than the low fat wheel-housed animals. No instance of gastric ulcer was observed in home cage animals in either diet condition. No evidence of hepatic lesions was observed in either control or wheel-housed animals. Blood glucose levels were significantly higher in the high-fat wheel-housed rats ($F(3,56)=5.60$; $p<0.01$; Tukey-test) and lowest in the regular diet wheel-housed animals. The temporal course of the blood glucose changes appeared to be that of a gradual decline over days of 1-h feeding. Rectal temperature (YSI Instruments) indicated that fat-supplemented activity-stress rats had slightly (but not significantly) higher core temperatures than low fat activity-stress animals (36.3 ± 0.9 °C and 34.3 ± 1.9 °C, respectively). Home cage control rats averaged 37.5 (±1.7) °C. The energy intake in the low fat groups (0.08%) was approximately 4.25 kcal/g of food per day, while that of the fat-supplemented group was approximately 7.35 kcal/g of food per day.

Discussion. These data reveal an important etiological factor in experimental activity-stress-induced gastric disease and suggest that dietary fat may have a prophylactic effect in the development of this disease. It is important to note that both groups of activity-stress animals (high fat and low fat diet groups) exhibited similar and elevated levels of activity. In fact, any observed activity differences between these groups were not statistically significant. Thus, the major difference between the groups was not one of activity, but rather one of diet. Supplementing the diet with 32.5% fat resulted in a marked reduction of ulcer incidence (3 out of 15 fat-supplemented diet rats showed ulcer disease as opposed to 15 out of 15 low fat diet animals), frequency, and severity. Whether this phenomenon is a local effect in the gut or a systemic effect (suggested by the blood glucose data) remains to be determined.

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Little-excitable transitional cells in the rabbit sinoatrial node: a statistical, morphological and electrophysiological study

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Summary. It is demonstrated that systolic and diastolic depolarization rate are correlated with the percentage of myofilaments in the cells of the rabbit sinoatrial node. It appears that, in the rabbit sinoatrial node, little-excitable transitional cells exist in the zone of propagation at the septal side of the typical nodal cells.

In a correlative electrophysiological and ultrastructural study of 42 cells in the rabbit sinoatrial node, we have found that this structure has a symmetrical morphology. From the ultrastructural point of view it comprises typical nodal cells, surrounded by transitional nodal cells which gradually become atrial cells at the periphery. However, its electrophysiological activation pattern is asymmetrical, showing a preferential conduction pathway of the impulse, diagonally upward to the crista terminalis[2]. This preferential pathway seems to be caused by the existence of a zone of little-excitable cells at the septal border of the sinoatrial node, rather than by poor electrical coupling[3]. It is clear from electrophysiological and electronmicroscopical observations that typical nodal cells have the following features: a low percentage of myofilaments, a low upstroke velocity of the action potential, and a prominent rate of diastolic depolarization. Atrial cells bordering the sinoatrial node have opposite features. The transitional cells between the atrial cells and the typical nodal cells are intermediate both in the percentage of myofilaments and in the rate of diastolic depolarization. It was observed that the rate of diastolic depolarization and the percentage of myofil-

Mean value of 6 variables (±SE) after distribution of 30 cells from the sinoatrial region over 4 classes of cell types by stepwise discriminant analysis

	Typical nodal cells	Normally excitable transitional cells	Little-excitable transitional cells	Atrial cells
Number of cells	9	10	10	1
Percentage of myofilaments	27 ± 1.5	34 ± 1.8	40 ± 2.7	41
Orientation of myofilaments (%)	21 ± 0.9	37 ± 2.0	54 ± 2.1	80
Organization of myofilaments (%)	37 ± 1.9	58 ± 1.4	68 ± 2.3	88
Rate of systolic depolarization (V/s)	10 ± 1.1	18 ± 1.5	12 ± 0.8	28
Rate of diastolic depolarization (mV/s)	50 ± 1.2	31 ± 0.7	14 ± 0.6	15
Maximal diastolic potential (mV)	−66 ± 1.6	−63 ± 2.6	−60 ± 4.4	−63

The percentage of myofilaments is the percentage of intracellular space occupied by myofilaments. The orientation and organization of the myofilaments are given as summed scores in percentages. Orientation: 100%, myofilaments in one direction only; 0%, myofilaments occurring in all directions. Organization: 100%, all myofilaments organized in myofibrils; 0%, all myofilaments occur isolated only.

aments have an inverse relation in the rabbit sinoatrial node[4]. This is not true for the relation between the percentage of myofilaments and the upstroke velocity of the action potential.

It is the aim of this study to investigate whether it is relevant to divide the transitional cells into 2 different functional groups – the normally excitable and the little-excitable transitional cell groups – and to establish the spatial distribution of the 2 groups in relation to the typical nodal cells. To this end we attempted to discriminate between cell types using both electrophysiological and morphological variables as discriminating variables.

Materials and methods. Sinoatrial node preparations were made as described previously[2]. Action potentials were recorded by the conventional glass-microelectrode technique. At the end of the electrophysiological part of the experiments the tissue was marked iontophoretically with Alcian Blue at the sites where the action potentials had been recorded. Afterwards, these marked sites were excised (pieces 50–80 × 100 µm) for electronmicroscopical examination[4]. In 42 such pieces we measured: a) the intracellular volume percentage of myofilaments, b) the regularity of orientation of the myofilaments, c) the degree of organization of the myofilaments, d) the maximal upstroke velocity ($\dot{V}_{max}$) of the action potential and e) the rate of diastolic depolarization ($\dot{V}_{diast}$).

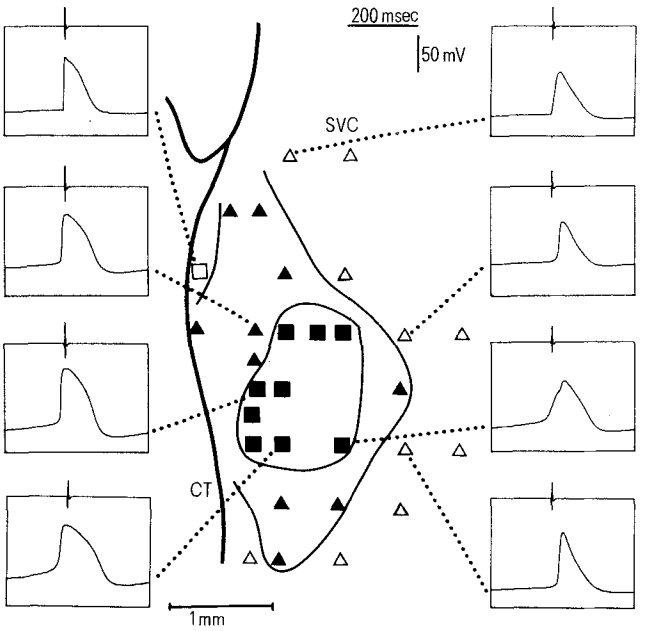
The percentage of myofilaments (a), the organization of the myofilaments (c), the upstroke velocity (d) and the rate of diastolic depolarization (e) were measured as described previously[4]. The quantification of the regularity of orientation of the myofilaments (b) for each of the 42 sites was done using the same 20 micrographs as had been used for the quantification of the myofilaments[4]. The orientation was scored on a 3-point scale noting that myofilaments could be seen cut in 3 different directions; longitudinally, obliquely and transversely. Micrographs with all myofilaments running in the same direction scored 3, those with myofilaments running in 2 directions scored 2 and micrographs with myofilaments in 3 directions scored 1 point. The results for 20 micrographs per site were summed and transformed to a percentage scale.

Definition of the cell types. Since electrophysiological differences may be observed in ultrastructurally identical parts of a sinoatrial node preparation[4], we classified 4 cell types only on the basis of electrophysiologica variables.

Atrial cells and typical nodal cells. We ranked the 42 cells from high to low upstroke velocity. Further we ranked these cells from low to high diastolic depolarization rate. We selected the 3 topmost cells of both lists and called them atrial cells ($\dot{V}_{max}$ 50±9.0 (SE) V/sec; $\dot{V}_{diast}$ 7±3.8 (SE) mV/sec). The 3 lowermost cells of both lists were called typical nodal cells ($\dot{V}_{max}$ 3±0.4 (SE) V/sec; $\dot{V}_{diast}$ 52±1.2 (SE) mV/sec).

Transitional cells. We selected from the 42 cells those with moderate diastolic depolarization rate (20–30 mV/sec). From those cells we selected the 3 cells with the highest upstroke velocity ($\dot{V}_{max}$ 26±2.4 (SE) V/sec; $\dot{V}_{diast}$ 27±1.8 (SE) mV/sec) and called them normally excitable transitional cells. From the same group of cells (20–30 mV/sec diastolic depolarization rate) we further selected the 3 cells with the lowest upstroke velocity, which were called little-excitable transitional cells ($\dot{V}_{max}$ 5±1.0 (SE) V/sec; $\dot{V}_{diast}$ 21±0.9 (SE) mV/sec).

Thus we used 12 out of 42 cells to define our 4 cell types. Apart from the 2 electrophysiological variables, the percentage of myofilaments, the orientation of the myofilaments and the organization of the myofilaments were variables in the discrimination procedure. The remaining 30 cells were divided over the 4 defined cell types as described below.



Spatial distribution of 30 cells over 4 cell types by classification after stepwise discriminant analysis. SVC; superior vena cava, CT crista terminalis; □, atrial cell; ■, typical nodal cell; ▲, normally excitable transitional cell; △, little-excitable transitional cell.

Stepwise discriminant analysis. Stepwise discriminant analysis was applied to the 5 variables (BMDP7M-Stepwise Discriminant Analysis, Health Sciences Computing Facility, University of California, Los Angeles, PDP-11 Version V2.0, Software Development Inc., Middlebury, Vermont 05753, USA). Briefly, discriminant analysis is a technique to distinguish between 2 or more groups of cases, using discriminant functions, i.e. linear combinations of the discriminating variables. In the stepwise discriminant analysis the discriminating variables are entered into the discriminant functions one at a time, taking at each step the variable which gives the largest improvement in discrimination power. The discriminant functions thus found may be used to classify new cases (in this study the remaining 30 cells), of unknown a priori classification. The statistical details are described by Jennrich[5].

Results and discussion. Stepwise discriminant analysis revealed that 3 of the 5 variables were selected as relevant. The 1st variable selected was the rate of diastolic depolarization. The 2nd variable was the rate of systolic depolarization and the 3rd was the percentage of myofilaments. The 30 cells were distributed over the 4 classes on the basis of these 3 variables (table). Our main aim was to decide whether the distinction between the 2 types of transitional cells is correct.

From microscopy we know that the transitional cells are situated between the typical nodal cells and the atrial cells. Since the computer had no information at all about the location of the 30 cells to be analyzed, it is very interesting to notice that the cells were assigned to the 4 classes in such a way that neighbouring cells were found within the same class (fig.). The 9 typical nodal cells form 1 group in the center of the preparation. There are no cells from other classes between them. The normally excitable transitional cells surround the typical nodal cells on all sides. One atrial cell is located at the crista terminalis side of the sinoatrial node. The selection of only 1 atrial cell is caused by the fact that few impalations were performed at the outer margins of the node. However, in many other experiments we

always observed atrial action potentials at all outer margins of the sinoatrial node[2,3]. The little-excitable transitional cells are predominant at the septal side of the typical nodal cells.

The table gives information about the values of all 5 variables after the distribution of the 30 cells over the 4 classes by stepwise discriminant analyis. The maximal diastolic potentials in the 4 classes are also given. These do not differ significantly. From the figure and the table we conclude that there is good reason to distinguish a 4th class of cells within the rabbit sinoatrial node. Little-excitable transitional cells are intermediate between atrial cells and the other 2 cell types with respect to all 3 tested morphological variables. However, their rate of diastolic depolarization is as low as that of atrial cells. Their rate of systolic depolarization is as low as that of typical nodal cells. Their low excitability causes the zone of block within the rabbit sinoatrial node. It explains the fact that the ultrastructure of the rabbit sinoatrial node is symmetrical, while the activiation pattern is asymmetrical.

1 The authors thank Joop Houtkooper and Wilbert van Meerwijk for statistical advice.

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Photoperiodicity in the male rosefinch[1]

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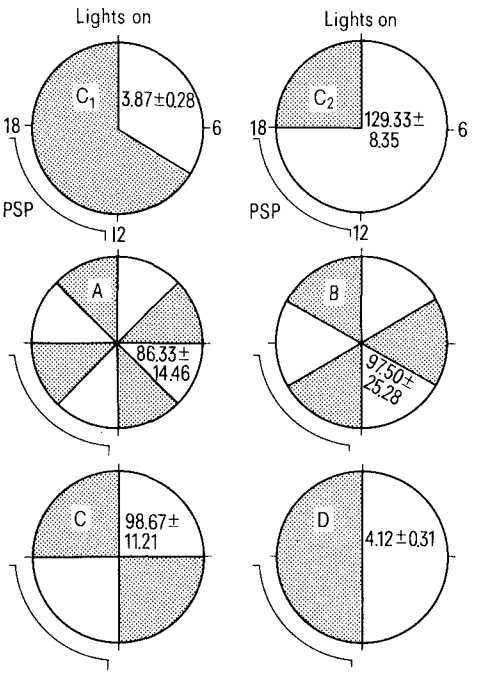
Summary. In male rosefinches *(Carpodacus erythrinus)* 12 h non-stimulatory photoperiods (12L:12D) were as effective in stimulating testicular recrudescence, as were long photoperiods (18L:6D) when presented as several intermittent symmetrical photoperiods. The results are discussed with regard to the hypothesis that photoperiodic effects in birds are mediated by circadian rhythms.

As yet most of the experimental evidence for photoperiodic gonadal responses in birds pertains to the temperate seasonal breeders[3,4]. However, recently we could demonstrate that the reproductive rhythms in a few migratory birds, overwintering on the Indian subcontinent (tropics/subtropics), are controlled by circadian rhythms, which are manipulated by light/dark cycles[5–10]. Here, we will report the results from the experiments performed on common Indian rosefinches *(Carpodacus erythrinus)* to determine the effect of symmetrical, intermittent light on testicular growth. This species uses longer photoperiods to induce its reproductive activities[11,12], and gonadotropic activity depends upon circadian period of light-sensitivity as shown by resonance and night-interruption experiments[7,8]. The present experiments were aimed to investigate: a) whether multiple light flashes given during the so-called photosensitive (=photoinducible) phase (PSP; 12 h following lights 'on' or 'dawn') were more effective than a single light flash, and b) whether photostimulated testis growth depends upon the duration of light within the PSP.

Materials and methods. Groups (n=4 each) of adult male rosefinches (previously maintained on 8 h light and 16 h darkness, 8L:16D) were exposed to different programmed photoperiods (3L:3D, 4L:4D, 6L:6D and 12L:12D) adding up to a total of 12 h light per day, at an intensity of about 300 lux at perch level, but differing in the number of light/dark cycles by which the 12 h light was given; besides, a group was exposed to 18L:6D as long-day controls. Laparotomy at this time showed that they had regressed testes (combined testicular weight, CTW=about 4 mg); simultaneously a group (n=4) was sacrificed and the testes were fixed in fixative; this served as initial control. After a 30-day exposure to the experimental conditions, all the finches were sacrificed and testes were fixed in fixative. Testes were fixed in fixative (a solution of 95% ethanol:glacial acetic acid:40% formalin:water; 50:10:10:30) for 2 days, transferred into 70% ethanol for an additional 2 days, and then weighed to nearest 1 mg on an ADCO



Gonadal responses of male rosefinches after 30 days (except C_1) in the photoperiodic cycles indicated. Each circle is a separate group and once around is a 24-h period. The stippled area represents scotophase. PSP represents photosensitive phase within a 24-h period, demonstrated by night-interruption experiments[8]. Group C_1 is the initial controls from 8L:16D; C_2, 18L:6D; A, 3L:3D; B, 4L:4D; C, 6L:6D; D, 12L:12D. All experiments started initially with 4 individuals; 3 birds, 1 each from C_2, A, and C, died during the experimental period and the data were not included in our statistical analysis. Within each group is indicated the mean testes weight ±SE. Testes weight in any of the stimulated groups were higher ($p < 0.001$) than initial or 12L:12D; there was, however, no significant difference among the stimulated groups.