Experimental studies on the control and regulation of feeding in the common carp, *Cyprinus carpio* (L.).

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ABSTRACT

The aims of this work were three-fold, to investigate the patterns of food intake exhibited by carp (*Cyprinus carpio*) feeding on an operant regime, to investigate the role of the nucleus posterior thalamicus (NPTh) in the control of food intake, and to investigate the role of gut peptides in the control of food intake.

A range of techniques were employed in pursuit of these objectives. Behavioural studies were used to investigate feeding patterns, and to assess the effects of small electrolytic lesions placed in the region of the NPTh. Histological and physiological techniques were employed to investigate the cellular morphology of the NPTh and its relationship with both hindbrain and telencephalon. Pharmacological and behavioural techniques were employed to obtain a dose response characteristic for the peripheral administration of bombesin and its effect on food intake.

Animals held individually and feeding on an operant regime showed no indication that they were feeding in bouts. Satiety was seen to increase exponentially or logarithmically over the course of a feeding session.

Small lesions of the NPTh caused a highly significant and variable period of anorexia or hypophagia. On recovery of feeding behaviour in lesioned animals, satiety was seen to increase in a linear fashion over the course of a feeding session.

Histologically the cell bodies within the NPTh are seen to be morphologically homogenous. Physiologically it is probable that the NPTh efferent fibres are of small diameter and unmyelinated, projecting to the vagal and facial lobes of the medulla. There is some evidence that the NPTh operates as a single unit providing the same function in both internal and external chemosensory systems. The hypothesis is advanced that the NPTh has a role in the determination of palatability in both internal and external chemosensory systems.

Intra-peritoneal administration of the peptide bombesin was shown to reduce food intake in animals feeding on an operant regime, in a dose dependent manner. This result is very similar to the effects of bombesin administration seen in the higher vertebrates. A further result of bombesin administration demonstrated in these experiments is the appearance of a bout feeding pattern, effects of bombesin administration on the micro-structure of food intake have not been reported in other species.

I hereby declare that this thesis is my own work, except where the contrary is specifically indicated. No other registrations for an award of either the CNAA or any university occurred during the period of this research programme. Advanced studies undertaken with this programme of research included attendance of seminars and conferences.

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GENERAL INTRODUCTION

Food intake and feeding behaviour in general have been studied in a wide range of animals although mammalian studies have tended to dominate the available literature. Those studies carried out in fish have tended to concentrate on commercially important groups such as the salmonidae. Fish may well become an increasingly important source of high quality protein, and as such an increasingly cultivated and farmed animal. An understanding of the factors which control food intake in fish will assume an ever greater importance as increased demand for fish protein leads not only to greater pressure on the need for efficient farming techniques, but also to the farming of an ever greater variety of fish species.

This thesis attempts to investigate some of the features associated with the control of food intake in the carp *Cyprinus carpio*. Feeding is a highly complex behaviour which depends upon a great many variables ranging from the physiological to the psychological and the social, for this reason the environment under which feeding behaviour is investigated must be well controlled. Section I of this thesis describes a computer controlled operant feeding experiment and investigates the pattern of food intake exhibited by individually housed carp feeding under such a regime.

Sections II and III of this thesis deal with the role of the hypothalamus in the control of food intake. The hypothalamus is a region of the brain known to be involved with the control of food intake in many species, and section II investigates the effects of

hypothalamic lesions on food intake in carp. The nucleus posterior thalamicus (NPTh) is a discrete hypothalamic nucleus which has been shown anatomically to project to hindbrain gustatory areas. Section II specifically investigates the effects of lesions placed within the NPTh. Section III contains a more detailed investigation of the anatomy of the NPTh and its physiological relationship with the hindbrain.

Peripheral factors also exert a controlling influence on food intake and a number of peptides are now known to be of great importance, one such peptide is bombesin. Section IV deals with the effects of intra-peritoneally administered bombesin on food intake in the carp.

SECTION I

INTRODUCTION

There has been a great deal of work carried out on the normal feeding patterns and rates of various fish species including carp (Garcia and Adelman 1985, Kevern 1966, Wright and Eastcott 1982a,b), rainbow trout (Grove, Loizides and Nott 1978, Landless 1976), brown trout (Elliott 1979, Elliott and Persson 1978), perch (Craig 1978, Elliott and Persson 1978) and goldfish (Rozin and Mayer 1964,1961).

Some of these studies have been field-based and some have been laboratory-based using operant conditioning techniques. Many of the estimates of food consumption made in the field rely on various assumptions, in addition they may well involve sampling and the sacrifice of some individuals. Bajkov (1935) derived an equation to estimate the daily food consumption of fish. The technique involves taking two samples of, for example, 100 fish each. The first sample are killed and the average stomach contents are calculated. The second sample are retained and each hour 10 fish are killed and their average stomach contents calculated. In this way the rate of decline of stomach contents is obtained. This enables a number (n) to be obtained which represents the time taken for the stomach contents to move into the intestine. If all food passes from the stomach to the intestine in 24 hours and the fish is feeding by day and by night then the average stomach contents = the average daily consumption. For slower or faster rates of digestion then the equation shown overleaf may be used.

$$D = A(24/n)$$

Where: D = daily consumption

A = average stomach contents at sampling time.

n = the number of hours as derived by the technique described above

This equation has formed the basis for many estimates of food consumption. It has been criticised however by Elliott and Persson (1978). They described two methods for accurately estimating daily food consumption which take into account the possibility that gastric evacuation is exponential - which is thought to be true for many species of fish. Their first method assumes a constant rate of feeding, and the second that food intake decreases with time. A problem with both methods is that the rate of feeding may vary between meals eaten by the same fish and between fish of similar size (Elliott 1975). If this variation occurs between sampling then a compromise needs to be adopted. In addition if feeding is discontinuous between samples then the food consumption will be under estimated. It is thus of great importance to understand the normal feeding patterns exhibited by the fish under consideration in these field trials. If the fish feed in bouts, as is generally accepted for trout (Landless 1976), but is debatable in carp (Rozin and Mayer 1961, 1964, Wright and Eastcott 1981a,b), then the technique might be inaccurate. If the fish feed continuously then the experimenter needs to know whether the rate is constant or declines with time. Elliott and Persson (1978) tested the adequacy of their model for fish which fed for only a part of the inter-sample interval. They found that the

test gives good estimates of food consumption if the inter-sample intervals are shorter than 3 hours. Longer intervals gave worsening estimates of food consumption. They conclude that the accuracy of their methods does depend on the particular pattern of feeding in evidence. In order to accurately determine feeding patterns for a particular species it is necessary to consider the evidence from both field studies and laboratory-based investigations. Feeding is not the result of a simple physiological condition but may have many causations including the social facilitation of feeding as described by Olla and Samet (1974). Any study of feeding behaviour carried out in 'field conditions', where such social aspects may be important, will be usefully supplemented by laboratory-based studies where all environmental parameters (and hence to a large extent physiological parameters) are controlled by the experimenter. In this way the experimenter may, as a result of controlling the environment, control the physiological factors influencing food intake and hence attempt to draw conclusions as to the internal mechanisms by which food intake is controlled.

Much of the recent work in this area which has a bearing on feeding patterns in fish, has in general been concerned with those aspects of fish feeding which might have commercial applications, particularly in areas such as fish farming. As a result many of the species studied have been those which are considered to be commercially important such as trout (Landless 1976), salmon (Brett 1971) and plaice (Jobling 1980). The main aim of research of this type has been in establishing those factors which allow a controlled feeding regime leading to the maximum conversion of food mass into fish biomass,

These studies have thus concentrated on factors such as the maximum amount of food eaten by such fish, the effect of temperature on food intake, the time taken for the return of appetite after feeding to satiation, the effect of calorific content of the foodstuff, and the effect of fish size on conversion of food mass into fish biomass. Knowledge of these factors allows efficient and cost effective farming of commercially important species.

The investigation of the features mentioned above has however shed some light on the physiological events underlying fish feeding. The findings on temperature effects show that irrespective of species, up to a certain point raising ambient temperature raises food consumption and *vice versa* (Rozin and Mayer 1961, Brett and Higgs 1970, Gwyther and Grove 1981). This effect has been attributed in large part to the fact that a rise in temperature increases the rate of gastric evacuation (Garcia and Adelman 1985). The relationship between appetite and gastric emptying rate has been studied by a number of workers (Brett 1971, Grove, Loizides and Nott 1978, Grove and Crawford 1980, Jobling 1981), who find that faster gastric evacuation leads to a quicker return of appetite and hence to an overall higher rate of feeding.

Having demonstrated a correlation between gastric evacuation rate and food intake, several investigations attempted to establish which factors other than temperature had an effect on gastric evacuation rate. Several mathematical models describing the gastric emptying curve are currently in existence. These models attempt to describe the amount of food left in the stomach in terms of the elapsed time

after cessation of feeding. Two of the most widely used models are the exponential model (Grove and Crawford 1980) and the square root model (Hopkins 1966) these are shown below.

Exponential model $\ln W_T = 4.925 - 0.69T$

Square root model $\sqrt{W_T} = 9.09 - 1.259T$

where: W_T = the amount of food remaining in the stomach (expressed as a percentage weight of the original meal) at a specified time after feeding.

T = the time in days.

These equations allow predictions as to the emptying time of the stomach, in the case of the exponential relationship the prediction is made that the emptying time of the stomach is the same for all sizes of meal (when expressed in percentage terms). In the case of the square root model the emptying time will increase in proportion to meal size to the power 0.5. Jobling (1981) has undertaken a re-analysis of much of the published feeding data and finds that in most cases the square root model proves to be a better fit to this data than the exponential model. Significantly, data obtained for the stomachless teleost *Blennius pholis* (Grove and Crawford 1980), were best described by the exponential model even after Jobling's re-analysis. Furthermore a later study on gastric evacuation in the carp (Garcia and Adelman 1985), found that mean alimentary canal contents decreased at an exponential rate, and that this relationship held true over a 5 month period during which the field samples were taken. These conclusions relating as they do to meal size, raise the

question of which parameters are we to use when measuring meal size. Two basic parameters of meal size are the surface area of the meal and the volume of the meal. Elliott (1972) found that rates of gastric evacuation in the brown trout were unaffected by variations in the size of a number of different food organisms, in other words in this species at least, surface area of meal has no bearing on gastric evacuation rate. This finding was echoed for carp in the study cited above by Garcia and Adelman (1985), they found that although the composition of gut contents was fairly uniform at any one sampling time there was considerable variation in the relative abundance of the predominant items in the diet between sampling dates. The clearest conclusion at the moment is that in many species gastric evacuation is best described as a function of the square root of the volume of the ingested meal with respect to time. There are however notable exceptions to this conclusion which in spite of careful re-analysis seem to be characterised by an exponential relationship, these exceptions appear to be those species which do not possess a morphologically well defined stomach.

Clearly the question of gut evacuation rates is not a simple one and it seems quite likely that it will not prove possible to derive a single model which adequately describes the process in all species. This is not a surprising finding when one considers the range of anatomical and physiological differences exhibited by the species of fish investigated in the studies mentioned above.

The analysis of factors such as gastric evacuation rate, temperature effects, and the return of appetite have all provided much good

evidence on which to base predictive estimates of the best times at which to feed fish held in aquaculture in order to obtain the highest conversion rates of fish food to fish biomass. This area of research has not surprisingly concentrated on those species considered to be commercially important, these types of fish are for the most part carnivorous animals in which the feeding pattern has been shown to be comprised of discrete meals or bouts. This should not be a surprising result in the case of carnivorous fish (such as the salmonidae) which possess a well defined storage stomach. Landless (1976) proposes a positive feedback control model to explain the feeding patterns of fish of this type. In this model the carnivore will, under the influence of positive feedback, feed very rapidly once feeding is initiated, this will allow the animal to consume as much food as possible in as short a time as possible. The degree of feedback is controlled in this case by the fullness of the stomach, which fills rapidly over the duration of the feeding bout. Once the stomach is full this brings about an end to the feeding period until some gastric evacuation has occurred and food becomes available once more.

In studies carried out on cyprinid species the presence of meals or bout feeding is debatable. In the case of the goldfish *Carassius auratus* Rozin and Mayer (1961) found no evidence for the presence of bout feeding. Similarly in the case of the carp *Cyprinus carpio* Garcia and Adelman (1985) also found no evidence for the presence of bout feeding. However, Wright and Eastcott (1982) also working on the carp *Cyprinus carpio* found that these animals did feed in bouts. No accurate time information is given for these bouts only that they were inconsistent with respect to duration and time of occurrence. If

the explanation of feeding patterns given by Landless (1976) is accepted one would expect that cyprinid species, which have no well defined storage stomach, would feed in a large number of very small bouts due to the fact that only a small volume of the gut is available to be filled. To this extent the findings of Wright and Eastcott appear to be in keeping with the positive feedback driven process of food intake outlined above. Their findings are however at odds with the results obtained by other workers in this area. Many of the studies mentioned above employed an operant regime in which the animals under investigation had to activate some form of trigger mechanism in order to gain access to food. Several studies have been carried out on cyprinid species (Wright and Eastcott (1982a,b), Rozin and Mayer (1961, 1964)). Wright and Eastcott drew many of their conclusions from studies which involved fish held in group tanks and sharing access to a single operant mechanism, in such a situation an operant response by any one individual would result in the delivery of food pellets which might have been consumed by any of the fish in the group. Behaviourally this situation is much more complex than that in which fish are housed individually. For this reason the studies described in section one of this thesis are based on the operant conditioning of individually held carp performing a simple operant response in order to obtain a precise quantity of food.

The use of animals in conditioning experiments has been a powerful analytical tool for many years. The type of conditioning used has in general been dictated by the type of behaviour under investigation. Under naturally occurring circumstances behaviour may be said to be elicited or emitted. Elicited responses tend to be those that can be

reliably induced by a particular stimulus. These responses tend to be reflexive in nature, the Mauthner cell driven tail flick of a startled goldfish for example. Emitted responses in contrast may not be elicited by any particular stimulus, an example here might be the locomotor activity of animals placed in an activity box. Elicited and emitted responses may be modified by conditioning. There are two types of conditioning; classical (or respondent) conditioning and operant (or instrumental) conditioning. Classical conditioning tends to be concerned with modifying the eliciting conditions for behaviour, whereas operant conditioning tends to be concerned with modifying the processes by which the consequences of present behaviour determine future behaviour. Operant conditioning has been found to be a particularly powerful tool in the analysis of the effects of certain drugs on behaviour. The use of fish in conditioning experiments is not unusual and many experiments have been carried out which demonstrate that fish are quite capable of being trained to perform both operant and conditioned responses. Bull (1928) for example was able to show that the blenny (*Blennius gattorugine*) could be conditioned to leave a small pot in its aquarium and proceed to a particular location awaiting the introduction of food as soon as it detected a rise in temperature of one or two degrees. Goldfish have also been shown to perform at least as well as other more commonly used experimental animals under complex schedules of reinforcement (Breuning and Wolach 1981). With regard to operant conditioning studies there is a wealth of data which testifies that many species of fish have been trained to lever press or perform some other task in order to receive a reward, usually food (Landless 1976, Grove Loizides and Nott 1978, Gwyther

and Grove 1981).

Recent technological advances have contributed greatly to the ease and accuracy of operant techniques particularly the availability of cheap and powerful microcomputers which in conjuction with suitable electronic interfaces may be used to drive sophisticated food delivery mechanisms. These factors are important when establishing a particular schedule of reinforcement for animals trained on an operant regime. It is necessary to construct the experiment with an appropriate schedule for the behavioural feature under investigation. Reinforcement schedules fall into four main categories.

1. The fixed interval schedule - on this schedule food becomes available at regular intervals provided the animal makes the appropriate response. Irrespective of an animals behaviour between reinforcements the first response after the fixed time interval has elapsed is maximally reinforced. Under this type of schedule animals may receive maximum reinforcement merely by responding just after the end of the prescribed time interval, not needing to respond during the interval.

2. Fixed-ratio schedule - On ratio schedules a fixed number of responses must be made before the animal receives any reinforcement. In this case frequency of reinforcement is determined by frequency of responding. This type of schedule would tend to promote a high regular rate of responding.

3. Variable-interval schedule - This schedule is again a time

dependent schedule, however in this case the time interval between reinforcements is variable and is distributed around a quoted mean time. This type of schedule will generate a very high and sustained rate of responding due to the uncertainty of the reinforcement.

4. Variable-ratio schedule - This schedule presents each reinforcement after a variable number of responses again number of required responses is grouped about a quoted mean number. This schedule will once again generate a high regular rate of responding until the demands of the schedule become so great that the response wanes.

Obviously when investigating complex behavioural relationships such as those between learning and memory, it is quite likely that a combination of the above schedules will be used. There is good evidence that goldfish will respond well even in procedurally complex operant schedules (Couvillon 1984).

It should be emphasised that in terms of the observed frequency of behaviour the schedule of reinforcement is more important than the reinforcement itself. It is quite possible that the schedule of reinforcement may well have an effect on the behaviour under investigation (Morse, McKearney and Kelleher 1977) particularly if the `cost` of obtaining the reward is set too high. The problem of matching reward to behavioural cost underlies the concept of the ecological control of satiety (Collier 1985). This concept arises from the fact that it is possible to show that when foraging animals encounter opportunities to procure meals that differ in cost or

benefit, they almost always procure low-cost or high-benefit meals. To feed optimally an animal must minimise the costs of foraging and maximise the benefits. The behavioural consequence of raising the cost of foraging for food is to reduce the number of meals taken, but to increase the meal size (Collier 1985).

In any operant experiment designed to measure 'normal' feeding patterns care must be taken not to set reinforcement schedules incorrectly thereby setting the cost of obtaining food too high or too low, and hence affecting meal size or duration. Obviously in these situations it is not possible to evaluate the metabolic costs to an animal of its normal foraging behaviour, and a degree of subjective judgement must be employed when deciding upon a schedule of reinforcement which approximates to the costs of normal foraging behaviour. In the case of operant studies the cost of the meal relates to the difficulty of the task and the number of times it must be repeated before the reinforcement is received.

In the case of the carp, normal foraging behaviour is represented by a spectrum of activity patterns. A commonly observed feeding sequence is for the animal to draw a quantity of the substrate into the mouth and by manipulation of the substrate with the palatal organ 'sieve' out any food items. Using this technique carp may be seen to graze at a steady rate for long periods of time. It was felt that this method of feeding would not be metabolically costly and would result in some small reward for many of the initiated feeding manouvres. As a result it was decided that a fixed ratio (1:1) reinforcement schedule should be employed. This type of ratio will allow a small reward for each

feeding response thus avoiding very high rates of responding and, provided the task is not too diffcult will be relatively low cost. The task chosen has been described in the materials and methods section below and is one which fits quite easily into the normally observed activity patterns of carp held under the conditions also described below.

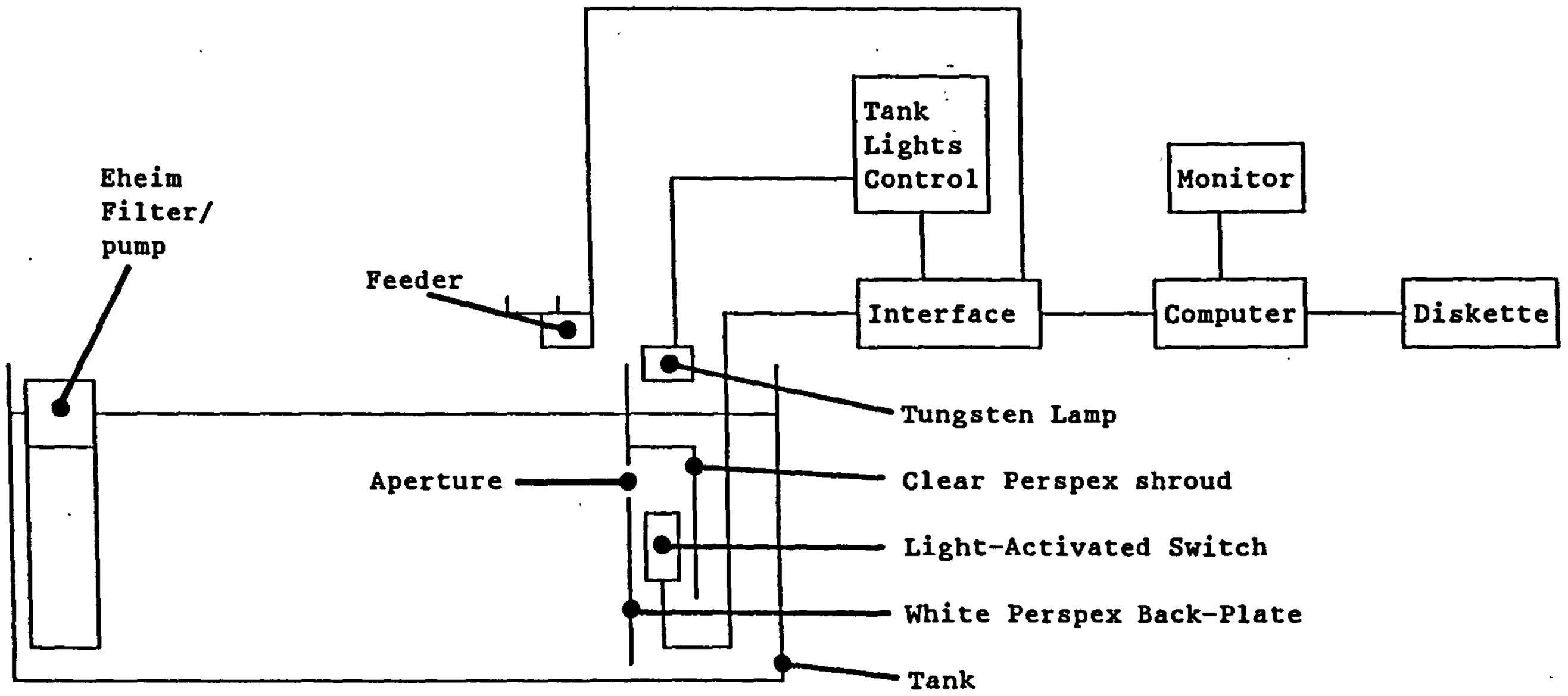
MATERIALS AND METHODS

Carp *(Cyprinus carpio)* of between 100 and 300 grams were obtained from a reputable independent supplier (Mr. Martin Moore). For the purposes of these experiments animals were held in glass tanks (60cm x 30cm x 38cm), water was circulated by means of an Eheim 2009 combined filter and pump. Tanks were subjected to a 12 hour light-dark cycle. The dark phase of the cycle was in fact a low light level condition as opposed to a complete black-out. Tank temperature was measured each day and varied between 19°C and 23°C. The feeders were controlled by a BBC model B microcomputer which also recorded the time to the nearest second, of every feeding event in each of up to eight tanks. The feeders were highly accurate and consistently delivered a single pellet for each actuation (Beach, Baker and Roberts 1986). Food was available to the carp for the first seven hours of each light phase. The food in use was Ewos Baker carp and trout holding food number 5. The apparatus is shown diagrammatically in figure 1.

In order to obtain a pellet of food the carp passed its head through a 6cm diameter aperture in a white Perspex sheet at one end of the tank, thereby casting a shadow onto a light-activated-switch (LAS). This event was detected by the computer which logged the time of the event and operated the feeder delivering a single pellet of food. Many animals spent several seconds poised over the LAS, to avoid repetitive stimulation of the LAS a 'dead-time' of 4 seconds was

Figure 1.

Diagrammatic representation of the apparatus used for all operant feeding experiments.


Eheim
Filter/
pump
Feeder
Tank
Lights
Control
Monitor
Interface
Computer
Diskette
Tungsten Lamp
Aperture
Clear Perspex shroud
Light-Activated Switch
White Perspex Back-Plate
Tank

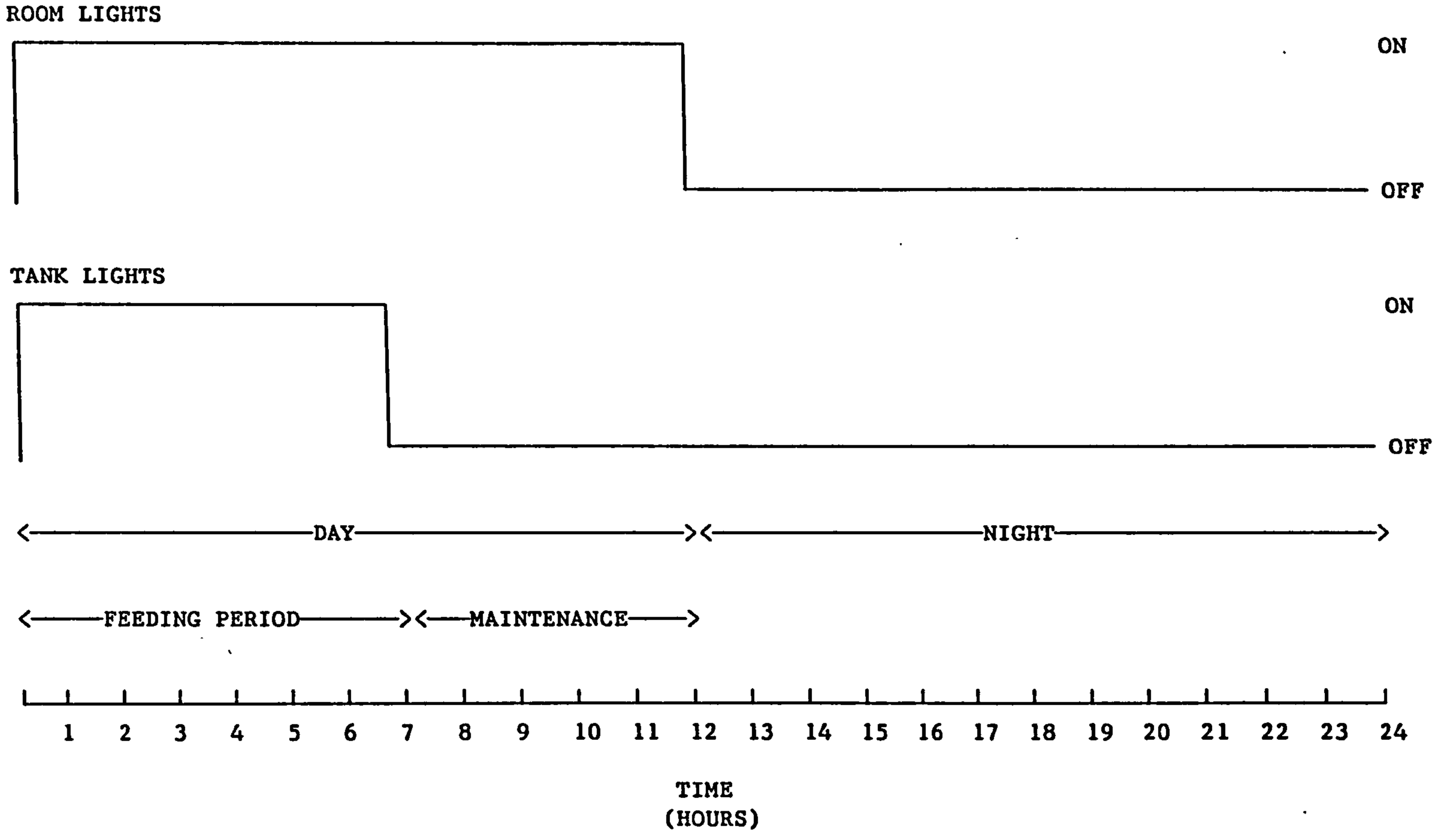
introduced by the controlling software, this meant that once activated, any further input from that LAS was ignored for the next 4 seconds. This strategy did not cause any problems due to missed counts since visual observation of the fish showed that they never fed at a rate fast enough to allow them to return for a subsequent pellet within the 4 second dead time.

Most of the animals used learned the task within 4-5 days of being placed in the experimental tank, requiring no experimenter-dispensed food pellets. Once the response had been learned a five day settlement period was allowed during which time the feeding response was consolidated and any food deficit incurred during the learning phase was redressed. Subsequent to this period an experimental period was begun during which time the weight of food consumed by each fish on each day was noted, this was achieved by the simple expedient of removing the food hopper from the feeder and weighing its contents at the end of the feeding period. In addition the computer recorded the precise time of each feeding event onto a floppy diskette for later analysis.

The timing cycle of room lights and tank lights allowed the onset of the light period to provide the cue that food was available to the animals in each tank, in addition the last 5 hours of the light phase were available for routine maintenance activities such as tank cleaning, fish weighing and the weighing of the food left in the feeder hoppers. Under this regime the animals had at least 12 hours to recover from any stress caused by maintenance activities. Figure 2 shows a timing diagram of the lighting shedule. Initially each fish

Figure 2.

Timing diagram showing the light-dark cycle used for the room lights, and the lighting cycle used for the tank lights.


ROOM LIGHTS
ON
OFF
TANK LIGHTS
ON
OFF
DAY
NIGHT
FEEDING PERIOD
MAINTENANCE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
TIME
(HOURS)

was weighed on a regular basis of every third day. However, over a period of a few weeks it became clear that food intake was declining. It was felt that this might be due to the stress associated with frequent weighing of the animals, for this reason the frequency of fish weighing was reduced to a rate of once a week. Weighing animals at a frequency of once a week did not cause a detectable decline in food intake. Water in each tank was replaced twice a week with fresh water pre-mixed to the correct temperature. Filters were removed and cleaned once a week.

In order to check that all the pellets demanded by each animal were consumed the tanks were checked each day for the presence of uneaten pellets. Any such pellets were removed and the tank was then monitored for a period of days for further evidence of uneaten food. Some animals developed the practice of demanding pellets which were not subsequently eaten, these animals were removed from the experiment and replaced with naive animals, this was necessary in less than 10% of cases.

In all cases direct visual observation of the experimental tanks during feeding sessions was possible. Visual observations of some of the experiments were also made by means of a video camera and a remote recorder held in an adjacent room. Remote viewing of feeding sessions was useful in that it allowed the monitoring of feeding behaviour to take place without the possibly disruptive presence of an observer.

Features of the design, construction and performance of the feeder have been dealt with in detail elsewhere (Beach, Baker and Roberts 1986, bound at the end of this volume) and will not be included here.

Data for this section was collected from a total of 33 animals. It was felt that in order to obtain a coherent set of data, feeding results from consecutive days should be used for the results analysis. The number of consecutive days should be as large as possible and the same for each animal. On inspection of the complete set of results it proved possible to extract coherent data from ten animals, each with ten consecutive days of uninterrupted feeding. Data was subjected to 5 types of analyses.

1. The weight of food eaten each day by each animal was measured over a period of ten consecutive days. This allowed verification of the fact that animals kept under this 7-hour feeding regime could establish a steady state level of feeding on a day to day basis. In addition a group of five animals were allowed to feed under a regime whereby food was available for only 3 hours per day. Animals were given several days to get used to the new feeding regime and then food weight data was collected over a period of 8 consecutive days. These data were analysed using a sign test to determine whether these animals could increase food intake under the 3 hour regime sufficiently to attain feeding levels previously attained under the 7 hour feeding regime.

2. Cumulative curves of feeding responses were recorded for each animal over a period of ten consecutive days. This allowed the

detection of any major differences in feeding patterns exhibited by each animal on a day to day basis.

3. Log survivor analysis was used to detect the possible presence of bout feeding. Log survivor analysis has been used to analyse 24 hour feeding patterns (Slater 1974), however more recent work (Willner and Towell 1982) has demonstrated its efficacy in detecting bout feeding in brief feeding sessions. Log survivor analysis depends upon the production of an inter-response time (IRT) frequency histogram. The IRT frequency histogram may be transformed to a survivor function, which shows the proportion or number of IRT's greater than any given IRT. A further transform produces a log survivor function. Experiments using this technique have shown that the log survivor function typically falls off steeply, very often in a straight line, which at a well defined break point changes to a much shallower slope. The assumption underlying this type of analysis is that IRT's shorter than the break point represent gaps between responses within a continuous bout whilst IRT's greater than the break point represent gaps between bouts. In other words, if a log survivor curve exhibits a well defined break point, then the feeding behaviour that generated that curve was organised into discrete bouts of feeding. In effect this type of analysis is tuned to detect the presence of two populations of intervals one grouped about a relatively short IRT representing intra-bout intervals, and a second (if present) grouped about a relatively longer IRT representing inter-bout intervals. The data used for each animal in this analysis was summed over a ten day period for that animal, this technique generates a single log survivor function for each animal based on data from ten consecutive

days of feeding.

4. The number of feeds in each half hourly interval was plotted as a function of elapsed time from the beginning of the feeding period. This analysis is similar to the one presented by Tugendhat (1960) for the stickleback, and demonstrates the distribution of feeds over the complete feeding period. Data for this section was plotted as the number of counts recorded (ordinate scale) in each half hourly interval for the duration of the feeding period (abcissa scale). In order to achieve statistically significant results, the data used in this analysis was pooled from the maximum number of consecutive days of uninterrupted feeding for each of the 33 animals used in this experiment. An average value for the number of feeding events in each 30 minute time bin was calculated. The curve of best fit was calculated using functions provided as part of the Graphwriter II software package used to plot these results.

5. In addition to the analyses described above a record was also kept of the weight gain of animals feeding under this regime, this allowed the calculation of conversion rates of food mass into fish biomass. In the context of this experiment fish biomass is taken as the wet weight of the animal concerned. The temperature of the water in the experimental tanks was also measured each day to allow the analysis of any possible correlation between temperature and feeding level. Data analysis was carried out with the aid of a BBC microcomputer. Except where stated below software used for these analyses was developed by the author and/or the author's supervisor. Graphs were generated using an ICL M50 personal computer and Lotus software`s

Graphwriter II application, graphs were plotted on a Mannesmann Tally 910 laser printer.

RESULTS

1. Daily food consumption.

Figures 3 to 5, and table 1.

Inspection of the data presented in this section of the results shows that superficially the fish fall loosely into three groups based on the day to day feeding patterns in evidence; those that show what might be described as a steady baseline (Figure 3), those that exhibit a slightly more variable baseline of food intake (Figure 4), and those that show an almost periodic oscillation between days of very high food intake and days of much lower intake (Figure 5). In the results presented in Figure 5 it may be seen that the period of oscillation varies between 3 and 5 days. This data shows the high degree of variability in the daily amounts of food consumed by these animals.

The results obtained from the reduced feeding period experiment are shown in table 1, which shows a comparison of the amount of food eaten during 8 consecutive days under the 7 hour regime with the amount eaten during 8 consecutive days under the 3 hour regime. As may be seen, a large increase in average feeding rate is achieved under the 3 hour regime. To determine whether there was a significant reduction in the amount of food eaten under the 3 hour regime, the data representing the difference in food intake under the two regimes were analysed using a one-tailed sign test. For the purpose of the sign test, the null hypothesis (H_0) was that the median of the

Figure 3.

Data from 3 animals showing a steady baseline feeding pattern. The data presented here was collected over ten consecutive days for each animal.

Daily Food Consumption

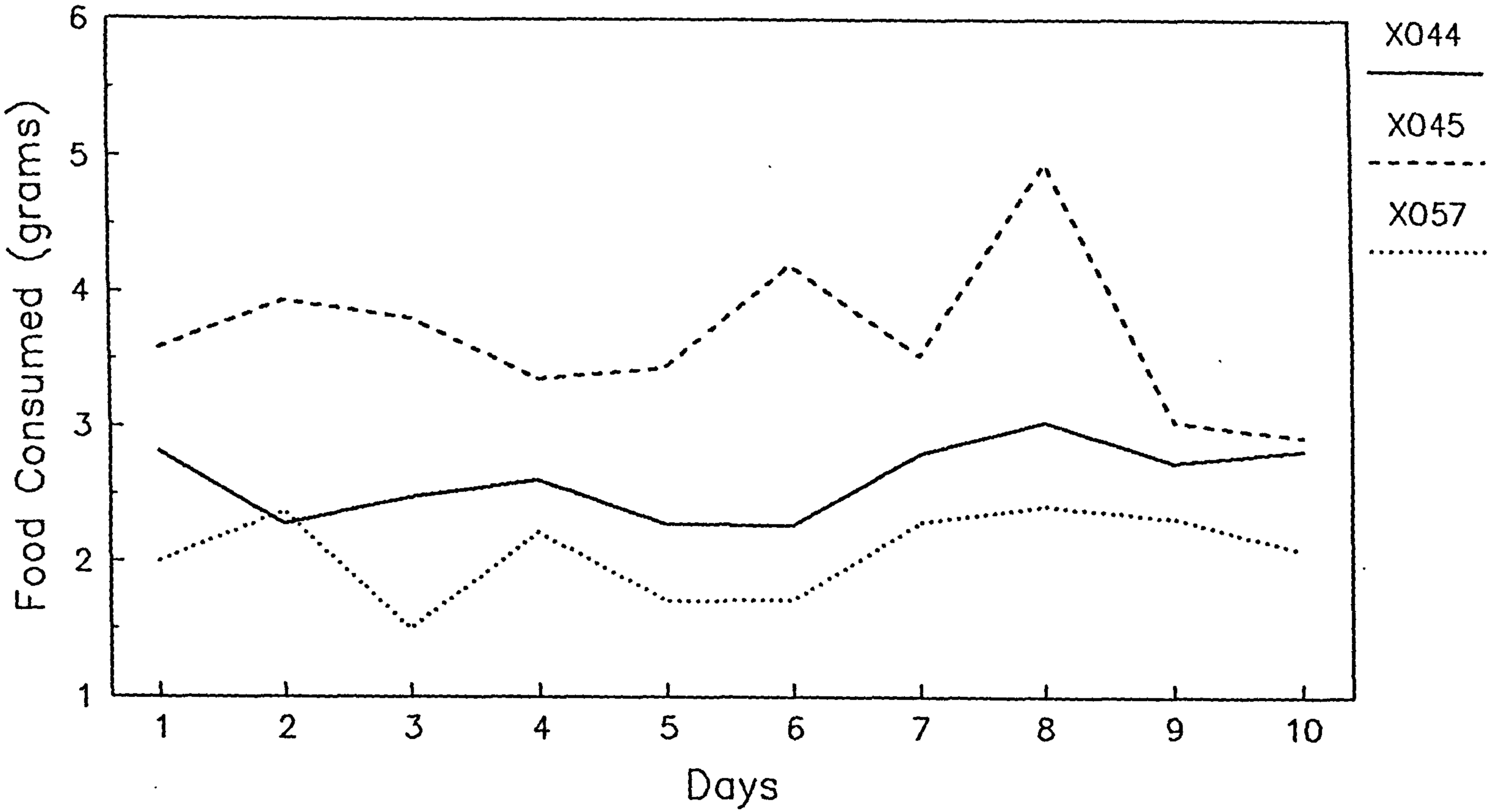


Figure 4.

Data from 4 animals showing a variable baseline feeding pattern. The data presented here was collected over ten consecutive days for each animal.

Daily Food Consumption

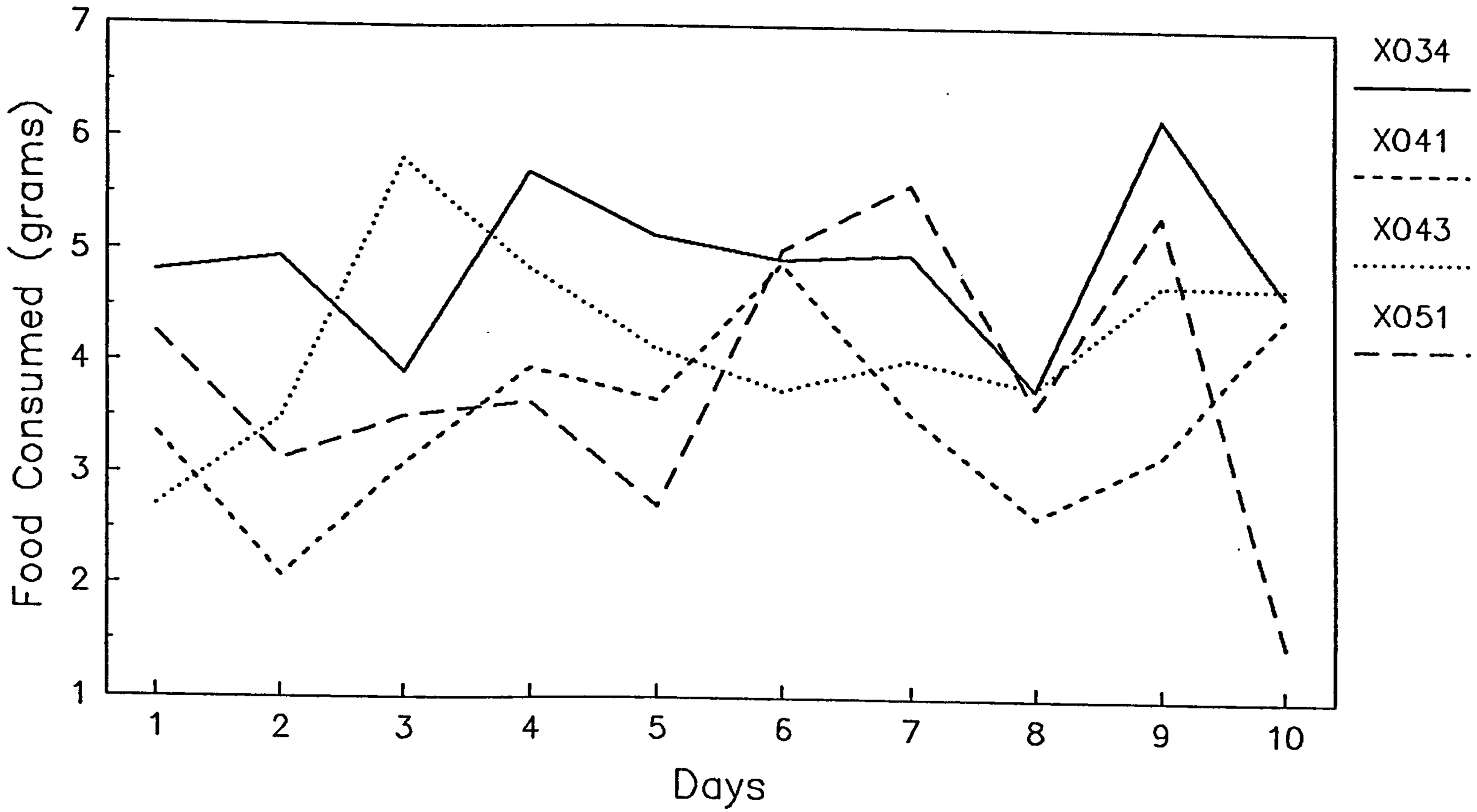
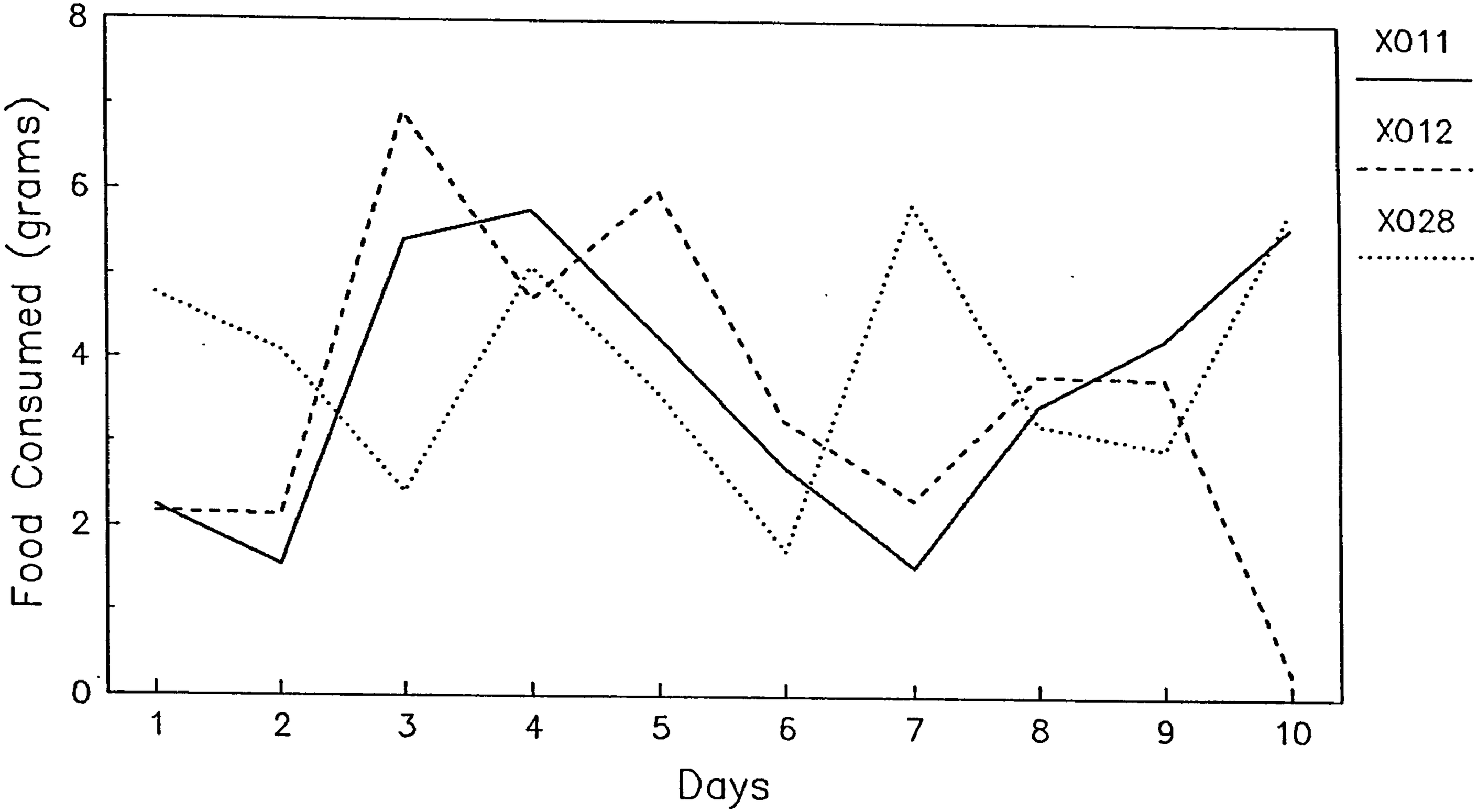


Figure 5.

Data from 3 animals showing a cyclic baseline feeding pattern.

The data presented here was collected over ten consecutive days for each animal.

Daily Food Consumption



differences was zero, and the alternative hypothesis (H_1) was that the median of the differences was negative. The sign test showed a probability under H_0 of 0.188, in other words there was a slight, but not highly significant deficiency of food intake under the 3 hour regime. These results show that as food availability time is decreased, feeding rate increases so as to maintain previous levels of food intake.

Table 1

Fish Number	Total amount eaten over 8 days		Difference	Average feeding rate g hr^{-1}	
	7 hours	3 hours	3hrs-7hrs		
	grams	grams	grams	7 hrs	3 hrs
CP08	18.45	14.55	-3.9	0.33	0.61
CP11	23.64	20.62	-3.02	0.42	0.86
CP12	22.64	18.55	-4.09	0.40	0.77
CP13	27.39	25.18	-2.21	0.49	1.05
CP14	22.17	25.21	+3.04	0.40	1.05

2. Daily cumulative feeding response patterns.

Figures 6 to 15.

This set of data allows us to examine more closely the individual patterns of feeding established by each animal on a daily basis. The advantage of plotting data as a cumulative sum (or cusum) is that relatively small changes in rate are effectively highlighted and are thus more easily detected. The technique used was to plot 5 days data for each of the ten animals which have been analysed in detail in these experiments. In each case the five days which had the most similar number of feeding events in total were used, this enabled an analysis as to whether on those days in which similar amounts were eaten the fish displayed a similar cusum. The cusum curves although varied between animals, are often consistent for any one animal. Animals X011 (figure 6), X044 (figure 7) and X045 (figure 8) show a strong tendency towards a cusum which is consistent within an individual's data set, these data contrast markedly with that for X012 (figure 9), X028 (figure 10), and X057 (figure 11) which did not show a great deal of consistency in the cusum data. Animals X034 and X043 (figures 12 and 13) demonstrate a pattern in which the feeding rate slows gradually over the entire feeding period producing a cusum similar in form to a hyperbolic, whereas X041 (figure 14) shows an almost constant rate of feeding over the seven hour period producing a cusum in the form of a straight line. X051 (figure 15) shows a rate which is constant for five to six hours but then falls abruptly almost to zero, the cusum in this case is in the form of two straight lines intersecting at about the five hour point.

Figure 6.

Cumulative sum data from 5 days for animal XO11. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X011

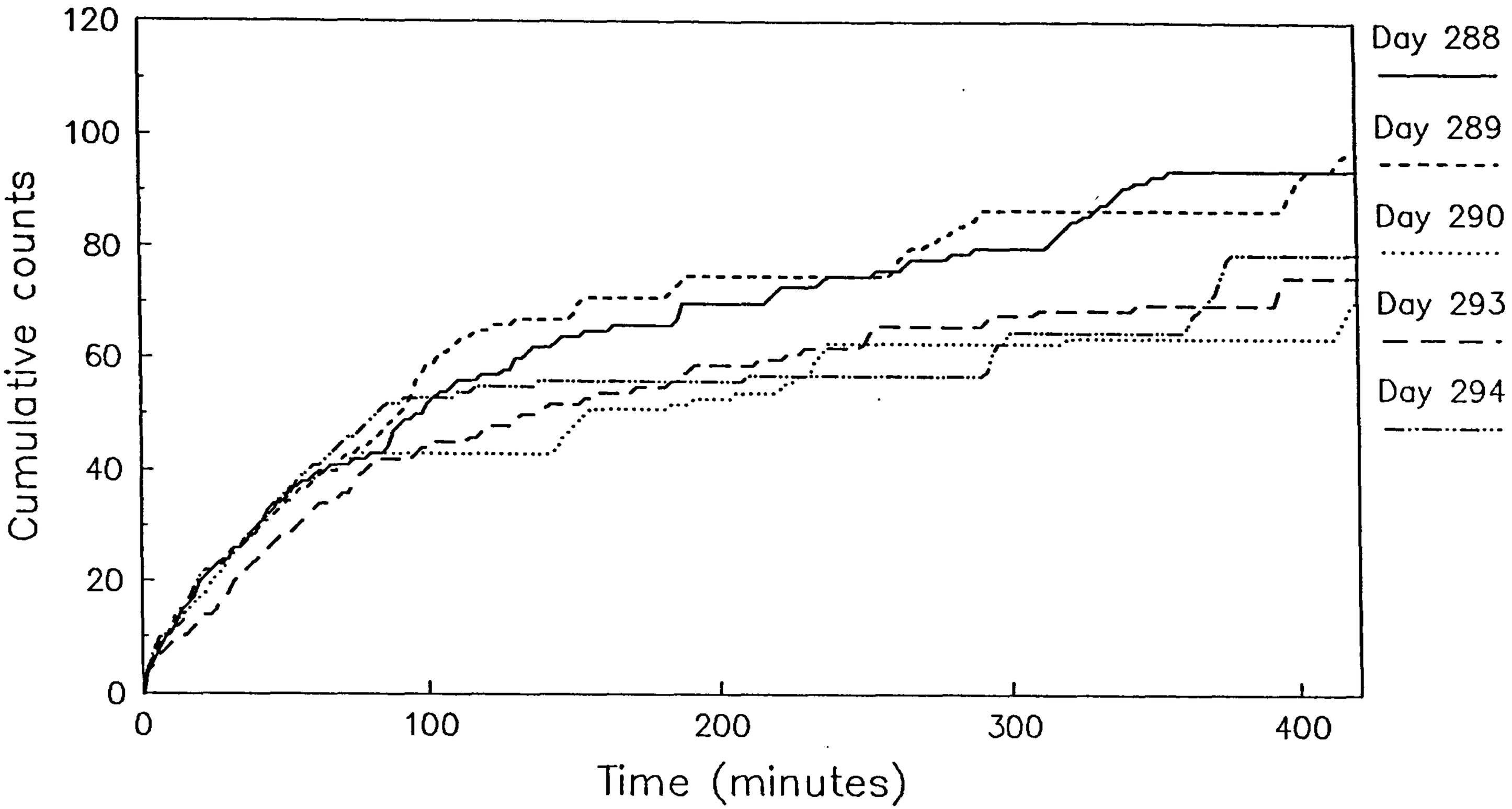


Figure 7.

Cumulative sum data from 5 days for animal XO44. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X044

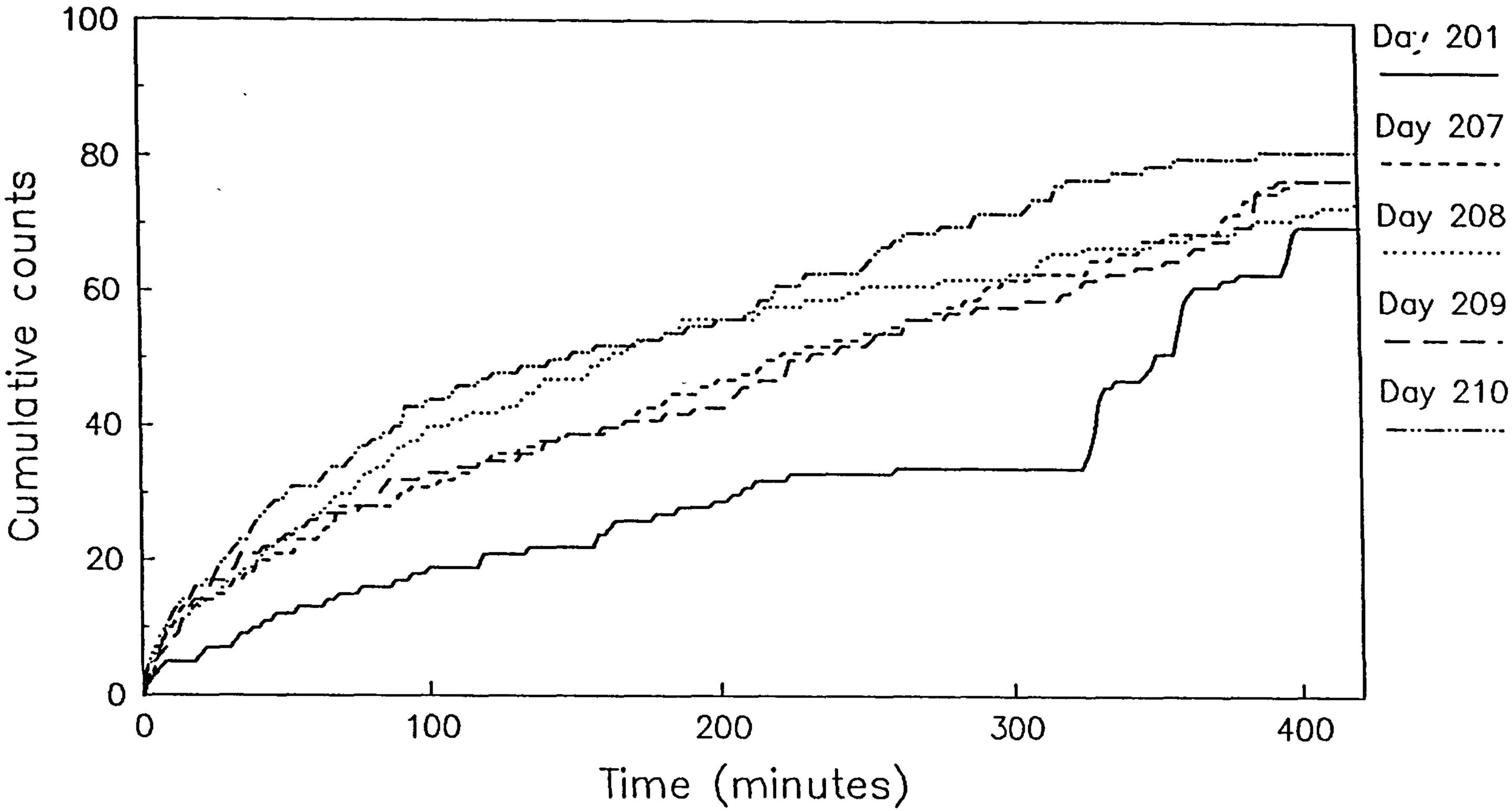


Figure 8.

Cumulative sum data from 5 days for animal XO45. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X045

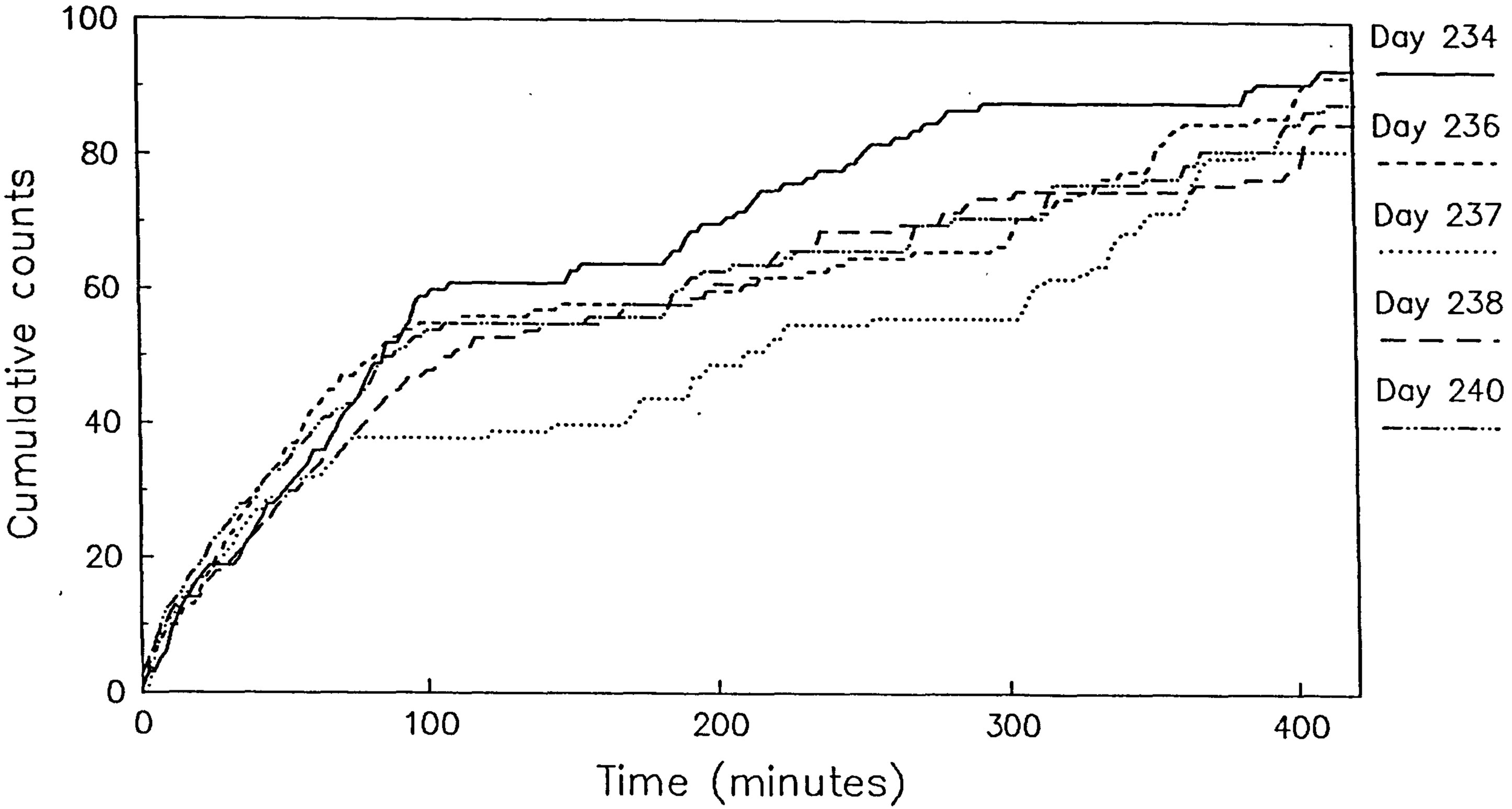


Figure 9.

Cumulative sum data from 5 days for animal XO12. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X012

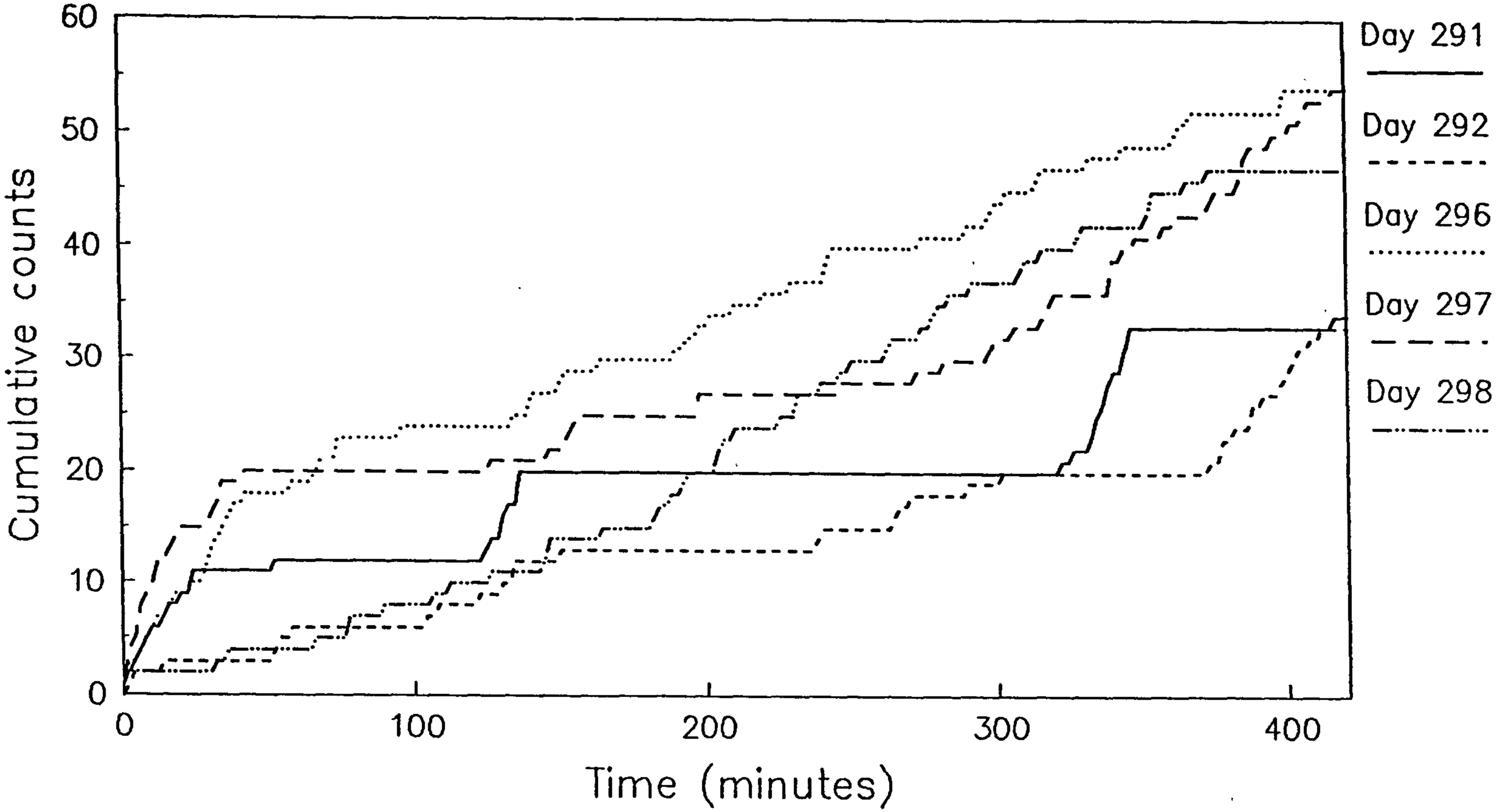


Figure 10.

Cumulative sum data from 5 days for animal X028. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X028

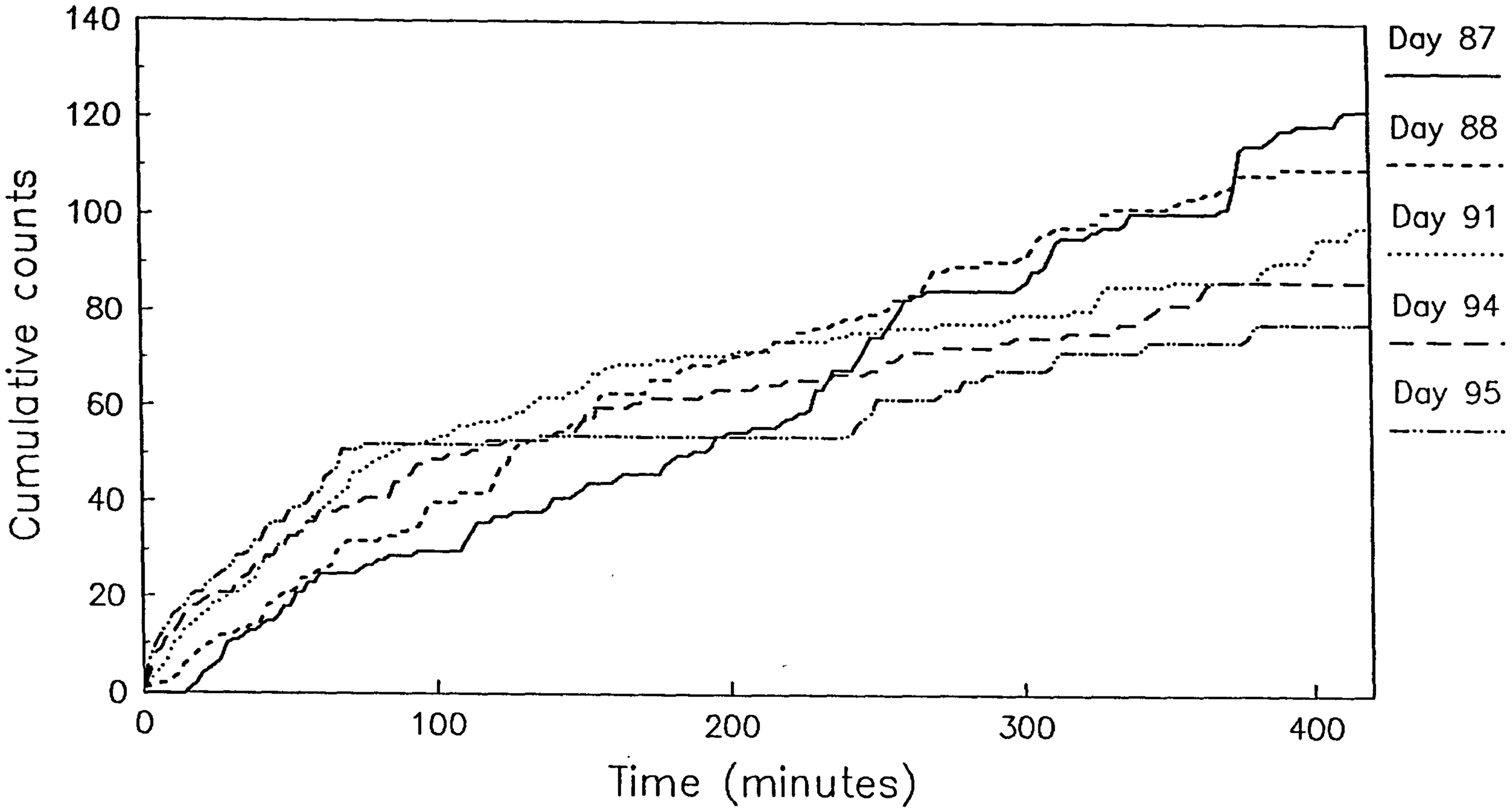


Figure 11.

Cumulative sum data from 5 days for animal XO57. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X057

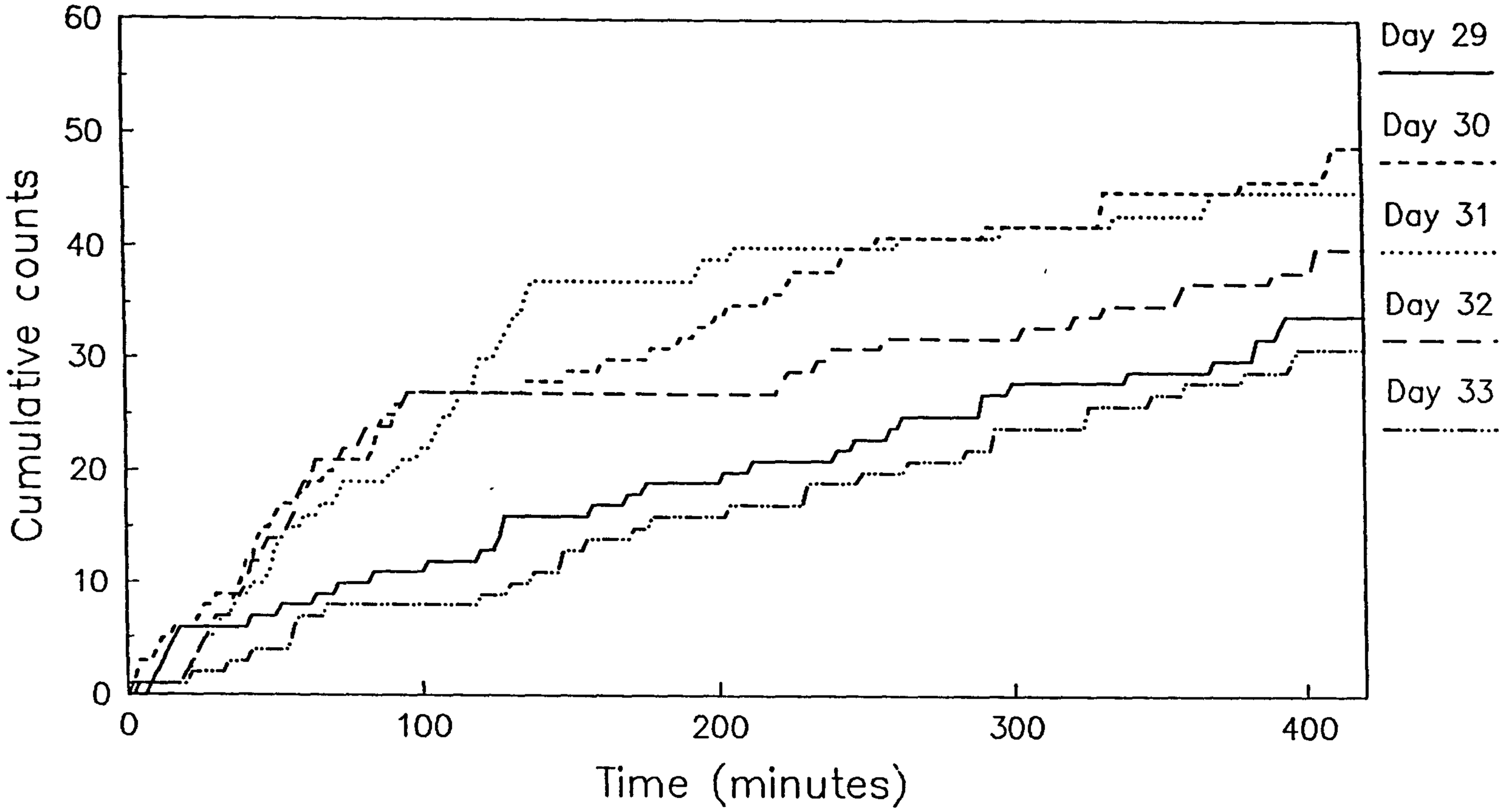


Figure 12.

Cumulative sum data from 5 days for animal XO34. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X034

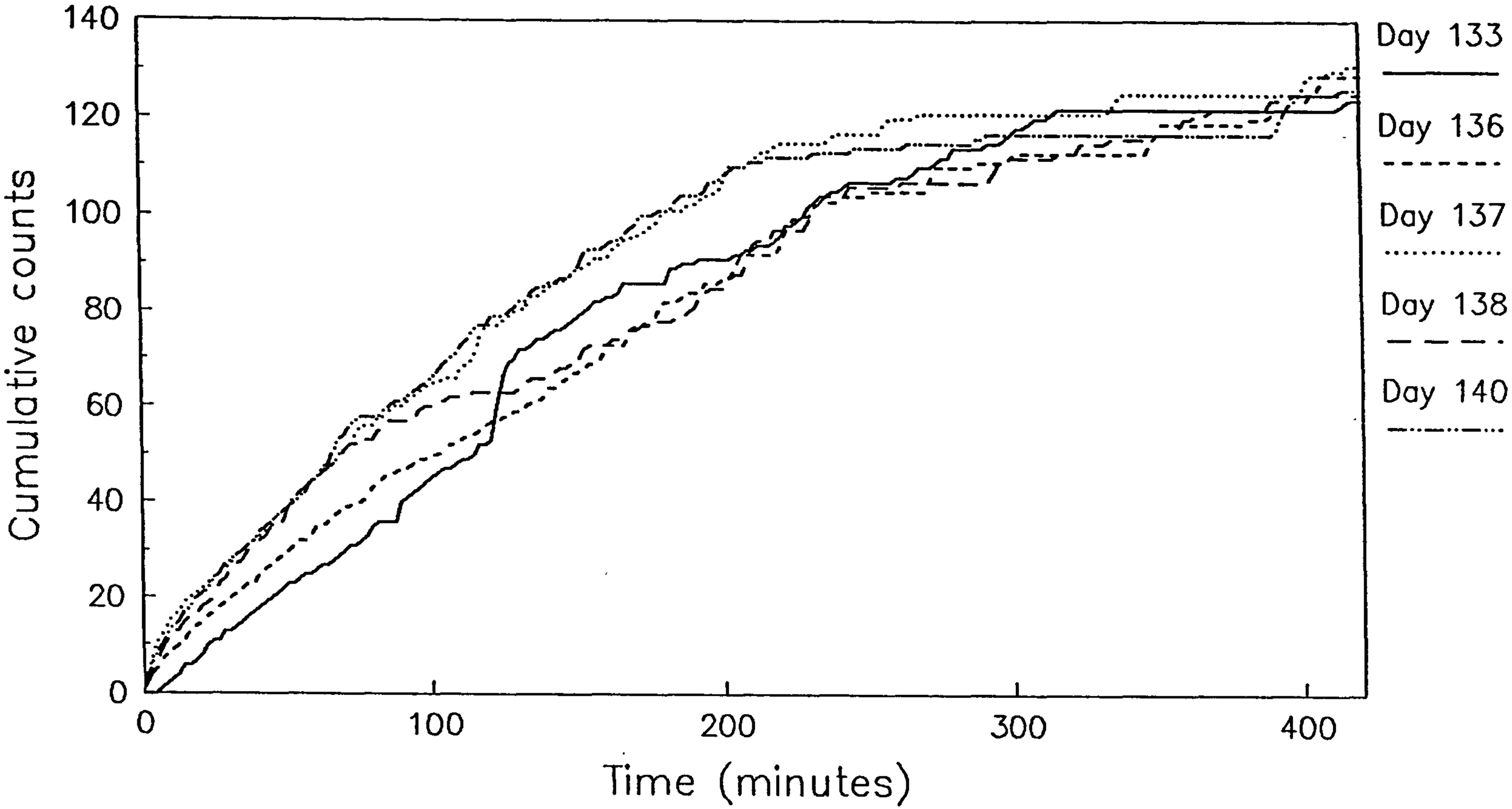


Figure 13.

Cumulative sum data from 5 days for animal X043. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X043

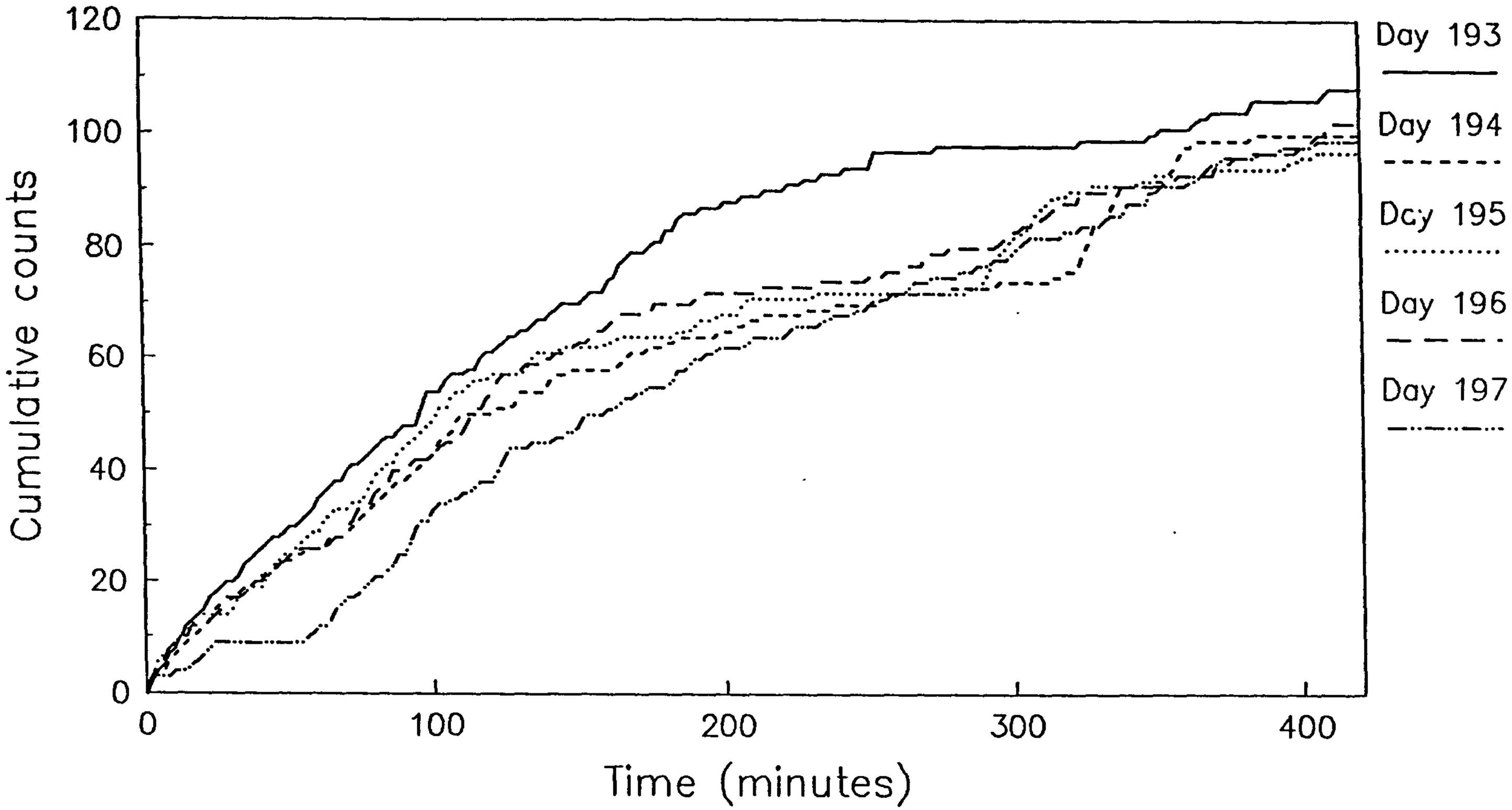


Figure 14.

Cumulative sum data from 5 days for animal XO41. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X041

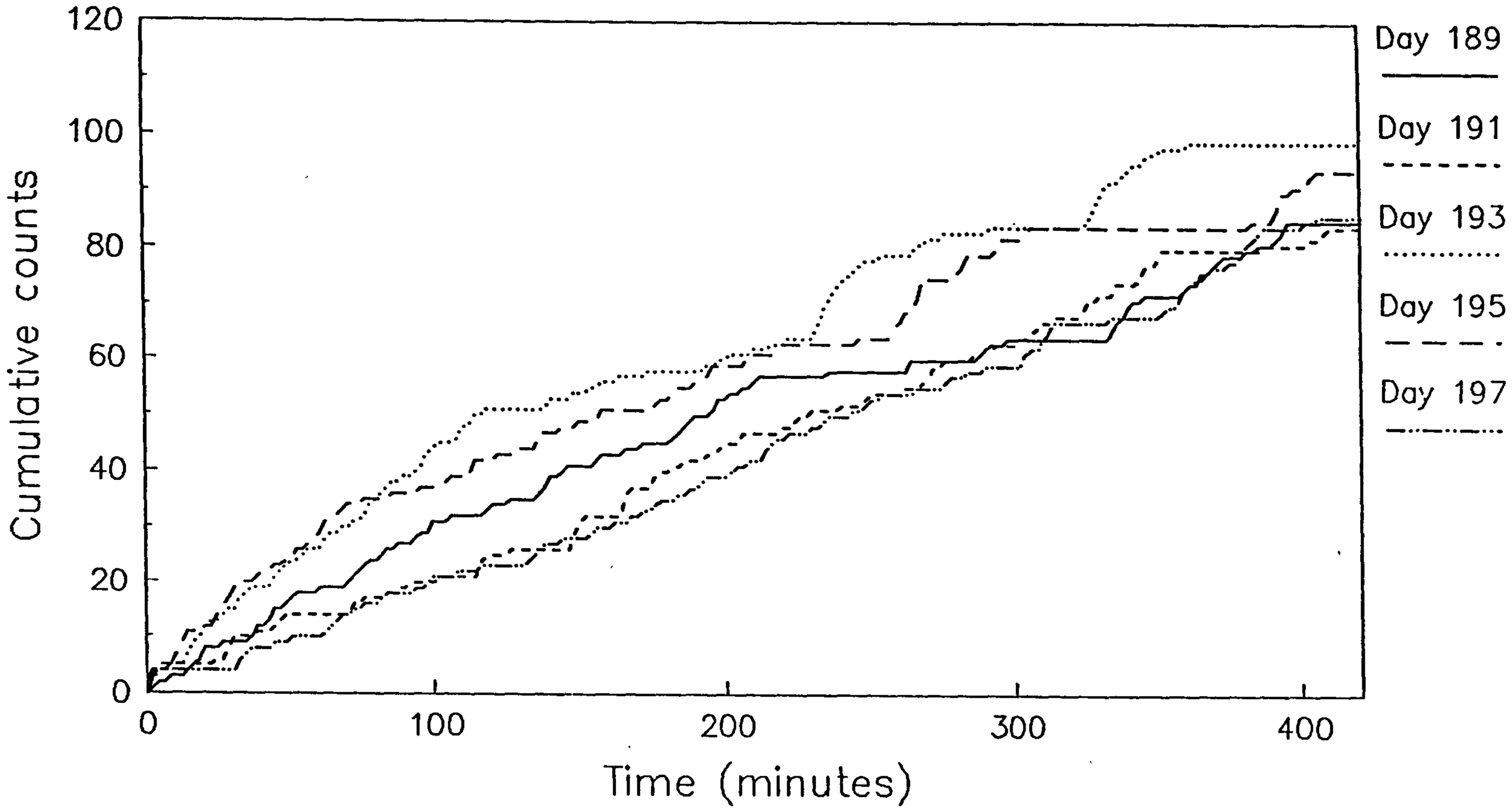
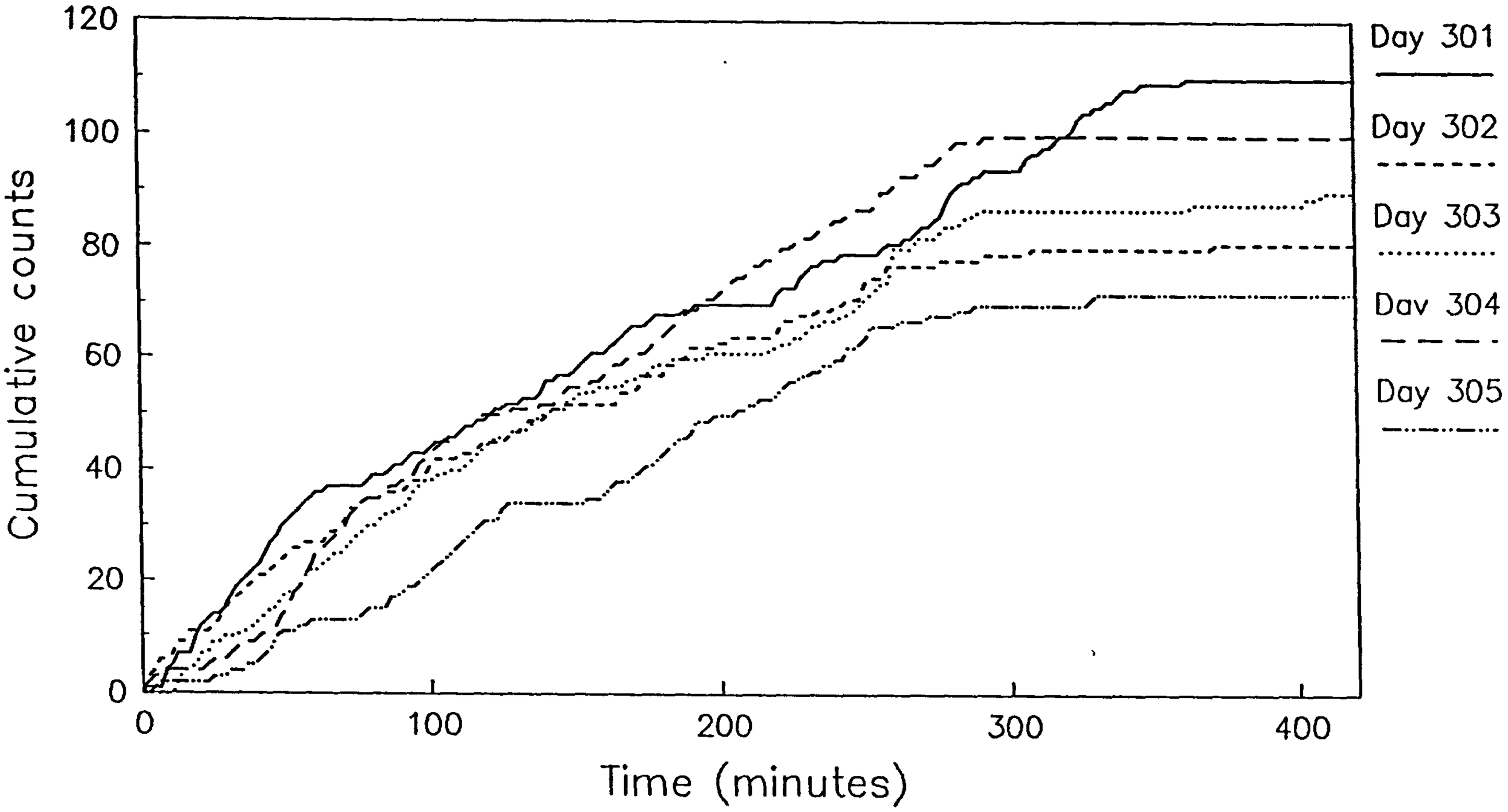


Figure 15.

Cumulative sum data from 5 days for animal XO51. Data presented here was taken from 5 days showing a similar number of feeding events.

Cumulative feeding patterns for X051



3. Log survivor analysis.

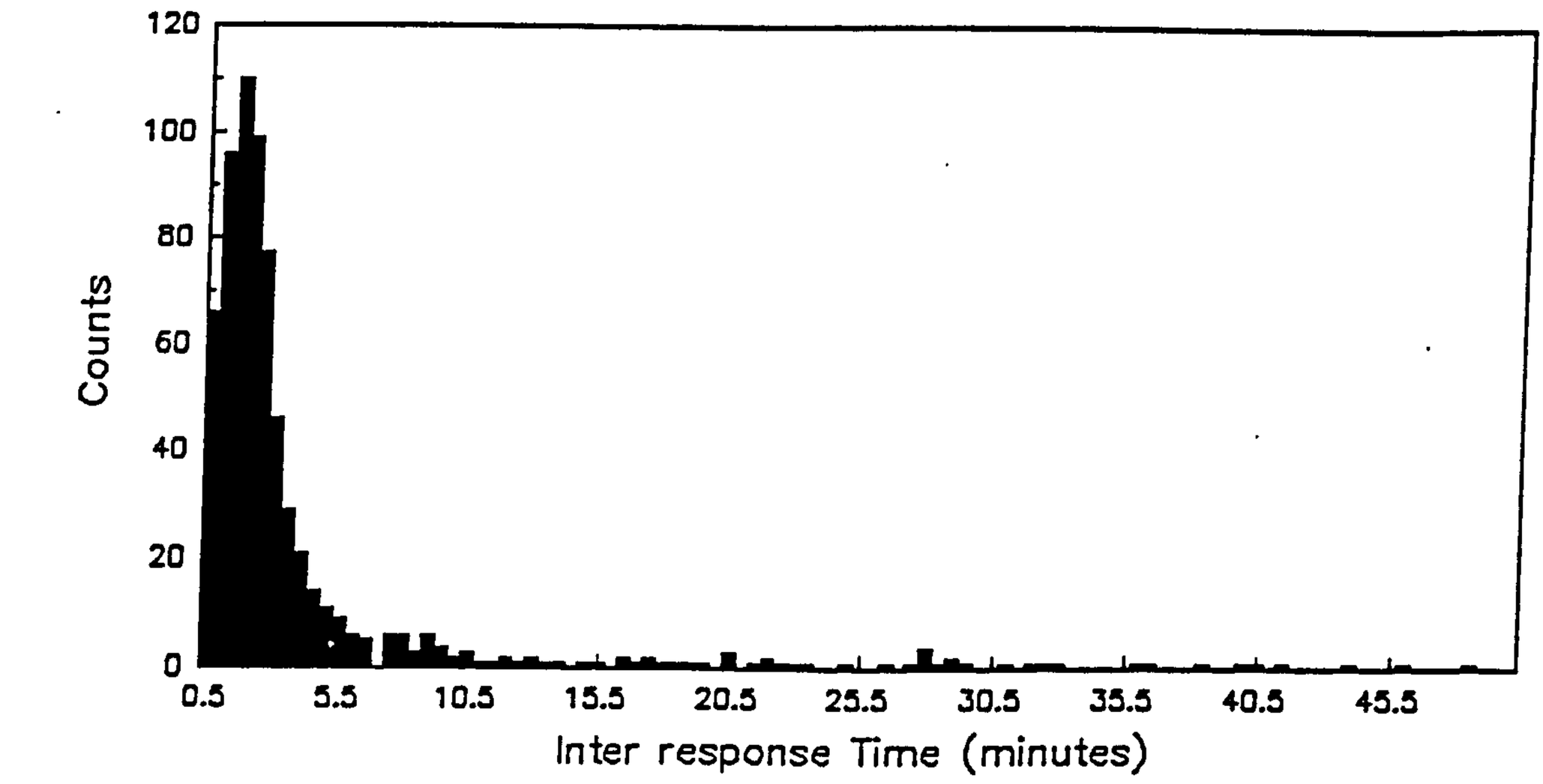
Figures 16 to 20 show sample data from five of the animals used in this experiment.

The top half of each figure shows an interval histogram which is used to generate the log survivor function shown in the lower half of the figure. Inspection of the data presented in these figures shows that it is not possible with any degree of certainty to detect well defined break points in any of the log survivor functions shown. The form of the curves is one which tends to progress asymptotically towards the abcissa. The underlying trend in interval distribution is thus one which does not show intervals grouped around discrete values but rather the longer IRT's are evenly distributed along the abcissa. These results show quite clearly the absence of bout feeding.

Figure 16.

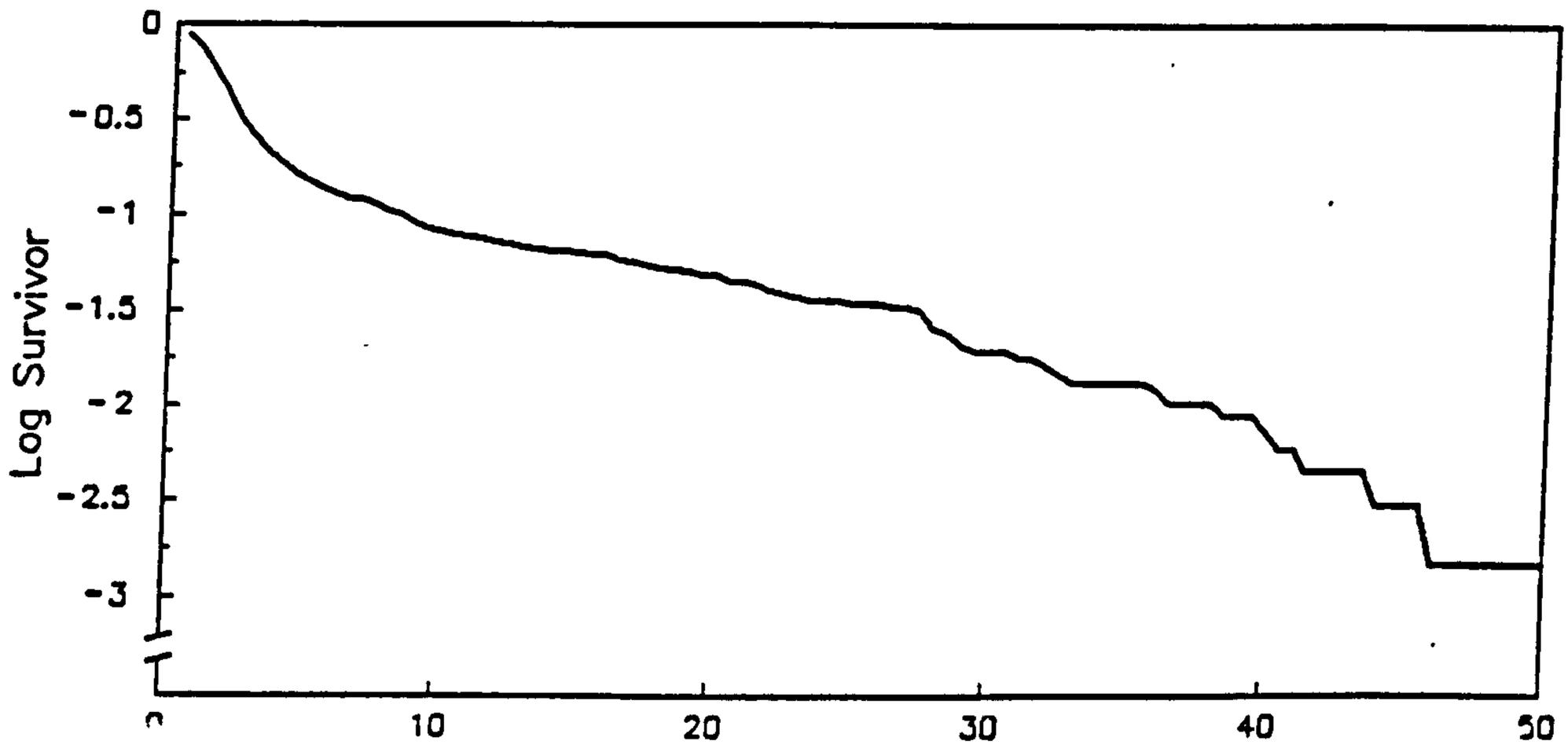
Interval histogram (top) and log survivor function (bottom) for animal XO11. The data presented here is pooled from 10 days.

X011 Interval Histogram



Pooled data for 10 days

X011 Log Survivor Analysis

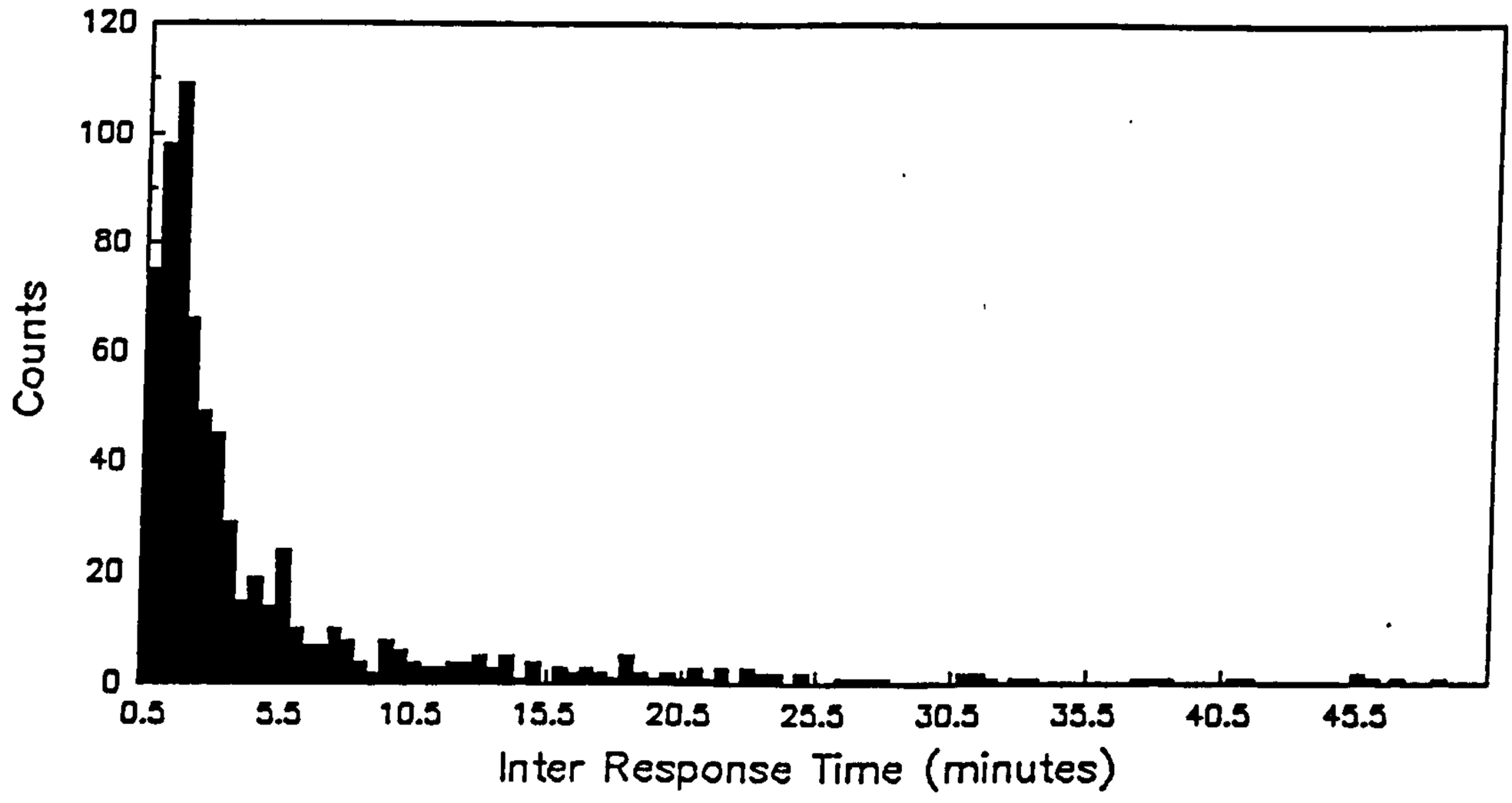


Log survivor function generated from the interval histogram shown above

Figure 17.

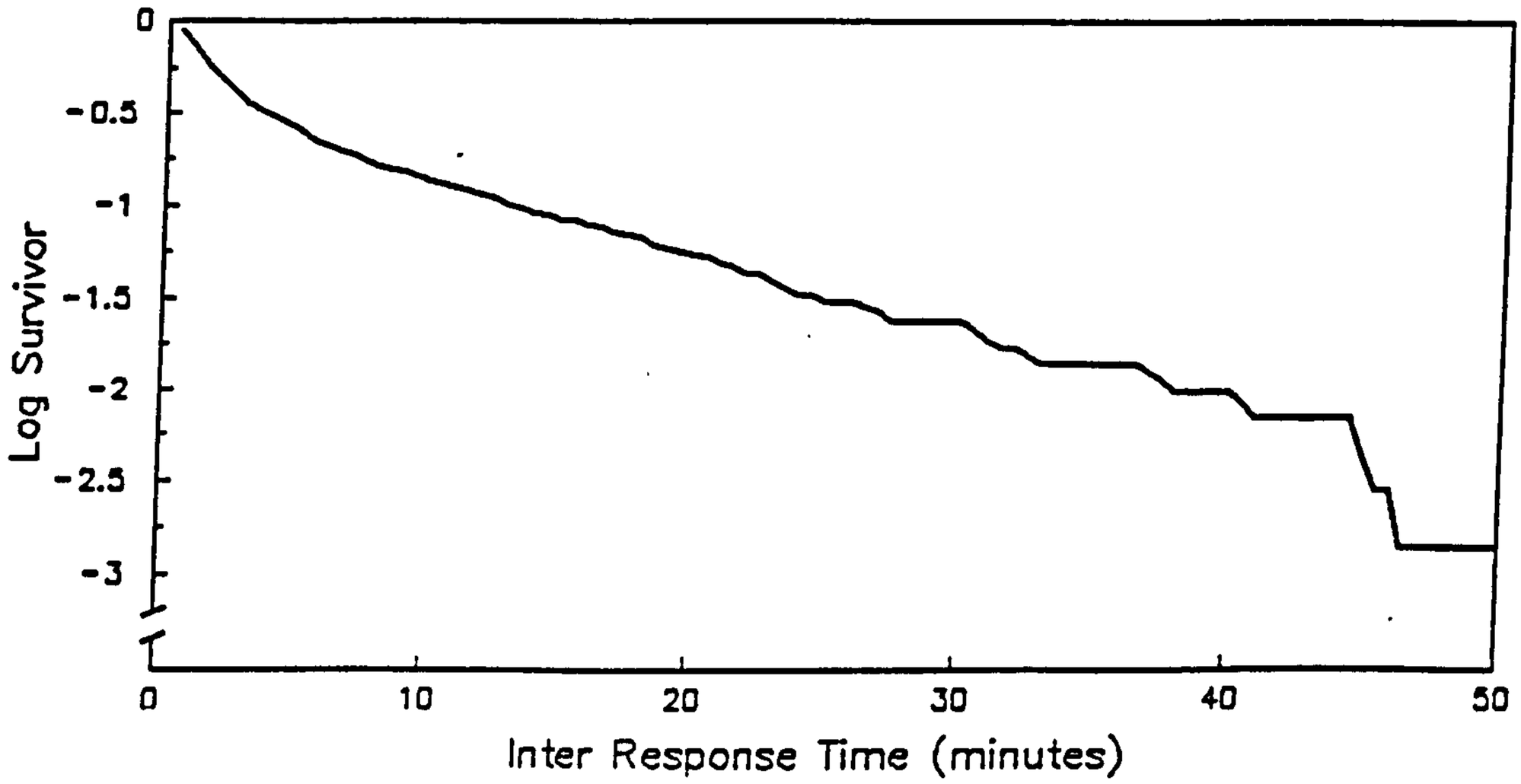
Interval histogram (top) and log survivor function (bottom) for animal XO12. The data presented here is pooled from 10 days.

X012 Interval Histogram



Pooled data for 10 days

X012 Log Survivor Analysis

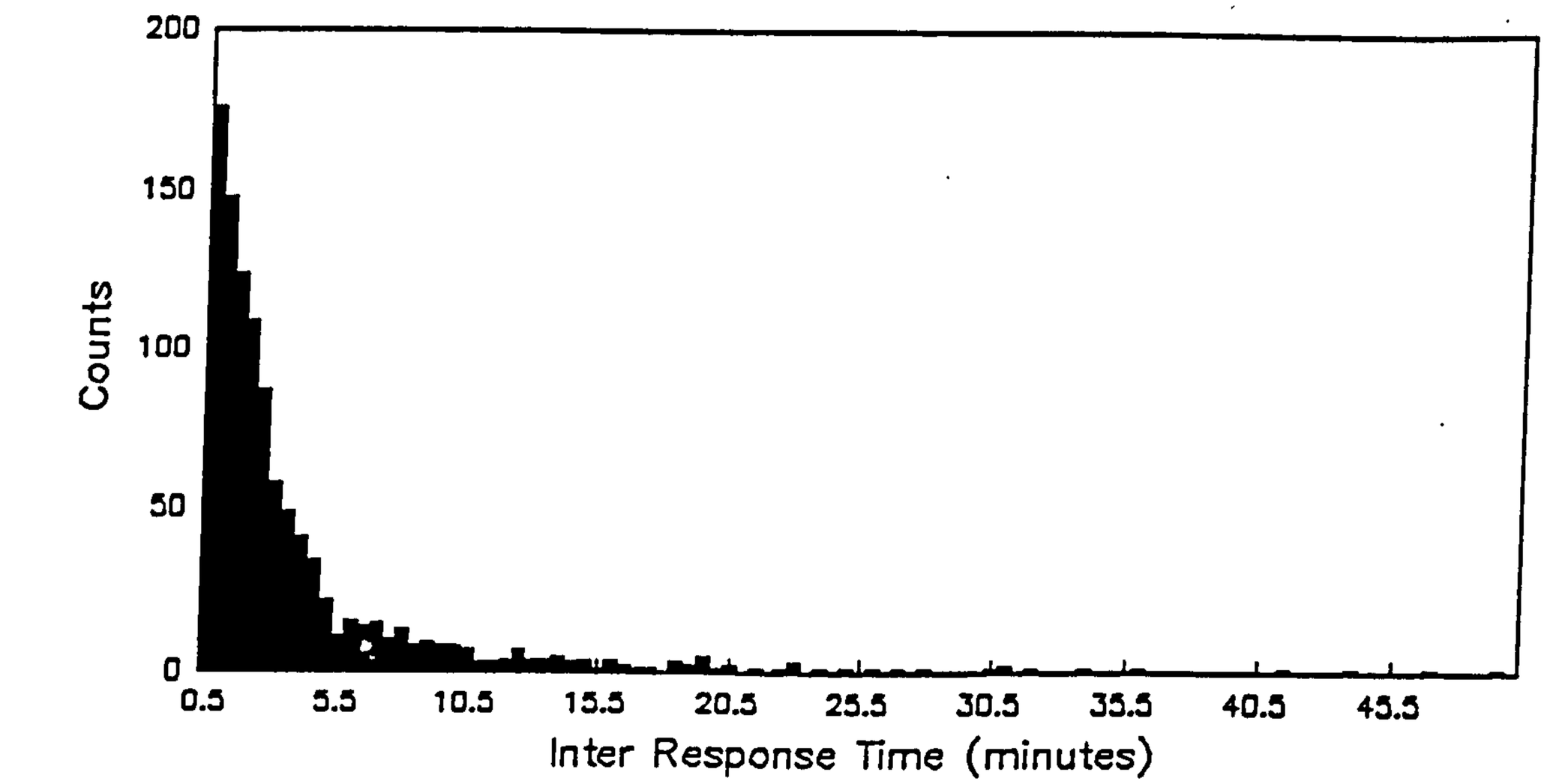


Log survivor function generated from the interval histogram shown above

Figure 18.

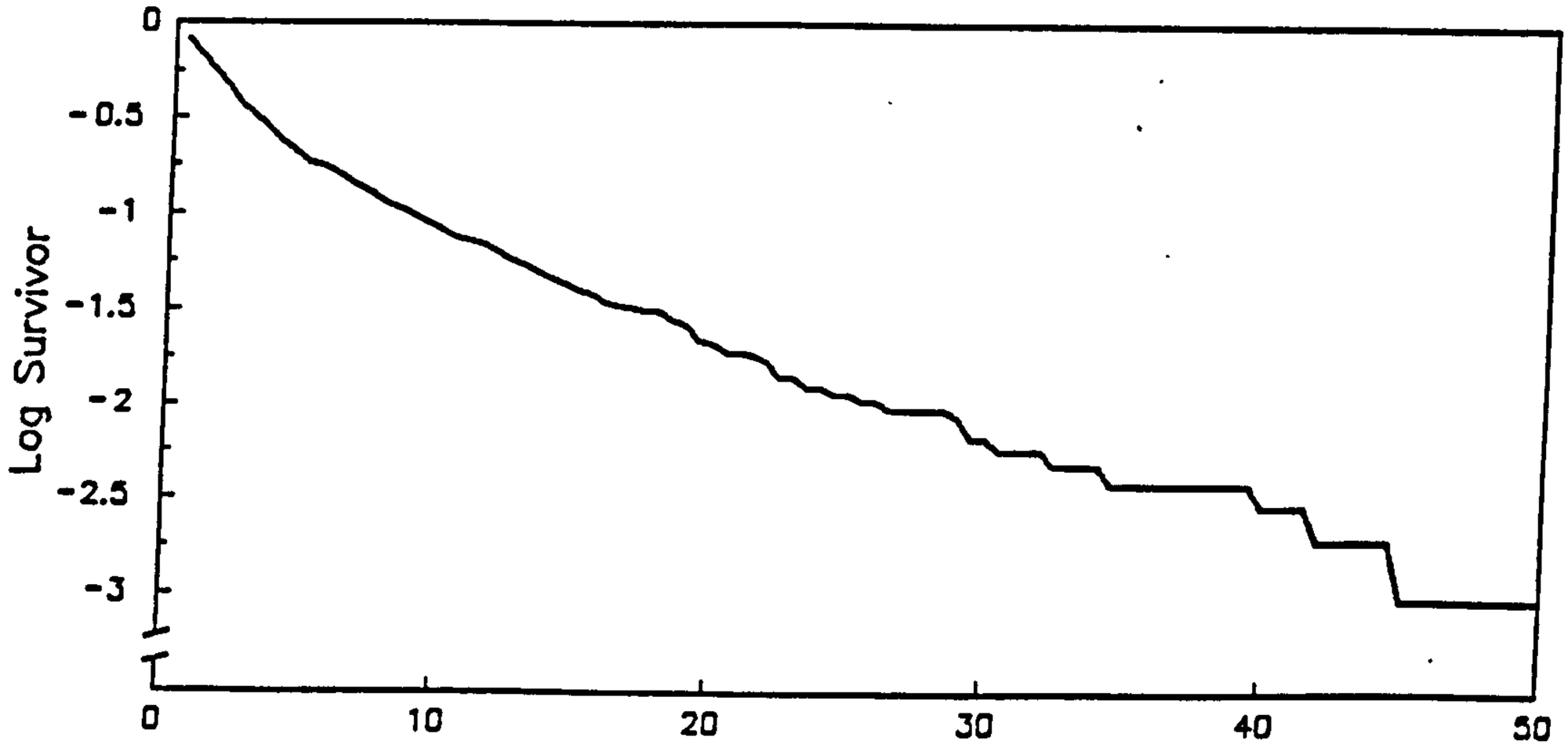
Interval histogram (top) and log survivor function (bottom) for animal XO28. The data presented here is pooled from 10 days.

X028 Interval Histogram



Pooled data for 10 days

X028 Log Survivor Analysis

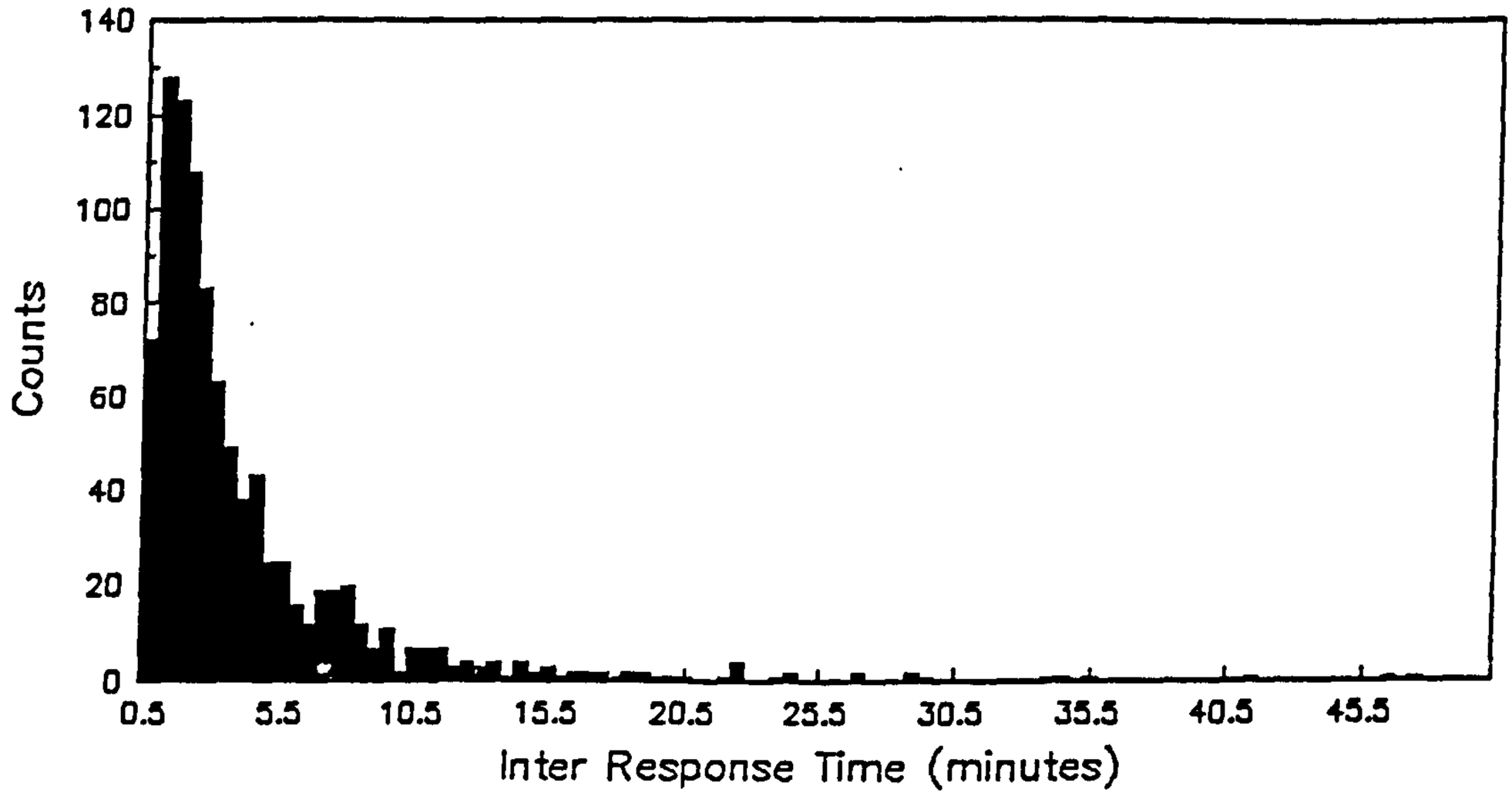


Log survivor function generated from the interval histogram shown above

Figure 19.

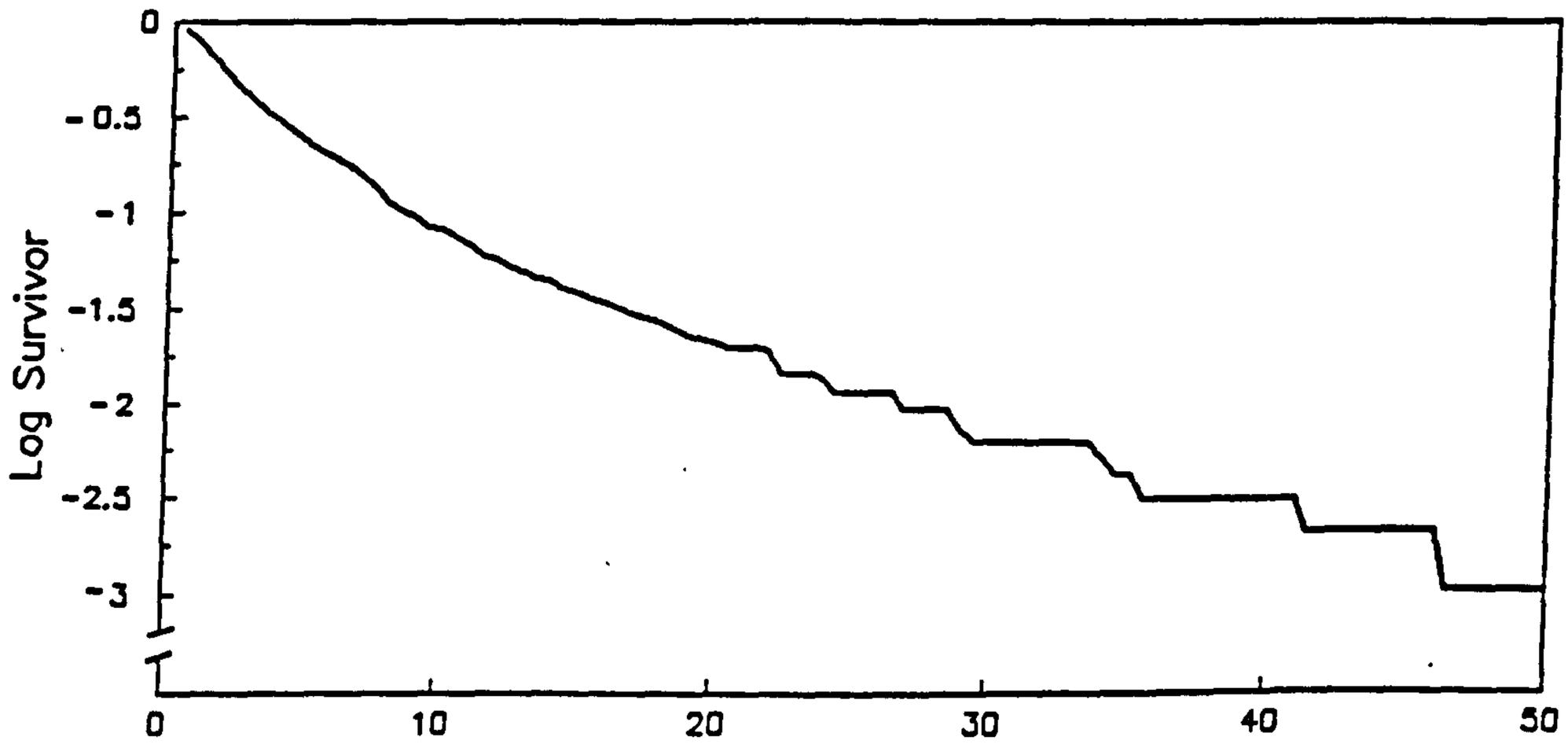
Interval histogram (top) and log survivor function (bottom) for animal XO41. The data presented here is pooled from 10 days.

X041 Interval Histogram



Pooled data for 10 days

X041 Log Survivor Analysis

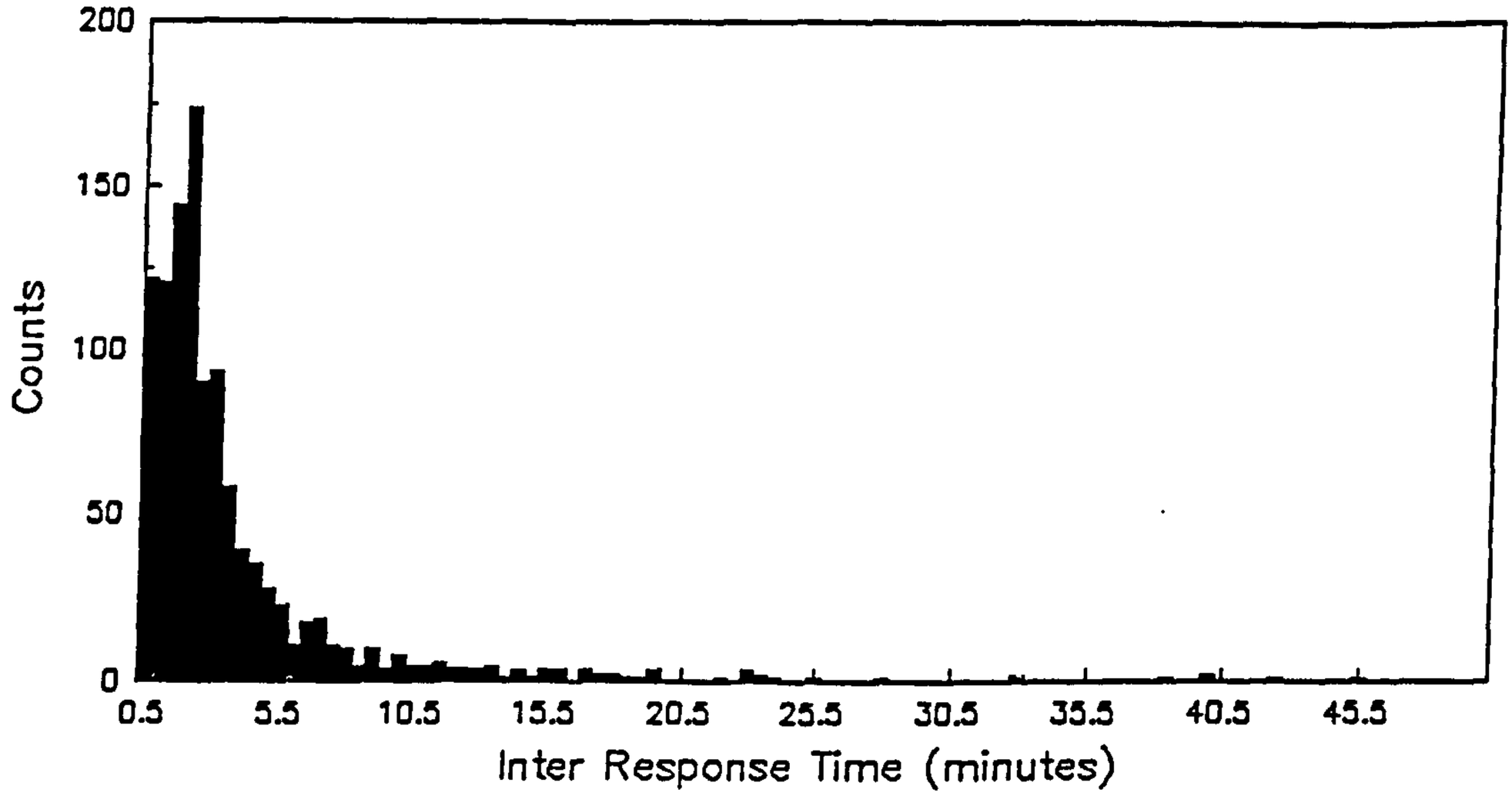


Log survivor function generated from the interval histogram shown above

Figure 20.

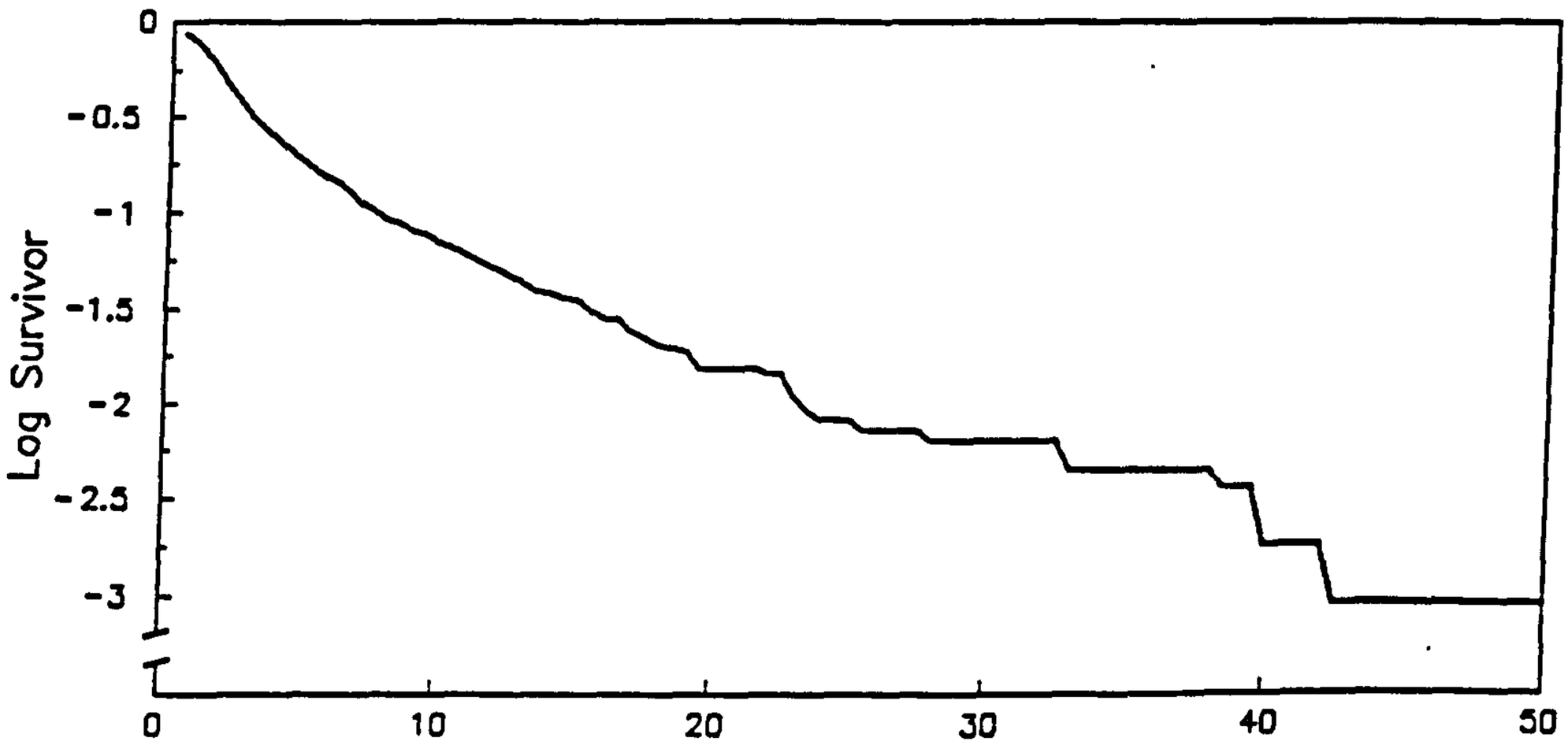
Interval histogram (top) and log survivor function (bottom) for animal X043. The data presented here is pooled from 10 days.

X043 Interval Histogram



Pooled data for 10 days

X043 Log Survivor Analysis



Log survivor function generated from the interval histogram shown above

4. Elapsed time analysis.

Figures 21 and 22 show data summed for all animals used in this experiment.

A curve fitting exercise carried out on this data showed that there are two lines of equally good fit, an exponential function and a logarithmic function. For both curves r^2=0.97. The equations for these curves are shown below

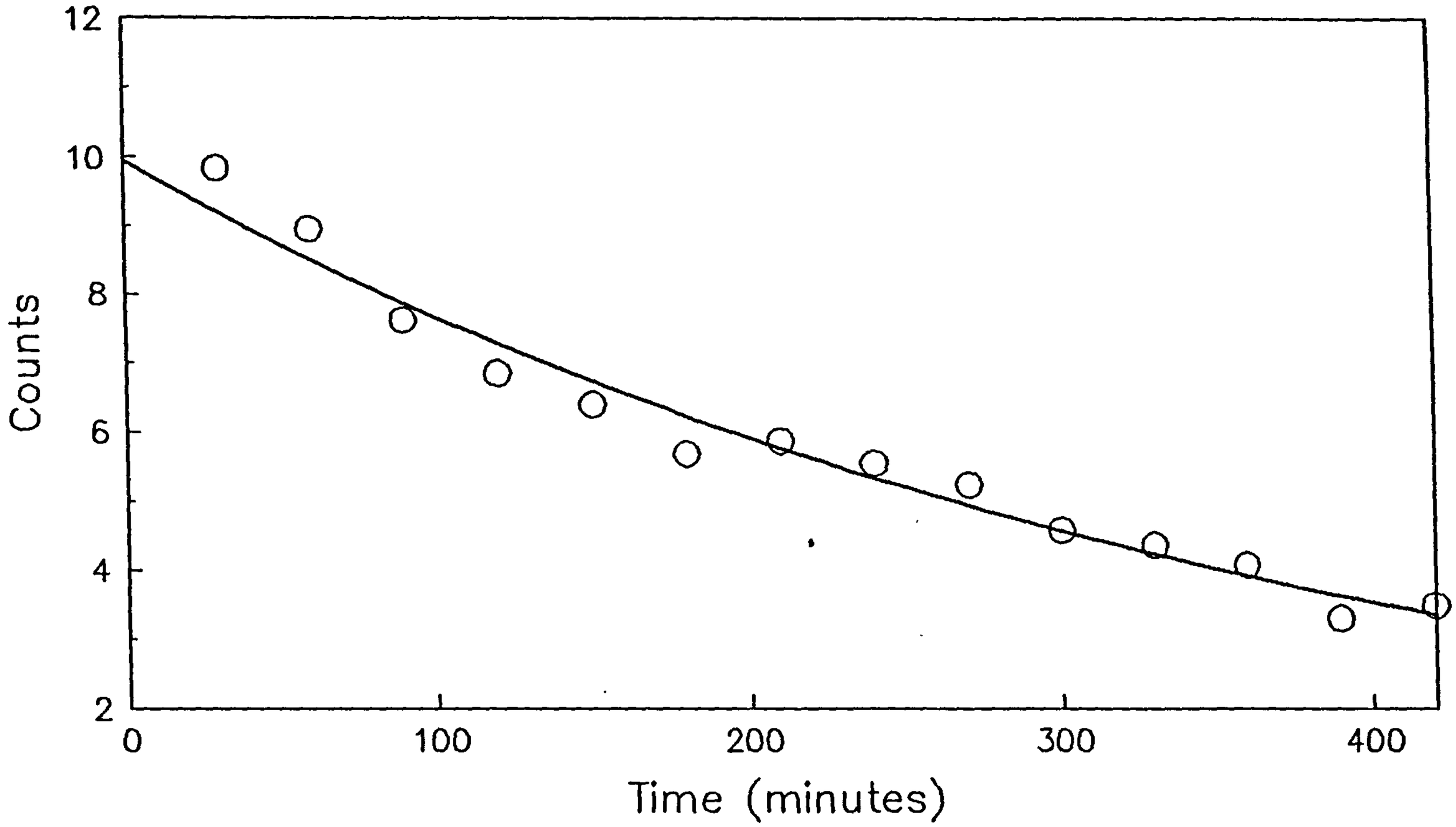
Exponential	$y=ae^{bx}$	where a = -0.0026, b = 99.48
Logarithmic	y=a+b(lnx)	where a = 187, b = -24.87

This data is useful in that the decline in food intake will closely mirror the increase in level of satiety, and it therefore gives an indication of the changes in satiety during the feeding period.

Figure 21.

Distribution of feeding events in each half-hourly interval fitted with an exponential function. Data presented here is the average for all animals from 10 consecutive days.

All Fish

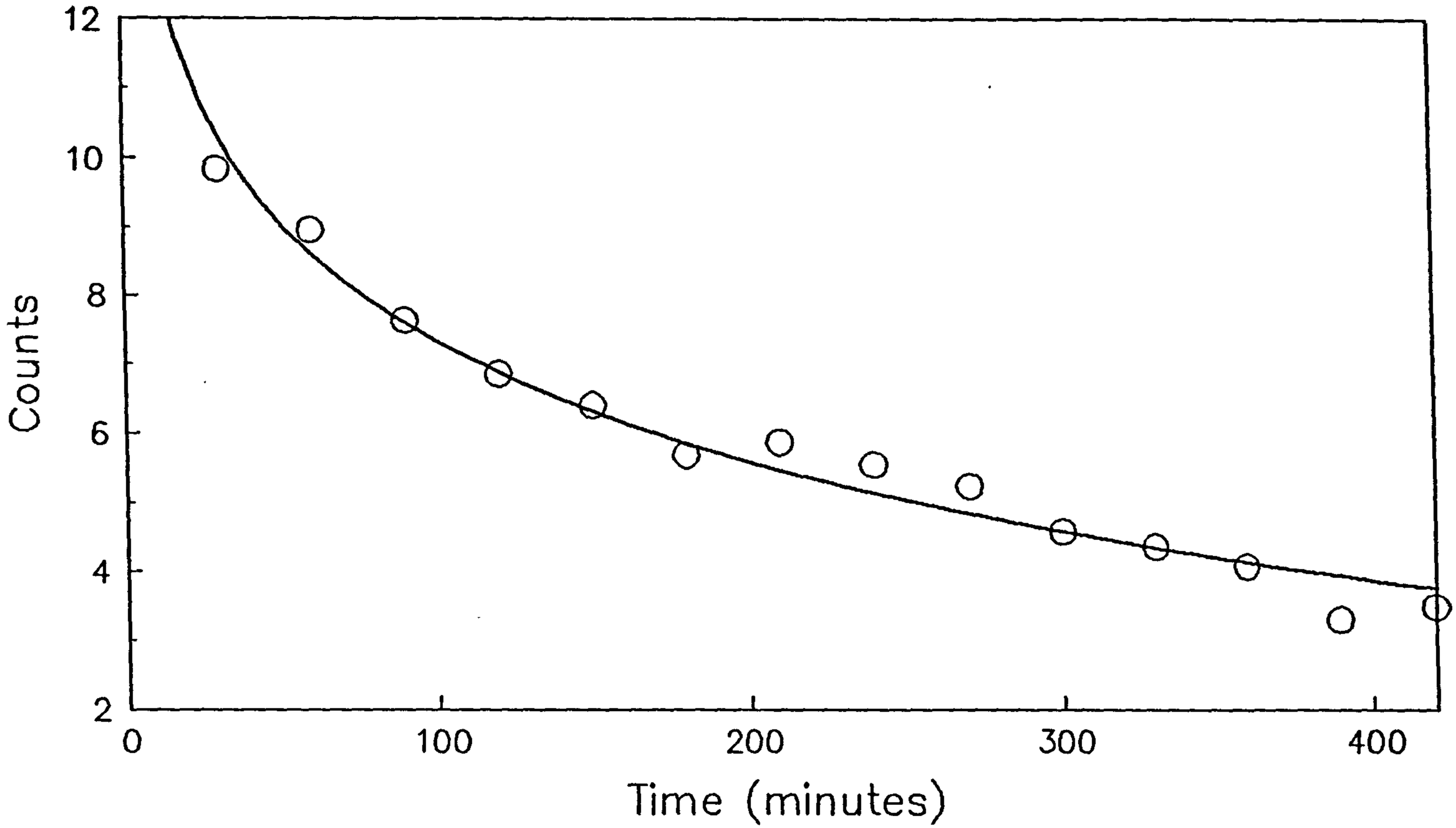


Pooled data for 33 animals
Fitted with exponential function

Figure 22.

Distribution of feeding events in each half-hourly interval fitted with a logarithmic function. Data presented here is the average for all animals from 10 consecutive days.

All Fish



Pooled data for 33 animals
Fitted with logarithmic function

5. Conversion of food mass to fish biomass.

Tables 2, 3, 4, 5, and 6.

The results in this section were obtained from the daily measurements of weight of food eaten and tank temperature, and the weekly measurement of fish weights. These results may be compared with published data obtained from animals on hand-feeding regimes, and are thus useful as a guide to the relative efficacy of operant feeding regimes in converting food mass to fish mass.

Table 2 shows an analysis of the composition of food used in this experiment.

Table 2

Trout floating plain SIZE 5.

Protein:	40%
Oil:	6%
Fibre:	2%
Ash:	12.5%
Metabolic Energy:	2.64 Mcal kg^{-1}
Vitamin A:	15000 iu kg^{-1}
Vitamin D3:	1500 iu kg^{-1}
Vitamin E:	75 iu kg^{-1}

Table 3 shows the Food Conversion Ratios obtained from this experiment. FCR is defined as the weight gain of the fish (wet weight) per gram of dry food consumed.

Table 3

Animal Number	Number of days	Total mass of food eaten (g)	Total weight gained by animal (g)	Food Conversion Ratio
XO11	13	32.05	15.5	0.48
XO12	8	34.09	18.7	0.56
XO28	26	84.04	41.7	0.50
XO34	19	76.65	35.85	0.48
XO41	21	79.04	46.93	0.59
XO43	20	97.6	51.93	0.53
XO44	17	51.88	23.1	0.44
XO45	81	322.51	113.49	0.35
XO51	15	47.88	26.55	0.55
XO57	21	43.42	34.26	0.79

Table 4 shows the values obtained for protein efficiency ratio (PER). PER is a measure of the efficiency with which dietary protein is converted to body protein, and is defined as the gain in weight of the animal per gram of crude protein consumed (Osborne Mendel and Ferry 1919).

Table 4

Animal Number	Number of days	Total mass of protein eaten (g)	Total weight gained by animal (g)	Protein Efficiency Ratio
XO11	13	12.82	15.5	1.21
XO12	8	13.64	18.7	1.37
XO28	26	33.62	41.7	1.24
XO34	19	30.66	35.85	1.12
XO41	21	31.62	46.93	1.48
XO43	20	39.04	51.93	1.33
XO44	17	20.75	23.1	1.11
XO45	81	129	113.49	0.88
XO51	15	19.15	26.55	1.39
XO57	21	17.37	34.26	1.97

A useful measure of growth is the 'Specific Growth Rate' (SGR) this is defined as the percentage gain in weight of the animal per day. Feeding level is defined as the amount of food eaten each day (dry weight) as a percentage of fish wet weight. The values of these variables were calculated from the data presented in table 3, and are shown in table 5 below. Tank temperature data is included in table 5, since SGR and feeding rate are temperature dependent.

Table 5

Animal	SGR	Feeding level %	Temperature °C
XO11	0.62	1.25	23
XO12	0.82	1.45	23
XO28	1.01	1.8	23
XO34	1.62	3.05	23
XO43	2.1	3.35	22
XO44	1.35	2.75	22
XO41	1.93	2.75	21
XO45	1.35	2.8	20
XO51	1.08	1.8	20
XO57	1.2	1.35	20

Table 6 shows the data shown in table 5 averaged and presented on a by-temperature basis.

Table 6

Temperature °C	Average feeding level %	Average SGR
23	1.89	1.02
22	3.05	1.72
21	2.75	1.93
20	1.98	1.21

DISCUSSION

Inspection of the data presented for the weight of food consumed each day (figures 3 to 5) shows a high degree of variability in the patterns exhibited. The main difference between the data in figures 3 to 5 and that recorded by Rozin and Mayer (1961) for the goldfish *Carassius auratus*, is the absence of the anorexic periods seen by Rozin and Mayer. In the experiments reported here there was no evidence of periods of anorexia. It is not easy to draw quantitative conclusions from these results. The purpose of presenting data in this manner is to show that as with the goldfish *Carassius auratus*, the carp *Cyprinus carpio* does not exhibit a consistent pattern of food intake in terms of the weight of food consumed each day. These results do highlight the need for a more fundamental appraisal of the distribution of feeding events during each feeding session. The simple measurement of weight of food consumed each day does allow us to be sure that under this type of feeding regime fish will establish a feeding response pattern which allows the maintenance of growth and weight gain. An important feature of the design of this experiment was that the task was not so difficult as to prevent fish from eating sufficient food, nor should it be so easy that fish can perform it accidentally thereby obtaining pellets which were not intentionally 'pressed' for. As mentioned above the possibility of unintentionally obtained pellets, and pellets which were demanded but not eaten was avoided by the use of two visual observation checks. The first of these was the inspection of the tanks each day for the presence of uneaten pellets, the second was the use of video-taped recordings of many of the feeding sessions. Using these techniques we

were reassured that the data presented here was obtained from animals that did not 'overpress', and which consumed pellets at the time they were delivered from the feeder.

The results from the reduced (3 hour) feeding period when compared to the original (7 hour) feeding period show quite clearly the ability of these animals to increase their rate of feeding in response to the shortening of the feeding period. The slight deficit in total food consumption seen under the 3 hour regime may well be explained by the possibility that the animal is unable to increase it's rate of feeding beyond a certain point, due perhaps to some physical rate limiting step in the ingestive process. As discussed above carp do not possess a well defined stomach, at high rates of ingestion the capacity of this part of the digestive tract to store food will become the rate limiting step once rate of ingestion gets close to or exceeds rate of gastric evacuation. Looked at in another way this is equivalent to saying that at high rates of ingestion, gastric evacuation may be the major factor controlling food intake in the carp, this does not however appear to be the case under conditions that allow carp to feed to satiation at lower rates of ingestion.

The data from the daily cumulative feeding response patterns (figures 6 to 15) shows that the animals used fell into two groups with regard to the patterns exhibited in the cusum data; the larger group (seven out of the ten animals) demonstrated a highly consistent cusum on those days on which similar amounts of food were eaten, the second and smaller group (the remaining three out of the ten animals) showed a cusum that was highly variable on days on which similar amounts of

food were eaten. The fact that these animals proved capable of developing a feeding pattern which was repeated each day, and was sufficient to produce a rate of growth which compares highly favourably with carp feeding under a non-operant regime (see below for analysis), indicates that a degree of learning has taken place with regard to the amount of time available each day for food consumption. This feature of the behaviour is demonstrated quite clearly when the results from the 3 hour food availability experiment are considered. The ability of the animal to carry out this type of learning is of obvious benefit when one considers the cost-benefit analysis of acquiring food which has been demonstrated to play a role in the ecological control of food intake as postulated by Collier (1985).

The results of the log survivor analysis (figures 16 to 20) clearly demonstrate the absence of bout feeding in carp under these experimental conditions. It should of course be remembered that the assessment of breakpoints in these curves is carried out subjectively. Reference to published data of this type (Slater 1974, Willner and Towell 1982) which do show clearly defined breakpoints in the log survivor function vindicates the judgement that this data does not demonstrate a bout feeding pattern. With regard to the postulation of Landless (1976) of a positive feedback mechanism of food intake control, the results here lead to the conclusion that in carp the immediate or short term control of food intake is not mediated by a positive feedback mechanism. This finding is not surprising given the fact that carp are not active predators.

The results from the elapsed time analysis (figures 21 and 22) are used as an indication of the changes in satiety which take place over the course of a feeding period. These results indicate that satiety rises exponentially or logarithmically during the course of the feeding periods used in this experiment. It should be noted that there is a tendency for food intake to show a slight increase during the middle hours of the feeding period and then again towards the end. Initial analyses of these data showed that curve fitting exercises which employed a polynomial function could obtain an almost perfect fit to the data presented here. In addition, statistically significant values for goodness of fit were obtained using polynomial analysis even when sample sizes (that is, number of days) were low. Polynomial functions are of the form:

$$y=ax+bx^2+cx^3+dx^4+ex^5+fx^6.....$$

The form of a polynomial is such that it becomes possible to obtain highly significant curve fittings to a very wide range of complex curves by simply adding more factors to the equation. The result of this feature is that the polynomial is not a particularly powerful tool in the analysis of the curves presented here.

The data relating to the conversion of food mass into fish mass (tables 2 to 6) are interesting in that they provide some information as to the possible effects of foodstuff composition on feeding rates and weight gain. There has been a great deal of discussion as to what an optimum level of protein content in commercially available fish food should be, this is largely due to the finding that weight gain

is directly proportional to dietary protein level. This relationship has been demonstrated to be true for carp over the whole range of crude protein content from 0.4% to 55% (Ogino and Saito 1970). The same study showed that the optimum protein level for carp was 38% at 23^{o}C, for feed with a metabolisable energy content of 3.7 Mcal kg^{-1}. Table 7 shows the range of values quoted for optimum protein content and metabolisable energy values for carp food stuff.

Table 7

crude protein %	ME Mcal kg^{-1}	Temperature ^{o}C	Author
38	3.7	23	Ogino and Saito 1970
35	3.4	20	Ogino Chiou Takeuchi 1976
38.4	2.7	25	Sin 1973
33	3.06	25	Sin 1973
35	3.82	28	Jauncey 1982

It is clear from table 7 that the food used in the experiments described in this thesis is slightly above the optimum range of protein content quoted for temperatures between 20^{o}C and 28^{o}C. The slightly higher protein content, will to some extent, be offset by the lower metabolisable energy content of the food used.

Inspection of table 7 shows that there is some evidence (Sin 1973) that a raised protein content may be offset by a lower ME.

It has been argued that the food conversion ratio (FCR), defined as the weight gain of the fish (wet weight) per gram of dry food consumed, may be used as an indicator of the nutritional status of an animal (Jauncey 1982). Table 3 shows the range of food conversion ratios obtained from this experiment, these ratios are slightly lower than the published range of between 0.6 and 1.5 (Jauncey 1982). Although FCR may be seen as an indicator of nutritional status, a possibly better indication is given by a measure of the efficiency with which dietary protein is converted to body protein. This is particularly true in the fishes, where muscle tissue is by far the major body component. Since protein is the major component in muscle tissue it makes up between 65% and 85% of the body mass. One measure of protein conversion is the protein efficiency ratio (PER). The PER is defined as the gain in weight of the animal per gram of crude protein consumed (Osborne Mendel and Ferry 1919). The values for the PERs obtained in this experiment may be seen in table 4 (mean value 1.3), they are in close agreement with values obtained elsewhere, Meske and Pfeffer (1977) reported a PER value of 1.28 for carp fed on commercially available trout food. It may be concluded that the food utilised in the experiments described above is perfectly adequate for the maintenance of good growth rates, and that it seems feasible to compare results obtained here with those obtained by other authors. It is possible to compare growth rates obtained from the animals used in the experiments described here with those obtained from carp feeding under different conditions in other experiments. A useful

measure of growth is the 'Specific Growth Rate' (SGR) this is defined as the percentage gain in weight of the animal per day. Jauncey (1982) has recorded the SGR for carp feeding over a range of temperatures from 20°C to 25°C and on three different feeding levels, feeding level is defined as the amount of food eaten each day (dry weight) as a percentage of fish wet weight. Feeding levels in the results presented by Jauncey (1982) were fixed at 3% 6% or 9% and the experimental regime was a hand feeding as opposed to an operant one. The results obtained here are from fish feeding under an operant regime and as a result the feeding level as defined above will be established not before hand by the experimenter, but by the experimental animal itself at the time that the feeding takes place. A comparison of results should provide an insight as to the relative 'efficiencies' as measured by the SGR of the two methods of feeding. For the purposes of this comparison, reference to the data presented above shows that it is safe to assume that animals feeding under the operant regime described above were able to feed to satiation in the allotted feeding period. Another feature of the operant regime is that one might expect animals to be feeding at the optimum level to maintain satiety on a day-to-day basis given that food is available (during the allotted feeding period) on demand. Table 5 shows the results of the analysis of SGR, and table 6 shows the results averaged and presented on a by-temperature basis. These results compare very favourably with those provided by Jauncey (1982) for carp feeding at a level of 3%, SGRs of 1.22 at 23°C, 1.2 at 22°C, 1.18 at 21°C and 1.13 at 20°C were reported. What is immediately clear is that the preferred feeding levels adopted by animals feeding on an operant regime is almost always below 3%, in addition specific

growth obtained on these feeding levels is much higher than would be obtained under the non-operant regime adopted by Jauncey. Clearly an operant regime, if appropriately designed and implemented is capable of optimising the conversion of food protein content into fish biomass when compared to other feeding regimes. It must be remembered however that the optimum level in terms of food utilisation may not be the feeding level at which maximum growth is obtained. Huisman (1976) has shown for carp that although the optimum feeding level at 23°C is 2.2% per day, the feeding level which produces maximum growth is 6.5% per day. In commercial terms the feeding level employed for any particular fish species will depend upon the relative costs of the diet and the culture system.

As described in the introduction to this section much of the literature on food intake in fish has concentrated on gastrointestinal factors as being the most important in controlling food intake and Colgan (1973) has produced a model in which the feeding rate is determined by gastric volume and systemic need. In this model the motivation for an animal to feed is deemed to be hunger, and hunger is presumed to arise from the interaction of the amount of food in the stomach and the metabolic debt (gastric volume, and systemic need). Hunger is presumed to be zero when the stomach is full (irrespective of any systemic need), greater than zero when the stomach is less than full, and to generally increase with systemic need and decrease with gastric volume. This model draws heavily on a 'set-point' based theory in which a systemic need requires some reference point against which the degree of need may be compared. Set-point or homeostatic theories have been discussed in relation to

the control of body weight in fish and there seems to be little direct experimental evidence to support such a theory (Toates 1981). One of the main drawbacks of such theories is that they tend to argue in favour of the regulation of a single physiological variable as being the dominant factor in the control of food intake. The lipostatic model (Kennedy 1953) proposed that depot fat was the regulated variable in the rat and that rates of food intake and energy expenditure were its controls. The glucostatic model (Mayer 1955) proposed that the utilisation of glucose was maintained at a constant level by initiating or inhibiting eating. The aminostatic theory (Mellinkoff, Frankland, Boyle and Greipel 1956) in the same way proposed that the maintenance of levels of plasma amino acids were responsible for the initiation or inhibition of food intake.

Homeostatic theories inevitably raise the question as to the mechanisms of monitoring and control of physiological parameters, and in particular at what sites in the body these functions are carried out. In mammals there is evidence of a role for both peripheral sites such as the liver (Russek 1981), and central sites such as the hypothalamus (Gunion and Tache 1990) and some hindbrain areas (Flynn 1989, Ewart, Jones and Primi 1990). In fish a number of lesion and electrical stimulation studies have been used to demonstrate a role for central regions in appetitive behaviour (Grimm 1960, Demski 1973, Savage and Roberts 1975, Roberts and Savage 1978).

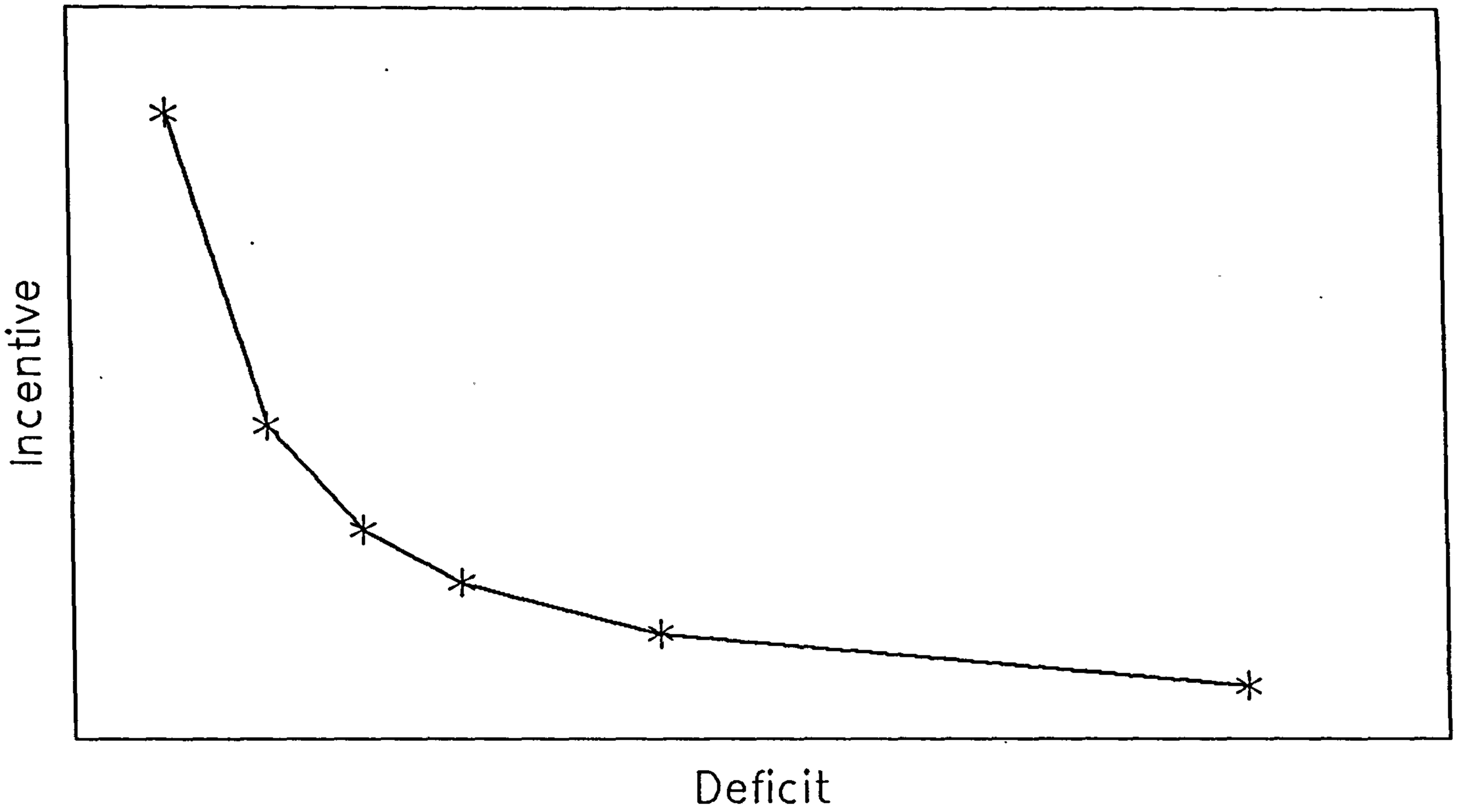
It is accepted that amongst the higher vertebrates food intake is under multifactorial control (Fletcher 1984 for review). The results presented above show quite clearly that this is also the case with

the carp, *Cyprinus carpio*. Multifactorial regulation of food intake raises the possibility that non-homeostatic theories (or at least some aspects of them) may be required in order to adequately describe the control of food intake. Non-homeostatic theories have been reviewed and classified into 3 categories; ecological, psychological, and computable (Kissileff and Van Itallie 1982). Ecological mechanisms have been mentioned above and rely on a cost-benefit analysis of the energy expended in obtaining food and the energy content of the food itself. Psychological theories rely on reinforcement or reward, the basic tennet being that feeding once initiated will continue all the while an animal is rewarded (as a result of certain sensory qualities of the food). Computable theories rely on the fact that food intake can be predicted from certain physiological variables, a number of variables are potentially involved; rate of stomach emptying, glucose levels, depot fat levels, plasma amino acid levels. It is clear that in order to adequately describe a model relating the factors controlling food intake it will be necessary to include aspects of each of the non-homeostatic categories listed above. It is thus necessary to include factors which are both internal and external to the animal. An important concept which relates internal and external factors is the motivational isocline (McFarland and Sibly 1975, Sibly 1975) in which the tendency to feed is related to the product of the food incentive value and the deficit at that time, see figure 23. The main drawback with this model is that incentive value and deficit are seen as two independent variables when in reality it is likely that the perceived incentive value will depend in part on the current physiological condition of the animal (ie. it may depend on the deficit). The

Figure 23.

Illustration of the concept of a motivational isocline. For all points on the isocline the product of the x and y values subtended by that point is a constant. All points on the curve thus represent points of equal level of motivation, where level of motivation is defined as the product of deficit and incentive. The motivational isocline thus predicts that if deficit is doubled the incentive required to produce the original level of motivation is halved.

Motivational Isocline

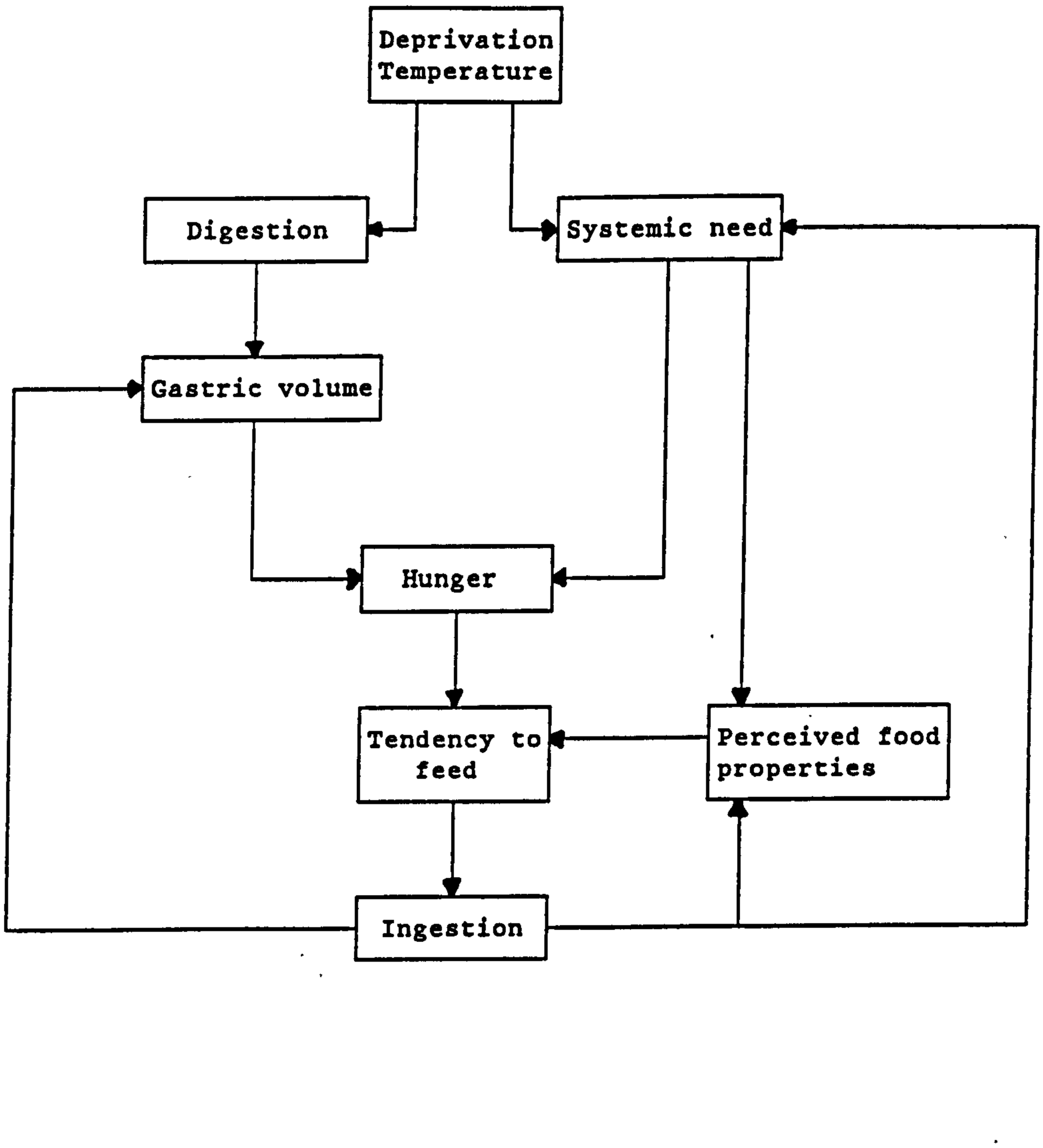


concept does not however rely on set-point theories.

It is possible to derive a model which is based on that proposed by Colgan (1973) see figure 24. The main difference between the model proposed here and that of Colgan is that this model does not equate the tendency to feed with hunger. In this model tendency to feed is seen as separate from hunger, thereby allowing external factors such as the perceived incentive value of the food to exert an effect on the tendency to feed. Furthermore, this model allows that the incentive value of the food items will be modified by systemic factors which may include levels of lipid, glucose and amino acids. This feature of the model is particularly interesting since it provides a mechanism to explain the presence of bout feeding in carnivores and its absence in carp (a non-carnivorous species). The appearance of bout feeding would depend simply on the nature of the link between perceived food properties and tendency to feed. In an animal exhibiting bout feeding, if the effect of ingestion were to enhance the perceived properties of food items making them appear highly palatable, this might have a positive effect on the tendency to feed, thus raising the feeding rate. This situation would persist until some other controlling factors such as increased gut fullness or lowered systemic need turned off the bout. In the carp the nature of the link between ingestion and perceived food properties may well be such that no positive effect is exerted on the tendency to feed. In this case the perceived food properties may be used simply as a veto of the tendency to feed, thereby acting as a safeguard against the ingestion of noxious substances. Feeding patterns in carp appear to be explicable using a system of negative feedback controls,

Figure 24.

Model showing the relationships of the factors involved in the control of food intake.


Deprivation
Temperature
Digestion
Systemic need
Gastric volume
Hunger
Tendency to
feed
Perceived food
properties
Ingestion

whereas those patterns seen in carnivorous fish are best explained by the action of a positive feedback element operating in the presence of food. Although carp have no well defined storage stomach, the foregut or anterior intestine has a finite capacity and the fullness of this part of the digestive system may well, during a very high rate of feeding limit food intake in the short term. Rozin and Mayer (1961) tested the possibility of this effect by feeding goldfish under an operant regime on pelleted fish food which had been calorifically diluted by 50% with the addition of kaolin. The results of this study showed quite clearly that goldfish increase their food intake by the correct amount to maintain a certain level of nutrient intake over a given feeding period. This result was not attributable to preference effects since in free feeding 'choice' experiments fish showed a preference for the normal pelleted food over the kaolin adulterated food. In addition the feeding periods on diluted food did not lead to the emergence of any different feeding patterns, that is to say the emergence of bout feeding was not reported. Interestingly, in the majority of similar studies of other species a similar result was noted in that animals ate more frequently when fed on calorifically diluted food and gastric evacuation was also found to be faster. This correlates well with the prediction of a square root model of gastric evacuation where evacuation is related to the volume of the ingested meal. The fact that gastric evacuation proceeds more rapidly leads to a much quicker return of appetite in fish feeding on a calorifically reduced diet. In carnivorous fish this is manifested as a decrease in the time between feeding bouts (Jobling 1981, Grove Loizides and Nott 1978). It appears therefore that in carnivorous fish a bout feeding style is maintained but the pattern is altered

and closely mirrors the pattern of gastric emptying. In other words the fullness of the gut would appear to be the controlling factor in food intake in these animals, and on a diluted food the gut merely fills and empties more rapidly. It appears that in both carnivorous and 'stomachless' animals the practice of providing calorifically diluted feedstuff leads to an increase in frequency of the normally observed feeding pattern. Although experiments using calorifically diluted food were not carried out a reduced period of food availability was employed in order to determine whether fish could increase their rate of intake sufficiently to maintain a constant feeding level. The results showed quite clearly that animals were able to almost double their average rate of food intake (as measured in g hr^{-1}), and showed only a slight and insignificant deficit in amount of food eaten ($p < 0.85$). The conclusion from this experiment is similar to that drawn by Rozin and Mayer using diluted foodstuff and it is that the state of fullness of the anterior part of the gut does not constitute the rate limiting step in food intake in carp feeding at the rates demonstrated here. It is however interesting to note that the slight deficit in food intake noted here may well be seen to become significant if food intake rate were to be raised slightly higher by further reducing the time available for feeding, or reducing the energy value of the food. Unfortunately these experiments were not carried out.

It seems possible that the difference in pattern in food intake between carp and more carnivorous species may well be explained by the hypothesis put forward by Landless (1976) that the inter-specific differences in feeding patterns is due to the absence or presence of

a storage stomach together with a positive feedback control over food intake. If the stomach of an animal does not empty into the next portion of the gut at a rate at least equal to or close to the rate of food intake then eventually food intake must slow down or even cease until some of the stomach contents have evacuated. This of course could lead to the observed feeding pattern being structured into bouts where bout length and distribution would depend on the relative rates of filling and emptying of the stomach. In an animal such as the carp the stomach is represented quite simply by a swelling of the anterior part of the gut. Unless the rate of emptying of the fore-gut is high it is obvious that under rapid feeding conditions the rate of evacuation of this part of the gut will become the rate limiting step in food intake. The absence of positive feedback during the feeding period will tend to keep feeding rates lower in the presence of food thus promoting a rate of intake which may be lower than the rate of evacuation of this part of the gut. It is quite possible for fish with well defined stomachs and carp to feed at the same level (in terms of percentage of body weight ingested) over a period of time but for the observed pattern (in terms of the microstructure) to be very different, the feeding level being determined by levels of systemic need which may be monitored and controlled by the organism over a relatively long period of time (possibly hours), and the feeding pattern being determined by 'local' conditions such as gut fullness and food palatability which are effective in exerting control over a relatively short period of time (minutes).

The control of food intake may be seen to operate over wide time scales, it is hardly surprising therefore that both central and peripheral sites have been implicated. Quite clearly an animal must be able to alter its rate of intake almost instantaneously if it is to take advantage of available food and avoid potentially toxic items in the environment. This rapid reaction is most likely to be based on the gustatory qualities of the food and be mediated centrally via sensory and motor systems. In addition an animal must regulate systemic levels of essential compounds such as glucose, lipids and amino-acids and the regulation of these variables will of necessity take place over a relatively long timescale. It is quite possible therefore that levels of these variables will be controlled via peripheral organs such as the liver. With regard to the sites involved in the control of food intake in carp, there is evidence for both peripheral sites (Beach, McVean, Roberts and Thorndyke 1988) and central sites (Beach and Roberts 1985). An investigation and discussion of the possible sites at which food intake may be controlled in the carp will appear in the subsequent sections of this thesis.

SECTION II

INTRODUCTION

There is a large body of work on the role of the hypothalamus in the control of food intake, this work dates back many years and has largely been concerned with mammalian species. Some of the earliest studies in this area demonstrated, using lesioning techniques, that discrete areas of the hypothalamus could be associated with a particular behaviour. The demonstration that ventro-medial hypothalamic (VMH) lesions led to hyperphagia in the rat (Hetherington and Ranson 1940), and that lesions in the lateral hypothalamic (LH) regions produced aphagia in the rat (Anand and Brobeck 1951) led to a 'dual centre' hypothesis (Stellar 1954) whereby food intake was under the control of two discrete areas of the hypothalamus, a medial satiety centre and a lateral feeding centre. The dual centre hypothesis has been the subject of considerable controversy largely due to the fact that many of the effects of hypothalamic lesions can be shown to be the result of damage to fibres of passage rather than to discrete nuclei (Ungerstedt 1971). In addition the concept of a VMH satiety centre has been criticised on the grounds that the effects of VMH lesions may be reversed by sub-diaphragmatic vagotomy and that the observed hyperphagia is therefore largely due to a disturbance in insulin secretion (Powley and Opsahl 1974). The centre concept has received much attention and continues to do so in more recent times (Bernardis 1985, Kyrkouli, Stanley and Leibowitz 1987). Not surprisingly, the bulk of research into the role of the hypothalamus in the control of food intake has concentrated on mammalian species, as a result very little is known of the role of the hypothalamus in the control of

feeding in fish. Much of the published data has been based on electrical stimulation techniques (Demski 1973, Savage and Roberts 1975), and electrical lesioning or physical ablation techniques (Roberts and Savage 1978, Savage 1969). One of the important features of these studies has been the finding that, although the hypothalamus is undoubtedly involved in the control of food intake, there is no evidence to suggest a high level of homology either morphologically or functionally between mammalian and teleostean species.

With regard to the sensory aspects of food intake two families of fish stand out as having evolved elaborate gustatory systems, the *Cyprinidae* and the *Siluridae*, studies on these two families having dominated the investigation of the neural basis of gustation. As early as 1905 Herrick accurately described the central pathways of the gustatory systems in several Cyprinid and Silurid species. More recent studies have generally confirmed the earlier results and added further to the anatomical details of these pathways (Morita, Ito and Masai 1980, Morita, Murakami and Ito 1983). More recently still, work carried out by Baker (1987) using a very sensitive Horse-radish peroxidase (HRP) technique for neuroanatomical tract-tracing, has shown the involvement of two areas of the hypothalamus in both the goldfish (*Carassius auratus*) and the common carp (*Cyprinus carpio*). The technique involved the injection of HRP into the facial lobe and vagal lobe areas of the hindbrain. Two areas of retrogradely labelled cells were detected in two zones of the diencephalic inferior lobes. The more ventral area was in the region of the nucleus lobo-bulbaris (NLB) and the second more dorsal group was in the region of the nucleus posterior thalamicus (NPTh). These findings provided good

evidence of an anatomical projection from the hypothalamus to the primary gustatory centres of the hindbrain. Of the two hypothalamic nuclei labelled in the anatomical studies the NPTh is the most compact, the NLB tends to be a much more diffuse nucleus. Figure 25 shows a diagram of a transverse section through the carp brain at the level of the NPTh. Figure 26 shows a transverse section through the area of the NPTh, the NPTh is clearly visible at the centre of the photomicrograph. It is possible using electrical stimulation of the vagal or facial lobes of the hindbrain to antidromically excite neurons in the NPTh. The signal recorded as a result of such stimulation is long lasting and highly characteristic of the area in and around the NPTh (Beach and Roberts 1985), and an example of such a signal is shown in figure 27. The relative ease with which an electrode may be located within the NPTh allows for the placement of very small, accurately located lesions. The ability to functionally locate such discrete lesions is very important in restricting lesion damage to the area of interest. Earlier lesioning experiments (Roberts and Savage 1978), carried out in those areas of the hypothalamus where it was not possible to employ functionally located lesions, relied on the use of stereotactic techniques. Lesions placed stereotactically have been very large (6 - 7.5 mC) when compared to the size of the lesions used in the experiments described here (120 μC), and as a result they may have caused damage to several hypothalamic nuclei. The fact that the NPTh could be functionally located, allowed the accurate placement of small lesions within the NPTh which did not cause any visible damage to the surrounding areas of the hypothalamus. This situation allows a precise investigation into the effects on food intake of damage to a single nucleus in the

hypothalamus which is known to project to the primary gustatory areas in the hindbrain. Although functional localisation of the NPTh proved to be a major advantage in terms of accurately locating that nucleus, the technique does have a drawback in that it involves the electrical stimulation of another area of the brain. Electrical stimulation of the brain will cause some degree of damage, and in this case that damage is within part of the known functional gustatory system. Careful controls need to be implemented to ensure that any observed effects of NPTh lesions are not in part (or in whole) due to damage sustained by the hindbrain during stimulation.

Figure 25.

Transverse section through the carp diencephalon illustrating the relative positions of the nuclei in the area of the Nucleus Posterior Thalamicus. NLB nucleus lobo-bulbaris, NPTh nucleus posterior thalamicus, NPGL nucleus preglomerulosus pars lateralis, NPGM nucleus preglomerulosus pars medialis, NSPg nucleus sub-preglomerulosus.

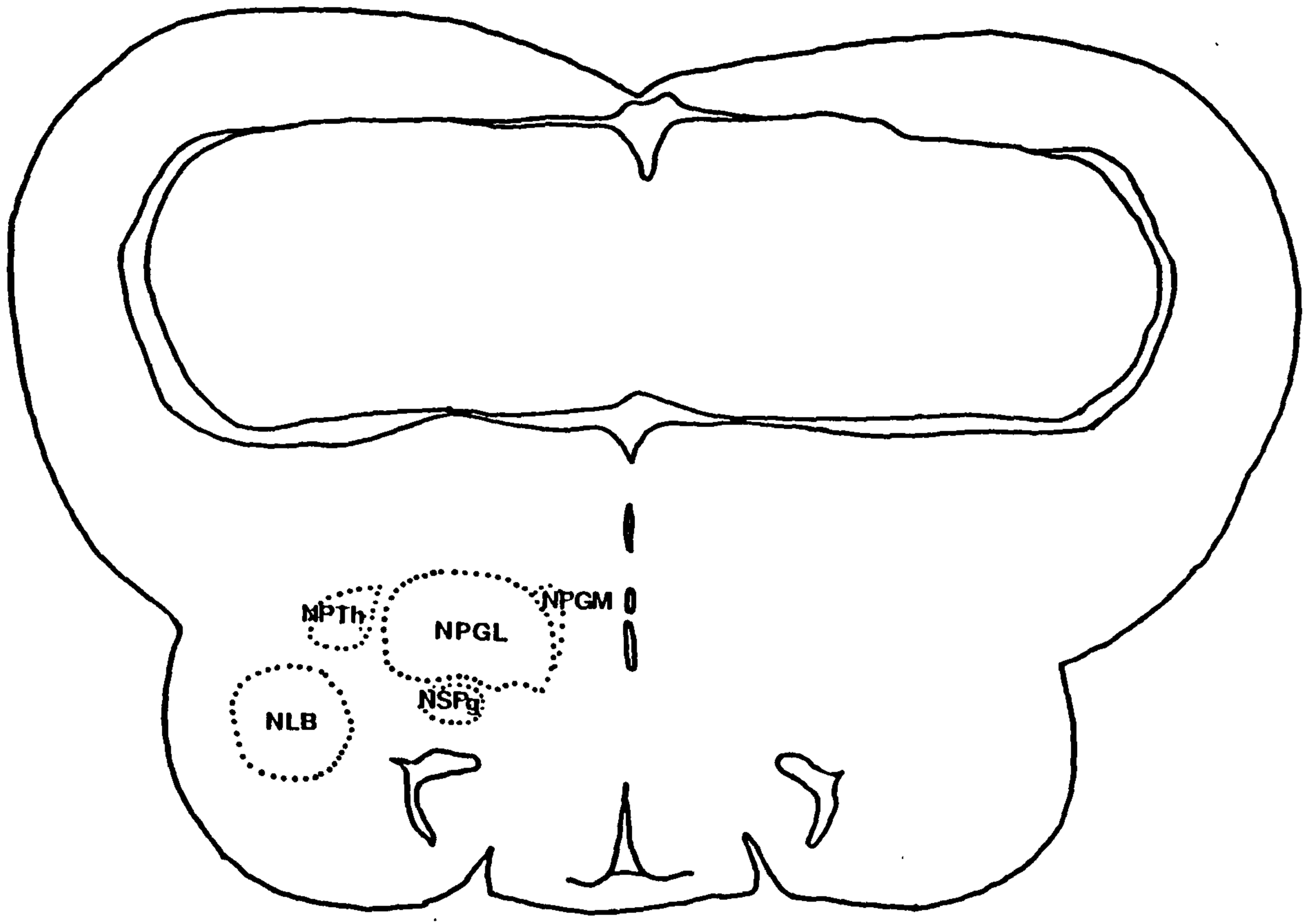

NPTh
NPGL
NPGM
NSPg
NLB

Figure 26.

Photomicrograph of the area represented in figure 25. The nucleus posterior thalamicus is indicated by the arrowhead. Scale bar 100µm.

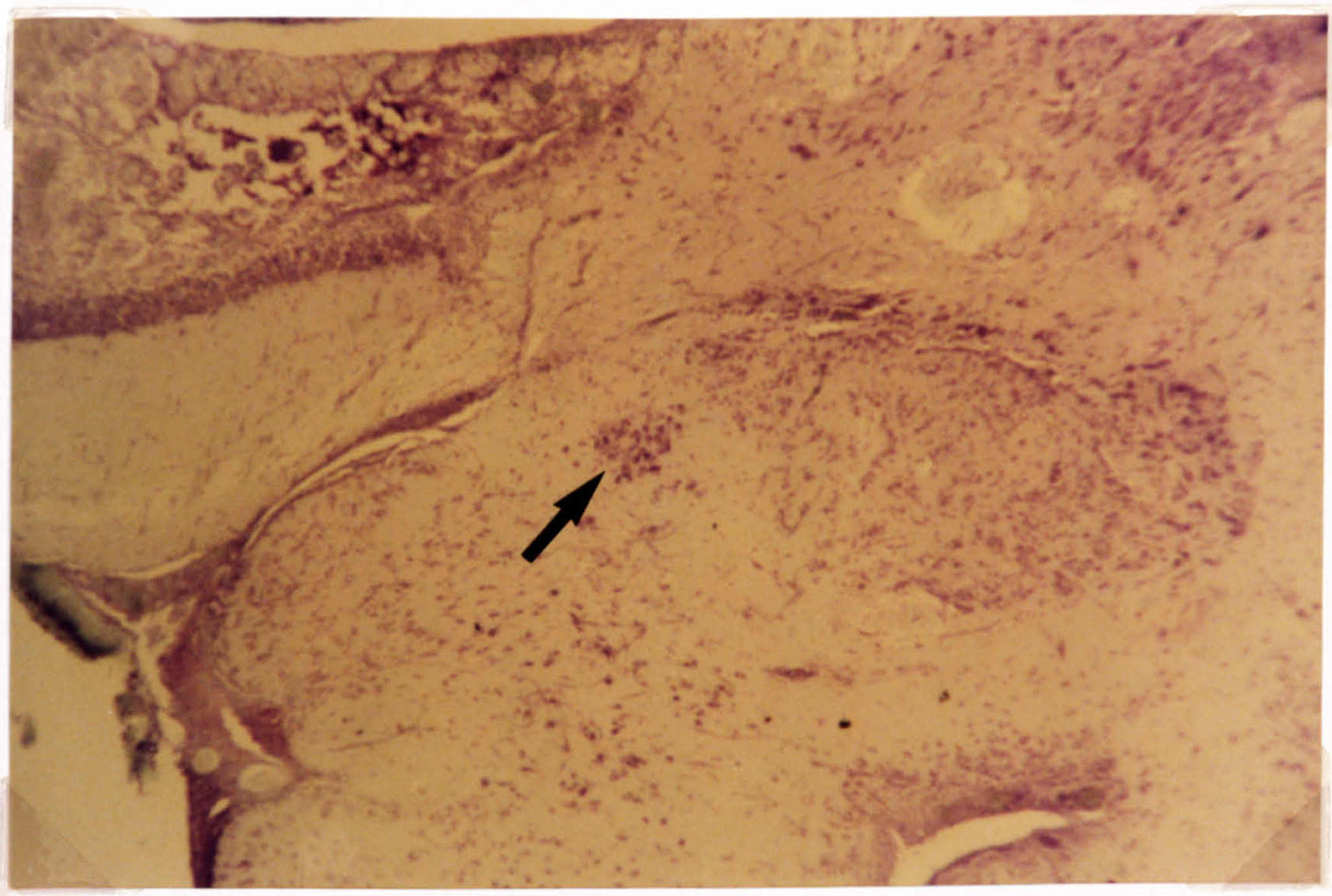
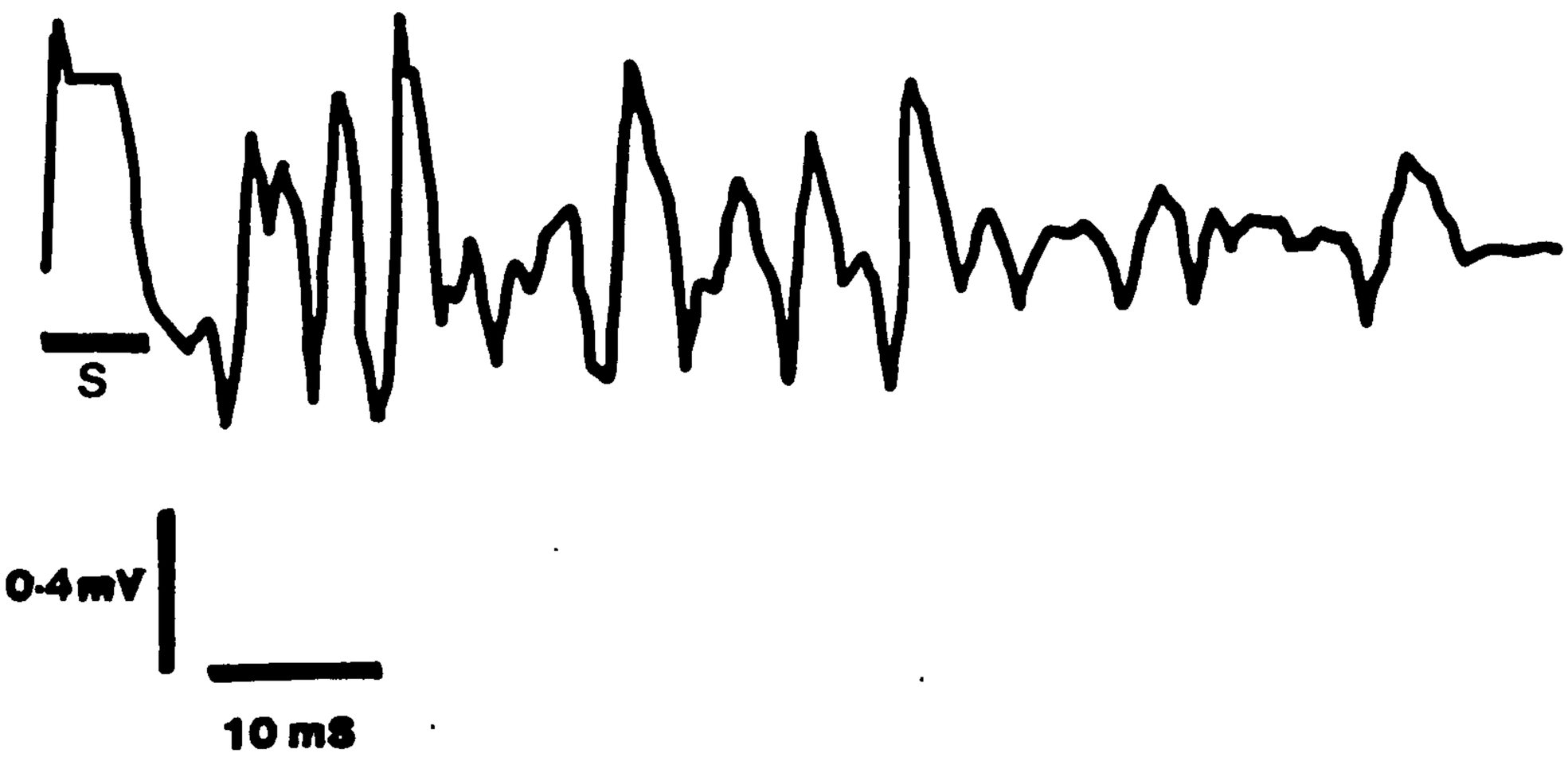


Figure 27.

Example of a signal recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. The stimulus artefact is marked by the horizontal bar labelled S.

Signal recorded from the nucleus posterior thalamicus in response to ipsilateral vagal lobe stimulation



MATERIALS AND METHODS

The operant regime used to test the effects of NPTh lesions was the same as that described in the materials and methods of section I. Animals were held in the operant tanks for a period of a few days in order to acclimatise to the new conditions. After this period there followed a period of at least ten days during which time feeding data was recorded as described in section I. This data provided a 'baseline' set of data against which the subsequent experimental data could be compared. After the ten day period, animals were removed from their tanks and hypothalamic lesions were introduced as described in the 'lesioning procedure' section below.

Lesioning procedure

The lesioning procedures described in this section rely on the use of monopolar recording/lesioning electrodes and bipolar stimulating electrodes. Monopolar electrodes were obtained from Digitimer UK Ltd. (Welwyn Garden City, England), these were of the tungsten-in-glass type, and a full specification may be seen in table 8. The bipolar stimulating electrode pairs were constructed `in-house` using tungsten or stainless steel wire, which was electrolytically etched to a fine point and then insulated with Clark's epoxylite resin (Clark Electromedical Instruments, Pangbourne, England). It was found that Digitimer stimulating electrodes (NL05) suffered less tip damage after passing a lesioning current than did the recording electrodes (NL02). As a result of their greater robustness the NL05 electrodes were employed as recording electrodes in those situations where the recording electrode was to be used to produce an electrolytic lesion.

Table 8

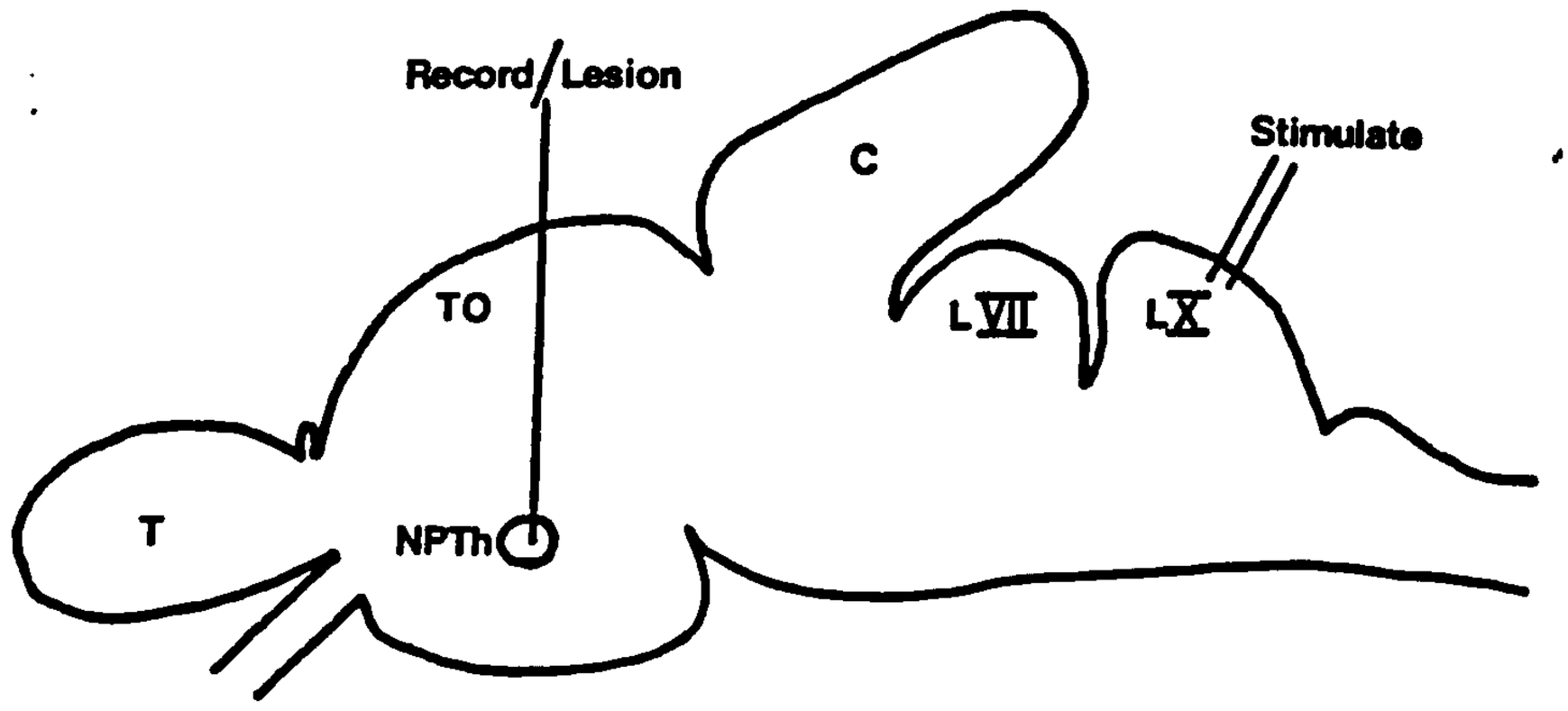
	NL02 Recording	NL05 Stimulating
Shank diameter	125 µm	as NL02
Overall length	35 mm	as NL02
Insulated length	20 mm	as NL02
Taper	2^{o}	as NL02
Exposed tip length	15 µm	20-25 µm
Tip plating	platinum black on gold	no tip plating
Impedance at 1kHz	200 kΩ	1MΩ

Figure 28 shows the anatomical locations of stimulated and lesioned sites.

Physiological recordings were carried out in a 92cm x 70cm x 66cm Faraday cage. The base of the Faraday cage was covered by a 10 mm thick stainless steel plate, and the whole was situated on top of a concrete 'steady bench'. The carp was held in a topless perspex box by means of a contoured body clamp. Aeration of the gills was achieved by way of a mouth tube which carried a constant stream of water from a header tank mounted above the Faraday cage. The

Figure 28.

Diagrammatic sagittal section through the carp brain showing the relative positions of the stimulated and recorded/lesioned sites. T telencephalon, TO optic tectum, NPTh nucleus posterior thalamicus, C cerebellum, LVII facial lobe, LX vagal lobe.


Record/Lesion
C
Stimulate
TO
LVII
LX
T
NPTh

header tank contained a solution (1:10 000) of ethyl m-aminobenzoate methanesulphonate (Sigma, England) anaesthetic. Waste fluid collected in the base of the perspex box and then drained away. The steel base of the Faraday cage allowed the use of Eclipse magnetic based retort stands for equipment mounting purposes. The dissecting microscope was mounted such that the area of operation could be viewed at all times during the experiment. A Barr and Stroud LS10 cold light source with a fibre optic neck was used to illuminate the experimental area.

Electrodes were mounted in Prior micro-positioners which were clamped onto Eclipse magnetic based stands. The pre-amplifier head stage was suspended from the roof of the Faraday cage a short distance above the animal's head. Electrical connections were made with short 'flying leads' terminated in 1.02 mm diameter Cambion gold pins.

Prior to lesioning fish were anaesthetised by immersion in a 1:5000 anaesthetic solution (ethyl m-aminobenzoate methanesulphonate). When opercular activity had ceased animals were removed from the anaesthetic, and the top of the skull removed using a small circular saw. The fish was then wrapped in tissue paper soaked in physiological saline, transferred to the recording apparatus and clamped in place. With the aid of an Olympus zoom (0.7x - 4x) dissecting microscope excess fatty tissue was removed from the skull cavity by means of a vacuum driven aspirator. With the adipose tissue removed the dorsal surface of the brain was clearly visible, and the placement of recording and stimulating electrodes was relatively easily achieved. Stimulation and recording was provided by Neurolog rack mounted modular equipment, and recorded signals were dispayed on

a Tektronix D13 storage oscilloscope, and if necessary recorded onto magnetic tape using a Racal Store 4DS DC tape recorder for later analysis. Signals recorded onto magnetic tape were later replayed, and displayed on a Gould OS4040 digital oscilloscope, these traces were plotted onto paper using a Bryans 26000 A4 X-Y plotter.

Once the animal had been prepared and placed in the apparatus a pair of stimulating electrodes was driven into the vagal lobe on one side, such that the tips just penetrated the surface. A ground electrode consisting of a scintered silver/silver chloride pellet was placed just inside the aperture in the skull, and in contact with the body fluid. A recording electrode was held vertically above the aperture in the skull and was mounted in the final stage of a Clark's hydraulic microdrive connected to a micro-positioner. The micro-positioner was used to place this electrode initially on the surface of the tectum, and the hydraulic microdrive subsequently used to advance the electrode through the brain. The use of this technique allowed measurement of the depth of the tip of the recording electrode relative to the surface of the brain to the nearest 0.002mm. The recording electrode was driven down into the brain to a depth of 2 mm (measured from the point at which contact was made with the surface of the tectum). The stimulating circuitry was switched on and constant current pulses of 10μA intensity and 1ms duration were delivered at a rate of 1Hz by way of the pair of stimulating electrodes located in the ipsilateral vagal lobe. The recording electrode was driven slowly down through the brain until a characteristic evoked NPTh response was seen (figure 27). Experience showed that signals of this type were only found within the region of

the NPTh under these experimental conditions. Once the recording electrode had been located in the region of the NPTh the depth was adjusted until the maximum sized signal was obtained. If no satisfactory signal was found the recording electrode was withdrawn, moved a small distance across the tectal surface and another attempt to record an NPTh signal was made. With practise it usually proved possible to locate a satisfactory signal on the first attempt or within three attempts. This was an important aspect of the experiment as it was desirable to keep all unnecessary damage to the brain to a minimum. Having located a satisfactory signal the depth of the electrode tip was noted, and a tape recording of the signal made for future reference. The connections of the recording electrode were then rearranged such that it was connected to a second stimulus isolator, and a lesioning current was passed cathodally via the electrode into the recorded site. Lesions produced in this experiment were 120 μC in size (10μA x 12 seconds). Cathodally produced lesions tend to be more variable in size than the coulombically equivalent anodal lesions (Moore 1981), this is due to the fact that cathodal lesions tend to release gas during current passage and as a result there may be loss of electrical contact, and hence the duration of passage of lesioning current may be uncertain. The reason for utilising cathodally produced lesions for this study was that anodal lesioning current, although producing more regular lesions in terms of size and shape do tend to cause the deposition of metal ions in the tissue. The amount of metal deposited by anodal current varies with the type of electrode, however all metal electrodes including platinum do deposit metal ions. It was essential that any observed effects of the experiment were the result of the physical presence of

the lesion and not to some toxic effect of the deposited metal.

The entire procedure described above was repeated to produce a second lesion on the other side of the brain.

Having produced the lesions the cranial cavity was packed with Sterispon absorbable gelatin sponge. The flap of bone that had been removed to create the aperture in the skull was scraped clean of skin and dried with tissue paper, as was the area of the skull immediately surrounding the aperture. The flap of bone was laid back in its original place and Permabond C cyanoacrylate adhesive was applied to the join. Subsequent localised application of methyl methacrylate ensured that the cement hardened immediately. The area of exposed bone was treated with Permabond cement, this formed a watertight seal over the whole area.

The flow of respiratory water was then changed to clean water containing no anaesthetic. As soon as the animal started to exhibit opercular movements it was removed from the experimental apparatus and returned to the tank from which it had been taken. Careful visual observation of the animal was maintained until it had fully recovered from the anaesthetic, this usually took about 30 minutes. Any animals which showed evidence of motor or postural abnormalities after recovery were sacrificed immediately on the grounds that these types of abnormality are often an indication of intra-cranial bleeding. These experiments were conducted at such a time as to allow several hours between the end of the surgical procedure and the start of the next feeding period.

On being returned to the experimental tank animals were left in the experiment for a further period of at least ten days during which time feeding data was recorded daily. At the end of this period animals were removed, sacrificed by anaesthetic overdose, and the brain removed. Each brain was fixed in Bouin's fluid, dehydrated, embedded in paraffin wax, and serially sectioned at a thickness of 15 μm. Sections were stained with cresyl fast violet. In this way it proved possible to recover the exact anatomical location of the lesioned sites. A survival period of approximately ten days was found to be an optimum time in that most lesioned animals had resumed feeding by this time, many at near normal levels. In addition ten day old lesions were still easy to locate, and it was still possible to make a reasonable estimate of the original extent of the damage. The histologic events following the placement of electrolytic lesions have been discussed by Moore (1981), they include the immediate appearance of a central core of necrotic tissue, followed by the migration of phagocytic cells into the lesioned area. Figure 29 shows the appearance of an electrolytic lesion 1 day after its placement. Figures 30 and 31 show the appearance of electrolytic lesions after 16 days and 18 days respectively.

The classification of animals in this experiment into control and experimental groups did not take place until the lesions had been histologically recovered, and is described below.

Figure 29.

Photomicrograph of a transverse section through the diencephalon at the level of the nucleus posterior thalamicus (NPTh). A 1 day old 120 µC electrolytic lesion is shown located at the anterior pole of the NPTh. The lesion is clearly visible as the darkly stained object at the centre of the photomicrograph. The cells of the anterior NPTh are labelled with an arrowhead. Scale bar 100 µm.

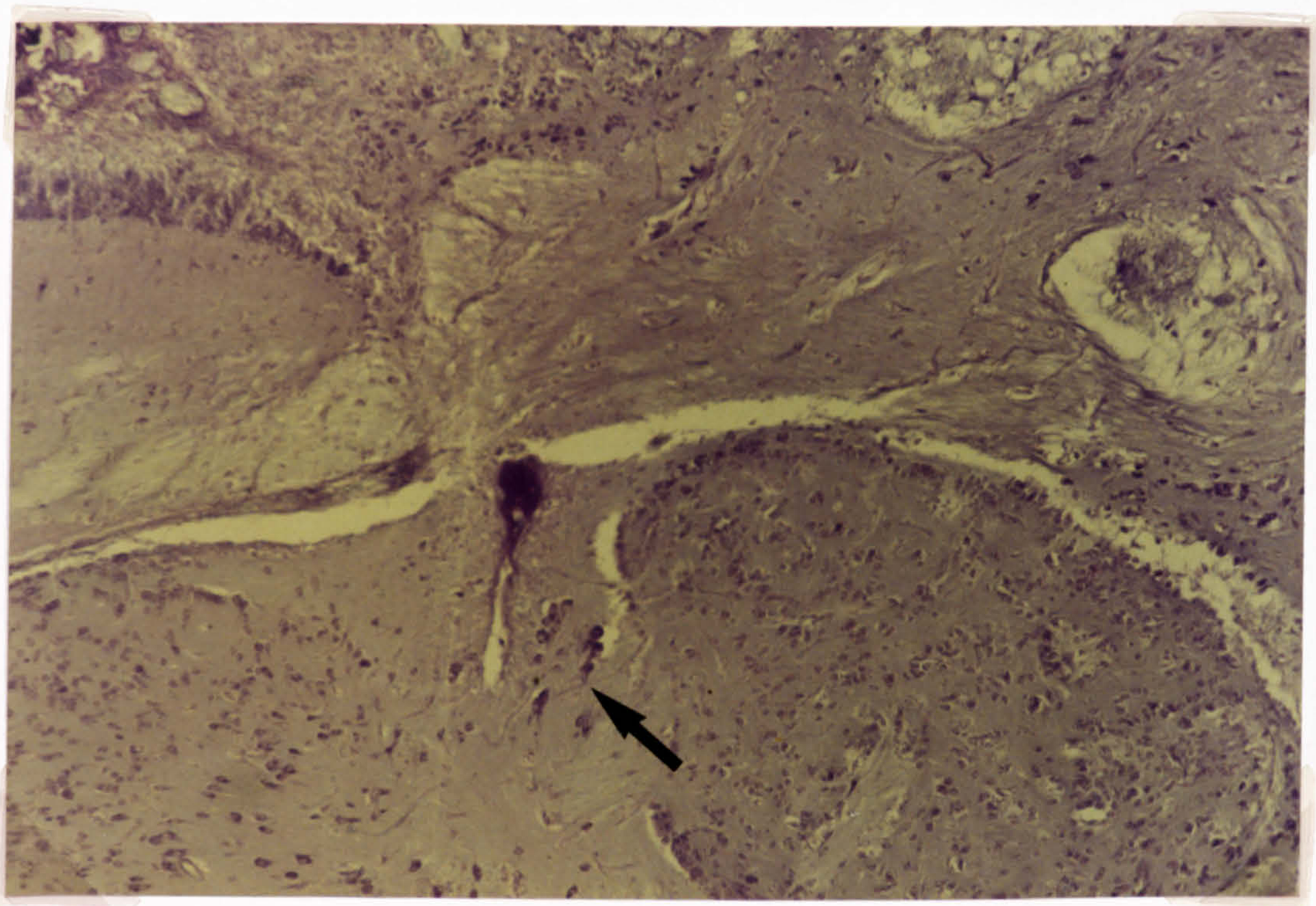


Figure 30.

Photomicrograph of a transverse section through the diencephalon at the level of the nucleus posterior thalamicus (NPTh). A 16 day old 120 µC electrolytic lesion (L) may be seen located ventromedially within the NPTh (dotted outline). Scale bar 100 µm.

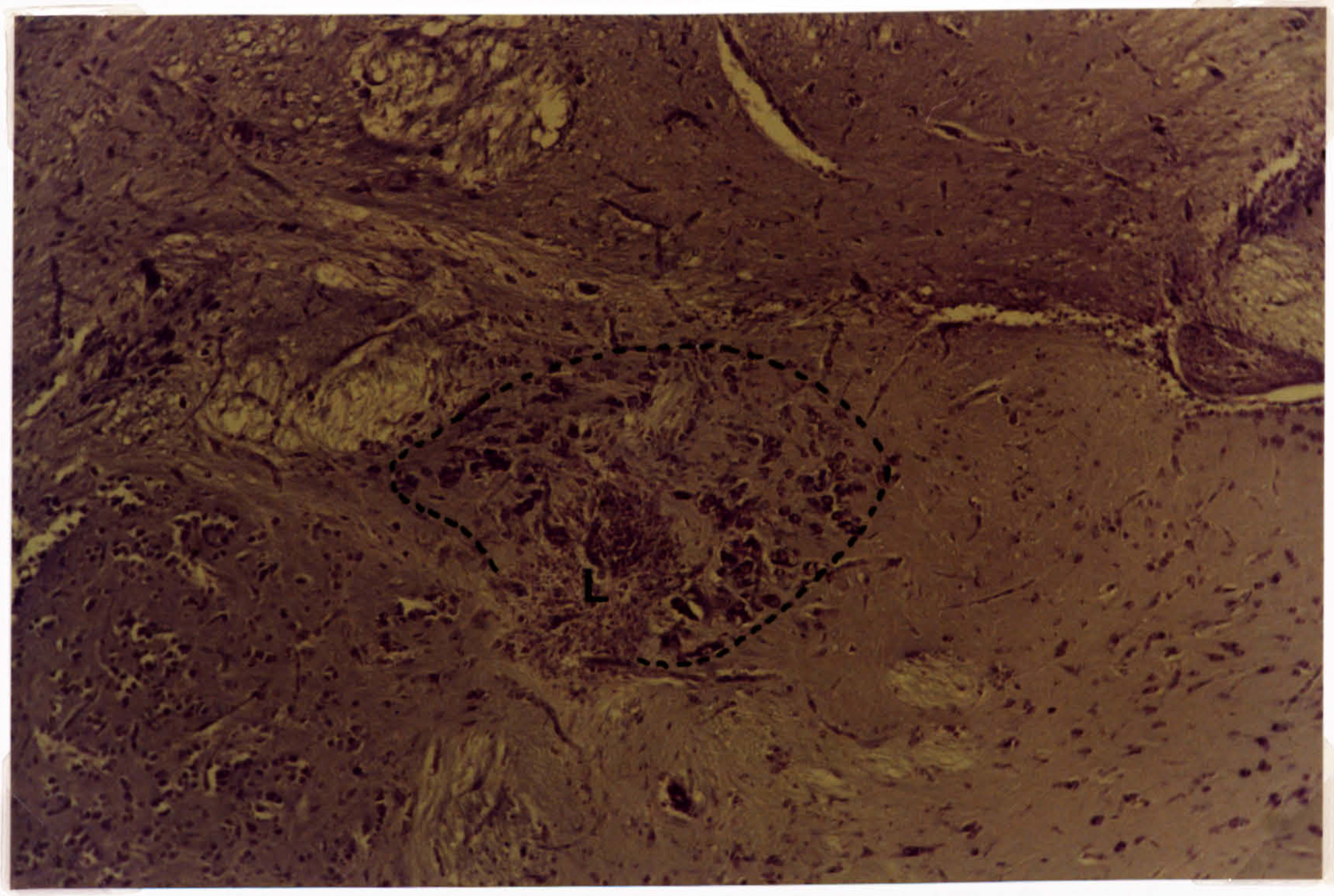
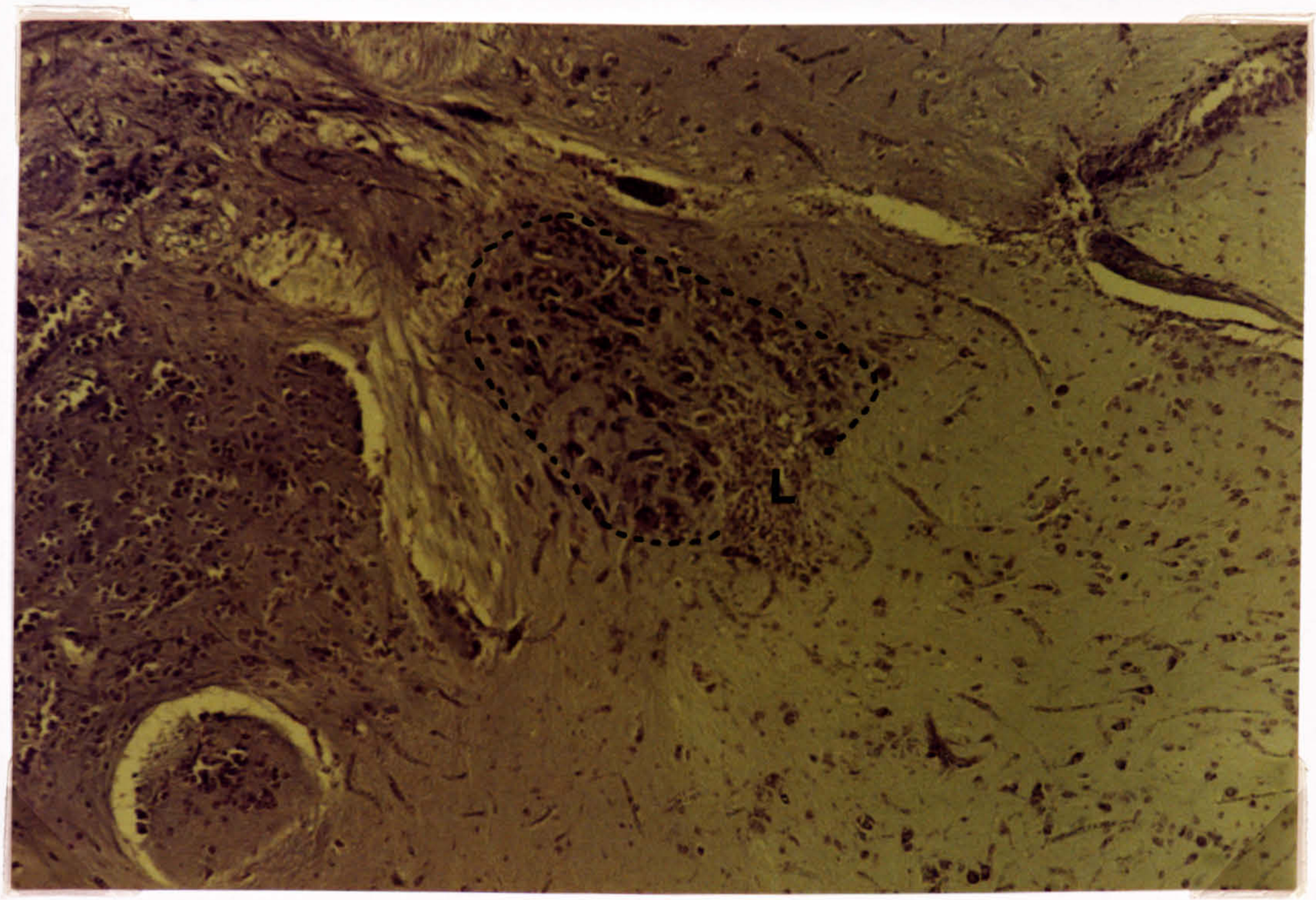

L

Figure 31.

Photomicrograph of a transverse section through the diencephalon at the level of the nucleus posterior thalamicus (NPTh). An 18 day old 120 µC electrolytic lesion (L) may be seen located ventrolaterally within the NPTh (dotted outline). Scale bar 100 µm.


L

1. Group one.

This group consisted of those animals which underwent the full surgical procedure described above. In addition an electrolytic lesion (120μC) was introduced into each side of the brain, no attempt was made to locate hypothalamic structures using any form of electrical or other stimulation. Lesions were introduced at depths of between 3.2 and 3.5mm. The exact lesion depth in each animal was adjusted slightly to allow for slight differences in animal weight (lighter animals receiving slightly shallower lesions). These lesions were placed 'freehand' (that is to say with no attempt at a functional localisation) in order that any hindbrain damage caused by electrical stimulation and its consequent effect on food intake could be avoided. This allowed the investigation of the effects of hindbrain stimulation on food intake separately in another group of animals. Although not intended to be in any highly specific location, these lesions were placed such that they should be located in the region of the dorsal hypothalamus. The depth estimates were based purely on previous experimental experience. Subsequent histological investigation of the brains of these animals showed that in no case were any lesions associated with the NPTh or any of its surrounding areas. These lesions were thus designated nonspecific lesions. This group of animals served as a control for the effects of surgery. The total number of animals in this group was 7.

2. Group two.

As in group 1 these animals underwent the full surgical procedure. In addition electrical stimulation of the ipsilateral vagal lobe was employed in an attempt to functionally locate the NPTh. Stimulus parameters used for this experiment were:-

Pulse rate	1 Hz
Pulse duration	2 ms
Pulse amplitude	100 μA

During the experiment if no evoked NPTh activity had been recorded over the course of 5 recording electrode tracks, the attempt to functionally locate the NPTh was abandoned, and a 120 μC lesion was placed as close as possible to the suspected region of the nucleus. This procedure was necessary in order to eliminate the possibility of excessive hypothalamic damage being caused by multiple recording electrode tracks. In all cases where this procedure was necessary subsequent histological investigation failed to identify any lesioned sites in or around the NPTh. Again these lesions were considered to be nonspecific, and this group of animals served as a control for the effects of hindbrain stimulation. The total number of animals in this group was 3.

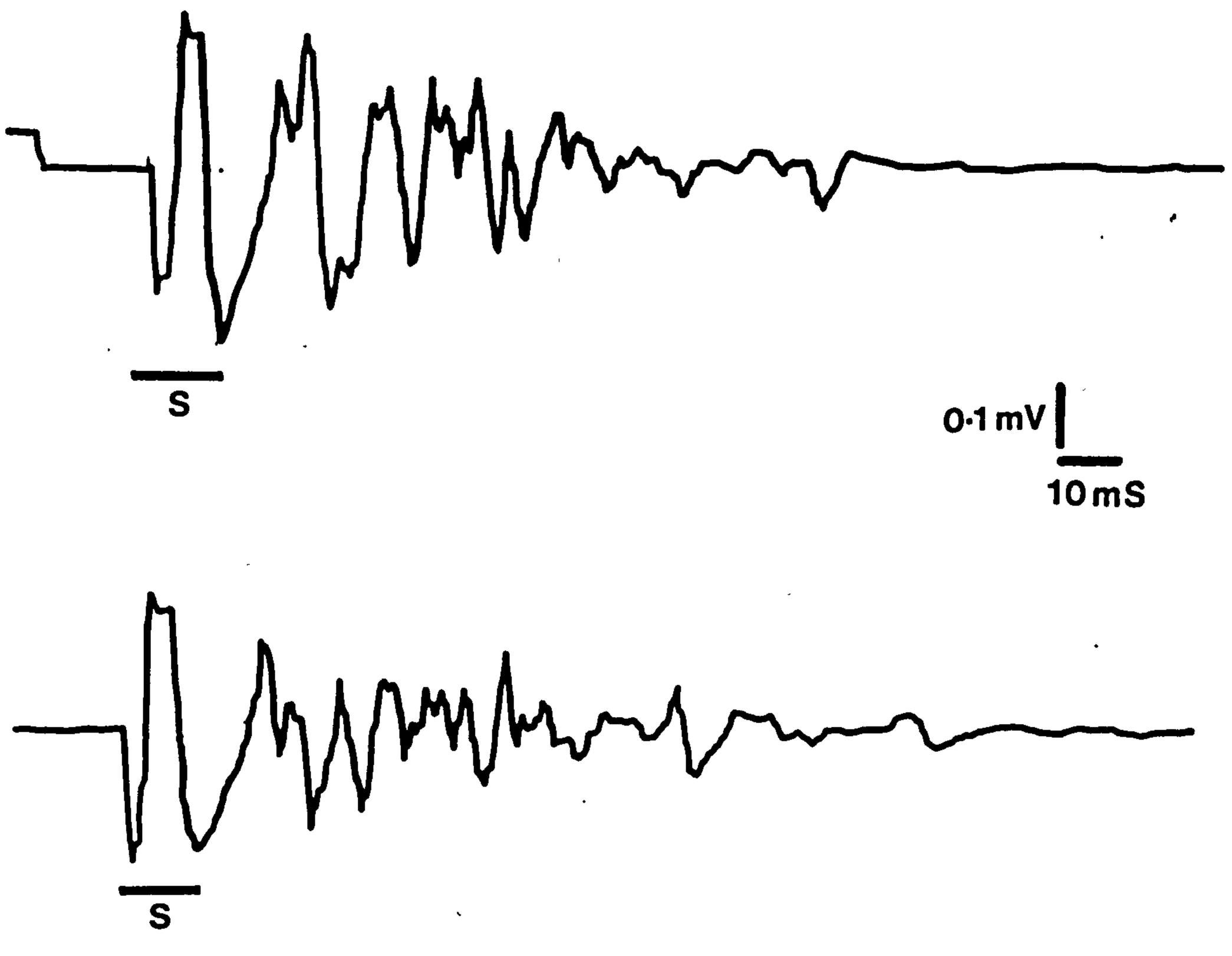
3. Group three.

Animals in this group received the same treatment as the animals in group 2. The animals in this group were those animals where a large evoked signal was recorded from the NPTh on one or both sides of the brain, figure 32 shows the signals recorded from animal X036. A 120 μC cathodal lesion (12 seconds x 10 μA) was placed at the site of the evoked signals. These lesions were deemed to be `NPTh specific`, they were histologically recovered and their locations in (or around) the NPTh were noted. The animals in this group comprised the experimental animals. The total number of animals in this group was 6.

All NPTh lesions produced in this experiment were recovered histologically, damaged areas on both sides of the brain were plotted with the aid of a camera lucida drawing tube. Drawings were later digitised and stored on a microcomputer, the computer used for this purpose was an Elonex PC-88 Turbo with an attached Bioquant II Hipad Digitizer. The software used to store and subsequently analyse the data was Bioquant System IV from R and M Biometrics (1985). This system allowed the area of any structure seen in the histological sections to be drawn and calculated, thereby enabling the calculation of the extent of damage to the NPTh caused by the lesions. The total area of the NPTh was recorded in all of the sections in which it appeared, multiplication of the total area by the section thickness allowed the volume of the NPTh to be calculated. Subsequently in all of the sections in which the lesion was apparent, the total area of overlap between the lesioned area and the NPTh was recorded, as before multiplication of this area by the section thickness allowed

Figure 32.

Signals recorded from animal X036 prior to lesion placement in the hypothalamus. Top trace signal recorded from right hand side, bottom trace signal recorded from left hand side. The stimulus artefacts are marked by the horizontal bars labelled S.


S
0·1 mV
10 mS
S

the volume of the damaged parts of the NPTh to be calculated. This procedure was repeated on both sides of the brain. In this way it was possible to express the extent of the damage caused by the lesions, as a percentage of the total bilateral volume of the NPTh.

In the case of those animals in group 3 which resumed feeding before sacrifice, data was recorded and subjected to a log survivor analysis as described in section I, this allowed a check for the possible appearance of a bout feeding style following NPTh lesions.

Following hypothalamic lesions precautions were necessary to ensure that changes in food intake were not due to motor or visual dysfunction. Animals which had been returned to their tanks post-operatively were monitored by means of a video camera attached to a remote monitor in an adjacent room. Post-operative feeding sessions were also video taped to allow later off-line inspection of fish behaviour. These procedures were necessary to establish that animals were not exhibiting abnormal behavioural patterns. Animals showing abnormal behaviour were removed from the experiment and sacrificed immediately. In addition tanks containing lesioned animals were carefully inspected each day for the presence of uneaten pellets. Again this procedure was important for the detection of any behavioural dissociation between the performance of the operant task and the consumption of the delivered pellet. A problem with any experiment of this type is that the lesions may have in some way impaired the animal's ability to perform an operant task rather than the mechanisms of satiety or food intake control. This was not considered to be the case in this experiment since in 5 out of the 6

NPTh lesioned animals operant responses were made during the first feeding session after lesioning had taken place. This phenomenon was observed even in the animal which subsequently showed the highest degree of aphagia; fish XO37 made 46 responses during the first post-lesion session, and then no more until sacrifice at 12 days post-lesion. A final precaution was the use of hand feeding, at intervals of 3 or 4 days a few food pellets were dropped into the experimental animal's tanks. These pellets were dropped in such a way as to be fully visible to the animal, pellets delivered in this way were never seen to be eaten, and were subsequently siphoned from the experimental tanks.

RESULTS

The daily feeding data for the three groups of animals is shown in figures 33 to 35. Using this data it was possible to determine for each group, whether a significant difference existed between the amount of food eaten in the ten days prior to lesioning and the amount eaten in the ten days after lesioning. The figures used for this analysis are shown in table 9. A Mann-Whitney U-test (one-tailed) was used for this purpose. A one-tailed test is appropriate since the change in food intake was anticipated to be in the direction of a deficit. This was born out on initial investigation of the results which showed that in every case animals ate less food in the 10 days post-lesion period than in the 10 days pre-lesion.

In the first test the fish in group 1 were ranked alongside the fish in group 2. The null hypothesis (H_0) states that there is no difference in deficit between these two groups. Using the data shown in table 9, the probability under H_0 is found to be greater than 0.583. The conclusion is that the data do not give evidence which justify rejecting H_0. In other words, the combined effects of the surgery and the hindbrain stimulation required to locate the NPTh, do not produce a significantly greater deficit than the procedure required to place the nonspecific lesions. It is thus possible to put the animals in groups 1 and 2 together as a single control group, this new control group together with the animals in group 3 were analysed again using a Mann-Whitney U-test. The results from this analyses show that in this case it is possible to reject the null hypothesis at a level of significance of better than 0.001 for a one-

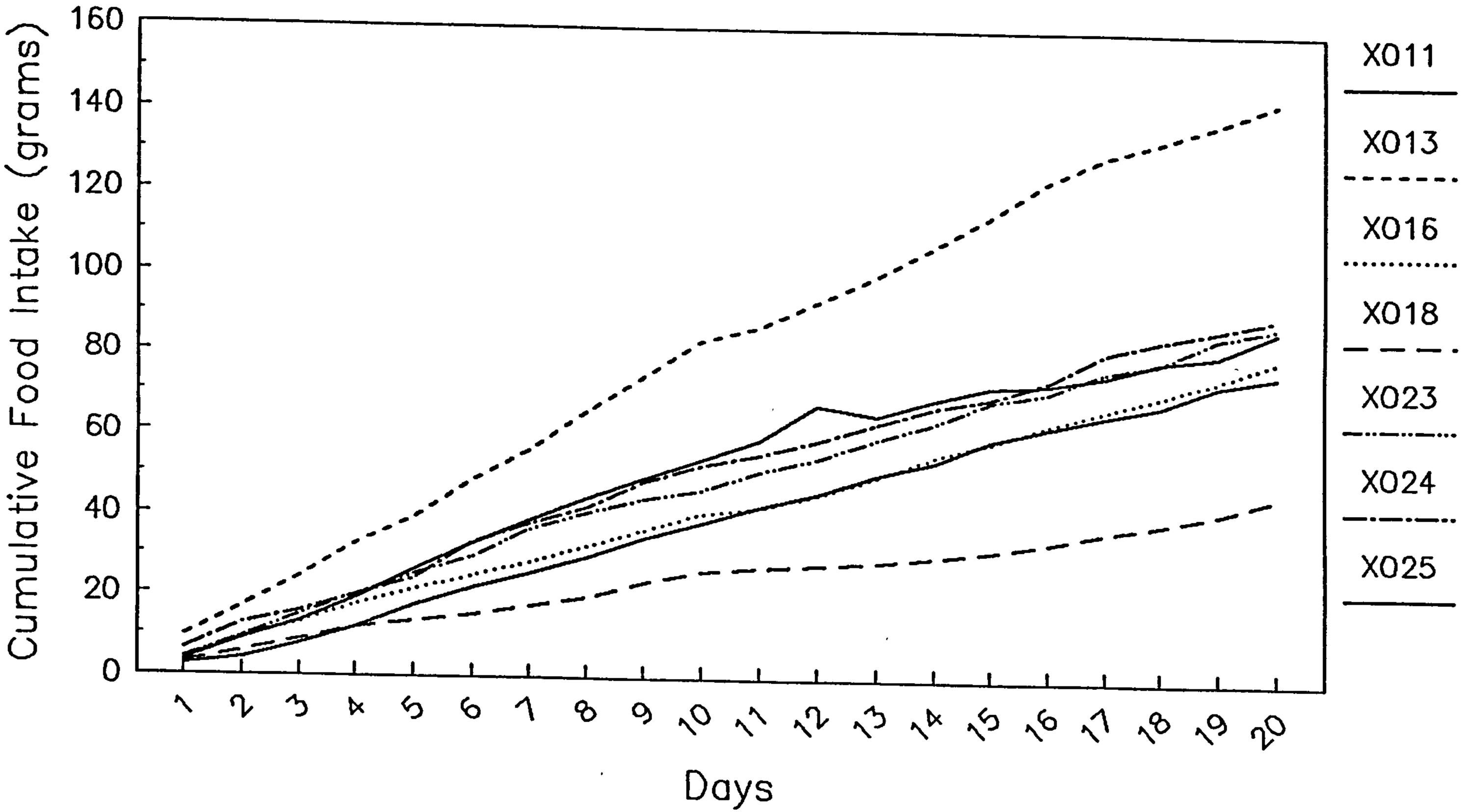
Table 9

	Fish number	Pre-lesion intake 10 day total grams	post-lesion intake 10 day total grams	deficit grams	deficit %
	XO11	38.53	37.99	0.54	1.4
	XO13	83.54	59.99	23.55	28.2
	XO16	40.72	39.63	1.09	2.6
GROUP 1	XO18	26.34	20.06	6.28	23.8
	XO23	46.65	41.97	4.68	10
	XO24	52.57	38.09	14.48	27.5
	XO25	54.05	33.54	20.51	37.9
	XO30	43.7	21.48	22.22	50.8
GROUP 2	XO31	30.1	25.11	4.99	16.6
	XO32	36.01	18.85	17.16	47.6
	XO29	35.78	13.95	21.83	61
	XO35	52.78	0.33	52.45	99.4
GROUP 3	XO36	39.43	2.22	37.21	94.4
	XO37	45.35	1.6	43.75	96.5
	XO38	38.53	20.8	17.73	46
	XO50	25.32	8.53	16.79	66.3

Figure 33.

Shows the cumulative daily food intake for the animals in group 1 (first control group).

Group 1

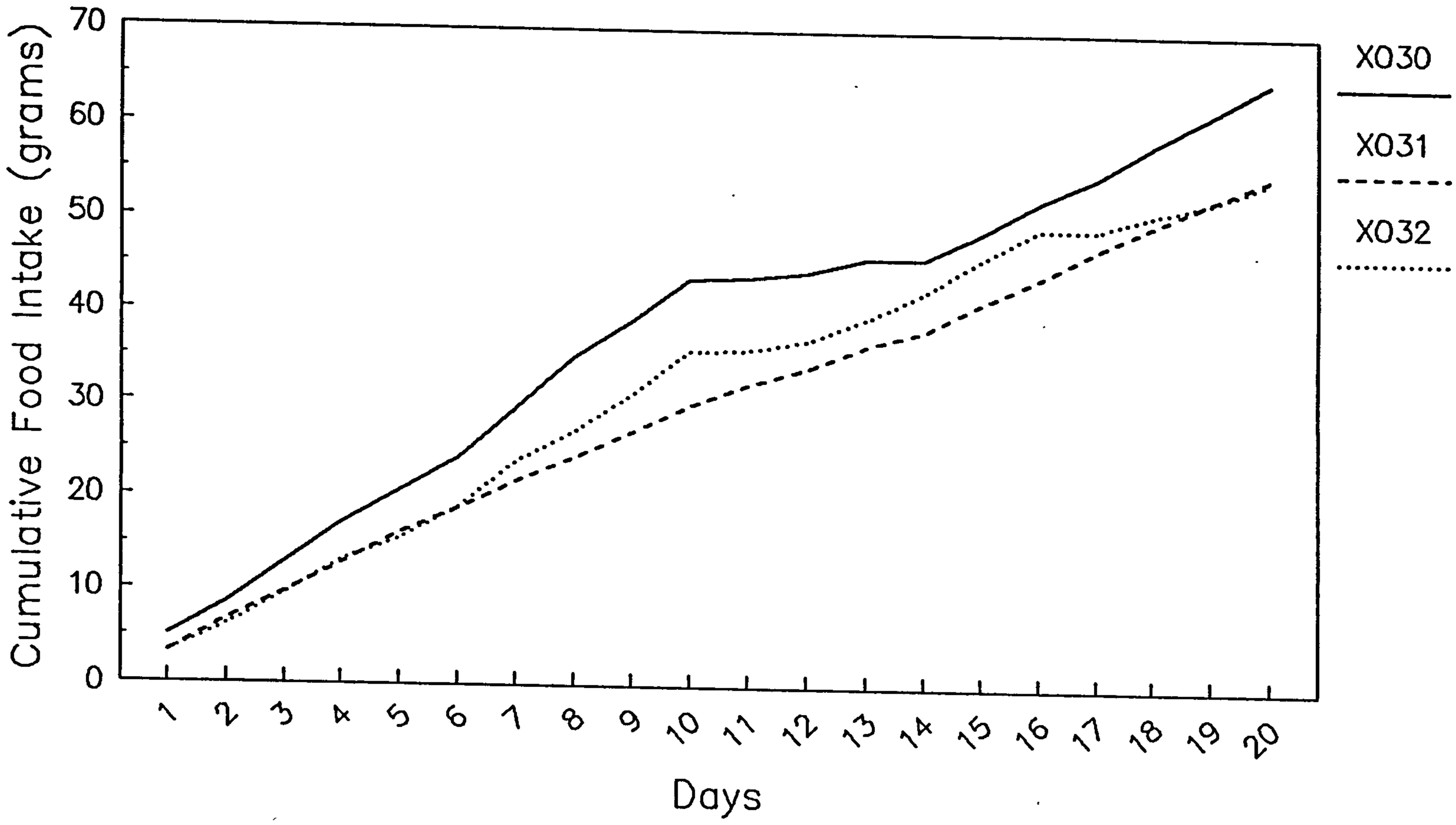


All animals lesioned on day 10

Figure 34.

Shows the cumulative daily food intake for the animals in group 2 (second control group).

Group 2

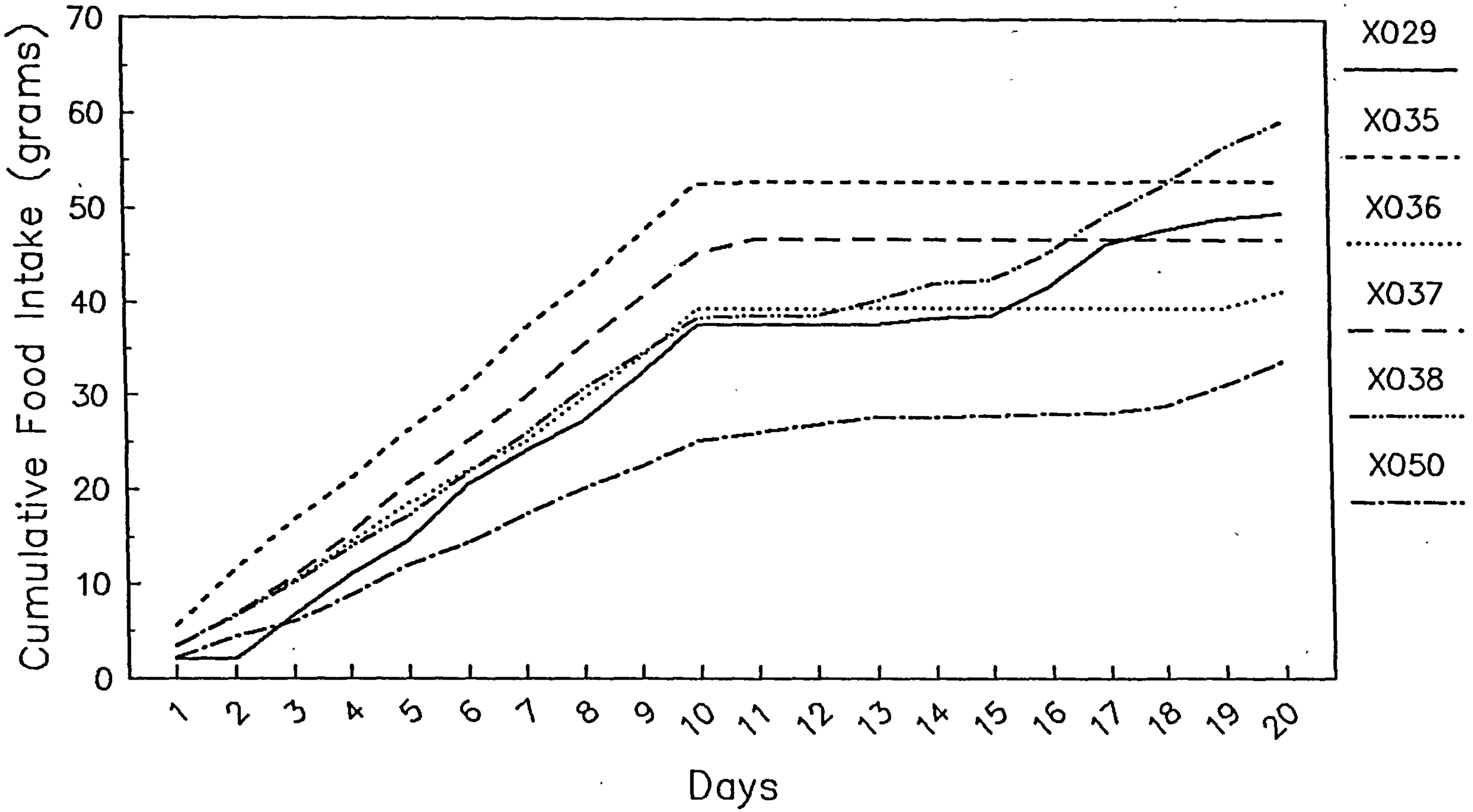


All animals lesioned on day 10

Figure 35.

Shows the cumulative daily food intake for the animals in group 3 (experimental group).

Group 3



All animals lesioned on day 10

tailed test. Thus, lesions placed in the NPTh caused a highly significant deficit in food intake in animals feeding under the operant regime when compared with animals without NPTh lesions feeding under the same regime.

Having established a relationship between NPTh damage and a deficit in food intake, it was necessary to investigate the possibility of a correlation between the amount of damage and the degree of deficit. With regard to the degree of deficit, table 9 shows a percentage deficit figure expressing the difference in food intake between the pre- and post-lesion periods as a percentage of the amount eaten in the pre-lesion period. The degree of NPTh damage was assessed using the technique described in the materials and methods section. The results for NPTh damage are shown in table 10. Table 11 shows the animals from group 3 ranked in descending order of post-lesion food intake deficit compared with the rank order for degree of NPTh damage. Clearly no correlation between degree of damage and degree of deficit may be established.

In addition to the lack of correlation between degree of damage and food intake deficit, it is equally difficult to relate the observed deficits to damage in a particular area of the NPTh. Figures 36 through 43 show transverse sections of the NPTh and the lesions from all the animals in group 3. In addition figure 44 shows the antero-posterior distribution of lesions in all animals in group 3. It is clear from inspection of these figures that damage in many different regions of the NPTh may cause a deficit in food intake.

Figure 36.

Shows the outlines of the lesion (filled area) and the nucleus posterior thalamicus in transverse section for animal X035 left hand side. Sections are shown sequentially anterior to posterior, reading from left to right and top to bottom. Dorsal aspect to top of page, medial aspect to left of page. Scale bar 100 µm.

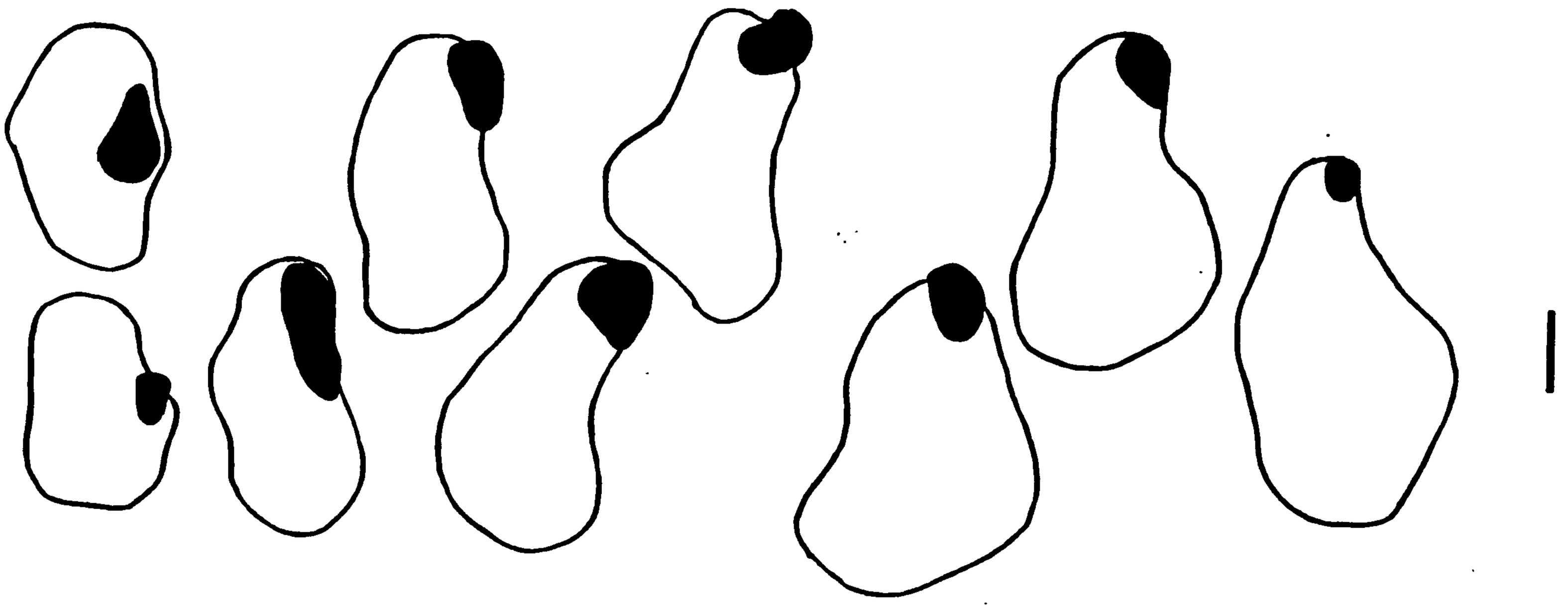


Figure 37.

Shows the outlines of the lesion (filled area) and the nucleus posterior thalamicus in transverse section for animal X037 left hand side. Sections are shown sequentially anterior to posterior, reading from left to right and top to bottom. Dorsal aspect to top of page, medial aspect to left of page. Scale bar 100 µm.

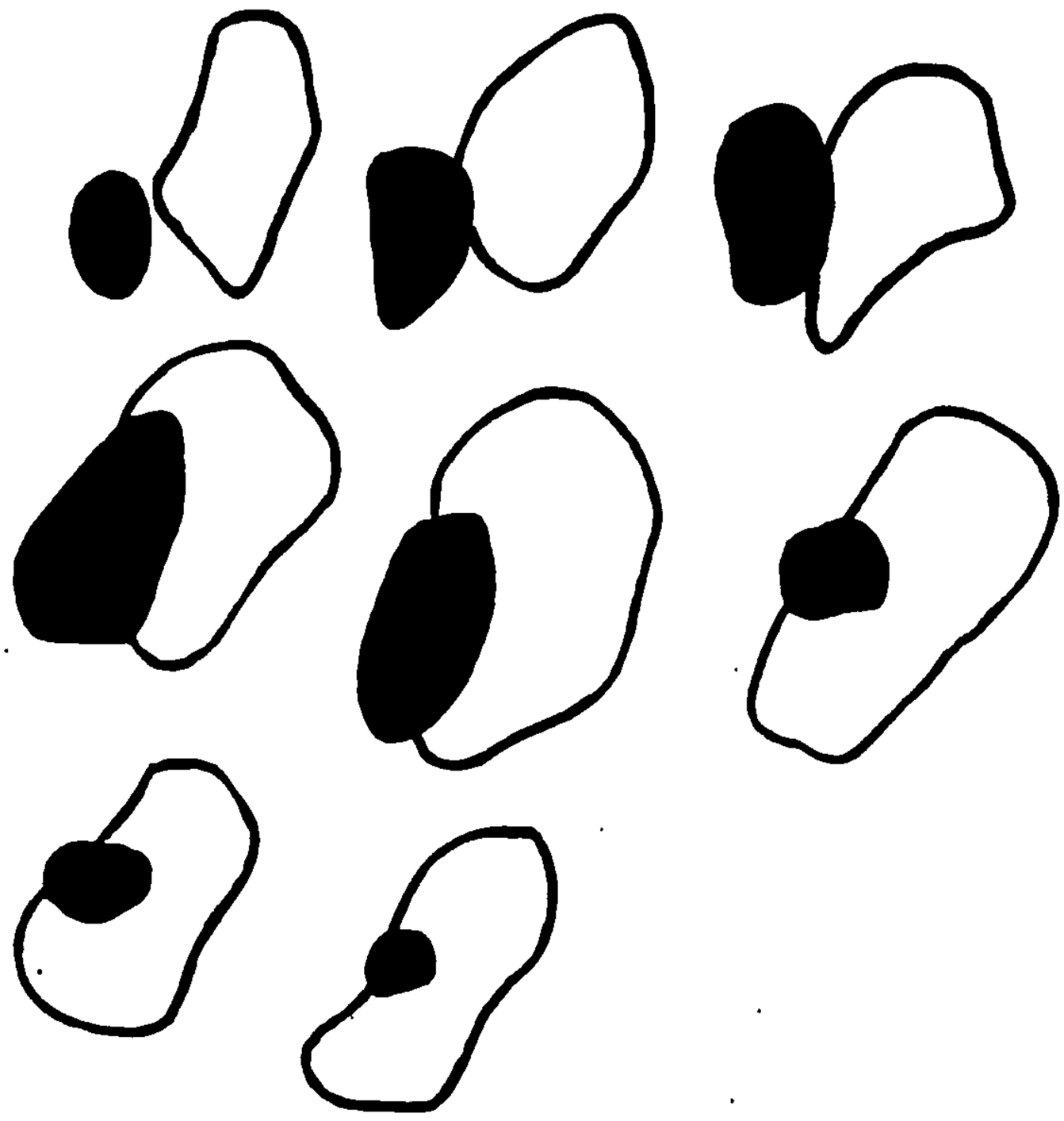


Figure 38.

Shows the outlines of the lesion (filled area) and the nucleus posterior thalamicus in transverse section for animal X036 left hand side. Sections are shown sequentially anterior to posterior, reading from left to right and top to bottom. Dorsal aspect to top of page, medial aspect to left of page. Scale bar 100 µm.

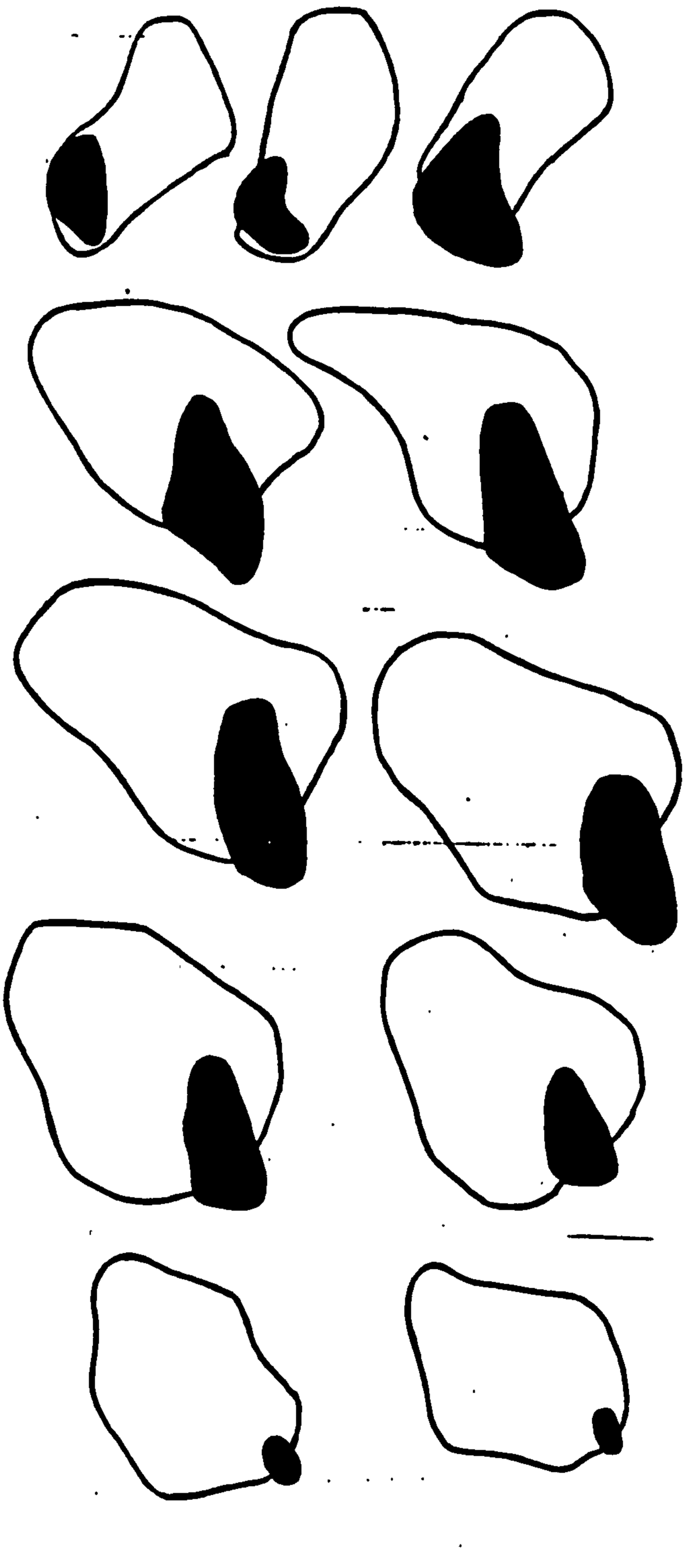


Figure 39.

Shows the outlines of the lesion (filled area) and the nucleus posterior thalamicus in transverse section for animal X036 right hand side. Sections are shown sequentially anterior to posterior, reading from left to right and top to bottom. Dorsal aspect to top of page, medial aspect to right of page. Scale bar 100 µm.

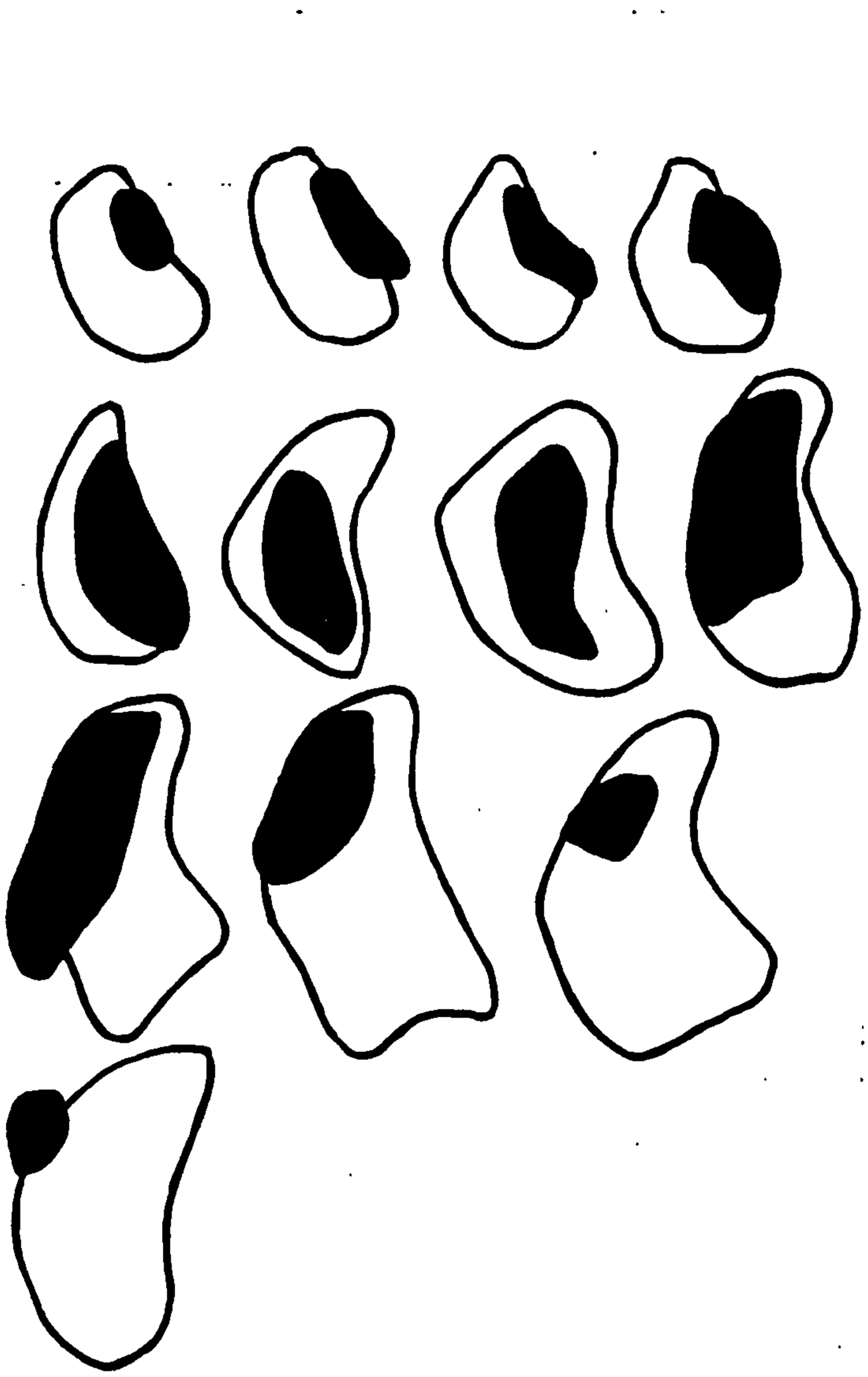


Figure 40.

Shows the outlines of the lesion (filled area) and the nucleus posterior thalamicus in transverse section for animal XO50 left hand side. Sections are shown sequentially anterior to posterior, reading from left to right and top to bottom. Dorsal aspect to top of page, medial aspect to left of page. Scale bar 100 µm.

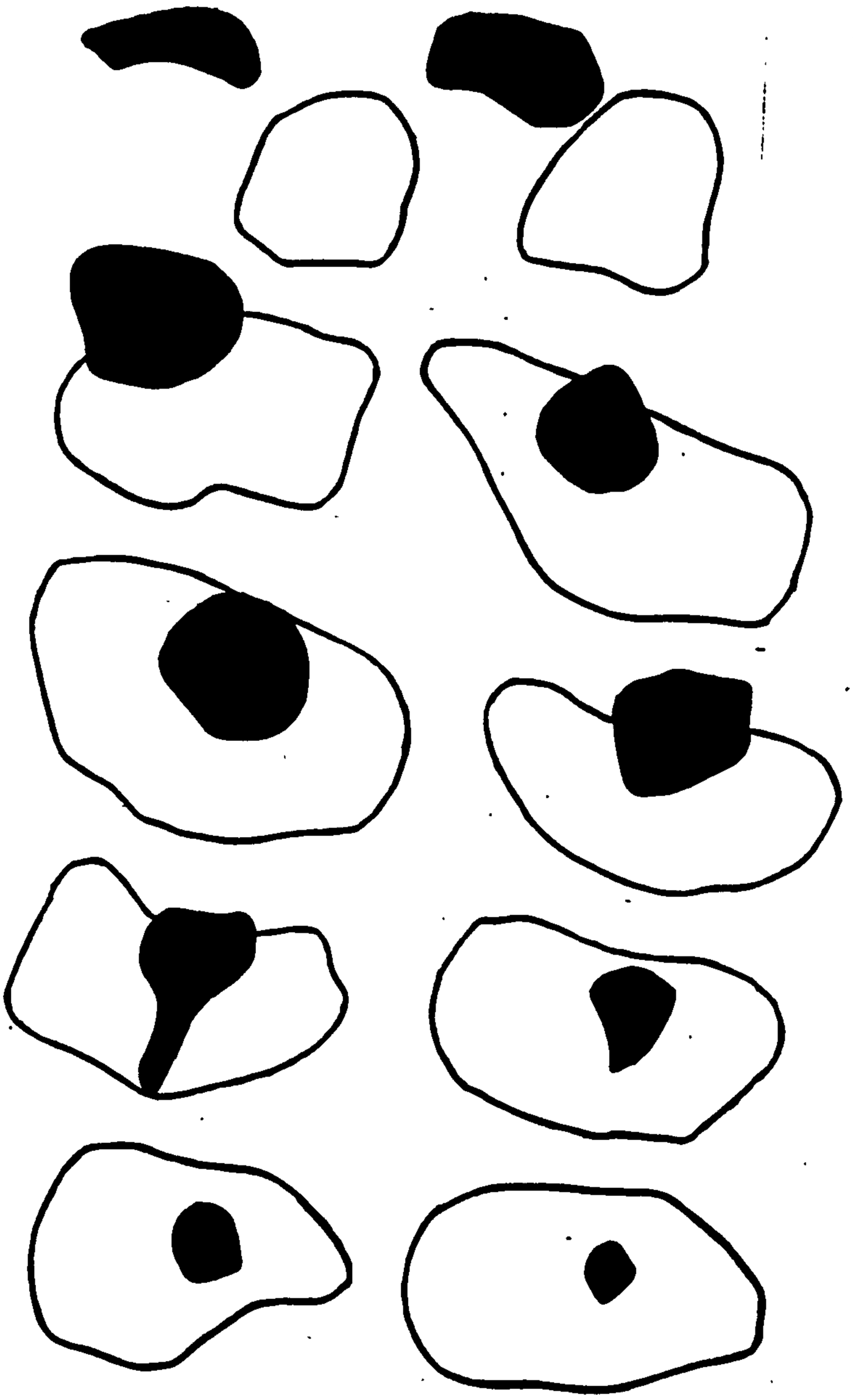


Figure 41.

Shows the outlines of the lesion (filled area) and the nucleus posterior thalamicus in transverse section for animal XO29 left hand side. Sections are shown sequentially anterior to posterior, reading from left to right and top to bottom. Dorsal aspect to top of page, medial aspect to left of page. Scale bar 100 µm.

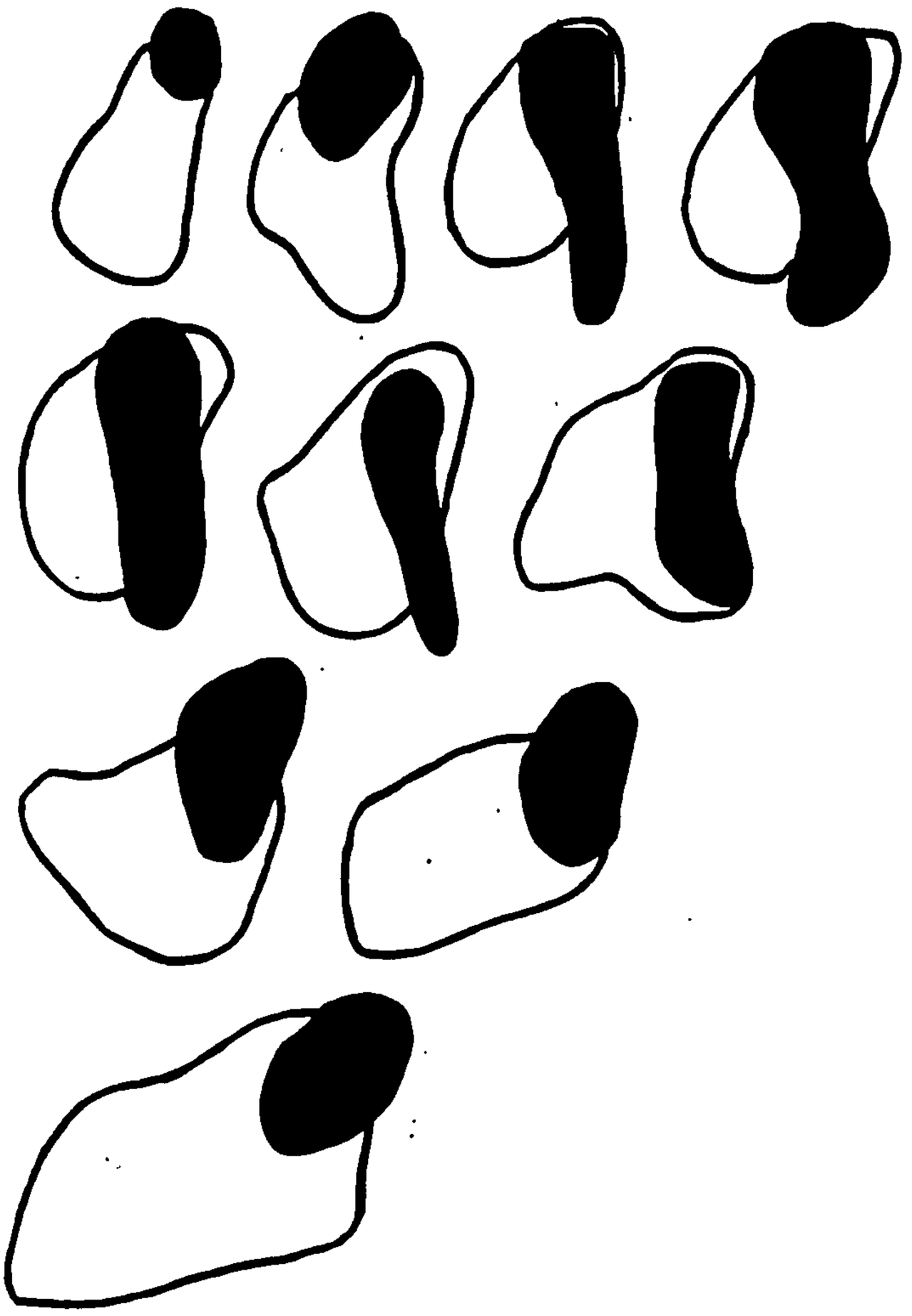


Figure 42.

Shows the outlines of the lesion (filled area) and the nucleus posterior thalamicus in transverse section for animal X038 left hand side. Sections are shown sequentially anterior to posterior, reading from left to right and top to bottom. Dorsal aspect to top of page, medial aspect to left of page. Scale bar 100 μm.

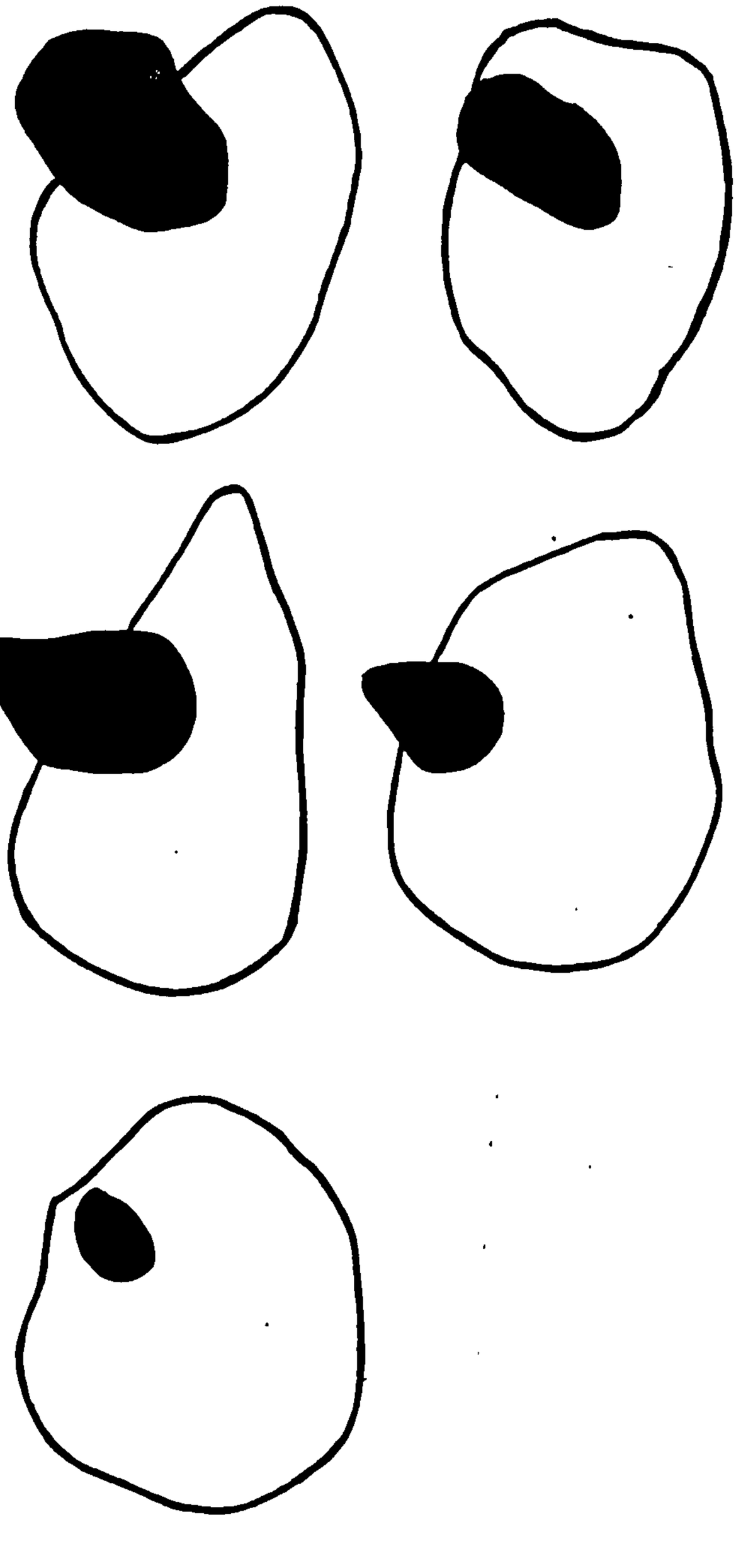


Figure 43.

Shows the outlines of the lesion (filled area) and the nucleus posterior thalamicus in transverse section for animal XO38 right hand side. Sections are shown sequentially anterior to posterior, reading from left to right and top to bottom. Dorsal aspect to top of page, medial aspect to right of page. Scale bar 100 µm.

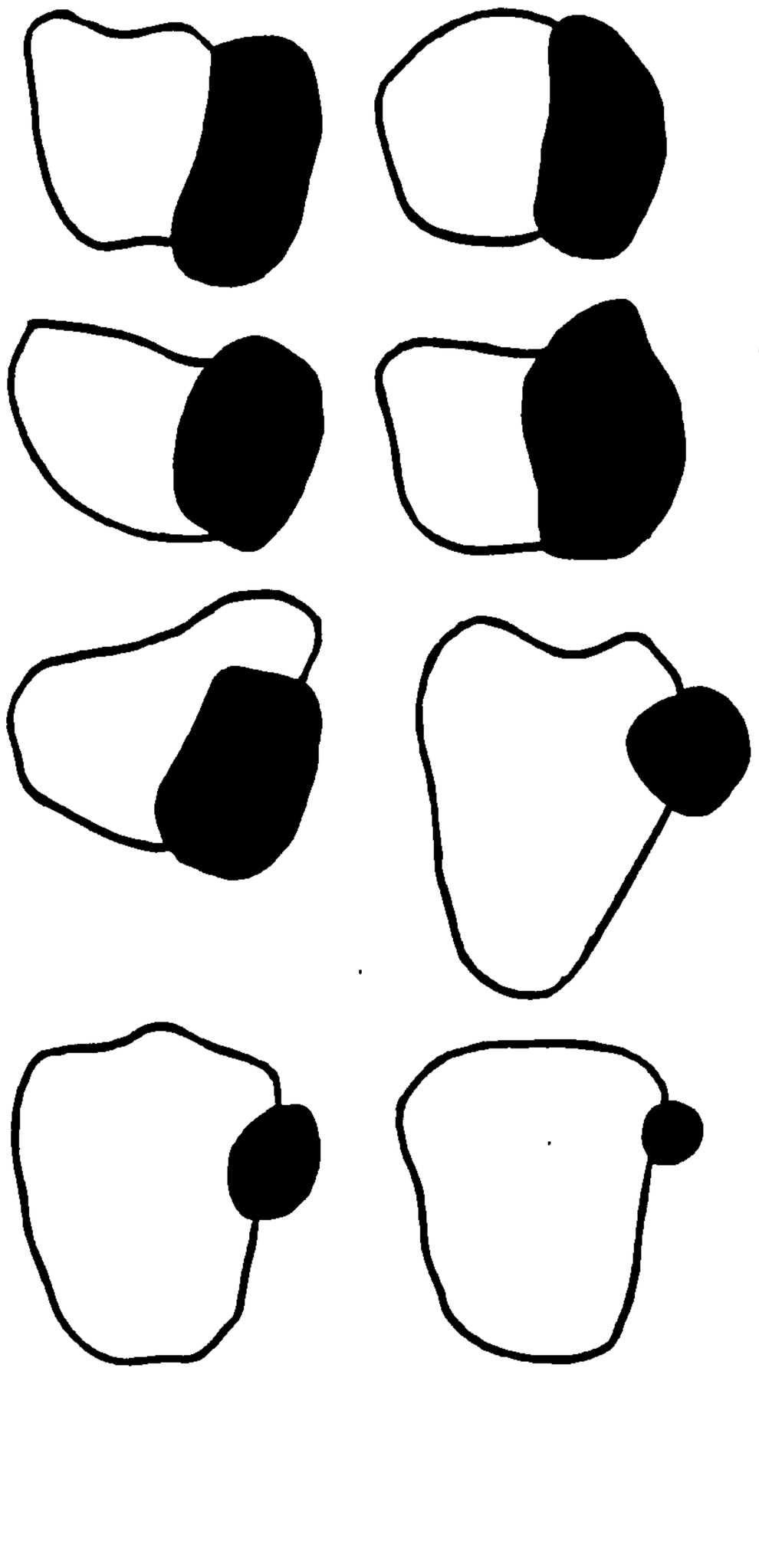
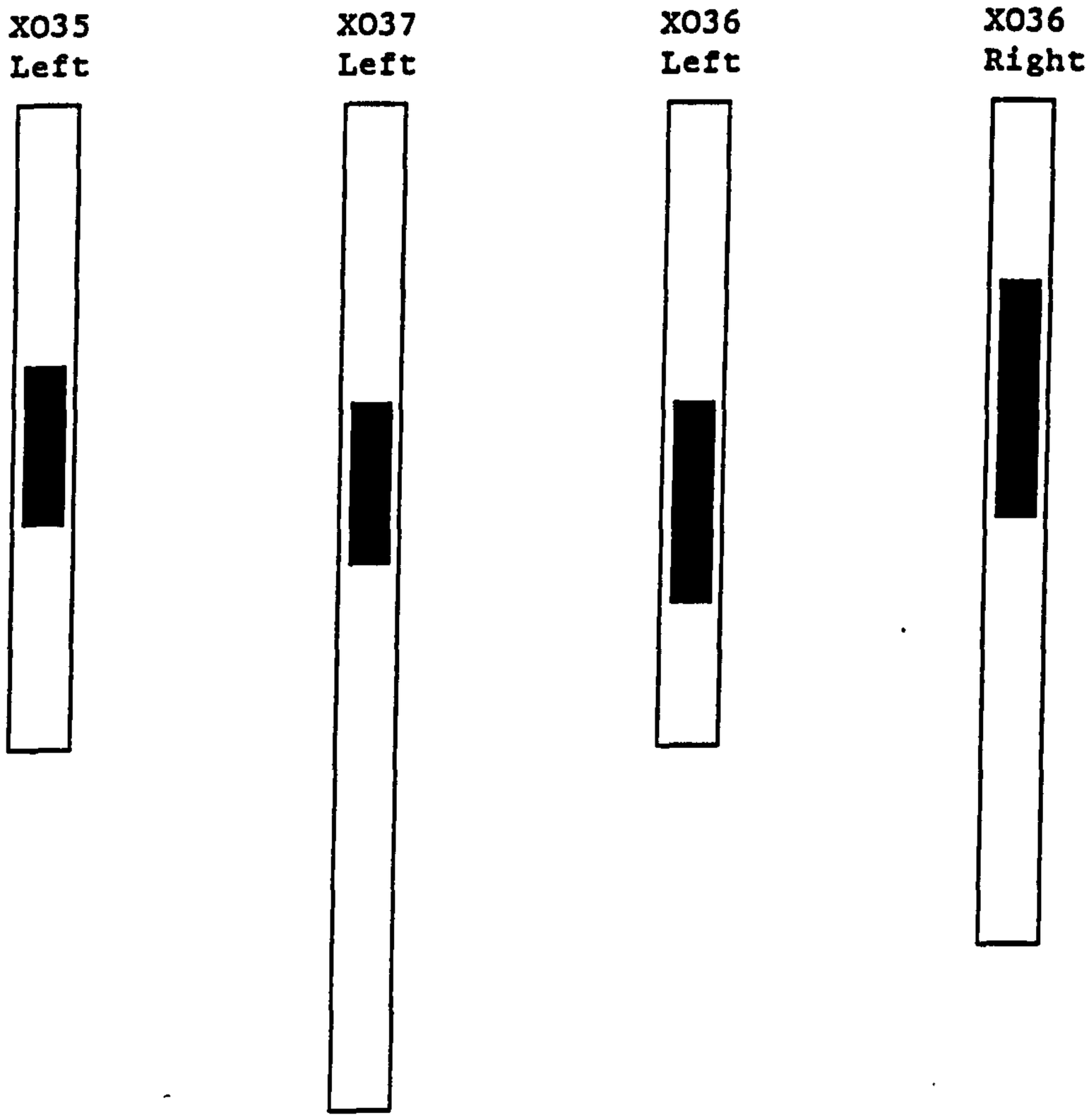


Figure 44.

Diagrammatic representation of the antero-posterior distribution of the lesions for animals in group 3. The open rectangle represents the antero-posterior length of the nucleus posterior thalamicus, and the filled rectangle the length of the lesioned area. This figure does not show dorso-ventral or medio-lateral positions of the lesioned area.

POSTERIOR



ANTERIOR

Table 10

Fish Number	Left side NPTh Vol. mm^3 ($x10^{-2}$)	Left side Damage Vol. mm^3 ($x10^{-3}$)	Right side NPTh Vol. mm^3 ($x10^{-2}$)	Right side Damage Vol. mm^3 ($x10^{-3}$)	Bilateral Damage %
XO29	1.27	1.13		0	4.45
XO35	2.39	0.735		0	1.53
XO36	1.53	1.25	1.58	1.38	8.45
XO37	1.66	0.253		0	0.76
XO38	1.43	0.729	1.38	0.752	5.27
XO50	1.76	1.24		0	3.49

Table 11

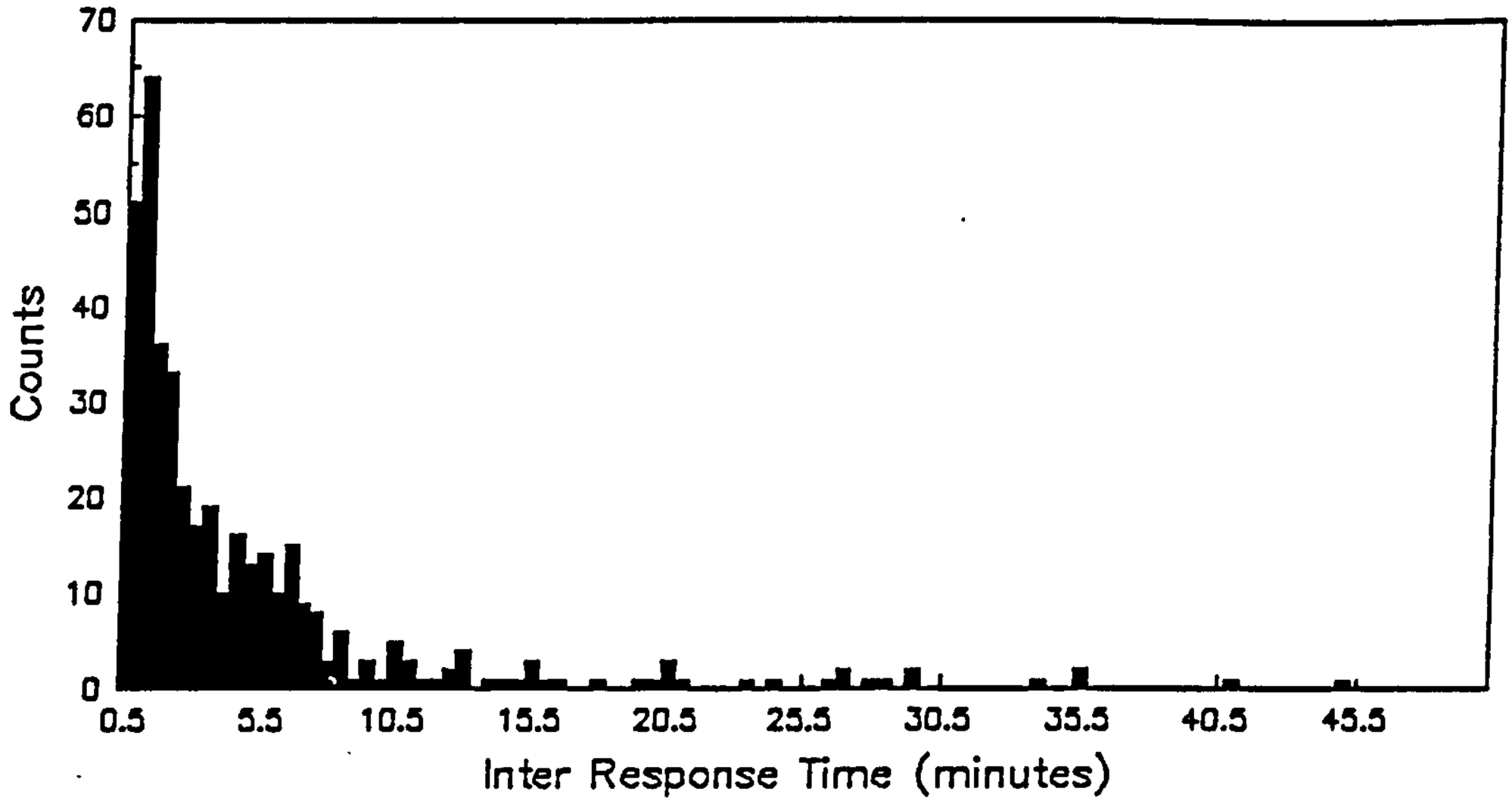
Rank Order of food intake deficit	Rank order of NPTh damage	Fish No.
1	5	XO35
2	6	XO37
3	1	XO36
4	4	XO50
5	3	XO29
6	2	XO38

Of the NPTh lesioned animals 5 of the 7 resumed feeding prior to sacrifice, feeding data was collected from these animals, this allowed an analysis of whether NPTh damage had altered the 'normal' feeding patterns described in section I. The data from lesioned animals was analysed using the log survivor technique as described in section I, and these results are presented in figures 45 to 49. The data was also plotted as a feeding rate distribution see figure 50. It was possible to compare the results from these sets of data with those obtained from the operant experiments described in section I. In the case of the log-survivor analysis, with the possible exception of animal XO36, the results do not show the presence of bout feeding. This result is consistent with the results seen in section I, and shows that bout feeding does not `emerge` once post-lesion feeding resumes.

Figure 45.

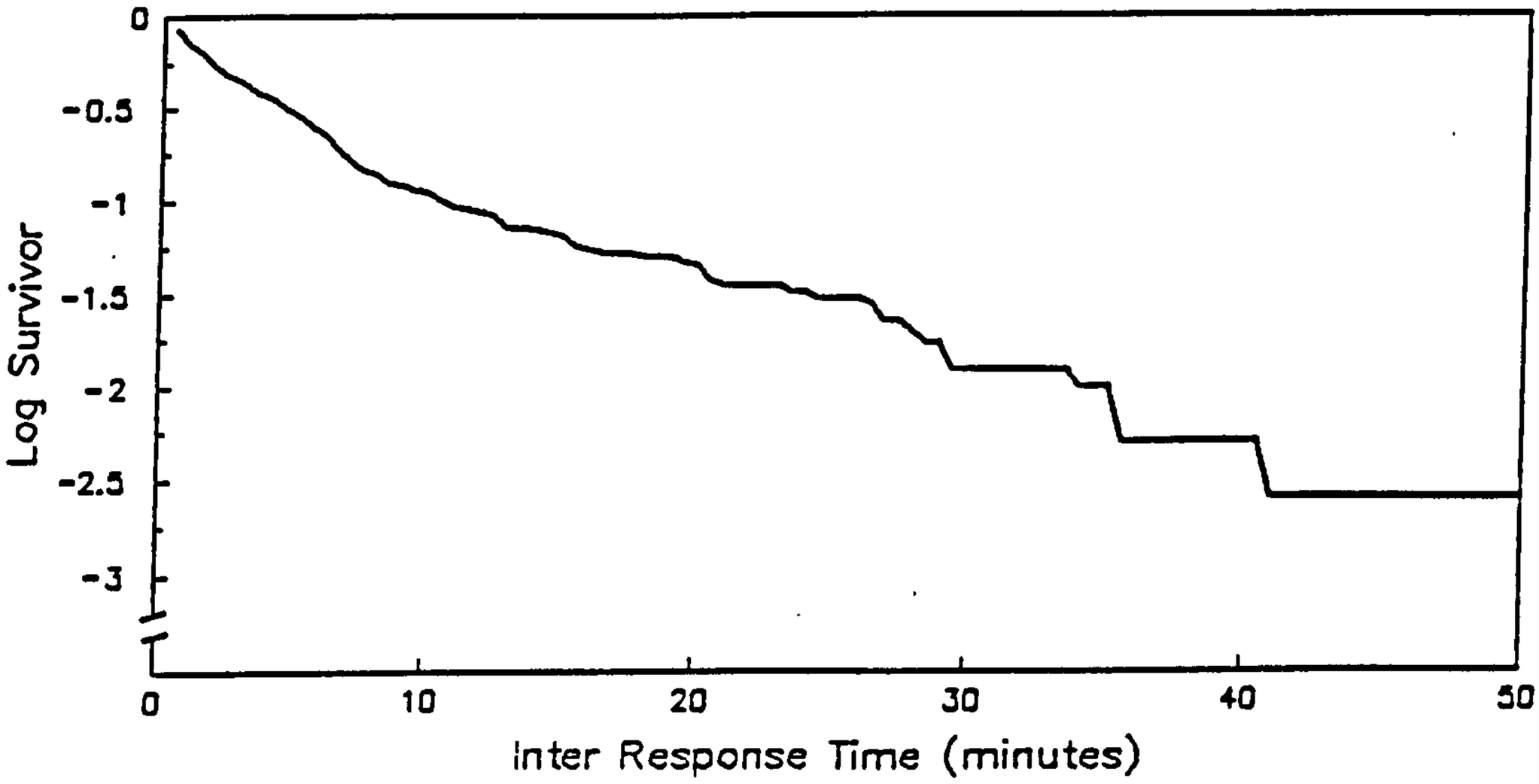
Interval histogram (top) and log survivor function (bottom) for animal XO29. The data presented here is pooled from 8 days following recovery of feeding after hypothalamic lesions.

X029 Post Lesion Feeding Interval Histogram



Pooled data for 8 days

X029 Post Lesion Log Survivor Analysis

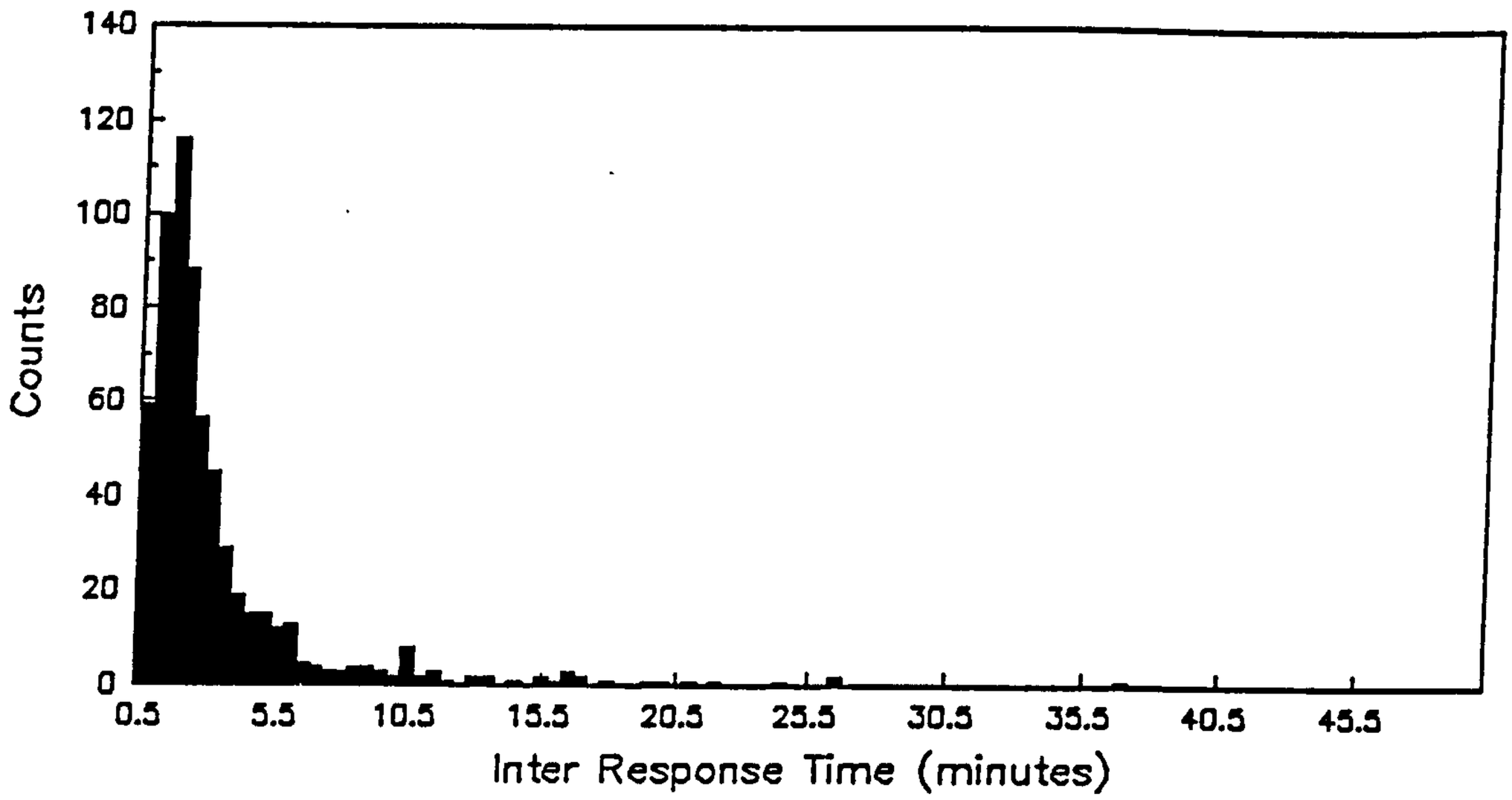


Log survivor function generated from the interval histogram shown above

Figure 46.

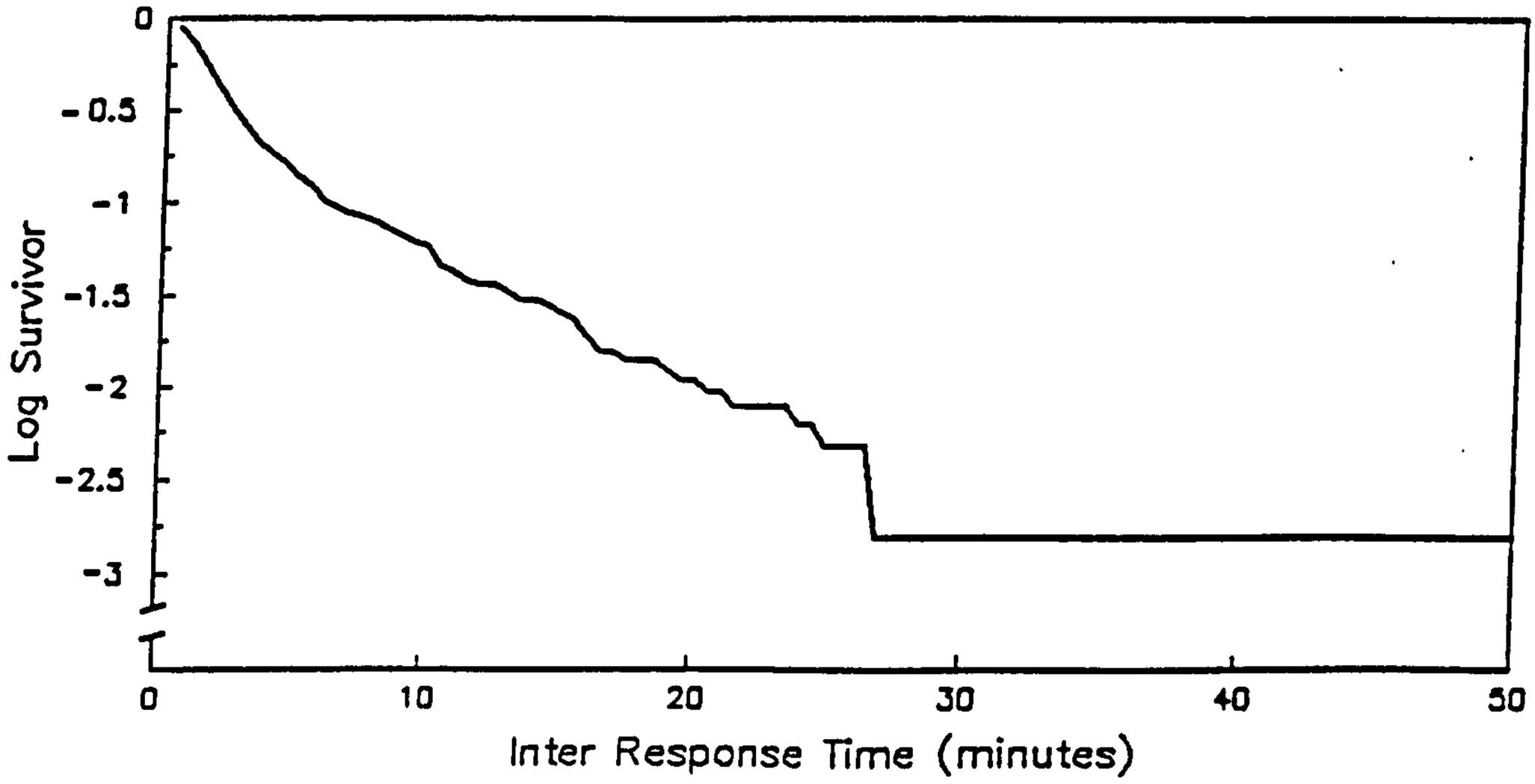
Interval histogram (top) and log survivor function (bottom) for animal XO35. The data presented here is pooled from 5 days following recovery of feeding after hypothalamic lesions.

X035 Post Lesion Feeding Interval Histogram



Pooled data for 5 days

X035 Post Lesion Log Survivor Analysis

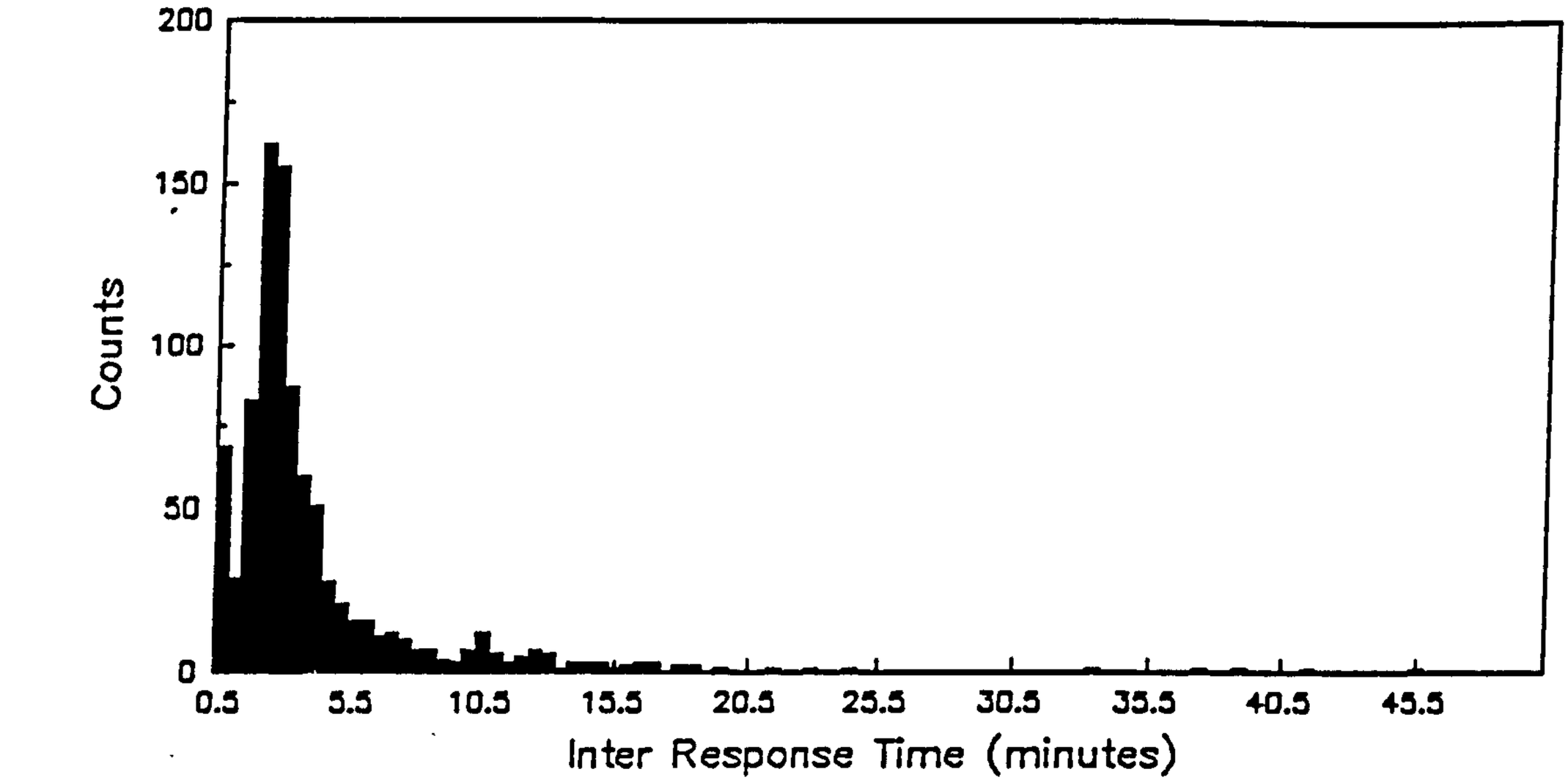


Log survivor function generated from the interval histogram shown above

Figure 47.

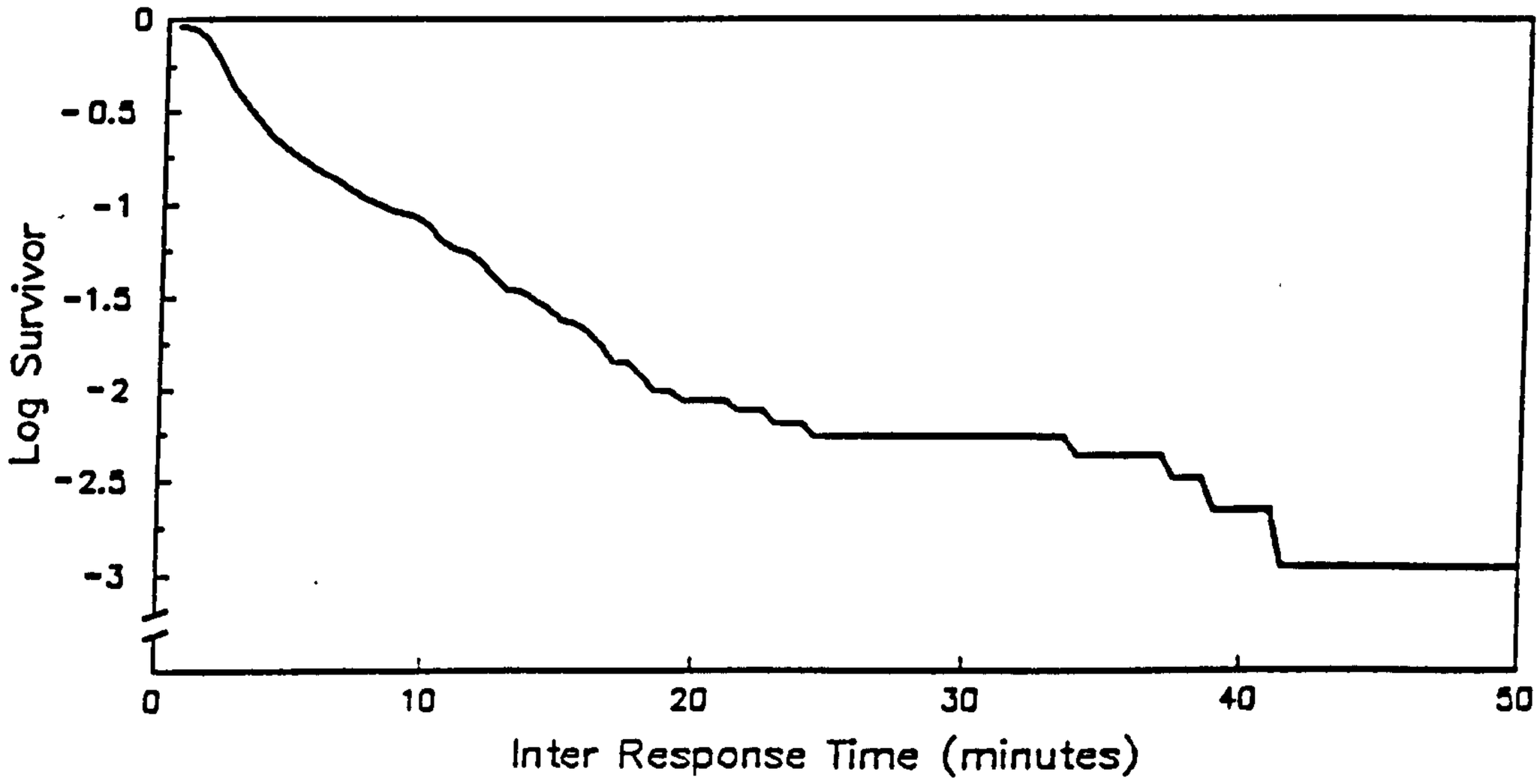
Interval histogram (top) and log survivor function (bottom) for animal X036. The data presented here is pooled from 9 days following recovery of feeding after hypothalamic lesions.

X036 Post Lesion Feeding Interval Histogram



Pooled data for 9 days

X036 Post lesion Log Survivor Analysis

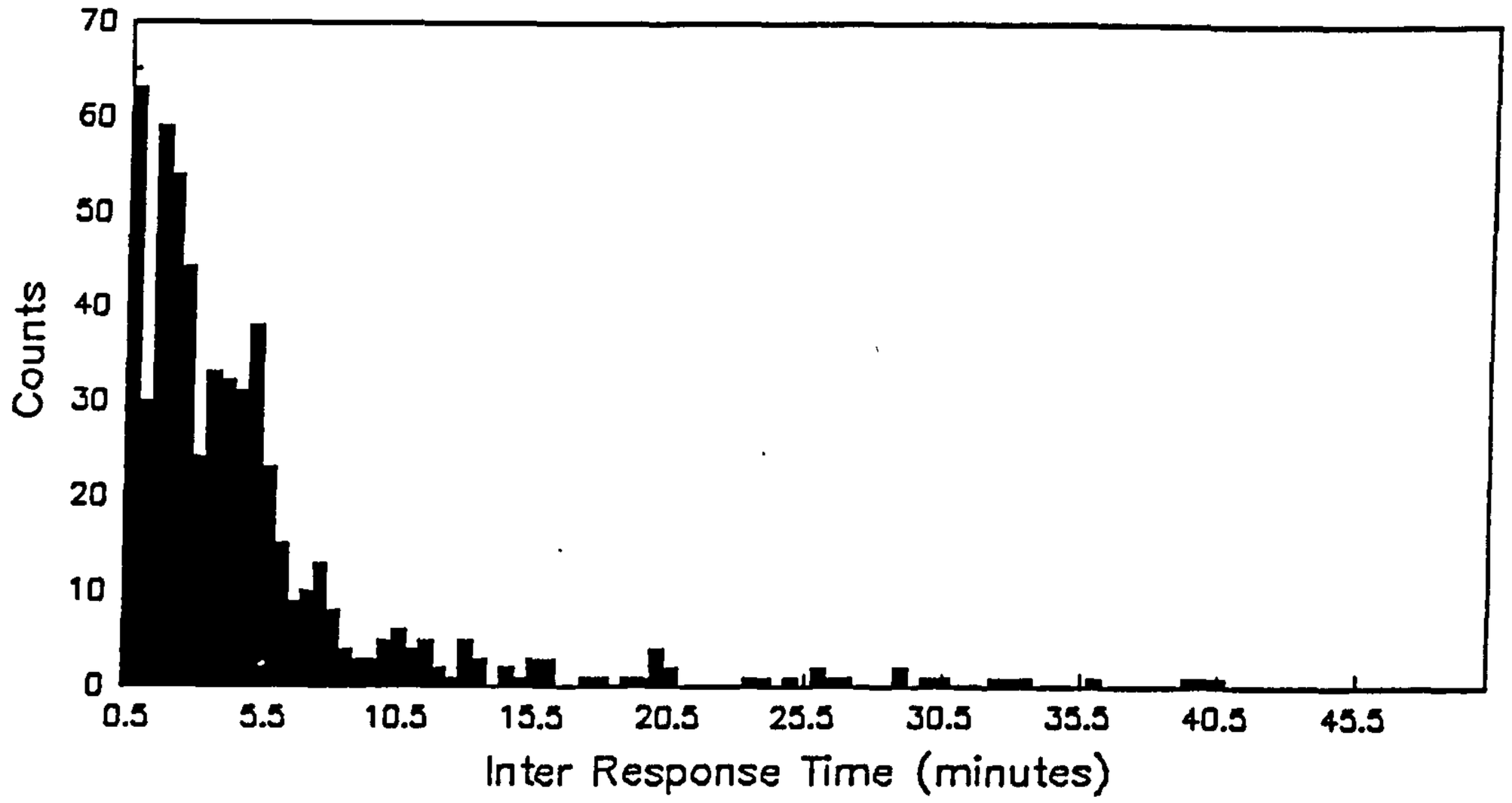


Log survivor function generated from the interval histogram shown above

Figure 48.

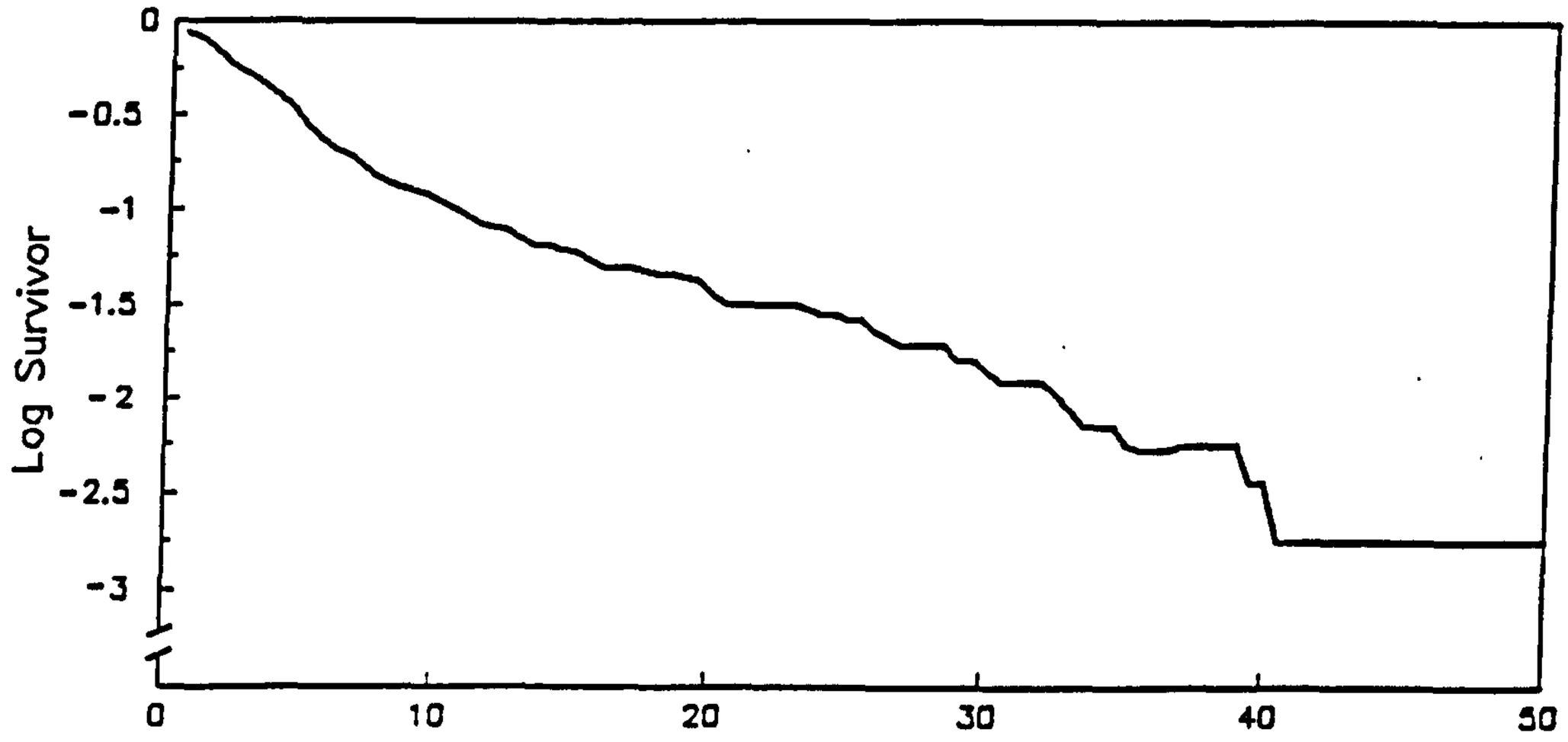
Interval histogram (top) and log survivor function (bottom) for animal XO38. The data presented here is pooled from 8 days following recovery of feeding after hypothalamic lesions.

X038 Post Lesion Feeding Interval Histogram



Pooled data for 8 days

X038 Post Lesion Log Survivor Analysis

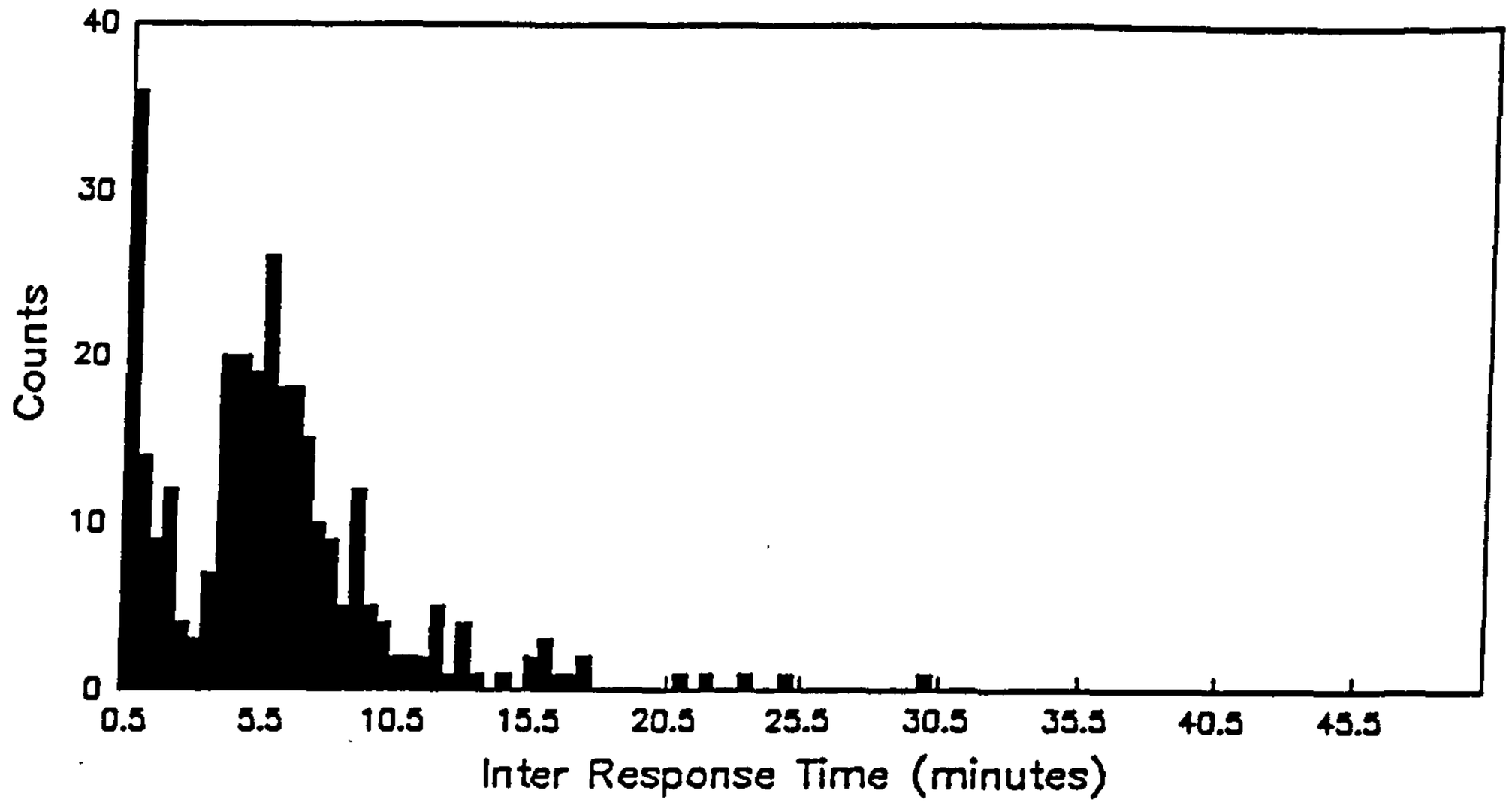


Log survivor function generated from the interval histogram shown above

Figure 49.

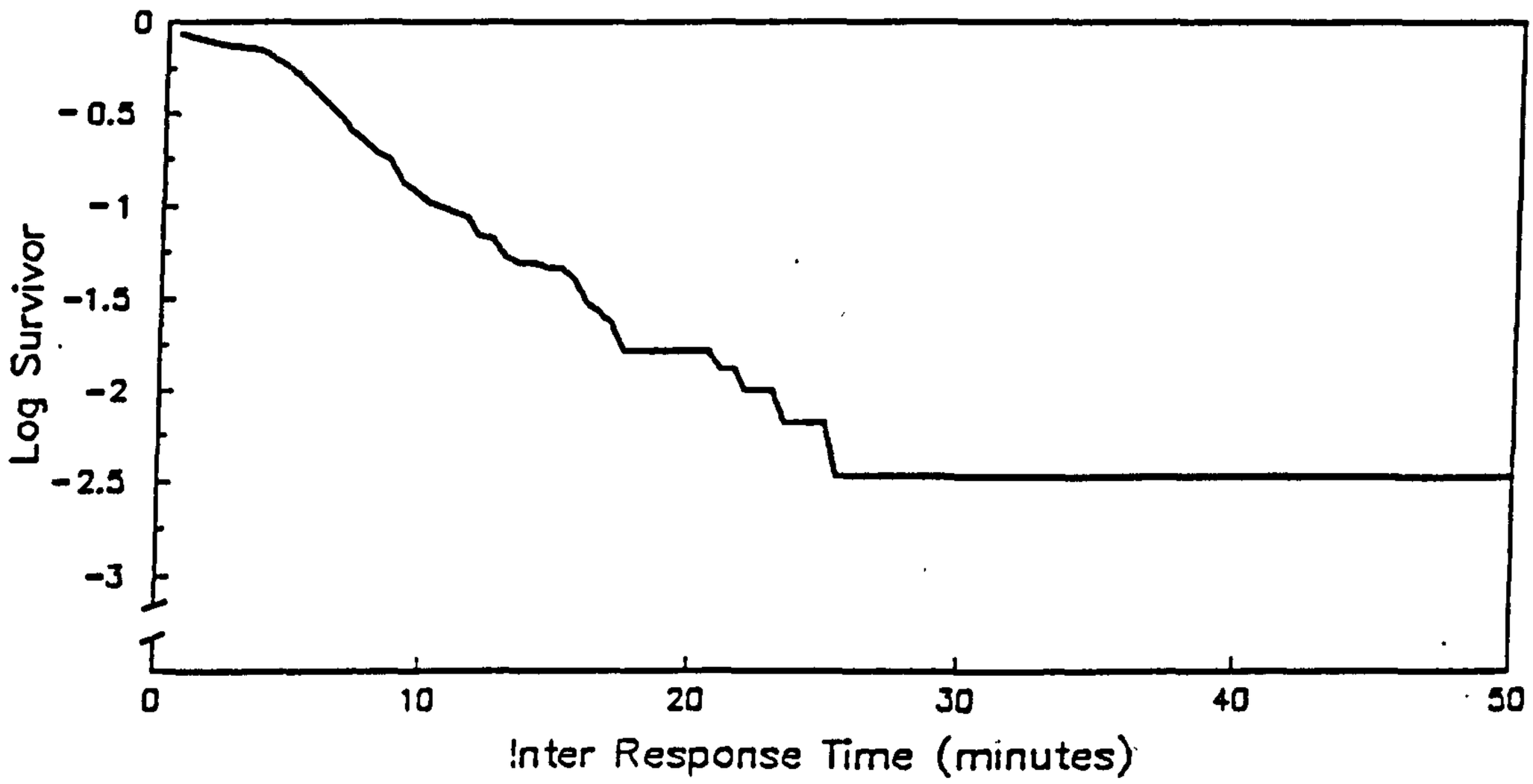
Interval histogram (top) and log survivor function (bottom) for animal XO50. The data presented here is pooled from 4 days following recovery of feeding after hypothalamic lesions.

X050 Post Lesion Feeding Interval Histogram



Pooled data for 4 days

X050 Post Lesion Log Survivor Analysis

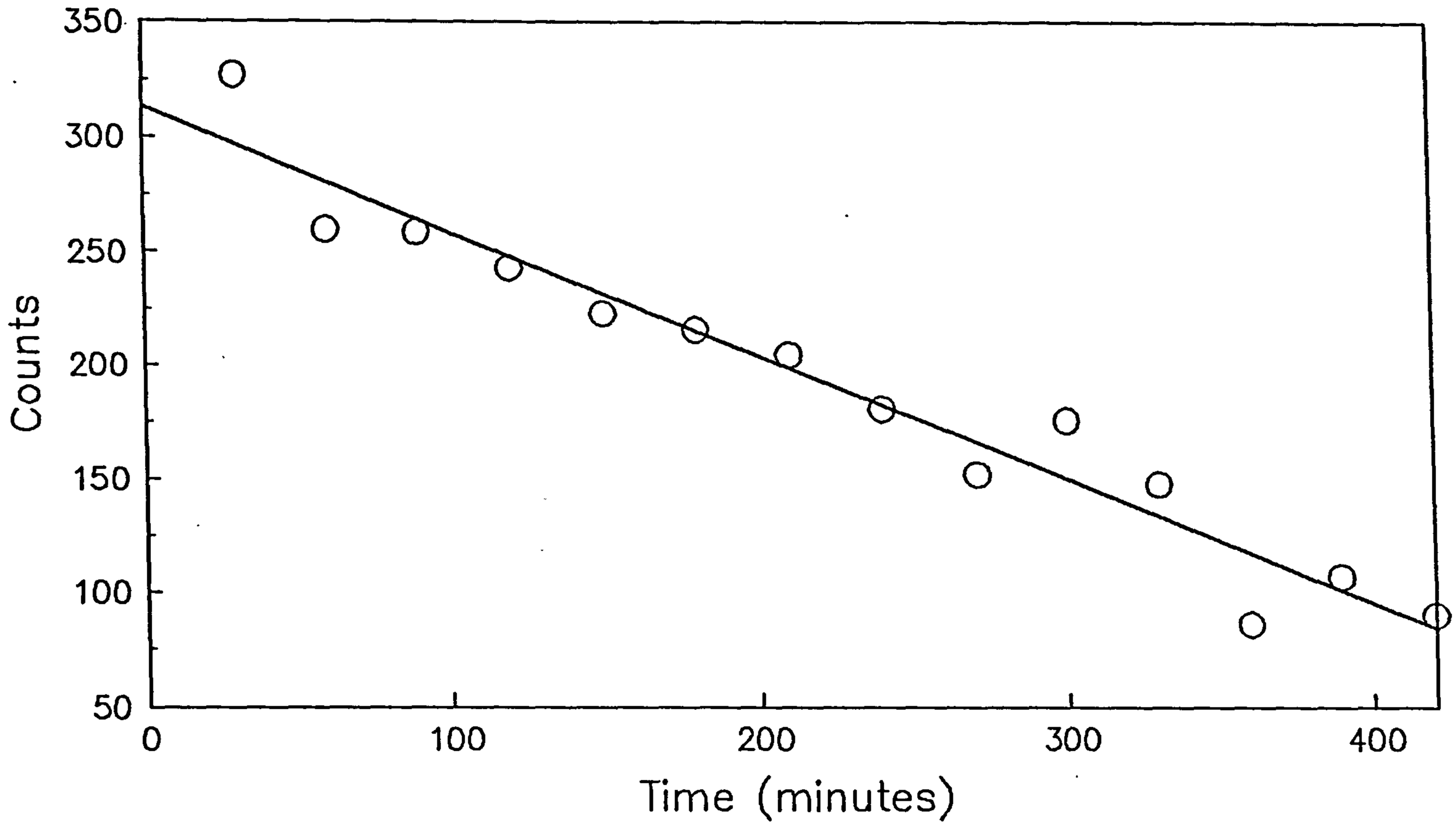


Log survivor function generated from the interval histogram shown above

Figure 50.

Distribution of feeding events in each half-hourly interval fitted with a linear function. Data presented here is summed from all animals used for the log survivor analysis.

All Fish Post-Lesion Feeding
Real-Time Distribution



Data summed from 5 animals

In the case of feeding rate with respect to time, the data from the lesioned animals (group 1) was best fitted by a linear function (r^2=0.95), an exponential function was less significant in it's goodness of fit (r^2=0.90), as was a logarithmic function (r^2=0.89). This result may be compared to the result of similar analyses carried out on the combined results from the animals in groups 2 and 3 (the control groups), the data from these animals was best fitted by a logarithmic function (r^2=0.94), furthermore the post-lesion feeding exhibited by these animals was markedly non-linear (for a linear function r^2=0.75). This result demonstrates that the animals which did not receive NPTh lesions showed a pattern of food intake over time very similar to that seen in the experiments described in section I of this thesis. Data presented in section I showed that feeding rate with respect to time was best described by an exponential or logarithmic function (r^2=0.97 in both cases). It appears that the effect of damage to the NPTh was to cause a shift from a logarithmically or exponentially declining rate of intake to a linearly declining rate of intake, the microstructure of food intake remained the same, that is to say the emergence of bout feeding was not observed. Average feeding rate measured in grams hour^{-1} was also obtained for the NPTh lesioned animals and the results may be seen in table 12. Although no good statistical test was available due to the low number of samples, the feeding rates for the pre-lesion period did appear to be slightly higher than during the post-lesion period. The difference in rates is almost certainly due to the fact that as feeding behaviour was resumed in the NPTh-lesioned animals, the amount of food eaten during the first few days of feeding recovery was lower than on pre-lesion days. The inclusion of data

from the first few days of feeding recovery has biased the feeding rate results towards a slightly lower level.

Table 12

Fish Number	Pre-lesion feeding rate g hr^{-1}	Post-lesion feeding rate g hr^{-1}
XO29	0.59	0.30
XO35	0.76	0.62
XO36	0.57	0.54
XO38	0.49	0.37
XO50	0.39	0.39

DISCUSSION

The results show quite clearly that destruction of part of the NPTh on one or both sides of the brain can lead to a highly significant reduction in food intake over and above the reduction caused by the trauma of the surgical techniques employed. Interestingly it is not possible on the basis of these results to locate a particular region of the nucleus which appears to be important. Figures 36 to 43 show that many of the lesions leading to a reduction in food intake are located on the edge of the nucleus and do in fact encroach into the surrounding tissue on lateral, dorsal, medial and ventral aspects. A high proportion of the recovered lesions lie in the medial part of the NPTh, however there were no instances of damage to the nucleus preglomerulosus (NPG) detected in any of these cases. It is not possible to relate degree of food intake deficit to degree of damage to the NPTh, however an aspect of this type of `damaged area` analysis is the accurate determination of the boundary of the lesioned area. Accurate determination of lesion boundaries is particularly relevant in experiments where the lesions involved are very small, since a small error in determining the edge of the lesion may lead to a large over or under estimation of the size of the lesioned area. The lesions produced in this experiment were of the order of 100 μm in diameter, and are small when compared to the size of the NPTh. There are advantages in the use of small lesions, most notably that damage to surrounding areas and in particular to fibres of passage is potentially minimised. In addition, where a lesion is much smaller than the 'target' nucleus, the effects of damage to a particular part of the nucleus may possibly be investigated. The

results presented here, although demonstrating damage in discrete areas of the NPTh without collateral damage to other nuclei, do not provide evidence for differential effects of lesions placed in different areas of the NPTh. This finding may be interpreted to mean that the NPTh is not functionally subdivided, this conclusion tends to support the findings regarding cell size and morphology distributions presented in section III, namely that the nucleus is composed of a morphologically single population of cell bodies. The finding that NPTh lesions produce a significant deficit in food intake is entirely in keeping with those lesioning studies carried out in this area already, Roberts and Savage (1978) were able to conclude that lesions of the lateral lobe of the hypothalamus produced a long-lasting aphagia in the goldfish. The lesions utilised in the experiments described in this thesis were small and localised almost exclusively within the NPTh. The NPTh lies close to the NPG and the nucleus subpreglomerulosus (NSPG), and it is of some interest to note that experiments involving electrical stimulation of the hypothalamus cite the area of the NSPG in the goldfish (Savage and Roberts 1975), and the nucleus rotundus (a homologue of the NSPG) in the cichlid *Lepomis* (Demski and Knigge 1971) as being the areas most likely to cause feeding behaviour when electrically stimulated. It is quite possible that the NPTh (or its homologue) has been involved as an active site in the lesioning and stimulation studies quoted above. The use of a functional localisation technique as described here has allowed the identification of the NPTh in particular as an important part of the controlling system for food intake.

The analysis of the data from those animals which resumed feeding prior to sacrifice was important in that it allowed an insight into the possible role of the NPTh. The finding that there is no apparent change in the microstructuring of food intake as determined by a log survivor analysis suggests that the NPTh is not involved in the determination of the microstructure of food intake. If as is postulated in section I, the microstructure of food intake is determined by the morphology, rate of emptying and available volume of the gut, then it would appear that these variables are adequately integrated at levels lower than the NPTh and that this nucleus is not involved in the moment to moment regulation of food intake. Although it appears that the NPTh is not involved with the gastric consequences of food intake, it is quite possible that it is involved with the cephalic consequences of food intake. In 5 of the 6 animals which received NPTh lesions, operant responses were made, and food was consumed on the first feeding session after the lesions had been placed, followed subsequently by a period of aphagia or hypophagia (as compared to pre-lesion feeding data). This type of result raises the possibility that the food used in the experiment may have become aversive to the NPTh-lesioned animals. If it were the case that the result of NPTh damage was to produce a taste aversion, this might indicate that the normal function of the NPTh is to provide some indication as to the palatability of food items.

Perhaps the most striking result of the analysis of feeding data from the NPTh lesioned animals was the change from non-linear to linear in the shape of the food intake curve. The main feature of a non-linear curve is that the slope of the curve at any point is proportional to

the value on the y axis at that point. In the case of a curve which represents growth in satiety it is thus possible to say that the level of satiety produced by the ingestion of each pellet will depend on the amount of food already ingested, in other words pellets eaten at the start of a feeding period will produce a lower level of satiety than those eaten at the end. In the case of a satiety curve in the form of a straight line (the slope of which is the same at all points on the y axis) the level of satiety produced by the ingestion of each pellet is constant, that is to say the effect on satiety of pellets eaten at the end of a feeding period is the same as that at the beginning. These results imply that the NPTh may be involved in some way in determining both the perceived palatability and the effect on satiety of ingested food stuffs. It is possible that the growth of satiety during the course of a feeding period may in part be due to a reduction in the perceived palatability of the food stuff being ingested. The fact that animals recover some form of feeding response a short time after NPTh damage has been sustained, presumably when the systemic factors promoting food intake outweigh any possible aversive effects caused by damage to the NPTh, indicate that the NPTh plays only a minor role in the control of food intake. It is true to say that the NPTh does not appear to play a part in the moment to moment control of food intake, nor does it appear to control food intake to the exclusion of other regulatory systems. The NPTh may well play a highly significant role in the situation where an animal is feeding in a novel environment sieving possible food items from the substratum, in such a situation it is important that the animal does not ingest potentially harmful substances. The decision as to whether a substance is harmful may only be based on

the taste of a potential food item and the animal's past experience, the NPTh appears to be a candidate for at least part of such a decision making system, this hypothesis is discussed further in section III of this thesis, where the results of morphological and physiological investigations of the NPTh are presented.

SECTION III

It is quite clear from the preceding section that the nucleus posterior thalamicus (NPTh) plays an important role in the control of food intake. This section will describe a series of experiments aimed at investigating in more detail the anatomical and physiological features of the NPTh. Although the work is presented in two sections it is important to realise that anatomical and physiological studies should be treated as complementary views of the same system. There has been very little published work relating to the NPTh, and much of the information regarding this nucleus has been obtained as the result of experiments carried out in these laboratories (Baker and Beach 1985, Beach and Roberts 1985, Baker 1987).

ANATOMICAL INVESTIGATION

INTRODUCTION

The hypothalamus of the fishes has been extensively investigated and there is considerable anatomical diversity across the evolutionary spectrum, indeed even within the teleosts there is still considerable diversity. The situation tends to be confused by the use of a nomenclature which although correct within a particular group or family, may not be indicative of the homology of similarly named nuclei in other groups. This type of misnomer is evident in the case of the nuclei of the glomerular complex. The corpus glomerulosum pars anterior in many species is continuous with the pars rotunda and is characterised by the presence of glomeruli (Sakamoto and Ito 1982), whereas species which belong to the cypriniformes do not have the glomeruli in the similarly named nucleus (Ito, Murakami and Morita

1982). The nomenclature used here is based on that used by Roberts (1973) in an atlas of the goldfish brain, accordingly the areas of the nucleus preglomerulosus are assigned to the hypothalamus (rather than the ventral thalamus) and the nucleus posterior thalamicus is based on the description given by Sheldon (1912).

The NPTh has been anatomically implicated in the neural pathways associated with control of food intake in the carp *Cyprinus carpio*. It has been labelled retrogradely following horse-radish peroxidase (HRP) injections into both facial and vagal lobes of the medulla oblongata (Baker 1987), and into the telencephalon (Murakami, Ito and Morita 1986) the NPTh thus appears to provide afferent fibres to both the telencephalon and the gustatory centres of the hindbrain. The NPTh of the crucian carp *Carassius carassius* has also been shown to be retrogradely labelled following injections of HRP into the vagal and facial lobes (Morita, Murakami and Ito 1983).

It has been suggested that HRP injections into facial and vagal lobes label different regions of the NPTh (Baker 1987). It has also been shown in the goldfish *Carassius auratus* that facial lobe injections of HRP give rise to bilateral retrograde label in the NPTh, and vagal lobe injections give rise to ipsilateral retrograde label in the NPTh (Morita, Murakami and ito 1983). Morita, Murakami and Ito (1983) state that the NPTh is composed rostro-medially of large round cells and caudally of medium sized fusiform cells. These findings raise the possibility that the NPTh is composed of two sub-populations of neurons one associated with the facial lobe, and one with the vagal lobe. The experiments described in this section were designed to

investigate the possibility of the existence of sub-populations of neurons within the NPTh.

MATERIALS AND METHODS

The experiments described in this section were carried out in the laboratories of the department of human anatomy at the University of Oxford. The resources required for these experiments were very kindly made available by Dr. G. E. Baker.

The techniques used in these experiments were the standard histological procedures used elsewhere for the preparation of brain tissue for light microscopy. Animals were sacrificed by anaesthetic overdose. The brain was removed and fixed in Bouin's fluid, dehydrated, embedded in paraffin wax, and serially sectioned at 15 or 30 μm, sections were taken in either the transverse plane (2 animals) or the horizontal plane (2 animals). Sections were stained with cresyl fast violet. The nucleus posterior thalamicus (NPTh) was located in both transverse and horizontal sections and cell body profiles of all cells deemed to be within the NPTh were drawn with the aid of a microscope fitted with a camera lucida drawing tube. In tissue stained with cresyl fast violet the nucleolus stains much more darkly than the surrounding areas and is thus highly visible. In order to avoid drawing multiple profiles from the same cell body only those profiles which possessed a clearly visible nucleolus were drawn in any one tissue section. There has been much debate over the problems associated with estimating particle or cell numbers based on profile counting techniques (Royet 1991). It has been pointed out however, that the most accurate technique of determining the number of particles (or cell bodies) in an object is to base the profile counts on completely serially reconstructed objects (Coggeshall

1992). All the results obtained from the experiments described in this section were based on complete serial reconstructions of the NPTh. Drawings were later digitised and stored on a microcomputer, the computer used for this purpose was an Elonex PC-88 Turbo with an attached Bioquant II Hipad Digitizer. The software used to store and subequently analyse the data was Bioquant System IV from R and M Biometrics (1985). This system allowed the outline and area of any particular structure to be drawn and calculated, in addition it allows the calculation of a shape factor. The shape factor is a fraction for estimating the amount by which the shape of a structure varies from a circle (Capowski 1988). The equation for the calculation of shape factor (F) is given below:

$$F = (4\pi A)/p^2$$

Where: A = Area of structure.

p = perimeter of structure.

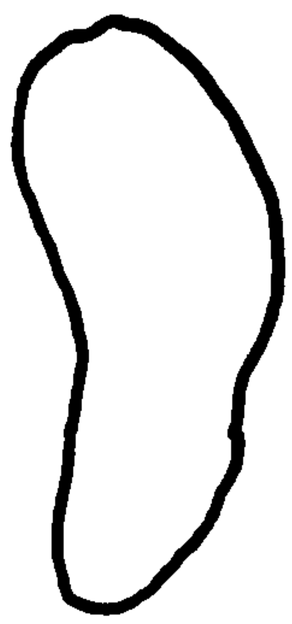
Shape factor is a fraction and has no units. The value of F for a circle is 1.00, and for a line it is 0.00. Examples of shape factor may be seen in figure 51.

Calculation of shape factor is of importance in the determination of the presence of sub-populations within the NPTh particularly where those sub-populations may differ in shape (Morita, Murakami and ito 1983).

Figure 51.

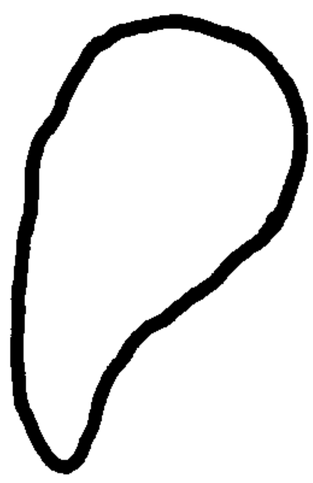
Example shape factors. Shape factor for a circle is 1.00 and that for a straight line is 0.00.

0·347



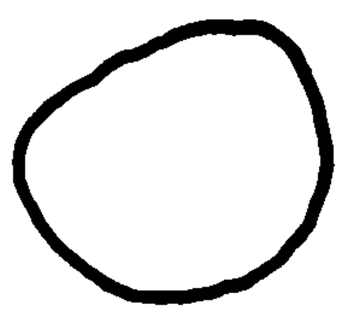
0·601

0·701



0·729

0·734



0·942

The results were analysed and presented as listed below.

1. Cell number - the number of cell bodies in the NPTh in each section was measured, this was carried out in both horizontal and transverse sections.

2. Cell size - the average size of the cell bodies in the NPTh in each section was measured, this was carried out in both horizontal and transverse sections.

3. Shape factor - the average shape factor of the cell bodies in the NPTh in each section was measured, this was carried out in both horizontal and transverse sections.

4. Cell size frequency - a frequency distribution of the cell body sizes seen in both transversely and horizontally sectioned brains was plotted.

5. Cell shape factor frequency - a frequency distribution of the cell body shape factors seen in both transversely and horizontally sectioned brains.

6. Relative shape factor - a statistical analysis of the relative shape factors seen between horizontally and transversely sectioned material was carried out. This analysis was used to test the null hypothesis (H_0) that there was no difference in shape factor between cells of the NPTh when viewed in transverse and horizontal section. The test employed was the randomisation test for two

independent samples (Siegel 1956). A significant difference in shape factor between the horizontal and transverse profiles might indicate the existence of a population of non-spherical cells aligned in some non-random manner.

RESULTS

The nucleus posterior thalamicus (NPTh) may be seen as a well defined closely packed area of cell bodies located dorsally in the hypothalamus. It is bounded on its medial aspect by the nucleus pre-glomerulosus pars lateralis, and on its lateral aspect by a relatively cell free zone (see figures 26, 29, 30 and 31). As measured in these experiments the overall dimensions of the NPTh are 360 µm to 405 µm in its rostro-caudal extent, and 255 µm to 270 µm in its dorso-ventral extent. With regard to the number of cell bodies in the NPTh, estimates based on the counting of cell body profiles are summarised in table 13 below.

TABLE 13

Animal Number	Section plane	Number of cells
XX21	Transverse	350
XX22	Transverse	401
XX23	Horizontal	364
XX24	Horizontal	440

1. Cell number.

The results from these data do not show evidence of the subdivision of the NPTh into discrete regions based on numbers of cells in either the transverse or the horizontal planes. Inspection of figure 52

shows a reduction in the number of cell profiles recorded at 240 μm from the anterior pole of the nucleus. Although such a reduction in numbers of cell profiles may be interpreted as the result of the NPTh being structured into an anterior and a posterior lobe, the effect is not seen in the data presented in figure 53. Furthermore, a great many NPTh preparations were examined histologically, and there was no indication of any form of lobed structuring within the nucleus. Interpretation of this type of data is particularly difficult since the distribution of cell bodies as seen in histological preparations will depend to a large extent on the exact plane of the section (Royet 1991).

Figures 52 and 53 show the number of cells recorded in each serial transverse section through the NPTh.

Figures 54 and 55 show the number of cells recorded in each serial horizontal section through the NPTh.

2. Cell size.

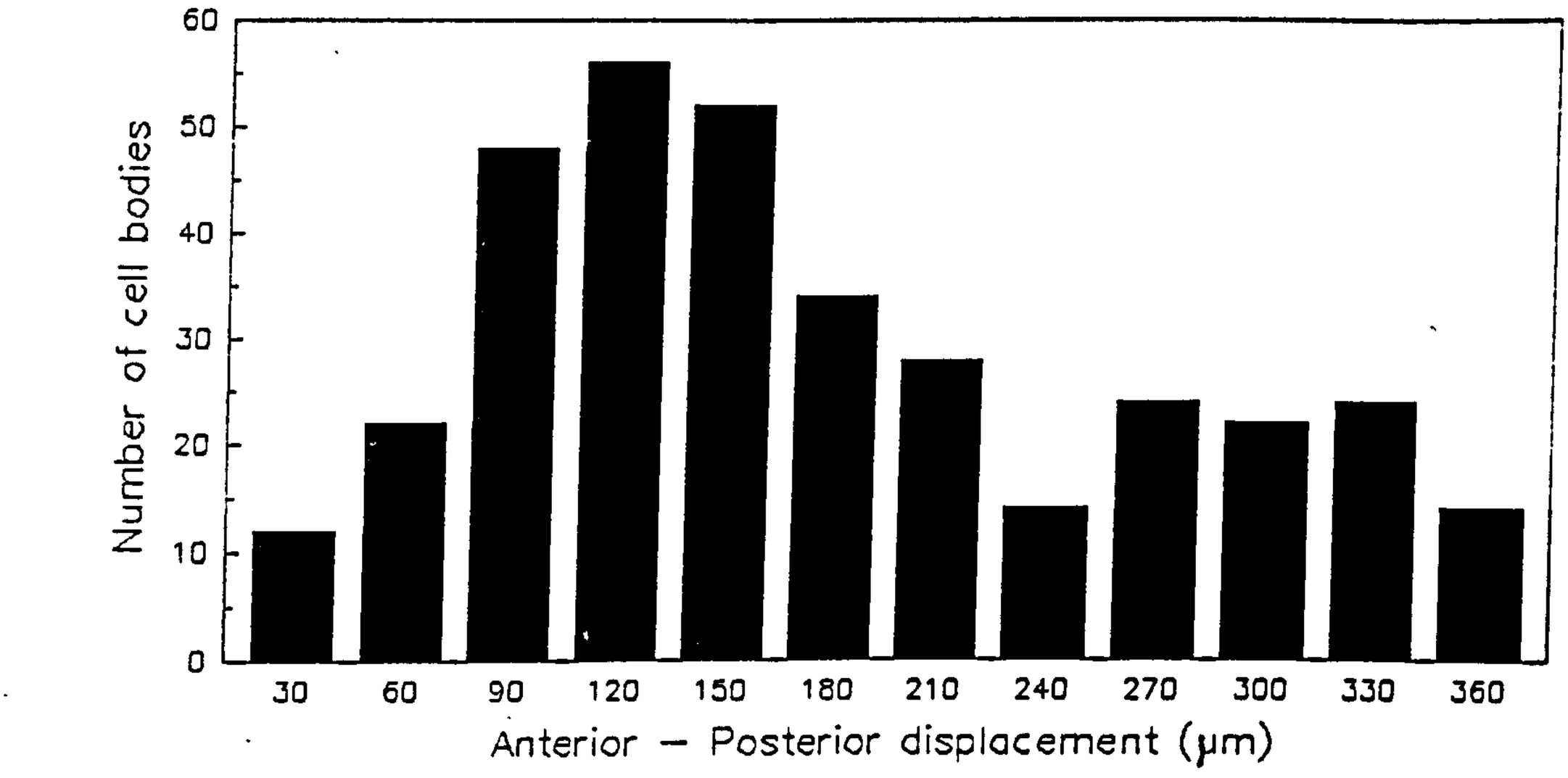
In each tissue section the average cross-sectional area of all the cell profiles which contained a visible nucleolus was recorded. The use of this technique was intended to demonstrate the existence of sub-populations of neurons within the NPTh based on a differentiation in size. This method of presenting the data would demonstrate size differentiated cell populations only if they were spatially separated in either the dorso-ventral or rostro-caudal dimensions. The data presented here shows no evidence that the NPTh is composed of sub-populations of neurons differentiated on the basis of size and

Figure 52.

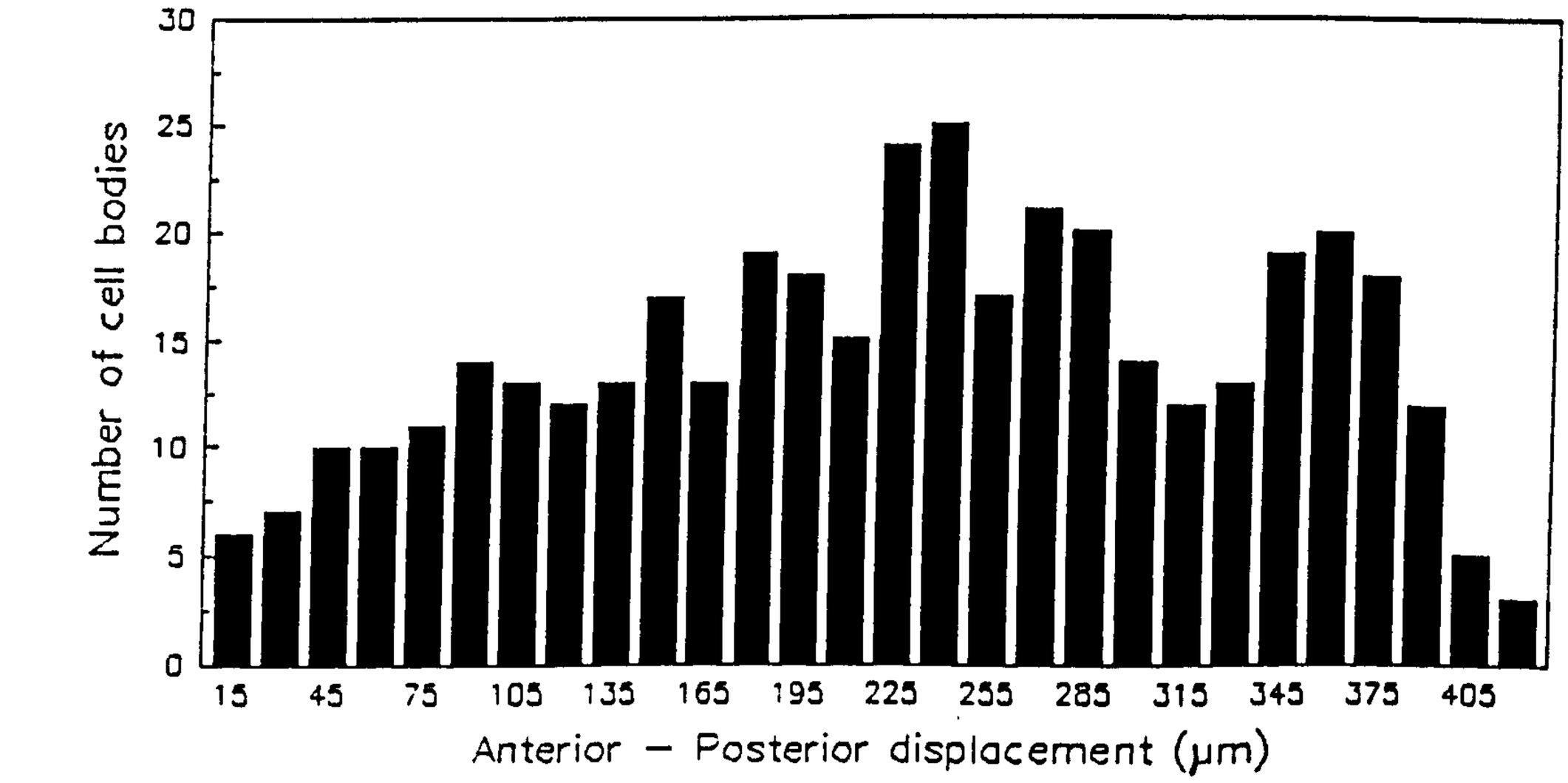
Shows the distribution of cell bodies throughout the NPTh as seen in serial transverse sections taken from fish XX21.

Figure 53.

Shows the distribution of cell bodies throughout the NPTh as seen in serial transverse sections taken from fish XX22.



Fish XX21



Fish XX22

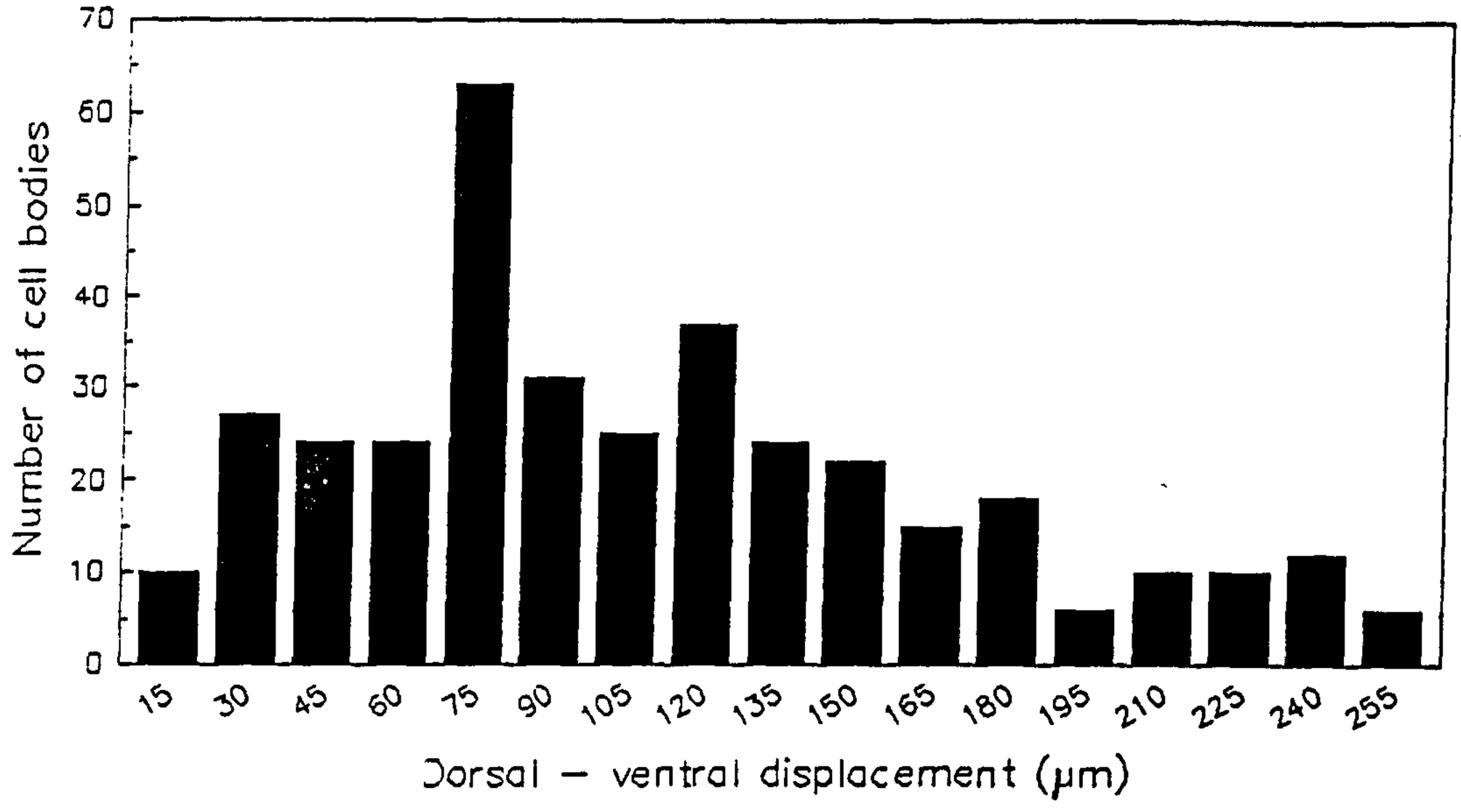
Figure 54.

Shows the distribution of cell bodies throughout the NPTh as seen in serial horizontal sections taken from fish XX23.

Figure 55.

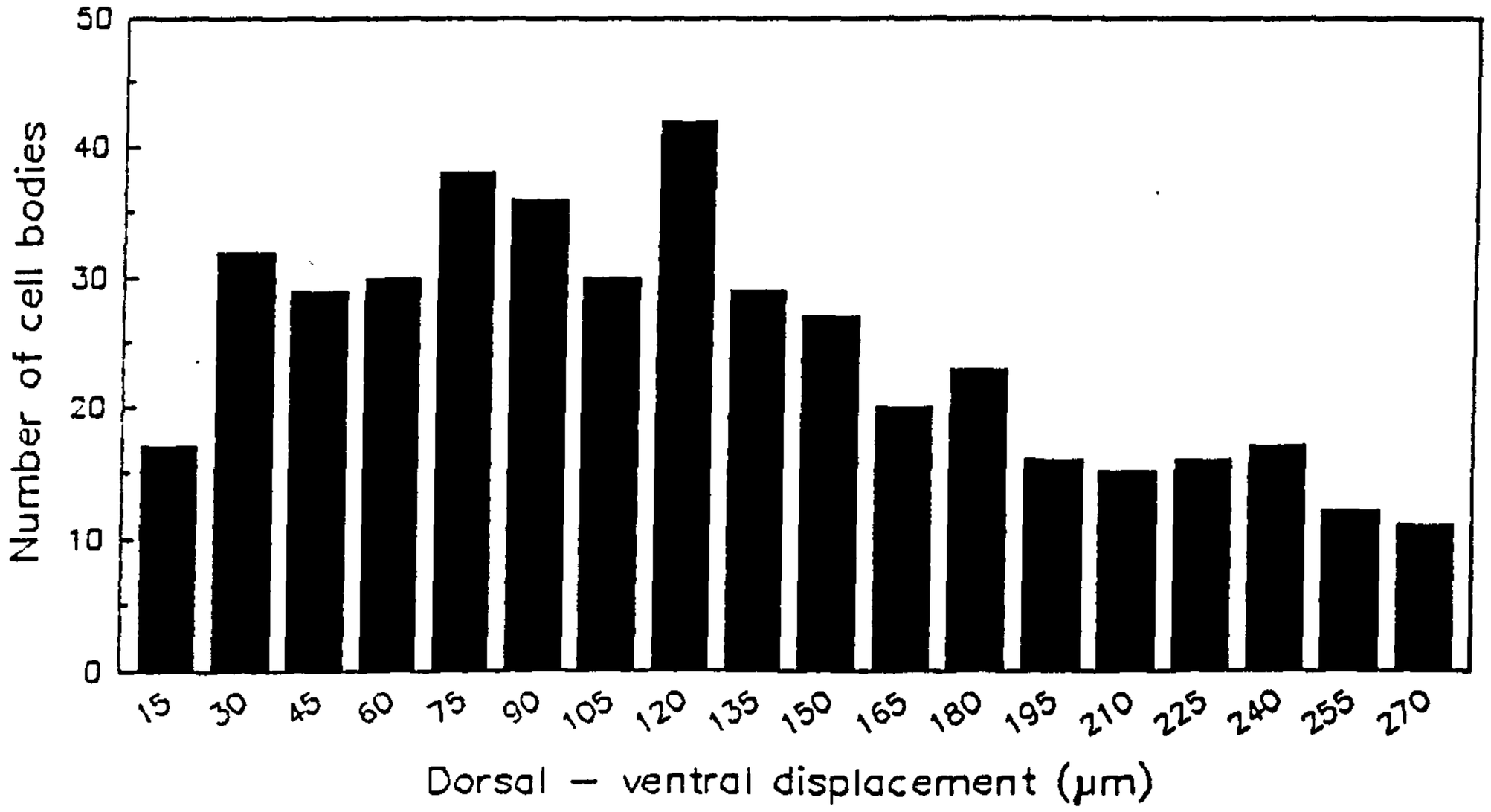
Shows the distribution of cell bodies throughout the NPTh as seen in serial horizontal sections taken from fish XX24.

Cell body distribution
Horizontal section



Fish XX23

Cell body distribution
Horizontal section



Fish XX24

spatial separation.

Figures 56 and 57 show the average size of the cells recorded in each serial transverse section through the NPTh.

Figures 58 and 59 show the average size of the cells recorded in each serial horizontal section through the NPTh.

3. Shape factor.

In each tissue section the average shape factor of all the cell profiles which contained a visible nucleolus was recorded. The use of this technique was intended to demonstrate the existence of sub-populations of neurons within the NPTh based on a differentiation in shape. This method of presenting the data would demonstrate shape differentiated cell populations only if they were spatially separated in either the dorso-ventral or rostro-caudal dimensions. The data presented here shows no evidence that the NPTh is composed of sub-populations of neurons differentiated on the basis of shape and spatial separation.

Figures 60 and 61 show the average shape factor of the cells recorded in each serial transverse section through the NPTh.

Figures 62 and 63 show the average shape factor of the cells recorded in each serial horizontal section through the NPTh.

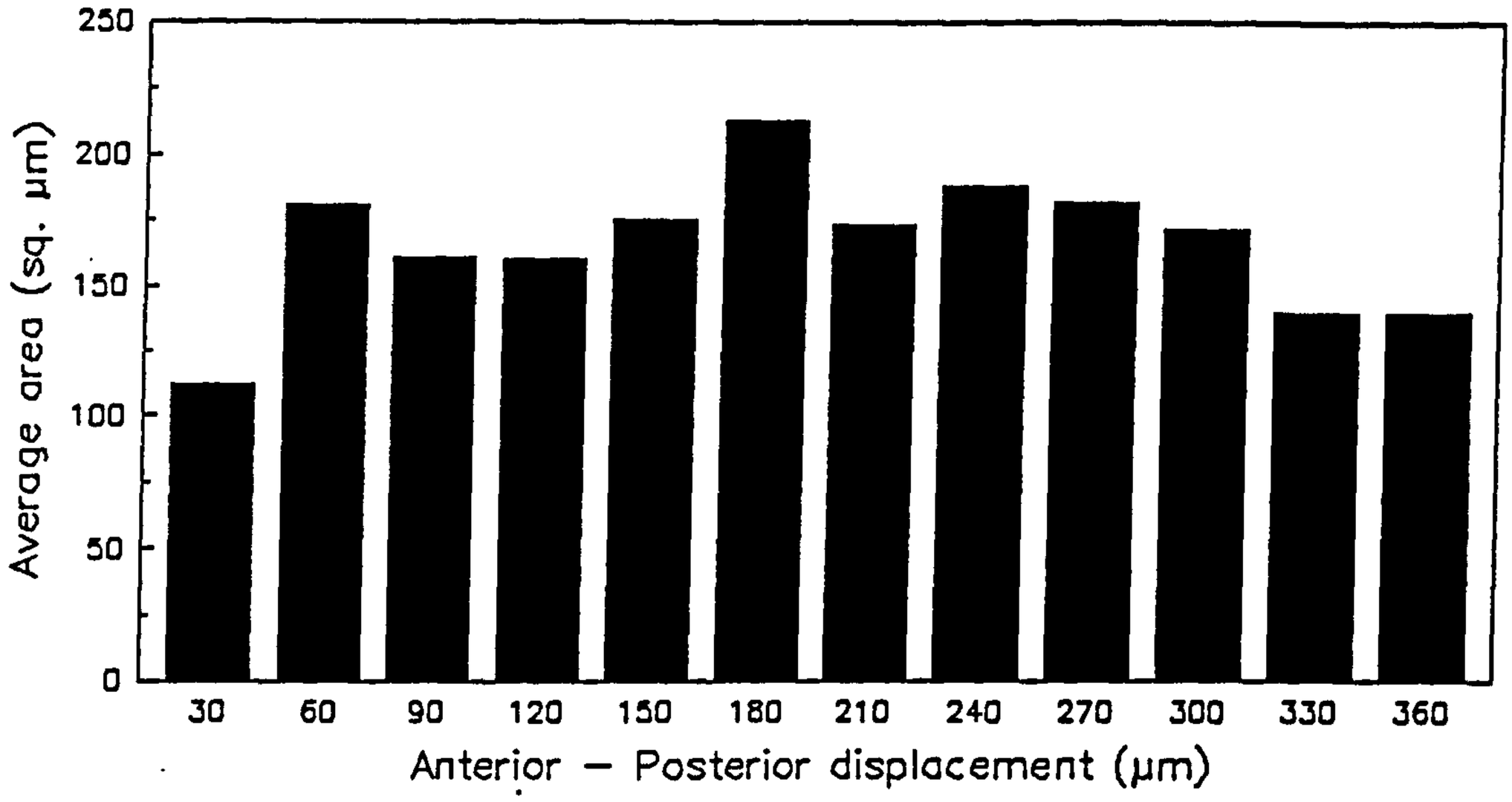
Figure 56.

Shows the distribution of cell body size (cross-sectional area) throughout the NPTh, as seen in serial transverse sections taken from fish XX21.

Figure 57.

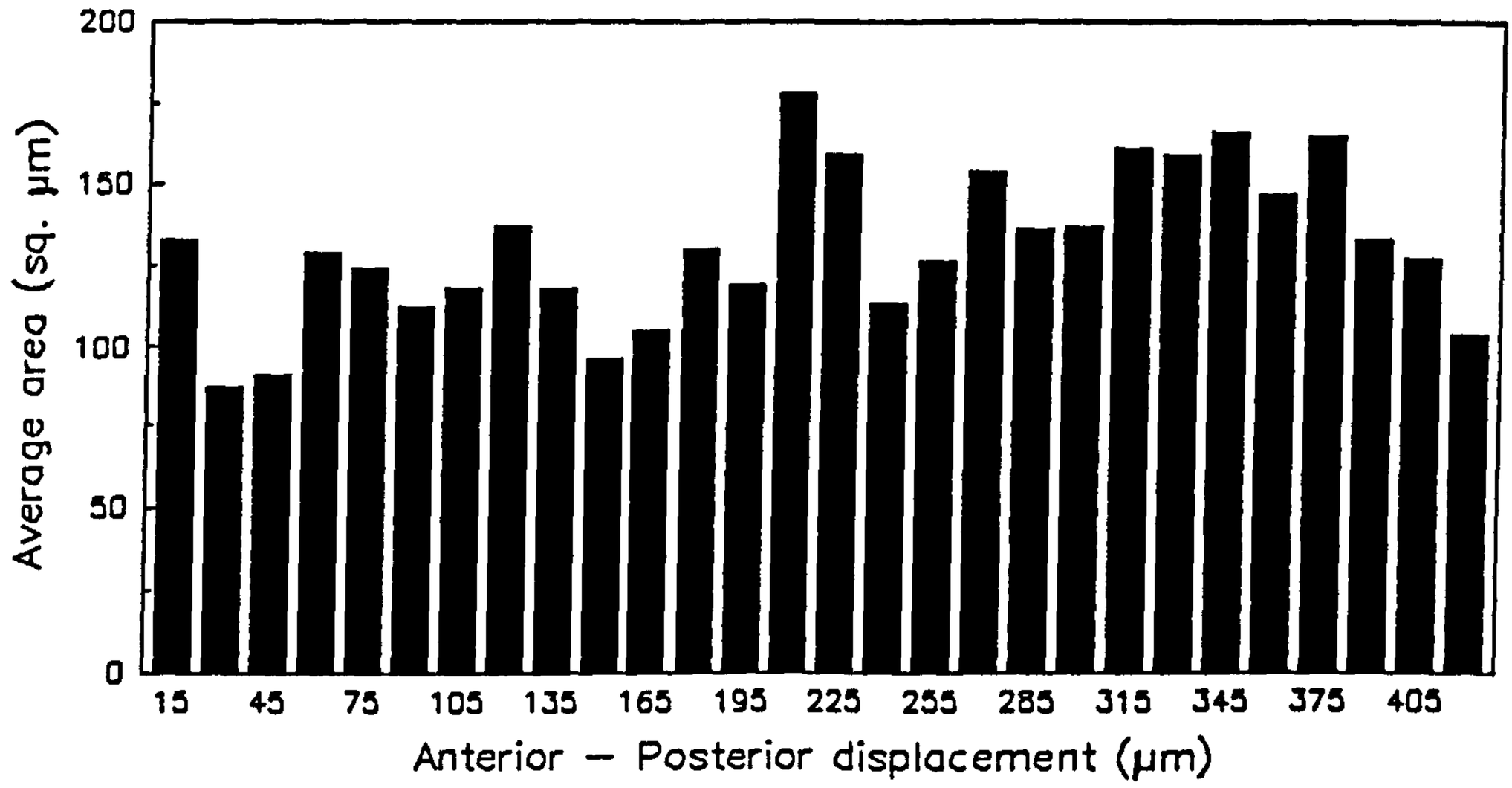
Shows the distribution of cell body size (cross-sectional area) throughout the NPTh, as seen in serial transverse sections taken from fish XX22.

Average cell body size
Transverse section



Fish XX21

Average cell body size
Transverse section



Fish XX22

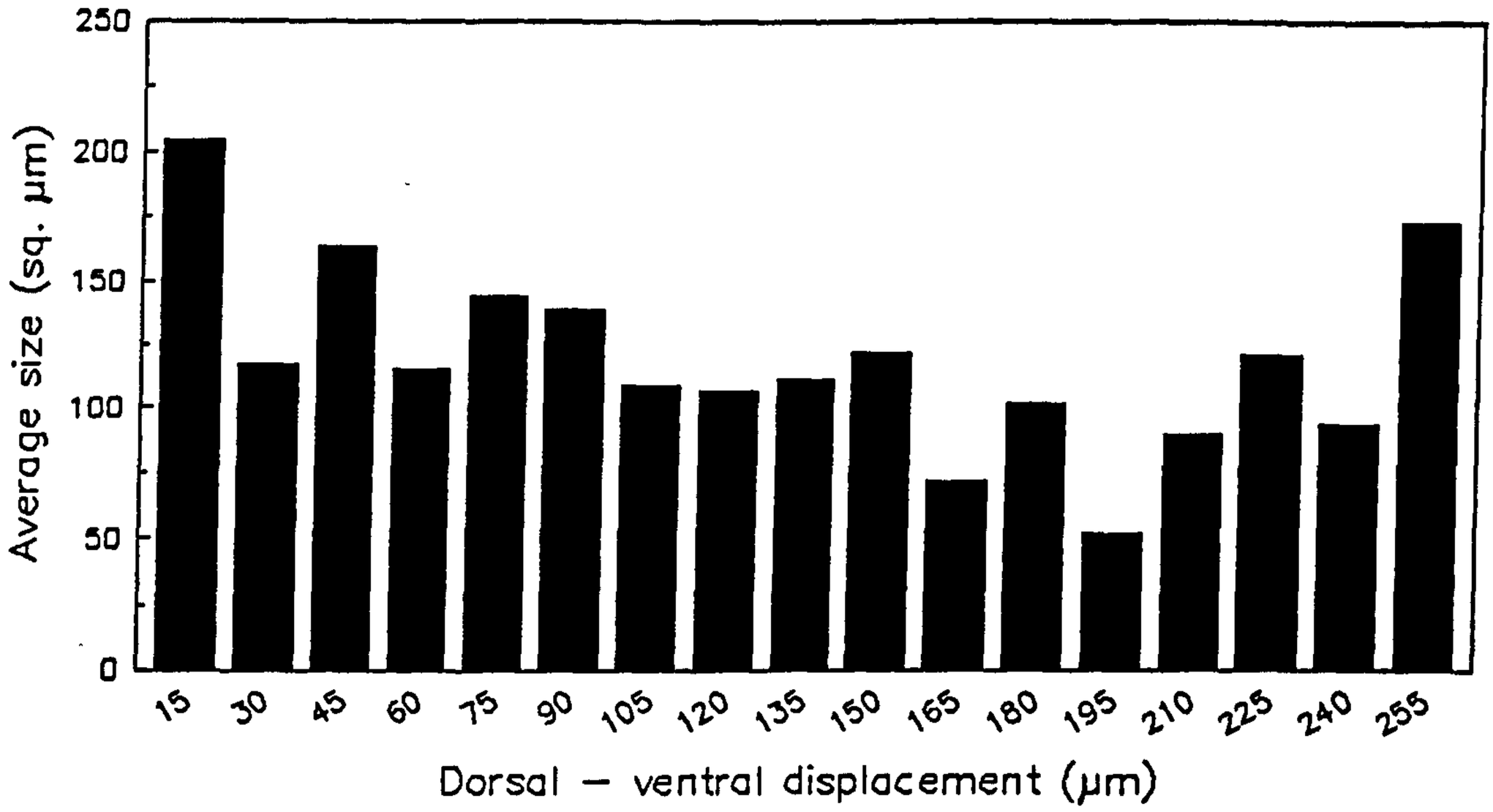
Figure 58.

Shows the distribution of cell body size (cross-sectional area) throughout the NPTh, as seen in serial horizontal sections taken from fish XX23.

Figure 59.

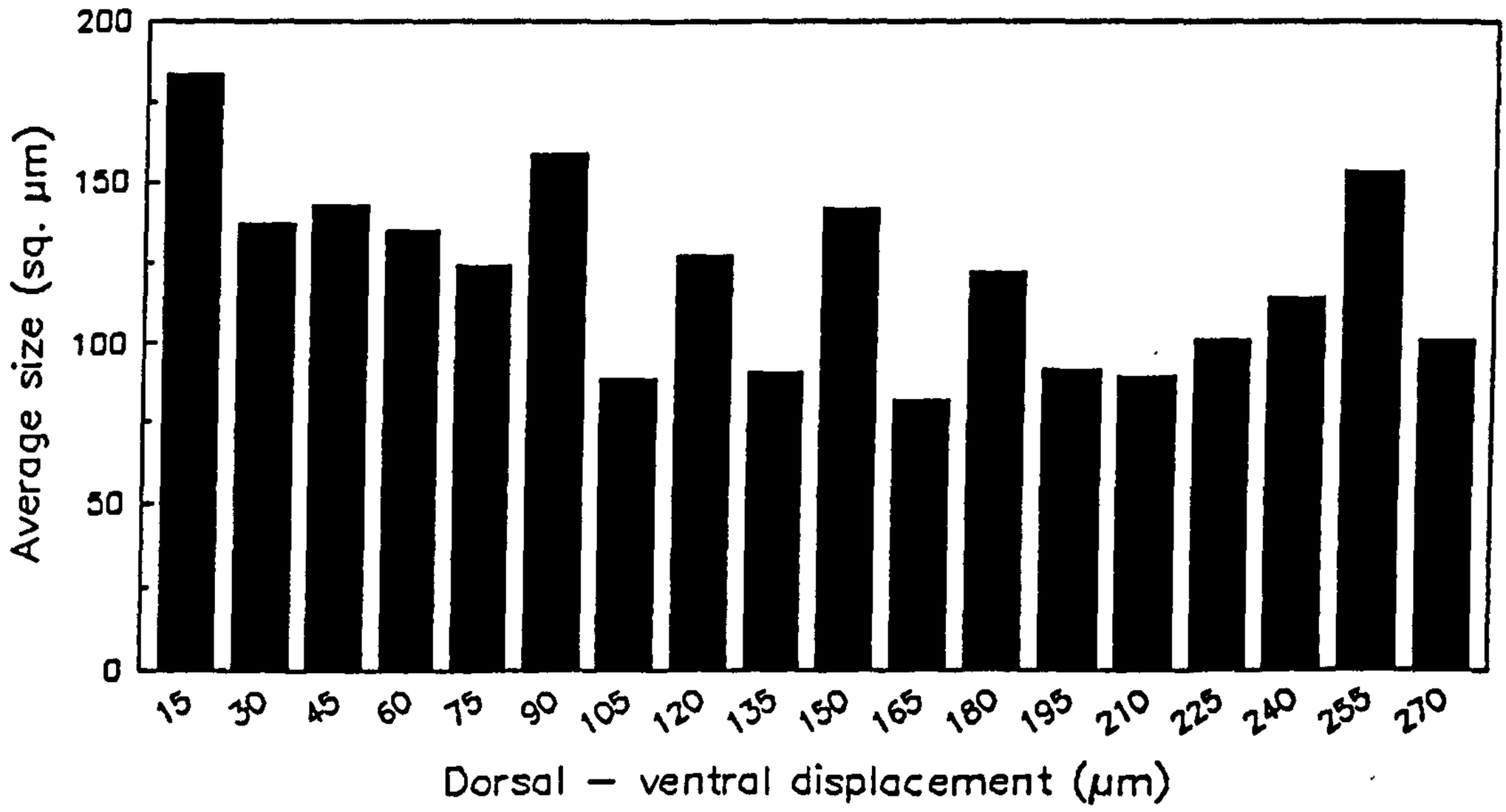
Shows the distribution of cell body size (cross-sectional area) throughout the NPTh, as seen in serial horizontal sections taken from fish XX24.

Average cell body size
Horizontal section



Fish XX23

Average cell body size
Horizontal section



Fish XX24

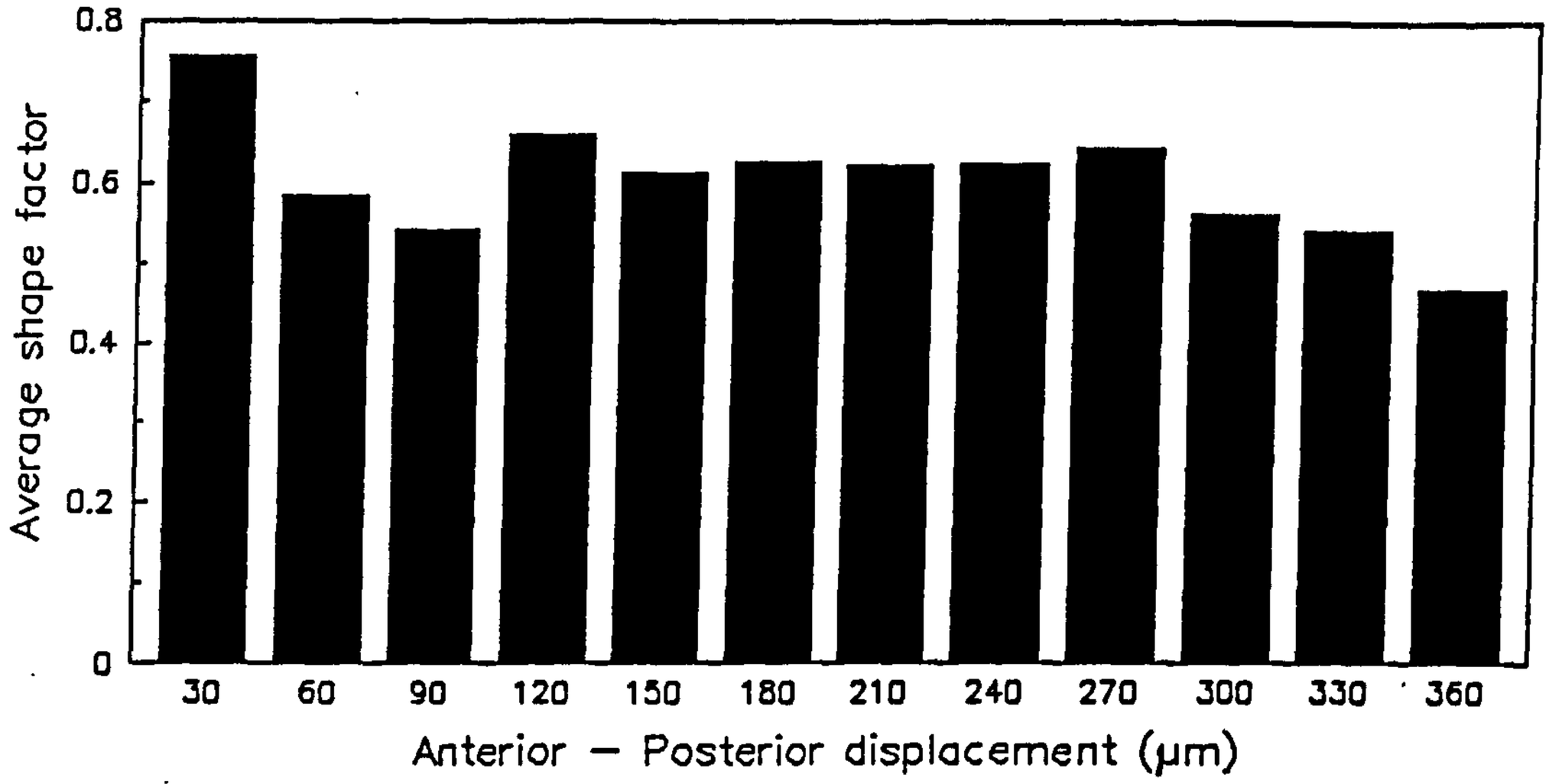
Figure 60.

Shows the distribution of cell body shape factor throughout the NPTh, as seen in serial transverse sections taken from fish XX21.

Figure 61.

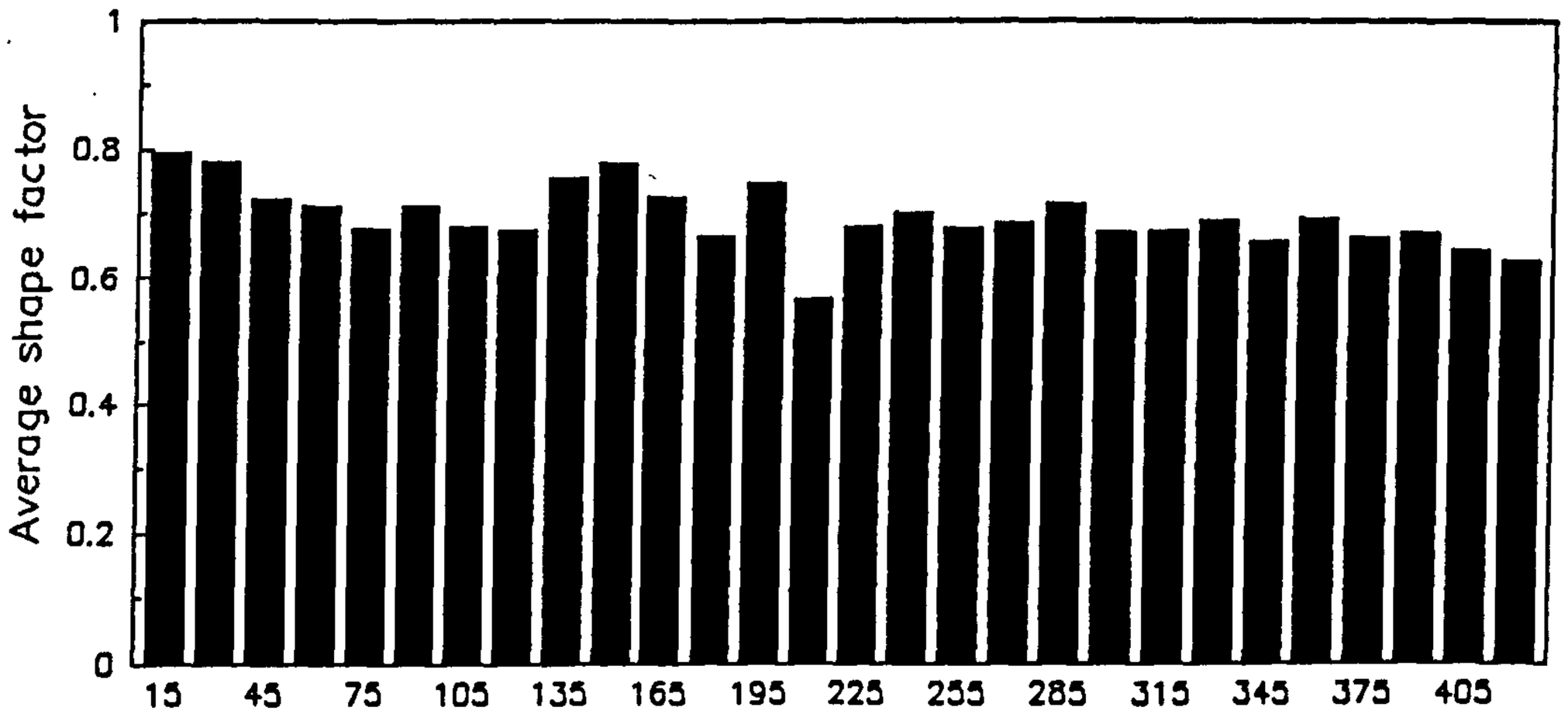
Shows the distribution of cell body shape factor throughout the NPTh, as seen in serial transverse sections taken from fish XX22.

Average cell body shape
Transverse section



Fish XX21
Shape factor for a circle = 1.00
Shape factor for a line = 0.00

Average cell body shape
Transverse section



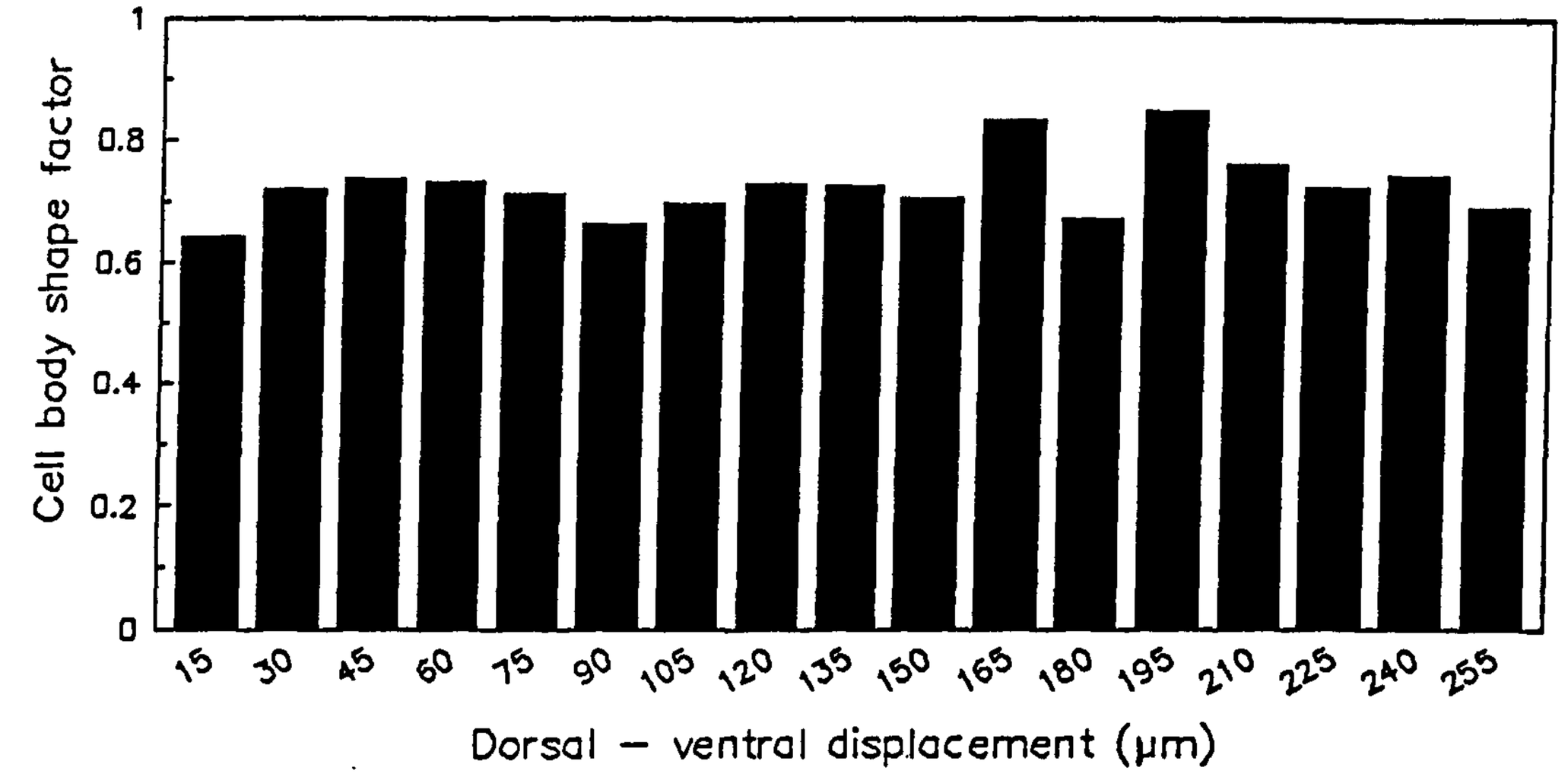
Fish XX22
Shape factor for a circle = 1.00
Shape factor for a line = 0.00

Figure 62.

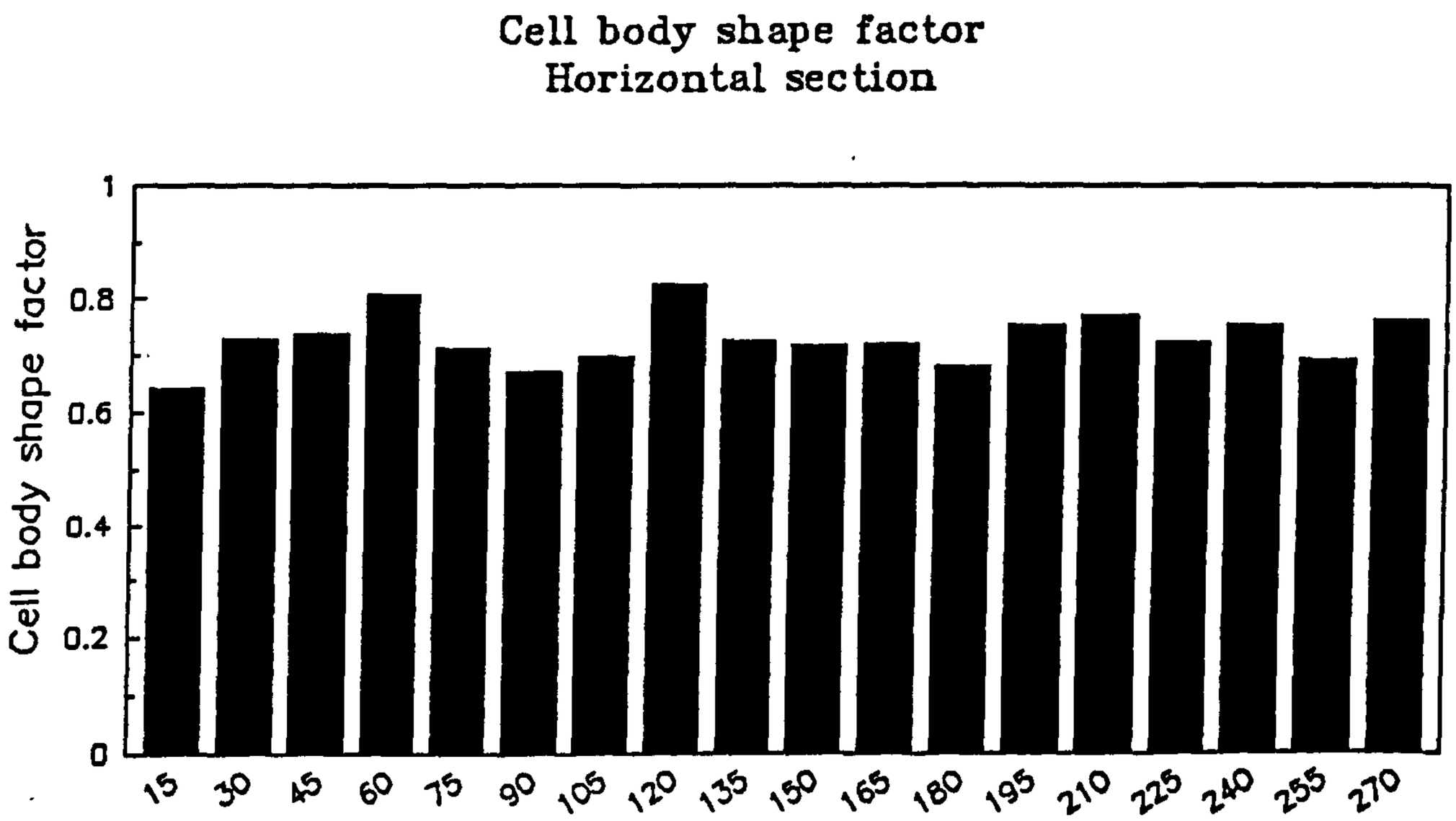
Shows the distribution of cell body shape factor throughout the NPTh, as seen in serial horizontal sections taken from fish XX23.

Figure 63.

Shows the distribution of cell body shape factor throughout the NPTh, as seen in serial horizontal sections taken from fish XX24.



Fish XX23
Shape factor for a circle = 1.00
Shape factor for a line = 0.00



Fish XX24
Shape factor for a circle = 1.00
Shape factor for a line = 0.00

4. Cell size frequency.

The data presented in this section shows the frequency distribution of cell body cross-sectional area. This type of analysis is necessary to test for the possibility that there are sub-populations of neurons within the NPTh which may be differentiated on the basis of cell body size, but which are not spatially separated within the nucleus. In all cases the frequency histograms appear to be in the form of a skewed normal distribution. The presence of sub-populations based on cell body size would be manifest as multiple peaks on the frequency distribution histograms. The results in this section provide no evidence for the existence of sub-populations of neurons within the NPTh differentiated on the basis of cell body size.

Figures 64 and 65 show the size frequency distribution of the cells recorded in each serial transverse section through the NPTh.

Figures 66 and 67 show the size frequency distribution of the cells recorded in each serial horizontal section through the NPTh.

5. Cell shape factor frequency.

The data presented in this section shows the frequency distribution of cell body shape factor. This type of analysis is necessary to test for the possibility that there are sub-populations of neurons within the NPTh which may be differentiated on the basis of cell body shape factor, but which are not spatially separated within the nucleus. The presence of sub-populations based on cell body shape factor would be manifest as multiple peaks on the frequency distribution histograms. Inspection of figure 68 shows the possibility of multiple peaks in

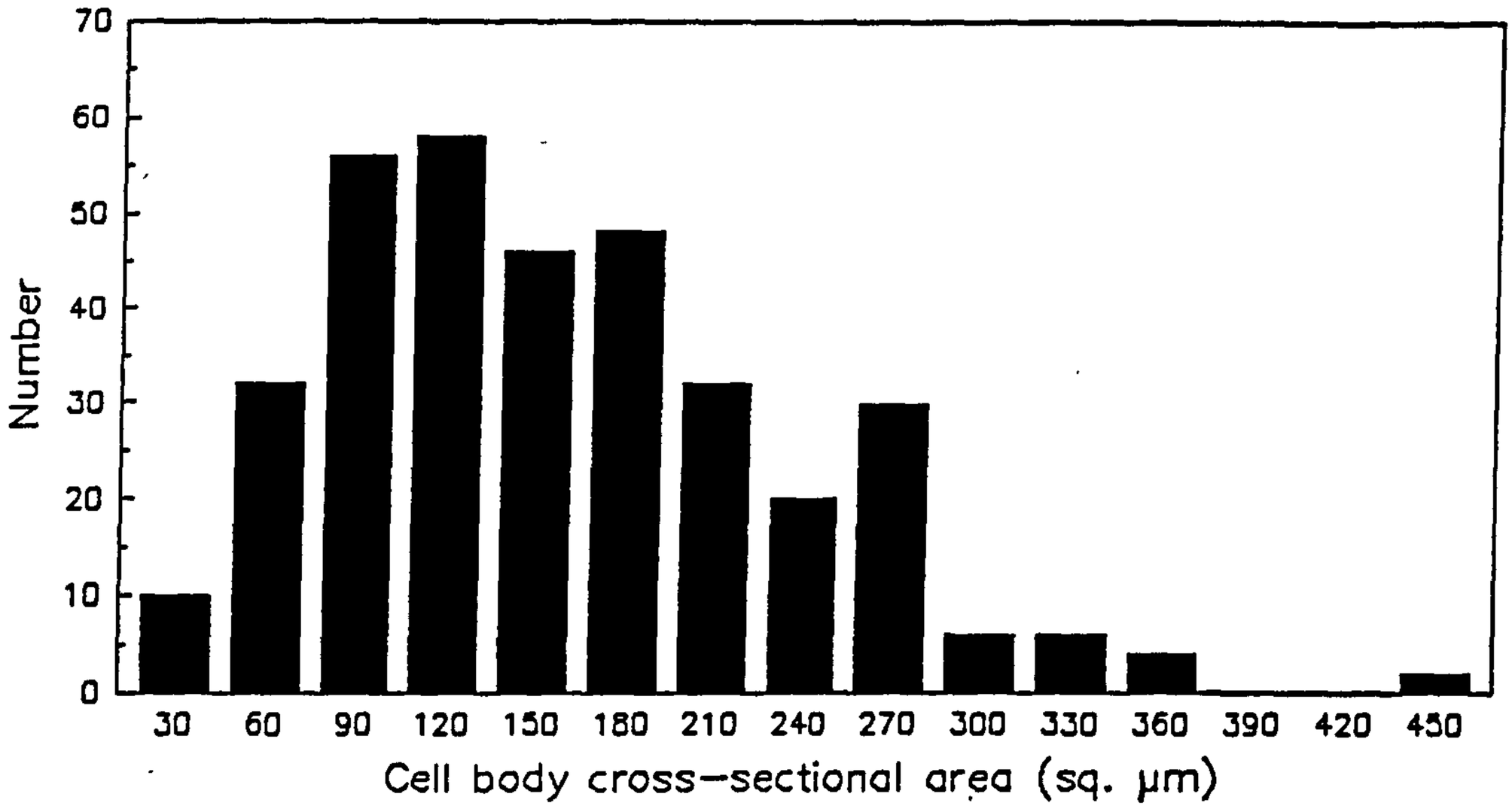
Figure 64.

Shows the frequency distribution of cell body cross-sectional area within the NPTh, as seen in serial transverse sections taken from fish XX21.

Figure 65.

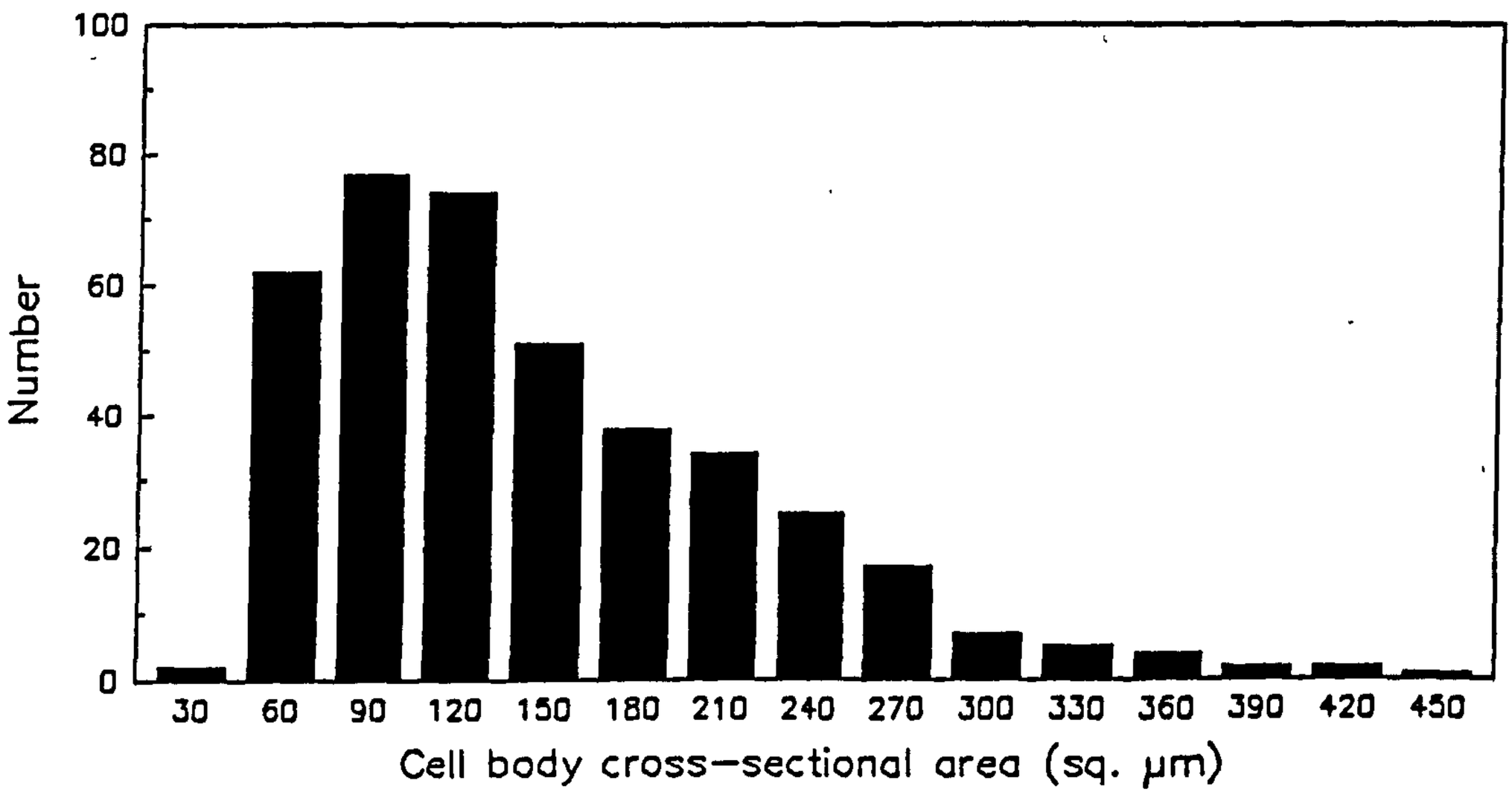
Shows the frequency distribution of cell body cross-sectional area within the NPTh, as seen in serial transverse sections taken from fish XX22.

Frequency distribution of cell size
Transverse section



Fish XX21

Frequency distribution of cell size
Transverse section



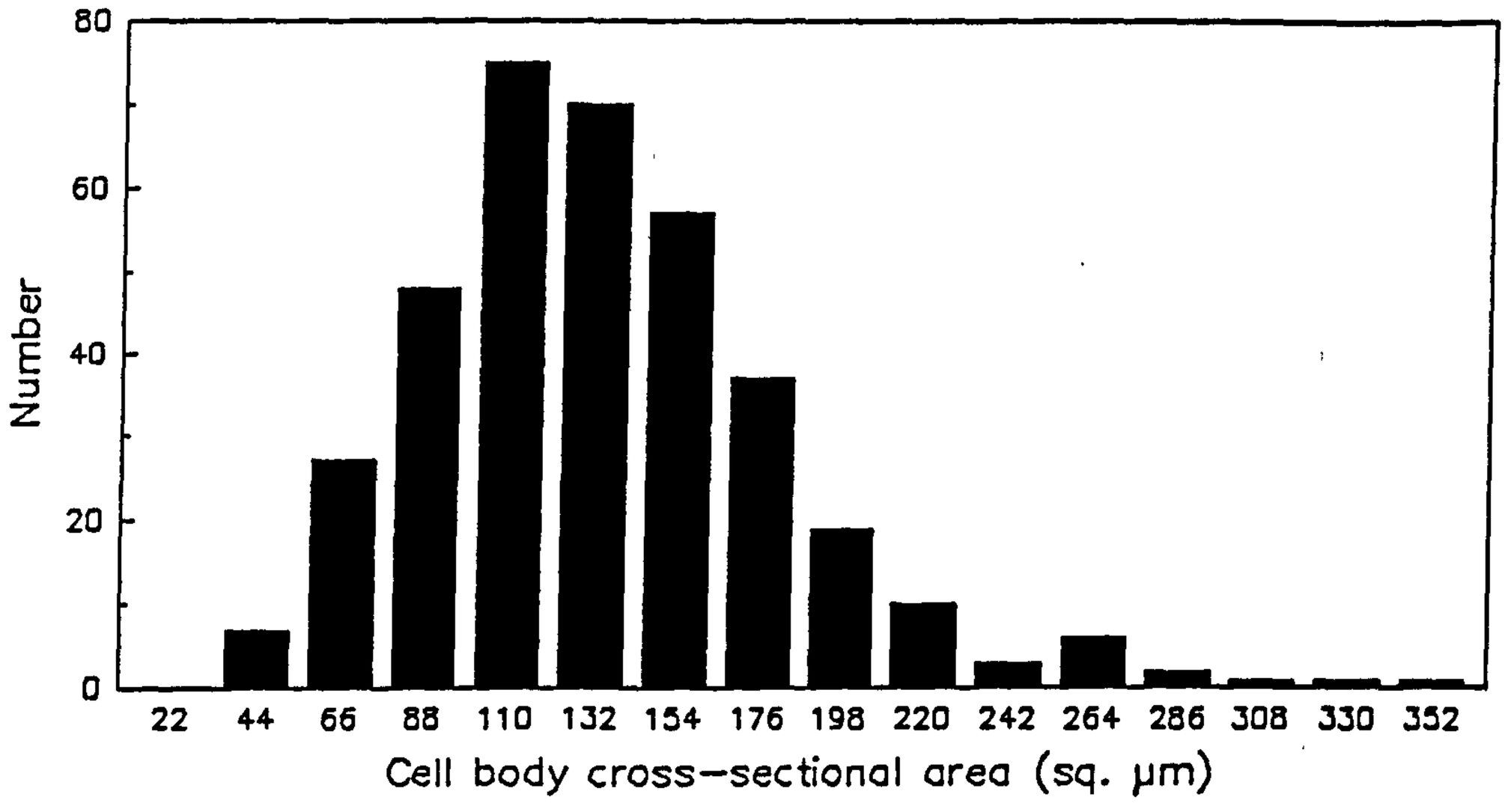
Fish XX22

Figure 66.

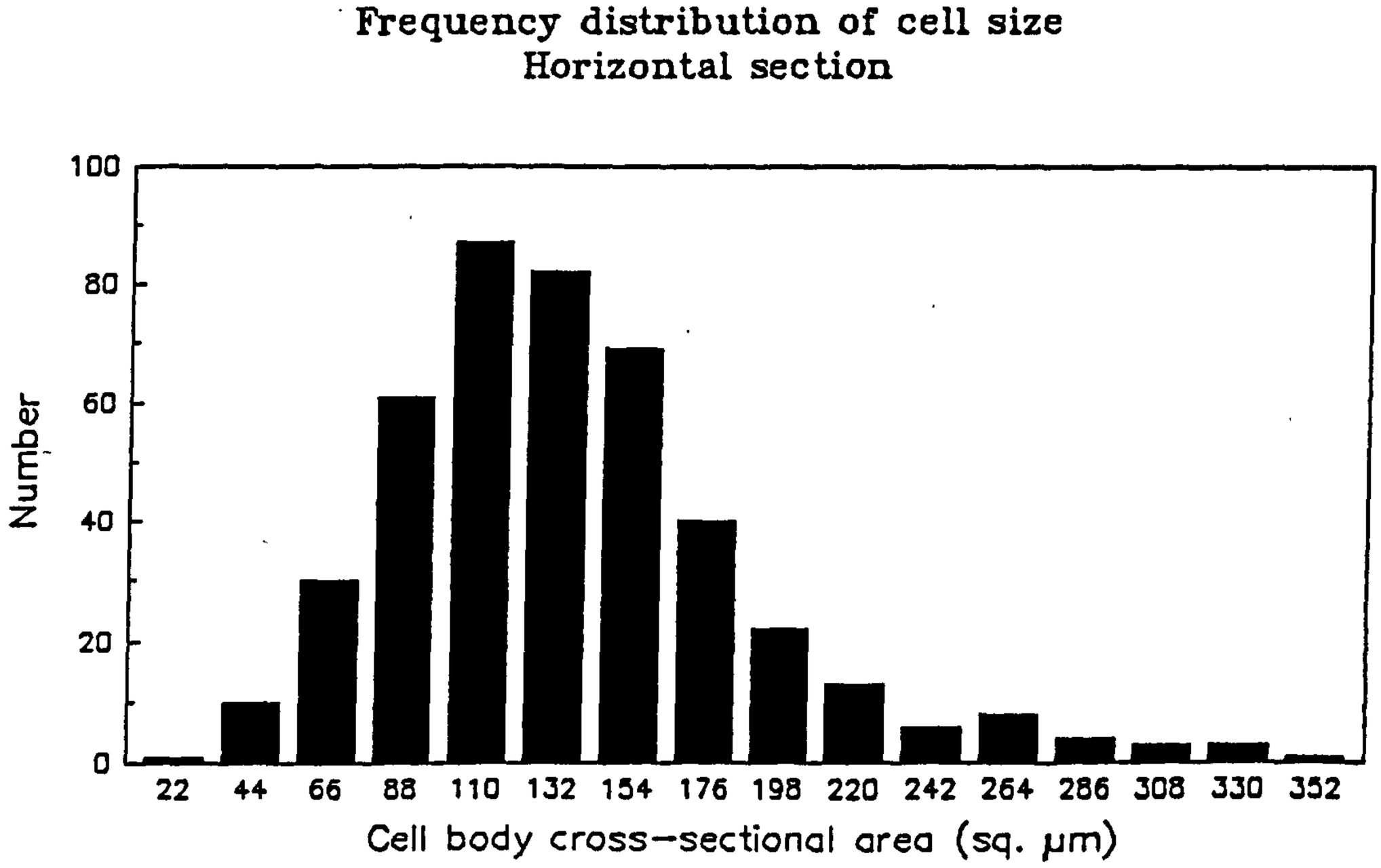
Shows the frequency distribution of cell body cross-sectional area within the NPTh, as seen in serial horizontal sections taken from fish XX23.

Figure 67.

Shows the frequency distribution of cell body cross-sectional area within the NPTh, as seen in serial horizontal sections taken from fish XX24.



Fish XX23



Fish XX24

the frequency histogram. The presence of multiple peaks was not seen in figure 69, and unfortunately this type of analysis was not carried out on a sufficiently large number of specimens to allow verification of the effect. Data from the horizontally sectioned material (figures 70 and 71) was in the form of a skewed normal distribution, and thus showed no evidence of multiple populations of neurons differentiated on the basis of shape factor. The results in this section do not provide good evidence for the existence of sub-populations of neurons within the NPTh differentiated on the basis of cell body shape factor.

Figures 68 and 69 show the shape factor frequency distribution of the cell bodies recorded in each serial transverse section through the NPTh.

Figures 70 and 71 show the shape factor frequency distribution of the cell bodies recorded in each serial horizontal section through the NPTh.

6. Relative shape factor.

The results presented in sections 1 to 5 above indicate that based on the morphological features of the cell bodies the NPTh is most likely composed of a single population of neurons. The analysis used in this section was designed to test for the possibility that there was a significant difference between the shape factor of cell body profiles seen in transverse section and those seen in horizontal section. Using the randomisation test for two independent samples a value of 2.475 was obtained for t. Reference to statistical tables for the

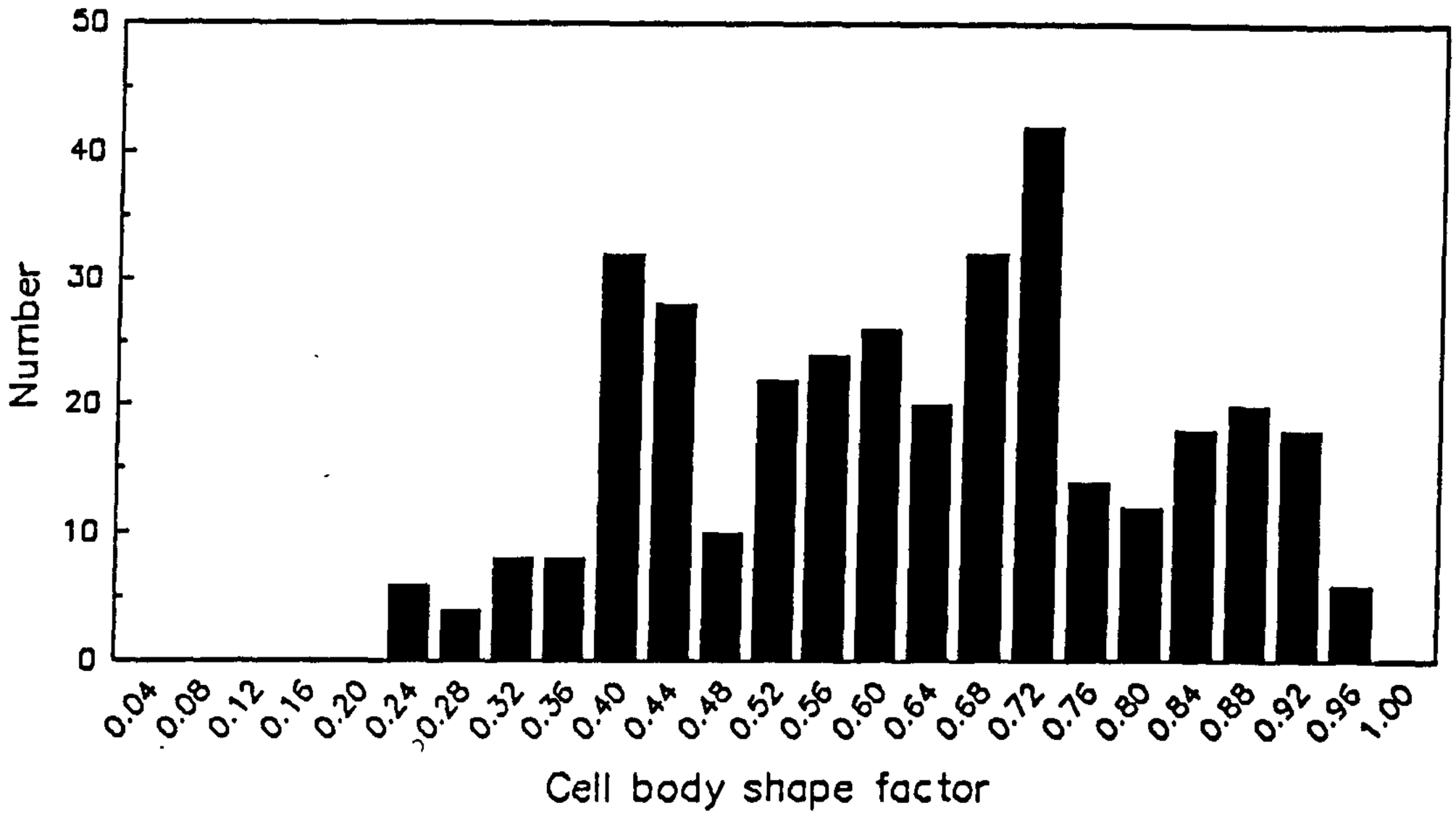
significance of t shows a probability under H_0 of 0.01 for a one-tailed test and 0.02 for a two-tailed test. There is thus a highly significant difference in shape factor between cells of the NPTh when viewed in transverse section and when viewed in horizontal section. This result indicates that the cell bodies of the NPTh are slightly elongated and tend to be aligned with the longest axis positioned dorso-ventrally.

Figure 68.

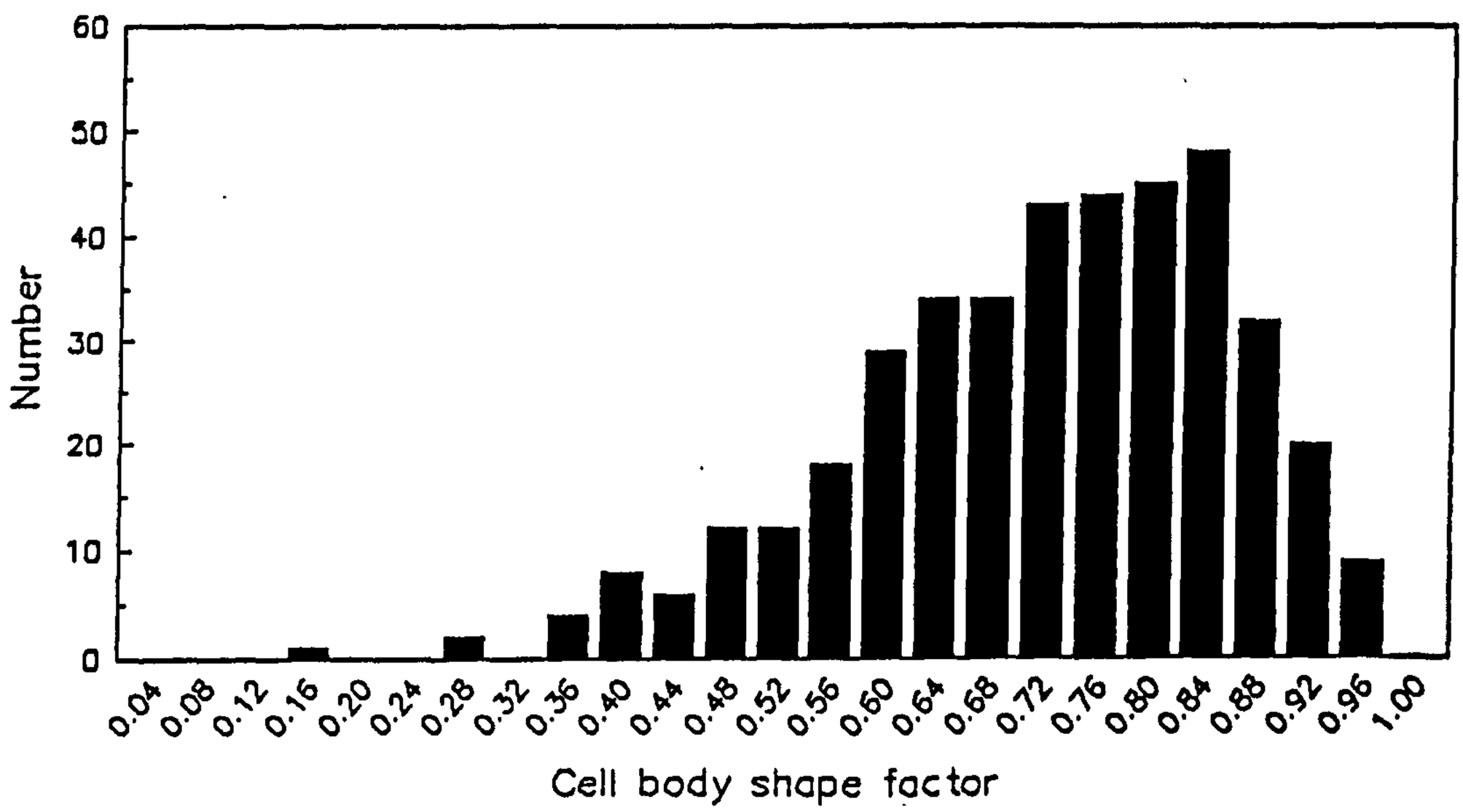
Shows the frequency distribution of cell body shape factor within the NPTh, as seen in serial transverse sections taken from fish XX21.

Figure 69.

Shows the frequency distribution of cell body shape factor within the NPTh, as seen in serial transverse sections taken from fish XX22.



Fish XX21



Fish XX22

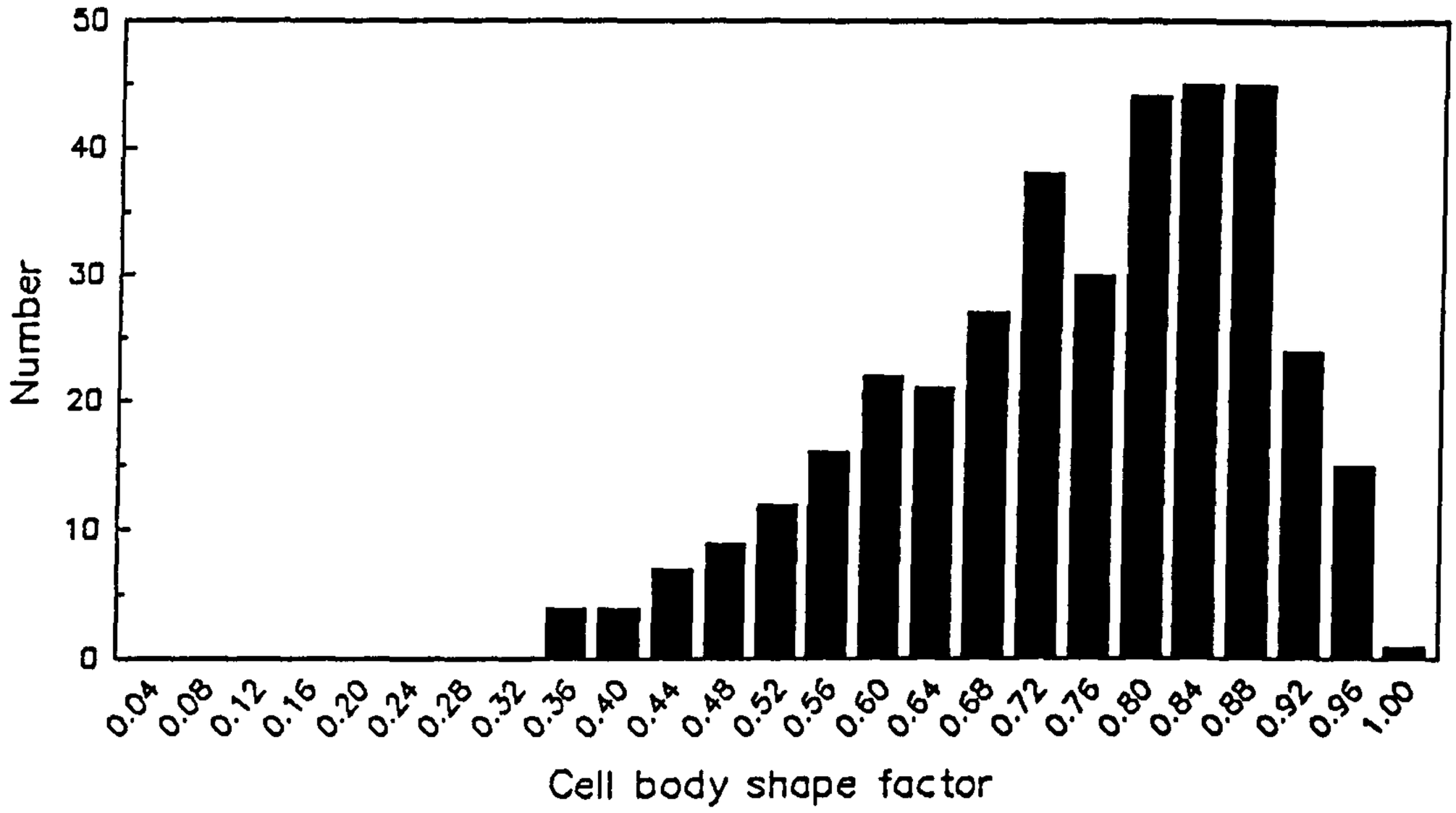
Figure 70.

Shows the frequency distribution of cell body shape factor within the NPTh, as seen in serial horizontal sections taken from fish XX23.

Figure 71.

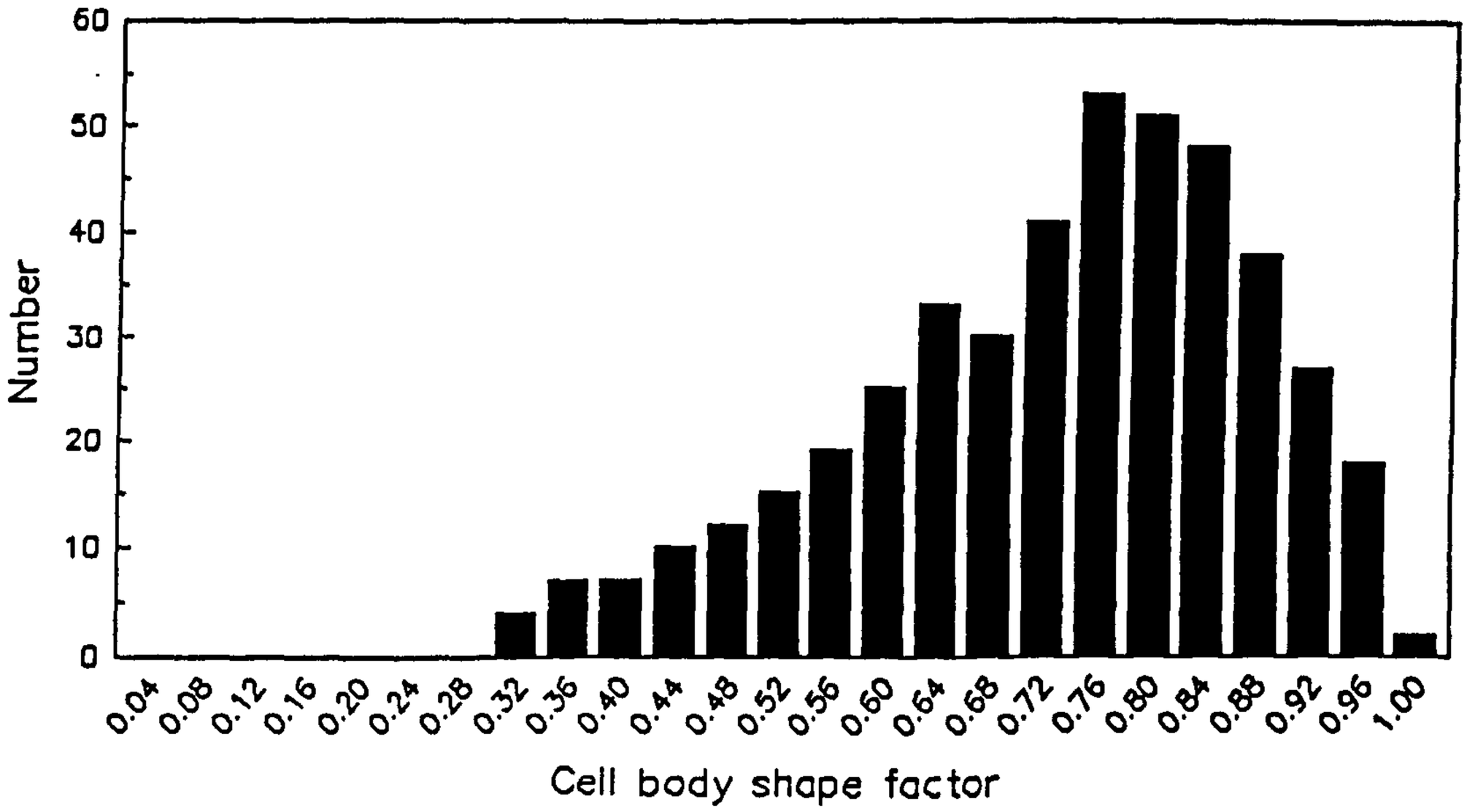
Shows the frequency distribution of cell body shape factor within the NPTh, as seen in serial horizontal sections taken from fish XX24.

Frequency distribution of cell shape
Horizontal section



Fish XX23

Frequency distribution of cell shape
Horizontal section



Fish XX24

DISCUSSION

The main aims of the experiments conducted here were to provide a morphological description of the nucleus posterior thalamicus (NPTh), and to investigate the presence of a morphological correlate of the possible dual functionality of this nucleus. The NPTh makes an efferent projection to two very distinct and different regions in the medulla oblongata, the facial lobe associated with the VIIth cranial nerve, and the vagal lobe associated with the Xth cranial nerve (Baker 1987). The facial and vagal lobes, although associated with the same sensory modality (chemoreception) are instrumental in different aspects of feeding behaviour. The facial lobe receives afferent input from the taste buds and tactile receptors situated on the external surface of the fish's body, and is involved in the accurate localisation of food items. The vagal lobe receives afferent input from the taste buds located on the palatal organ in the roof of the mouth, and is involved with the swallowing stages of food ingestion (Atema 1971). A central structure such as the NPTh which is associated with two different aspects of feeding behaviour might be expected to show some degree of anatomical differentiation. Anatomical differentiation may be represented in a number of ways, differences in the arrangement of afferent or efferent fibres for example, it might also be apparent as morphological differences between cells within the nucleus. Morphological sub-populations might be expected to appear in a variety of ways, either being differentiated on the basis of cell size or cell shape, in addition sub-populations where present may be localised within different regions of the nucleus or be mixed and distributed throughout the

body of the nucleus. The data analyses employed in this study were designed to detect any of the possible ways in which morphological sub-populations of cell bodies may be manifested. The plots showing cell size and shape factor recorded on a section by section basis were designed to demonstrate the presence of sub-populations differentiated on the basis of shape and/or size being located in different regions of the NPTh. Inspection of figures 56 to 63 shows quite clearly that there was no zonation of cell bodies within the NPTh on the basis of size or shape. The plots showing frequency distributions of shape and size were designed to demonstrate the presence of morphological sub-populations which might overlap throughout the body of the NPTh. Inspection of figures 64 to 71 show no evidence for the presence of sub-populations differentiated on the basis of size or shape but overlaid within the NPTh. Quite clearly on the basis of the morphological variables employed in these analyses the cell bodies of the NPTh comprise an homogenous population. This finding stands in contrast to the finding of Morita, Murakami and ito (1983) that the NPTh was composed of large round cells and medium-sized fusiform cells, and that these cell types were located in different regions of the nucleus. The obvious contradiction between these results might be explained on the basis of generic differences, the results of Morita *et al* (1983) being based on studies of the crucian carp *Carassius carassius*. In addition to the search for morphological differentiation within the NPTh an investigation into the possibility of the non-random alignment of cell bodies was conducted. The result of this investigation is presented in section 6 of the results and shows that cell bodies within the NPTh tend to be slightly elongated in the dorso-ventral plane. The effect is that of

a number of elongate structures stacked in register when viewed in the transverse plane. A simple explanation for the existence of cell bodies stacked in a regular manner is that such an arrangement may be used to maximise the number of individual units which may be accommodated in any given volume. A second benefit of regularly arranged units is that of enhanced 'cable management'. Any nuclear structure which is to have a large number of external connections, and is to function as a coherent unit must be arranged in such a way that the coherency of the activity within the nucleus is not lost due to large differences in conduction latencies associated with different regions of that nucleus. The structure of the NPTh may indicate that it functions as a coherent ensemble of neurons. The fact that the cell bodies within the NPTh all appear to be morphologically similar may also point to a nucleus serving a single function. Whilst it is often true to say that neurons with different morphologies serve different functions, it is not necessarily true to say that neurons with similar morphologies serve similar functions. Obviously the assumption that the NPTh is functionally homogenous must, in the absence of additional evidence, remain speculative. Should the hypothesis that the NPTh serves a single function be true, the fact that the NPTh has a direct relationship with both facial and vagal gustatory lobes of the hindbrain raises the question as to which features of chemoreception and gustation are common to both internal and external taste systems. An obvious factor which must be common to both facial and vagal gustatory systems is that of the palatability of potential food items. As has been shown in the experiments described in section II there is some evidence that the NPTh may play a role in determining the palatability of food items.

PHYSIOLOGICAL INVESTIGATION

INTRODUCTION

The experiments described in this section rely largely on the technique of antidromic activation of the nucleus posterior thalamicus (NPTh). The NPTh may be reliably activated antidromically by means of electrical stimulation of the facial and/or the vagal lobes of the medulla oblongata (Beach and Roberts 1985). Antidromic responses may be elicited by stimulation of the peripheral part of the axon, evoking a potential which travels towards the cell body. An antidromic potential may `invade` the cell body where it may be recorded intra- or extracellularly. Since antidromic activation involves backward conduction of an impulse along the axon towards the cell body it may be assumed to occur only in experimental conditions. Although antidromically elicited activity does not provide physiologically relevant data in the same way that orthodromically elicited activity does, it may allow some deductions as to the physical parameters of the pathways and nuclei involved. For example, antidromic stimulation will allow the estimation of conduction velocites, in addition the rectifying nature of chemical synapses means that antidromically activated pathways must be mono-synaptic, and finally manipulation of the parameters of the electrical stimulation required to produce an evoked response allows the calculation of the chronaxie for the stimulated tissue. Chronaxie is a feature of electrically capacitive systems such as neuronal membranes, and for such membranes it has been empirically shown that chronaxie approximates to 0.7 x membrane time constant (Ranck 1981).

The time constant of a membrane is given by the equation:

membrane time constant = $r_m c_m$

where r_m = membrane resistance,

and, c_m = membrane capacitance.

Thus knowledge of the chronaxie (and hence the membrane time constant) may allow an insight as to which elements are being activated during the passage of the stimulating current. Knowledge of which elements are being stimulated is important in determining what the stimulus parameters should be, and how the stimulation should be applied. The basic features of electrical stimulation which must be addressed are; whether stimulation should be constant current or constant voltage; whether it should be via bipolar or monopolar electrodes, and if monopolar whether it should be via the anode or the cathode.

Wherever possible, electrical stimulation should be provided from a constant current supply rather than a constant voltage supply. It is current (the movement of charged particles) which causes membranes to depolarise. If constant voltage stimulation is employed the amount of current passing through the stimulated site may vary greatly during the course of the stimulation. Due to the electrolytic nature of the events taking place during electrical stimulation it is likely that the electrical resistance at the electrode-tissue interface will alter during stimulation. Under constant voltage conditions any changes in resistance at the electrode-tissue interface which occur

during the course of the stimulation will lead to a change in current flowing at that point. If changes in electrical current flow during stimulation are to be avoided constant current stimulation must be employed.

The use of bipolar or monopolar stimulation determines to a large extent which neural structures are stimulated. For the stimulation of axons or bundles of axons it has been shown that the extracellular voltage gradient in the direction of the axon is the salient parameter, if the voltage gradient is transverse to the axon then stimulation requires a much higher current (Rushton 1927). Bipolar stimulation parallel to the direction of the axons is thus suitable for use where axonal processes are the preferred stimulation target. A feature of bipolar stimulation is that of anode block (Ranck 1975). Anode block occurs when the degree of hyperpolarisation produced at the anode may be large enough to block the impulses generated at the cathode. Anode block may be avoided by adjusting the polarity of the electrode pair such that impulse conduction in the required direction is achieved. Another important feature of bipolar electrodes is the spacing of the electrode tips. In those cases where the separation of the electrode tips is greater than two length constants of the fibres being stimulated then the two electrodes may be considered to be independent, where the separation of the electrode tips is less than two length constants then their effects interact (Ranck 1981). It has been argued that for central nervous system (CNS) stimulation monopolar cathodal stimulation is preferred. Monopolar electrodes allow much better localisation of the stimulation since they stimulate only a discrete number of neural units in a well defined

shell around the electrode tip (Jankowska and Roberts 1972). It has also been demonstrated that cathodal stimulation requires between 3 and 7 times less current than does anodal stimulation (BeMent and Ranck 1969). Obviously consideration must be given to the method and parameters used when electrical stimulation of neural tissue is employed, this is particularly important when the stimulated sites are within the CNS. CNS tissue is highly anisotropic and it is impossible to control the flow of electrical current around the extracellular space, this problem is particularly marked in the *in vivo* preparation as used in the experiments described below. Many of the technical considerations discussed above are of particular importance when neural systems are to be stimulated orthodromically in order to emulate the normal function of the system or to obtain some physiologically relevant response. It is not the intention in these experiments to claim that the signals evoked antidromically from the NPTh are necessarily physiologically relevant, but rather that the shape, latency and duration of the evoked signals may shed light on the relationship between the NPTh and the hindbrain gustatory areas, and possibly on the relationships between the cells of the NPTh itself.

MATERIALS AND METHODS

The investigations described in this section all involved electrical stimulation of and recording from central sites. The surgical techniques used to prepare the animal and the equipment used to provide electrical stimulation and recording facilities were as described in the materials and methods of section II of this thesis. The following experiments were carried out:

1. Electrical stimulation of a hindbrain vagal lobe

This preparation allowed the investigation of several features of the relationship between the hindbrain and the nucleus posterior thalamicus (NPTh). The experiment consisted of the placement of bipolar stimulating electrodes into the anterior half of the vagal lobe on one side of the medulla such that the tips just penetrated the surface. The electrical stimulation used for this preparation was provided by constant current pulses of 100 μA amplitude and 2 ms duration at a frequency of 1 Hz. The stimulating electrodes used were constructed in-house and consisted of stainless steel wire etched to a fine point and insulated with epoxylite resin (Clark electromedical instruments, Reading UK). The tips of the electrode pair were of the order of 0.5 mm apart.

In its simplest form the preparation allowed the measurement of the latency between vagal lobe stimulation and the evoked response in the NPTh. The duration of the evoked activity was also measured.

In addition this preparation was used to carry out a stimulus-response experiment. The recruitment of activity in the NPTh was recorded as stimulus amplitude was increased from a level which elicited no activity in the NPTh to a level at which elicited NPTh activity was maximal.

This preparation was also used to carry out a strength-duration study of several of the units observed in the evoked NPTh signal. It proved possible to calculate the chronaxie of the stimulated tissue in the vagal lobe. A strength-duration curve is generated by recording some response from a system (usually an all-or-nothing response such as a `spike` event) and varying the strength and duration of constant current stimulating pulses. In this way it is possible to plot the strength versus the duration of the stimulating pulse required to give a constant response (the appearance of a spike event) (Ranck 1981). Within the evoked NPTh signal it is possible to identify a number of extracellularly recorded spikes. The strength-duration curve associated with these spikes may be generated by reducing the duration of the stimulating pulse until the extracellular spike disappears from the evoked signal, the amplitude of the stimulating current may then be gradually increased until the spike re-appears in the evoked response, these two new values of stimulus duration and amplitude form new x-y coordinates on the strength-duration curve. The strength-duration curve allows the calculation of the chronaxie for that part of the nervous system being stimulated. Chronaxie is defined as the time on a strength-duration curve at twice the rheobase current. Rheobase current is the current required to elicit a response when a very long pulse length is used.

This preparation was also used to map the activity in the NPTh in response to ipsilateral vagal lobe stimulation. Several recording tracks were made across a transverse plane through the region of the NPTh. On each track a Neurolog NL02 recording electrode was advanced down through the hypothalamus in 100 μm steps by means of a hydraulic microdrive. Evoked activity in response to 16 stimulating pulses was recorded at each 100 μm step. Finally in order to mark the course of each electrode track a small electrolytic lesion (50 μC) was made at the top and bottom of each track. These lesions allowed the subsequent histological location of recording tracks. At the end of the experiment animals were sacrificed immediately by anaesthetic overdose, the brain was removed and processed for light microscopy according to the technique outlined in section II of this thesis. A total of 31 animals were used in these experiments.

2. Electrical stimulation of the hindbrain facial lobe

This preparation was used to perform the evoked activity latency measurements and the evoked activity mapping as described above. In order to carry out these experiments the facial lobe was stimulated on one side of the mid-line ipsilateral to the recorded sites in the hypothalamus. The stimulation parameters were the same as those used for vagal lobe stimulation (constant current pulses of 100 μA amplitude and 2 ms duration at a frequency of 1 Hz), as were the stimulating electrodes. A total of 7 animals were used in these experiments.

3. Electrical stimulation of the telencephalon

This preparation was used to investigate the effects of concurrent telencephalic stimulation on activity evoked in the NPTh in response to ipsilateral vagal lobe stimulation. Initially ipsilateral vagal lobe stimulation was used to locate evoked activity within the NPTh. With a recording electrode remaining at the site of the vagally elicited response the vagal lobe stimulation was turned off and a further set of stimulating electrodes were introduced into the ipsilateral telencephalon. Electrical stimulation was applied to the telencephalon via the newly implanted electrodes, these electrodes were moved to successive sites in the telencephalon until evoked activity was recorded from the electrode in the NPTh at the site of the vagally evoked activity. In this way it was possible to locate sites within the NPTh at which activity could be evoked in response to ipsilateral stimulation of both telencephalon and vagal lobes. It was thus possible to investigate the effects of concurrent telencephalic and vagal lobe stimulation on the evoked activity at a common site within the NPTh. The parameters used for both vagal and telencephalic stimulation were as described in 1 above. A total of 8 animals were used in these experiments.

RESULTS

As has been described elsewhere in this thesis, activity evoked in the NPTh in response to electrical stimulation of both the facial and the vagal lobes is highly characteristic. The main features of the extracellularly recorded evoked response are its long latency, its long duration and its large amplitude (in some instances up to 0.5 mV peak to peak). Due to its large amplitude it was often possible to extracellularly record the evoked signal over a wide area. Broadly speaking the evoked response is comprised of a large number of rapid (approximately 1 ms duration) positive-going spike events. The polarity of the evoked response remained constant when the recording electrode was moved through the region of the NPTh in either the dorso-ventral or the medio-lateral planes.

1. Electrical stimulation of a hindbrain vagal lobe

Latency and evoked activity duration measurements.

The results for this experiment are summarised in table 14. Inspection of the results shows that the mean latency of the evoked response was 10.66 ms and the mean duration of the response was 58.7 ms.

The approximate distance covered by the antidromic signal in travelling from the vagal lobe to the NPTh was determined by reference to serially sectioned histological sections and was found to be in the order of 3.5 mm. The range of conduction velocities

Table 14

Animal No.	Recording track	Latency ms	Duration ms
XX12	A	8.5	65
XX24	A	10	40
XX24	B	11	50
XX24	C	9	50
XX24	D	11	55
XX25	A	15	80
XX25	B	16	80
XX26	A	9	47
XX26	B	9.5	55
XX26	C	10	47
XX26	D	15	35
XX27	A	10	40
XX27	B	11	50
XX27	C	12.5	52
XX30	A	10	80
XX30	B	9	55
XX30	C	10	72
XX31	A	7	60
XX31	B	10	60
XX31	C	9.5	80
XX31	D	11	80
	Mean	10.66	58.7

determined from this experiment were between 0.33 ms^{-1} and 0.05 ms^{-1} the slower figure relies on the assumption that the late activity seen at the end of the evoked NPTh response was elicited by the vagal lobe stimulation and was not due to any regenerative activity from within the NPTh.

Stimulus-response experiments

The results from these experiments are shown in figures 72 to 89. These figures clearly show the incremental recruitment of additional spike events into the evoked response as the stimulus amplitude is increased from 10 μA to 30.5 μA. In addition a decrease in latency of the earliest peaks may be seen as the stimulus amplitude is increased this is often a feature of the antidromic activation of neurons (Lipski 1981). Most of the additional activity recruited as a result of increased stimulus amplitude occurs after the early peaks of the evoked response. There is also a considerable enhancement of the early peaks as the result of increased stimulus amplitude.

Strength-duration measurements

The strength-duration results are shown in figures 90 and 91, these results were obtained using two extracellularly recorded `spike` events taken from each of two recording tracks within the same animal.

Figure 72.

Shows 12 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 10 μA, frequency 1 Hz.

Figure 73.

Shows 14 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 13.25 μA, frequency 1 Hz.

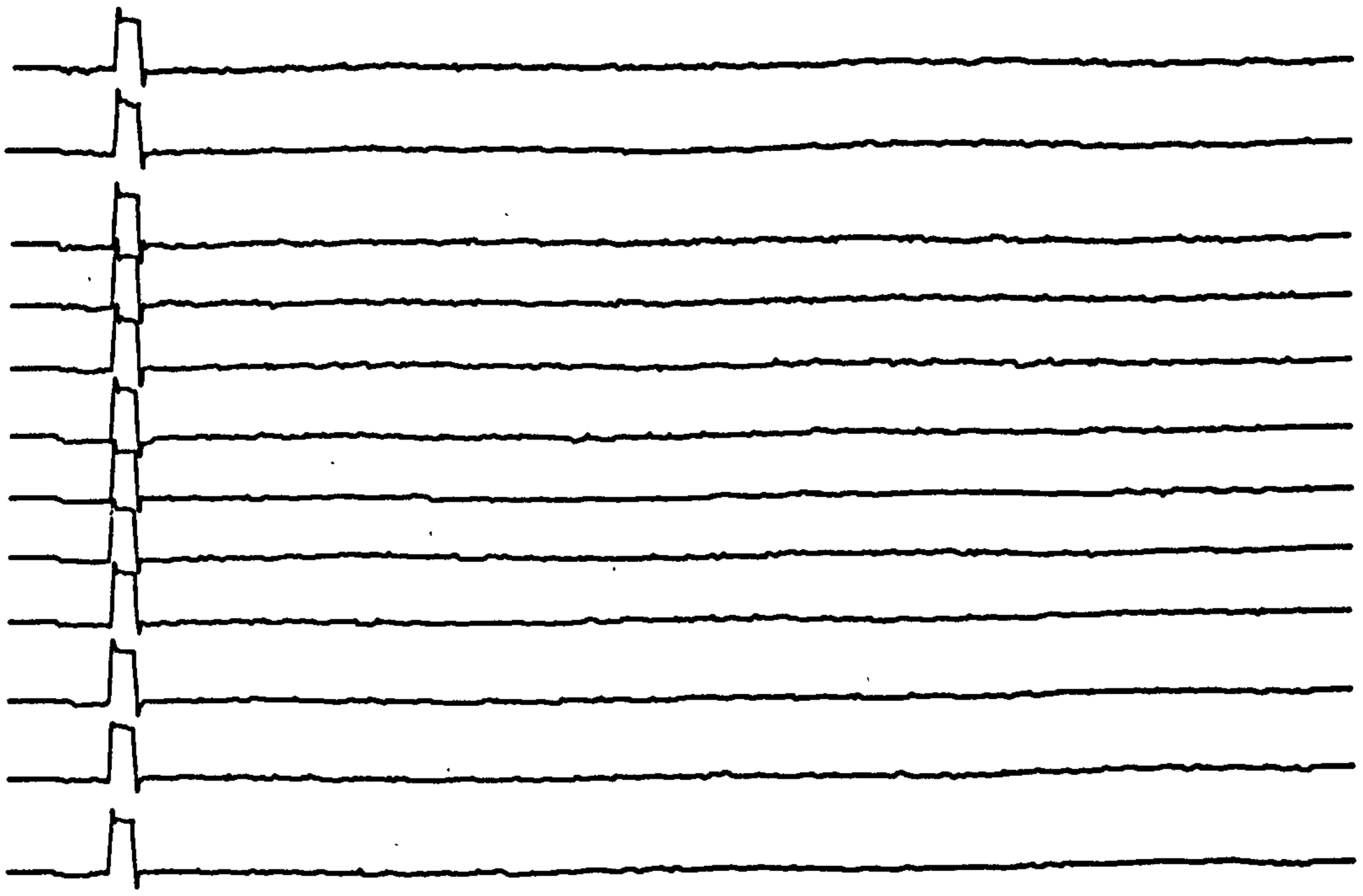

0·2 mV
2mS

Figure 74.

Shows 10 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 14.25 μA, frequency 1 Hz.

Figure 75.

Shows 10 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 15.5 μA, frequency 1 Hz.

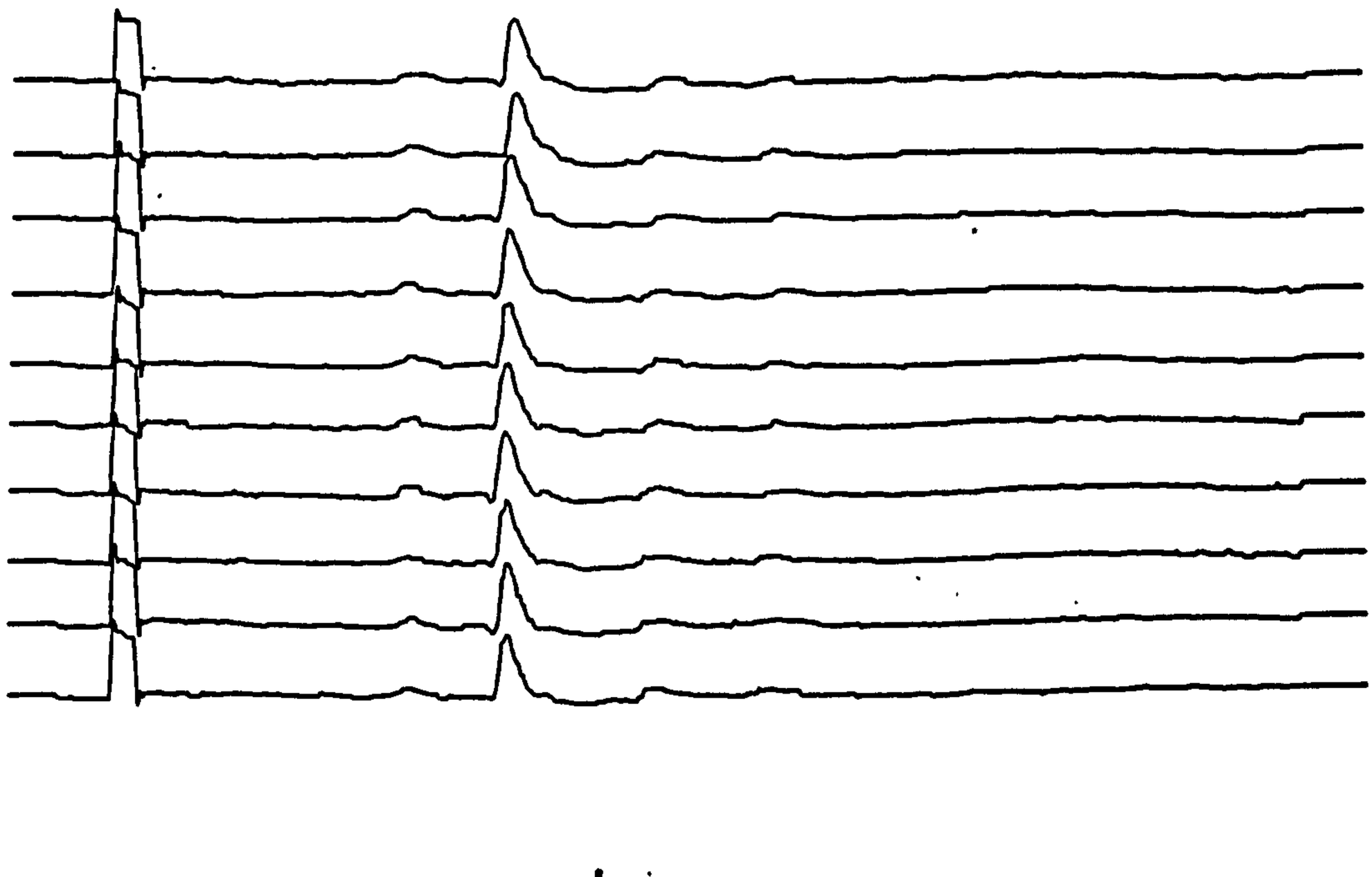

0·2 mV
2mS

Figure 76.

Shows 10 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 16.5 μA, frequency 1 Hz.

Figure 77.

Shows 10 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 17.5 μA, frequency 1 Hz.

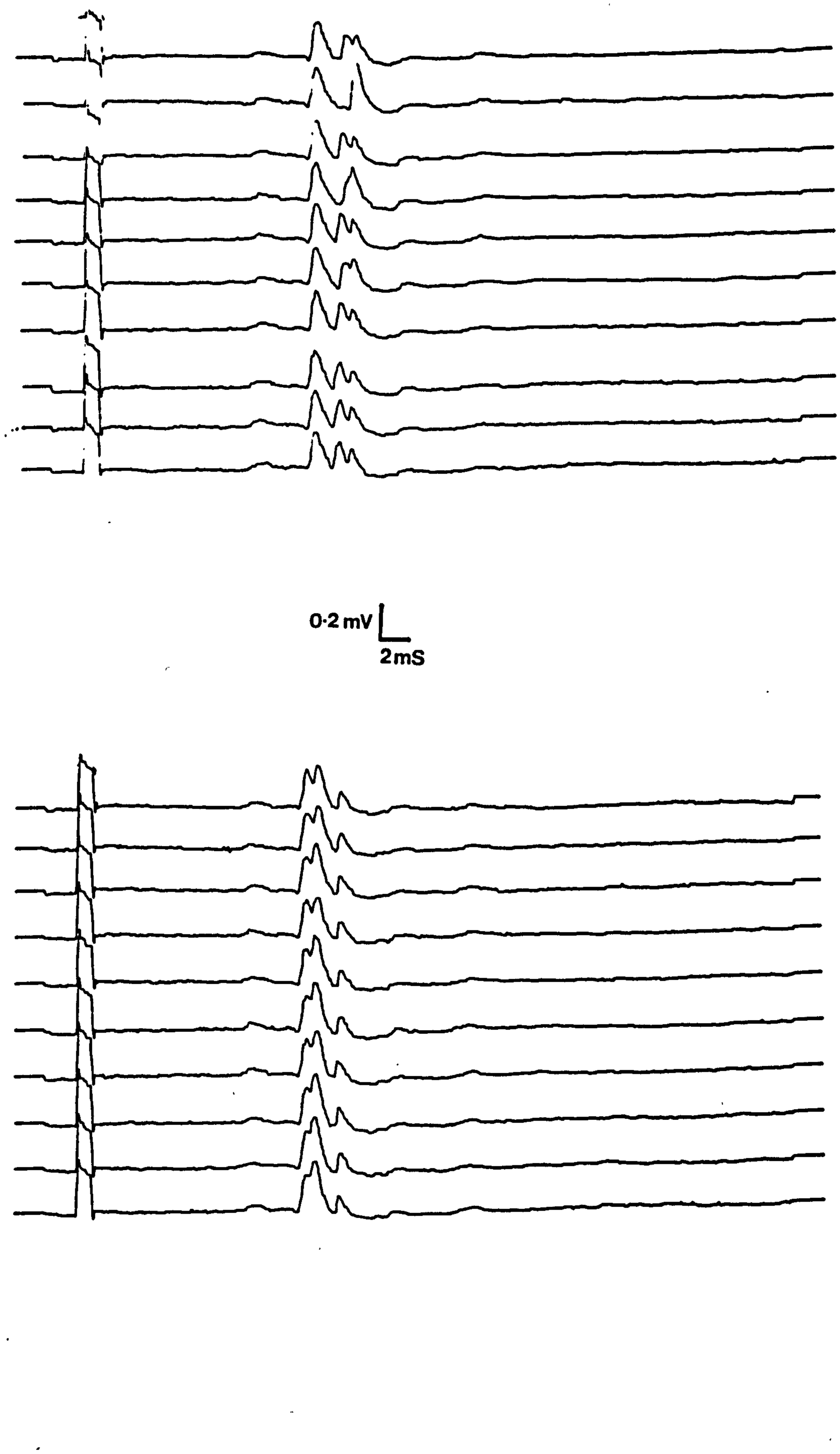

0·2 mV
2mS

Figure 78.

Shows 10 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 18.5 μA, frequency 1 Hz.

Figure 79.

Shows 10 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 19.75 μA, frequency 1 Hz.

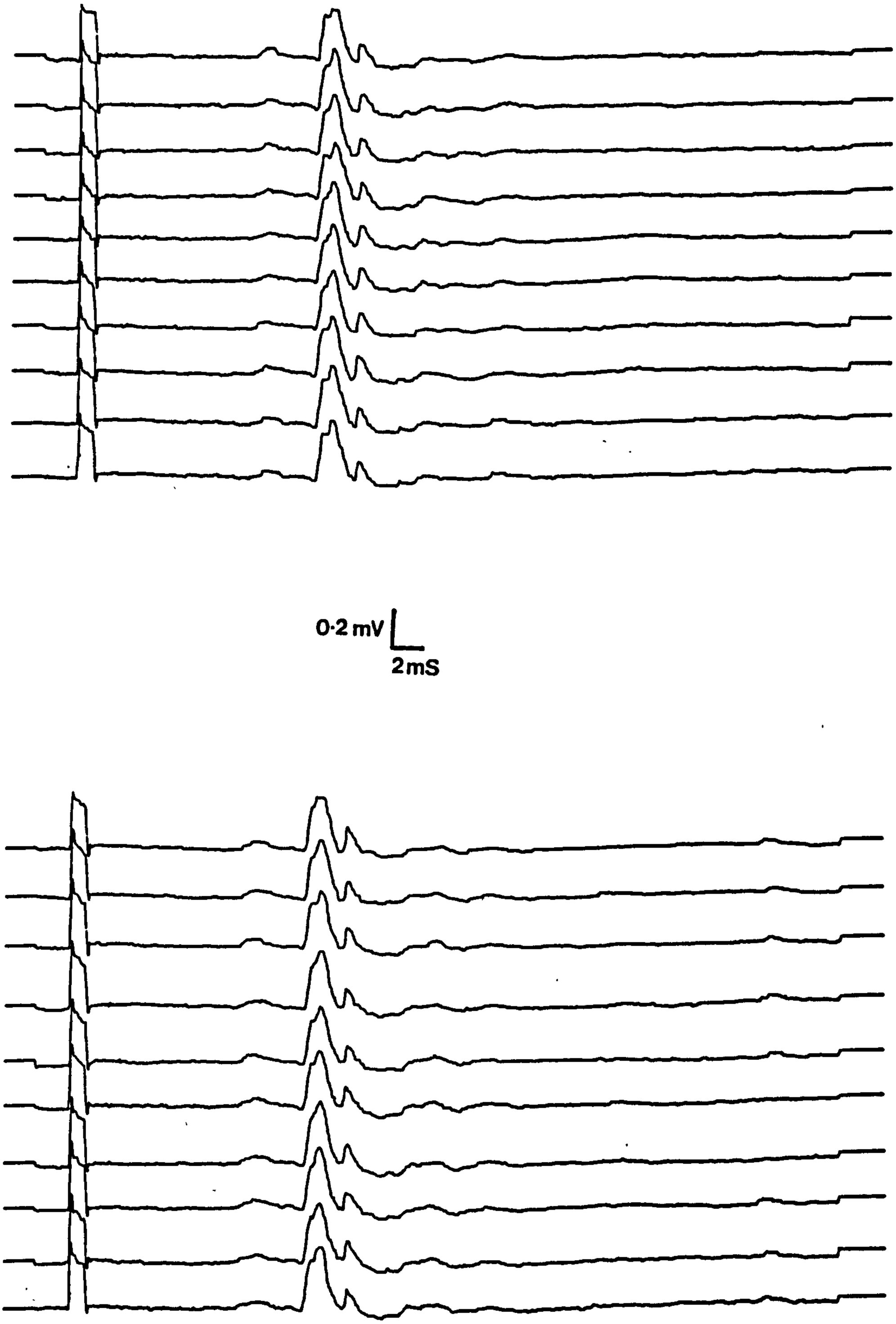

0·2 mV
2mS

Figure 80.

Shows 12 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 20.75 μA, frequency 1 Hz.

Figure 81.

Shows 14 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 21.75 μA, frequency 1 Hz.

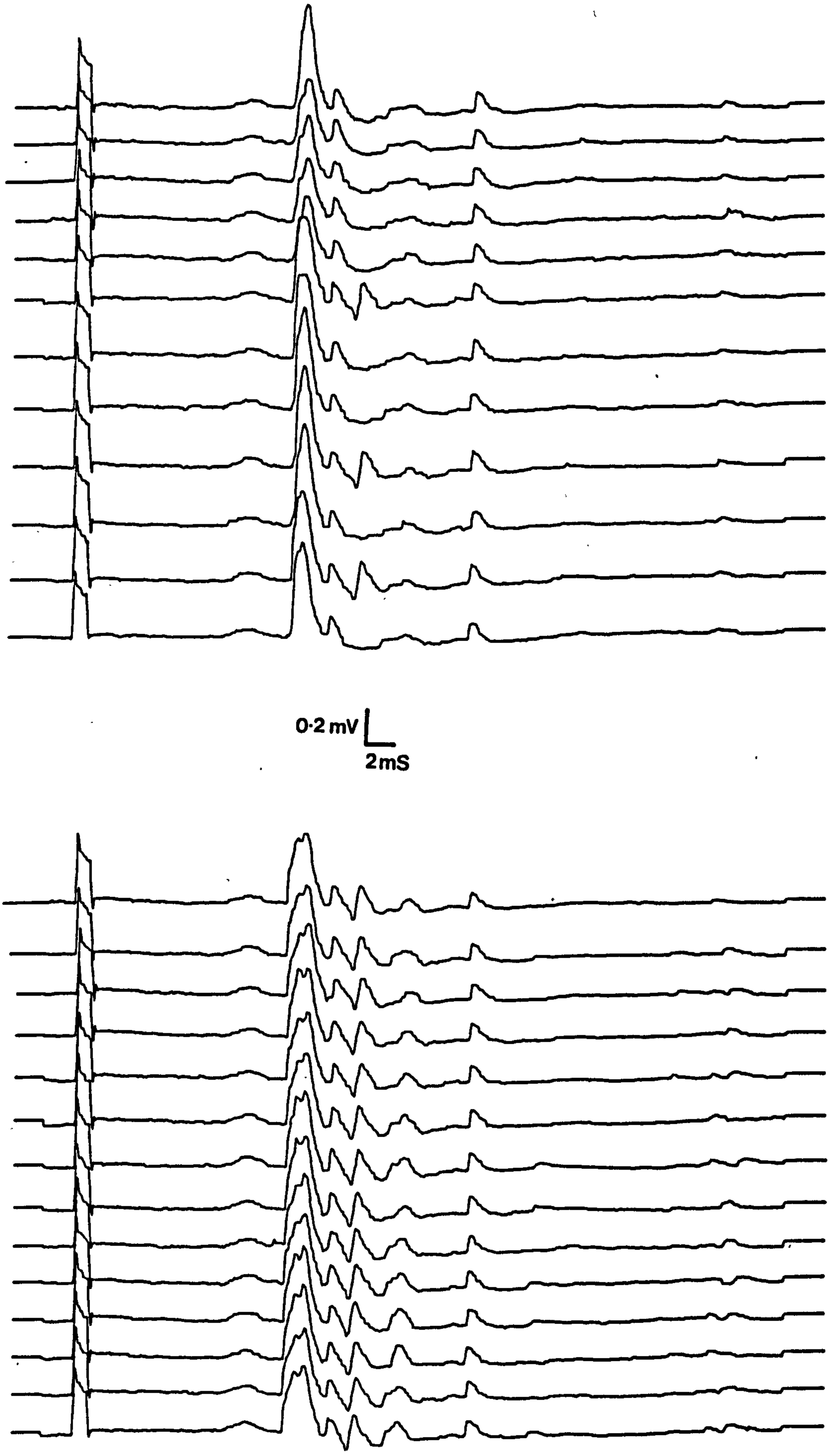

0·2 mV
2mS

Figure 82.

Shows 15 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 23 μA, frequency 1 Hz.

Figure 83.

Shows 16 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 24 μA, frequency 1 Hz.

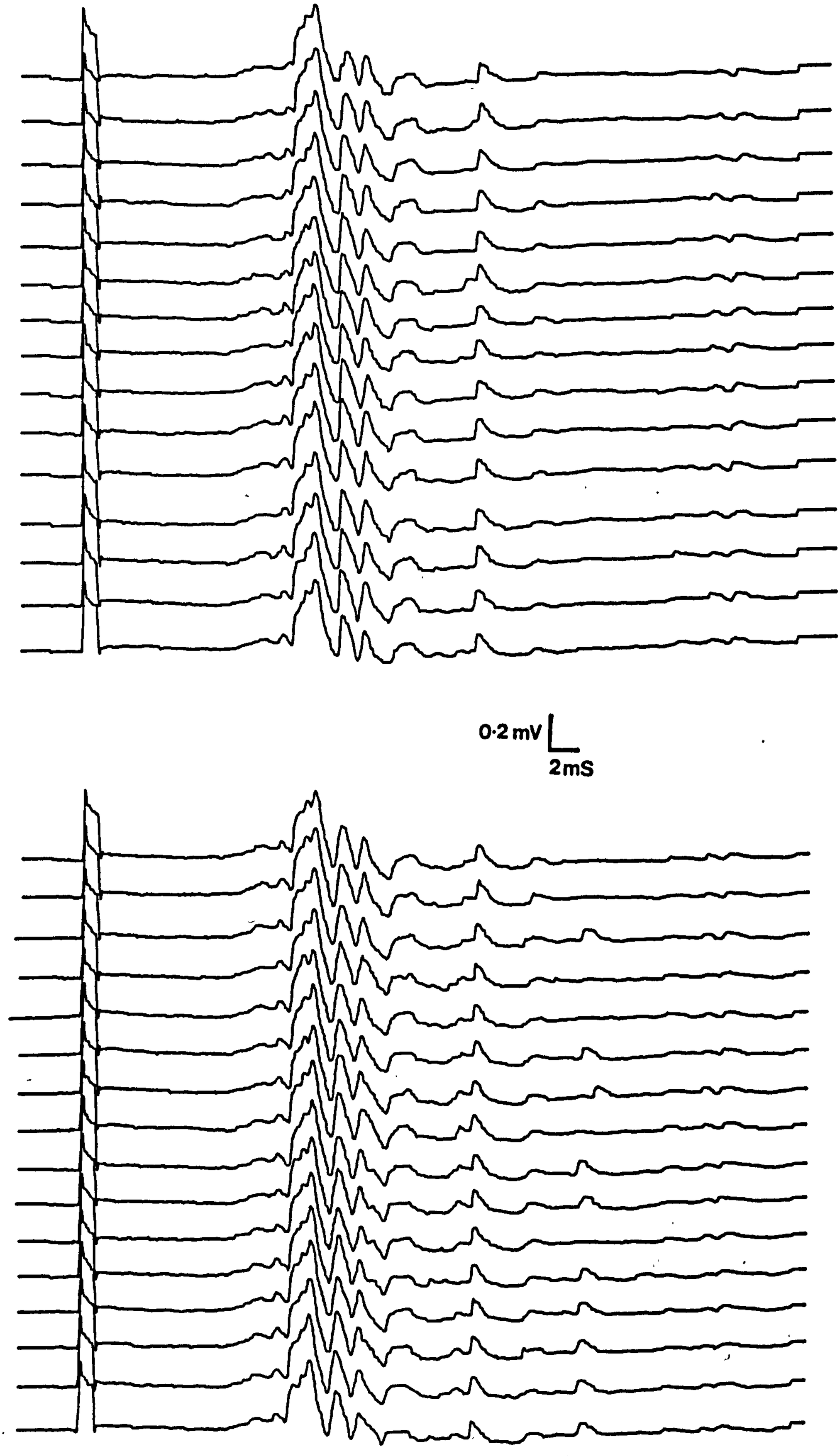

0·2 mV
2mS

Figure 84.

Shows 7 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 25 μA, frequency 1 Hz.

Figure 85.

Shows 10 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 26.25 μA, frequency 1 Hz.

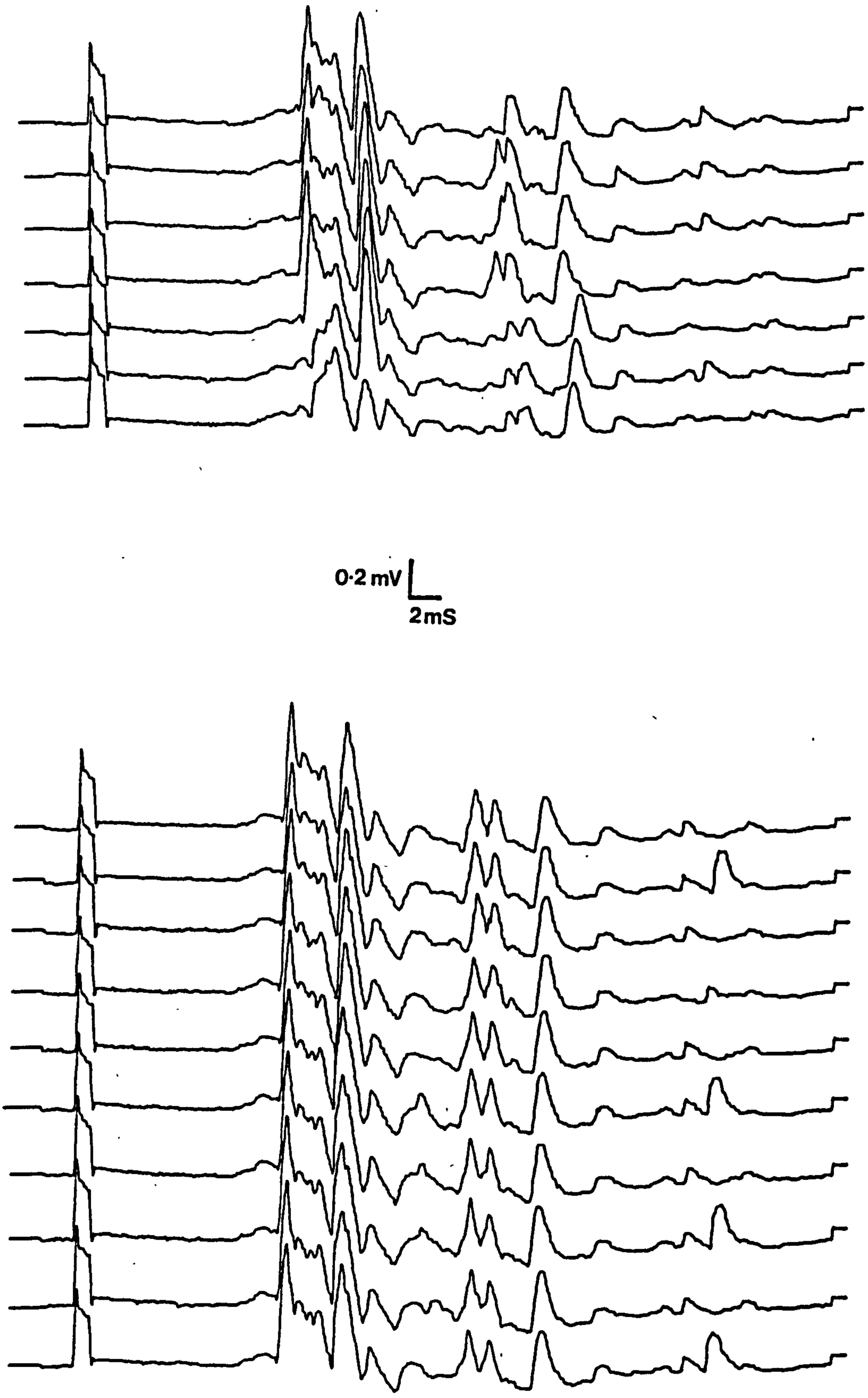

0·2 mV
2mS

Figure 86.

Shows 8 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 27.25 μA, frequency 1 Hz.

Figure 87.

Shows 8 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 28.25 μA, frequency 1 Hz.

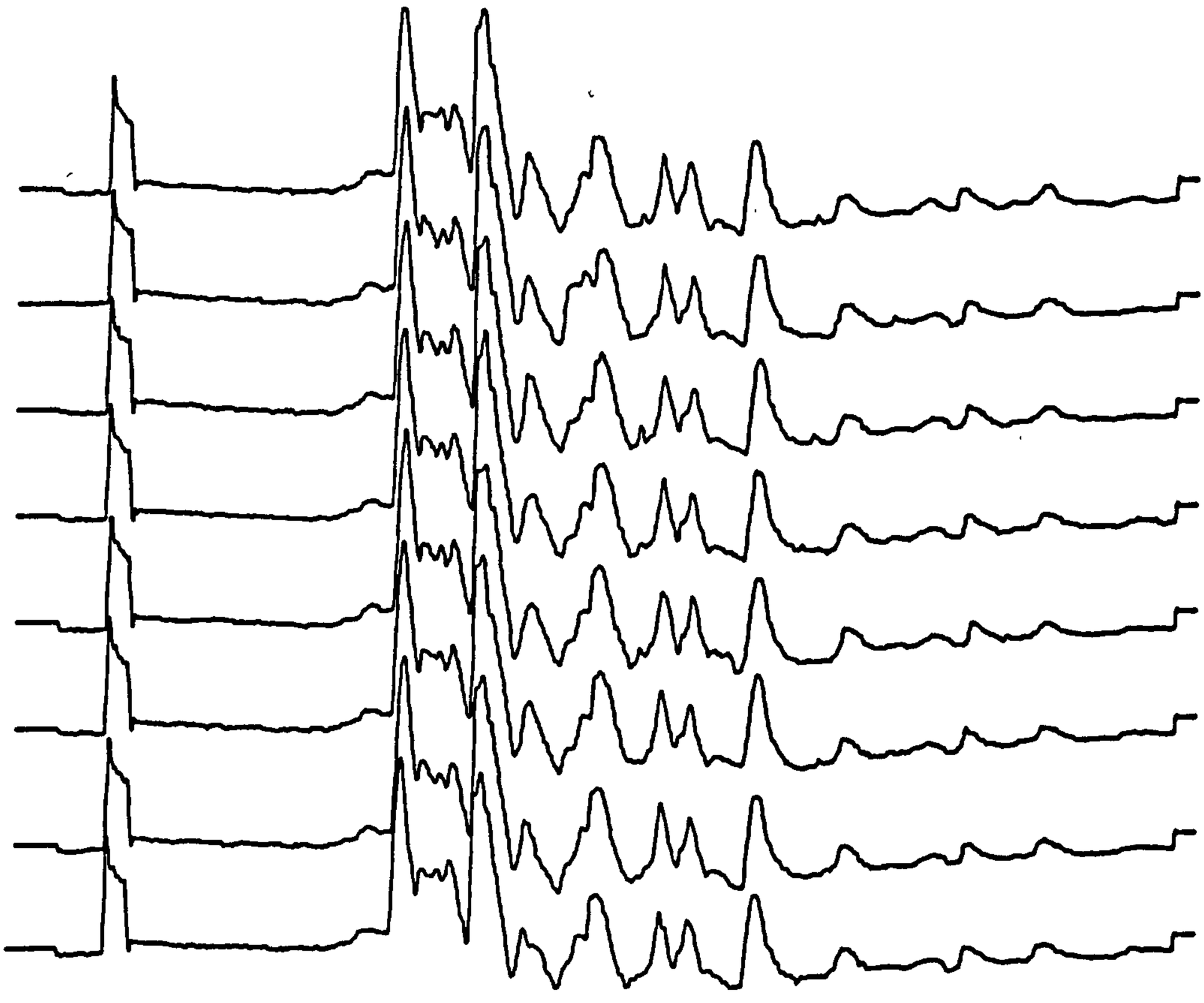

0·2 mV
2mS

Figure 88.

Shows 8 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 29.5 μA, frequency 1 Hz.

Figure 89.

Shows 8 traces recorded from the nucleus posterior thalamicus in response to electrical stimulation of the ipsilateral vagal lobe. Stimulation was via bipolar electrodes, and stimulus parameters were: pulse width 2 ms, pulse amplitude 30.5 μA, frequency 1 Hz.

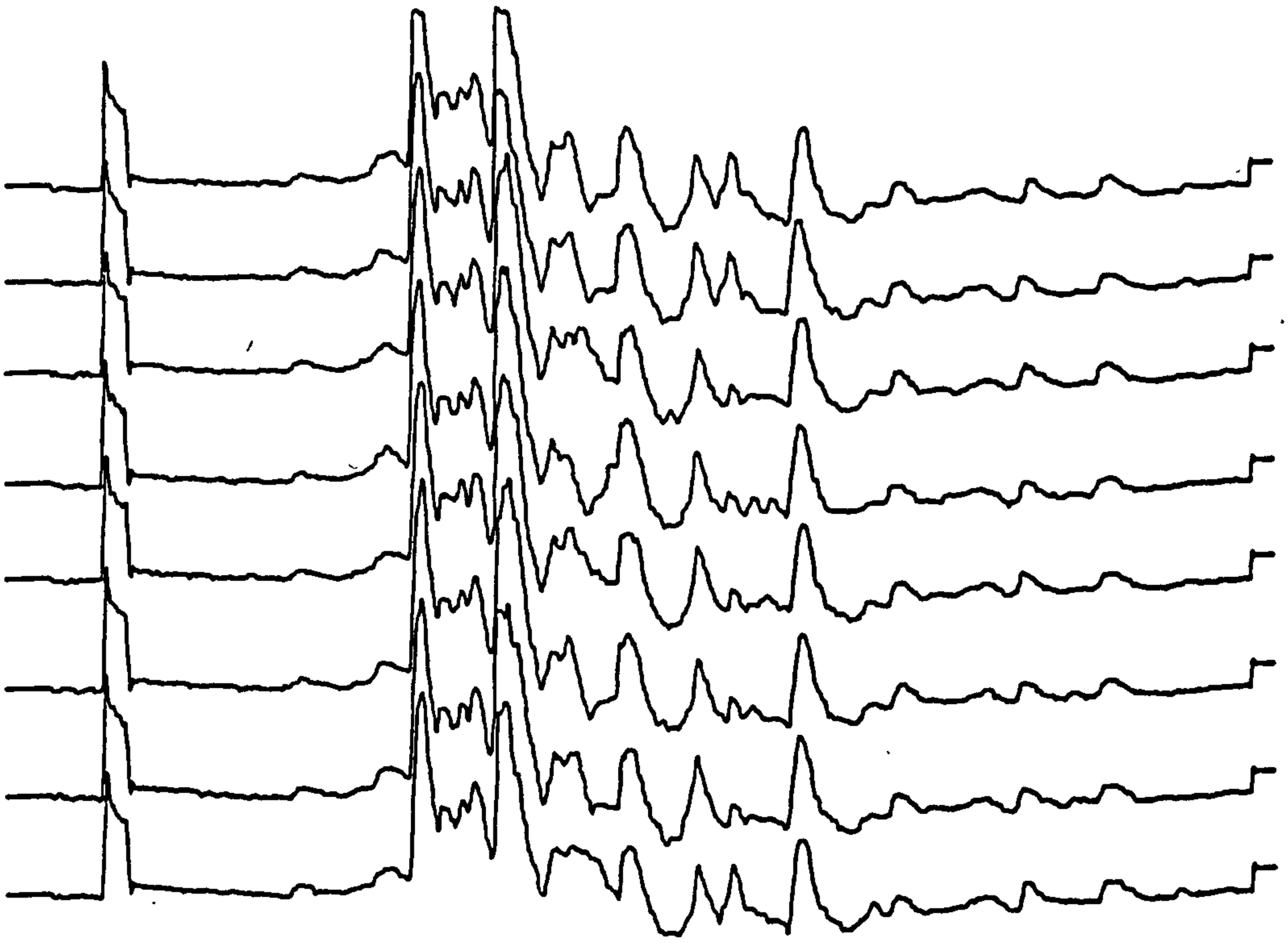

0·2 mV
2mS

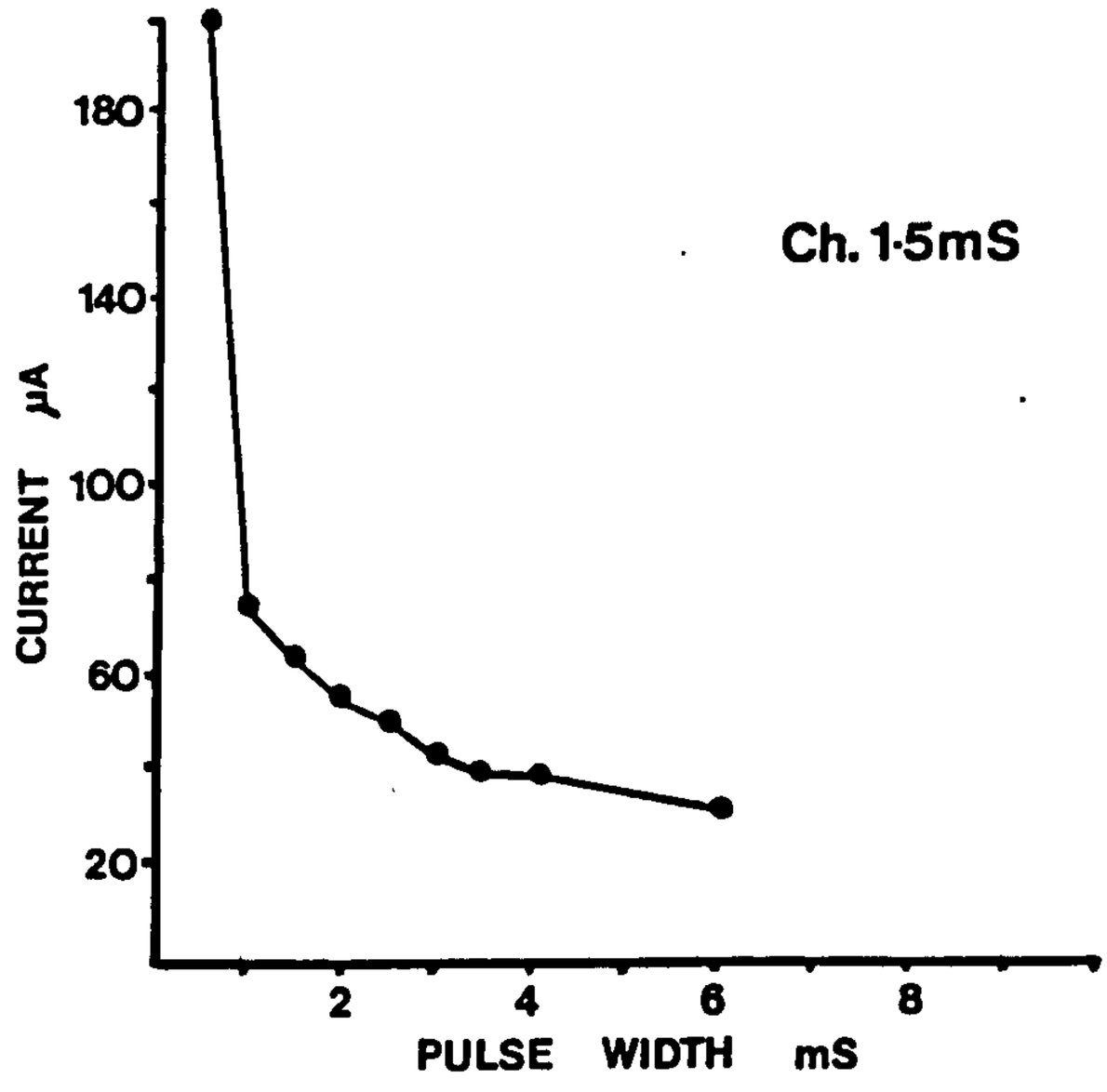
Figure 90.

Shows the strength-duration relationship for two extracellularly recorded spike events from electrode track A in fish XX10. The value of the calculated chronaxie (Ch.) is quoted on each graph.

XX10

Track A

Unit 1



Unit 2

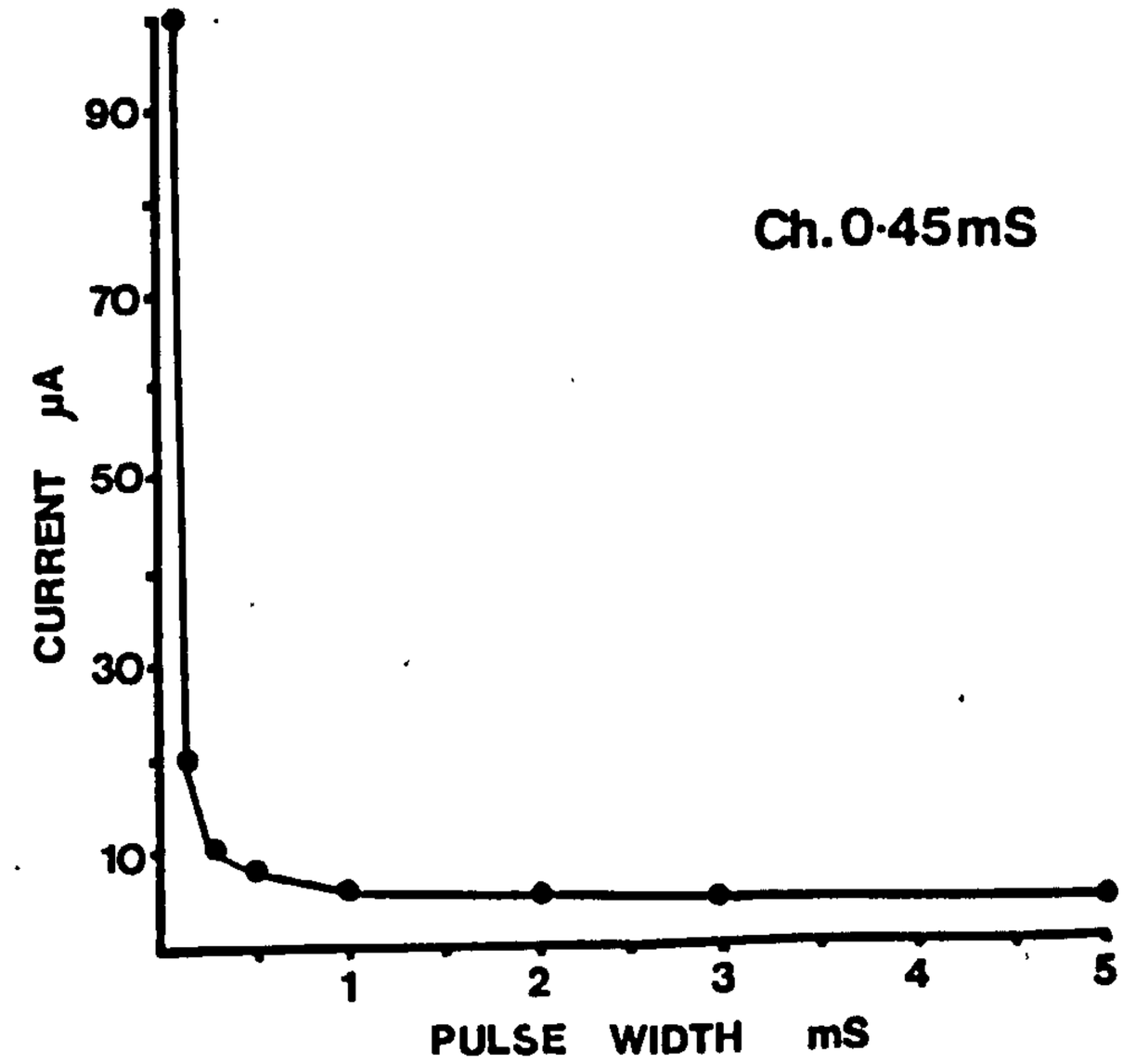


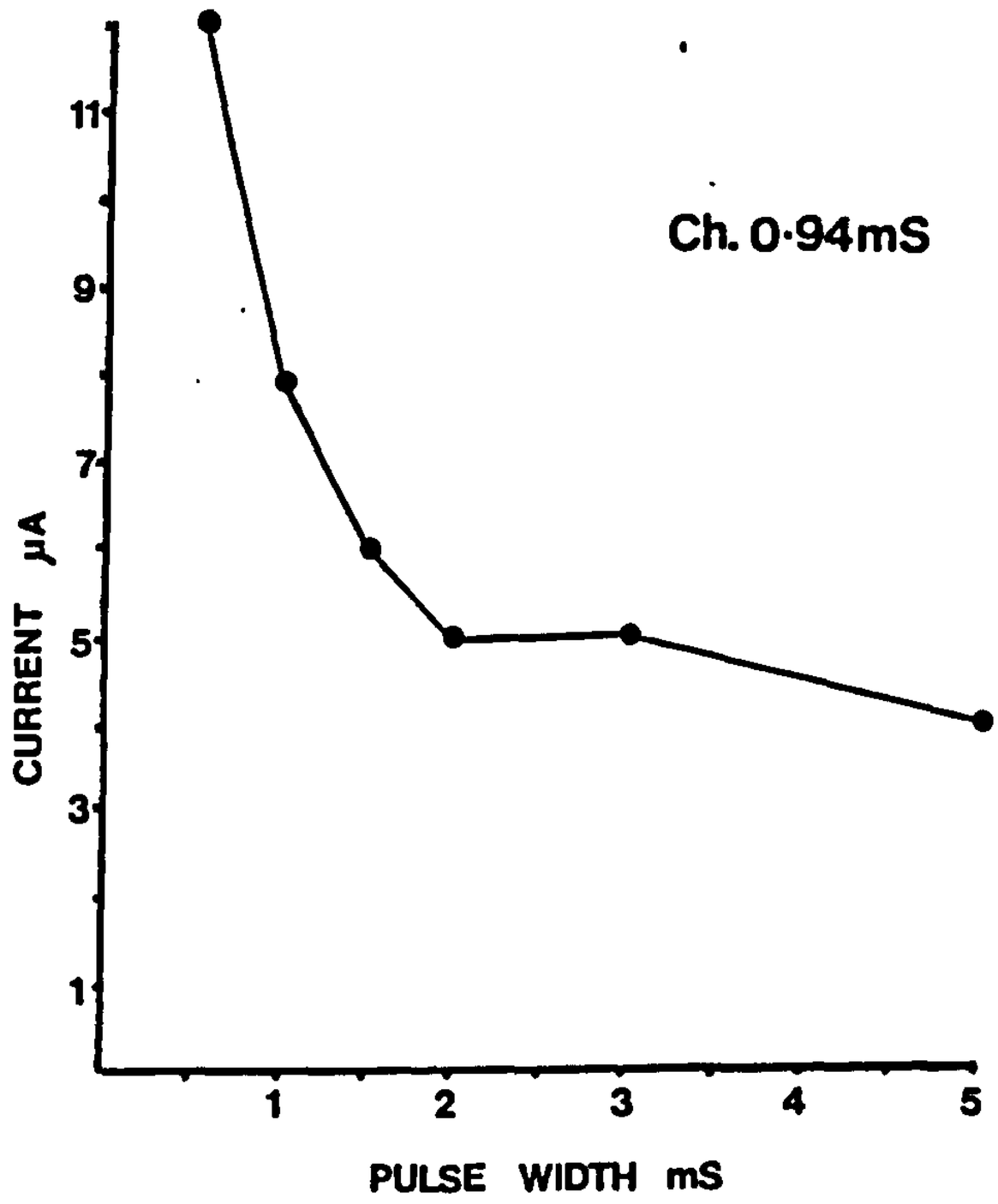
Figure 91.

Shows the strength-duration relationship for two extracellularly recorded spike events from electrode track B in fish XX10. The value of the calculated chronaxie (Ch.) is quoted on each graph.

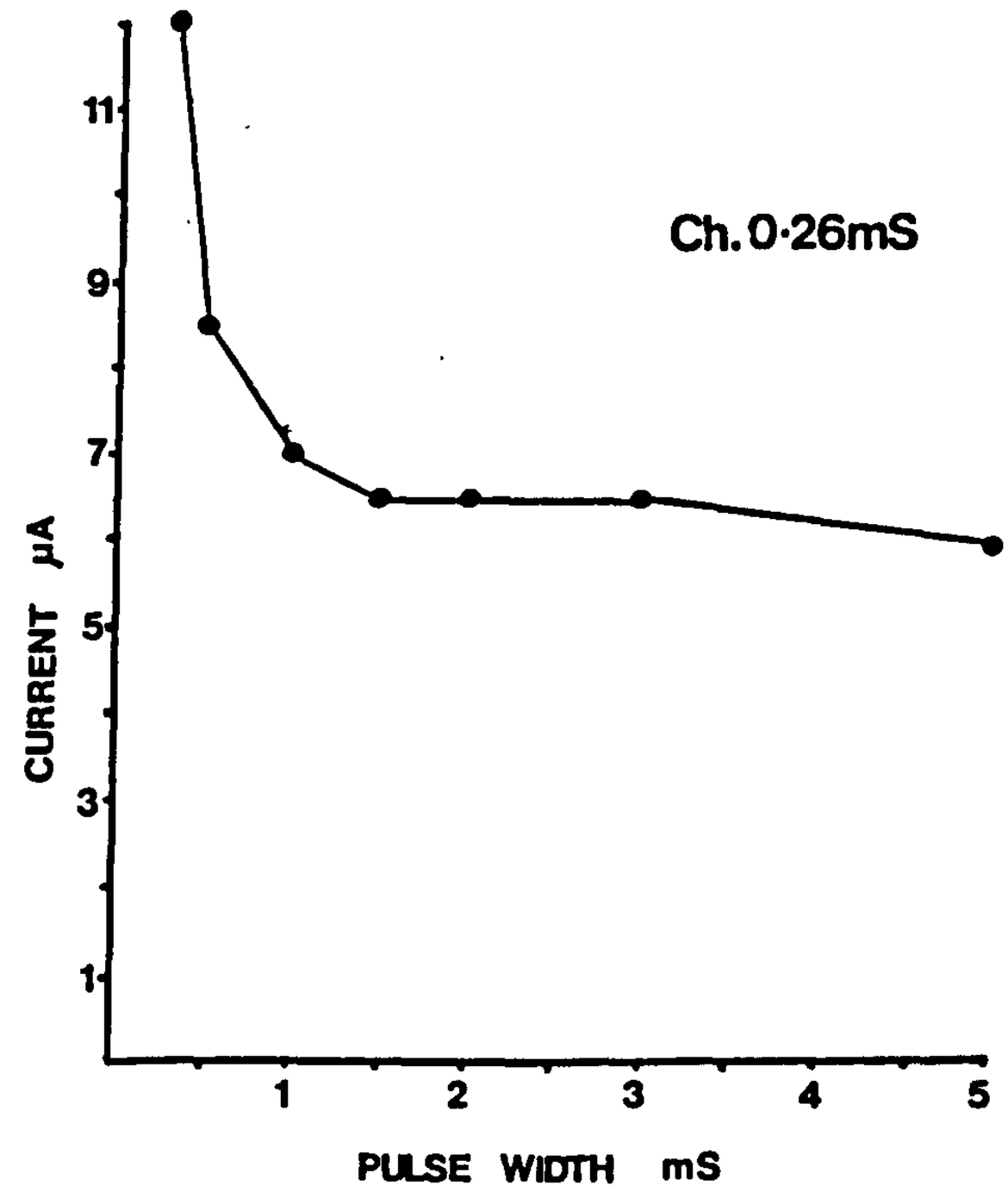
XX10

Track B

Unit 1



Unit 2



Evoked activity mapping

The mapping of evoked activity within the NPTh in response to vagal lobe stimulation is shown in figures 92 to 95. Figure 92 shows a diagrammatic representation of the right half of the brain of animal XX29 at the level of the NPTh. Figures 93 to 95 show the signals recorded at the marked points on the electrode tracks in figure 92.

Figure 92.

Part of a transverse section through the diencephalon at the level of the Nucleus posterior thalamicus (NPTh). The vertical lines marked A C E represent the lines of successive electrode tracks through the region. Evoked responses to ipsilateral vagal lobe stimulation were recorded at each marked point on each track. Numbers show the depth in mm from the tectal surface. LR, lateral recess of the III ventricle, NPG, nucleus preglomerulosus, NSPg, nucleus sub-preglomerulosus.

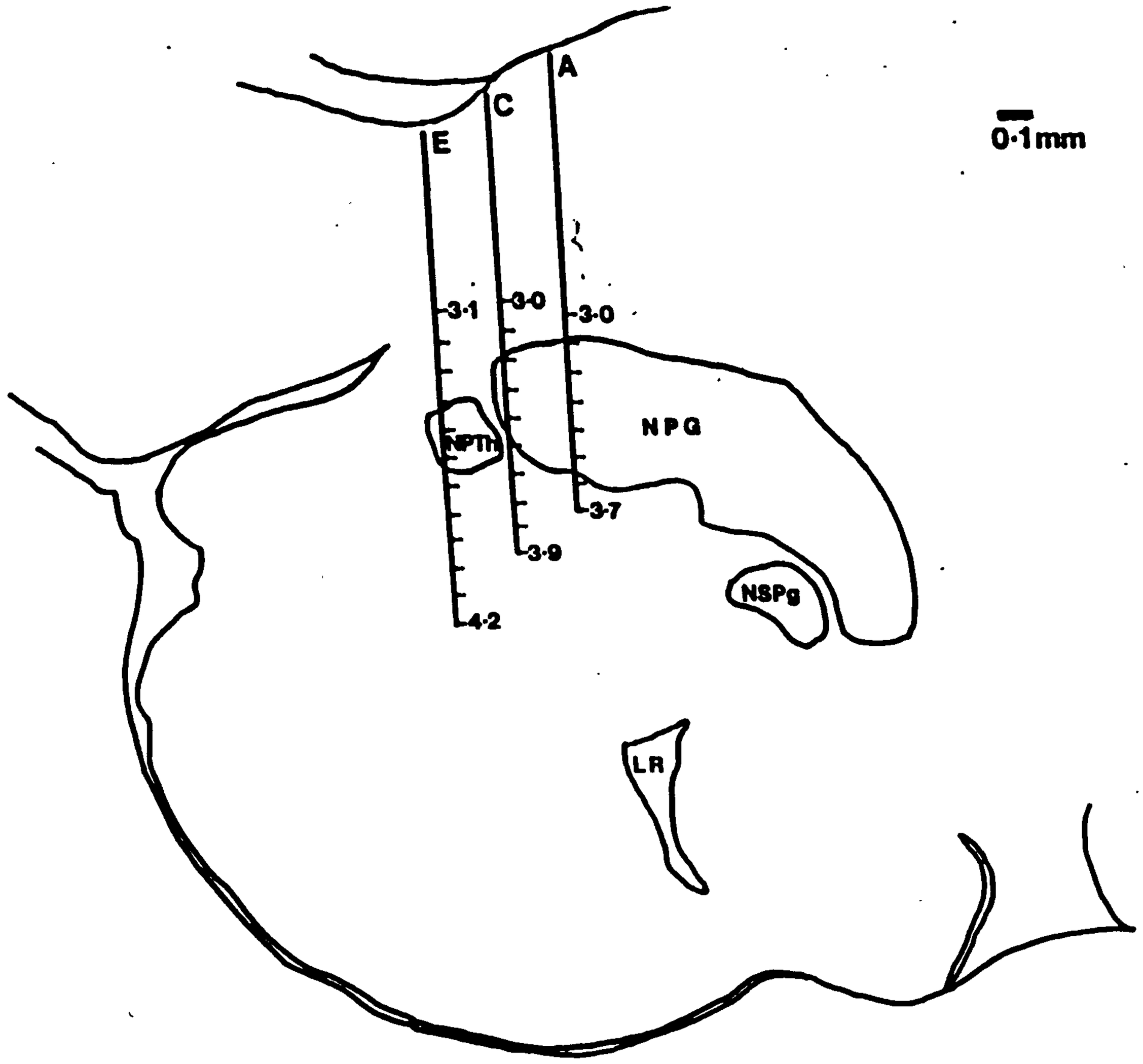

A
C
E
0·1mm
3·1
3·0
3·0
NPG
NPTh
3·7
3·9
NSPg
4·2
LR

Figure 93.

Shows the signals recorded from electrode track A in figure 92. Signals are shown from successive 0.1 mm depth increments reading from top to bottom and starting at a depth of 3.0 mm. Depth measurements are relative to the tectal surface. Scale bars are horizontal 5 ms, vertical 0.05 mv. All traces are the averaged response to 16 stimulus presentations.

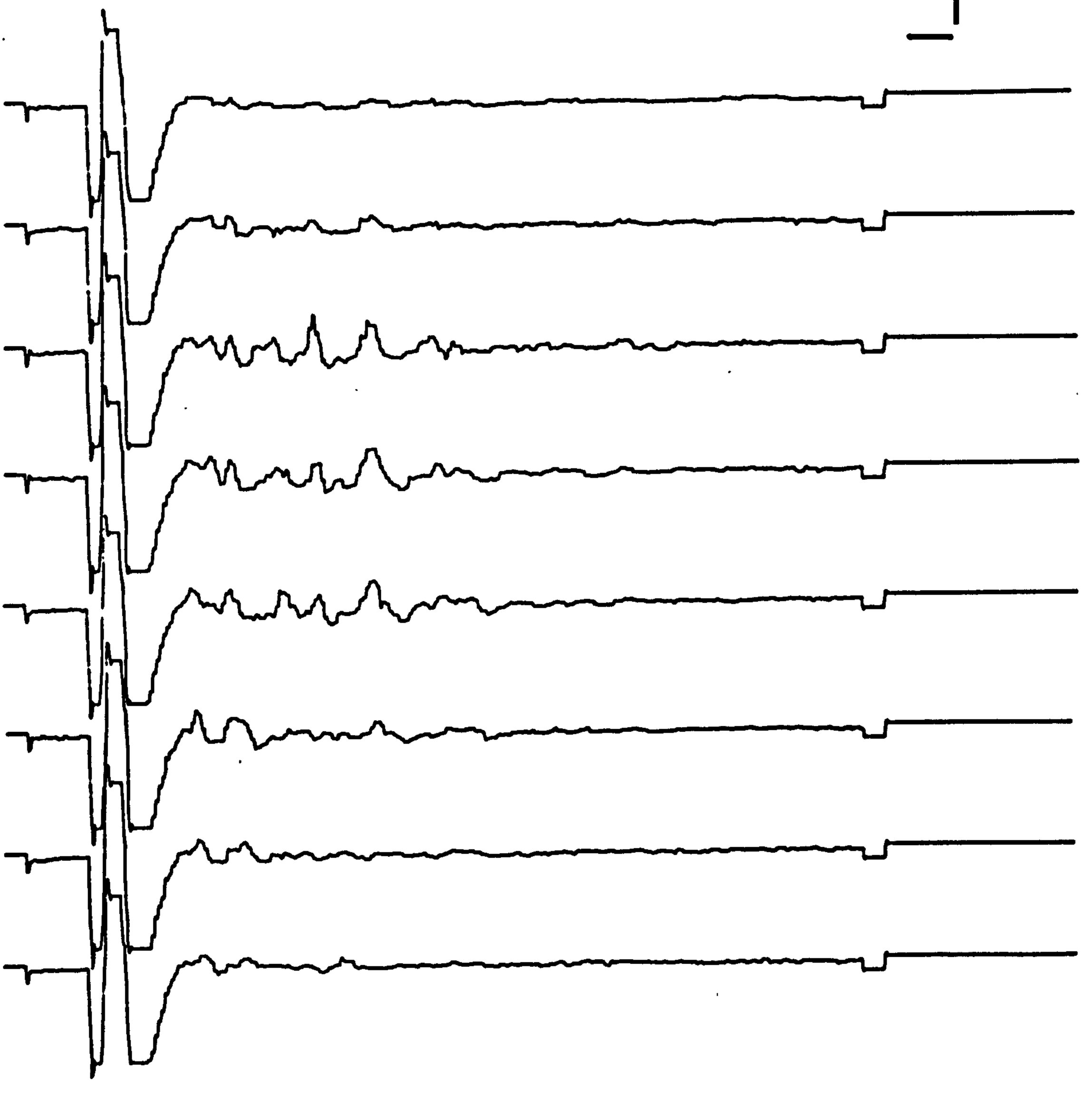


Figure 94.

Shows the signals recorded from electrode track C in figure 92. Signals are shown from successive 0.1 mm depth increments reading from top to bottom and starting at a depth of 3.0 mm. Depth measurements are relative to the tectal surface. Scale bars are horizontal 5 ms, vertical 0.05 mv. All traces are the averaged response to 16 stimulus presentations.

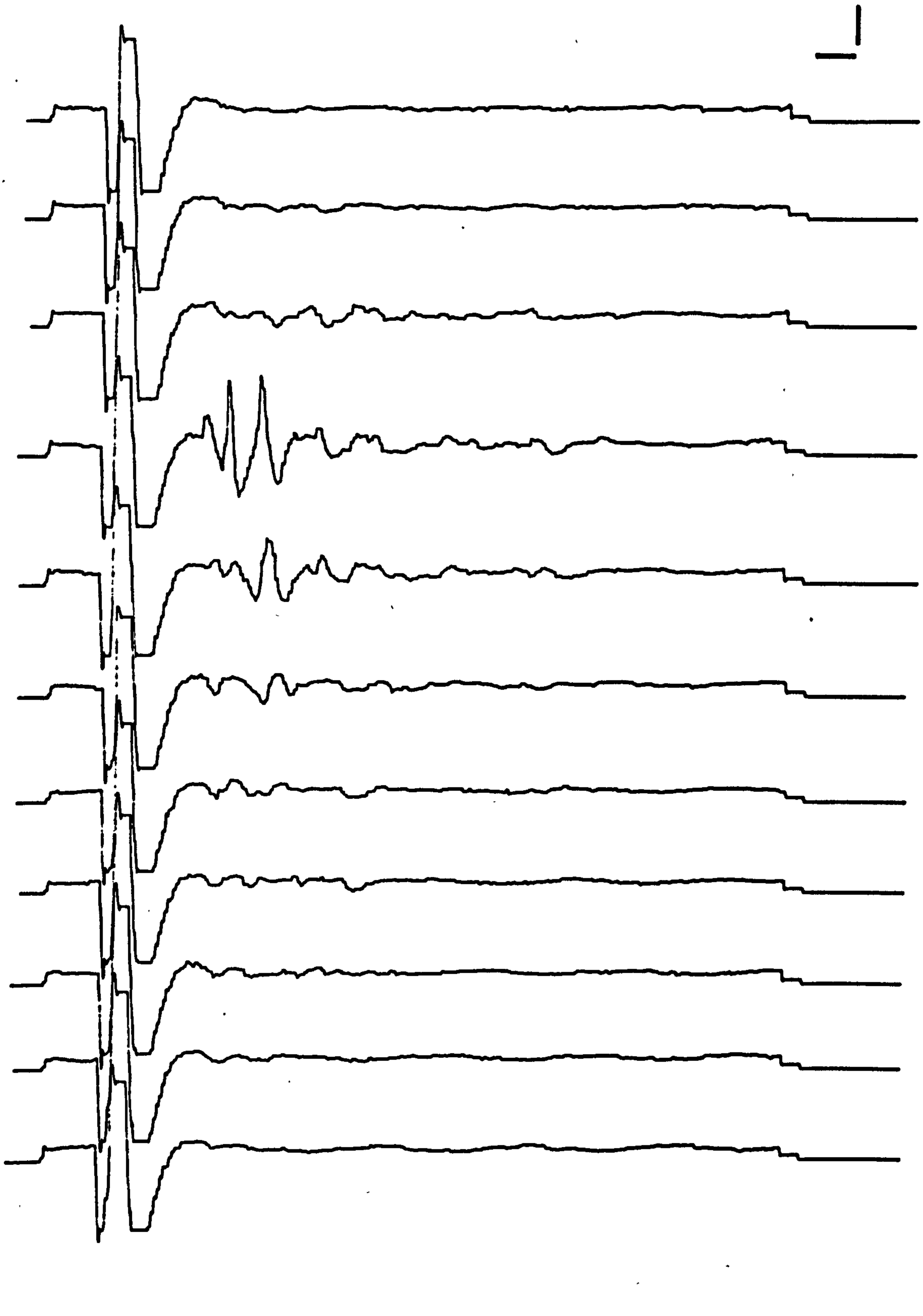


Figure 95.

Shows the signals recorded from electrode track E in figure 92. Signals are shown from successive 0.1 mm depth increments reading from top to bottom and starting at a depth of 3.0 mm. Depth measurements are relative to the tectal surface. Scale bars are horizontal 5 ms, vertical 0.05 mv. All traces are the averaged response to 16 stimulus presentations.

2. Electrical stimulation of the hindbrain facial lobe

Latency and evoked activity duration measurements.

The results for this experiment are summarised in table 15.

Inspection of the results shows that the mean latency was 14.3 ms and the mean duration was 64 ms.

Table 15

Animal No.	Recording track	Latency ms	Duration ms
XV01	C	17.5	60
XV01	D	16	70
XV01	E	16	70
XV01	F	17.5	70
XV05	A	13.5	37
XV06	A	12.5	85
XV06	B	9	75
XV06	C	12.5	45
	Mean	14.3	64

The approximate distance covered by the antidromic signal in travelling from the facial lobe to the NPTh was determined by reference to serially sectioned histological sections and was found

to be in the order of 3.5 mm. The range of conduction velocities determined from this experiment were between 0.24 ms^{-1} and 0.04 ms^{-1} the slower figure relies on the assumption that the late activity seen at the end of the evoked NPTh response was elicited by the facial lobe stimulation and was not due to any regenerative activity from within the NPTh.

Evoked activity mapping

The mapping of evoked activity within the NPTh in response to facial lobe stimulation is shown in figures 96 to 100. Figure 96 shows a diagrammatic representation of the right half of the brain of animal XV01 at the level of the NPTh. Figures 97 to 100 show the signals recorded at the marked points on the electrode tracks in figure 96.

Figure 96.

Part of a transverse section through the diencephalon at the level of the Nucleus posterior thalamicus (NPTh). The vertical lines marked C D E F represent the lines of successive electrode tracks through the region. Evoked responses to ipsilateral facial lobe stimulation were recorded at each marked point on each track. Numbers show the depth in mm from the tectal surface. LR, lateral recess of the III ventricle, NPG, nucleus preglomerulosus, NSPg, nucleus sub-preglomerulosus.

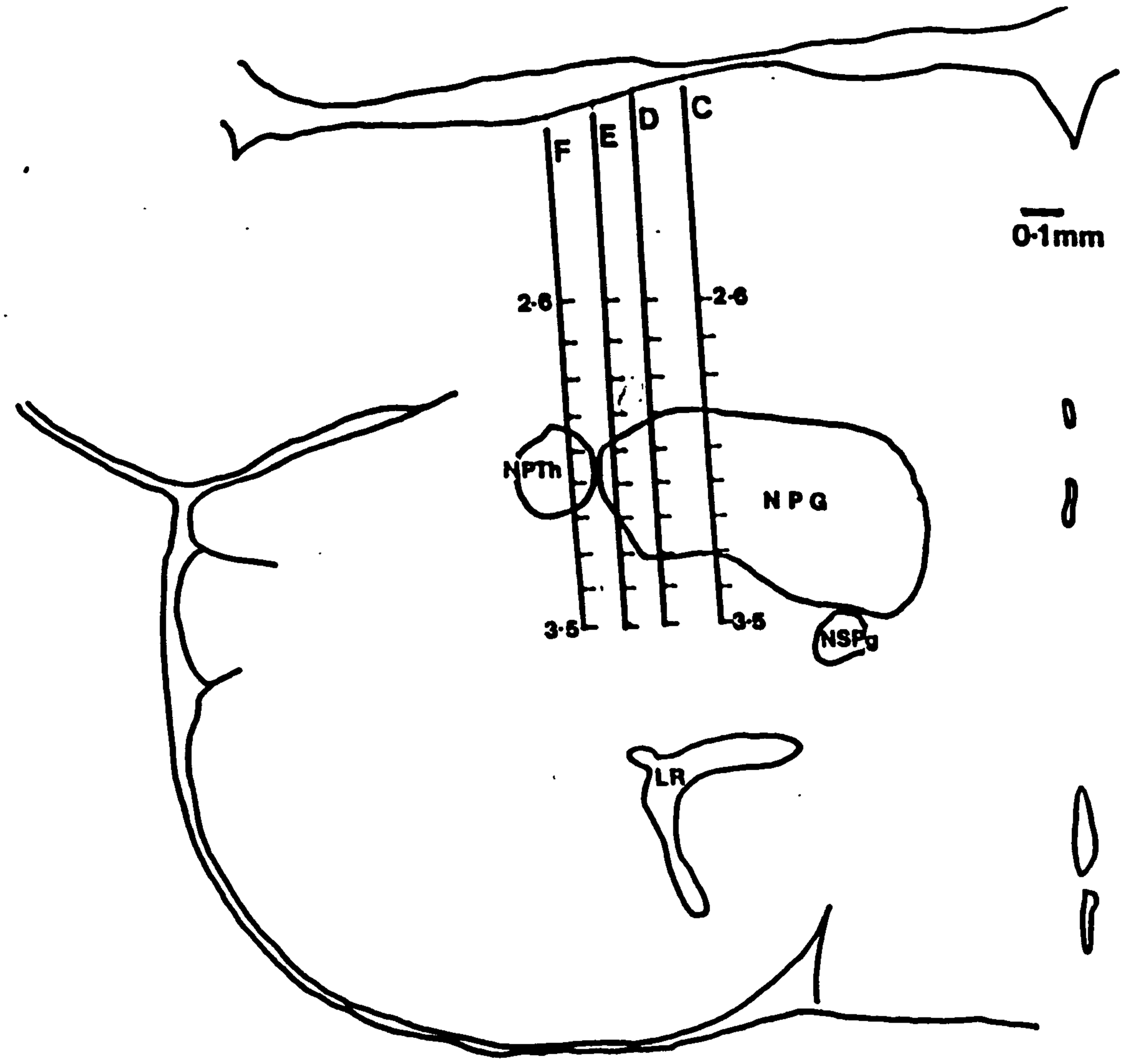

F
E
D
C
0·1mm
2·6
2·6
NPTh
NPG
3·5
3·5
NSPg
LR

Figure 97.

Shows the signals recorded from electrode track C in figure 96. Signals are shown from successive 0.1 mm depth increments reading from top to bottom and starting at a depth of 2.6 mm. Depth measurements are relative to the tectal surface. Scale bars are horizontal 5 ms, vertical 0.1 mv. All traces are the averaged response to 16 stimulus presentations.

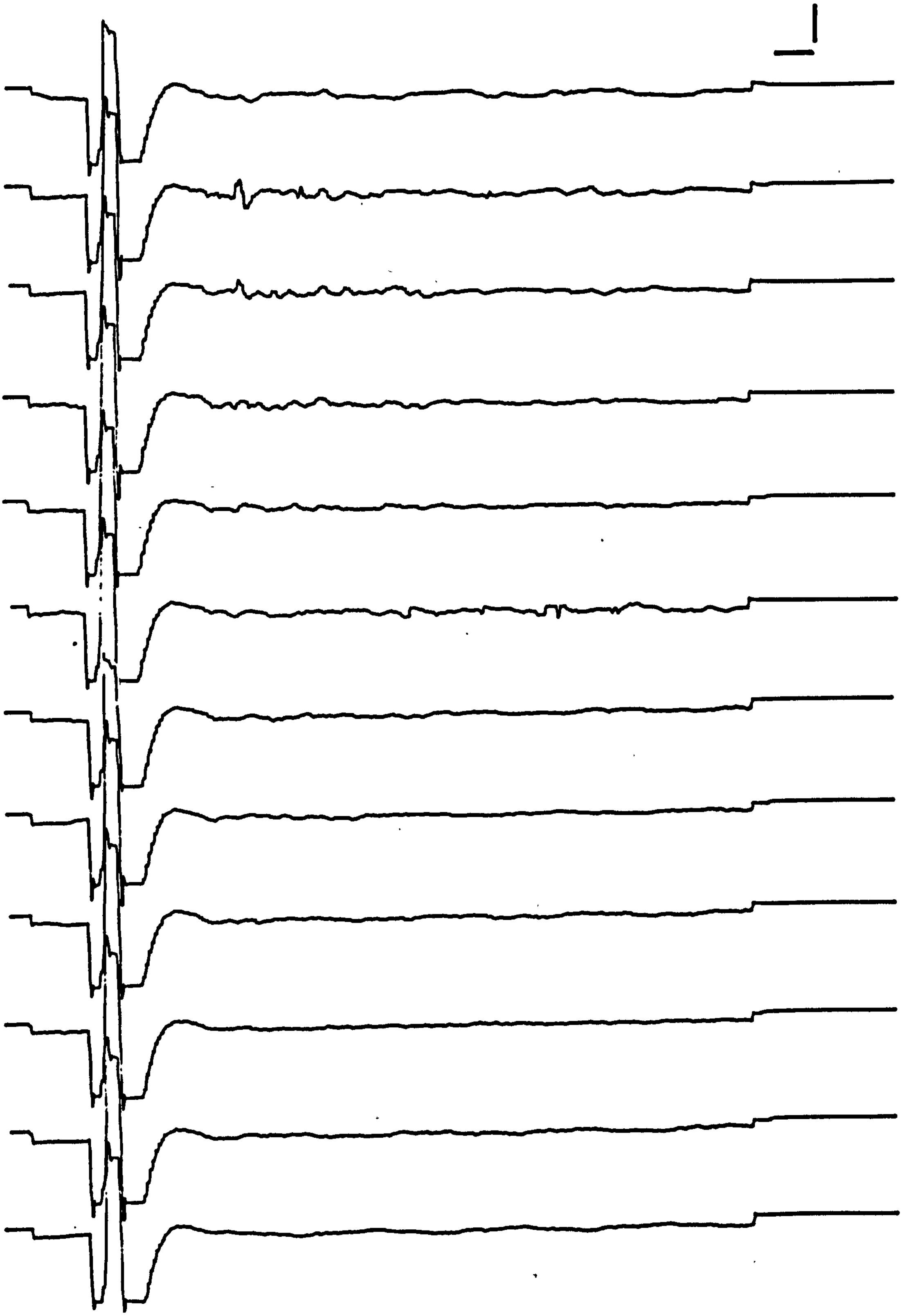


Figure 98.

Shows the signals recorded from electrode track D in figure 96. Signals are shown from successive 0.1 mm depth increments reading from top to bottom and starting at a depth of 2.6 mm. Depth measurements are relative to the tectal surface. Scale bars are horizontal 5 ms, vertical 0.1 mv. All traces are the averaged response to 16 stimulus presentations.

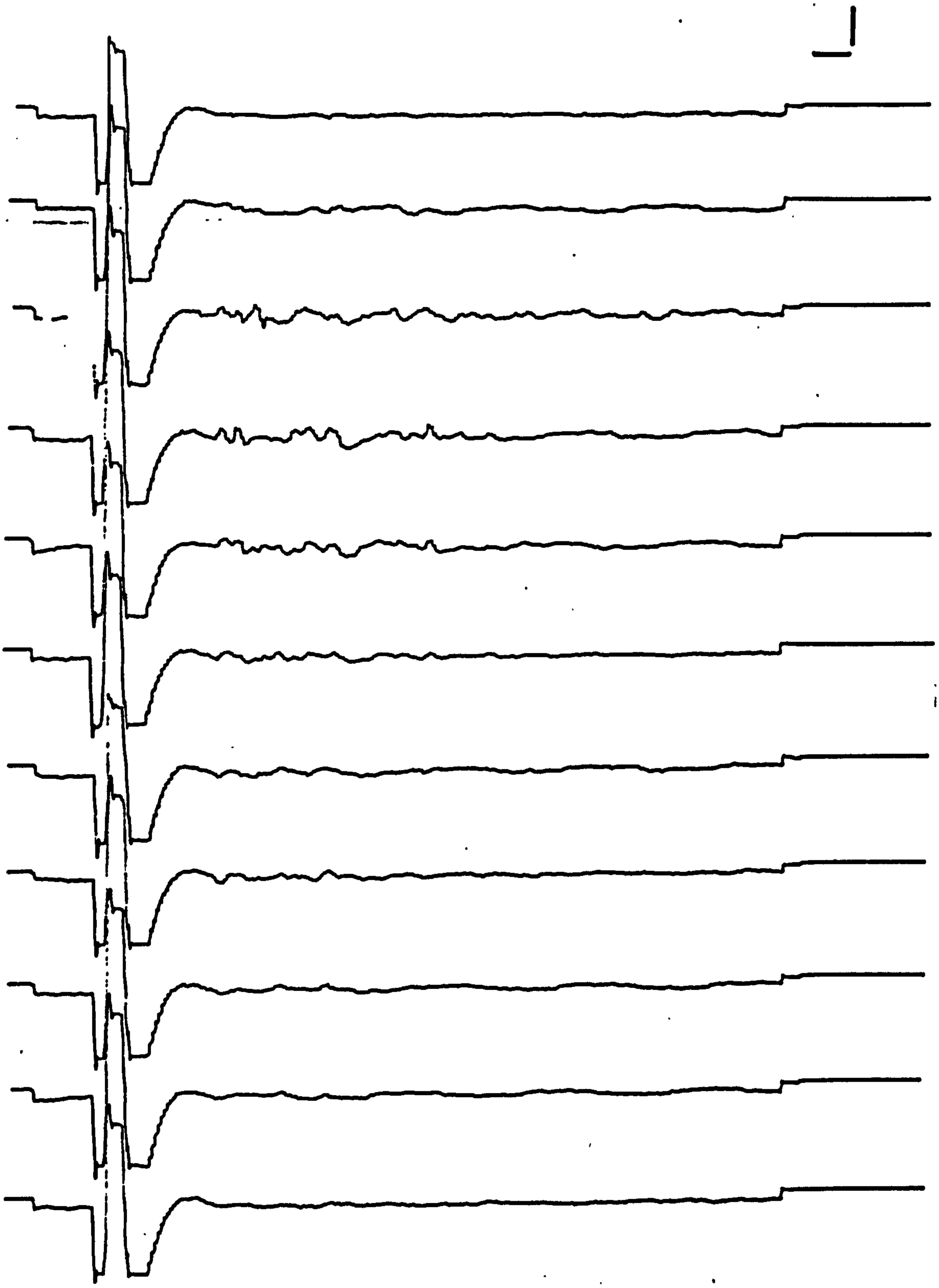


Figure 99.

Shows the signals recorded from electrode track E in figure 96. Signals are shown from successive 0.1 mm depth increments reading from top to bottom and starting at a depth of 2.6 mm. Depth measurements are relative to the tectal surface. Scale bars are horizontal 5 ms, vertical 0.1 mv. All traces are the averaged response to 16 stimulus presentations.

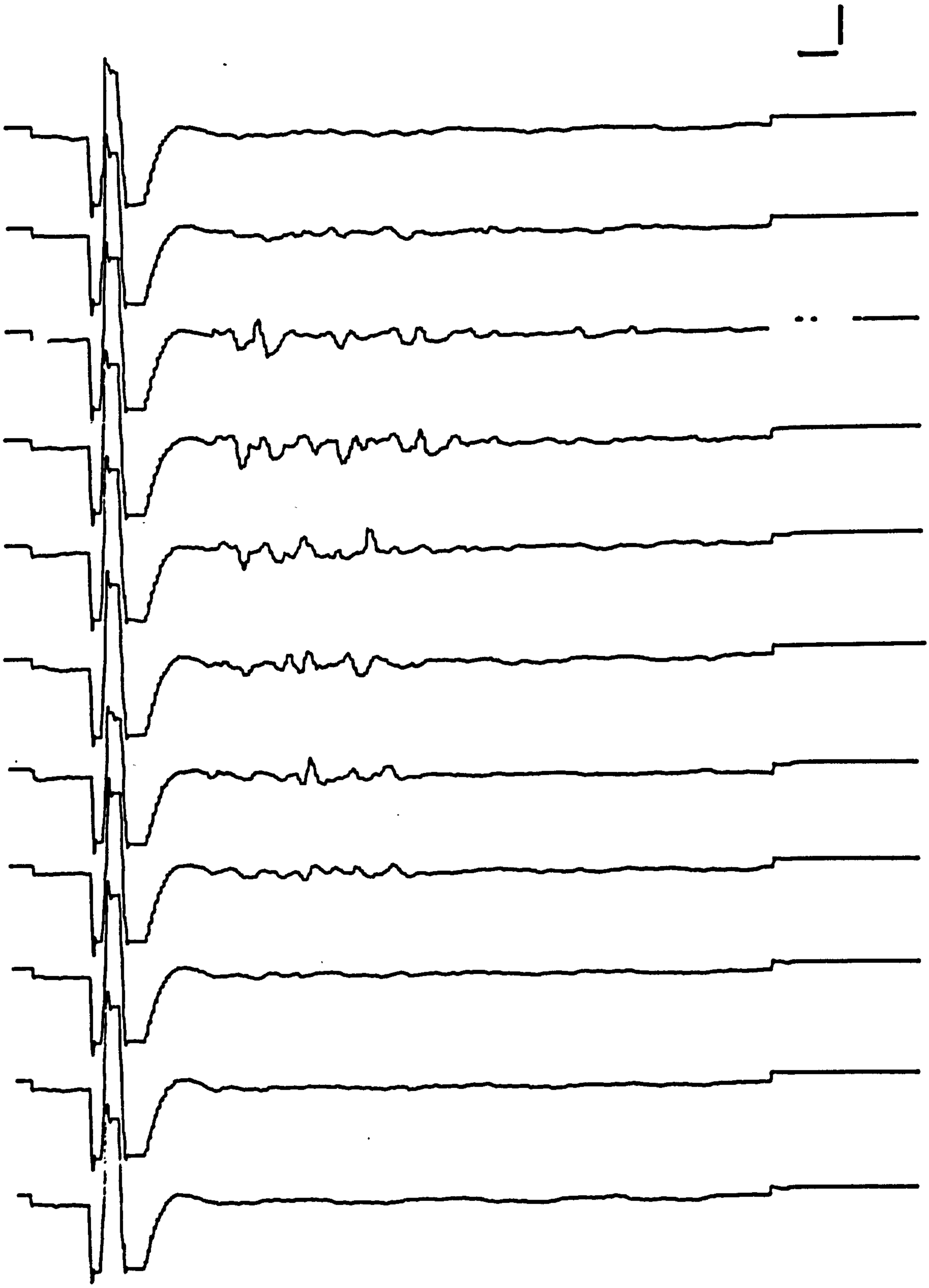
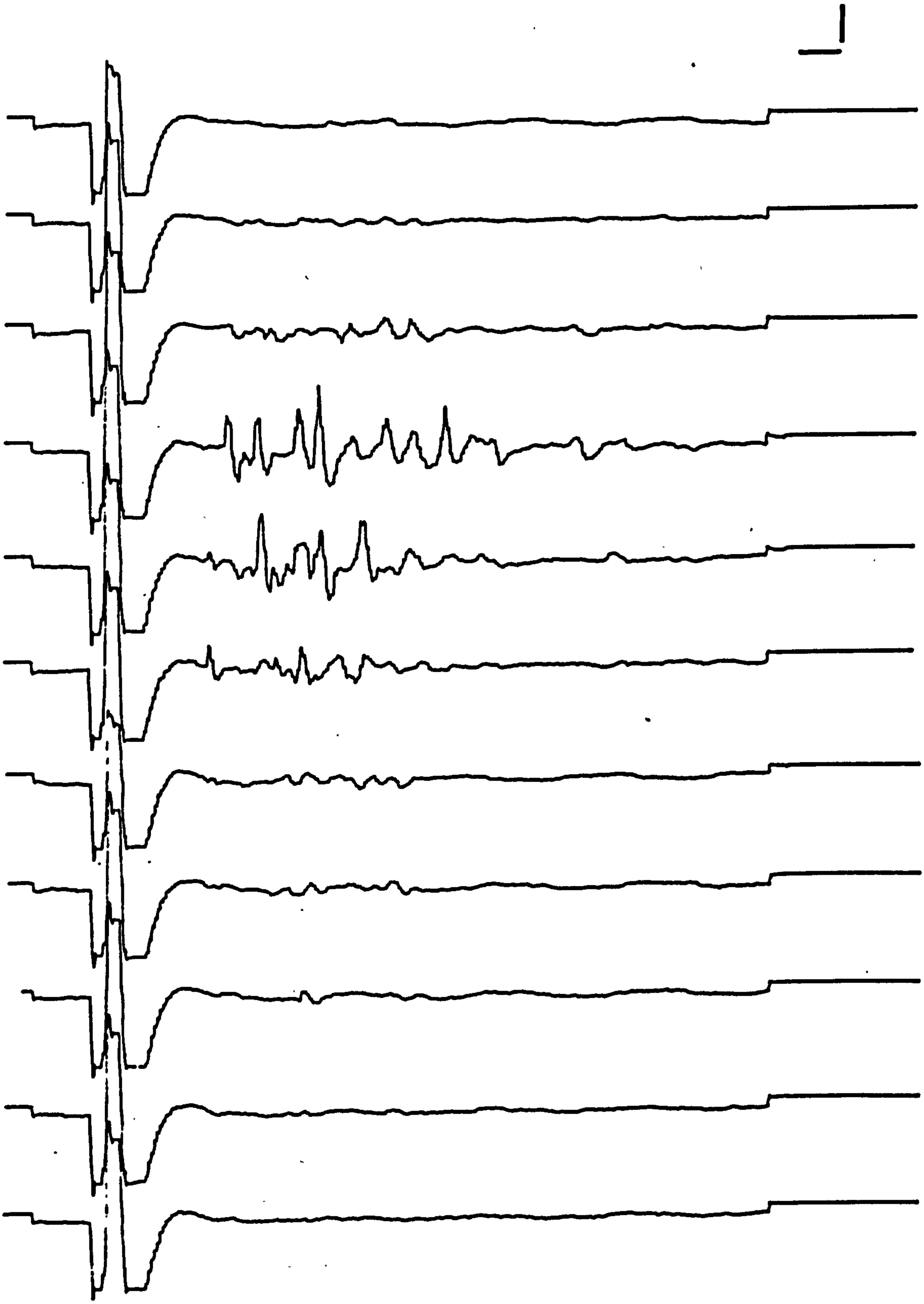


Figure 100.

Shows the signals recorded from electrode track F in figure 96. Signals are shown from successive 0.1 mm depth increments reading from top to bottom and starting at a depth of 2.6 mm. Depth measurements are relative to the tectal surface. Scale bars are horizontal 5 ms, vertical 0.1 mv. All traces are the averaged response to 16 stimulus presentations.



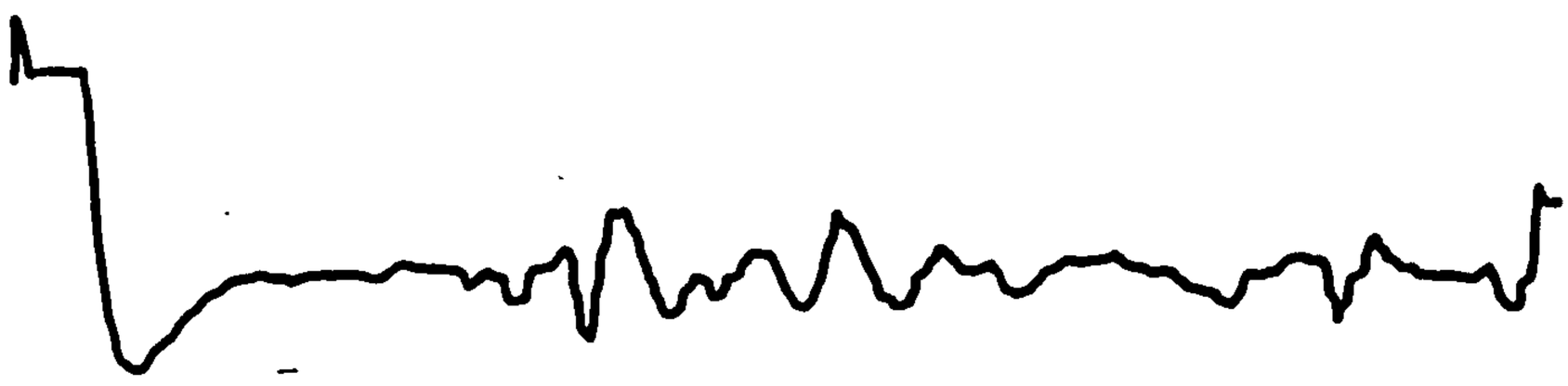
3. Electrical stimulation of the telencephalon

Figure 101 shows the response evoked by ipsilateral vagal lobe stimulation, the response evoked at the same site by ipsilateral telencephalic stimulation, and the response evoked at the same site by concurrent ipsilateral stimulation of both telencephalon and vagal lobe. It also shows for reference purposes the algebraic summation of the responses from vagal only and telencephalic only stimulation. Summation was achieved by means of a neurolog signal averaging unit and is clearly distinct from the physiological response to concurrent stimulation. Unfortunately the anatomical location of the recorded site was not histologically verified it was not therefore possible to ascribe the activity unequivocally to the NPTh.

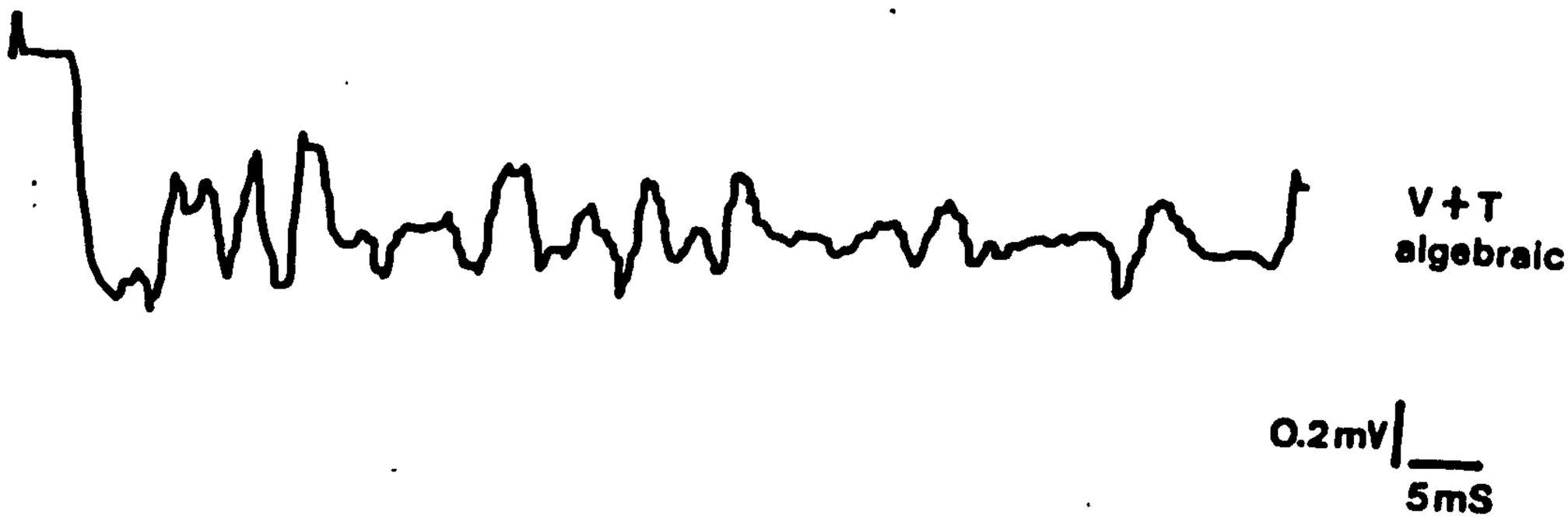
Figure 101.

Shows signals recorded from the same site in the region of the nucleus posterior thalamicus. The top trace shows the response to electrical stimulation of the ipsilateral vagal lobe only. The second trace shows the response to electrical stimulation of the ipsilateral telencephalon only. The third trace shows the response to concurrent electrical stimulation of both the ipsilateral telencephalon and the ipsilateral vagal lobe. The fourth trace shows the algebraic summation of the evoked responses seen in the top two traces, this was achieved by means of a neurolog NL710 signal averaging unit. All signals shown are the averaged responses from 16 stimulus presentations.


V only


T only


V+T
concurrent


V+T
algebraic
0.2mV
5mS

DISCUSSION

The vagal lobe stimulation experiments indicate that the conduction velocity of the fastest recorded activity is very slow at only 0.33 ms^{-1}. Conduction velocities of less than 2 ms^{-1} are likely to be associated with unmyelinated fibres with diameters of less than 1 um (Rushton 1951). Rushton calculated that the critical fibre diameter below which myelination conferred no conduction velocity advantage was 1 μm. It should be remembered however that myelinated fibres of less than 1 um diameter have been demonstrated in mammalian central nervous system (CNS) (Waxman and Bennet 1972). If the late activity in the evoked response was due to the vagal lobe stimulation this would indicate a conduction velocity of only 0.05 ms^{-1}, and if the theoretical calculations of Rushton (1951) hold true this would indicate transmission by fibres of less than 0.05 μm diameter. It is quite likely that the late activity evoked in the NPTh is regenerative and is seen as the result of interactions between cells of the NPTh. There are a number of possible anatomical arrangements which might well give rise to such activity.

The possibility exists that axons arising from cell bodies within the NPTh send a collateral fibre the dendritic areas of other cells within the nucleus. Provided there are no gross impedance mismatches the antidromic spikes propagating from the vagal lobe area will invade the axon collateral, and there will thus in effect be orthodromic activity occurring locally within the nucleus. In a situation where orthodromic activity has been established it is reasonable to assume that long term activity may be set up as the

result of activity within dendritic fields. Obviously orthodromic activity is much more likely than antidromic activity to reflect the pattern of activity seen in the NPTh as the result of normal physiological activity.

Within the NPTh, cells may be functionally coupled together by way of gap junctions. Gap junctions have been identified as low resistance pathways between cells which allow the exchange of diffusable molecules. Gap junctions in effect provide a hydrophilic channel between cells such that cells are electrotonically coupled (Gilula 1979). A major feature of systems coupled by way of gap junctions is the rapid electrotonic spread of an action potential from one cell to another without a significant time delay, this property allows cells coupled in this way to function as a syncytial unit (Bennet 1977).

Another mechanism known to produce concerted activity amongst groups of cells is that of electric field effects. It is possible for the excitability of one neuron to be directly altered as the result of extracellular currents generated by electrical activity in adjacent cells (see Korn and Faber 1979 for review). An important feature of such `coupling` is that no specific anatomical junction need exist between the cells in question. The effect requires that the electrical currents generated as the result of activity in one cell be channelled across the membrane of another cell thereby altering the polarisation of that cell`s membrane. Since it is the spaces around cells which provide a pathway for the flow of electric current, field effects are critically dependent on cell orientation and on the electrical properties of the extracellular medium. Two

specific features of field effects have been demonstrated within the vertebrate CNS. The first of these features is that cells lying adjacent to each other may be `coupled` as the result of electrical field effects, this coupling may lead to an increased synchronisation of the activity of cells within a particular region (Korn and faber 1980). A second feature of field effects is the possible triggering of events which would not be triggered purely as the result of synaptic activity alone, for example the excitation caused by `anode-break` or `post-anodal rebound` at the offset of a strongly hyperpolarising synaptic input (Faber and Korn 1983).

Any or all of these effects might be responsible for the long-lived activity seen in the NPTh as the result of antidromic activation. All of the specific physiological phenomena described above rely on the presence of specific anatomical features. A detailed morphological investigation of the NPTh at the electron microscopical level might demonstrate the presence of one or all of the anatomical features necessary to facilitate the effects discussed. Unfortunately no electron microscopic investigations were carried out.

With regard to activity elicited within the NPTh as the result of facial lobe stimulation; it has not proved possible to differentiate facially evoked responses from the vagally evoked responses on the basis of waveform shape. There is a slight difference in latency between the vagally and facially elicited activity. The facially elicited activity is approximately 4 ms delayed in comparison with the vagally elicited activity. There may be several explanations for this difference. The length of the conduction pathway may be longer

between facial lobe and NPTh than that between vagal lobe and NPTh. Assuming that conduction velocities in the two pathways are the same and are the maximum observed in this experiment (0.33 ms^{-1}) the facial lobe - NPTh pathway would only have to be approximately 100 μm longer than the vagal lobe - NPTh pathway to account for the difference in latencies. An alternative explanation for the difference in signal latencies might be that, although the conduction pathway lengths are very similar the fibres of the facial lobe - NPTh pathway are of a smaller diameter than those of the vagal lobe - NPTh pathway. There is not enough evidence presented either here or in the anatomical studies carried out by other authors (Baker 1987) to determine the path lengths or the axon diameters for the fibre tracts being stimulated in these experiments. Furthermore it is not possible to determine whether the facial lobe - NPTh and vagal lobe - NPTh pathways are in fact collaterals arising from the same population within the NPTh. As the discussion of the anatomical investigations above shows there is no morphological evidence for the existence of sub-populations of neurons within the NPTh. The possibility therefore exists that both descending pathways are in fact collaterals of a common fibre tract.

In some cases it proved possible to identify the existence of large extracellular spikes within the complex activity of the evoked NPTh response. As described above these spike events were used to generate strength-duration curves. Strength-duration curves allow the determination of a chronaxie value for the stimulated tissue. Empirical studies have shown that chronaxies within certain value ranges may be associated with stimulation of different types of

tissue, for example stimulation of myelinated fibres has been shown to generate chronaxie values of 30 - 700 μs (membrane time constants of between 45 μs and 1 ms), and the time constant of the cell body and most dendrites is in the 1 - 10 ms range (Ranck 1975). The problem in interpreting the data presented in this section of the results is that of determining which elements are being stimulated. The measured chronaxie values fall largely between the two ranges quoted for myelinated fibres and cell bodies or dendrites. It is probably easiest to state which elements are not being stimulated rather than which are being stimulated, thus it is probable that myelinated fibres, cell bodies and dendrites within the vagal lobes are not responsible for producing the spike events seen in the evoked NPTh response.

The experiments which show the mapping of evoked activity onto the structures of the hypothalamus are of interest in that they demonstrate the range over which evoked activity may be extracellularly recorded. The distance over which activity will be recorded extracellularly will depend to a large extent on the electrical properties of the extracellular matrix. The results presented here show that activity may be recorded over a distance of up to 200 μm although the peak amplitude of the recorded activity declines sharply with distance. The results presented here are disappointing in that the distribution of the reconstructed recording tracks is mainly medial to the NPTh and therefore lies within the confines of the lateral nucleus preglomerulosus (NPG). Unfortunately, although tracks more lateral than those illustrated were recorded it was not possible to verify their courses histologically. Although

shrinkage of the tissue as a result of histological processing was not directly calculated, the mapping of recorded sites onto histological drawings was possible since the original separation of the marker lesions was known.

Several of the lesioning experiments reported in section II of this thesis in which large amplitude evoked signals had been recorded from the NPTh showed that the recorded activity had been located on the lateral borders of the nucleus. An interesting feature of the area of the NPTh is a relatively cell-free zone located lateral to the nucleus. It is quite possible that a dendritic field exists lateral to the NPTh, and that some of the evoked activity recorded from the NPTh originates in this area.

The experiments described here which involve the recording of evoked activity in the NPTh as the result of electrical stimulation of the telencephalon demonstrate that there are sites in the region of the NPTh which may be antidromically activated from both hindbrain and forebrain. It has been shown that the NPTh sends efferent fibres to the telencephalon (Murakami, Ito and Morita 1986) as well as to the medulla (Baker 1987). The NPTh is thus in a central position, and may be able to influence activity in both forebrain and hindbrain areas. The precise anatomical location of the recorded sites in this experiment were never histologically verified. It should be noted that in all cases the hypothalamic sites which show a response to both telencephalic and vagal lobe stimulation were initially located using vagal lobe stimulation, it is thus possible to be sure that recorded sites were located in the region of the NPTh. The nature and

the importance of the effects that the NPTh might have on the forebrain need to be weighed in the light of the evidence that the telencephalon is not necessary for the successful performance of feeding behaviour (Savage 1969). This observation together with the evidence that the telencephalon receives projections from the NPTh indicates that although the telencephalon may be involved in the regulation of food intake in some way it does not exert a major influence on the decision to feed. Whilst it may be argued that the telencephalon is not involved in the decision to feed, it is quite possible that it is involved in a decision to not feed. For example, in the situation where a potentially toxic substance is encountered it is possible that neural activity within the telencephalon, initiated by input from the NPTh, is required to prevent ingestion or swallowing. This hypothesis would be in keeping with the evidence that the telencephalon is involved with associations involving avoidance, but is not the site of such associations (Savage 1969).

Based on the anatomical and physiological evidence presented in this section and in section II, it is possible to postulate a role for the NPTh in the regulation of food intake. The NPTh is a functionally and morphologically homogenous nucleus providing a single function, that of palatability discrimination, this function is provided for both facially and vagally mediated gustatory stimuli. The determination of palatability will of necessity depend on factors such as the association of any particular gustatory stimulus with unpleasant consequences of ingestion, this associative aspect of palatability determination may well be mediated via the telencephalon. The question as to the palatability of any particular gustatory stimulus

will also depend on an animals eating history, this is to say that palatability is a subjective phenomenon and is not fixed at a particular `level` for a particular type of food. The operational level of palatability will depend to a large extent on afferent information regarding conditions in the peripheral nervous system and the gut. Much of the information regarding the gut will be carried by the vagus nerve terminating in the vagal lobe of the medulla. The vagal lobe is a highly complex and highly structured region and some of the features of its organisation, the columnar arrangement of cell bodies for example, are reminiscent of the organisation seen in the higher CNS levels of other vertebrates (Baker 1987). The highly organised nature of the vagal lobe implies that much of the `processing` of afferent gustatory stimuli is carried out locally in the hindbrain. Higher levels within the carp brain such as the superior secondary gustatory nucleus (SSGN) have been shown to receive afferent fibres from both vagal and facial lobes (Herrick 1905), and it is at these levels that the integration of information from both vagal and facial lobes may be possible. Although the NPTh afferents are not clear it may well have access to both facial and vagal information. Although the NPTh is not a highly structured morphological substrate, there does appear to be some regenerative neural activity within the nucleus which may be the result of some internal connectivity or spatial relationship between the neurons. Efferent information from the NPTh to the medulla is probably carried over a mono-synaptic, unmyelinated, small fibre diameter pathway to both vagal and facial lobes, possibly with both lobes being innervated by collateral fibres. Within the vagal and facial lobes the afferent signal from the NPTh may form an input to a local

network of neurons thereby providing a weighting factor for that network such that the final output from the network includes a `palatability factor`.

SECTION IV

INTRODUCTION

For many years now certain peptides have been implicated in the control of food intake and satiety, and there is now a very large literature on this subject. Much of the early work concentrated on the effects of the peptide cholecystokinin (CCK) and its effects in various mammalian species. More recently a number of different peptides and peptide fragments have attracted interest. The aim of this section is to investigate the effects on food intake in carp of the peptide bombesin.

Bombesin is a representative of a large family of bio-active peptides which may be isolated from the skin of various amphibian species. In 1970 Nakajima, Tanimura and Pisano described the occurrence of a hypertensive undecapeptide in the skin of the frog *Rana pipiens*. This peptide was named ranatensin owing to its source and effect on blood pressure. The amino acid sequence of ranatensin is shown in table 16. Subsequent work led to the isolation of bombesin and alytesin from extracts of the skin of two European discoglossid frogs *Bombina bombina* and *Alytes obstetricans* (Anastasi, Erspamer, and Bucci 1971) (see table 16 for sequences). Since its discovery bombesin has been shown to display a wide range of biological activity the effects on blood pressure are similar to those displayed by ranatensin; in rats, cats, dogs and rabbits pressure is raised, and in the monkey pressure is lowered (Erspamer 1988). Bombesin possesses many other actions in addition to its "tensin" activity. Many of the additional effects of bombesin are mediated peripherally and include stimulation of gastric acid secretion (Bertaccini, Erspamer and Impicciatore 1973),

stimulation of gall bladder contraction and pancreatic enzyme secretion (Erspamer, Improta, Melchiorri and Sopranzi 1974), and disruption of peristaltic activity (Caprilli, Melchiorri, Improta, Vernia and Frieri 1975). In addition to its peripheral gastro-intestinal effects, bombesin demonstrates central effects on food intake (Gibbs, Fauser, Rowe, Rolls, Rolls and Maddison 1979), thermoregulation (Wunder, Hawkins, Avery and Swan 1980) and memory (Flood and Morley 1988).

Table 16

Bombesin

pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Alytesin

pGlu-Gly-Arg-Leu-Gly-Thr-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Ranatensin

pGlu-Val-Pro-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2

Carboxyl-terminal decapeptide of the gastrin-releasing-peptides

Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Neuromedin B (NMB)

Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2

Note: pGlu=pyroglutamic acid; Met-NH_2=methionine amide.

It is now clear that there are a large number of bombesin-like peptides grouped into well defined families based upon the presence of common amino acid sequences at the carboxyl terminal of the peptide. The 'authentic' bombesins possess the characteristic carboxyl terminal sequence Gly-His-Leu-Met-NH_2. The ranatensin family which includes ranatensin C from *Rana catesbeiana* and ranatensin R from *Rana rugosa* (Yasuhara, Ishikawa and Nakajima 1979) are characterised by the carboxyl terminal sequence Gly-His-Phe-Met-NH_2. A group of peptides related to the ranatensins is the litorins, these share the same carboxyl terminal tetrapeptide as the rantensins and are isolated from the skin of the Australian hylid frogs of the genus *Litoria* (Anastasi, Erspamer and Endean 1975). A third family of bombesin-like peptides is the phyllolitorins, these differ from all other bombesin-like peptides in that the histidine residue usually found at position three from the carboxyl terminal is replaced by a serine residue. The phyllolitorins are isolated from South American frogs of the genus *Phyllomedusa*, and are characterised by the sequence Gly-Ser-Phe(Leu)-Met-NH_2 at the carboxyl terminal (Yasuhara, Nakajima, Nokihara, Yanaihara, Yakaihara, Erspamer and Falconieri Erspamer 1983). The closest mammalian counterparts to the peptides described above are the gastrin-releasing-peptides (GRP) (Spindel 1986) and the neuromedins (Minamino, Kangawa and Matsuo 1983). The gastrin-releasing-peptides belong to the bombesin family and neuromedin B (NMB) is typical of the ranatensin family (see table 16 for sequences).

The majority of the work in this area has concentrated on the effects of the bombesin-like peptides on mammalian species, and the search for true mammalian counterparts to these peptides. Recent studies have reported the finding that in the rat there are two receptor subtypes for mammalian bombesin-like peptides which show different relative affinities for GRP and NMB (Battey and Wada 1991). In contrast to the large amount of work on mammalian species there have been only a small number of studies concerned with the effects of such peptides in the fishes. These studies have used frog skin bombesin peptides since native fish bombesin is not readily available. Recent work has however demonstrated that native fish bombesin-like peptides do exist, and that they may be purified and retain a bio-active capacity (Thorndyke, Reeve and Vigna 1990). Quite clearly the bombesin-like peptides have been highly conserved during evolution. Those studies which have been carried out in the fish have tended to corroborate the results seen from the mammalian studies demonstrating that bombesin exhibits bio-active properties at both central (Kavaliers and Hawkins 1981) and peripheral sites (Thorndyke and Holmgren 1990). In addition studies in fish have shown that some of the behavioural consequences of bombesin administration noted in mammals are also seen in fish (Beach, McVean, Roberts and Thorndyke 1988).

MATERIALS AND METHODS

The operant apparatus used in this experiment was the same as that described in section I in terms of the operant task and the feeding periods and lighting regime. Each fish after being placed in the experimental tank was allowed a period of at least ten days to establish a baseline feeding pattern under these conditions.

After this acclimitisation period the trial period was begun. On each experiment day fifteen minutes before the feeding period was due to begin (i.e. fifteen minutes before room lights and tank lights came on) all fish, including control trial animals were weighed. This was to allow the calculation of standardised injection doses for both control and experimental animals. Ten minutes before the feeding period began each fish was removed from the tank and injected intra-peritoneally using a Hamilton 100µl glass syringe fitted with a 21 gauge needle. Experimental animals received bombesin, and control animals received Young's freshwater teleost saline (YFTWS) injections. Immediately after the injection had taken place the fish was returned to the tank and allowed to complete the feeding period without further disturbance.

The injection routine for any one animal was in the form of paired injections such that, on one day the animal received a dose of BBS, and then after a period of at least two days the fish received an injection of YFWTS (or *vice versa*). In this way each animal acted as a control for itself.

The doses of bombesin used in this investigation were 7 $\mu g\ kg^{-1}$, 0.7 $\mu g\ kg^{-1}$ and 0.07 $\mu g\ kg^{-1}$. The appropriate dose for each animal was arrived at by using a stock solution of 1mg ml^{-1} bombesin and varying the volume injected according to the weight of the animal. In practise injected volumes range from 44 µl for the smallest animal used, up to 136 µl for the largest animal used. The bombesin used for this experiment was obtained from Bachem (UK) Ltd. *of Saffron Walden, Essex. The structure of the bombesin supplied by* Bachem was as shown above in table 16, the molecular weight was 1620.1, the peptide content was 75.34% (+/- 3%), and the peptide purity was >99%. A total of 10 animals were used in this study.

RESULTS

The results for this section are presented graphically in figures 102 to 111. Results are grouped into 5 sections, these are described below.

Section 1 Bombesin 7 μgkg^{-1}

figures 102 and 103.

These figures show the results from two of the individuals used in this trial and are the results obtained using a dose of bombesin of 7 μgkg^{-1}, a total of 10 animal trials were used for this section.

Inspection of figures 102 and 103 shows quite clearly that BBS at this dose leads to an initial delay in the onset of feeding when compared to control trials.

Section 2 Bombesin 0.7 μgkg^{-1}

figures 104 and 105.

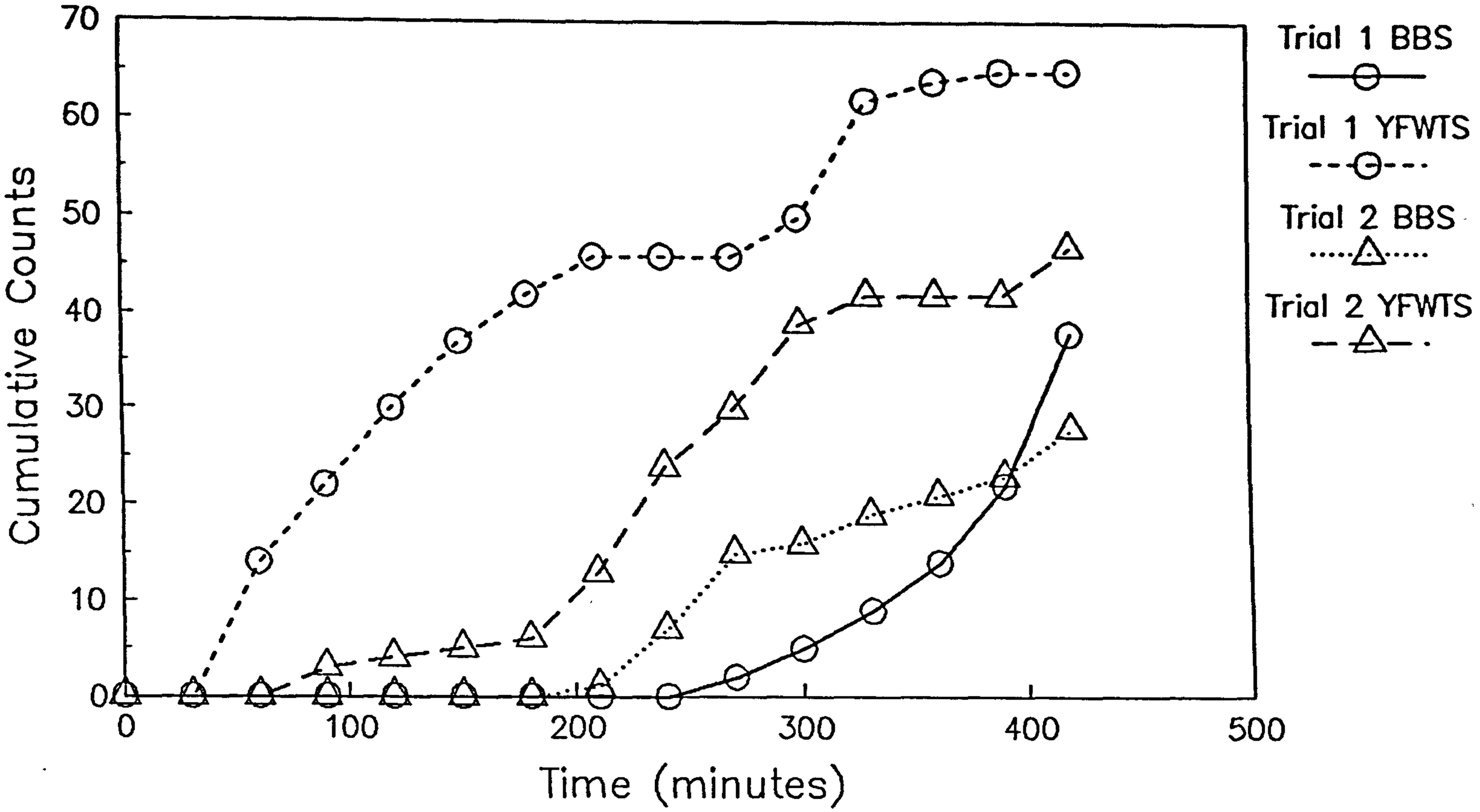
These figures show the results from two of the individuals used in this trial and are the results obtained using a dose of bombesin of 0.7 μgkg^{-1}, a total of 10 animal trials were used for this section.

Inspection of these figures shows that in the majority of trial pairings the onset of feeding under bombesin treatment is delayed, at

Figure 102.

The cumulative feeding results from two trials of intra-peritoneally administered bombesin (BBS) at 7 μgkg^{-1}. The results of BBS injections were paired with the results of control injections of Young's freshwater teleost saline (YFWTS). The same individual was used for both BBS and YFWTS injections. These results were obtained from animal CP07.

7 µg/kg BBS

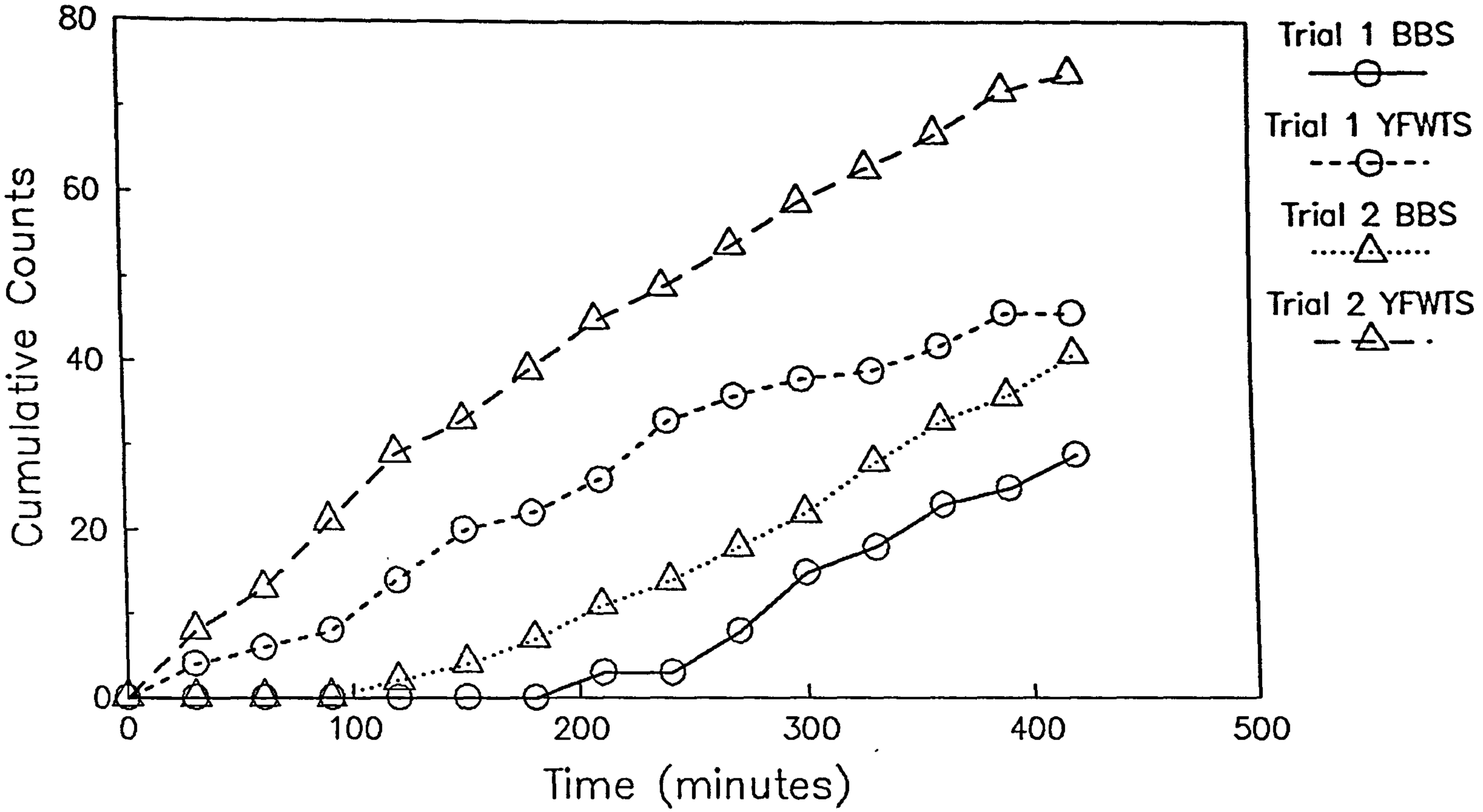


Fish number CP07

Figure 103.

The cumulative feeding results from two trials of intra-peritoneally administered bombesin (BBS) at 7 μgkg^{-1}. The results of BBS injections were paired with the results of control injections of Young's freshwater teleost saline (YFWTS). The same individual was used for both BBS and YFWTS injections. These results were obtained from animal CP10.

7 µg/kg BBS

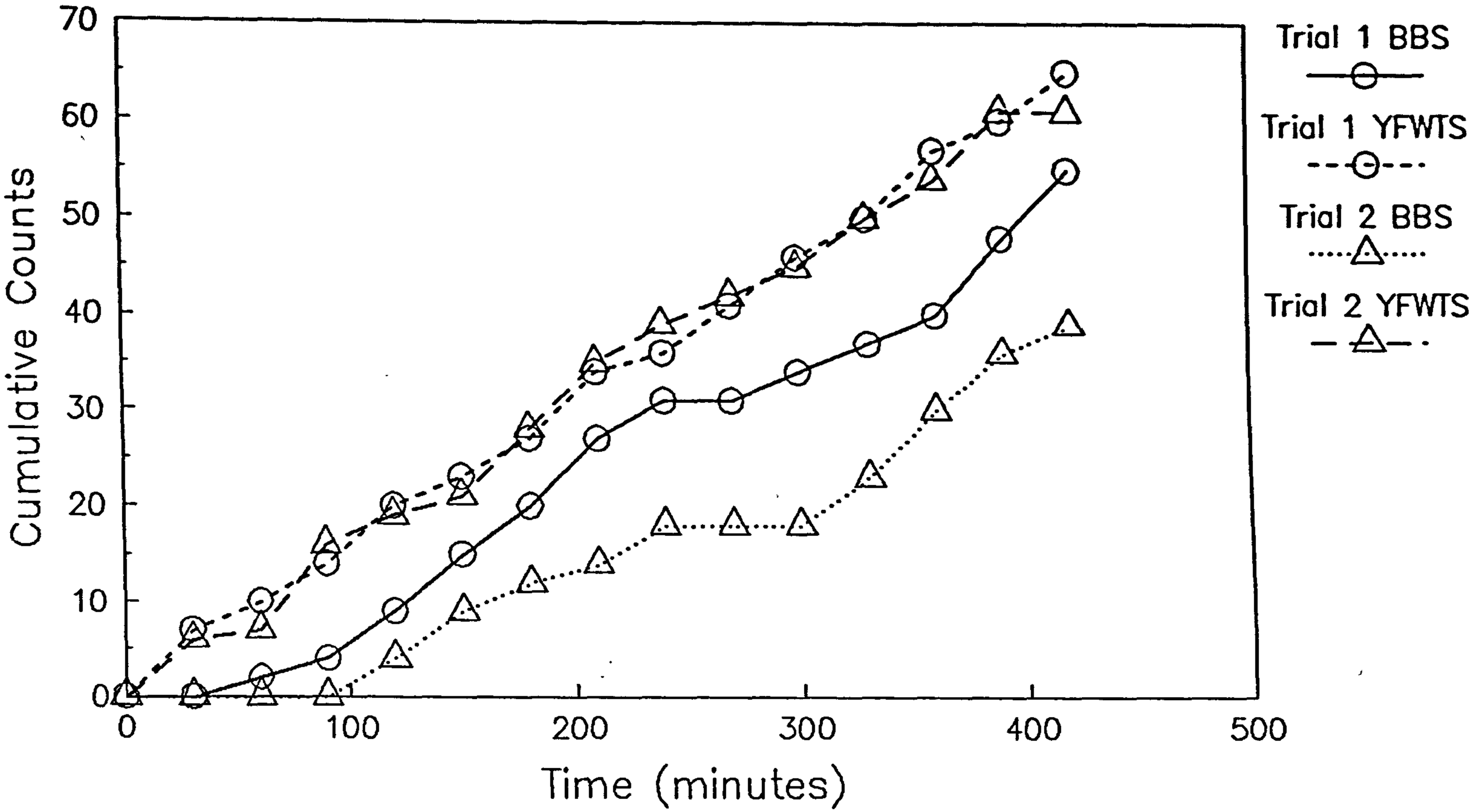


Fish number CP10

Figure 104.

The cumulative feeding results from two trials of intra-peritoneally administered bombesin (BBS) at 0.7 μgkg^{-1}. The results of BBS injections were paired with the results of control injections of Young's freshwater teleost saline (YFWTS). The same individual was used for both BBS and YFWTS injections. These results were obtained from animal CP11.

0.7 µg/kg BBS

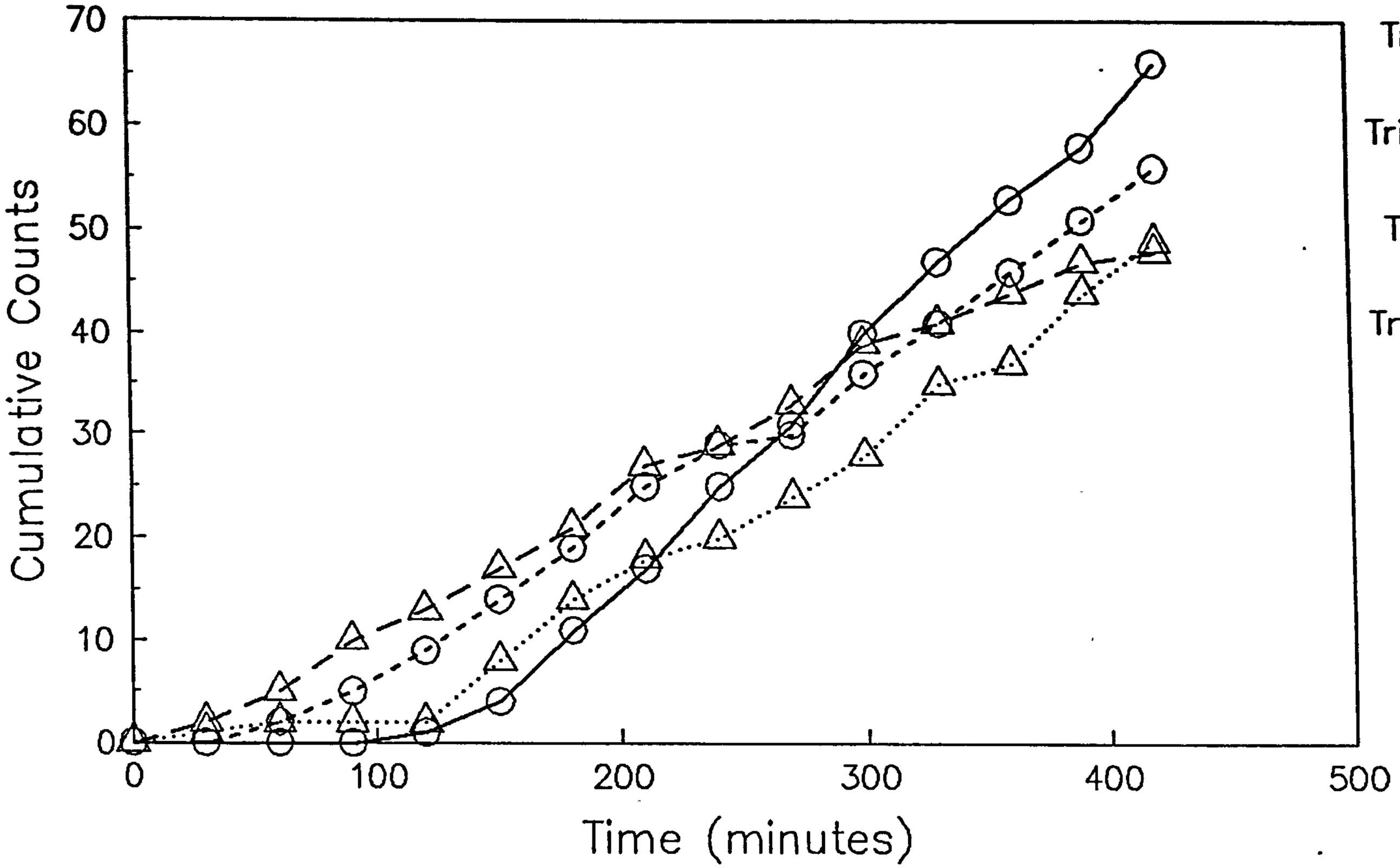


Fish number CP11

Figure 105.

The cumulative feeding results from two trials of intra-peritoneally administered bombesin (BBS) at 0.7 μgkg^{-1}. The results of BBS injections were paired with the results of control injections of Young's freshwater teleost saline (YFWTS). The same individual was used for both BBS and YFWTS injections. These results were obtained from animal CP12.

0.7 µg/kg BBS



Fish number CP12

this dose however the delay is much less marked than that seen in the higher dose experiment. In addition, the difference in total amount of food eaten over the duration of the feeding period, between trial and control days, is very much less than with the higher dose of bombesin.

Section 3 Bombesin 0.07 μgkg^{-1}

figures 106 and 107.

These figures show the results from two of the individuals used in this trial and are the results obtained using a dose of bombesin of 0.07 μgkg^{-1}, a total of 11 animal trials were used for this section.

Inspection of these figures shows the minimal effects of bombesin administration at this dose.

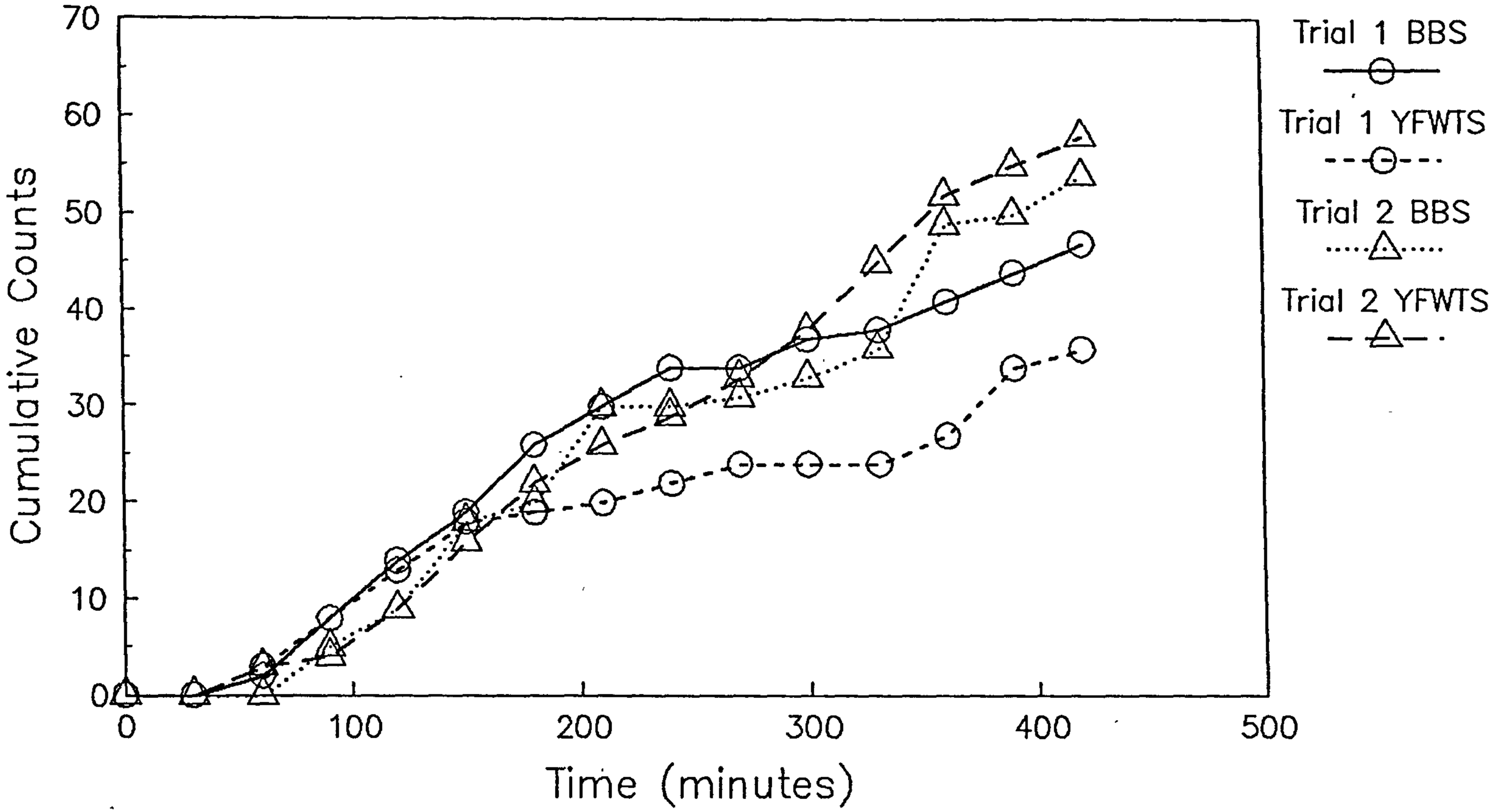
Section 4 Bombesin results summary and dose-response calculation

Since the aim of this experiment was to produce a dose-response curve for the effects of bombesin on food intake the difference in food intake between bombesin treated and YFWTS treated animals had to be measured at some point. The problem immediately arose as to which point in the 7 hour feeding period should serve as a reference point against which food intake under the two treatments could be measured. A cursory look at the graphs shows that no single point in time provides a satisfactory solution to this problem, the generated cusums vary from dose to dose and from animal to animal. In any case

Figure 106.

The cumulative feeding results from two trials of intra-peritoneally administered bombesin (BBS) at 0.07 μgkg^{-1}. The results of BBS injections were paired with the results of control injections of Young's freshwater teleost saline (YFWTS). The same individual was used for both BBS and YFWTS injections. These results were obtained from animal CP12.

0.07 µg/kg BBS

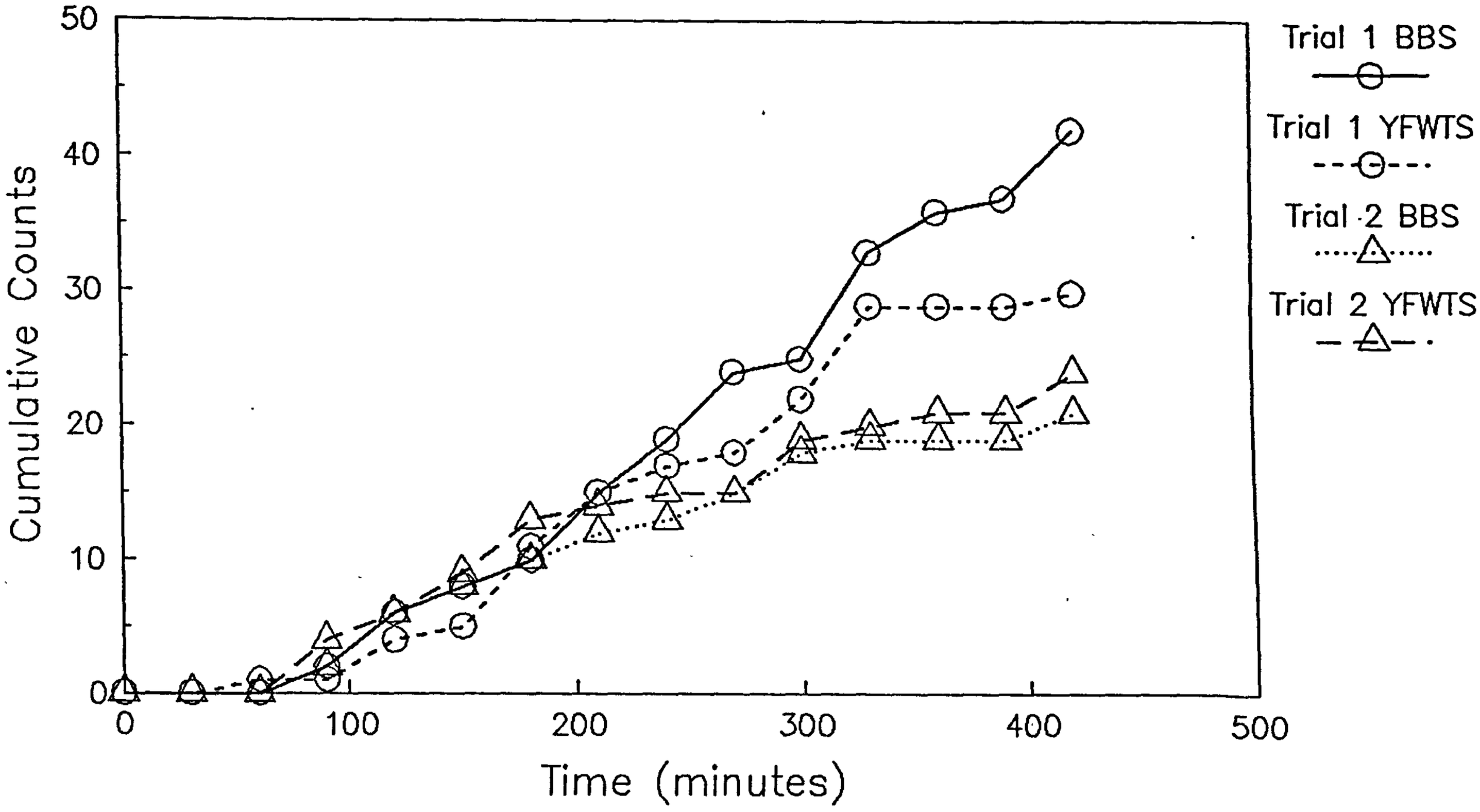


Fish number CP12

Figure 107.

The cumulative feeding results from two trials of intra-peritoneally administered bombesin (BBS) at 0.07 μgkg^{-1}. The results of BBS injections were paired with the results of control injections of Young's freshwater teleost saline (YFWTS). The same individual was used for both BBS and YFWTS injections. These results were obtained from animal CP19.

0.07 µg/kg BBS



Fish number CP19

the use of a particular point in time would only enable the conclusion to be stated in terms of the effects of bombesin intake at a particular number of minutes post injection. The solution to this analysis problem was to divide the 7 hour feeding period into half-hourly time bins. For each time bin the difference in the cumulative number of pellets eaten by the bombesin and YFWTS treated fish for all trial pairs at that dose were analysed using the Wilcoxon matched-pairs signed-ranks test. This analysis gives for every dose, at half-hourly intervals, a statistic which measures any significant difference in food intake between the two treatments. This allows the change in significance of the differences between the treatments to be plotted over the whole 7 hour period, in this way the different time spans over which the different doses are effective are accurately reflected. Statistical methods used for this analysis employed two-tailed tests since it was not possible to state beforehand whether bombesin administration would enhance or depress food intake. The results of this analysis are shown in tables 17 (bombesin at 7 μgkg^{-1}), 18 (bombesin at 0.7 μgkg^{-1}) and 19 (bombesin at 0.07 μgkg^{-1}). Data from these three tables is presented graphically in figure 108. For the purposes of this experiment values of $p > 0.05$ were considered to demonstrate no significant difference between treatments. As may be seen, at all doses where an effect of bombesin adminstration was observed this was to reduce food intake. It appears from figure 108 that the maximal effects of bombesin were not apparent until some way into the feeding period, this feature is an artefact of the statistical test, and is caused by the low number of feeding events at the earliest stages of the feeding period.

Table 17

Bombesin 7 μgkg^{-1}

Time (mins)	significance of bombesin induced effect (p)
30	0.02
60	>0.01
90	>0.01
120	>0.01
150	>0.01
180	>0.01
210	>0.01
240	>0.01
270	>0.01
300	>0.01
330	0.01
360	>0.01
390	0.02
420	0.02

Table 18

Bombesin 0.7 μgkg^{-1}

Time (mins)	significance of bombesin induced effect (p)
30	0.05
60	0.05
90	>0.05
120	0.02
150	0.01
180	0.01
210	0.02
240	0.01
270	0.05
300	>0.05
330	>0.05
360	>0.05
390	>0.05
420	>0.05

Table 19

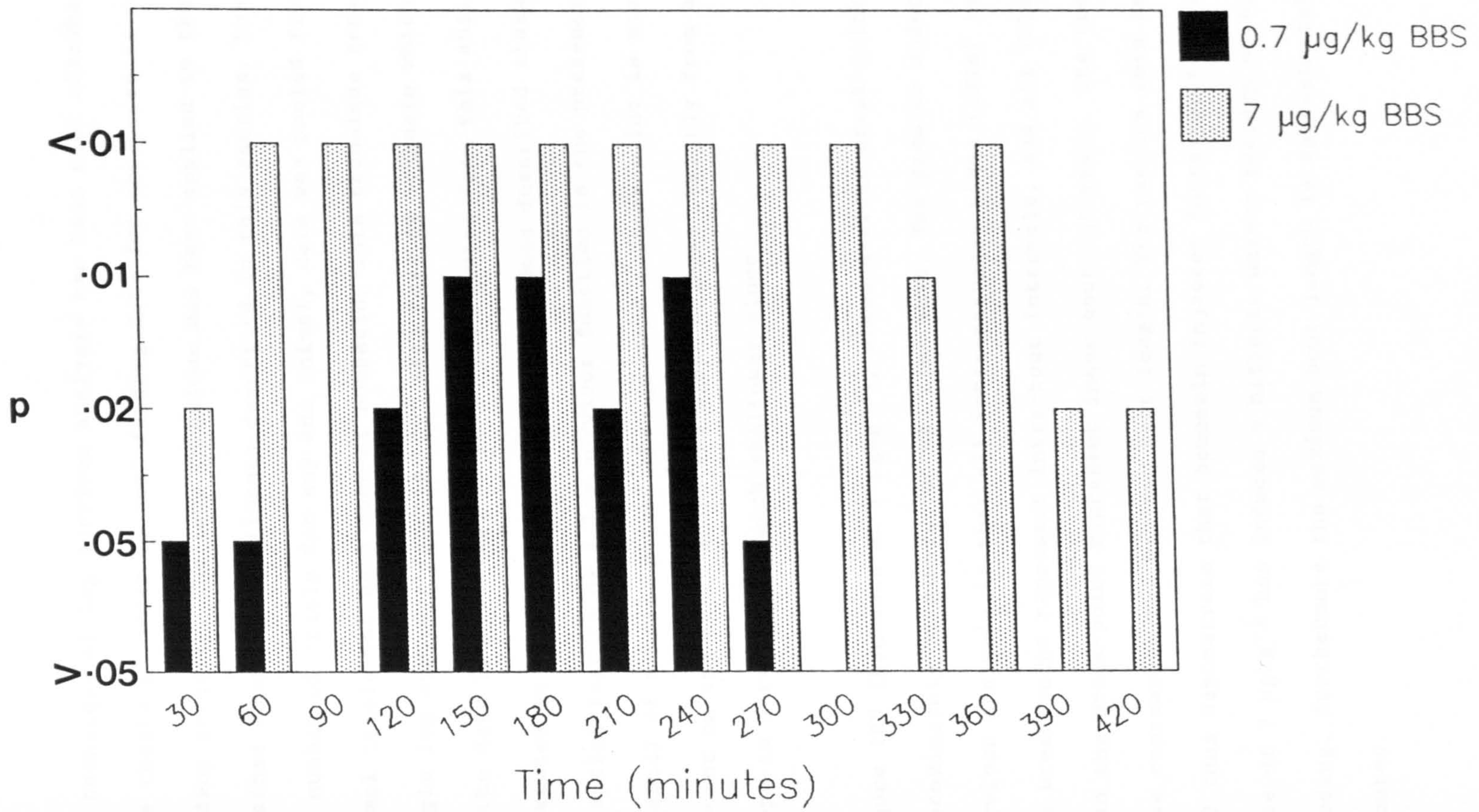
Bombesin 0.07 $\mu g kg^{-1}$

Time (mins)	significance of bombesin induced effect (p)
30	N too small
60	>0.05
90	>0.05
120	>0.05
150	>0.05
180	>0.05
210	>0.05
240	>0.05
270	>0.05
300	>0.05
330	>0.05
360	>0.05
390	>0.05
420	>0.05

Figure 108.

Shows the level of significance of the bombesin (BBS) induced deficit at each half-hourly point throughout the 7 hour feeding period. The lowest dose of BBS used produced no significant deficit at any point during the feeding period.

Significance of BBS induced feeding deficit with respect to time



0.07 µg/kg BBS produced no significant deficit

Section 5 Effects of bombesin on the microstructure of food intake

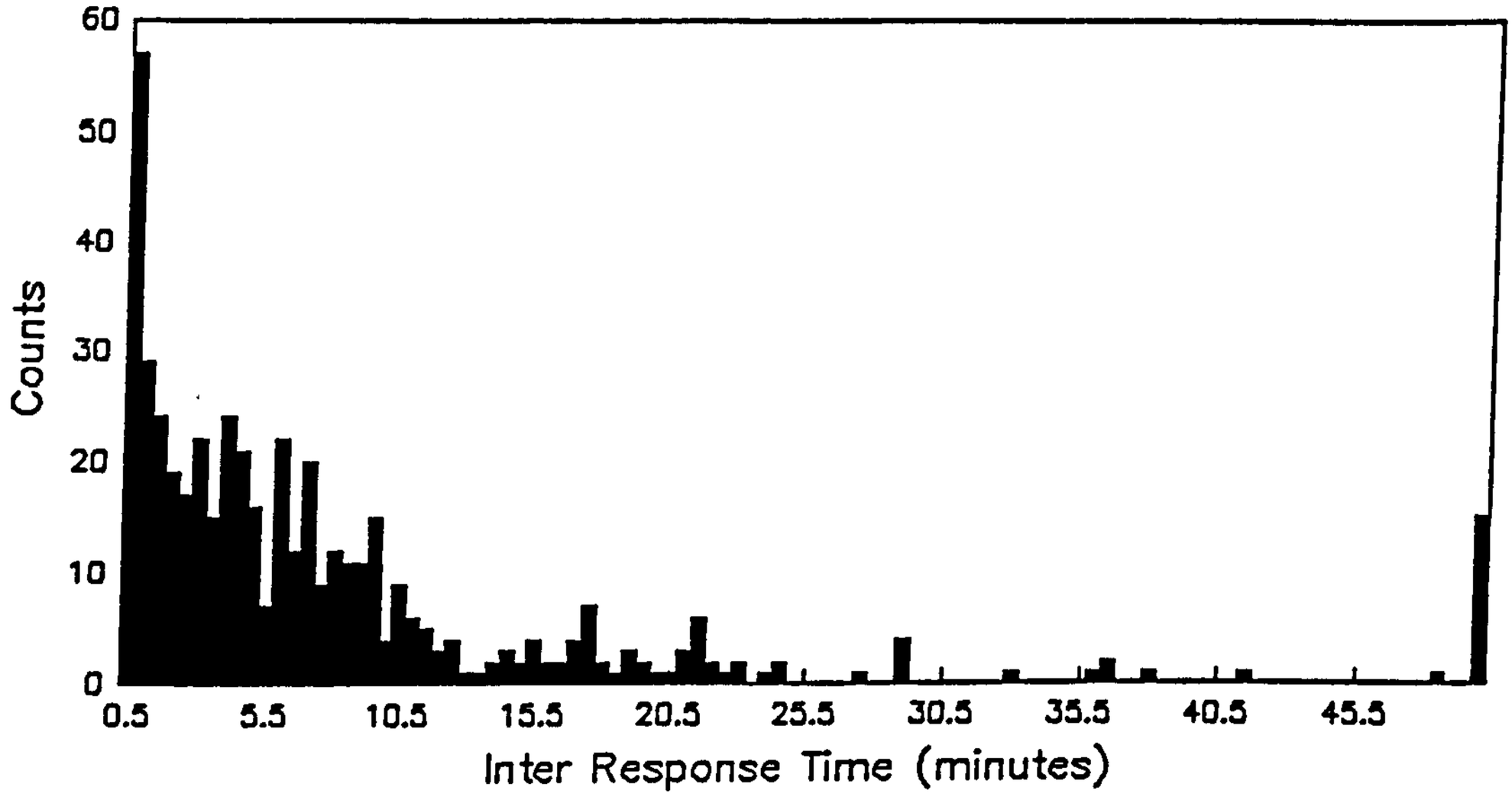
The technique of log survivor analysis has been used elsewhere in this thesis in order to test feeding data for the presence of bout feeding activity. The same technique has been applied to the data obtained from the experiments described in this section. Due to the low number of trials for any one animal, data was pooled from all animal trials for each dose of bombesin. This technique generates a single log survivor function for each dose of bombesin which is based on the data from several animals. The results from this analysis are presented in figures 109 to 111. As has been described elsewhere the essential feature of a log survivor function is the presence (or absence) of a break-point. When present a break-point is manifest as a point on the curve where the slope changes abruptly from a steep slope to a relatively much shallower slope.

Figure 109 (bombesin at 7 μgkg^{-1}), shows a clear break-point at approximately 23 minutes on the time axis. The premise underlying log survivor analysis is that all inter-response times (IRTs) less than the break-point represent intra-bout intervals, and all IRTs greater than the break-point represent inter-bout intervals. The break-point thus represents the maximum bout length. The results seen in figure 109 thus demonstrate that bombesin injected intra-peritoneally at a dose of 7 μgkg^{-1} has induced a hitherto unseen pattern of bout feeding, furthermore the maximum bout length is approximately 23 minutes.

Figure 109.

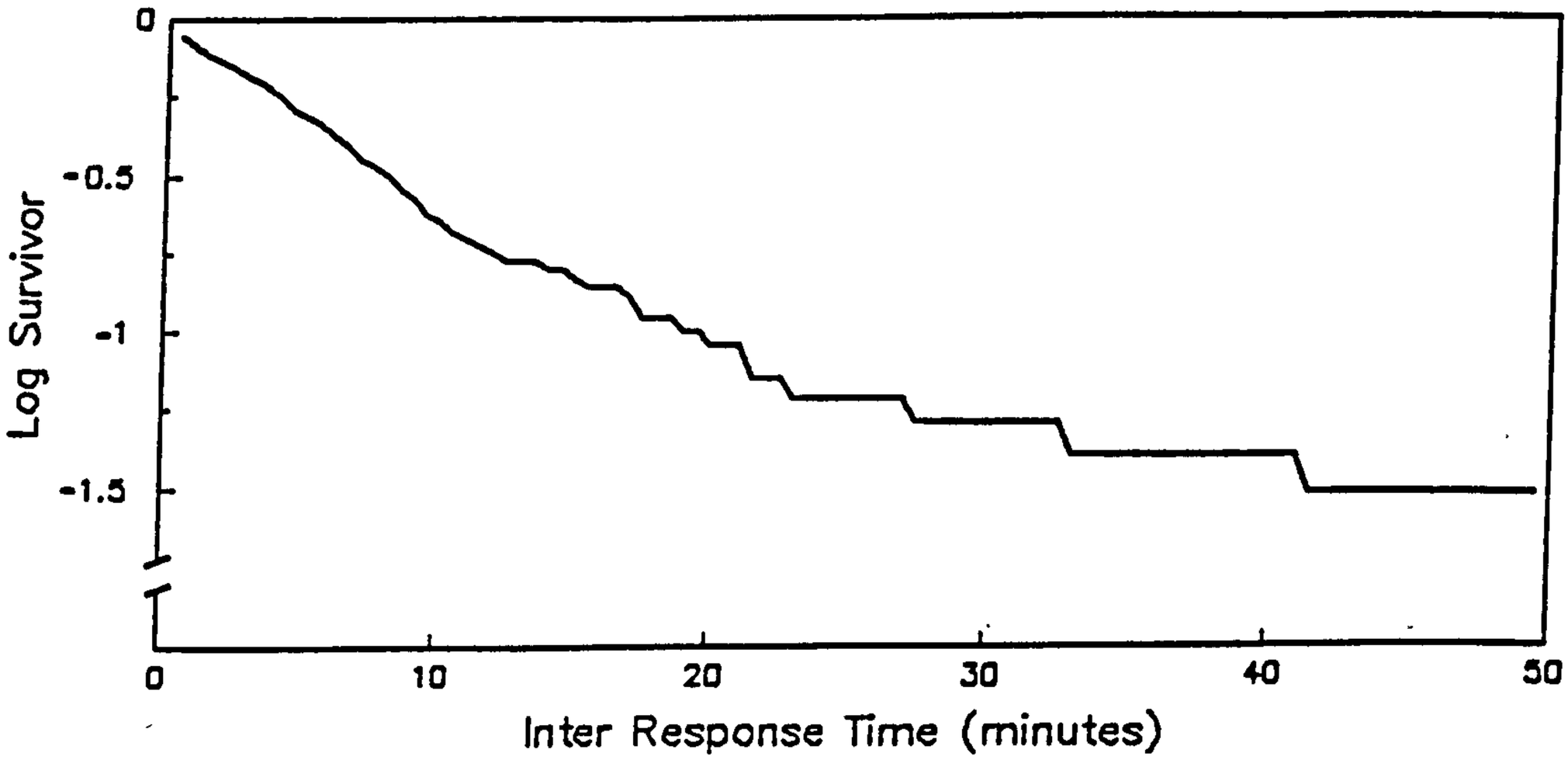
Interval histogram (top) and log survivor function (bottom) for feeding events following intra-peritoneally administered bombesin (BBS) at 7 μgkg^{-1}. The data presented here is pooled from 12 trials.

All Fish Interval Histogram



Pooled data for 12 fish days.
BBS 7 µg/kg

All Fish Log Survivor Analysis



Log survivor function generated from the interval histogram shown above
BBS 7 µg/kg

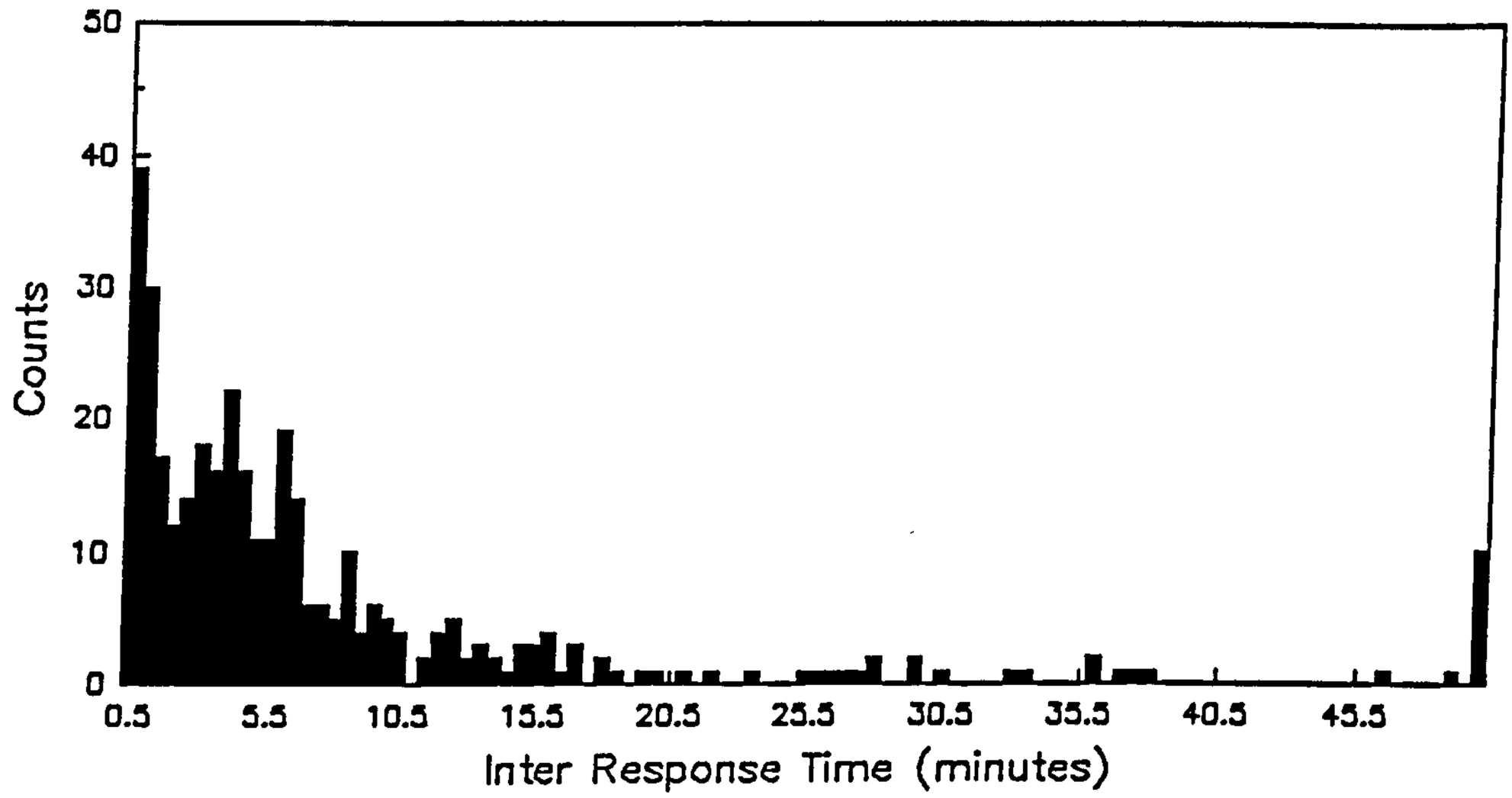
Figure 110 (bombesin at 0.7 μgkg^{-1}), shows the possibility of a break-point at approximately 38 minutes on the time axis. Although this break-point is much less marked than that seen in figure 109 it is still reasonably clear. Thus as with the higher dose, bombesin at 0.7 μgkg^{-1} has induced a pattern of bout feeding, the bouts seen at this dose are however longer with a maximum value of approximately 38 minutes.

Figure 111 (bombesin at 0.07 μgkg^{-1}), shows no evidence of a break-point in the log survivor function. Unlike the higher doses, bombesin at 0.07 μgkg^{-1} does not induce a pattern of bout feeding.

Figure 110.

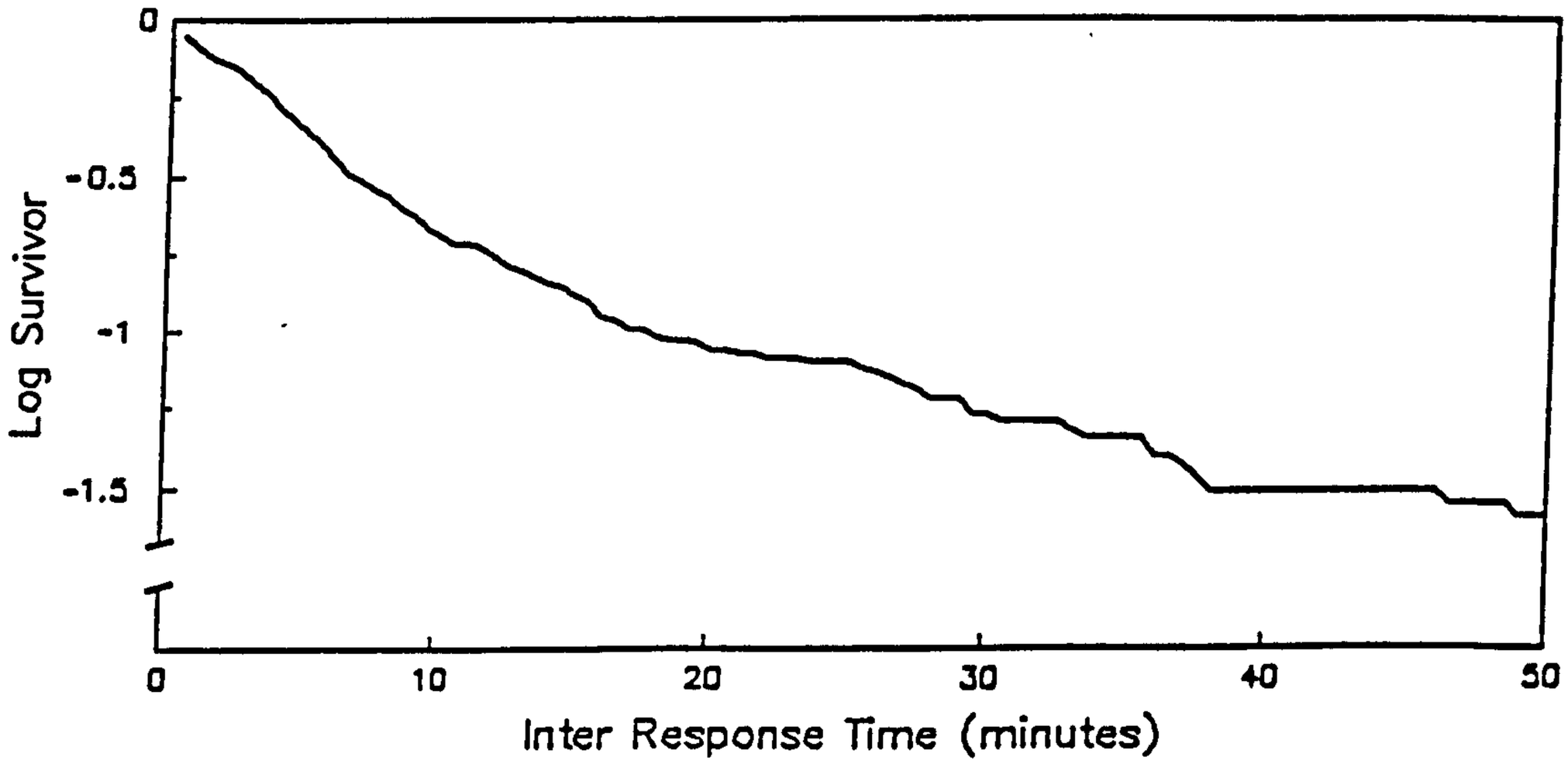
Interval histogram (top) and log survivor function (bottom) for feeding events following intra-peritoneally administered bombesin (BBS) at 0.7 μgkg^{-1}. The data presented here is pooled from 8 trials.

All Fish Interval Histogram



Pooled data for 8 fish days
88S 0.7 µg/kg

All Fish Log Survivor Analysis

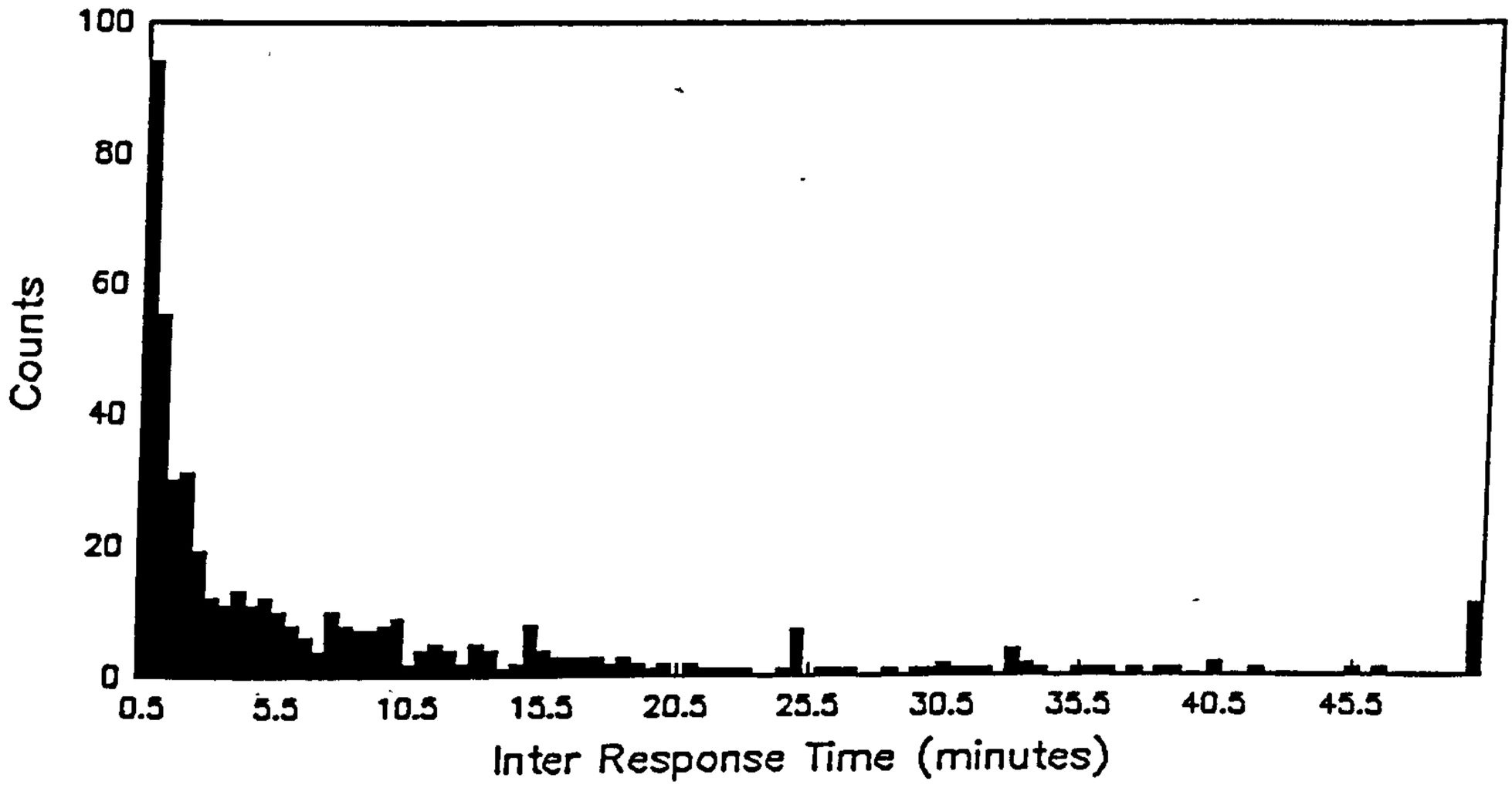


Log survivor function generated from the
interval histogram shown above
88S 0.7 µg/kg

Figure 111.

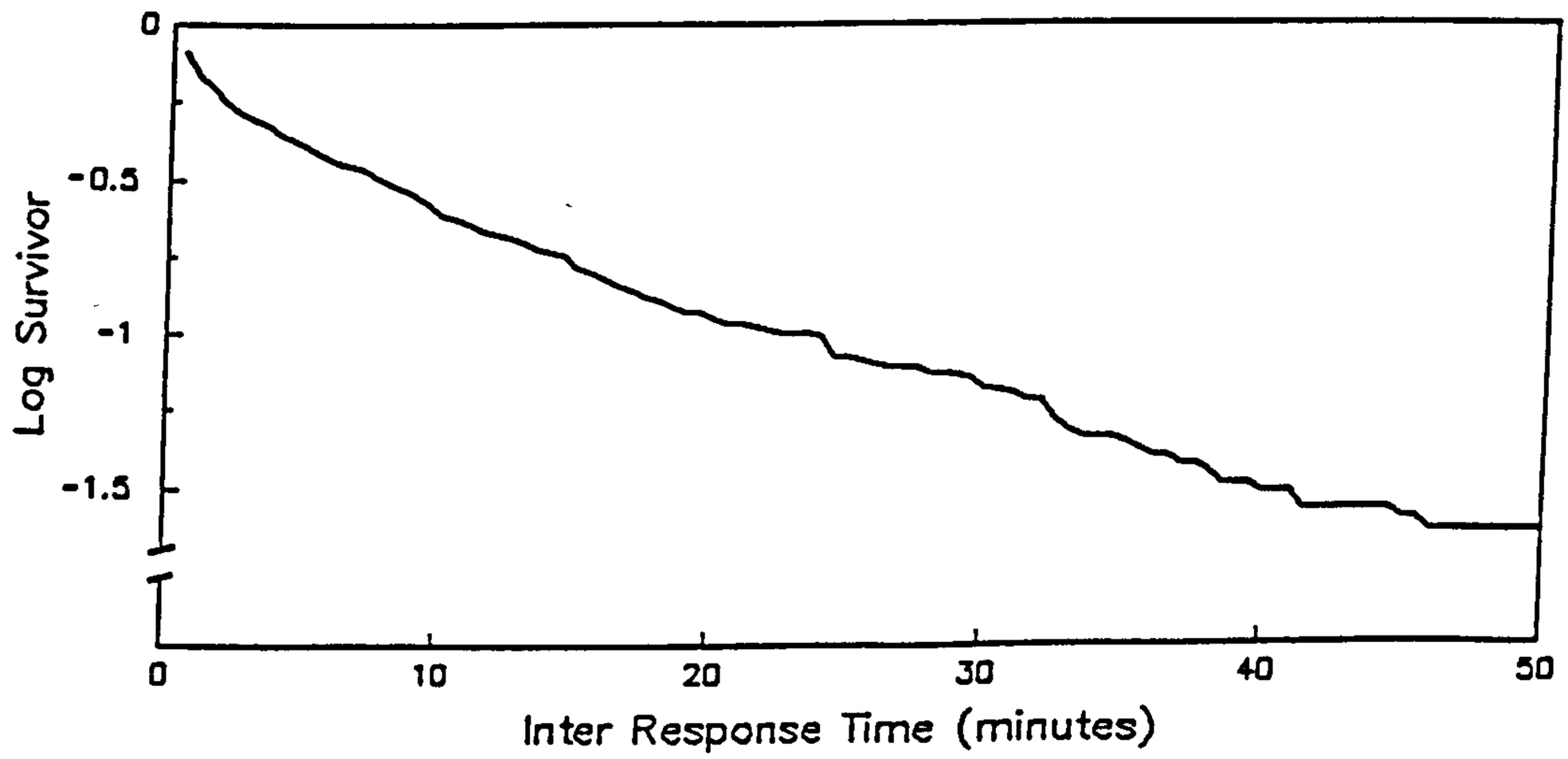
Interval histogram (top) and log survivor function (bottom) for feeding events following intra-peritoneally administered bombesin (BBS) at 0.07 μgkg^{-1}. The data presented here is pooled from 10 trials.

All Fish Interval Histogram



Pooled data for 10 fish days
BBS 0.07 µg/kg

All Fish Log Survivor Analysis



Log survivor function generated from the interval histogram shown above
BBS 0.07 µg/kg

DISCUSSION

A feature of the work on the effects of bombesin and bombesin-like peptides is that there has been remarkably little research carried out in fish species. This lack of research means that much of the discussion of the results presented in this section will of necessity attempt to draw parallels with the results seen in mammalian studies. Whilst there is little alternative to this course of action, other than outright speculation, it is imperative to avoid relying upon a too detailed mammalian model, particularly in light of the evidence that the effects of bombesin may differ markedly even between mammalian species (Erspamer 1988).

The results presented earlier show quite clearly that intra-peritoneally administered bombesin depresses food intake in food deprived animals (17 hours deprived) in a dose dependent manner. This result is broadly in line with the findings of studies carried out on the rat (Gibbs Fauser Rowe Rolls Rolls and Maddison 1979), the doses shown to produce a significant effect in the rat ranged from 2 μgkg^{-1} to 8 μgkg^{-1}, a dose range which is of the same order of magnitude as the maximally effective dose used in the experiments described here. There has been considerable debate as to the mechanism of the anorexic effect in rats. It has been argued that bombesin produces its effects as the result of its being a satiety factor (Gibbs *et al* 1979), in this respect bombesin is viewed as showing a similar effect to that of peripherally administered cholecystokinin (CCK). CCK has been shown to be an anorexic agent in a number of species including rats (Gibbs Young and Smith 1973),

rabbits (Houpt Anika and Wolff 1978), monkeys (Gibbs Falasco and McHugh 1976) and man (Sturdevant and Goetz 1976). Bombesin has been directly implicated in the CCK satiety mechanism, since it has been shown that levels of CCK in the blood plasma and cerebrospinal fluid of dogs are not only related to each other, but are also modulated by bombesin such that intra-ventricular infusion of bombesin raises blood plasma levels of CCK (Banks 1980). There is thus a possibility that bombesin achieves its anorexic effects indirectly via the anorexic effects of CCK, if this is indeed the case then the question as to the mechanism of action of CCK arises.

There has been a great deal of discussion as to whether the effects of CCK are mediated centrally or peripherally. It is now clear that CCK can produce its anorexic effects both centrally and peripherally. Evidence for a central site of action comes from the finding that intra-cerebroventricular administration of CCK decreases food intake in sheep (Della-Fera and Baile 1979). Experiments carried out by Smith Jerome Cushin Eterno and Simansky (1981) show that the satiety effect of intra-peritoneally administered CCK was blocked by abdominal vagotomy, specifically lesions of just the gastric branch of the vagus were sufficient to block CCK induced anorexia. These authors conclude that CCK produces satiety by activating vagal afferent nerves through a direct effect on afferent terminals or receptors. The possibility is also raised that there may be an effect on smooth muscle which stimulates gastric vagal afferent receptors. These findings have been explained by postulating that the area postrema (AP) is the major site of action (Van der Kooys 1984), AP is a blood-brain barrier deficient organ situated in the floor of the

fourth ventricle, and it provides a major termination zone for the vagus nerve. Lesions of this area attenuate the reduction in food intake seen in rats after intra-peritoneal administration of CCK. Whilst this finding suggests a role for the AP in mediating the effects of CCK administration on food intake, great care must be exercised in the interpretation of these results. There is some evidence that the AP in conjunction with the nucleus of the solitary tract is the locus for co-ordinating glucoregulatory signals with ingestive behaviour, and that lesions of the AP disrupt glucoprivic feeding (Bird, Cardone and Contreras 1983).

Although it is possible to postulate sites of action for an anorexic effect of CCK (and by implication bombesin) it is still the case that the induced anorexia may be due not to physiological satiety but to some other non-specific effect such as malaise.

It is apparent that a major problem associated with the measurement of satiety in a behavioural paradigm is the possible absence of a particular behavioural sequence which may be ascribed purely to satiety. It is quite likely that the behavioural sequence seen as a result of satiety is characteristic of other states which could also diminish food intake, states such as sleepiness or malaise. It appears that what is required is a test or tests which discriminate general effects from the specific effect of satiety. Deutsch (1982) describes an experiment which avoids this problem by employing rats operating under an intra-cranial self stimulation (ICSS) regime. It is known that rats working for brain reward stimulation at some anatomical loci reduce their rate of bar pressing when satiated.

Deutsch (1982) was not able to demonstrate a reduction in bar pressing in response to CCK infusion, and therefore ascribed the anorexic effects of CCK to an induced malaise rather than to satiety. In addition Van der Kooy (1984) states that the anorexic effects of peripherally administered CCK are likely to be due to the effects of malaise. This statement is based on the finding that the effects of CCK appear to be mediated through the AP and that this area is known to be a chemoreceptive site underlying the noxious effects of several drugs. It is highly likely that the AP fulfils a similar role in the goldfish *Carassius auratus*, where it is known that the primary afferent fibres of the abdominal branches of the vagus nerve terminate in the AP (Morita and Finger 1987).

Whilst it is true that CCK may exert its anorexic effects as a result of malaise and that bombesin may act via a CCK mediated pathway, it is likely that bombesin may also produce its effects directly or at least in some non-CCK mediated fashion. Evidence for a non-CCK mediated effect in mammals comes from the fact that in the dog, truncal vagotomy does not prevent the bombesin stimulated release of gastrin (Rayford, Guzman, Hill and Thompson 1978). In addition it is known that hypophysectomy and adrenalectomy do not abolish the effects of bombesin on gastrin release, and it has been postulated that the gastrin response to bombesin is mediated via the central nervous system acting through the sympathetic nerves to the stomach or its vasculature (Morley, Allen, Levine and Silvis 1982).

Bombesin has been shown to have an excitatory effect on gastro-intestinal smooth muscle *in vitro* in the Atlantic cod (*Gadus morhua*)

and the rainbow trout (*Oncorhynchus mykiss*) (Thorndyke and Holmgren 1990). In these species bombesin has been shown to cause contraction of stomach smooth muscle when applied on its own, and to potentiate the excitatory effects of acetycholine (Ach) when applied at the same time as Ach. The potentiation of the effects of Ach by bombesin appear to be due to a direct effect of bombesin rather than to an inhibition of cholinesterase activity, this conclusion is based on the fact that bombesin also causes a potentiation of the effects of the Ach analogue carbachol.

The case for bombesin as a satiety factor is argued against strongly by Deutsch (1982) on the grounds that the effects of bombesin are more likely to be the result of a generalised aversion possibly associated with a non-specific decrease in arousal level. It does appear that in the rat at least, peripherally administered bombesin has been shown to produce both aversion (Deutsch and Parsons 1981) and satiety (Kulkosky, Gray, Gibbs and Smith 1981). In addition to the effects of peripherally administered bombesin on food intake, there have been several studies based on centrally administered bombesin which have shown a decrease in food intake (Gibbs, Fauser, Rowe, Rolls, Rolls and Maddison 1979, Kyrkuoli Stanley and Leibowitz 1985). Kyrkouli *et al* were able to show that injections of bombesin at various sites in the hypothalamus led to a decrease in food intake, an increase in grooming and low levels of activity, and a decrease in resting and sleeping. McCoy and Avery (1990) argue that these responses constitute a behavioural index of satiety and are in marked contrast to the behavioural index of animals suffering from malaise. Although it has not been possible to demonstrate a

behavioural index of satiety (or malaise) in the carp, a specific decrease in operant responding has been demonstrated, this type of response has been seen as indicative of satiety in the rat (Babcock, Livovsky and Avery 1985).

A further documented effect of central BBS administration is thermoregulatory changes, these have been demonstrated in rats (Wunder, Hawkins, Avery and Swan 1980) and the teleost fish *Catostomus commersoni* (Kavaliers and Hawkins 1981). In both these cases the effect was interpreted in terms of a lowering of a set point for body temperature.

A hitherto unreported effect of the peripheral administration of bombesin has been the demonstration of a bout feeding pattern. This effect was most apparent in those experiments employing the highest dose of bombesin (7 μgkg^{-1}) where the maximum length of a feeding bout was seen to be approximately 24 minutes. It is particularly difficult to determine the mechanism by which the appearance of a bout feeding pattern is brought about. Visual observation of those animals which received bombesin injections showed that they did not exhibit `abnormal` behavioural responses, that is to say no motor or postural disturbances were noted. It should be remembered that in animals such as the fish, the behavioural repertoire is very limited when compared to higher vertebrates such as the rat. A limited behavioural repertoire makes the development of a behavioural index of satiety (or malaise) very difficult. Although the most likely cause of the bout feeding pattern seen in these experiments is the effect of bombesin on the gastro-intestinal tract (Thorndyke and

Holmgren 1990), it is not possible based on the experiments described here, to determine whether these effects cause malaise or satiety.

The demonstration of the many effects of centrally and peripherally administered bombesin in a wide range of species (Walsh 1987) indicates the widespread presence of receptors which bind bombesin-like peptides. The presence of bombesin receptors raises the question of the existence of endogenous bombesin-like peptides. Bjenning and Holmgren (1988) used an immuno-histochemical technique to study the distribution of bombesin in the gut of representatives of several fish families across a wide evolutionary range. Although absent in the hagfish (*Myxine glutinosa*), bombesin was found to be present in the gut tissue of species ranging from the spiny dogfish (*Squalus acanthias*), to the gar pike (*Lepisosteus platyrhincus*) and the carp (*Cyprinus carpio*). The carp showed a very high level of bombesin-like immmunoreactivity in the rectum and the proximal two thirds of the intestine. A wide range in the strength of the immuno-reaction across the evolutionary spectrum was noted, this finding may indicate the presence of more than one bombesin-like neuropeptide in the fish. Given the multiplicity of the effects of bombesin and the difficulty of establishing a behavioural index of satiety in fish, it is not possible on the basis of the experiments conducted here to elucidate a possible mechanism for the anorexic effect of peripherally administered bombesin in the carp *Cyprinus carpio*, nor for the appearance of bout feeding in these animals. The results presented here do however provide yet another example of a behavioural effect of peripherally administered bombesin in a relatively primitive vertebrate. This type of result adds weight to the argument of McCoy

and Avery (1990), that bombesin has a role as an integrative peptide functioning as a peripheral and central satiety inducing agent. McCoy and Avery have argued that bombesin should be classed as an integrative peptide according to the definition of this type of peptide by Hoebel (1985) as one which, "acts in the body and in the brain to integrate physiological and psychological functions". The basic tenet of the integrative peptide hypothesis is that there are three types of mechanism responsible for homeostasis in any organism, physiological, behavioural and cognitive. Peptides which operate within the physiology-behaviour-cognition axis are candidate integrative petides. From the foregoing discussion it may be seen that bombesin is a peptide which acts very much within the physiology-behaviour-cognitive axis, furthermore it has been highly conserved during evolution, has both central and peripheral effects and has been shown to be effective across an enormous range of vertebrate species.

SYNOPSIS OF RESULTS

Recent advances in technology and the availability of cheap micro-computers has made the fine-grained analysis of the feeding behavior of individually housed carp possible. This study employed a high resolution analysis of the feeding patterns exhibited by carp feeding on an operant regime. The results of this experiment show quite clearly the absence of bout feeding.

A technique of functional localisation of a hypothalamic nucleus has been developed. This technique was used to locate and subsequently lesion the nucleus posterior thalamicus (NPTh). Lesions of the NPTh have been shown to cause a highly significant decrease in the food intake of carp feeding on an operant regime. These studies have demonstrated a role for the NPTh in the control of food intake in the carp.

The morphology of the cell bodies of the NPTh has been investigated. The relationship of the NPTh to both hinbrain and forebrain areas has also been investigated using antidromic activation of the nucleus. The NPTh is seen to be morphologically homogenous and to project to the medulla via a slow conduction velocity pathway. It is postulated that the NPTh performs a common function for both the internal and external chemosensory systems, and that this function is related to palatability discrimination.

The effects of the peripheral administration of bombesin have been investigated. Bombesin has been shown to reduce the food intake of carp feeding on an operant regime, in a dose dependent manner. A bombesin induced reduction in food intake is also evident in higher vertebrates, and demonstrates that although the central structures associated with feeding in the fish are highly developed and have no direct homology with similar systems in the higher vertebrates, certain fundamental pharmacological principles apply across a wide evolutionary range. The highest doses of bombesin have also led to an alteration in the microstructure of food intake such that a bout feeding pattern is seen to emerge. There appears to be no evidence from studies carried out in other species that peripheral administration of bombesin causes a change in the microstructure of food intake.

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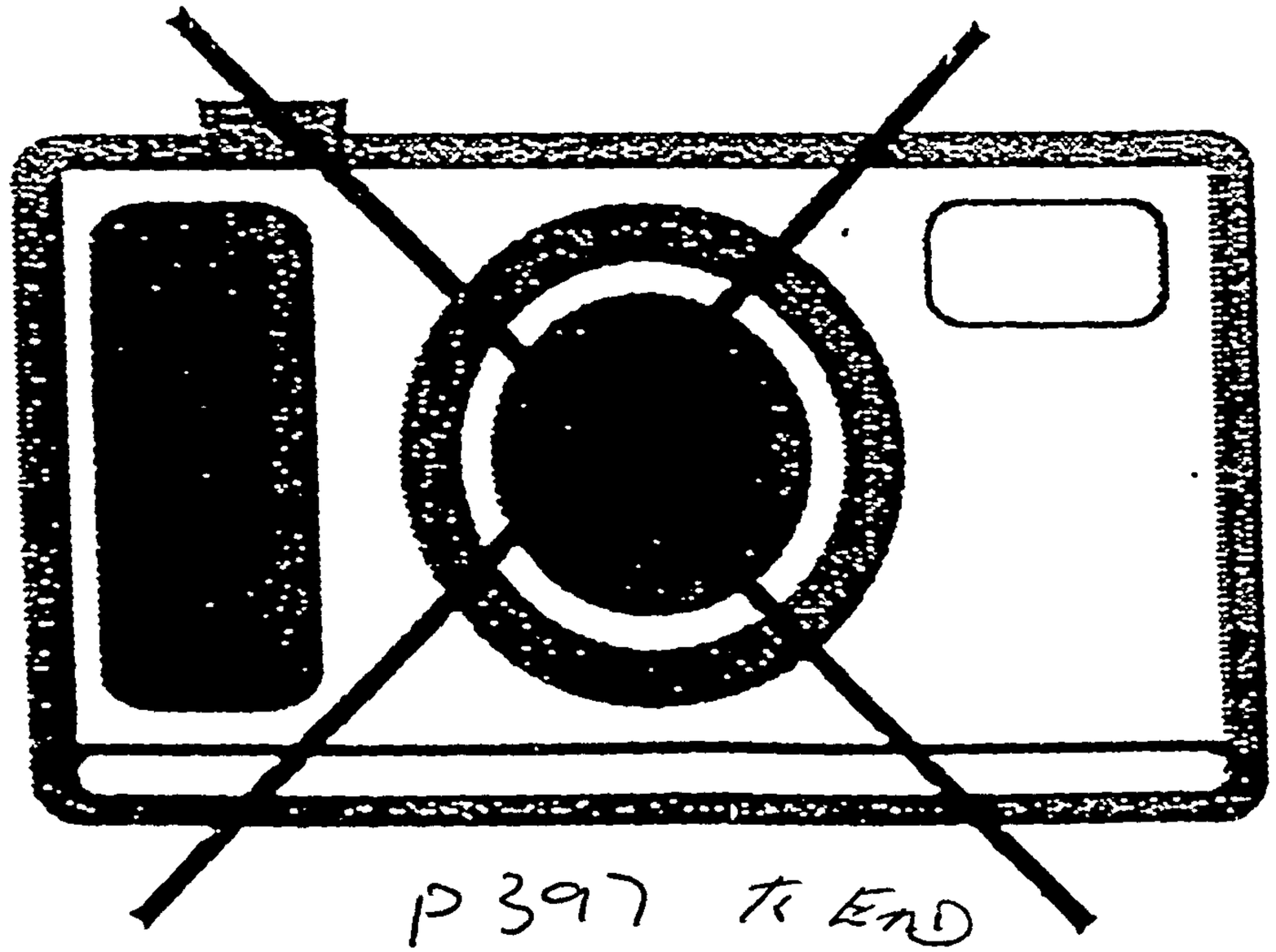

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