administration of 2 ml of the anti-secretin antibody (No. 21) undiluted at high antibody concentrations, and on days 27 and 70 at low concentrations.

Anti-secretin antibody significantly diminished HCl-stimulated volume by 61.4% ($p < 0.01$), bicarbonate by 62% ($p < 0.001$), and protein output by 63% ($p < 0.005$) respectively (figure 1). All parameters returned to pretreatment levels, when the antibody 'concentration' was 30% (day 27) or less (day 70) of the immediate post injection value.

In contrast, pancreatic secretion following a test meal did not show any significant changes at any time after antibody administration (figure 2).

The data show that the secretin antibody used blocks the biological activity of exogenous and endogenous secretin. The pancreatic response to intraduodenal acid was reduced by over 60%. The residual pancreatic secretion could be due to pancreozymin[8,9] and peptide hormones such as VIP[10]. Alternatively, it is possible that the antibodies used did not completely block the biological activity of secretin.

The observation that there was no reduction of pancreatic response to a mixed liquid meal after antibody injection suggests that secretin is not involved in stimulating pancreatic secretion after the type of meal used in this experiment.

1 This work was supported by grant No. 3.816-0.76 of the Swiss National Foundation for Scientific Research.
2 We express our thanks to Miss G. van Hees, Mrs L. Jecker and Dr F. Meyer for their excellent technical assistance, Dr W.W. Rittmann for the surgical support, Dr G.A. Stalder for general advice, Dres R. Studer and D. Gillessen for providing secretin 'Roche', and Mrs C. Frei for secretarial work.
3 I. Fahrenkrug and O.B. Schaffalitzky de Muckadell, Eur. J. clin. Invest *7*, 201 (1977).
4 K. Gyr, L. Kayasseh, W.W. Rittmann, J. Girard and G.A. Stalder, Gut *19*, 355 (1978).
5 L. Kayasseh, W.L. Haecki, K. Gyr, G.A. Stalder, W.W. Rittmann, F. Halter and J. Girard, Scand. J. Gastroenterol. *13*, 385 (1978).
6 J. Girard, J.B. Baumann, G. van Hees, L. Kayasseh, K. Gyr and G.A. Stalder, Schweiz Med Wschr. *107*, 542 (1977).
7 L. Kayasseh, K. Gyr, G.A. Stalder, W.W. Rittmann and J. Girard, Hormone Res *9*, 176 (1978).
8 R.W. Spence, L.R. Celestin and R.F. Harvey, Gut *17*, 920 (1976).
9 D.D. Reeder, H.D. Becker, N.J. Smith, Ph.L. Rayford and J.C. Thompson, Ann. Surg. *178*, 304 (1973).
10 S.R. Bloom, S.J. Mitchell, G.R. Greenberg, N. Christofides, W. Domschke, S. Domschke, P. Mitznegg and L. Demling, Gut *18*, A963 (1977).

Regularly firing neurones in the rat suprachiasmatic nucleus

G.A. Groos and J. Hendriks

Department of Physiology and Physiological Physics, Department of Psychology, University of Leiden, Wassenaarseweg 62, Leiden (The Netherlands), 3 May 1979

Summary. The spontaneous discharge of some suprachiasmatic neurones in vivo and in vitro was found to exhibit a very constant interspike interval. In vivo these cells were comparatively rare and appeared to be mutually coupled. These findings are discussed in relation to coupled oscillator theories of circadian rhythm generation.

In mammals the suprachiasmatic nuclei of the hypothalamus (SCN) are generally thought to be involved in the photic entrainment of circadian rhythms. Moreover, they play an important role in the generation of selfsustained circadian oscillations[1]. Although gradually more insight is being obtained with respect to the physiology of entrainment[2,3] little is known about the putative oscillating mechanism of the SCN. In the present paper, we report the presence of regularly firing neurones in the rat SCN in vitro and in vivo. This finding will be discussed in relation to Pavlidis' coupled oscillator model of circadian rhythm generation[4].

Materials and methods. Hypothalamic slices were prepared from 16 albino rats which were decapitated after light halothane anaesthesia. From each brain 1 or 2 thin transverse slices (approx. 400 µm thick) containing the optic chiasm and SCN were dissected out. Within 12 min after decapitation, these slices were incubated in an in vitro incubation chamber[5]. The slice was bathed from below in a glucose/saline bicarbonate buffered medium, while the upper surface was exposed to a warm, humid atmosphere of 95% O_2 and 5% CO_2. The medium was equilibrated with this gas mixture to give a pH of approximately 7.3 at 37 °C. Flow rates varied between 1 and 2 $ml \cdot min^{-1}$. The composition of the incubation medium was: NaCl: 124 mM; KCl: 5 mM; KH_2PO_4: 1.24 mM; $MgSO_4$: 1.3 mM; $CaCl_2$: 1 mM; $NaHCO_3$: 26 mM; glucose: 10 mM. The temperature in the chamber was regulated at 37 ± 0.1 °C. Glass micropipettes either filled with Wood's metal and platinized at the tip or filled with 3 M KCl were positioned in the SCN. In addition single units were recorded in 42 anaesthetized rats using conventional recording techniques[2]. Power spectra of spike trains were computed according to the method of French and Holden[6]. Recording sites in both in vivo and in vitro experiments were verified histologically to be within the SCN by means of electrolytic or pontamine sky blue marking.

Results and discussion. The slices could be kept in good condition for recording for over 8 h after decapitation. Action potentials were recorded reliably for extended periods of time from 98 spontaneously active SCN units in vitro. 82 of the neurones had stationary randomly varying firing rates or showed a bursing spike pattern.

A remarkable observation was the presence of 16 regularly firing SCN cells (RFC's) exhibiting a very constant interspike interval (figure 1 A and C). These patterns do not represent an injury discharge as the firing rates were lower than those commonly observed in injured cells, while the frequency remained stable over extended periods of time. Moreover, the behaviour of these cells did not change when the electrode was slowly moved away from the cell and back. The interval distributions of all RFC's were Gaussian and typically had small coefficients of variation (range: 0.05–0.2, e.g. figure 1, B). Only cells with variation coefficients smaller than 0.2 were classified as regular. These small coefficients are only slightly larger than those found for molluscan beating neurones[7]. At present, however, it is unclear whether the RFC's in our sample are endowed with a similar intrinsic pacemaker mechanism as beating pace-

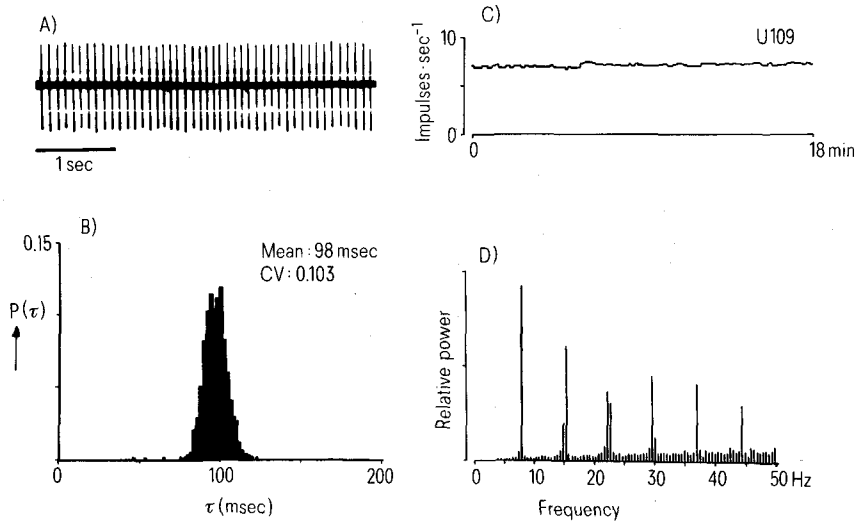

Fig. 1. Examples of 2 regularly firing cells in the in vitro SCN. In A the spike trace of 1 cell is shown with the corresponding interspike interval distribution (B). The stability of the firing rate over time for another cell is illustrated in C. The peaks in the power spectrum (D) computed for this neuron reflect the periodicity of discharge while the continuous part of the spectrum can be ascribed to Gaussian jitter of this periodic process.

makers, or whether synaptic processes are essential for their behaviour.

Although the discharge rate varies between individual RFC's (range: 4.2–21.7 sec^{-1}) the frequency of any particular RFC remained constant as a function of time (e.g. figure 1, C). The small fluctuation of the interspike intervals is reminiscent of an ideal pacemaker jittered by a Gaussian process[8]. The Gaussian interval distribution (figure 1, B) and the power spectrum of the spike process (figure 1, D) indicate this possibility.

The type of RFC described here has also been observed in hippocampal nerve cell cultures although in this structure they appear to be absent in vivo[9]. To assess the possibility that the RFC's in the SCN described here are similarly present in the in vitro preparation only, we recorded single unit activity in the SCN of anaesthetized rats. In a total number of 397 spontaneously active SCN units we only encountered 2 RFC's (figure 2) using the same classification criteria as those stated for the in vitro preparation. Interestingly these cells were located very close to each other as they could be recorded simultaneously with 1 micro-electrode. Moreover, their periodic spike processes exhibited a very strong phase relationship as an actionpotential in one of the units was always immediately preceded by a spike in the other. Neither the period nor the phase of these RFC's were affected by changing the illumination level of the retina. It is conceivable that this lack of responsiveness to visual stimulation resulted from the depressive effect of anaesthesia. It should be noted, however, that visual SCN cells receiving a direct retinal input are not significantly depressed by urethane[2], the anaesthetic used in the present in vivo experiments. Besides the SCN we also encountered 1 RFC among 258 units in the preoptic area and another among 315 neurones in the anterior hypothalamic area.

One interpretation of the scarcity of RFC's in vivo is that the specific condition of the neurones and their interconnections in vitro seems to make their occurrence more likely, as seems the case in the hippocampus[9]. On the other hand the fact that regular firing is not uncommon in the SCN units in vitro and that similar RFC's encountered in the in vivo SCN may be mutually coupled is interesting in the light of coupled high frequency oscillator models as the basis for circadian rhythms[4]. In a neural structure such as the SCN one could conceive of such a mechanism as a network of synaptically connected, short period RFC's. However, coupled oscillator models require a large number of light-sensitive oscillators to generate a circadian rhythm

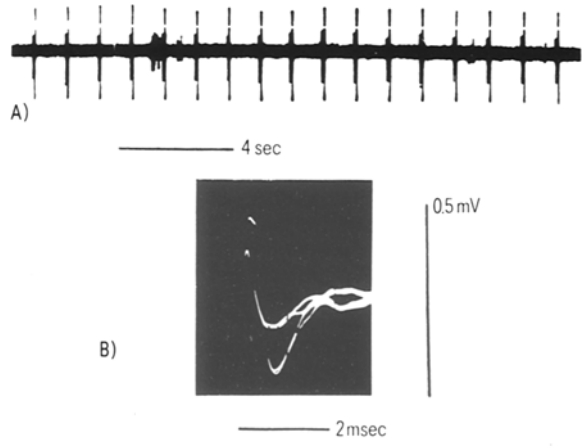

Fig. 2. Discharge pattern of 2 coupled, regularly firing neurones in the SCN in vivo. These cells were recorded simultaneously with the smaller spike immediately preceding the larger one (A). This is illustrated in *B* where a single pair of spikes triggered the oscilloscope set at a faster time base.

from high frequency oscillators. Consequently the apparent scarcity of SCN units in vivo exhibiting regular discharge patterns, their insensitivity to changes in retinal illumination and the fact that they are not confined to the SCN which is the major brain structure involved in circadian rhythm generation make it less attractive to apply the coupled oscillator model to the rat SCN. Further study of the response of RFC's to temperature changes[4] or deuterium oxide[10] is needed, however, to establish the usefulness of this model more firmly.

1 M. Menaker, J.S. Takahashi and A. Eskin, A. Rev. Physiol. *40*, 501 (1978).
2 G.A. Groos and R. Mason, Neurosci. Lett. *8*, 59 (1978).
3 S. Daan, Annls N.Y. Acad. Sci. *290*, 51 (1977).
4 T. Pavlidis, Bull. Math. Biol. *40*, 675 (1978).
5 C.F. Doré and C.D. Richards, J. Physiol. *239*, 83 P (1974).
6 A.S. French and A.V. Holden, Kybernetik *8*, 165 (1971).
7 D. Junge and G.P. Moore, Biophys. J. *6*, 411 (1966).
8 A.V. Holden, Models of the stochastic activity of neurones. Springer-Verlag, Berlin 1976.
9 M.B. Shtark, V.I. Stratievsky, A.S. Ratushnjak, L.V. Voskrensenskaja and N.P. Karasev, J. Neurobiol. *7*, 551 (1976).
10 P.C. Caldarola and C.S. Pittendrigh, Proc. natl Acad. Sci. USA *71*, 4386 (1974).