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Behavioral Satiety Sequence (BSS) for the Diagnosis of Drug Action on Food Intake

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HALFORD, J. C. G., S. C. D. WANNINAYAKE AND J. E. BLUNDELL. *Behavioral satiety sequence (BSS) for the diagnosis of drug action on behavior.* PHARMACOL BIOCHEM BEHAV **61**(2) 159–168, 1998.—The Behavioral Satiety Sequence (BSS) is the name given to the orderly transitions of eating, activity grooming and resting measured during the postingestive period. Because the BSS is considered to reflect the operations of natural physiological processes underlying satiety, the sequence can be used to discriminate between different drugs (and other manipulations) that reduce food intake via these natural physiological mechanisms or those that do so by interference. The BSS is only produced by the presence of a caloric load in the gut, and the preabsorptive satiety factors (such as CCK) the caloric load triggers. The BSS is most accurately defined by continuous observation rather than time or event sampling techniques [Partial Time Sampling (PTS) or Momentary Time Sampling (MTS)]. Continuous observation also allows the true duration and true frequency of each behavior to be analyzed. Continuous observation can be used to determine if the profiles associated with the reduction in food intake is caused by nausea, sedation, hyperactivity, or altered palatability of food. At the present time is it possible to identify a number of drugs whose suppression of food intake is associated with the disruption or preservation of the BSS. Drugs that increase synaptic 5-HT activity such *d*-fenfluramine, fluoxetine, and sibutramine all preserve the BSS and advance the onset of resting. The 5-$HT_{1b/2c}$ agonists mCPP and TFMPP and the 5-HT_{1b} agonist CP-94,253 produce similar effects. However, the 5-HT_2 agonist DOI and the 5-$HT_{1a/1b}$ agonist RU-24969 disrupt the BSS by inducing hyperactivity as does amphetamine. The 5-HT_2 agonist MK-212 disrupts the BSS by inducing sedation. Selective dopamine agonists, at low doses, such as SKF-38393 (DA_1) and LY-171555 (DA_2) also preserve the BSS. However, detailed behavioral analysis of the effects of many recently discovered putative satiety factors remains to be carried out. © 1998 Elsevier Science Inc.

Behavioral Satiety Sequence Food intake Locomotion Resting Serotonin (5-HT) Dopamine (DA)
Continuous observation Obesity drugs *d*-Fenfluramine Sibutramine Fluoxetine

A plethora of drugs exist that inhibit food intake when administered to experimental animals. One of the most contentious issues in the psychopharmacology of appetite concerns the identification of mechanisms underlying an observed reduction of eating. It cannot be denied that many chemicals could suppress eating by producing adverse physiological effects such as pain, or illness, or by altering neurochemical systems or behavioral dispositions so as to prevent the normal expression of appetite. Because animals cannot report their aversive side effects to the experimenter, the maintenance of a normal structure in strings of behavioral acts may be used as a means of verifying normal physiology. For over 20 years researchers have argued that the structure of behavior can be used as a marker of the nonphysiological effects of drugs on food intake (8).

The utility of the BSS rests initially on the validity of behavior as an indicator of toxic, pathologic, or nonphysiological events. The use of behavior to reflect the operation of associated physiological events has a large history. Wiepkema (70) noted how the physiological status (fed vs. deprived) of an animal altered both the feeding and nonfeeding elements of animal behavior. Blundell (9) argued that the structure of animal feeding behavior reflected the operation of contextual variables (e.g., food, drug, environment, physiological state) influencing food intake. Therefore, if environmental features were held constant, the structure of animal behavior could thus be used to reflect the natural operation of physiological processes involved in the modulation of food intake. It followed that anorectic drugs acting on distinct neurochemical mechanisms could be distinguished by their differing effects on behavior (6). Monitoring animal behavior, inducing feeding, and nonfeeding activities could provide a powerful biobehavioral assay of drug action on appetite. Using this biobehavioral as-

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say of feeding avoids problems of model validity, as it does not rely on modeling the human condition, but uses the animal's natural behavioral register as the yardstick of relevance. The credibility of the BSS itself has been largely established through studies of Smith and colleagues (2,26)

Description of the Behavioral Satiety Sequence (BSS)

The most detailed behavioral account of drug action on appetite can be gained by using the Behavioral Satiety Sequence (2) (BSS). Bindra and Blond (5) and Bolles (14) first observed that grooming occurred after eating and before resting. The BSS is a clearly identifiable stochastic progression of behavioral trends over time (not a strict deterministic sequence). The progression of the BSS is from an initial phase of eating, through peaks of active and grooming behavior, to an eventual phase of predominately resting behavior. The BSS appears robustly related to the processes satiation (meal termination) and the development of satiety (postingestive inhibition of eating). Sham feeding, the process of draining ingested food from a rats stomach, does not produce the BSS. The effect of sham feeding is reversed by an injection of the gut satiety factor cholecystokinin (2,26) (CCK). Conversely, antagonism of CCK blocks the development of satiety and the BSS (22). The BSS is not produced by saccharin ingestion, but can be produced by glucose preloads (47). Therefore, expression of the BSS relies on the presence of a caloric load within the gut, and the stimulation of satiety factors that this gastric load produces. A bout of prefeeding in animals advances the onset of resting while preserving the temporal components of the sequence. Reductions in food intake produced by adulterating food with quinine or injecting the animal with lithium chloride are not accompanied by the expression of the BSS (2,13). Hypophagia brought about by aversive factors such as unpalatable food or nausea do not produce the BSS. Thus, the behavioral profile of the BSS is linked only with the natural operation of satiety. Variations of this sequence have been described in the mouse, rhesus monkeys, and human infants (25,27,61).

Methodological Aspects of the BSS

The equipment needed to study the BSS is standard to most behavioral pharmacology laboratories. The researcher's basic needs are an observation arena, video cameras, video recorder, monitor, and a PC with a behavioral coding package. The procedural details of the Leeds studies can be found in Halford and Blundell (30,36) and Halford et al,. (38). We have also published a detailed account of our BSS methodology in Current Protocols in Neuroscience (36). For this review we will concentrate on methodological aspects that the authors consider essential to ensure accurate recording of the behavioral sequence.

The animal should be placed in an observation arena of sufficient size to allow full expression of the BSS. Rats are generally monitored under low-intensity red light in the dark phase of their daily light/dark cycle. During this phase they consume up to 80% of their food (62,63,68). This provides a high feeding baseline, and it is important to note that the effect of drugs on food intake vary, depending on the point in the light/dark cycle in which the animals are tested. For example, the hypophagic effect of the three anorectic compounds, fenfluramine, amphetamine, and phenlypropanolamine are significantly enhanced during the dark phase of the light cycle (19). Neurochemical fluctuations associated with circadian rhythms may thus contribute to the variability in drug action observed between varying experimental designs. This laboratory is the first to analyze the effects of many drugs on the temporal structure of rat behavior during the dark phase. A multiangled view of behavior is essential, as it eliminates the ambiguity experienced when coding behavior from a single angle (10,20). Video recording observation sessions allows the assessment of coding accuracy to ensure the quality of the behavioral data (inter- and intrarater reliability tests). The strength of behavioral observation rests on the competence of the observer. In addition, to code behavior, either live or from tape, the observer requires a PC with a data collection program capable of downloading data to a statistical analysis package (36).

The operational definitions of behavior currently used in our laboratory are shown in Table 1. This category system is designed to be mutually exclusive (behavior can be placed in one category only) and also exhaustive (all behavior is recorded). We have divided activity into sniffing, locomotion, and rearing to better define any drug-induced disruptions caused by increases in any one of these active behaviors. Similarly, other behavior categories could be subdivided. For example, grooming behavior can be defined into distinct subcategories such as coat grooming, facial grooming, and genital licks. Such classifications have been used by other researchers using the BSS to examine the nature of hyperphagia (39,40). In addition, an animal's position within the observation arena, in relation to the food, or the number of times the animal return to the food bowl (whether it eats or not), may indicate the nature of drug induced anorexia.

Comparisons among BSS experiments indicate that studies vary with regards to the phase of the light/dark cycle used for observation, the dose of the anorectic drug chosen, and the use of fasting as a prelude to eating. Another important difference is the form of data collection used during behavioral coding. Arrington (3) stated that the essential function of observation is accurate measurement of the incidence of specific behavioral acts or patterns under specific conditions. Additionally, Altmann (1) stated that the choice of sampling re-

TABLE 1

THE EIGHT BEHAVIORAL CATEGORIES USED FOR BEHAVIORAL ANALYSIS

Eating	Biting, gnawing, or swallowing food from wet mash dish directly or from front paws.
Drinking	Licking the spout water bottle.
Grooming	Licking of the body, feet, and genitals. Scratching of coat or head with hind leg. Stroking whiskers with paws. Biting of the tail.
Locomotion	Walking around cage or circling. Movements involving all four limbs.
Rearing	Front paws raised from the tank floor and either placed on the side of the tank or placed in front of the body.
Sniffing	Rapid wrinkling of nose (twitching of vibrissae) directed at some aspect of the environment. Head movement. Rear limbs immobile.
Resting (inactive)	Relaxed position with head curled to body or resting on the bottom of the cage, stretched out either on side or belly. Animal Inactive.
Other	None of the above.

Based on Antin et al. (2), Halford and Blundell (33,35).

stricts the kinds of behavior studied. In the study by Antin et al. (2), behavior was recorded using a form of "one–zero" or Partial Interval Sampling (PIS). If behavior occurs at any time during an interval "one" is scored. "Zero" is scored if the behavior is not present during that interval. In Antin's study the intervals were 1 min long and occurred every 2 min. Sampling procedures produce data in the form of modified frequency scores (58). The test of their accuracy is the extent to which these modified frequencies represent the true frequency and duration of behavior. Many studies have shown that PIS, when compared to a complete behavioral record, systematically overestimates true duration and underestimates true frequency of behavior (1,4,54,64–67).

Instantaneous or Momentary (1,54) Time Sampling procedures (MTS) are generally the preferred observational methods employed in contemporary BSS studies. The animal's behavior is noted at a fixed instant every 15 or 30 s, allowing many animals to be monitored at the same time. In addition to this economy, unlike PIS, the MTS estimation of true duration as represented in the modified frequency score is unbiased (48,58). Figure 1 shows 5 min of actual animal behavior, taken from one of our animal studies. Table 2 summaries the true duration and true frequency of each behavior, and their modified frequencies as produced by a simulation of a 15-s interval MTS procedure. It should be noted that MTS fails to detect all 14 occurrences of rearing and six occurrences of locomotion.

Various authors have described three distinct problems with MTS. First, the modified frequencies it produces systematically underestimate the true frequency of behavior (56). Altmann (1) noted that to reliably assess the true frequency of behavior the subject should be monitored continuously or the interval chosen should be shorter than the shortest duration of any behavior (for rats in the BSS this could be less than 1 s). But is the measurement of true frequency of behavior so important in the BSS? Disruption to the BSS caused by the fragmentation of behavior as a result of hyperactivity are better characterized by frequency. It has been shown that drugs can increase the duration of a behavior while decreasing its frequency (a sign of mild sedation). This dichotomy can only be picked up with continuous observation techniques. So the nature of behavior should be defined in both duration and frequency.

Second, although MTS provides an unbiased account of true duration, the estimation of behavioral duration in the 5-min time bins (which make up the period levels of the analysis) is open to a large degree of random error (see Table 2). This, and the limited number of modified frequency scores is each time bin (only 20 or 10 observations in total per animal) make standard parametric analysis difficult (15,55). ANOVA is not an ideal method of analyzing frequency data collected by continuous observation. The third problem with MTS is an observation bias we have termed "event over state observer bias." An "event-like" behavior is one that is very frequent but short in duration, such as locomotion or rearing (1). Conversely, a "state-like" behavior is one that occurs infrequently but has a long duration, such as resting. During the MTS procedure the observer does not actually code the behavior at a set instant (like a still photograph), but looks at the animal for up to 1 s before passing to the next. It is not unlikely that an animal may change its behavior. If the transition from a "state" to an "event," or vice versa, an "event" to a "state" behavior occurs, the active ("event") behavior is more likely to be coded as it is visually more prominent.

Measures Derived From and Analysis of the BSS

Food intake. Food intake can either be measured by weighing the difference in the food bowl before and after the observation or it can be measured automatically during the observation. Various forms of automated feeding devices are available.

Eating parameters. Eating parameters describe the relationship between changes in the amount of food eaten and changes in the various structural elements of eating behavior. Continuous observation is required to identify those parameters. Mean local eating rates (LER) (g/min), mean intake per eating behavior episode (MBI) (g), and mean eating bout length (MBL) (s) can be calculated. In earlier studies these parameters have been shown to be a sensitive indicator of

Actual continuously recorded data with time sampled simulation.

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SAMPLE DATA          23.1.92        BSS "KEETH" EXAMPLE.
RN AC TIME(SECS)
1  s  8.39          RESULTS OF ANALYSIS.
1  r  .82
1  l  .72
1  r  .7
1  l  .61
1  r  .76
1  g  .7
1  r  .72
1  s  .71
1  r  .81
1  g  .66
1  l  .72
1  r  .77
1  s  .59
1  g  1.21
1  r  1.32
1  s  74.53
1  l  .7
1  r  1.54
1  g  .65
1  s  88.76
1  l  .44
1  r  .7
1  g  .5
1  r  .49
1  r  .6
1  s  1.64
1  r  .76
1  g  71.23
1  l  .82
1  r  .66
1  s  .76
1  r  .66
1  g  86     < 5 mins g=34.35
```

FIG. 1. Simulation of 15-s momentary time sampling on 5 min of continuously observed behavior.

TABLE 2

COMPARISON OF THE COMPUTATION DERIVED FROM THE REAL DATA IN FIG. 1 USING CONTINUOUS SAMPLING (A) AND INTERVAL (PARTIAL) SAMPLING (B)

(a) Continuous Analysis—Actual Frequency and Duration

Behavior	Frequency	Duration (s)
Sniffing	7	176.36
Rearing	14	11.31
Locomotion	6	4.01
Grooming	7	109.32

(b) 15-s Interval Sample—Generated Modified Frequency Scores

Behavior	Modified Frequency
Sniffing	11
Rearing	0
Locomotion	0
Grooming	0

The partial sampling fails to detect the actual composition of the behavioral data.

drug action on behavior (7). Continuous automatic weighing of the food bowl across the period allows the plotting of changes in these parameters over time.

Microstructural analysis of behavior (bidimensional profiles). To analyze the change in behavior over time, data from the 40-min continuous record of each animal can be divided into 5-min periods (or time bins). The true frequency and true duration of each individual behavior in each period (or bin) is calculated. Profiles are plotted for each behavior over the 40-min period. Drug effects on each individual behavior are analyzed using analysis of variance. For each behavior a 2×8 repeated measures analysis of variance is normally carried out with drug (two levels—control and drug) and time period (eight levels—periods 1 to 8) as the two factors. The authors note ANOVA is not an ideal method of analyzing frequency data.

Interpreting the BSS

The BSS, compiled from a true experiment showing the orderly changes in eating, grooming, and resting is shown in Fig. 2.

Enhanced satiety. The effect of a 5-min bout of prefeeding (in which the animal consumes 50% of its normal intake), indicates the advancement of the elements of the sequence as full satiety is reached earlier. After the prefeeding period the food is immediately replaced and observation begins. The prefeeding procedure results in a shift in the BSS profile to the left (Fig. 3, upper left panel). Eating behavior is reduced and finishes earlier in the profile. Resting behavior increases and also starts earlier in the profile (13,30,32,34,35). Anorectic drugs believed to enhance satiety produces a similar shift in the profile. However, some procedures (including the actions of certain drugs) may inhibit food intake via mechanisms unrelated, at least in part, to the operation of physiological satiety. The effect of such (nonphysiological) manifestations are suggested in the altered pattern of the BSS (see Fig. 3).

Nausea. Inducing nausea in animals with lithium chloride results in a distinct reduction in the frequency of eating but not necessarily in eating duration. In fact, like severe sedation, the frequency of all observed behavior appears to be reduced. Eating is consequently a slow extended process, and the BSS does not develop normally (13).

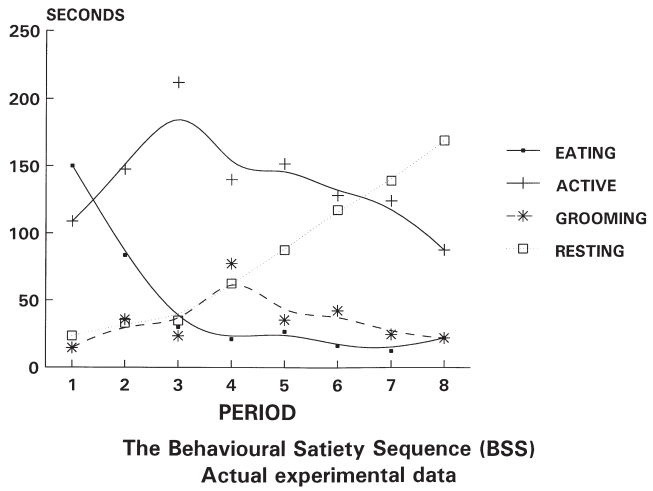

FIG. 2. The Behavioral Satiety Sequence (BSS)—From our control data.

Sedation. Mild sedation, such as that induced by pimozide or low doses of MK-212 does not alter food intake itself, but affects eating behavior. The drugs reduce the frequency of eating behavior, which leads to prolonged eating duration (7,30). Sedation is seen in a reduced local eating rate (LER) and increases in the mean eating bout intake (MBI) and the mean eating out length (MBL). In the BSS, the onset of resting may be delayed due to a prolonged period of slow eating. However, the overall structure of the BSS remains. However, severe sedation, such as that produced by an anorectic dose of MK-212, completely disrupts the BSS (38) (Fig. 3). The initial stage of the behavioral profile is dominated by resting, not eating behavior. Eating appears to be suppressed or displaced by resting. Eating behavior, along with some active and grooming behaviors, slowly increasing to modest levels in the later periods of the observation (Fig. 3, upper right panel).

Hyperactivity. Increases in activity, like increases in sedation, can only be considered disruptive if they interfere with the structure of the BSS (Fig. 2). Small increases in locomotion produced by certain drugs may not disrupt the expression of the BSS. An increase in any active behavior that delays the onset of resting and fragments eating behavior into numerous short bouts extending across the whole observation period can be considered disruptive (11,35). Such effects have been produced by *d*-amphetamine (Fig. 3, lower left), RU-24969, DOI, and various centrally administered dopamine agonists (10,12,29,45,50,53,59,60).

Palatability. Mild dosing of a test diet with quinine decreases food intake and reduces eating rate but increases the number of feeding bouts. Eating bouts become very brief as the animal finds the quinine taste aversive and avoids any prolonged contact with the food (2,13,29). If the quinine adulteration is stronger, the animal will do little more than repeatedly sniff the diet. In the BSS, bouts of eating behavior and/or sniffing behavior are increased, with repeated returns to the food bowl. Sniffing behavior consequently delays the onset of resting, and the BSS is disrupted (Fig. 3, lower right). When presented with quinine-adulterated food the animal engages in food-oriented behavior. The presence of food-oriented behavior is in contrast to the distraction from food caused by hyperactivity. With aversive or unpalatable food, the animal remains motivated to eat, but does not consume sufficient food to generate the classical BSS profile.

Drugs and the BSS

Table 3 shows the pharmacological action and the action on the BSS of the drugs mentioned in this section. The BSS profiles of some of the drugs mentioned in this section are shown in Figs. 4 and 5.

Blundell and McArthur (12) were the first to use the BSS to compare the behavioral effects of the catecholaminergic agent, amphetamine with the serotonergic agent, fenfluramine. In simple food-intake tests, both drugs produced equal and highly potent reductions in food intake, but their temporal profiles of behavior were quite distinct. Amphetamine was observed to disrupt the natural sequence of behaviors by increasing activity and postponing resting. Therefore, amphetamine did not produce the classical BSS profile. However, fenfluramine advanced the cessation of eating behavior and the onset of resting, thus producing a classical BSS. This suggested that fenfluramine reduced food intake by acting directly on satiety mechanisms. These results have been replicated a number of times (10,29,31) (see Fig. 4). However, not all studies have confirmed these results. Montgomery and

Willner (51) and Willner et al. (71) reported that fenfluramine increased activity and suppressed the onset of resting. The methodology of these two studies differed from contemporary studies, confirming the original hypothesis in a number of ways. First, in these studies animals were observed during the light phase of the light–dark cycle (see previous). Second, these studies used a mixer of *d*-fenfluramine and *l*-fenfluramine isomers (racemic fenfluramine) and not the active isomer d-fenfluramine alone. Finally, these studies used MTS and not continuous behavioral observation (see previous). Initially, it was argued that the action of *d*-fenfluramine was incompatible with satiety. However, this conclusion was overturned by the results of a subsequent long-term study indicating that repeated dosing with d-fenfluramine clearly preserved the BSS (49).

The selective serotoninergic reuptake inhibitor fluoxetine has also been shown to preserve the BSS (30,35,49,71) (see Fig. 4) in a similar way to *d*-fenfluramine (31). However, chronic administration of fluoxetine disrupts the BSS profile (49). Disruption may occur because fluoxetine and its active metabolite nor-fluoxetine, have very long half-lives, leading to a continuous build-up of drug and metabolites to a steady-state level. The effects of fluoxetine on food intake can be blocked by the 5-HT antagonist metergoline (30). However, metergoline did not reverse all the effects of fluoxetine on the BSS profile. Continuous analysis of behavior revealed that following metergoline preadministration, fluoxetine still suppressed the frequency of all behaviors. Additionally, the duration of eating increased. Fluoxetine also appeared to be affecting behavior by a non-5-HT mechanism. These effects are similar to the effects of the neuroleptic pimozide. Thus, the BSS defined by continuous monitoring demonstrates a fluoxetine–5-HT-mediated effect on satiety but also a non-5-HT (possibly dopaminergic) induction of mild sedation. It is possible that higher doses of fluoxetine cause hypophagia via both these mechanisms. This could explain why some researchers have found fluoxetine-induced hypophagia difficult to block using solely 5-HT antagonists, (28,72) and why chronic fluoxetine does not produce a behavioral profile consistent with the BSS (48).

Other selective serotonin reuptake inhibitors such as sertraline, femoxitine, and paroxetine have been shown to pre-

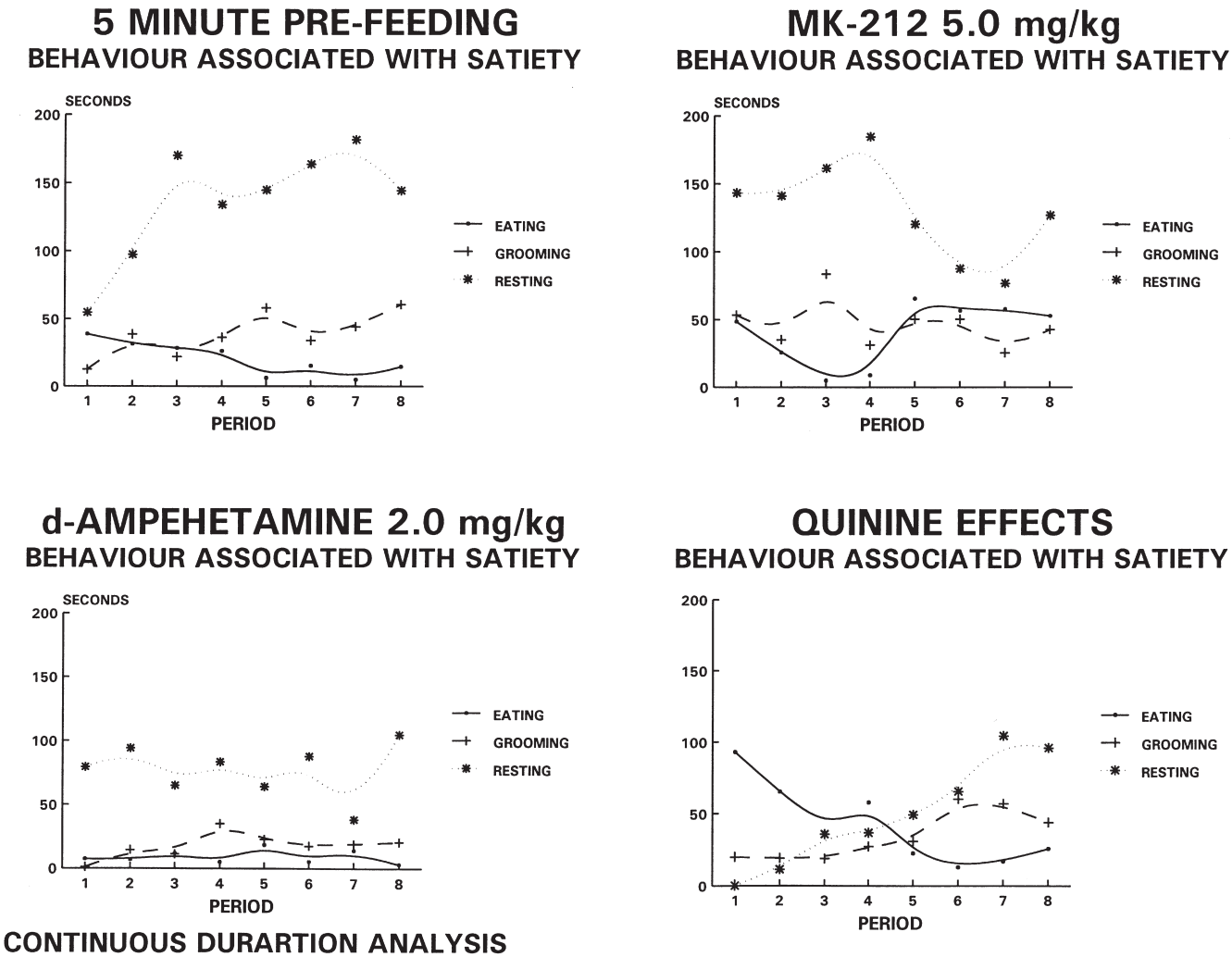

FIG. 3. The effects of prefeeding, seadtion (MK-212), hyperactivity (*d*-amphetaime), and unpalatability (quinine) on the BSS.

serve the BSS (33,49,60). These studies provide further evidence of the role of 5-HT in modulating the process of satiety and the expression feeding behavior. Sibutramine, a 5-HT and nor-adrenergic (NA) reuptake inhibitor, and potential anti-obesity compound, also preserves the BSS (Fig. 4) (37). Therefore, general activation of NA receptors does not appear to disrupt the expression of the BSS.

5-HT Receptor Subtypes and the BSS

Although it is established that pharmacological manipulation of 5-HT reduces food intake while preserving the BSS, which 5-HT receptors mediate these effects? The 5-HT receptors implicated in satiety are 5-HT_{1b} and 5-HT_{2c} [see (21) for review], although a relative lack of highly selective 5-HT agonists and antagonists makes the investigation of the precise role of 5-HT_{1b} and 5-HT_{2c} sites difficult. For example, agonists of the 5-HT_{2c} receptor generally have affinity for other 5-HT_2 receptors such as 5-HT_{2a} and 5-HT_{2b}, or 5-HT_{1b} receptors. DOI is a 5-HT_2 receptor agonist. Despite activating 5-HT_{2c} receptors, DOI does not produce a classic BSS. Instead, DOI disrupts behavior by inducing hyper activity (45,60). It is likely that DOI-induced hypophagia is mediated by 5-HT_{2a} receptors (60). MK-212 is another 5-HT_2 receptor that is supposed to reduce food intake via activation of the 5-HT_{2c} subtype (44). However, MK-212 produces a profile of severe sedation (39) (see previous, Fig. 3). Resting dominates in the early stages of the profile, with eating and other behaviors increasing slowly over time. The precise role of the various 5-HT_2 receptors in the BSS remains to be determined.

TFMPP and mCPP are 5-HT_{2c} agonists that do not appear to possess great affinity for other 5-HT_2 receptors. However, TFMPP and mCPP do have affinity for the other (5-HT_{1b}) "satiety" receptor (41–43). TFMPP and mCPP have both been shown to preserve the structure of the BSS and to advance the onset of resting (29,45,60). TFMPP, unlike mCPP, has also been shown to produce a small increase in locomotion. This does not disrupt the BSS at a dose that reduces food intake by 50% (29). Curzon (17,18) suggested that while reduction in food intake induced by TFMPP and mCPP was mediated by the 5-HT_{2c} receptor, activation of the 5-HT_{1b} receptor was necessary to produce the effect.

The 5-HT_{1b} receptor agonist RU-24969 is a possible tool to explore the role of 5-HT_{1b} receptors without activating 5-HT_{2c}

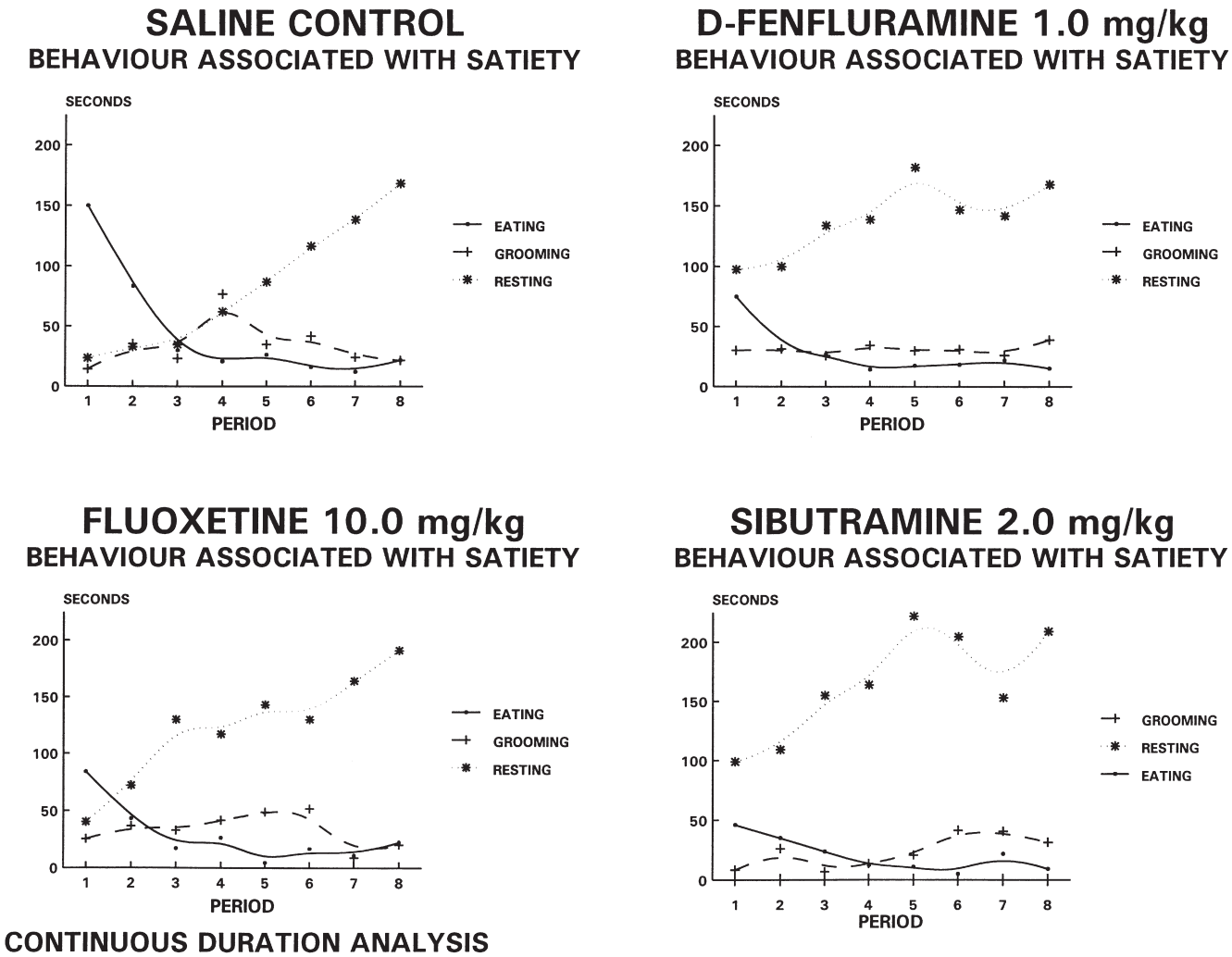

FIG. 4. The similar effects of equianorectic doses of *d*-fenfluramine, fluoxetine, and sibutramine on the BSS.

receptors, even though there is an affinity of RU-24969 for 5-HT_{1a} receptors. As RU-24969 reduces food intake and 5-HT_{1a} activation increases food intake (23,69), it is likely that the activation of 5-HT_{1b} receptors mediates hypophagia. As RU-24969–induced hypophagia is blocked by the 5-HT antagonist metergoline, this appears to confirm the involvement of the 5-HT_{1b} receptor (43). Despite this, RU-24969 has been shown to disrupt the expression of the BSS by inducing hyperactivity (predominantly locomotion), even at low anorectic doses (10,35,45). RU-24969–induced hyperactivity had previously been documented and appears to be dopamine mediated (43) (blocked by haloperidol), although RU-24969 does not appear to directly activate DA receptors. Other authors have provided strong evidence that RU-24969 induces locomotion via a 5-HT–DA interaction. The nigrostriatal and mesolimbic DA systems project into the globus pallidus (GP), which in turn, outputs towards the motor system. RU-24969 induces locomotion in GP-lesioned rats, an effect that is not blocked by haloperidol (52). In contrast, haloperidol blocks RU-24969–induced locomotion following chemical degeneration of 5-HT neurons. This supports the previous assertion that the motor response to RU-24969 is not DA mediated, but implicates DA systems in the control of the 5-HT locomotor response. "Downstream" somato-dendritic 5-HT_{1a} receptors may mediate this effect, but this is by no means proven. CP-94,253 is a more selective 5-HT_{1b} agonist that has only recently become available (46). Unlike RU-24969, CP-94,253 produces a classical BSS in which the onset of resting is advanced (35) (see Fig. 5). This appears to be the first study to demonstrate that activation of the 5-HT_{1b} receptor alone is a sufficient condition to induce satiety in the rat.

Other Compounds That Preserve the BSS

The BSS is not only produced by increasing CNS 5-HT levels or directly activating hypothalamic 5-HT_{1b} and 5-HT_{2c} receptors. Activation of peripheral 5-HT receptors, possibly in the gut, suppressed food intake and preserved the BSS (24). This may be related to the role of peripheral 5-HT in gastric motility and stomach emptying. Other peripheral satiety factors such as CCK (see previous) bombesin and enterostatin have also been shown to preserve the BSS. The behavioral effects of the ob-gene product, ob-protein (leptin) have yet to be reported.

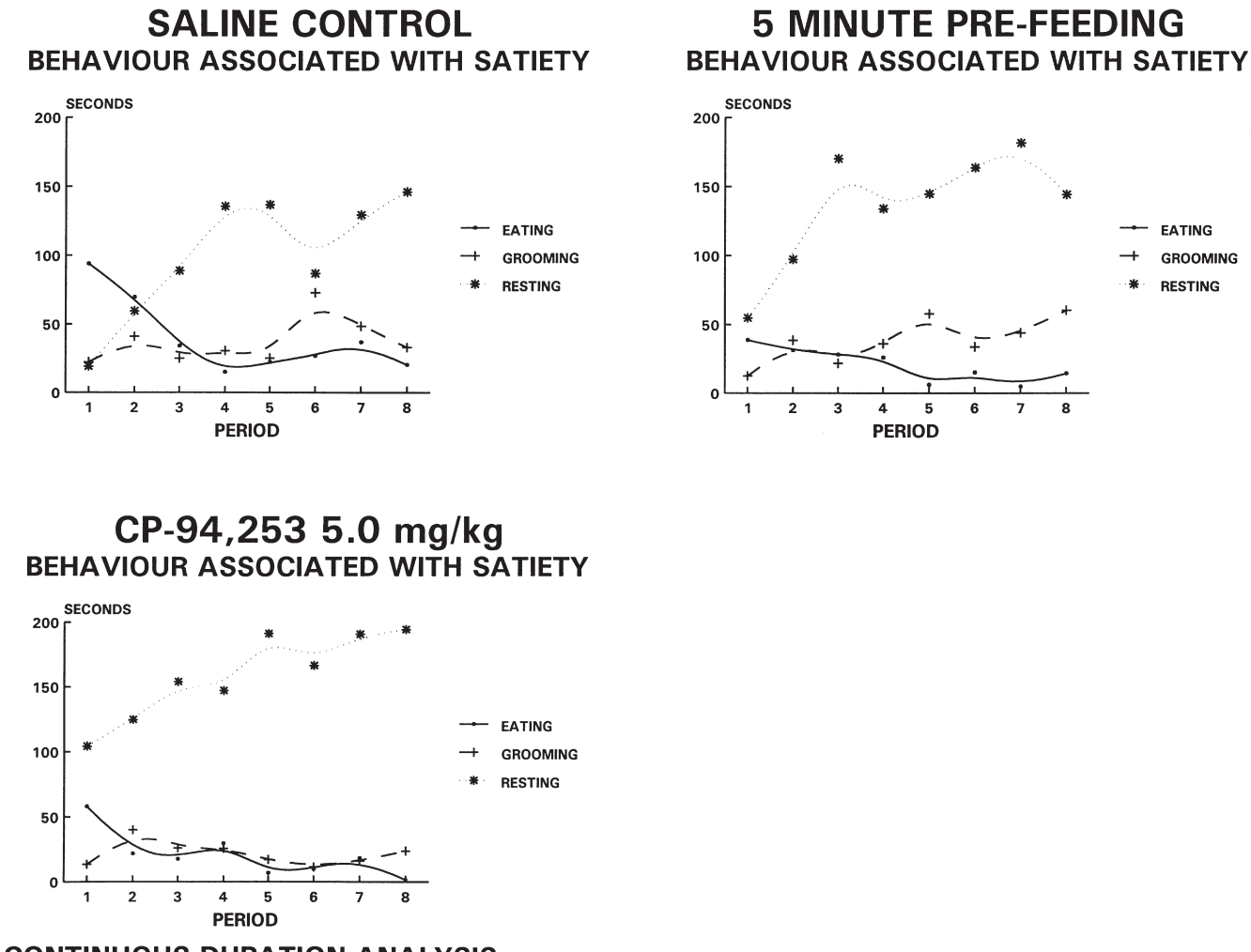

FIG. 5. The effects of the selective 5-HT_{1B} agonist CP-94,253 on the BSS, compared to saline control and prefeeding.

Other CNS appetite systems have been studied. Phillips (53) and colleagues noted that low doses of the DA_1 receptor agonist SKF-38393, and the DA_2 receptor agonist LY-171555, advanced the onset of resting. At higher, anorectic doses, these dopamine agonists increased locomotion, without disrupting the BSS. The profile produced by the DA agonists was similar to that produced by prefeeding. The BSS can sensitively discriminate between the effects of D_1 and D_2 receptor activation. For example, microstructural analysis revealed that D_1 agonist SKF 38393 reduced the total episodes of feeding bouts, thereby reducing the total duration of feeding and the local eating rate (16). This is markedly different to the action of the D_2 agonist N-0437, which achieves its anorectic effect almost exclusively through selectively reducing LER (57). It is notable that the hypophagic effect of the DA_1 agonist SKF-38393 is blocked by the 5-HT antagonist metergoline (73). This provides some evidence of a 5-HT–DA interaction in the satiety response produced by SKF-38393. Further studies are needed to separate the roles DA receptors in locomotion and satiety, and how these DA systems may be linked to 5-HT systems.

The effect of various CNS appetite control systems [in the paraventricular nucleus (PVN), and adjacent nuclei of the hypothalamus] on food intake have been documented. A large number of neurotransmitters and neuropeptides have been shown to either significantly decrease or increase food intake when injected into these "appetite regulatory" centers. For example neuropeptide Y (NPY), galanin, and glucagon like peptide (GLP-1) all potently effect food intake. Additionally, the selective agonism of PVN $NA\alpha_1$ autoreceptors produces a marked reduction in food intake. There are, as yet, no detailed descriptions of how manipulation of such CNS appetite systems affect actual feeding behavior. How do these neurochemicals modify the differing dimensions of feeding and related behavior (eating rate, eating bout size, duration and frequency of eating, and the profile of the BSS)?

FUTURE DIRECTIONS

To continue this work, newer, more selective neurochemicals that act on target appetite systems and on-going research to further characterize feeding behavior are both required. There are many aspects of behavior that could be utilized by researchers to more accurately characterize satiety, nausea, sedation, hyperactivity, and changes in palatability. Such measure could include direct measurement of food bowl weight over the full observation period to reveal changes in eating rates (g/min) decrease over time. Changes in locomotion could be measured in speed (cm/min) indicting changes in intensity of activity. Some behaviors in the BSS change qualitatively over time. Sniffing changes in character from a very active seeking behavior, to an almost passive behavior, occurring between resting bouts. Hartley et al. (39,40) have exploited the change in grooming behavior over time. Facial licks and coat grooming early in the sequence are replaced by genital licks. These changes in the qualitative nature of the behavior, could be quantified and used to further characterized sedation and various types of hyperactivity. Changes in an animals location within the observation tank, with reference to the location to the food, or number of returns to the food bowl may reflect the animal's underlying motivation to eat or conflict between the desire to eat and the adversiveness of the food. The inclusion of newer measures of feeding and related behavior could help us better define the nature of disruption to feeding. Additionally, this process requires detailed analysis differing severity of behavioral disruption. Disruption induced by nausea, palatability changes, sedation, and hyperactivity needed to be studied at a range of anorectic (and subanorectic) doses. This will ultimately provide researchers with a great diagnostic power to determine the nature of drug induced anorexia.

Once a BSS or disruption profile has been isolated, researchers can then determine the pharmacology that underlies the observed behavior. Using selective antagonists we can

TABLE 3

THE PHARMACOLOGY AND EFFECT OF DRUGS STUDIED ON THE BSS

Drug	Action	Effect on BSS
CCK	Agonizes vagal CCK receptors in gut	enhanced
d-Fenfluramine	5-HT releaser and reuptake inhibitor	enhanced
Fluoxetine	5-HT (and DA) reuptake inhibitor	enhanced
Sertraline	5-HT reuptake inhibitor	enhanced
Paroxetine	5-HT reuptake inhibitor	enhanced
Femoxitine	5-HT reuptake inhibitor	enhanced
DOI	$5\text{-}HT_2$ receptor agonist	disrupted
MK-212	$5\text{-}HT_2$ receptor agonist	disrupted
mCPP	$5\text{-}HT_{1B/2C}$ receptor agonist	enhanced
TFMPP	$5\text{-}HT_{1B/2C}$ receptor agonist	enhanced
RU-24969	$5\text{-}HT_{1A/1B}$ receptor agonist	disrupted
CP-94,253	$5\text{-}HT_{1B}$ receptor agonist	enhanced
Sibutramine	NA and 5-HT reuptake inhibitor	enhanced
Amphetamine	DA and NA releaser and reuptake inhibitor, DA receptor agonist NA_α receptor antagonist	disrupted
SKF-38393	DA_1 receptor agonist	enhanced
LY-171555	DA_2 receptor agonist	enhanced

identify the underlying physiological and/or neurochemical mechanisms by which a drug produces its effect. Antagonists may reverse all or some effects of the drug on the behavioral profile. This will ultimately allow researchers using the BSS to identify systems and receptors mediating satiety, sedation, hyperactivity, nausea, and palatability.

SUMMARY

The BSS, first identified in its modern form by Smith's group at Cornell, is widely recognized as a behavioral representation of the physiological processes of satiety set in operation by food ingestion. The BSS has been used to confirm the role of the gut peptide CCK in satiety. The BSS has been used to identify drugs that enhance satiety. The investigation of macro- and microstructural analysis of feeding behavior permits identification of differing mechanisms of drugs action not apparent in pharmacological studies of food intake alone. The continuous and complete analysis of behavior is superior to partial (or interval) sampling techniques and produces the most accurate and detailed profile of the BSS. This type of behavioral 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. This can occur before the mechanisms of action of the drug on physiological or neurochemical elements have been elucidated. Thus, the BSS has utility as a powerful diagnostic tool in the preclinical identification of drugs with potential therapeutic value in the treatment of obesity. The BSS also provides a scientific procedure to advance the understanding of the physiology and neurochemistry of feeding underlying pharmacological manipulation.

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