original epidemiologic evidence for the origin of Lyme arthritis. We postulate that an arthropod transmits the causative agent at the site where a skin lesion may appear. Those patients who develop cryoglobulins (primarily IgM to begin with) are at risk to develop subsequent Lyme arthritis, and this finding therefore has prognostic value. The presence of cryoglobulins (now both IgM and IgG) in almost all patients with active arthritis and their decrease or disappearance when the joint involvement has subsided, again support a pathogenetic role for circulating immune complexes in Lyme arthritis. Further support for this hypothesis can be obtained through the serial study of the individual patients, and possibly through the identification in the cryoprecipitates of an important antigen, or even of the causative agent itself (*5*).

Note added in proof: The presence of cryoglobulins in this illness may sometimes be associated with neurologic abnormalities (cranial nerve palsy, sensory radiculopathy, or aseptic meningitis) or with myocardial conduction abnormalities (*6*).

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4. However, these and other complement components may still be present, and we are currently looking for them by more sensitive methods than double immunodiffusion.
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Licking Behavior: Evidence of Hypoglossal Oscillator

Abstract. *Action potentials and slow waves were recorded from the hypoglossal nucleus of rats during licking of water from a drinking tube. Periods of licking and of rhythmic neural activity were usually highly correlated, as were their frequencies. Neural activity sometimes continued after cessation of licking; at other times, it stopped during a short interruption of licking and resumed in rhythm with licking. These observations are consistent with an oscillatory model of the control of licking.*

Mammalian licking behavior is a highly stereotyped, rhythmic process. The tongue and jaw movements in such behavior represent an especially clear example in a mammal of the stereotyped rhythmic motor activity that repeatedly has been observed in invertebrates (*1*). A central oscillator has been proposed as the basis for the observed rhythmicity; the typical rate of sustained licking in the laboratory albino rat is five to seven licks per second (*2, 3*). From this model it is predicted that rhythmic neural activity can continue while motor output is temporarily suspended. We report data on action potentials and slow waves recorded from the hypoglossal nucleus, which contains the cell bodies of the motoneurons that are efferent to the muscles of the tongue, during licking by conscious, unrestrained animals. The neural and behavioral responses corresponded both in periodicity and in general pattern. During interruptions of licking, we found evidence for the oscillatory nature of the control of licking behavior.

Data were obtained from six male albino rats (Charles River) between 3 and 6 months of age, weighing 300 to 500 g. Under barbiturate anesthesia, an electrode device that permitted multiunit and, occasionally, single-unit recording (*4, 5*) was implanted above the hypoglossal nucleus or surrounding hindbrain areas (*5*). All recordings were made at least 7 days after implantation from animals that were deprived of water for 23 hours. The rats, while in their living cages, were placed individually into an electrically shielded enclosure for recording of water licking and hypoglossal activity (*6*). Licking behavior was monitored by means of circuitry which produced a pulse when tongue contact with the drinking tube was terminated.

Multiunit responses were recorded

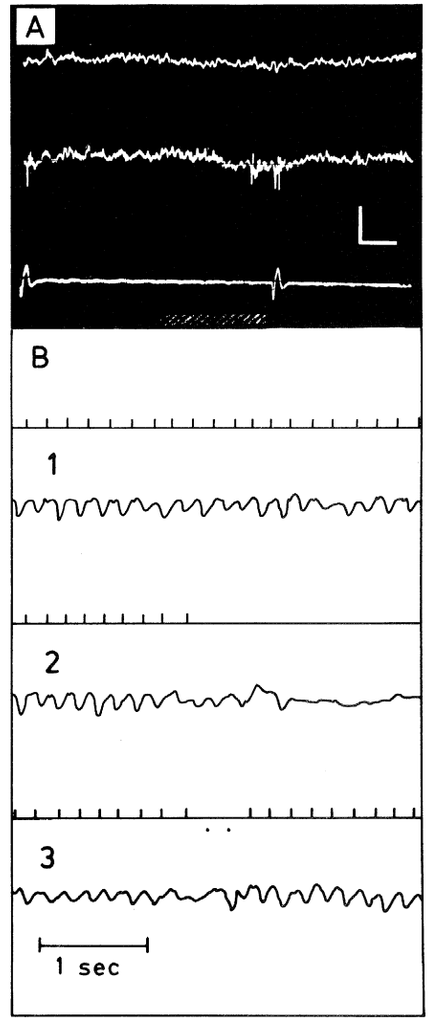

Fig. 1. (A) Neuronal activity recorded simultaneously with licking in an unanesthetized rat. Upper and middle traces are multiunit activity recorded in the hindbrain during two successive licks. The upper trace was recorded from a control electrode in the principal vestibular nucleus; the middle trace, from an electrode in the radiations of the hypoglossal nucleus. The voltage calibration for upper and middle traces is 200 μv. The lower trace, from an electrical sensor on the drinking tube, shows two pulses that correspond to termination of tongue contact. The horizontal bar below the lower trace shows the lick contact duration of 70 msec expected for the observed licking rate of 5.5 licks per second (*2, 6*). Calibration time for all three traces is 25 msec. (B) Licking patterns and slow waves from the hypoglossal nucleus. The upper trace of each pair consists of lick termination pulses; the lower trace is simultaneously recorded slow-wave bioelectric activity of the hypoglossal nucleus. All traces were produced from tape-recorded activity as displayed on an inkwriter. Action potentials were filtered by the frequency response of the inkwriter (0 to 25 hertz). In trace pair 1, steady licking is accompanied by rhythmic waves from the hypoglossal nucleus. In pair 2, cessation lick contact precedes the disappearance of the slow waves by about 500 msec. In pair 3, rhythmic licking shows a brief pause. The expected times of the missing lick termination pulses are marked by dots. There is corresponding disruption of slow waves from the hypoglossal nucleus.

from the hypoglossal nucleus and its centripetal axons (radiations) in two rats (Fig. 1A). Both slow-wave activity and action potentials preceded termination of tongue contact during each lick of an uninterrupted series of licking responses (Fig. 1A, middle trace). The electrode location in the two rats was in the rostral portion of the radiation of the hypoglossal nucleus, where electrical stimulation produces tongue retraction in the rat and cat (*7*). Electrodes that were not in hypoglossal loci did not yield slow-wave and action potential activity during various phases of tongue contact with the drinking tube (Fig. 1A, top trace).

The relationship between discrete licks and hypoglossal motoneural activity was evaluated by producing computer-generated histograms of neural activity that were timed with respect to licks during periods of drinking. The modal interlick interval (interval between termination of successive licks) was 170 to 180 msec for rat 1 (5.5 to 5.9 licks per second) and 180 msec for rat 2 (5.5 licks per second). About two-thirds of the interlick intervals were 160 to 190 msec. Neural activity was examined by using the lickometer pulse as a trigger. The histograms were generated from the action potentials as recorded from the hypoglossal nucleus (Fig. 2, B and D). For rat 1, the modal hypoglossal activity occurred 140 msec after the end of the first lick, 30 to 40 msec before the end of the second lick. For rat 2, the modal timing of action potentials was 160 to 170 msec after the end of the first lick and 10 to 20 msec before the end of the second lick.

In all six animals, slow-wave activity was associated with each lick; multiunit or single unit activity was observed only in rats 1 and 2. The slow-wave activity and lick terminations had a consistent temporal relationship (Fig. 1B). Slow waves did not always terminate at the end of a series of licks but sometimes continued for several cycles (Fig. 1B, trace pair 2). The continuation of slow waves in the absence of tongue contact may indicate aborted licks (*8*), which are particularly common at the beginning of a long pause in licking. During brief pauses in licking (Fig. 1B, trace pair 3), aborted licks appear to be less typical. In the illustrated trace, the licking sequence was interrupted for the equivalent of two interlick intervals, with a concomitant disruption of slow-wave activity. The first lick after the pause was at a time expected from the earlier licking frequency. The data in trace pairs 2 and 3 are consistent with the hypothesis that activity of a licking oscillator had continued after tongue protrusion had ended or had been suspended.

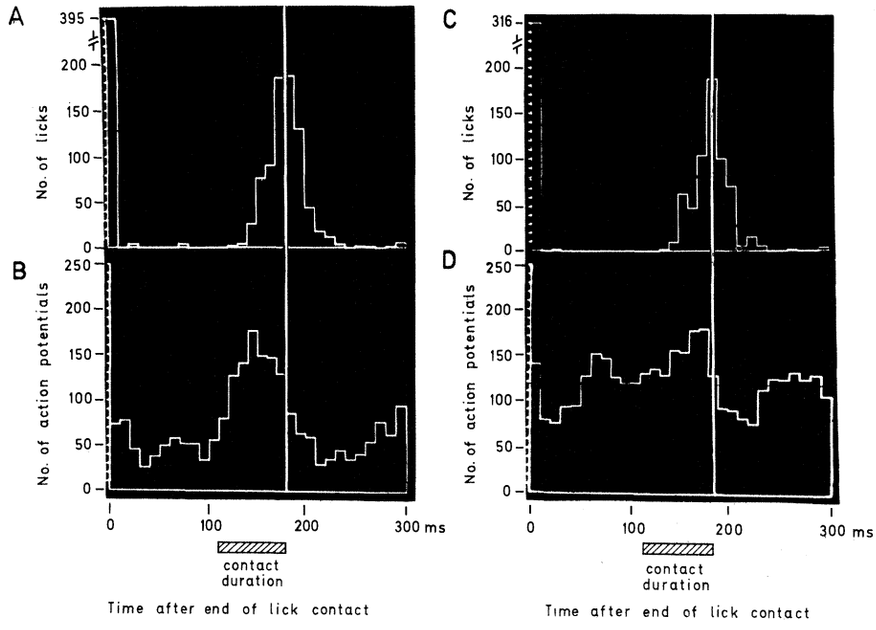

Fig. 2. Histograms of action potentials from hypoglossal nucleus and lick termination pulses. The termination of contact of the first of a pair of licks is the trigger for computer logging. Interlick interval histograms are shown for 395 pairs of licks for rat 1 (A) and 316 pairs of licks for rat 2 (C). All bins are 10 msec. The large bars at zero time are the first licks of all pairs. Lines are drawn through the modal interlick intervals, which are 170 to 180 msec for rat 1 and 180 msec for rat 2. Histograms of action potentials recorded simultaneously with the lick data in (A) and (C) are shown for rat 1 (B) and rat 2 (D). The modal activity of action potentials (140 msec for rat 1 and 170 msec for rat 2) precedes modal lick termination (lines). The histogram in (B) is based on 1107 action potentials and that in (D), on 962 action potentials.

The location of the proposed oscillator may or may not be within the hypoglossal nucleus, but there is a network of neurons within the nucleus that could control the activity of the motoneurons from which we were recording. The phenomena illustrated in Fig. 1B were observed in all six of the animals.

It is not surprising that the motoneurons of the hypoglossal nucleus show activity that is tightly and consistently coupled with tongue motion in freely behaving animals. Mechanical deformation of the tongue or jaw is related to the activity of hypoglossal motoneurons in anesthetized animals (*9*). However, attempts to elicit rhythmic licking by electrical stimulation of cortical or pontine loci in anesthetized cats (*10*) have produced rhythmic activity much slower than the typical four per second licking rate of the domestic cat. Studies of ongoing neural activity in unanesthetized animals, through the use of environmental controls such as restriction of access to the drinking tube, various schedules of deprivation, and different types of liquid reinforcers may be the methods of choice for studying this rhythmic behavior. Behavioral measures of licking in isolation are not adequate for understanding the physiological basis of this rhythmic behavior because neural events, such as those which continue during pauses in licking (Fig. 1B), are not measured. Licking behavior in the rat can be affected by many factors in the internal and external environment of the animal (*11*). The neural basis of this response plasticity should be studied by recording from various parts of the sensory-motor pathways involved in gustation and control of food ingestion.

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5. Platinum-iridium wires (60 μm) that were coated with Teflon except at the tip were inserted into the hypoglossal nucleus for recording of neural activity during licking and into other bulbar regions for control recordings. Placement of the tips of recording electrodes in the hypoglossal nucleus was achieved by recording antidromic responses to electrical stimulation of the tongue during the implantation procedure. A ground connection was provided by bare stainless steel wires that were wrapped around stainless steel screws implanted in the skull. The wires from the recording electrodes and ground wire were connected to a miniature plug (Amphenol 223-1205) that was cemented to the skull.
6. The steel enclosure was 45 cm high, 30 cm wide, and 46 cm long. The skull-mounted plug was connected to an Amphenol 223-1105 socket that had a field effect transistor (TI 2N5045) in line with each recording electrode lead. A flexible multiconductor cable ran from the field effect transistors to a mercury slip-ring commutator (Scientific Prototype Co.). The rat could reach all parts of the cage without losing slack in the cable. From the commutator, signals were conventionally amplified and recorded on FM magnetic tape. A stainless steel drinking tube with a 3.6-mm opening was attached through a silicone rubber stopper to a glass bottle filled with tap water. The end of the drinking tube was placed 4 mm outside a 1.3-cm hole that was centered opposite an elliptical opening in the back wall of the cage. This arrangement, which approximated the high-restriction licking environment described by L. Marowitz and B. P. Halpern [*Physiol. Behav.* **11**, 259 (1973)], required the rat to protrude its tongue to make contact with the liquid-filled drinking tube and thereby prevented contact with the lips. Tongue contact by the rat was detected by a solid-state drinkometer circuit (<1-μa short-circuit current) that emitted a brief pulse within 1 msec of the end of each lick contact. A single tongue contact with the drinking tube at the observed licking rates was expected to last 70 msec [B. P. Halpern, in *Olfaction and Taste*, D. A. Denton and J. P. Coghlan, Eds. (Academic Press, New York, 1975), vol. 5, pp. 47–52]. Each lick was reinforced with approximately 5 μl of tap water at room temperature. The lickometer pulses were tape-recorded on a second channel. The neural and lickometer responses were analyzed off-line with a digital computer (PDP 8-E) under a LAB-8/E PST program.
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Classical Nictitating Membrane Conditioning in the Awake, Normal, Restrained Cat

Abstract. *Present knowledge of its central nervous system makes the cat a desirable subject for studies of brain-behavior relationships. Response frequencies and latency characteristics in conditioning and control groups indicate that the response of the nictitating membrane can be classically conditioned in a new restraint system in which detailed brain and behavior measures can be easily obtained.*

The cat has long been a favored subject for studies of basic neural processes in both the brain and spinal cord and has perhaps the best understood of all mammalian nervous systems. Thus, it would be desirable to analyze simple learning processes in the cat nervous system. However, the cat's inherent dislike of restraint has precluded the development of an easily used and readily quantifiable behavioral paradigm in the cat such as was developed by Gormezano and his collaborators (*1*) for the rabbit and used for brain recording in more recent work (*2*). Behavioral studies of the cat have frequently been performed with free-ranging or minimally restrained subjects. Other experimenters have used long periods of adaptation, paralyzed preparations, or invasive measures such as skull bolts to achieve adequate restraint for behavioral measurements (*3*). The need for such procedures has precluded the use of large numbers of subjects in studies requiring recording of discrete responses, such as leg lift or eyelid closure. We now report our successful experimental efforts at showing unequivocal evidence of classical conditioning of the discrete, easily quantifiable, nictitating membrane (NM) response in cats restrained in a system requiring no adaptation.

Sixty adult, mongrel cats were randomly assigned to squads of four subjects with three squads in each of five experimental or control conditions. One subject was randomly eliminated from each condition after one subject died during the study. Each subject was prepared the day before training by shaving the region around the right eye and suturing a 6-0 monofilament nylon loop into the outer edge of the membrane. Stainless steel wound clips placed 0.5 cm above and below the eye allowed delivery of the 100-msec, 3-ma a-c shock unconditioned stimulus (UCS). The conditioned stimulus (CS), a 72-db sound pressure level (SPL), 1000-hertz, 500-msec tone was delivered through a speaker in the ventilated, dimly lit, deactivated refrigerator shells that served as experimental chambers. A 65-db SPL white-noise background was continuously present. The response of the NM was recorded by hooking a thread to the NM loop. The thread was coupled to the arm of a minitorque potentiometer mounted on the restraint box. Membrane extensions were recorded on a polygraph (Grass), and response latency was also measured by a digital recorder. A response was any NM movement of at least 0.5 mm starting within the appropriate scoring interval.

The details of the restraint system will be available elsewhere (*4*). Briefly, the animal is placed in a box patterned after the Gormezano rabbit restrainer (*1*). The box is of heavy Plexiglas with a lid and a movable back plate that allows the cat's body to be completely enclosed while the cat's head protrudes through a slanted front plate. A stock comes down over the top of the neck and is secured at a point allowing free neck movement but not allowing the head to be pulled back. The chin rests in a slot on a plate projecting from the front of the box below the neck stock. A bite bar attached to the chin plate is positioned through the mouth behind the lower canine teeth and is tightened sufficiently to preclude head turning but not to produce discomfort. Two nylon releasable cable ties, which fit through slots in the chin plate, are tightened around the head, in front of and behind the ears. The ties are the major head restraint and leave room for skull electrode implants or surgery that might be desired. The cat is thus held sufficiently securely to permit eyelid or nictitating membrane movements to be measured by a transducer mounted on the box. The cat accepts this restraint for as long as 2 hours with little struggling. No appreciable increase in resistance to being placed in the apparatus has developed for as long as 9 days in our studies. The animal's legs could also be made accessible if holes were made in the bottom of the restrainer.

All subjects received nine daily experimental sessions. The first session was an adaptation session, during which no stimuli were given, and which was used to assess the random response rate by recording as though stimuli had been delivered. Six acquisition (or control) ses-