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The neuron as a dynamic electrogenic machine: modulation of sodium-channel expression as a basis for functional plasticity in neurons

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Neurons signal each other via regenerative electrical impulses (action potentials) and thus can be thought of as electrogenic machines. Voltage-gated sodium channels produce the depolarizations necessary for action potential activity in most neurons and, in this respect, lie close to the heart of the electrogenic machinery. Although classical neurophysiological doctrine accorded 'the' sodium channel a crucial role in electrogenesis, it is now clear that nearly a dozen genes encode distinct sodium channels with different molecular structures and functional properties, and the majority of these channels are expressed within the mammalian nervous system. The transcription of these sodium-channel genes, and the deployment of the channels that they encode, can change significantly within neurons following various injuries. Moreover, the transcription of these genes and the deployment of various types of sodium channels within neurons of the normal nervous system can change markedly as neurons respond to changing milieus or physiological inputs. As a result of these changes in sodium-channel expression, the membranes of neurons may be retuned so as to alter their transductive and/or encoding properties. Neurons within the normal and injured nervous system can thus function as dynamic electrogenic machines with electro-responsive properties that change not only in response to pathological insults, but also in response to shifting functional needs.

Keywords: action potential; electrogenesis; ion channel; sodium channel; neural plasticity

1. INTRODUCTION

In the many decades since Sherrington (1906) described the central nervous system as an 'enchanted loom', we have learned much about the ways in which the ten billion neurons within the brain and spinal cord communicate with each other, in the aggregate forming a computer that is more complex and flexible than any device produced by man. Each neuron functions relatively independently in integrating incoming information, so as to generate a sequence of regenerative electrical impulses (action potentials) that conveys its message to other neurons. In this regard, neurons can be thought of as electrogenic machines. According to the Sherringtonian view, the richness of function of the enchanted loom rests on the changing and complex patterns of action potentials generated by these neuronal electrogenic machines in response to activity at synapses, some having excitatory effects and others having inhibitory ones, which impinge upon them. The synaptic mechanisms responsible for these excitatory and inhibitory effects have been a topic of detailed study, and we now understand not only the steady-state behaviour of synapses, but also appreciate that synaptic activity is a dynamic processs that can be altered by mechanisms that include both sprouting and pruning, and also facilitation, potentiation and depression. Indeed, synaptic plasticity is increasingly well understood, and is considered to be an important substrate for the adaptive mutability that characterizes the nervous system's actions.

The unique and important capability of neurons to communicate via series of action potentials suggests that, as we think about these cells as electrogenic machines and consider their information-processing capabilities, we must not neglect the molecules necessary for a fundamental aspect of their electrogenicity, the ability to generate action potentials. The pivotal studies of Hodgkin & Huxley (1952) taught us that, in most neurons, voltage-gated sodium channels are responsible for the regenerative depolarization that underlies the action potential. This crucial role of sodium channels is reflected, for example, by their aggregation in high density within the cell membrane at the initial segment of motor neurons, where the axon takes its origin from the neuronal cell body (Waxman & Quick 1978), and is also apparent in the clustering of sodium channels in high densities within the axon membrane, at the nodes of Ranvier, which serve as way-stations for saltatory conduction along myelinated axons (Ritchie & Rogart 1977; Waxman 1977; Shrager 1989). Electrophysiological evidence indicates that, in at least some types of neurons, action potentials are initiated by the sodium channels

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that are clustered at the initial segment and secondarily invade the somatodendritic component of the neuron and the axon trunk (Coombs *et al.* 1957; Fatt 1957; Fuortes *et al.* 1957; Dodge & Cooley 1973).

The heroic epoch of electrophysiology that was ushered in by Hodgkin & Huxley's (1952) discoveries has been followed by an equally enlightening era of molecular neuroscience. Much has been learned, over the past few years, about the molecular structure and function of neuronal sodium channels. Although students of neuroscience have traditionally been taught about 'the' sodium channel, it is now clear that a repertoire of multiple sodium channels with different molecular properties is available for deployment within neurons. Moreover, it is becoming increasingly clear that the expression of sodium channels within some types of neurons is state dependent and dynamic, and that, because sodium channels are essential players in electrogenesis, this molecular plasticity can endow neurons with significant functional plasticity. Although modulation of sodium-channel expression is only beginning to be understood, the available evidence provides hints suggesting that it may represent a widespread motif. This paper reviews current progress in this field and suggests that, as a result of plasticity of sodium-channel expression, neurons can functionally remodel their action-potential-generating apparatus, so that we can think of them as dynamic electrogenic machines.

2. PLASTICITY IN THE LOCALIZATION OF NEURONAL SODIUM CHANNELS

The complex and non-uniform pattern of distribution of sodium channels within the mature neuron is perhaps best exemplified by the myelinated axon where there are sharp gradients of sodium-channel density. Within the axon membrane, the distribution of sodium channels is non-homogeneous. Sodium channels are clustered in high densities ($>10^3\,\mu m^{-2}$) in the axon membrane at the node of Ranvier, where they are required for the generation of action potentials. In contrast, the number of sodium channels falls rapidly in extranodal regions and there is a much lower density of sodium channels ($<25\,\mu m^{-2}$) in the internodal axon membrane, beneath the myelin sheath (Ritchie & Rogart 1977; Waxman 1977; Shrager 1989). Figure 1 demonstrates this non-uniform distribution of sodium channels.

Although sodium channels are deployed within the neuronal membrane in a highly non-uniform manner, with relatively sharp borders between channel-rich and -poor domains, the pattern of sodium-channel distribution is not immutable. It changes markedly, in fact, both during development and in response to some pathological insults. During the development of the myelinated axons, for example, there is a distinct phylogenetic sequence, whereby the premyelinated axon initially displays a uniform membrane structure and, subsequently, clusters of sodium channels develop close to the time when myelin is first laid down (Waxman & Foster 1980; Wiley-Livingston & Ellisman 1980; Waxman *et al.* 1982; Dugandzija-Novakavic *et al.* 1995; Vabnik *et al.* 1996). Later in myelination, the internodal part of the axon membrane also matures. Sodium-channel expression is maintained at a nearly constant level until the formation of compact myelin, which provides capacitative and resistive shielding and is accompanied by a suppression of sodium-channel expression in the underlying internodal axon membrane (Waxman 1987).

The highly differentiated membrane organization of the mature axon is not fixed or invariant. Figure 2 presents an electron micrograph, which shows that the internodal axon membrane retains the capability for significant molecular plasticity. Following loss of the overlying myelin, the formerly internodal axon membrane can reorganize, so as to acquire a density of sodium channels that is much higher than normal (Foster *et al.* 1980). The acquisition of increased numbers of sodium channels within the demyelinated axon membrane has functional significance, since it endows the bared membrane with the capability to conduct action potentials, even in the absence of myelin (Bostock & Sears 1976, 1978; Waxman & Brill 1978). This plasticity of the axon membrane, and the resultant recovery of axon potential conduction in chronically demyelinated axons, appear to contribute to the clinical recovery that occurs, for example, during remissions in disorders such as multiple sclerosis (Waxman 1998).

3. MULTIPLE SODIUM CHANNEL GENES ARE EXPRESSED WITHIN NEURONS

Following the molecular cloning of the first three sodium channels from the brain by Numa and his colleagues (Noda *et al.* 1986*a*), it has become apparent that nearly a dozen, molecularly distinct voltage-gated sodium channels are encoded by different genes within mammals (see, for example, Noda *et al.* 1986*b*; Kayano *et al.* 1988; Auld *et al.* 1988; Suzuki *et al.* 1988; Schaller *et al.* 1995). At least eight putative sodium-channel genes are expressed within neurons. Notably, multiple sodium-channel genes are expressed within even reasonably well-defined groups of neurons. One of the best-studied examples of this is provided by dorsal root ganglion (DRG) neurons (figure 3). These primary sensory neurons express the messenger RNAs (mRNAs) for at least six presumed sodium channels. The sodium-channel mRNAs expressed within DRG neurons include high levels of transcripts for the α-I and Na6 sodium channels, which are also present at high levels within many other neuronal cell types within the central nervous system (Black *et al.* 1996). In addition, DRG neurons express high levels of four additional presumed sodium-channel mRNAs, which are not detectable at significant levels, or are present at only low levels, in other neuronal cell types: (i) PN1–hNE, a sodium channel that is expressed preferentially in DRG neurons (Toledo-Aral *et al.* 1997), produces a fast transient tetrodotoxin (TTX)-sensitive current in response to sudden depolarizations and a persistent current that is evoked by slow depolarizations close to resting potential (Cummins *et al.* 1998); (ii) SNS–PN3, which is expressed preferentially in small DRG and trigeminal neurons, encodes a slowly inactivating TTX-resistant sodium current when expressed in oocytes (Akopian *et al.* 1996; Sangameswaran *et al.* 1996); (iii) NaN, expressed preferentially in C-type and trigeminal neurons, exhibits an amino-acid sequence that, while only 47% similar to SNS–PN3, predicts that it

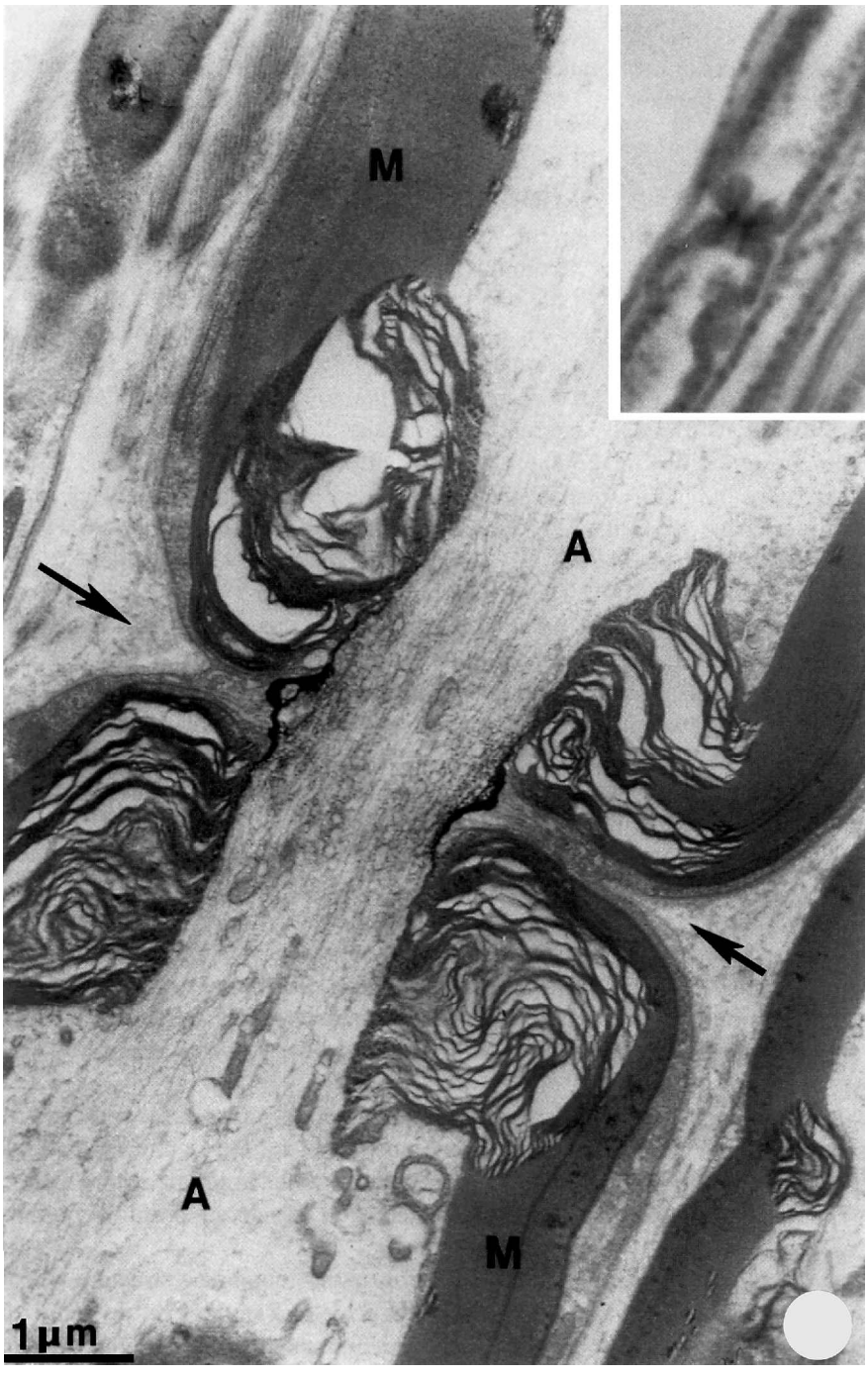

Figure 1. Electron micrograph showing cytochemical staining of a normal myelinated fibre from guinea-pig sciatic nerve with ferric ion and ferrocyanide. There is selective staining of the axon membrane at a node of Ranvier (arrows), while the internodal axon membrane remains unstained. This stain provides a marker for regions of the axon membrane expressing a high density of sodium channels. A, axoplasm; M, myelin; ×16 000. Modified from Quick & Waxman (1976).

encodes a distinct TTX-resistant sodium channel (Dib-Hajj *et al.* 1998*a*), its sequence has been confirmed by Tate *et al.* (1998), patch-clamp studies in transgenic mice in which SNS–PN3 has been knocked out indicate that NaN channels produce a persistent TTX-resistant sodium current with substantial overlap between activation and steady-state inactivation curves, suggesting that it is active near resting potential (Cummins *et al.* 1999); (iv) NaG, originally cloned from astrocytes and at first thought to be a glial cell-specific sodium channel (Gautron *et al.* 1992), is also expressed at high levels within DRG neurons (Black *et al.* 1996) and at low levels within other neurons of neural crest origin but not within other types of neurons (Felts *et al.* 1997*a*), some authorities have noted that NaG mRNA is also present in lung, pituitary and bladder, and encodes relatively few positively charged amino-acid residues in the putative voltage sensor region, and have expressed doubt as to whether NaG functions as a voltage-dependent sodium channel (Akopian *et al.* 1997).

The physiological signatures of only a few of these sodium-channel subtypes within DRG neurons have been determined. Analysis of the function of each of these channels is complicated by results that suggest their currents interact in a complex manner during electrogenesis (see, for example, Rizzo *et al.* 1996; Cummins & Waxman 1997; Schild & Kunze 1997). Nevertheless, there is electrophysiological evidence that indicates that differences in the currents produced by the different types of sodium channels are functionally important. For example, the available evidence from a 'top-down' approach indicates that selective expression of different channel

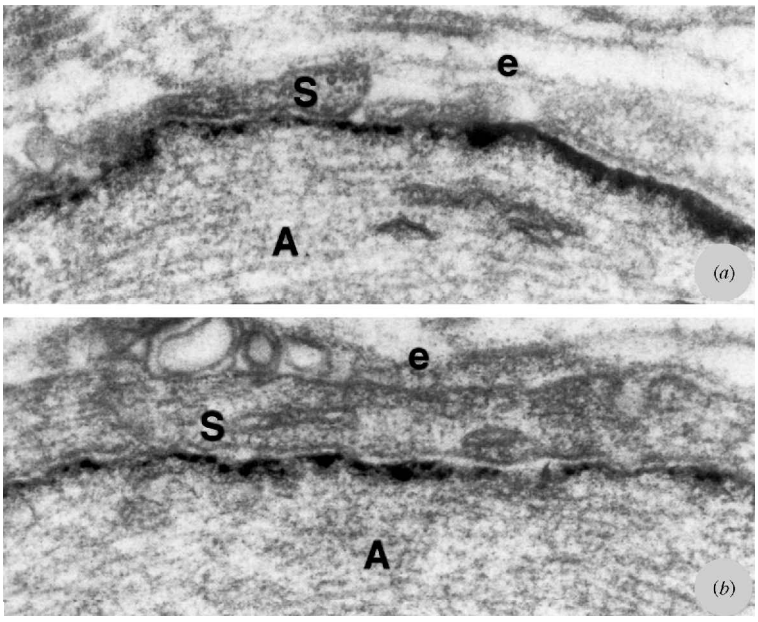

Figure 2. Molecular plasticity of the axon membrane. These micrographs illustrate the acquisition, by chronically demyelinated (formerly internodal) axon membrane, of node-like staining with ferric ion and ferrocyanide (compare to figure 1). The development of higher-than-normal sodium-channel densities in the demyelinated axon membrane permits it to support action potential conduction in the absence of myelin, thus providing a basis for restoration of impulse conduction that contributes to clinical remissions in disorders such as multiple sclerosis. A, demyelinated axon; S, Schwann cell; e, extracellular space; ×65 000. Modified from Foster *et al.* (1980).

subtypes, in groups of DRG neurons with different sensory functions, endows them with different transductive and/or encoding properties. Figure 4 displays whole-cell patch-clamp recordings of sodium current, from a cutaneous afferent DRG neuron and a muscle afferent DRG neuron (Honmou *et al.* 1994). The sodium currents in these two subtypes of DRG neurons differ in terms of kinetics and voltage dependence (figure 4*c*,*d*); this appears to provide at least a partial basis for the different action potential characteristics that are displayed by these cells (figure 4*e*,*f*).

Physiological signatures have also been established by a 'bottom-up' approach for some of the sodium-channel transcripts that are expressed in DRG neurons, such as the PN1–hNE channel. When expressed in mammalian cell lines that lack other ion channels, the properties of PN1–hNE can be examined in isolation. In the expression system provided by HEK 293 cells, PN1–hNE encodes a sodium channel characterized by slow closed-state inactivation. As a result of this, PN1–hNE channels can be activated by slow depolarizations close to resting potential, a property that poises them to amplify depolarizing signals such as generator potentials (Cummins *et al.* 1998). It seems likely that PN1–hNE channels do, in fact, subserve this function within DRG neurons (possibly together with Na6 channels, which, as described below, can also activate in response to slow, small depolarizations; Vega-Saenz DeMiera *et al.* 1997; Raman *et al.* 1997; Tanaka *et al.* 1999). PN1–hNE channels are localized at the distal tips of neurites arising from sensory neurons *in vitro* (Toledo-Aral *et al.* 1997). Although their localization *in situ* has not yet been determined, a distal localization at the sensory terminals would place PN1–hNE channels close to the trigger zones which produce trains of action potentials in response to generator potentials. Such a spatial localization, close to sensory transduction zones, would meet functional needs since it would take advantage of the tuning of these channels, to amplify slow depolarizing inputs (Cummins *et al.* 1998).

There are four putative sodium-channel transcripts that appear to be selectively distributed in DRG neurons. The presence of neuronal type-specific sodium channels may not be unique to DRG neurons. Indeed, current interest in these sensory neurons, and their accessibility for experimental study outside of the spinal cord in isolation from other neuronal cell types, have facilitated the cloning of SNS–PN3 and NaN, and the identification of PN1 and NaG, as sodium channels that are preferentially expressed within them. It is an intriguing possibility that other, neuron-specific, sodium channels are expressed preferentially within other nuclei or neuronal cell groups within the brain and spinal cord. This, together with alternative splicing (Sarao *et al.* 1991; Gustafson *et al.* 1993; Schaller *et al.* 1992) and RNA editing, could confer unique electrophysiological properties on various types of neurons, and would further increase the complexity that sodium-channel diversity endows on the nervous system.

4. SODIUM-CHANNEL GENE EXPRESSION IS A DYNAMIC PROCESS

It is now well established that sodium-channel expression in neurons is not static. On the contrary, it is a highly dynamic process. During the course of development, the level of expression of some sodium channels increases, while expression of others (e.g. α-III) concomitantly decreases in most parts of the nervous system (Beckh *et al.* 1989; Brysch *et al.* 1991; Waxman *et al.* 1994; Felts *et al.* 1997*b*).

At least some of these developmental changes appear to reflect the regulatory effects of neurotrophins and other growth factors on the transcription of various sodium-channel genes. These effects are complex. For example, nerve growth factor (NGF) has opposing actions on expression of the α-SNS and α-III sodium-channel genes, upregulating the former and down-regulating the latter in mature DRG neurons *in vitro* (Black *et al.* 1997) and *in vivo* (Dib-Hajj *et al.* 1998*b*). At

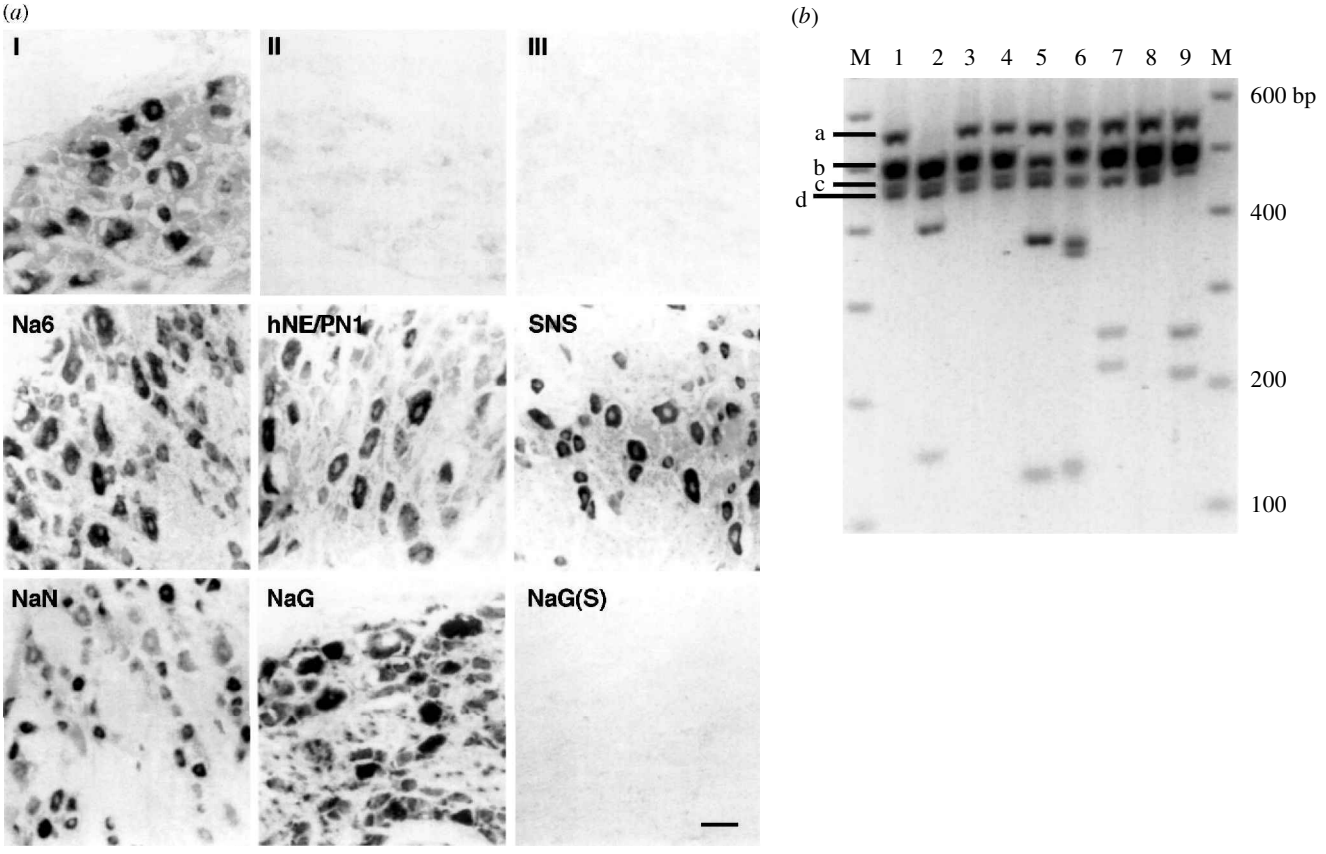

Figure 3. (*a*) Dorsal root ganglion neurons express multiple sodium channels. The micrographs illustrate sodium-channel α-subunit mRNAs visualized in sections from adult rat DRG by *in situ* hybridization with subtype-specific antisense riboprobes. mRNAs for α-I, Na6, hNE–PN1, SNS, NaN and NaG are present at moderate-to-high levels in DRG neurons. Hybridization signal is not present with sense riboprobes, for example, for NaG (S). Scale bar, 40 µm. Modified from Black *et al*. (1996) and Dib-Hajj *et al*. (1998*a*). (*b*) Restriction mapping confirms the expression of multiple Na channels in DRG neurons. 'M' lanes contain 100-bp ladder marker. Lane 1 contains amplification products (bands a–d) from domain 1 in DRG cDNA. Lanes 2–9 show the result of cutting this DNA with *Eco*RV, *Eco*N1, *Aval*, S*phl*, *Bam*H1, A*flII*, X*bal* and *Eco*R1, which are specific to subunits α-I, -II, -III, Na6, PN1, SNS, NaG and NaN, respectively. Reproduced with permission from Dib-Hajj *et al*. (1998*a*).

least some of the effects of NGF on sodium-channel expression are mediated by pathways involving protein kinase A (Kalman *et al*. 1990), but there is evidence indicating that NGF regulates the expression of different types of sodium channels via different signal transduction pathways, some of which are protein kinase A independent (D'Arcangelo *et al*. 1993).

Basic fibroblast growth factor (bFGF) has been found to stimulate sodium-channel expression in PC12 cells (Pollack *et al*. 1990). Brain-derived growth factor (BDNF) does not alter sodium current expression to a significant degree in DRG neurons, although it has significant effects on GABA receptor expression in these cells (Oyelese *et al*. 1997) and increases sodium-channel mRNA and sodium-current expression in PC12 sublines engineered to express trkC receptors (Fanger *et al*. 1995). Glial-derived growth factor (GDNF) strongly modulates the expression of NaN in $IB4^+$ DRG neurons, which are known to express the ret receptor (Fjell *et al*. 1999). Consistent with this, intrathecal adminstration of GNDF partially protects against the decrease in conduction velocity that is observed in c-fibres following axotomy (Bennett *et al*. 1998). Multiple neurotrophins and growth factors thus appear to have effects on DRG neurons, probably via multiple signalling pathways, and it is possible that sodium-channel expression in these cells reflects combinatorial effects of multiple factors.

Interestingly, the effects of neurotrophins on expression of sodium channels may be time dependent and thus could be activity dependent. Toledo-Aral *et al*. (1995) have demonstrated, for example, that pulsed administration of NGF (for periods as short as 1 min) can induce the selective expression of the PN1 sodium-channel subunit (but not the α-II subunit) in PC12 cells via a signalling pathway requiring immediate early genes. It is not yet known whether the precise pattern of episodic exposure to neurotrophins has an effect on channel expression.

Electrical activity itself may modulate the expression of sodium channels within excitable cells. Catterall and colleagaues (Sherman *et al*. 1985; Offord & Catterall 1989) have shown that electrical activity, cAMP levels and intracellular calcium all modulate the expression of sodium channels in muscle cells. And elevation of intracellular calcium by exposure to the calcium ionophore A23187 has been shown to modulate sodium-channel mRNA and sodium-current expression in neuroblastoma cells (Hirsh & Quandt 1996). Sashihara *et al*. (1996) have demonstrated that deafferentation of the olfactory bulb, via surgical transection of the olfactory nerve, results in a

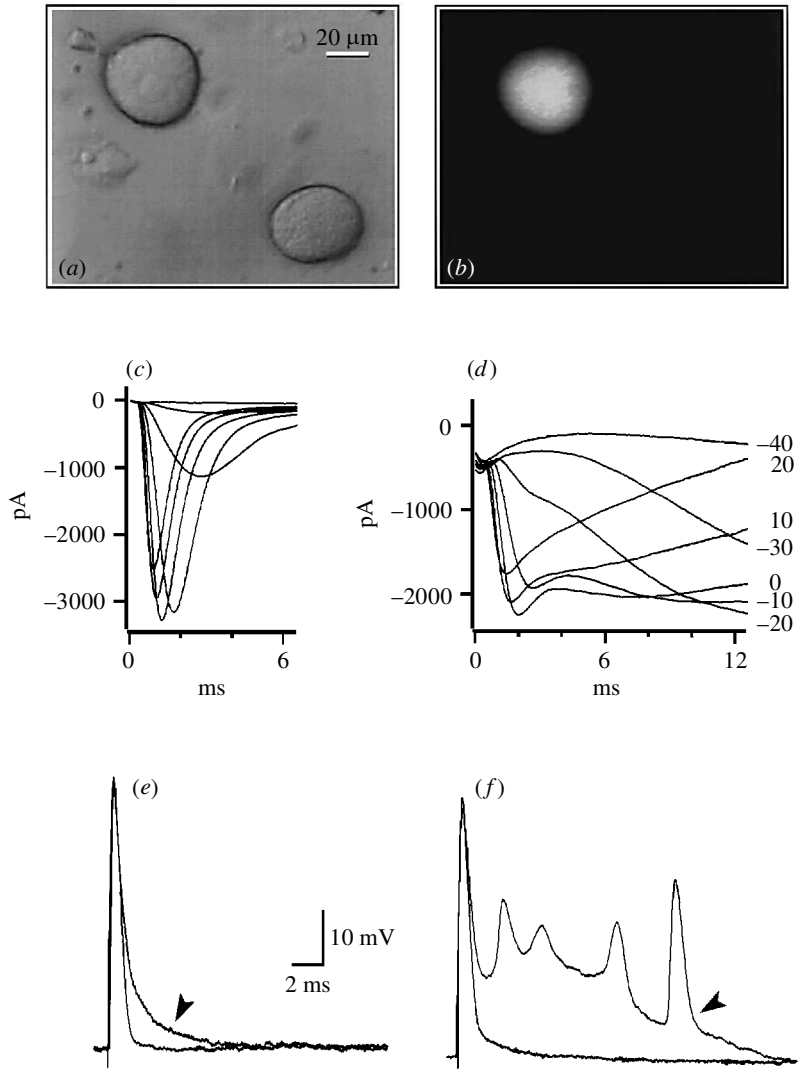

Figure 4. Different sodium currents are expressed in functionally different types of DRG neurons. (*a*) DRG neurons in cell culture. (*b*) Fluorescence microscopy following intracutaneous injection of Fluorogold facilitates identification of cutaneous afferent neurons which are brightly labelled. (*c*, *d*) Whole-cell patch-clamp records demonstrate kinetically different Na^+ currents in muscle (*c*) as compared to cutaneous afferent (*d*) DRG neurons. Deployment of different ensembles of sodium channels in these different types of neurons endows them with different electrogenic properties that can be seen following blockade of potassium channels with 4-aminopyridine; note the different action potential characteristics (arrowheads) following potassium-channel blockade in muscle afferent axons (*e*) compared to cutaneous afferents (*f*). Modified from Honmou *et al.* (1994).

downregulation of α-II sodium-channel mRNA in tufted and mitral cells. This effect is not due to denervation *per se*, but rather appears to be due to a change in the level of synaptic activity since similar changes occur following cauterization of the naris of newborn rats, which abolishes access to olfactory stimuli without denervating the olfactory bulb (Sashihara *et al.* 1997).

Little is currently known about the control mechanisms responsible for modulation of sodium-channel expression. Alternatively spliced sodium-channel mRNAs that encode truncated proteins have been found (Plummer *et al.* 1997; Oh & Waxman 1998) and, since they may encode non-functional channel fragments, might participate in the control of channel expression. The selective expression of functional sodium channels, primarily in excitable cells, suggests the presence of mechanisms that can suppress their expression. Maue *et al.* (1990) and Kraner *et al.* (1992) have demonstrated the presence of a 28 base-pair (bp) silencer element, located in the 5′-flanking region of the α-II sodium-channel gene, that is active only in cells that do not express this gene, suggesting that it is responsible for restricting the expression of α-II channels to specific cell types. The precise mechanism of action of this putative silencer element is not understood. It is not yet clear whether similar control mechanisms participate in the regulation of expression of other sodium-channel genes.

5. REBUILDING THE ELECTROGENIC MACHINE AFTER INJURY: SODIUM-CHANNEL EXPRESSION CAN BE MALADAPTIVE

In some cases, plasticity of sodium-channel expression can be maladaptive. In early studies on motor neurons,

Eccles and his colleagues (Eccles *et al*. 1958) demonstrated that, following axonal transection, there are changes in somatodendritic excitability which appear to represent the deployment of increased numbers of sodium channels within the neuronal membrane. More recent electrophysiological studies have confirmed these findings (Kuno & Llinas 1970) and have firmly established that the abnormal somatodendritic excitability is sodium dependent (Sernagor *et al*. 1986; Titmus & Faber 1986), providing additional evidence for a change in sodium-channel deployment in neurons following axonal injury. Other studies have demonstrated increased sodium-channel immunoreactivity within the injured axonal tips of neuromas (Devor *et al*. 1989; England *et al*. 1994, 1996). One mechanism that could account for these changes is a shift, following axotomy, in the vectorial transport of sodium channels (Titmus & Faber 1990; Devor 1994).

Molecular techniques have recently allowed us to ask whether, in addition to accumulation of abnormally large numbers of sodium channels following injury, different types of sodium channel are deployed within neurons following axonal transection. Our initial experiments demonstrated that, following axonal transection, there is an upregulation of several sodium-channel genes in adult DRG neurons, including a striking upregulation of the previously silent α-III sodium-channel gene (Waxman *et al*. 1994). These changes are not due to an overall increase in protein synthesis. Using *in situ* hybridization and RT-PCR, we have more recently shown (Dib-Hajj *et al*. 1996, 1998*a*) that, in addition, there is a downregulation of the α-SNS and NaN sodium-channel genes (figure 5). There is evidence that some of these changes in sodium-channel expression persist for months following injury (Dib-Hajj *et al*. 1996; Cummins & Waxman 1997).

These changes in sodium-channel gene expression are paralleled by distinct changes in the voltage-sensitive sodium currents that can be recorded in DRG neurons. Following axonal transection, there is a change in the properties of the fast, TTX-sensitive sodium current in these cells (figure 6). Specifically, there is a switch from a slowly repriming current (i.e. a current that recovers slowly from inactivation; $\tau \approx 60$ ms) to a more rapidly repriming current ($\tau \approx 15$ ms) (Cummins & Waxman 1997). It has been suggested that the emergence of the rapidly repriming current is due to the upregulation of α-III channels (Cummins & Waxman 1997), but this hypothesis has not yet been definitively tested. There is also a downregulation of TTX-resistant sodium current in these cells following axotomy (Rizzo *et al*. 1995; Cummins & Waxman 1997), consistent with the downregulation of SNS–PN3 and NaN sodium-channel transcripts (figure 7).

Several arguments suggest that these changes should predispose DRG neurons to fire spontaneously, or at inappropriately high frequencies, following injury. First, increased densities of sodium channels at sites of action potential generation, in themselves, should lower threshold (Waxman & Brill 1978; Matzner & Devor 1992). Second, overlap between steady-state activation and inactivation curves of different types of sodium channels, together with weak voltage dependence of TTX-resistant sodium channels, may confer instability on the neuronal membrane. Coexpression of combinations of

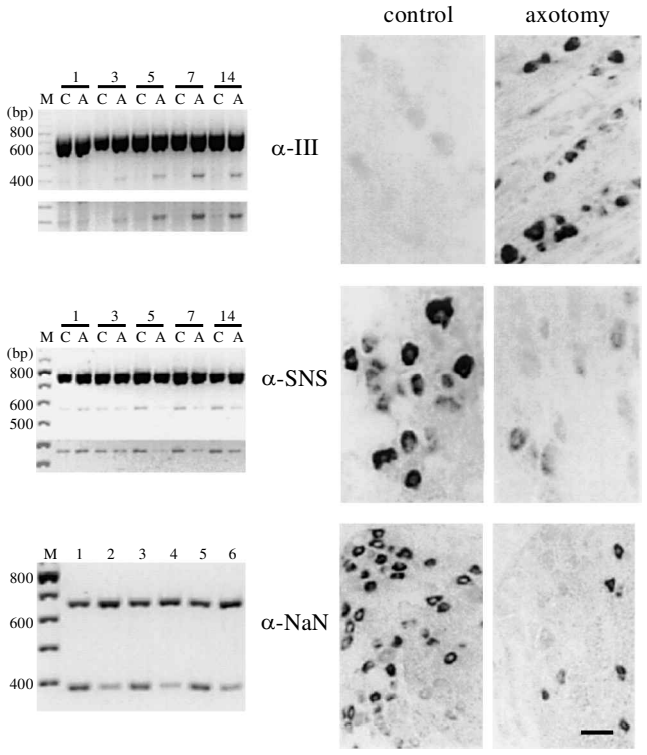

Figure 5. Sodium channels α-III (*a*) are upregulated, and SNS (*b*) and NaN (*c*) are downregulated, in DRG neurons following transection of their axons within the sciatic nerve. The *in situ* hybridizations (right-hand side) show α-III, SNS–PN3, and NaN mRNA in control DRG, and at five to seven days post-axotomy. RT-PCR (left-hand side) shows products of co-amplification of α-III and SNS together with β-actin transcripts in control (C) and axotomized (A) DRG (days post-axotomy indicated above gels), with computer-enhanced images of amplification products shown below gels. Co-amplification of NaN (392 bp) and GAPDH (6076 bp) shows decreased expression of NaN mRNA at seven days post-axotomy (lanes 2, 4, 6) compared with controls (lanes 1, 3, 5). (*a*, *b*) Modified from Dib-Hajj *et al*. (1996); (*c*) modified from Dib-Hajj *et al*. (1998*a*).

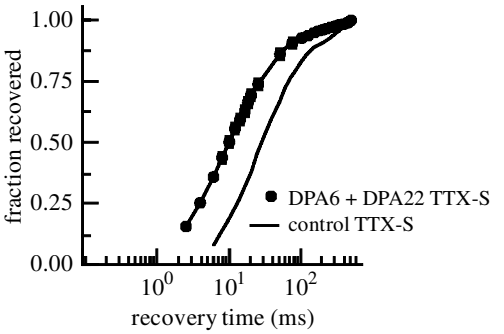

Figure 6. A rapidly repriming sodium current, not detectable in normal DRG neurons, emerges in these cells following axotomy. The graph shows recovery of TTX-sensitive sodium current from inactivation as a function of time, for DRG neurons following axonal transection (six and 22 days post-axotomy (DPA), results pooled) and for control, uninjured controls. Note the leftward shift in the recovery curve, which is due to the emergence of a rapidly repriming sodium current in the axotomized neurons. Modified from Cummins & Waxman (1997).

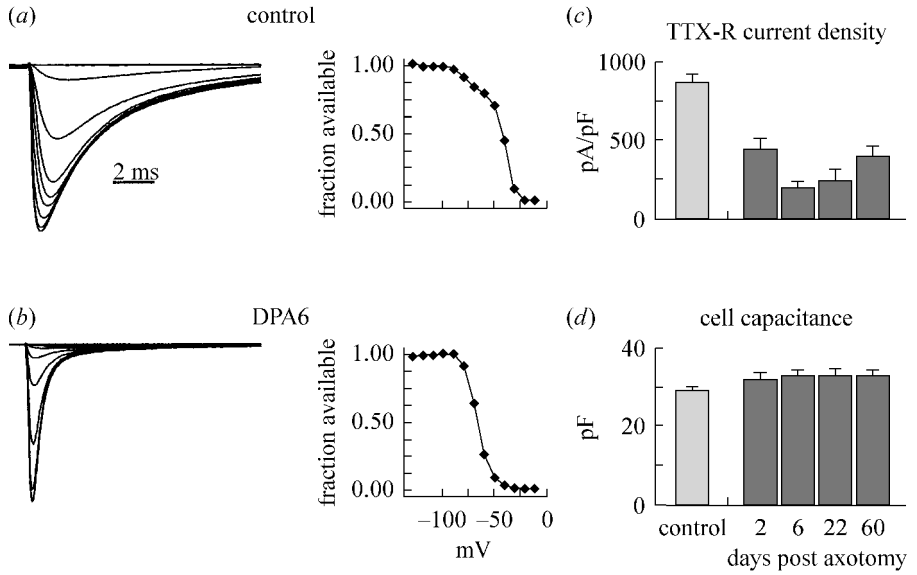

Figure 7. Slow, TTX-resistant sodium currents in small DRG neurons are downregulated following axotomy. (*a*, *b*, left-hand side) Whole-cell patch-clamp recordings from representative control (*a*) and axotomized (*b*, 6 DPA) DRG neurons. Note the loss of the TTX-resistant, slowly inactivating component of sodium current following axotomy. Steady-state inactivation curves (*a*, *b*, right-hand side) show loss of a component characteristic of TTX-resistant currents. (*c*) Attenuation of TTX-resistant sodium current persists for at least 60 DPA. (*d*) Cell capacitance, which provides a measure of cell size, does not change significantly following axotomy (modified from Cummins & Waxman 1997).

several types of channels, whose window currents bracket each other, would be predicted to permit subthreshold oscillations in voltage, supported by TTX-resistant channels, to activate other sodium channels, thus producing spontaneous activity (Rizzo *et al.* 1996). Third, because TTX-sensitive sodium current in DRG neurons following axotomy recovers more rapidly from inactivation than normal TTX-sensitive sodium currents in these cells (Cummins & Waxman 1997), injured DRG neurons would be expected to display a reduced refractory period, and to fire at higher-than-normal frequencies. Fourth, a low-threshold, persistent sodium current, which appears to be partially activated close to resting potential, is present in DRG neurons (Baker & Bostock 1997). Persistent sodium channels are known to participate in setting the resting potential in optic nerve axons (Stys *et al.* 1993). Loss of the channels responsible for the persistent current (which are likely to include TTX-resistant channels) in DRG neurons and their axons following axotomy could produce a hyperpolarizing shift in resting potential which, by relieving resting inactivation, might increase the amount of TTX-sensitive sodium current available for electrogenesis in response to rapid depolaraizations (Cummins & Waxman 1997). Finally, changes in sodium-channel expression in injured DRG neurons may be accompanied by changes in the expression of other ion channels that participate in shaping their excitability. There is evidence for a downregulation of potassium-channel expression in DRG neurons following axotomy (Everill & Kocsis 1999; Ishikawa *et al.* 1999) and this, too, would be expected to produce hyperexcitability.

Although most of the studies on plasticity in sodium-channel expression after neuronal injury to date have focused on DRG neurons, it is possible the hyperexcitability also develops as a result of altered sodium-channel expression following other types of insult, and in other types of neurons following injury. It is now clear that sodium-channel densities can increase in DRG neurons as a response to inflammation in their projection fields (Tanaka *et al.* 1998; Gould *et al.* 1998), and it has been demonstrated that this is due, at least in part, to changes in sodium-channel gene expression (Tanaka *et al.* 1998). Changes in sodium-channel expression have been observed within neurons whose axons have lost their myelin in the tiaep rat, a mutant in which oligodendrocytes degenerate (Black *et al.* 1999). Sashihara *et al.* (1992) observed the production of larger-than-normal numbers of sodium channels in the brains of genetically seizure-susceptible mice. Bartolomei *et al.* (1997) and Gastaldi *et al.* (1997) observed transient changes in neuronal sodium-channel mRNA expression that persisted for approximatly 24 h following kainate-induced seizures in a rat model of epilepsy, and suggested that these changes could lead to alterations in excitability. Vreugdenhil *et al.* (1998) observed a shift in voltage dependence in sodium currents within hippocampal neurons in a model of epileptic kindling, consistent with the idea that there is a switch in the pattern of expression of sodium-channel genes. There is evidence that persistent sodium channels constitute a particularly important substrate for the sustained depolarizations associated with epileptiform activity (Segal 1994; Segal & Douglas 1995). Thus, even in the absence of a global upregulation of sodium channels, a selective upregulation of sodium channels producing persistent sodium currents could increase neuronal excitability and contribute to epileptogenesis.

As noted above, it is well established that sodium-channel gene expression changes in DRG neurons following injury to their axons (Waxman *et al.* 1994; Dib-Hajj *et al.* 1996, 1998*a*). Similar changes have been observed in facial motor neurons following axonal transection (Iwahashi *et al.* 1994). It thus seems reasonable to ask

whether sodium-channel expression is altered in some groups of corticospinal neurons following injury to their axons. If this hypothesis is correct, excitability of these cells might be altered following spinal cord injury, which can transect or otherwise injure corticospinal axons as they travel downwards within the spinal cord. It has been demonstrated that there are changes in the relative densities of TTX-sensitive and TTX-resistant sodium currents in afferent neurons innervating the urinary bladder following experimental spinal cord injury (Yoshimura & deGroat 1997). Changes of this type in axotomized corticospinal neurons could alter their excitability and might at least in part explain the post-traumatic epilepsy seen in some patients following spinal cord injury.

6. REBUILDING THE ELECTROGENIC MACHINE TO MEET FUNCTIONAL NEEDS: STATE DEPENDENCE AND ELECTROGENIC RETUNING IN NON-PATHOLOGICAL NEURONS

Most of the developmental and pathological changes in sodium-channel expression described above occur over a relatively long time-scale (a few days to a few months). The electrophysiological state of neurons, in contrast, changes over a shorter time-scale. This leads us to ask: When a neuron in the non-pathological nervous system passes from one functional state to another, for example from a relatively quiescent state (generating action potentials at low frequencies) to a bursting (high-frequency discharge) state, does it use a fixed repertoire of pre-existing sodium channels in different ways? Or does it rebuild itself by deploying a new and different ensemble of sodium channels so as to retune its electrogenic machinery?

One model for studying this question is provided by the magnocellular neurosecretory cells within the supraoptic nucleus of the hypothalamus. The supraoptic magnocellular neurons send their axons to the neural lobe of the pituitary. In their basal state, these cells are relatively quiescent, firing at low frequencies (<3 impulses s^{-1}) and irregularly, but they respond to changes in osmotic stimuli by generating bursts of action potentials which trigger the release of vasopressin (Walters & Hatton 1974; Mason 1980). Earlier studies had shown that the magnocellular neurons possess an intrinsic regenerative mechanism (Andrew & Dudek 1983; Hatton 1990), which can be triggered by endogenous osmosensitivity mediated by mechanosensitive channels (Ollet & Borque 1993) and by synaptic inputs from circumventricular neurons which are also osmosensitive (Richard & Borque 1992). Moreover, action potential activity in magnocellular neurons of the rat was known to be sodium dependent and TTX sensitive, indicating that it is mediated by sodium channels (Andrew & Dudek 1983; Inenaga *et al*. 1993; Li & Hatton 1996). Thus, in generating action potentials, the magnocellular neurons activate sodium channels in their membranes. But does the membrane of these cells contain the same, or a different, ensemble of channels in the quiescent and bursting states? My colleagues and I recently tested the hypothesis that the transition from the quiescent to the bursting state includes a rebuilding of these cells' electrogenic membrane, so that it contains a different repertoire of sodium channels (Tanaka *et al*. 1999). To test this hypothesis, we studied magnocellular neurons under normal conditions and following salt loading, which is known (Jones & Pickering 1969; Balment *et al*. 1980) to expose supraoptic neurons to a milieu of elevated extracellular osmolality which triggers bursting.

In our first experiments (Tanaka *et al*. 1999), we studied sodium-channel gene expression in the supraoptic nuclei of adult rats using isoform-specific riboprobes for *in situ* hybridization. We first studied the control supraoptic nucleus (from rats that had not been salt loaded), and observed that low levels of the mRNA for the α-II and Na6 sodium-channel α subunits are present within magnocellular neurons. Significant levels of α-I and α-III mRNA could not be detected in these cells. We next salt-loaded animals, and observed a distinct upregulation of the α-II and Na6 mRNAs (figure 8). These observations (Tanaka *et al*. 1999) showed that in response to salt loading, expression of α-II and Na6 sodium channels is upregulated at the transcriptional level, i.e. the expression of the α-II and Na6 sodium-channel genes is increased.

The expression of ion channels and receptors within the cell membrane is controlled at transcriptional, translational and post-translational levels (Ginty *et al*. 1992; Sharma *et al*. 1993; Sucher *et al*. 1993; Hales & Tyndale 1994; Black *et al*. 1998). As a next step we therefore had to determine whether changes in gene transcription were paralleled by increases in sodium-channel protein. To do this we used immunocytochemical and immunoblotting methods with antibody SP20, directed against a conserved region of sodium channels (Westenbroek *et al*. 1989, 1992), to examine the expression of sodium-channel protein in the magnocellular neurons (Tanaka *et al*. 1999). As shown in figure 9*a*,*b*, these studies showed that, following salt loading, there is a distinct increase in sodium-channel immunoreactivity within these neurons. Consistent with these results, there is an increase in the density of the 230 kDa immunoreactive band characteristic of sodium channels (see Westenbroek *et al*. 1989) in membrane preparations from the supraoptic nucleus of salt-loaded rats (figure 9*c*).

These observations demonstrated that the transcription of α-II and Na6 sodium-channel mRNA in magnocellular neurons is upregulated in response to osmotic changes, and further showed that this results in an increased level of sodium-channel protein in these cells, a change which could support a remodelling of their electrogenic machinery. To demonstrate that there was, indeed, a functional change of these cells, however, it was necessary to further show that these channels were inserted into the cell membrane where they could alter the electrogenic properties of these cells. We therefore used patch-clamp recording to study sodium currents in magnocellular neurons (Tanaka *et al*. 1999). These recordings demonstrated the presence of two distinct sodium currents in control magnocellular neurons. The first was a fast transient sodium current, which contributes to the rapid upstroke of the action potential. In addition, because slower sodium currents with thresholds closer to resting potential are known to contribute to burst activity in some types of neurons including supraoptic neurons (Li & Hatton 1996), we searched for them by applying slow (0.23 mV ms^{-1}) ramp depolarizations in magnocellular neurons. These experiments

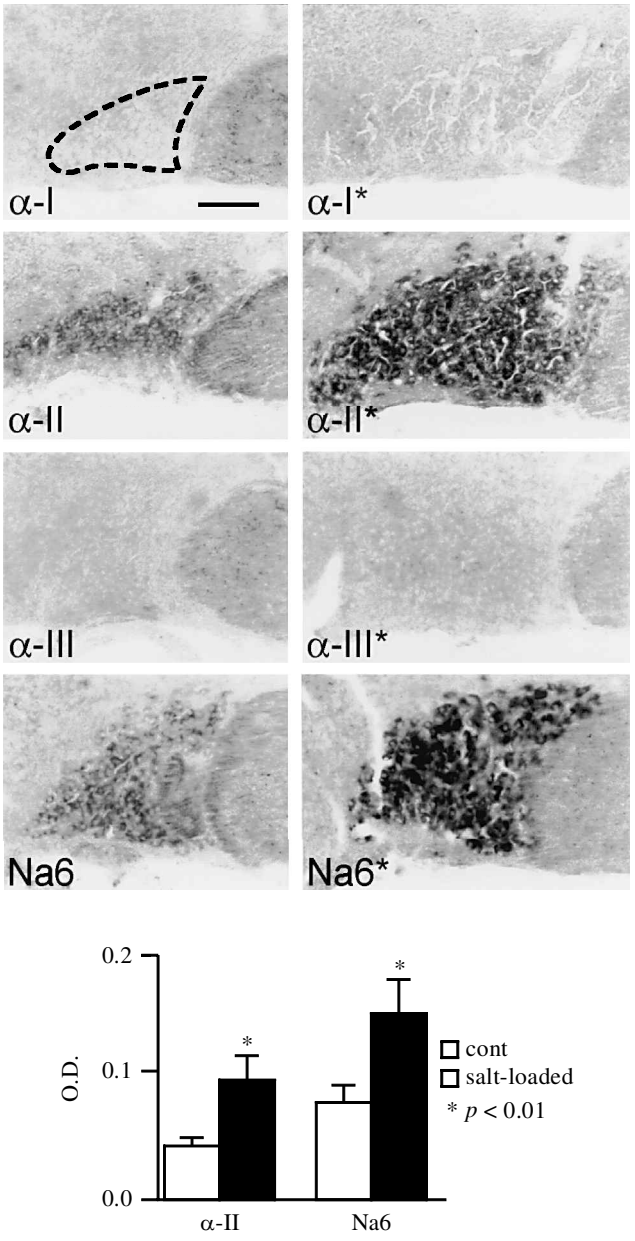

Figure 8. Upregulation of α-II and Na6 sodium channel mRNA in supraoptic magnocellular neurons following salt loading. The micrographs, from control (left column) and salt-loaded (right column) rats, were digitally enhanced to show *in situ* hybridization with subtype-specific riboprobes for Na channel subunits α-I, α-II, α-III and Na6. α-I and α-III mRNA are not detectable, and low levels of α-II and Na6 mRNA are present in the control supraoptic nucleus (no asterisks). Expression of the α-II and Na6 transcript is upregulated following salt loading (asterisks). Optical densities from unenhanced micrographs (graph) provide a quantitative measure of mRNA levels and show a significant increase in α-II and Na6 mRNA following salt loading. * denotes $p < 0.01$. Scale bar, 100 µm. Modified from Tanaka *et al.* (1999).

demonstrated the presence of TTX-sensitive 'ramp' sodium currents which were activated by slow depolarizations close to resting potential. Both of the currents, the fast transient current and the slow ramp current, were increased in salt-loaded magnocellular neurons. There was an increase of 20% in the density of the fast transient sodium current in salt-loaded rats. In contrast,

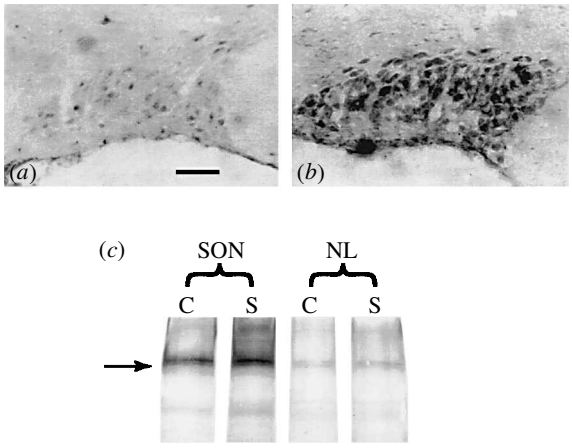

Figure 9. Sodium-channel immunoreactivity with SP20 antibody is increased in the supraoptic nucleus following salt loading (*b*) compared to controls (*a*). Immunoblotting (*c*) shows a 230 kD band (arrow) that is denser in the salt-loaded (S) supraoptic nucleus (SON) than in the control (C). There is a less pronounced increase in density of this band in the salt-loaded pituitary neural lobe (NL), which contains the terminals of the axons of the supraoptic neurons. Scale bar, 100 µm. Modified from Tanaka *et al.* (1999).

however, the ramp current density was approximately 50% larger in salt-loaded neurons (figure 10), an increase that was significantly larger than the increase in the fast transient sodium current. The two sodium currents were thus both increased, but to significantly different degrees.

The presence of α-II and Na6 sodium channels in the magnocellular neurons appears to provide a molecular substrate for the fast transient and slow ramp sodium currents in these cells. It is known from studies in other neuronal cell types, such as Purkinje cells, that the Na6 sodium channel can produce a persistent or ramp current (Vega-Saenz DeMiera *et al.* 1997; Raman *et al.* 1997). The α-II channel, in contrast, has been shown to produce a fast transient current (Noda *et al.* 1986*b*; Auld *et al.* 1988). The different voltage dependence and kinetics of these two channels appear to permit them to collaborate in electrogenesis, the Na6 current being evoked by small, slow depolarizations close to threshold and thus serving to amplify depolarizing inputs, and the α-II current underlying the rapid depolarizing upstroke of the action potential. The disproportional increases in the two currents encoded by these two channels would be expected to lower the threshold for action potential generation by exogenous stimuli and thus appears to effect a retuning of the electrogenic membrane of these neurons.

These changes demonstrate that the molecular structure of excitable membranes, even in the absence of disease states, is subject to modulation in some types of neurons. In a corresponding manner, the functional (electrogenic) properties of neurons, even within the normal nervous system, are subject to modulation. Some neurons, such as the magnocellular cells of the supraoptic nucleus, can incorporate different mixtures of sodium channels into their electrogenic machinery, so as to retune it in response to changing environmental stimuli or different functional requirements. This molecular and functional remodelling adds a dynamic non-synaptic

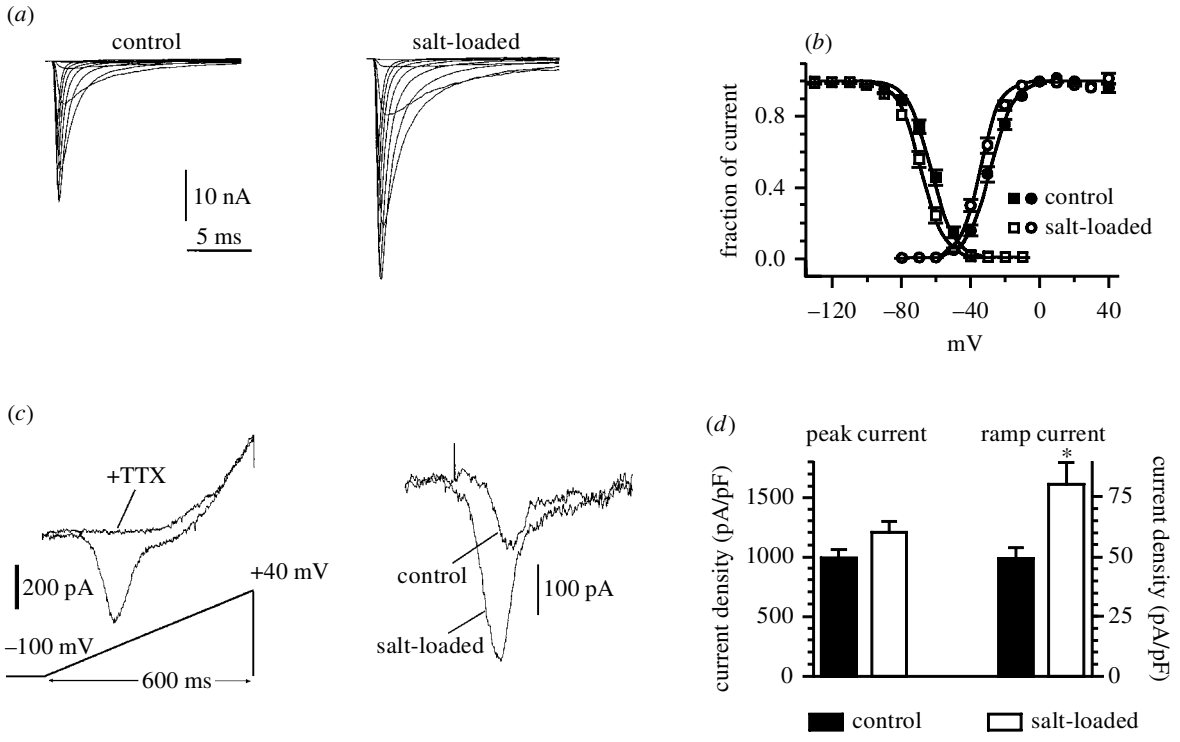

Figure 10. Differential increases in two sodium currents in supraoptic neurons following salt loading. (*a*) Families of traces are shown from representative supraoptic neurons acutely isolated from control (left panel) or salt-loaded (right panel) rats. The currents were elicited by 40-ms test pulses to various potentials from −60 to 30 mV. Cells were held at −100 mV. (*b*) Normalized activation (circles) and steady-state inactivation (squares) curves show only small differences between control (filled symbols) and salt-loaded (open symbols) neurons. Curves are fits to Boltzmann functions. Steady-state inactivation was measured with 500-ms inactivating prepulses, in cells held at prepulse potentials ranging from −130 to −10 mV prior to a test pulse of 0 mV for 20 ms. Error bars indicate s.e. (*c*) Ramp currents are elicited in supraoptic neurons by slow voltage ramps (600 ms ramp extending from −100 to +40 mV). The left panel shows that TTX (250 nM) blocks the ramp current in salt-loaded supraoptic neurons, thus demonstrating that this current is produced by sodium channels. The right panel shows the TTX-sensitive ramp currents in representative control and salt-loaded supraoptic neurons. Leak currents recorded after application of 250 nM TTX were subtracted. Note the larger ramp current in salt-loaded neurons. (*d*) The peak and ramp current densities (estimated by dividing the maximum currents by the cell capacitance) are larger following salt loading; the increase is proportionately greater for the ramp currents. Error bars indicate s.e.; * denotes $p < 0.005$. From Tanaka *et al.* (1999).

element to circuits containing these cells. These neurons not only use their electrogenic machinery in different ways to generate different patterns of electrical activity—they can rebuild it.

7. REBUILDING THE ELECTROGENIC MACHINE: A ROLE IN LONG-TERM POTENTIATION AND DEPRESSION?

It is now well established that the properties of some neural circuits can be altered in an activity-dependent manner via processes such as long-term potentiation and long-term depression, which are best understood in terms of underlying changes in synaptic strength. Might 'downstream' events also contribute to changes in transmission along these circuits? Synaptic currents are integrated at action potential trigger zones such as the initial segment where, as noted above, sodium channels are clustered. The available evidence indicates that the threshold for action potential initiation is, in part, a function of sodium conductance and thus of the density, single-channel conductance and kinetics of sodium channels (Matzner & Devor 1992; Colbert *et al.* 1997; Jung *et al.* 1997). At these trigger zones, even relatively small changes in the densities of various types of channels, including sodium channels, might be expected to alter electroresponsiveness, thus shaping the input–output function for the neuron.

Sodium channels serve, in some types of neurons, to amplify synaptic depolarizations (Stuart & Sakmann 1995; Lipowsky *et al.* 1996). This has been especially well studied in dendrites (Huguenard *et al.* 1989; Regehr *et al.* 1993). As a result of the high input impedance of dendrites, even a small sodium conductance would be expected to produce large potential changes within them (Jack *et al.* 1983; Miller *et al.* 1985; Perkel & Perkel 1985), and the sensitivity of the amplification factor to changes in sodium-channel density would thus be expected to be especially high within dendrites. It is an intriguing possibility that, in addition to changes in synaptic efficacy due to alterations in transmitter release or effects on receptors (see, for example, Lissin *et al.* 1998; Turrigiano *et al.* 1998; O'Brien *et al.* 1998), changes in membrane excitability reflecting altered sodium-channel expression may contribute to activity-dependent changes in the physiological properties of neuronal circuits. A logical consequence of this would be the involvement of sodium channels in forms of neuronal plasticity that have been thought of as 'synaptic'. Consistent with this speculation, computer simulations have demonstrated that a switch in sodium-channel properties (positive shift in the voltage

dependence of activation) can reproduce changes in threshold that have been experimentally observed in a model of operantly conditioned motor neuron plasticity (Halter *et al.* 1995). As noted above, there is, in fact, evidence for activity-related regulation of sodium-channel expression in some types of neurons, suggesting that electrogenic properties of these cells may be modulated in an activity-dependent manner. It could be relatively straightforward to determine whether, in addition to changes in synaptic strength due to alterations in transmitter release or efficacy, alterations in sodium-channel expression and resultant changes in threshold or other aspects of action potential generation contribute to activity-dependent changes in neuronal circuit properties.

8. THE NEURON AS A DYNAMIC ELECTROGENIC MACHINE

It is becoming increasingly clear that the neuron is not only an electrogenic machine—it is a dynamic electrogenic machine. Sodium channels, which lie at the heart of neuronal electrogenicity, are now known to be encoded by a family of genes, and the heterogeneity of the proteins that they encode imparts an important richness to the electroresponsive behaviour of neurons. We have begun to understand that the expression of sodium-channel genes is not a fixed process; it is mutable and, in at least some types of neurons, it is highly dynamic. This is manifested by molecular changes, in sodium-channel expression, which occur not only following various injuries to neurons, but also as neurons move between various functional states. These molecular changes may have important functional implications, since they are reflected in the electrogenic tuning that underlies the normal and pathological generation of electrical activity within neurons. In pathological situations, this may present some novel therapeutic opportunities, since the distinct molecular structure of different sodium channels may make them amenable to selective blockade, modulation, up- or downregulation.

The full range of plasticity in the building, and rebuilding, of neuronal electrogenic machinery within the normal nervous system remains to be determined. At a minimum, we have learned that neurons are not fixed or static electrogenic devices; on the contrary, the electroresponsive properties of some neurons appear to be state dependent, and the mutability of their excitable membranes suggests that incoming messages may be processed in a highly dynamic way via neuronal algorithms that change over time. It remains to be learned whether changes in sodium-channel expression contribute, together with changes in transmitter release and/or efficacy associated with long-term potentiation and/or depression, and with other activity-related changes in neural activity, to learning and memory. Given the tools currently available, which permit the examination of the genes, transcripts, proteins and physiological properties that shape electrogenic behaviour in neurons, we will undoubtedly learn much more about these questions in the relatively near future.

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