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EVIDENCE FOR CENTRAL TIMING OF RHYTHMICAL MASTICATION

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SUMMARY

1. The origin of the co-ordination and rhythm of mastication provoked by electrical stimulation of the putamen and corticobulbar pathways was studied in the rabbit.

2. Bursts of activity were recorded from the mandibular and hypoglossal nerves and the hypoglossal nucleus, which were in phase with the observed masticatory movements. Discharges occurred alternately in nerves to jaw-opening and jaw-closing muscles.

3. The rate of burst discharges was not altered by paralysis, or by large variations in the stimulation frequency.

4. Regularly recurring bursts of activity continued to occur in the hypoglossal nucleus in response to random frequency stimulation after severing branchial nerves, cervical nerves and the spinal cord of paralysed rabbits.

5. Mechanical deformation of the brain of vascular or respiratory origin was discounted as the origin of the rhythm.

6. It is concluded that mastication is controlled by a brain-stem pattern generator which can be activated by adequate inputs from certain higher centres and, as concluded in other studies, from the oral cavity itself.

INTRODUCTION

The co-ordination and rhythmicity of mastication have been traditionally attributed to the alternate activation of two simple brain-stem reflexes (Sherrington, 1917; Rioch, 1934; Kawamura, 1964, 1967; Kidokoro, Kubota, Shuto & Sumino, 1968). These are the jaw-opening reflex, activated by tooth pressure or tactile stimulation of wide areas of the mouth and lips, and the jaw-closing reflex which follows stretch of the elevator muscles during opening (Sherrington, 1917; King, Minz & Unna, 1955). The introduction of a food bolus into the mouth was thought to initiate

a self-perpetuating cycle by producing jaw opening. The consequent stretching of the elevator muscles would produce jaw closure on to the bolus, again producing jaw opening by stimulation of periodontal and soft tissue receptors.

Repetitive electrical stimulation of the motor cortex and of subcortical structures in experimental animals has been shown by many workers to evoke rhythmical jaw and tongue movements which resemble normal mastication (Ferrier, 1886; Miller, 1920; Bremer, 1923; Magoun, Ranson & Fisher, 1933; Rioch, 1934; Kawamura & Tsukamoto, 1960; Sumi, 1969; Lund & Dellow, 1970). Rioch (1934) postulated that electrical stimulation of corticofugal pathways selectively excited jaw-opening motoneurones. The rhythm of mastication would then result from intermittent inhibition of tonic jaw opening during reflexly initiated jaw closure. In contrast, Magoun *et al.* (1933) suggested that stimulation of suprabulbar sites activated a rhythmic bulbar centre controlling mastication. Support for this view has recently been provided by Sumi (1970).

The following experiments were conducted to test the hypothesis that the rhythmicity and co-ordination of mastication are ordered within the brain stem. A preliminary report of these results has been presented (Lund & Dellow, 1969).

METHODS

Twenty-one male New Zealand albino rabbits weighing 2·3–5·2 kg were anaesthetized with a 20 % solution of urethane (8 ml./kg I.V.), tracheotomized and fixed in a stereotaxic apparatus. Deep colonic temperature was monitored and maintained at $38 \pm 1°$ C.

Surgical preparation

In six animals in which recordings were to be made from the trigeminal nerve, the zygomatic arch and superficial belly of the masseter muscle were removed on one side to reveal the masseteric nerve. The mylohyoid nerve was exposed by removing the remainder of the masseter, the ascending ramus and angle of the mandible.

In another six rabbits the hypoglossal nerve was identified in the neck and prepared for recording. All nerves were cut as far distally as possible, and surrounded by pools of warmed paraffin oil.

Nine additional rabbits were prepared for recording from the hypoglossal nucleus in the following manner. An occipital craniotomy and removal of the arch of the atlas were followed by severing of the spinal cord at the level of C2. Nerves C1, C2, XII, XI, X, IX and VII were cut bilaterally and the exposed surface of the medulla covered with 2 % agar in Ringer–Locke solution. Bilateral parietal craniotomy and removal of the occipital poles of the cerebrum provided access to the trigeminal nerves, which were exposed by removing bone from the base of the tentorium. These nerves were then severed. Arterial blood pressure was continuously recorded and maintained when necessary at the level prevailing before section of the spinal cord with an intravenous infusion of methoxamine hydrochloride in 10 % dextrose saline.

In all twenty-one animals, gallamine triethiodide (Flaxedil, 5 mg/kg.hr I.V.) was

administered either before recording was commenced or after data had been gathered in the unparalysed state. Animals were then respired by a positive pressure respirator at a volume and frequency sufficient to maintain the end-tidal CO_2 concentration at about 4·5 %.

Stimulation

Rhythmical masticatory movements and bursts of neuronal activity in the branches of the trigeminal nerve, hypoglossal nerve and nucleus were evoked by stereotactic electrical stimulation of the putamen or the corticobulbar pathways from their point of origin down to the level of the pons. All records of activity in the branches of the trigeminal nerve were made while using 10 sec trains of stimulation of 40 Hz, 1 msec pulse duration and a voltage 1·5 times threshold. The effect of the various input parameters described below on the frequency of masticatory discharges was calculated from records of hypoglossal nerve and nucleus activity.

Trains were generally of 10 sec duration, although when the stimulation frequency was below 20 Hz it was sometimes necessary to prolong the period of stimulation because latencies of greater than 10 sec were encountered. Pulses were always of 1 msec duration. Threshold voltages for the production of rhythmical activity (T 1) were found for each stimulation point, using a constant frequency stimulus of 40 Hz. Voltages 1·5, 2 and 3 times this value (T 1·5, T 2, T 3) were employed for subsequent stimulation trials. The trains of constant frequency stimulation (5–500 Hz) were delivered by a Grass S 4 stimulator and isolation unit.

Random frequency stimulation, with each pulse of constant voltage and duration, was obtained by amplifying the output of an Electra model 63 white-noise generator, which was then used to trigger the stimulator. The average frequency of stimulation could be adjusted by varying the amplifier gain or the frequency control of the stimulator. Trains of average frequency 4·2–680 Hz were used.

Interval histograms of sample stimulus trains were exponential in form although there were a greater number of short intervals than predicted for an ideal random process. However, the mean of the distribution approximately equalled the standard deviation (e.g. at an average stimulation frequency of 52·4 Hz, the mean interval between pulses being 19·1 msec, the S.D. was ± 20·7 msec). Each stimulus train was therefore accepted as a random process.

Using both methods of stimulation, multiple trials separated by 40 sec intervals were carried out both before and after paralysis.

Electrical recordings

Discharges were recorded from the hypoglossal nerve trunk and the branches of the trigeminal nerve by silver-wire bipolar electrodes and from the hypoglossal nucleus by fine concentric bipolar electrodes, shaft diameter 0·25 mm and tip separation 0·5 mm. Each recording electrode was connected serially to a Grass P 15 preamplifier, a Type 2A 63 plug-in amplifier of a Tektronix 565 oscilloscope and an Ampex 4 channel tape-recorder. The stimulus train and, during some experiments, the femoral arterial blood pressure or e.c.g. were also recorded on tape. Tapes were later replayed for analysis or photography.

Data analysis

The neural recordings from the hypoglossal nerve and nucleus were integrated by a Beckman Type R Dynagraph and each burst of activity was represented by a single pulse at a point in time when the level of activity exceeded an arbitrary

threshold (see Fig. 1). Frequency analyses, autocorrelograms, and cross-correlation histograms between neural bursts and heart beats were computed by hand as for a point process (Gerstein & Kiang, 1960; Perkel, Gerstein & Moore, 1967).

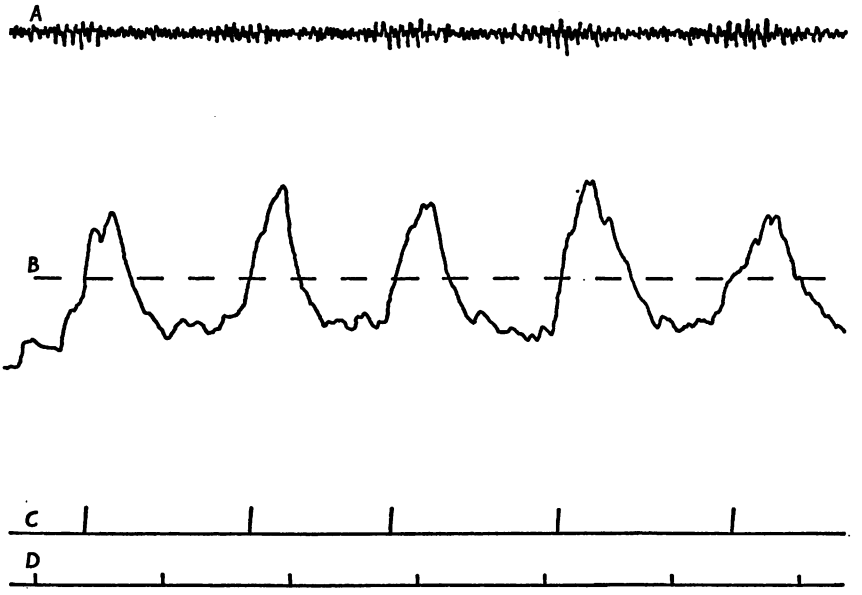

Fig. 1. Tracing from a polygraph recording on curvilinear paper. *A*, discharge from XIIth nucleus, integrated in *B* and reduced to a point process (*C*). *D*, time calibration 0·2sec.

RESULTS

During central stimulation, bursts of activity which were in phase with the observed masticatory movements were recorded from masseteric and mylohyoid branches of the trigeminal nerve and from the hypoglossal nerve or nucleus. Jaw opening was always the first movement observed following a latent period from the beginning of stimulation which varied from approximately 200 msec to greater than 10 sec at low stimulation frequencies. Usually one or two chewing movements and discharge bursts followed the cessation of stimulation, although sometimes the masticatory activity was lost before the end of the stimulus train.

Activity in the masseteric nerve was associated with jaw closing, and in the mylohyoid it was synchronous with jaw opening. Paralysis with gallamine did not abolish these discharges (Fig. 2). When recording from these two branches of the trigeminal nerve, activity was always seen initially in the neurogram recorded from the mylohyoid nerve after a latency of the same order as that recorded before paralysis. Discharges recurred alternately in these nerves to jaw-opening and jaw-closing muscles

throughout the period of activity. Recordings made after paralysis from the hypoglossal nerve and nucleus similarly appeared unaltered in latency, in frequency of masticatory discharges or in regularity. Throughout these experiments, no differences were observed between ipsilateral and contralateral stimulation. The following results are all taken from recordings made from the hypoglossal nerve and nucleus.

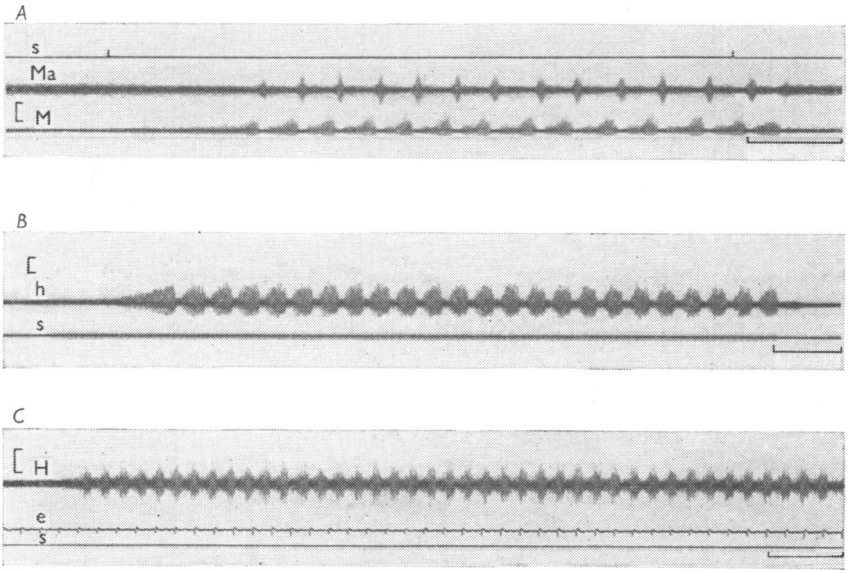

Fig. 2. Neurograms recorded from three paralysed animals while stimulating (*A*) cortical white matter, 30 V, (*B*) pedunculus cerebri, 9·9 V, (*C*) internal capsule, 12 V. The frequency of stimulation was 40 Hz and of pulse duration 1 msec. Calibrations, 200 μV and 1 sec. Neurograms: Ma, Masseteric; M, Mylohyoid; h, hypoglossal; H, hypoglossal nucleus; s, stimulus; e, e.c.g.

Input–output relationships

Constant frequency stimulation in paralysed animals

The relationship between stimulation frequency and the discharge frequency is shown graphically in Fig. 3. Above a certain input frequency the output frequency (bursts/sec) increased with the stimulus frequency to reach a limiting value which varied from animal to animal within the range 2·4–4·8 bursts/sec. In the example shown, this plateau was attained in a very abrupt manner, while in some other animals it was approached more gradually and reached at a stimulus frequency between 10 and 30 Hz. Output later decreased beyond an input frequency of between 80 and 400 Hz.

Increasing the input voltage increased the range of stimulation frequencies at both the low and high ends of the freqency spectrum which

would elicit a response. The limiting output rate was slightly higher with voltages of T 2 and 3 than at T 1·5.

Random frequency stimulation in paralysed animals

As previously stated, the stimulus trains were found to be approximately random, and incapable of providing the animal with timing information. In contrast, the output recorded from the hypoglossal nerve

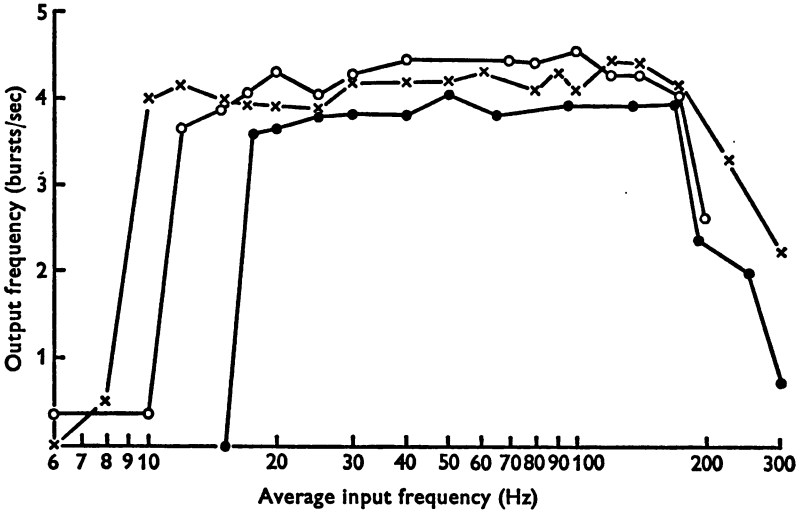

Fig. 3. Semilog. plot of the relationship between the stimulation frequency, using regular trains of monophasic pulses, and the rate of occurrence of bursts of neural activity. The stimulus was delivered to the internal capsule. Three stimulus strengths were used: T 1·5, 13·5 V (●); T 2, 18 V (○); and T 3, 27 V (×).

and from the hypoglossal nucleus still consisted of regular bursts of activity, as illustrated in Fig. 4 and verified in the autocorrelation analyses (Fig. 5). The discharge pattern did not change if the respirator was turned off for the period of stimulation.

In a number of the cross-correlation analyses there were no intervals following each QRS complex during which bursts of neural activity tended to occur (e.g. Fig. 5*A*), which would indicate independence between the two events. However, other cross-correlation histograms exhibited peaks (Fig. 5*B*, *C*), but these varied in phase and magnitude from sample to sample in the same animal.

The average stimulus frequency and the discharge frequency (Fig. 6) have the same type of three-phase relationship as that previously described for constant frequency stimulation in the same animal (Fig. 2). While these two figures are not directly comparable because the regular and

random frequency stimulation trials were not run alternately, the limiting output frequency was approximately 4 bursts/sec in both cases. Again, increasing the voltage extended the range of effective stimulation frequencies but had less effect on the limiting output.

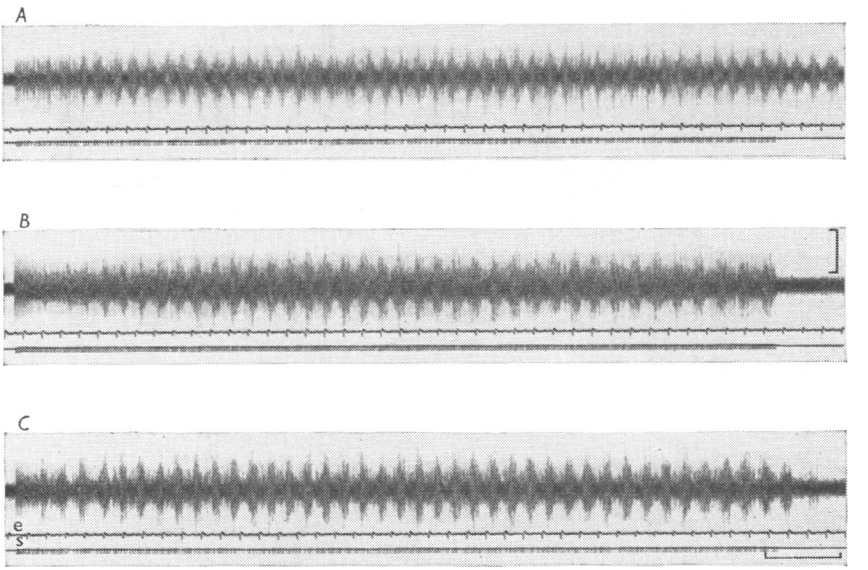

Fig. 4. Recordings from the hypoglossal motor nucleus made during monopolar, monophasic stimulation of the internal capsule (*A*) T 3 (24 V), average frequency 152 Hz; (*B*) T 2 (16 V), average frequency 285 Hz; (*C*) T 1·5 (12 V), average frequency 221 Hz. e, e.c.g.; s, stimulus. All three records were taken from the same animal. Calibrations, 200 μV and 1 sec.

Comparison before and after paralysis

When multiple stimulus trials were run before and 15 min after the administration of the first dose of gallamine, no real differences could be seen in the input–output relationship diagrams (Fig. 7). The addition of proprioceptive feed-back does not therefore appear to affect the basic transfer characteristics of this masticatory pattern generator.

DISCUSSION

The following criteria were used by Gloor (1960) to prove that electrically stimulated masticatory movements are of true neural origin: absence of frequency following, latency, post-stimulatory continuation and ineffective stimulation points. These criteria are similarly fulfilled by the bursts of

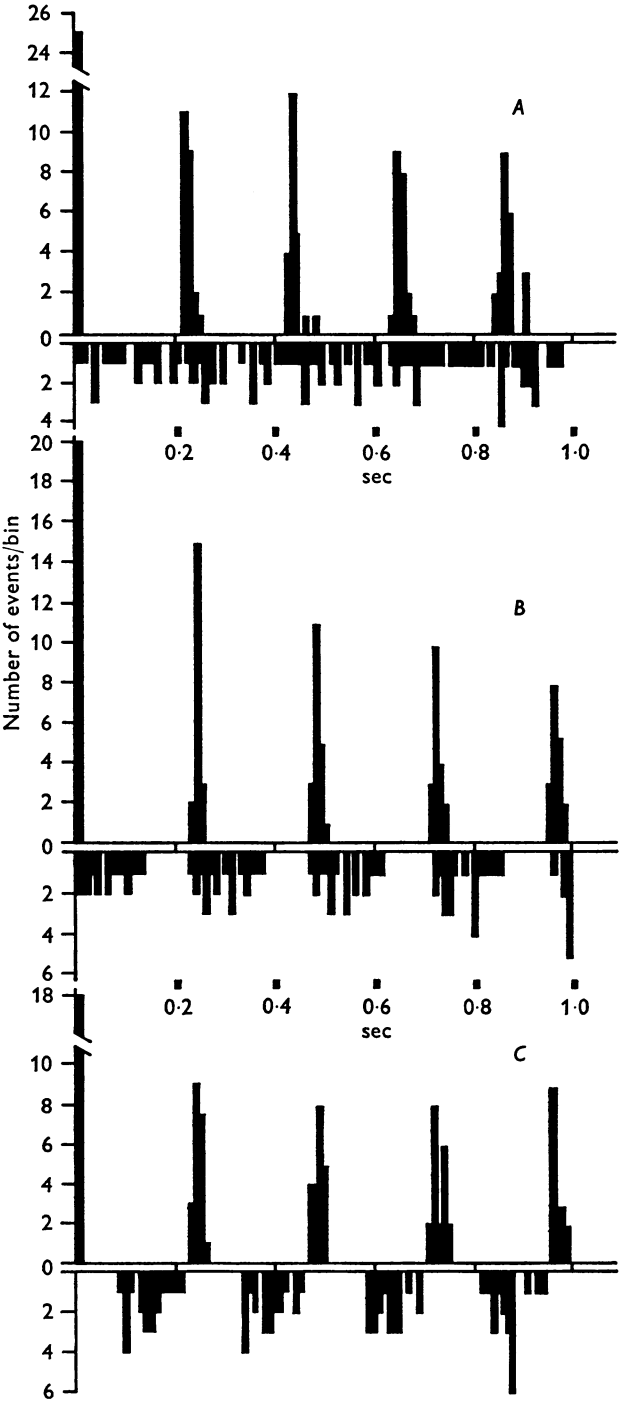

Fig. 5. For legend see opposite page.

neural activity recorded before and after paralysis in the present experiments. However, proof that the central nervous system alone can generate the pattern of neuronal activity which results in rhythmical mastication demands that all timing information supplied by proprioceptors in the orofacial region be removed. This was accomplished in the present study by paralysing the animals with a dose of gallamine triethiodide which has been shown to block both intrafusal and extrafusal muscle fibre activity

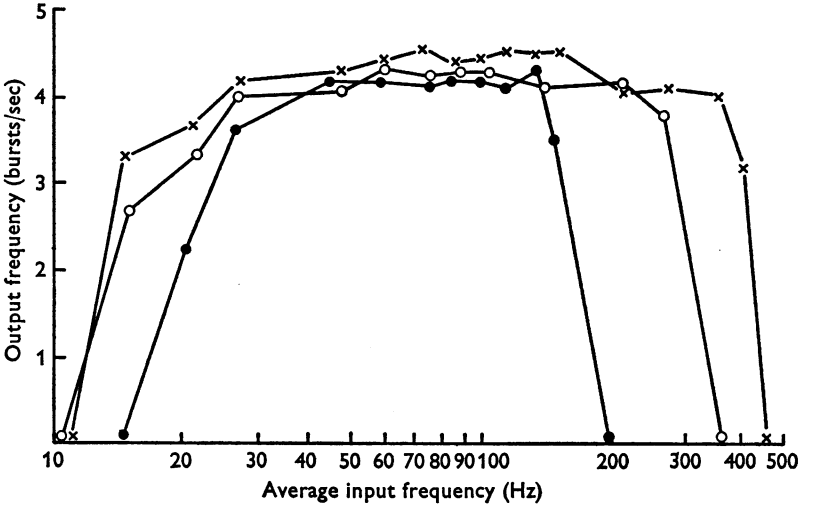

Fig. 6. Semilog. plot of the relationship between the average stimulation frequency using random trains of monophasic pulses and the rate of occurrence of bursts of neural activity. The stimulus was delivered to the internal capsule but not at the same locus as in Fig. 3, which was compiled using data from the same animal. The three stimulus voltages used were T 1·5, 6·75 V (●); T 2, 9 V (○); and T 3, 13·5 V (×).

by Carli, Diete-Spiff & Pompeiano (1967). Rhythmic masticatory discharges in response to stimulation were not abolished, confirming the findings of Sumi (1970), who reported that rhythmical masticatory discharges in single hypoglossal fibres were unaffected by paralysis.

Severing branchial and cervical nerves and the spinal cord did not prevent regular masticatory discharges from occurring in the hypoglossal

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Legend to Fig. 5.

Fig. 5. Analyses of neurograms shown in Fig. 4, calculated for the 5 sec of stimulation time in which the neural pattern was most regular. The cross-correlation histograms between heart beats and the following bursts of neural activity are shown beneath the autocorrection histograms of the integrated neurograms. Both analyses were continued for 1 sec following the initial event. Bin width 20 msec.

nucleus, removing the possibility that afferent inputs due to unblocked intrafusal fibres, peripheral respiratory or vascular mechanoreceptors were responsible for the rhythmic output.

Egger & Wyman (1969) have demonstrated that the rhythmic stepping seen by Sherrington (1913) in the deafferented hind limbs of cats was, in fact, an artifact caused by patterning in the electrical stimulus used to provoke the movements. This cannot be the explanation for the rhythmical bursts of activity observed in the present experiments, because the stimulus was of random frequency.

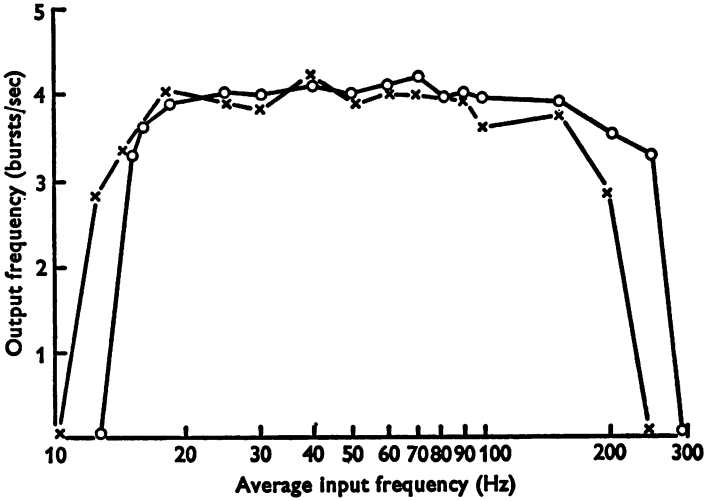

Fig. 7. Semilog. plot of the relationship between the average stimulation frequency and the output bursts before (○) and after (×) paralysis with gallamine. The subcortical white matter was stimulated with random trains of monophasic pulses at a voltage 1·5 times threshold (10·5 V).

Finally, timing information could conceivably be supplied by vascular pulsations within the brain. Werner & Mountcastle (1963) suggested that oscillatory mechanical irritation may have been the cause of regularity in the discharge pattern of thalamic neurones. They rejected this hypothesis because no frequency dependence could be demonstrated between neural discharge patterns and events in the cardiac or respiratory cycles. Similarly, it does not appear that such mechanical factors are of any importance in the patterning of mastication. The discharges persisted in the partly deafferented preparation when the respirator was turned off and the apparent dependence of the neural discharges on events in the cardiac cycle shown in certain analyses probably arises by chance. Perkel *et al.* (1967) showed that independent pace-makers of nearly the same frequency exhibit peaks in the cross-correlation histogram, the magnitude

and phases of which vary from sample to sample, as found in the present study.

Corticobulbar fibres do not synapse directly with branchial motoneurones in the rabbit (Haartsen, 1962), but the long latencies encountered between the start of the stimulus train and the first burst of neural activity (greater than 80 msec) would not appear to be explained solely by delay caused by passage through a long direct chain of neurones. Rather, it would seem that, during this time, reverberating circuits are set up within the brain stem which eventually produce a patterned output. Reverberation could also be responsible for masticatory discharges continuing for some seconds after the cessation of stimulation.

The initial increase in discharge rate as the frequency of stimulation was increased (see Figs. 3 and 6) is best explained by a summation of neural elements along the multisynaptic pathway leading to the branchial motoneurones. The postulated masticatory pattern generator subsequently becomes extremely insensitive to a wide range of changes in both the instantaneous and average frequency of the stimulus pulses. During this phase the available bulbar interneuronal pool is probably being maximally excited thereby producing a stable output pattern. The reduction in rate of discharge and its eventual cessation when the stimulation frequency is very high probably occurs as a consequence of fatigue.

It appears that, as originally postulated by Magoun *et al.* (1933), mastication is patterned within the central nervous system. There is also ample evidence that this patterning occurs within the brain stem (Bremer, 1923; Magoun *et al.* 1933; Lund & Dellow, 1970) and is activated by higher centres or from the oral cavity. Bremer (1923) observed rhythmical mastication in chronically decerebrate rabbits in response to oral stimuli, while Monnier & Willi (1947) found that human medullo-spinal anencephalics could carry out the closely related activity of rhythmical sucking. These oral stimuli do not act by initiating the alternation of jaw reflexes previously discussed. Rhythmical bursts of activity have been recorded from the hypoglossal nerves of lightly anaesthetized, paralysed rabbits when a small balloon was distended in the mouth (J. P. Lund & P. G. Dellow, unpublished results). Summation occurred between central and oral stimuli.

It is concluded that mastication is controlled by a brain-stem pattern generator which can be activated by adequate inputs from higher centres or from the oral cavity.

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