Motor Pattern Production in Reciprocally Inhibitory Neurons Exhibiting Postinhibitory Rebound

Abstract. *Pairs of neurons which inhibit each other can produce regular alternating bursts of impulses if they also exhibit postinhibitory rebound (PIR). Computer studies show that stable patterns occur spontaneously in systems of pacemaker neurons with PIR, and can be triggered in systems of nonpacemakers without requiring tonic excitation. The repetition rates of these patterns are determined largely by the PIR parameters. The patterns resist perturbation by phasic synaptic inputs, but can be modulated or turned off by tonic inputs. One pair of PIR neurons can be entrained by another pair with a different repetition rate to produce more complex firing patterns.*

Many cyclic behaviors are caused by alternating bursts of impulses in motor neurons that innervate antagonistic sets of muscles; such motor patterns are often generated by neuronal mechanisms entirely intrinsic to the central nervous system (*1*). The hypotheses proposed to explain them fall into two classes: oscillator hypotheses and network hypotheses (*2*). The oscillator hypotheses invoke a single neuron, having special membrane properties, as the primary determinant of the pattern (*3*). The network hypotheses state that the synaptic connections among neurons primarily determine the pattern; no single neuron controls it, nor is any neuron required to have unusual membrane properties. We report here the results of quantitative studies of a particular network hypothesis: reciprocal inhibition in neurons exhibiting postinhibitory rebound.

Reiss (*4*) and others (*5*) have used models of neurons to investigate the characteristics of neuronal networks capable of producing motor patterns. A pair of carefully adjusted reciprocally inhibitory model neurons can be made to produce trains of alternating, single impulses (*4*). We have found that such a system can also exhibit irregular alternations of sustained firing. But in order to obtain alternating bursts with constant periods, Reiss (*4*) and others (*5*) have had to introduce an extra state variable, which simulates "fatigue" or accommodation by decreasing a neuron's excitability as a result of its antecedent activity. These model systems require tonic excitatory input, and the alternating pattern is stable only through a relatively narrow range of parameters (*5*). Moreover, the characteristics of accommodation have not been drawn from experimental findings in the neurons being modeled, but rather have been stringently dictated by the requirements of the model.

An alternative source of regularity in these systems is the postinhibitory rebound (PIR) that occurs in many neurons following experimental hyperpolarization or hyperpolarizing synaptic inhibition (*6*). After such a period of inhibition, the neuron is for a time more excitable than normal (Fig. 1). If PIR exceeds threshold, it may trigger one or more impulses; the train of impulses within a typical burst first accelerates, then gradually decelerates. For a particular neuron, the degree and duration of the increased excitability depend on the strength and duration of the preceding inhibition.

It has been suggested that PIR can lead to stable, alternating bursts in reciprocally inhibitory model neurons (*7*) and interneurons in *Aplysia* (*6*). We have investigated the consequences of PIR in reciprocally inhibitory pairs of model neurons, using a digital-computer simulation. Our results confirm that PIR can stabilize alternating burst patterns in such systems. We have further investigated the requirements for initiating, maintaining, and terminating sustained motor patterns and have studied the recovery of these systems after perturbation. Also, we have determined minimal requirements for coordinating reciprocally inhibitory pairs of neurons of differing intrinsic repetition rates.

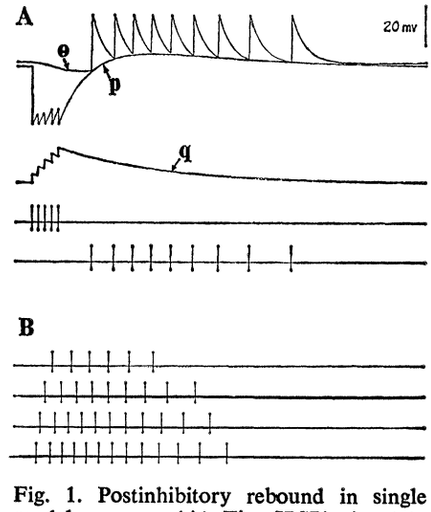

Fig. 1. Postinhibitory rebound in single model neurons. (A) Five IPSP's (next to bottom trace) lower threshold (θ) and also cause rebound of membrane potential (p). The second trace shows the time course of q, the PIR variable. For clarity, action potentials are suppressed, and are shown in the bottom trace. (B) Response of model neuron to three, four, five, and six IPSP's, delivered at a time corresponding to the beginning of each record.

We modeled the effects of PIR in individual neurons without attempting to incorporate the underlying ionic or molecular mechanisms. Starting with an earlier neuron model (*8*) that uses membrane potential and threshold, we added a third state variable, $q(t)$. In the model, we augment $q(t)$ whenever an inhibitory postsynaptic potential (IPSP) arrives, so that $q(t)$ is sensitive to both the size and the frequency of the recently arriving IPSP's. By continuously lowering threshold and depolarizing the membrane, $q(t)$ enhances the excitability of the neuron by an amount that gradually decreases as $q(t)$ decays exponentially.

The response of this model neuron to a short barrage of IPSP's is a burst of impulses which exhibits acceleration and deceleration resembling those seen in real neurons. The latency, duration, frequency, and number of impulses in the burst depend in a similar way for real and simulated neurons on the size and number of preceding IPSP's (Fig. 1) (*9*).

If two model neurons with PIR are linked by reciprocal inhibitory synapses, their behavior depends on whether they are pacemakers or are silent in the absence of synaptic input. Within a wide range of parametric values, pacemaker neurons will spontaneously fire alternating bursts (Fig. 2A). Both PIR and reciprocal inhibition are required to establish a regularly repeating firing pattern, whose characteristics are determined largely by the PIR parameters.

If the neurons are not pacemakers, but have relatively large PIR, they will burst indefinitely once triggered by synaptic input (Fig. 2, B to D), which may consist only of a single EPSP (excitatory postsynaptic potential) or IPSP delivered to one member of the

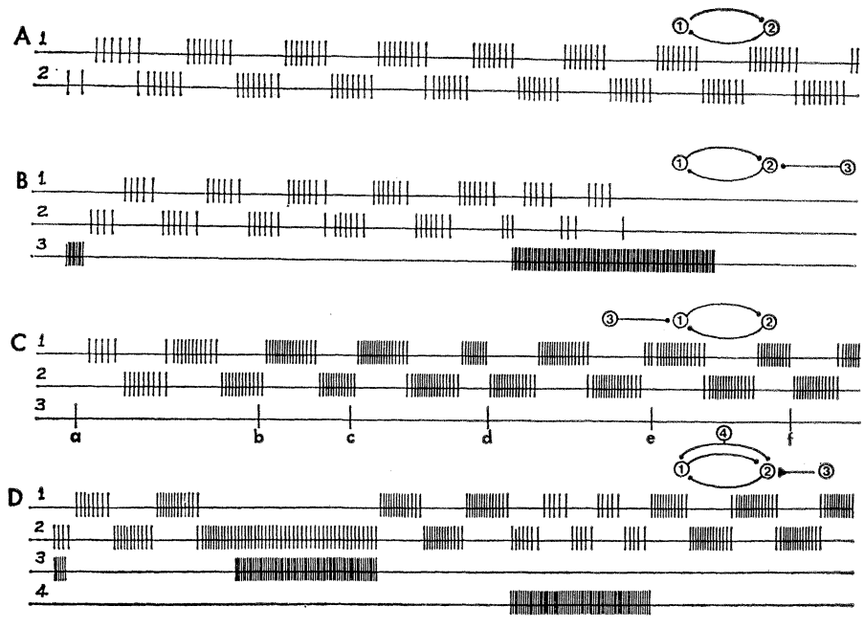

Fig. 2. Impulse trains in pairs of reciprocally inhibitory neurons with PIR. In the circuit diagrams a dark circle indicates an inhibitory synapse, the triangle indicates an excitatory synapse, and the numbers refer to the lines of impulse traces. (A) Spontaneous development of burst pattern in two pacemaker neurons; the pattern continues indefinitely. (B) Nonpacemaker neurons. The burst pattern is triggered by a brief barrage of small IPSP's to neuron 2; the pattern continues until stopped by longer barrage of IPSP's with diminishing amplitude, produced by the same fiber. See text. (C) Phase-dependent effects of a single, large IPSP delivered to neuron 1 at the times indicated in the third trace. The initial IPSP (a) triggers the motor pattern. An IPSP arriving toward the end of the silent period (b and c) increases the intensity of subsequent bursts in both neurons. An IPSP early in the burst (e) has little effect; an IPSP arriving later in the burst (d and f) terminates the burst and so resets the motor pattern. (D) Modification and resetting of pattern by sustained input. An excitatory barrage to one neuron interrupts the pattern, which resumes after excitation stops. Weak inhibition of both neurons decreases the number of spikes in each burst and increases the rate of alternation; the pattern is subsequently restored.

pair. Neurons with relatively small PIR require a tonic excitatory input to both neurons to produce alternating bursts. The tonic input to such pairs can modulate the frequency as well as sustain the output. When the excitation stops, the bursts die out within a few cycles.

In pairs of nonpacemaker neurons able to maintain regular bursting in the absence of tonic driving, activity can be turned off again by a relatively long, rapid barrage of small IPSP's. These IPSP's must decrease in amplitude at a rate slower than the decay of PIR, or else the neurons will subsequently rebound. A brief barrage in a single such fiber can initiate bursts, and a long barrage in the same fiber can terminate the activity (Fig. 2B). In pairs of pacemaker neurons, activity can be turned off temporarily by simultaneous inhibition of both neurons, but for nondecreasing IPSP's the pattern resumes when the inhibitory input stops.

Synaptic inputs to one neuron of a pair temporarily disrupt the pattern. Exciting one neuron can drive it to regular firing and inhibit the other neuron, but the normal alternation reappears once the excitation stops. Brief inhibition of one neuron during its burst will terminate the burst and reset the timing of the pattern in both neurons (Fig. 2C), as in endogenous burster neurons (*2*, *3*). Brief inhibition early in the period between bursts has no apparent effect, but inhibition later in the silent period makes the subsequent burst longer and more intense (Fig. 2C).

If both neurons of a pair are weakly inhibited, the firing frequency within each burst will decrease while the inhibition lasts (Fig. 2D). Similarly, common excitation raises the firing frequency (*10*).

Pairs of neurons with different intrinsic repetition rates can be entrained by surprisingly simple synaptic connections between the pairs. We find that two sets of neurons can be entrained by a single inhibitory synapse whose IPSP's are one-tenth the size of the reciprocal IPSP's. If the free-running repetition rate of the postsynaptic pair is between 30 percent slower and 18 percent faster than that of the presynaptic pair, it will be entrained. More discrepant pairs require stronger synaptic interaction, more than one coordinating fiber, or both.

Although the membrane properties responsible for PIR are not precisely known (*6*), the phenomenon itself occurs widely and is easily demonstrated and measured. Postinhibitory rebound alone is capable of producing bursts of impulses having a characteristic internal structure; when combined in reciprocally inhibitory networks, neurons having PIR produce patterns of alternating bursts without the necessity for any single neuron to generate the basic repetition rate of the pattern.

Although it is difficult to distinguish experimentally between driving oscillator and network mechanisms, our results suggest that, in particular cases, an experimental search for driving oscillator neurons may be fruitless. In such motor systems, it may be possible to demonstrate reciprocal inhibition of antagonistic neurons together with PIR in the same neurons; these properties constitute a sufficient condition for the production of stable motor patterns.

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Neurophysiol. **25**, 56 (1962)] showed PIR in an overstretched crayfish stretch receptor. E. R. Kandel and W. A. Spencer [*ibid.* **24**, 243 (1961)] demonstrated PIR following synaptic inhibition in hippocampal neurons of cats, and E. R. Kandel, W. T. Frazier, and H. Wachtel [*ibid.* **32**, 496 (1969)] showed similar bursts in *Aplysia* neurons. Postinhibitory rebound is a synonym for postanodal exaltation (PAE), a term used by P. Anderson and J. C. Eccles [*Nature (Lond.)* **196**, 645 (1962)], and is often treated as a synonym for the anodal break response analyzed in squid axons by A. L. Hodgkin and A. F. Huxley [*J. Physiol. (Lond.)* **116**, 497 (1952)]. Cellular mechanisms and early work are reviewed by T. Narahashi [*J. Cell. Comp. Physiol.* **64**, 73 (1964)]. P. G. Sokolove [*Biophys. J.* **12**, 1429 (1972)] discusses a possible mechanism for PIR in tonically active cells.

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9. M. V. S. Siegler, G. J. Mpitsos, W. J. Davis, *J. Neurophysiol.*, in press; figures 15, 16, and 17 illustrate PIR phenomena in a marine mollusk. Postinhibitory rebound in crustacean ganglia is shown by B. Mulloney and A. I. Selverston (*J. Comp. Physiol.*, in press) and A. I. Selverston and B. Mulloney (*ibid.*, in press). We emphasize that the results reported here do not depend on the detailed assumptions or implied mechanisms of the model; they follow essentially from the phenomenon of burst production following cessation of synaptic inhibition, as shown in Fig. 1.
10. Note the paradoxical effect of inhibition in increasing the rate of alternation.
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Brain Catechol Synthesis: Control by Brain Tyrosine Concentration

Abstract. *Brain catechol synthesis was estimated by measuring the rate at which brain dopa levels rose following decarboxylase inhibition. Dopa accumulation was accelerated by tyrosine administration, and decreased by treatments that lowered brain tyrosine concentrations (for example, intraperitoneal tryptophan, leucine, or parachlorophenylalanine). A low dose of phenylalanine elevated brain tyrosine without accelerating dopa synthesis. Our findings raise the possibility that nutritional and endocrine factors might influence brain catecholamine synthesis by controlling the availability of tyrosine.*

The rate at which the rat brain synthesizes serotonin varies with its tryptophan concentration (*1*); this, in turn, depends upon the ratio of the plasma tryptophan concentration to the sum of the concentrations of other neutral amino acids that compete with tryptophan for transport into the brain (*2*). The administration of insulin to, or the consumption of carbohydrates by, fasting rats increases this plasma ratio, and thereby accelerates brain serotonin synthesis (*2, 3*); in contrast, the consumption of high-protein rat chow elevates neither the plasma ratio nor the concentration of the indoleamine neurotransmitter in the brain (*2*).

There is abundant evidence that treatments that increase the physiological activity of catecholamine-containing cells cause parallel changes in the activity (*4*), and, ultimately, the concentration (*5*) of the enzyme tyrosine hydroxylase. Such evidence supports the concept that the rate-limiting factor controlling brain catecholamine synthesis can be tyrosine hydroxylase activity. We now present evidence that treatments which increase or decrease brain tyrosine concentrations can produce parallel changes in the rate at which the brain synthesizes catechols. Since the published Michaelis constant (K_m) for tyrosine of the tyrosine hydroxylase in brain homogenates [0.14 m*M* for whole rat brain (*6*), and 0.1 m*M* for sheep caudate nuclei (*7*)] appears to be high relative to brain tyrosine concentrations [approximately 0.08 m*M* (Table 1)], our data suggest that brain tyrosine concentration constitutes an additonal factor controlling catechol synthesis.

Brain catechol synthesis was estimated by measuring the accumulation of dopa during the hour after administration of the decarboxylase inhibitor RO4-4602 (*8*). We found that a dose of 800 mg/kg caused brain dopa concentrations to rise linearly for at least 1 hour, from unmeasurably low levels to approximately 250 ng/g. This treatment also elevated brain tyrosine by 20 to 40 percent, but had no significant effect on brain dopamine or norepinephrine concentrations during the interval examined.

Table 1. Effect of tyrosine or tryptophan administration on accumulation of dopa in rat brain. Rats received the decarboxylase inhibitor RO4-4602 (800 mg/kg intraperitoneally) and, after 15 minutes, tyrosine or tryptophan (50 mg/kg, intraperitoneally) or their diluent; they were killed 1 hour after the first injection. Data are given as means ± standard errors.

Treatment	Tyrosine (μg/g)	Dopa (ng/g)	*N*
None	14.7 ± 0.46	250 ± 10	28
Tyrosine	26.6 ± 0.80*	283 ± 10†	20
Tryptophan	12.1 ± 0.40*	170 ± 18*	10

* Differs from control, $P < .001$. † Differs from controls, $P < .05$.

Male rats, weighing 150 to 175 g (Charles River Breeding Laboratories), were given free access to a 26 percent protein diet (Charles River rat and mouse formula) and water and maintained under light (Vita-Lite, Duro-Test Co., North Bergen, N.J., 300 μw/cm^2) between 8:00 a.m. and 8:00 p.m. daily. In most experiments groups of rats were injected intraperitoneally with a solution of RO4-4602 between 10:00 a.m. and 11:00 a.m., and then were given injections of various amino acids or their diluent 15 minutes thereafter. The rats were killed 60 minutes after the first injection. Brains were weighed and assayed for tyrosine (*9*), dopa (*10*), and, in some cases, dopamine (*11*) and norepinephrine (*12*). Dopa concentrations were corrected for column recoveries averaging 72 percent.

The administration of a low dose (50 mg/kg) of tyrosine caused, after 45 minutes, an 81 percent increase in brain tyrosine and a 13 percent increase in the accumulation of dopa ($P < .05$) (Table 1). The same dose of tryptophan caused an 18 percent fall in brain tyrosine and a 32 percent decrease in dopa accumulation ($P < .001$). The failure of brain dopa accumulation to rise or fall as a linear function of tyrosine concentration after the administration of tyrosine or tryptophan suggests that the effects of one or both of these treatments on tyrosine hydroxylation involved more than simply changing the brain tyrosine concentration (for example, tryptophan might have inhibited tyrosine hydroxylase).

Brains of rats given leucine (100 mg/kg intraperitoneally), another neutral amino acid believed to be in the same transport group as tyrosine (*13*) contained significantly less tyrosine and dopa than the controls (Table 2); in contrast, the administration of similar doses of histidine, alanine, or lysine affected neither brain tyrosine nor the accumulation of brain dopa (Table 2).