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The behavioral satiety sequence in pigeons (*Columba livia*). Description and development of a method for quantitative analysis

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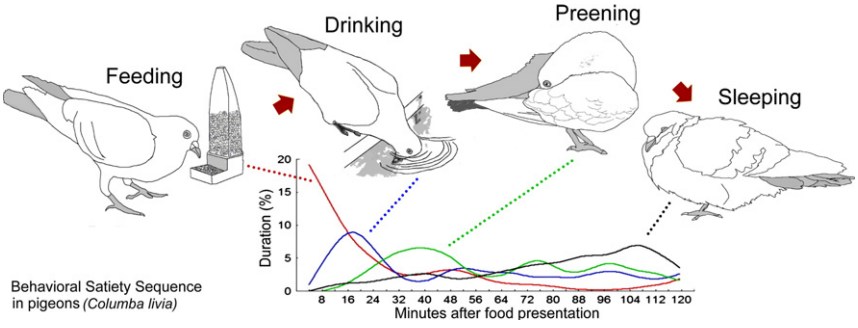
HIGHLIGHTS

- Feeding prompts a reliable and consistent behavioral satiety sequence in pigeons.
- Temporal/sequential traits of post-meal drink–preen–sleep BSS were quantitatively described.
- BSS temporal structure is similar after different feed-evoking stimuli and food intakes.
- Though similar in profile, central controls of BSS timing may be different in pigeons and rats.

GRAPHICAL ABSTRACT



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ABSTRACT

The postprandial event known as the specific dynamic action is an evolutionarily conserved physiological set of metabolic responses to feeding. Its behavioral counterpart, a sequence of drinking, maintenance (e.g., grooming) and sleep-like behaviors known as the behavioral satiety sequence (BSS), has been thoroughly described in rodents and has enabled the refined evaluation of potential appetite modifiers. However, the presence and attributes of a BSS have not been systematically studied in non-mammalian species. Here, we describe the BSS induced in pigeons (*Columba livia*) by 1) the presentation of a palatable seed mixture (SM) food to free-feeding animals (SM + FF condition) and 2) re-feeding after a 24-h fasting period (FD24h + SM), which was examined by continuous behavioral recording for 2 h. We then compare these patterns to those observed in free-feeding (FF) animals. A set of graphic representations and indexes, drawn from these behaviors (latency, time-to-peak, inter-peak intervals and the first intersection between feeding curves and those of other BSS-typical behaviors) were used to describe the temporal structure and sequential relationships between the pigeon's BSS components. Cramér–von Mises-based statistical procedures and bootstrapping-based methods to compare pairs of complex behavioral curves were described and used for comparisons among the behavioral profiles during the free-feeding recordings and after fasting- and SM-induced BSS. FD24h + SM- and SM + FF-induced feeding were consistently followed by a similar sequence of increased bouts of drinking, followed by preening and then sleep, which were significantly different from that of FF birds. The sequential and temporal patterns of the pigeon's BSS were not affected by differences in food intake or by dissimilarity in motivational content of feeding stimuli. The present data indicated that a BSS pattern can be reliably evoked in the pigeon, in a chronological

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succession and sequence that strongly resembled that observed in rodents and primates. This pattern can be quantitatively described and compared using different suitable and coordinated behavioral measures, enabling further studies on the comparative and evolutionary aspects of the mechanisms that shape the post-consummatory behavioral flux in amniotes.

1. Introduction

Feeding behavior was followed by conspicuous metabolic/thermal changes (the thermal effect of feeding or "specific dynamic action", [1,2]) which is a functional trait that is widely shared by vertebrates and invertebrates. Beyond the robust changes in metabolic and gastrointestinal functions, the postprandial state in mammals was also associated with intense modifications in cardiovascular (e.g., [3,4]), renal (e.g., [5]), and HPA activity [6], which are thought to be of relevance as risk factors for cardiovascular diseases and diabetes (e.g., [7–9]).

The behavioral counterpart of these physiological changes is a remarkable sequence of maintenance (grooming and preening) behaviors and then sleep/rest, which are known as the behavioral satiety sequence (BSS, [10–12]). Water intake is a major (although not compulsory) component of the periprandial events in mammals [13–15], while post-meal quiescence was observed in invertebrates (e.g., *Caenorhabditis elegans*, [16]) as well as in vertebrates, including rats (e.g., [17,18]), mice (e.g., [19]), rhesus monkeys [20] and humans [21–23]. Most studied in the rat, the temporal patterns and sequential arrangement of the BSS components are thought to reflect the natural and physiological process of satiety and have been used in the last four decades as an important method to evaluate the behavioral selectivity and specificity of changes in food intake induced by drugs or changes in the palatability of foods (e.g., [10–12,24,25]). Thus, measures of changes in the temporal and sequential relationships among BSS components may be valuable as tools to dissect the functional interactions between systems related to energy homeostasis, hydrosaline balance and sleep–waking states.

Although some attributes of sleep, feeding and drinking behavior control systems appear to be phylogenetically conserved in amniotes (e.g., [26–30]), the relationships between these behaviors as parts of the feeding and post-prandial continuum are mostly unknown in non-mammalian species. Quantitative descriptions of postprandial behaviors in vertebrate taxa other than rodents could enable further studies on the comparative and evolutionary aspects of the mechanisms that shape the post-consummatory behavioral flux. Birds show intense postprandial changes in thermal and metabolic indices [2,31–33], and these changes were associated with increases in gastrointestinal distension in pigeons (*Columba livia*) [34,35]. Fasting-induced feeding is followed by increased drinking and then, within 30–45 min, by intense sleep-like episodes that exhibit EEG patterns that are comparable to spontaneous sleep in this species [30,36,37].

In addition to being habitual subjects in the neurobiology lab (and thus possessing abundant documentation on their behavioral, neuroanatomical and neurochemical brain attributes), feral rock pigeons are relatively free from artificial selection for particular growing or feeding traits and may be relevant to comparative functional studies on the relationships between feeding, drinking and sleep behaviors in the context of the BSS. We have recently shown that an ongoing, tonic and inhibitory influence of central 5-HT circuits may integrate feeding, drinking and resting behaviors in pigeons, so that feeding-induced 5-HT_{1A}-receptor-mediated changes in the activity of central serotonergic neurons induced drinking and sleep behaviors [30,38] in a pattern that resembled the rodent BSS. However, a detailed description and the tools available for the study of satiety-like sequences are lacking in pigeons. In the present report, we sought to describe the postprandial behaviors after fasting and palatable food feeding and to develop indices and statistical behavioral approaches to assess the sequential and temporal structures of postprandial behaviors that allow for studies on BSS in pigeons. In companion papers, the calibration of a palatable food-based test protocol and the effects of hyper- and hypophagy-inducing serotonergic drugs and neuropeptides on the pigeon's BSS are examined.

2. Material and methods

2.1. Animals

All of the experimental procedures described below were conducted in strict adherence to the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the local Committee for Ethics in Animal Research (CEUA–UFSC, protocol: PP00133/2007 and PP00524/2010). Adult domestic pigeons (*C. livia* of both sexes, 360–520 g bw, raised at the central vivarium of the Universidade Federal de Santa Catarina) that were brought to the lab vivarium and maintained in individual cages at a temperature of 23–25 °C on a 12:12 light–dark cycle (lights on at 07:00 a.m.; fluorescent day-light lamps, resulting in 80–90 lx light intensity) and with free access to food (pigeon chow, formulation for growing birds, SUPRA Ltda, Itajaí, SC, referred to here as regular chow, RC) and tap filtered water were used throughout the experiments. The pigeons were adapted to lab conditions for at least 10 days. In the week preceding the experiments, the pigeons had access (for 90 min in 3 alternated days in their own home cage) to a seed mixture (SM), consisting of oatmeal (*Avena sativa*, 57%), millet (*Panicum millaceum*, 30%) and sunflower seeds (*Helianthus annuus*, 13%). This mixture was shown in preliminary tests to be highly palatable to the pigeons: it evoked prompt and intense intake and was preferred by the pigeons, compared to the RC.

2.2. Behavioral and ingestive recordings and analysis

The experiments were conducted in the bird's own home cage. After presentation of the food in any protocol, digital video recordings (Microsoft®, VX80) were continuously taken from the bird (for different periods of time and specified at each experiment described below). The latency to the first event, and the duration and frequency of drinking, feeding, preening, locomotor/exploratory and sleep behaviors were scored using the EthoWatcher® software ([39]; which is freely available at www.ethowatcher.ufsc.br). These behavioral units, defined in Table 1, have been previously described (e.g., [38,40]) and are shown in a movie clip available on the internet ([41]; http://dx.doi.org/10.1016/j.regpep.2007.12.003). Transcriptions of these recordings were carried out by 3 observers (WAS, ACA, GIH, Kappa's inter-observer concordance index = 0.80; Kappa's intra-observer concordance index: WAS = 0.93, ACA = 0.94, GIH = 0.80). SM and RC foods were delivered in identical opaque plastic cups and water was provided in plastic bottles. Food and water were weighed after different periods of time according to the experiment. The experiments were performed between 13:00 and 17:00 h during the illuminated part of the light/dark cycle when the ingestive behavior was usually higher than that observed in the morning hours [42,43].

The behavioral scores for each consecutive 4-min time bin (total duration and frequency of each behavior in a given bin) were used to describe the temporal profile of the behavioral change. Typical durations of each of the recorded behaviors varied widely: drinking bouts lasted up to 24 s in a free feeding condition, while sleep bouts could reach up to 210 s under the same conditions (see Fig. 2E–H). To better depict the temporal relationships between these behaviors, the

Table 1
Behavioral catalog.

Behavior	Description
Feeding	A bout of pecking movements directed at the feeder, including brief (< or = 3 s) inter-pecking intervals, during which the animal adopted an upright posture, showed swallowing and beak movements, and then started pecking again.
Drinking	Pigeons drink by suction of water through the beak; a drinking bout was recorded for the interval between each beak immersion in and its removal from the water reservoir's spout.
Preening	Rubbing the beak or lower limbs over or between the feathers.
Exploratory/locomotor	Locomotion (at least a complete hind limb step, jumps to or from the perch) and exploratory behavior (angular or ballistic, to-and-fro and stretching movements of the head and neck directed in every direction, occurring in the absence of locomotion, known as peeping behavior [64]) were recorded.
Alert immobility	A quiet upright waking posture with the animal standing on both legs, with both eyes showing fast blinks, but no head/body exploratory movements.
Sleep	Sleep-like behavior was recorded when the pigeon showed chest and neck plumage puffed up and one or both eyelids showing slow blinks or remaining steadily closed for at least 3 s. This may occur with the animal in a crouching position or standing on one or both legs. This category included both drowsiness and sleep states, which have been observed after large, fasting-evoked meals and were associated with slow wave sleep-typical signals (SWS), with a low-frequency, high-amplitude EEG and decreased EMG activity and rapid eye movement sleep (REMS): characterized by a posture identical to the SWS with sporadic and sudden downward head drops followed by slow return to an upright posture, closed eyes (one or both), low-activity EMG, fast and high amplitude EOG activity indicative of large eye movements, and a high-frequency low-amplitude EEG [30,38,42].

raw data of each animal were expressed as the percentage of time spent in a given behavior (in each 4-min bin) relative to the total duration of the behavior in the total recording period. This was calculated using the formula $RD_{i\ bin} = (AD_{i\ bin} * 100) / TD$, where $RD_{i\ bin}$ is the relative duration of a given behavior in the i^{th} time bin, $AD_{i\ bin}$ is the absolute duration (in seconds) of that behavior in the i^{th} time bin and TD is the total duration of that behavior in the total recording period sleep.

From the individual raw and RD data, the scores for each animal were calculated for indices related to the temporal structure of these behaviors and the sequential relationships between them. These indices included the latency for each behavior (the time, in seconds, to the first occurrence of the behavior) and the time-to-peak (TTP) of each behavior (the time bin when the behavior first reached its maximum duration in the session). These indices were intended as measures of the absolute position in time and in sequence of each behavioral item, aiming to assess potential changes in their order and time of appearance after food presentation (latency) and the time to reach its maximum (TTP).

To measure changes in the temporal relationships between feeding and BSS behaviors, we measured the inter-peak interval (IPI) between the peaks of feeding and drinking, feeding and preening, and feeding and sleeping (in seconds). To verify for changes in the relative prevalence of feeding upon other behaviors throughout the recording period, we calculated the first intersection (ItS) between feeding and drinking, between feeding and preening, and between feeding and sleeping curves. The ItS was calculated from the RD transformed data, as the first time bin when the relative duration of feeding was 5% lower than that of drinking, preening or sleeping. We also scored an intersection when the relative durations of the 2 behaviors were equal to each other for at least 5 consecutive time bins.

2.3. Data analysis and graphical presentation

In all of the experiments, food and water intake, as well as the behavioral data (hourly sums or totals for the entire recording sessions) were analyzed using 2-way repeated-measures ANOVA, with the experimental conditions as factor and the different periods of time (1st and 2nd hours of recording, consecutive re-tests) as repeated measures (using Statistica 8.0, Statsoft, Tulsa, Oklahoma, USA) followed by post-hoc Duncan's tests when appropriate. Most of the behavioral indices failed to show a significant Gaussian distribution (as indicated by Shapiro–Wilk's W-test for sample normality of distribution) and some of the tests also failed to be homoscedastic within a given time bin (as judged by applying a Brown–Forsythe modification of the Levene test to the data). Thus, latency, IPI, ItS and TTP data were analyzed using a non-parametric test (Kruskal–Wallis ANOVA by ranks) followed by a post-hoc Mann–Whitney *U*-Test when appropriate. Pearson's product–moment tests were used to probe for correlations between intake and behavioral data.

A graphical representation of the temporal changes in the recorded behaviors used the medians (minus 25% and plus 75%) of the relative durations of feeding, drinking, preening and sleeping in each experimental group (the other behaviors were removed for the purpose of clarity). Also in the interest of clearness, a least square estimate (distance-weighted least squares fitting) for each curve was plotted in these graphs. The curves were calculated using a stiffness parameter (=zero) and a 2nd order polynomial regression to avoid excessive smoothing of the raw data.

The use of parametric or non-parametric ANOVA approaches to compare pairs of curves may be troublesome, particularly when numerous repeated measurements describing the treatment effects are unidentified nonlinear functions of, e.g., time. In this report, comparisons between two curves of a given behavior throughout the recording period (e.g., free-feeding versus 24-h fasting condition) were performed using the Cramér–von Mises statistic procedure (HL-test, [44]) with bootstrapping. Briefly, this method tested for differences between two regression curves (e.g., curves that represented the population of 4-min-bin data of the controls and of a given experimental group over 2 h). This test assumes that Y and Z are, respectively, the population of scores in two experiments (e.g., Y = durations of feeding in free-feeding animals, and Z = durations of feeding in pigeons presented with SM food) at the different periods of time (X). In the durations of feeding, Y and Z, are assumed as being functions of X, such as f(X) and g(X). Comparison of the two treatment effects was equivalent to checking if $g(X) = f(X)$, and the HL-test is used to test for an statistically significant similarity between these two functions. The null hypothesis was rejected if the proportion of bootstrap statistics (5000 bootstrapping samples in the present study) exceeding HL was less than or equal to the nominal level. For a more detailed description of this test, please refer to the Supplementary material, Appendix.

2.4. Evoking the BSS: Ingestive and behavioral responses to a 24 h-food deprivation period and to a "palatable" food

To probe for different protocols to observe the sequences of post-prandial behaviors, we compared the intake and behaviors in free-feeding animals to those observed in two conditions of increasingly higher motivation to feed. Six pigeons (5 males, 1 female, 340–400 g bw at the beginning of the experiments) were adapted to lab conditions for 15 days prior to the experiments. Their behaviors were recorded in their home-cages for 2 consecutive hours (from 14:00 to 16:00 h) in 3 sessions (sessions 7 days apart and distributed to the birds according to a Latin-squared design) in the following conditions: 1) the pigeons were maintained in a free-feeding regimen (for at least 7 days) and had their RC food cup replaced by a new cup with fresh RC (FF sessions); 2) the pigeons were in a free-feeding regimen (for at least 7 days) and had their RC food cup replaced by a new cup with fresh RC and were

simultaneously presented to a similar cup with fresh PF (FF + SM sessions); and 3) the pigeons were deprived of food for 24 h and then given access to cups containing fresh RC and SM (FD24h + SM sessions). Food and water were weighed 1 and 2 h after food presentations and the PF cup was removed at the end of the 4th hour.

3. Results

In experiment 1, food intake increased significantly after SM plus RC presentation to free-feeding pigeons (FF + SM) as well as to pigeons subjected to 24-h food deprivation (FD24h + SM; nutritional state effect: $F(2,30) = 8.53$, $p = 0.001$; time after food presentation effect: $F(1,30) = 72.61$, $p < 10^{-6}$; interaction: $F(2,30) = 17.37$, $p < 10^{-6}$) (Fig. 1A). Total food intake increased in the 1st hour and was similar to FF controls in the subsequent period in both conditions. SM intake in the 1st and 2nd hour was significantly higher than the RC in all nutritional conditions (data not shown). Food intake after FD24h was also higher compared to that observed in FF + SM animals. No differences in water intake were observed across the different nutritional states or among the different hours of recording (Fig. 1B). Significant changes

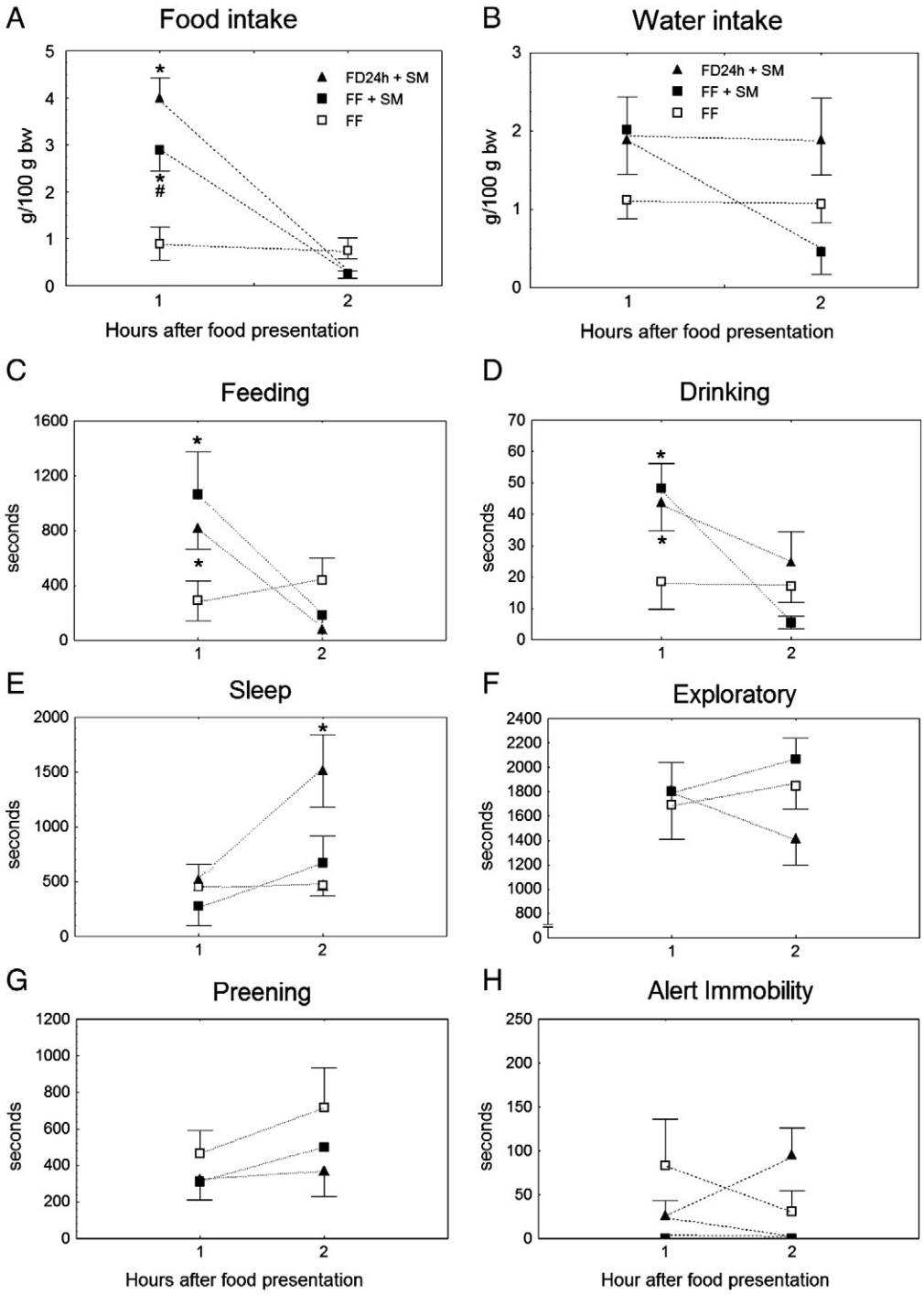

Fig. 1. Food/water intake (A–B) and duration (C–H) of ingestive and non-ingestive behaviors in free-feeding pigeons (FF), in free-feeding pigeons after food presentation to the seed mixture (FF + SM), and in 24-h-food deprived animals presented to the seed mixture and regular chow (FD24h + SM). Data were expressed as the mean ± SEM. (*) $p < 0.05$ compared to the FF data in the 1st recording hour. (#) $p < 0.05$ compared to the FD24h + SD results.

in the duration of feeding [time period: $F(1,30) = 12.44$, $p < 0.001$; nutritional state: non-significant (NS); interactions: $F(2,30) = 5.32$, $p = 0.01$], and drinking [time period: $F(1,30) = 10.25$, $p < 0.003$; nutritional state: NS; interactions: $F(2,30) = 3.29$, $p = 0.05$] were observed.

In the FF + SM and FD24h + SM conditions, feeding and drinking were significantly higher than those of the FF animals and were also higher in the 1st compared to the 2nd hour (Fig. 1C, D). Sleep duration [nutritional state: $F(2,30) = 4.61$, $p = 0.01$; time period: $F(1,30) = 7.55$, $p < 0.01$; interactions: (NS)] increased only in the FD24h + SM animals in the 2nd (as compared to FF pigeons), while preening, exploratory/locomotor, and alert immobility were not significantly changed (Fig. 1E–H; Table 2). Pearson's test indicated a strong positive correlation between total food intake and sleep duration ($r^2 = 0.72$, in the 1st hour after food presentation) only in the FD24h + SM animals.

Latency to start feeding [Kruskal–Wallis test; $H(2,18) = 14.76$, $p = 0.0006$], preening [$H(2,18) = 5.49$, $p = 0.05$] and sleeping [$H(2,18) = 11.28$, $p = 0.003$] was affected by the nutritional state while latency to drink remained unchanged (Fig. 2A). Feeding started significantly earlier in the FF + SM and FD24h + SM animals compared to the FF animals. Latency to the 1st preening episode was increased in the FD24h + SM animals, and the 1st sleep episode occurred later in the FF + SM and FD4h + SM pigeons compared to the FF pigeons. Moreover, FD24h + SD animals increased their latency to start preening and decreased their latencies to start sleeping when compared to the FF + SM animals. No significant differences between the latencies for feeding, drinking, preening and sleep were observed in the FF animals, while feeding occurred significantly earlier than drinking and sleep in the FF + SM animals [$H(3,24) = 14.85$, $p = 0.002$] and earlier than drinking, preening and sleep in the FD24h + SD animals [$H(3,24) = 13.83$, $p = 0.003$] (Fig. 2A, Table 2).

The amount of time to reach the peak (TTP) of feeding [$H(2,18) = 12.12$, $p = 0.002$], preening [$H(2,18) = 10.21$, $p = 0.006$] and sleep [$H(2,18) = 9.18$, $p = 0.01$] after food presentation was also changed in the different nutritional states (Fig. 2B). TTP decreased for feeding and increased for preening. Furthermore, sleep increased significantly for all conditions compared to the FF animals, while drinking reached its maximum at similar times in all conditions. Compared to the FF + SM animals, the time to reach preening peak increased significantly in the FD24h + SD animals, and their latencies for the peak of

Table 2
Ingestive and behavioral responses to fasting and palatable food in pigeons.

	Free-feeding (FF) (n = 6)	FF + seed mixture (n = 6)	24-h food deprivation (n = 6)
Food intake (g/100 g bw)			
1st hour	0.89 ± 0.34	2.88 ± 1.08[a,b]	3.99 ± 1.17[a]
2nd hour	0.75 ± 0.45	0.23 ± 0.20	0.23 ± 0.19
Feeding (duration, s)			
1st hour	292 ± 142	1064 ± 75[a]	818 ± 349[a]
2nd hour	436 ± 369	118 ± 23[a]	50 ± 65[a]
Latency (s)	2321 (529, 2710)[c]	185 (144, 221)[a]	56 (39, 61)[a]
TTP (bins)	12.5 (12, 3)	2.5 (3, 2)[a]	2 (1, 3)[a]
Water intake (ml/100 g bw)			
1st hour	1.11 ± 0.32	2.01 ± 1.31	1.89 ± 1.05
2nd hour	1.06 ± 1.08	0.46 ± 0.37	1.80 ± 1.00
Drinking (duration, s)			
1st hour	25.56 ± 8.46	48.31 ± 21.75[a]	43.74 ± 24.99[a]
2nd hour	17.05 ± 13.59	5.48 ± 5.05	24.66 ± 24.02
Latency (s)	863 (442, 2053)	1387 (881, 2306)	822 (580, 3065)
Time to peak (bins)	8.50 (3.00, 9.00)	8.0 (4, 12)	5 (4, 6)
Preening (duration, s)			
1st hour	758 ± 219	311 ± 223	608 ± 772
2nd hour	715 ± 534	496 ± 369	374 ± 359
Latency (s)	304 (143, 529)	251 (98, 694)	986 (863, 1025)[a]
TTP (bins)	4 (4, 8)	13 (12, 14)[a]	9.5 (7, 12)[a]
Sleep (duration, s)			
1st hour	516 ± 125	276 ± 441	520 ± 405
2nd hour	470 ± 247	669 ± 599	1512 ± 807
Latency (s)	870 (184, 1281)	2880 (2533, 4560)[a]	1854 (1263, 2131)[a]
TTP (bins)	7 (6, 9)	19.5 (16, 22)[a]	10 (7, 14)[a]
Exploratory (duration, s)			
1st hour	1908 ± 166	1783 ± 513	1733 ± 408
2nd hour	1845 ± 463	2062 ± 432	1415 ± 531
Alert immobility (duration, s)			
1st hour	81 ± 53	0.0	26 ± 41
2nd hour	30 ± 57	0.0	95 ± 75
IPI feeding/drinking (bins)	−4 (−12, −2)	2.5 (2, 8)[a]	2.5 (1, 4)[a]
IPI feeding/preening (bins)	−7.5 (−8, −5)	11 (9, 12)[a]	6.5 (5, 10)[a]
IPI feeding/sleep (bins)	−6,5 (−7, −4)	17 (7, 19)[a]	8.5 (6, 11)[a]
ItS feeding/drinking (bins)	4 (2, 9)	4 (4, 10)	4 (3, 4)
ItS feeding/preening (bins)	1.5 (1, 3)	6.5 (5, 13)[a]	8.5 (7, 9)[a]
ItS feeding/sleep (bins)	4 (1, 6)	12 (10, 16)[a]	9.5 (7, 14)[a]

[a] $p < 0.05$ as compared to FF data in the 1st recording hour.
[b] $p < 0.05$ as compared to FD24h + SD results.
[c] Values of latency, inter-peak interval (IPI), time-to-peak (TTP) and intersections (ItS) are expressed as medians (minus 25% and plus 75%), while food/water intake and duration of behaviors as expressed as mean ± SEM.

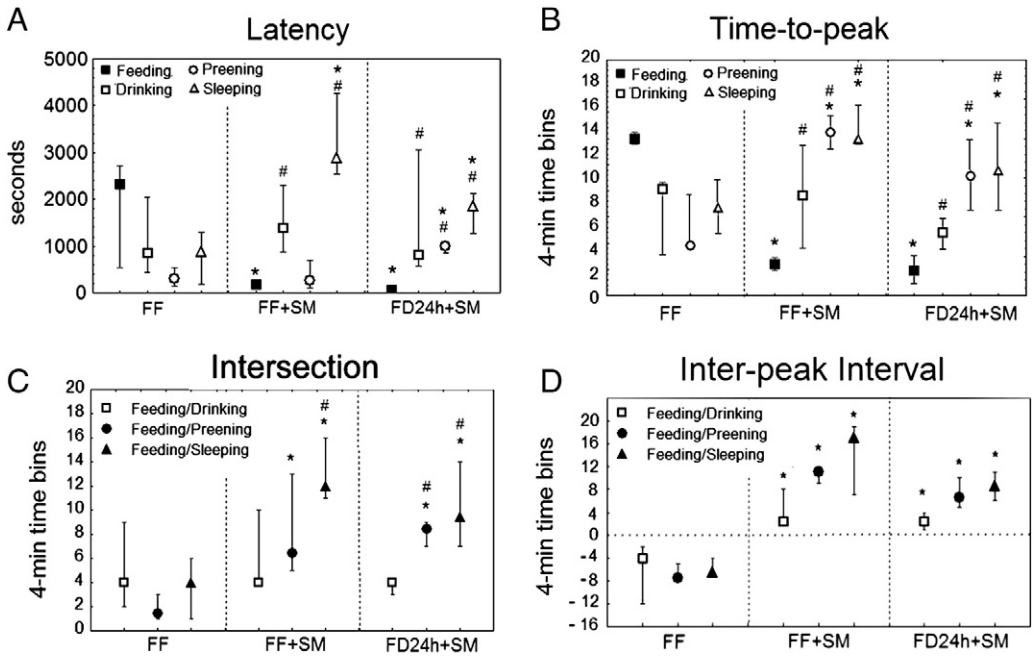

Fig. 2. Latency to the first occurrence (A), time-to-peak (B), intersection point (C), and inter-peak intervals (D) of ingestive and non-ingestive behaviors in free-feeding pigeons (FF) in free-feeding pigeons after food presentation to the seed mixture (FF + SM) and in 24-h-food deprived animals presented to the seed mixture and regular chow (FD24h + SM). Data were expressed as the median (symbols) plus the 75th percentile and minus the 25th percentile (whiskers). (*) $p < 0.05$ compared to the FF data and (#) $p < 0.05$ compared to the feeding score in the same experimental condition.

sleeping were decreased. The peaks of drinking, preening and sleep behaviors occurred significantly later than the peaks of feeding in FF + SM [$H(3,24) = 13.36$, $p = 0{,}004$] and FD24h + SD animals [$H(3,24) = 13.58$, $p = 0{,}003$], and the peaks of preening and sleep appeared significantly after the peaks of drinking in both contexts (Fig. 2B, Table 2).

Despite the significant changes in feeding latency and time to the feeding peak, the intersection point (ItS) between feeding and drinking was not changed in the different experimental conditions (Fig. 2C). However, ItS of the feeding–preening curves [$H(2,18) = 8.98$, $p = 0.01$] and feeding–sleep curves [$H(2,18) = 11.20$, $p = 0.003$] was significantly postponed in both FF + SM and FD24h + SD conditions compared to the FF animals (Figs. 2C, 4E–G); the ItS between feeding and drinking curves occurred significantly earlier than the ItS of feeding and sleep in the FF + SM and in the FD24h + SD but not the FF birds. The inter-peak intervals (IPI) between feeding and drinking [$H(2,18) = 6.82$, $p = 0.03$], between feeding and preening [$H(2,18) = 12.25$, $p = 0.002$], and between feeding and sleep [$H(2,18) = 11.93$, $p = 0.002$], which were all negative and similar in the FF animals, were significantly changed in the FF + SM and FD24h + SD pigeons (Fig. 2D and Table 2).

The temporal progression and peaks of the different behaviors throughout the recording sessions are depicted in Figs. 3 and 4A–G as the distance-weighted least squares line curves; the smoothing effect of these procedures on feeding and sleep individual data and in the medians can be observed in Fig. 3. Comparisons between pairs of curves of behaviors in different nutritional conditions using the Cramér–von Mises-based H-L test (Fig. 4A–D) indicated significant differences in the feeding curves of FF + SM ($p < 0.0001$) and FD24h + SD ($p < 0.0001$) animals compared to FF animals, but the FF + SM and FD24h + SD curves were similar ($p < 0.479$). There were no significant differences in the drinking curves in the different nutritional conditions, while the preening curves of FF + SM ($p < 0.0001$) and FD24h + SD ($p < 0.0001$) animals were significantly different from the FF animals. The sleep behavior curves of the FF + SM ($p < 0.027$) and FD24h + SD ($p < 0.0001$) pigeons were significantly different from that of the FF animals, and the FF + SM sleep curve was also different from that of the FD24h + SD animals ($p < 0.0001$). Comparisons using Cramér–von Mises test are further described in the Supplementary material (Appendix 1).

The evolution of feeding, drinking, preening and sleeping curves in each experimental condition is shown in Fig. 4E–G and is expressed as the percentage of time spent in that behavior in each 4-min bin relative to the total duration of the behavior. In these figures, the point of intersection between the feeding and sleeping curves (median minus 25% and plus 75% of 6 animals) is indicated, showing a significant increase in the FF + SM and FD24h + SD conditions. A noticeable peak of drinking appears after the first third of the feeding curve in the FF + SM and FD24h + SD curves, while the preening and sleep curves gradually waxes throughout the recording period, resulting in peaks that are more evident in the last third of the recordings. Furthermore, as indicated by the analysis of the IPI, ItS and TTP indices, the peaks of preening and sleep cannot be temporally segregated (visually or statistically); and only the latency of the first sleep event was significantly higher than that of preening. The individual data from the only female in the experimental group did not differ significantly from those of the 5 males (as judged by an outlier analysis using the group means ± 2× standard deviation as the exclusion criterion), and thus, they were included in the analyses.

4. Discussion

The analyses carried out for the total hourly pooled data indicated that the intense bouts of feeding evoked by both the FF + SM and FD24h + SM conditions affected drinking duration but failed to change the total water intake as well as the duration of exploratory or preening behaviors. Moreover, the time spent in the sleep behavior increased significantly only in the FD24h + SM condition, despite the fact that food intake in both conditions was distinctly different from the FF controls. Accordingly, a significant positive correlation between food intake and sleep duration was observed only in the FD24h + SM animals. From these coarse analyses, the presence of a clear-cut BSS similar to that of rodents [10,11,19] could not be easily perceived in pigeons.

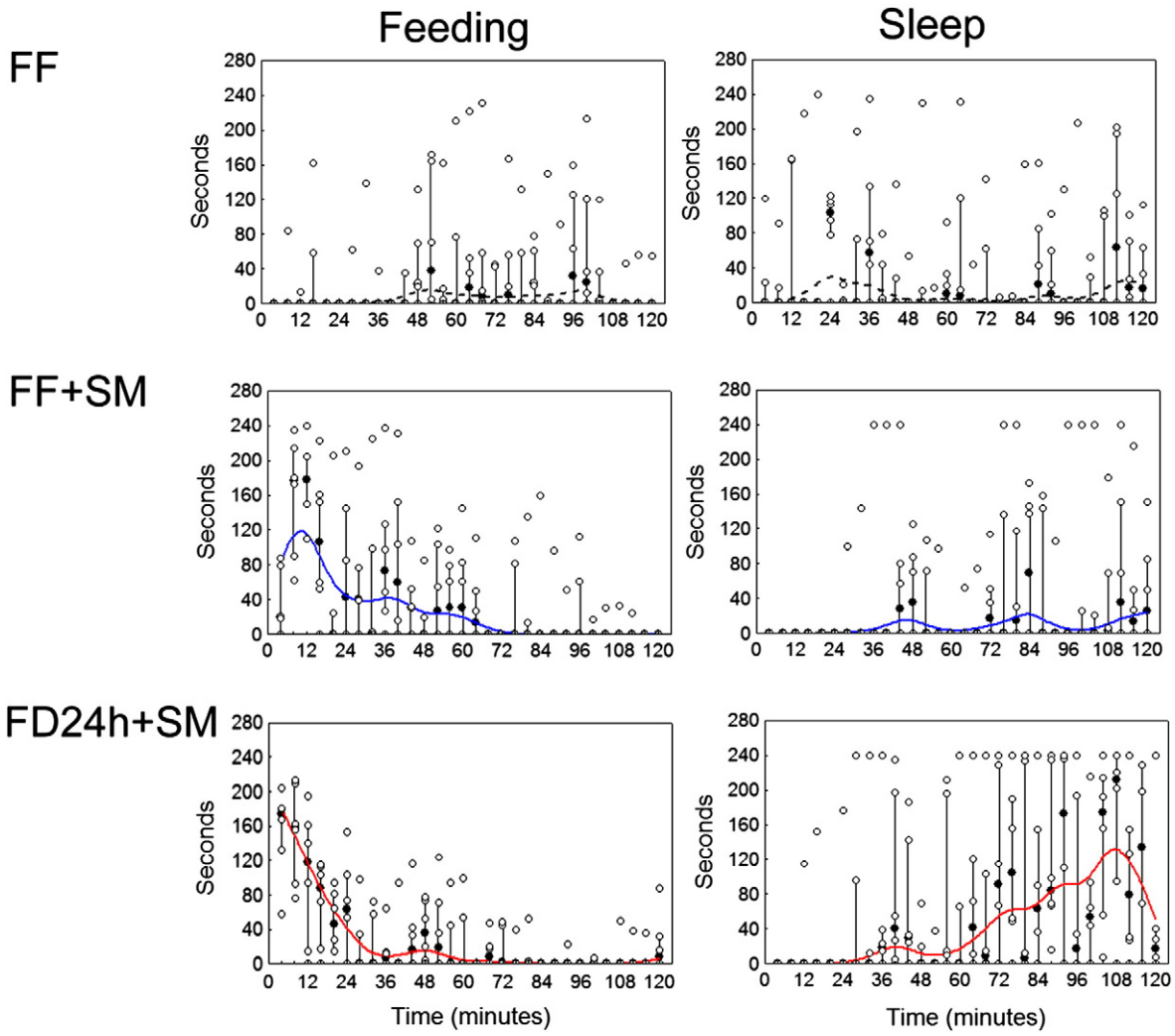

Fig. 3. Time course of the recorded behaviors during the 2 h subsequent to food presentation: each line represented the least square estimate (distance-weighted least squares fitting) for the median (filled circles, minus 25% and plus 75%) of the durations of each behavior (in seconds) of feeding, drinking, preening and sleeping in each experimental condition. Empty circles represented the individual data of the subjects in each time bin.

Furthermore, the relationship between the total food intake and magnitude of the subsequent resting state in the pigeon could not be fully compared to those of mammals. In rats, a positive correlation between the size of a meal and the duration of the electrographic signals of sleep during the following inter-meal interval has been shown [45,46]. However, food deprivation for 3, 6 or 12 h in rats evoked significantly different 1-h total food intakes and durations in feeding behavior in the early part of the BSS 1-h test but failed to affect the total amount of resting, grooming or exploratory behaviors compared to free-feeding rats eating a palatable mash [47]. Thus, at least in the range of intakes evoked by short fasting periods and presentation of palatable food, the magnitude of the post-prandial signs in pigeons appeared to be more susceptible to differences in meal size compared to rodents.

Conversely, the more fine-grained temporal/sequential description of these behaviors indicated that feeding after fasting, as well as after the mere presentation of a palatable food to a free-feeding animal, onsistently evoked sequential and temporal patterns of post-consummatory behaviors in the pigeon that were similar to those observed in primates and rodents. These animals showed definite post-prandial sequences of increased drinking and preening and then increased resting similar to the mammalian BSS. In contrast to the FF animals, latency to drink and the peak of drinking were placed ahead of feeding latency and of the feeding peak in FD24h + SM and FF + SM animals, which was similar to the results for the preening and sleep peaks. Thus, in both conditions, a definite, statistically verifiable sequence of feeding, drinking, preening and resting occurred. The temporal relationships among the components of this sequence were also changed in a similar fashion after fasting- and palatable food-evoked feeding compared to FF animals. Drinking behavior peaks increased at similar time intervals from the peak of feeding and crossed the declining feeding curve after comparable periods of time. Drinking, preening and sleep peaks occurred after feeding at equivalent time intervals and were predominant over the curve of feeding behavior at similar time points in both feeding-evoking protocols.

These data indicated that, beyond the differences in food intake and in the magnitude of feeding and sleeping total durations, FD24h + SM and FF + SM-evoked feeding was followed by a BSS-like pattern with comparable sequential and temporal parameters. These findings suggested that the mechanisms that establish the pace of BSS events in pigeons may not be as affected by the differences in the amount of food consumed or by the different motivational processes mediating feeding consummatory responses, which are driven by the homeostatic state (food reward in food-deprived pigeons) or by hedonic mechanisms (in satiated animals). Thus, it is apparent from these data that interconnected but distinct mechanisms control the magnitude of the BSS components and their time course in the pigeon. Nevertheless,

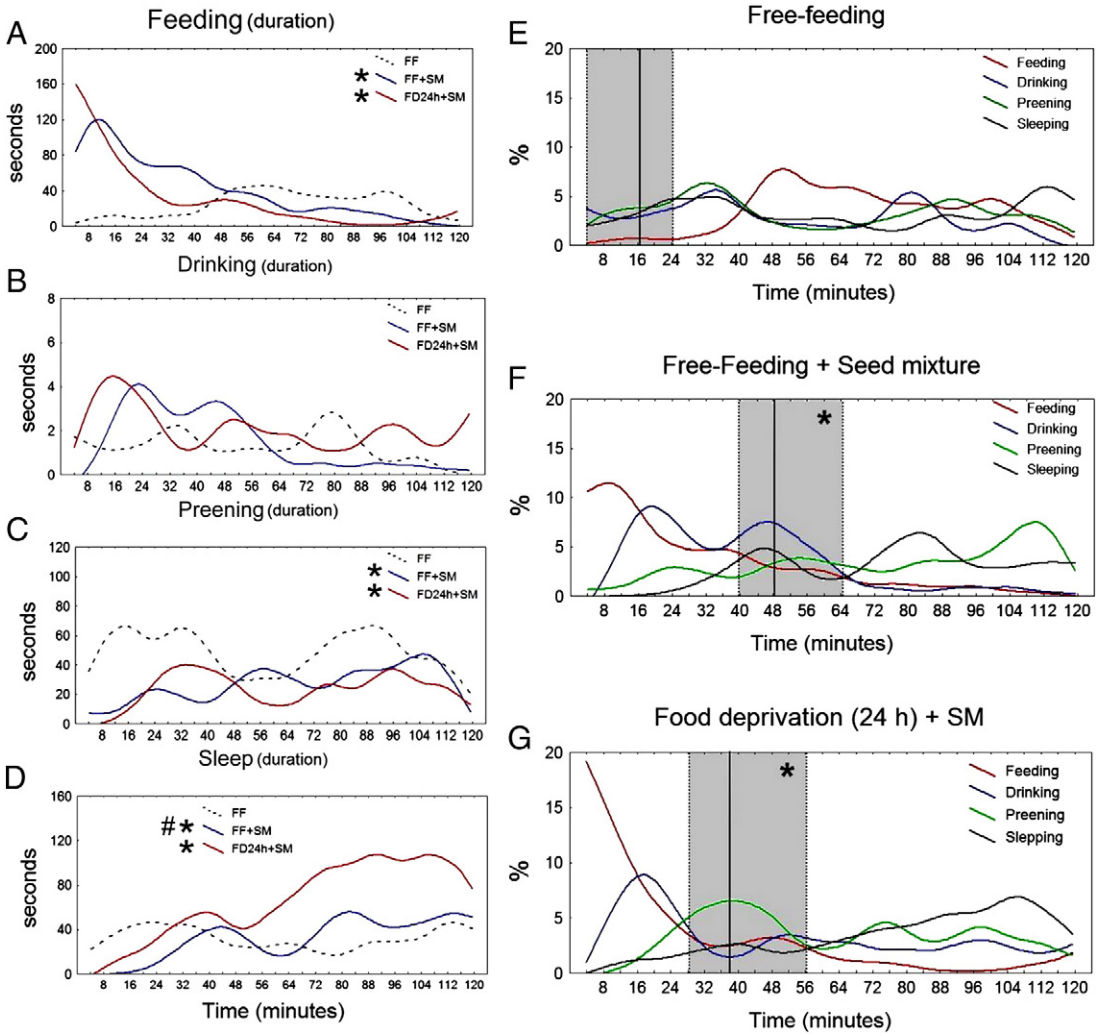

Fig. 4. Temporal changes in the recorded behaviors during the 2 h subsequent to food presentation: each line represented a least square estimate (distance-weighted least squares fitting) for the median (minus 25% and plus 75%) of the durations of each behavior (in seconds, A–D) or of the relative durations (in % of each 4-min bin, E–G) of feeding, drinking, preening and sleeping in each experimental condition. In A–D figures, the (*) indicated $p < 0.05$ in the Cramér–von Mises H-L test compared to the FF data, while (#) indicated $p < 0.05$ compared to the FD24h + SM animals. The shadowed regions in E, F and G indicated the median (straight line) minus 25% (left dotted line) and plus 75% (right dotted line) for the intersections between feeding and sleeping. In E–G, (*) denotes $p < 0.05$ compared to the FF data.

similar to pigeons, the mere presentation of a palatable mash to free-feeding rats increased feeding and evoked a noticeable BSS response. However, different fasting conditions in rats (3, 6 or 12 h) produced different feeding profiles and a delay in the BSS, as observed by the occurrence of transition points between eating and resting, which were postponed in food-deprived animals compared to non-deprived animals [47]. Although, the total amount of postprandial sleep appeared to be more sensitive to the food intake amount in pigeons compared to rats (see above). In addition, the temporal structure of the BSS in rat appears to be more sensitive to different volumes of feeding compared to pigeons, suggesting that mechanisms controlling the timing of BSS components may be different in pigeons and rats.

Prandial drinking has been suggested to be an integral part of the meal in rats [14] and accounts for nearly two-thirds of the daily total fluid intake in humans and other mammals (e.g., [15,48–50]). Prandial drinking is evoked by specific, pre-absorptive food-related signals in rodents [13,51,52]. Peri- and postprandial drinking are frequently recorded but usually not analyzed within the BSS context in rodent studies, most likely due to the low probability of occurrence of this behavior during the tests [10,47,53]. A low incidence of short drinking episodes was also observed after FD24h + SM and FF + SM-evoked feeding in pigeons and these procedures failed to change the total hourly water intake. Furthermore, the high positive correlation between 24-h food intake and water intake observed in pigeons (e.g., [43,54]) could not be demonstrated in our 2-h long recordings. Nevertheless, a detailed analysis of the drinking and feeding in free-feeding pigeons [55] revealed that 50% of the total time spent drinking occurred within 1 min following a feeding bout and that 70% of all drinking occurred between 3 min before and 3 min after feeding. Furthermore, the total duration of drinking increased, and a distinctive peak of drinking was detected at 12–24 min after the test food was offered. A rather rigid temporal link between feeding and drinking behaviors has been suggested by the stability of the latency to drink, the drinking TTP, the feeding–drinking ItS and the feeding–drinking IPI indices in both high-feeding conditions. It is apparent that a feeding episode could concentrate the drinking events in the late, waning period of the feeding event and that the mechanisms that establish the pace of the feeding–drinking relationships during the pigeon's BSS are not affected by different food intakes or by different feeding-inducing stimuli.

Importantly, the peaks of feeding, drinking and sleeping, but not of preening, are noticeably different in the high intake conditions compared to the FF conditions: a conspicuous early peak of preening was only observed in the FD24h + SM BSS curves. Nevertheless, the use of the IPI, ItS and TTP indices helped to reveal significant feeding-evoked

changes in the subsequent preening behavior in FF + SM, which were not apparent from the mere observation of the line plots or raw data. The results derived from these analytical tools indicated that preening may be an integral component of the BSS in pigeons. The close temporal proximity between peaks of preening and of sleeping may add support to the notion that preening may be a comfort behavior in birds and is associated with the anticipation of reinforcing (pleasant) events or with states of relaxation and de-activation [56–59]. Together with the evidence that fasting-induced feeding is followed by a period of low-frequency high-amplitude hippocampal EEG typical of SWS and drowsiness states, as well as of paradoxical sleep periods in the pigeon [36,37], the present data suggested that the preening and sleeping scores may be both associated with a general resting or relaxed state. Although preening is a major component of the pigeon's BSS, its corresponding behavior in rats (grooming) showed a more separate discernible peak amidst the waning of feeding and waxing of resting (see, e.g., [10,11,53]). Moreover, grooming occurred after eating and before resting, which was shown in initial reports of its occurrence as a major postprandial event in rodents [60,61].

Comparisons between the behavioral curves based on Cramér–von Mises statistics (the H-L test, [44]) helped to extend these conclusions in interesting ways. Despite differences in the total food intake between the FF + SM and FD24h + SM conditions, these tests indicated that the feeding curves were the same in both experiments. In addition, these tests were sensitive to the effects of the FF + SM-induced feeding on the preening and sleep behaviors evoked (which were dismissed by an hourly analysis). These results provided support to the findings of the lPI, ItS and TTP measures, which cross-validated H-L test findings. Comparing the curves of the ongoing behavioral data poses statistical challenges, including incomplete longitudinal observations, small samples, and inter-subject variability in temporal patterns. The Cramér–von Mises statistical approach did not require the limiting normality assumptions of other commonly used tests, which might result in a loss of efficiency and lead to the rejection of meaningful or useful data. In addition, the bootstrap method used in this study was a nonparametric approach and thus avoided the usual underlying distribution assumptions, demonstrating excellent coverage probability for small sample sizes [44,62,63]. Thus, it appears that this procedure may be suitable to interpret complex curves derived from behavioral flux and to detect significant behavioral changes that the coarse-grained temporal analysis and two other popular statistical tests (Wilcoxon and T-tests, see Appendix 1 in the Supplementary material) failed to discover.

In conclusion, the present data indicated that a BSS pattern could be reliably evoked in the pigeon in a chronological succession and sequence that strongly resembled that observed in rodents, and that this pattern could be quantitatively described and compared by different, suitable and coordinated behavioral measures. However, before the present observations can fuel studies on the comparative, evolutionary aspects of the mechanisms that shape the post-consummatory behavioral flux, systematic studies aimed at behavioral calibration (e.g., [53]) and pharmacological validation (e.g., [10]) must be carried out for the pigeon's BSS. These experiments will be reported in following companion papers to reveal potentially conserved and species-specific traits of the mechanisms controlling postprandial behaviors in amniotes.

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Disclosure of conflict of interest statement

All authors here state that they have no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence the work here submitted.

Role of the funding source

The funding agencies CNPq, CAPES and FAPESC had no influence or role in the study design, in the collection/analysis/interpretation of the data, in the writing of the report, or in the decision to submit the paper for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.physbeh.2013.08.031.

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