phosphorus and sulphur peaks associated with chromatin, as reported by other authors[13,14]. Calcium produced prominent peaks in the chromosomes (figure), indicating its presence in large concentrations. This is of particular interest in view of the permanently condensed nature of dinoflagellate chromosomes, as previous work[20,21] has suggested that high calcium levels are associated with the condensation of chromatin in mammalian nuclei.

The major conclusion to be drawn from this work is that high levels of the transition metals, iron, nickel, copper and zinc are present in the chromosomes (probably in an organic complex) of the dinoflagellates *A. carterae, G. foliaceum* and *P. micans*. It will be of considerable interest to determine if a similar situation exists in other dinoflagellates, or even in other members of the Protozoa.

1 L.P.K. gratefully acknowledges the financial support of the SRC.

2 J.D. Dodge, in: Chromosomes of the Algae. Ed. M.B.E. Godward. Arnold, London 1966.
3 P.J. Rizzo, J. molec. Evol. *8*, 79 (1976).
4 P.J. Rizzo and E.R. Cox, Science *198*, 1258 (1977).
5 D.F. Kubai and H. Ris, J. Cell Biol. *40*, 508 (1969).
6 A.R. Loeblich III, J. Protozool. *23*, 13 (1976).
7 J.R. Allen, T.M. Roberts, A.R. Loeblich III and L.C. Klotz, Cell *6*, 161 (1975).
8 M. Daune, in: Metal Ions in Biological Systems, vol. 3. Ed. H. Sigel. Dekker, New York 1974.
9 V.A. Skrinska, L. Messineo, R.L.R. Towns and K.H. Pearson, Experientia *34*, 15 (1978).
10 A.R. Spurr, J. Ultrastruct. Res. *26*, 31 (1969).
11 A.J. Morgan, T.W. Davies and D.A. Erasmus, Micron *6*, 11 (1975).
12 H. Shuman, A.V. Somlyo and A.P. Somlyo, Ultramicroscopy *1*, 317 (1976).
13 J.D. Duckett and D. Chescoe, Cytobiologie *13*, 22 (1976).
14 B.A. Weavers, J. Microsc. 97, 331 (1973).
15 J.A. Chandler, B.G. Timms and M.S. Morton, Histochem. J. *9*, 103 (1977).
16 B.A. Silverberg, P.M. Stokes and L.B. Ferstenberg, J. Cell Biol. *69*, 210 (1976).
17 H. Skaar, E. Ophus and B.M. Gullvag, Nature *241*, 215 (1973).
18 S.M. Saifullah, Mar. Biol. *44*, 299 (1978).
19 M.A. Anderson, F.M.M. Morel and R.R.L. Guillard, Nature *276*, 70 (1978).
20 I.L. Cameron, R.L. Sparks, K.L. Horn and N.R. Smith, J. Cell Biol. *73*, 193 (1977).
21 D. Mazia, in: Cell Cycle Controls. Ed. G.M. Padilla, I.L. Cameron and A.M. Zimmerman. Academic Press, New York 1974.
22 T.A. Hall, in: Physical Techniques in Biological Research, vol. 1A, 2nd edn, p. 157. Ed. G. Oster. Academic Press, New York 1971.

## Registration of feeding behaviour in rats by recording food approach behaviour

W.J. Rietveld, F. ten Hoor, M. Kooij and W. Flory

*Department of Physiology, Department of Physiological Psychology, University of Leiden, Wassenaarseweg 62, Leiden (The Netherlands), 27 December 1978*

*Summary.* A method is described in which food approach behaviour of rats is recorded to study feeding behaviour. Between rats, differences in food approach behaviour were observed. For each rat, food approach behaviour was constant over a long period of time. This allows conversion of approach behaviour data into quantified feeding behaviour. Examples of long-term feeding behaviour and of reproducibility of food intake are given.

The investigation of 24 h eating rhythmicity in rats requires reliable but uncomplicated apparatus for monitoring food intake. For our studies on changes in 24 h eating rhythmicity during hypothalamic hyperphagia in rats[1,2], we developed simple, inexpensive food hoppers provided with approach detectors so that food approach can be monitored continuously. This method has the advantage of being relatively inexpensive and avoids time-consuming measurement of food intake. However, it will be clear that food approaches are not necessarily representative of food intake. Unless each entry into the food hopper is followed by ingestion of the same amount of food and no spillage occurs, the method does not provide reliable data on actual food intake, and gives only information on the temporal pattern of feeding behaviour. On the other hand, if food approach can be monitored and is found to correspond to conventionally measured food intake, the method can give detailed information on the feeding behaviour in rats.

In order to evaluate the experimental set-up, we studied 15 rats for a period of about a year, continuously monitoring food approach and measuring food intake by weighing the food at intervals varying from 0.5 to 6.0 days.

*Methods.* 15 adult female Wistar rats (body weight at the start of the experiment $285 \pm 23$ g) were individually housed in a light and temperature controlled room with an average humidity of 60%. Each cage contained a pellet box constructed as described elsewhere[1,2].

In short, for each approach to the food a perspex flap had to be pushed away by the animal. The movement of the flap resulted in the activation of an approach detector (Pepperl and Fuchs, Mannheim FRG), the output of which was fed into a microcomputer type DEC LSI-11 configuration for temporary storage. In addition to the eating activity, a number of items of general information (number of

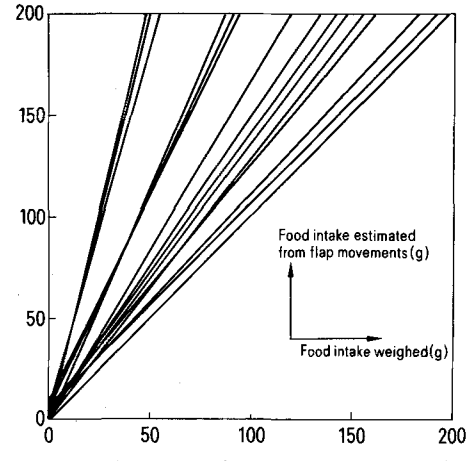


Fig. 1. Regression lines for each rat weighed food intake and food intake calculated from the number of food hopper flap movements and average pellet weight. The slopes of the regression lines range from 1.03 to 5.49, indicating differences in food approach behaviour between rats.

animal, light or dark period) could be stored simultaneously and each half-hour the data were automatically transmitted to a PDP 11/45 computer for further analysis. Food consisted of commercially available pellets with an average weight of $0.124 \pm 0.024$ g. Actual food consumption was measured over periods of 0.5–6.0 days by weighing the food reservoir at the beginning and at the end of each period. The weighing program was carried out several times during the whole experiment, which lasted from 8 to 11 months.

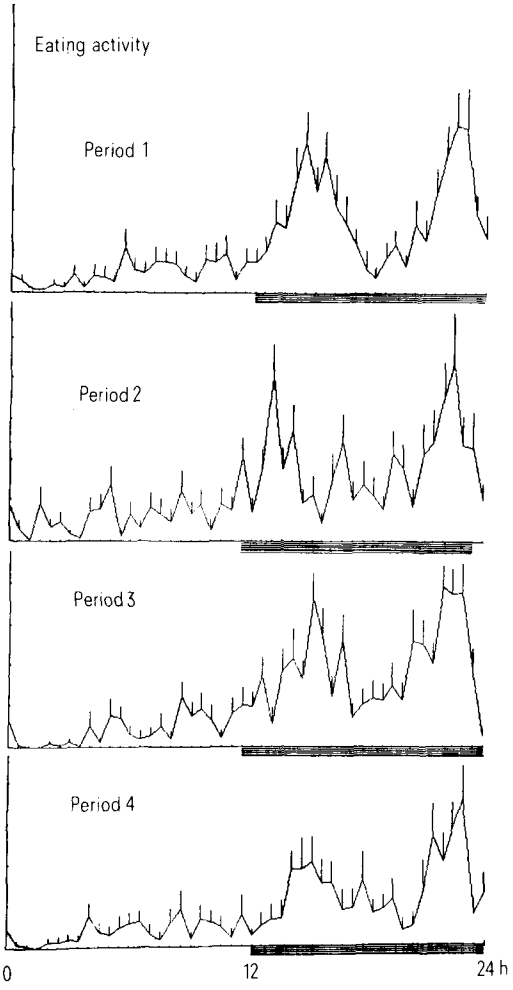


Fig. 2. Mean values of corresponding 0.5 h eating activities measured for rat No. 03 on 20 successive days (see table). Vertical bars indicate 1 SEM. Measurements were repeated during 4 time periods which fall within those indicated in figure 3. The characteristic eating activity pattern for each rat remains essentially the same for a period of 10 months.

For each animal, food consumption was compared to the total number of approaches totalled over the same period of time. In a number of cases, the animal's behaviour was observed and analysed with the aid of a closed circuit television system.

*Results and discussion.* The relationship between actual (weighed) food intake and the number of approaches recorded during the measuring periods appeared to be essentially linear for each rat. When food consumption as estimated by multiplying the number of flap movements with the average pellet weight, was compared to the actual consumption of food, we found correlation coefficients ranging from 0.90 to 0.99.

The slope of the regression lines ranged from 1.03 to 5.49 (figure 1). For each rat, however, the slope was remarkably reproducible during the whole experimental period (table). Analysis of the TV-observed behaviour revealed various approaches to food intake. A number of rats open the flap and eat a pellet. Others play around with the flap in a very rigid manner before taking the pellet. The most peculiar pattern observed was that of a rat who, using its mouth, repeatedly swung the flap before finally taking one pellet.

Pressing a door-flap in order to take a food pellet requires an operant conditioning process which will take place during the first days of housing. Individual differences in motor behaviour during this acquisition period will determine the ultimate food approach behaviour. The findings that this behaviour appears to be quite stable over a long time period (probably due to the continuous reinforcement) and that the relationship between actual food intake and food intake as estimated from flap movements is essentially linear, make the apparatus suitable for monitoring feeding habits in rats. Examples of the possibilities of the method are shown in figures 2 and 3. In figure 2 the mean values of corresponding half-hour eating activities measured over 20 day periods are plotted against time; the 20 day periods chosen fall within those mentioned in the table for rat Nr. 03. It is shown that in addition to the remarkable reproducibility of the slope of the regression line which relates weighed and estimated food intake (table), the pattern of eating activity for each rat also remains essentially similar for a long time period. Figure 3 gives for the same rat a plot of the total amount of food consumed per light (lower graph) and dark period against time, for a period of 10.5 months. Apart from the marked differences between day and night levels of eating activity, an oscillation with a period of 5 days is observed. Since a definite influence of the estrous cycle on food intake behaviour has been described[3], this 5 day oscillation may run parallel with the estrous cycle. Figure 3 also shows a long-term fluctuation, the eating activity being highest in March and April and lowest in August. Whether this fluctuation reflects an endogenous circannual rhythm, or has been induced by external circumstances, is the subject of further investigation. We conclude that, with the aid of

Slopes and y-axis intercepts with 90% confidence intervals of regression lines relating weighed food intake and food intake calculated from number of food hopper flap movements and average pellet weight

| Rat number | Period | Slope | 90% interval | Intercept | 90% interval |
|---|---|---|---|---|---|
| 03 | 1 | +1.23 | +1.11–+1.35 | +0.56 | − 0.20–+ 1.32 |
| | 2 | +1.41 | +1.27–+1.55 | +0.36 | − 0.64–+ 1.36 |
| | 3 | +1.26 | +1.06–+1.45 | +0.04 | − 1.21–+ 1.30 |
| | 4 | +1.33 | +1.27–+1.39 | +0.77 | − 0.90–+ 4.45 |
| 04 | 1 | +3.79 | +3.40–+4.18 | −8.23 | −11.06–+ 5.41 |
| | 2 | +3.87 | +2.98–+4.75 | −1.27 | −06.85–+ 4.31 |
| | 3 | +4.32 | +3.24–+5.41 | −7.76 | −14.72–− 0.79 |
| | 4 | +3.43 | +2.83–+4.04 | +6.40 | −19.07–+31.86 |

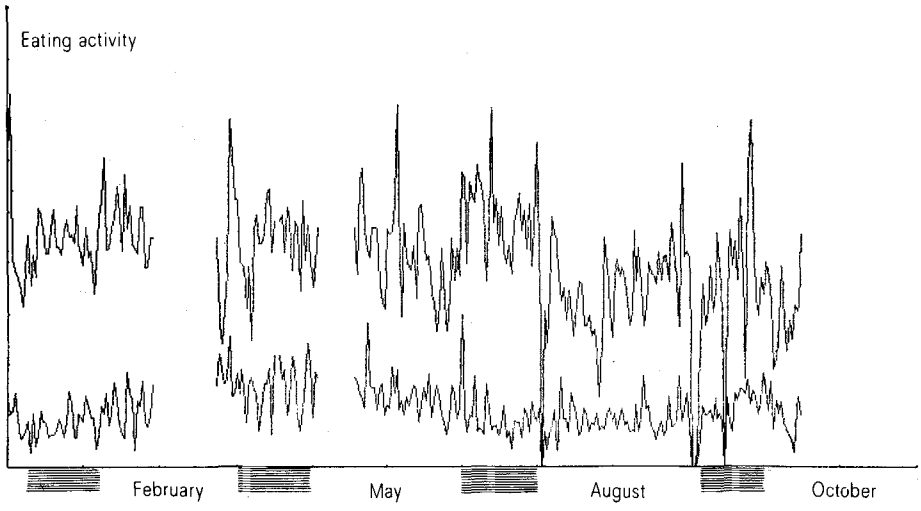


Fig. 3. Total amount of food consumed per light (lower graph) or dark period plotted against time for 10.5 months. The hatched areas indicate the periods during which the data presented in the table and figure 2 were obtained. The observed 5 day fluctuations in eating activity may run parallel with the estrous cycle. In addition a longterm fluctuation - peak in March/April, dip in August - can be observed.

simple food hoppers, recording of the eating activity of rats is very feasible. Automatic data sampling and storage as well as suitable computer evaluation of these data makes the method a valuable and reliable one for the investigation of eating behaviour in rats.

1 W.J. Rietveld, F. ten Hoor, M. Kooij and W. Flory, Physiol. Behav. *21*, 615 (1978).
2 W.J. Rietveld, F. ten Hoor, M. Kooij and W. Flory, Physiol. Behav. *22*, 549 (1979).
3 M.B. ter Haar, Horm. Behav. *3*, 213 (1972).

## Amylase secretion from rat parotid glands as dependent on co-operation between sympathetic and parasympathetic nerves[1]

B. Asking, Ursula Delfs, N. Emmelin and P. Gjörstrup

*Institute of Physiology, University of Lund, Sölvegatan 19, S-223 62 Lund (Sweden), 19 January 1979*

*Summary.* A slow, long-lasting 'degeneration secretion' from the parotid gland was brought about in anaesthetized rats by section of the auriculo-temporal nerve 16–19 h in advance. This parasympathetic background activity greatly increased the secretion of amylase elicited by sympathetic nerve stimulation.

Although sympathetic nerve fibres surround the acini of most salivary glands, the salivary flow response to electrical stimulation of the cervical sympathetic trunk is mostly remarkably small, even when the pronounced vasoconstriction elicited at the high stimulation frequencies required for secretion is prevented pharmacologically[2]. Experiments on dogs and cats show, however, that the flow is much increased, and otherwise subliminal frequencies become effective, when the sympathetic nerve is stimulated while the gland is exposed to a slow stream of parasympathetic impulses, corresponding to the physiological situation in the waking state[2,3]. In these experiments, only the volumes of saliva produced were taken into account, but recent observations on the parotid gland of the rabbit indicate that also the secretion of amylase on sympathetic stimulation is promoted by simultaneous parasympathetic activity[4].

After section of the auriculo-temporal nerve in rats, acetylcholine release from the degenerating axons causes a period of 'degeneration secretion' of saliva from the parotid gland; it starts after 14–22 h and lasts for 7–8 h[5]. In the present experiments, this was made use of to provide the slow, prolonged parasympathetic background activity on which sympathetic stimulation was superimposed to evoke secretion of amylase. As in the rabbit, but not in the dog or cat, the parotid gland of the rat is rich in amylase, which is secreted particularly in response to sympathetic stimulation[6,7].

*Methods.* In 10 male rats of a Wistar strain (weights 290–400 g) the right auriculo-temporal nerve was cut in ether anaesthesia and 16–19 h later chloralose (50 mg/kg) was given i.v. after induction with ether. Anaesthesia was then maintained by injecting pentobarbitone (2–5 mg/kg) i.v. when required. A tracheal cannula was inserted and the right parotid duct cannulated. Drops of saliva of a size of 10 µl were recorded and collected in samples of 12–14 drops each, which were stored frozen until analysed for amylase[8]. The cervical sympathetic trunk was exposed, placed on a bipolar electrode and stimulated at 0.3, 0.5 and 1.0 Hz, using a pulse duration of 2 msec and supramaximal intensity. Stimulation at 1.0 Hz was repeated after i.v. injection of atenolol 2 mg/kg. The 10 rats all showed degeneration secretion, but only in 6 of them was it sufficiently rapid, constant and long-lasting to allow collection of all the stimulation samples and interposed controls, altogether 10–12 samples, occupying a time period of 3.5–4.5 h.

*Results and discussion.* The results of the 6 experiments are summarized in the figure. The saliva produced during the degeneration secretion contained amylase in a concentration similar to that reported for saliva secreted during electrical stimulation of the auriculo-temporal nerve in normal rats[7]. Superimposed sympathetic stimulation markedly increased the amylase content of the saliva. A frequency as low as 0.3 Hz raised it almost 4 times, from