Retrograde Invasion of Lobster Stretch Receptor Somata in Control of Firing Rate and Extra Spike Patterning

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SUMMARY AND CONCLUSIONS

1. Extra spikes may be interleaved in the otherwise rhythmic discharge pattern of the lobster stretch receptor neuron, about 2 ms after an expected spike. A constant input to the neuron is maintained by injecting current intrasomatically. The axon recovers its excitability while the retrograde invasion of the soma and dendrites is still in progress, which provide electrotonic currents to reexcite the axon.

2. While extra spikes in the axon often arise from a prolonged somatic (dendritic?) depolarization, they may also arise from a delayed retrograde invasion of the soma.

3. Failure of retrograde invasion may cause a sudden jump in the rate of rhythmic discharge, demonstrating the role of the soma-dendritic afterhyperpolarization in the regulation of rhythmic firing rate.

4. The history of repetitive firing is often important. Because extra spikes often first appear during a decline in firing rate, turning on and then off, an additional current may sometimes activate the extra spike mode, thus doubling the resting firing rate in a metastable manner. Another metastable state is associated with failure of retrograde invasion.

5. Extra spikes augment the high end of the frequency-current curve in some receptor neurons; in other cases, the extra spikes are seen only at low rhythmic firing rates, dropping out as current reaches intermediate values to create a paradoxically high instantaneous firing rate.

6. The results suggest that both the extent and the speed of active retrograde invasion of the soma and dendrites are likely candidates for pathophysiological mechanisms, since they may control whether extra spikes are generated.

INTRODUCTION

The basic discharge pattern of most neurons is rhythmic, i.e., action potentials (spikes) often recur at a relatively fixed interspike interval provided that the input to the neuron is held constant. One usually mimics sustained physiological inputs to the neuron by substituting steps of current injected through an intracellular recording microelectrode (6). Once a certain minimum current is reached, most neurons begin to discharge rhythmically. Further increase in the current raises the firing rate proportionally, i.e., the neuron acts as a current-controlled oscillator with a threshold. Very short interspike intervals, e.g., 2 ms, thus require massive inputs if this current-to-frequency conversion mechanism is to produce them.

Many types of neurons, ranging from invertebrate receptors through human spinal motoneurons (6), exhibit a phenomenon called “double spikes” where very short interspike intervals occur without massive inputs. Indeed, one way in which double spikes typically arise is during an otherwise normal rhythmic discharge. The rhythmic pattern, e.g., a spike every 100 ± 5 ms, is occasionally interrupted by a very short interspike interval, e.g., 2 ms. Thus, one has a paradoxically high instantaneous firing rate for relatively low (and unchanging) input levels.

In the case of cat spinal motoneurons and motor cortex neurons, one can observe certain events which underlie the extra spikes (6, 10-12). In these types of neurons, as in many others, each spike is followed by a brief humplike event which peaks a few milliseconds after the spike. These “postspike humps” are different sizes in different cells (and often
Retrograde Control of Firing Pattern

In crustacean stretch receptor neurons (20, 26, 44), as in other neurons (10, 14, 24, 46), spikes usually originate from a trigger zone at the axon's initial segment. The retrograde invasion of the soma from the initial segment often involves some delay as the small axon attempts to depolarize the large soma past threshold (17, 25), and an inflection can often be seen on the rising phase of the intrasomatically recorded spike. When invasion fails, a smaller spike is seen intrasomatically from the electrotonic spread of the initial segment spike. This is called an A spike: the full-sized spike, representing active retrograde invasion (27), is descriptively called the B spike, and the delays in retrograde invasion which exaggerate the inflection on the rising phase of the full-sized intrasomatic spike are called AB delays. The extent to which active (as opposed to electrotonic) invasion of the dendrites is involved in the B component is poorly understood. There is, of course, the possibility that the cell may not repolarize uniformly, e.g., the initial segment or soma might repolarize sooner than the dendrites, thus attracting currents from the dendrites where depolarization remains.

The role of dendrites in postspike humps is inferential in motoneurons (39, 42), but Grampp (26–30) has added good evidence for the role of dendrites in the case of the lobster stretch receptor. Retrograde invasion of the dendrites was not completed at the time that the initial segment trigger zone recovered its excitability; i.e., the remaining depolarization in the dendrites served to reexcite the cell (27, 29).

The purpose of the present experiments is to further explore reexcitation phenomena in a neuron in which it is possible to record simultaneously for long periods from both the axon and the soma, thus allowing a more precise description of the reexcitation, its effects on the sensitivity of the current-to-firing rate conversion performed by the neuron, and its historical dependencies. We now show that an after-depolarization is but one of a number of ways in which extra spikes arise, that there are often extra spikes in the axons when one would not suspect them from the traditional intrasomatic recordings, that extra spikes and failure of retrograde invasion may both give rise to metastable alterations in the neuron's input-output relations, and that absolute sensitivity regions may occur in frequency-current curves because of extra spike properties.

Methods

We used the slowly adapting muscle receptor neuron (2, 23) of the spiny lobster, Panulirus interruptus. Panulirus physiological solution was composed of 480 mM NaCl, 13 mM KCl, 17 mM CaCl₂, 10 mM MgSO₄, 1 mM NaSO₄, 0.36 g/liter glucose, and 0.75 g/liter TES (Sigma) buffer, with NaOH added to adjust to pH 7.45. Lobsters were kept in running seawater.

The receptor muscles and the associated nerve were removed and placed in physiological solution. The two ends of the muscle pair were mounted in forceps and a moderate stretch applied. The cut end of the nerve was pinned to the bottom of the Sylgard-coated petri dish and a 60-µm suction electrode used to record from the tonic receptor's axon. Fine dissecting pins were used to stretch the connective tissue on the perimeter of the triangle of tissue near the receptor neurons. The connective tissue immediately on top, and around, the tonic receptor neuron was often enzymatically treated (32) by ejecting physiological solution containing less than 1 mg/ml pronase from a 40-µm pipette. After immediately rinsing with normal physiological solution (two complete solution changes), a delayed increase in resting firing rate was usually observed within 10 min; the rhythmic nature of the discharge did not alter. Such changes are consistent with a central relaxation in muscle connective tissue which allowed greater stretch to be applied to proximal dendrites. We noted no obvious differences in membrane or repetitive-firing properties between treated and untreated preparations.

We recorded intracellularly with a double-barrelled micropipette filled with 3 M KCl, usually of 20–60 MΩ resistances. The electrode blanks were made using two layers of heat-shrinkable tubing. First, a short piece was placed around the individual 1.4 mm OD capillary tubing near one end; the opposite ends of the two such tubes were then placed parallel to one another, being held about 0.5 mm apart by the shrunk tubing spacers. Two additional pieces of shrink
tubing were then used over the previous ones to secure the two tubes together. This blank was then placed in a Narashige vertical micropipette puller; with the heat on but the pull off, one end of the blank was rotated 180–360° to fuse together the central portion. After cooling, the electrode was pulled in the usual way. This procedure was efficient in producing double-barrelled electrodes with minimal cross coupling; although the application of Vaseline to the open end of the pipettes was used to prevent evaporation of KCl solution, it was not typically necessary to prevent cross coupling by wetting of the external surfaces of the two barrels. One barrel was used for current injection, which we often monitored with a virtual ground circuit. Steps and slow triangular waveforms of injected current were used to stimulate muscle stretches. All data was tape recorded (0- to 2,500-Hz bandpass) for later analysis and photography.

We increased the temperature of the receptor (4, 22, 28) by heating a plate beneath the petri dish; we cooled by careful replacement of solution.

RESULTS

We found lobster receptor neurons to exhibit prominent double spikes with very short (and often fixed) interspike intervals in the 1- to 4-ms range, analogous in their timing patterns to the phenomenon in mammalian neurons (6, 12). These “extra” spikes during rhythmic discharge occurred at room temperature (20°C) in some cells; in others, we used elevated temperatures (28–33°C) to augment the probability of observing extra spikes (28). Except as noted, the rhythmic waveform of the discharge pattern was maintained to a wide range of injected currents. Temperature effects were reversible.

Simultaneous recordings from the axon via the suction electrode were of great importance; if we had judged the occurrence of extra spikes only by their retrograde invasion of the soma (as one currently does in mammalian neurons), we would have greatly underestimated the occurrence of extra spikes in lobster receptors. As can be seen in Fig. 1, extra spikes in the axon can correlate with at least five different configurations of the intrasomatic waveform and not merely the low postspike humps so prominent in the mammalian literature.

Intrasomatic voltages associated with axonal extra spikes

Extra spikes may be associated with low postspike humps (Fig. 1C), apparently in a causal relationship; they do not necessarily arise from the peak of the hump as in spinal motoneurons and motor cortex neurons of cat (5, 6, 10–12). Often, retrograde invasion was unreliable and only a bump was seen intrasomatically corresponding to the extra spike in the axon.

High postspike humps (or “shoulders”) as are seen in Purkinje cells (18, 19) and inferior olive neurons (16) may generate extra spikes in lobster receptors; while the extra spike may fully invade the soma at low firing rates (Fig. 1A), the extra spike invasion can fail during higher rhythmic firing rates caused by higher currents (Fig. 1B).

One also sees extra spikes when the soma spike broadens (Fig. 1D, E) because of the delayed retrograde invasion indicated by the AB inflection on the rising phase of the spikes; again, examination of the intrasomatic record cannot predict when an extra spike is, or is not, being generated in the axon.

One may also see A-only double spikes (Fig. 1F and Fig. 5E). This occurs after the B spike has failed at high current: as the A spike starts to recover in height during descending currents (Fig. 5D, E), it may develop a slight afterdepolarization from which an extra spike can arise (28). This demonstrates that full retrograde invasion of the first spike of the doublet is not necessary for the generation of extra spikes, but the increase in spike height and the slight afterdepolarization suggests partial invasion.

Often, more than one of the extra spike patterns of Fig. 1 could be seen during the 1- to 8-h intracellular study of an individual neuron.

Historical factors

The recent repetitive firing history of the neuron is important in determining whether double spikes are seen. The extra spikes arising from low humps (Fig. 1C) often occurred after the first few spikes terminating a silent period. When scanning with triangle-shaped stimuli, we often first observed double spikes during the descent from high firing rates (Fig. 2B); the spikes had more prominent humps or shoulders during descent than during ascent of the triangular waveform of injected current (Fig. 2A). This sequence reliably repeated after a brief silence.

At slightly higher temperatures, double spikes were seen during ascent as well, again only at low currents. If the extra spikes were ignored, one saw the typical linear firing rate versus current relationship (Fig. 2C). The extra spikes in the low end of the range elevated the total spikes per second and, thus, gave rise to the same paradoxical fall of firing rate with increasing drive as is seen in cat spinal motoneurons, where extra spikes also disappear with increasing driving currents (6, 36). Other receptors exhibited double spikes only in midrange (e.g., Fig. 1D, E) or only at higher rates, as in cat motor cortex neurons (11–12). The stereotyped nature of the extra
Intrasomatic potential is recorded from lobster receptor neuron with one barrel of micropipette, and steps or triangular waveforms of current are injected through the other barrel to produce rhythmic firing. Samples are shown of spikes from such rhythmic firing. Suction electrode records spike propagated down axon (variable gain, from 0.2 to 2.0 mV, negative up). A: double spikes from a neuron heated to 28.9°C where the extra spike invades the soma at low currents but not at medium current, producing higher rhythmic rates (B). C: another neuron at 31.6°C where extra spikes arise from a low postspike hump following the silent period after sustained stimulation. D: same neuron earlier at 32.6°C with a narrow spike at low firing rates but a broadened spike at intermediate firing rates (E) during a slow ramp of injected current; the marked AB inflection on the rising phase of the intrasomatic spike (E) indicates that the delayed retrograde invasion of the soma from the axonal trigger zone has provided a current source to reexcite the axon following its refractory period. Again, one cannot usually tell from examination of the somatic record whether or not an extra spike has been generated in the axon. F: in yet another neuron (29.7°C) one sees A-only double spikes. This occurs after the B spike has failed at high currents and after the A-only rhythmic firing (as in Fig. 2A B). As the A spike starts to recover in height during descending currents, it may develop a slight afterdepolarization from which extra spikes arise, indicating that only partial, and not full, retrograde invasion of the soma is necessary for the generation of extra spikes. Same neuron as in Fig. 3A and Fig. 5, where spike height change can be seen.

spike interval may be seen in the scatter plot of Fig. 2D.

Most phenomena elicited during triangle waves can also be seen during adaptation to a step of injected current. Spike shapes often changed as in Figs. 1A and B, 1D and E, or 2A and B; in Fig. 3A, extra spikes are seen following the second and subsequent rhythmic spikes of the response to the current step but not following the first spike, whose shoulder is not as prominent as in later spikes.

Metastable double-spoke patterns

The increasing duration of the soma spike (together with shoulders, etc.) with sustained activity as seen in Figs. 1D, E, 2A, B, and 3A suggests that "fatigue" may paradoxically increase firing rates by making extra spikes possible (45). This factor may account for the dramatic example of historical effects seen in Fig. 3B. Double spikes appear in the axon on return to the original current level, where there were only single spikes before and during the brief step. This might be called a "metastable" state rather than a latch-up, as the double spike pattern spontaneously reverted to ordinary rhythmic firing within a few seconds.

Delayed retrograde invasion

The prominent AB inflection seen on the rising phase of spikes in many types of cells is often exaggerated by the stress of short interspike intervals; this is usually interpreted as a delayed retrograde invasion of the soma by the spike originating at an axonal trigger zone. As shown in Fig. 1D, E, extra spikes in the axon may be seen
when there is sufficient AB delay; the soma spike has evidently reexcited the initial segment. As the extra spikes do not retrogradely invade, the intrasomatic recording gives no indication of their generation, again emphasizing the importance of the axonal monitor. Slightly enhanced AB delay was correlated with the metastable double spikes in Fig. 3B.

Failure of retrograde invasion

When the B component is delayed enough, it will often fail, leaving an A spike (presumably the trigger zone's spike, attenuated by the cable and geometrical coupling factors). The firing pattern is greatly affected by failure of somatic invasion but in a manner quite different from the extra spikes generated by merely delayed invasion. Rather than extra spikes, one sees sudden jumps in the rate of rhythmic firing on failure of the B spike. Sustained repetitive firing in the receptor neuron, e.g., due to a step of injected current as in Fig. 4A, may bring about a progressive decrease in the B spike and then sudden failure. There is sudden shortening of the interspike interval in the middle of the current step in Fig. 4A when the B invasion fails.

As in other types of neurons (10, 15, 37), the afterhyperpolarization following the B spike in lobster receptor neurons is much more pronounced than that following an A spike, suggesting that the retrograde invasion might play a prominent role in controlling rhythmic firing rate (6). When the B spike recovers following a period of A-only rhythmic firing at a high rate (as
shown in Fig. 4B for a different neuron), its afterhyperpolarization substantially lengthens interspike intervals.

Another metastable state which may be observed in lobster receptor neurons is associated with retrograde invasion failures. At a current where the neuron has shifted into A-only rhythmic firing, one may apply a brief hyperpolarizing current pulse to convert back to B spikes; one will often observe B spike rhythmic firing even on return to the previous current level. Similarly, following initial penetration with the micropipette, we sometimes observed small spikes which could be converted by a brief hyperpolarization into full-sized spikes; this B spike could be converted back into A-only firing by a brief depolarizing pulse (and would sometimes convert spontaneously).

Combination of extra spikes and A-only rhythmic firing

While scanning with a triangular-current waveform, one would often see double spikes at some currents and A-only jumps in rhythmic firing rates at others. As noted in the presentation of Fig. 1, double spikes occur at low currents in some cells, on both ascent and descent in others, etc. If the peak current is high enough, one will often see A-only rhythmic firing at the high end of the triangle. Figure 5 illustrates a cell (at 29.7°C) with double spikes at minimal currents on ascent, then rhythmic firing, then double spikes again, then A-only rhythmic firing at peak currents, then A-only double spikes as spike height begins to increase, followed by standard AB spike rhythmic firing during the remainder of descent. Elaborate timing patterns may arise from alternations of B failure (short A-only rhythmic intervals) and AB delay (extra spikes at even shorter intervals); an example is shown in Fig. 6 during ascent only.

DISCUSSION

Edwards and Ottoson (20), and later Grampp (26, 27), used extracellular recording to show that all of the spikes of the multiple spike clusters from Homarus stretch receptor neurons originated from the initial segment; we saw nothing which would suggest that our expected and extra spikes originated from different sites. Thus the interpretation of our records will proceed from the assumption that the normal trigger zone is being reexcited.

Axons do not typically reexcite themselves because, by the time a particular segment recovers its excitability, the spike has propagated far enough away so that electrotonic spread is insufficient to reexcite that segment. The boundary between excitable and inexcitable membrane propagates as a wave some distance behind the spike’s depolarizing wave front. However, when a spike propagates into a region where the conduction velocity slows or the spike broadens (25, 40, 43), the recovering membrane may now come within effective electrotonic distance of a spike still in progress and hence becomes reexcited,
resulting in a spike propagating backwards. "Reflection" effects have been reported in Aplysia neurons (46), lobster cardiac ganglion neurons (34), frog (45) and cat (36a) dorsal root ganglion cells, and in focally demyelinated axons (36a), as well as in heart muscle (49).

The retrograde invasion of the soma and dendrites provides a number of opportunities for reexcitation: while the dendrites might often be the source of the reexcitation current (27), so might the soma (Fig. 1D,E). Once the source of depolarizing current exists due to slowed invasion or broadened spikes, the time course of the recovery of excitability becomes the important issue (5), together with the effectiveness of the electrotonic spread (31). These mechanisms must be considered separately from the historical factors which bring them into play.

**Historical factors**

The conditions which augment extra spike production would seem to form two broad groups: 1) those associated with stressed repetitive firing such as fatigue; and 2) those associated with unstressed repetitive firing. That stress may augment extra spike production via AB delays is apparent; it is less obvious how silence may augment extra spikes or depolarizing afterpotentials (Fig. 1C).

Tagini and Camino (45) stimulated frog peripheral nerves at sustained 20–100/s rates and recorded the spike trains arriving in dorsal root axons; during sustained stimulation, they saw extra spikes several milliseconds following expected spikes. From timing considerations, the extra spikes seemed to arise from near the axonal
bifurcation leading to the dorsal root ganglion. Intracellular recording in the ganglion cells showed AB delays developing there during sustained spike trains; while they did not have simultaneous recordings from the axon, their intrasomatic spikes were often delayed enough to correlate with the time at which the extra spike typically arose nearby. They observed the extra spikes in 25% of their large myelinated axons but not in small axons, perhaps because of the shorter refractory periods associated with large fibers; the extra spikes have also been seen in cat dorsal root ganglion (36a).

Our extra spikes associated with AB delays were never seen without an immediate history of substantial repetitive firing; if seen during steps, they were only later in the step; if seen during triangular waves, they were seen after repetitive firing had been in progress.

At the other end of the “exercise” spectrum, one sees the extra spikes which follow the first spikes to break a silence (Fig. 1C). There is, as
yet, no study of the underlying afterdepolarizations as a function of past silence. Somewhat related are the cases where extra spikes are only seen at the low end of the current range, e.g., the spinal motoneurons (5, 6, 36) and external cuneate nucleus neurons (7) whose extra spikes drop out as one attempts to drive the firing rate up with more input. These cases may have the paradoxical frequency-current curves such as in our Fig. 2C with its negative sensitivity region. It is not presently possible to say how threshold recovery curves and afterdepolarization time course intersections (e.g., Fig. 1 of ref 7) shift to effect this change.

This discussion might suggest that extra spikes at higher rhythmic firing rates are associated with AB delays, while those at lower rhythmic rates are associated with depolarizing afterpotentials. This is not always the case even in our lobster receptor neurons, and the extra spikes of fast pyramidal tract neurons of cat (10, 11) also serve as a counterexample. The pyramidal tract neuron extra spikes are not seen at the low rhythmic firing rates, but arise sporadically at intermediate firing rates. Further increases in current cause extra spikes to appear after each rhythmic spike. These extra spikes clearly arise from large humps which follow the rapid repolarizing phase of the spike, not from AB delays (11, 12). If depolarizing humps are due to the retrograde invasion of the dendrites, changes in humps are thus likely to be associated with alterations in the extent or speed of retrograde invasion (just as in the case of the delayed soma spikes in Fig. 1E).

Thus, the historical factors associated with extra spike production form several groups (high versus low rate history) as do the underlying phenomena (AB delays versus postspike shoulders and humps), but postspike humps may be associated with extra spike production at both low and high rhythmic rates.

An additional historical factor concerns the tendency of large postspike humps (and thus extra spikes associated with postspike humps) to begin following the second, rather than the first, spike of a rhythmic train elicited by a step of depolarizing current in cat spinal motoneurons (5) and cat motor cortex neurons (11), thus raising the question of whether the first spike serves to "prime" an underlying mechanism. On a few occasions (Fig. 3A) we observed humps or shoulders which substantially increased in size following the second spike; this may or may not be analogous to the mammalian phenomena.

**Extra spikes versus postpriming repetitive firing**

Because it facilitates analysis, it is sometimes useful to distinguish between spikes generated by the usual rhythmic firing mechanism, where firing rate is proportional over a broad range to the generator currents, and extra spikes which clearly arise from an event associated with the antecedent spike (6). Where this event (AB delay, shoulder, hump, etc.) has a time course on the order of the duration of the spike and its refractory period, one may clearly make such a distinction between rhythmic mode spikes and extra spikes. Where there are longer lasting depolarizing afterpotentials, they may add to the original generator currents in such a way as to obscure the distinction.

In comparing our work on the *Panulirus* stretch receptors to previous work on *Homarus* and crayfish stretch receptors (19, 20, 25, 29), it would seem as if the *Panulirus* neurons exhibit more pronounced inflections on both the rising phase of the spike and during falling phase and afterdepolarizations; furthermore, they do not often exhibit the slower afterdepolarizations which have complicated earlier analyses. In the original definitive treatment by Eyzaguirre and Kuffler (21), extra spikes were seen even following A-only spikes; the subsequent studies of Granpp (29) defined a "labile" depolarizing afterpotential which could be seen even after A-only spikes. Eyzaguirre and Kuffler (21) postulated a "local response" of the soma to underlie these slow events. Our rare instances of A-only double spikes (Fig. 1F and Fig. 5E) and the apparent increase in afterdepolarization which accompanied the transition from A-only rhythmic firing (Fig. 5D) to A-only double-spike rhythmic firing (Fig. 5E) would seem analogous.

In earlier investigations, slower afterdepolarizations have been prominent and led to bursts of many extra spikes where only A spikes were seen in the soma (21, 29, 30). Our *Panulirus* experiments seem to have often straddled the threshold conditions for extra spike production; we only infrequently observed multiple extra spikes. This was of considerable assistance in our analysis as it allowed us to compare extra spike cases with closely analogous cases where no extra spike was seen but where the underlying shoulders, humps, and AB delays were visible. The lack of slower underlying events, as noted above, meant that we could more clearly associate an extra spike with these other events on a one-for-one basis.

This experimental advantage, however, should not obscure the fact that multiple spike bursts can and do arise from a cumulative effect of preceding activity. Besides the previously cited examples in crustacean stretch receptors, postpriming repetitive firing (where repetitive activity outlasts the short train of evoked priming spikes) may be seen in focally demyelinated mammalian nerve fibers (36a); Adrian and Bryant (1) have analyzed the mechanism of the postpriming repetitive fir-
ing in muscle fibers of myotonic goats as involving a generator current from extracellular potassium accumulation; Kandel and Spencer (38) saw summating afterdepolarizations (analogous to the slow ones of Homarus) and extra spikes which outlasted the current step which started the activity. Dramatic cases of postpriming repetitive firing are those in drug-treated crustacean stretch receptors (47, 48) where a single priming spike can cause a sustained soma depolarization which generates a long train of axon spikes. Thus, the mechanism of such postpriming repetitive firing could be extra spikes produced in a one-for-one fashion from postspike humps, in the vicious-cycle manner observed in some cases (6, 12); it can also simply be accumulating depolarizing aftereffects (e.g., potassium accumulation producing a prolonged generator current) serving to drive a rhythmic firing mode mechanism. In the former case, blocking a single spike would abort the entire train; such a maneuver would have little effect in the latter case. Some cases of postpriming repetitive firing are suggestive of combinations of the two mechanisms (36a).

**Extra spike mechanisms**

While determinations of the identity of the underlying ionic currents and their kinetic behavior would be most useful, the spatial interactions would seem to be a major underlying mechanism themselves: in the same manner as the electrotonic spread of current in an axon may be said to be the mechanism of spike propagation (regardless of whether the upstroke of the spike is mediated by sodium or calcium), so the electrotonic and active propagation of the spike in the axon-soma-dendritic complex may be said to often be the mechanism of reexcitation and extra spikes. This is not to deny the possibility of recurrent EPSPs, extracellular potassium accumulations, or variants on Hodgkin-Huxley membrane kinetics in the generation of postspike humps in some cases; it is merely to emphasize the important role which may be played by geometrical and physiological inhomogeneities in an electrotonically interacting region. Our work, together with the recent theoretical efforts (25, 40, 43, 43a), has shown that reexcitation may be manifested in several different ways and that there are several (sometimes opposing) sets of historical implications for the functioning of the neuron.

Our AB delay waveforms are quite analogous to those seen in the computer simulations of spike propagation through a region of sudden enlargement of an axon (25, 43). One must, of course, implicate a changeable physiological parameter (see discussion in ref 8) to vary the extent of AB delay which we observe rather than anatomical variation of the axon-soma enlargement. In the theoretical and experimental situations examined thus far, the extra spike associated with an AB delay waveform does not seem to successfully invade the enlargement itself because of the spike in progress there. Because of this, the extra spike cannot create still another extra spike by the same mechanism. On the other hand, the extra spikes associated with postspike humps (and other less dramatic types of depolarizing afterpotentials) seem capable of regenerative vicious cycles; the extra spike retrogradely invades in a manner superficially analogous to the original spike and may itself possess a postspike hump. Certainly in cat spinal motoneurons (5) and cortical neurons (12), this postspike hump following an extra spike can sometimes be large enough to itself elicit another extra spike; the cycle may seem to repeat to create a tight cluster of spikes.

The difference between the AB delay reflections which seem limited to one extra spike and the postspike-hump style regenerative cycles of many extra spikes could be related to the number of "compartments" involved in the retrograde invasion process, e.g., the two-compartment axon-soma interaction versus the hypothesized three-compartment axon-soma-dendrite interaction. As was noted in the INTRODUCTION, the postspike humps are thought to be the consequence of depolarization remaining in the dendrites following the fast repolarization of the axon and soma. The ascent of the hump would presumably (42) be a consequence of the increasing resistance of the soma membrane following the spike (the returning current $I$ from the spike remaining in the dendritic tree would produce an increasing $I R$ drop across the soma resistance $R$). The fast repolarization of the soma and axon would allow for rapid recovery of excitability in the trigger zone, so that it might be possible for the hump to intersect the falling threshold curve (5). This type of cycle has the capability of being self-regenerative because it allows reexitation by extra spikes as well as expected spikes. The two or three compartments need not, of course, necessarily be the aforementioned examples.

Extra spikes have been tentatively identified (6, 12, 13) as the source of some of the stereotyped high-frequency bursting firing patterns seen in deafferented CNS neurons (41) and in cortical neurons within epileptogenic foci (9, 13, 50); one would presume that the mechanisms underlying extra spikes have been somehow augmented in these situations. Our results would suggest that both the extent and the speed of active retrograde invasion of the soma and dendrites are likely candidates for pathophysiological mechanisms, since they may control whether extra spikes are generated.
Implications for input-output curves

The input-output curve for a neuron is somewhat simpler when the neuron does not utilize spikes: Graubard (32) has shown in spikeless lobster stomatogastric neurons that they still exhibit a threshold presynaptic depolarization below which no postsynaptic potential occurs, above which the postsynaptic response increases in a linear fashion with presynaptic depolarization. In neurons such as lobster receptors, the output synapses are located at such electrotonic distances from the input sites that propagating spikes seem to be necessary to communicate between input and output sites in the neuron. The properties of the repetitive firing mechanism are a primary determinant of the input-output relation rather than just the synaptic release curve: there is a threshold depolarization below which no signal is sent, above which a repetitive firing is produced at a rate proportional to suprathreshold currents. There is characteristically a stepwise jump at the bottom of the frequency-current curve, as the neuron will begin firing rhythmically at an elevated rate (in cat spinal motoneurons, this minimal rhythmic rate is clearly related to the duration of the afterhyperpolarization, cf. ref 39a). The sensitivity of the repetitive firing process (the slope of the frequency-current curve, measured in impulses per second per nanoampere) may exhibit sudden alterations (6, 35), some of which are due to the extra spike process appearing (6, 11, 12) or disappearing. Our N-shaped negative sensitivity region (Fig. 2C), due to the extra spikes dropping out with increasing currents, constitutes one of the more dramatic of the many examples of sensitivity alteration in the literature.

REFERENCES


36a _Howe, J. E., Calvin, W., H., and Loeper, J. D._ Impulses reflected from dorsal root ganglia and from focal nerve injuries. _Brain Res._ 116: 139–144, 1976.


