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Review

Behavioural satiety sequence (BSS): Separating wheat from chaff in the behavioural pharmacology of appetite

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ABSTRACT

The history of anti-obesity drug development is far from glorious, with transient magic bullets and only a handful of agents currently licensed for clinical use. In view of recent progress in our understanding of the multiplicity of signalling pathways involved in appetite regulation, and the resultant deluge of reports on the anorectic efficacy of novel therapies, it seems timely to stress the need to differentiate treatments that suppress intake by primary means from those that only indirectly achieve this endpoint. The current article reviews the conceptual history of the behavioural satiety sequence (BSS), also known as the behavioural sequence of satiety, post-ingestive satiety, and the postprandial satiety sequence. Early research confirmed that natural satiation, produced by a caloric load on the gut, is associated with a predictable transition from feeding through grooming to resting. Although many less naturalistic manipulations are also capable of reducing food intake, very few do so without disrupting the normal structure of this feeding cycle. Thus, while CCK and D-fenfluramine reduce intake by accelerating but otherwise maintaining the integrity of the BSS, other anorectic interventions disrupt the BSS through response competition (e.g. D-amphetamine), nausea/discomfort (e.g. lithium chloride) and/or interference with taste-mediated positive feedback (e.g. quinine adulteration of the diet). A substantial literature now strongly supports the specific involvement of serotonin 5-HT_{1B} and 5-HT_{2C} receptor subtypes in satiety and in the anorectic effect of agents such as fenfluramine and fluoxetine. Recent BSS analyses have also identified rather selective anorectic profiles for the dual noradrenaline and 5-HT reuptake inhibitor sibutramine, the orexin-1 receptor antagonist SB-334867, and the broad spectrum opioid receptor antagonist naloxone. However, similar analyses have offered little/no support for the anorectic potential of the gut peptide PYY_{3-36} while the acute anorectic efficacy of cannabinoid CB1 receptor antagonist/inverse agonists appears largely to be secondary to response competition. In contrast, studies with low-dose combinations of naloxone and CB1 receptor antagonist/inverse agonists have very recently confirmed the potential of drug polytherapies not only in appetite suppression but also in attenuating/eliminating unwanted side-effects. In sum, as BSS analysis offers a reliable means of differentiating the wheat (primary anorectics) from the chaff (secondary anorectics), it should form an integral part of early phase testing in any anti-obesity drug screening programme.

Contents

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1. Introduction

A child of the 'fifties', behavioural pharmacology really only began to focus on patterns of spontaneous behaviour towards the end of the following decade (Glick and Goldfarb, 1976). Nowhere was this more apparent than in research on the psychobiology of aggression where, until the mid-60s, behavioural tests were limited to single outcome measures e.g. attack frequency (Rodgers, 1981). However, based on the pioneering work of Barnett (1960), Grant and Mackintosh (1963) and Scott (1966), researchers began to appreciate that overt attack – while important – was merely the culmination of a complex, choreographed, sequence of offensive and defensive acts and postures. They also understood that the whole pattern of 'agonistic' interaction was itself embedded within an even broader repertoire (or context) of non-agonistic social and non-social behaviours (e.g. Silverman, 1965; Krsiak and Steinberg, 1969; Miczek and Grossman, 1972; Blanchard et al., 1975). These conceptual developments, especially when linked with emerging video technology, provided the means to generate truly comprehensive profiles for the behavioural effects of physiological and pharmacological manipulations (e.g. Rodgers and Waters, 1985). Rather than being limited to endpoint analysis (e.g. suppression of attack), researchers could begin to address the question as to how particular endpoints had been reached. Thus, concurrent assessment of treatment effects on a range of behaviours within one and the same test would indicate whether such effects were merely secondary to sedation, ataxia or even psychomotor stimulation. Furthermore, if the treatment effect was found to be behaviourally-selective, the profile could be further interrogated to determine whether specific components of offensive behaviour (e.g. appetitive vs consummatory elements) were particularly sensitive to manipulation of the independent variable. Answers to these sorts of question led to the refinement of hypotheses concerning behavioural and/or molecular mechanism.

Remarkably similar issues and developments were evident in early work on the neurobiology of appetite. In the introduction to a now classic paper on feeding in wild and laboratory rats, Barnett (1956) stressed the fact that nearly all research up to that time had been on the end-products of feeding (i.e. the amount of food consumed) with little attention to 'behaviour in the strict sense'. An acknowledged exception to this generalisation was a landmark 'behaviouristic study of the activity of the rat' (Richter, 1922) which detailed a characteristic behavioural sequence in which feeding is followed by a brief period of grooming and then a longer period of resting. Although broadly similar observations were later reported by Bolles (1960) in his study of grooming in the rat, they had relatively little impact until their insightful application in the early-mid 1970s to the suspected role of the gut peptide cholecystokinin (CCK) in appetite.

2. The behavioural sequence of satiety

The earliest suggestion that CCK may function as a natural satiety signal came from studies by Smith and colleagues at Cornell, in which systemic administration of the peptide was consistently shown to suppress feeding (Gibbs et al., 1973a,b; Holt et al., 1974; Liebling et al., 1974; Smith et al., 1974). However, aware that the cessation of feeding is *per se* an ambiguous measure of satiety, the authors went on to use detailed behavioural analysis to further define the appetite-regulating effect of CCK. The results, reported by Antin et al. (1975), confirmed the work of Richter and Bolles in defining a characteristic 'behavioural sequence of satiety' in food-deprived rats presented with a liquid diet. They further demonstrated that, while absent in sham-feeding animals and in animals confronted with quinine-adulterated diet, this natural sequence is fully elicited by intraperitoneal injection of CCK (40 Ivy dog units/kg). These observations not only confirmed the direct relationship between the behavioural sequence of satiety and the natural process of satiation but, prophetically, also emphasised a crucial difference between two manipulations that produced otherwise identical end-points, i.e. whereas intake was suppressed both by diet adulteration and CCK, only the latter elicited the complete satiety sequence. Given its implications, but possibly a result of the popularity of automated analysis of the macro- and micro-structure of feeding (for review, see Blundell et al, 1979; Clifton, 1994, 2000; Smith, 2000a; Smith, 2000b), it is somewhat surprising that this new behavioural methodology was not more widely adopted until the early 1980s.

The case for detailed behavioural profiling in appetite research was very clearly outlined by Blundell and McArthur (1981) who used the concept of 'behavioural flux' (i.e. behaviours do not exist in isolation but are embedded in a constellation of related activities) to draw attention to the need to better differentiate primary and secondary effects of experimental treatments. They argued that by focusing on what superficially is the behaviour of interest (i.e. food intake), a rich fund of potentially crucial information is lost. More specifically, when feeding, animals do not eat continuously but break off to engage in other actions (e.g. drinking, exploration; Wiepkema, 1971a), while the natural process of satiation leads to a gradual though predictable transition from eating, through grooming and exploration, to resting. Thus, drugs could in principle reduce food intake physiologically by accelerating the satiety sequence or non-physiologically by interfering with its structural integrity. The advantages of the new profiling approach were exemplified using time-lapse videoanalysis of 24 h feeding tests with two anorectic agents, D-amphetamine and DL-fenfluramine. As an initial step towards the development of contemporary satiety sequence protocols, Blundell and McArthur differentiated 3 elements of the *feeding cycle* (appetitive, feeding and satiety phases) and, within each, recorded time spent on activity, grooming, drinking and resting. The vehicle control profile fully confirmed earlier research demonstrating the existence of the satiety sequence. The principal effect of amphetamine was to disrupt the feeding phase by markedly stimulating general activity, grooming and behavioural switching i.e. anorexia as a secondary effect. In contrast, fenfluramine selectively reduced time spent feeding while enhancing postprandial resting i.e. anorexia as a primary effect. This differentiation between two otherwise similar anorectic profiles was reminiscent of the earlier distinction between the anorectic effects of CCK and food contamination (Antin et al., 1975).

Somewhat surprisingly, satiety sequence methodology did not feature prominently in the literature between 1981 and 85. Two exceptions were studies by Kulkosky et al. (1982) on the satiety-enhancing effects of systemic (but not intraventricular) bombesin, and by Kushner and Mook (1984) who employed glucose and saccharin to confirm the earlier findings of Antin et al. (1975) on the role of post-ingestive factors in the satiety sequence. However, it is generally agreed (e.g. Dourish and Hutson, 1985; Halford et al., 1998) that the stimulus which provided the much-needed kickstart to the research career of satiety sequence methodology was the now widely-cited calibration study by Blundell et al. (1985) Extending the observational techniques used by Blundell and McArthur (1981), these authors not only recorded food intake and a range of related measures (latency to eat; duration of eating; number of eating bouts; local eating rate) but also the frequency and duration of many non-feeding behaviours seen during the feeding

tests (rearing; walking; sniffing; grooming; resting; drinking). Furthermore, they used the duration data to plot (by 10 min timebin) the timecourses of eating, grooming and resting over the 1 h test sessions. In this pioneering manner, detailed information was obtained on food intake, feeding and non-feeding behaviours, and the entire satiety sequence. Consistent with earlier observations, the results showed that, under control conditions, animals displayed the full satiety sequence in which feeding dominated the early part of the test but gradually gave way to grooming and resting. Prefeeding (to the extent of 70% of normal 1 h intake) fulfilled predictions in leading not only to a marked reduction in food intake/time spent feeding but also a clear acceleration (shift to the left) in the satiety sequence. In contrast to this profile of natural satiety, two other 'anorectic' manipulations that reduced intake to the same extent as prefeeding (circa 50%) had very different behavioural signatures. Thus, whereas 'prefeed anorexia' was associated with increased latency and a short duration of quite rapid feeding, 'lithium anorexia' was brought about by reduced the number of eating bouts and a markedly reduced rate of eating and 'quinine anorexia' by a large number of brief eating bouts involving a slow rate of eating. These contrasting profiles confirmed that changes in behavioural structure can provide a means of diagnosing whether a suppression of food intake is produced through a normal physiological process or by physiological disturbance (lithium nausea) or food contamination (bitter-tasting diet). In this manner, the 1985 calibration study extended earlier observations (i.e. CCK vs quinine; fenfluramine vs amphetamine) on the utility of satiety sequence methodology in differentiating primary and secondary anorexia.

Over the past 25 years, the behavioural satiety sequence (BSS), as it has become more widely known (Montgomery and Willner, 1988), has been used to assess the behavioural specificity of numerous appetite-modulating manipulations (procedural, pharmacological and/or genetic). Prior to a brief overview of these findings (see also Halford et al., 1998), it seems appropriate to outline a typical BSS protocol.

3. Typical BSS protocol

Despite quite wide inter-laboratory variation in methodological detail (Table 1), the basic structure of the BSS has been repeatedly observed since the pioneering studies of Richter (1922), Bolles (1960) and Antin et al. (1975). Unsurprisingly, current protocol at Leeds closely follows that pioneered by Blundell and McArthur (1981); Blundell et al. (1985); and Halford and Blundell (1997), is essentially very straightforward, and closely adheres to the rudiments of good laboratory practice. Fundamental to the protocol is consistency, ranging from the source of experimental animals, through extensive habituation, to well-designed experiments conducted in a controlled environment. These simple but crucial practices help minimise non-specific confounds arising from stress and/or unwanted distractions during testing. Stress *per se* is well-known to suppress food intake while distractions caused by movement and/or noise in the test room (or nearby) would naturally reduce the time available for other behaviours, including feeding (see Latham and Blundell, 1979).

Fig. 1 illustrates the basic BSS protocol as currently used in the Leeds laboratory. Readers are invited to compare this protocol to that described earlier by Halford and Blundell (1997); much is the same, but there are some notable differences e.g. absence of food deprivation, more extensive habituation, statistically-confirmed stability of basal food intake, and prolonged bodyweight tracking.

3.1. Acclimatisation

On arrival from a reputable commercial supplier, animals (adult male Lister hooded rats, circa 200–250 g) are caged in groups of 4 or 5 and maintained under a 12 h reversed light cycle (lights off: 0700 h) in a climate-controlled environment (temperature: 21 ± 1 °C; relative humidity: 50 ± 5%). A daily routine of handling and weighing (c. 0900 h) is initiated at this time and maintained until 7 days following the final test session (see Section 3.4). Group-housing is used for the first week in order to facilitate acclimatisation to local laboratory conditions (light cycle, background sounds and smells, new personnel), while the reversed light cycle acknowledges the nocturnal nature of rodents (e.g. Barnett, 1956) and permits behavioural testing during the active part of the 24 h cycle. After the first week of group-housing, animals are transferred to individual cages which facilitates bodyweight tracking as well as initial familiarisation with the test diet. It is important to note that animals can still see, hear and smell their conspecifics in adjacent cages and are therefore not socially isolated. Additional daily stimulation is provided in the form of routine handling and weighing. Towards the end of the second week of individual housing (i.e. third week following arrival in the lab), animals are individually exposed in their home cages to glass food pots containing samples of the test diet. This diet is simply a hydrated form of the maintenance diet (Bantin & Kingman Universal Diet UK; 1 g dry = 3.125 g mash; digestible energy value = 4.48 kJ/g) that is made up freshly early on test days. In contrast to pelleted chow, mash is highly palatable (thereby negating the need for food deprivation; Ishii et al., 2003b), while its consistency minimises spillage and reduces any tendency towards food hoarding (Halford et al., 1998). The home cage is used for this familiarisation process (3 h daily on 2 consecutive days) in order to encourage sampling of a new food but in a familiar context. Despite the *new object reaction* (Barnett, 1956), all animals consume at least some mash during these introductory sessions.

3.2. Habituation

Formal habituation commences in week 4 and comprises 5 daily 1 h sessions run under pseudo-experimental conditions, i.e. under experimental conditions but without the independent variable. For each of these sessions (run under dim red light during the mid-dark phase), non-deprived animals are treated with drug vehicle in a neutral environment

Table 1
Some common sources of inter-laboratory variation in BSS methodology.

Subjects
• Species, strain, gender, age
• Housing (grouped, individual)
• Maintenance light cycle (normal, reversed)
Subject preparation
• Acclimatisation to laboratory environment and lab personnel
• Familiarisation with test diet
• Habituation to handling, injection and all test procedures
• Objective confirmation of stable basal food intake
• Nutritional status (deprived, free-feeding)
Test environment
• Home cage or neutral arena
• Test diet (pellet, powder, mash, liquid)
Test conditions
• Test period relative to light cycle (light phase, dark phase)
• Illumination level (normal, dim, dark)
• Test duration (40 min, 60 min, 90 min, 4 h)
• Background noise level (low, moderate, high)
• Interruptions/distractions
Behavioural scoring
• Observer awareness (blind to treatment?)
• Observer accuracy and reliability (reported, not reported)
• Live or from video record
• Time-sampling or continuous monitoring
• Sampling rate
• Measures recorded (all or BSS only)
BSS format
• Number of rats displaying given behaviour when sampled
• Proportion of each timebin devoted to eat, groom, rest
• Number of observations of given behaviour/unit time
• Percentage of observations of a given behaviour in each timebin
• Actual duration (s) of given behaviour within each timebin

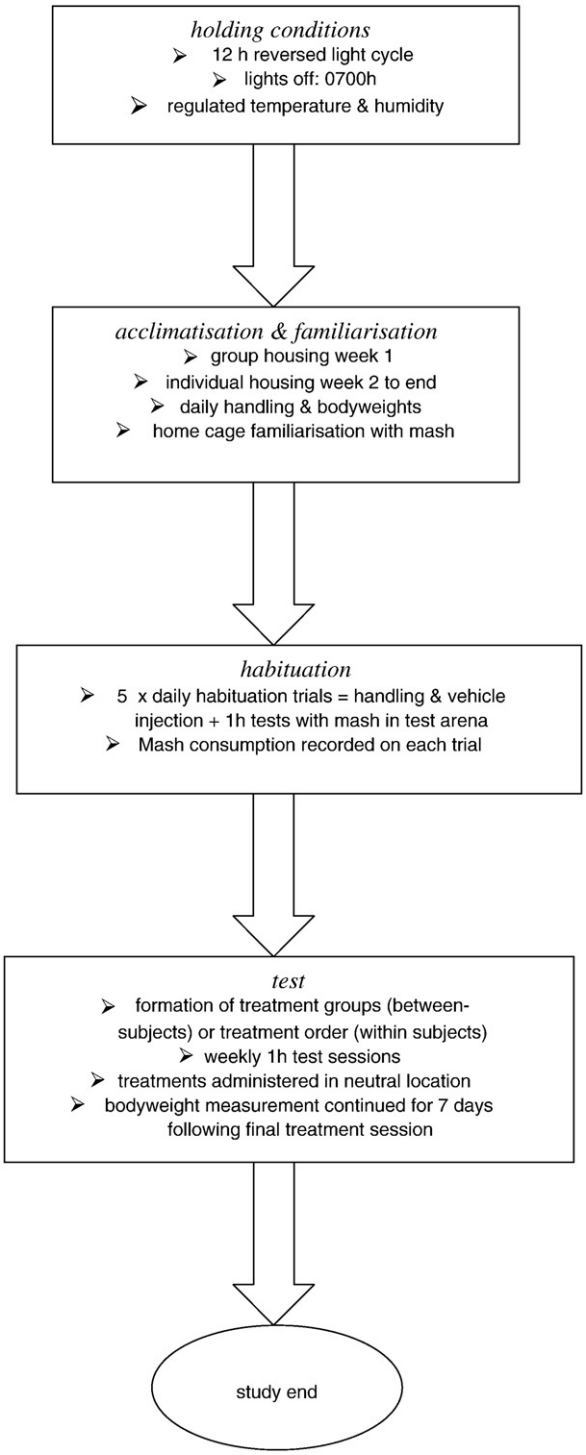

Fig. 1. Basic BSS protocol as currently used in the Behavioural Neuroscience Laboratory (Institute of Psychological Sciences) at The University of Leeds, UK.

(neither holding room nor test laboratory) following which they are replaced in their home cages (chow removed) and returned to the holding room. After the study-appropriate injection-test interval (ITI), rats are transported in their home cages over the short distance (20 m) between the holding room and test laboratory, and introduced into the test arena with preweighed mash and tapwater *in situ*. The test arena is a large glass vivarium (60×30×45 cm), the floor of which is lightly covered with wood shavings; use of home cage bedding may help to attenuate any initial neophobic response. The glass food pot containing preweighed test diet is secured to the centre of the floor with an annular metal mounting, while a water bottle is suspended from one of the end walls. Mash consumption is accurately measured (accounting for any spillage) on each of these 5 trials, with the data used to (i) statistically confirm the development of stable intake patterns prior to the test phase and, for between-subjects designs, (ii) form treatment groups with statistically-equivalent basal intake scores. For the latter, care is also taken to ensure that these matched groups are equivalent not only in terms of basal food intake but also in starting bodyweight. As would be expected, intake scores are significantly lower on the first couple of habituation trials (c. 10–15 g) but thereafter typically stabilise at c. 20 g. Control food pots are used (both on habituation and test trials) to objectively confirm the invariably minimal loss of food mass (typically <0.2%) through evaporation alone.

3.3. Experimentation

The experimental phase commences within 72 h of the final habituation trial, and the design can be either between- or within-subjects. In view of the well-documented individual variation in food intake and behaviour, our preference is for the within-subjects format in which animals serve as their own controls in a Latin-Square design. However, this is not always possible. For example, although a 7 day inter-test interval (wash-out period) is routinely employed, this may not be sufficient to negate carryover effects from compounds with long half-lives and/or active metabolites. Where such is suspected from pharmacokinetics and/or functional timecourse data, it is wiser to employ a between-subjects design. Irrespective of study design, however, non-deprived animals are randomly assigned to treatment, with appropriate counterbalancing of treatment order both within and between test days. As for habituation sessions, test sessions are run under dim red light during the mid-dark phase of the cycle. Following the study-appropriate ITI, animals are individually placed in the test arena with preweighed mash and ad lib water supply. The 1 h test sessions are recorded onto DVD by two low-light-sensitive monochromatic videocameras, one positioned in front of and the other directly above the arena. The videosignal is fed via an image merger to a nearby monitor and DVD recorder. Food pots (plus spillage) are retrieved and reweighed at the end of each test session, and animals returned to their home cages/holding room.

3.4. Behavioural scoring and dependent variables

Halford et al. (1998) discuss at length the different approaches that may be used to score behavioural test sessions and in particular the advantages of continuous monitoring over various forms of time-sampling. Suffice it here to say that, while certainly more labour-intensive in the short-term, the preferred method of continuous monitoring provides a very much more complete (latency, frequency, duration) and hence more accurate record of behaviours displayed. Furthermore, the scores for intake and feeding behaviour can be used to generate derivative measures including the average rate of eating and the average length of eating bouts. A complete list of dependent variables currently used in our laboratory is presented in Table 2. It should be noted that this default 'ethogram' can and must be considered flexible in that certain drugs or other manipulations (e.g. food contamination, gene knockout) may induce behaviours that are normally never seen or which are extremely rare. The experimenter must therefore be alert to such possibilities and, as necessary, modify the ethogram and rescore the study (for a good example, see Tallett et al, 2007a).

Behavioural scoring from DVD record is typically performed blind to treatment condition by a highly trained observer (intra-rater reliability ≥ 0.9) using ethological software (Hindsight; Weiss, 1995) that permits real-time scoring by direct keyboard entry to a PC or laptop. Once the entire experiment has been scored, data can be readily downloaded to a statistical package (such as Statistica or SPSS) for further processing. In addition to analysing the total 1 h scores,

Table 2
Measures routinely scored in BSS studies in the Leeds Laboratory. s = seconds, g = grams, f = frequency, d = duration.
Table adapted from Halford et al. (1998), Rodgers et al. (2001); Tallett et al. (2009b).

Measure	Definition
Latency to locate food	Time in s from start of session to first contact with food pot
Latency to commence eating	Time in s from first contact with food source to first eating episode
1 h food intake	Mash consumption in g, accommodating any spillage
Eating	Biting, gnawing, or swallowing food from food pot or forepaws (f and d)
Drinking	Licking the spout of the water bottle (f and d)
Grooming	Licking of the body, feet and genitals; stroking of face and whiskers with forepaws; biting the tail (f and d)
Scratching	As distinct from grooming; repetitive ipsilateral hindpaw scratching of flanks, neck and head (f and d): a low probability behaviour but seen to occur at unusually high levels in response to CB1 receptor antagonist/inverse agonists
Locomotion	Walking around the cage or circling; movements involving all four limbs (f and d)
Rearing	Forepaws raised from cage floor; either supported against a wall or free-standing (f and d)
Sniffing	Rapid wrinkling of nose/twitching of vibrissae directed at a feature of the environment; head movements with rear limbs immobile (f and d)
Resting	Sitting or lying in a relaxed position with head curled to body or resting on floor; animal inactive (f and d)
Stop	Previously called 'freezing', but does not meet formal definitions of the classical defence response: refers to cessation of and subsequent return to ongoing behaviour (f and d)
Eating rate	Food consumed in g divided by time spent eating in min
Eat bout length	Time spent eating in s divided by total frequency of eating episodes

treatment effects on behavioural timecourses are studied by dividing the test session into 12×5 min timebins. Although such analyses are routinely performed on the frequency and duration scores for all recorded behaviours (apart from those with low probability under current test conditions e.g. drinking), they are particularly useful for the visualisation of treatment effects on the BSS. Although different methods can be employed to illustrate the BSS (e.g. frequency of observation, absolute duration, percent timebin; see Table 1), we prefer to plot the absolute durations of eating, grooming and resting for each 5 min timebin. To enhance clarity, separate charts are prepared for each treatment condition comprising the experiment. When used *in conjunction with* the more routine behavioural analyses described above, the BSS profiles can help to determine whether treatment effects on food intake are behaviourally-selective, whether they are associated with a normal behavioural structure, and whether they are accompanied by a temporal shift (i.e. acceleration or delay) in the sequence. Visualisation of shifts to the left or right in the BSS can be aided by the use of vertical lines to denote roughly the point at which resting begins to dominate feeding in the behavioural repertoire (see Fig. 2 for example). It should however be emphasised that these visual aids are neither mathematically-derived nor statistically evaluated. As the BSS is a stochastic (i.e. probabilistic) sequence, and since animals will repeatedly (but less and less often) return to the food pot during the process of satiation, the identification and quantification of unambiguous eat-rest transitions *for individual subjects* has proven anything but straightforward (Goodson and Blundell, unpublished).

Fig. 2 also emphasises a crucial point made previously by many authors (e.g. Barnett, 1956; Wiepkema, 1971a; Antin et al., 1975; Latham and Blundell, 1979; Blundell and McArthur, 1981; Blundell et al., 1985), and this is the fact that feeding in mammals is a discontinuous process — it alternates with periods in which the animal engages in non-feeding behaviours e.g. exploration. Barnett (1956) went so far as to suggest that the function of this variable behaviour pattern is to make possible the rapid learning of topography, and especially the whereabouts of food, water and shelter. Thus, even during the 'peak' feeding response (e.g. time periods 1–5, Fig. 2), animals are seen to spend only up to 35% of their time feeding which logically means that they break off to engage in other behaviours (see also Wiepkema, 1971a). Clearly, as the session progresses and they become increasingly sated, animals spend increasing amounts of time in non-feeding activities. As such, treatments that primarily influence these non-feeding activities will only secondarily influence feeding (Blundell and McArthur, 1981).

Finally, in addition to food intake and behaviour, bodyweight data (collected from the start of the study through to one week following the final treatment) are used to confirm the equivalence of testday bodyweights, to monitor the health status of animals throughout a study (via normal growth curves), and to detect any longer-term effects of acute drug treatment on weight gain. Most of these analyses are carried out using actual bodyweights and/or scores for absolute weight gain. However, finer-grain analyses (growth curves by treatment condition) are made possible by converting absolute weight gain scores to daily changes expressed as a percentage of test day bodyweight.

4. Pharmacology of the BSS

4.1. Further calibration studies

As summarised in Table 3, many calibration studies have now confirmed that the BSS is produced only by the presence of a caloric load in the gut (Antin et al., 1975; Kushner and Mook, 1984; Montgomery and Willner, 1988) and/or by preabsorptive satiety factors e.g. CCK

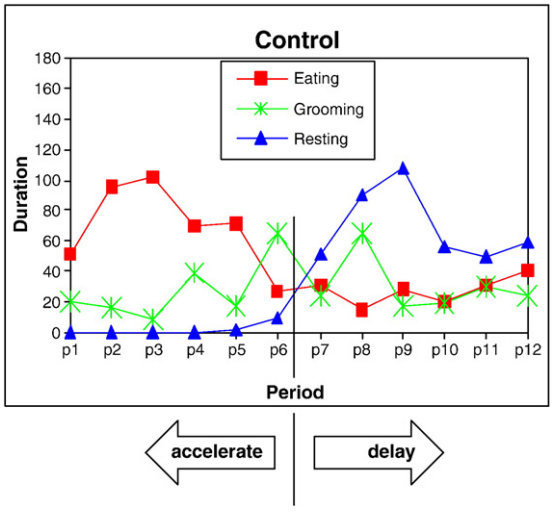

Fig. 2. The behavioural satiety sequence (BSS) in undeprived adult male Lister hooded rats tested for 1 h with palatable mash ($n = 10$). Data shown are mean duration (s) scores for each of the three behaviours (eat, groom, rest) in each of 12×5 min timebins comprising the 1 h test. As shown by the horizontal arrows, the vertical line (representing the transition from eating to resting i.e. behavioural satiety) would shift to the left (accelerate) with an anorectic agent and to the right (delay) with an appetite stimulant. Behavioural selectivity of treatment would be indicated by preservation of the sequence despite acceleration or delay.
Adapted from Rodgers et al. (2002).

Table 3
'Calibration' studies on the BSS. A summary of studies demonstrating the credentials of the BSS as a valid behavioural tool for the study of satiety, and as a means to differentiate primary and secondary treatment effects on food intake.

Independent variable	Main finding	Reference/s
Sham-feeding	Full BSS not elicited	Antin et al. (1975)
Glucose vs saccharin feeding	Full BSS to glucose but not to saccharin	Kushner and Mook (1984)
Sucrose feeding	Concentration-dependent full BSS	Montgomery and Willner (1988)
Presatiation	'Dose'-dependent acceleration of BSS	Blundell et al. (1985); Dourish (1992); Cooper and Francis (1993); Kitchener and Dourish (1994); Halford et al. (1998); Ishii et al. (2003b); Lievens et al. (2009)
Fasting	'Dose'-dependent delay in BSS	Ishii et al. (2003b)
Lithium chloride (nausea)	Disruption of BSS, and slowed rate of eating	Blundell et al. (1985); Ishii et al. (2004); Lievens et al. (2009)
Quinine contamination of diet	Disruption of BSS (repeated food sampling, digging)	Antin et al. (1975); Blundell et al. (1985); Halford et al. (1998); Ishii et al. (2003c); Lievens et al. (2009)
Cholecystokinin (CCK)	Marked acceleration/short-circuiting of BSS	Antin et al. (1975); Dourish (1992); Ishii et al. (2005b); Lievens et al. (2009); Verbaeys et al. (2009)
D-amphetamine	Disruption of BSS via response competition (hyperactivity)	Blundell and McArthur (1981); Halford et al. (1998)
Dark phase vs light phase testing	Clear BSS apparent under both conditions but dark phase testing reduces postprandial resting and delays the eat-rest transition	Tallett et al. (2009c)

(Antin et al., 1975; Dourish, 1992; Ishii et al., 2005b; Lievens et al., 2009; Verbaeys et al., 2009). Fasting has been found to delay the BSS (shift to the right) to an extent dependent upon the duration of food deprivation (Ishii et al., 2003b). Crucially, from the viewpoint of anti-obesity drug development, the BSS is also influenced in a predictable way by presatiation, with numerous reports confirming a load-dependent acceleration of the sequence (shift to the left; Blundell et al., 1985; Dourish, 1992; Cooper and Francis, 1993; Kitchener and Dourish, 1994; Halford et al., 1998; Ishii et al., 2003b; Lievens et al., 2009). Clearly, selective anorectic treatments, i.e. those that reduce food intake by primary action on the mechanisms of appetite regulation, would be expected to have this behavioural signature. In contrast, non-selective manipulations reducing intake only through secondary/indirect means would be expected to disrupt the sequence as, e.g., shown by studies on quinine-induced disruption of the taste-mediated positive feedback on feeding (Wiepkema, 1971b; Antin et al., 1975; Blundell et al., 1985; Halford et al., 1998; Ishii et al., 2003c; Lievens et al., 2009), lithium-induced nausea (Blundell et al., 1985; Ishii et al., 2004; Lievens et al., 2009), and amphetamine-induced response competition (Blundell and McArthur, 1981; Halford et al., 1998). On a purely practical level, we have recently confirmed the superficially paradoxical finding that the BSS is most clearly apparent in animals that have been held on a normal light cycle and tested under normal laboratory illumination during the light phase of the 24 h cycle. Clarity here refers to a transition between eating and resting that occurs roughly half-way through a normal 1 h test session and which, in theory, allows for the detection of bidirectional shifts in the timing of the BSS. In contrast, the eat-rest transition occurs much later in animals held on a reversed light cycle and tested under dim red light during the dark/active phase of the cycle (Tallett et al., 2009c). While a late transition is probably optimal for the detection of accelerations in the BSS, its utility is somewhat compromised for treatments that delay the sequence.

4.2. 5-HT, its receptors and the BSS

In view of the early link between serotonin (5-HT) and appetite (Blundell, 1977), it is perhaps unsurprising that BSS methodology has been used most extensively in research on this indoleamine transmitter and its various receptor subtypes. Much of the early work in this area (see Halford et al, 1998) involved drugs that either release 5-HT (e.g. D-fenfluramine) or inhibit its reuptake (e.g. fluoxetine, sertraline, sibutramine). Consistent with the proposed role of 5-HT in promoting satiety, both early and more recent studies with these agents in rats and mice have overwhelmingly confirmed that elevated levels of synaptic 5-HT inhibit food intake and accelerate with BSS without disrupting its integrity (see Table 4).

As more selective pharmacological and genetic tools have become available, increasingly sophisticated questions have been asked about the specific receptor subtypes involved in mediating the effects of 5-HT on the BSS. As summarised in Tables 5 and 6, studies in rats and mice have consistently shown that agonists for 5-HT_{1B} and/or 5-HT_{2C} receptors (i.e. CP-94253, RU 24969, *m*CPP, TFMPP, Ro-60-0175. Ro-4590334) inhibit food intake and accelerate the BSS (Simansky and Vaidya, 1990; Kitchener and Dourish, 1994; Halford and Blundell, 1996b; Hewitt et al., 2000; Lee et al., 2002, 2004; Clifton et al., 2005). The ability of metergoline (a non-selective 5-HT receptor antagonist) to block the hypophagic but not the behavioural effects of fenfluramine (Vickers et al., 1996) and fluoxetine (Halford and Blundell, 1996a) had initially suggested possible differential mediation of 5-HT effects on intake and behaviour. However, this proposal has since been

Table 4
Summary of the effects of serotonin (5-HT), 5-HT releasers and 5-HT reuptake inhibitors on the BSS.

Independent variable	Main findings	Reference/s
D- and/or DL-fenfluramine	Acceleration of BSS (both rats and mice) Disruption of BSS (abolition of resting phase)	Blundell and McArthur (1981); Vickers et al. (1996); Webster et al. (2001); Halford et al. (1998); Vickers et al. (1999); Hewitt et al. (2000); Lee et al. (2004) Montgomery and Willner (1988); McGuirk et al. (1992)
Fluoxetine	Acceleration of BSS	Clifton et al. (1989); Willner et al. (1990); McGuirk et al. (1992); Halford and Blundell (1996a)
Sertraline	Acceleration of BSS	Simansky and Vaidya (1990)
Paroxetine and femoxetine	Acceleration of BSS	Halford and Blundell (1993)
Sibutramine	Acceleration of BSS	Halford et al. (1995); Halford et al. (1998); Tallett et al. (2009b)
Serotonin (systemic)	Acceleration of BSS	Edwards and Stevens (1991)

Table 5
Effects of 5-HT receptor agonists, 5-HT receptor knockout and overexpression of the 5-HT transporter (5-HTT) on the BSS. Drug administration is acute and systemic (intraperitoneal or subcutaneous) unless otherwise specified. PVN = paraventricular nucleus of the hypothalamus. 5-HTT = 5-HT transporter.

Independent variable	Main finding/s	Reference/s
5-HT_{1A} agonists		
8-OH-DPAT	Disruption of BSS (stereotypy)	Simansky and Vaidya (1990)
8-OH-DPAT (intra-PVN)	Acceleration of BSS	Lopez-Alonso et al. (2007)
5-HT_{1B} agonists		
CP-94253	Acceleration of BSS	Halford and Blundell (1996b); Lee et al. (2002)
RU 24969	Acceleration of BSS but also	Simansky and Vaidya (1990); Hewitt et al. (2000)
	Disruption of BSS (hyperactivity)	Kitchener and Dourish (1994)
5-$HT_{1B/2C}$ agonists		
*m*CPP	Acceleration of BSS	Simansky and Vaidya (1990); Kitchener and Dourish (1994); Hewitt et al. (2000); Lee et al. (2004)
TFMPP	Acceleration of BSS	Simansky and Vaidya (1990); Kitchener and Dourish (1994)
5-HT_2 agonists		
DOI	Disruption of BSS (hypoactivity)	Simansky and Vaidya (1990); Kitchener and Dourish (1994)
MK-212	Disruption of BSS (sedation)	Halford et al. (1997)
5-HT_{2C} agonists		
Ro-60-0175	Acceleration of BSS	Hewitt et al. (2000)
Ro-60-0175 (intra-PVN)	No clear effect on BSS	Lopez-Alonso et al. (2007)
Ro-4590334	Acceleration of BSS	Clifton et al. (2005)
5-HT_{1B} receptor null mutants	BSS similar to wildtype	Lee et al. (2004)
5-HT_{2C} receptor null mutants	BSS delayed relative to wildtype	Vickers et al. (1999); Hewitt et al. (2000)
5-HTT overexpression	BSS similar to wildtype	Pringle et al. (2008)

rejected on the grounds that the effects of 5-HT_{1B}, 5-HT_{2C} and 5-$HT_{1B/2C}$ receptor agonists on both measures are blocked by antagonists preferential for these receptors (Hewitt et al., 2000; Lee et al., 2002). Although the relatively normal BSS phenotypes of 5-HT_{1B} null mutants, 5-HT_{2C} null mutants and 5-HTT (5-HT transporter)-overpressing mice is suggestive of developmental compensation, these animals nevertheless display abnormal responses to pharmacological challenge (Table 6). Thus, relative to wildtype controls, 5-HT_{1B} knockout mice show reduced sensitivity to the anorectic and BSS-accelerating effects of *m*CPP (Lee et al., 2004) while 5-HT_{2C} knockout mice display reduced sensitivity to the anorectic and BSS-accelerating effects of D-fenfluramine (Vickers et al., 1999). Furthermore, while showing no difference in anorectic response to low-dose fenfluramine, 5-HTT overexpressing mice show hypersenstivity to the behaviourally-disrupting effects of high dose fenfluramine (Pringle et al., 2008).

4.3. Peptides and the BSS

The ability of the gut peptide CCK to induce (or short-circuit) the BSS, originally reported by Antin et al. (1975), has since been replicated by several laboratories (Dourish, 1992; Ishii et al., 2005b; Verbaeys et al., 2009; Lievens et al., 2009). Reciprocally, in an elegant series of studies (on what was termed the *postprandial satiety sequence*; see also Rusk and Cooper, 1989a,b; Cooper et al., 1990; Cooper and Francis, 1993; Kitchener and Dourish, 1994), Dourish (1992) demonstrated that systemic administration of either a CCK_A (devazepide) or CCK_B (L365,260) receptor antagonist increased feeding behaviour, delayed the onset of resting, and induced a shift to the right in the postprandial sequence (Table 7). Another early peptide study involved bombesin which, when administered systemically, inhibited food intake and feeding behaviour without disrupting the normal structure of behaviour (Kulkosky et al., 1982). However, while intake and feeding behaviour were also suppressed following intracerebroventricualr infusion of the peptide, the satiety sequence was grossly disrupted by excessive scratching and grooming. Very recently, Cooke et al. (2009) have reported that two other closely related gut peptides, neurotensin and xenin, suppress food intake when administered ICV to rats and systemically to mice. Although there was no discernible BSS in any of the test conditions (including saline control), both peptides were seen to markedly increase resting. As the effects on resting were apparent before any appreciable amount of feeding, it seems likely that this 'anorexia' was secondary to sedation.

In 2002, another gut peptide, $PYY_{3–36}$, hit the media headlines as a putative natural satiety signal (Batterham et al., 2002). In contrast to earlier studies demonstrating the potent *orexigenic* effects of $PYY_{3–36}$ when administered centrally (e.g. Gerald et al., 1996; Wyss et al., 1998; Iyengar et al., 1999), intraperitoneal (IP) administration of 3–100 μg/kg

Table 6
Further BSS studies on 5-HT receptor subtype involvement in the regulation of appetite. Metergoline — non-selective 5-HT receptor antagonist; WAY 100635 — selective 5-HT_{1A} receptor antagonist; GR 127935 — 5-$HT_{1B/1D}$ receptor antagonist; SB 242084 — selective 5-HT_{2C} receptor antagonist. 5-HTT — 5-HT transporter.

Manipulation	Main finding/s	References
Fenfluramine anorexia and acceleration of BSS	Anorexia, but not BSS advance, blocked by metergoline; no effect of WAY 100635. Antagonists devoid of intrinsic effect	Vickers et al. (1996)
Fluoxetine anorexia and acceleration of BSS	Anorexia, but not BSS advance, blocked by metergoline. Antagonist devoid of intrinsic effect	Halford and Blundell (1996a)
CP-94253 anorexia and acceleration of BSS	Both effects blocked by GR 127935 and SB 242084 but not WAY 100635. Antagonists devoid of intrinsic effect	Lee et al. (2002)
Ro 60-0175 anorexia and acceleration of BSS	Both effects blocked by SB 242084	Hewitt et al. (2000)
*m*CPP anorexia and acceleration of BSS	Both effects blocked by SB 242084 but not GR 127935	Hewitt et al. (2000)
RU 24969 anorexia and acceleration of BSS	Both effects blocked by GR 127935	Hewitt et al. (2000)
5-HT_{2C} receptor null mutants	Reduced sensitivity to effects of D-fenfluramine on intake and BSS	Vickers et al. (1999)
5-HT_{1B} receptor null mutants	Reduced sensitivity to anorectic and BSS effects of *m*CPP	Lee et al. (2004)
5-HTT overexpression	No difference to wildtypes in the anorectic and BSS effects of low-dose fenfluramine, but enhanced sensitivity to disruptive effects of high doses	Pringle et al. (2008)

Table 7
Effects of non-5-HT manipulations on the BSS. Drug administration is acute and systemic (intraperitoneal or subcutaneous) unless otherwise noted. ICV = intracerebroventricular; NAcc = nucleus accumbens.

Independent variable	Main finding/s	Reference/s
Devazepide (CCKA receptor antagonist)	Delay in the BSS	Dourish (1992)
L-365,260 (CCKB receptor antagonist)	Delay in the BSS	Dourish (1992)
Bombesin (systemic or ICV)	Acceleration of BSS (systemic); disruption of BSS by excessive grooming (ICV)	Kulkosky et al. (1982)
Bretazenil (benzodiazepine receptor partial agonist)	Enhanced feeding, followed by more rapid progression through BSS	Clifton and Cooper (1996)
$GABA_A$ receptor α2-knockout mice	BSS similar to wildtype	Morris et al. (2009)
$GABA_A$ receptor α2 (H101R) mice	BSS similar to wildtype	Morris et al. (2009)
Midazolam (benzodiazepine receptor full agonist)	Increased feeding in α2-knockout mice (but not wildtype)	Morris et al. (2009)
L-838417 (subtype-selective $GABA_A$ receptor agonist)	Increased feeding in wildtype and α2(H101R) mice	Morris et al. (2009)
Hippocampal lesions	No effects on BSS	Clifton et al. (1998)
SKF-38393 (dopamine D1 receptor agonist) or N-0437 (dopamine D2 receptor agonist)	No effects on BSS	Rusk and Cooper (1989a,b); Cooper et al. (1990)
SKF-38393 and/or LY-171555(D2 agonist):NAcc	Locomotor stimulation	Phillips et al. (1995)
Simmondsin (jojoba extract)	Disruption of BSS (hyperactivity)	Lievens et al. (2009)
Naloxone (opioid receptor antagonist)	Acceleration of BSS	Kirkham and Blundell (1984); Tallett et al. (2008a)
Orexin-A (hypothalamic neuropeptide; ICV)	Hyperphagia and delay of BSS	Rodgers et al. (2000, 2001)
SB-334867 (orexin1 receptor antagonist)	Blocks effects of orexin-A on intake and BSS Intrinsic anorectic effects and acceleration of BSS	Rodgers et al. (2001) Rodgers et al. (2001); Ishii et al. (2004, 2005a,b)
$PYY_{3–36}$ (gut peptide)	No effects on food intake or BSS at IP doses up to 100 μg/kg But (with very high 50 μg/kg intravenous dose) Anorexia and acceleration of BSS; effects blocked by Y2 receptor antagonist BIIE0246	Ishii et al. (2003a); Tschop et al. (2004); Boggiano et al. (2005); Holch (2009) Scott et al. (2005)
Neurotensin, xenin, neuromedin U (ICV)	Absence of BSS in controls; behavioural disruption (sedation/xenin and neurotensin; grooming/neuromedin U)	Cooke et al. (2009)
MTII (melanocortin 3/4 receptor agonist)	Disruption of BSS (excessive grooming)	Webster et al. (2001)
Rimonabant (cannabinoid CB1 receptor antagonist)	Disruption of BSS (excessive scratching and grooming)	Tallett et al. (2007a); see also Webster et al. (2003)
AM 251 (cannabinoid CB1 receptor antagonist)	Disruption of BSS (excessive scratching and grooming)	Tallett et al. (2007b)
Rimonabant plus naloxone, or AM 251 plus naloxone	Low-dose additive interaction: anorexia, acceleration of BSS and naloxone antagonism of rimonabant- or AM 251-induced compulsive scratching	Tallett et al. (2008b); Tallett et al. (2009a)
Sibutramine plus rimonabant	No low-dose interactions on intake, BSS or scratching	Tallett et al. (2010a)
Sibutramine plus naloxone	Intra-additive interaction: effects of low-dose combinations on intake and behaviour lower than predicted from the sum of individual effects	Tallett et al. (2010b)

was found to have potent *anorexigenic* effects in rats (Table 7). Unfortunately, consistent replication of the latter effect has proved extremely elusive (Tschop et al., 2004; Boggiano et al., 2005). In an initial study of $PYY_{3–36}$ in our own laboratory, not only did we fail to find any significant effect on food intake or BSS over the reportedly active dose range, we actually observed a transient increase in time spent feeding (Ishii et al., 2003a). These findings prompted a series of full dose–response experiments (Holch, 2009) in which the putative anorectic effects of the peptide were systematically examined as a function of: study design (between-subjects vs within-subjects), nutritional status (free-feeding vs fasted), test diet (chow vs mash), test environment (home cage vs test arena), test duration (30 min–4 h) and time of testing (light phase vs dark phase). In only one of 9 dose–response experiments, and at one dose level only, was a weak anorectic-like effect detected. Fig. 3 illustrates the total lack of effect of the peptide (3–100 μg/kg IP) on food intake and the BSS in one of these unpublished but wholly representative studies. In this context, it is pertinent to note that although Scott et al. (2005) did find both anorectic and BSS-accelerating effects of systemically-administered $PYY_{3–36}$ in rats, this result was obtained only following bolus *intravenous* (not intraperitoneal) infusion of a high dose of the peptide (50 μg/kg). Thus, despite the initial publicity, major inconsistency would appear to obviate a role for $PYY_{3–36}$ in the treatment of obesity. Consistent with this conclusion, a Phase II clinical trial on the potential of intranasally-delivered $PYY_{3–36}$ as an anti-obesity treatment was prematurely halted due to significant nausea and vomiting (Gantz et al., 2007). That said, the biotech company 7TM Pharma has reported encouraging anorectic and/or weight loss effects with Obinepitide (TM 30338; a synthetic analogue of both $PYY_{3–36}$ and pancreatic polypeptide) in animal models and in Phase I/II clinical trials (Melnikova and Wages, 2006; Li and Cheung, 2009; Sato et al., 2009).

Given the large number of hypothalamic neuropeptides involved in appetite regulation, it is most surprising that only one has thus far been subject to BSS analysis. Just over a decade ago, and virtually simultaneously, several research groups announced the discovery of two closely related hypothalamic peptides, orexin-A/hypocretin-1 and orexin-B/hypocretin-2 (see Rodgers et al, 2002 for detailed review). The orexins (*orexis*-Greek, meaning appetite) were so-named because they stimulated feeding when administered centrally. As summarised in Table 7, we replicated the hyperphagic effect of orexin-A (ICV) in 2000, additionally showing that moderate doses delayed the BSS while higher doses disrupted the sequence (Rodgers et al., 2000). A later study (Rodgers et al., 2001) reported that both effects of orexin-A were blocked by low doses of the selective orexin-1 receptor (OX1R) antagonist SB334867. At somewhat higher doses, the OX1R receptor antagonist exhibited intrinsic anorectic activity, both suppressing mash intake and accelerating the BSS. These effects were repeatedly confirmed in several subsequent studies that contrasted the profile of SB 334867 with that of lithium (Ishii et al., 2004) and CCK-8S (Ishii et al., 2005b) and which also reported a reduction in weight gain following single acute dosing (Ishii et al., 2005a). Although more recent work has tended to focus on the role of the orexins in the sleep–wake cycle, the lateral hypothalamic orexin system is very well placed to orchestrate the diverse subsystems (including arousal and vigilance) involved in foraging in potentially hostile environments (for further elaboration, see Rodgers et al., 2002).

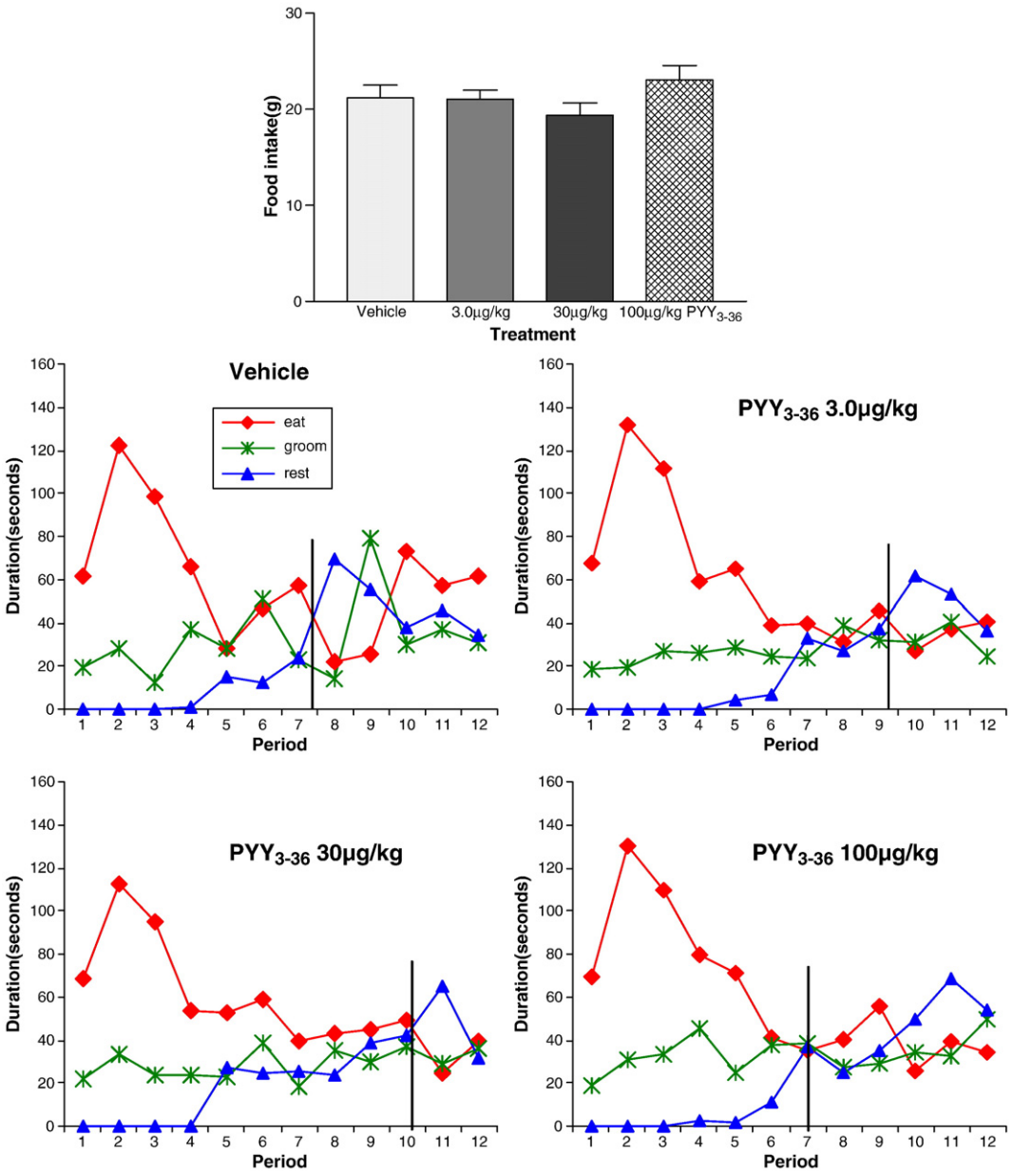

Fig. 3. Lack of effect of acute PYY_{3-36} (3–100 µg/kg, IP, 15 min before testing) on food intake (top panel) and the BSS (lower panels) in adult male rats presented with palatable mash for 1 h. The design of this study was between-subjects ($n=10$). Vertical lines represent the transition from eating to resting. Furthermore, no consistent anorectic response was evident over the same dose range in 9 other studies in which experimental design, nutritional status, test environment, test diet, test duration and light cycle were systematically assessed (Ishii et al., 2003a; Holch, 2009). See text for further details.

4.4. Cannabinoids, opioids and the BSS

Beyond 5-HT and peptides, two of the most widely researched neurohumoral systems involved in appetite regulation and energy homeostasis are the endogenous opioids and the endocannabinoids. It is beyond the scope of this short review to rehearse all the relevant empirical evidence. Rather, the interested reader is invited to consult the bibliographies of opioid and cannabinoid papers listed in Table 7. Suffice it (for current purposes) to say that, despite voluminous literatures on the anorectic effects of opioid receptor antagonists (e.g. naloxone and naltrexone) and cannabinoid CB1 receptor antagonist/inverse agonists (e.g. rimonabant, AM 251), very little attention has until recently been paid to the influence of these agents on the BSS. As suggested in earlier work by Kirkham and Blundell (1984), we have recently found that naloxone produces very potent anorectic effects under our test conditions, effects that do not disrupt behaviour but which accelerate the BSS (Tallett et al., 2008a). In contrast, the acute anorectic effects of the CB1 receptor antagonist/inverse agonists rimonabant (Tallett et al., 2007a) and AM 251 (Tallett et al., 2007b) appear to be secondary to response competition in that both agents severely disrupt the BSS via the induction of compulsive scratching and grooming (see also Webster et al., 2003). Consistent with this interpretation, both the anorectic and scratching/grooming syndrome seen in response to acute treatment with rimonabant show tolerance with repeat dosing (Vickers et al., 2003; Webster et al., 2003). It is interesting to note in passing that similar disruption of the BSS by excessive grooming has also been reported following ICV infusion of bombesin (Kulkosky et al., 1982) and by both systemic and central administration of the melanocortin MC4 receptor agonist MTII (Webster et al., 2001) and neuromedin U (Cooke et al., 2009). In view of current interest in the potential advantages of drug polytherapy for obesity (e.g. Adan et al., 2008; Vemuri et al., 2008; Greenway et al., 2009), we have most recently found not only additive

anorectic effects for low-dose combinations of naloxone and either rimonabant (Tallett et al., 2008b) or AM 251 (Tallett et al., 2009a), but also that co-treatment with the opioid receptor antagonist blocks the scratching response to low doses of the CB1 receptor antagonist/inverse agonists. In this context, it is pertinent to note that not all 'obvious' co-treatment strategies yield positive outcomes e.g. sibutramine + rimonabant (Tallett et al., 2010a) or sibutramine + naloxone (Tallett et al., 2010b). These studies further emphasise the substantial value of detailed behavioural profiling to basic research on the pharmacology of appetite.

In addition to peptides, opioids and cannabinoids, Table 7 summarises the effects of miscellaneous manipulations that either disrupt or have no effect upon the BSS. These examples further illustrate the discriminative value of detailed behavioural analysis in the study of treatment effects on food intake.

5. Concluding remarks

Echoing earlier arguments, Blundell and Alikhan (1990) nicely summarised the basic philosophy of the BSS approach as follows: 'the fact that a chemical compound reduces the number of grams of food consumed by experimental animals does not automatically identify that compound as an anorexic drug. The animal may be rendered sick, physiologically disabled or behaviourally impaired. The manner of the expression of behaviour contributing to the suppression of intake is crucial to a proper interpretation of the action of the compound' (p. 204). In their more recent review of pharmacological research on the BSS, Halford et al. (1998) poignantly drew attention to the fact that none of the then recently-discovered appetite-related peptides (e.g. neuropeptide Y, galanin, glucagon-like peptide and leptin) had yet been subject to detailed behavioural analysis. Over two decades later, and despite the obvious utility of the BSS, it is sobering to realise that the same claim is as valid today as it was back in 1998. None of these substances, nor any of the appetite-modulating signals discovered in the interim (e.g. agouti-related protein, amylin, ghrelin, melanin concentrating hormone, melanocortin, nesfatin-1, obestatin, pancreatic polypeptide PP, peptide YY, xenin), have been researched using the detailed behavioural profiling typical of BSS analysis. However, until such analyses are conducted, the jury will remain most definitely 'out' regarding the behavioural selectivity of their effects on food intake and/or weight gain and, hence, their potential relevance to the treatment of obesity and related disorders. With many potentially blind alleys ahead, not only is there considerable scope for the wastage of millions of dollars/euros but the disappointment of physicians and their patients.

Behavioural research of the type advocated in this article certainly appears time-consuming. However, it should not have gone unnoticed that, *from one and the same experiment*, an extremely rich database is generated for treatment effects on food intake, the frequency and duration of feeding and non-feeding behaviours, the structure of feeding behaviour (BSS) and bodyweight gain. As research methodologies evolve beyond the demonstrably flawed 'quick-fix' approach, it should be obvious that in-depth behavioural analysis at an early stage will actually foreshorten drug development programmes by helping to more rapidly identify the serious contenders (the 'wheat') and eliminating the no-hopers (the 'chaff'). To this end, BSS analysis (defined here as incorporating the detailed analysis of all behaviours displayed during feeding tests) should be seen as an integral component of early screening programmes. As such, we fully endorse the view that 'This type of behavioural analysis can sensitively discriminate between drugs that preserve or disrupt the BSS. This procedure therefore provides a methodology for the preliminary identification of drugs that appear to reduce food intake via processes linked to natural mechanisms of satiety' (Halford et al., 1998; p167). Of course, substances that succeed in meeting the BSS challenge following acute treatment should be further examined in this model following subchronic dosing in order to demonstrate maintained anorectic and weight loss efficacy. Still promising compounds should then ideally be subject to even more thorough behavioural assessment, e.g. meal patterning analysis, macronutrient preference and dietary-induced hyperphagia as well as studies on impulsivity and reward (hedonics and motivation). A more thorough understanding of exactly how diverse agents produce the same endpoint (reduced intake) should ultimately translate into more rational, effective and (probably) multitarget interventions for appetite control.

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