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Progressive ratio responding in an obese mouse model: Effects of fenfluramine

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ABSTRACT

The progressive ratio schedule of operant responding is a well utilised task for assessing the rewarding aspects of abused drugs and natural rewards including food. Interestingly, progressive ratio paradigms have mainly been neglected in the field of animal research in obesity. Among the most widely studied mouse models of obesity is the leptin-deficient *ob/ob* mouse, characterised by hyperphagia and obesity. To date there are no studies on the behaviour of these mice in progressive ratio responding, thus we sought to validate the utility of the progressive ratio paradigm in obese mice and demonstrate its sensitivity to an anorectic drug challenge.

Ob/ob mice and their lean controls were tested in fixed ratio paradigms of different demand, extinction learning, and progressive ratio schedules with linear and exponential increments, followed by an anorectic drug challenge with fenfluramine (5 and 10 mg/kg).

Obese animals showed equal fixed ratio-acquisition and -responding for ratios 1 and 3, but displayed lower responding in ratios 6 and 9. Interestingly, obese animals showed equal motivation to respond in progressive ratio schedules. Fenfluramine dose-dependently induced anorectic effects in both genotypes and reduced progressive ratio responding significantly.

This study, for the first time, describes motivational food intake in an operant progressive ratio paradigm in *ob/ob* mice. Leptin deficiency did not alter appetitive learning or motivation in the progressive ratio. The utility and sensitivity of the progressive ratio task for studies on motivational food intake was demonstrated by a challenge with the anorectic agent fenfluramine.

1. Introduction

The progressive ratio schedule of operant responding was first described by Hodos (1961). Since then the progressive ratio has become a well utilised task for assessing the rewarding aspects of abused drugs and natural rewards including food in rats and to a lesser extent in mice (Cason et al., 2010; Katz, 1990; Richardson and Roberts, 1996; Roberts et al., 2007; Stafford et al., 1998). As in most operant behaviours, the progressive ratio requires the animal to respond, by nosepoking or lever pressing, to a certain stimulus to obtain a reward; e.g. sucrose, cocaine. In contrast to fixed ratio paradigms, in the progressive ratio schedule the response demand increases on a trial-by-trial basis within a single session by a pre-determined and often exponential increment (Arnold and Roberts, 1997). The session length is determined by the animal's motivation to obtain another reward and by the reinforcer potency (Griffiths et al., 1978; Hodos and Valenstein, 1962; Richardson and Roberts, 1996), as the session ends when a ratio was not completed within a certain amount of time (timeout interval, e.g. 15 min). Analysis of the breaking point (the last ratio fulfilled before time-out) and the number of rewards obtained, gives information on the strength of the reinforcer and the motivational drive of the animal.

The strong link between food intake, reward, motivation and obesity is well known (Berthoud, 2006; Davis et al., 2007; Lutter and Nestler, 2009), but interestingly progressive ratio paradigms have mainly been neglected in the field of animal research in obesity. Obesity is a world wide problem with increasing prevalence and the search for efficient substances causing appetite suppression and weight loss is a major target of today's pharmaceutical research (Bloom et al., 2008). The mechanisms underlying obesity, food intake and appetite control have been studied extensively in both, animals and humans but remain still unclear in

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many aspects (Morton et al., 2006). Two important neuromodulators involved in energy homeostasis, feeding and body weight regulation are leptin and serotonin. (Leibowitz and Alexander, 1998; Zhang et al., 1994). Leptin, an adipocytic and brain derived hormone encoded by the *ob* gene, binds to its receptor in the arcuate nucleus of the hypothalamus to suppress food intake by inhibition of the orexigenic and stimulation of the anorexigenic neurons (Friedman, 2000). One animal model of obesity specifically exploits the role of leptin namely the *ob/ob* mouse, which was first discovered over 60 years ago (Ingalls et al., 1950). This strain has an autosomal recessive mutation of the *ob* gene on chromosome 6 leading to a complete leptin protein deficiency (Zhang et al., 1994). *Ob/ob* mice are among the most widely studied mouse models of obesity and the metabolic syndrome (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). In these animals leptin deficiency leads to a lack of suppression of orexigenic neurons and decreased activation of anorexigenic pathways in the arcuate nucleus, resulting in extremely increased food intake, reduced activity and obesity. These may severely alter the behavioural repertoire of these animals.

The serotonergic [5-hydroxytryptamine (5-HT)] system of the brain is known to play an important role in appetite control and the regulation of food intake [for review see: (Blundell, 1977, 1992; Simansky, 1996; Vickers and Dourish, 2004)]. Fenfluramine stimulates central release of serotonin and inhibits serotonin reuptake [for review see:(Garfield and Heisler, 2009; Rothman and Baumann, 2002)], and thereby decreases food intake mainly via the serotonin 2C (Miller, 2005; Vickers et al., 1999, 2001), 1A and1B receptor (Lucas et al., 1998; Neill and Cooper, 1989; Simansky and Nicklous, 2002; Vickers et al., 1996).

Our recent data in appetitive learning tasks allowed us to gain confidence that *ob/ob* mice, despite their overt phenotype, are able to perform complex behavioural tasks appropriately (Finger et al., 2010). However, to our knowledge, no studies exist on the behaviour of leptin-deficient obese mice in progressive ratio responding. Thus we sought to demonstrate the utility and sensitivity of the progressive ratio schedule of operant behaviour in *ob/ob* mice and their lean littermates. To this end, we explored possible differences between genotypes in acquisition learning, motivational responding and extinction behaviour in a series of operant responding tasks including fixed ratio paradigms of different demand, extinction learning, and progressive ratio schedules with linear and exponential increments. Subsequently, we conducted an anorectic drug challenge with fenfluramine to assess if there was a differential sensitivity to its effects in the progressive ratio paradigm. Thus these series of experiments have the potential to significantly add to our knowledge of how *ob/ob* mice can be used in operant-based progressive ratio responding and inform future studies of novel anorectic compounds on motivated behaviour in a widely used animal model of obesity.

2. Materials and methods

2.1. Animals

In this study male *ob/ob* mice ($n = 8$) and their lean controls ($n = 8$) (Harlan, UK) were used. On the date of arrival mice were five weeks old. Animals were housed in standard holding cages ($33 \times 15 \times 13$ cm^3) in groups of four, with a separation of experimental groups. The holding room was temperature (21 ± 1 °C) and humidity ($55 \pm 10\%$) controlled and under a 12-h light/dark cycle (lights on 7.45 am). Water was available ad libitum throughout the whole study.

2.2. Food and body weight

Animals received standard lab chow (2018S Teklad Global 18% Protein Rodent Diet) on a free-feeding schedule for four days after arrival. For the operant habituation and testing animals were kept on a food restriction schedule. To maintain a body weight of 85% of the free-feeding body weight, animals received pre-weighed portions of standard lab chow inside their homecages after testing. The amount of chow fed was adjusted to the variations in body weight. Between 9 am and 10 am animals were removed from their homecages and weighed (precision 0.1 g).

2.3. Apparatus

Operant testing was performed in eight standard mouse operant chambers (MED-307A-B2; Med Associates Inc., St. Albans, VT), each located in a sound attenuating cubicle. Chambers (15.9 cm × 14.0 cm × 12.7 cm interior dimensions) were equipped with a stainless steel grid floor, a ventilating fan, a houselight, one illuminated nosepoke response hole and a food magazine. Responses were measured by ultra-sensitive photo beams at the food receptacle and the nosepoke hole. Responding at the nosepoke hole was recorded and reinforced by the delivery of a 20 mg peanut-butter flavoured sucrose pellet (Test Diet, Richmond, IN, USA) into the magazine from a 20 mg-pellet dispenser (Med Associates). Animals were always tested in the same operant chambers and assignment was counterbalanced by genotype. Boxes were not cleaned between animals, but un-eaten sucrose parts and dust were removed from food receptacles and a fresh sheet of absorbent paper was placed into the steel pan below the grid floor between runs. All test chambers were controlled by a PC using Med-PC software.

2.4. Experimental procedures

2.4.1. Habituation

Each animal was handled for 5 min three times per day for two days. Mice were then habituated to the operant chamber for two days and received five sucrose rewards pellets per animal in the homecage over night prior to testing. Magazine training was conducted on two consecutive days. Animals were placed into the operant chamber for 15 min twice per day with sucrose pellets freely available in the magazine and eating was recorded by the experimenter. The nosepoke response hole was closed with adhesive tape during habituation and following non-contingent training.

2.4.2. Non-contingent reward delivery

Animals received two 15 min sessions with non-contingent food delivery on day seven. Each animal was placed into the assigned operant chamber and the houselight was turned on at the start of the trial. Mice then had to initiate the trial by entering the food magazine with the head. The houselight was then turned off and after 30 s one reward pellet was delivered into the magazine, the houselight was turned on until another magazine entry was recorded. A new pellet was only given if the mouse had entered the food magazine after reward delivery.

2.4.3. Fixed ratio

Animals received one day of two 15 min training sessions in the fixed ratio schedule 1 (FR1), where the nosepoke hole was primed at the start of the session with a small chow pellet to initiate and increase nosepoking. Animals were placed into the chamber, the houselight was turned on and mice had to enter the magazine to initiate the trial. Then the houselight was turned off and the light (conditioned stimulus, CS) inside the nosepoke hole was turned on awaiting a response. One nosepoke then triggered the delivery of one sucrose pellet (unconditioned stimulus, US) into the magazine, the nosepoke light was turned off, the houselight was turned on and an entry into the magazine was required. Further nosepoke responding without previous entry into the magazine did not trigger the delivery of another reward. Within the two primed FR1 sessions, all animals formed the CS-US association.

From the next day on, mice received two daily 30 min FR1 sessions using the programme described above, but without priming of the nosepoke holes. Mice were trained in the FR1 setting for five consecutive days receiving a total of 10 sessions. Subsequently response demands were increased to FR3, FR6 and FR9. Again, mice received two daily sessions for five consecutive days in each training stage.

2.4.4. Linear progressive ratio

Following fixed ratio testing, mice were trained in a progressive ratio 3 (PR3) schedule (Hayward and Low, 2007). The number of required responses to obtain a reward was increased in steps of 3 for every subsequent reward ($r = 3n + 3$, with n as the number of reinforcers already received). Mice therefore had to fulfil linear response requirements of 3, 6, 9, 12, 15…. .The last ratio completed before a timeout period of 15 min elapsed was defined as the breakpoint. As each mouse had a time interval of 15 min to complete every ratio, the end of the testing session was individually determined by the mouse's performance. Mice were immediately removed after timeout and placed back into the homecage. When all animals from one run had timed out, boxes were prepared for the second set of animals. Animals were trained in the PR3 schedule for two days with two sessions daily. As testing times exceeded 120 min for some animals, all mice were tested once per day in the PR3 programme from day three on to avoid interference of inconsistent testing times with performance levels.

For 16 consecutive days animals were tested in the PR3 schedule in an effort to reach stable baseline performance. The criterion for stability was set at a maximum