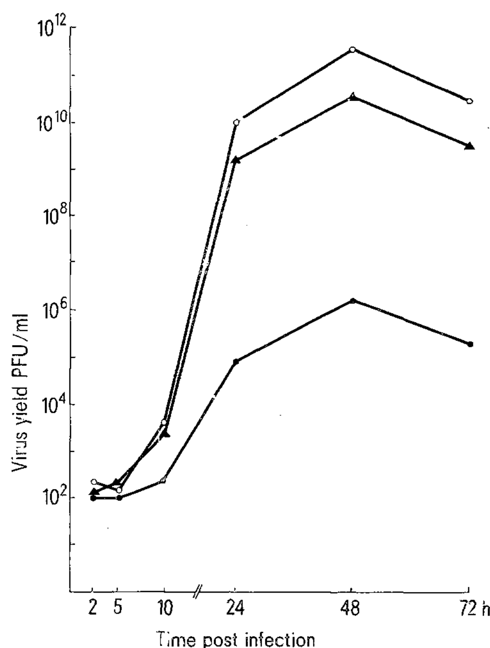

Fig. 1. Growth cycle of SFV in treated and untreated L_{929} cells. Control untreated cells (●); cells treated with yolk sac fluid (○); cells treated with allantoic fluid (▲).

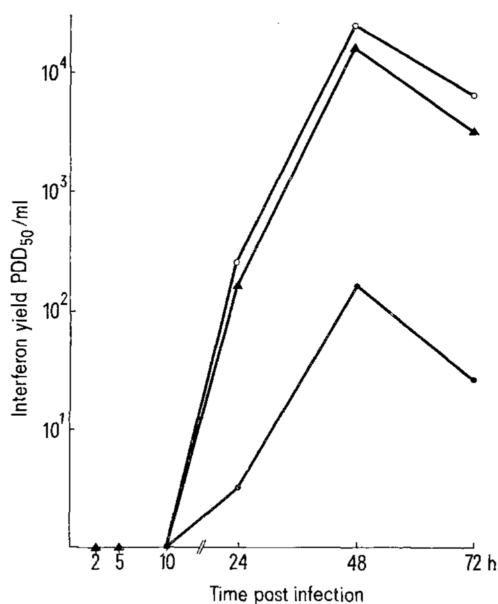

Fig. 2. Interferon production in treated and untreated L_{929} cells during SFV growth. Control untreated cells (●); cells treated with yolk sac fluid (○); cells treated with allantoic fluid (▲).

Reports in the literature[6,7] reveal that egg fluids have an inhibitory effect on interferon. It was thus desirable to clarify whether a blocking effect on the endogenic interferon, formed during viral replication, was responsible for the stimulation of SFV.

The effect of egg fluids on interferon synthesis was the first parameter to be explored. The data obtained (Figure 2) indicate that egg fluids, far from having a suppressive effect on interferon production, actually increased its yield, possibly as a consequence of enhanced virus titer.

An inhibitory effect on the antiviral action of interferon might be the alternative mechanism for the stimulatory phenomenon observed. Therefore, interferon, obtained from West Nile virus-infected mice brain, was titrated in L_{929} cells treated by the enhancing factor and in untreated control cells, using VSV as a challenge virus. Only a slight inhibition (about 2-fold) of interferon activity was observed after egg fluids treatment, a result which cannot explain the 10^5-fold enhancement in virus production.

These experiments indicate that the mechanism of the enhancement phenomenon is not based mainly on an inhibitory effect of the endogenic interferon. It is possible that the enhancing factor acts as a stimulator in one of the stages of viral multiplication.

The precise chemical nature of the enhancing factor, present in egg fluids, has not yet been determined. However, investigation of some of its physical and chemical properties have eliminated the possibility of its being a protein, lipid or nucleic acid. This conclusion is based on the fact that the enhancing factor preserved its activity after heating at 100 °C for 60 min, and even after exposure to 120 °C at a pressure of 1.5 atmospheres for 20 min. This factor is neither dialyzable nor can it be sedimented at $123,000 \times g$ for 4 h, and retains its activity after treatment at different pH values over a range from pH 1 to pH 10 for 72 h. Exposure to proteolytic enzymes, such as trypsin or pronase, and to lipase, RNase or DNase, did not lead to any decrease in the enhancing activity.

Résumé. Un agent stimulant la reproduction des virus a été trouvé dans les liquides des œufs fécondés, non-infectés. Cet agent a stimulé la multiplication des virus Semliki Forest jusqu'à 10^5 fois dans les cellules L_{929}. Nous avons constaté que le mécanisme de ce phénomène stimulant n'est pas basé principalement sur l'effet suppressif de l'interféron endogénique.

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Lateral Hypothalamic Self-Stimulation and Post-Stimulation Eating

We have shown that eating can be elicited by electrical stimulation of the neocortex and hippocampus, as well as by KCl-induced single waves of spreading depression in these structures[1–3]. In rats the eating in all cases occurs reliably with a latency of 2–6 min and is usually preceded by short bouts of shaking (especially upon hippocampal stimulation). We have suggested that propagated steady potential change is the common underlying event that

somehow leads to eating, since it accompanies both stimulation-induced limbic and neo-cortical afterdis charges, as well as KCl-induced spreading depression in these structures[3].

Similar so-called post-stimulation effects were previously reported upon stimulation of the hippocampus (drinking) by MCLEAN[4], and thalamus (eating) by MAIRE[5]. NEAL MILLER[6] summarized unpublished work by COONS and KRASNE demonstrating post-stimulation eating with onset latencies of about 4 min, preceded by shaking, upon stimulation of the anterior thalamus, lateral septal nucleus and ventromedial hypothalamus. MORGANE[7] also found poststimulation feeding upon stimulating the ventromedial hypothalamus, and SMITH et al.[8] upon stimulating the thalamus. Post-stimulation feeding was subsequently reported with stimulation of the septum[9,10] and substantia nigra[11]. The reports of eating occuring between closely-spaced trains of hippocampal stimulation in rats[12] and hamsters[13], probably represent the same phenomenon.

This type of post-stimulation eating has not been reported (as far as we know) upon stimulation of the lateral hypothalamus, which, instead, has been identified with the dual effects of stimulus-bound consummatory behavior (feeding and other behaviors elicited during the stimulation) and self-stimulation. Hence, we decided to specifically test for lateral hypothalamic post-stimulation eating.

Fourteen 300–350 g male Sprague Dawley rats were stereotaxically implanted with bipolar stimulating electrodes (Plastic Products Co., stainless steel, 0.2 mm tip diam.) aimed at the lateral hypothalamus. They were then tested for post-stimulation eating and self-stimulation. The electrical stimulation consisted of 0.1 msec rectangular pulses at variable frequency (50–100 Hz) delivered by a constant current stimulator. Self-stimulation in a Skinner-box was tested at various current levels using 0.1–0.3 sec trains of stimulation. 12 animals exhibited both self-stimulation as well as post-stimulation eating from the same electrode.

The post-stimulation eating was very similar to that observed upon electrical stimulation of the hippocampus: it occured at latencies from 2–5 min and was generally preceded by short bouts ($\sim$1 sec) of shaking. The animals ate between 1–2 g of solid food; drinking was observed less frequently. Post-stimulation eating was elicited at relatively high current levels, far above the threshold for self-stimulation (eg. 600–1550 μA vs 100–200 μA). This differentiates lateral hypothalamic post-stimulation eating from lateral hypothalamic stimulus-bound eating, which requires current levels below the threshold for self-stimulation[14]. Furthermore, post-stimulation eating appeared optimally only after repeated stimulation trials (5–10 trains). Although post-stimulation eating could be induced with 0.1–0.3 sec trains of stimulation, longer trains (eg. 5 sec) were more effective.

The animals were allowed to self-stimulate under continuous reinforcement schedules at high current levels that were previously found to be effective for inducing post-stimulation eating. 3 animals, with relatively high reward threshold and unstable, low rates of self-stimulation, intermittently stopped lever-pressing and showed post-stimulation eating preceded by bouts of shaking. The other 9 rats exhibited longer pauses in lever-pressing (1–4 min) along with bouts of shaking, but eating was observed rarely, and only during the first one or two self-stimulation sessions. During later sessions, instead of eating they returned to the lever to commence self-stimulation. This latter observation explains why post-stimulation eating has not been reported concomitantly with self-stimulation of the lateral hypothalamus. Apparently the animals 'prefer' to self-stimulate rather that eat. This is not surprizing, since it has been reported that food deprivated animals show a similar preference[15], and that lateral hypothalamic stimulation which elicits stimulus-bound eating, will instead induce lever-pressing after the animal has had extensive self-stimulation experience[16]. Another possible reason why lateral hypothalamic post-stimulation eating has not been recognized is that animals showing evidence for convulsive activity concomitant with self-stimulation or exhibiting unstable lever pressing due to pauses, are frequently discarded from further investigation.

Post-stimulation feeding has been reported concomitant with self-stimulation of the hippocampus[12,13]. This can be explained by the low hippocampal threshold for stimulation-induced afterdischarges, and by the fact that this structure, unlike the lateral hypothalamus, is not a primary focus for self-stimulation[17,18], thus, allowing eating to preponderate.

Lateral hypothalamic post-stimulation feeding seems to be indentical with hippocampal, septal, thalamic and neocortical post-stimulation and spreading depression-induced feeding, which may all be due to a common process (which we suggest to be steady potential changes, since these accompany both spreading depression and limbic and neocortical afterdischarge).

On the bases of our studies and those of others, it seems likely that stimulation of any part of the brain that activates sufficient massive input to limbic structures (hippocampus and amygdala) will induce the sequence of events (afterdischages and /or spreading depression) that somehow lead to the eating response. It follows that neurochemical depolarizing agents injected into the brain could induce feeding via the same route; i.e. by triggering discharge into limbic structures.

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Zusammenfassung. Es konnte gezeigt werden, dass elektrische Stimulation des lateralen Hypothalamus mit einer Verzögerung von 2–6 min Fressen auslöst. Selbstreizversuche zeigten, dass diese Stimulation auch belohnend wirkt. Ähnlich verzögertes Fressen kann auch durch Reizung oder spreading depression in anderen Hirngebieten ausgelöst werden. Es wird vermutet, dass alle diese Phänomene auf einem gemeinsamen Mechanismus basieren.

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Cuticular Gas in Marine Decapod Crustacea

A film of gas, perhaps no more than a few microns thick, on the body surface has been suggested as the basis of the pressure sensitivity shown by certain marine crustacea[1], but until recently it has not been possible to establish the existence of such minute volumes of gas experimentally. The ultrasonic techniques currently available are either non-specific for gas[2] or designed to detect moving bubbles using the Doppler shift principle[3,4]. An alternative method[5,6], based on measurement of the harmonic waveform distortion caused by gas in an acoustic field has been developed in the Department of Electronic and Electrical Engineering at the University of Birmingham. This technique, which has the advantage of being specific for gas, is thus suitable for the detection and estimation of small, stationary gas volumes in a liquid medium, and has been used here for the detection of gas on the body surface of decapod crustacea.

Materials and method. Experiments were carried out with the transmit and receive acoustic transducers arranged about 5–7 mm apart (Figure 1a) and immersed in kerosene to avoid electrolysis at the metallic surfaces of the transducers. An acoustically 'transparent' test cell, containing water boiled at reduced pressure to remove any residual air bubbles, was positioned between the two transducers and the apparatus calibrated by generating a known volume of gas electrolytically on the end of a fine copper wire, insulated to the tip and held in the acoustic field. Legs removed from living or freshly killed specimens of two pressure-sensitive species of crab, *Carcinus maenas*[7] and *Macropipus holsatus*[8], were mounted in a rubber

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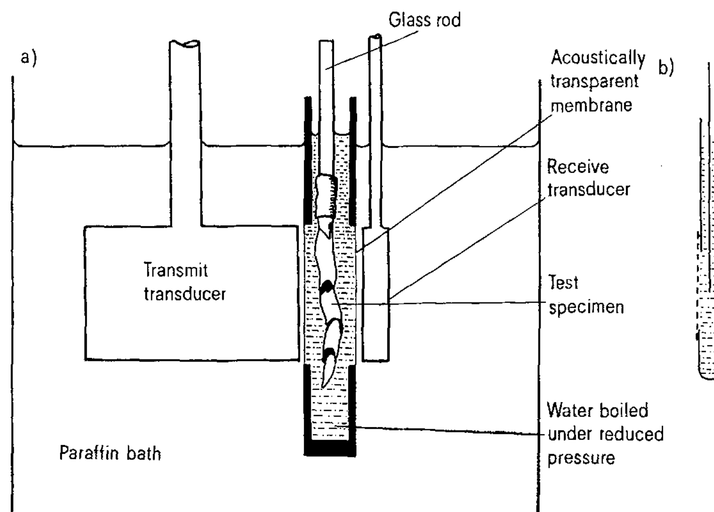

Fig. 1. a) Diagram of the apparatus. The transmit transducer was made from paired lead-zirconate plates mounted on aluminium alloy blocks and enclosed in an aluminium alloy housing while the receiver contained a single plate embedded in araldite in an aluminium alloy block. Kerosene was preferred to water as an acoustic coupling fluid to avoid generating gas electrolytically on the metallic surface of the transducers. The specimen under investigation was lowered into a test cell with acoustically-transparent membrane walls, and containing water boiled under reduced pressure to remove air bubbles. The fundamental and harmonic waveforms were displayed on an oscilloscope and the harmonic distortion component amplified and rectified for monitoring on a pen recorder when required. b) Diffusion cell with dialysis membrane collar.