Effects of Ion Channel Activity on Development of Dorsal Root Ganglion Neurons

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ABSTRACT: Studies of mouse dorsal root ganglion neurons *in vitro* demonstrate that ion channel function and regulation can influence a wide range of developmental processes. The work suggests that much as exposure to different trophic factors, the pattern of impulse activity a neuron experiences can have significant structural and functional effects during development. Studies concerning effects of ion channel activity on growth cone motility, axon fasciculation, synaptic plasticity, myelination, and intracellular signaling pathways regulating gene expression are presented in the context of changes in endogenous firing patterns during development. J Neurobiol 37: 158–170, 1998

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A wide range of processes are influenced by impulse activity during development, including neuronal phenotype, neurite outgrowth, cell–cell adhesion, axon fasciculation, pathfinding, axon–glia associations, myelination, synapse remodeling, and neuronal death. These activities encompass some of the major processes of nervous system development, and this implies that neural activity can interdigitate with regulatory mechanisms controlling gene expression, cytoskeletal dynamics, and signal transduction.

Ion channel regulation, through changes in expression or channel kinetics or conductance, can have a profound effect on neuronal excitability and spontaneous or evoked activity. Such changes in firing patterns provide instructive information to the developing neuron regarding the developmental or functional state of the cell. However, the mechanism by which temporally varying membrane depolarizations are transduced and integrated within the neuron to control gene expression and other neuronal responses is not well understood. This review illustrates some of the ways in which action potentials influence key developmental processes in mouse dorsal root ganglion (DRG) neurons in culture, and places the effects into context with what is known of the patterns of impulse activity in these neurons *in utero*. Finally, it addresses the mechanisms that regulate intracellular signaling according to temporal features of membrane depolarization.

SPONTANEOUS ACTIVITY DURING DEVELOPMENT OF DRG NEURONS

Detailed information on patterns of spontaneous activity during embryonic development, especially of mammalian organisms, is limited because of the technical difficulty of performing electrophysiological recordings on fetal animals. The information available suggests that early in development spontaneous activity is slow and characterized by long interburst intervals (Fitzgerald 1987; Meister et al., 1991; Gu and Spitzer 1995). Later in development as afferent and efferent circuits become functional, the pattern of firing becomes more vigorous and

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phasic as it is modulated by sensory evoked and synaptic activity.

Dorsal root ganglion neurons are not spontaneously active in the postnatal or adult periods, but they are spontaneously active during later phases of development (Fitzgerald, 1987). Spontaneous impulse activity begins when the axon terminals reach the periphery, even before they form functional end organs or synaptic connections with the central nervous system (CNS). Initially, the frequency of impulse activity is low (<0.5 Hz), but the rate of firing increases with later stages of development [1–10 Hz at embryonic day 19 (E19)] (Fitzgerald and Fulton, 1992). After sensory end-organs differentiate and neurons form central synapses, impulse activity changes to higher frequency and stimulus evoked bursts (10–20 Hz). Thus, impulse activity in DRG neurons falls into three phases: (a) a preimpulse activity period, (b) a low-frequency spontaneous activity period, and (c) a final phase of high-frequency, phasic and stimulus evoked firing. These three phases parallel transitions in major developmental events.

PRE-IMPULSE ACTIVITY PERIOD: A PHASE OF NEURITE OUTGROWTH

The onset of spontaneous electrical activity may represent a significant milestone in nervous system development, marking the transformation of a neurite outgrowth phase into a synaptogenesis and neurotransmission phase. Prior to development of electrical excitability, DRG neurons are undergoing a period of neurite outgrowth (Fitzgerald and Fulton, 1992). Consistent with outgrowth during this preimpulse phase, time-lapse microscopy has shown that action potentials induced by electrical stimulation of mouse DRG neurons in culture cause rapid collapse of growth cones (Fields et al., 1990).

The frequency of impulse activity is an important variable in the effects of action potential activity on growth cone motility. Growth cones of DRG neurons can tolerate very low-frequency impulse activity, but the inhibitory effects increase directly with the frequency of firing (between 2.5 and 10 Hz) (Fields et al., 1990). Phasic stimulation is more effective at inducing growth cone collapse than stimulation with the same number of impulses delivered at constant frequency. The increased efficacy of higher frequency and phasic activity patterns in inhibiting growth cone motility in culture correlate with neurite outgrowth during developmental periods accompanied by low-frequency or no spontaneous activity.

Inhibition of DRG growth cone motility by action potentials requires activation of voltage-sensitive sodium channels, and calcium influx through voltage-sensitive calcium channels. Inhibition of growth cone motility as a result of calcium influx appears to be a rather general phenomenon. Many types of stimuli that activate calcium influx through voltage- or ligand-gated ion channels will induce growth cone collapse in a wide variety of neurons (Cohan et al., 1987; Mattson and Kater, 1987; Fields and Nelson, 1994), including invertebrate neurons (Cohan and Kater, 1986).

PERIOD OF SPONTANEOUS ACTIVITY: MODIFICATION OF ION CHANNELS, GENE EXPRESSION, AND DEVELOPMENT OF SYNAPTIC FUNCTION

After DRG neurons are stimulated to fire action potentials for more than 24 h, growth cones regenerate and neurite outgrowth continues at normal rates in culture (Fields et al., 1990). The mechanism responsible for accommodation of growth cones to the inhibitory effects of electrical stimulation involves changes in expression of voltage-sensitive calcium channels.

Whole-cell patch recording in mouse DRG neurons reveals pronounced and prolonged down-regulation of voltage-sensitive calcium currents in neurons stimulated for 24 h or more, but the different types of calcium currents are affected differently (Li et al., 1996). LVA currents are rapidly (<24 hrs) diminished by >80%, and they recover completely by 120 h poststimulation. The HVA currents are suppressed 50%, and they recoversubstantially within 24 h poststimulation. This decrease in conductance is paralleled by a decrease in the number of calcium channels in the membrane, as shown by radioactive binding assay with the L-type calcium channel antagonist PN-200.

The time course of calcium channel down-regulation parallels the time course of changes in growth cone motility during continuous stimulation (Li et al., 1996; Fields et al., 1993). Although cytoplasmic calcium can reach equally high levels in collapsing and accommodated growth cones during action potential stimulation, the slower rate of calcium increase in neurons after chronic electrical stimulation is compatible with growth cone motility (Fields et al., 1993). The biological significance of this change in growth

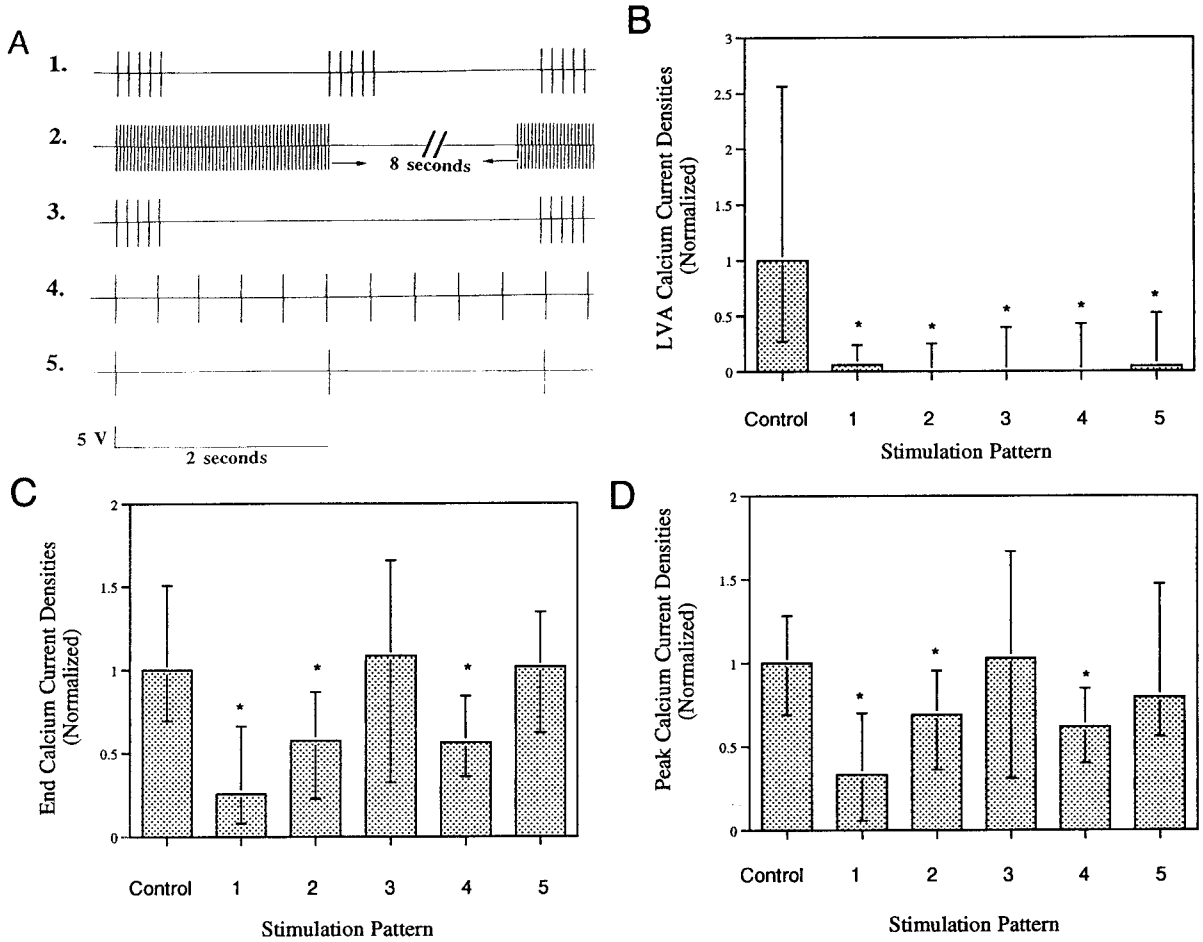

Figure 1 Different patterns of impulse activity modulate distinct calcium currents differently. (**A**) The effects of chronic stimulation with five different patterns were compared. These consisted of: (**1**) 10-Hz stimulus bursts of 0.5 s delivered every 2 s, (**2**) 30-Hz stimulation for 2 s delivered every 10 s, (**3**) 10-Hz stimulus bursts of 0.5 s delivered every 4 s, (**4**) 2.5-Hz stimulation, (**5**) 0.5-Hz stimulation. (**B**) Most patterns of stimulation produced a long-term reduction in the LVA current, including single-action potentials delivered at 2-s intervals (**5**). The end (**C**) and peak (**D**) HVA currents were not affected by 0.5 Hz stimulation (**5**), but 2.5 Hz stimulation did produce a significant decrease (**4**). Phasic stimulation with an average frequency of 2.5 Hz (**1**) produced more down regulation than tonic stimulation at the same average frequency (**4**). The efficacy of the phasic stimulation varied inversely with the interval between bursts (**1** vs. **3**). The activity-dependent reduction in calcium current was associated with reduced numbers of calcium channels, and resulted in slower increases in intracellular calcium in response to electrical depolarization. (From Li et al., *J. Neurophysiol.* **76:**2595–2607, 1996.)

cone sensitivity to action potentials may be that accommodation permits outgrowth necessary for sprouting, synaptic remodeling, or regeneration after the period of target recognition and synaptogenesis.

Different patterns of activation have different effects on the various calcium currents (Fig. 1). This suggests the possibility for more subtle regulation of ion channel expression and concomitant functional effects by the pattern of impulse activity in developing neural circuits. Tonic stimulation at frequencies as low as 0.5 Hz down-regulate the LVA current in DRG neurons, but HVA currents are not affected. HVA currents are reduced significantly by 2.5 Hz stimulation, but phasic stimulation with the same average frequency produces more down-regulation than tonic 2.5 Hz stimulation. The efficacy of phasic stimulation varies inversely with the interval between bursts. For example, phasic stimulation

with 0.5-s bursts (at 10 Hz) repeated at 4-s intervals has no effect, but the same stimulus repeated at 2-s intervals down-regulates the HVA current. Similar activity-dependent reductions in calcium currents in other neurons have been described using chronic membrane depolarization with elevated extracellular potassium (Delorme and McGee 1986; Delorme et al., 1988; Franklin et al., 1992; Garcia et al., 1994) or electrical stimulation *in situ* (Hong and Lnenicka, 1995). Brief chemical depolarization at daily intervals increases HVA currents in cultured hippocampal neurons without affecting LVA currents (Garcia et al., 1994).

The different effects of various impulse patterns on distinct calcium currents suggests another way in which firing pattern can have selective effects on specific neuronal responses. The comparative insensitivity of the regulatory mechanism controlling HVA calcium currents to low-frequency stimulation and stimulus bursts separated by longer intervals may be consistent with the functional role of calcium current through L-type calcium channels in activity-dependent development. The low-frequency spontaneous action potential firing characteristic of early phases of development (Fitzgerald, 1987; Meister et al., 1991) would have minimal effect on expression of L-type channels. These currents may be particularly important in linking such processes as neuronal outgrowth, selective gene expression (Thompson et al., 1995; Fields, 1996), and neuronal differentiation (Brosenitsch et al., 1998) to the functional activity of the neuron. In contrast, the sensitivity of LVA currents to a wide range of neural impulse patterns implies that impulse activity in general tends to lower the calcium current through these channels. Even very low-frequency stimulation at 0.5 Hz can cause sizable long-term suppression of the LVA current. Activity-dependent down-regulation of LVA currents may have greater relevance to synaptic functions or control of repetitive firing in neurons.

Target-derived and developmentally programmed changes are important in triggering the transition from an outgrowth phase to a synaptogenesis phase, but electrical excitability seems to be an important factor in this progression. DRG neurons become electrically active after contact with target structures, and many neurons including motoneurons begin to secrete transmitter rapidly after contact with their target (Greensmith and Vrbova, 1996). The change from a growing phase to a transmitting phase not only involves changes in growth cone responses, but is accompanied by a wave of new gene expression affecting cytoskeletal dynamics, ion channels, and proteins associated with synaptic transmission. Transmembrane ion fluxes resulting from depolarization could have a role in triggering these changes in gene expression. Indeed, the changes in expression of ion channels in electrically active neurons may be a necessary adaptation for functional activity of neurons after synaptogenesis. Greensmith and Vrbova (1996) suggested that the target confers upon the motoneurone the ability to cope with the levels of excitation that exist within the mature CNS. They theorized that motoneurons that fail to make this transformation from a growing phase into a transmitting phase within a critical period of development may remain immature and unable to cope with the demands placed upon them within the electrically active mature CNS. Ion channels not only help signal this transition through their activity, but they participate in the transition through activity-dependent changes in their expression or physiological properties. In addition to inhibiting neurite outgrowth, the commencement of ion channel activity can trigger a new wave of gene expression influencing neuronal survival (Choi, 1987; Koike Martin and Johnson, 1989) and differentiation (Ip and Zigmond, 1984; Hodaie et al., 1995).

PERIOD OF STIMULUS-EVOKED AFFERENT ACTIVITY: A PHASE OF SYNAPTIC REMODELING

Connections between DRG neurons and motor neurons are largely genetically specified, but in vertebrates the interplay between genetically predetermined and experientially regulated influences on nervous system development is essential for normal development of many brain structures and functions. Studies of associative learning and conditioning inspired the theory that the coincidence of activation between an afferent and sufficient activation of the postsynaptic neuron provided the logical condition for initiating an increase in the efficacy of the connection (Hebb, 1949). Dissonant firing of an afferent and the postsynaptic neuron should promote weakening of the afferent (Stent, 1973).

Consistent with these theories, studies on an *in vitro* preparation of mouse DRG neurons projecting to ventral spinal cord neurons revealed that stimulated afferents become strengthened and unstimulated afferents on the same postsynaptic target neuron become weakened (Nelson et al., 1989). This activity-dependent mechanism could be an important factor in remodeling synaptic connections according to appropriate functional activity.

The molecular mechanism for the activity-dependent changes in synaptic strength are not well understood, but transmembrane flux of calcium and activation of the *N*-methyl-D-aspartate (NMDA) channel have important roles. Exposure to amino-5-phosphonovaleric acid (APV), an antagonist of the NMDA channel, prevents the competitive advantage associated with electrical stimulation in the cell culture preparation of spinal cord and DRG neurons (Fields et al., 1991). NMDA channel activation was first implicated in developmental processes of synaptic restructuring in the visual system (Cline et al., 1987; Kleinschmidt et al., 1987; Cline and Constantine-Paton 1989; Gu et al., 1989; Scherer and Udin, 1989). However, APV also suppresses spontaneous impulse activity, which could be important in allowing the postsynaptic neuron to reach the level of ''sufficient activation'' proposed by Hebb (1949). Indeed, when postsynaptic activity is suppressed in an *in vitro* preparation of spinal cord neurons by methods that do not directly block NMDA receptor function, the activity-dependent change in synaptic strength is also blocked (Fields et al., 1991). This blockade does not affect the coincident activation of converging afferents, suggesting that triggering the process that strengthens the coincidentally active afferent requires more than activation of the NMDA receptor. Something associated with the general level of postsynaptic membrane depolarization must also have an important influence in triggering the cell biological processes that lead to changes in synaptic strength. Postsynaptic calcium influx is one of these factors, because plasticity can be restored in the presence of APV simply by increasing the concentration of extracellular calcium. This suggests that activity-dependent plasticity of DRG neurons on spinal neurons is mediated through a mechanism interacting with intracellular calcium via calcium influx through NMDA and non-NMDA channels.

ACTIVITY-DEPENDENT REGULATION OF CELL-ADHESION MOLECULES (CAMS): AN IMPORTANT MOLECULAR MECHANISM REGULATING STRUCTURE AND FUNCTION THROUGHOUT DEVELOPMENT

Cell adhesion molecule expression is highly regulated during nervous system development to control cell migration, neurite outgrowth, fasciculation, and synaptogenesis. Recent work has shown that expression of different CAMs is regulated by distinct patterns of neural impulses *in vitro*, and the results are consistent with changes in firing pattern accompanying DRG development and cell biological functions involving specific CAMs. These cell-surface proteins appear to be key molecules in linking changes in membrane depolarization to a wide range of developmental processes controlling nervous system structure and function (Fields and Itoh, 1996).

The calcium-dependent CAM cadherin promotes initiation of neurites, neurite outgrowth, and growth cone motility (Riehl et al., 1996). N-cadherin is down-regulated during periods of cell migration (Nakagawa and Takeichi, 1995; Barami et al., 1994) and myelination (Cifuentes-Diaz et al., 1994; Shibuya et al., 1995; Scherer, 1996). N-cadherin is a major component of the synaptic complex in central (Beesley et al., 1995) and peripheral synapses (Cifuentez-Diaz et al., 1994). It has been proposed that the distribution of specific cadherins at different CNS junctions may promote synaptogenesis with appropriate targets expressing complementary cadherins (Fannon and Coleman, 1996). Furthermore, the subcellular localization of cadherins bordering the active zones at some CNS synapses suggests possible involvement in structural/functional modifications of synaptic junctions (Fannon and Coleman, 1996).

Recent research shows that electrical impulse activity can regulate expression of N-cadherin in DRG neurons and myotubes. The normal developmentally regulated decrease in N-cadherin in developing myotubes (Hahn and Covault, 1992) can be prevented by blocking activity with *d*-tubocurare in cell culture (Fredette et al., 1993), suggesting that development of impulse activity could trigger down-regulation of N-cadherin expression in muscle. Electrical stimulation of mouse DRG neurons in culture results in rapid down-regulation of N-cadherin mRNA, and the magnitude of the reduction is directly proportional to the stimulus frequency (Itoh et al., 1997). Remarkably low frequencies are effective in down-regulating N-cadherin expression in DRG neurons (0.1 Hz). This suggests that the functional significance of high levels of N-cadherin expression must pertain to early developmental events, prior to the onset of spontaneous activity.

Members of the immunoglobulin (Ig) superfamily of membrane glycoproteins comprise a large family of developmentally important cell adhesion molecules that are involved in neurite outgrowth (Appel et al., 1995), axon pathfinding (Sretavan et al., 1994; Honig and Rutishauser, 1996), fasciculation (Magyar-Lehmann et al., 1995), myelination

(Seilheimer et al., 1989; Wood et al., 1990), synaptogenesis (Jorgensen, 1995), and synaptic plasticity in vertebrates (Luthi et al., 1995; Muller et al., 1996) and invertebrates (Peter et al., 1994; Davis et al., 1996; Schuster et al., 1996a,b). Chemical depolarization with KCl or neurotransmitters can increase expression of the cell adhesion molecule L1 in N2a or cerebellar cells in culture, without affecting another member of the Ig superfamily, NCAM (Scherer et al., 1992). In *Aplysia,* the invertebrate homologue of NCAM, apCAM, also undergoes decreased expression after exposure to neurotransmitter (Mayford et al., 1992). Studies of DRG neurons *in vitro* have shown that electrical stimulation causes down-regulation of the CAM (Itoh et al., 1995), but this effect depends upon stimulation at an appropriate frequency. Stimulation at 0.1 Hz down-regulates L1 mRNA and protein significantly, but higher-frequency stimulation (1 Hz) or unstimulated axons show high levels of L1 expression. Expression of NCAM is not altered significantly by these frequencies of stimulation. The activity-dependent changes in N-cadherin and L1 in DRG neurons are associated with changes in major developmental processes known to be influenced by these molecules (Fig. 2).

Fasciculation and defasciculation are critical processes in axon pathfinding and synaptogenesis, and there is substantial evidence that reduced L1 expression promotes defasciculation. This can in turn promote synaptogenesis and facilitate changes in direction of neurite outgrowth at critical anatomical loci. Inhibition of L1 function partially defasciculates nerve roots and enhances the growth of axons over myotubes (Landmesser et al., 1988; Dahm and Landmesser, 1988). Stimulation at 0.1 Hz causes marked defasciculation of mouse DRG neurites in culture, but stimulation at 1 Hz, a frequency of activity that does not alter L1 expression, has no effect on neurite fasciculation (Itoh et al., 1995).

Depolarization that decreases expression of apCAM in *Aplysia* is also associated with defasciculation, which is thought to promote synaptogenesis in association with facilitation of the gill withdrawal reflex (Mayford et al., 1992). If expression of the NCAM homologue in *Drosophila* (fasciclin II) is increased by genetic manipulation, axons of the RP3 motor neuron fail to defasciculate and leave the common motor pathway at a crucial choice point to innervate their appropriate muscles (Lin and Goodman, 1995). Mutants characterized by hyperactivity show a decrease in presynaptic expression of fasciclin II and a concomitant increase in presynaptic sprouting (Davis et al., 1996).

Reduction of L1 and N-cadherin mRNA by low-frequency action potential firing are compatible with the transition from a neurite outgrowth phase to a synaptogenesis phase, which follows the onset of spontaneous activity in these neurons *in vivo.* The results of different frequencies of electrical stimulation of mouse DRG neurons in cell culture suggest that L1 and N-cadherin levels would be relatively high prior to development of spontaneous electrical activity in DRG neurons *in vivo,* but would lower soon after axons become spontaneously active. N-cadherin and L1 promote outgrowth of neurites from several types of neurons (Lemmon et al., 1992). Relatively high expression of these two CAMs prior to spontaneous impulse activity would promote neurite outgrowth and fasciculation. Studies *in vitro* and correlations with development suggest that target-derived factors are important in inducing the onset of spontaneous activity in DRG neurons. Electrical excitability of DRG neurons is markedly increased by exposure of neurons to conditioned medium derived from muscle cultures (Chen et al., 1987). Low-frequency spontaneous activity starts at about the time peripheral endings reach the subepidermis (Reynolds et al., 1991; Fitzgerald, 1987) and this coincides with defasciculation of the terminals to form a diffuse subepidermal plexus (about E17 in rat) (Fitzgerald and Fulton, 1992). Central DRG terminals, which had reached the spinal cord by E12, finally begin to extend collaterals to the gray matter (E15) (Ziskind-Conhaim, 1990; Fitzgerald, 1991). Defasciculation of peripheral terminals and penetration of central axons into the spinal cord may be promoted by down-regulation of L1 and N-cadherin induced by the low-frequency spontaneous activity. Spontaneous activity in DRG neurons declines in later stages of development as high-frequency, sensory-evoked activity develops (Fitzgerald and Fulton, 1992). This period is accompanied by the formation of peripheral sensory end organs (E14–19), consolidation of the subepidermal plexus of DRG terminals (E17 to birth) (Reynolds et al., 1991), and synaptogenesis of the central terminals of DRG neurons with dorsal horn and motoneurons (beginning about E17) (Kudo and Yamada, 1987; Ziskind-Conhaim, 1990) (Fig. 2).

Myelination begins in the latest stages of DRG development and continues into the postnatal period. The involvement of impulse activity in myelination by Schwann cells has not been addressed, but research in the CNS suggests that impulse activity could be an important factor in myelination. However, the results of experiments on this question are open to various interpretations, and the molecular mechanisms are un-

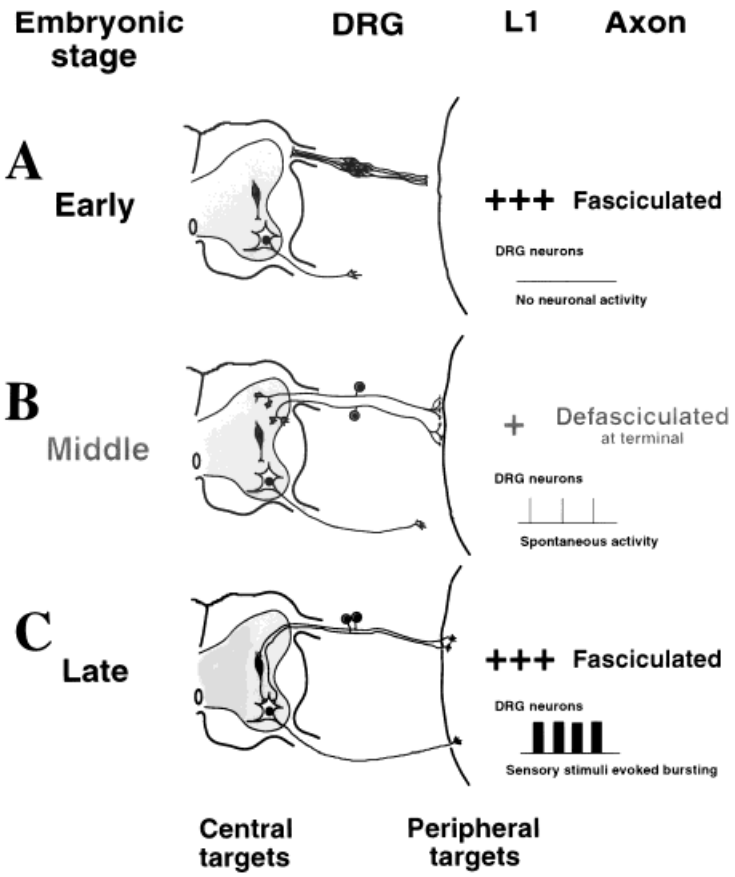

Figure 2 Changes in spontaneous impulse activity correlate with major transitions in DRG development, which are compatible with the changes in expression of L1 and defasciculation induced by comparable patterns of impulse activity *in vitro* (Itoh et al., 1995). (**A**) During the period of neurite outgrowth DRG neurons are electrically inexcitable. Under these conditions, *in vitro* levels of L1 and N-cadherin are high, which would promote axon outgrowth and fasciculation. (**B**) Spontaneous impulse activity begins when terminals reach the subepidermis and axon terminals defasciculate. Low-frequency stimulation *in vitro* down-regulates L1 (Itoh et al., 1995) and N-cadherin (Itoh et al., 1997) expression, and causes axon defasciculation. (**C**) Later in development, after the formation of central synapses and sensory end organs, spontaneous impulse activity ceases and is replaced with high-frequency, phasic impulse activity. During this phase, axon terminals refasciculate and myelination begins. *In vitro,* stimulation at higher frequencies down-regulates N-cadherin but not L1 expression, and produces no effects on fasciculation or myelination. [Modified from Fitzgerald and Fulton (1992).]

known. Early studies showed that mice reared in the dark develop fewer myelinated axons in the optic nerve compared with normally reared mice (Gyllensten et al., 1963), and early eye opening increases the level of myelin protein expression in the optic nerve (Tauber et al., 1980). In a recent study, intraocular injections of tetrodotoxin (TTX) during a narrow developmental time frame resulted in inhibited myelination in the optic nerve of mice (Demerens, 1996). Incubation of cell cultures, derived from mouse cerebral hemispheres, in TTX or high concentrations of KCl reduced myelination *in vitro* (Demerens, 1996). A complementary increase in myelination was produced by incubating cultures in alpha-scorpion toxin, which increases repetitive firing by slowing sodium channel inactivation. Demerens et al., (1996, p. 9887) concluded that ''it is the action potential itself which is responsible for the onset of myelination.''

In contrast to these studies, intraocular injections of TTX over a similar developmental period showed no effect on myelination in another study (Colello et al., 1995). This is consistent with results of experiments on mouse spinal cord explants, which undergo normal oligodendrocyte development and

myelination in the presence of TTX (Shrager and Novakovic, 1995). It has been suggested that activity blockade may alter myelination as a result of changes in the number of oligodendrocytes rather than as a direct effect on initiation of myelination (Colello et al., 1995), because proliferation of oligodendrocyte progenitor cells is influenced by the activity-dependent release of platelet-derived growth factor (PDGF) from astrocytes in optic nerve (Barres and Raff, 1993). Second, ion channels in oligodendrocytes can influence proliferation and myelination, and glial channels may be affected by pharmacological treatments. For example, inhibition of potassium channels (presumed to be on glia) by incubation in tetraethylammonium ion (TEA) can eliminate myelination in spinal cord explants without altering axonal conduction (Shrager and Novakovic, 1995).

Although Schwann cells are closely associated with DRG axons when they are firing at low frequency, myelination does not begin until the prenatal period when the firing pattern increases to higher frequency (Fitzgerald and Fulton, 1992). In recent studies, we tested whether myelination would be inhibited by a frequency of axonal firing characteristic of firing patterns prior to the onset of myelination. These studies showed that myelination was inhibited significantly on axons firing at 0.1 Hz, compared with axons firing at 1 Hz or unstimulated (Stevens et al., 1996, 1997). Antibodies against L1 have been shown to block the initiation step in myelination of DRG axons by Schwann cells in culture (Seilheimer et al., 1989; Wood et al., 1990). The results suggest that myelination is inhibited by the reduction in axonal L1 expression produced by low frequency impulse activity characteristic of the rates of spontaneous activity in the premyelinating phase of development.

These results showing genes expressing different CAMs are regulated in response to different patterns of action potentials suggest that neural impulse activity may represent one cue, working in combination with other intrinsic and extrinsic signals, to influence neurite outgrowth, fasciculation, synaptogenesis, and myelination during development. How expression of different genes could be regulated by specific temporal patterns of action potential firing is an intriguing question.

CONTROL OF GENE EXPRESSION BY TEMPORALLY VARYING MEMBRANE DEPOLARIZATION

There is considerable information on the signal transduction pathways controlling gene transcription in response to activation of membrane receptors by extracellular ligands, but it is not clear how different temporal patterns of membrane depolarization could regulate specific genes. This is a particularly important problem in the nervous system where information is coded in the temporal pattern of impulse activity. The concentration of second messengers generated by different patterns of impulse activity together with differences in activation thresholds for calcium-dependent signaling reactions are important factors in controlling cellular responses to stimulation (Ghosh and Greenberg, 1995; Clapham, 1995). Second, subcellular heterogeneity in the concentration dynamics of second messengers or their substrates is thought to contribute to stimulus-response specificity (Bading et al., 1993; Bootman and Beridge, 1995). However, the temporal dynamics of the second messenger calcium and integration by downstream intracellular signaling components also contribute to specific activation of appropriate signaling pathways to activate genes in response to appropriate patterns of neuronal firing (Fields et al., 1997).

Studies combining imaging of intracellular calcium concentration in DRG neurons with reverse transcriptase–polymerase chain reaction (RT-PCR) measurements of the expression of IE genes evoked by electrical stimulation have shown that under dynamic conditions, i.e., outside stimulus conditions that produce saturating or steady-state levels of intracellular messengers, the interval of time between bursts of calcium can be more important than the amplitude of the calcium increase (Sheng et al., 1993). These studies show that transcription of the c-*fos* gene is not dependent on a sustained increase in intracellular calcium, because transcription of this gene can be activated by many types of action potential patterns that do not result in a residual calcium increase. Conversely, a large increase in intracellular calcium is not necessary for gene transcription, because even the smallest possible increase in calcium levels, that produced by a single action potential, can activate gene expression if presented at appropriate intervals (10 s). This stimulus produces a minimal (about 20 n*M*) transient increase in cytoplasmic calcium. Finally, a large increase in intracellular calcium does not stimulate gene transcription if it is presented at intervals that are too long: 2 min between 12 action potential bursts (Sheng et al., 1993) or 5 min between 90 action potential bursts (Fields et al., 1997). Thus, the temporal features of calcium influx are critical in the mechanism regulating gene transcription (Fields and Nelson, 1994).

Further support for the conclusion that temporal differences, rather than calcium concentration differences, can be important in controlling gene expression derives from the observation that remarkably low frequencies of stimuli can be effective in regulating expression of many different genes and ion channels in DRG neurons in culture. The t-type calcium current in DRG neurons can be decreased by stimulus frequency as low as 0.5 Hz (Li et al., 1996). Expression of c-*fos* (Sheng et al., 1993) and the CAM L1 (Itoh et al., 1995) and N-cadherin (Itoh et al., 1997) are regulated by single action potential stimulation delivered at 10-s intervals, a stimulus that would cause minimal change in the concentration of intracellular signaling molecules.

The importance of the interval between bursts may be relevant to activity-dependent regulation of genes during development of the nervous system. In developing *Xenopus* spinal neurons, differences in the frequency of spontaneous calcium transients correlate with discrete effects of impulse activity on development (Gu and Spitzer, 1995). Calcium transients at a frequency of 6–9/h inhibit neurite outgrowth, and calcium transients at a frequency of $\geq$2/h or greater are required for maturation of potassium channels and the expression of GABA. No relation is evident between these developmental processes and the peak calcium concentration, duration or time integral of the calcium transients. In *Drosophila,* multiple training sessions with a rest interval between them (spaced training) produce stronger, longer-lasting memory than the same number of training sessions with no rest interval (massed training) (Yin et al., 1995).

Recent experiments have shown that two intracellular pathways converging on the promoter region of the c-*fos* gene can be differentially activated by different patterns of action potentials. By virtue of differences in their activation and inactivation kinetics, the transcription factor cAMP-responsive element binding protein (CREB) and mitogen-activated protein kinases (MAPK) pathways are activated by distinct patterns of action potentials (Fields et al., 1997). Levels of CREB phosphoryated at Ser-133 increase rapidly in response to brief action potential stimulation but remain at high levels several minutes after an action potential burst (Bito et al., 1996; Fields et al., 1997). These kinetics limit the fidelity with which this transcription factor can discriminate bursts of action potentials repeated at short intervals. The MAPK pathway is also stimulated by action potentials of appropriate temporal patterns in DRG neurons, but the effective patterns are quite different. Bursts of action potentials separated by long intervals (5 min) do not activate the MAPK pathway effectively, but will increase CREB phosphorylation. This is a consequence of the more rapid dephosphorylation rate of MAPK in comparison to CREB. High expression of *c-fos* is dependent on the combined activation of the MAPK pathway and phosphorylation of CREB. For this reason, bursts of action potentials repeated at intervals longer than the rate at which MAPK becomes inactivated by dephosphorylation (5 min) fail to stimulate transcription of the c-*fos* gene (Fields et al., 1997).

Nonlinearities and complex behavior could result from a convolution of many different signaling and transcriptional processes under dynamic conditions that may be saturated or not activated in steady-state stimulus conditions (Fields, 1996). This could include parallel protein kinases, phosphatases, other transcription factors, coactivators, or transcription factor binding proteins. Different temporal patterns of stimulation might also regulate CREB binding through dimerization with related bZIP repressor proteins (Meyer and Habener, 1993), or through interdependencies between multiple DNA binding proteins (Robertson et al., 1995; Nakajima, 1996; Thompson et al., 1995). Each of these processes may have kinetic features or threshold effects that limit their involvement to certain patterns of stimulation.

CONCLUSIONS

This review places results of experiments on a highly reduced preparation of mouse DRG neurons into context with the normal developmental sequence *in vivo.* The reduced preparation provides the necessary experimental control to isolate specific interactions between patterned ion channel activation and developmental processes such as gene expression, growth cone motility, fasciculation, synaptic plasticity, and myelination. The experimental access has enabled identification of specific molecular processes mediating the interaction between impulse activity and these developmentally relevant functions, including ion channel regulation, calcium fluxes, signal transduction cascades and transcription factors that regulate gene transcription, CAMs, and the involvement of NMDA receptors and proteases in activity-dependent plasticity. The correlations and generalizations stemming from these *in vitro* experiments provide hypotheses regarding biological significance *in vivo,* which must be tested directly.

In general, the results show that the pattern of

impulse activity a neuron experiences can have significant influences on a wide range of developmentally relevant functions. It is well established that structured neural impulse activity is important in regulating synaptic plasticity. Several lines of evidence now indicate that prior to the formation of synapses, spontaneous impulse activity can also have important developmental effects through regulation of gene expression and activation of signaling cascades sensitive to ion fluxes through membrane channels. The transition from an inexcitable to an excitable phase often precedes synaptogenesis, and it appears to mark a critical developmental threshold. Many developmental processes are affected by this change in functional state. The onset of spontaneous activity, when it is stimulated by factors associated with appropriate targets, can signal over large distances the formation of an appropriate functional connection. Alternatively, the onset of spontaneous impulse activity in the natural course of maturation of an excitable membrane also marks the developmental progression from an outgrowth to a neurotransmitting phase, which is accompanied by profound changes in gene expression to support the new functional activities. Some of the changes necessary for this functional phase are incompatible with an outgrowth phase, and changes in the functional activity can reciprocally influence the maturation or development of the neuron. The temporal features of impulse activity contain instructive information that is not lost when the signal is transduced across the membrane from electrical to biochemical states. This information is decoded in part by differences in dynamic properties of the system of intracellular signaling reactions linking ion channel function to appropriate biological responses.

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