Behavioral satiety sequence: an experimental model for studying feeding behavior

Sequência comportamental de saciedade: um modelo experimental para o estudo do comportamento alimentar

Lisiane dos Santos OLIVEIRA[1]
Sandra Lopes de SOUZA[2]
Raul MANHÃES-DE-CASTRO[3]

ABSTRACT

Feeding behavior is controlled by interactions between psychobiological and physiological systems. In rats, there is a sequence in the feeding behavior that is characterized by similar movements at the beginning and end of a meal, known as the behavioral satiety sequence. In the sequence, eating is followed by grooming and other activities, and ends with resting. The objective of this systematic review is to evaluate the use of the behavioral satiety sequence as an experimental model for the study of feeding behavior. A systematic search of the electronic databases MedLine, Lilacs, SciELO, Cochrane Library and PubMed was done from November 2007 to January 2008, using combinations of the keywords "behavioral," "satiety" and "sequence". Ninety articles were found and, of these, fifteen articles were selected for the review. The studies demonstrated the efficacy of using behavioral satiety sequence to evaluate the effects of some types of manipulations on feeding behavior. With this study method it was also possible to observe different factors that can interfere with feeding behavior, such as sedation, malaise or intake inhibition, by increasing satiety. Behavioral satiety sequence offers solid tools for gaining a better understanding of how treatment can influence feeding behavior.

Indexing terms: Feeding behavior. Resting. Satiety response.

RESUMO

O comportamento alimentar é controlado por interações entre sistemas psicobiológicos e fisiológicos. Em ratos, existe uma sequência no comportamento alimentar que é caracterizada por movimentos similares no

[1] Universidade Federal de Pernambuco, Centro Acadêmico de Vitória. R. Alto do Reservatório, s/n., Bela Vista, 55608-580, Vitória de Santo Antão, PE, Brasil. Correspondência para/*Correspondence to*: L.S. OLIVEIRA. *E-mail*: <lisianenutricao@yahoo.com.br>.
[2] Universidade Federal de Pernambuco, Centro de Ciências Biológicas, Departamento de Anatomia. Recife, PE, Brasil.
[3] Universidade Federal de Pernambuco, Centro de Ciências da Saúde, Departamento de Nutrição. Recife, PE, Brasil.

início e no término de uma refeição, conhecida como sequência comportamental de saciedade. Na sequência, o ato de comer é seguido pela limpeza e outra atividades, terminando com o descanso. O objetivo dessa revisão sistemática é avaliar o uso da sequência comportamental de saciedade como um modelo experimental para o estudo do comportamento alimentar. Uma busca sistemática das bases de dados MedLine, *Lilacs, SciELO, Biblioteca* Cochrane e PubMed *foi realizada, no período de novembro de 2007 a janeiro de 2008, usando combinações das palavras chaves* "behavioral", "satiety" *e* "sequence". *Noventa artigos foram encontrados e, desses, quinze artigos foram selecionados para a revisão. Os estudos mostraram a eficácia do uso da sequência comportamental de saciedade para a avaliação dos efeitos de alguns tipos de manipulações sobre o comportamento alimentar. Com esse método de estudo, também é possível observar diversos fatores que podem intervir no comportamento alimentar, assim como sedação, mal-estar ou inibição do consumo por aumento da saciedade. A sequência comportamental de saciedade oferece sólidas ferramentas para obter um entendimento melhor de como um tratamento pode influenciar o comportamento alimentar.*

Termos de indexação: *Comportamento alimentar. Descanso. Resposta de saciedade.*

INTRODUCTION

Appetite control is based on a psychobiological system and reflects the synchronous operation of events and processes involved with this system[1]. An interaction exists between psychological events (hunger perception, cravings, hedonic sensations), behavioral operations (intake of meals, snacks, energy and macronutrients), peripheral physiologic and metabolic events, and the levels of neurotransmitter and metabolic interactions in the brain that control feeding behavior[2].

The feeding behavior of animals is an adaptive response, arising from demands of the internal environment and is modulated by limitations imposed by the external environment[3]. Neural events trigger and guide behavior, but each behavioral act involves a response in the peripheral physiological system[1]. These physiological responses are termed satiety signals, and can be represented by the satiety cascade[1].Some concepts must be defined to understand feeding behavior better. Satiety involves the events subsequent to food intake that suppress hunger and maintain an inhibition toward eating for a particular period of time while hunger can be regarded as the need to eat or a period in which satiety signals are absent[1]. Between hunger and satiety there is satiation, a group of processes that determine meal termination[1]. The coordinated effects of satiation and satiety control the size and frequency of eating episodes, thereby defining the eating pattern[1].

Studies have demonstrated that some behaviors in animals follow specific patterns[4,5]. Thus, after eating, an adult rat presents a period of grooming and locomotor activity[5]. After this period, the animal rests or sleeps[4]. Following these observations, a behavioral sequence was identified that is associated with satiety, because the cessation of eating is not a sufficient condition for the complete appearance of this behavioral sequence[6]. Thus the sequence was named Behavioral Satiety Sequence (BSS)[6].

In 1975, the BSS was used for the first time as an experimental model for the study of satiety[6]. This work confirmed the association of postingestive behavior with satiety, and it is still considered a landmark for the consolidation of BSS as a technique for the study of feeding behavior.

In rodents, BSS is characterized as an eating phase, followed by grooming and locomotor activities and ending with a resting phase[6,7]. Eating itself is characterized by biting, gnawing, or swallowing food directly from a dish or from the front paws[6,7]. This action is one of the elements of feeding behavior that is related to the biological need of getting nutrients.

Grooming is characterized by licking of the body, feet and genitals, by scratching the coat or head with the hind leg, by stroking whiskers with the paws and biting the tail[6,7]. These actions normally occur after eating[7]. Locomotion involves movements with the participation of the four limbs and rearing (front paws raised from the cage floor, either supported against a wall or free

standing)[6,7]. Locomotor activity is related to exploratory behavior[8]. It consists of acts and postures that allow the animal to acquire information and to become familiar with its environment[8]. Changes of activities related to BSS can interfere with feeding behavior[6]. The increased duration and/or frequency of non-feeding activities associated with BSS can delay the start of the resting period and fragment eating into numerous, short episodes[3,7].

Resting is characterized by inactivity. The animal sits or lies in a relaxed position with its head curled close to the body or resting against the floor[6,7]. Resting is the final posture assumed in the BSS[6,7]. The appearance of the resting posture in BSS is a condition caused by satiety[7]. This fact was demonstrated by changes of palatability, reduced food intake and because resting does not occur[9]. It has also been verified that the onset of resting can be anticipated by the prefeeding period[10].Drug-induced changes can alter BSS and make resting occur before eating[6].

The objective of this systematic review was to evaluate, through studies published in indexed journals, the efficiency of the BSS method for studying feeding behavior, as well as what type of behavioral interference related to satiety can be highlighted with the BSS method, since the study of food intake alone will not allow such verification.

METHODS

A systematic search of the literature was done from November 2007 to January 2008 in the electronic databases MedLine (National Library of Medicine), Lilacs (*Literatura Latino-americana e do Caribe em Ciências da Saúde*), SciELO (Scientific Electronic Library Online), Cochrane Library and PubMed. This search focused on studies published from 1975 to 2008 that used BSS. The literature search used combinations of the keywords "behavioral", "satiety" and "sequence". In order to define the literature for this review, the following inclusion criteria were established: a) studies that used the BSS method; b) studies with rats or mice; c) articles that considered the time when BSS was evaluated, such as eating, grooming and/or resting behavior durations. Articles that did not disclose eating, grooming and/or resting behavior durations or that had problems in the statistical analyses, such as no significance values or no confidence intervals, were excluded.

RESULTS AND DISCUSSION

The initial search of the databases returned fifteen articles in MedLine, two articles in the Cochrane Library and ninety articles in PubMed. The two articles of the Cochrane Library were discarded because they did not concern BSS. The fifteen articles found in Medline were also present in PubMed. Of the ninety articles found in PubMed, thirty were discarded after analysis of the abstracts because they did not concern BSS. After reading the abstracts, sixty articles were selected and after complete analysis of each article, only fifteen articles met the inclusion criteria mentioned earlier. The results tabulated for this study were the murine species, phase of the light/dark cycle, duration of BSS assessment, type of diet or treatment and method of administration, amount of food consumed and duration of eating, grooming and resting behaviors. The duration of eating, grooming and resting behaviors were presented as means or percentage ± standard deviation. The articles were categorized according to type of manipulation: nutritional, pathological or pharmacological, and are summarized in Tables 1, 2 and 3, respectively.

All articles used analysis of variance to compare the groups. The studies demonstrated the ample applicability and usefulness of BSS for evaluating feeding behavior. In particular, these studies show that BSS analysis is a simple method used for establishing the microstructure of feeding. That is, to define the duration and/or frequency of each behavior associated with food intake.

Table 1. Effects of nutritional manipulations on the behavioral satiety sequence.

Authors	Species	Phase of the light/dark cycle	Time	Treatment/ diet	n	Food intake (g)		Latency (s)		Duration of eating(s) or (%)		Duration of grooming(s) or (%)		Duration of rest (s) or (%)	
						M	SD	M	SD	M	SD	M	SD	M	SD
(9)	Rat Lister hooded	Light	1h	control	10	n/a		31	8	725s	30	233s	30	279s	119
				quinine 0.015%		n/i		35	8	786s	100	239s	32	113s	68
				quinine 0.04%		reduction		25	8	603s	77	273s	19	5s*	4
				saccharine 0.2%		n/i		15	2	704s	25	236s	30	240s	111
				saccharine 0.3%		n/i		21	5	702s	33	259s	32	277s	145
(11)	Rat Wistar	Light	1h	AIN-93/P 14	8	n/i		n/i		18.9%	1.8	12.4%	1.4	54.9%	4.2
				AIN-93/ P50 (1º dia)						32.2%*	5.5	10%	2.2	39.2%*	8.7
				AIN-93/ P50 (2º dia)						18.3%	2.2	13.7%	1.3	50.5%	2.8
				AIN-93/ P50 (14ª dia)						13.9%	1.2	13.7%	2	60.4%	3.2
(10)	Rat Lister hooded	Light	1h	Control A	12	n/i		19	4	828s	44	293s	38	511s	114
				Prefeeding 3min		n/i		30	6	729s[a]	41	316s	40	606s	144
				Prefeeding 6min		n/i		44[a]	7	637s[a]	50	331s	32	617s	137
				Prefeeding 9min		n/i		48[a]	22	598s[a]	54	305s	35	588s	161
				Control B		16.9	1.3	21	4	585s	68	346s	53	426s	143
				Fasting 3h		17.7	1.2	26	8	677s	51	317s	38	365s	110
				Fasting 6h		21.7*	1.0	24	6	788s[b]	50	345s	69	232s	66
				Fasting 12h		22.2*	1.0	21	7	778s[b]	63	374s	73	332s	135

Data are means of the duration in seconds or mean of the percent duration and SD; (AIN-93): diet formulated for rodents by the American Institute of Nutrition in 1993 (35); (P14): Diet with 14% protein; (P50): diet with 50% protein; n/a: not applicable to the group; n/i: data not informative in the original reference; *significant difference (p>0.05) in relation to the control group.

[a]significant difference (p>0.05) in relation to the control group A, [b]significant difference (p>0.05) in relation to the control group B; M: media; SD: standard deviation.

Table 2. Effects of pathological state on the behavioral satiety sequence.

Authors	Species	Phase of the light/dark cycle	Time	Treatment/diet	n	Food intake	via	Food intake (g)		Latency	Duration of eating(s) or (%)		Duration of grooming(s) or (%)		Duration of rest (s) or (%)	
								M	SD		M	SD	M	SD	M	SD
(12)	Rat Sprague-Dawley	Light	1h	Saline 1st hour	8	0,9%	i.p.	5.5	1.2	n/i	10	1	10	3	52	4
				Saline 3rd hour				3.3	0.3		7	1	13	3	58	9
				MDP 1st hour	8	1.5mg/Kg		6.3	1.0		10	2	7	3	54	4
				MDP 3rd hour				2.2	0.6		4	1	5*	1	82*	3

Data are means of the duration in seconds or the mean percentage of the duration and SD; n/i: data not informative in the original reference, *significant difference (p>0.05) in relation to the saline group in the respective schedule.

M: media; SD: standard deviation.

Table 3. Effects of pharmacological manipulations on the Behavioral Satiety Sequence.

Authors	Species	Phase of the light/dark cycle	Time	Treatment/ diet	n	Dose	Food intake		Latency (s)		Duration of eating(s) or (%)		Duration of grooming(s) or (%)		Duration of rest (s) or (%)	
							M	SD	M	SD	M	SD	M	SD	M	SD
(18)	Rat Lister hooded	Light	1h	Control	12	n/a	14.02g	0.96	23s	n/i	804s	49	305s	51	700s	159
				Orexina-A	12	3.33µg	19.99g*	1.15	12s	n/i	905s	62	429s	52	298s	63
					12	10µg	18.92g*	1.11	12s	n/i	823s	45	461s	49	417s	79
					12	30µg	19.26g*	1.09	16s	n/i	928s	69	474s	42	464s	135
(16)	Rat Lister hooded	Light	1h	Control	10	n/a	21.25g	0.8	25s	4	659s	32	263s	24	593s	106
				LiCl	10	90mg/Kg	12.36g*	0.98	22s	3	991s*	66	138s*	20	828s	119
				SB-334867	10	10mg/Kg	16.41g*	1.46	26s	4	575s	33	236s	41	858s	144
					10	30mg/Kg	12.06g*	1.35	37s	7	467s	44	154s	36	1251s*	185
(19)	Rat Lister hooded	Light	1h	Control	10	n/a	n/a		21s	5	705s	43	194s	29	1007s	105
				SB-334867	10	30mg/Kg	reduction		15s	4	418s*	41	160s	31	1634s*	129
				Control	10	n/a	n/a		23s	4	719s	50	278s	30	480s	146
				CCK-8S	10	5µg/Kg	reduction		199s*	65	1151s*	87	154s*	21	855s*	107
(18)	Rat Lister hooded	Dark	1h	Control	10	n/a	n/i		38s	12	n/i		n/i		n/i	
				naloxone	10	1mg/Kg	reduction, 54%		58s	12	Reduction		Not altered		Not altered	
					10	2.5mg/Kg	reduction, 61%		37s	11	Reduction		Not altered		Reduction	
					10	5.0mg/Kg	reduction, 65%		41s	12	Reduction		Not altered		Not altered	
(27)	Rat Sprague-Dawley	Light	90 min	Control	8	n/a	5.41g	0.46	n/i		33%	3	n/a		34%	7
				Antagonist Y2 PYY 3-36	8	5mg/Kg	5.49g	0.44			31%	3	Not altered		38%	5
					8	50mg/Kg	3.58*g	0.35			22%*	2	Not altered		63%*	4
				Antagonist Y2 + PYY 3-36	8	5+50mg/Kg	6.24g	0.64			34%	5	Not altered		35%	9
(28)	Rat Lister hooded	Dark	40 min	Control	12	n/a	8.0g	0.4	n/i		360s	31	207s	46	628s	114
				Fluoxetine (ISRS)	12	10mg/Kg	4.1g*	0.9	n/i		279s	57	176s	32	1127s*	162
				Metergoline	12	1mg/Kg	7.9g	0.7	n/i		419s	72	99s	20	1129s*	142
				Fluoxetine + metergoline	12	10+1mg/Kg	7.3g	0.8	n/i		506s*	77	105s	20	1051s*	114
(30)	Rat Lister hooded	Dark	40 min	Control	12	n/a	8.92g	1.31	n/i		405s	61	371s	47	511s	62
				MK-212	12	5mg/Kg	4.33g*	0.81	n/i		320s	48	371s	69	1042s*	129
(31)	Rat Lister hooded	Dark	40 min	Control	12	n/a	7.2g	0.9	n/i		318s	38	298s	46	802s	149
				CP-94.253	12	5mg/Kg	3.1g*	0.6	n/i		160s*	26	173s	33	1274s	175
				Control	12	n/a	9.1g	0.94	n/i		530s	39	248s	42	398s	137
				RU-24969	12	1mg/Kg	6.22g*	0.79	n/i		440s*	42	264s	44	305s	107
(29)	Mice	Light	40 min	Control	12	n/a	1.82g	0.18	n/i		n/a		n/a		n/a	
				RO-60-0175	12	3mg/Kg	1.55g	0.31	n/i		Reduction		Not altered		Increase	
				Fenfluramine	12	10mg/Kg	1.18g*	0.23	n/i		Reduction		Not altered		Increase	
					12	3mg/Kg	1.00g*	0.19	n/i		Reduction		Not altered		Increase	
(32)	Rat Lister hooded	Dark	40 min	Control	12	n/a	n/a		n/i		n/a		n/a		n/a	
				CP-94.253	12	1.25mg/Kg	not altered		n/i		Reduction		Not altered		Not altered	
					12	2.5mg/Kg	reduction 37%		n/i		Reduction		Not altered		Increase	
					12	5mg/Kg	reduction 78%		n/i		Reduction		Reduction		Increase	
(33)	Mice	Light	40 min	Control	12	n/a	n/a		n/i		n/a		n/a		n/a	
				VER23779	12	3mg/Kg	reduction				Reduction		Reduction		Reduction	
					12	10mg/Kg	reduction				Reduction		Reduction		Reduction	

Dates presented are the means of the duration in seconds or mean percent of duration and SD; n/a: not applicable to the group; n/i: the original reference is not informative; (reduction): reduction of the duration of the behavior in relation to the control group [values not available in the original article]; (not altered): no significant differences in relation to the control group [values not present in the original article].

*significant difference (p>0.05) in relation to the control group; M: media; SD: standard deviation.

In the first category of studies where the effects of nutritional manipulations on the temporal feeding pattern were examined (Table 1), it was clear how hunger and satiety states[10], diet palatability[9], and satiation power of the ingested diet[11] affect feeding behavior. Thus, adulteration of food with quinine[9], a bitter substance, reduced food intake without affecting eating duration and abolished the resting behavior usually observed at the end of BSS. Since satiety is associated with sleeping or inactivity, the absence of resting indicates lack of satiation. This observation underlies the fact that the taste of food is a crucial determinant of feeding and that this factor must be considered when the anorexic or orexigenic properties of a drug are studied or when the characterization of a drug response involves its administration through drinking water.

BBS has also been used to examine the effect of a high-protein diet on satiety[11], and in particular on food intake, the rate of feeding and the relation between food intake and eating duration. In this study, animals were fed either standard chow or a high-protein diet and their feeding behavior was evaluated daily. On the first day, the animals fed the high-protein diet exhibited a reduction in food intake and rate of feeding, as well as an increased eating duration and a decreased resting duration. From the second to the fourteenth day, no differences between the two groups in the temporal BSS pattern were observed. The reduced resting duration in the first day indicated that a high-protein diet delays the appearance of satiety but this initial aversion to the high-protein diet is followed by adaptation.

Finally, it has been demonstrated that the interval between meals can affect food intake and the appearance of satiation[9]. Specifically, it was observed that the longer the fasting period, the greater the food intake and the duration of eating, and that the longer the pre-feeding period, the smaller the latency to begin eating and the feeding duration. These results indicate that a smaller interval of time between meals can reduce the motivation to eat the next meal. Using this experimental approach, one can obtain information about the temporal display of feeding behavior, and identify the level of control in which individual behaviors are affected by nutritional manipulations.

In the second study category (Table 2), BSS was used to analyze how a pathological state can interfere with food intake. In one of these studies[12], the effects of muramyl dipeptide on feeding behavior were analyzed. Muramyl dipeptide is the minimally active subunit of bacterial peptidoglycan, which is abundantly released during infections by gram-positive bacteria[13,14], and has been associated with reduced food intake during infection[15]. In this study, none of the parameters of BSS, including the amount of ingested food, eating duration and feeding rate were altered within the first two hours after the administration of muramyl dipeptide. However, three hours after the administration of muramyl dipeptide there was a reduction in grooming duration and prolonged resting period. These behavioral changes are similar to those that appear during illness. This study also evaluated the cumulative food intake over a 24-hours period. This analysis demonstrated that the inhibitory effects of muramyl dipeptide on food intake extend from the third to the tenth hour after its administration. Collectively, these data suggest that the hypophagic effect of illness induced by a bacterial infection results from a change in the physiological mechanisms involved in the regulation of satiety.

In relation to the changes in feeding behavior associated with a pathological state, it has also been observed that the discomfort caused by the administration of Lithium Chloride (LiCl) is related to diminished food intake, reduced grooming duration and longer eating period[16]. Reduced food intake correlated with increased eating duration, thereby resulting in reduced feeding rate. LiCl-induced anorexia is associated with behavioral signs of malaise such as reduced activity, low food intake rate and BSS disruption. These observations corroborate previous studies[12], indicating that anorexia is related to the discomfort caused by sickness.

BSS has been extensively used in pharmacological studies that aimed to identify new therapeutic targets for the treatment of eating disorders and its consequences, such as obesity, as well as to get an insight into the mechanism involved in the control of feeding behavior, both at the central and peripheral levels (Table 3). In relation to the first point, it has been recently demonstrated that orexins participate in the regulation of feeding behavior by stimulating food intake[16]. Orexin-A and orexin-B are neuropeptides derived from prepro-orexin. Both peptides exert their actions through the activation of orexin-1 and orexin-2 receptors but orexin-A binds with greater affinity to the orexin-1 receptor[17]. Intracerebroventricular administration of orexin-A leads to increased food intake. The fact that this hyperphagic effect is not associated with increased meal duration indicates that the orexigenic properties of orexin-A are due to its capacity to increase feeding rate[18]. On the other hand, the intraperitoneal administration of SB-334867, an antagonist of the orexin-1 receptor, reduces food intake and eating duration and increases resting duration[16,19]. These results indicate that orexin stimulates food intake through its interaction with orexin-1 receptors.

The intestinal hormone cholecystokinin is a satiety signal with anorexic effects[7,20-22]. The administration of an equi-anorectic dose of the natural satiety-related signal cholecystokinin octapeptide (CCK-8S) induced reduced food intake, along with increased latency to begin eating, increased eating and resting duration, and reduced grooming duration. Collectively, these observations indicate that cholecystokinin reduces satiety before eating begins and stimulates satiety after eating begins. Analysis of the effects of other pharmacological or nutritional manipulations on the pattern of BSS induced by the administration of cholecystokinin might be of interest for the dissection of other variables related to satiety.

Endogenous opioids are also involved in the regulation of appetite. Systemic or central administration of these peptides induces hyperphagia[23]. The physiological mechanisms underlying the orexigenic effects of opioids were investigated by the use of naloxone, an opioid receptor antagonist[18]. The administration of this compound was shown to reduce both food intake and eating duration in control rats, without affecting the latency to start eating. Thus, opioids clearly regulate the satiety process but, in disagreement with a generally accepted idea, they do not seem to be involved in the motivation to eat.

BSS has also been used to analyze the anorexic effects of the YY3-36 peptide[24,25]. This peptide is released into the gastrointestinal tract after meals[26]. Examination of the BSS pattern after the administration of the YY3-36 peptide showed significant reductions in food intake and in eating duration. In addition, there was as an increase in resting duration, which is consistent with delayed onset of satiety. These results indicate that the YY3-36 peptide reduces food intake because it promotes satiety[27]. The stimulation of type 2 pre-synaptic receptors for neuropeptide Y (NPY) inhibits the release of NPY and GABA from hypothalamic arcuate nucleus neurons. Given that NPY is a potent orexigenic peptide and that YY3-36 peptide also stimulates NPY type-2 receptors[24], the possibility exists that the inhibitory effects of YY3-36 on food intake are mediated by NPY. In agreement with this hypothesis, the administration of a Y2-receptor antagonist alone did not alter BSS but prevented the anorexic effect of YY3-36[27].

BSS has been extensively used for the study of the effects of serotonin on food intake. Although all the studies performed so far agree that the inhibitory effects of serotonin on food intake are related to its capacity to advance satiety, some discordant results exist in relation to how serotonin affects the other behavioral components of the feeding microstructure. For example, the administration of fluoxetine, a selective serotonin reuptake inhibitor, reduces food intake but does not change eating duration or increases resting period[28]. In contrast, the administration of fenfluramine, a serotonin reuptake inhibitor which also stimulates the release of serotonin, leads to an inhibition of food intake which is associated with reduced eating duration and increased resting period[29]. Moreover, metergoline, a serotoninergic

receptor antagonist, does not alter food intake or eating duration, but it does increase the resting period[28]. When fluoxetine was administered together with metergoline, eating and resting duration increased but food intake did not change[28], indicating that metergoline inhibited the effect of fluoxetine on the inhibition of food intake.

These controversial results can be explained by the fact that serotonin interacts with 14 different receptors which have been classified into different families according to their pharmacological, molecular and functional properties. Among these, serotonin 5-HT1B and 5-HT2C receptors have been specifically recognized as mediators of serotonin-induced satiety[29,30]. Thus, RU-24969, an agonist of the 5-HT-1A and 5-HT-1B receptors, reduces food intake and eating duration without changing the resting period[31]. Similarly, the administration of CP-94,253, a selective 5-HT1B receptor agonist, reduced food intake and eating and grooming durations but, in contrast to the administration of RU-24969, increased resting duration[32]. These findings clearly demonstrate that 5-HT-1B receptors modulate the inhibitory effects of serotonin on food intake.

On the other hand, initial studies indicated that the administration of MK-212, a 5-HT-2 receptor agonist, reduces food intake and increases resting duration but does not change eating duration[30]. These data indicate that serotonin 5-HT-2 receptors regulate feeding behavior through the stimulation of satiety. Subsequent studies with RO-60-0175 and VER23779, two selective 5-HT-2C receptor agonists, confirmed that the specific stimulation of 5-HT2C receptors inhibits food intake by reducing feeding time and increasing resting time[29,33]. Collectively, these and other analyses of the BSS in association with pharmacological studies using selective serotonin receptor compounds, indicate that the anorexic action of serotonin is mediated by separate receptor subtypes. Thus, while the reduced meal size consecutive to the administration of serotonin would depend on the stimulation 5-HT1B receptors, the reduction in feeding rate would result from the selective stimulation of 5-HT2C receptors.

For BSS analysis, all the examined studies relied on a standard procedure in which a food-deprived animal is placed in an observation arena with ad libitum access to food and water for a period of 45-60 min. Food deprivation is used to obtain a high feeding baseline while the objective of the observation arena is to provide a larger enclosure than the animal's house-cage to allow the expression of all the BSS behavioral parameters. The experimental protocol used differed, however, between the different laboratories in some respects. These included the phase of the light/dark cycle in which the test was performed, the duration of the fasting period and how the behavioral data were analyzed and presented.

Most living organisms exhibit behavioral and physiological rhythms, including those associated with sleep, feeding and energy homeostasis. Therefore, the expression of BSS can vary depending on the phase of the light/dark cycle in which the analysis is performed. Initially, the BSS was monitored essentially during the light phase[7], but more recently analysis of the BSS during the dark phase has been preferred[18,19]. In the first studies in which BSS was analyzed during the light phase, the animals were submitted to a fasting period of 17-20 hours[3,7]. The objective of this procedure was to reduce the eating latency and to stimulate the onset of the characteristic BSS behaviors. However, rats are nocturnal and, consequently, most of its activity, including eating, occurs during the dark cycle. During this phase, rats consume up to 80% of their daily food intake[34]. Thanks to the development of monitoring technology, it is possible to document BSS during the dark phase using special video recorders and red light. The use of this methodology allows not only BSS analysis under more natural conditions, but also has the advantage of reducing the fasting period. Currently, studies done during the dark phase usually use a four-hour food deprivation period[6,28,30,35].

The data gathered in these studies were analyzed in different ways. Some authors calculated the frequency of each behavior, e.g.,

the number of episodes of each behavioral category per time bin (usually 5min), whereas other authors expressed the duration of each behavior in relation to the total length of the observation period (45-60min).

Only six articles of the fifteen articles presented data on feeding latency (Tables 1, 2 and 3). That is, the interval of time between the presentation of food and the moment at which the animal actually starts eating. Pre-satiated rats present longer latency periods to begin feeding[9]. Since food ingestion reduces the stimulation to start a new meal, feeding latency can be a measure of the animal's motivation to eat.

Food intake is also an important factor for the interpretation of BSS. By the measurement of food intake and the duration of eating, that is, the time the animal actually spends consuming food, the feeding rate can be determined by calculating the ratio between food intake (g) and eating duration (min). It is also possible to quantify the mean intake per eating episode through quantification of the relationship between food intake (g) and the number of eating episodes.

CONCLUSION

BSS is a noninvasive, inexpensive and highly efficient method for analyzing feeding behavior. The effects of pharmacological and nutritional manipulations on the natural physiological regulation of food intake can be evaluated using BSS. It is regrettable that many studies that aim to characterize the orexigenic or anorexigenic properties of a drug use the amount of ingested food as the only feeding behavior variable. Although a reduction or an increase in food ingestion reflects an effect on appetite, the measurement of food intake alone does not allow the determination of whether these changes are due to a change of the physiological mechanisms regulating food intake or to nonspecific effects, such as sedation, hyperactivity, malaise or enhanced satiety. BSS is a solid experimental tool for improving our understanding of the complex psychological and physiological processes involved in the regulation of feeding behavior.

CONTRIBUTORS

All authors contributed substantially to the paper, including literature review, organization of the manuscript, translation and critical review.

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