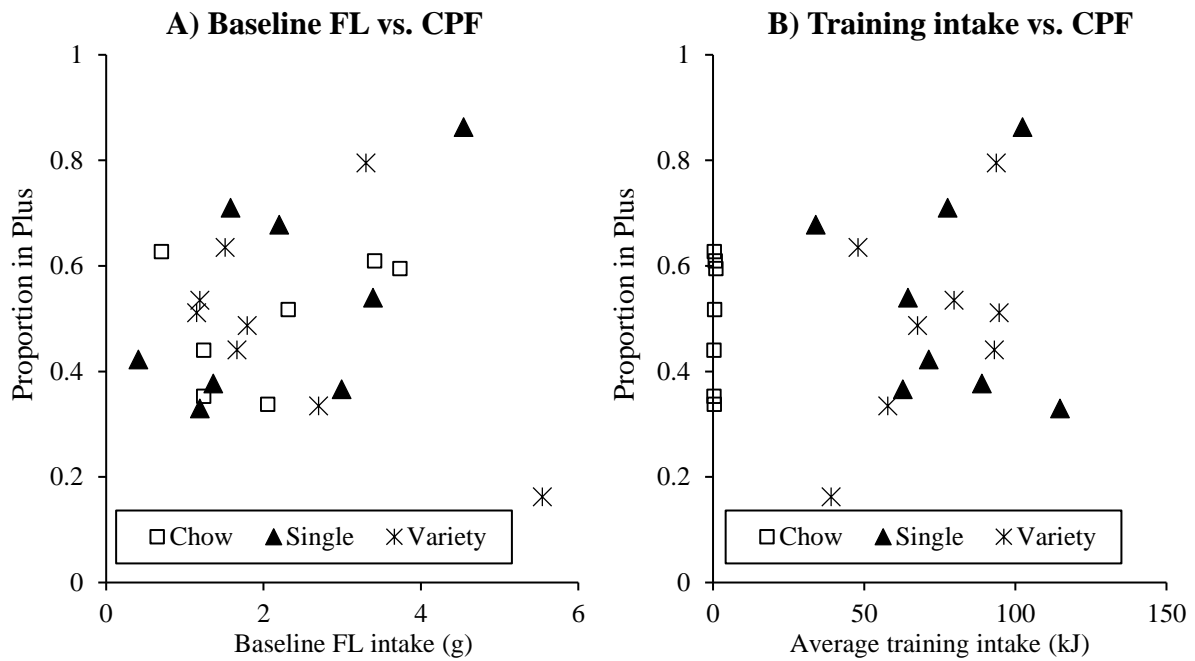
Figure 3.1.4. Correlational analyses in Experiment 3.1. Panel A: baseline FL intake did not correlate significantly with CPF on Test 2 overall, nor for any group individually. Panel B: Training consumption did not correlate significantly with CPF in Test 2 (see text for details).

3.1.3. Discussion

The first aim of this experiment was to test whether the variety effect observed in

Chapter 2 would transfer to a familiar but less palatable alternative, chow. The all but

complete absence of consumption in Test 1 indicated clearly that this was not the case. The

few rats that ate any chow at all in this test came equally from the 3 groups, and their

consumption was unaffected by context. That non-deprived rats did not eat chow in an

environment previously containing far more palatable foods is perhaps unsurprising, but was

important to test given the effect of the variety manipulation in Chapter 2. It is also consistent with past CPF studies that have failed to show CPF when chow is the test food (Petrovich et al., 2007a, 2007b, 2012).

Subsequently, rats were re-trained and then CPF tests were repeated with Froot Loops as the now palatable alternative food. Surprisingly, no CPF effect in the *Variety* group was found, unlike in the experiments reported in Chapter 2. One possibility is degradation of the Plus context from Test 1, where the Plus context test (containing chow) was effectively an extinction session, since few rats ate. However, this should have been outweighed by the three subsequent Plus re-training sessions, in which rats continued to eat large amounts of palatable food. As in previous experiments, rats ate more FL on the second test than the first, yielding interaction and main effects involving test order. However, in the present experiment the increase from the first to the second test day cancelled out context effects, whereas in past experiments the effect of context prevailed in the *Variety* group. A speculative reason for why test order had stronger effects in the present experiment is that the two FL tests were held on consecutive days, not with a single day of rest as in Chapter 2.

The failure to observe CPF at the group level is likely to have undermined attempts to explore individual differences in the effect. CPF did not correlate with baseline FL intake or with average consumption during Plus context training sessions. Two limitations are worth noting, however. First, with only 23 animals, the present study was likely underpowered to detect significant correlations. Additionally, these associations were explored as a secondary aim, and were tested over and above between-group differences in terms of conditioning training (Chow/Single/Variety) and test order counterbalancing, which demonstrably affected test results.

3.2. Experiment 3.2. CPF after assessing preferences for the training foods

The first aim of Experiment 3.2 was to compare preferences for the three palatable foods used in training. Evaluating preferences for each animal ensured that rats in the *Single* group could be presented with a preferred food in Plus context training sessions that were held subsequently. This was unlike previous experiments, where foods were assigned arbitrarily. Although rats in the *Single* group ate substantial amounts during training, we reasoned that identifying preferences prior to conditioning might reduce variability in this group and, potentially, enhance the CPF effect. As in Experiment 3.1, measures of baseline FL intake were taken prior to training, in the form of three days of individual consumption tests. Three sessions (rather than one) were used to yield a more reliable estimate of individual variability, given that consumption in a single session might vary due to other factors, such as anxiety. As well as baseline FL intake, Experiment 3.2 tested rats' preference for the training food versus the test food, FL. Correlations between this preference index and the CPF effect were also explored to assess whether contrast between the two foods would relate to the ability of the context to promote eating of the alternative food. For example, a food eaten in relatively small amounts during training might still be preferred to FL; conversely, a food less preferred than FL might still be eaten in large quantities. Correlations allowed for these possibilities to be evaluated.

3.2.1. Method

Subjects

Twenty-four adult female Sprague-Dawley rats were used. Their experimental history and housing conditions were as described in Experiment 3.1, except that rats were housed 4 (rather than 6) per cage. Mean body weight was 277g (range 253 – 339g) at the beginning of

the experiment. Rats had *ad-libitum* access to chow and water throughout all experimental procedures.

Apparatus

The preparation of the contexts was as described previously in Chapter 2. The pre-feeding chambers were those described in Experiment 2.1.

Procedure

Baseline Froot Loop (FL) intake

Rats were familiarised to FL in three 30-min individual feeding tests held on consecutive days in the pre-feeding chambers. Twenty FL were provided in a glass Petri dish in each session. Consumption was summed over the 3 sessions to establish a measure of baseline FL intake.

Training food preference tests

Two days after baseline FL testing, rats were familiarised with the foods used in training. Thus, 2 Oreos, 10 Burger Rings and 10g Banana Bread were distributed in each home cage. Rats ate these foods readily and no traces were left on the following morning. Over the next three days, rats' relative preferences for these foods were measured in three 30-min choice preference tests. On each test, rats were placed in the pre-feeding chambers with two of the foods presented in separate Petri dishes 10 cm apart. The order of these tests (i.e., Oreos vs. Rings; Oreos vs. Banana Bread; Banana Bread vs. Rings) was counterbalanced. As outlined below, these tests found that Rings were the least preferred food for most rats. Therefore, on the afternoon of the third preference test, rats were pre-exposed to another savoury palatable food, Sausage Roll (Coles®; 11.1 kJ/g, see Appendix A), followed by two additional preference tests comparing consumption of this food to Oreos and to Banana

Bread. The order of these two 30-min tests was counterbalanced. Rats were then allocated to *Chow*, *Single*, and *Variety* groups (each $n = 8$) that did not differ significantly in baseline FL intake or body weight ($F < 1$; one-way ANOVAs).

Training

Context conditioning consisted of nine exposures each to the Plus and Minus contexts. A massed training procedure was used in which rats were exposed to both Plus and Minus contexts each day in two separate sessions beginning at 1000 and 1300 hrs, respectively. For half the rats, the Plus context was always exposed in the morning and the Minus context in the afternoon; the other rats received the reverse configuration. Therefore, time of day served as an additional contextual cue, and training was shortened to 9 days. The Plus context contained chow for the *Chow* group, while the *Single* group received their second-preferred food as determined by the second set of preference tests (3 Sausage roll, 3 Banana Bread, 2 Oreos). We opted not to provide the most-preferred food so that the *Single* and *Variety* group would be better matched. The *Variety* group received cycling access to Banana Bread, Oreos, and Sausage roll, with different orders used for subsets of rats.

Tests

Rats were rested for one day after the completion of training. Tests 1 and 2 used a procedure in which rats were pre-fed FL for 10-min immediately prior to a 20-min test in the Plus or Minus context. For both Test 1 and Test 2, the first test session was held in the afternoon and, after a day of rest, the second test was held the following morning. This was in keeping with the timing of training sessions and ensured that test order was counterbalanced.

Rats then received three days of re-training, consisting of three exposures each to Plus and Minus contexts. Since data from Tests 1 and 2 were highly variable, no pre-feeding was conducted in Tests 3 and 4, which compared consumption of FL (Test 3) and the training

82

foods (Test 4) in 20-min tests. The procedure was otherwise identical to Tests 1 and 2. For the training food test, the *Chow* and *Variety* group were presented with their second-preferred training food, based on preference test results.

Preference test

Finally, rats received a 30-min choice preference test in pre-feeding chambers between FL and one of the palatable foods presented in the Plus context during training. The purpose of this test was to assess whether variability in CPF for the *Variety* and *Single* groups might be explained by the relative palatability of FL to the food/s paired with the Plus context. Given the *Variety* group received three foods in the Plus context, this group received their second-preferred palatable food as identified prior to training, a food to which they had received seven-to-eight exposures at the time of test. The *Single* group had received fifteen exposures to their food, whereas all rats had received nine exposures to FL across three baseline tests and six CPF tests. Therefore, although exposure to the foods was not matched perfectly, both FL and the comparison palatable food were familiar at the time of the test.

The *Chow* group was not of central interest for this analysis but was included and also received their second-preferred food (which had been exposed 2-4 times previously). In total, nine rats received Sausage Roll, seven received Banana Bread and eight received Oreos. A preference was calculated by expressing the percentage of total intake (in grams) consumed of palatable food; i.e. preference = [g palatable food]/[g palatable food + g Froot Loops]*100.

3.2.2. Results

Baseline FL intake

The total consumption of FL in the three pre-exposure sessions was 5.09 ± .38g [SEM]; these data are shown in Figure 3.2.1. The Shapiro-Wilk test for normality was not significant ($W = .96, p = .41$).
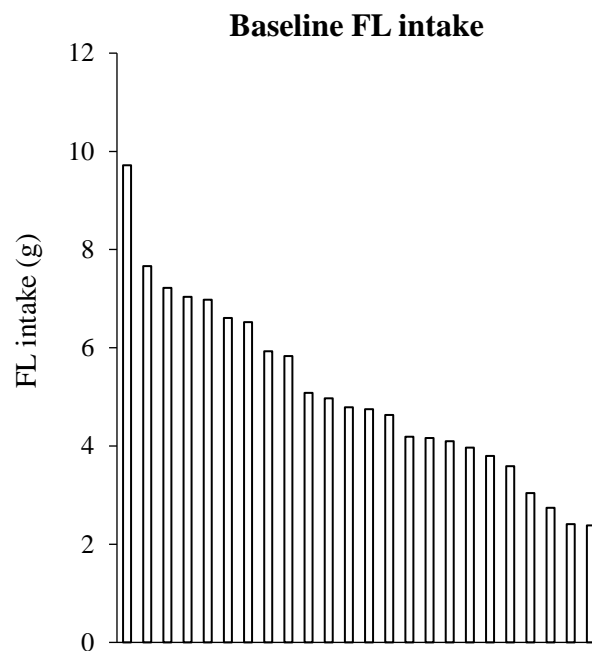
Figure 3.2.1. Baseline FL intake in Experiment 3.2. Total consumption in three 30-min sessions is shown. Bars denote consumption by individual rats.

Palatable food preference tests

Preferences were evaluated in each choice test by comparing the weight of each food eaten. Although the foods differed in energy density, calculating preferences in grams and in kJ yielded the same result in 71 out of 72 tests (24 rats x 3 tests). The first set of tests comparing Banana Bread, Oreos and Rings found that the latter were clearly less preferred

than both Oreos (by 21/24 rats) and Banana Bread (by all 24 rats). Most rats preferred Banana Bread to Oreos (20/24 rats). When consumption across the three preference tests was summed, rats ate 49.3 ± 2.5% [SEM] of food as Banana Bread; 34.1 ± 2.8% as Oreos and 16.6 ± 1.8% as Rings.

The second set of preference tests compared consumption of Sausage roll to Banana Bread and Oreos in two separate tests, with order counterbalanced. Banana Bread was preferred to Sausage roll by a majority of rats (17/24) but 13/24 rats preferred Sausage roll to Oreos. By incorporating rats' preference for Banana Bread vs. Oreos in the initial set of preference tests, it could be determined that Banana Bread was the most-preferred, middle-preferred and least-preferred food for 15, 8, and 1 rat, respectively. Oreos were the most-preferred food for 4 rats, the middle-preferred food for 7 rats, and the least-preferred for 13 rats. Sausage roll was the most-preferred food for 5 rats, the middle-preferred food for 9 rats, and the least-preferred food for 10 rats. Because Sausage roll was clearly a more equivalent alternative, it was retained alongside Banana Bread and Oreos for context conditioning.

Training

Training consumption in the Plus context is shown in Figure 3.2.2 and was analysed in a 3 x (9) (group x [session]) mixed-ANOVA. This analysis found no linear or quadratic changes in intake over sessions (both $F < 1$) and no group x session linear interaction trend ($F < 1$). However, the group main effect was significant ($F(2, 21) = 42.87$, $p < .001$). Pairwise comparisons using the Tukey HSD correction found that consumption was significantly higher in the *Variety* and *Single* groups than in the *Chow* group (both $p < .001$), and did not differ significantly from each other ($p = .99$). The most likely reason for the relatively flat gradient appears to be rats' exposure to the palatable foods during pre-training preference tests. Therefore, foods were familiar and readily eaten from the first training day.

Experiment 3.2: Training Consumption
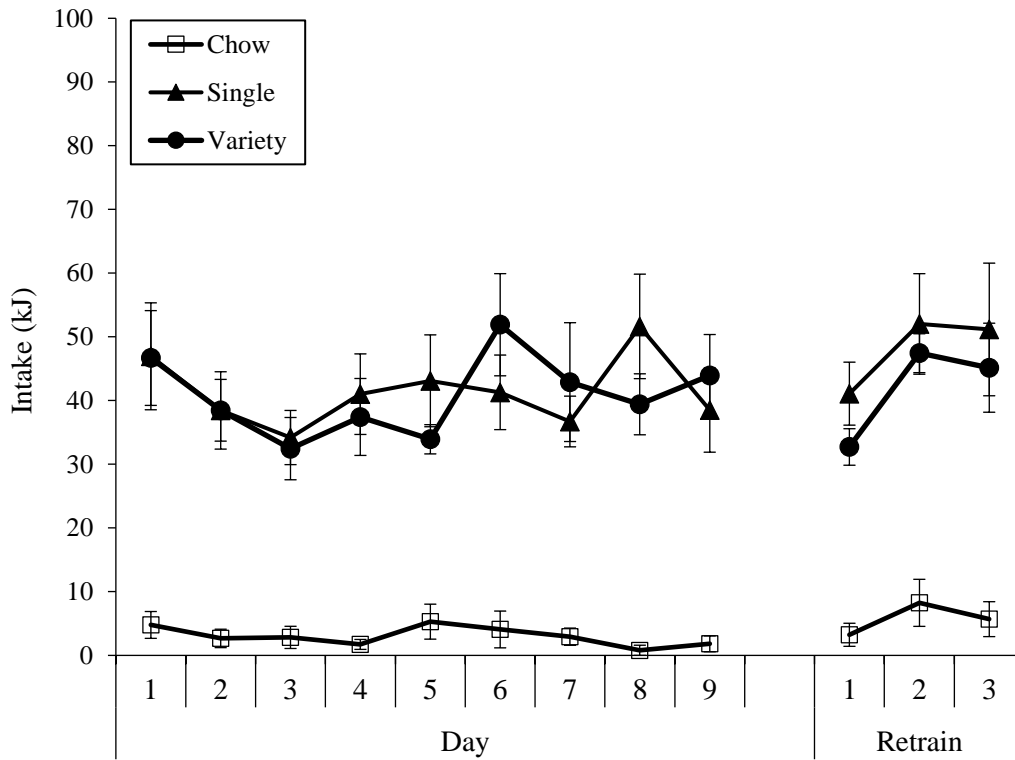
Figure 3.2.2. Training consumption in Experiment 3.2. Unlike previous experiments, consumption remained stable over sessions.

Tests

Results of the four CPF tests are displayed in Figure 3.2.3. Consumption in the Plus and Minus contexts at each test was analysed in 3 x 2 x (2) mixed-ANOVAs (group x test order x [context]).

Test 1: 10-min pre-feed (individual cages) → 20-min context test (Figure 3.2.3.A)

Consumption of FL in pre-feeding did not differ according to group, test order, context, or any of their interactions (largest $F(2, 18) = 1.68$, $p = .22$). Analysis of consumption in the contexts found no main effect of context, no group x context interaction, and no other significant interaction effects (largest $F(1, 18) = 1.90$, $p = .19$).

Test 2: 10-min pre-feed (home cage) → 20-min context test (Figure 3.2.3.B)

Since in Test 2 rats were pre-fed in the home cage, and rats were not housed in condition-matched cages, pre-feeding consumption of FL could not be compared between groups. However, rats ate considerably more in the 10-min pre-feeding period of Test 2 compared to the Test 1 pre-feeding conducted in feeding cages (compare Panels A and B of Fig. 3.2.3.B). This appeared to excessively suppress consumption in the contexts. Analysis found no main effect of context and no interactions with group or test order (all $F < 1$). The main effect of group was marginally significant ($F(2, 18) = 3.21$, $p = .064$) and the main effect of test order was significant ($F(1, 18) = 5.37$, $p = .033$). This latter result reflected greater overall intake by rats tested in the Plus->Minus sequence than those tested Minus->Plus.

Test 3: 20-min context test with FL (Figure 3.2.3.C)

Test 3 omitted pre-feeding, since this manipulation appeared to hamper rather than improve the sensitivity of the test. Consequently, consumption in the contexts increased substantially relative to Tests 1 and 2. However, analysis found no significant main or interaction effects (largest $F(2, 18) = 2.03$, $p = .16$).

Test 4: 20-min context test with training food (Figure 3.2.3.D)

Analysis found no significant main of context ($F < 1$), no context x group interaction ($F(2, 18) = 2.37$, $p = .12$) but a significant context x test order interaction ($F(2, 18) = 6.44$, $p = .021$). No other main or interaction effects were significant (largest $F(1, 18) = 2.07$, $p = .17$). The context x test order interaction was driven by increasing consumption over tests, such that rats tested Minus->Plus tended to eat more in the Plus context while those tested Plus->Minus context did the reverse.
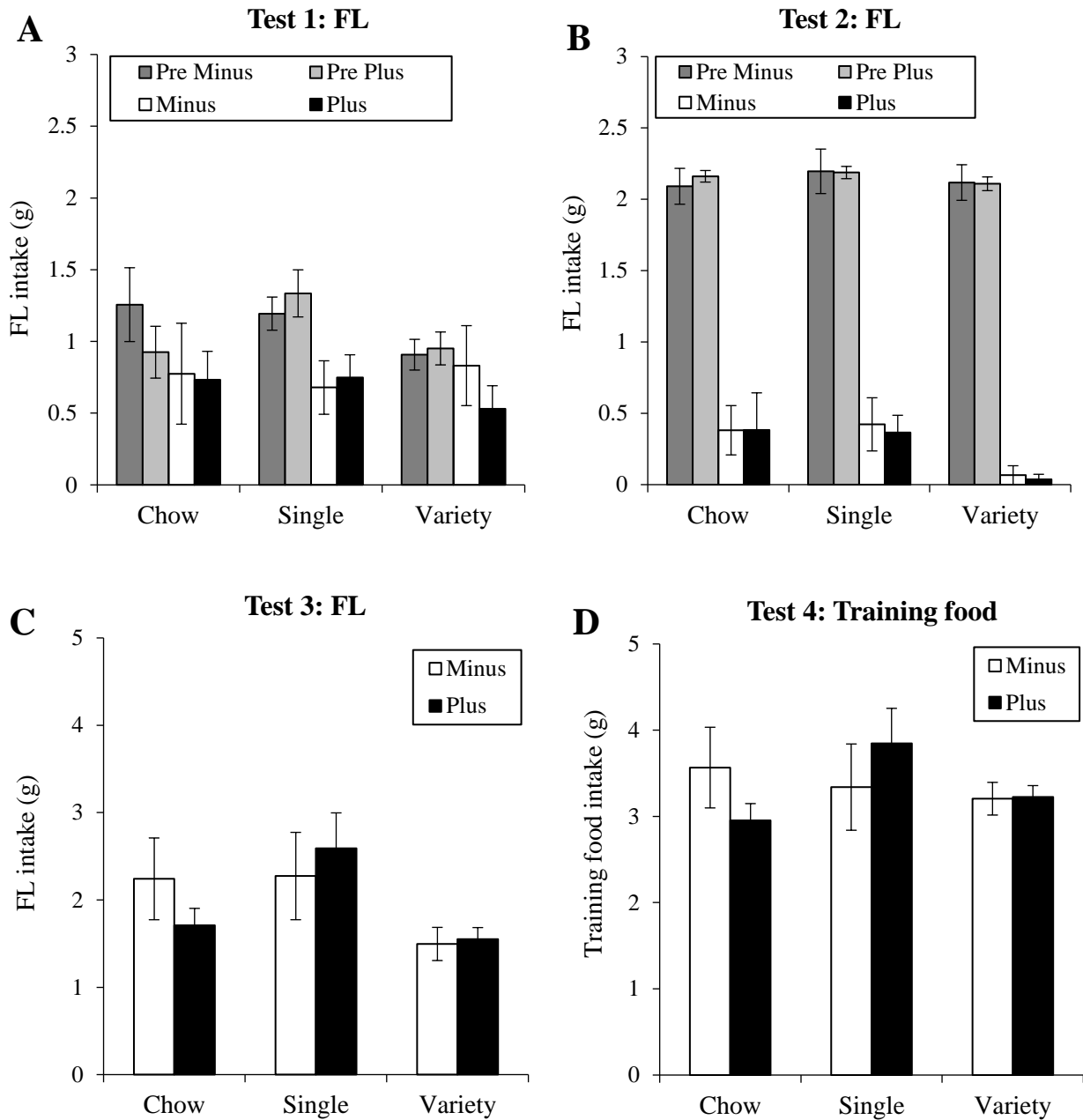
Figure 3.2.3. Experiment 3.2 test results. Pre-feeding did not yield a CPF effect in Test 1 (Panel A) or Test 2 (Panel B), when animals were pre-fed in the home cage. CPF was not observed when pre-feeding was omitted in Test 3 (Panel C), or when the training foods were tested (Panel D). *N.B.* On Test 4 the *Chow* and *Variety* group were presented with their middle-preferred palatable food as determined by the pre-training preference tests.

Correlations with CPF

Correlational analyses used data from Test 3, since test conditions were most comparable to earlier experiments. Baseline FL intake and CPF, shown in Figure 3.2.4.A, did not correlate significantly overall ($r_s = .26$, $n = 24$, $p = .23$), nor for any group individually (*Chow*: $r_s = -0.67$, $n = 8$, $p = .071$; *Single*: $r_s = .31$, $n = 8$, $p = .46$; *Variety*: $r_s = .24$, $n = 8$, $p = .57$). Training consumption and CPF, shown in Figure 3.2.4.B, did not correlate significantly overall ($r_s = .31$, $n = 24$, $p = .15$), nor for the *Chow* ($r_s = .14$, $n = 8$, $p = .74$) or *Single* groups ($r_s = -0.29$, $n = 8$, $p = .49$). However, for the *Variety* group, CPF was stronger in rats with higher training intakes ($r_s = .79$, $n = 8$, $p = .021$). Baseline FL intake and training consumption did not correlate significantly overall nor for any group individually (largest $r_s = .64$, $n = 8$, $p = .09$, for *Single* group).
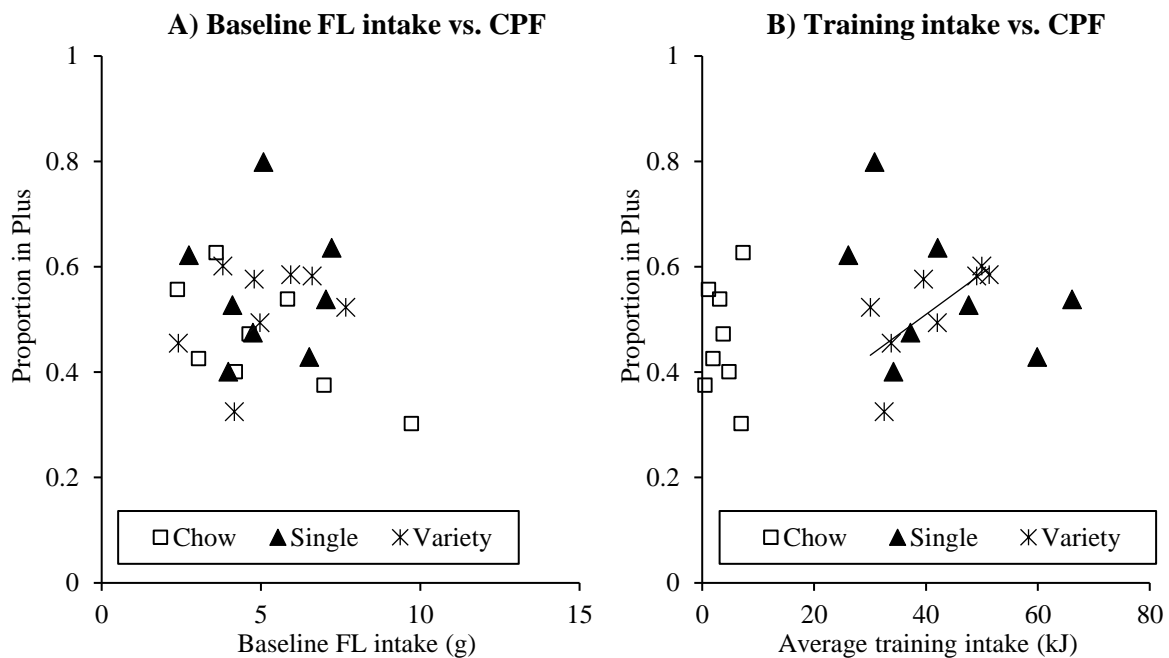
Figure 3.2.4. Correlational analyses in Experiment 3.2. Panel A: CPF did not correlate with baseline FL intake. Panel B: training intake and CPF correlated positively for the *Variety* group solid trendline) but not for other groups.

Preference for the training food versus FL

A surprising result was the failure to detect CPF across multiple tests. In addition, the correlational analyses described above found no evidence of a relationship between the two measures of eating behaviour that were hypothesised to account for some variability in the effect. Therefore, we next hypothesised that rather than evaluating measures of absolute consumption (baseline FL and training consumption), what might influence CPF is the extent to which rats preferred the alternative test food to the expected training food. This was the purpose of the preference test described earlier.

Most rats in the *Single* (7/8) and *Variety* (6/8) groups ate more of their training food than FL in this test. These preferences (preference = [g training food] / [g training food + g FL] x 100) are displayed in Figure 3.2.5.A. One-sample *t*-tests confirmed that each group's mean preference was significantly greater than 50% (*Single*: $t(7) = 2.92$, $p = .022$; *Variety*: $t(7) = 3.54$, $p = .009$) with no significant difference between groups ($F < 1$). In this test the *Chow* group were presented with their middle-preferred palatable food from preference tests. Although 6/8 rats in this group ate more of this food than of FL, mean preference did not differ significantly from 50% ($t(7) = 1.87$, $p = .10$).

As shown in Figure 3.2.5.B, we next assessed whether preference for the training food over FL was associated with CPF for *Single* and *Variety* groups, since these rats received palatable food in the Plus context. Higher preferences for the training food over FL were associated with a *weaker* CPF effect in Test 3 ($r_s = -0.64$, $n = 16$, $p = .007$). This correlation was in the same direction for both *Single* and *Variety* groups, but was only statistically significant in the latter (*Variety* group: $r_s = -0.71$, $n = 8$, $p = .047$; *Single* group: $r_s = -0.57$, $n = 8$, $p = .14$), a result that likely reflects low statistical power.
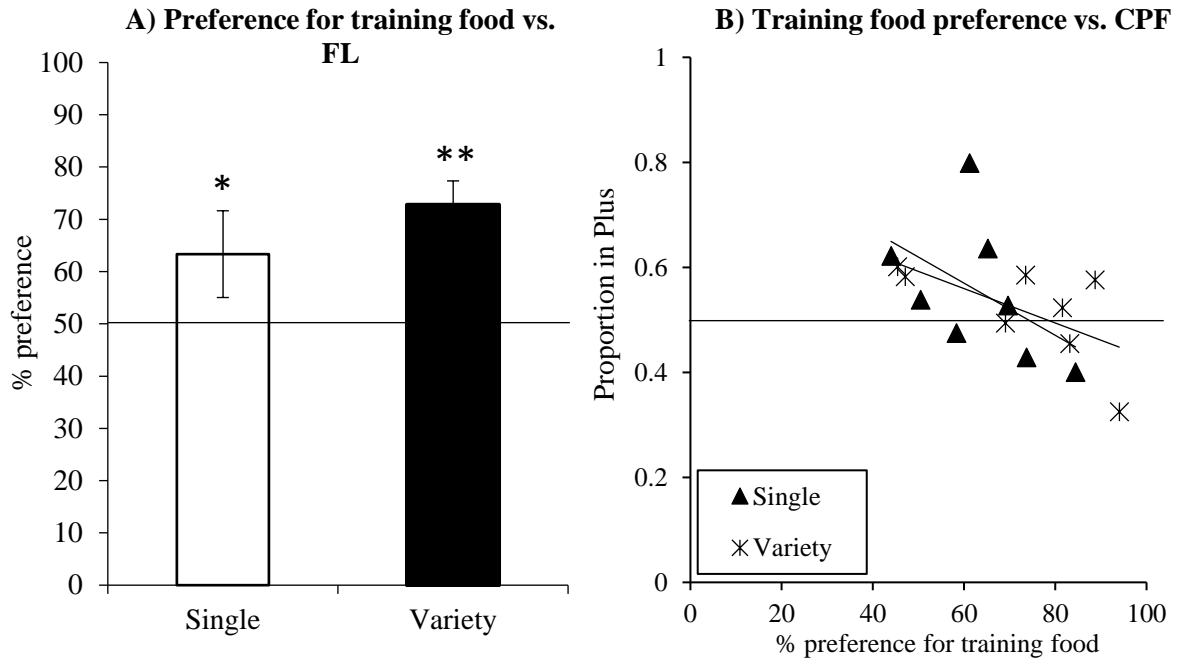
Figure 3.2.5. Preference test results in Experiment 3.2. Panel A: Both *Single* and *Variety* groups exhibited a significant preference for the training food. The *Variety* group received their middle-preferred food for this test. Panel B: Greater preference for the training food over FL was associated with a weaker CPF effect in Test 3. $*p < .05$; $**p < .01$, one-sample t-tests with 50% as the reference value.

3.2.3. Discussion

Experiment 3.2 began with a series of tests assessing preferences for the palatable foods to be used in training. These preference tests had two effects. First, they identified that Rings were relatively less preferred to the other foods, leading to the substitution of a more equally preferred alternative. Importantly, this would not appear to undermine the results of prior experiments using Rings (Experiments 2.2 and 3.1), since inspection of those data indicated that subsets fed Rings still escalated intake over training and ate substantially more than rats fed chow (see Appendix B). The second consequence was that they allowed for individual preferences to be established. This was most relevant for the *Single* group, since it could now be ensured that this group did not received a less-preferred food during training, a possibility that was not controlled in previous experiments.

Although preference tests were informative for identifying differences in relative palatability and ensuring the *Single* group was trained with a relatively preferred food, they appeared to abolish the CPF effect. Thus, multiple exposures to the palatable foods in a distinct environment (the pre-feeding cages) may have undermined the salience of the intended discrimination between Plus and Minus contexts that was trained subsequently. Indeed, training data differed notably from past experiments in that consumption by *Single* and *Variety* groups did not increase over sessions, but instead remained stable at a level around half that seen in Experiment 3.1 (~50 vs. ~100 kJ/rat/day), which used rats of comparable size. The fact that consumption was high on the first training session is likely to reflect rats' previous exposure to the foods. By contrast, the failure to further increase consumption over training might relate to the shift to twice-daily training, such that rats now received palatable food at a predictable time every 24-h and not in an unpredictable fashion every 24-72 hrs as in previous experiments. Some support for this idea comes from a study in which rats given access to sucrose solution every fourth day consumed significantly more per day than those given unrestricted access (Eikelboom & Hewitt, 2016).

Although there was again little evidence to suggest that CPF was predicted by baseline FL consumption or training intake, a preference test between the training food and FL yielded two notable results. The first was that *Single* and *Variety* groups exhibited a clear preference for the training food over FL. This test occurred in an environment where both foods had previously been eaten multiple times, suggesting that consumption was unlikely to have been affected by contextual influences. In addition, the number of prior exposures to the training food and FL was approximately equal – if not matched perfectly. Therefore, these data indicate that the presentation of FL at test marked a shift to a less preferred alternative, albeit one that was still eaten readily. The second key result was that the extent of this preference meaningfully related to CPF for groups (*Single* and *Variety*) trained with palatable

food. Thus, preference for the training food correlated negatively with CPF on Test 3 for these groups, such that rats that more strongly preferred their training food to FL tended to consume a lower proportion of FL in the Plus context. Put differently, rats that exhibited a stronger CPF effect on FL tended to more equally prefer FL to their training food. Together, these results indicate that the relative palatability of the training and test foods is an important determinant of CPF.

3.3. Experiment 3.3: Compensation during training

Given that the procedural changes implemented in Experiments 3.1 and 3.2 appeared to undermine the CPF effect, Experiment 3.3 reverted to a simpler design. The main change was that the *Chow* group was omitted, given data from this group had proven relatively uninformative, thus providing a more focused comparison between the *Single* and *Variety* groups. In addition, a larger sample was used to increase statistical power for correlational analyses. Another question addressed was the extent to which rats compensated for the palatable foods eaten in the Plus context training sessions by reducing their intake of chow in the home-cage. An inability to proportionately reduce chow consumption – i.e. inadequate compensation – would suggest that chronic exposure to food cues increases longer-term energy intake. Analysis of this was precluded in prior experiments because rats given palatable food (*Single* and *Variety* groups) were mixed in home cages also containing *Chow* animals that never received palatable food. In addition, the number of cages per experiment (4-6) was insufficient for statistical analysis. By focusing on *Single* and *Variety* groups within a larger experiment, each of these limitations were overcome. Thus, we measured chow intake on each day of training, as described further below, and compared total energy intake on days that began with Plus and Minus sessions.

3.3.1. Method

Subjects

Forty-eight female adult Sprague-Dawley rats were sourced from Animal Resource Centre (Perth, Australia). At the beginning of the experiment mean body weight was 226g (range: 210-240g). Rats were group-housed ($n = 4$/cage) in temperature- and humidity-regulated ventilated cages within a colony room maintained on a 12:12 dark:light reverse light cycle (lights off at 0900h). Thus, these rats differed from those in Experiments 3.1 and 3.2 in that, first, they were experimentally naïve and, second, they were maintained on a reverse light cycle. The latter was to encourage greater consumption during Plus sessions, since training sessions were now held soon after the onset of the dark cycle when rats consume a majority of daily food intake (Spiteri, 1982). Chow and water were freely available in the home cage across the experiment. During a one-week acclimation phase body weight and per-cage chow intake were recorded daily. The experiment was run in two cohorts that received identical treatment until the time of the first test, as described below. This factor of 'cohort' was included in initial statistical analyses but did not interact with context or group (both $F < 1$); therefore, it was removed for final analyses.

Apparatus

The preparation of the contexts and pre-feeding cages were as described in preceding experiments. Based on the results of Experiment 3.2, the training foods were Oreos, Banana Bread, and Sausage roll. The alternative test food was FL.

Procedure

Baseline FL intake

Rats were first familiarised with FL in the home-cage by scattering 30 FL in the bedding of each cage. All rats were observed to sample FL and inspection of the bedding the following day confirmed all were eaten. Beginning two days later, two 30-min FL consumption tests were held in individual feeding chambers. These tests were held on consecutive days between 1000 and 1200 hrs, i.e. during the early portion of the dark phase. The measure of baseline intake was the sum of consumption in these two sessions. Rats were then allocated to *Single* or *Variety* groups (each $n = 24$) that were matched on body weight and baseline FL intake.

Training

Training began six days after baseline FL tests. Over twelve days rats received intermixed 30-min exposures to a Plus and Minus context (one per day, six to each in total). Rats were trained in two 12-rat squads run in the same order each day between 0930 – 1230h. The order of Plus and Minus sessions was such that there were no more than two consecutive days in the same context (order: MPPMMPMMPPMP). The Minus context contained no food. In Plus sessions rats in the *Single* group received one of the three palatable foods ($n = 8$ per food), whereas the *Variety* group was exposed to each food in a cycling, counterbalanced order. Each day, the contents of the chow hopper in each cage were measured while rats were undergoing context conditioning. This provided an index of home-cage chow consumption, which was combined with intake of palatable foods in the previous day's Plus session (or nothing on Minus sessions) to provide total energy intake for each of the twelve cages.

Tests

The primary data of interest were from 30-min CPF tests of FL consumption in the Plus and Minus contexts. For *Cohort 1* this was preceded by a test of the training foods; these data were uninformative and are discussed only briefly. Next, these rats were re-trained for six days, with three Plus and three Minus sessions in the order MPPMMP, prior to the CPF Test of FL. For *Cohort 2* the first CPF test used FL and explored the time-course of the effect by measuring intake after 10, 20, and 30 min, as described in Experiment 2.1. The Petri dish and any FL scattered on the floor were quickly removed, weighed, and returned to the cage, usually taking 15 s per box. Rats remained in the context throughout the test. CPF tests began 2 days after the end of training and the order in which contexts were tested was counterbalanced. Preliminary examination of test data confirmed that the between-subjects factor of 'Cohort' (1 vs. 2) did not interact with the main effect of context or group, or their interaction (all F <1); it was therefore removed from final analyses.

3.3.2. Results

Baseline FL intake

Rats ate an average of 2.38 ± .15g [SEM] of FL in the two baseline FL sessions, as shown in Figure 3.3.1. The Shapiro-Wilk test for these data was significant (W = .945, p = .024), indicating that the distribution did not fit a normal distribution.

Experiment 3.3: Baseline FL intake
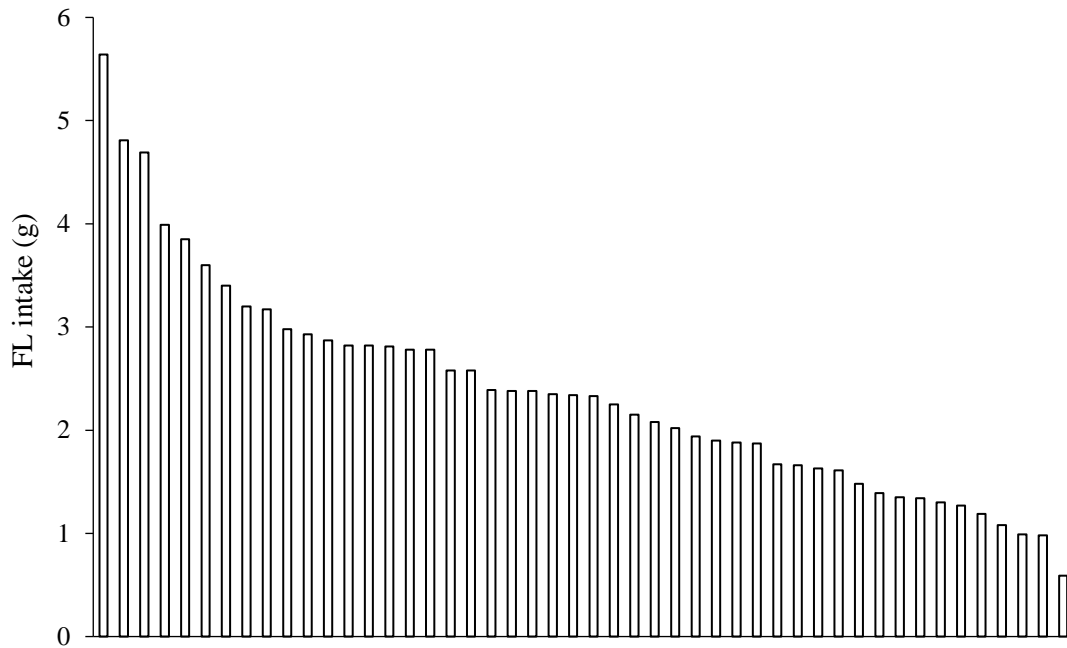
Figure 3.3.1. Baseline FL intake in Experiment 3.3. Data are total consumption by individual rats in two 30-min tests held on consecutive days.

Training

Consumption during training is shown in Figure 3.3.2 and was analysed in a 2 x (6) mixed-ANOVA (group x [session]). This analysis found significant linear ($F(1, 46) = 219.81$, $p < .001$) and quadratic trends ($F(1, 46) = 12.34$, $p = .001$) that each interacted with group (linear interaction trend: $F(1, 46) = 8.79$, $p = .005$; quadratic interaction trend: $F(1, 46) = 12.64$, $p = .001$). These interactions appeared to reflect the continued linear increase by the *Variety* group and an inverted-U pattern by the *Single* group, which increased consumption more rapidly early in training before levelling off on day 12. Indeed, analysis of consumption on day 12 found that groups differed significantly ($F(1, 46) = 5.69$, $p = .021$). Since rats were tested in cohorts that included both groups, and all other factors were counterbalanced, this might reflect habituation to the palatable food in this group. Analysis of consumption during the three re-training sessions given to *Cohort* 1 found no significant change over sessions

(linear trend: $F < 1$), no group x time interaction ($F < 1$) and no main effect of group ($F(1,$ 22) = 2.32, $p = .14$) (data not shown).

Experiment 3.3: Training Consumption
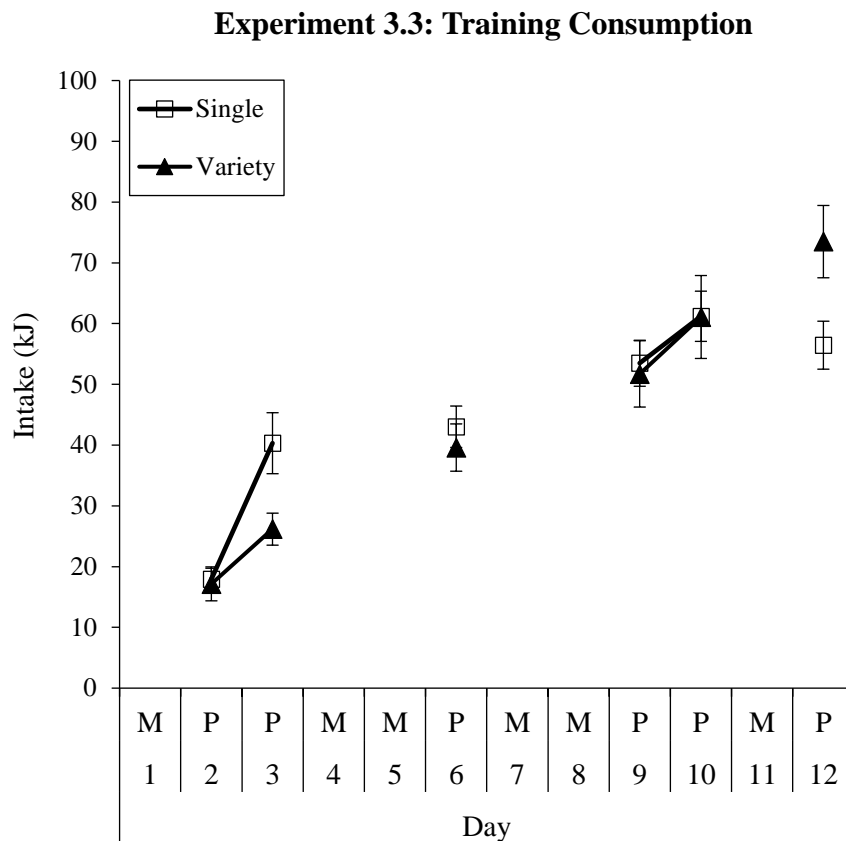
Figure 3.3.2. Training consumption in Experiment 3.3. Consumption increased at a similar rate between groups, save for a modest decline in consumption by the *Single* group on day 12.

Energy intake during training

Energy intake derived from home-cage chow and palatable food in the Plus context across the 12 days of training is shown in Figure 3.3.3. The first analysis compared total energy intake on Plus and Minus days in a (2) x (6) ([day type: Plus or Minus] x [session: 1-6]) repeated-measures ANOVA. This analysis showed a significant linear trend for session ($F(1, 11) = 40.61$, $p < .001$) and a significant linear interaction trend between session and day

type ($F(1, 11) = 11.66$, $p = .006$). The main effect of day type, however, was not significant ($F(1, 11) = 3.18$, $p = .10$). The trend effects suggested that energy intake significantly increased over the course of training, but that the increment of change differed over successive Minus and Plus days. When Minus and Plus days were considered separately, analyses showed that energy intake increased significantly over the 6 Plus days (linear trend: $F(1, 11) = 47.70$, $p < .001$), but decreased significantly over the 6 Minus days (linear trend: $F(1, 11) = 5.63$, $p = .037$).

Next, in order to assess the extent to which rats compensated for palatable food intake on Plus sessions, the same (2) x (6) repeated-measures ANOVA was applied to home-cage chow consumption. This analysis found a trend toward a main effect of day type ($F(1, 11) = 4.07$, $p = .069$), suggesting a tendency toward lower chow intake on days beginning with a Plus session. The linear trend for session was not significant ($F(1, 11) = 2.27$, $p = .16$) but there was a significant day type x session linear interaction trend ($F(1, 11) = 6.30$, $p = .029$). This indicated that while chow intake decreased over the 6 Minus days (already analysed above) it did not change significantly over the 6 Plus days ($F < 1$). Consequently, the proportion of total energy intake derived from palatable food intake increased consistently from the first to the sixth Plus session (mean ± SEM: 7.0 ± .8%; 13.2 ± 1.2%; 14.9 ± .8%; 17.9 ± 1.7%; 20.8 ± 1.4%; 22.7 ± 1.1%).

A final point of interest was that chow intake on Minus days appeared to vary according to whether a Plus or Minus day preceded it. For example, in Figure 3.3.3, home-cage chow intake appeared lower on days 4, 7, and 11, which were preceded by 1 or 2 Plus days, whereas rats ate more chow on days 1, 5, and 8 – days preceded by periods where only chow was available. To test this, chow consumption was compared between Minus days preceded by a Plus session (days 4, 7, 11) and those preceded by a Minus session (days 1, 5, 8) in a (2) x (3) repeated-measures ANOVA. Confirming our observations, the main effect of

'preceding day' was significant ($F(1, 11) = 27.53$, $p < .001$): chow intake was significantly lower on Minus days preceded by one or more Plus sessions. There were no other significant main or interaction effects (largest $F(2, 22) = 2.38$, $p = .12$).

Experiment 3.3: Energy intake during training
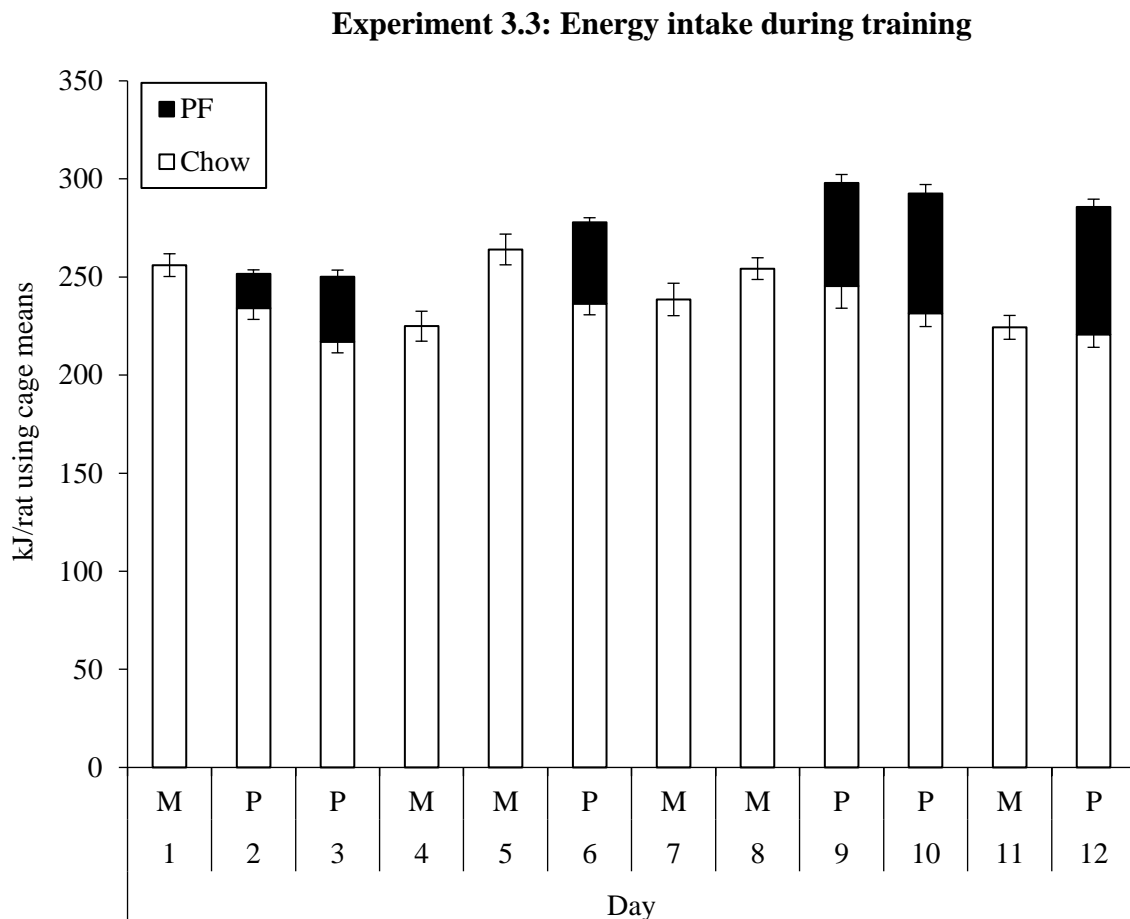
Figure 3.3.3. Total energy intake during training in Experiment 3.3. Energy intake increased over the 6 Plus days and decreased over the 6 Minus days. *NB.* PF = Palatable foods in Plus training sessions.

Test

Test results are shown in Figure 3.3.4. Data are presented collapsed for each group alongside the subsets of each group that were tested Minus->Plus and Plus->Minus. Consumption was analysed in a 2 x 2 x (2) mixed-ANOVA (group x test order x [context]).

The context main effect was significant ($F(1, 44) = 8.58$, $p = .005$) indicating greater consumption in the Plus than in the Minus context. The context x test order interaction and test order main effects were significant ($F(1, 44) = 15.38$, $p < .001$ and $F(1, 44) = 7.39$, $p = .009$, respectively), with the latter result reflecting higher intake by rats tested Minus->Plus than Plus->Minus. There was a trend toward a group x test order interaction ($F(1, 44) = 3.62$, $p = .064$) with no other significant effects (all $F < 1$).

The absence of a group x context interaction was unexpected, given this was a reliable result in Chapter 2 To explore this and clarify the nature of the test order interaction effect, we applied separate 2 x (2) (test order x [context]) mixed-ANOVAs to each group. For the *Single* group, the main effect of context was not significant ($F(1, 22) = 2.66$, $p = .12$) but there was a significant test order x context interaction ($F(1, 22) = 5.79$, $p = .025$). For the *Variety* group, the context main effect was significant ($F(1, 22) = 6.31$, $p = .020$) as well as the context x test order interaction ($F(1, 22) = 9.86$, $p = .005$). As shown in Figure 3.3.4, rats tested M->P ate significantly more in the Plus context (*Single* group: $F(1, 11) = 8.09$, $p = .016$; *Variety* group: $F(1, 11) = 14.76$, $p = .003$), whereas rats in both groups tested P->M showed no difference in consumption between the contexts (both $F < 1$). In summary, although the context main effect was significant in the *Variety* but not the *Single* group, the difference between these effects (i.e., the interaction) was not statistically significant.
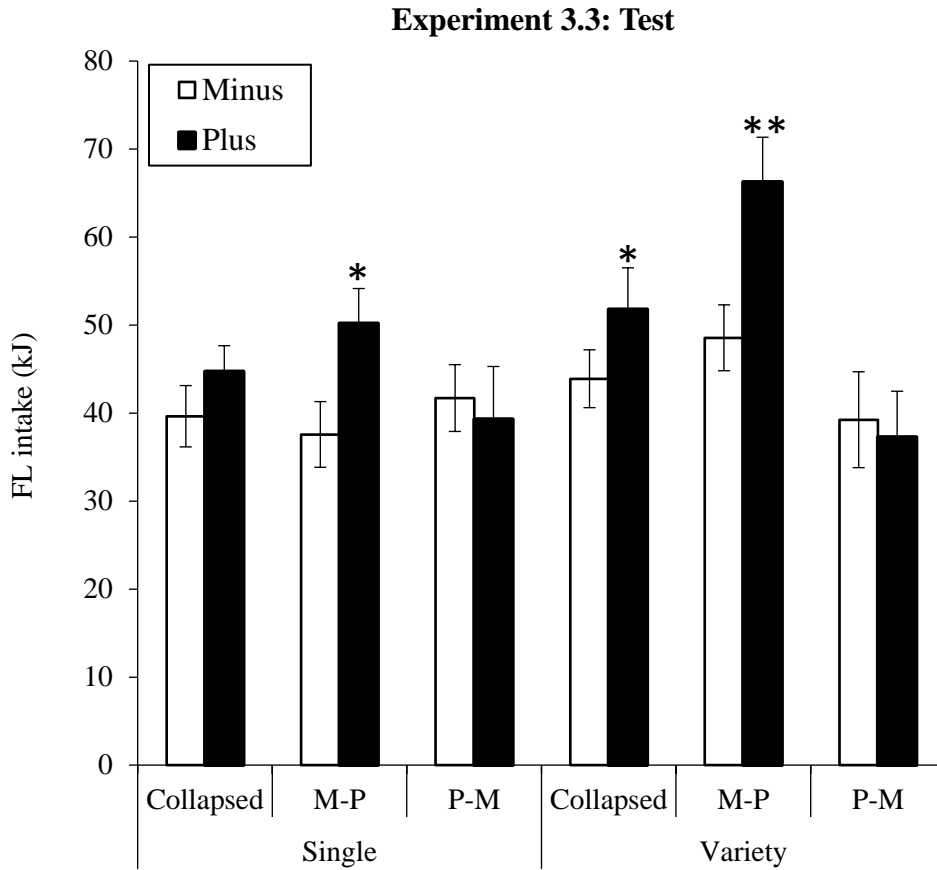
Experiment 3.3: Test

Figure 3.3.4. Experiment 3.3 test results. An overall CPF effect indicated greater consumption in the Plus context; however, these results were moderated by test order (see text for details). *$p < .05$; **$p < .01$ for context effects within cohorts.

Supplementary tests

Cohort 1: Training food test

As described, *Cohort 1* received a preliminary test of the training foods, in which the *Single* group received 15g of their training food and the *Variety* group received 5g of each of the three training foods. This latter decision appeared to undermine analysis of context effects, however, by fostering far greater intake in the *Variety* group relative to the *Single* group (overall group means = 91.00 ± 7.2 kJ and 58.12 ± 5.1 kJ, respectively). Thus, when consumption in the contexts was analysed in a 2 x 2 x (2) mixed-ANOVA (group x test order

x [context]), only the group main effect was significant ($F(1, 20) = 12.86$, $p = .002$), but no other main or interaction effects were significant (largest $F(1, 20) = 1.32$, $p = .27$).

Cohort 2: Time-course of CPF

Intake in 10-min bins is shown in Figure 3.3.5 and was analysed in a 2 x 2 x (2) x (3) mixed-ANOVA (group x test order x [context] x [bin]). This analysis found that consumption differed significantly between bins ($F(2, 40) = 26.08$, $p < .001$) and that this effect interacted with context and test order (3-way interaction: $F(2, 40) = 4.04$, $p = .025$) with no other significant interaction effects (largest $F(2, 40) = 2.48$, $p = .096$). To explore these effects, separate analyses examined consumption in each 10-min bin. Considering Figure 3.3.5 suggests that the second 10-min bin was where context effects were most pronounced. The main effect of context was not significant in the first or third 10-min bin ($F < 1$ and $F(1, 20)$ = 1.77, $p = .20$, respectively) but was significant in the second 10-min bin ($F(1, 20) = 5.19$, p = .034). In this bin there was also a significant group main effect ($F(1, 20) = 9.54$, $p = .006$) indicating greater overall consumption in the *Variety* group. The main effect of test order was significant in each bin ($F(1, 20) = 4.99$, $p = .037$; $F(1, 20) = 5.78$, $p = .026$) and $F(1, 20) =$ 7.72, $p = .012$), indicating higher consumption in rats tested Minus->Plus than Plus->Minus. No other main or interaction effects were significant (largest $F(1, 20) = 3.76$, $p = .067$).

Time course of CPF
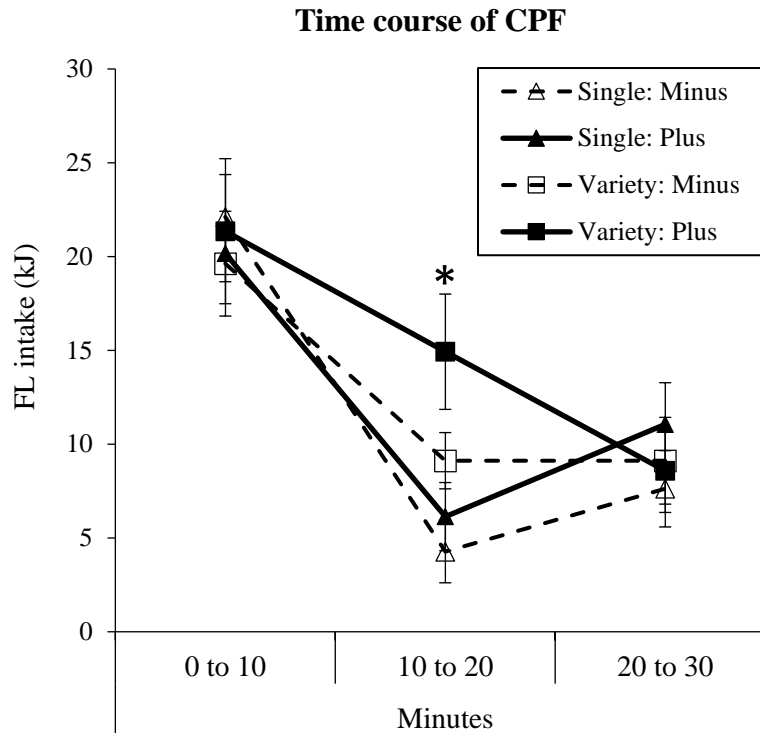
Figure 3.3.5. Time-course analysis of Cohort 2 in Experiment 3.3. The CPF effect was significant only in the second 10-min bin, with consumption beginning high and ending low in both groups and contexts. *$p < .05$ for main effect of context.

Correlations with CPF

No significant correlations were found between baseline FL intake, average training intake, and CPF expressed as a proportion (largest $r_s = .24$, $n = 48$, $p = .098$, for baseline FL vs. training intake). When *Single* and *Variety* groups were analysed separately, training intake and baseline FL intake correlated positively for the *Variety* group ($r_s = .657$, $n = 24$, $p < .001$) but no other correlations were statistically significant (largest $r_s = .324$, $n = 24$, $p = .13$).

3.3.3. Discussion

The first key result from Experiment 3.3 was that rats showed incomplete compensation for the palatable foods eaten during training, leading to an increase in energy intake on these days. Studying total energy intake in CPF experiments is often precluded by the restriction both of home-cage chow and of the food paired with food cues or contexts. Each were free to vary in the present experiment, allowing comparison of chow intake on Plus versus Minus days. Increased total energy intake was suggested by the fact that although rats tended to eat slightly less chow on days beginning with a Plus session, this suppression did not account for the amount of food eaten in the Plus context. However, there was also evidence that chow consumption on Minus days was lower when a Plus session had been held the previous day. This may reflect a longer term compensatory mechanism for the substantial amounts of palatable food eaten in the Plus context.

Although the observation that palatable foods enhance energy intake in rats is by no means novel, few studies have shown this when there is only very limited access, as in the present experiment (i.e. 30-min on alternate days, on average). Given that this experiment calculated energy intake on a per-cage basis, richer data could be obtained from individually housed animals given similar training in future experiments. An interesting question is whether compensation would be poorer in the *Variety* group due to their exposure to multiple foods of varying energy densities, as predicted by some theories of variety (Martin, 2016). Whereas this experiment considered the effects of palatable food exposure within-subjects (i.e. Plus vs. Minus days), comparisons with groups fed only chow or fed palatable food only in the home-cage would determine whether context conditioning of this type contributes meaningfully to long-term energy intake.

Test results found that consumption of FL, a food never exposed in the contexts during training, was significantly greater in the Plus context. Unlike experiments in Chapter 2, however, the CPF effect did not interact with group. Additionally, consumption in the contexts was significantly moderated by the order in which they were tested: if tested first in the Minus context, rats in both groups displayed a robust CPF effect and ate more on their second test (in the Plus context). By contrast, rats tested first in the Plus context ate comparable amounts of FL in both tests. It may be noted that for these rats, a CPF effect required inhibiting consumption of the now-recently-exposed food on the second (Minus) test. An important note is that context was a significant determinant of consumption despite test order effects. Thus, the main effect of context was significant for the *Variety* but not for the *Single* group. Nonetheless, in the absence of a significant interaction between context and group, this result cannot be concluded to indicate a stronger effect in the *Variety* group.

The time-course analysis in Cohort 2 suggested that the overall CPF effect was driven by greater consumption in the Plus context in the middle portion of the test. Thus, consumption was high in all conditions in the first 10-min, and low in all conditions in the final 10-min. This bears some resemblance to a past study in which a CPF effect across a 20-min test was driven by greater consumption during the 11-20 min period (Petrovich et al., 2007b). Finally, the training food test administered to Cohort 1 found no CPF effect in either group. For the *Variety* group this was almost certainly explained by the simultaneous provision of all 3 foods, which dramatically enhanced consumption and appeared to obscure any influence of context. However, the lack of a CPF effect in the *Single* group in Cohort 1 is more difficult to explain, since Experiment 2.1 found an effect on the training food after an equivalent period of training (12 sessions). Finally, no significant associations were found between baseline FL intake, training consumption, and the extent of CPF, despite a substantially larger sample.

3.4. General discussion

The general aim of the experiments presented in Chapter 3 was to further explore characteristics of CPF as observed in Chapter 2. Three results from these experiments suggested that the relative palatability of the training versus test food is an important determinant of whether CPF can transfer to alternative foods. First, the 'variety' effect reported in Chapter 2 was not evident when a familiar but less palatable alternative food – chow – was tested in Experiment 3.1. This finding is consistent with most previous CPF experiments to test chow, in which floor effects have been observed (Petrovich et al., 2007a, 2007b, 2012; Reppucci & Petrovich, 2012), except when very long (4-24h) tests are used (Boggiano et al., 2009). Second, Experiment 3.2 found that most rats preferred their training food to FL. Third, this experiment found that variability in this preference correlated negatively with CPF on the alternative test food, FL. Thus, CPF when testing FL tended to be weaker in rats that more strongly preferred their training food, and stronger in rats for which the foods were more equally preferred. These results each implicate contrast processes in the specificity of CPF.

The second aim of these experiments was to test whether there would be meaningful individual differences in various aspects of rats' eating behaviour that would correlate with CPF. Our two variables – baseline FL and average training consumption – were effectively measures of short-term food intake: how much palatable food would rats eat in a limited amount of time? Notably, these variables rarely correlated, suggesting that the propensity to overeat FL at baseline did not necessarily continue in training with other palatable foods. In any event, the clear result was that neither variable correlated with the CPF effect in any group. The large sample used in Experiment 3.3 appears to rule out the possibility that the failure to detect significant correlations was because of inadequate statistical power. Thus, overeating in the Plus context was not related to variability in initial consumption of FL, nor

how much food was previously eaten in that environment. One way in which this could be

explored further in future work would be to administer more baseline tests to allow for more

stable 'binge-like' patterns of consumption to emerge, since the experiments here used only

1-3 sessions. However, the results of Experiment 3.2 indicate this approach might interfere

with subsequent context conditioning. In contrast to measures of absolute consumption,

measuring animals' hedonic responses to foods would seem an interesting measure to

compare with the strength of CPF effects.

A caveat for the null results observed for the correlational analyses is that the overall

CPF effects were generally weaker than in Chapter 2. For Experiments 3.1 and 3.2 these

appeared due to the addition of the chow test (3.1) and due to the extensive pre-exposure to

the training foods (3.2). By contrast, Experiment 3.3 found an overall effect of context that

did not interact with group. The only evidence for an effect of variety was in tests of simple

effects that suggested a CPF effect only in the *Variety* group. However, as discussed, the

effect of context was not *significantly* greater in this group relative to the *Single* group. In any

case, the variability in CPF effects was what originally prompted us to investigate individual

differences.

Chapter 4: Unexpected failures to detect CPF

4.0. Introduction

The experiments reported in this chapter were undertaken to examine how motivational processes and procedural variables might contribute to CPF. The primary aim was to test experimental manipulations that might yield clearer CPF effects. Our first hypothesis was that this might be achieved by a simple motivational change: increasing hunger during training. Thus, Experiment 4.1 trained rats under food-restriction, following many reported CPF studies (Petrovich, 2013), but unlike experiments in Chapters 2 and 3 where rats were non-deprived, and compared CPF in different deprivation states during testing. Experiment 4.2 tested CPF following a longer delay between training and test, while also assessing the effects of extinction of the Plus context. These manipulations were intended to remove or reduce test order effects, an added source of variability in past experiments, by increasing consumption of the alternative food, FL, at test. In contrast to the motivational and incentive influences tested in Experiments 4.1 and 4.2, Experiment 4.3 precluded test order effects by testing CPF in a between-subjects design that removed the need for multiple tests. However, none of these experiments produced a CPF effect. The possible reasons for this are discussed with reference to incentive contrast.

4.1. Experiment 4.1: CPF following food restriction during training

So far all of the experiments reported here, save for Experiment 2.1, have studied CPF without the use of food restriction. The provision of unrestricted access to chow throughout all procedures contrasts with the approach commonly adopted in previous CPF experiments; in these studies rats are usually food-restricted during training and tested only after a period of re-feeding in the home-cage when body weight returns to free-feeding levels (Petrovich,

2013). Food restriction is, of course, standard practice in animal models of appetitive conditioning, and encourages initial learning and sustained responding in Pavlovian and instrumental tasks involving food reward. However, it is by no means necessary for learning to occur. The results reported in Chapters 2 and 3 and those from another study (Boggiano et al., 2009) show that food cues exposed to non-deprived animals can still exert some influence over feeding behaviour. Additionally, eating in the absence of hunger may provide a more valid model of *overeating*. Nevertheless, the effects reported in previous chapters were modest in size, despite their statistical significance. We reasoned that increasing the strength of conditioning during training should yield stronger effects in CPF tests. The most obvious means to achieve this was to implement food-restriction during training.

The resulting within-subject design of Experiment 4.1 was one in which animals were trained deprived and initially tested satiated. The three primary groups were those included in Chapters 2 and 3: *Chow*, *Single*, and *Variety* rats. A fourth group received exposure to the three palatable training foods and to the contexts in an unpaired fashion. This *Unpaired* group controlled for non-associative factors that could feasibly affect food intake during tests, such as experience with eating large amounts of palatable food within limited periods of time; or mere exposure to multiple foods. Given that the *Chow* groups included in Chapters 2 and 3 controlled for neither of these factors, the *Unpaired* group appeared to better match *Single* and *Variety* groups.

The question of primary interest was how training under food restriction, and the consequent increase in consumption in the Plus context, would affect the transfer of CPF to an alternative food. We hypothesised that greater consumption in training might produce stronger associations between the Plus context with both the act of eating and the relief of hunger, increasing consumption of alternative foods at test. The first test in Experiment 4.1 assessed FL consumption in satiated rats so that, aside from training deprivation state,

conditions were comparable to experiments in Chapters 2 and 3. In two subsequent tests, however, rats were acutely food-deprived in order to assess the effects of deprivation state at test. Thus, Test 2 measured intake of FL, whereas Test 3 examined consumption of the training foods, with deprivation state now consistent with training conditions.

4.1.1. Method

Subjects

Forty adult, experimentally naïve, female Sprague-Dawley rats were used. Rats were bred in-house at the School of Psychology, University of Sydney. The early life history was identical for all rats until the beginning of the present experiment, at which point they were 10 weeks old and weighed an average of 251g (range: 190 – 316g). Rats were group-housed ($n = 2$ (two cages) or $n = 4$ (nine cages)) in temperature- and humidity-controlled ventilated cages within a colony room maintained on a reverse light cycle (lights off 0845-2045h).

Apparatus

The preparation of the contexts and foods were as described in Chapter 3.

Procedure

Adaptation to food restriction

Over a 1-week acclimation phase rats were weighed daily. Next, rats were familiarised with the test food, Froot Loops (FL), in individual feeding cages in two 30-min sessions held on consecutive days. A day later home-cage chow was removed at 1700 hrs and thereafter chow was provided only between 1500 and 1700 hrs each day. Daily consumption per cage was measured and rats were given five days to adjust to this schedule prior to training.

Design

Rats were allocated to *Unpaired*, *Chow*, *Single*, or *Variety* groups (each $n = 10$) that did not differ in terms of body weight and consumption of FL in familiarisation sessions. Since the *Unpaired* group were to receive palatable food in the home cage in training, these rats were housed in condition-matched cages (two cages of 4 and one cage of 2 rats). The other three groups were distributed among the remaining cages.

Training

Training lasted 12 days and consisted of six 30-min intermixed exposures to Plus and Minus contexts, as in previous chapters (order: MPPMMPPMPMMP). Table 4.1 shows the training procedure on Plus and Minus days. Rats were run in four 10-rat squads; the *Unpaired* rats were one squad and the other three contained a mix of *Chow*, *Single*, and *Variety* rats. The order in which these squads were trained was varied over days (days 1-4: order ABCD; days 5-8: DCAB; days 9-12: BDAC). In the Plus context the *Single* group received either Oreos ($n = 3$), Sausage roll ($n = 3$) or Banana Bread ($n = 4$); the *Variety* group received cycling access to all three of these foods in varying orders; while the *Chow* group received chow. The Minus context contained no food for any group.

The *Unpaired* group received equivalent exposures to the two contexts, except that neither contained food. Instead, on Plus days the *Unpaired* group was given access to one of the three palatable foods for 30-min in the home-cage. The specific food provided each day was varied, as for the *Variety* group, such that all *Unpaired* rats were exposed to the three palatable foods. The total amount of food provided to each home cage was equivalent, per rat, to that provided to rats in *Single* and *Variety* groups. After 30 min, fragments of food were located and weighed to estimate intake. The bedding was then changed to ensure no traces of food or odours remained beyond this period. On these Plus days home-cage palatable food

access occurred at least 1 h before or 1 h after exposure to one of the two (empty) contexts, and the two contexts were exposed equally on Plus and Minus days. Therefore, this group was matched in terms of exposure to the palatable foods, exposure to the contexts, and handling, but consumption was not paired with either environment.

Table 4.1. Daily training procedures for Experiment 4.1.

PLUS:	8:30	9am – 12pm			~3-5pm
Variety	BW taken	Contexts (3 runs of 10 rats; 30-min each)			Home-cage chow
Single					
Chow					
Unpaired		**Home-cage PF** (or contexts)	←at least 60-min→	**Contexts** (or home-cage PF)	
MINUS:	8:30	9am – 12pm			~3-5pm
Variety	BW taken	Contexts (4 runs of 10 rats; 30-min)			Home-cage chow
Single					
Chow					
Unpaired					

Tests

After training rats were given unrestricted access to chow in the home cage for five days. All tests lasted 30 min and the order in which contexts were tested was counterbalanced. The two halves of each test were separated by a single day of rest in the home cage. Tests 1 and 2 measured FL consumption under sated and deprived conditions, respectively. Test 2 (deprived) began two days after the end of Test 1 and involved removing home-cage chow at 1700 hrs on the evening prior to tests, as had previously occurred during training. After Test 2 rats received two re-training sessions over three days (Minus – rest – Plus), during which time the 2-h restricted feeding schedule was returned for the remainder of the experiment. Finally, Test 3 measured consumption of the training foods in the contexts.

For this test the *Variety* and *Unpaired* rats were tested with the food they received on the last Plus re-training day.

4.1.2. Results

FL familiarisation

On average, rats ate a total of 3.98 ± .29g [SEM] of FL in the two familiarisation sessions. This corresponded to approximately 13 FL pieces.

Body weight

On average, food restriction reduced rats to 95.8 ± .5% [SEM] of their free-feeding weight during training, with no differences between groups (one-way ANOVA: $F < 1$). Returning rats to unrestricted chow after training increased body weight to 107.0 ± .8% of weights prior to food restriction, on average. Again, groups did not differ on this measure ($F < 1$).

Training

Consumption during Plus sessions is shown in Figure 4.1.1 and was analysed in a 3 x (6) ANOVA (group x [session]) comparing *Chow*, *Single*, and *Variety* groups. The *Unpaired* group was not included in this analysis since consumption for individual rats could not be determined. However, average consumption is presented in Figure 4.1.1 for comparison. Analysis found significant linear ($F(1, 27) = 110.22$, $p < .001$) and quadratic trends ($F(1, 27) = 21.38$ $p < .001$) which each interacted with group ($F(2, 27) = 20.55$, $p < .001$ and $F(2, 27) = 10.53$, $p < .001$, respectively). There was also a group main effect ($F(2, 27) = 28.79$, $p < .001$). Follow-up contrasts using the Tukey HSD correction found that the *Single* and *Variety* groups each ate significantly more than the *Chow* group (both $p < .001$). Of interest, average

consumption over training was significantly greater in the *Single* group than in the *Variety* group ($p = .016$). However, additional analysis found groups did not differ on the last Plus session ($F < 1$). Notably, consumption by the *Unpaired* group in the home cage appeared at least as high as *Single* and *Variety* groups.

Experiment 4.1: Training
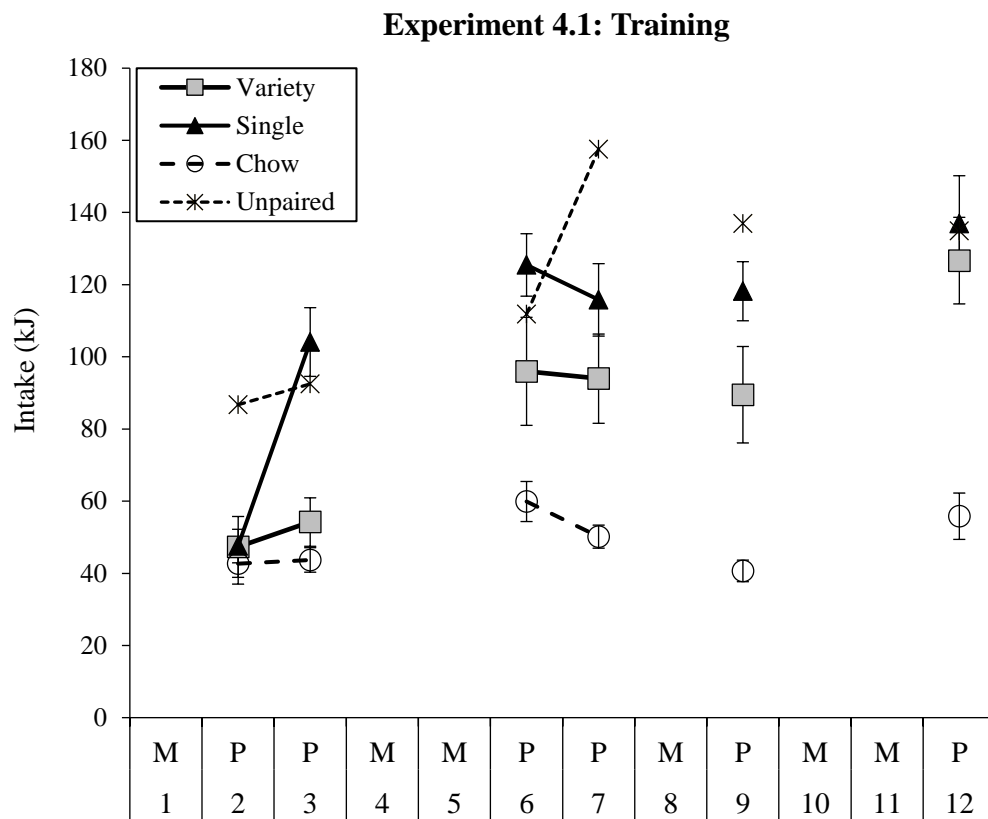
Figure 4.1.1. Training consumption in Experiment 4.1. *Single* and *Variety* groups increased their consumption of palatable food/s, while the *Chow* group's chow intake was consistent across training. *Unpaired* rats received home-cage access to palatable food.

Tests

Results from the three CPF tests are shown in Figure 4.1.2. The primary analysis compared CPF between the *Chow*, *Single* and *Variety* groups in 3 x 2 x (2) mixed-ANOVAs (group x test order x [context]) at each test. To facilitate comparison across the three tests, compiled data are presented together in Panels A, B, and C of Figure 4.1.2. Figures splitting

these compiled data between test order cohorts are shown in Appendix D. Consumption by the *Unpaired* group was compared to the *Variety* group in additional analyses described below.

Test 1 (FL, sated)

Consumption in Test 1 is shown in Figure 4.1.2.A. Analysis found no main effect of context ($F(1, 24) = 1.45$, $p = .24$) and no context x group interaction ($F(2, 24) = 1.27$, $p = .30$). However, there was a significant context x test order interaction ($F(1, 24) = 21.20$, $p < .001$). To explore this result further, Minus-Plus and Plus-Minus cohorts were analysed in separate 3 x (2) (group x [context]) ANOVAs. These analyses showed that rats tested Minus->Plus ate significantly more in the Plus context ($F(1, 12) = 104.66$, $p < .001$) while rats tested Plus->Minus ate significantly more in the Minus context ($F(1, 12) = 107.91$, $p < .001$). Therefore, consumption increased from the first to the second test day, irrespective of which context was tested. No other main or interaction effects were significant (largest $F(2, 24) = 2.01$, $p = .157$).

Test 2 (FL, deprived)

Consumption of FL in the contexts under food deprivation is shown in Figure 4.1.2.B. Analysis found a significant main effect of context ($F(1, 24) = 7.59$, $p = .011$) indicating higher consumption in the *Minus* context, on average. The context x test order interaction was significant ($F(1, 24) = 23.08$, $p < .001$), but the context x group interaction was not ($F < 1$). No other main or interaction effects were significant (largest $F(1, 24) = 3.14$, $p = .09$). Separating the data according to test order confirmed that, as in Test 1, increasing consumption over tests drove the context x test order interaction. However, this effect was statistically significant for the Plus-Minus cohort ($F(1, 12) = 22.84$, $p < .001$) but not statistically significant for the Minus-Plus cohort ($F(1, 12) = 2.80$, $p = .12$).

Test 3 (Training food, deprived)

Consumption of the training food under food deprivation is shown in Figure 4.1.2.C. Consumption in grams was converted to kJ to account for differing energy densities. The main effect of context was not significant ($F(1, 24) = 2.37$, $p = .14$) and did not interact with group ($F < 1$). There were significant interactions between context and test order ($F(1, 24) = 16.44$, $p < .001$) and context, group, and test order ($F(2, 24) = 5.50$, $p = .011$). The main effect of group was significant ($F(2, 24) = 28.89$, $p < .001$) but not the main effect of test order or the group x test order interaction (largest $F(2, 24) = 2.40$, $p = .11$). The group main effect reflected significantly greater intake in *Single* and *Variety* groups relative to the *Chow* group (Tukey HSD correction: both $p < .001$), with no difference between the former two groups ($p = .99$). Split-file analyses showed that unlike Tests 1 and 2, decreasing consumption over tests drove test order interactions. Thus, the cohort tested Plus->Minus ate significantly more in the Plus context (context main effect: $F(1, 12) = 12.42$, $p = .004$) with no context x group interaction ($F(2, 12) = 2.92$, $p = .092$), whereas the cohort tested Minus->Plus tended to eat more in the Minus context ($F(1, 12) = 4.28$, $p = .061$) with no group x context interaction ($F(2, 12) = 3.09$, $p = .083$).
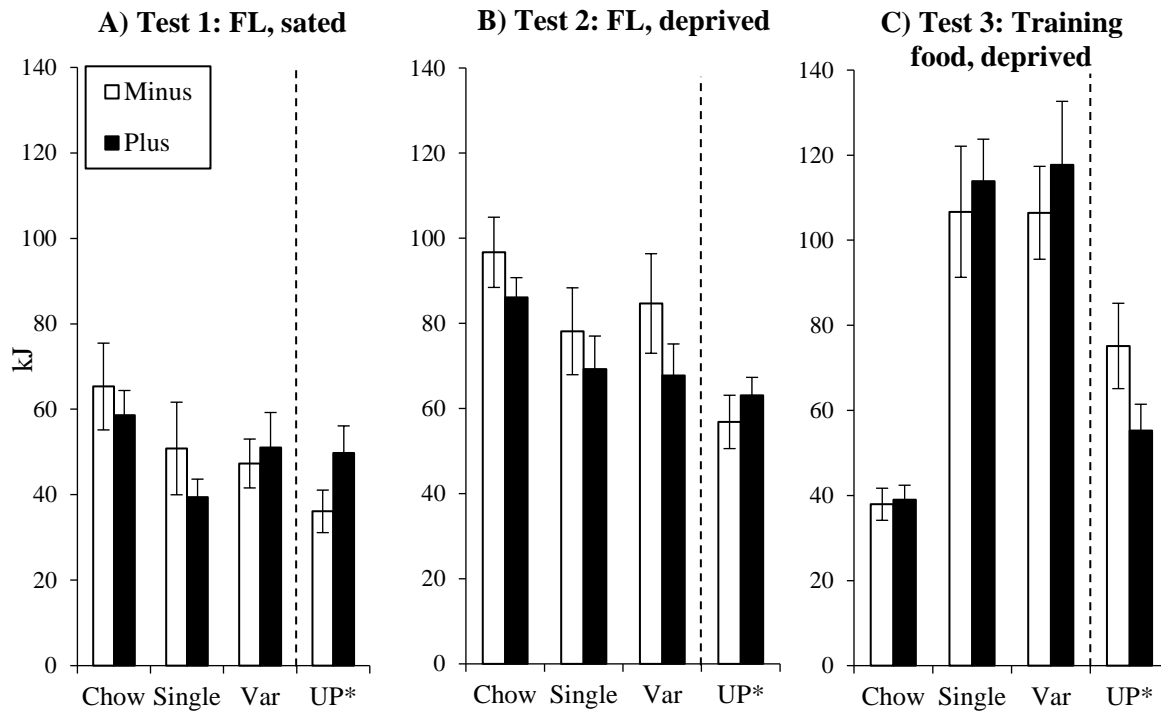
Figure 4.1.2. Experiment 4.1 test results. In Test 2 (B: FL, deprived), a significant main effect of context indicated rats ate *less* in the Plus context. No effect of context was found in Test 1 (A: FL, sated) or Test 3 (C: training food, deprived). *UP = Unpaired rats. The 'Minus' and 'Plus' tests denote this group's first and second test, respectively.

Correlational analyses

The above results were contrary to hypotheses and, in general, indicated high variability. Whereas Test 1 found no evidence that the Plus context modulated consumption of FL when rats were sated, more interesting data were obtained from the two food-deprived tests: The Plus context suppressed FL intake in Test 2, yet appeared to weakly enhance consumption of the training foods in Test 3, at least for *Single* and *Variety* groups. To explore the relationship between these two tests, we calculated the proportion measure used in Chapter 3 (CPF proportion = Plus / (Minus + Plus)) and correlated this measure between the two tests, as shown in Figure 4.1.3. Analyses indicated a significant negative correlation between CPF on Tests 2 and 3 (r_s = -0.48, n = 30, p = .007), such that rats that ate a lower proportion of FL in the Plus context in Test 2 tended to exhibit a stronger CPF effect on the

training food. This correlation was significant in the *Single* and *Variety* groups (r_s = -0.87, n = 10, p = .001; r_s = -0.64, n = 10, p = .048, respectively), but not the *Chow* group (r_s = .03, n = 10, p = .93). These results indicated that for groups trained with palatable food/s, a stronger CPF effect with the training food was associated with a weaker CPF effect on FL. This was not an artefact of test order, which was preserved (but counterbalanced) across the two tests. Further analyses found that CPF on Test 1 also correlated negatively with Test 3 (r_s = -0.544, n = 30, p = .002) while CPF on the two FL tests – Tests 1 and 2 – correlated positively (r_s = 0.548, n = 30, p = .002).
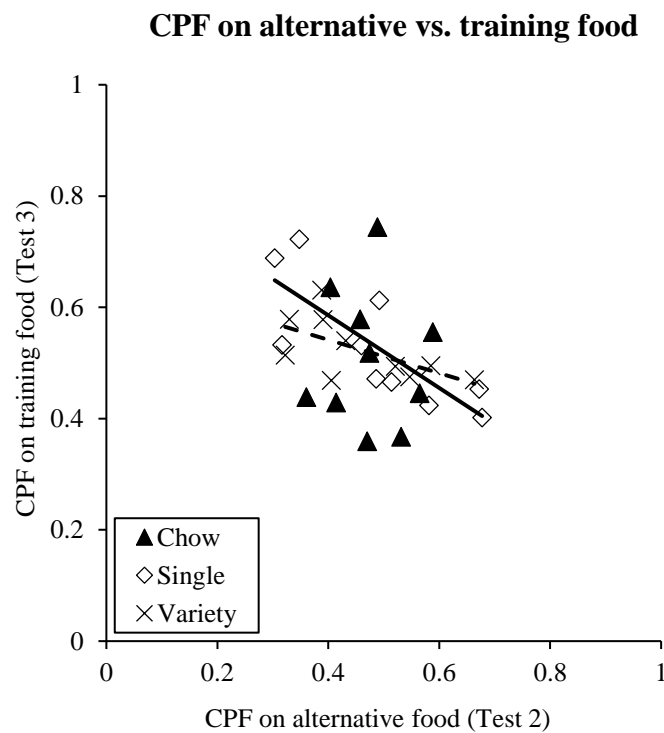
Figure 4.1.3. Correlational analyses in Experiment 4.1. For *Single* and *Variety* groups (solid/dashed lines for trends, respectively) but not the *Chow* group (no trendline), a stronger CPF effect on FL in Test 2 associated with a *weaker* CPF effect on the training food in Test 3.

Comparison with the Unpaired group

The *Unpaired* group were matched to the *Variety* group in terms of their exposure to multiple palatable foods and to the contexts, except that exposure was explicitly unpaired. To

compare these groups, the *Variety* group's consumption in the Plus context was assessed against the *Unpaired* group's average consumption in the two context tests using independent samples *t*-tests. No differences were found on Tests 1 or 2 when FL were the test food (both *t* < 1). On Test 3, the *Variety* group ate significantly more in the Plus context than the *Unpaired* group ($t(18) = 3.15$, $p = .006$). Although this suggested a between-group CPF effect, consumption was also significantly higher in the *Variety* group's Minus test than in the *Unpaired* group ($t(18) = 3.17$, $p = .005$) (see Figure 4.1.2.C). Further analyses that considered the *Unpaired* group's data in terms of test order effects are described in Appendix D.

4.1.3. Discussion

Experiment 4.1 tested whether training rats under mild food restriction would enhance the transfer of CPF to an alternative food. Unsurprisingly, food restriction substantially increased consumption of palatable foods for *Single* and *Variety* groups, relative to previous experiments. While the general pattern over training was comparable to past experiments, one notable aspect of the data was that food deprivation appeared to foster greater learning about the contexts in the *Chow* group, given their consumption of ~3g chow per Plus session. Consequently, it is likely that unlike previous experiments, in which satiated *Chow* rats often ate no chow at all, the Plus and Minus contexts carried distinct associations with food and no food, respectively.

Increasing consumption during training was hypothesised to produce a stronger association of the Plus context with the act of eating and the rewarding post-ingestive consequences of consuming palatable foods. In turn, the question of interest was whether this would facilitate the consumption of an alternative food in CPF tests. Contrary to predictions, the Plus context was without effect on FL intake in Test 1, when rats were tested in a state of

relative satiety. This was most surprising in the *Variety* group, given the CPF effects previously described. Tests 2 and 3 explored whether CPF would manifest when deprivation state was similar to training conditions by testing after overnight food deprivation. Test 2 found that FL consumption was *suppressed* in the Plus relative to the Minus context, and that this effect did not differ significantly between groups. Consumption of the training food under deprived conditions did not vary between contexts in Test 3.

One interpretation of the context effect in Test 2 is that training under food deprivation modulated the content of learning and produced stronger associations between the Plus context and the specific sensory qualities of the training foods, irrespective of whether these were palatable (*Single*/*Variety*) or bland (*Chow*). At test, then, placement in the Plus context generated a stronger specific expectation of the training food, suppressing consumption and preventing generalisation to the alternative, FL. By contrast, the Minus context, free of associations with food, allowed greater consumption. Although contrary to our hypotheses, the failure to observe CPF on an alternative food is consistent with Reppucci and Petrovich (2012), who found that a cue paired with pellets did not modulate consumption of chow that was available simultaneously, regardless of whether rats were satiated or deprived at test. Reppucci and Petrovich (2012) also found no effect on the training food when animals were deprived at test, consistent with the present Test 3.

A procedural decision that might explain the present results is that the training foods and test food (FL) were initially exposed in different motivational states. Past work shows that the incentive value of food, as evaluated by instrumental responding, is moderated critically by the motivational state in which it is experienced (Balleine & Dickinson, 1998b). Here, rats were non-deprived for FL familiarisation sessions and ate ~2g in 30-min. By contrast, when subsequently food-deprived, they ate over 3g of the training foods in the first Plus session – and far more thereafter. Consequently, food restriction afforded greater

opportunity for learning about the satiating properties of the training foods in addition to their palatability, unlike FL, for which learning was likely constrained to palatability. Therefore, it seems likely that the incentive value of FL was lower than the training foods, on account of their being pre-exposed prior to food restriction, blocking the ability of the Plus context to increase consumption in Test 1. This effect was stronger in Test 2, perhaps because food restriction enhanced attention to the satiating properties of the available food (and, possibly, generating a stronger expectation of the training food). Without prior experience of eating FL while food-deprived, consumption of FL was suppressed further.

Despite the absence of significant CPF effects, there were significant correlations between the three tests. While CPF correlated positively between the two FL tests, each of these tests correlated negatively with CPF on the training food test. This was clearest when comparing the two tests held under food-restriction (Tests 2 and 3). In *Single* and *Variety* groups trained with palatable food, rats that exhibited stronger CPF for FL on Test 2 tended to display weaker CPF for the training food in Test 3. Put differently, the degree to which FL were avoided in the Plus context in Test 2 associated with a stronger CPF effect on the training food in Test 3. This might suggest that the ability of CPF to transfer to alternative foods is influenced by how strongly the Plus context was associated with the training food. This adds to the result in Experiment 3.2 that animals with stronger preferences for their training food over FL tended to exhibit a weaker CPF effect on FL. Together, these results indicate that the contrast between the training and alternative test food is an important determinant of CPF.

In summary, results suggest that the ability of CPF to transfer to alternative foods is modulated by deprivation state at test. Another variable that will be important to explore more systematically in future work is animals' deprivation state when they are initially exposed to the foods that are to be tested. In the present experiment, our decision to match

procedures closely with previous chapters – i.e., to pre-expose FL to satiated rats – may have prevented CPF effects by reducing the incentive value of the test food.

4.2. Experiment 4.2: CPF after a longer training-test delay, with or without extinction of the Plus context

Experiment 4.2 began with the hypothesis that presenting FL in CPF tests could be considered an example of the kind of procedure that can produce incentive contrast: alterations in behaviour observed when a reward is replaced with a more or less desirable alternative (positive and negative contrast; Flaherty, 1996). Specifically, the presentation of FL in CPF tests appeared analogous to *successive* contrast procedures in which animals are repeatedly exposed to one reward before a sudden shift to a more- or less-preferred alternative. Thus, the substantially increased consumption FL by *Chow* rats, relative to their minimal intake of chow during training, would appear to reflect positive contrast. Conversely, negative contrast appeared to accurately describe the tendency for *Single* and *Variety* groups to eat less FL in CPF tests than palatable food in training (irrespective of CPF effects in the latter group). Support for this notion comes from Experiment 3.2: Rats clearly preferred their training foods to FL, and stronger preferences for the training food were associated with a smaller CPF effect on FL.

We reasoned that negative contrast might contribute to the test order effects reported in previous experiments, which have reflected initially low consumption that increases over successive tests. Therefore, manipulations that attenuate negative contrast should reduce test order effects and enhance CPF. Importantly, evidence indicates that negative contrast is attenuated as the delay between experiencing the two contrasting rewards increases. For example, the suppression in consumption – i.e. negative contrast – produced by a shift from

32% to 4% sucrose solution is reduced, and eventually abolished, as the delay between exposure to the two is increased (Flaherty, 1996). In addition, flavour conditioning studies have shown that after pairing neutral flavours with saccharin, responding for the flavour presented in water alone is suppressed when tested the day after conditioning (i.e. negative contrast) but is substantially greater when tested after a delay of 7 or 14 days (Holmes, Hutton-Bedbrook, Fam, & Westbrook, 2016). Most experiments in this thesis so far have tested CPF 1-2 days after the end of training, with slightly longer delays (5-6 days) in the two experiments using mild food deprivation (Experiments 2.1 and 4.1). Each of these delays are shorter than most previous studies of CPF, in which more stringent food deprivation typically requires 1-2 weeks of re-feeding in the home cage to allow rats to regain body weight.

Therefore, Experiment 4.2 tested CPF after a longer delay between training and test to examine whether this would reduce negative contrast at test. This could occur via several mechanisms. First, a delay involving extended access only to chow in the home cage might increase the incentive value of FL when presented at test. Second, the memory of the specific food/s paired with the Plus context might decay over a longer delay (Holmes et al., 2016), leaving a more general association with food that would more easily generalise to an alternative food at test. Thus, the first hypothesis in Experiment 4.2 was that both *Single* and *Variety* groups would show CPF on an alternative food after a longer delay.

Experiment 4.2 also tested CPF when the association of the Plus context with food was extinguished in some groups by now placing the rats in the Plus context when it no longer contained food. Our rationale for testing the effects of extinction was because there would appear to be many instances where individuals deliberately choose not to buy or consume food in environments where 'bad' food choices were once made, or when confronted with cues signalling food. Given that extinction comprises the learning of new inhibitory associations and not the erasure of original learning (Bouton, 2011), the key

question was how consumption would be affected when an alternative food was presented in the Plus context after extinction. The effects of extinction training on CPF do not appear to have been studied previously. However, research on Pavlovian-to-Instrumental transfer (PIT) indicates that although extinction suppresses specific PIT under some circumstances (Delamater, Schneider, & Derman, 2017; see also Delamater, 1996), the effects of extinction are sensitive to context shifts and rarely produce a complete suppression in responding to cues (Laurent, Chieng, & Balleine, 2016; Bezzina, Lee, Lovibond, & Colagiuri, 2016).

Of particular interest was whether the effects of extinction would differ for *Single* and *Variety* groups. Thrailkill et al. (2014) reported that the rate of extinction of an instrumental response did not differ significantly between a 'Variety' group for which responding had previously earned multiple food rewards and a group for which responding always earned the same reward. Our focus here was not on behaviour during extinction of the Plus context (indeed, there was relatively little that could be measured), but rather how this treatment would affect consumption when an alternative food was subsequently made available.

Our interpretation of the 'variety effect' reported in Chapters 2 and 3 was that exposure to multiple foods during training encoded a broader representation of food reward that encompassed the alternative food, FL. Therefore, extinction of the Plus context should suppress intake of FL at test for the *Variety* group. On the other hand, extinction for the *Single* group should target the specific characteristics of the training food and not FL, allowing the context to promote consumption of this food. We therefore hypothesised that extinction would abolish the CPF effect in the *Variety* group and yield a CPF effect in the *Single* group. As described earlier, CPF effects were also predicted in both groups tested after a delay with no extinction. Table 4.2 shows the 2 x 2 factorial design used to assess these questions.

Table 4.2. Experimental design in Experiment 4.2. P = Plus context paired with food; M = Minus context paired with no food.

Group	Phase 1 (12 days)	Phase 2 (12 days)	Phase 3: Test
Single-Delay	6 x P→1 food, 6 x M→no food	-	FL (in P + M)
Single-Ext	6 x P→1 food, 6 x M→no food	6 x P→no food	FL (in P + M)
Var-Delay	6 x P→3 foods, 6 x M→no food	-	FL (in P + M)
Var-Ext	6 x P→3 foods, 6 x M→no food	6 x P→no food	FL (in P + M)

4.2.1. Method

Subjects

Twenty-four adult female Sprague-Dawley rats (Animal Resource Centre, Perth) were used. Rats had previously completed a Pavlovian conditioning study in which discrete stimuli were paired with reward pellets. Neither the pellets nor stimuli were used in the present experiment. At the beginning of this experiment rats weighed an average of 316g (range 262 – 378g). Rats had free access to chow and water throughout all experimental procedures.

Apparatus

The contexts, pre-feeding chambers, training foods and test food (FL) were as described in Chapter 3.

Procedure

FL familiarisation

Rats were familiarised to FL in two 30-min consumption tests held on consecutive days. Rats were placed into individual feeding cages during these tests with 20-25 FL available in a glass Petri dish.

Phase 1: Training

Phase 1 of training began five days after FL familiarisation. During this period rats underwent a preliminary test of anxiety in the Elevated Plus Maze (EPM); these data were uninformative and are presented in Appendix E. After EPM testing rats were allocated to *Single* or *Variety* groups that were matched on body weight, FL consumption, and EPM anxiety (percent open arm time). Over the subsequent 12 days of training rats received intermixed 30-min exposures to Plus and Minus contexts once per day (order: MPPMMPPMPMMP). As previously described, the same palatable food was available in the Plus context for the *Single* group, while the *Variety* group received each of the three foods in a cycling, counterbalanced fashion. Subsets of the *Single* group received each of the three training foods (Oreos, Banana Bread, Sausage Roll; $n = 4$/food).

Phase 2: Delay or Extinction

After the twelfth training session rats remained in home cages for a single day. At this point the *Single* and *Variety* groups were divided into two sub-groups that would either receive the delay or extinction manipulations in Phase 2 of training. This manipulation yielded 4 groups (each $n = 6$) within a 2 x 2 factorial design (i.e., Single vs. Variety and Delay vs. Extinction), as shown in Table 4.2 above. The four groups are herein called *Single-Delay*, *Single-Ext*, *Var-Delay*, and *Var-Ext*, and did not differ on EPM anxiety or average consumption during training, as confirmed in 2 x 2 ANOVAs (all $F < 1$).

Phase 2 lasted twelve days. The two extinction groups (*Single-Ext* and *Var-Ext*) were given six 30-min exposures to the Plus context with no food available. These extinction sessions were held on alternate days. During extinction sessions, rats in the *Delay* groups (*Single-Delay* and *Var-Delay*) were brought into the test room but remained in home cages.

Phase 3: Test

CPF tests began two days after the sixth extinction session (for the extinction groups), and 14 days after the end of Phase 1 context conditioning. Consumption of FL was compared in two 30-min tests in the Plus and Minus contexts. The order of these two tests was counterbalanced and they were separated by a day of rest in the home-cage.

4.2.2. Results

FL familiarisation

During the two FL familiarisation sessions rats ate an average of 3.77 ± .33g [SEM].

Phase 1: Training

Consumption in grams was converted to kJ to account for differences in energy density between the foods and is displayed in Figure 4.2.1. A 2 x (6) (group x [session]) mixed-ANOVA found that consumption rose significantly over Plus sessions (linear trend: $F(1, 22) = 40.50$, $p < .001$) at a decreasing rate (quadratic trend: $F(1, 22) = 12.63$, $p = .002$). These trends did not interact with group (both $F < 1$) and the main effect of group was not significant ($F < 1$).

Experiment 4.2: Training
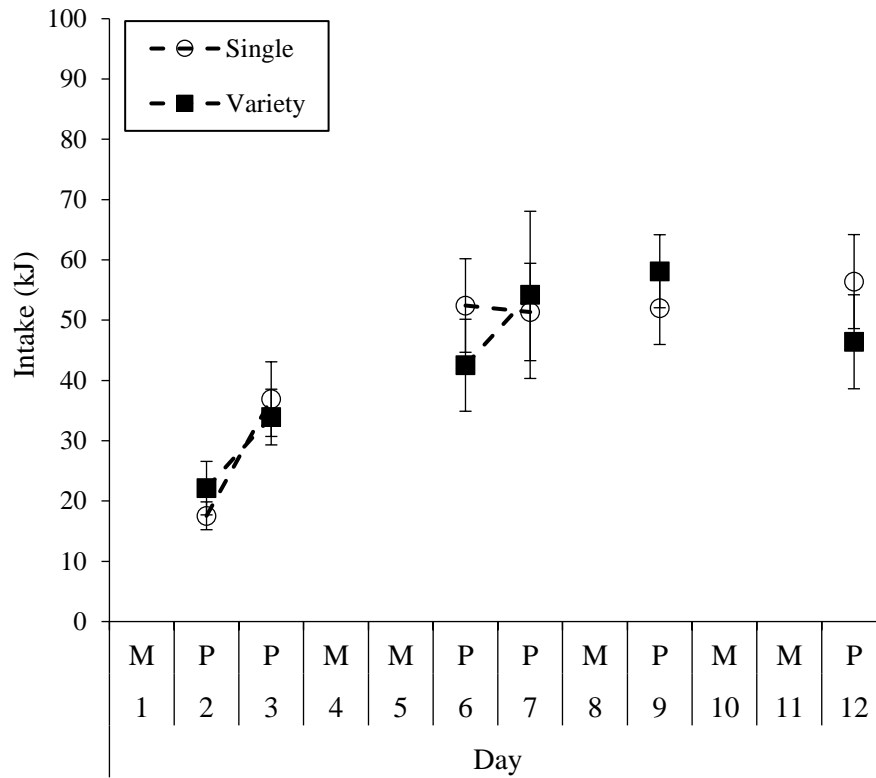
Figure 4.2.1. Training consumption in Experiment 4.2. Consumption of palatable foods rose over Plus sessions (P) and did not differ between *Variety* and *Single* groups. No food was available in the Minus (M) context.

Phase 3: Test

Test data are shown in Figure 4.2.2. They were initially analysed in a 4-way mixed-ANOVA, with group (2 levels), extinction (2 levels), and test order (2 levels) as between-subjects factors, and context (2 levels) as within-subjects factor. This preliminary analysis found no significant main or interaction effects of test order (largest $F(1, 16) = 3.01$, $p = .10$), confirming our initial hypothesis with respect to testing after a delay. To facilitate interpretation of the data, test order was removed and the analysis repeated as a 2 x 2 x (2) mixed-ANOVA (group x extinction x [context]). This found a significant main effect of group ($F(1, 20) = 5.32$, $p = .032$) indicating greater consumption by *Variety* than *Single* groups, and marginally significant interactions between group and context ($F(1, 20) = 4.25$, p

= .052) and group, context and extinction ($F(1, 20) = 3.86$, $p = .064$). These interactions

suggested that the difference between contexts varied according to Phase 1 training (*Single* or

Variety), and extinction of the Plus context in Phase 2. No other main or interaction effects

were significant (largest $F(1, 20) = 2.49$, $p = .13$).

To explore these interaction effects, data were split by the group factor and separate 2

x (2) ANOVAs (extinction x [context]) were applied to *Variety* and *Single* groups. In the two

Single groups, there was no main effect of context and no interaction with extinction (both *F*

< 1). Within the *Variety* groups the main effect of context was significant ($F(1, 10) = 7.73$, *p*

= .019) as well as the context x extinction interaction ($F(1, 10) = 6.54$, $p = .028$), indicating

that the context effect differed for *Var-Delay* and *Var-Ext* groups. Thus, a final analysis

tested the effect of context within these two groups in separate within-subjects ANOVAs.

The *Var-Delay* group showed no difference in consumption between contexts ($F < 1$),

whereas the *Var-Ext* group ate significantly more in the Minus than in the Plus context ($F(1,$

$5) = 15.10$, $p = .012$).

Experiment 4.2: Test
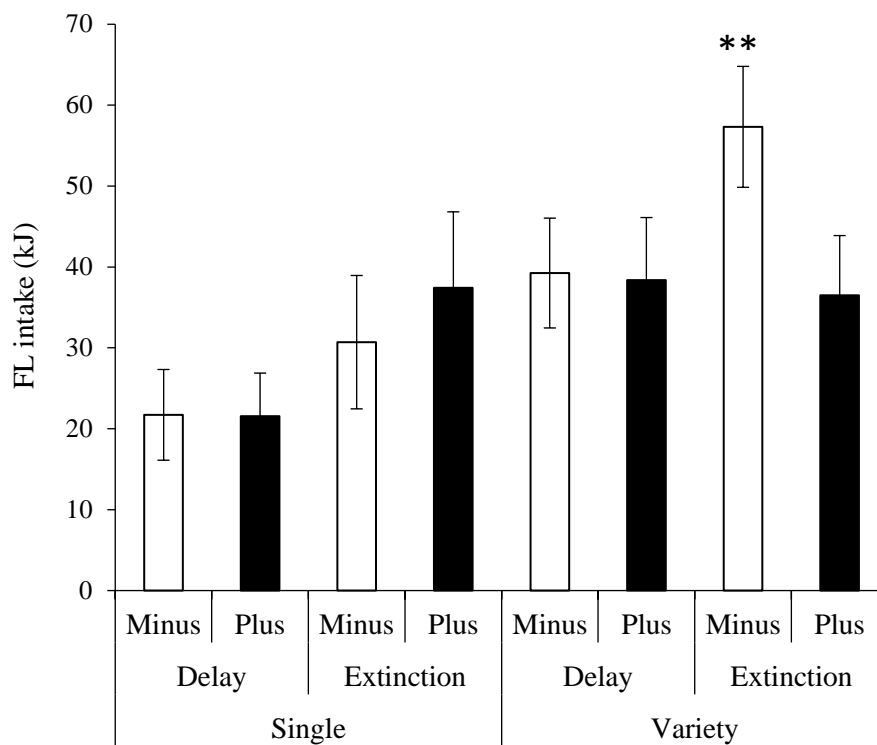
Figure 4.2.2. Experiment 4.2 test results. After regular training, tests occurred after a 2-week delay which for two groups included extinction of the Plus context. The *Var-Ext* group ate significantly more in the Minus context (**$p = .012$ for Plus vs. Minus); see text for details.

Contrast effects

The longer delay introduced between training and test was hypothesised to reduce negative contrast and increase consumption of FL at test. To visualise the difference between training and test intake, a difference score was calculated by subtracting test consumption in each context (in kJ) from rats' average training consumption in the last three Plus training sessions. It is important to note that because this transformation involved subtracting a constant (training intake) for each rat, it had no effect on the relative difference between Plus and Minus contexts within each group. Nor did it alter between-group differences, because training consumption did not differ significantly between the four groups. Therefore, the purpose of presenting these 'contrast scores' in Figure 4.2.3 is simply to view test intake as

the relative change from training consumption. What this figure shows clearly is that consumption in both contexts was suppressed for all groups, except for the *Var-Ext* group's Minus test, in which rats ate similar amounts as in training.
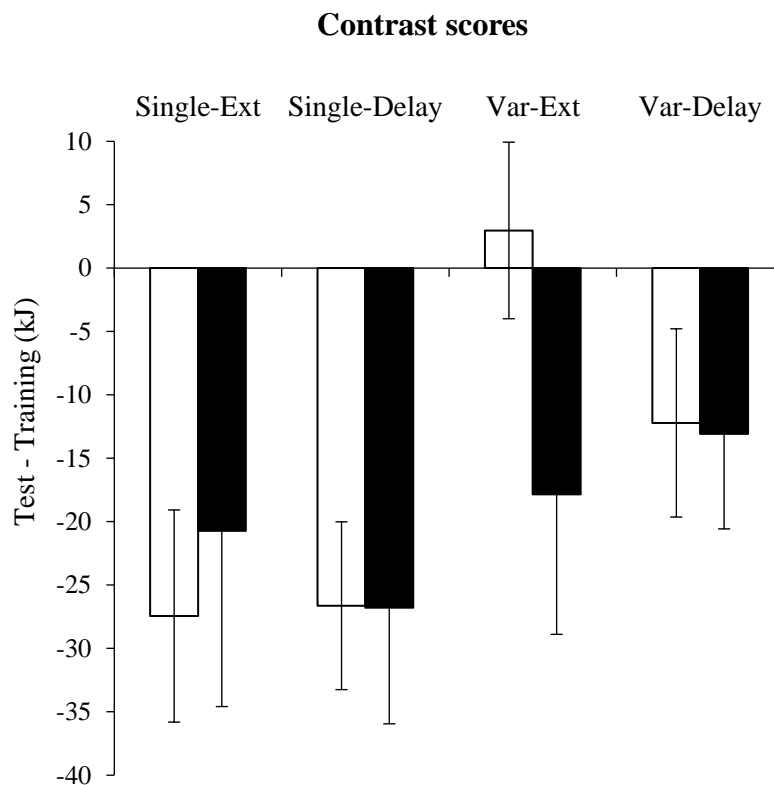
Contrast scores

Figure 4.2.3. Contrast scores in Experiment 4.2. Bars express test consumption relative to training. This figure is for illustrative purposes only, and shows that relative to training, consumption of FL at test was suppressed in every case except for the *Var-Ext* group's Minus test.

4.2.3. Discussion

Experiment 4.2 interspersed a longer delay between training and test to explore whether this would reduce the negative contrast at test produced by the presentation of an alternative food. The rationale for these predictions was that a delay would (1) enhance the incentive value of the test food, FL, and (2) decay the memory of the specific sensory

characteristics of the training food paired with the Plus context, leaving a more general association with food that would facilitate consumption of FL. Consequently, we hypothesised that by fostering larger intake of FL at test, a delay might prevent test order effects driven by initially low consumption. Despite the absence of significant test order interactions, consumption in tests was still suppressed relative to training, suggesting that negative contrast did not account for test order effects.

We hypothesised that a delay would produce a stronger CPF effect for rats given *Variety* training and, of particular interest, yield a CPF effect for those given *Single* training. These predictions were not supported, with similar consumption across contexts in both groups. The most likely reason for the null effects in these groups is that the delay weakened the ability to discriminate between Plus and Minus contexts. More interesting data were obtained in the two *Extinction* groups, for which tests compared intake in a context paired with no food six times and another paired with food and then extinguished over six sessions. For the *Single-Ext* group, we reasoned that extinction of the specific associations with the training food might allow consumption of FL at test; this hypothesis was not supported, with no effect of context within this group.

For the *Var-Ext* group we hypothesised that extinction should abolish CPF if the test food, FL, is contained within the association of food reward encoded to the Plus context during training (as suggested by CPF effects in previous chapters). Rather than abolishing the effect, however, the *Var-Ext* group ate significantly more in the *Minus* context. All six rats in this group exhibited this pattern, leading to a robust effect despite the small sample. Although extinction targeted the Plus context, it is interesting that consumption in this environment by *Var-Ext* animals was comparable to both the *Var-Delay* and *Single-Ext* groups (see Fig. 4.2.2). Instead, what drove the significant difference was enhanced consumption in the Minus

context, where *Var-Ext* rats ate an amount comparable to their training intake (see Fig. 4.2.3) and more than other groups.

One possibility is that during extinction, the Plus context's associations with food generalised to the Minus context, which was exposed in initial training (Phase 1) but not during extinction training (Phase 2). Future work could test whether continued exposures to the Minus context during extinction of the Plus context would abolish the effect reported here. This would also address whether the delay worsened the discriminability of the contexts, an important possibility given that *Single-Delay* and *Var-Delay* groups did not exhibit CPF. However, if such a retrospective association between the Minus context and food drove consumption at test, additional explanation is required for why this was specific to rats exposed to variety, given that *Var-Ext* and *Single-Ext* groups ate comparable amounts of palatable food during Stage 1 training and received an equivalent amount of extinction. One possibility is that despite no differences in total intake, the provision of multiple foods was more arousing and/or conditioned a stronger hedonic association for the *Var-Ext* group. In Phase 2, then, experiencing no food in the Plus context was more surprising for the *Var-Ext* group, leading to deeper extinction. Yet this account should predict a suppression in consumption in the Plus context at test − not enhanced consumption in the Minus context.

The interaction between extinction and variety prompts further questions. While the present study involved training followed by extinction, it would be interesting to test for similar effects after swapping these stages, in which case the design would resemble a latent inhibition procedure (i.e. pre-expose the Plus context multiple times with no food). A related manipulation would be to intersperse reinforced and nonreinforced Plus sessions in training; that is, to test whether partial reinforcement produces a stronger effect on consumption. These would inform whether *any* manipulation of the association of the Plus context with food disrupts its ability to potentiate feeding. This would constitute variety in terms of the

135

availability of food, rather than in types of food. Finally, a limitation of the present design is that CPF was not tested prior to extinction to confirm that initial training produced similar effects to previous experiments. Although training consumption was comparable to Experiment 3.3, which found CPF effects when testing after an identical training procedure, this was not verified and may have differed in this experiment.

4.3. Experiment 4.3: CPF in a between-subjects design

A recurring finding in Experiment 4.1 and earlier chapters has been main and interaction effects involving test order. Driving these effects is the reliable tendency for rats to eat more on consecutive tests. Although order effects are accounted for in all analyses, and main effects of context often outweigh the influence of this factor, a simpler method would involve only a single test. As discussed in Chapter 1, the Holland-Petrovich method often uses between-subject designs wherein rats receive Paired or Unpaired presentations of a cue and pellets, in which the former group show greater consumption of pellets in a single test. Data from Experiment 4.1 suggested that consumption in the Plus context was greater for the *Variety* group than for *Unpaired* rats given home-cage exposure to the foods. Experiment 4.3 extended this observation using a between-subjects design in which four groups were trained to associate a single context with multiple palatable foods (*Variety*), one palatable food (*Single*), one bland food (*Chow*) or no food (*Nothing*). As in past experiments, the CPF test measured consumption of a familiar and alternative food never previously exposed in the context. We predicted higher consumption in rats given *Variety* training than in the other three groups. To the extent that consumption by *Chow* and *Single* groups is driven by positive and negative contrast effects, respectively, intake by the former group should exceed the

latter, as suggested by earlier experiments (e.g. Experiments 2.2 and 2.3). The *Nothing* group was expected to consume an amount intermediate to these two groups.

4.3.1. Method

Subjects

Thirty-two adult, female, experimentally naïve Sprague-Dawley rats were sourced from Animal Resource Centre (Perth, Australia). Housing conditions were as described in Experiments 4.1 and 4.2. Rats acclimated to the colony room and were weighed and handled regularly for one week prior to experimental procedures. Mean body weight at the beginning of the experiment was 236g (range: 187 – 280g). Rats were maintained on unrestricted access to chow and water throughout all experimental procedures.

Apparatus

The individual feeding cages and preparation of the training context was as described previously. While previous experiments trained two contexts, here rats were assigned one of the contexts (rough/smooth floor with rosewater/vanilla odour with striped/smooth walls) as their training context. The training foods and test food (FL) were as described for Experiments 4.1 and 4.2.

Procedure

FL familiarisation

Rats were familiarised to the test food, FL, in two 30-min sessions in individual feeding cages on consecutive days. Subsequently rats were allocated to four 8-rat groups that did not differ significantly in body weight or in baseline FL intake (one-way ANOVA; both $F < 1$). Rats remained in home cages for two days prior to training.

Training

Training lasted 9 days and involved a 30-min exposure to the context once per day. Rats were trained in three squads of 12, 12, and 8 rats in the same order each day, and with all groups represented in each squad. The four experimental groups differed in terms of what type of food was available in the context. The *Chow* group were presented with chow, which was also available *ad-libitum* in the home-cage. The *Single* group received Oreos ($n = 3$), Banana Bread ($n = 3$) or Sausage Roll ($n = 2$), while the *Variety* group were exposed to each of these foods, one per session, in a cycling and counterbalanced order. For the *Nothing* group the context contained an empty dish, ensuring that the context was associated with no food. This group was included because in past experiments it was unclear to what extent rats in *Chow* groups associated the Plus context with food (as eating occurred sporadically and not in every session).

Test

The total length of the feeding test was 2 h and measured consumption of FL in the training context. Consumption was measured every 30-min using the method described in previous experiments (Experiments 2.1 and 3.3). The data of main interest were consumption after 30-min, since this was comparable to the duration of training sessions and to tests in earlier experiments. In addition, we were interested in whether group differences would emerge over a longer test that allowed rats to overcome any initial avoidance of the surprising alternative food. The longer duration of the test required it to be split over 2 days to keep the timing similar to training. Therefore, the first training squad were tested two days after the last training session, and the second and third squads tested on the following day. Preliminary analyses of test data included 'test day' and found no significant main or interaction effects

involving this factor (largest $F(1, 24) = 2.4$, $p = .13$). It was therefore excluded from final analyses.

4.3.2. Results

FL familiarisation

On average, rats ate a total of $2.84 \pm .2$g [SEM] of FL in the two familiarisation sessions.

Training

Consumption during the 9 days of training is shown in Figure 4.3.1 and was analysed in a 3 x (9) (group x [session]) mixed-ANOVA. The data suggested a comparable increase in *Single* and *Variety* groups that levelled off by day 9. The only difference between the two appeared within the first 3 sessions, when the *Variety* group was exposed to a new palatable food each day. Consumption of *Chow* was lower but consistent over the nine sessions. These observations were supported by significant linear ($F(1, 21) = 57.30$, $p < .001$) and quadratic trends for session ($F(1, 21) = 17.63$, $p < .001$) and a significant group x session linear interaction trend ($F(2, 21) = 10.66$, $p = .001$). Post-hoc contrasts (Tukey correction) indicated that consumption by the *Variety* and *Single* groups did not differ from each other ($p = .99$) but each consumed more than the *Chow* group (both $p = .004$).
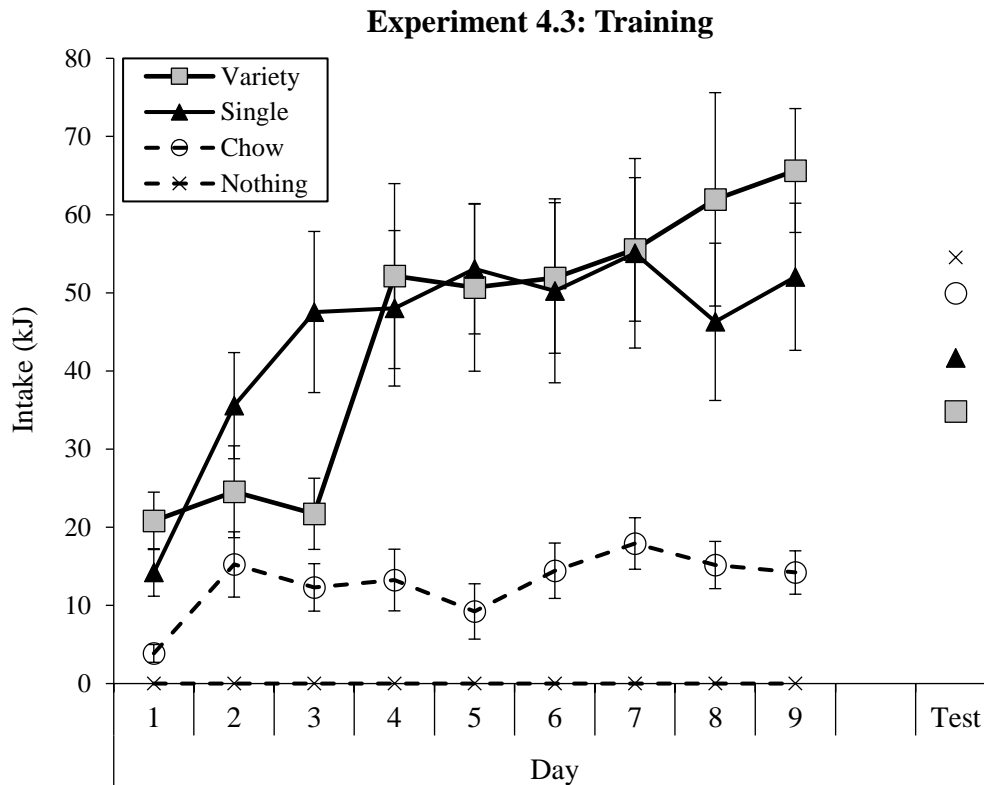
Figure 4.3.1. Training consumption in Experiment 4.3. *Single* and *Variety* groups increased their consumption of palatable food at a comparable level, whereas the *Chow* group consumed a steady but low amount of chow. Consumption in the first 30-min of the test is shown on the right-hand side of the plot for comparison.

Test

Consumption across the 2-h test is shown in Figure 4.3.2, with 30-min intakes also displayed in Figure 4.3.1 for comparison with training data. The main analysis was a 4 x (4) mixed-ANOVA (group x [bin]) including consumption from each 30-min period. Results found significant linear ($F(1, 28 = 97.51, p < .001$) and quadratic trends for bin ($F(1, 28) = 12.31, p = .002$), indicating that consumption declined over the four 30-min periods, but at a decreasing rate. Neither of these trends interacted with group (linear interaction trend: $F(3, 28) = 1.58, p = .22$; quadratic trend $F < 1$) and the main effect of group was not significant ($F(3, 28) = 1.05, p = .39$). Nor was the main effect of group significant for any individual 30-min period (largest $F(3, 28) = 1.22, p = .32$).
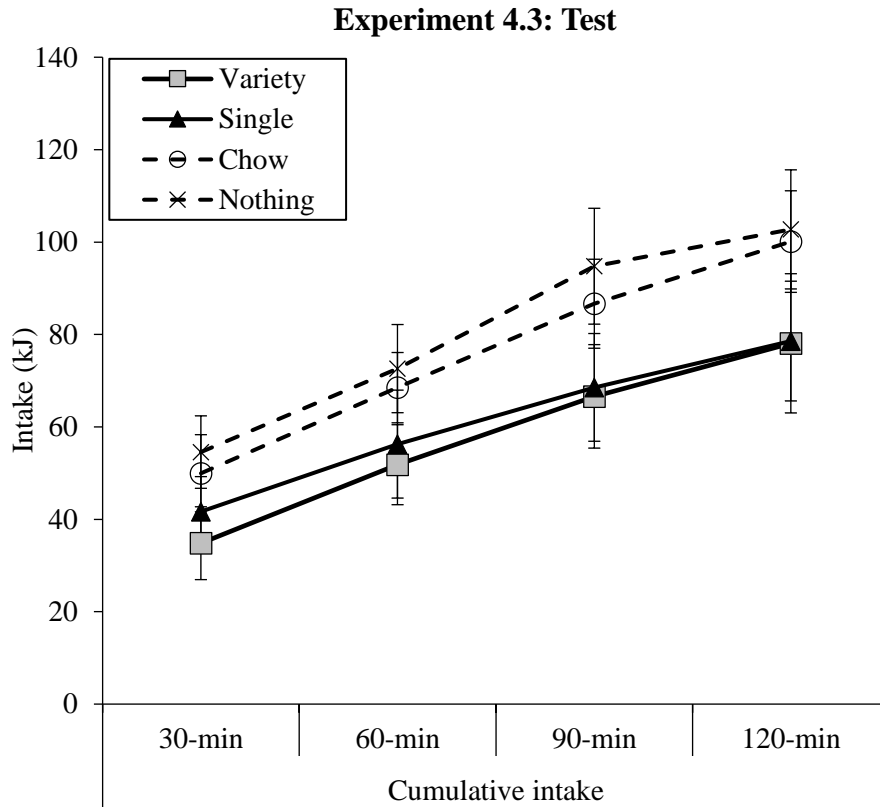
Experiment 4.3: Test

Figure 4.3.2. Experiment 4.3 test results. No significant group differences were found at any time point during the 2-h CPF test.

Contrast effects

The pattern of differences between the group means was in the opposite direction to that predicted. In addition, it appeared that at the group level, test and training consumption were inversely related. As shown in Figure 4.3.1, the order of consumption at the end of training (*Variety > Single > Chow > Nothing*) was reversed for the first 30-min period of the CPF. We sought to quantify this change from training to test as a measure of contrast. To provide a stable estimate of training consumption, and because the *Variety* group received a different food on each Plus session, consumption (in kJ) in the last 3 training sessions was averaged. This average was subtracted from FL intake (in kJ) in the first 30-min of the test, such that a positive difference score reflected greater consumption at test than in training.

These scores are shown in Figure 4.3.3. (Note that there was no training consumption to subtract for the *Nothing* group, and their data are simply FL intake after 30-min.)

A one-way ANOVA comparing the difference scores for *Chow*, *Single*, and *Variety* groups found a significant main effect of group ($F(2, 21) = 10.52$, $p = .001$). Post-hoc contrasts using the Tukey HSD correction found that the difference score for the *Chow* group significantly exceeded that for the *Single* ($p = .011$) and *Variety* ($p = .001$) groups, with no difference between the latter two groups ($p = .45$). To assess whether the change from training to test was significant for each group, one-sample *t*-tests compared the contrast scores to 0 (i.e. indicating equal consumption in training and test). The *Chow* group's contrast score was significantly greater than 0 ($t(7) = 3.59$, $p = .009$), whereas for the *Variety* group there was a non-significant trend toward a negative score ($t(7) = 2.16$, $p = .068$). The *Single* group's contrast score was also negative but not statistically significant ($t(7) = 1.51$, $p = .17$).
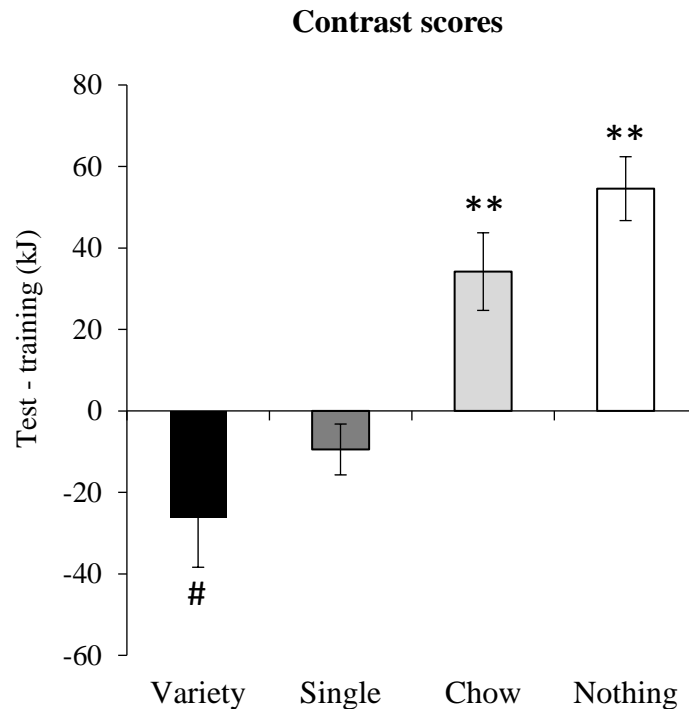
Figure 4.3.3. Contrast scores in Experiment 4.3. FL consumption at test (at 30-min) is expressed relative to training consumption (average of the last 3 training sessions). $**p < .01$; $\#p = .07$ for one-sample t-tests comparing group mean to 0.

4.3.3. Discussion

Experiment 4.3 adopted a between-subjects design to test CPF on an alternative food. The single context to which rats were exposed in training contained one or three palatable foods; chow; or nothing. The pattern of consumption between *Chow*, *Single*, and *Variety* groups over 9 consecutive days was comparable to previous experiments in which exposures to the Plus context occurred every 24-72 hours and were intermixed with Minus sessions. Once again, the CPF test assessed the effects of this environment on consumption of another palatable food, FL. Contrary to predictions, no group differences were found, and consumption by the *Variety* group relative to other groups was in the opposite direction to that hypothesised on the basis of past experiments. In fact, the two groups for which the context was paired with palatable food appeared to consume less than the other groups.

Intake increased relatively steadily over the 2-h test and there was no evidence that after an initial period of high consumption, the context promoted further eating only in some groups.

Calculation of contrast scores was prompted by the observation that the pattern of mean differences at test was opposite to that observed in training. Analysis of these scores highlighted the differing effects of presenting FL at test. The *Chow* group significantly increased their intake of FL at test relative to their training chow consumption, a difference that was significantly larger than for the *Single* and *Variety* groups, which tended to consume less in test than of their palatable food/s in training. This pattern is suggestive of positive contrast for the *Chow* group, and negative contrast for the *Variety* and *Single* groups. Consumption was highest of all in rats for which the context had always been empty and for which the CPF test marked the first opportunity to eat in this environment (*Nothing* group). This result was surprising since this constituted the sort of 'Minus' context exposure present in all previous experiments. However, past studies have demonstrated that contexts associated with the absence of food (Roitman et al., 2001) or cues signalling the imminent termination of food availability (Galarce & Holland, 2009; Holland & Hsu, 2014) can stimulate food intake to at least as great an extent to food-paired cues.

The result most at odds with our hypotheses was the low consumption by the *Variety* group. Why might contexts paired with multiple palatable foods enhance consumption of other foods in within-subjects designs (as in previous chapters), but not in the present between-group design? In addition, why was the decline in consumption from training to test most evident in this group? The answer to the former question might relate to the absence of a Minus context as a comparison environment. Generally, the CPF effects previously reported for the *Variety* group were not driven by dramatically enhanced intake in the Plus context relative to other groups, but by a larger difference between the Plus and Minus contexts. Another procedural difference was the present experiment administered nine consecutive

'Plus' sessions, unlike previous experiments in which Plus sessions occurred every 24-72 hours in an unpredictable sequence intermixed with Minus sessions. Therefore, the apparent negative contrast effect in these groups might suggest that the regularity of exposure to palatable food made the presentation of an alternative food more surprising at test. It would be interesting to compare the effects of our standard training involving intermixed Plus/Minus sessions with a separate group exposed only to the Plus context on the same days.

4.4. General discussion

The experiments in Chapter 4 tested whether manipulating motivational and procedural factors would enhance CPF effects by reducing the contribution of test order effects and negative contrast. The procedural changes introduced were unsuccessful in achieving these aims. Experiment 4.1 found that the transfer of CPF to an alternative food was not enhanced after training with mild food restriction. Whereas Experiment 4.2 found no test order effects after a 2-week delay, a clear negative contrast effect persisted in the form of suppressed test intake relative to training. This result suggested that contrast effects are not uniquely responsible for test order effects in our preparation. In Experiment 4.3 *Single* and *Variety* groups suppressed consumption at test relative to training in a single test designed to preclude test order effects. All of these manipulations abolished the CPF effect in the *Variety* group that was found reliably in previous chapters. Instead, results were often in the reverse direction to those hypothesised.

Experiment 4.1 implemented mild food deprivation during training in order to enhance intake in the Plus context and, consequently, the salience of conditioning. The key result from this experiment was that when rats were tested mildly hungry, as in training, the Plus context suppressed consumption of an alternative food, FL, relative to the Minus

context. The propensity to avoid FL in the Plus context on this test was associated with the extent to which the Plus context promoted consumption of the training foods in a subsequent test. The suppression in FL intake in Test 2 of this experiment may relate to the fact that animals had never previously eaten FL while food-deprived. Therefore, a key future direction is to assess the effects of animals' motivational state when first exposed to the test food.

Whereas Experiment 4.1 tested the effects of a general shift in motivational state, Experiment 4.2 extended the delay between training and test to target the nature of the association of the Plus context with food and the incentive value of the test food. The predicted larger CPF effects were not found in either *Variety* or *Single* groups tested after a 2-week delay, nor in *Single* rats for which the Plus context was extinguished. However, extinction of the Plus-food association enhanced consumption in the Minus context for rats given *Variety* training. This result indicates that the effects of extinction differed for animals exposed to variety, unlike previous studies using instrumental tasks (Thrailkill et al., 2014). However, additional work is required to determine whether this effect is sensitive to additional exposures to the Minus context during extinction.

To explore CPF between-groups, Experiment 4.3 exposed rats to a single context that differed in terms of the presence or absence and type of food it contained. The predicted difference between *Variety* group relative to the *Single*, *Chow* and *Nothing* groups was not observed; if anything, intake appeared higher in the latter two groups, though this difference was not statistically reliable. The general pattern, however, indicated that training and test consumption were inversely related; that is, rats that ate more in training (*Single* and *Variety* groups) ate less FL at test. This appeared another example of negative contrast, consistent with preference tests from Experiment 3.2 (where rats preferred the training food to FL) and test results from Experiment 4.1 (where rats CPF for the training food vs. FL tests correlated negatively). This possibility could be examined in future experiments in which the training

and test foods are fully counterbalanced, though this might raise logistical issues with respect to interpreting consumption at test.

For the most part, the present experiments considered incentive contrast to explain the overall change in consumption from training to tests. However, contrast may have operated on a more local level; specifically, between the last day of training – which was always a Plus session – and the first day of CPF testing. A possibility arising from this observation is that when presenting FL in the Plus context, negative contrast might be stronger when this context is tested first (i.e. Plus→Minus rats) than when it is tested second (i.e. Minus→Plus). This possibility is explored in Appendix C through a re-examination of data from Chapters 2 and 3. Results found no evidence that the degree of negative contrast from the end of training to the first test day differed according to whether the Plus or Minus context was tested first. This is consistent with a study showing that negative contrast produced by a shift from 32% to 4% sucrose solution was not altered by the context in which the 4% solution was tested (Flaherty, Hrabinski, & Grigson, 1985). In summary, results from the present chapter and Appendix C suggest that negative contrast and test order effects are largely independent processes in our preparation.

It is also worth noting that consideration of contrast effects was made within the broader aim of studying CPF and its transfer to alternative foods. The present training and test foods were selected to vary on several dimensions (sweetness, fattiness, texture, etc.) that might be seen as inopportune for the study of contrast. Additionally, since these training foods are likely at the upper limit of palatability in the rat, using more moderately palatable foods might yield clearer results when tests rely on consumption in multiple environments. One logical option in this regard would be sugar solutions of varying concentrations, as successfully used in many studies of consummatory contrast (Flaherty, 1996). Finally, an important direction for future research is to test CPF in *Single* and *Variety* groups when the

change from training to test involves presentation of a more-preferred food (i.e. positive contrast), rather than the apparent negative contrast as in the present experiments.

A final point relates to the general failure to detect robust CPF effects. Each experiment in this Chapter adopted relatively minor procedural changes hypothesised to improve CPF, yet effects were in each case abolished. One implication of these collective results is that CPF is not a readily observable nor common behaviour, but instead a response that is produced only by a confined set of experimental parameters. It is worth noting, however, that our methods were tailored toward testing the transfer of CPF to alternative foods. Including more tests of the training foods would have better informed whether null results were explained by the overall fragility of CPF or by its apparent specificity under these conditions (i.e., its failure to transfer to other foods). Repeating the current designs using parameters from previously published methods will be important in delineating these possibilities.

Chapter 5: Effects of diet-induced obesity on CPF

5.0. Introduction

Schacter's (1968) claim, that obesity results from eating in response to external rather than internal cues, has been highly influential. Despite limitations of the research on which this claim was based (see Chapter 1), there is ample evidence to suggest that responding toward food cues is altered in obesity. Relative to their lean counterparts, overweight and obese individuals exhibit attentional biases toward food cues in visual attention tasks (Hendrikse et al., 2015), enhanced salivary responses to olfactory food cues (Ferriday & Brunstrom, 2011; Jansen, Stegerman, Roefs, Nederkoorn, & Havermans, 2010) and consume more food following exposure to food cues (adults: Werthmann et al., 2011; children: Jansen et al., 2003). In some cases, however, effects are evident on self-report and physiological measures but not in actual consumption (Ferriday & Brunstrom, 2011).

Similar effects have been reported in animal models of obesity. Johnson and Kenny (2010) found that obese animals continued to eat palatable food in the presence of a cue paired with an aversive outcome (shock) that inhibited consumption in lean control rats. Outcome devaluation studies indicate that obesity impairs the ability to adjust food-seeking behaviour when the value of the food reward that is earned is manipulated (Furlong, Jayaweera, Balleine & Corbit, 2014; see also Horstmann, Dietrich, Mathar, Pössel, Villringer, & Neumann, 2015). In apparent contrast to impaired sensitivity to devaluation, animals made obese by diets high in fat and/or sugar often show *reduced* rates of responding and/or breakpoints in instrumental tasks (Davis, Tracy, Schurdak, et al., 2007; Tracy, Wee, Hazeltine, & Carter, 2015; Kendig, Boakes, Corbit, & Rooney, 2014) and reduced consumption of fluid rewards in Pavlovian tasks (Reichelt, Morris, & Westbrook, 2014). Other results indicate that individual differences in food-seeking behaviour might pre-empt

obesity. For example, propensity to gain weight on obesogenic diets has been shown to be predicted by baseline differences in conditioned responding to Pavlovian food cues (Robinson et al., 2015) and by willingness to work for food reward in instrumental tasks (la Fleur, Vanderschuren, Luijendijk, Kloeze, Tieskema, & Adan, 2007).

Whereas the above studies used Pavlovian and instrumental response measures, few animal models of obesity have tested for differences in consumption. Differences in eating patterns between cafeteria diet- and chow-fed rats have been assessed in the home-cage (Martire, Holmes, Westbrook, & Morris, 2013), but it appears that no published studies have compared obese and lean animals in terms of feeding in response to a food-paired cue; i.e., CPF. This was the aim of Experiment 5.1. The experiment began with a five-week diet intervention in which one group received unrestricted access to a high-fat, high-sugar supplement (*HFHS* group) in addition to chow and water, while the *Control* group was fed only chow and water. Previous work from our lab indicated that this diet intervention significantly accelerates weight gain (Furlong et al., 2014) and produces higher fat mass (Kendig & Corbit, unpublished observations) relative to control animals.

Following the diet intervention both groups were maintained on unrestricted chow and water for context conditioning, in which rats were trained to associate a 'Plus' context with a palatable food – Banana bread – and a Minus context with no food. Tests 1 and 2 measured consumption of the training food; we hypothesised a modest CPF effect in the *Control* group and an enhanced effect in the *HFHS* group. Test 3 measured consumption of an alternative food, Fruit Loops (FL), to assess the specificity of CPF in the manner described in previous chapters. Given that the Plus context was paired with only one food, we hypothesised no CPF effect on an alternative food in the *Control* group, since these rats were analogous to *Single* groups in previous chapters. However, following past reports that obesogenic diets promote habitual control over behaviour (Furlong et al., 2014) and impair

sensory-specific satiety (Reichelt, et al., 2014) – measures indicating performance that is divorced from the specific outcome of responding – we reasoned that the *HFHS* group might be less affected by which specific food was available in the Plus context, and exhibit CPF on an alternative food. Finally, metabolic measures were recorded after the diet intervention (fasting blood glucose) and at cull (fat mass); these were correlated with CPF test data.

5.1. Method

Subjects

Twenty-four experimentally naïve, adult (3-4 months old) male hooded Wistar rats (University of Adelaide, Australia) were used. Males were used in order to examine the generality of results from previous chapters, which used females, and because previous work from our lab using this diet intervention used male rats (Furlong et al., 2014). Rats were group-housed ($n = 4$/cage) throughout the experiment on a 12:12 light:dark cycle (lights on at 0700h) in temperature- and humidity-controlled ventilated cages. Prior to the experiment rats were handled and weighed regularly over a 1-wk period, before being allocated randomly to *Control* or *HFHS* groups (each $n = 12$). At the beginning of the experiment mean body weight was 373 ± 6.7g [SEM] for the *Control* group and 377.8 ± 8.5g for the *HFHS* group; group means did not differ significantly ($F < 1$).

Apparatus

The high-sugar, high-fat dietary supplement was sweetened condensed milk (SCM; 3.25 kJ/g, 67% sugar, 10% protein, 22% fat, Nestlé®). SCM was diluted 3 parts to 1 with water and was provided in 300 ml plastic bottles with ball bearing sipper spouts (Lab Products Inc., www.labproductsinc.com) that were inserted into each *HFHS* home-cage. Chow and water were available *ad-libitum*. The preparation of the contexts and pre-feeding

chambers were as described previously. The food presented in the Plus context during training was Banana bread (BB), and the alternative food presented in Test 3 was Froot Loops (FL), as described previously.

Procedure

Diet intervention (Days 1-36)

During the diet intervention SCM bottles were weighed, cleaned and replenished daily for the *HFHS* cages. Every fourth day rats were weighed, cage bedding was changed, and chow and water consumption was measured. At 2000h on Day 36, SCM bottles were removed for the three *HFHS* cages and chow was removed from all cages. After a 12-h fast, fasting blood glucose were measured using an Accu-Chek© Glucometer by removing the tail tip using a sterile scalpel blade. Rats were then returned to *ad-libitum* chow (with continuing free access to water) for the remainder of the experiment

Training (Days 39-50)

The 12-day training phase began three days after fasting glucose was measured and consisted of six 30-min exposures each to distinctly marked 'Plus' and 'Minus' contexts in an intermixed fashion (order: MPPMMPPMPMMP), once per day. Sessions were held between 1400-1700h each day. During Plus sessions, approximately 15g of BB was provided in a Petri dish centred against the side-wall of the chamber; no food was provided in the Minus context. During this phase all rats were placed for 15-min on two occasions in the pre-feeding cages that were later used for the tests; these cages were empty during this familiarisation procedure.

Test 1 (Days 53 and 54)

After the twelfth training session rats remained in home-cages for two days. Test 1 began on the following day and measured consumption of the training food, BB, in the Plus and Minus contexts in separate 30-min tests. The two test days were held on consecutive days and the order in which the contexts were tested was counterbalanced.

Test 2 (Days 56 and 57)

After Test 1 rats were rested in the home cage for a day prior to Test 2. Here, a pre-feeding manipulation was adopted, as described in Experiments 2.1 and 3.2, in which rats were given 20-min access to 20g BB in individual feeding cages, immediately prior to a 10-min test in the Plus or Minus context with a new dish of BB provided. The two context tests were held on consecutive days and test order was counterbalanced in the same arrangement as in Test 1.

Test 3 (Days 59 and 60)

After Test 2 rats were given a day of rest in the home-cage for a day prior to Test 3. On the rest day, 20 Froot Loops (FL) were scattered in each home cage and all rats were observed to sample them within a 15-min period. Beginning the following day, two 30-min tests on consecutive days measured FL consumption in the Plus and Minus contexts, with no pre-feeding conducted. Test order was counterbalanced such that rats were tested in the reverse order to that used in Tests 1 and 2.

Cull (Day 85)

Seven weeks after the end of the diet intervention, rats were culled by intraperitoneal injection of sodium pentobarbital (Lethabarb ©). Retroperitoneal, epididymal and visceral fat

pads were excised and weighed. Fat mass in grams was expressed relative to terminal body weight (g/kg).

5.2. Results

Body weight

Body weight gain during the diet intervention is shown in Figure 5.1.1 and was analysed in a 2 x (10) (group x [time]) mixed-ANOVA. This analysis showed that body weight significantly increased over time (linear trend: $F(1, 22) = 651.09$, $p < .001$) and was significantly greater in the *HFHS* group (group x time linear interaction trend: $F(1, 22) = 81.65$, $p < .001$). The *HFHS* group gained a significantly greater percentage of starting body weight ($20.87 \pm 0.94\%$ [SEM]) than the *Control* group ($10.81 \pm .92\%$) ($F(1, 22) = 58.73$, $p < .001$). The difference in absolute body weight was statistically significant from Day 12 onwards (day 12: $F(1, 22) = 4.37$, $p = .048$, thereafter all $p < .02$).

Throughout CPF training and testing rats were weighed every 4-5 days, during which time both groups had unrestricted access to chow and water in home cages, and equivalent opportunity to consume palatable food in training and CPF tests. However, these conditions produced distinct effects on weight; whereas *HFHS* rats stabilised, *Control* rats continued to gain weight such that percent body weight change from the end of the diet intervention until cull was significantly greater for the *Control* group (mean change: $7.87 \pm 1.0\%$ [SEM]) than for the *HFHS* group (mean change: $-0.45 \pm 0.77\%$) ($F(1, 22) = 42.35$, $p < .001$). Independent samples *t*-tests found that the group difference was statistically significant throughout context conditioning and at Test 1 (smallest $t(22) = 2.10$, $p = .047$ at the time of Test 1). The difference was not statistically significant when rats were weighed on their rest day between Tests 2 and 3 ($t(22) = 1.69$, $p = .10$) nor at cull ($t(22) = .75$, $p = .46$).

Experiment 5.1: Body weight
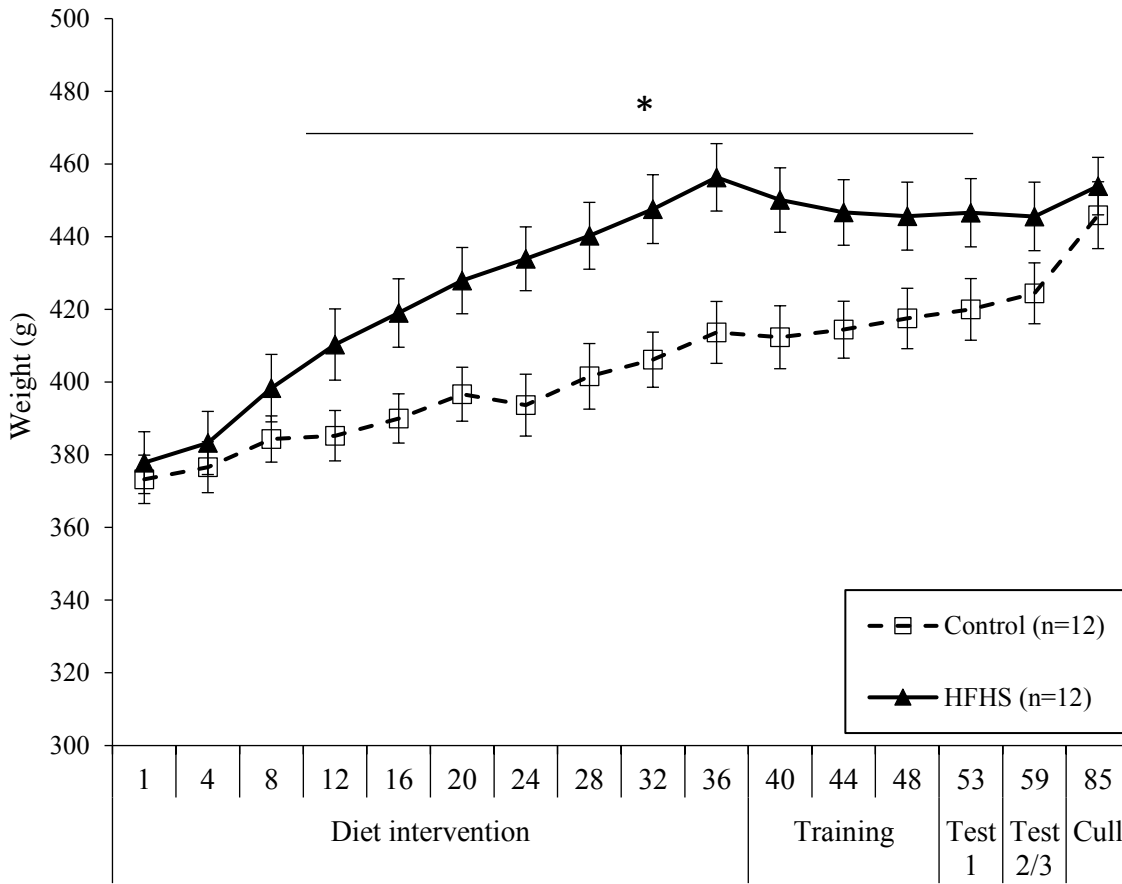
Figure 5.1.1. Body weight gain in Experiment 5.1. The addition of sweetened condensed milk (SCM) to the diet accelerated body weight in the *HFHS* group, with statistically significant differences from day 12 of the diet intervention to 17 days after its conclusion (*all $p < .05$, independent samples *t*-tests), after which groups did not differ significantly. Note that time-points are irregular after training.

Fasting glucose

After the diet intervention, mean fasting glucose levels were $5.86 \pm .13$ [SEM] mMol/L for the *Control* group and $6.03 \pm .10$ mMol for the *HFHS* group. This difference was not statistically significant ($F(1, 22) = 1.08$, $p = .31$).

Energy intake

Total energy intake was estimated on a per-cage basis, providing three data points per group for statistical analyses. Figure 5.1.2 presents average energy intake (kJ/rat/day) across

the diet intervention, since consumption patterns did not change meaningfully over time. *HFHS* cages consumed most energy from SCM and ate little chow, with their total energy intake 14% higher, on average, than *Control* cages. One-way ANOVA confirmed this difference was statistically significant ($F(1, 4) = 19.97$, $p = .011$).
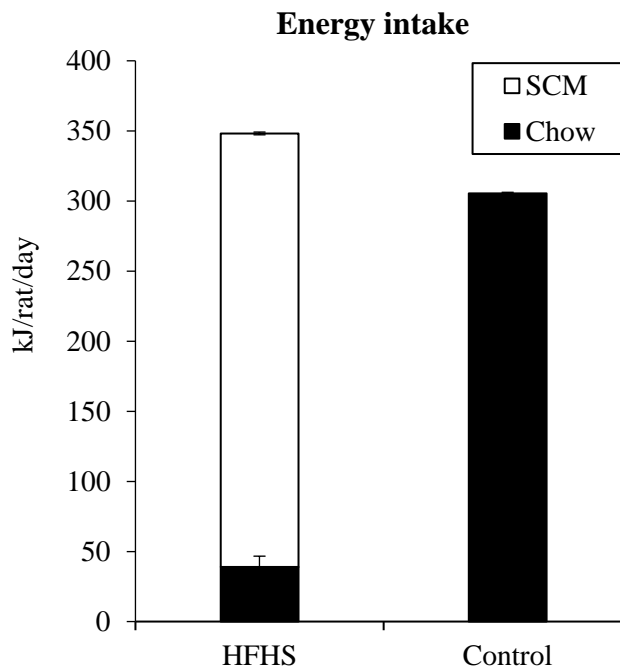
Figure 5.1.2. Energy intake during the diet intervention of Experiment 5.1. The provision of SCM in addition to chow and water significantly increased energy intake in the *HFHS* relative to the *Control* group.

Training

Consumption (g) of BB in training is shown in Figure 5.1.3 and was analysed in a 2 x (6) (group x [session]) mixed-ANOVA. Consumption increased significantly over sessions (linear trend: $F(1, 22) = 216.51$, $p < .001$) at a decreasing rate (quadratic trend: $F(1, 22) = 11.85$, $p = .002$) with no interaction between these trends and group (largest $F(1, 22) = 2.39$, $p = .14$). The main effect of group was significant ($F(1, 22) = 14.81$, $p = .001$), indicating

higher consumption in the *Control* than *HFHS* group, on average (6.76 ± .31g [SEM] vs. 5.12 ± .29g).
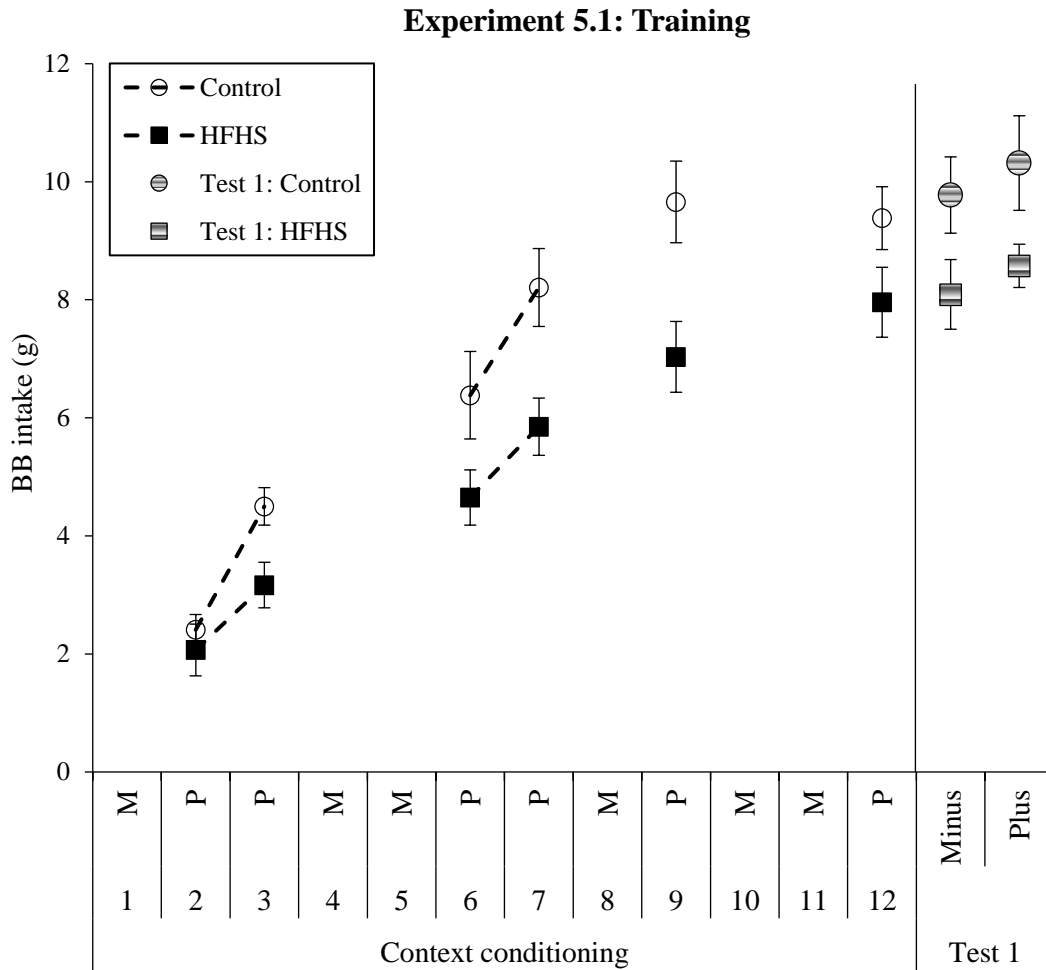
Figure 5.1.3. Experiment 5.1 training intake and Test 1 results. Consumption of BB increased significantly in both groups but was greater, on average, in the *Control* group. The difference between contexts in Test 1 was not statistically significant.

Test 1

Consumption from Test 1 is shown alongside training data in Figure 5.1.3, given that test conditions in the Plus context were identical to training. Consumption was analysed in a 2 x 2 x (2) (group x test order x [context]) mixed-ANOVA. Although consumption appeared

somewhat higher in the Plus context, the context main effect was not significant ($F(1, 20) =$ 1.54, $p = .23$) with no context x group interaction ($F < 1$). A significant main effect of group ($F(1, 20) = 5.75$, $p = .026$) indicated that, as in training, consumption was higher in *Control* rats. There was a trend toward a main effect of test order ($F(1, 20) = 4.10$, $p = .057$) suggestive of higher consumption overall in rats tested in the Minus context first (and Plus context second). The context x test order and context x group x test order interaction effects were not significant ($F(1, 20) = 3.42$, $p = .079$ and $F(1, 20) = 3.88$, $p = .063$, respectively).

Test 2: Pre-feeding

Whereas Test 1 was a straightforward design in keeping with most of our previous experiments, in Test 2 we added a pre-feeding manipulation to ask whether the *HFHS* group might be more resistant to satiety and exhibit an elevated CPF effect. During pre-feeding the *Control* group ate an average of 7.71 ± .54g [SEM] of BB, while the *HFHS* group ate 4.90 ± .41g. A 2 x (2) mixed-ANOVA (group x [context]) applied to pre-feeding intakes found this difference was statistically significant (group main effect: $F(1, 22) = 20.78$, $p < .001$) but did not vary according to whether the pre-feeding was prior to a Minus or Plus test (context main effect: $F < 1$) and with no group x context interaction ($F < 1$).

Consumption during the 10-min context tests is shown in Figure 5.1.4 and was analysed with a 2 x 2 x (2) mixed-ANOVA (group x test order x [context]). This analysis found no main effect of context ($F < 1$), no context x group interaction ($F(1, 20) = 1.99$, $p = .17$) and no other main or interaction effects (all $F < 1$). To account for variability in consumption, as well as the group difference in pre-feeding (*Control > HFHS*), we next conducted two further analyses. The first was to express consumption in the contexts as a ratio of pre-feeding prior to that test; i.e. Minus ratio = Minus context / Minus pre-feeding and Plus ratio = Plus context / Plus pre-feeding. However, this transformation had little

effect, since analysis in a 2 x 2 x (2) mixed-ANOVA revealed no main effect of context ($F <$ 1), no context x group interaction ($F(1, 20) = 1.71$, $p = .21$), nor any other main or interaction effects (largest $F(1, 20) = 2.08$, $p = .17$; data not shown).
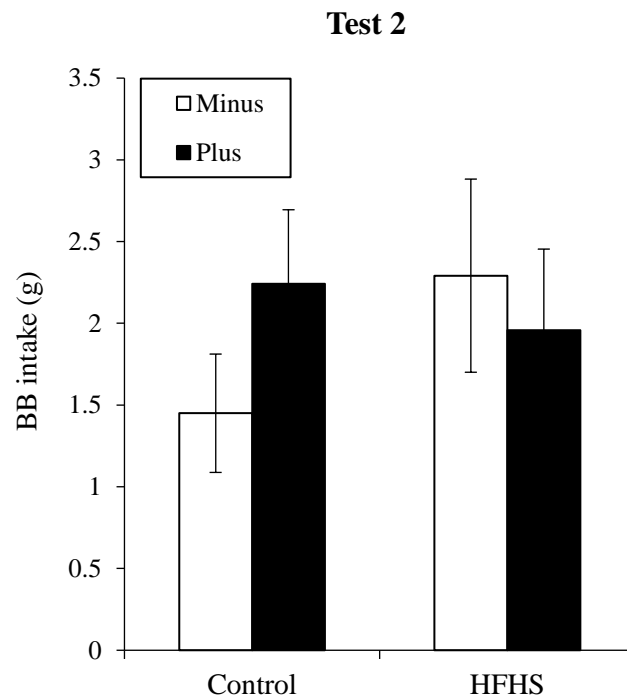
Figure 5.1.4. Results of Test 2 in Experiment 5.1. After 20-min pre-feeding, no effect of context and no context x group interaction was found in the 10-min context test.

The second set of additional analyses tested the influence of pre-feeding by correlating consumption in each pre-feeding session with consumption in the subsequent context test. Scatter plots of these relationships are presented in Figures 5.1.5A and B. We reasoned that in the absence of any external influence on feeding, consumption in two consecutive periods of time should be expected to correlate. This relationship could conceivably be positive over short periods of time (e.g. rats that eat more in pre-feeding continue to eat more) or negative over longer periods of time (e.g. greater intake in pre-feeding produces more satiety and inhibits eating). Importantly, these putative correlations

should be weakened by any external stimulus that modulates feeding. Thus, we hypothesised that the correlation between pre-feeding and context consumption should be weaker on the Plus test, to the extent that this environment acquired the capacity to enhance feeding. By contrast, a stronger association between pre-feeding and context intake might be expected for the Minus test, where animals may be more sensitive to internal satiety signals in an environment without past associations with food.

Analyses indicated that in the Minus context test (Fig. 5.1.5.A), pre-feeding and context consumption correlated negatively ($r(24) = -0.41$, $p = .043$), such that rats that ate more during pre-feeding tended to eat less during the Minus context test. By contrast, pre-feeding and context consumption did not correlate significantly in the Plus context test (Fig. 5.1.5.B; $r(24) = 0.017$, $p > .05$). The difference between correlations approached statistical significance (Fisher's r to z transformation: $z = -1.47$, $p = .07$, one-tailed test). This pattern was consistent within *HFHS* and *Control* groups, though no correlations were statistically significant (*HFHS* group: $r(12) = -.26$, $p = .41$ for the Minus test and $r(12) = .06$, $p = .85$ for the Plus test; *Control* group: $r(12) = -.50$, $p = .10$ for the Minus test and $r(12) = -.09$, $p = .77$ for the Plus test).
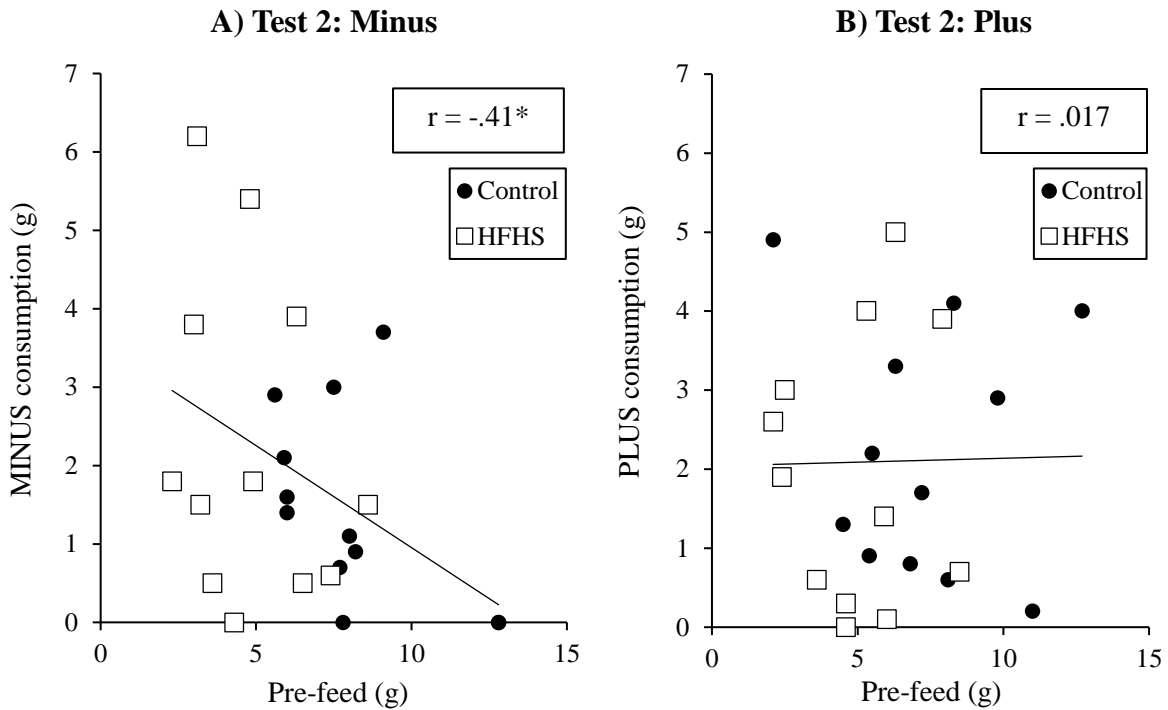
Figure 5.1.5. Correlational analyses from Test 2. In the Minus (Panel A) but not the Plus (Panel B) test, pre-feeding and context consumption correlated negatively.

Test 3: Froot Loops

Test 3 measured consumption of an alternative food, FL. Consumption is shown in Figure 5.1.6 and was analysed in a 2 x 2 x (2) ANOVA (group x test order x [context]). This analysis found no main effect of context and no context x group interaction (both $F < 1$). A significant context x test order interaction was found ($F(1, 20) = 23.17$, $p < .001$) reflecting the tendency for consumption to increase from the first to second test, regardless of which context was tested first. Unlike Tests 1 and 2, the main effect of group was not significant ($F(1, 20) = 1.60$, $p = .22$) and no other main or interaction effects were significant (all $F < 1$).
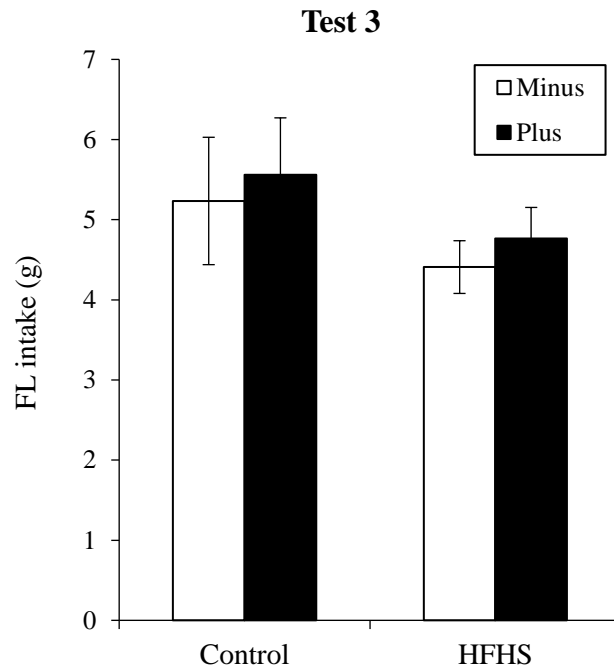
Figure 5.1.6. Results of Test 3 in Experiment 5.1. Consumption of an alternative palatable food, FL, did not differ between contexts or groups.

Fat mass

Fat mass data are displayed in Figure 5.1.7, adjusted for body weight. One-way ANOVA found that despite no difference in terminal body weight between groups ($F < 1$), the *HFHS* group had significantly higher total g/kg fat ($F(1, 22) = 4.80, p = .039$). Further analyses found this effect was statistically significant for epididymal fat ($F(1, 22) = 9.35, p = .006$) and visceral fat ($F(1, 22) = 5.54, p = .028$), but not retroperitoneal fat ($F(1, 22) = 1.82, p = .191$). There were no significant correlations between fat mass (total g/kg) or fasting blood glucose with CPF test data when all rats were analysed together (largest $r(24) = .16, p = .46$), nor when *Control* and *HFHS* groups were considered separately (largest $r(12) = .45, p = .14$).
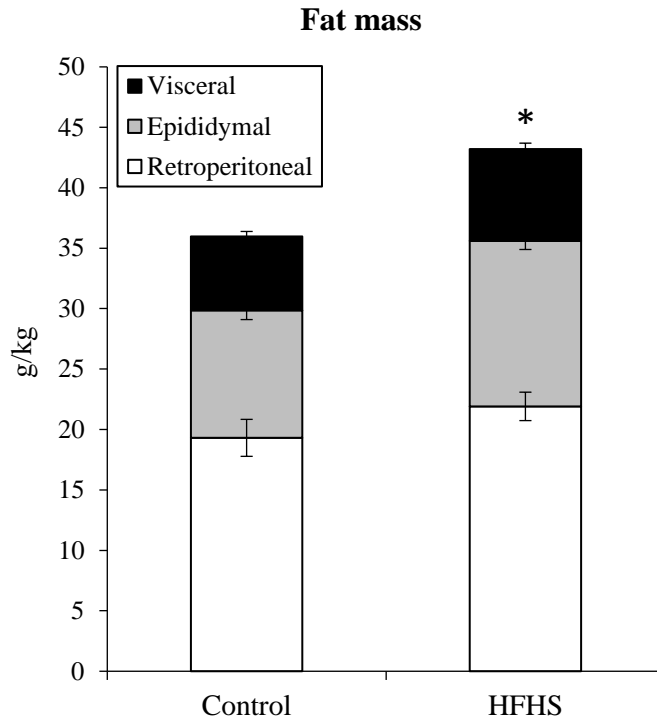
Fat mass

Figure 5.1.7. Fat mass at cull in Experiment 5.1. The *HFHS* group had significantly more total g/kg fat than the *Control* group (*p < .05).

5.3. General discussion

Experiment 5.1 tested the effects of diet-induced obesity on CPF and found three key results. First, there was no evidence that animals made obese by chronic access to a high-fat, high-sugar diet showed any greater tendency to exhibit CPF relative to controls. Second, the *HFHS* group suppressed their consumption of palatable foods relative to *Controls* across training and CPF tests. This result corroborates past research and has several implications for future study of CPF in obesity models. Third, correlational analyses indicated that the Plus context disrupted sensitivity to pre-feeding in Test 2, despite the absence of a significant overall difference between the contexts. The extent to which this result comprises CPF will be discussed with reference to the only previous study, to our knowledge, to have applied correlational analyses in this way (Galarce et al., 2007).

The diet intervention consisted of unrestricted access to sweetened condensed milk for 5 weeks and was successful in enhancing energy intake and producing a group difference in body weight that was statistically significant on day 12 of the diet and thereafter. By the end of the diet intervention *HFHS* rats were approximately 10% heavier than their *Control* counterparts, but did not differ in their fasting blood glucose levels. Though this suggests the metabolic effects of the diet intervention were relatively mild, they were persistent: the body weight difference was statistically significant well into behavioural testing, during which time both groups were fed only chow, together with relatively brief access to the palatable foods used for CPF training and tests. The restoration of the body weight difference between groups might relate to the consistently greater consumption of BB and FL by the *Control* group. Despite this, at cull the *HFHS* group exhibited higher body fat stores, a result which parallels a prior study from our laboratory showing that animals given unrestricted access to 10% sucrose solution for 8 weeks retained higher fat stores after 6 weeks of behavioural testing on food deprivation (Kendig et al., 2014). The present experiment shows that this 'persisting adiposity' effect holds under conditions where food deprivation is not introduced.

The 12-day training procedure was based on two experiments that found CPF effects (Experiments 2.1 and 3.3), suggesting conditions were appropriate for observing CPF. Thus, exposure to the Plus context provided an opportunity to consume a new palatable food, whereas in the Minus context rats were without food for the first time (albeit for relatively short periods). The clear result from training was that the *HFHS* group ate significantly less than the *Control* group. This effect was not due to initial neophobia toward BB in the *HFHS* group, since groups did not differ on the first training session, nor to a failure of the *HFHS* group to increase consumption over time, since training data showed no interaction between group and session. This result suggests that eating BB in the Plus context was rewarding for both *HFHS* and *Control* groups. It seems unlikely that *Control* animals ate more because of

greater hunger, since animals were not food deprived in training. Rather, these results suggest that previous unrestricted access to palatable SCM for the *HFHS* group reduced the incentive value of BB in the Plus context relative to the *Control* group, who previously ate only chow and water.

Test 1 measured consumption in the Minus context under training conditions; i.e., in a 30-min test with no pre-feeding. The modest difference in consumption between contexts, although in the predicted direction, was not statistically significant. The group difference in consumption seen in training persisted into the tests, with significantly lower intake by *HFHS* rats, across both contexts. In Test 2 rats were pre-fed in a neutral environment in order to test whether further intake would be stimulated by placement in the Plus but not the Minus context. Pre-feeding again revealed a group difference (*HFHS < Control*), but this was not evident in the subsequent context tests. The pattern of data suggested a CPF effect in *Control* but not *HFHS* animals; again, however, no effects were statistically significant when analysing group means in the contexts alone. Test 3 continued the aim of previous chapters to explore the specificity of CPF. Contrary to hypotheses, no effect was found in the *HFHS* group, whose overall consumption of FL was now not statistically different to the *Control* group.

The absence of significant differences between the Plus and Minus contexts was not a central concern because our primary aim was to compare *HFHS* and *Control* groups. Indeed, given the CPF effects reported in previous experiments were statistically significant but modest in size, we reasoned this might be ideal for detecting the larger effects hypothesised for the *HFHS* group. A similar rationale was adopted in a paper examining the effects of ghrelin function in the ventral hippocampus on CPF (Kanoski et al., 2013; Experiment 4). Using a training paradigm modified from Weingarten (1983), Kanoski et al. food-deprived rats and then exposed them to a CS+ cue that signalled the delivery of their five-daily meals,

and a CS- cue that did not predict food. In satiated tests, feeding in response to the CS+ and CS- did not differ under vehicle; by contrast, the CS+ initiated significantly more meals than the CS- after ghrelin administration to the ventral hippocampus. The authors explained that their training procedure was chosen to be sub-threshold, such that only modest effects were expected in the absence of the experimental manipulation (in this case, pharmacological). In the present experiments, however, results found no support for augmented CPF in the *HFHS* group after their exposure to an obesogenic diet.

The inclusion of pre-feeding in Test 2 offered another means of assessing the influence of the contexts on consumption: correlations. Analyses showed that when the Minus context was tested, consumption in pre-feeding and in the context correlated negatively: Rats that ate more in pre-feeding tended to eat less in the context, and *vice-versa*. In the Plus context test, however, there was no relationship between intake in pre-feeding and in the context. Thus, despite no significant difference between the contexts when analysed in isolation, this analysis suggests that the Plus context disrupted sensitivity to pre-feeding, since rats' consumption in this environment was unrelated to the amount just eaten in pre-feeding. Similar results were found in a study using discrete cues (Galarce et al., 2007), in which consumption during a 5-min pre-feeding and 5-min CPF test did not correlate when presenting a cue that previously signalled the available reward (i.e., a consistent CS). By contrast, when an alternative cue or no cue was presented – conditions where consumption fell and, therefore, no CPF effect was found – pre-feeding and test consumption correlated *positively*. Thus, both the present results and those of Galarce and colleagues (2007) suggest that food-paired cues or environments reduce sensitivity to the amount just eaten, whereas pre-feeding consumption exerts a stronger influence on feeding under control conditions[15]. It

---

[15] The fact that this correlation was negative in the present experiment and positive in Galarce et al. (2007) may relate to differences in pre-feed duration (20-min vs. 5-min) and reward type (Banana bread vs. 4% sucrose or maltodextrin solution). Each of these factors would appear to foster greater intake and satiety in the present

is noteworthy that these patterns did not appear to differ between *HFHS* and *Control* animals in the present study. This forms an important line of enquiry for future research.

The suppressed consumption by the *HFHS* group throughout training and Tests 1-2 warrants further discussion. Why did animals now withdrawn from their previously unrestricted access to highly palatable SCM consume *less* palatable food than control animals, despite being significantly heavier? As outlined in the introduction to this chapter, this initially counterintuitive result is consistent with past work in animal models of obesity. Reichelt et al. (2014) reported lower consumption of flavoured sugar solutions in rats made obese by unrestricted access to a cafeteria diet. We (Kendig et al., 2014) found that animals given chronic access to 10% sucrose solution showed depressed response rates in instrumental training relative to their leaner control counterparts. Tracy et al. (2015) found that animals made obese by six weeks' unrestricted access to a high-fat diet showed lower breakpoints in a progressive ratio test of instrumental responding, suggestive of reduced motivation[16]. Notably, this deficit was not present in rats that were pre-exposed to the reinforcer (sucrose pellets) prior to the diet. This result suggests that pre-exposing the training and test foods prior to the diet intervention may have precluded the lower overall consumption by the *HFHS* group. However, it does not appear to explain the failure to detect CPF.

The present results suggest several directions for future study of CPF as it relates to obesity. First, it will be interesting to assess CPF after longer and/or more severe diet interventions, given that our 5-wk manipulation did not impair fasting glucose and produced

---

experiment, such that rats were satiated at the point of the CPF test. In Galarce et al. (2007), the positive correlation under no-cue or inconsistent CS conditions could be because rats were not fully satiated on the reward. Therefore, the association might be tracking individual differences in preference for the reward.

[16] Animals fed the high-fat diet for 8 weeks also failed to display a conditioned place preference for a compartment paired with the sucrose pellets, suggesting the reward was less valued by these obese rats.

only modest increases in energy intake and weight gain (~14% and ~10% above controls, respectively). Therefore, it is possible that feeding behaviour in the presence of food cues might become more dysregulated in a state of more severe obesity produced by longer periods on the diet (e.g. 90 days; Kanoski & Davidson, 2010) and/or by the provision of 'cafeteria' diets that can enhance energy intake over fivefold (e.g. Tran & Westbrook, 2015). In addition, while this experiment tested whether CPF was enhanced in diet-induced obese animals, the converse is important to address: are animals that exhibit a larger CPF effect at baseline more susceptible to weight gain on obesogenic diets (cf. Robinson et al., 2015)? Further, testing CPF before and after a diet intervention might better reveal the effects of the diet, while addressing the issue of reinforcer pre-exposure identified by Tracy et al. (2015). Another interesting possibility would be to increase deprivation state at test, to see whether this would selectively enhance the ability of the Plus context to drive consumption in control but not obese animals.

A final broader point relates to the issue of blunted response rates in animal models of obesity. In most previous studies, exposure to the diet is (necessarily, for the study of obesity) confounded with the obesity it produces. It would be highly interesting to test CPF in animals previously given sufficiently *intermittent* access to a cafeteria or high-fat, high-sugar diet so that they do not gain weight faster than controls. First, it would address the extent to which suppressed performance – whether measured in terms of consumption or instrumental responding – is due to experience with highly palatable food or to the metabolic consequences of excess weight gain. Second, unlike the distributed consumption of palatable food produced by unrestricted access to cafeteria diets (Martire et al., 2013), the rapid increase in short-term intake produced by intermittent or 'binge' procedures (e.g. Berner, Avena, & Hoebel, 2008; Eikelboom & Hewitt, 2016) might well produce larger effects on CPF, which also tests consumption within limited time periods.

Chapter 6: General discussion

This thesis studied the conditions under which rats' short-term food intake was enhanced in environments paired with palatable food. By doing so, experiments sought to better understand *cue-potentiated feeding* (CPF): when and how is food intake affected by external factors? Understanding the boundary conditions of CPF is important to model the ways in which societies replete with food, and food cues, drive overeating. Study of CPF at the behavioural level has received less attention than the neural and hormonal processes that underlie the effect, in part because experiments aimed at the latter mechanisms have, sensibly, used a relatively narrow range of conditioning and test parameters. Consequently, however, the generality of the effect is less well investigated.

This thesis used an animal model in which the associations between external cues and food could be controlled strictly and measured systematically. The general experimental approach was to pair distinct environments, or contexts, with palatable food, and then measure food consumption under various conditions. Within this framework, experiments were designed to address research questions relating to (1) the extent to which CPF is general or specific; (2) whether susceptibility to CPF can be tracked by individual differences in eating behaviour; and (3) how the effect is moderated by motivational state and methodological parameters. Each of these issues has implications for how prevalent CPF is in day-to-day life.

6.1. Specificity

A major question explored was the extent to which food cues affect consumption in a specific or general manner. The answer to this issue has implications for whether the effects

of food cues are confined to certain circumstances (i.e. a specific cue and a specific, conditioned food) or whether they enhance consumption more broadly. To date, almost all CPF experiments have suggested the former: food-paired cues or contexts enhance consumption only of the food they have previously signalled, and not alternative foods such as chow, novel pellet varieties, or flavoured solutions previously predicted exclusively by other cues (Delamater & Holland, 2008; Galarce et al., 2007; Petrovich et al., 2007a, 2007b, 2012, but see Boggiano et al., 2009). Therefore, most evidence indicates that CPF is specific, suggesting that presenting food cues evokes a specific representation of the paired food rather than a general desire to eat (Delamater & Holland, 2008; Petrovich, 2013, Johnson, 2013).

The present thesis tested the specificity of CPF under a broader range of conditions than those explored previously. We hypothesised that the failure to see CPF on the alternative foods listed above might relate to properties of the foods, such as low palatability (for chow) and neophobia (for novel pellets) rather than the nature of conditioning to the food-paired cue or context. Therefore, we asked whether the predictive "Plus" context would enhance intake of an alternative food that was palatable, familiar, and not previously predicted by other cues. The clear result across experiments was that even when these criteria were satisfied, the Plus context did not enhance intake of the alternative food. This was the case both when the test food was Froot Loops (used in most experiments) and Banana Bread (Experiment 2.1), suggesting that the absence of an effect was unrelated to which specific food was presented at test. Moreover, the absence of CPF was not due to floor effects: *Single* group rats readily ate the test food, but intake was not modulated by the surrounding environment. Additional tests confirmed that, as in previous studies, the Plus context did not enhance intake of chow (Experiment 3.1) or of a novel palatable food (Experiment 2.3) in the *Single* group.

Another important point was that within the *Single* groups, across experiments, CPF did not vary between subsets trained with the different foods. A difference between subsets – e.g. those trained with savoury versus sweet foods – could indicate that the failure to detect CPF on alternative foods related to the similarity between the training and test food; that is, to generalisation decrement. Data provided no support for this hypothesis (see Appendix B*)*. However, since we deliberately chose foods that varied significantly on several dimensions in order to meaningfully capture "variety", this possibility could be explored more systematically, particularly given the importance of the relative palatability of the training and test foods suggested by the results of Experiment 3.2 (where preference for the training food correlated negatively with CPF on FL) and Experiment 4.1 (where CPF on the training food and CPF on FL correlated negatively).

A caveat for these results is that because experiments focused on the transfer of CPF to alternative foods, the training foods themselves were often not tested. Consequently, it might be argued that the results from the *Single* group provide only partial support for the specificity of CPF by characterising the conditions under which the effect did *not* occur on alternative foods. However, Experiment 2.1 found an effect on the training and not on an alternative food, when these were Froot Loops and Banana Bread, respectively. It appears unlikely that this would have differed in subsequent experiments when additional foods were used. In summary, the present experiments add to existing research by demonstrating that contexts paired with a single food fail to enhance intake of alternative foods, even when these are familiar and palatable. This is generally consistent with studies of food cravings in people, which tend to be specific (Cornell et al., 1989; Federoff et al., 2003), but which may transfer to foods that are sufficiently similar (Ferriday & Brunstrom, 2008, 2011).

6.2. Specificity and variety

As discussed in Chapter 2, a key feature of 'obesogenic' environments is that they contain an enormous variety of food products. While many studies have focused on the increases in energy intake and body weight produced by simultaneous access to multiple foods (Sclafani & Springer, 1976; Treit et al., 1983; Rolls et al., 1981, 1983), none have studied variety in relation to food cues. By confining access to variety to a distinct context, the present experiments appear to be the first to model how such environments affect consumption. The novel result is that variety-paired contexts enhanced intake of other foods, both familiar and novel, which were never previously consumed within it. We interpreted this effect to suggest that exposure to multiple tastes and textures within the Plus context formed an association that generalised more readily to the test scenario when an alternative food was available. Thus, the consumption of Froot Loops (typically the alternative food) was elevated by the Plus context for animals trained with a variety of foods because the sensory characteristics of this food were more closely related to the representation of food reward encoded during training than for the *Single* group. The mechanisms thought to drive variety's short-term effects on consumption, such as sensory-specific satiety (Rolls et al., 1983) and compromised flavour-nutrient learning (Martin, 2016), do not appear to explain these results, given comparable training consumption between *Single* and *Variety* groups and the fact that exposure to the alternative test food was equivalent between groups.

There are several ways in which the present 'variety effect' could be studied further. Our variety manipulation could have been made more extreme by providing a new palatable food on every Plus session, rather than cycling access to three foods. Testing CPF under these conditions would clarify the contribution of habituation processes that likely occurred with repeated exposures to the same foods during training (Epstein et al., 2009). It is also

important to note that we manipulated variety mostly in terms of taste and texture (and, to some extent, energy density), but not palatability, which was consistently high. Therefore, there is scope to study the effects of variety on a broader spectrum of palatability. For example, an interesting possibility would be to pair a context with a relatively bland but nutritive food (e.g. chow mash) that was, over sessions, either presented alone, or rendered relatively more or less palatable by adding saccharin or quinine. Additionally, given that we do not always choose to eat when in food-paired environments, identifying the effects of partially-reinforced cues or contexts on consumption will be informative. Indeed, Experiment 4.2 found that extinction of the Plus context only affected rats given variety training, such that consumption was *lower* in this environment for *Variety* rats but no different in *Single* rats. Here, of course, reinforced and non-reinforced exposures were blocked and not intermixed.

6.3. Individual differences and vulnerable populations

Experiments also explored whether the vulnerability to overeating triggered by food cues differed on an individual level. Chapter 3 examined whether CPF related to individual differences in training intake and baseline consumption of the test food. These variables rarely correlated, indicating that they captured relatively unique aspects of eating behaviour. Nonetheless, neither variable predicted susceptibility to CPF. Thus, the ability of the Plus context to enhance intake of FL appeared unrelated to the amount of previous consumption in that context, and to animals' initial consumption of the test food. Although Experiments 3.1 and 3.2 were likely underpowered to detect significant correlations, Experiment 3.3 found no relationship between these variables and CPF in a larger sample. It is worth noting, however, that there is tension between group effects and individual differences, such that detecting

significant group effects requires minimising within-group variance, while individual differences might be obscured by group treatments. Thus, our aim of tracking individual differences in CPF may have been better served using designs with no group variable.

Whereas Chapter 3 studied pre-existing individual differences within a single population, Chapter 5 applied a diet intervention to form two distinct populations of normal weight and obese animals. Following past reports showing that responding to a conditioned food cue predicted susceptibility to diet-induced obesity (Robinson et al., 2015), we explored whether CPF would be augmented as a *consequence* of obesity. The propensity to overeat in the Plus context did not differ between obese and normal-weight animals; however, obese animals consistently ate significantly less palatable food across tests, suggestive of a general decrease in motivation produced by chronic access to palatable food, or perhaps a carryover effect of positive energy balance from the diet intervention.

Therefore, the present results found no evidence that CPF was predicted by measures of eating behaviour, on an individual level, or body weight, at a group level. These results are consistent with a past study in which CPF did not differ between 'binge-prone' and 'binge-resistant' rats (Boggiano et al., 2009). In addition, a recent meta-analysis reported that the effects of food cue exposure on eating and weight-related outcomes in humans "…*generalize across individual differences in BMI, age, dietary restraint and gender*" (Boswell & Kober, 2016, p. 169), where 'generalize' was meant to indicate that effect sizes were unaffected by variability in any of these four factors. Nonetheless, further work on individual differences in animal models will be valuable because of the potential to study how variance in responding to food cues contributes to the *genesis* of obesity (e.g. Robinson et al., 2015) in a way that is less feasible in humans. Variability in metabolic and physiological responses to food-cue exposure appear promising measures in this regard.

6.4. Effects of motivational state and procedural variables

6.4.1. Training factors

A relatively novel aspect of our training protocol was that rats were usually not food-restricted. Even in the two experiments where access was restricted – Experiments 2.1 and 4.1 – animals remained at a relatively mild deprivation state (~95% of free-feeding weights). Therefore, the present results show that food deprivation is not required for food cues to acquire the ability to potentiate feeding in later tests. Of course, the extent to which rats were in fact 'satiated' at the time of training sessions cannot be verified – only that consumption during Plus context training sessions was driven less by hunger than if food restriction had been employed. Further, it is not argued that deprivation state is unimportant; in fact, Experiment 4.1 indicated that deprivation state was a critical determinant of CPF when testing an alternative food, since rats ate *less* in the Plus context under acute food restriction. This result appeared to suggest that hunger enhanced the surprise of experiencing an alternative food in the Plus context and inhibited consumption, but was also likely to reflect the fact that the incentive value of the test food was lower on account of it being pre-exposed prior to food-deprivation.

A second novel aspect of the training protocol was that the amount of food provided in Plus context training sessions was unrestricted[17]. As discussed above, one reason was so that variability in consumption could be correlated with CPF. Another was so that the contribution of training consumption to total energy intake could be derived (see Future Directions below). However, an additional possibility was that unrestricted access encoded associations of the Plus context not only with the taste of the food but, in cases where large

---

[17] 'Unrestricted', of course, means an amount of food almost certainly more than rats will consume within a session – though experimenters are occasionally proven wrong.

amounts were eaten, short-term satiety toward the end of the session. One implication is that consumption within the Plus context at test could be suppressed for rats that had previously eaten larger amounts during training, leading to association of the context with satiety (Booth, 1972). Yet as discussed, no significant relationship between training intake and CPF was found (Chapter 3). In addition, several previous studies have shown CPF effects when the amount of food available is substantial, but capped (e.g. 7g of pellets; Petrovich et al., 2007a, 2007b) or apparently unrestricted (Boggiano et al., 2009). Furthermore, it has proven difficult to replicate experiments reporting conditioned satiety, at least in humans (Yeomans, 2012).

6.4.2. Contexts versus discrete cues

The experiments in the main body of this thesis used contexts as food-paired stimuli. However, a series of experiments modelling CPF with discrete cues is reported in Appendix F. There are several important ways in which conditioning might proceed differently for these two classes of stimuli.

Training features

The above comment on conditioned satiety alludes to a key difference between the use of contexts and discrete cues to model CPF: studies using contexts involve consumption of a greater amount of food than when discrete cues are conditioned. In the latter case, multiple CS+ presentations in daily training sessions require that the amount of food be limited to encourage sustained responding and tight temporal pairing of the cue with consumption. Typically, a single CS+ presentation is rewarded with one or two 45-mg pellets

or a 0.1-0.2ml delivery of liquid reward[18]. The number of reinforced CS+ presentations per daily training session has ranged from 2 (McDannald et al., 2005) to 4 (Dailey et al, 2016; Holland et al., 2001) to 6 (Holland & Gallagher, 2003) to 8 (Holland et al., 2002; Petrovich et al., 2002, 2005; Petrovich, Hobin, & Reppucci, 2012) to 10 (Sherwood et al., 2015) to 16 (Delamater & Holland, 2008) to 20 (Walker et al., 2012). Even in the latter studies, the total amount of food only amounts to a few grams per day – far less than in studies using contexts, such as the present experiments.

The most obvious implication of this difference is that satiety exerts a larger role in studies using contexts than discrete cues. Another more intriguing possibility is that the distribution of eating behaviour differs: whereas discrete cues prompt orientation and approach to the food location and intake of the morsel, exposure to contexts with ample food available presumably allows for more natural and repeated bouts of meal patterning to develop. Although it is clear that both approaches can successfully produce CPF, an interesting question is whether cues and contexts produce effects via distinct consumption patterns in the free-feeding conditions used to test CPF. For example, do discrete cues produce CPF by fostering a great number of smaller and/or shorter meals, whereas contexts promote fewer but larger meals?

Pre-feeding during test

Another difference between context and cue models of CPF is that the latter typically include pre-feeding manipulations prior to testing the cues. Because initial placement in the conditioning chamber itself elicits eating (!), pre-feeding enhances the sensitivity of the test by reducing variability in acute hunger between rats and increasing the likelihood that

---

[18] One exception is a study in which daily training sessions consisted of two CS+ presentations that each terminated with the delivery of 50 pellets to the magazine (Cole et al., 2015).

consumption is driven by associative properties of the cue and not of the context – or simply by palatability of the food (Petrovich, 2013; Johnson, 2013). By contrast, in studies using contexts the animals typically have not been pre-fed (Boggiano et al., 2009; Petrovich et al., 2007a, 2007b), perhaps because consumption in the conditioning chamber is precisely what is of interest. However, pre-feeding might still be useful by bringing down what would otherwise be high levels of consumption. Though most experiments in this thesis omitted pre-feeding, the few tests to include it yielded mixed results. Experiment 2.1 demonstrated a CPF effect after pre-feeding, whereas Experiments 3.2 and 5.1 found that adding pre-feeding did not 'reveal' effects that were not evident in other tests without pre-feeding. (Pre-feeding is also included in the discrete cue experiments reported in Appendix F.)

In these instances, additional analyses tested whether the correlations between pre-feeding and context consumption differed between the Plus and Minus context tests. The rationale was that the strength of this correlation should be weaker on the Plus context test, to the extent that this environment acquired the ability to override satiety and prolong consumption. Some evidence for this hypothesis was reported in Chapter 5, where pre-feeding and context consumption correlated negatively on the Minus context test, with no relationship between the two on the Plus test[19]. However, this pattern of results was not found in other experiments. One potential reason for these inconsistent results might be opposing influences on consumption of satiety and preference for the test food. Presumably, the effects of short-term satiety should produce a negative correlation between pre-feeding and context consumption, if greater consumption in pre-feeding reduces additional consumption in the context. However, if preference for the test food is what guides consumption, the correlation

---

[19] These correlations reflect variability in consumption that, ideally, should reflect animals' desire to eat and not stress or anxiety. In turn, an important logistical issue is where pre-feeding should occur. Measuring pre-feeding intake by individual rats requires placement in another 'context' that, ideally, has been pre-exposed at some point prior to tests, but not to the extent that conditioning to the intended Plus and Minus contexts is affected.

between pre-feeding and context intake might be positive: rats that eat more in pre-feeding subsequently continue to eat more in the context test, while those with lower preference for the food tend to eat less in both periods. The duration of pre-feeding and test sessions is also likely to be important, with consumption perhaps dictated by preference in short sessions and by satiety over longer sessions.

A complication introduced by pre-feeding is the potential for unequal consumption between groups (e.g. lower intake by *HFHS* rats in Experiment 5.1) or chance differences between tests (e.g. higher intake prior to the Plus test in Experiment 2.1). Indeed, the only paper to our knowledge to report similar correlational analyses did so as a means of accounting for differences in pre-feeding rates between different cue tests (Galarce et al., 2007). Nonetheless, continued exploration of these correlations would seem to fit well with what is tested by pre-feeding designs: can a food-paired cue or context sustain or prolong consumption *despite* satiety on the test food? A final point is that testing these correlations required animals to be shifted from group-housed home cages to individual chambers for pre-feeding. To mitigate the effects of stress and/or novelty, rats were exposed to these chambers multiple times prior to tests. While the contribution of stress cannot be dismissed entirely, the success of habituation was suggested by a decline in urination and defecation, progressive ease of handling upon retrieval of rats from the chamber, and by their ample consumption during pre-feeding at test.

6.5. Future directions

6.5.1. Motivational state and meal size

The observations made in this chapter suggest several research questions. It will be relevant to test whether the present variety effect reported in Chapters 2 and 3 replicates when only limited access to palatable food is provided in training. More generally, future experiments should clarify whether cues paired with small versus large amounts of food exert different effects on CPF, given these have been a feature of cue and context experiments, respectively, but never compared directly. This would delineate the contributions of taste versus satiety to CPF. Since the effects of reward size might vary according to whether or not animals are food-deprived for training, these factors could be manipulated systematically to integrate the present results (no food restriction, unlimited food in the Plus context) with most past research (food restriction, limited amounts of food). In addition, whether or not animals are food-deprived when first exposed to the test food appears an important determinant of the food's incentive value (as suggested by Experiment 4.1). Understanding how hunger, meal size and satiety interact with food cues is important because people learn about and encounter food cues across a range of hunger states in everyday life, and the form of food intake stimulated by cues might vary widely (e.g. snacks versus meals). By demonstrating CPF effects in rats given free access both to chow in the home cage and to the food provided in the Plus context, the present experiments go some small way toward this aim.

6.5.2. Does CPF increase the risk of obesity over the long-term?

A major goal for future CPF research should be to clarify if and how exposure to food cues over the longer-term increases energy intake and weight gain. This hypothesis is

appealing because food cues are ubiquitous and examples of the temptation they produce are easy to imagine. That food-paired stimuli can initiate and/or enhance short-term food intake is not at issue, as shown presently and in much past animal research (Petrovich, 2013), and in a recent meta-analysis of studies in humans (Boswell & Kober, 2016). However, what is not well understood is how these 'cued' meals operate in the context of total energy intake (Berthoud, 2012). An important question is to identify whether snacks or meals initiated or extended by food cues lead to smaller intake in subsequent meals. Is this putative compensation complete, particularly when cued meals are likely to be energy-dense and highly palatable[20] (see also Levitsky, 2005)? Is compensation evident immediately, or revealed only over a longer period? A recent study using autocorrelations found that peoples' food intake did not correlate reliably between one day and the next (Levitsky et al., 2017).

The present thesis did not test this issue directly but instead explored several ways in which food cue exposure affected compensation over the short-term. As described above, correlational analyses appear a promising method for analysing the acute effects of food cues after pre-feeding. Another approach taken in Experiment 3.3 was to explore whether total energy intake differed on training days beginning with a Plus versus a Minus context training session. Results indicated that the increase in palatable food intake over Plus sessions was not accompanied by a proportional decrease in chow intake in the home-cage after each session. Despite the consequent increase in energy intake over Plus days, some evidence of compensation was suggested by a modest decrease in chow intake over the intermixed Minus days. This result is interesting to compare with a study by Reppucci and Petrovich (2012), who exposed rats to a food cue at the start of a 4-hour test in which both pellets, the cued food, and chow were available. In addition to a CPF effect in the form of greater pellet intake

---

[20] 'Healthy' foods can be signalled too, of course, but this would seem far less common.

in rats given Paired training, inadequate compensation was suggested by the fact that chow intake did not differ between groups in the 4-hr test or in the home cage over the following 20-h.

A limitation of Experiment 3.3 and past attempts to examine compensation of this kind is that they have not separated the effects of the food cue from exposure to the foods *per se*. Identifying whether food cues have additive stimulatory effects on consumption beyond those of the foods they signal will be important to determine. The only test of this was in Experiment 4.1, where the *Unpaired* group ate similar amounts of the palatable foods in the home-cage as eaten by the *Variety* group in the Plus context. Nonetheless, results suggested that monitoring home-cage chow intake was a sensitive measure of compensation for short-term access to palatable food, and changes in this measure over time. Tracking incremental changes are important because differences in energy intake as little as 100 kcal/day may meaningfully affect long-term weight gain (Hill, Wyatt, Reed, & Peters, 2003).

Two final points are important to consider. First, evaluating the effects of food cues should not be restricted to consumption. Not only is eating outside the home more frequently associated with higher energy intake (Kant & Graubard, 2004), but these meals also tend to contain more saturated fat and less fibre, calcium, and iron (Guthrie, Lin, & Frazao, 2002). Therefore, exposure to food cues might still pose risk to overall health if the foods we are tempted to buy are poorer in nutritional value. Second, food cues can elicit cephalic phase responses in anticipation of a meal (Weingarten & Powley, 1981; Nederkoorn, Smulders, & Jansen, 2000). For example, a recent study in humans found that pictures of palatable food blunted the blood glucose response to a meal without affecting the amount eaten, suggestive of an improved metabolic response (Brede et al., 2017). Therefore, comparing the

consumption produced by food cues with the metabolic and hormonal responses that are

evoked appears an important direction for future research on CPF in animal models.

6.5.3. How does CPF relate to the length of training?

Inherent in studying whether chronic exposure to food cues enhances weight gain is

understanding how CPF varies with different amounts of training. A recent study in humans

found that pairing chocolate consumption with a specific time of day significantly enhanced

desires to eat at that time after 15 but not 5 conditioning sessions (van den Akker,

Havermans, & Jansen, 2017). Aside from this experiment, it does not appear that training

amount has been systematically manipulated in CPF studies in either rodents or humans.

Instead, almost all rodent CPF experiments have used moderate amounts of training –

typically fewer than 20 sessions[21]. Whereas this amount of Pavlovian training is

demonstrably able to produce learning, it is currently unknown at what point cues acquire the

ability to potentiate feeding and how this changes over extended training. Although observing

CPF after extremely limited training may seem unlikely, there is evidence for one-trial

learning in aversive paradigms (e.g. Fanselow, 1990) and some evidence from appetitive

procedures (e.g. Parkes et al., 2014).

Of course, much is already known about how instrumental and Pavlovian

conditioning are affected by extended training. Goal-directed performance of an instrumental

response becomes impaired with extended training (Adams, 1982; Lingawi & Balleine,

2012), after chronic access to high-fat, high-sugar diets (Furlong, Jayaweera, et al., 2014;

---

[21] The longest training phase appears to have been by Lovibond (1980) who administered 50 exposures to food-paired contexts across a total of 100 days of conditioning.

Kendig et al., 2013) and is acutely impaired in environments previously paired with palatable food (Kendig, Cheung, Raymond, & Corbit, 2016; see Appendix G). This suggests the interesting possibility that the specificity of CPF might wane over extended training. On the other hand, Pavlovian cues retain the ability to selectively increase performance of instrumental responses which earn the same outcome even after extended training (Holland, 2004). Crucially, however, this form of specific Pavlovian-to-instrumental transfer (PIT) can exist alongside a general increase in responding produced by Pavlovian cues, whether or not this cue shares a common outcome with instrumental responses (Corbit & Balleine, 2005; Holland, 2004).

Therefore, a parametric analysis manipulating the amount of training would be highly interesting in terms of the size and specificity of CPF. Given PIT, CPF and sensitivity to devaluation can feasibly be tested within the same experiment[22] (e.g. Delamater & Holland, 2008), comparisons between these tasks would also be informative. For example, do animals that exhibit the strongest CPF effect also show enhanced PIT, or impaired sensitivity to devaluation? This might inform how distinct incentive versus general motivational processes interact to produce CPF. More broadly, understanding if and how CPF changes over extended training are vital for extrapolating the relevance of the effect to overeating and obesity, given the extensive opportunities for stimulus-food associations to be formed and strengthened over the lifespan.

---

[22] If not the same test!

6.6. Limitations

6.6.1. Sex

With one exception (Experiment 5.1), all experiments used female rats; it will be important to replicate the key results from this thesis in males, in light of evidence of sex differences in context renewal paradigms (e.g. Anderson & Petrovich, 2015). Notably, food intake is suppressed during the proestrous phase of the 4-5-day oestrous cycle in the rat (Blaustein & Wade, 1976; Butera, 2010). We did not measure or control for oestrous cycle during these experiments and, therefore, this may have formed an added source of variability in consumption. However, a recent meta-analysis found no evidence of greater variability in female than male rodents across a range of behavioural and neuroscientific studies (Becker, Prendergast, & Liang, 2016). Our use of female rats might also be viewed as valuable following a recent call for greater use of female mammals in preclinical research, at least in the USA (Clayton & Collins, 2014), given that males are used over five times as often as females in most biological sciences (Beery & Zucker, 2009).

6.6.2. Test order effects

With one exception (Experiment 4.3), all testing in the present experiments was within-subjects with the aim of increasing statistical power and allowing for CPF to be indexed in each animal. The latter point was particularly important for correlational analyses. However, the consequent need to counterbalance test order produced frequent interactions with the context main effect of interest. These interactions, which do not appear to have been an issue in past CPF experiments, almost always reflected increasing consumption over repeated tests when an alternative food was presented, and were unrelated to whether the

food was novel or familiar and, if familiar, to whether pre-exposure occurred proximally to testing (e.g. Experiments 2.1 and 5.1) or prior to training (most other experiments). Additional analyses of selected test data in Appendix C suggested that test order effects were unrelated to differences in incentive contrast from training to the first test. Instead, results indicated simply that consumption of a palatable food increased with repeated exposures. Critically, the ability of the Plus context to moderate consumption despite this influence was evident only in *Variety* groups.

6.6.3. What factors constrain CPF effects?

The CPF effects reported in this thesis were modest in absolute size despite statistical significance. There are at least two reasons why this may not be a central concern. First, as discussed earlier, small increases in energy intake may be precisely what fosters long-term weight gain if organisms fail to adequately compensate at other times. Additionally, training conditions that do not produce large CPF effects may be opportune for detecting effects of pharmacological or other behavioural interventions that are hypothesised to enhance feeding in response to external cues (e.g. Kanoski et al., 2013). Nonetheless, the moderate size of CPF appears in keeping with past literature, and warrants discussion of several factors that are likely to constrain effects.

General satiety

CPF tests are typically brief and occur after pre-feeding consisting of *ad-libitum* chow access for several days prior to tests (at a minimum) and, often, acute pre-feeding of the test food. A physical upper limit in consumption – that is, gastric capacity and satiety – might constrain the potential for large effects that require further eating.

Sensory-specific satiety

In addition to the general form of satiety described above, CPF effects are also working against sensory-specific satiety in designs where pre-feeding is used. Here, CPF is the continuation of feeding despite satiety on that very food. As well as studies of resistance to satiation (described in Chapter 1), more recent learning experiments have used pre-feeding to probe the content of instrumental conditioning. For example, pre-feeding comprises one means of outcome devaluation that is used to assess the extent to which performance of an instrumental response is under goal-directed control (e.g. Balleine & Dickinson, 1998b). Thus, after pre-feeding a food, goal-directed control over behaviour is suggested if performance of an instrumental response earning that food is reduced relative to the performance of another response earning a separate food that has not been devalued. Or, when only a single lever is trained, performance is compared with another test session in which a control food (one not earned by any trained response) is pre-fed.

To confirm pre-feeding has been effective, researchers often employ consumption tests in which, after pre-feeding, animals are presented with either the devalued or non-devalued food (Corbit & Balleine, 2005). A desired outcome is lower intake of the devalued food, since results otherwise indicate either that devaluation was ineffective or that sensory-specific satiety was not intact. Fortunately, sensitivity to devaluation in terms of consumption – i.e. reduced consumption of the devalued relative to the non-devalued food – is typically highly robust. For example, a recent study found that rats reduced their consumption of the devalued food even five hours after pre-feeding, and regardless of whether pre-feeding occurred in the instrumental training context or another location (Parkes, Marchand, Ferreira, & Coutureau, 2016).

A recent study from our laboratory showed that sensitivity to devaluation was impaired in contexts paired with palatable food (Kendig et al., 2016; Appendix G). Although the primary focus of this study was instrumental performance, we included a consumption test to verify that pre-feeding was effective. For this test rats were pre-fed one of the two rewards earned in instrumental training (pellets or sucrose solution) for an hour, and were then placed into the 'Plus' context paired with palatable food, where pellets were available. Pellet intake was significantly lower in animals pre-fed pellets than in those pre-fed sucrose, indicating that sensory-specific satiety was intact even in an environment paired with palatable food. Therefore, the inhibitory effects of sensory-specific satiety appear to be another factor that constrains the ability of food-paired cues to enhance consumption. Indeed, rats pre-fed pellets in our study still ate ~0.5g pellets in the consumption test; this small amount may yet have exceeded consumption in another context not paired with food, although this was not tested (Kendig et al., 2016; Appendix G).

Incentive contrast

Several results in the present thesis suggested that CPF was modulated by incentive contrast between the training and test foods. Our decision to test a palatable alternative food led to ample consumption in CPF tests; nonetheless, there was evidence that this constituted a form of negative contrast for *Single* and *Variety* groups. This was most clearly suggested by the fact that these groups ate less FL in tests than of their training foods, constraining the size of effects. In addition, Experiment 3.2 found that *Single* and *Variety* groups ate more of the training food than FL in a choice preference test, and that the extent of this preference correlated negatively with CPF when testing FL. Finally, Experiment 4.1 found that CPF on the training food and on FL correlated negatively. Clearer understanding of the latter result might be obtained by counterbalancing the order of training food and alternative food tests;

however, this could introduce additional variability or unforeseen effects that might undermine CPF.

Incentive contrast was also suggested by an inverse relationship between rats' prior exposure to palatable food and their subsequent consumption of FL at test. Thus, *Chow* groups without prior access to palatable food in the contexts (and the *Nothing* group in Experiment 4.2) tended to eat more in tests, overall, than *Single* and *Variety* groups given palatable foods during training. These results appear to reflect positive contrast: presentation of Froot Loops, a food far more palatable than the chow (or no food) previously available, was highly salient and drove high consumption. At the other end of the spectrum, Experiment 5.1 found that chronic access to sweetened condensed milk persistently suppressed consumption of palatable food, a result suggestive of negative contrast. Collectively, these results highlight a gap between the present animal model and the human experience: whereas most people are familiar with a wide variety of intensely palatable foods, exposure to FL for *Chow* rats marked a rare opportunity to consume something more palatable than chow (or indeed, anything other than chow).

Comparison conditions

A final factor contributing to modest effects is that there is little disincentive for animals *not* to eat when in control conditions or in the presence of a control cue. This is particularly relevant for the present experiments given their use of palatable 'junk' foods. One way to view this is that at test, the salient sensory features of the test food comprised an additional cue that competed with the surrounding Minus context. Indeed, the ability of food to act as a cue is readily demonstrated in priming studies in which a small morsel of food prompts further consumption (e.g. Boggiano et al., 2009; Cornell et al., 1989), food craving literature in humans (Boswell & Kober, 2016) and in research on portion size effects, where

manipulating the amount of food available can shape the amount eaten (e.g. Ello-Martin, Ledikwe, & Rolls, 2005; see also Tordoff, 2002, for analogous results in rats). An interesting question for future research is whether a food-paired cue or context would enhance consumption more than would a small morsel of the same food.

6.7. Concluding remarks

This thesis studied how short-term food intake by rats is moderated in environments associated with palatable food. Results extend current knowledge by characterising the conditions under which CPF is manifest. In particular, they indicate that overeating may be especially common in the many modern food environments that now contain an abundant variety of foods. However, the nature of learning for stimuli or contexts paired with a single type of food appears to consist of a highly specific association with the sensory properties of that food that does not transfer to alternatives, even when these are familiar and palatable. Situating these acute effects on food consumption within the larger framework of total energy intake is a key future direction for CPF research, and one which might be fruitfully advanced using methods where more aspects of consumption are free to vary, as in the present experiments. Continued study of CPF should not be discouraged by the fact that effects are often subtle and sensitive to experimental parameters. To the contrary, there are numerous advantages for the continued use of animal models to study factors influencing the size and specificity of CPF over the short-term, and its contribution to overeating and obesity over the longer term.

References

Adams, C. D. (1982). Variations in the sensitivity of instrumental responding to reinforcer devaluation. *The Quarterly Journal of Experimental Psychology*, *34*(2), 77-98. http://dx.doi.org/10.1080/14640748208400878

Ahn, S., & Phillips, A. G. (2012). Repeated cycles of restricted food intake and binge feeding disrupt sensory-specific satiety in the rat. *Behavioural brain research*, *231*(2), 279-285. https://doi.org/10.1016/j.bbr.2012.02.017

Anand, B. K., & Brobeck, J. R. (1951). Localization of a "feeding center" in the hypothalamus of the rat. *Proceedings of the society for experimental biology and Medicine*, *77*(2), 323-325. https://doi.org/10.3181/00379727-77-18766

Anderson, L. C., & Petrovich, G. D. (2015). Renewal of conditioned responding to food cues in rats: Sex differences and relevance of estradiol. *Physiology & Behavior, 151*, 338-344. http://doi.org/10.1016/j.physbeh.2015.07.035

Balleine, B., Davies, A., & Dickinson, A. (1995). Cholecystokinin attenuates incentive learning in rats. *Behavioral neuroscience*, *109*(2), 312. http://dx.doi.org/10.1037/0735-7044.109.2.312

Balleine, B. W., & Dickinson, A. (1998a). Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. *Neuropharmacology*, *37*(4), 407-419. https://doi.org/10.1016/S0028-3908(98)00033-1

Balleine, B. W., & Dickinson, A. (1998b). The role of incentive learning in instrumental outcome revaluation by sensory-specific satiety. *Animal Learning & Behavior*, *26*(1), 46-59. doi: https://doi.org/10.3758/BF03199161

Barnett, S. A., & Spencer, M. M. (1951). Feeding, social behaviour and interspecific competition in wild rats. Behaviour, 229-242. http://www.jstor.org/stable/4532728

Bayer, E. (1929). Beiträge zur Zweikomponententheorie des Hungers. *Zsch. F. Psychol.*, *112*, 1-53.

Becker, J. B., Prendergast, B. J., & Liang, J. W. (2016). Female rats are not more variable than male rats: a meta-analysis of neuroscience studies. *Biology of sex differences*, *7*(1), 34. https://doi.org/10.1186/s13293-016-0087-5

Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neuroscience & Biobehavioral Reviews*, *35*(3), 565-572. https://doi.org/10.1016/j.neubiorev.2010.07.002

Bellisle, F. (1979). Human feeding behavior. *Neuroscience & Biobehavioral Reviews*, *3*(3), 163-169. https://doi.org/10.1016/0149-7634(79)90006-X

Berner, L. A., Avena, N. M., & Hoebel, B. G. (2008). Bingeing, self- restriction, and increased body weight in rats with limited access to a sweet- fat diet. *Obesity*, *16*(9), 1998-2002. doi:10.1038/oby.2008.328

Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). Dissecting components of reward: 'liking', 'wanting', and learning. *Current opinion in pharmacology*, *9*(1), 65-73. https://doi.org/10.1016/j.coph.2008.12.014

Berthoud, H. R. (2007). Interactions between the "cognitive" and "metabolic" brain in the control of food intake. *Physiology & Behavior, 91(5)*, 486-498. http://doi.org/10.1016/j.physbeh.2006.12.016

Berthoud, H. R. (2012). The neurobiology of food intake in an obesogenic environment. *Proceedings of the Nutrition Society, 71(04)*, 478-487. doi:10.1017/S0029665112000602

Bezzina, L., Lee, J. C., Lovibond, P. F., & Colagiuri, B. (2016). Extinction and renewal of cue-elicited reward-seeking. *Behaviour research and therapy*, *87*, 162-169. https://doi.org/10.1016/j.brat.2016.09.009

Bindra, D. (1978). How adaptive behavior is produced: a perceptual-motivational alternative to response reinforcements. *Behavioral and Brain Sciences*, *1*(1), 41-52. https://doi.org/10.1017/S0140525X00059380

Birch, L. L., McPhee, L., Sullivan, S., & Johnson, S. (1989). Conditioned meal initiation in young children. *Appetite*, *13*(2), 105-113. https://doi.org/10.1016/0195-6663(89)90108-6

Blaustein, J. D., & Wade, G. N. (1976). Ovarian influences on the meal patterns of female rats. *Physiology & behavior*, *17*(2), 201-208. https://doi.org/10.1016/0031-9384(76)90064-0

Boakes, R. A. (1977). Performance on learning to associate a stimulus with positive reinforcement. *Operant-Pavlovian interactions*, 67-97.

Boggiano, M. M., Dorsey, J. R., Thomas, J. M., & Murdaugh, D. L. (2009). The Pavlovian power of palatable food: lessons for weight-loss adherence from a new rodent model of cue-induced overeating. *International Journal of Obesity, 33(6)*, 693-701. doi:10.1038/ijo.2009.57

Bolles, R. C. (1967). Theory of motivation. New York, New York: Harper & Row.

Booth, D. A. (1972). Conditioned satiety in the rat. *Journal of comparative and physiological psychology*, *81*(3), 457. http://dx.doi.org/10.1037/h0033692

Booth, D. A. (1981*). Hunger and satiety as conditioned reflexes.* In: Weiner, H., Hofer, M. A. and Stunkard, A. J. (Eds.) *Brain, Behavior and Bodily Disease. Research Publications: Association for Research in Nervous and Mental Disease*, 59 (pp. 143-160). New York, NY: Raven Press.

Boswell, R. G., & Kober, H. (2016). Food cue reactivity and craving predict eating and weight gain: a meta- analytic review. *obesity reviews*, *17*(2), 159-177. doi:10.1111/obr.12354

Bouton, M. E. (2011). Learning and the persistence of appetite: Extinction and the motivation to eat and overeat. *Physiology & Behavior, 103(1)*, 51-58. http://doi.org/10.1016/j.physbeh.2010.11.025

Bouton, M. E., Todd, T. P., Miles, O. W., León, S. P., & Epstein, L. H. (2013). Within-and between-session variety effects in a food-seeking habituation paradigm. *Appetite, 66*, 10-19. http://doi.org/10.1016/j.appet.2013.01.025

Brede, S., Sputh, A., Hartmann, A. C., Hallschmid, M., Lehnert, H., & Klement, J. (2017). Visual food cues decrease postprandial glucose concentrations in lean and obese men without affecting food intake and related endocrine parameters. *Appetite*, *117*, 255-262. https://doi.org/10.1016/j.appet.2017.07.001

Butera, P. C. (2010). Estradiol and the control of food intake. *Physiology & behavior*, *99*(2), 175-180. https://doi.org/10.1016/j.physbeh.2009.06.010

Calvin, J. S., Bicknell, E. A., & Sperling, D. S. (1953a). Establishment of a conditioned drive based on the hunger drive. *Journal of Comparative and Physiological Psychology*, *46*(3), 173. http://dx.doi.org/10.1037/h0060708

Calvin, J. S., Bicknell, E. A., & Sperling, D. S. (1953). Effect of a secondary reinforcer on consummatory behavior. *Journal of comparative and physiological psychology*, *46*(3), 176. http://dx.doi.org/10.1037/h0063489

Capaldi, E. D., Davidson, T. L., & Myers, D. E. (1981). Resistance to satiation: Reinforcing effects of food and eating under satiation. *Learning and Motivation*, *12*(2), 171-195. https://doi.org/10.1016/0023-6690(81)90017-5

Capaldi, E. D., & Myers, D. E. (1978). Resistance to satiation of consummatory and instrumental performance. *Learning and Motivation*, *9*(2), 179-201. https://doi.org/10.1016/0023-6690(78)90019-X

Capaldi, E. D., & Myers, D. E. (1979). Resistance to satiation as a function of three satiation procedures. *Bulletin of the Psychonomic Society*, *14*(1), 53-56. https://doi.org/10.3758/BF03329398

Clayton, D. A. (1978). Socially facilitated behavior. *The Quarterly Review of Biology*, *53*(4), 373-392. https://doi.org/10.1086/410789

Clayton, J. A., & Collins, F. S. (2014). NIH to balance sex in cell and animal studies. *Nature*, *509*(7500), 282-3. PMID: 24834516

Cole, S., Mayer, H. S., & Petrovich, G. D. (2015). Orexin/Hypocretin-1 Receptor Antagonism Selectively Reduces Cue-Induced Feeding in Sated Rats and Recruits Medial Prefrontal Cortex and Thalamus. *Scientific Reports, 5*, 16143-16143. doi:10.1038/srep16143

Corbit, L. H., & Balleine, B. W. (2005). Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. *Journal of Neuroscience*, *25*(4), 962-970. https://doi.org/10.1523/JNEUROSCI.4507-04.2005

Cornell, C. E., Rodin, J., & Weingarten, H. (1989). Stimulus-induced eating when satiated. *Physiology & behavior*, *45*(4), 695-704. https://doi.org/10.1016/0031-9384(89)90281-3

Dailey, M. J., Moran, T. H., Holland, P. C., & Johnson, A. W. (2016). The antagonism of ghrelin alters the appetitive response to learned cues associated with food. *Behavioural brain research*, *303*, 191-200. https://doi.org/10.1016/j.bbr.2016.01.040

Dally, J. M., Clayton, N. S., & Emery, N. J. (2008). Social influences on foraging by rooks (Corvus frugilegus). *Behaviour, 145*(8), 1101-1124. doi:10.1163/156853908784474470

Dashiell, J. F. (1937). Fundamentals of general psychology. Boston, Massachusetts: Houghton Mifflin Co.

Davidson, T. L., Kanoski, S. E., Walls, E. K., & Jarrard, L. E. (2005). Memory inhibition and energy regulation. *Physiology & behavior*, *86*(5), 731-746. https://doi.org/10.1016/j.physbeh.2005.09.004

Davidson, T. L., Sample, C. H., & Swithers, S. E. (2014). An application of Pavlovian principles to the problems of obesity and cognitive decline. *Neurobiology of Learning and Memory, 108*, 172-184. http://doi.org/10.1016/j.nlm.2013.07.014

Davis, J. D. (1981). Has a meal trigger been found? *Behavioral and Brain Sciences*, *4*(4), 580-581. doi: https://doi.org/10.1017/S0140525X00000303

De Castro, J. M. (1996). How can eating behavior be regulated in the complex environments of free-living humans? *Neuroscience & Biobehavioral Reviews*, *20*(1), 119-131. https://doi.org/10.1016/0149-7634(95)00047-I

De Castro, J. M. (1997). Socio-cultural determinants of meal size and frequency. British Journal of Nutrition, 77(S1), S39-S55. https://doi.org/10.1079/BJN19970103

De Castro, J. M. (2010). The control of food intake of free-living humans: putting the pieces back together. *Physiology & Behavior, 100(5),* 446-453. http://doi.org/10.1016/j.physbeh.2010.04.028

Delamater, A. R. (1996). Effects of several extinction treatments upon the integrity of Pavlovian stimulus-outcome associations. *Learning & behavior*, *24*(4), 437-449. https://doi.org/10.3758/BF03199015

Delamater, A. R., & Holland, P. C. (2008). The influence of CS-US interval on several different indices of learning in appetitive conditioning. *Journal of Experimental*

Psychology: Animal Behavior Processes, 34(2), 202. http://dx.doi.org/10.1037/0097-7403.34.2.202

Delamater, A. R., Schneider, K., & Derman, R. C. (2017). Extinction of specific stimulus–outcome (SO) associations in Pavlovian learning with an extended CS procedure. *Journal of Experimental Psychology: Animal Learning and Cognition*, *43*(3), 243. http://dx.doi.org/10.1037/xan0000138

Dickinson, A. (1985). Actions and habits: the development of behavioural autonomy. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *308*(1135), 67-78. doi:10.1098/rstb.1985.0010

Drew, G. C. (1937). The Recurrence of Eating in Rats after apparent Satiation. *Journal of Zoology*, *107*(1), 95-106. doi: 10.1111/j.1469-7998.1937.tb08503.x

Eikelboom, R., & Hewitt, R. (2016). Intermittent access to a sucrose solution for rats causes long-term increases in consumption. *Physiology & behavior*, *165*, 77-85. https://doi.org/10.1016/j.physbeh.2016.07.002

Ello-Martin, J. A., Ledikwe, J. H., & Rolls, B. J. (2005). The influence of food portion size and energy density on energy intake: implications for weight management. *The American journal of clinical nutrition*, *82*(1), 236S-241S. PMID: 16002828

Epstein, A. N. (1960). Reciprocal changes in feeding behavior produced by intrahypothalamic chemical injections. *American Journal of Physiology--Legacy Content*, *199*(6), 969-974. PMID: 13697000

Epstein, L. H., Temple, J. L., Roemmich, J. N., & Bouton, M. E. (2009). Habituation as a determinant of human food intake. *Psychological Review, 116(2)*, 384-407. http://dx.doi.org/10.1037/a0015074

Fanselow, M. S. (1990). Factors governing one-trial contextual conditioning. *Learning & behavior*, *18*(3), 264-270. https://doi.org/10.3758/BF03205285

Fedoroff, I., Polivy, J., & Herman, C. P. (2003). The specificity of restrained versus unrestrained eaters' responses to food cues: general desire to eat, or craving for the cued food? *Appetite, 41(1)*, 7-13. http://doi.org/10.1016/S0195-6663(03)00026-6

Ferriday, D., & Brunstrom, J. M. (2008). How does food-cue exposure lead to larger meal sizes? *British Journal of Nutrition*, *100*(6), 1325-1332. https://doi.org/10.1017/S0007114508978296

Ferriday, D., & Brunstrom, J. M. (2011). 'I just can't help myself': effects of food-cue exposure in overweight and lean individuals. *International Journal of Obesity*, *35*(1), 142. doi:10.1038/ijo.2010.117

Fischel, W. (1927). Beitrage zur Soziologie des Haushuhns. Biol. Zentralbl, 47, 678-95.

Flagel, S. B., Watson, S. J., Akil, H., & Robinson, T. E. (2008). Individual differences in the attribution of incentive salience to a reward-related cue: influence on cocaine sensitization. *Behavioural brain research*, *186*(1), 48-56. https://doi.org/10.1016/j.bbr.2007.07.022

Flaherty, C. F. (1996). *Problems in the behavioural sciences, No. 15. Incentive relativity. New York, NY*: Cambridge University Press.

Flaherty, C. F., Hrabinski, K., & Grigson, P. S. (1990). Effect of taste context and ambient context changes on successive negative contrast. *Animal Learning & Behavior*, *18*(3), 271-276. https://doi.org/10.3758/BF03205286

Flaherty, C. F., & Largen, J. (1975). Within-subjects positive and negative contrast effects in rats. *Journal of Comparative and Physiological Psychology, 88(2)*, 653. http://dx.doi.org/10.1037/h0076416

Friedman, M. I. (1981). Metabolic explanations of eating behavior. *Behavioral and Brain Sciences*, *4*(4), 583-584. https://doi.org/10.1017/S0140525X00000339

Furlong, T. M., Jayaweera, H. K., Balleine, B. W., & Corbit, L. H. (2014). Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum. *Journal of Neuroscience*, *34*(14), 5012-5022. https://doi.org/10.1523/JNEUROSCI.3707-13.2014

Galarce, E. M., Crombag, H. S., & Holland, P. C. (2007). Reinforcer-specificity of appetitive and consummatory behavior of rats after Pavlovian conditioning with food reinforcers. *Physiology & Behavior, 91(1)*, 95-105. http://doi.org/10.1016/j.physbeh.2007.01.021

Galarce, E. M., & Holland, P. C. (2009). Effects of cues associated with meal interruption on feeding behavior. *Appetite, 52(3)*, 693-702. http://doi.org/10.1016/j.appet.2009.03.009

Galarce, E. M., McDannald, M. A., & Holland, P. C. (2010). The basolateral amygdala mediates the effects of cues associated with meal interruption on feeding behavior. *Brain research*, *1350*, 112-122. https://doi.org/10.1016/j.brainres.2010.02.042

Galef, B. G., & Laland, K. N. (2005). Social learning in animals: empirical studies and theoretical models. AIBS Bulletin, 55(6), 489-499. https://doi.org/10.1641/0006-3568(2005)055[0489:SLIAES]2.0.CO;2

Galloway, A. T., Addessi, E., Fragaszy, D. M., & Visalberghi, E. (2005). Social facilitation of eating familiar food in Tufted Capuchins (Cebus apella): does it involve behavioral coordination? *International Journal of Primatology*, *26*(1), 181-189. https://doi.org/10.1007/s10764-005-0729-7

Grant, D. P., & Milgram, N. W. (1973). Plasticity of normal feeding: situational and individual factors. *Canadian Journal of Psychology/Revue canadienne de psychologie*, *27*(3), 305. http://dx.doi.org/10.1037/h0082481

Grossman, S. P. (1960). Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. *Science*, *132*(3422), 301-302. doi:10.1126/science.132.3422.301

Guerin, B. (1993). Social facilitation. Cambridge (England): Cambridge University Press.

Guerin, B. (2010). Social facilitation. Corsini Encyclopedia of Psychology. 1–2. Retrieved from doi:10.1002/9780470479216.corpsy0890.

Guthrie, J. F., Lin, B. H., & Frazao, E. (2002). Role of food prepared away from home in the American diet, 1977-78 versus 1994-96: changes and consequences. *Journal of nutrition education and behavior*, *34*(3), 140-150. https://doi.org/10.1016/S1499-4046(06)60083-3

Harb, M. Y., Reynolds, V. S., & Campling, R. C. (1985). Eating behaviour, social dominance and voluntary intake of silage in group- fed milking cattle. *Grass and Forage Science*, *40*(1), 113-118. doi: 10.1111/j.1365-2494.1985.tb01727.x

Hardman, C. A., Ferriday, D., Kyle, L., Rogers, P. J., & Brunstrom, J. M. (2015). So many brands and varieties to choose from: does this compromise the control of food intake in humans? *PloS one, 10(4)*, e0125869. https://doi.org/10.1371/journal.pone.0125869

Harlow, H. F. (1932). Social facilitation of feeding in the albino rat. *The Pedagogical Seminary and Journal of Genetic Psychology*, *41*(1), 211-221. http://dx.doi.org/10.1080/08856559.1932.9944151

Harlow, H. F., & Yudin, H. C. (1933). Social behavior of primates. I. Social facilitation of feeding in the monkey and its relation to attitudes of ascendance and submission. *Journal of Comparative Psychology*, *16*(2), 171. http://dx.doi.org/10.1037/h0071690

Hendrikse, J. J., Cachia, R. L., Kothe, E. J., McPhie, S., Skouteris, H., & Hayden, M. J. (2015). Attentional biases for food cues in overweight and individuals with obesity: a systematic review of the literature. *Obesity reviews*, *16*(5), 424-432. doi:10.1111/obr.12265

Herman, C. P., & Polivy, J. (1984). A boundary model for the regulation of eating. In Stunkard, A. K., & Stellar, E. (Eds), *Research Publications-Association for Research in Nervous and Mental Disease*, *62* (pp. 141-156). New York, NY: Raven Press.

Herman, C. P., & Polivy, J. (2005). Normative influences on food intake. *Physiology & behavior*, *86*(5), 762-772. https://doi.org/10.1016/j.physbeh.2005.08.064

Herman, C. P., & Polivy, J. (2008). External cues in the control of food intake in humans: the sensory-normative distinction. *Physiology & Behavior, 94(5)*, 722-728. http://doi.org/10.1016/j.physbeh.2008.04.014

Hetherington, M. M. (2007). Cues to overeat: psychological factors influencing overconsumption. *Proceedings of the Nutrition Society*, *66*(1), 113-123. https://doi.org/10.1017/S0029665107005344

Hill, J. O., Wyatt, H. R., Reed, G. W., & Peters, J. C. (2003). Obesity and the environment: where do we go from here? *Science*, *299*(5608), 853-855. doi:10.1126/science.1079857

Hoffman, H. S., Stratton, J. W., & Newby, V. (1969). The control of feeding behavior by an imprinted stimulus. *Journal of the experimental analysis of behavior*, *12*(6), 847-860. doi: 10.1901/jeab.1969.12-847

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacology Biochemistry and Behavior*, *54(1)*, 21-30. https://doi.org/10.1016/0091-3057(95)02126-4

Holland, P. C. (2004). Relations between Pavlovian-instrumental transfer and reinforcer devaluation. *Journal of Experimental Psychology: Animal Behavior Processes*, *30*(2), 104. http://dx.doi.org/10.1037/0097-7403.30.2.104

Holland, P. C. (2014). Stimuli associated with the cancellation of food and its cues enhance eating but display negative incentive value. *Learning & Behavior, 42(4)*, 365-382. doi:10.3758/s13420-014-0154-x

Holland, P. C., Hatfield, T., & Gallagher, M. (2001). Rats with basolateral amygdala lesions show normal increases in conditioned stimulus processing but reduced conditioned potentiation of eating. *Behavioral neuroscience*, *115*(4), 945. http://dx.doi.org/10.1037/0735-7044.115.4.945

Holland, P. C., & Gallagher, M. (2003). Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus- potentiated feeding and Pavlovian- instrumental transfer. *European Journal of Neuroscience*, *17*(8), 1680-1694. doi: 10.1046/j.1460-9568.2003.02585.x

Holland, P. C., & Hsu, M. (2014). Role of amygdala central nucleus in the potentiation of consuming and instrumental lever-pressing for sucrose by cues for the presentation or interruption of sucrose delivery in rats. *Behavioral neuroscience*, *128*(1), 71-82. http://dx.doi.org/10.1037/a0035445

Holland, P. C., & Petrovich, G. D. (2005). A neural systems analysis of the potentiation of feeding by conditioned stimuli. *Physiology & behavior*, *86*(5), 747-761. https://doi.org/10.1016/j.physbeh.2005.08.062

Holland, P. C., Petrovich, G. D., & Gallagher, M. (2002). The effects of amygdala lesions on conditioned stimulus-potentiated eating in rats. *Physiology & Behavior*, *76*(1), 117-129. https://doi.org/10.1016/S0031-3384(02)00688-1

Holmes, N. M., Hutton-Bedbrook, K., Fam, J., & Westbrook, R. F. (2016). Incentive contrast effects regulate responding to a flavor presented in compound with a saccharin unconditioned stimulus in rats. *Journal of Experimental Psychology: Animal Learning and Cognition*, *42*(3), 233. http://dx.doi.org/10.1037/xan0000101

Horstmann, A., Dietrich, A., Mathar, D., Pössel, M., Villringer, A., & Neumann, J. (2015). Slave to habit? Obesity is associated with decreased behavioural sensitivity to reward devaluation. *Appetite*, *87*, 175-183. https://doi.org/10.1016/j.appet.2014.12.212

Hoyenga, K. T., & Aeschleman, S. (1969). Social facilitation of eating in the rat. *Psychonomic Science*, *14*(5), 239-239. https://doi.org/10.3758/BF03332815

Hsia, L. C., & Wood-Gush, D. G. M. (1984). Social facilitation in the feeding behaviour of pigs and the effect of rank. *Applied Animal Ethology*, *11*(3), 265-270. https://doi.org/10.1016/0304-3762(84)90033-6

James, W. T. (1953). Social facilitation of eating behavior in puppies after satiation. *Journal of Comparative and Physiological Psychology*, *46*(6), 427. http://dx.doi.org/10.1037/h0056028

James, W. T. (1954). Secondary reinforced behavior in an operant situation among dogs. *The Journal of genetic psychology*, *85*(1), 129-133. doi: 10.1080/00221325.1954.10532866

James, W. T., & Cannon, D. J. (1955). Variation in social facilitation of eating behavior in puppies. *The Journal of genetic psychology*, *87*(2), 225-228. http://dx.doi.org/10.1080/00221325.1955.10532934

James, W. T., & Gilbert, T. F. (1955). The effect of social facilitation on food intake of puppies fed separately and together for the first 90 days of life. *The British Journal of Animal Behaviour*, *3*(4), 131-133. https://doi.org/10.1016/S0950-5601(55)80050-0

Jansen, A., Stegerman, S., Roefs, A., Nederkoorn, C., & Havermans, R. (2010). Decreased salivation to food cues in formerly obese successful dieters. *Psychotherapy and psychosomatics*, *79*(4), 257-258. https://doi.org/10.1159/000315131

Jansen, A., Theunissen, N., Slechten, K., Nederkoorn, C., Boon, B., Mulkens, S., & Roefs, A. (2003). Overweight children overeat after exposure to food cues. *Eating behaviors*, *4*(2), 197-209. https://doi.org/10.1016/S1471-0153(03)00011-4

Johnson, A. W. (2011). Melanin concentrating hormone (MCH) influences cue-driven food intake under conditions of satiety. *Appetite*, *57*, S21.

Johnson, A. W. (2013). Eating beyond metabolic need: how environmental cues influence feeding behavior. *Trends in Neurosciences, 36*, 101-109. https://doi.org/10.1016/j.tins.2013.01.002

Kanarek, R. B. (1981). Some limitations of homeostatic explanations of feeding behavior. *Behavioral and Brain Sciences*, *4*(4), 584-585. https://doi.org/10.1017/S0140525X00000340

Kanoski, S. E., & Davidson, T. L. (2010). Different patterns of memory impairments accompany short-and longer-term maintenance on a high-energy diet. *Journal of Experimental Psychology: Animal Behavior Processes*, *36(2)*, 313-319. http://dx.doi.org/10.1037/a0017228

Kanoski, S. E., Fortin, S. M., Ricks, K. M., & Grill, H. J. (2013). Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biological psychiatry*, *73*(9), 915-923. https://doi.org/10.1016/j.biopsych.2012.07.002

Kant, A. K., & Graubard, B. I. (2004). Eating out in America, 1987–2000: trends and nutritional correlates. *Preventive Medicine, 38(2)*, 243-249. http://doi.org/10.1016/j.ypmed.2003.10.004

Keeling, L. J., & Hurnik, J. F. (1996). Social facilitation and synchronization of eating between familiar and unfamiliar newly weaned piglets. *Acta Agriculturae Scandinavica A-Animal Sciences*, *46(1),* 54-60. http://dx.doi.org/10.1080/09064709609410924

Kendig, M. D., Boakes, R. A., Rooney, K. B., & Corbit, L. H. (2013). Chronic restricted access to 10% sucrose solution in adolescent and young adult rats impairs spatial memory and alters sensitivity to outcome devaluation. *Physiology & behavior*, *120*, 164-172. https://doi.org/10.1016/j.physbeh.2013.08.012

Kendig, M. D., Cheung, A. M., Raymond, J. S., & Corbit, L. H. (2016). Contexts Paired with Junk Food Impair Goal-Directed Behavior in Rats: Implications for Decision Making in Obesogenic Environments. *Frontiers in behavioral neuroscience*, *10:216*. doi:10.3389/fnbeh.2016.00216

Kendig, M. D., Rooney, K. B., Corbit, L. H., & Boakes, R. A. (2014). Persisting adiposity following chronic consumption of 10% sucrose solution: Strain differences and behavioural effects. *Physiology & behavior*, *130*, 54-65. https://doi.org/10.1016/j.physbeh.2014.03.021

Kennedy, G. C. (1950). The hypothalamic control of food intake in rats. *Proceedings of the Royal Society of London B: Biological Sciences*, *137*(889), 535-549. https://doi.org/10.1098/rspb.1950.0065

La Fleur, S. E., Vanderschuren, L. J. M. J., Luijendijk, M. C., Kloeze, B. M., Tiesjema, B., & Adan, R. A. H. (2007). A reciprocal interaction between food-motivated behavior and diet-induced obesity. *International journal of obesity*, *31*(8), 1286-1294. doi: 10.1038/sj.ijo.0803570

Laurent, V., & Balleine, B. W. (2015). Factual and counterfactual action-outcome mappings control choice between goal-directed actions in rats. *Current Biology*, *25*(8), 1074-1079. https://doi.org/10.1016/j.cub.2015.02.044

Laurent, V., Chieng, B., & Balleine, B. W. (2016). Extinction Generates Outcome-Specific Conditioned Inhibition. *Current Biology*, *26*(23), 3169-3175. https://doi.org/10.1016/j.cub.2016.09.021

Le Magnen, J. (1981). The metabolic basis of dual periodicity of feeding in rats. *Behavioral and Brain Sciences*, *4*(4), 561-575. doi: https://doi.org/10.1017/S0140525X00000236

Levin, B. E., Dunn-Meynell, A. A., Balkan, B., & Keesey, R. E. (1997). Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *273*(2), R725-R730.

Levitsky, D. A. (2005). The non-regulation of food intake in humans: hope for reversing the epidemic of obesity. *Physiology & Behavior, 86(5)*, 623-632. http://doi.org/10.1016/j.physbeh.2005.08.053

Levitsky, D. A., Limb, J. E. R., Wilkinson, L., Sewall, A., Zhong, Y., Olabi, A., & Hunter, J. (2017). Lack of negative autocorrelations of daily food intake on successive days challenges the concept of the regulation of body weight in humans. *Appetite*, *116*, 277-283. https://doi.org/10.1016/j.appet.2017.04.038

Lingawi, N. W., & Balleine, B. W. (2012). Amygdala central nucleus interacts with dorsolateral striatum to regulate the acquisition of habits. *Journal of Neuroscience*, *32*(3), 1073-1081. https://doi.org/10.1523/JNEUROSCI.4806-11.2012

Lovibond, P. F. (1980). Effects of long-and variable-duration signals for food on activity, instrumental responding, and eating. *Learning and Motivation*, *11*(2), 164-184. https://doi.org/10.1016/0023-9690(80)90011-9

Looy, H., & Weingarten, H. P. (1992). Facial expressions and genetic sensitivity to 6-n-propylthiouracil predict hedonic response to sweet. *Physiology & behavior*, *52*(1), 75-82. https://doi.org/10.1016/0031-9384(92)90435-5

Looy, H., Callaghan, S., & Weingarten, H. P. (1992). Hedonic response of sucrose likers and dislikers to other gustatory stimuli. *Physiology & behavior*, *52*(2), 219-225. https://doi.org/10.1016/0031-9384(92)90261-Y

Martin, A. A. (2016). Why can't we control our food intake? The downside of dietary variety on learned satiety responses. *Physiology & Behavior, 162*, 120-129. http://doi.org/10.1016/j.physbeh.2016.04.010

Martire, S. I., Holmes, N., Westbrook, R. F., & Morris, M. J. (2013). Altered feeding patterns in rats exposed to a palatable cafeteria diet: increased snacking and its implications for development of obesity. *PloS one*, *8*(4), e60407. https://doi.org/10.1371/journal.pone.0060407

Mayer, J. (1955). Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis. *Annals of the New York Academy of sciences*, *63*(1), 15-43. doi: 10.1111/j.1749-6632.1955.tb36543.x

McDannald, M. A., Saddoris, M. P., Gallagher, M., & Holland, P. C. (2005). Lesions of orbitofrontal cortex impair rats' differential outcome expectancy learning but not conditioned stimulus-potentiated feeding. *Journal of Neuroscience*, *25*(18), 4626-4632. https://doi.org/10.1523/JNEUROSCI.5301-04.2005

Meyers, A. W., & Stunkard, A. J. (1980). Food accessibility and food choice: A test of Schachter's externality hypothesis. *Archives of General Psychiatry*, *37*(10), 1133-1135. doi:10.1001/archpsyc.1980.01780230051007

Miller, N. E. (1955). Shortcomings of food consumption as a measure of hunger; results from other behavioral techniques. *Annals of the New York Academy of Sciences*, *63*(1), 141-143. doi: 10.1111/j.1749-6632.1955.tb36553.x

Morgan, M. J. (1974). Resistance to satiation. *Animal Behaviour*, *22*(2), 449-466. https://doi.org/10.1016/S0003-3472(74)80044-8

Morgan, M. J. (1979). The concept of drive. *Trends in neurosciences*, *2*, 240-242. https://doi.org/10.1016/0166-2236(79)90093-6

Myers Ernst, M., & Epstein, L. H. (2002). Habituation of responding for food in humans. *Appetite, 38(3)*, 224-234. http://doi.org/10.1006/appe.2001.0484

Nederkoorn, C., Smulders, F. T. Y., & Jansen, A. (2000). Cephalic phase responses, craving and food intake in normal subjects. *Appetite*, *35*(1), 45-55. https://doi.org/10.1006/appe.2000.0328

Nielsen, B. L. (1999). On the interpretation of feeding behaviour measures and the use of feeding rate as an indicator of social constraint. *Applied Animal Behaviour Science*, *63*(1), 79-91. https://doi.org/10.1016/S0168-1591(99)00003-9

Nielsen, B. L., Lawrence, A. B., & Whittemore, C. T. (1995). Effect of group size on feeding behaviour, social behaviour, and performance of growing pigs using single-space feeders. *Livestock Production Science*, *44*(1), 73-85. https://doi.org/10.1016/0301-6226(95)00060-X

Parent, M. B. (2016). Cognitive control of meal onset and meal size: Role of dorsal hippocampal-dependent episodic memory. *Physiology & behavior*, *162*, 112-119. https://doi.org/10.1016/j.physbeh.2016.03.036

Parkes, S. L., De la Cruz, V., Bermúdez-Rattoni, F., Coutureau, E., & Ferreira, G. (2014). Differential role of insular cortex muscarinic and NMDA receptors in one-trial appetitive taste learning. *Neurobiology of learning and memory*, *116*, 112-116. https://doi.org/10.1016/j.nlm.2014.09.008

Parkes, S. L., Marchand, A. R., Ferreira, G., & Coutureau, E. (2016). A time course analysis of satiety-induced instrumental outcome devaluation. *Learning & behavior*, *44*(4), 347-355. https://doi.org/10.3758/s13420-016-0226-1

Pavlov, I. P. (1927). Conditioned reflexes; an investigation of the physiological activity of the cerebral cortex. London: Oxford University Press.

Petrovich, G. D. (2013). Forebrain networks and the control of feeding by environmental learned cues. *Physiology & Behavior, 121*, 10-18. http://doi.org/10.1016/j.physbeh.2013.03.024

Petrovich, G. D., Hobin, M. P., & Reppucci, C. J. (2012). Selective Fos induction in hypothalamic orexin/hypocretin, but not melanin-concentrating hormone neurons, by a learned food-cue that stimulates feeding in sated rats. *Neuroscience, 224*, 70-80. http://doi.org/10.1016/j.neuroscience.2012.08.036

Petrovich, G. D., Holland, P. C., & Gallagher, M. (2005). Amygdalar and prefrontal pathways to the lateral hypothalamus are activated by a learned cue that stimulates eating. *Journal of Neuroscience*, *25*(36), 8295-8302. https://doi.org/10.1523/JNEUROSCI.2480-05.2005

Petrovich, G. D., & Lougee, M. A. (2011). Sex differences in fear-induced feeding cessation: Prolonged effect in female rats. *Physiology & behavior*, *104*(5), 996-1001. https://doi.org/10.1016/j.physbeh.2011.06.020

Petrovich, G. D., Ross, C. A., Holland, P. C., & Gallagher, M. (2007a). Medial prefrontal cortex is necessary for an appetitive contextual conditioned stimulus to promote eating in sated rats. *Journal of Neuroscience, 27*(24), 6436-6441. https://doi.org/10.1523/JNEUROSCI.5001-06.2007

Petrovich, G. D., Ross, C. A., Gallagher, M., & Holland, P. C. (2007b). Learned contextual cue potentiates eating in rats. *Physiology & Behavior, 90*(2), 362-367. http://doi.org/10.1016/j.physbeh.2006.09.031

Petrovich, G. D., Ross, C. A., Mody, P., Holland, P. C., & Gallagher, M. (2009). Central, but not basolateral, amygdala is critical for control of feeding by aversive learned cues. *Journal of Neuroscience*, *29*(48), 15205-15212. https://doi.org/10.1523/JNEUROSCI.3656-09.2009

Petrovich, G. D., Setlow, B., Holland, P. C., & Gallagher, M. (2002). Amygdalo-hypothalamic circuit allows learned cues to override satiety and promote eating. *Journal of Neuroscience*, *22*(19), 8748-8753. PMID: 12351750

Phillips, C. J. C. (2004). The effects of forage provision and group size on the behavior of calves. *Journal of Dairy Science*, *87*(5), 1380-1388. https://doi.org/10.3168/jds.S0022-0302(04)73287-7

Pickering, C., Alsiö, J., Hulting, A. L., & Schiöth, H. B. (2009). Withdrawal from free-choice high-fat high-sugar diet induces craving only in obesity-prone animals. *Psychopharmacology*, *204*(3), 431-443. https://doi.org/10.1007/s00213-009-1474-y

Raynor, H. A., & Epstein, L. H. (2001). Dietary variety, energy regulation, and obesity. *Psychological Bulletin, 127*(3), 325. doi:10.1037//0033-2909.127.3.325

Reichelt, A. C., Morris, M. J., & Westbrook, R. F. (2014). Cafeteria diet impairs expression of sensory-specific satiety and stimulus-outcome learning. *Frontiers in psychology*, *5*, 852. doi:10.3389/fpsyg.2014.00852

Reppucci, C. J., & Petrovich, G. D. (2012). Learned food-cue stimulates persistent feeding in sated rats. *Appetite, 59*(2), 437-447. http://doi.org/10.1016/j.appet.2012.06.007

Robinson, M. J., Burghardt, P. R., Patterson, C. M., Nobile, C. W., Akil, H., Watson, S. J., Berridge, K. C., & Ferrario, C. R. (2015). Individual differences in cue-induced motivation and striatal systems in rats susceptible to diet-induced obesity. *Neuropsychopharmacology*, *40*(9), 2113. doi:10.1038/npp.2015.71

Rodin, J. (1981). Current status of the internal–external hypothesis for obesity: What went wrong? *American Psychologist*, *36*(4), 361. doi: 10.1037/0003-066X.36.4.361

Roitman, M. F., Van Dijk, G., Thiele, T. E., & Bernstein, I. L. (2001). Dopamine mediation of the feeding response to violations of spatial and temporal

expectancies. *Behavioural Brain Research, 122*(2), 193-199. http://doi.org/10.1016/S0166-4328(01)00189-9

Rolls, B. J., Rowe, E. A., Rolls, E. T., Kingston, B., Megson, A., & Gunary, R. (1981). Variety in a meal enhances food intake in man. *Physiology & Behavior, 26*(2), 215-221. http://doi.org/10.1016/0031-9384(81)90014-7

Rolls, B. J., Van Duijvenvoorde, P. M., & Rowe, E. A. (1983). Variety in the diet enhances intake in a meal and contributes to the development of obesity in the rat. *Physiology & Behavior, 31*(1), 21-27. http://doi.org/10.1016/0031-9384(83)90091-4

Ross, S., & Ross, J. G. (1949a). Social facilitation of feeding behavior in dogs: I. Group and solitary feeding. *The Pedagogical Seminary and Journal of Genetic Psychology*, *74*(1), 97-108. doi: 10.1080/08856559.1949.10533484

Ross, S., & Ross, J. G. (1949b). Social facilitation of feeding behavior in dogs: II. Feeding after satiation. *The Pedagogical Seminary and Journal of Genetic Psychology*, *74*(2), 293-304. doi: 10.1080/08856559.1949.10533498

Rubenstein, D. I., Barnett, R. J., Ridgely, R. S., & Klopfer, P. H. (1977). Adaptive advantages of mixed-species feeding flocks among seed-eating finches in Costa Rica. *Ibis*, *119*(1), 10-21. doi: 10.1111/j.1474-919X.1977.tb02040.x

Saper, C. B., Chou, T. C., & Elmquist, J. K. (2002). The need to feed: homeostatic and hedonic control of eating. *Neuron*, *36*(2), 199-211. https://doi.org/10.1016/S0896-6273(02)00969-8

Scarborough, B. B., & Goodson, F. E. (1957). Properties of stimuli associated with strong and weak hunger drive in the rat. *The Journal of genetic psychology*, *91*(2), 257-261. http://dx.doi.org/10.1080/00221325.1957.10533053

Schachter, S. (1968). Obesity and eating. *Science, 161*(3843*)*, 751-756. http://dx.doi.org/10.1126/science.161.3843.751

Schachter, S. (1971). Some extraordinary facts about obese humans and rats. *American Psychologist*, *26*(2), 129. http://dx.doi.org/10.1037/h0030817

Schallert, T., Pendergrass, M., & Farrar, S. B. (1982). Cholecystokinin-octapeptide effects on eating elicited by "external" versus "internal" cues in rats. *Appetite*, *3*(2), 81-90. https://doi.org/10.1016/S0195-6663(82)80001-9

Sclafani, A., & Springer, D. (1976). Dietary obesity in adult rats: similarities to hypothalamic
    and human obesity syndromes. *Physiology & behavior*, *17*(3), 461-471.
    https://doi.org/10.1016/0031-9384(76)90109-8

Sherwood, A., Holland, P. C., Adamantidis, A., & Johnson, A. W. (2015). Deletion of
    Melanin Concentrating Hormone Receptor-1 disrupts overeating in the presence of
    food cues. *Physiology & behavior*, *152*, 402-407.
    https://doi.org/10.1016/j.physbeh.2015.05.037

Siegel, P. S., & Macdonnell, M. F. (1954). A repetition of the Calvin-Bicknell-Sperling study
    of conditioned drive. *Journal of comparative and physiological psychology*, *47*(3),
    250. http://dx.doi.org/10.1037/h0062891

Sobik, L., Hutchison, K., & Craighead, L. (2005). Cue-elicited craving for food: a fresh
    approach to the study of binge eating. Appetite, 44(3), 253-261.
    https://doi.org/10.1016/j.appet.2004.12.001

Spiteri, N. J. (1982). Circadian patterning of feeding, drinking and activity during diurnal
    food access in rats. *Physiology & behavior*, *28*(1), 139-147.
    https://doi.org/10.1016/0031-9384(82)90115-9

Strobel, M. G. (1972). Social facilitation of operant behavior in satiated rats. *Journal of
    comparative and physiological psychology*, *80*(3), 502.
    http://dx.doi.org/10.1037/h0032997

Stroebele, N., & De Castro, J. M. (2004). Effect of ambience on food intake and food choice.
    Nutrition, 20(9), 821-838. https://doi.org/10.1016/j.nut.2004.05.012

Swinburn, B., Egger, G., & Raza, F. (1999). Dissecting obesogenic environments: the
    development and application of a framework for identifying and prioritizing
    environmental interventions for obesity. *Preventive Medicine, 29(6)*, 563-570.
    https://doi.org/10.1006/pmed.1999.0585

Temple, J. L., Giacomelli, A. M., Roemmich, J. N., & Epstein, L. H. (2008). Dietary variety
    impairs habituation in children. *Health psychology: official journal of the Division of
    Health Psychology, American Psychological Association, 27(*1 Suppl*)*, S10.
    doi:10.1037/0278-6133.27.1

Thrailkill, E. A., Epstein, L. H., & Bouton, M. E. (2015). Effects of inter-food interval on the variety effect in an instrumental food-seeking task. Clarifying the role of habituation. *Appetite, 84*, 43-53. http://doi.org/10.1016/j.appet.2014.09.015

Toates, F. M. (1981). The control of ingestive behaviour by internal and external stimuli—A theoretical review. *Appetite*, *2*(1), 35-50. https://doi.org/10.1016/S0195-6663(81)80035-9

Tolman, C. W. (1964). Social facilitation of feeding behaviour in the domestic chick. *Animal Behaviour*, *12*(2), 245-251. https://doi.org/10.1016/0003-3472(64)90008-9

Tolman, C. W., & Wilson, G. F. (1965). Social feeding in domestic chicks. *Animal Behaviour*, *13*(1), 134-142. https://doi.org/10.1016/0003-3472(65)90083-7

Tordoff, M. G. (2002). Obesity by choice: the powerful influence of nutrient availability on nutrient intake. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *282*(5), R1536-R1539. doi:10.1152/ajpregu.00739.2001

Tracy, A. L., Davis, J. F., Heiman, J. U., Schurdak, J. D., Clegg, D. J., & Benoit, S. C. (2007). Effect of high-fat diet-induced obesity on the efficacy of food and drug reinforcers. *Appetite*, *49*(1), 335. https://doi.org/10.1016/j.appet.2007.03.203

Tracy, A. L., Wee, C. J., Hazeltine, G. E., & Carter, R. A. (2015). Characterization of attenuated food motivation in high-fat diet-induced obesity: critical roles for time on diet and reinforcer familiarity. *Physiology & behavior*, *141*, 69-77. https://doi.org/10.1016/j.physbeh.2015.01.008

Tran, D. M., & Westbrook, R. F. (2015). Rats fed a diet rich in fats and sugars are impaired in the use of spatial geometry. *Psychological science, 26*(12), 1947-1957. https://doi.org/10.1177/0956797615608240

Treit, D., Spetch, M. L., & Deutsch, J. A. (1983). Variety in the flavor of food enhances eating in the rat: a controlled demonstration. *Physiology & Behavior, 30(2)*, 207-211. http://doi.org/10.1016/0031-9384(83)90007-0

Valle, F. P. (1968). Effect of exposure to feeding-related stimuli on food consumption in rats. *Journal of Comparative and Physiological Psychology*, *66*(3p1), 773. http://dx.doi.org/10.1037/h0026541

van den Akker, K., Havermans, R. C., & Jansen, A. (2017). Appetitive conditioning to specific times of day. *Appetite*, *116*, 232-238. https://doi.org/10.1016/j.appet.2017.05.014

Walker, A. K., Ibia, I. E., & Zigman, J. M. (2012). Disruption of cue-potentiated feeding in mice with blocked ghrelin signaling. *Physiology & behavior*, *108*, 34-43. https://doi.org/10.1016/j.physbeh.2012.10.003

Warwick, Z. S., & Schiffman, S. S. (1991). Flavor-calorie relationships: Effect on weight gain in rats. *Physiology & Behavior, 50(3)*, 465-470. http://doi.org/10.1016/0031-9384(91)90531-R

Warwick, Z. S., & Weingarten, H. P. (1996). Flavor-postingestive consequence associations incorporate the behaviorally opposing effects of positive reinforcement and anticipated satiety: implications for interpreting two-bottle tests. *Physiology & behavior*, *60*(3), 711-715. https://doi.org/10.1016/0031-9384(96)00087-X

Weingarten, H. P. (1983). Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science, 220(4595)*, 431-433. doi:10.1126/science.6836286

Weingarten, H. P. (1984a). Meal initiation controlled by learned cues: Basic behavioral properties. *Appetite*, *5*(2), 147-158. https://doi.org/10.1016/S0195-6663(84)80035-5

Weingarten, H. P. (1984b). Meal initiation controlled by learned cues: Effects of peripheral cholinergic blockade and cholecystokinin. *Physiology & behavior*, *32*(3), 403-408. https://doi.org/10.1016/0031-9384(84)90254-3

Weingarten, H. P. (1985). Stimulus control of eating: Implications for a two-factor theory of hunger. *Appetite*, *6*(4), 387-401. https://doi.org/10.1016/S0195-6663(85)80006-4

Weingarten, H. P., & Elston, D. (1990). The phenomenology of food cravings. *Appetite*, *15*(3), 231-246. https://doi.org/10.1016/0195-6663(90)90023-2

Weingarten, H. P., & Elston, D. (1991). Food cravings in a college population. *Appetite*, *17*(3), 167-175. https://doi.org/10.1016/0195-6663(91)90019-O

Weingarten, H. P., & Martin, G. M. (1989). Mechanisms of conditioned meal initiation. *Physiology & behavior*, *45*(4), 735-740. https://doi.org/10.1016/0031-9384(89)90287-4

Weingarten, H. P., & Powley, T. L. (1980). A new technique for the analysis of phasic gastric acid responses in the unanesthetized rat. *Laboratory animal science*, *30*(4 Pt 1), 673-680. PMID: 7421114

Weingarten, H. P., & Powley, T. L. (1980b). Ventromedial hypothalamic lesions elevate basal and cephalic phase gastric acid output. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *239*(3), G221-G229. PMID: 7435576

Weingarten, H. P., & Powley, T. L. (1981). Pavlovian conditioning of the cephalic phase of gastric acid secretion in the rat. *Physiology & behavior*, *27*(2), 217-221. https://doi.org/10.1016/0031-9384(81)90260-2

Werthmann, J., Roefs, A., Nederkoorn, C., Mogg, K., Bradley, B. P., & Jansen, A. (2011). Can (not) take my eyes off it: Attention bias for food in overweight participants. *Health Psychology*, *30*(5), 561. http://dx.doi.org/10.1037/a0024291

Woods, S. C., & Ramsay, D. S. (2000). Pavlovian influences over food and drug intake. *Behavioural brain research*, *110*(1), 175-182. https://doi.org/10.1016/S0166-4328(99)00194-1

Yeomans, M. R. (2012). Flavour–nutrient learning in humans: An elusive phenomenon? *Physiology & behavior*, *106*(3), 345-355. https://doi.org/10.1016/j.physbeh.2012.03.013

Zamble, E. (1973). Augmentation of eating following a signal for feeding in rats. *Learning and Motivation*, *4*(2), 138-147. https://doi.org/10.1016/0023-9690(73)90026-X

Appendix A: General method

Contexts

The contexts trained as food-paired stimuli were decorated conditioning chambers shown below. Tactile, visual and olfactory cues were manipulated to create two distinct contexts in the configuration shown below. The context on the left has a smooth Perspex floor and spotted wallpaper (which also covered the front panel). The folded paper towel inserted at the edge of the bedding tray was scented with vanilla essence using a pipette. The context on the right has a rough sandpaper floor insert, striped wallpaper, and was scented with rosewater essence. In some experiments, peppermint odour was used in place of rosewater essence (with no discernible effect on results). Odours were sourced from Queen®, Australia, and were diluted 10% v/v in tap water.
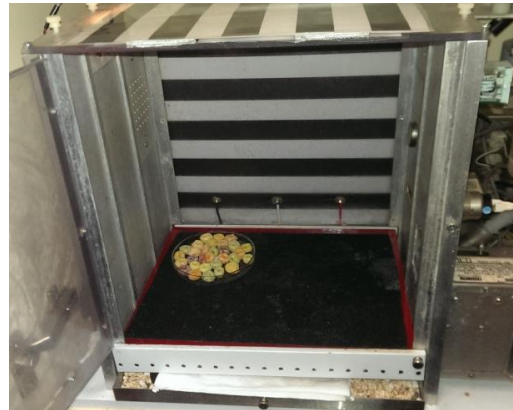
The allocation of these configurations as Plus and Minus contexts was always counterbalanced. Analyses found no differences in training or test data as a function of which environment served as which context. The above pictures show the contexts prior to a CPF test where Froot Loops were the test food. In between consecutive runs, odour solutions were refreshed and the floor inserts were removed and cleaned quickly. The perimeter of the chamber and the underlying bedding tray were checked carefully for fragments of food.

Foods

The following table shows nutritional information for the foods used in this thesis:

| Food | Manufacturer | Energy density (kJ/g) | Distribution of kJ by macronutrient | | | Description |
			Carbohydrate	Protein	Fat	
Chow: Rat and Mouse Cubes®	Specialty Feeds (WA, Australia)	14.23	65	23	12	12 mm diameter pellets (wheat/barley based)
Froot Loops®	Kellogg's (MI, USA)	16.33	90	6	4	Sweet cereal (corn, wheat, oats) with 'fruit' flavour
Oreos®	Nabisco (NJ, USA)	20.33	58	4	38	Chocolate sandwich biscuit with vanilla-flavoured cream
Banana Bread®	Coles (VIC, Australia)	13.5	59	6	35	Dense banana-flavoured loaf cake
Burger Rings®	Smith's (NSW, Australia)	21.93	45	5	50	Ring shaped corn/rice snack with savoury 'burger' flavour
Sausage roll®	Coles (VIC, Australia)	11.1	40	13	47	Processed meat wrapped in puff pastry
Mini jam roll® (Expt. 2.3)	Coles (VIC, Australia)	11.95	88	5	7	Thin sponge cake, rolled and filled with strawberry jam
Sweetened condensed milk (Expt. 5.1)	Nestlé (VA, USA)	14.4	67	11	22	Thick, sugar-sweetened milk with water removed

The foods used in training were broken up so as to be presented in approximately similar sizes, as shown below. For the foods that were not homogenous, rats occasionally ate only one component of the food (e.g. the cream but not the biscuit of the Oreo, or the meat but not the pastry component of the Sausage roll). This was particularly evident in the latter training sessions. There was no evidence that test data were any different for these animals.

Figure A1. Petri dishes prepared for a Plus training session. From left to right: Chow, Oreos, Burger Rings, Banana bread, Sausage roll (after a training session).

Froot Loops

Sausage roll

Mini Jam Roll

Appendix B: Subsets of the *Single* group

Introduction

This appendix explores whether CPF differed between subsets of the *Single* group trained with Oreos, Banana bread, Sausage roll, and Rings. Data were collapsed from Experiments 2.2 ($n = 11$), 2.3 ($n = 14$), 3.1 ($n = 8$), and 3.3 ($n = 24$), forming a total of 57 rats given *Single* training. Of these, 20 were trained with Oreos, 20 with Banana bread, 17 with Sausage roll and 5 with Rings. In Experiments 2.2 and 3.1, Rings were used as the savoury food; they were replaced with Sausage roll in other experiments when choice preference tests in Experiment 3.2 indicated they were relatively less preferred than Banana bread and Oreos.

Test data

CPF for these four subsets is shown in Figure B1, and was initially compared in a 4 x (2) (food x [context]) mixed-ANOVA. This analysis found no main effect of context, no main effect of food type, and no context x food interaction (all $F < 1$). Next, CPF was compared between the two subsets trained with sweet (Banana bread and Oreos) and savoury foods (Rings and Sausage roll) in a 2 x (2) (flavour x [context]) mixed-ANOVA. This analysis found no significant main or interaction effects (all $F < 1$). Therefore, there was no evidence that within the *Single* group, CPF varied according to which specific food was paired with the Plus context, nor whether this food was savoury or sweet.

Subsets of Single group
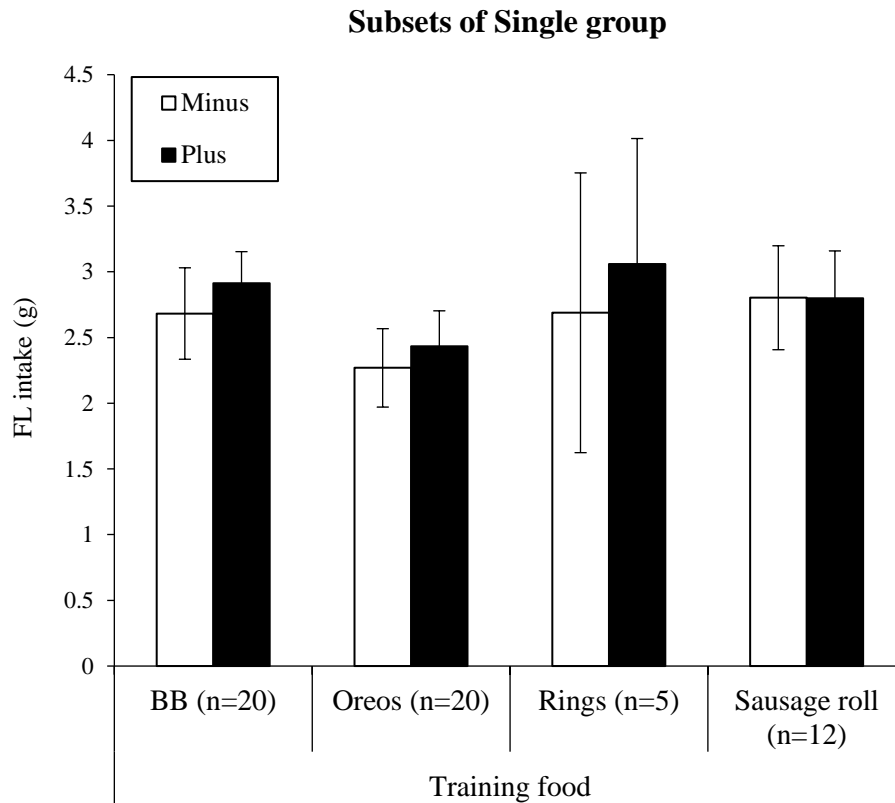
Figure B1. CPF by food subset. No differences were found according to what food was paired with the Plus context. *NB.* BB = Banana bread.

Training data

Average consumption during training (kJ) is shown in Figure B2, which also includes intake by the *Chow* rats in these experiments ($N = 29$). A one-way ANOVA comparing these five groups found significant differences between them ($F(4, 81) = 55.42$, $p < .001$). The results of pairwise comparisons using the Tukey HSD correction are overlaid on Figure B2; groups not sharing a common letter differed significantly (all $p \leq .013$). The important result from this analysis is that all subsets of the *Single* group ate significantly more than *Chow* rats, even though Rings were found to be less preferred to Banana bread and Oreos in Experiment 3.2. Results also indicated greater consumption by subsets trained with sweet foods.

Training intake

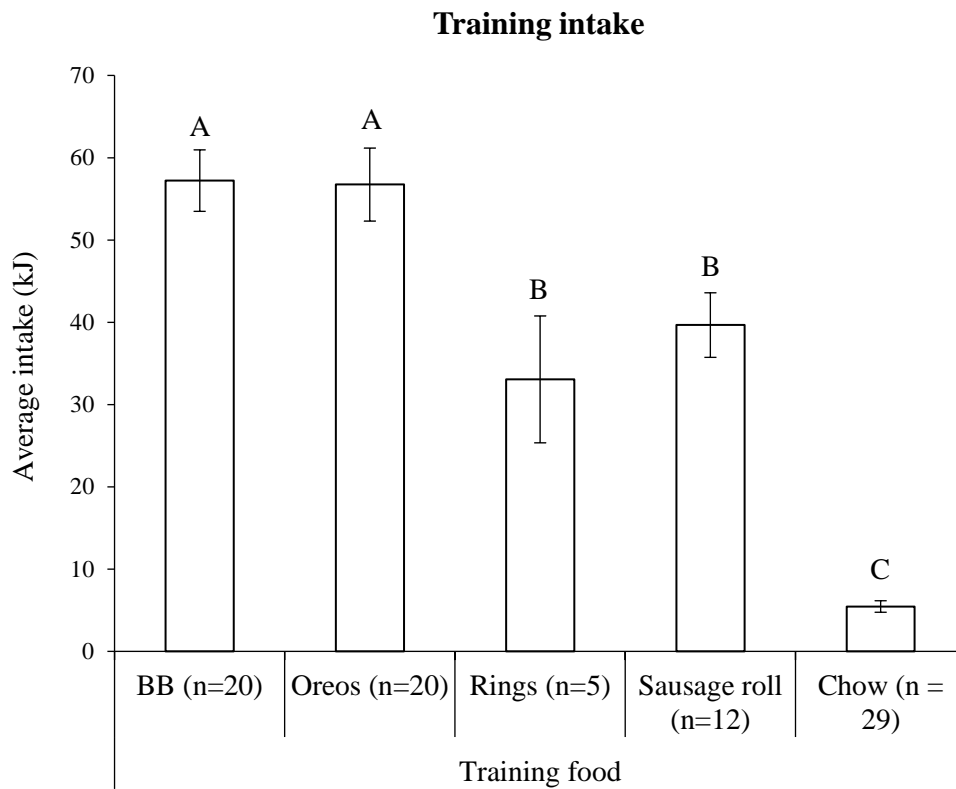*Figure B2.* Training intake by subset. All subsets of the *Single* group ate significantly more than *Chow* rats. Those trained with sweet foods ate more than those trained with savoury foods. *NB.* BB = Banana bread.

Introduction

Chapters 2 and 3 found that when a palatable alternative food, FL, was presented in CPF tests, consumption increased for rats previously fed chow and decreased for those fed palatable food (*Single* and *Variety* groups), relative to training consumption. This pattern of results was interpreted as examples of positive and negative contrast, respectively. Experiment 3.2 confirmed that *Single* and *Variety* groups preferred their training food to FL, and found that higher preferences for the training food associated with a weaker CPF effect when testing FL. This prompted us to explore whether negative contrast might explain the test order interactions observed in several experiments, since these effects reflected the tendency for FL consumption to increase over tests, regardless of context.

This appendix examines whether negative contrast influenced test order effects by affecting consumption in rats tested Plus→Minus more than those tested Minus→Plus. The rationale was that negative contrast might be stronger when the Plus context was tested first than second, since in the latter case FL had been exposed in the preceding Minus context test. Another point of interest was whether negative contrast between training and the first test day explained group differences. For example, if the *Variety* group exhibited less negative contrast than the *Single* group on the first test session, this might explain the CPF effect in the former group. To assess these possibilities, test data from Experiments 2.2, 2.3, and 3.3 were examined in closer detail. These were experiments in which overall CPF effects were found when testing FL.

Data presentation

Since the key questions were in terms of test order effects and the change from training, test data are presented in chronological order, rather than collapsed according to

Plus and Minus context. Additionally, average training consumption during the final three Plus context sessions is shown, in kJ, for comparison with CPF tests. The final three sessions were averaged to provide a more stable estimate of training consumption and to average over the three foods provided to the *Variety* group. An important note in this regard is that the pattern of results did not differ according to whether grams or kilojoules were used. Indeed, this would not be expected because the energy density of Froot Loops (16.33 kJ/g) was very similar to the average energy density of the three training foods used for *Single* and *Variety* groups (~15.31 kJ/g for average of Oreos, Banana bread, and Sausage Roll) and chow (14.23 kJ/g).

The figures below show consumption at the end of training and on the first and second CPF test days. Groups are plotted in separate panels for clarity, and data are presented separately for the test order cohorts (Plus-Minus or Minus-Plus). Therefore, data points for 'Test 1' show consumption in the Plus context for the Plus-Minus cohort and in the Minus context for the Minus-Plus context.

Key predictions

The main index of contrast was taken as the change from training to test day 1. A difference in the slope of the line between Plus-Minus and Minus-Plus cohorts would suggest that negative contrast from training to test was moderated by which context was tested first. Comparing Test 1 and Test 2 shows the change in consumption between the two CPF test days. A null effect of context would be reflected in parallel slopes between Test 1 and Test 2 for Plus-Minus and Minus-Plus cohorts, whereas a perfect 'X' shape would reflect no effect of order and a main effect of context (among several other more complicated possibilities).

Experiment 2.2

Figure C1 presents test consumption plotted in the manner described above. The *Chow* group exhibited substantial positive contrast by consuming more FL in Test 1 than their prior chow consumption in training. The degree of positive contrast appeared comparable between test order cohorts. Consumption did not increase further from the first to the second CPF test day. Both *Single* and *Variety* groups exhibited substantial negative contrast from training to test. Importantly, test order cohorts did not appear to differ in the extent of negative contrast in either group, since the slope of the lines was comparable from training to Test 1. The CPF effect in the *Variety* group is reflected in the differential change from Test 1 and Test 2 between test order cohorts. Whereas the Minus-Plus cohort substantially increased FL intake in their second (Plus) test, the Plus-Minus context suppressed intake in their second (Minus) test. In the *Single* group, the change in intake from Test 1 to Test 2 was similar for both test order cohorts; thus, context did not modulate consumption. (NB: The Minus-Plus cohort of the *Single* group appeared to eat more overall. This appeared a chance result of counterbalancing, since training consumption was somewhat higher in this group.)
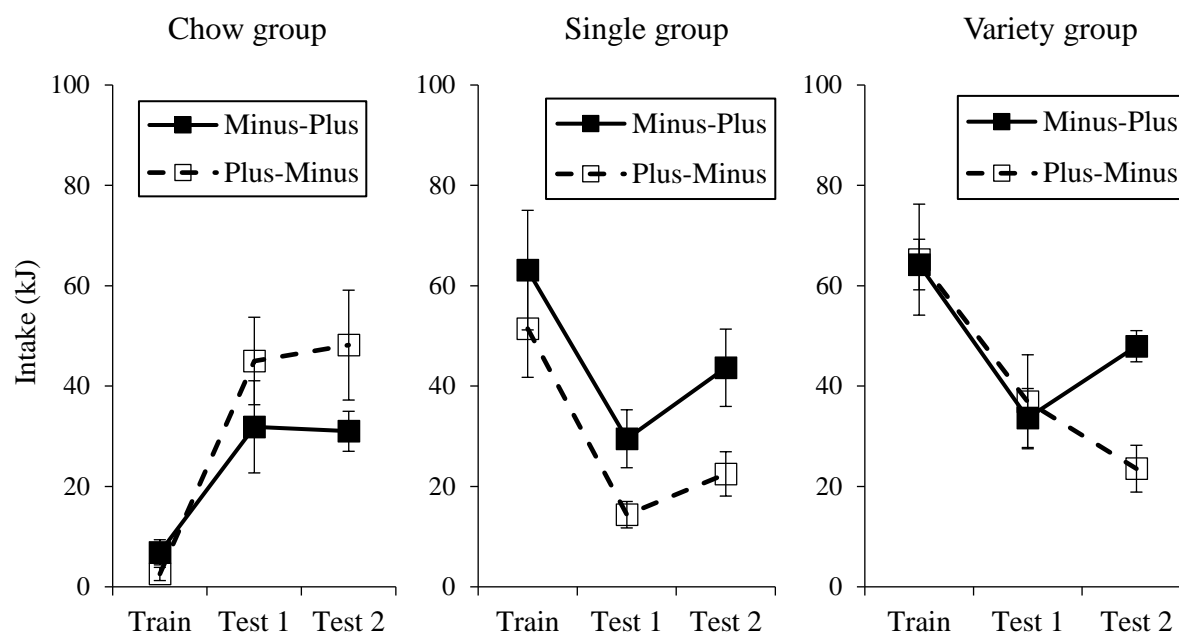
Figure C1. Test data from Experiment 2.2 re-plotted in chronological order and separated by test order cohort.

Experiment 2.3

Experiment 2.3 replicated the variety effect in Experiment 2.2 when chow was available in the Minus context during training. Consumption from the first set of CPF tests, when FL were presented, is shown in Figure C2, expressed chronologically and separated by test order cohort for the three groups. The pattern of change relative to training consumption for each group is comparable to those for Experiment 2.2. Thus, the overall change from training to Test 1 indicates positive contrast for the *Chow* group and negative contrast for the *Single* and *Variety* groups. Once again, there is little in this data to suggest the change from training to Test 1 differed between Plus-Minus and Minus-Plus cohorts for any of the three groups. As in Experiment 2.2, the *Chow* group showed little increase from Test 1 to Test 2, while both test order cohorts of the *Single* group displayed a comparable, substantial increase

in consumption from Test 1 to Test 2. The context effect in the *Variety* group, once again, is evident in the differential change from Test 1 to Test 2 for the two test order cohorts. Consumption was higher in the Plus context regardless of when this context was tested.

EXPERIMENT 2.3
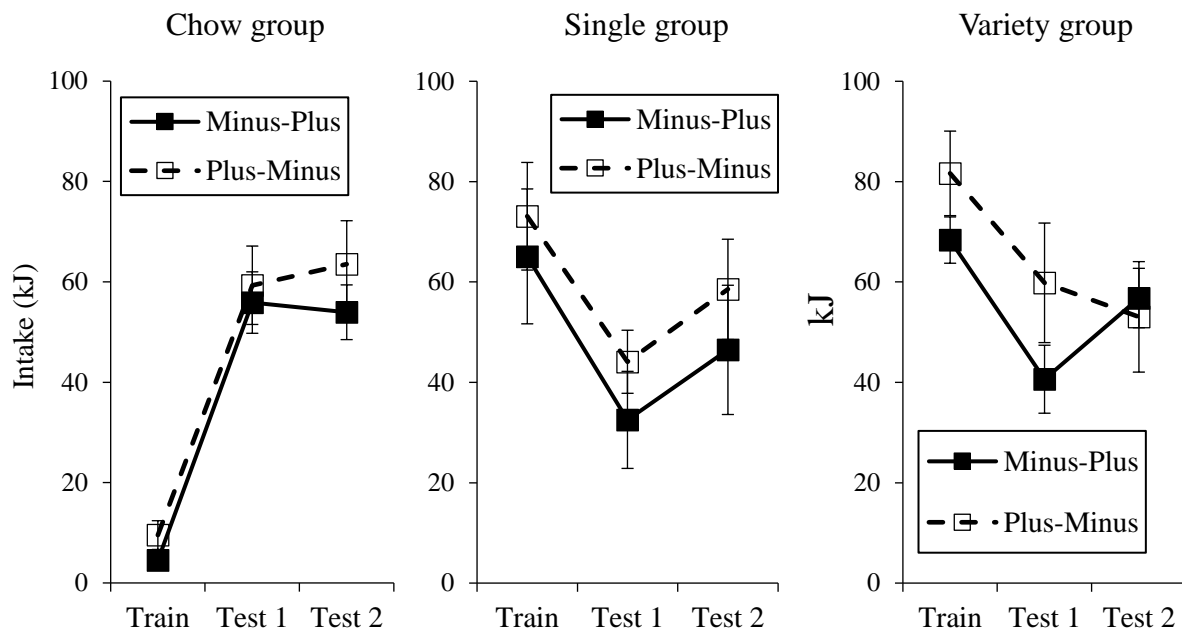
Figure C2. Test data from Experiment 2.3 presented in chronological order and according to test order cohort.

Experiment 3.3

Experiment 3.3 examined whether CPF was predicted by baseline consumption of FL and training palatable food intake. No *Chow* group was included in this experiment, which also employed a shorter training period than the above experiments (12 rather than 20 total sessions). Analyses indicated an overall effect of context that did *not* interact with group; however, tests of simple effects found that the CPF effect was only statistically significant in the *Variety* group. Figure C3 shows that the negative contrast effects observed in past

experiments were again present, with suppressed intake on Test 1 relative to training. Comparison of Minus-Plus and Plus-Minus cohorts did not suggested differences in the extent of contrast for either group. Notable aspects of these data were that in the *Single* group, the Test 1-Test 2 change appeared greater for the Minus-Plus cohort, explaining the marginally significant main effect of context that was found when data were collapsed ($p =$ .08). By comparison, the Plus-Minus cohort of the *Variety* group failed to suppress consumption in their second (Minus) test, potentially explaining the absence of a group x context interaction in this experiment.

EXPERIMENT 3.3
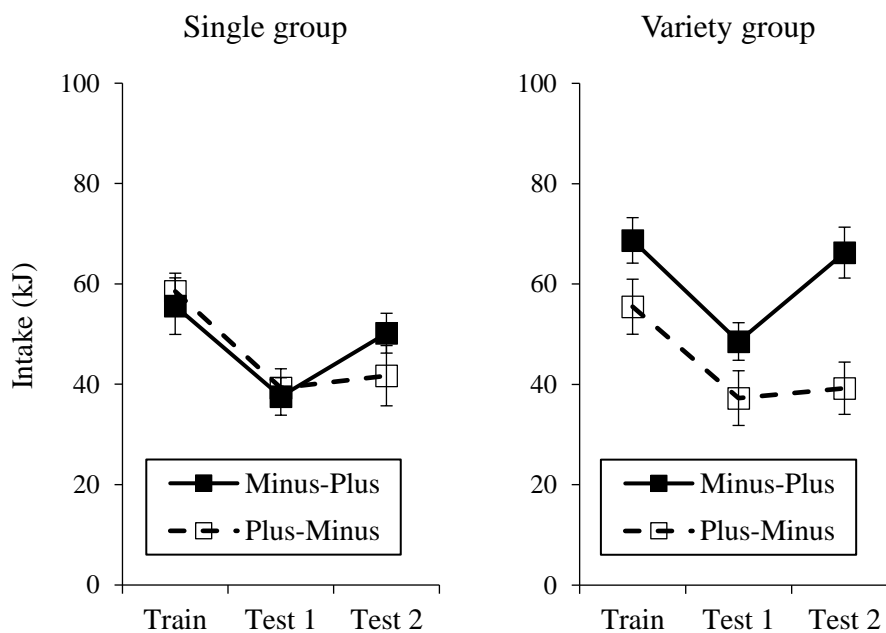
Figure C3. Test data from Experiment 3.3 for *Single* and *Variety* groups presented according to test order cohort.

Summary

This re-examination of test data found no evidence that the negative contrast produced by the presentation of an alternative food varied according to the order in which the Plus and Minus contexts were tested. Rather, all rats in *Single* and *Variety* groups suppressed intake of FL relative to their training consumption on the first CPF test session, regardless of where this test occurred. The change from the first to second CPF test session, however, differed markedly between groups. FL intake remained high in *Chow* rats regardless of test order – an unsurprising result given this marked their first opportunity to consume a highly palatable food in the contexts, in which little conditioning had occurred. FL intake reliably increased in *Single* groups in both test order cohorts, reflecting the failure of the contexts to influence consumption. Only the *Variety* group's consumption was sensitive to context more than to test order, a result demonstrated by the opposite or non-parallel slopes of the test order cohorts between Tests 1 and 2 in each of the above examples.

Appendix D: Test order effects in Experiment 4.1

The figures below show CPF test data from Experiment 4.1 separated according to test order cohort; that is, the subsets of each group tested Minus→Plus and Plus→Minus ($n = 5$/subset in each group of 10 rats).

Test 1

In Test 1 rats were satiated and tested with FL. Analyses found significant interactions between test order and context indicating increasing consumption over the two tests, irrespective of which context was tested first. The increase from the first to the second test was comparable for the *Unpaired* group relative to the three groups that received conditioning.
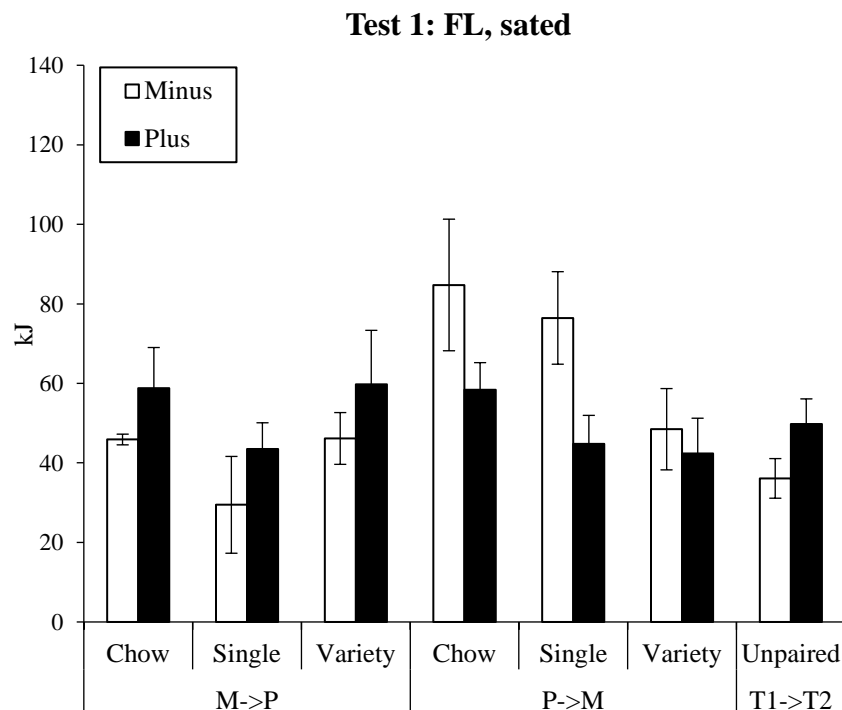
Figure D1. Test 1 of Experiment 4.1 by order cohorts.

Test 2

In Test 2 rats were food-deprived and presented with FL. Analyses found significant interactions between test order and context. Although this interaction once again reflected the tendency for consumption to increase over tests, the effect was more pronounced in the cohort tested P→M than in those tested M→P.

Test 2: FL, deprived
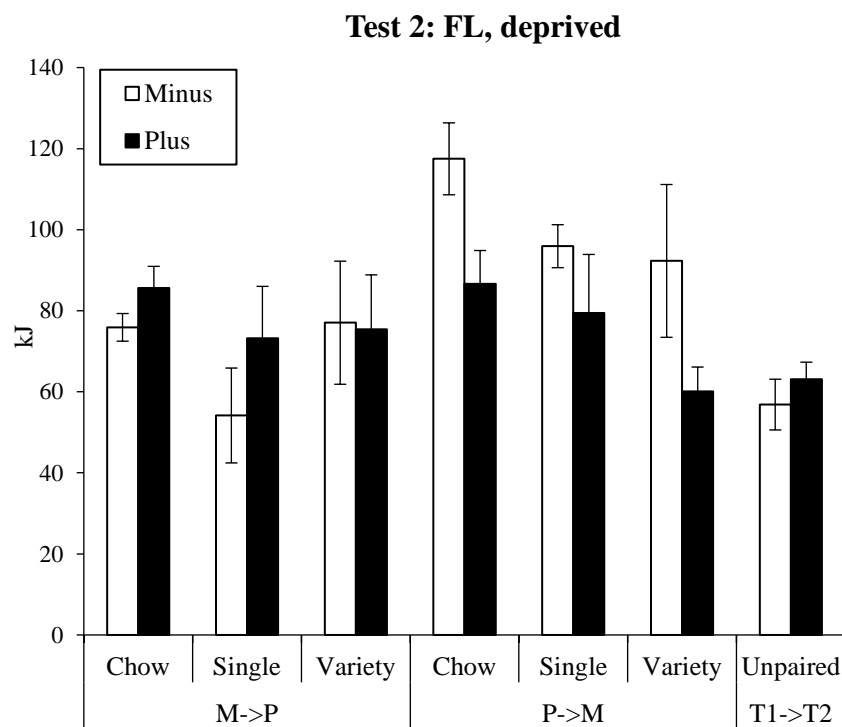
Figure D2. Test 2 of Experiment 4.1 by test order cohort.

Test 3

In Test 3 rats were food-deprived and presented with the training food. *Variety* and *Unpaired* rats received whatever palatable food they were fed on the re-training session held after Test 2. A significant test order x context interaction was found on this test; however, on this occasion the tendency was for consumption to *decrease* from the first to the second test day, regardless of which context was tested.

Test 3: Training food, deprived
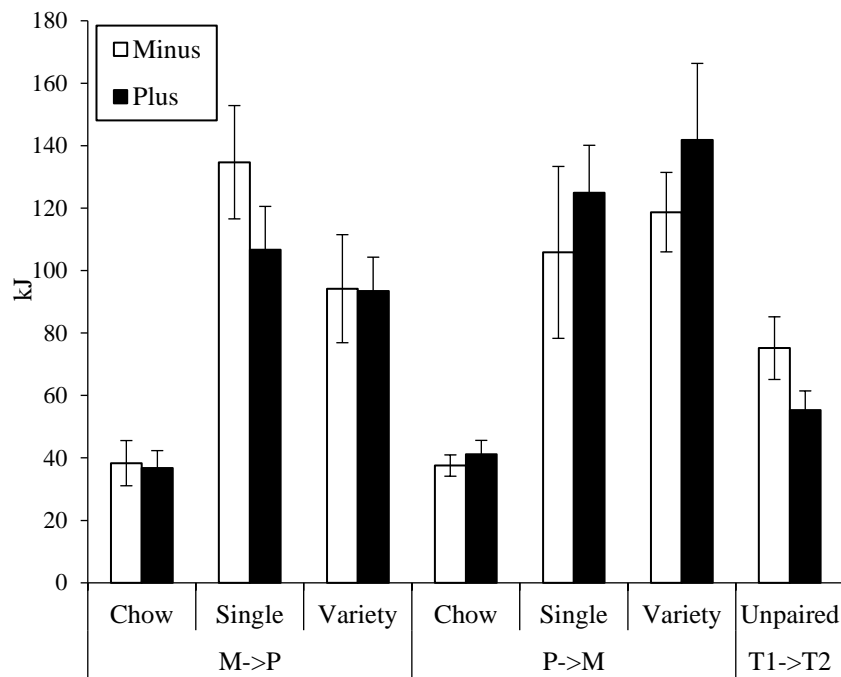
Figure D3. Test 3 of Experiment 4.1 by test order cohort.

Unpaired group

During training the *Unpaired* group were exposed to both contexts, neither of which were paired with food. Test data for this group, therefore, indexed the increase in consumption in consecutive tests in the absence of conditioning. Intake in the *Unpaired* group's first and second test halves is depicted in Figures D1-D3 by unfilled and filled bars which for other groups denote Minus and Plus context consumption, respectively. In each case, the change in consumption from the first to the second test appeared comparable for the *Unpaired* group across the three tests. To quantify this, a difference score for the change from the first to second test day (regardless of which context was tested) was calculated for each rat. The difference scores for the *Unpaired* group were then compared to the average of the

Single and *Variety* groups' difference scores, since these latter two groups were most equivalent in terms of palatable food exposure. Importantly, the increase from the first to the second test day did not differ significantly between *Unpaired* and [*Single*/*Variety*] groups on any of the three tests (Test 1 and Test 3: $t < 1$; Test 2: $t(28) = 1.58$, $p = .13$). There was, therefore, no indication that pairing of palatable food with the Plus context altered the tendency for consumption to change over repeated tests relative to rats for which the contexts were unpaired with palatable food.

Appendix E: Elevated Plus Maze testing in Experiment 4.2

Introduction

Experiment 4.2 assessed whether a measure of baseline anxiety would predict CPF in later tests. For this purpose, rats were tested in an Elevated Plus Maze prior to training. This test is a widely-used assay of general anxiety (Hogg, 1996) that measures rats' tendency to explore a raised X-shaped maze in which two arms are exposed and two are enclosed with walls. After an initial period of exploration, rats typically avoid the open arms of the maze; the amount of open arm time is taken as a measure of anxiety (more open arm exploration indicating *less* anxiety).

Apparatus

The X-shaped apparatus was elevated 1m above the ground. The four arms of the maze were 450mm long and 100mm wide and intersected at a central platform (100 x 100mm). The two 'closed' arms were enclosed with walls (400mm high) of red Perspex, and were perpendicular to the two 'open arms' with no walls. The apparatus was contained in a well-lit room separate to where other feeding procedures were held.

Procedure

Testing in the EPM began two days after FL familiarisation. Rats received a single 5-min test, with 8 rats tested on day 1 and 16 rats tested on day 2. Rats were placed in the centre of the maze facing an open arm; recording began as soon as the experimenter left the room. A video camera located above the apparatus recorded behaviour, which was later scored by a trained observer (MK) using OdLog® software (Macropod software). The dependent measure was time spent in the two open arms of the maze, expressed as a percentage of the total time exploring the closed and open arms. Time spent in the middle intersection of the closed and open arms was excluded.

Results

The average percent of open arm time was $28.76 \pm 2.6\%$ [SEM], with high variability (6 – 58%). Percent open arm time was correlated with baseline FL intake, consumption in the first training session, average training intake, and CPF expressed as a proportion (as described in Chapter 3). Pearson correlations were tested since the distributions of percent open arm time and baseline FL intake did not violate assumptions of normality (Shapiro-Wilk test: $W = .96$, $p = .52$ and $W = .95$, $p = .27$, respectively). Higher open arm time in the EPM was weakly positively correlated with baseline FL intake ($r(24) = .36$, $p = .09$), suggesting that more anxious rats tended to eat less FL in baseline tests. However, open arm time did not correlate significantly with training consumption on the first Plus session ($r(24) = .30$, $p = .15$), average training intake ($r(24) = .25$, $p = .24$), or CPF as a proportion ($r(24) = -.32$, $p = .13$). Therefore, there was no evidence that baseline anxiety was reliably associated with training consumption or CPF test results.

Appendix F: CPF using discrete cues

Introduction

Experiments in this appendix tested the specificity of CPF when discrete cues were trained as food-paired stimuli. The training procedure was similar to the Holland-Petrovich method described in Chapter 1: in daily conditioning sessions, hungry rats were given intermixed exposures to one auditory cue that was paired with food reward (CS+) and another that was never reinforced (CS-). One important difference was that rather than deliver reward at the offset of a 10-s CS presentation (e.g. Petrovich, 2013), we used 2-min CS presentations and delivered rewards at variable periods during the CS+, similar to previous reports (Holland & Gallagher, 2003; Galarce et al., 2007). In each experiment, a period of re-feeding in the home cage separated the end of training from CPF tests. The primary measure of CPF in these experiments was consumption in the presence of the CS+ relative to the CS-. However, the inclusion of pre-feeding manipulations allowed for consumption in this period to be correlated with cue intake, as described in Chapter 5.

Experiment F1: No effect on Chow but an effect on FL.

To assess the specificity of CPF after training with discrete cues, Experiment F1 tested whether a cue paired with reward pellets would enhance consumption of two alternative foods that varied in palatability: regular chow, and Froot Loops (FL). Past studies have consistently shown that consumption of chow is unaffected by cues paired with more palatable foods (Petrovich et al., 2007a, 2007b, 2012; Reppucci & Petrovich, 2012; see also Experiment 3.1 in the present thesis), with one exception over a longer test (4-24h; Boggiano et al., 2009). In most studies, however, chow has been tested after verifying a CPF effect on the paired food (e.g. Petrovich et al., 2007a, 2007b) or when the paired food is also available

(e.g. Reppucci & Petrovich, 2012). Experiment F1 prioritised a test of chow intake immediately after training and then assessed whether CPF would transfer to a more palatable alternative food, Froot Loops (FL), as described previously.

Method

Subjects

Subjects were 16 adult female Albino Wistar rats previously used in a flavour conditioning experiment that involved consumption of flavoured maltodextrin solutions under mild food deprivation. Rats were given free access to food and water for two weeks prior to the start of the present experiment, when average body weight was 362g (range 308 – 413g). Rats were group-housed ($n = 8$/cage) in large plastic tubs with wire lids, kept in a temperature- and humidity-controlled colony room maintained on a normal light:dark cycle (lights on 0700-1800h). Chow was freely available unless described below. Water was available in the home-cage at all times.

Apparatus

Training and testing were conducted in 8 identical operant chambers (Med Associates; East Fairfield, VT) contained within sound- and light-attenuating shells. These were the chambers used in the context experiments reported in previous chapters, without the decorative floor and wall inserts. The floor consisted of steel bars and a bedding tray containing wood shavings was inserted underneath. The top and side walls of the chambers were made of Plexiglas. A recessed magazine was centred on one wall of the chamber between two retractable levers (which were not used). A 3 W, 24 V houselight mounted on the top-centre of the wall opposite the magazine provided illumination and remained on throughout all experimental procedures. A computer equipped with MED-PC software

controlled the equipment and recorded magazine entries. Two auditory cues (clicker and white noise) were allocated as the CS+ and CS- in a counterbalanced fashion. The food used as the reward during training was 45mg pellets (Dustless Precision Pellets, grain-based formula; Bioserv, USA).

Procedure

Training

A restricted feeding schedule was introduced one week prior to training (12g per rat, per day). During this week rats were weighed and handled regularly. Daily training sessions were held between 0900-1200h and consisted of two consecutive 8-rat squads. The clicker was the CS+ for half of the rats in each squad and the noise was the CS+ for the other half. Training began with one magazine training session, in which 30 pellets were delivered to the magazine on a random-time schedule in a 30-min session. All rats ate at least 25 pellets. Training in proper began the following day and lasted for 10 sessions. Each session was 48 min in length and involved four 2-min presentations of the CS+ and four 2-min presentations of the CS-. The order of stimuli presentation was intermixed randomly such that there could be no more than two consecutive trials of the same type. Each trial began with a 2-min pre-CS period followed by the onset of the CS+ or CS-. During CS+ presentations pellets were delivered to the magazine on a variable-time (VT) 30s schedule. In practice, the median number of pellets dispensed per session was 14 (or 3.5 per CS+). No pellets were delivered on CS- trials. At CS offset, a variable 1-3 min inter-stimulus interval began. The duration of magazine entry time was recorded during CS+, CS- and pre-CS periods. On Days 9 and 10, a 2-min non-reinforced probe trial for the CS+ was inserted to gauge conditioned responding. Rats were fed 30-40 min after returning to the colony room following daily training sessions.

After the 10th training session rats were allowed unrestricted access to chow in the home cage for six days.

Test 1: Chow

Test 1 measured consumption of chow in four 10-min tests held on consecutive days. The CS+ and CS- cues were tested on Days 1 and 2 in counterbalanced order. Rats were placed into the test context with a Petri dish containing 20g chow placed against the side-wall of the chamber, adjacent to the magazine. Test foods were presented in a separate location to the magazine to reduce the contribution of conditioned approach to CPF (but see Holland et al., 2002). For CS tests, the cue played continuously after a 2-min pre-CS period for the remaining 8 min of the test. Consumption was measured to the nearest .01g. Magazine entry time was recorded in 30-s bins. Days 3 and 4 compared chow intake in the context when no stimuli were presented with consumption in a different context (the pre-feeding chambers described in Chapters 2, 3, and 5), to which rats had been familiarised during training on two occasions for 30 min. The order of Tests 3 and 4 was counterbalanced.

Test 2: Froot Loops

After Test 1 rats were returned to food restriction for three days. On the first day, 30 Froot Loops (FL) were scattered in the bedding of each home cage to familiarise rats to their taste: all rats were observed to sample them within a 10-min period. After two re-training sessions, free access to food was restored for two days before Test 2. Test 2 was identical to Test 1, except that consumption of FL and not chow was measured.

Test 3: Pre-feeding

Following Test 2 rats were returned to the food deprivation schedule and, after a 10-day hiatus, given five further training sessions. After the fifth session, free access to chow was returned for two days before Test 3. This test compared consumption of FL during the

presence of the CS+ and CS- on consecutive days, with test order counterbalanced. Rather than test the context on a separate day as during Days 3 and 4 of Test 2, we adopted a pre-feeding procedure in which rats were first placed in the context for 8-min with FL available and with no stimuli presented. Rats were then briefly removed and, after collecting FL and inserting another dish filled with FL, returned to the context for an 8-min CS test where the cue played continuously.

Results

Body weight

Food restriction reduced rats to $93.7 \pm 0.5\%$ [SEM] of free-feeding weights by the first day of training. On average, rats weighed $91.7 \pm 1.0\%$ of their free-feeding weights during training. Re-feeding in the home cage increased body weight in all animals such that rats weighed $100.5 \pm 1.4\%$ of their free-feeding weights at Test 1, and $100.8 \pm 1.5\%$ of free-feeding weights at Test 2. By Test 3, body weights had increased slightly above starting weights ($109.8 \pm 1.7\%$).

Training

Magazine entry time (s/min) across training is shown in Figure F1.1 and indicated successful conditioning to the CS+. Planned contrasts compared responding to the CS+ probe on days 9 and 10 with pre-CS and CS- periods, averaged across days 9 and 10. The CS+ probe significantly elevated responding relative to the CS- ($F(1, 14) = 23.01, p < .001$) and the pre-CS period ($F(1, 14) = 50.60, p < .001$). Responding to the probe was significantly lower than during reinforced CS+ trials, however ($F(1, 14) = 12.70, p = .003$). None of these differences interacted with stimulus counterbalancing (largest $F(1, 14) = 1.60, p = .23$). This pattern was sustained over the two periods of re-training. The CS+ probe test on the final day

of re-training significantly elevated responding relative to the CS- ($F(1, 14) = 30.27$, $p < .001$) and the pre-CS period ($F(1, 14) = 10.29$, $p = .006$) and did not differ significantly from responding during reinforced CS+ presentations ($F(1, 14) = 1.20$, $p = .29$). None of these contrasts interacted significantly with stimulus (largest $F(1, 14) = 1.91$, $p = .19$).

Experiment F1: Training
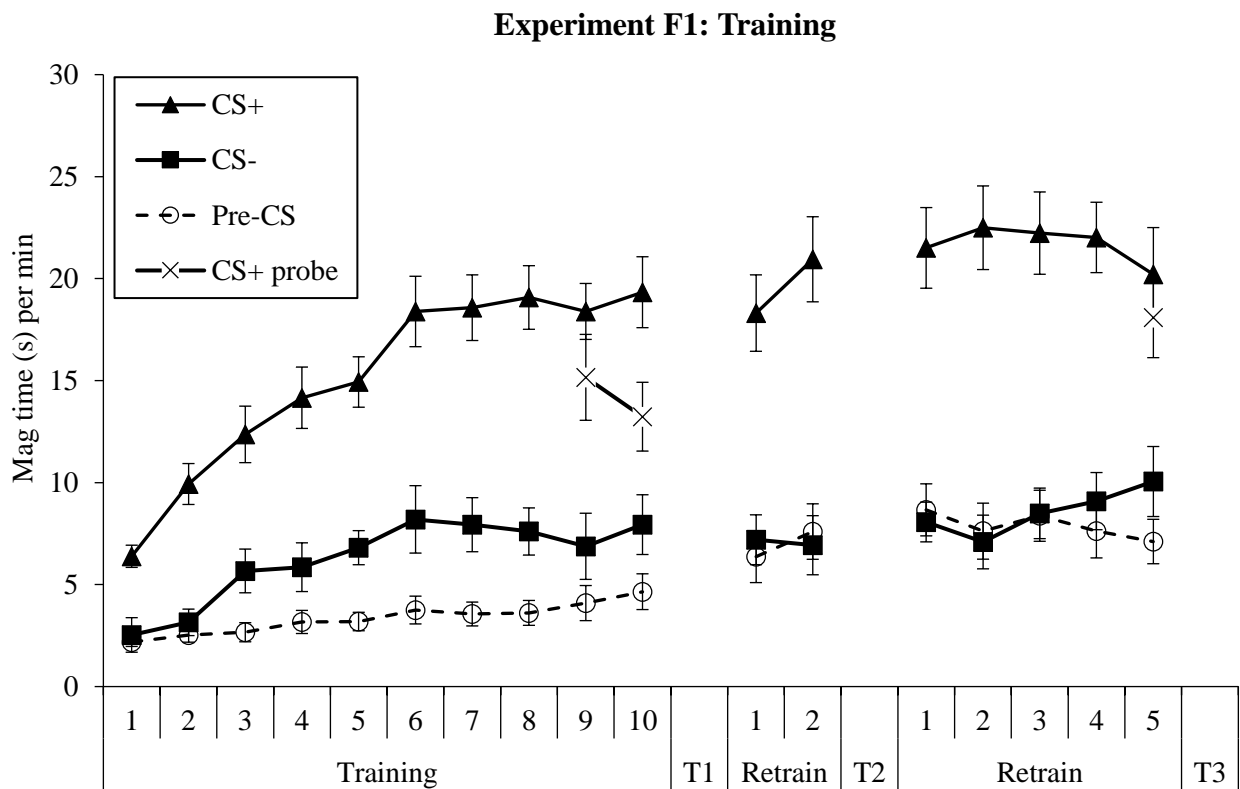
Figure F1.1. Magazine responding during training. Responding to the CS+ increased more rapidly than to the CS- and remained high throughout retraining. Responding to probe CS+ trials was robust and significantly above pre-CS and CS- presentations (see text for details).

Test 1: Chow

Consumption during the four tests (CS+, CS-, context alone, different context) is shown in Figure F1.2 and was analysed with a set of three orthogonal contrasts. Contrast 1 compared consumption between the CS+ and CS- tests, while Contrast 2 compared the two

context tests (Training vs. Different context). Contrast 3 compared the two cue tests to the two context tests, i.e. [CS+ and CS-] versus [Training context and Different context]. The respective coefficients were, therefore: [1 -1 0 0], [0 0 1 -1], [1 1 -1 -1]. None of these contrasts were significant (Contrast 1: $F < 1$; Contrast 2: $F < 1$; Contrast 3: $F(1, 12) = 1.59$, $p = .23$). However, the difference between CS+ and CS- tests (Contrast 2) interacted with stimulus type ($F(1, 12) = 8,61$ $p = .013$) and test order ($F(1, 12) = 6.01$, $p = .031$). This was driven by the tendency for the clicker to promote greater consumption than noise, regardless of whether it served as CS+ or CS-. The cue x test order interaction reflected the tendency for greater intake on the *first* test, regardless of which cue was tested. Finally, although the onset of the CS+ produced a temporary increase in magazine entry relative to other tests, this washed out over the duration of the test such that total magazine entry time did not differ between the CS+, CS-, and context tests ($F < 1$).

Test 2: Froot Loops

Consumption during Test 2 is shown in Figure F1.2 and was analysed in the same fashion as Test 1. The only significant result was an interaction between the CS+ and CS- tests (Contrast 2) and test order ($F(1, 12) = 21.00$, $p = .001$). This result reflected higher intake on the second FL test, regardless of which cue was tested. Consequently, consumption was significantly greater in the CS+ test if this cue was tested second ($F(1, 7) = 5.91$, $p = .045$) and significantly greater in the CS- test if this cue was tested second ($F(1, 7) = 18.56$, $p = .004$). This pattern of order effects is consistent with the context experiments in the main body of this thesis. No other results were statistically significant (largest $F(1, 14) = 2.71$, $p = .13$). Magazine entry time did not differ significantly between the three tests held in the conditioning context ($F < 1$).

Experiment F1: Tests 1 and 2
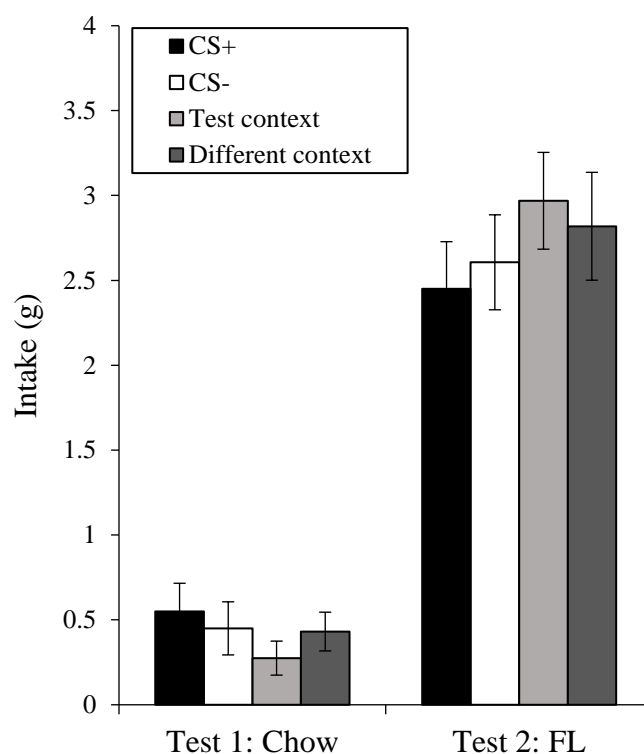
Figure F1.2. Test 1 and Test 2 results. No significant differences were found in either test.

Test 3: Pre-feeding

Consumption during pre-feeding and the cues is displayed in Figure F1.3. Rats ate 3.09 and 2.78 g of FL prior to the CS+ and CS- tests, respectively; this difference was not statistically significant ($F(1, 15) = 1.70$, $p = .21$). Consumption in cue tests was analysed in a 2 x 2 x (2) mixed-ANOVA (stimulus x test order x [cue]). This analysis found a significant main effect of cue ($F(1, 12) = 5.27$, $p = .04$) indicating higher consumption in the CS+ than CS- test. This result did not interact with stimulus ($F(1, 12) = 2.65$, $p = .13$), test order ($F < 1$) or their interaction ($F < 1$). There was a main effect of test order ($F(1, 12) = 10.48$, $p = .007$), reflecting lower intake in the cohort for which the CS+ was tested first. Total magazine entry time did not differ between the CS+ and CS- tests ($F(1, 12) = 1.91$, $p = .19$). The correlation

between pre-feeding and cue intake was not significant on either test (CS- test: $r(14) = -.212$, $p = .43$; CS+ test: $r(14) = -.024$, $p = .93$).

Experiment F1: Test 3
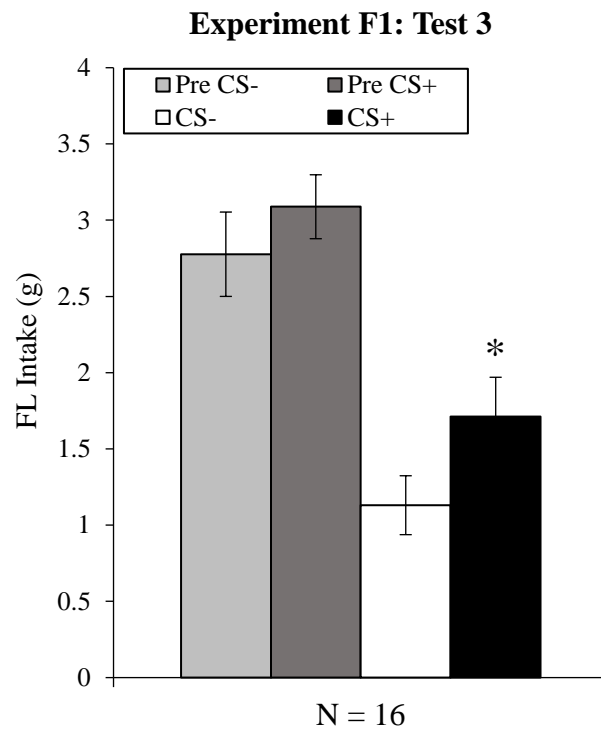
Figure F1.3. Test 3 results. Consumption of FL after pre-feeding in the context was significantly greater in the CS+ than CS- test (*$p = .04$, cue main effect).

Discussion

Experiment F1 tested whether a discrete cue paired with food reward for hungry rats would promote consumption of foods never paired with the CS+, chow and FL, when rats were satiated. Probe trials on the final two training sessions confirmed the success of conditioning, with significantly higher responding during the CS+ relative to CS- and pre-CS periods. Results of Test 1 showed clearly that chow consumption was not increased by the CS+ relative to the CS-, nor in the training context relative to a different context. The absence

of CPF and the generally low intakes are consistent with past studies that have tested CPF on chow (Petrovich et al., 2007a, 2007b, 2012; Reppucci & Petrovich, 2012).

Results of Test 2 indicated that presenting a more palatable alternative food increased consumption relative to chow. However, intake was comparable across the four test conditions and unaffected by cue. The interaction between test order and intake on the two cue tests was consistent with that reported in previous chapters and reflected the tendency for consumption to increase over multiple tests. One difference in procedure was that in the present experiment FL were pre-exposed a week prior to when they were tested in the presence of the cues. By contrast, in the context experiments in Chapters 2 and 3, FL were pre-exposed prior to training (i.e., 2-3 weeks prior to CPF tests). Therefore, whether FL are pre-exposed prior to or after training (or rather, distally or proximally to when they are tested) does not appear to explain test order effects.

The results of Tests 1 and 2 might suggest that the CS+ was without effect on consumption of these foods. An alternative possibility was that the test conditions were not sensitive to detect effects within a relatively short time frame. Test 3 adopted a pre-feeding procedure in which the cues were presented after the test food was pre-exposed in the context, in keeping with the typical Holland-Petrovich approach (Petrovich, 2013). Pre-feeding in the contexts removed the need for a separate test of consumption in the context alone, as in Tests 1 and 2. After ample consumption during pre-feeding, the CS+ promoted significantly greater intake than the CS-. Overall, intake in the presence of the cues was lower than in Test 2, suggesting that pre-feeding increased the sensitivity of the test by reducing high consumption driven by the palatability of FL.

In summary, results of Test 3 indicated that a cue paired with pellets increased consumption of a food with which it was never paired. This is a novel result that suggests that

CPF may not be specific to the paired food if an alternative food is presented that is familiar and palatable, but which has not previously been predicted by other cues as in previous studies (e.g. Delamater & Holland, 2008). A caveat for this result is that Test 3 was held after two other tests, which each involved periods of cycling food restriction and re-feeding in addition to re-training. Although training data indicated that responding to the CS+ and CS- cues was unaffected by these shifts, it is nonetheless possible that the CPF effect in Test 3 related to rats' prior exposure to the long-duration CSs used to test CPF. Replicating the result of Test 3 was, therefore, the primary aim of Experiment F2.

Experiment F2: Failure to replicate

Introduction

Experiment F2 prioritised the pre-feeding procedure used to test FL in Test 3 of Experiment F1. Chow was not tested because of the clear floor effect found in Test 1 of Experiment F1. Other minor procedural changes are detailed below.

Method

Subjects

Twenty-four adult, experimentally naïve female albino Wistar rats were used (Animal Resource Centre, Perth, Australia). They were group-housed (4 per cage) in temperature- and humidity-controlled ventilated cages with free access to laboratory chow and water. After a week of daily handling and acclimation to the laboratory, a restricted feeding schedule was introduced such that rats were fed a daily ration of 52 g per cage (13g/rat). Prior to food deprivation mean body weight was 239g (range: 217 – 266g).

Apparatus

The apparatus, stimuli, training and test foods were as described in Experiment F1.

Procedure

Training

There were two minor changes to the training protocol used in Experiment F1. First, the order of stimulus presentations in daily training sessions was now synchronised, since in Experiment F1 these were generated randomly for each box, and it was possible that the clicker was, on occasion, heard on noise trials and *vice versa*, reducing the efficacy of conditioning. The order of stimulus presentations was varied each day of training. Second, rats were trained for 16 sessions before the first CPF test. On sessions 8 and 16, a single 2-min CS+ probe trial was inserted to gauge conditioned responding; thus, no rewards were delivered during this trial. FL were pre-exposed in the home-cage on the first day of re-feeding after session 16. Two five-session blocks of re-training were held in between CPF tests, with a single probe trial included on the final day of each block.

CPF Tests

Three CPF tests with FL as the test food were held after 16, 21 and 26 training sessions. Rats were given unrestricted access to chow in the home-cage for at least 48 hours prior to each set of tests, and were returned to food deprivation for each re-training block. All tests applied the pre-feeding method from Test 3 of Experiment F1 and began by pre-feeding FL in the context immediately prior to a test where the CS+ or CS- cue played continuously. After pre-feeding rats were briefly removed from the chambers, FL were removed for later weighing, and a new dish of FL was placed in the chamber for the 8-min cue test. Pre-feeding was 8 min in Test 1 and 10 min in Tests 2 and 3. Whether the CS+ or CS- was tested first was counterbalanced. The two cue tests were spaced by a single day of rest in the home cage.

Results

Body weight

Food restriction reduced rats to $96.1 \pm 0.3\%$ of free-feeding weights on the first day of training, and $97.6 \pm 0.4\%$ of free-feeding weights when averaged over initial training (sessions 1-16). After re-feeding in the home cage, body weight increased to $104.6 \pm 0.6\%$ of free-feeding weights at Test 1. Despite subsequent periods of food restriction for re-training, body weight continued to increase at Test 2 ($117.0 \pm 0.6\%$) and Test 3 ($123.3 \pm 0.7\%$).

Training

Magazine entry time (s/min) is shown in Figure F2.1. As in Experiment F1, planned contrasts compared responding to the CS+ probe with pre-CS, CS-, and reinforced CS+ presentations on days 8 and 16. On Day 8, responding to the CS+ probe was significantly elevated relative to pre-CS periods ($F(1, 22) = 40.33$, $p < .001$) and CS- presentations ($F(1, 22) = 24.01$, $p < .001$) but significantly less than reinforced CS+ trials ($F(1, 22) = 65.77$, $p < .001$). The difference between pre-CS and probe responding was significantly greater in the cohort for which the noise was CS+ ($F(1, 22) = 5.04$, $p = .035$) but was statistically significant within both counterbalancing cohorts ($F(1, 11) = 17.97$, $p = .001$ and $F(1, 11) = 7.36$, $p = .02$ for noise and clicker cohorts, respectively). On Day 16, responding to the CS+ probe was significantly greater than during the pre-CS and CS- periods ($F(1, 22) = 21.45$, $p < .001$ and $F(1, 22) = 15.93$, $p = .001$) but significantly less than responding to reinforced CS+ presentations ($F(1, 22) = 8.43$, $p = .008$). None of these effects interacted with stimulus (largest $F = 1.05$). This pattern of responding was maintained throughout the two re-training blocks. On re-training day 5 and day 10, responding to the CS+ probe was significantly greater than pre-CS and CS- responding (day 5: $F(1, 22) = 34.08$, $p < .001$ and $F(1, 22) = 30.56$, $p < .001$ vs. pre-CS and CS-; day 10: $F(1, 22) = 30.44$, $p < .001$ and $F(1, 22) = 26.08$,

p < .001). These effects were significantly stronger for the cohort with noise as the CS+ (*F*(1, 22) = 5.48, *p* = .029 on day 5 and *F*(1, 22) = 5.45, *p* = .03); however, further analyses confirmed that clicker cohort still exhibited higher responding during the CS+ probe than pre-CS and CS- periods on both days 5 and 10 (smallest *F*(1, 11) = 7.01, *p* = .023). In sum, these data indicated rats successfully learned to discriminate between the CS+ and CS- and that this was consistent over the two blocks of re-training.
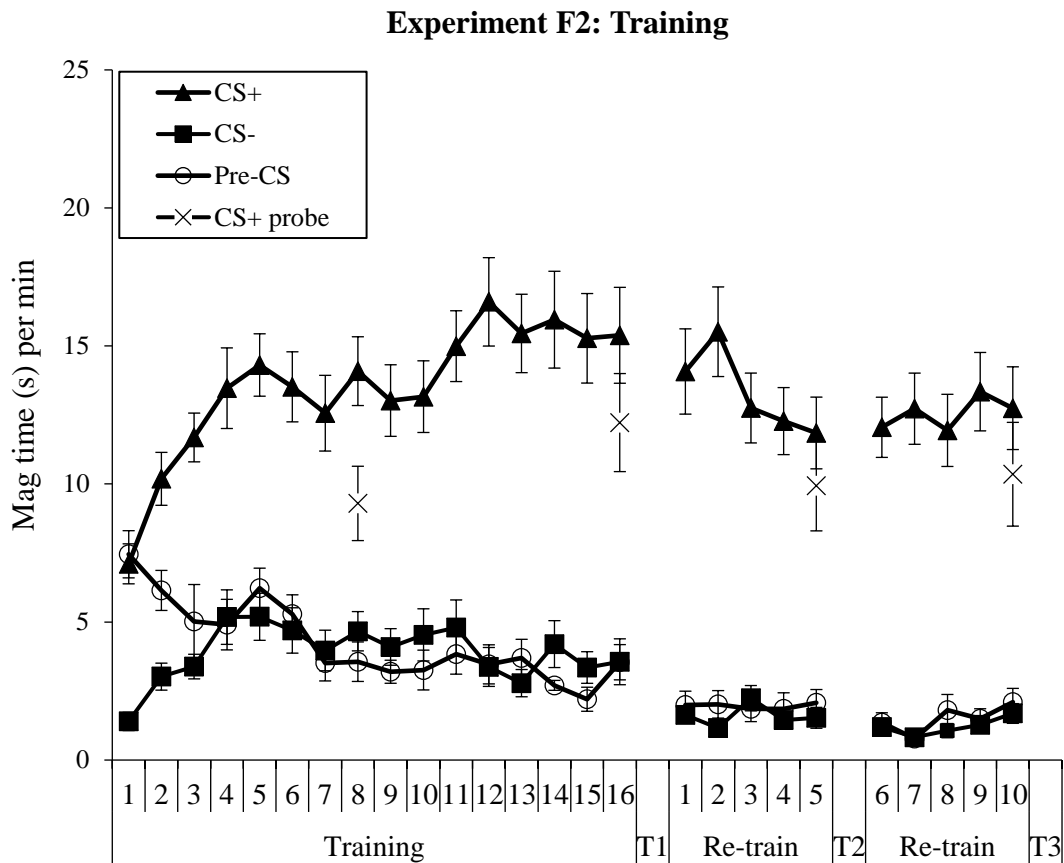
Experiment F2: Training

Figure F2.1. Magazine responding during training. Rats quickly learned to discriminate between the CS+ and CS- cues, with sustained high responding throughout training. Probe trials confirmed high levels of conditioned responding to the CS+ prior to each CPF test.

CPF Tests

Results from the three CPF Tests are shown together in Figure F2.2. Preliminary analyses confirmed that consumption in pre-feeding did not differ prior to CS- and CS+ tests on any test (largest $F(1, 23) = 1.18$, $p = .29$). Consumption in the presence of the cues was analysed in 2 x 2 x (2) (stimulus x test order x [cue]) mixed-ANOVAs. As detailed below, none of these tests produced a main effect of cue that would indicate CPF, though effects of stimulus counterbalancing and test order were suggested.

In Test 1 the main effect of cue was not significant ($F < 1$) but there were significant interactions between cue and stimulus ($F(1, 20) = 7.75$, $p = .011$) and cue and test order ($F(1, 20) = 5.24$, $p = .033$). No other effects were statistically significant (largest $F(1, 20) = 2.84$, $p = .108$). The cue x stimulus interaction reflected the tendency for both clicker-as-CS+ and noise-as-CS+ cohorts to eat more when the noise cue was presented. The effect of cue was not statistically significant within either cohort, however (clicker-as-CS+: $F(1, 10) = 3.86$, $p = .078$; noise-as-CS+: $F(1, 10) = 3.92$, $p = .076$). The cue x test order interaction was driven by greater consumption on the second test day, regardless of which cue was tested, consistent with Experiment F1 and the context studies in Chapters 2 and 3. Due to a computer error, magazine entry data were lost from the second day of Test 1. Therefore, total magazine entry time from the first test day was compared between the cohorts tested in the Plus and Minus context. No significant difference was found ($F < 1$).

In Test 2 the only significant result was an interaction between cue and test order ($F(1, 20) = 21.54$, $p < .001$) with no main effect of cue ($F < 1$) and no other significant main or interaction effects (largest $F(1, 20) = 2.19$, $p = .155$). Surprisingly, the test order x cue interaction was drive by *lower* consumption on the second test day, regardless of which cue was tested. The cohort given the CS+ test first tended to eat more in the presence of the CS+

than CS- ($F(1, 10) = 4.88$, $p = .052$) whereas no effect was found in the cohort given the CS-test first ($F < 1$). Analysis of magazine entry time found no significant main or interaction effects between cue type, test order, or stimulus (largest $F(1, 20) = 2.19$, $p = .15$). Finally, in Test 3 the main effect of cue was not significant ($F(1, 20) = 1.54$, $p = .23$) and no other main or interaction effects were significant (all $F < 1$).

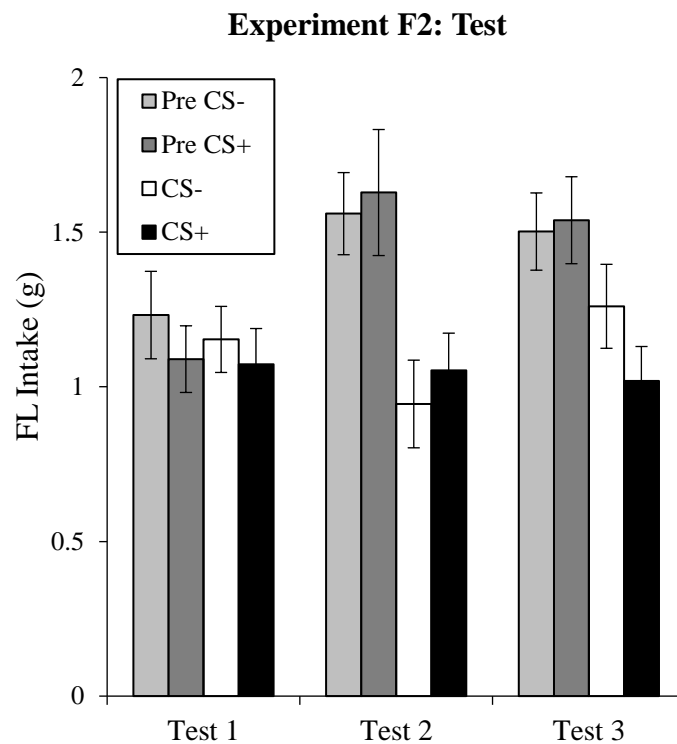**Experiment F2: Test**

Figure F2.2. Compiled test results. No main effect of cue was found on any of the three tests.

Correlations

The only significant correlation between pre-feeding and cue consumption was found when the CS+ was presented in Test 1 ($r(22) = .41$, $p = .04$). This indicated that rats with higher pre-feeding consumption tended to eat more subsequently when their CS+ was played.

Discussion

Experiment F2 failed to replicate the CPF effect found in Test 3 of Experiment F1, despite prioritising the pre-feeding method used in this test. Training indicated that rats quickly learned to discriminate between the CS+ and CS- cues. As in Experiment F1, rats were tested in a state of relative satiety after several days of access to free chow in the home-cage. None of the three CPF tests found evidence of enhanced feeding in the presence of the CS+ when testing FL. This was surprising given the results of Experiment F1. The discrepant results do not appear to be due to training, with 17 training sessions held prior to Test 3 in Experiment F1 and 16 held prior to the first test in Experiment F2.

Test 2 slightly increased the duration of pre-feeding to encourage greater consumption; while this was successful, no CPF effect was evident. Test 3 addressed the possibility that repeated experience with the test conditions – e.g. longer-duration CSs and exposure to a Petri dish of FL in the conditioning chamber – was necessary to allow the CS+ to enhance consumption, given that the CPF effect in Experiment F1 was found on the third test. This possibility was not substantiated. Nor does the failure to replicate appear to relate to different levels of deprivation at the time of tests, because in both experiments home-cage re-feeding returned rats above their *ad-libitum* body weights. In addition, the amount of training in the present experiment is comparable to previous experiments, which typically administer ~10 sessions (e.g. Petrovich et al., 2012).

Although inconsistent with Experiment F1, the finding that the CS+ cue had no effect on FL intake is consistent with past results showing that CPF is US-specific (Petrovich et al., 2007a, 2007b; Delamater & Holland, 2008; Galarce et al., 2007). Because the CS+ cue signalled only one food reward (pellets), the results are also consistent with the *Single* groups in the context experiments in Chapters 2-3, which failed to show CPF on alternative foods.

Importantly, those experiments found that CPF transferred to an alternative food in the *Variety* groups, for which the Plus context was paired with multiple foods. This raised the question of whether discrete cues paired with multiple rewards would enhance consumption of alternative foods.

Experiment F3: Variety effects using discrete cues

Experiment F3 aimed to replicate the 'variety effect' reported in Chapters 2 and 3 using discrete stimuli as the food-paired cues. Half the rats received *Single* training where their CS+ always signalled the same reward. For the *Variety* group, this cue was paired with three different rewards, with reward type varied over days (and not within-session), in keeping with the context experiments. The CPF test measured consumption of FL in the presence of the CS+ and CS- cues, on separate test days. Therefore, the *Single* group were a replication of Experiments F1 and F2 and were intended to clarify the discrepant results from those experiments. The inclusion of the *Variety* group assessed the generality of the results obtained from previous chapters using contexts.

Method

Subjects

Thirty-two adult, female, experimentally naïve Long-Evans rats were used (University of Adelaide, Australia). Albino Wistars were unavailable at the time of this experiment, necessitating a change in strain. Housing conditions were as described for Experiment F2. After acclimation to the laboratory and regular handling over two weeks, home-cage chow was removed and a daily ration of 44g per home cage (~11g/rat). At this

point mean body weight was 263g (range: 204 – 339g). The experiment was conducted in two identical replications, each with 16 rats (8 *Single* and 8 *Variety*).

Apparatus

The apparatus was as described in Experiment F2. In addition to reward pellets (14.20 kJ/g; Bioserv, USA), two liquid rewards were used: 20% w/v sucrose solution (3.18 kJ/g; table sugar in tap water) and 23% w/v chocolate flavoured Ensure® solution (7.80 kJ/g; Abbott, USA). The alternative test food was FL, as described previously. Given the stimulus interaction effects observed in Experiment F2, the clicker was replaced with a pure tone, informed by data from other Pavlovian conditioning experiments in our laboratory indicating that conditioning using this stimulus yielded results more equivalent to those when white noise was used.

Procedure

Pre-training

Prior to the removal of home-cage chow, rats were familiarised to the pre-feeding cages for 30-min. These cages were in a separate room to where training occurred. After the familiarisation session, 25 FL were scattered in each home cage and the experimenter verified that each rat sampled them within a 20-min period. On the next two days FL consumption was measured in pre-feeding cages in two 30-min sessions. Home-cage chow was then removed and the restricted feeding scheduled was introduced.

Group allocation

Rats were allocated to *Single* and *Variety* groups (each $n = 16$) that were matched on body weight at the time of food restriction (*Single* group: 264.2 ± 11.7g [SEM]; *Variety* group: 262.2 ± 11.6g [SEM]) and on total consumption in the two FL familiarisation sessions

(*Single* group: 3.29 ± .4g [SEM]; *Variety* group: 3.27 ± .3g [SEM]). The white noise and tone were assigned as CS+ and CS- in a counterbalanced fashion within each group. Rats in the *Single* group were randomly assigned to receive either pellets ($n = 6$), sucrose solution ($n = 5$) or Ensure solution ($n = 5$) paired with the CS+ during training. For the *Variety* group the CS+ was paired with all three rewards, but only one reward on any given day of training. The three rewards were presented in different orders for subsets of this group.

Training

Training began after four days of adaptation to the restricted feeding schedule with a magazine training session in which 20 rewards were delivered to the magazine on a random-time 60-s schedule. *Variety* rats received whatever reward they were scheduled to receive on the first day of training proper, which began the following day and consisted of 12 sessions held between 1400-1700h, once per day. The rats in each replication were run in two 8-rat squads, with *Single* and *Variety* rats equally represented. As in Experiments F1 and F2, sessions consisted of four 2-min CS+ and four 2-min CS- presentations, intermixed such that no more than two of the same stimulus type could occur consecutively. Each stimulus presentation was preceded by a 2-min pre-CS period. At CS offset there was a variable 1-4 min delay before the next pre-CS period. During CS+ presentations rewards were delivered to the magazine on a VT-30 schedule. A non-reinforced CS+ probe trial was inserted on Day 12 of training to gauge conditioned responding. Rats were fed in home-cages at least 30 min after the conclusion of daily training sessions.

Home-cage re-feeding

After the 12th training session rats were given unrestricted access to chow in the home-cage for 6 days.

CPF tests compared consumption of FL in the presence of the CS+ and CS-. The cues were tested on separate days with a single day of rest in the home-cage in between. The order in which the cues were tested was counterbalanced within each group. Each test session began with 20-min pre-feeding in the conditioning chamber with no stimuli presented. A longer pre-feeding period was chosen to foster higher intake and yield a more sensitive test of the cues. At the end of pre-feeding rats were briefly removed and the chamber inspected for FL. The dish of FL was then set aside to be weighed and a fresh dish was placed in each chamber. This typically took around 2-min for a squad of 8 rats. Cues were played continuously during the 10-min test phase.

Results

Body weight

On the first day of training, the *Single* group weighed 93.80 ± .33% [SEM] and the *Variety* group weighed an average of 95.59 ± .43% [SEM] of their free-feeding weights. This difference was statistically significant ($F(1, 30) = 10.80$, $p = .003$) but was a chance result, given rats were housed in group-mixed cages and had received identical treatment until that point. A 2 x (12) mixed-ANOVA (group x [session]) applied to percent free-feeding weights throughout training found a significant linear trend for session ($F(1, 30) = 67.02$, $p < .001$) that did not interact with group ($F < 1$), indicating that weight loss throughout training did not differ significantly between groups. The main effect of group was significant ($F(1, 40 = 11.42$, $p = .002$). Importantly, the re-feeding phase returned both groups to above their *ad-libitum* body weights, with no significant difference between groups at test (*Single* group: 103.69 ± 1.27% [SEM]; *Variety* group: 103.00 ± 1.38%; $F < 1$).

Training

Magazine entry time (s/min) across training is displayed in Figure F3.1, and suggested comparable conditioning between *Single* and *Variety* groups. The most important data from training were obtained on Day 12. As in Experiments F1 and F2, planned contrasts compared responding to the CS+ probe with responding during pre-CS, CS- and reinforced CS+ presentations. The CS+ probe significantly elevated responding relative to pre-CS levels ($F(1, 28) = 42.86$, $p < .001$) and to the CS- ($F(1, 28) = 41.06$, $p < .001$) but was significantly lower than during reinforced CS+ presentations ($F(1, 28) = 80.67$, $p < .001$). None of these contrasts interacted with group (all $F < 1$). Although the difference between pre-CS and probe trial responding was significantly greater in the cohort with noise as the CS+ ($F(1, 28) = 6.57$, $p = .016$), further analyses confirmed that the tone-as-CS+ cohort significantly increased responding during the CS+ probe relative to the CS- and pre-CS levels ($F(1, 14) = 13.07$, $p = .003$ and $F(1, 14) = 45.81$, $p < .001$). A final analysis restricted to the *Single* group verified that the three subsets trained with pellets ($n = 6$), sucrose solution ($n = 5$), and Ensure solution ($n = 5$) each significantly elevated responding to the CS+ relative to other periods during training (all $p < .01$).

Experiment F3: Training
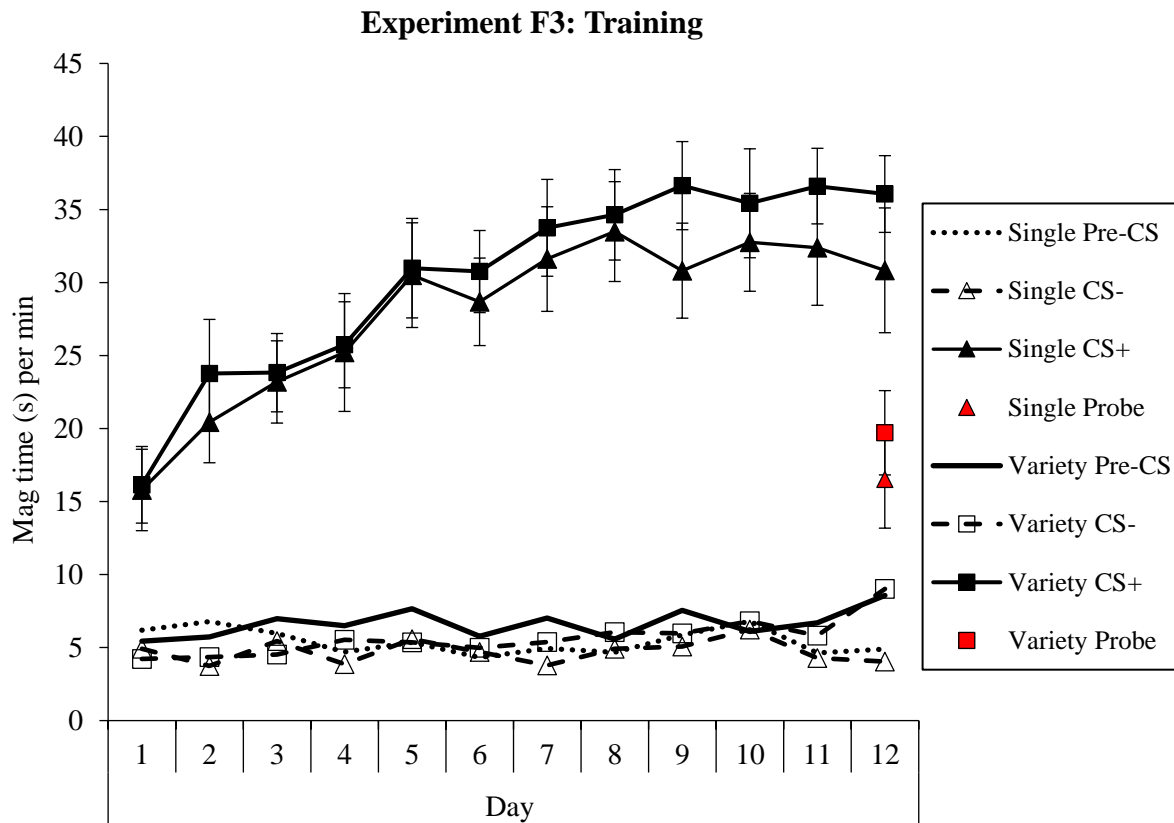
Figure F3.1. Training data. Conditioning was comparable for the *Single* and *Variety* groups, despite the latter receiving cycling access to three different rewards over sessions.

Test

Consumption

Compiled test data are displayed in Figure F3.2. Consumption during pre-feeding did not differ prior to the CS+ and CS- test, nor between groups (both $F < 1$). Consumption during the cue tests was analysed using a 2 x 2 x 2 (2) (group x test order x stimulus x [cue]) mixed-ANOVA. The main effect of cue was not significant ($F(1, 24) = 1.02, p = .32$) but there was a significant cue x stimulus interaction ($F(1, 24) = 6.46, p = .018$). No other main or interaction effects were significant (largest $F(1, 24) = 3.24, p = .09$). To clarify the nature of the interaction, separate analyses examined the cue effect within the cohorts trained with the noise and tone as CS+. The cue effect was significant within the noise cohort ($F(1,$

14) = 14.72, *p* = .002) but not significant in the tone cohort (*F*(1, 14) = 1.24, *p* = .28) with no

interaction with group in either cohort (both *F* < 1). The pellet, sucrose and Ensure subsets of

the *Single* group did not differ significantly (data not shown).

Experiment F3: Test
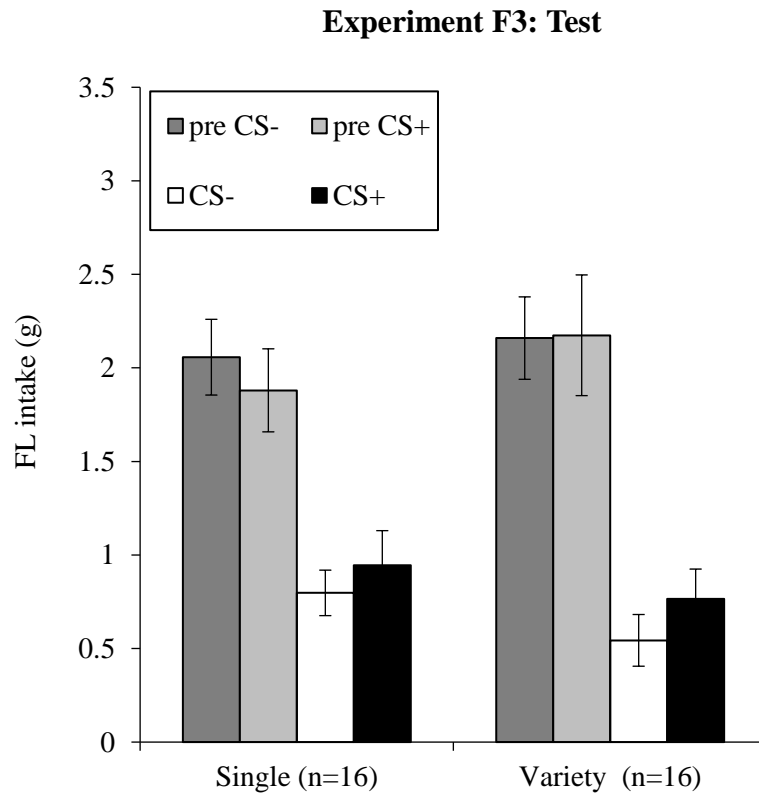
Figure F3.2. CPF test results. The overall CPF effect was not significant and did not vary
between groups.

Magazine time

Magazine entry times during the CS+ and CS- tests are displayed in Figure F3.3 and

were analysed in the same fashion as consumption data. This analysis found a significant

main effect of cue (*F*(1, 24) = 5.45, *p* = .028), indicating more magazine entry time in the

CS+ than CS- test. This result did not, however, interact with stimulus (*F*(1, 24) = 1.61, *p* =

.22) nor with group or test order (both $F < 1$) and no other effects were significant (largest

$F(1, 24) = 3.70$, $p = .06$).

Experiment F3: Magazine time
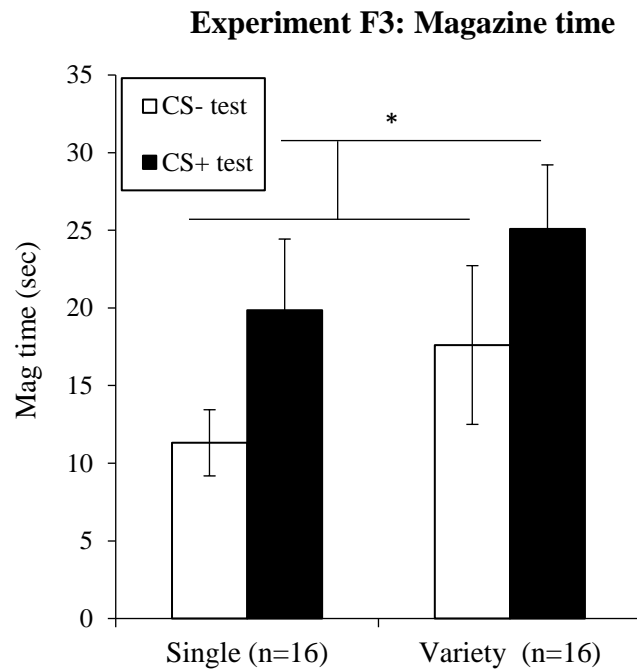
Figure F3.3. Total magazine entry time during the 10-min CS+ and CS- tests.

Correlations

Consumption during pre-feeding and cue periods did not correlate significantly on either the CS+ or CS- tests ($r(30) = -.054$, $p = .77$ and $r(30) = .21$, $p = .26$, respectively). After observing significantly greater magazine entry time during the CS+ test, we reasoned that this might have interfered with consumption, since FL were presented in another location. To test this, we examined whether magazine entry time and consumption correlated significantly on either the CS+ or CS- test. These correlations were not significant (Minus test: $r(30) = .19$, $p = .29$; Plus test: $r(30) = -.09$, $p = .62$).

Discussion

The two aims of Experiment F3 were to clarify the discrepant results from Experiments F1 and F2, and to test for a variety effect of the kind observed in Chapters 2 and 3. To this end, hungry rats underwent conditioning in which an auditory cue was paired with either the same reward (*Single* group) or a variety of rewards (*Variety* group), alongside a non-reinforced CS- cue. Conditioning data indicated no differences between groups at any stage of training, a result consistent with training consumption in the context experiments reported in previous chapters. Nor did *Variety* and *Single* groups differ in their conditioned responding during the probe trial held on the final day of training. This suggested that the associative strength of the CS+ was comparable between groups, despite its association with multiple rewards for the *Variety* group.

In keeping with the context experiments in Chapter 2, we focused on testing an alternative but familiar palatable food, FL. Ample consumption during pre-feeding indicated that rats readily recognised the availability of food from a new location (rather than at the magazine) and suggested that the subsequent cue test would be sensitive to detect differences between the CS+ and CS-. However, consumption was not enhanced by the CS+ in either group. This result did not appear to be a floor effect, given group means of over half a gram (~1.5 Froot Loops) in all test conditions. One observation from training data was that the difference between responding to the probe CS+ and the reinforced CS+ appeared greater than in previous experiments. This might suggest that responding during training was controlled as much by the delivery of a reward as by the CS+. Two possible reasons for the relatively weaker probe trial responding are that (1) the 12 training sessions administered here were fewer than the amount eventually trained in Experiments F1 and F2 (indeed, their data suggest extended training improved probe trial responding); and (2) whereas pellets were the reward for all rats in those experiments, most rats in this experiment were exposed

to liquid rewards delivered by the pumps. It is possible that the onset of the pumps was audible to the rats despite their location (outside the noise-attenuating shells) and that, consequently, animals used this sound to guide responding as well as the cue.

The interactions with stimulus counterbalancing were in the same direction as in Experiment F2 and suggested that the noise stimulus was more effective in potentiating feeding than the tone. One possibility is that the tone, though comparable in terms of its ability to be conditioned, became somewhat aversive when played continuously over a longer test. Alternatively, its effective salience may have declined more rapidly than the noise over the course of tests. In any event, the stimulus interactions did not vary according between groups, suggesting any differences between stimuli were unlikely to account for the overall null result.

The failure of the CS+ to enhance consumption of FL contrasted its effects on conditioned responding, with significantly higher magazine entry time during in the CS+ than in the CS- test. Because the test food was in a dish adjacent to the magazine, one possibility was that conditioned approach to the magazine elicited by the CS+ blocked the ability of the cue to elicit consumption; i.e., response competition. By this account, more time spent in the magazine should have correlated with lower consumption. Correlational analyses found no support for this hypothesis on the CS+ (or CS-) test, with magazine time during the CS+ test (~20-30s) comprising less than 5% of the total test duration (10-min)[23]. In addition, past work has demonstrated CPF both when the test food was presented in the food cup (as in training) and in a bowl in a separate location (Holland et al., 2002). Together, these results suggest that CPF was unlikely to have been precluded by response competition. In any event, our focus

---

[23] Response competition, of course, works both ways, such that consumption of FL limited conditioned magazine approach as much as the other way around. FL consumption may have 'won out' in the CS+ test, since conditioned responding in 10-min was less than in the 2-min CS+ probe on day 12. However, this is speculative because rats were hungry in training but satiated in tests, which would be likely to reduce magazine approach.

was on testing an alternative food which, if presented in the magazine, might be seen as a confound, given this would have marked the *Single* group's first exposure to a different reward in the magazine but the *Variety* group's fourth.

General discussion

The experiments in this appendix tested for CPF using discrete cues and found little evidence that an auditory CS+ enhanced intake of either palatable (FL) or less palatable foods (chow) relative to a CS-. The exception was in Test 3 of Experiment F1, where rats were pre-fed in the conditioning chamber prior to the cue tests. Experiment F2 failed to replicate this effect across multiple tests. Experiment F3 also failed to find CPF in the *Single* group, which now contained additional subsets of rats trained with sucrose and Ensure solutions in addition to those trained with pellets. The subsets of Ensure and sucrose-trained rats ruled out the possibility that the failure to detect CPF related to some specific aspect of the pellets, such as their relative palatability compared with FL. These results are consistent with the *Single* groups from the context experiments in this thesis and the broader literature in demonstrating that stimuli associated with a single food reward do not acquire the ability to increase consumption of alternative foods.

The *Variety* group in Experiment F3 was a novel attempt to study CPF using a discrete cue paired with multiple rewards across sessions. Discrimination between the CS+ and CS- during training and conditioned responding during the probe trial on day 12 did not differ significantly between *Variety* and *Single* groups, consistent with training consumption from the context experiments and with instrumental conditioning studies showing that response rates are not enhanced by variety when it is provided between sessions (Bouton et al., 2013; Thrailkill et al., 2014). By contrast, variety enhances responding when provided

within a session (Bouton et al., 2013; Thrailkill et al., 2014; Temple et al., 2008; Myers Ernst & Epstein, 2002), something not modelled in these experiments. If within-session variety exerts similar stimulatory effects on conditioned responding in Pavlovian preparations, cues or contexts paired with variety within a session might enhance CPF when food is presented at the location where it was presented in training.

Contrary to predictions and to the results of the context experiments in other chapters, Experiment F3 found no evidence that CPF on an alternative food was present in the *Variety* group. Although this might simply indicate that contexts are better able to promote consumption of other foods than are discrete cues, other possibilities are interesting to consider. One is that the variety effect reported with contexts followed training in which rats ate ample amounts of the three foods, receiving extensive exposure to their sensory characteristics and, presumably, experiencing some satiety following their consumption. The present cue studies, by contrast, presented only limited amounts of reward during daily training, perhaps suggesting this amount of exposure was insufficient to condition an association with the cue that could generalise to FL at test. This and other potential differences between cue and context models of CPF are discussed in Chapter 6.

The general method for these experiments was comparable to past studies: food-deprived rats received daily training sessions using a method similar to past studies (Holland & Gallagher, 2003; Galarce et al., 2007), and were tested using pre-feeding and cue tests of similar duration to those reported previously. The main difference was in our focus on testing alternative foods proximally to training. Consequently, a limitation of these experiments is that they did not verify that the CS+ enhanced consumption of the training food. This limits the conclusions that can be drawn about the specificity of CPF. Nonetheless, the close resemblance of the design to past work would suggest the parameters provided appropriate conditions to observe CPF if any effects existed. Therefore, these data indicate that under

these conditions, discrete stimuli successfully established as food-paired cues failed to promote consumption of alternative foods.

Appendix G: Contexts Paired with Junk Food Impair Goal-Directed Behavior in Rats: Implications for Decision Making in Obesogenic Environments

The following manuscript is published as:

Kendig, M. D., Cheung, M. K. A., Raymond, J. S., & Corbit, L. H. (2016). Contexts paired with junk food impair goal-directed behaviour in rats: implications for decision making in obesogenic environments. *Frontiers in Behavioral Neuroscience, 10,* 216.

This paper formed a significant component of my research program. It is distinct from the study of cue-potentiated feeding reported in the main body of this thesis but is relevant for aspects of its interpretation. I contributed to experimental design; ran the experiments with assistance from AMK and JSR; analysed the data; and wrote drafts of the manuscript with LHC. Permission to include the published material has been granted by the corresponding author (LHC).

Michael D. Kendig

frontiers
in Behavioral Neuroscience

Check for
updates

Contexts Paired with Junk Food Impair Goal-Directed Behavior in Rats: Implications for Decision Making in Obesogenic Environments

Michael D. Kendig , Ambrose M. K. Cheung , Joel S. Raymond and Laura H. Corbit *

School of Psychology, The University of Sydney, Sydney, NSW, Australia

The high prevalence of obesity and related metabolic diseases calls for greater understanding of the factors that drive excess energy intake. Calorie-dense palatable foods are readily available and often are paired with highly salient environmental cues. These cues can trigger food-seeking and consumption in the absence of hunger. Here we examined the effects of palatable food-paired environmental cues on control of instrumental food-seeking behavior. In Experiment 1, adult male rats received exposures to one context containing three "junk" foods (JFs context) and another containing chow (Chow context). Next, rats were food-deprived and trained to perform instrumental responses (lever-press) for two novel food rewards in a third, distinct context. Contextual influences on flexible control of food-seeking behavior were then assessed by outcome devaluation tests held in the JF, chow and training contexts. Devaluation was achieved using specific satiety and test order was counterbalanced. Rats exhibited goal-directed control over behavior when tested in the training and chow-paired contexts. Notably, performance was habitual (insensitive to devaluation) when tested in the JF context. In Experiment 2 we tested whether the impairment found in the JF context could be ameliorated by the presentation of a discrete auditory cue paired with the chow context, relative to a second cue paired with the JF context. Consistent with the results of Experiment 1, the devaluation effect was not significant when rats were tested in the JF context with the JF cue. However, presenting the chow cue increased the impact of the devaluation treatment leading to a robust devaluation effect. Further tests confirmed that performance in the chow context was goal-directed and that sensory-specific satiety in the JF context was intact. These results show that environments paired with palatable foods can impair goal-directed control over food-seeking behavior, but that this deficit was improved by a cue paired with chow. This has promising implications for assisting individuals in controlling their eating behavior in environments designed to dysregulate it.

Keywords: instrumental conditioning, Pavlovian conditioning, stimulus, habit, junk food, context, rat

INTRODUCTION

Obesity is now widespread across the developed and developing world, with the number of obese individuals recently estimated to exceed that of underweight people worldwide (World Health Organisation, 2016). A key driver of excess energy intake and long-term weight gain is the abundance of highly palatable and energy-dense foods. These products are typically

advertised with highly salient cues that are ubiquitous in day-to-day life and which are explicitly designed to influence consumption. For example, one study found that children ate significantly more after viewing advertisements for food than for non-food products, regardless of body weight, and that the amount eaten was positively correlated with how many adverts were recognized (Halford et al., 2004).

A substantial proportion of eating now occurs outside of the home, and these meals are associated with greater energy intake and lower micronutrient content (Stroebele and De Castro, 2004; Lachat et al., 2012). These external environments are riddled with stimuli designed to promote food purchase and consumption. While attending to food cues was highly adaptive in earlier periods of human history, relying too heavily on external cues may undermine body weight regulation in modern environments (Berthoud, 2007, 2012). Indeed, there is ample evidence for stimulatory effects of food cues on consumption in the short term. Animal models of *cue-potentiated feeding* show that cues paired with the delivery of food to hungry rats elicit consumption of this food when rats are no longer food-deprived (Weingarten, 1983; Petrovich, 2013), with similar effects found in people (e.g., Cornell et al., 1989). However, long-term effects of food-cue exposure on weight gain have not been established, in part due to the difficulty of testing this hypothesis. For example, in animal models the effects of food cues are sometimes tested within-subjects (Boggiano et al., 2009) and animals are commonly food deprived to encourage learning of the cue-food association, constraining body weight change (but see Reppucci and Petrovich, 2012).

Of course, food cues may affect eating behavior in ways other than prompting immediate consumption. Food is not always readily available in the presence of food cues; for example, when driving past a fast-food sign or walking through a shopping center food court. In these instances, cues may influence consumption via a series of cognitive processes involving where, what and how much food to procure. How food cues affect the decision-making processes that precede actual consumption is relatively less studied and was the focus of the present experiments. To explore this, we applied a framework based on principles of instrumental learning that distinguishes between behavior that is volitional (i.e., goal-directed) and that which is habitual (Dickinson, 1985). Performance of a goal-directed behavior, such as pressing a lever for food, relies on the contingency between the lever press (action) and food reward (outcome) and the fact that the food reward is currently valued. Therefore, manipulating the value of the reward should produce corresponding changes in performance of the action if the behavior is goal-directed, and no change or a reduced change if the behavior is under habitual control (Dickinson and Balleine, 1994). The outcome devaluation paradigm is a behavioral assay used to determine whether an action is under goal-directed or habitual control. The value of a reward is manipulated either by specific satiety or by inducing sickness (via lithium chloride) and performance of the action that earns the devalued outcome is compared either with conditions where the same outcome is valued or with a second action earning a different outcome for which value is intact (Adams and Dickinson, 1981; Balleine

and Dickinson, 1998). Goal-directed behaviors are sensitive to changes in outcome value and, therefore, manifest as a selective reduction of the action earning the devalued outcome. By contrast, behaviors under habitual control are insensitive to changes in outcome value and are evident in responding that is not selectively sensitive to manipulation of the outcome of responding.

Recent studies have shown that habitual control over behavior can be accelerated by chronic access to diets high in sugar and/or fat in rats (Kendig et al., 2013; Furlong et al., 2014) and that higher BMI was associated with reduced sensitivity to devaluation in people (Horstmann et al., 2015). Here we focused not on lasting changes produced by long-term diet but on whether contexts paired with highly palatable foods could alter sensitivity to devaluation. The general experimental procedure was modeled on that used in two studies demonstrating that contexts paired with drugs of abuse promoted habitual control over behavior. In the first, rats were injected with ethanol and placed in one distinct context and injected with saline then placed in another context, prior to instrumental training conducted in a third environment. Devaluation tests revealed that responding was insensitive to devaluation when rats were tested in the alcohol-paired context but goal-directed in the saline context (Ostlund et al., 2010). The second study used a similar procedure to demonstrate habitual control over behavior produced by contexts paired with methamphetamine (Furlong et al., 2015). Importantly, instrumental performance was reinforced with food rather than drug rewards and the animals were drug-free at test, indicating that the contexts, rather than acute intoxication, influenced the decision-making processes that promoted habitual responding. We adopted a similar experimental procedure to Ostlund et al. (2010) and Furlong et al. (2015) to assess whether junk food (JF)-paired contexts would disrupt sensitivity to outcome devaluation.

The two experiments reported here each began with Pavlovian context conditioning in which non-deprived rats received repeated exposures to one context paired with standard lab chow and another paired with highly palatable JFs. Rats were then food-deprived for instrumental training in a third context where two lever-press responses for two novel food rewards were trained. Sensitivity to outcome devaluation was then examined in the JF, chow and training contexts. Experiment 1 found that the JF context promoted habitual control over behavior. Experiment 2 attempted to reverse this effect by exploring whether the presentation of a discrete cue paired with chow and satiety would restore goal-directed control over behavior in the JF context.

EXPERIMENT 1

Materials and Methods

Subjects
All experimental procedures were carried out in accordance with the recommendations of the Australian code for the care and use of animals for scientific purposes 8th edition (2013),

and were approved by the Animal Ethics Committee at the University of Sydney. Twenty-eight adult male hooded Wistar rats were used. These animals were tested in two replications ($n = 16$ and $n = 12$) that underwent identical experimental procedures. Rats were sourced from the University of Adelaide, were experimentally naïve, and were group-housed ($n = 4$/cage) in temperature- and humidity-controlled ventilated cages in a colony room maintained on a 12:12 light:dark cycle (lights on 7 am–7 pm). Testing was conducted between 2–5 pm each day. Chow and water were available *ad libitum* during context conditioning, but food access was restricted during instrumental training (see below). Rats were handled regularly prior to the beginning of the experiment.

Apparatus

All behavioral procedures were conducted in operant chambers (Med-Associates, St. Alban, VT, USA) contained within light- and sound-attenuating shells. The top and side walls of these chambers were Plexiglas and the floor consisted of steel bars. A recessed magazine was centered on one wall of the chamber between two retractable levers. Illumination was provided by a houselight centered at the top of the wall opposite the levers. For context conditioning, visual, tactile and olfactory cues were used to form two distinct contexts that were paired with JFs and chow in a counterbalanced fashion. Thus, one context contained a smooth plastic floor insert, was scented with vanilla essence (10% v/v in water; Queen, Queensland) and had top and side walls decorated with black and white stripes. The second context was scented with peppermint odor (10% v/v in water; Queen, Queensland), had black spots on a white background surrounding the top and side walls, and contained a floor insert covered with rough sandpaper. Odors were pipetted onto folded paper towels that were inserted into the front edge of the bedding tray. Wall decorations were laminated sheets of paper fitted around the exterior of the chamber. Instrumental training was conducted in the same operant chambers with all cues removed to form a "training" context. The houselight was on during all context conditioning and instrumental training sessions. The rewards used in instrumental training were 45 mg pellets (grain-based formula, BioServ, USA) and 20% w/v sucrose solution (~0.1 ml per reward), which are both highly palatable to rats and greatly preferred to chow. Devaluation pre-feeding was conducted in individual acrylic cages with metal bar tops located in a separate room to operant chambers.

Procedure

Context conditioning

Context conditioning lasted for 14 days and consisted of seven, 1 h exposures each to the Chow and JF contexts in an alternating sequence (chow, JF, chow, JF, etc.). Laboratory chow (Specialty Feeds®; 14.23 kJ/g) was provided in the chow context. In the JF context three palatable foods were provided: Oreos (Nabisco, East Hanover, NJ, USA; 20.33 kJ/g), Pringles (Pringles, Battle Creek, MI, USA; 22 kJ/g), and Jelly Snakes (Nestlé, Australia, 14.2 kJ/g). The total weight of food available in JF and Chow sessions was approximately 15 g. Foods were presented in white ceramic dishes centered against the side wall of the chamber.

Food was weighed before and after the session to determine intake, which was converted from grams to kJ for analyses and summed for the three foods in the JF context.

Instrumental training

Immediately after day 14 of context conditioning, home-cage chow was removed and a restricted feeding schedule introduced wherein rats were fed 14–15 g of chow per rat each day. Instrumental training began 2 days after the last day of context conditioning with a magazine training session where 20 pellets and 20 sucrose rewards were delivered to the magazine on independent random-time 60 s schedules. The left and right levers were then assigned to earn these rewards in a counterbalanced fashion. For the first 6 days of instrumental training, left and right levers were trained in separate sessions that ended either after 30 rewards were earned or 45 min elapsed. The sessions were separated by a minimum of an hour and whether the pellet or sucrose outcome was trained first was alternated each day. For days 1 and 2 of training each lever press was rewarded (i.e., continuous reinforcement). Thereafter, the reinforcement schedule was increased to random-ratio (RR) 5 on days 3 and 4 and RR10 on days 5–7. On day 7 the two levers were trained in the same session. In this session the left lever was inserted until five rewards were earned and then retracted. After 10 s, the right lever was inserted until five rewards of the other outcome were earned. This sequence repeated until 30 rewards of each outcome were earned, or until 60 min had elapsed. This two-outcome procedure was used for all subsequent re-training days between tests. This procedure is similar to that used by Ostlund et al. (2010) but with a shorter delay between levers.

Devaluation tests

Devaluation tests were held in the JF, Chow and Training contexts. The order of these three tests was counterbalanced and test days were separated by a single day of re-training using the two-outcome procedure described for training day 7 above. Devaluation was achieved by specific satiety: rats were placed in individual feeding cages and allowed to consume pellets or sucrose solution *ad libitum* for 1 h. Approximately 15 g pellets or 30 g sucrose solution were provided during pre-feeding; rats never consumed more than these amounts. Rats were familiarized to pre-feeding cages on two occasions for 20-min during instrumental training (after daily sessions). The devalued outcome was held constant across tests and counterbalanced, such that pellets were devalued for half of the rats and sucrose solution was devalued for the other half. Immediately after devaluation treatment rats were transferred to the context (JF, Chow or Training) for a 15-min test. Levers were not inserted for the first 10 min of this test to promote attention toward the contexts. After 10 min, both levers were inserted simultaneously for a 5-min test. Presses were recorded but not reinforced.

Data analysis

Consumption of chow and JF in context conditioning sessions (kJ/rat) was analyzed using a (2) × (7) within-subjects ANOVA. The dependent measure during instrumental training was the response rate (lever presses/minute) averaged across pellet

and sucrose levers. Response rates across days were analyzed in a within-subjects ANOVA. Responding on devalued and non-devalued levers in the three context tests was compared using a within-subjects (2) × (3) ANOVA. Preliminary analyses included devalued outcome (sucrose or pellets) as an additional between-subjects factor but, as it did not interact with the context (Experiment 1) or cue (Experiment 2) effects of interest, we collapsed across this variable for subsequent analyses. Significant interaction effects were followed by tests of simple effects, results for which $p < 0.05$ were considered statistically significant.

Results

Context Conditioning

Consumption during training is shown in **Figure 1**. Rats rapidly increased their consumption of JF in the JF context but ate minimal chow in the Chow context. This was supported statistically by a significant effect of session (linear trend: $F_{(1,27)} = 84.92$, $p < 0.001$) and a significant context × session interaction ($F_{(1,27)} = 79.88$, $p < 0.001$) in a (2) × (7) ANOVA. Averaged over sessions, rats ate significantly more in the JF than Chow context (context main effect: $F_{(1,27)} = 149.30$, $p < 0.001$). Despite being non-deprived during this phase, by the end of context training, rats were consuming around eight times more energy in the JF context than in the Chow context.
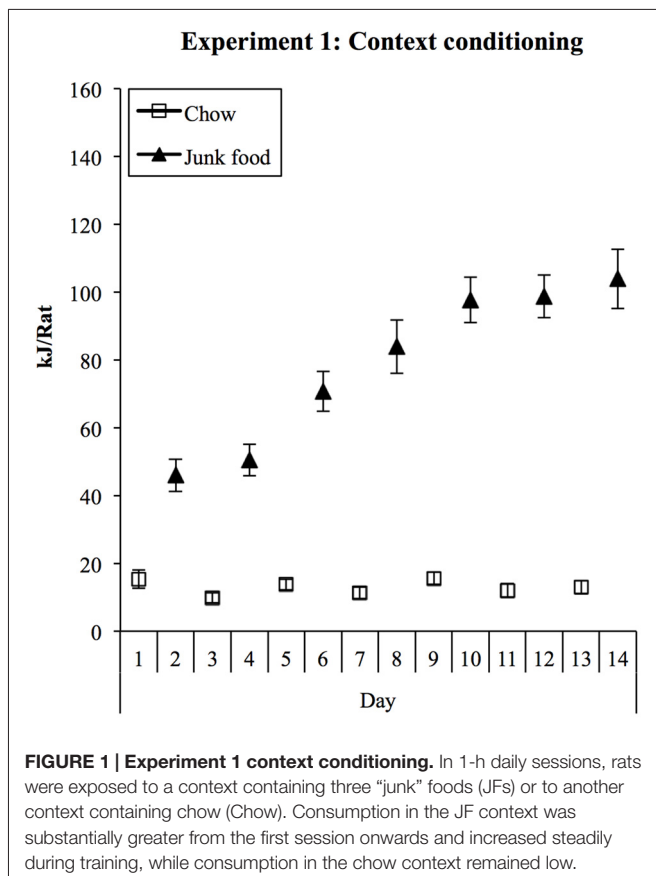
Instrumental Training

All rats learned both instrumental responses. Response rates are shown in **Figure 2** and significantly increased during training (linear trend: $F_{(1,27)} = 301.56$, $p < 0.001$). Two rats showed an extreme response bias by responding four times more on the pellet lever than the sucrose lever across training. Since this bias would likely obscure the devaluation effect, these rats were not included in test analyses.
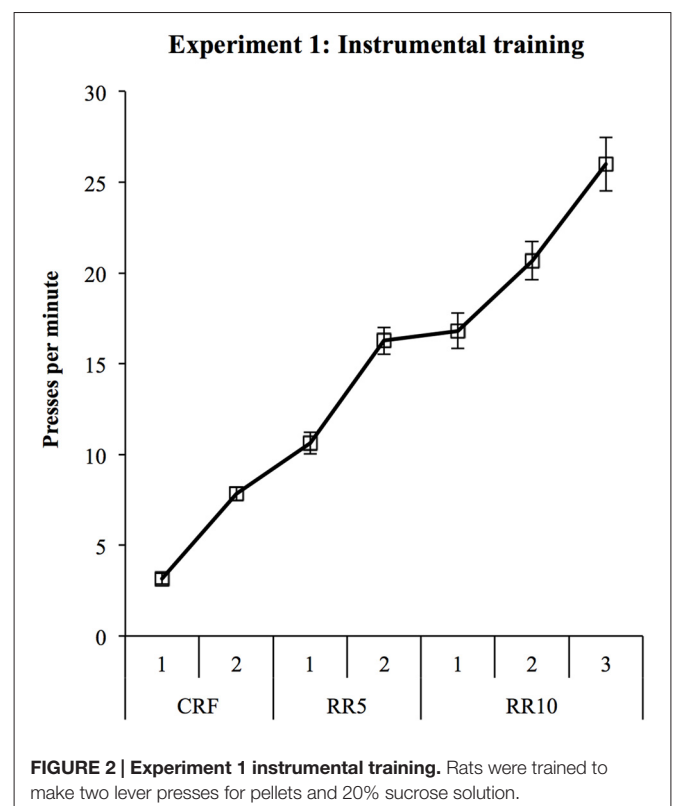
Devaluation Tests

Pre-feeding

Consumption during pre-feeding did not change significantly over the three test days ($F_{(2,48)} = 1.53$, $p = 0.227$). On average, rats pre-fed with pellets consumed 8.58 ± 0.29 g, while rats pre-fed with sucrose consumed 16.18 ± 0.54 g. However, when expressed as reward equivalents (1 pellet reward = 45 mg and 1 sucrose reward = 0.1 g), consumption was greater in pellet-fed rats (190.6 ± 0.6) than sucrose-fed rats (161.9 ± 5.4). In both cases, consumption far exceeded what rats earned in instrumental training sessions (30 rewards) and rats had stopped eating by the end of the 1-h period, indicating they were satiated.

Test

Compiled devaluation test data are displayed in **Figure 3** and were analyzed in a (2) × (3) ANOVA (devaluation × context). This analysis found a significant devaluation effect ($F_{(1,25)} = 5.47$, $p = 0.028$) that, critically, interacted with the context in which rats were tested ($F_{(2,50)} = 3.65$, $p = 0.033$). There were no differences in overall responding between contexts ($F < 1$).

FIGURE 1 | Experiment 1 context conditioning. In 1-h daily sessions, rats were exposed to a context containing three "junk" foods (JFs) or to another context containing chow (Chow). Consumption in the JF context was substantially greater from the first session onwards and increased steadily during training, while consumption in the chow context remained low.

FIGURE 2 | Experiment 1 instrumental training. Rats were trained to make two lever presses for pellets and 20% sucrose solution.
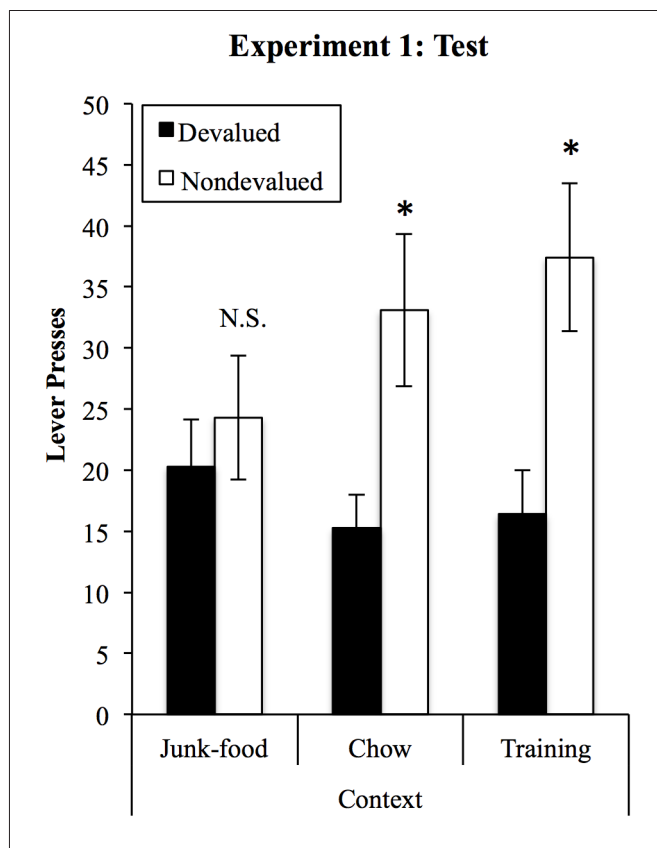
FIGURE 3 | A JF context impairs sensitivity to outcome devaluation.
Sensitivity to devaluation was tested in the three contexts, within-subjects and
in a counterbalanced order. After devaluation of one outcome by specific
satiety, rats selectively reduced responding on the lever that had earned that
outcome in training, but only in the chow and training contexts. When tested
in the JF context, performance was insensitive to devaluation, with no overall
difference in responding between contexts. *Indicates $p < 0.05$, N.S.,
non-significant.

Sensitivity to devaluation in each context was then assessed using
tests of simple effects. These found that rats showed significant
devaluation effects when tested in the Training ($F_{(1,25)} = 7.85$,
$p = 0.01$) and Chow contexts ($F_{(1,25)} = 6.27$, $p = 0.019$) but that
responding on the devalued and non-devalued levers did not
differ in the JF context ($F_{(1,25)} = 0.32$, $p = 0.576$).

Discussion

Experiment 1 trained non-deprived rats to associate one context
with highly palatable JFs and another with bland chow. After
repeated, alternating exposures to these environments, rats
were food-deprived and trained to perform two instrumental
responses for distinct food rewards in a third environment.
At test we assessed whether the ability to direct food-seeking
behavior according to the current value of those foods would be
affected by the context in which rats were tested. Rats showed
sensitivity to devaluation when tested in a context previously
paired with chow or in the environment in which instrumental
training occurred. The key finding from this experiment is
that these same rats were insensitive to devaluation when

tested in the context previously paired with palatable food.
Importantly, overall responding did not differ between the three
contexts, suggesting that this impairment was not driven by some
non-specific effect on overall responding. Rather, rats pressed
at a similar rate in this environment but were unable to adjust
behavior in accordance with the current value of the outcomes.
Therefore, contexts paired with highly palatable JFs undermined
goal-directed control over food-seeking behavior.

EXPERIMENT 2

Loss of goal-directed control over food-seeking behaviors could
be an obstacle to changing one's eating behavior. In Experiment 2
we explored whether additional conditioning manipulations
could ameliorate this impairment. We modeled our approach
on a body of literature studying the effects of discrete stimuli
paired with the extinction of previously learned associations,
often termed "e-cues", which are thought to serve as reminders of
extinction training and have been shown to promote expression
of extinction (Brooks and Bouton, 1993). Under most conditions,
extinction of an instrumental response does not erase original
learning but rather produces new learning that the response
no longer leads to reward. Because responding recovers under
a variety of circumstances (Bouton et al., 2012), interventions
that protect or strengthen extinction learning are important for
reducing these recovery phenomena, particularly in the context
of food-related behavior (Bouton, 2011). To this end, Brooks
and Bouton (1993) found that a visual cue presented during
extinction of a tone-food association (e-cue) attenuated the
spontaneous recovery of conditioned responding to the tone
when rats were tested 6 days later. Using a similar experimental
procedure, Brooks and Bouton (1994) found that presentation
of an e-cue prevented ABA renewal, a phenomenon where
a response learned in one context ("A") and extinguished in
a second context ("B") recovers with a return to the first
("A"). A recent study found similar effects of an e-cue on
ABA renewal in rats trained to nose-poke for alcoholic beer
(Willcocks and McNally, 2014). The typical interpretation of
these results is that the presentation of the e-cue facilitates
the retrieval of the extinction memory to buffer against
returned expression of the original learning (Brooks and Bouton,
1993).

Related to these findings, Ostlund et al. (2010) found that
the contextual promotion of habitual responding was reversed
by providing response-contingent feedback in the form of
outcome delivery. Together, these results suggest that where
two conflicting systems compete for behavioral control (original
learning vs. extinction, or goal-directed vs. habit systems), stimuli
that "remind" the rat of extinction or the devalued state of
the outcome can influence behavior to favor the cued learning.
Thus, in Experiment 2 rather than attempting to extinguish
the JF context, we examined whether a reminder of a relatively
unpalatable food; chow, could override the effects of the context
previously paired with the palatable JF and promote sensitivity
to devaluation. To this end, we presented discrete auditory
stimuli in the JF and Chow contexts so that consumption of JF
and Chow were paired with a "JF-cue" and a "Chow-cue" in

addition to the contexts. We then assessed whether presentation of the Chow-cue would improve sensitivity to devaluation in the JF context relative to when the JF cue was presented in this environment. An additional aim of Experiment 2 was to measure sensitivity to devaluation in terms of consumption as well as instrumental responding. We hypothesized that, just as the e-cue reminds rats of conditions of non-reinforcement (e.g., Willcocks and McNally, 2014), or as outcome delivery reminds animals of changes in outcome value following devaluation (Ostlund et al., 2010), presenting a cue previously paired with chow would remind rats of reduced palatability, and/or satiety, to enhance sensitivity to devaluation in the JF context.

Materials and Methods

Subjects

Twenty adult male Long-Evans rats were used. Animals were bred in-house at the Brain and Mind Centre at the University of Sydney, Australia, and were housed 2–4 per cage in ventilated cages contained in a temperature- and humidity-controlled room. The colony room was maintained on a 12:12 reverse dark:light cycle (lights off 9 am–9 pm). Behavioral testing occurred between 2–6 pm each day. During context conditioning rats had free access to chow and water in home cages. During instrumental training rats were fed approximately 12 g chow daily. Rats were handled regularly in the week prior to the start of the experiment.

Design

Context conditioning in Experiment 2 was identical to Experiment 1 except that a discrete auditory cue was also paired with each context. These cues were a white noise and pure tone and were paired with Chow and JF contexts in a counterbalanced fashion. Ten 2-min presentations of these stimuli occurred in every 1-h training session and were separated by a variable ITI (range: 1–4 min). To prevent hearing an inappropriate stimulus from adjacent boxes, rats were run in two groups of 10 rats according to stimulus type. Home-cage chow intake was monitored each day during context conditioning. On the day after the last context conditioning session, rats were pre-exposed in home cages for 2 h to pellets (Bioserv; grain-based formula) and 20% sucrose solution, the outcomes to be used for instrumental training. Food was then removed overnight, and from the following day the restricted feeding scheduled was introduced. Instrumental training was conducted as described for Experiment 1 except that two sessions of the two-outcome procedure were held prior to tests (rather than one).

The first two devaluation tests were conducted in the JF context and compared the effects of the JF and Chow cues (order counterbalanced). Devaluation was achieved by specific satiety as in Experiment 1. For the first 10 min of each test no levers were available and no stimuli were presented. After 10 min both levers were inserted for a 5-min choice extinction test. When levers were inserted, either the Chow- or JF-cue was turned on and played constantly for the remainder of the test. Lever presses were recorded in 1-min bins. On the following day rats received a single session of instrumental re-training using the two-outcome
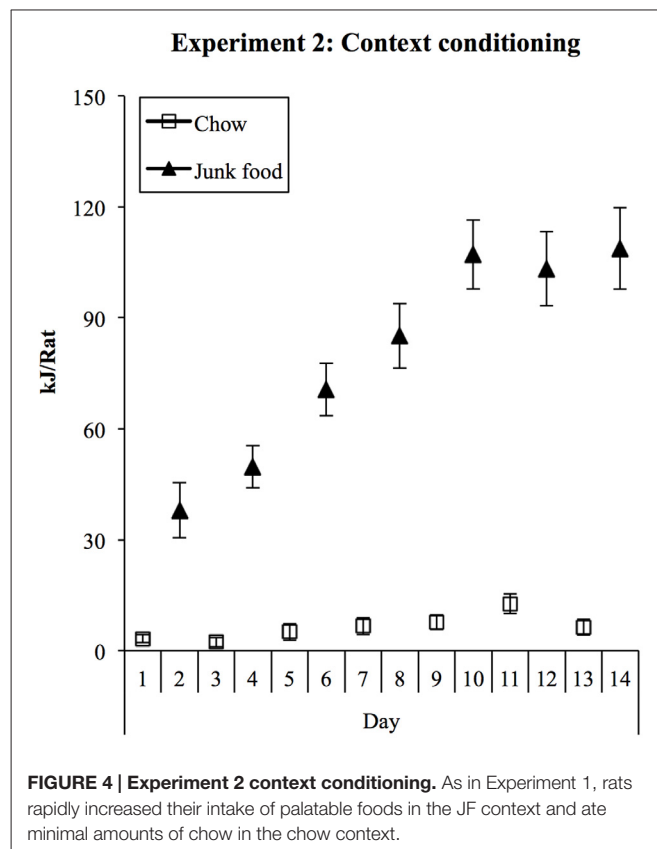
procedure described above. The second devaluation test was identical to the first except that rats tested with the chow cue in Test 1 now received the JF cue, and vice versa. Rats were then given 3 days of re-training prior to a second set of devaluation tests held in the chow context in order to confirm goal-directed responding in this context and test whether the presence of the JF cue was sufficient to impair sensitivity to devaluation. Rats pre-fed with pellets for Tests 1 and 2 were pre-fed with sucrose solution for these tests, and vice versa. The order in which JF- and Chow-paired cues were tested was counterbalanced, and for each rat was the reverse of the order used in tests 1 and 2.

We were also interested to examine whether the habitual performance in the JF-paired context could be explained by impaired sensitivity to sensory specific satiety in this context. Therefore, we examined whether rats would selectively reduce *consumption* of the pre-fed outcome in the JF-paired context. For this test, rats were pre-fed either with pellets ($n = 10$) or sucrose solution ($n = 9$) for 1-h in devaluation pre-feeding cages before a 10-min test of pellet consumption in the JF context with the JF-cue played continuously. Pellets were the test food for all tests due to the logistical difficulty of fixing a bottle of sucrose within operant chambers.

Results

Context Conditioning

Consumption in Chow and JF sessions during training is shown in **Figure 4**. As in Experiment 1, rats ate substantial amounts

FIGURE 4 | Experiment 2 context conditioning. As in Experiment 1, rats rapidly increased their intake of palatable foods in the JF context and ate minimal amounts of chow in the chow context.

of the palatable foods in the JF context but little chow in the Chow context. Consumption of all foods was converted to kilojoules, summed across the three foods in the JF context, and analyzed in a $(2) \times (7)$ repeated-measures ANOVA. This analysis showed a significant increase in consumption across sessions $(F_{(1,19)} = 40.28, p < 0.001)$ and a significant interaction between context and session $(F_{(1,19)} = 31.633, p < 0.001)$, indicating a greater increase in consumption in the JF- than Chow-paired context. Averaged over sessions, consumption was greater in the JF context $(F_{(1,19)} = 150.71, p < 001)$.

Each day, home-cage chow intake was measured when rats were in context conditioning sessions. Total energy intake was then calculated on a per-cage basis by adding home-cage chow intake to the total consumption in the context session by the rats in each cage. Consumption in each day's training session (kJ/rat) was added to home-cage consumption (kJ/rat) in the following 24-h. This resulted in a measure of 24-h energy intake for each of the six cages on each day of training. Subsequently, we compared total energy intake between chow and JF-training days to assess the extent to which rats compensated for the kJ consumed in JF sessions. Total daily energy intakes were analyzed in a within-subjects $(2) \times (7)$ ANOVA, with day type (JF- or Chow-paired day) and "session" as factors. This analysis found a main effect of "day type" $(F_{(1,5)} = 38.86, p = 0.002)$ indicating that energy intake was higher on days beginning with a JF-session. The difference in average total energy intake indicated by this result is shown in **Figure 5**. There was no
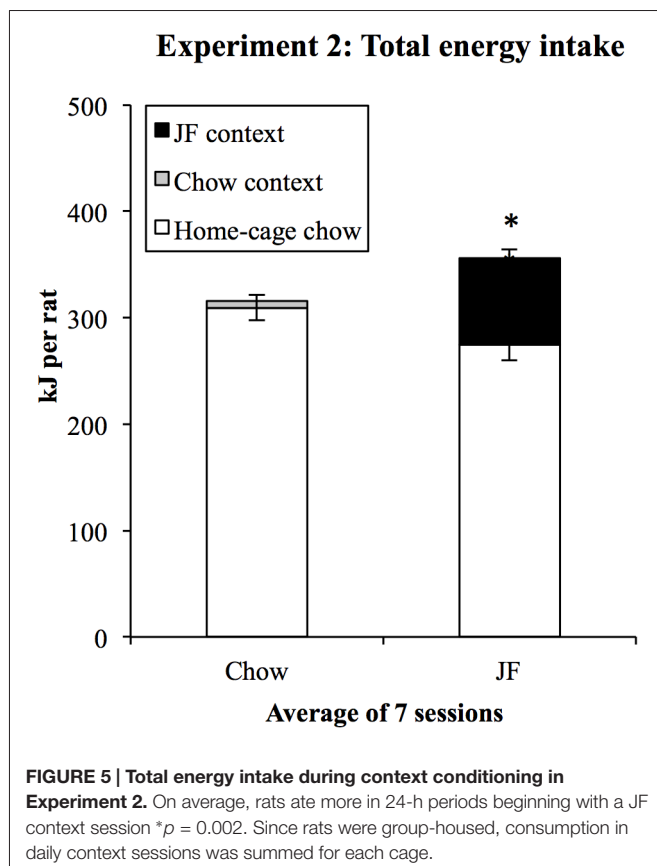
significant linear change in energy intake over days $(F_{(1,5)} = 6.17, p = 0.056)$.

Instrumental Training

Nineteen rats learned both instrumental responses; the 20th failed to respond for sucrose solution and therefore could not be tested. Average daily responding is displayed in **Figure 6**. Responding in the first block of training prior to the first test was analyzed in a within-subjects ANOVA. This analysis found a significant linear increase in response rates over sessions $(F_{(1,18)} = 154.19, p < 0.001)$. These response rates were maintained throughout subsequent re-training sessions.
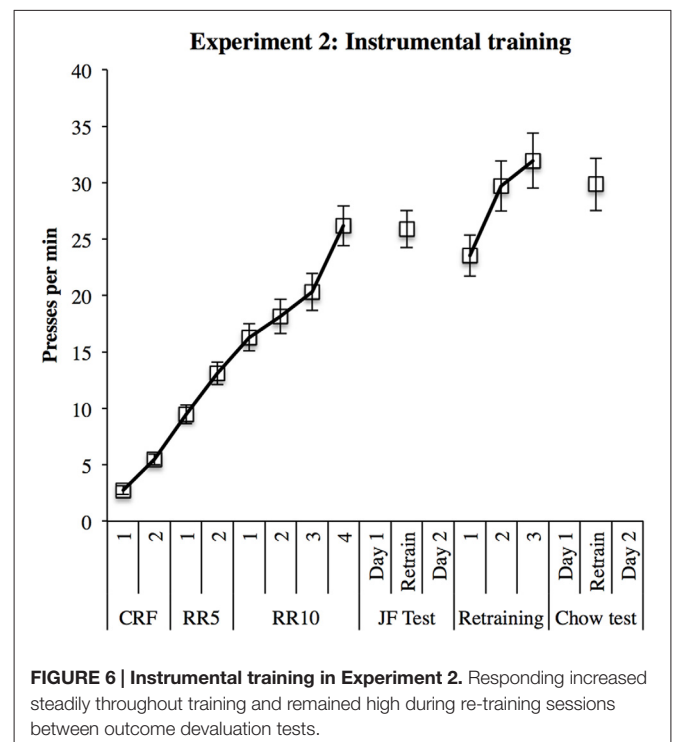
Tests

Pre-feeding

Familiarization to the pre-feeding cages was as described for Experiment 1. Rats were pre-fed either with sucrose solution or pellets for both JF context tests, and the other reward (pellets or sucrose) for both Chow context tests. On average, rats consumed 14.13 ± 0.87 g sucrose and 7.81 ± 0.45 g pellets; this was equivalent to 179.32 ± 8.43 pellet rewards and 144.53 ± 8.54 sucrose reward.

Effects of the JF- and Chow-cues on sensitivity to devaluation in the JF context

Presses on the devalued and non-devalued levers in the chow-cue and JF-cue test are shown in **Figure 7A** and were analyzed in a $(2) \times (2)$ within-subjects ANOVA. This analysis found a significant effect of devaluation $(F_{(1,18)} = 11.14, p = 0.004)$ and no main effect of cue $(F < 1)$. Importantly,

FIGURE 5 | Total energy intake during context conditioning in Experiment 2. On average, rats ate more in 24-h periods beginning with a JF context session *$p = 0.002$. Since rats were group-housed, consumption in daily context sessions was summed for each cage.

FIGURE 6 | Instrumental training in Experiment 2. Responding increased steadily throughout training and remained high during re-training sessions between outcome devaluation tests.
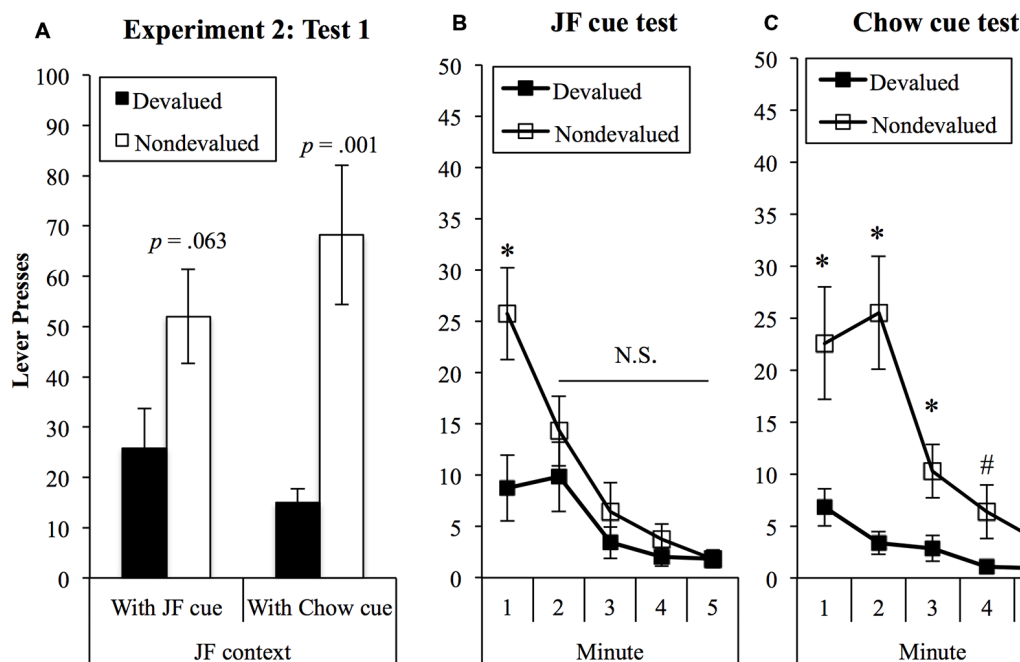
FIGURE 7 | Devaluation tests in the JF contexts. (A) Sensitivity to devaluation in the JF context was significantly improved when the chow cue was presented (interaction $p = 0.038$). p-values show tests of simple effects in each cue test. Analysis of bin data showed that sensitivity was lost rapidly in the presence of the JF cue **(B)** but remained statistically significant throughout the chow-cue test **(C)**. *$p < 0.05$; #$p = 0.057$.

there was a significant interaction between devaluation and cue ($F_{(1,18)} = 4.99$, $p = 0.038$), indicating that sensitivity to devaluation treatment varied according to whether the JF- or Chow-paired cue was present during the test. Simple effects analyses were then conducted to explore the nature of the interaction. These analyses found a significant devaluation effect when the Chow-cue was presented in the JF context ($F_{(1,18)} = 15.54$, $p = 0.001$) but not when the JF-cue was presented ($F_{(1,18)} = 3.93$, $p = 0.063$).

To explore the devaluation × cue interaction in greater detail, we examined 1-min bin data for JF-cue and chow-cue tests, shown in **Figures 7B,C**, respectively. Examining these data suggested that initial sensitivity to devaluation in both tests was rapidly lost in the presence of the JF-cue, but sustained by the chow-cue. To examine this, we added "bin" as a third factor with five levels to a 3-way within-subjects (5) × (2) × (2) ANOVA. This analysis found a significant 3-way interaction between cue, lever and bin ($F_{(4,72)} = 3.09$, $p = 0.021$), indicating that the difference between responding on devalued and non-devalued levers over the five bins varied between JF- and Chow-cue tests. In the JF-cue test, the devaluation effect was significant in the first minute ($F_{(1,18)} = 6.64$, $p = 0.019$) but not in minutes 2, 3, 4, or 5 (largest $F_{(1,18)} = 1.09$). By contrast, during the Chow-cue test the devaluation effect was significant during all five 1-min bins, save for a marginally significant result in minute 4 (minute 1: $F_{(1,18)} = 6.95$, $p = 0.017$; minute 2: $F_{(1,18)} = 16.02$, $p = 0.001$; minute 3: $F_{(1,18)} = 6.18$, $p = 0.023$; minute 4: $F_{(1,18)} = 4.13$, $p = 0.057$; minute 4: $F_{(1,18)} = 5.95$, $p = 0.025$).

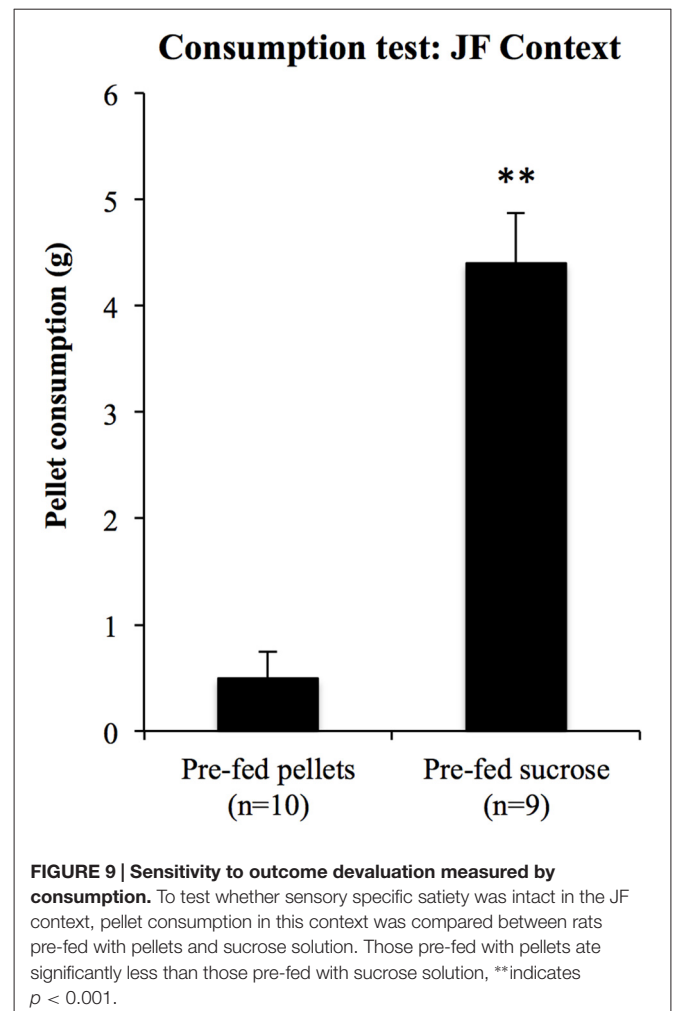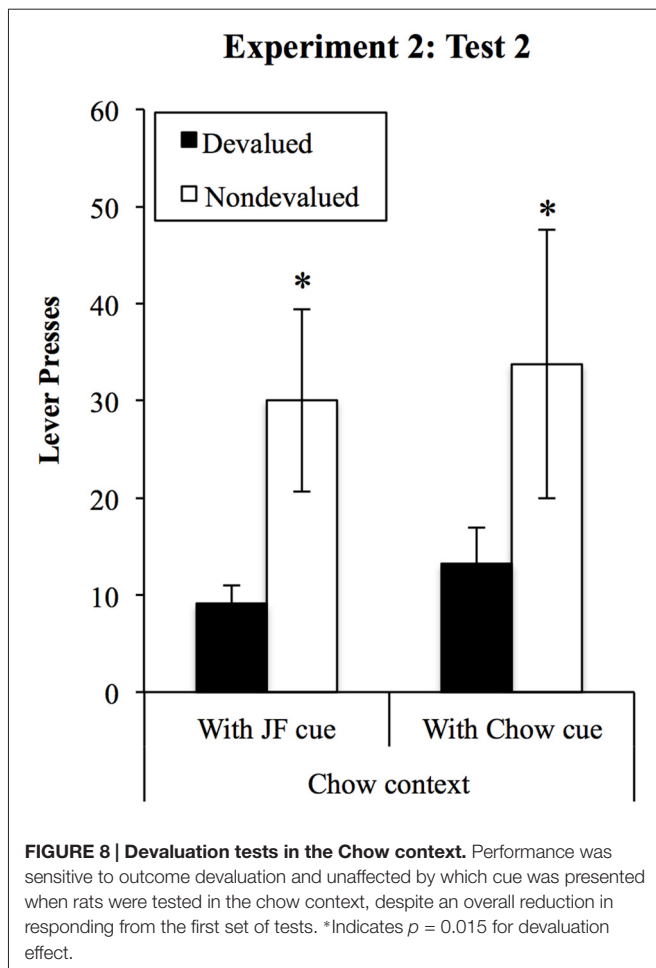### Sensitivity to devaluation in the Chow context

Responding on the devalued and non-devalued levers in the chow context tests is shown in **Figure 8**. The effects of the JF- and Chow-paired cues on performance were assessed using a (2) × (2) within-subjects ANOVA, with cue (JF and Chow) and lever (devalued vs. non-devalued). This analysis found a significant devaluation effect ($F_{(1,18)} = 7.17$, $p = 0.015$) but no effect of cue ($F < 1$) and no interaction between cue and lever ($F < 1$).

Sensitivity to devaluation measured with consumption in the JF context

For the consumption test in the JF context, 10 rats were pre-fed with pellets and nine were pre-fed with sucrose solution for 20 min prior to a 10-min test of pellet consumption in the JF context. Consumption during test is shown in **Figure 9**. Pellet consumption in the JF context was significantly reduced in rats pre-fed with pellets relative to those pre-fed with sucrose solution ($F_{(1,17)} = 57.29$, $p < 0.001$); thus, specific satiety itself was intact even when rats were tested in the JF context.

Discussion

In Experiment 2, we tested whether the effects of the JF context on sensitivity to devaluation would be affected by the presentation of discrete cues paired with JF and Chow. Results indicated that presenting the Chow cue in the JF context improved sensitivity to the devaluation treatment and promoted goal-directed performance across the 5-min of the test. By

FIGURE 8 | Devaluation tests in the Chow context. Performance was sensitive to outcome devaluation and unaffected by which cue was presented when rats were tested in the chow context, despite an overall reduction in responding from the first set of tests. *Indicates $p = 0.015$ for devaluation effect.

FIGURE 9 | Sensitivity to outcome devaluation measured by consumption. To test whether sensory specific satiety was intact in the JF context, pellet consumption in this context was compared between rats pre-fed with pellets and sucrose solution. Those pre-fed with pellets ate significantly less than those pre-fed with sucrose solution, **indicates $p < 0.001$.

contrast, when the rats were tested in the JF context with the cue that was present in this context during training, the devaluation effect was not statistically significant. Because numerically the impact of the JF context did not appear as complete as in Experiment 1, analysis of bin data characterized this effect further, showing that the devaluation effect was significant in the first minute of the test but not in minutes 2–5. The initial sensitivity to devaluation may be because the JF context was somewhat degraded at the beginning of the test due to the absence of the auditory cue that was present during training and which was likely to have been a salient element of the context. Thus, in the first 10 min of the test, the absence of the JF-cue rendered the context incomplete. As the onset of the cue "completed" the context, goal-directed behavior was then undermined, but this effect was not apparent until the second minute of the test. Future studies could examine the effects of the auditory cues alone (or other individual elements of the context) to examine their contribution to the observed effect.

Next, we confirmed that sensitivity to devaluation was intact in the Chow context and unaffected by the presentation of the JF- or Chow-cue. These tests were conducted separately and after a period of re-training, because our primary aim

was to test a means for restoring goal-directed control in the JF context after observing impaired performance in this environment. A consequence of this approach is that the order of the four devaluation tests was not fully counterbalanced. Not surprisingly, overall responding was lower in the chow context tests (compare **Figures 7A**, **8**) likely due to cumulative extinction of responding resulting from the multiple tests. Importantly, significant devaluation effects were still found in both tests.

Consumption of JFs in the JF context steadily increased over context conditioning sessions such that, by the seventh exposure to this context, rats consumed approximately 30% of their daily calories in a single hour. It is possible, then, that rats associated the JF context not only with palatable tastes, but also with satiety signals and—perhaps—resistance to this satiety. Indeed, studies of cue-potentiated feeding find that contextual food cues can promote consumption even in non-deprived rats that have been pre-fed with the test food (Petrovich et al., 2007). Therefore, we explored whether insensitivity to devaluation in the JF context could be explained by poorer sensory-specific satiety in this environment and to rule out whether altered expression of satiety specifically within the JF context undermined the effectiveness of

the devaluation treatment. Results of the consumption test in the JF context showed that this was not the case: rats pre-fed with pellets ate significantly less of that same food than did rats pre-fed with sucrose, indicating that consumption was sensitive to the current value of pellets. The impact of this treatment, however, did not translate into changes in instrumental performance.

GENERAL DISCUSSION

The present experiments sought to further understand how food cues alter food-seeking behavior in ways distinct from consumption. Rats learned to associate one context with the consumption of highly palatable JFs and another with chow, prior to instrumental training conducted in a third environment. We then compared whether these contexts would modulate sensitivity to outcome devaluation. Experiments 1 and 2 found that rats failed to show sensitivity to devaluation in an environment previously paired with consumption of palatable foods. By contrast, rats' performance was goal-directed when tested in the context previously paired with chow and in the training context. Presentation of a discrete cue previously associated with chow restored sensitivity to devaluation when rats were tested in the JF context. Importantly, these effects of context (Experiment 1) and of the chow-cue (Experiment 2) were not attributable to floor or ceiling effects in responding. Rather, it was the distribution of responding between devalued and non-devalued levers that was impaired by the JF context in Experiment 1. Likewise, presentation of the chow-cue in Experiment 2 significantly improved the ability to direct responding toward the non-devalued outcome.

The current findings are consistent with past studies showing similar impairments in sensitivity to devaluation in contexts paired with ethanol (Ostlund et al., 2010) and methamphetamine (Furlong et al., 2015). While rats ate more JF than chow, and thus may have associated eating freely available food with the JF context which may, in some way, have interfered with having to earn food, as noted above, rats continued to respond in the JF context, they just did so indiscriminately. Furthermore, given the similarity between the current results and those seen in drug-paired contexts, it seems unlikely that the results can be explained by previous consumption. The novel result of the present study is that presentation of a chow-cue significantly improved performance in the JF context.

Although caution should be taken when comparing across experiments, it is worth noting that the reduction in goal-directed control within the JF context appeared more complete in Experiment 1. Responding on devalued and non-devalued levers in the JF context was all but equivalent in Experiment 1, but in the comparable test in Experiment 2 (JF context with JF cue) the devaluation effect approached statistical significance ($p = 0.063$, see **Figure 7A**). We are confident, however, that this does not reflect inadequate statistical power: all testing was within-subjects, and the above result was generated from the data of 19 animals, which is highly powered to detect devaluation effects. Moreover, the most important result in

Experiment 2 was that performance in the JF-context was significantly improved by the presentation of the Chow-cue, as supported by a significant interaction between cue and devaluation. Here it may be useful to consider that, while goal-directed and habit-based control are conceptualized as distinct systems competing for control over behavior (Corbit, 2016), variability within them is still meaningful. Thus, the transition from goal-directed to habitual control over behavior does not occur instantaneously, but instead shifts gradually with extended training (Dickinson et al., 1995) and can be accelerated by exposure to drugs of abuse (e.g., Nelson and Killcross, 2006) or to high-sugar/high-fat diets (Kendig et al., 2013; Furlong et al., 2014). A relevant parallel to consider is that extinction-paired "E-cues" reduce, rather than completely block, the relapse from extinction produced by various manipulations (Brooks and Bouton, 1993, 1994; Willcocks and McNally, 2014). In the present studies, goal-directed behavior was significantly poorer in a context associated with highly palatable food and, in turn, was improved by a discrete cue paired with chow. These incremental changes in sensitivity to devaluation are relevant to food-seeking because the regulation of energy intake is as much a question of what and how much to eat as it is whether to eat or not (Wansink, 2004). Both of the present experiments demonstrated poorer sensitivity to devaluation in the JF context, despite differences in the extent of this impairment, while Experiment 2 demonstrated that the presentation of the chow-cue significantly improved performance.

The use of a "chow cue" in Experiment 2 drew from literature exploring how discrete cues paired with extinction protect against the recovery of the original response that occurs following various manipulations (e.g., renewal, reinstatement etc.; Bouton, 2002). However, an important difference in our approach was that the cue we used to "rescue" performance was not associated with extinction of the JF context but rather had been paired with another distinct environment paired with chow. It is worth noting that consumption of chow during context conditioning was minimal. Therefore, it is difficult to determine the extent to which rats associated the chow cue with chow consumption and the relative value of chow in a non-deprived state, or with an environment in which JFs were unavailable. However, it seems likely that any association formed with chow itself would be with its taste and relative palatability upon sampling, given consumption was appreciable, but low. Therefore, presenting this cue in the JF context may have primed memory of the less-palatable chow or, possibly, of the other elements of the chow context. Importantly, the chow cue was not simply a distraction in the JF context, since overall response rates were unaffected. Instead, instrumental responding was better distributed toward the currently-valued outcome in the presence of the chow cue, indicating some restoration of evaluative processes guiding instrumental performance. By contrast, the high levels of JF consumption in the JF context provided opportunity for the JF cue to become associated with the palatable taste and hedonic properties of the JFs and, potentially, with short-term satiety occurring toward the end of the 1-h conditioning session. Regardless, when this cue was

presented in the chow-context (in Test 2) responding was still goal-directed. In summary, our data indicate that the chow cue was effective in disrupting the influence of the JF context to promote goal-directed performance like that seen in the Chow-context, perhaps by retrieving some aspect of that that context, or the chow within it, to improve the efficacy of the devaluation treatment.

The current experiments demonstrated contextual influences on sensitivity to devaluation using a within-subjects design. This is an interesting complement to past research showing that chronic exposure to diets high in sugar, or sugar and fat, promotes habitual performance as assessed by outcome devaluation (Kendig et al., 2013; Furlong et al., 2014). Taken together, these results show that highly palatable foods can impair sensitivity to devaluation both transiently (i.e., the current results) and over the longer term. It is interesting to speculate that in people, repeated exposures to palatable food-paired environments might come to disrupt decision-making processes that alter what and how much individuals eat in these environments. In turn, this increases consumption of high-fat, high-sugar foods contained in these environments, predisposing individuals toward a more lasting expression of habitual behavior toward foods. This tentative suggestion bears some resemblance to the "vicious cycle" model of obesity posited by Davidson et al. (2005) which centers on environmental factors that produce and perpetuate hippocampal insult (see also Hargrave et al., 2016). Hippocampal effects would not appear to contribute to the present results, since sensitivity to devaluation is unaffected by lesions of the hippocampus (Corbit and Balleine, 2000) and the shift between goal-directed and habitual performance instead relies on functional changes to corticostriatal circuits (Corbit, 2016).

In summary, the key message from the present experiments is that decision-making processes can be altered by diet and environments associated with consumption of highly palatable foods. Entering an environment where a certain food type is routinely consumed may bias decision-making processes that mediate future food choices. In places where there has been a history of eating so-called JFs—for example, food courts—this conditioning history may predispose people toward poorer food choices and perpetuate consumption of JFs. This might manifest as a decision to buy food despite a recent meal; selecting a less healthy option; or continuing to eat when no longer hungry. Our data also suggest that relatively simple interventions, such as reminders of reduced food value or interrupting the automatic processing of JF cues, might assist individuals in restoring control in environments where control over eating behavior is compromised. Smartphone apps designed to encourage healthy food choices and prevent "binge" episodes are one example, though their efficacy is still unclear, at least in clinical populations (e.g., Fairburn and Rothwell, 2015). Other manipulations of the external environment may also be effective. For example, one study found that college students selected healthier food options when signs throughout a food court highlighted healthy rather than unhealthy foods (e.g., salads vs. burgers; Mollen et al., 2013). A specific hypothesis prompted by the present results is to test whether a chow-paired cue produces similarly beneficial effects in animals showing habit-based performance following chronic diet exposure of the kind described above.

AUTHOR CONTRIBUTIONS

MDK conducted experiments with assistance from AMKC and JSR. MDK and LHC drafted the manuscript with assistance from AMKC and JSR. Statistical analyses were performed by MDK. LHC conceived and directed the project. All authors approved the final submission of the manuscript.

FUNDING

REFERENCES

Adams, C. D., and Dickinson, A. (1981). Instrumental responding following reinforcer devaluation. *Q. J. Exp. Psychol.* 33, 109–121. doi: 10.1080/14640748108400816

Balleine, B. W., and Dickinson, A. (1998). The role of incentive learning in instrumental outcome revaluation by sensory-specific satiety. *Anim. Learn. Behav.* 26, 46–59. doi: 10.3758/bf03199161

Berthoud, H. R. (2007). Interactions between the "cognitive" and "metabolic" brain in the control of food intake. *Physiol. Behav.* 91, 486–498. doi: 10.1016/j.physbeh.2006.12.016

Berthoud, H. R. (2012). The neurobiology of food intake in an obesogenic environment. *Proc. Nutr. Soc.* 71, 478–487. doi: 10.1017/S0029665112000602

Boggiano, M. M., Dorsey, J. R., Thomas, J. M., and Murdaugh, D. L. (2009). The Pavlovian power of palatable food: lessons for weight-loss adherence from a new rodent model of cue-induced overeating. *Int. J. Obes. (Lond)* 33, 693–701. doi: 10.1038/ijo.2009.57

Bouton, M. E. (2002). Context, ambiguity and unlearning: sources of relapse after behavioral extinction. *Biol. Psychiatry* 52, 976–986. doi: 10.1016/s0006-3223(02)01546-9

Bouton, M. E. (2011). Learning and the persistence of appetite: extinction and the motivation to eat and overeat. *Physiol. Behav.* 103, 51–58. doi: 10.1016/j.physbeh.2010.11.025

Bouton, M. E., Winterbauer, N. E., and Todd, T. P. (2012). Relapse processes after the extinction of instrumental learning: renewal, resurgence and reacquisition. *Behav. Processes* 90, 130–141. doi: 10.1016/j.beproc.2012.03.004

Brooks, D. C., and Bouton, M. E. (1993). A retrieval cue for extinction attenuates spontaneous recovery. *J. Exp. Psychol. Anim. Behav. Processes* 19, 77–89. doi: 10.1037/0097-7403.19.1.77

Brooks, D. C., and Bouton, M. E. (1994). A retrieval cue for extinction attenuates response recovery (renewal) caused by a return to the conditioning context. *J. Exp. Psychol. Anim. Behav. Processes* 20, 366–379. doi: 10.1037/0097-7403.20.4.366

Corbit, L. H. (2016). Effects of obesogenic diets on learning and habitual responding. *Curr. Opin. Behav. Sci.* 9, 84–90. doi: 10.1016/j.cobeha.2016.02.010

Corbit, L. H., and Balleine, B. W. (2000). The role of the hippocampus in instrumental conditioning. *J. Neurosci.* 20, 4233–4239.

Cornell, C. E., Rodin, J., and Weingarten, H. (1989). Stimulus-induced eating when satiated. *Physiol. Behav.* 45, 695–704. doi: 10.1016/0031-9384(89)90281-3

Davidson, T. L., Kanoski, S. E., Walls, E. K., and Jarrard, L. E. (2005). Memory inhibition and energy regulation. *Physiol. Behav.* 86, 731–746. doi: 10.1016/j.physbeh.2005.09.004

Dickinson, A. (1985). Actions and habits: the development of behavioural autonomy. *Philos. Trans. R. Soc. Lond. Biol. Sci.* 308, 67–78. doi: 10.1098/rstb.1985.0010

Dickinson, A., and Balleine, B. W. (1994). Motivational control of goal-directed action. *Anim. Learn. Behav.* 22, 1–18. doi: 10.3758/bf03199951

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., and Boakes, R. A. (1995). Motivational control after extended instrumental training. *Anim. Learn. Behav.* 23, 197–206. doi: 10.3758/bf03199935

Fairburn, C. G., and Rothwell, E. R. (2015). Apps and eating disorders: a systematic clinical appraisal. *Int. J. Eat. Disord.* 48, 1038–1046. doi: 10.1002/eat.22398

Furlong, T. M., Jayaweera, H. K., Balleine, B. W., and Corbit, L. H. (2014). Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum. *J. Neurosci.* 34, 5012–5022. doi: 10.1523/JNEUROSCI.3707-13.2014

Furlong, T. M., Supit, A. S., Corbit, L. H., Killcross, S., and Balleine, B. W. (2015). Pulling habits out of rats: adenosine 2A receptor antagonism in dorsomedial striatum rescues meth-amphetamine-induced deficits in goal-directed action. *Addict. Biol.* doi: 10.1111/adb.12316 [Epub ahead of print].

Halford, J. C., Gillespie, J., Brown, V., Pontin, E. E., and Dovey, T. M. (2004). Effect of television advertisements for foods on food consumption in children. *Appetite* 42, 221–225. doi: 10.1016/j.appet.2003.11.006

Hargrave, S. L., Jones, S., and Davidson, T. L. (2016). The outward spiral: a vicious cycle model of obesity and cognitive dysfunction. *Curr. Opin. Behav. Sci.* 9, 40–46. doi: 10.1016/j.cobeha.2015.12.001

Horstmann, A., Dietrich, A., Mathar, D., Pössel, M., Villringer, A., and Neumann, J. (2015). Slave to habit? Obesity is associated with decreased behavioural sensitivity to reward devaluation. *Appetite* 87, 175–183. doi: 10.1016/j.appet.2014.12.212

Kendig, M. D., Boakes, R. A., Rooney, K. B., and Corbit, L. H. (2013). Chronic restricted access to 10% sucrose solution in adolescent and young adult rats impairs spatial memory and alters sensitivity to outcome devaluation. *Physiol. Behav.* 120, 164–172. doi: 10.1016/j.physbeh.2013.08.012

Lachat, C., Nago, E., Verstraeten, R., Roberfroid, D., Van Camp, J., and Kolsteren, P. (2012). Eating out of home and its association with dietary intake: a systematic review of the evidence. *Obes. Rev.* 13, 329–346. doi: 10.1111/j.1467-789X.2011.00953.x

Mollen, S., Rimal, R. N., Ruiter, R. A., and Kok, G. (2013). Healthy and unhealthy social norms and food selection. Findings from a field-experiment. *Appetite* 65, 83–89. doi: 10.1016/j.appet.2013.01.020

Nelson, A., and Killcross, S. (2006). Amphetamine exposure enhances habit formation. *J. Neurosci.* 26, 3805–3812. doi: 10.1523/JNEUROSCI.4305-05.2006

Ostlund, S. B., Maidment, N. T., and Balleine, B. W. (2010). Alcohol-paired contextual cues produce an immediate and selective loss of goal-directed action in rats. *Front. Integr. Neurosci.* 4:19. doi: 10.3389/fnint.2010.00019

Petrovich, G. D. (2013). Forebrain networks and the control of feeding by environmental learned cues. *Physiol. Behav.* 121, 10–18. doi: 10.1016/j.physbeh.2013.03.024

Petrovich, G. D., Ross, C. A., Gallagher, M., and Holland, P. C. (2007). Learned contextual cue potentiates eating in rats. *Physiol. Behav.* 90, 362–367. doi: 10.1016/j.physbeh.2006.09.031

Reppucci, C. J., and Petrovich, G. D. (2012). Learned food-cue stimulates persistent feeding in sated rats. *Appetite* 59, 437–447. doi: 10.1016/j.appet.2012.06.007

Stroebele, N., and De Castro, J. M. (2004). Effect of ambience on food intake and food choice. *Nutrition* 20, 821–838. doi: 10.1016/j.nut.2004.05.012

Wansink, B. (2004). Environmental factors that increase the food intake and consumption volume of unknowing consumers. *Annu. Rev. Nutr.* 24, 455–479. doi: 10.1146/annurev.nutr.24.012003.132140

Weingarten, H. P. (1983). Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science* 220, 431–433. doi: 10.1126/science.6836286

Willcocks, A. L., and McNally, G. P. (2014). An extinction retrieval cue attenuates renewal but not reacquisition of alcohol seeking. *Behav. Neurosci.* 128, 83–91. doi: 10.1037/a0035595

World Health Organisation. (2016). Obesity and overweight. Fact sheet no. 311. Available online at: http://www.who.int/mediacentre/factsheets/fs311/en/

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.