RHYTHMOGENESIS IN A HYBRID SYSTEM—
INTERCONNECTING AN OLIVARY NEURON TO AN
ANALOG NETWORK OF COUPLED OSCILLATORS

Y. YAROM
Department of Neurobiology, Life Sciences Institute, Hebrew University, Jerusalem 91904, Israel

Abstract—A hybrid system in which an olivary neuron was interconnected to an analog simulator was used to study possible mechanisms by which the combined behavior of individual olivary neurons evokes synchronized membrane potential oscillations in a large population of neurons. The analog system was composed of four identical, interconnected oscillating units, each of which was capable of producing a damped sinusoidal oscillation in response to a trigger signal. When the units were coupled to each other, a single trigger pulse to one unit could evoke sustained oscillations. The integrity of the connections within the system was required to maintain these oscillations. In the hybrid system the analog system was reciprocally connected to an olivary neuron in a slice preparation. As in the analog system, the hybrid system could generate sustained oscillations following a trigger pulse to one of the units, as well as following a low threshold Ca spike in the neuron. Activation of the low threshold Ca conductance in the olivary neuron was necessary to achieve both gain and in-phase activity within the hybrid system, and thereby sustain the oscillations. The ability of the hybrid system to generate sustained oscillation is frequency dependent. Sustained oscillations were readily obtained at a "preferred frequency" of 5.2 Hz (n = 7) which was independent on the parameters used by the simulator, or on the membrane potential of the neurons.

These results, which demonstrate the advantage of a new experimental approach developed to study rhythmogenesis in inferior olivary neurons, support the hypothesis that the inferior olivary nucleus, acting as an interconnected network of oscillating units, can generate an accurate subthreshold oscillation that serves as an internal time reference.

Rhythmic activity in the nervous system can be generated by two basically different types of mechanism: a pacemaker-like element having an inherent capability to generate rhythmic activity,1,2 or an interconnected network of excitable elements which can generate oscillating circuits. The well-documented synchronized rhythm generated by the neurons in the inferior olivary nucleus,3-5,8,11 appears to depend on a combination of both types of mechanism. In olivary neurons the interplay between various ionic conductances which are specifically distributed over the soma-dendritic membrane gives rise to the rhythmic activity, indicating that olivary neurons operate as pacemaker elements.8-10,20 Investigating the internal organization of the olivary nucleus, on the other hand, and the discovery of the gap junction between the olivary neurons7 suggested that electrotonic coupling synchronizes the activity of the neurons.11

In addition to the pacemaker-like activity, Linas and Yarom15 showed that the membrane potential of olivary neurons undergoes subthreshold fluctuations which are approximately sinusoidal in waveform and occur simultaneously in a large population of neurons. The frequency of these oscillations varies between 4 and 9 Hz, and their amplitude is in the range 3-10 mV (see also Ref. 4). Several lines of evidence suggest that these oscillations depend on the integrity of the olivary nucleus as well as on the properties of each neuron. For example, these oscillations were observed in only a small percentage of slice preparations but, in each of these oscillating preparations, they occur in most of the neurons encountered. Furthermore, the frequency of these oscillations and their amplitude are independent of the state of activity of any particular cell, but they are modified by either drug application or a massive stimulus, both procedures affecting a large population of neurons. In view of these observations, an hypothesis was presented, assuming that each olivary neuron can be looked upon as a generator of a damped, almost sinusoidal oscillation. Since these oscillators are electrotonically coupled, by interacting they can generate synchronized sustained oscillations. Thus, this mode of rhythm is assumed to be generated not only by the oscillatory properties of the neurons, but by the circuitry as well.

A novel experimental approach was utilized in the present work to study the oscillatory behavior of olivary neurons. Since according to the hypothesis the olivary nucleus is a network of coupled oscillators, an analog simulator composed of coupled oscillators was constructed. The simulator is characterized by its ability to generate sustained oscillations, even though each individual oscillator generates only damped oscillations. Inter-connecting the simulator

**Abbreviation:** LTS, low threshold spike.
to an olivary neuron in a slice preparation demonstrated that, in such a hybrid system the olivary neurons operate indistinguishably from any of the electrical oscillators. Furthermore, by analysing the responses of the hybrid system to either voltage changes or pharmacological treatment of the neuron, it was possible to determine which of the properties of olivary neurons are essential for the generation of rhythm and the oscillating frequencies “preferred” by the olivary neurons.

EXPERIMENTAL PROCEDURES

Physiological procedures

The physiological procedures used in this work are similar to those utilized in previous studies. The electrophysiological recordings were obtained using the submerged slice technique. The bathing solution had the following composition (in mM): NaCl, 124; KCl, 5; NaHCO₃, 26; KH₂PO₄, 1.2; CaCl₂, 2.4; MgSO₄, 1.3; and glucose, 10. All the experiments were carried out at constant temperature of 34 ± 1°C.

The intracellular recordings were performed with potassium acetate-filled microelectrodes (3 M; 30–60 MΩ), using a high input resistance bridge-amplifier. The data were stored on a HP 3964 tape-recorder and analysed on a Nicolet 4094 digital oscilloscope and on an Olivetti M-290 microcomputer, using commercially available software as well as that developed in the department.

The simulator

The simulator consists of four identical interconnected oscillating units. A block diagram of an oscillating unit and its connections is shown in Fig. 1A. The heart of each oscillating unit is an operational amplifier with a notch filter, tuned to 9 Hz, and controllable gain in its feedback loop. Such a unit will generate sustained oscillations, at a frequency of 9 Hz, provided that the closed-loop gain is equal to or greater than 1. It should be emphasized that in this arrangement the frequency is constant and predetermined by the notch filter. The feedback gain in each unit is determined by a variable gain amplifier which is introduced into the feedback loop in parallel with the notch filter. The gain of variable gain amplifier I is externally controlled by a d.c. source and by a trigger signal. Since a brief trigger signal momentarily increases the gain of variable gain amplifier I, it increases the loop gain, and thereby invokes damped sinusoidal oscillations. A second variable gain amplifier receives its input from a point which sums the activity in all the other oscillating units. The gain of variable gain amplifier II is also externally controlled and determines the extent of the coupling between the units. In this system, sustained oscillations will be generated if the equivalent overall gain (the feedback loop gain in each unit plus the contributions to the loop from the other units) exceeds a certain level (I). It should be noted that although the electronic components were carefully selected, the units are not identical. Thus, although the unit gain was simultaneously controlled, variation (in unit gain) among the units does exist.

A schematic illustration of the entire system is given in Fig. 1B. The unit gains of the four oscillating units are controlled simultaneously. Each unit is driven by two inputs: a trigger input and coupling input. The coupling input is actually the output of a summing point (Σ 1–4) which sums the activity of the other units. The summing points of all the units are also simultaneously controlled by the same coupling gain. The single output from each unit is connected to the summing points of the other three units. Since the two inputs are governed by switches, it is possible to determine whether a particular unit will receive an input signal, a trigger signal or both. The output switch (S₄), on the other hand, determines whether an output from any given unit will be transmitted to the other units. The trigger signal to the electrical units was generated by a rate-controllable trigger source which distributes a constant trigger signal either randomly to all the units or specifically to any given unit; S₅, a toggle switch connecting any given unit to the trigger source; OSC 1–4, the four oscillating units, whose gain (Gain) is simultaneously controlled; S₆, a toggle switch connecting the input into the oscillating units; Σ 1–4, summing points preceding the inputs of each of the oscillating units. The coupling gain (Coupling) is also simultaneously controlled; S₇, a toggle switch connecting the output of any given unit to the summing points of all the other units. The outputs from all the units are summed, translated into current and injected into an olivary neuron whose output (E) is fed back into the summing points of each of the units.

Fig. 1 Schematic presentation of the simulator. (A) A block diagram of an oscillating unit. OA, operational amplifier; T.T, twin tee system tuned to 9 Hz; VGA I, variable gain amplifier determines the closed loop gain and is controlled by a d.c. source (U.G.) and by a trigger signal (T.T); VGA II, another variable gain amplifier determines the coupling gain and is controlled by a d.c. source (C.G); Units 2–4, the output of the other oscillating units. (B) The organization of the hybrid system. Trigger source, a rate-controlled trigger source which distributes a constant trigger signal either randomly to all the units or specifically to any given unit; S₅, a toggle switch connecting any given unit to the trigger source; OSC 1–4, the four oscillating units, whose gain (Gain) is simultaneously controlled; S₆, a toggle switch connecting the input into the oscillating units; Σ 1–4, summing points preceding the inputs of each of the oscillating units. The coupling gain (Coupling) is also simultaneously controlled; S₇, a toggle switch connecting the output of any given unit to the summing points of all the other units. The outputs from all the units are summed, translated into current and injected into an olivary neuron whose output (E) is fed back into the summing points of each of the units.
rate specifically to any given unit. The generation of sustained oscillations, which is a threshold phenomenon, could be elicited by interconnecting the entire system and delivering a trigger signal to one of the units. The threshold is reached by setting the unit and coupling gain to a certain level—"threshold level". A detailed description of the electrical activity of the different units under various conditions is given in the Appendix.

The basic concepts of the hybrid system are also demonstrated in Fig. 1B. In order to achieve reciprocal connectivity, the outputs of the four units were summed, weighted, translated into current and injected through an intracellular microelectrode into an olivary neuron. The output of the neuron, as monitored by the microelectrode (Fig. 1E), was introduced into each of the electrical units at the summing point. With this arrangement, the connections between the olivary neuron and the electrical system are identical to the connections between each of the electrical units and the system. The weight of the combined output of the electrical units was adjusted to insure that the peak-to-peak amplitude of the sustained oscillating current should not exceed 2 nA. Special attention was given to maintain accurate bridge balance during experiments. For that purpose, a small square current pulse was occasionally delivered through the intracellular microelectrode during, as well as between, oscillations.

RESULTS

Sustained oscillations in the hybrid system

The activity in the hybrid system following a single triggering pulse is demonstrated in Fig. 2A. The top two traces are the activity in units 1 and 3. Units 2 and 4 were also connected and participated in the generation of sustained oscillations but their activity is not illustrated in the figure. The third trace is the activity in the olivary neuron as recorded by the intracellular microelectrode. The triggering signal, which was delivered to unit 1 and recorded as a fast negative deflection (arrow), initiated a process which led to a new stable state where all the components of the hybrid system generate synchronized sustained oscillations. This type of response, which has threshold characteristics, depends on the parameters of the unit and coupling gains as well as those of the interactions between the neuron and the simulator. The state of sustained oscillations will continue as long as the integrity of the system maintained and the parameters of the different gains remained unchanged (see Appendix). During the sustained oscillation, the amplitude of the olivary wave was in the range of 25–30 mV and, as a result, the peak of the oscillation occasionally reached threshold and action potentials were generated. Although the sustained oscillations in the olivary neuron followed the frequency dictated by the electrical system, the waveform was deformed and did not follow the sine wave generated by the electrical system.

The sustained oscillation evoked by the triggering signal is characterized by an initial phase, during which a slow build-up of oscillations was observed. The first and the last period of the initial phase are shown at higher gain and faster time scale in Fig. 2B and C, respectively. During the first period (Fig. 2B), a prominent phase shift exists between the wave in the olivary neuron (lower trace) and the electrical units (upper traces). This phase lag is actually expected whenever a sinusoidal current is introduced into an RC circuit such as offered by the neuron. It should

![Fig. 2. Sustained oscillation induced in the hybrid system by a single trigger signal delivered to unit 1. Simultaneous recording from units 1 and 3 (two upper traces) and from an olivary neuron (lower trace). Units 2 and 4 are also connected but are not shown. In A, a continuous recording illustrates the development of the oscillations (arrow denotes the triggering time). (B) The first three waves following the triggering signal displayed at higher gain (×2), and faster recording rate (×5). Note the phase shift of the response of the neuron in relation to the electrical units. (C) The last three waves before reaching the state of sustained oscillation. Note the accelerated rising phase of the waves recorded from the neuron (arrow) and the resultant reduction in phase shift.](image-url)
be mentioned that during this period all four electrical units were already locked in amplitude and phase (see Appendix). During the last period (Fig. 2C) of the initial phase, a progressive decrease in the phase shift of the olivary oscillations (lower trace), in relation to the activity in the electrical units (upper traces), was observed. This decrease in phase lag was accompanied by deformation of the waveform in the olivary neuron, characterized by an accelerated rising phase (arrow in Fig. 2C). The presence of an accelerated rising phase in the olivary neuron strongly suggests that an active process takes place, a process which contributes to the signal generated by the current input itself and, in turn, induces a reduction in the phase lag. Once a phase-locked condition was achieved, a state of sustained oscillation was quickly reached in the entire system. Therefore, synchronization between the different elements of the hybrid system seems to be a necessary prerequisite for the generation of sustained oscillations.

The critical role played by synchronization is demonstrated in Fig. 3, where the activity in the olivary neuron and one of the electrical units is shown. The hybrid system parameters were set to threshold level and spontaneous activity was induced in the olivary neuron by harmaline and serotonin treatment (see Ref. 15). This activity was characterized by prolonged subthreshold potentials which appear regularly with a mean interval of 1.2 s (Fig. 3A). At the time indicated by the arrow, the olivary neuron was connected to the electrical system. As a result, the next spontaneous response induced low amplitude oscillations in the olivary neuron as well as in the electrical system. The second spontaneous response, on the other hand, completely abolished the oscillations. The next three consecutive responses seem to occur with a preferred timing and therefore induce oscillations which, although failed to generate sustained oscillations, demonstrated progressively increasing amplitudes. This situation, where increases and decreases in oscillations were observed, could last for a rather long time or, as seen in Fig. 3B, might turn into a sustained state of oscillation, provided that a series of spontaneous responses occurred with exactly correct timing. It has been concluded, therefore, that the generation of sustained oscillations in a coupled system is strongly dependent on the ability of the elements of the system to achieve a state of synchronization.

The low threshold Ca conductance

According to the above description, a regenerative response in the olivary neuron contributed to the reduction of phase lag, thereby insuring complete synchronization between the olivary neuron and the electrical units. The most likely property of olivary neurons responsible for synchronization is the low threshold Ca conductance. Since both the activation and inactivation of this conductance occurs within a rather small potential range around the resting level, it generates graded regenerative responses as the result of relatively small depolarizations, as long as they were preceded by hyperpolarization. Thus, it is obvious that the hybrid system, which oscillates about the resting potential, provides favorable conditions for the activation of this low threshold Ca conductance.

The contribution of the low threshold Ca response to the generation of sustained oscillations was examined by shifting the membrane potential to either more negative or less negative values (relative to the resting level). Since the low threshold spike (LTS) has

---

Fig. 3. Sustained oscillations induced in the hybrid system by spontaneous rhythmic activity of the olivary neuron. (A, B) Continuous recording from unit 1 (upper trace) and an olivary neuron (bottom trace). The rhythmic activity in the neuron was induced by combined treatment with harmaline (5 mg/ml) and serotonin (10^-3 M). (A) Connecting the neuron to the simulator (arrow) induces low amplitude oscillations in both the electrical unit and the neuron. Note that occasionally the spontaneous responses act to inhibit the oscillations. (B) The same as in A, demonstrating that those potentials can induce sustained oscillations.
Rhythmogenesis in a hybrid system

a distinct threshold, and since it is completely inactive at potentials less negative than the resting level, d.c. voltage shift to either direction will prevent the generation of the LTS. These effects on the sustained oscillation of the hybrid system are demonstrated in Fig. 4. To avoid possible technical errors (see Discussion), the experiments were performed in the presence of harmaline (5 mg/ml) and CsCl (5 mM) which, by blocking several types of rectifications, induce linear current–voltage relations. Only the olivary activity (lower trace) and the activity in two of the coupled units (upper traces) are shown (Fig. 2). In the experiment illustrated in Fig. 4A, the neuron was held at hyperpolarizing level by a −0.3 nA d.c. injected through the microelectrode. Releasing the holding current (arrow), generated a LTS which was large enough to trigger a sodium-dependent spike and to activate the electrical oscillators (the fast sodium-dependent action potential does not contribute to the reciprocal connections because of the presence of low pass filters). Since the frequency generated by the olivary neuron was in the order of 5 Hz, while the frequency of the electrical system was 9 Hz, the first second following release from hyperpolarization appears chaotic, and the amplitude of the oscillation varied from wave to wave. This situation persisted until the rhythm generated by the olivary neuron subsided and the last (fifth) LTS (marked by an asterisk) coincided with the sine wave in the electrical units. From that time on, a state of sustained oscillation was quickly reached. Reintroducing the −0.3 nA d.c. into the olivary neuron (second arrow) induced, in addition to a drop in membrane potential, a dramatic reduction in the amplitude of the olivary neuron oscillations. As a result, the oscillations in the coupled electrical units slowly declined. Thus, it is obvious that most of the sine wave in the olivary neuron is generated by a voltage-dependent conductance.

The experiment demonstrated in Fig. 4B illustrates the blockade of sustained oscillation produced by depolarizing the olivary neuron. The sustained oscillations were induced by releasing the holding current (−0.3 nA d.c.; first arrow) (Fig. 4). At the time denoted by the second arrow, a +0.3 nA d.c. was injected into the olivary neuron. The resultant depolarization was again associated with a marked decrease in the amplitude of the oscillation in the neuron and, as a consequence, the oscillations in the coupled electrical units slowly subsided. Unlike the effect of hyperpolarization (Fig. 4A), the reduction in amplitude of the neuronal oscillations upon depolarization is also partially the result of a reduction in neuronal input resistance brought about by the onset of different types of delayed rectifications (see Ref. 20).

Fig. 4. Termination of sustained oscillations in the hybrid system induced by changes in the background potential level of the olivary neuron. (A, B) Continuous recordings of the activity in units 1 and 3 (upper traces) and the olivary neuron (lower trace; the lowest trace indicates the changes in the d.c. holding current). In both cases, oscillations were invoked by releasing −0.3 nA d.c. holding current in the neuron (denoted by the first arrow). While in A, oscillations were terminated by hyperpolarizing the neuron (second arrow), in B the same effect was induced by depolarizing the neuron (+0.3 nA d.c.; second arrow in B). Note that the release of the holding current in both cases was followed by a train of LTSs, and that the last LTS which occurred in phase with the electrical oscillations (denoted by an asterisk in A) seems to be the immediate trigger for the development of the sustained oscillations. The neuron was treated with harmaline (5 mg/ml) and CsCl (5 mM) in order to reduce various rectifications.
These experiments strongly suggest that the activation of the low threshold Ca conductance was essential for the generation of sustained oscillations. This possibility was further examined by exposing the oscillating hybrid system to octanol, which can produce complete depression of the LTS. The results of such an experiment are shown in Fig. 5A, which illustrates only the neuronal activity. After selecting threshold parameters, the hybrid system was pushed into sustained oscillation and 0.01% octanol was than added to the perfusate (not shown). As a result, a gradual reduction in the amplitude of the sustained oscillation in the olivary neuron was observed, followed by an abrupt fall in amplitude which, in turn, induced cessation of the sustained oscillations in the system. Close examination of the transition state (Fig. 5B) reveals that a failure to elicit a regenerative response, which was usually superimposed on the underlying sine wave, is the reason for the abrupt fall in the amplitude. Furthermore, comparing the LTS before addition of octanol (Fig. 5C) and immediately after the cessation of the oscillation (Fig. 5D) clearly demonstrates that octanol reduced the amplitude of the LTS by almost 50% (reduction in maximal rate of rise). Thus, the LTS is essential for the generation of sustained oscillations in the hybrid system. It should be mentioned that sustained oscillation can be elicited under conditions where the LTS is completely inactive, provided that either the unit gain or the coupling level be increased. Under such circumstances, however, the olivary neuron is a passive component which neither contributes to the oscillations nor can it interfere with their generation.

Preferred frequencies of oscillations

As demonstrated, the LTS of the inferior olive neuron actively participates in the generation of sustained oscillations by the hybrid system; therefore, it is expected that the ability of the hybrid system to generate sustained oscillations will be frequency dependent. The frequencies where the low threshold Ca conductance is most readily activated will be the frequencies "preferred" by the olivary neuron. At these preferred frequencies "gains" will be needed to evoke sustained oscillations. To investigate the ability of the hybrid system to generate sustained oscillations at different frequencies, the electronic units were modified so that their oscillating frequency could be altered (simultaneously in all units). Since this modification was carried out by introducing a different notch filter for each frequency, the number of steps is limited and a rather narrow range between 1 and 9 Hz was studied.

In determining the preferred frequencies, the following procedure was used. An olivary neuron was connected to the simulator. A set of unit and coupling gains (which did not induce oscillations upon triggering) was selected, the simulator was set to a given frequency, and the response of the hybrid system to a single triggering pulse, as reflected in the neuronal activity, was recorded. The frequency of the simulator was then changed and the system was triggered at the time of stimulation.
again. If sustained oscillations were not elicited (at any frequency), the unit gain was increased and the system was again tested. This procedure was repeated until sustained oscillations were generated at one of the simulator preset frequencies. At these gain settings, the duration of the oscillations induced by a single triggering pulse at different frequencies was measured and plotted as a function of the frequency.

The results of this type of experiment are shown in Fig. 6. The four records (Fig. 6A), which were obtained at the designated frequencies, represent the activity of the olivary neuron within the hybrid system following a single triggering pulse (first negative deflection) delivered to one of the electrical units. These records clearly demonstrate that the ability of the hybrid system to generate sustained oscillations, at a given set of gains, is frequency dependent. The fast decaying oscillatory responses triggered at low (3 Hz, upper trace) and high (8.7 Hz, lower trace) frequencies seem to be composed of two components: the passive response to the sinusoidal input current and the regenerative LTSs. At these frequencies the LTSs were activated only by the first several waves. In fact, a failure to generate a LTS was immediately followed by fast decay of the passive oscillatory response, evoked by the current input. The full size oscillations observed at 6.4 Hz, lasted 2 s and displayed a continuous decrease in the amplitude. This decrease led eventually to the abrupt failure to generate a LTS which was followed by a rapid decline of the oscillations. At intermediate frequency (5.1 Hz), the same triggering signal evoked sustained oscillations with constant amplitude which persisted as long as the system remained intact and the gains were unchanged.

The results of these experiments are summarized in the "tuning curve" of Fig. 6B (closed squares) which describes the duration of the oscillation as a function of the frequency. Only at three close frequencies (4.7, 5.1, and 5.7 Hz) did sustained oscillations develop. At other frequencies the oscillations did not last more than a few seconds. Although this apparent high sensitivity (narrow band tuning curve) could result from the method of quantification, it undoubtedly reveals that the olivary neuron preferred to operate at certain frequencies.

Control experiments verified that the frequency dependence of the hybrid system reflects the properties of the neuron and not the electrical units. In these experiments the responses of the same neuron with the same setting of unit and coupling gains were recorded in the presence of octanol (0.01%). The results, which are summarized in Fig. 6B (triangles), show a slight dependence of the oscillation on the frequency (a negative slope of 0.12). This dependence, which is due to imperfection of the electronic system and can be obtained with the simulator alone, suggests that the olivary preferred frequency is somewhat higher than the 5.5 Hz seen in this experiment. In other experiments the preferred frequency ranged between 4 and 6 Hz with an average of 5.2 Hz (n = 7).

The different tuning curves obtained at different gain settings are shown in Fig. 7A, B. The experiments were performed on the same neuron as the one used in the Fig. 6 experiments.
Fig. 7. The preferred frequency is independent of the gain setting or the membrane potential level of the neuron. (A) Two responses of the hybrid system, at the same frequency (6.4 Hz) and at two different unit gains, as recorded from the neuron. Note that at higher gain (upper trace) sustained oscillations ensued. (B) Frequency–duration curves of the same neuron at three different levels of gain settings. ■, the same curve as shown in Fig. A3B. □, △, frequency–duration curves of the same neuron at higher and lower gains, respectively. Note that although the shape of the curve was significantly changed, the preferred frequency remained unaltered. (C) Two responses of another hybrid system at the same frequency (6.4 Hz; note the different time scale) and at two different levels of membrane potential. The response shown in the upper trace was obtained during negative d.c. injection which induced 5 mV of hyperpolarization. The lower trace shows the response obtained at resting potential level. In both records, oscillations were elicited by a single triggering pulse delivered to unit 1. Note that spontaneous activity was seen after the termination of the oscillations. (D) Three frequency–duration curves of the same neuron as in C at three different levels of membrane potential. □, resting level; ■, △, 5 and 20 mV of hyperpolarization, respectively. Note that the curve obtained at −20 mV is similar to that obtained after octanol treatment and it does not show a preferred frequency.
increasing the probability of activating the LTS increases the oscillatory capabilities of the hybrid system in a similar way to that of the unit gain.

Although the differences in the preferred frequencies observed in the different hybrid systems were rather small, the duration of the oscillation varies more than 10 times (cf. Fig. 7D with C). This variability was attributed to the different preparations which probably represent a different number of coupled neurons. This possibility is supported by the observation that in those preparations where long oscillations were observed, spontaneous activity was occasionally encountered (Fig. 7C).

**DISCUSSION**

The working hypothesis of the present study is that the subthreshold membrane potential fluctuations observed in olivary neurons reflect both the unstable oscillations generated by each neuron and the electrotonic coupling between the neurons. Thus, an analog model consisting of elements with these two properties was built. This model demonstrated that these two properties are necessary and sufficient to generate sustained oscillatory activity like that observed in the inferior olivary nucleus. Furthermore, in such a model, an olivary neuron can replace any of the artificial elements without altering the inherent oscillatory property. In addition, it was demonstrated that synchronization is a necessary prerequisite for achieving a state of sustained oscillations. In the simulator by itself, this requisite is met by increasing either the unit gain or the coupling level. In the hybrid system, on the other hand, where an appreciable phase lag is generated by the RC circuit of the neuron, synchronization is achieved by the phase-accelerating activity of the LTS generated by each wave of the sinusoidal input current.

The most unlikely postulate assumed in constructing this simulator was to disregard the presence of a load effect in a coupled system. In fact, taking the load effect into account, a somewhat different mechanism regarding the generation of sustained oscillations could be considered. The basic assumption, underlying such a mechanism, is that each neuron in the olivary nucleus is capable of generating sustained oscillations rather than damped oscillations. However, in a coupled system each neuron introduces a large conductance load and, therefore, when a single neuron is triggered, the current which generates the oscillations will be shunted by the load, and the oscillations quickly subside. On the other hand, if all the neurons were synchronously active, the absence of a voltage difference across the coupling junction would eliminate the load effect, thereby preventing decline of the oscillations. Although this hypothesis is based on a different assumption, in both hypotheses sustained oscillations are strongly dependent on achieving a state of synchronized activity in the different elements of the network.

It is important to bear in mind that working with such a hybrid system introduces possible technical as well as conceptual errors. The hybrid system, in fact, translates a voltage signal into current, which is injected through a bridge amplifier. The voltage drop across the cell input resistance is recorded and reintroduced into the system. Actually, neurons with high input resistance and high gain in the voltage-to-current translation will introduce a positive feedback loop into the hybrid system. Such a positive feedback loop will in itself induce sustained oscillations even in a completely passive neuron. However, a neuron with a high input resistance usually has a long time constant which introduces a phase lag into the feedback loop, thereby reducing the possibility of generating sustained oscillations. Furthermore, limiting the current injected into the neuron to a maximum of 2 nA ensured that, during the state of sustained oscillations, the membrane voltage fluctuation generated by passive voltage drops across the membrane resistance would not exceed a peak-to-peak value of 40 mV assuming an average input resistance of 21 MΩ. In addition, experiments where the effect of membrane potential was examined were performed after membrane rectifications were reduced to a minimum (see Fig. 4). This precaution was essential since a voltage-dependent reduction in input resistance will reduce the feedback loop gain thereby decreasing the oscillations. Preventing membrane rectification insured that the results of membrane potential change were solely due to change in active properties of the neuron. It should be mentioned that similar results were readily obtained under normal conditions.

A positive feedback loop might also be introduced into the system by an unbalanced recording (improper bridge balance). Since current was introduced into the neuron as a sine wave, it was impossible to detect bridge balance problems. An under-balanced bridge will increase the voltage signal that is reintroduced into the electrical system, thereby increasing the gain of the feedback loop. Moreover, since the electrode time constant is in the order of 1 ms, the phase lag will be minimized and favorable conditions for the generation of sustained oscillations will be reached. An over-balanced bridge, on the other hand, will induce a 180° phase shift which will actively interfere with the generation of oscillations. To prevent such artifacts, the bridge balance was frequently examined (see Experimental Procedures).

Although the model behaves similarly to the inferior olivary nucleus, there are discrepancies between the two. For example the preferred frequency as determined in the hybrid system was 5.2 Hz. This value, which is in perfect agreement with previous reports, where the spontaneous occurring subthreshold oscillation has an average frequency of 5 Hz in slice preparation (Ref. 15; 6 Hz according to Benardo and Foster), or 4-6 Hz in the in vitro brainstem–cerebellum preparation is lower, however, than has been described for in vivo experiments.
The autocorrelations of olivary spontaneous activity (recorded as complex spikes at the cerebellar cortex) reveal a frequency of about 10 Hz (10.5 ranged 6–15 Hz; Ref. 18). The spontaneous activity seen after harmaline treatment was also in the range 8–10 Hz or 8–12 Hz.  

There are two explanations to account for this discrepancy. The single neuron in the hybrid system is unable to support higher frequencies. Similarly, the relatively small number of neurons that survived the slicing procedure can not generate the 10 Hz frequency. This explanation assumes that the frequency of oscillation depends to a certain extent on the number of coupled neurons. An alternative explanation assumes that the frequency of the oscillations is partially determined by neuroactive substances. Accordingly, the absence or the uncontrollable level of these substances in the in vitro preparation results in a lower frequency of oscillations.

Even if the alternative hypotheses given were correct and the discrepancies between the behavior of the hybrid system and the olive nucleus were significant, investigation of the behavior of the hybrid system led to significant conclusions and useful predictions. Not only does it enable us to determine the preferred frequency of oscillations, it gives us insight into the relative roles of the components which determine the frequency of oscillation.  

According to the model, the electrotonic coupling, even using a rather low coupling coefficient, is the major determinant of the oscillations. Thus, the coupling between olivary neurons should be considered not just as a device which synchronizes spikes but as the main control which dictates the functional state of the internal clock mechanisms. Accordingly, the dense GABAergic innervation of the olivary nucleus, which is postulated to modulate the coupling between these neurons, determines not only how many neurons will be synchronously active, but also whether the underlying timing device will be functional.

Finally, the novel approach used in the current study not only supports the working hypothesis as to the mechanism underlying the subthreshold activity of olivary neurons, it also highlights the role of electrotonic connections in coupled systems. In this regard it is important to note that this approach is not limited to the study of electrotonically coupled neurons but it is applicable to the study of other types of neuronal networks. In synaptically coupled neurons the electrical part of the hybrid system should be modified in a way that each unit will generate a synaptic current and by interacting with other units will compute a final current waveform which will be reintroduced into the biological part of the hybrid system. In such a system the role of synaptic efficiency and membrane properties in generating network specific activity will be examined. It is not unlikely that in the near future the electrical part of the hybrid system will be replaced by a computer which will interact, in real time, with the biological system, and thus provide a powerful tool to study complex behavior of neuronal networks.

Acknowledgements—I am most grateful to A. Adan who designed and built the analog simulator. Without his enthusiasm and knowledge this work would not have materialized. I would like to thank R. Werman for his contribution to the style of this manuscript and his encouragement throughout the work. This work was supported by a grant from The National Institute for Psychobiology in Israel, founded by the Charles E. Smith family.

REFERENCES


APPENDIX

The purpose of this appendix is to describe the functional properties of the simulator and to point out the differences as well as the similarities between this electrical device and the biological system. Accordingly, it is divided into two parts: the function of the basic controls of the simulator activity and the ensemble activity manifested as the generation of sustained oscillations.

Unit gain and coupling level

The properties of the simulator are governed by two main controls which determine the nature of the oscillations and a series of switches which enable selection of the number of units that participate in the system. The basic function of the two main controls of the simulator, the unit gain and the coupling level, are illustrated in Fig. A1. The increased oscillations induced by increasing the unit gain in an isolated oscillating unit (unconnected—S, and S, are open; see Fig. 1B) is demonstrated in Fig. A1A. Oscillations were evoked by a single triggering pulse (the first negative deflection in each trace) delivered to the same unit at four different levels of unit gain. As the gain increases (top to bottom), the time constant of decay increases and as a result the amplitude and duration of the oscillations increases. Further elevation of the unit gain will result in the generation of sustained oscillations (not shown).

For comparison, similar responses of an olivary neuron are demonstrated (Fig. A1B). The records were obtained by an intracellular microelectrode inserted into an olivary neuron in a slice preparation with tetrodotoxin (10^{-6} M) and harmaline (5 mg/ml) present in the superfusion solution. A hyperpolarizing current pulse (120 ms) of various intensities was delivered through the intracellular microelectrode at low current intensity (top trace); the hyperpolarizing response was followed by a single low threshold Ca spike, accompanied by a small subthreshold wave. As the current intensity was increased (top to bottom), a concomitant increase in the number of LTSs was observed. Since the LTSs exhibit a progressive decline in amplitude, the impression of a damped oscillation was obtained.

The effect of increasing the coupling level between the simulator units is demonstrated in Fig. A1C–E. Simultaneous recordings of the electrical activity in all the units, at three different levels of coupling, are presented. The three coupled units (three lower traces) were mutually connected, while the input to unit 1, which received the triggering signal (top trace), was disconnected (S, open; see Fig. 1B); thus, unit 1 serves as the source but does not receive the feedback signal. As the coupling between the units increases, the oscillations in the coupled units increase both in amplitude and duration. Two fundamental issues should be emphasized. (1) In this system the load effect, expected in a coupled system, does not exist. Therefore, even when the coupling was increased, the source, unit 1, remained unchanged. (2) Since each of the coupled units responds actively to the input signal and since all of them are interconnected, at the highest coupling level presented (Fig. A1E), the oscillations in the coupled units outlast the response in unit 1.

Generation of sustained oscillations

By interconnecting the entire system and delivering a trigger signal to one of the units, sustained oscillations could be generated, provided that the unit gain and the coupling level were properly set. This behavior is demonstrated in Fig. A2, which shows the activity induced by a triggering signal delivered to unit 1 (upper trace) and recorded simultaneously in all four units. The absence of feedback to unit 1 prevented the generation of sustained oscillations and only damped oscillations were observed (in Fig. A2A, as in Fig. A1C). After reconnecting the input to unit 1, without changing either the coupling level or the unit gain, the same triggering signal now generated sustained oscillations (Fig. A2B). The build-up of sustained oscillations is characterized by an initial phase during which the amplitude of the response in unit 1 decreases, while the amplitude of the responses in the coupled units increases until the activities in all the units are locked in phase and amplitude. From this time on, a progressive increase in amplitude in all the units is evident. The process then develops into its final form, the generation of sustained oscillations with constant amplitude.

A detailed description of the initial phase is shown in Fig. A2C, D. In order to examine the relations between the activity in the unit which serves as a source (unit 1) to one of the coupled units (unit 3), the records were superimposed. As mentioned above, under both conditions, when sustained oscillations were generated (Fig. A2D) and when they failed to occur (Fig. A2C), the initial phase is characterized by a reduction in amplitude in unit 1 and an increase in amplitude in unit 3. The difference between the two conditions is that, in one case (Fig. A2C), the phase difference persists and the amplitude in both units slowly declines, while in Fig. A2D a reduction in phase difference led to a slower reduction in amplitude and eventually to the build-up of
sustained oscillations. In short, accurate synchronization of the units seems to be a prerequisite for the generation of sustained oscillations.

Once sustained oscillations are generated they will be maintained if no interference is introduced. Interference is defined as a reduction in either coupling level or unit gain or disconnecting one or more of the units from the system. The records illustrated in Fig. A3 demonstrate the latter type of interference. The oscillations, recorded simultaneously in all four units, were evoked by a single triggering pulse delivered to unit 1. At the time designated by the first arrow, the input to unit 3 was disconnected (S, open; see Fig. 1B). As a result, the activity in this unit rapidly decayed while the oscillations in the other three units, although slightly reduced in amplitude, persisted. It should be mentioned that oscillations could not be induced in a system with the same set of parameters where only three units are connected (if, however, the unit or coupling gains were set to higher values, oscillations could be induced in a system of three or even two units). Thus, four units were needed to induce oscillation but, once generated, it could be supported by only three units. Only when the input to unit 4 was also disconnected (second arrow), did the oscillations in units 1 and 2 slowly subside.

The ability to produce sustained oscillations depends on the unit gain and the coupling level in such a way that a system with a high coupling level and low gain behaves similarly to a system with low coupling level and high unit gain. In fact, once a set of “threshold” parameters (a level of gain settings where a single trigger signal to unit 1 evoked sustained oscillations) was selected, they could be modified in a reciprocal way and the system would still maintain the same properties. However, in a system with high coupling level, the number of units becomes a crucial factor. In addition, if one or both of the parameters were slightly reduced, a single triggering signal would then fail to induce sustained oscillations. On the other hand, if two to four signals were delivered simultaneously to two to four units or, a series of signals, in a certain temporal arrangement, was delivered to one of the units, sustained oscillation could be generated. The simulator properties can be summarized as follows. (1) When oscillating units, each of which can generate damped oscillations and respond actively to input signals, are coupled, the system can generate sustained oscillations. (2) Synchronization of the oscillating units is a necessary condition for the development of sustained oscillations. (3) Maintenance of the oscillations depends on the integrity of the system.
Fig. A2. Sustained oscillations generated by the electrical simulator with a threshold setting of the unit and coupling gains. (A, B). The activity recorded in all four units following a triggering signal to unit 1. In A the input to unit 1 was unconnected (Si is open); therefore, the triggering signal evoked only damped oscillations. In B, after connecting the input to unit 1, the same triggering signal now elicits sustained oscillations. Note that the sustained oscillations develop simultaneously in all units and that the amplitude is larger than that induced by the triggering signal. (C, D) Two superimposed records of the activity in units 1 and 3, at higher gain (×6) and faster speed (×2), as in A and B, respectively. Note that when the sustained oscillations fail to occur (C), the phase difference between the two units persists while, when sustained oscillations are generated (D), a progressive decrease in phase shift is evident.

Fig. A3. Termination of the sustained oscillations by disconnecting the inputs to units 3 and 4. The sustained oscillations recorded in all four units were evoked by a triggering signal to unit 1, after selecting threshold parameters of the unit and coupling gains. At the first arrow the input to unit no. 3 was disconnected (S, opened), inducing a rapid and complete disappearance of the oscillations in that unit and a slight reduction in amplitude of oscillations in the other three units. By disconnecting the input to unit 4 (second arrow), the oscillations in that unit immediately subsided followed by a slower decline of oscillations in the other two units (units 1, 2).