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Regulation of Ion Channel Expression in Neural Cells by Hormones and Growth Factors

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Abstract

Voltage-and ligand-gated ion channels are key players in synaptic transmission and neuron–glia communication in the nervous system. Expression of these proteins can be regulated at several levels (transcriptional, translational, or posttranslational) and by multiple extracellular factors in the developing and mature nervous system. A wide variety of hormones and growth factors have been identified as important in neural cell differentiation, which is a complex process involving the acquisition of cell-type-specific ion channel phenotypes. Much literature has already accumulated describing the structural and functional characteristics of ion channels, but relatively little is known about the factors that influence their synthesis and cell surface expression, although this area has generated considerable interest in the context of neural cell development. This article reviews several examples of regulated expression of these channels by cellular factors, namely peptide growth factors and steroid hormones, and discusses, where applicable, current understanding of molecular mechanisms underlying such regulation of voltage-and neurotransmitter-gated ion channels.

Index Entries: Ligand-gated; voltage-gated; neurotransmitter receptors; neural development; glia; gene expression; transcription; nerve growth factor; steroid hormones.

Introduction

The transmission and integration of information in the nervous system depends on the ability of individual neurons to generate and propagate electric signals as well as to respond to neurotransmitters. In the central and peripheral nervous system, voltage-and ligand-gated membrane channels are responsible for the regulation of the membrane potential of neural cells and the process of cell-to-cell communication. Numerous lines of evidence indicate that

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expression of neurotransmitter sensitivity is central to synaptogenesis (reviewed in Role and Berg, 1996), and that changes in sensitivity to neurotransmitters are at least partly caused by changes in the expression of neurotransmitter receptors. As a consequence, a critical component of neuronal development and differentiation is the tightly controlled expression and function of a spectrum of ion channel proteins. These ion channels exist as a mosaic of homo- and hetero-oligomeric assemblies of single subunits (Hille, 1992), the composition and stoichiometry of which determine their physiological and biophysical properties. The cellular factors that control membrane channel subunit gene expression are thus of great interest, given the pivotal roles of ion channels not only in synaptic transmission between neurons but also in glia cell function (Kettenmann and Ransom, 1995). More importantly, emerging roles for ion channels are being elucidated in normal and abnormal cellular development in the brain (Dietzel, 1995; Ben-Ari et al., 1997), and in neurodegenerative conditions (George, 1995; Lindstrom, 1997).

One of the most challenging questions in understanding nervous system function is identifying the determinants of cell-specific subunit expression and the coordinate modulation of channel subunit gene expression in the brain. Many studies have so far shown that functional activity of ion channels can be regulated at multiple levels—gene transcription, RNA splicing and editing, posttranslational modifications, subunit assembly, directed expression in distinct cellular domains—both during development and in the mature brain. The expression of some ion channels is known to be tightly regulated during development (Dryer, 1994; Dietzel, 1995), however, the biochemical and molecular mechanisms that govern the expression of neural ion channels and subunit repertoire in specific cell types remain poorly understood. This is largely because of the complexity of the multigene families encoding channel subunits, the heterogeneity of the cell types expressing such genes, and the intricate network of growth factors that regulate neural cell development. For example, undifferentiated cell lines from the nervous system, when grown under proliferative conditions, express few functional channels (Mandel et al., 1988; Kubo, 1989; Lesser and Lo, 1995), whereas embryonic mouse central nervous system (CNS), progenitor cells treated with growth factors are electrically excitable (Vescovi et al., 1993), indicating an intimate relationship between developmental program and functional expression of ion channels.

Growth factors such as nerve growth factor (NGF) (Snider, 1994), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF) (Raff, 1989; McKinnon et al., 1990) exert profound effects on neural cell survival and differentiation through the activation of transmembrane receptor tyrosine kinases (Van der Greer and Hunter, 1994; Valenzuela et al., 1997). The biological effects of NGF are mediated by both the p75 receptor and the tyrosine receptor kinase Trk receptor family (Greene and Kaplan, 1995, and references therein), whose activation leads to a cascade of phosphorylation events. Ras proteins may then relay signals from the tyrosine kinases to the nucleus via complex signaling pathways, e.g. mitogen-activated protein (MAP) kinase pathway, which may ultimately culminate in altered gene expression in the nucleus through stimulating immediate early gene activity (Fig. 1).

The nervous system is also a target for steroid hormone action. Steroid hormones, such as thyroid hormones, have been shown to affect cell migration, dendrite and axon outgrowth, and gliogenesis (Bernal and Nunez, 1995). Gonadal steroids can also influence the survival, differentiation, and connectivity of specific neuronal populations in the brain (De Vries et al., 1984). Briefly, these hormones are believed to bind cognate intracellular receptors, which are then translocated as dimers to the nucleus, where they function as ligand-activated transcription factors of steroid-responsive genes (Fig. 1). Although this is an oversimplified view of steroid activity, it constitutes the traditional "genomic" action of steroid hormones (McEwen, 1991). There is now, however, a large

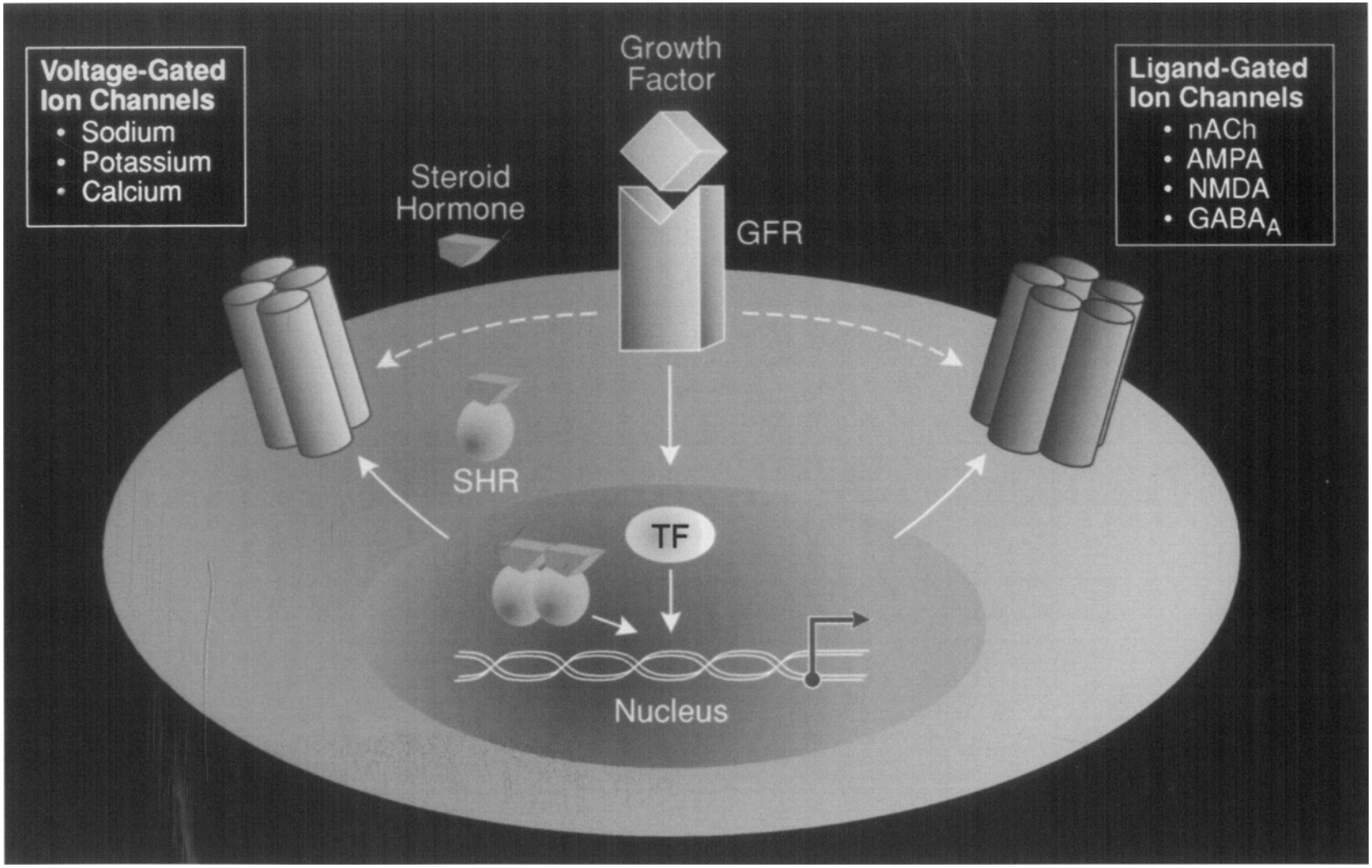

Fig. 1. General scheme of growth factor and steroid hormone action on voltage- and ligand-gated ion channel gene expression. Growth factors activate transmembrane receptor tyrosine kinases, which relay signals to the nucleus, stimulating the activity of transcription factors and promoting ion channel gene transcription. Protein products are then assembled at the cell surface. Steroid hormones bind intracellular receptors, which can also stimulate target gene transcription. Dashed arrows denote direct effect on channel activity or assembly. Ligand-gated ion channels are represented as a pentamer, although recent studies of glutamate receptors provide evidence in favor of a tetrameric arrangement *(see text)*. GFR: growth factor receptor; SHR: steroid hormone receptor; TF: transcription factor; nACh: nicotinic acetylcholine; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA: *N*-methyl-D-aspartate; GABA: γ-aminobutyric acid.

body of evidence indicating that steroids can also act directly on the neuronal membrane, most likely by binding to membrane receptors for neurotransmitters and modulating through a "nongenomic" mechanism, the activity of ion channels (Baulieu and Robel, 1995). Similarly, peptide growth factors can also modulate ion channel activity without involving *de novo* subunit synthesis (Fig. 1).

This review thus seeks to highlight some examples of cellular factor-induced regulation of voltage- and ligand-gated ion channels in the nervous system, and also to discuss the molecular mechanisms underlying such regulation.

Regulation of Voltage-Gated Ion Channels

Introduction

The coordinated and timely expression of the appropriate complement of voltage-gated ion channels during development is critical for the correct formation of a nervous system. Neuronal and glial precursor cells express a voltage-gated channel phenotype that is drastically different from that of differentiated neurons or glia (Sontheimer et al., 1989; Barres et al., 1990b; Ribera and Spitzer, 1992; Wiedehage et al., 1992; Dryer, 1994; Dietzel, 1995; Sah et al., 1997). This implies that important changes in ion channel function occur in the nervous system between the time of terminal division of precursor cells and the acquisition of a differentiated cellular phenotype. A large number of studies have analyzed how voltage-gated ion channel function changes during maturation of the vertebrate nervous system (Ribera and Spitzer, 1992; Vescovi et al., 1993; Dryer, 1994; Dietzel, 1995; Ben-Ari et al., 1997). Membrane ion channels and their activity are profoundly modified during neuronal and glial development, because of either an intrinsic program, and/or the action of cellular factors that can alter channel expression and function at several levels. Additionally, ion channels themselves can indirectly modulate gene expression through impulse-dependent activation of transcription factors (Gallin and Greenberg, 1995; Rosen et al., 1995). The signal transduction pathways that link membrane channel activity with nuclear events can also cause reciprocal modulation of ion channel expression in neural cells. In the present section this latter aspect of modulation of ion channel expression will not be discussed, rather we will focus on how cellular factors, including hormones, can affect voltage-gated ionic channel expression. A summary of the effects of distinct cellular factors on voltage-gated Na^+, K^+, and Ca^{2+} channel expression is shown in Table 1.

Na^+ Channels

Na^+ Channel Subunits and Expression

Functional voltage-gated Na^+ channels are widely expressed in the central and peripheral nervous system. Although these ion channels are predominantly involved in the generation and propagation of action potentials in nerve and muscle, they are also expressed in macroglial cells (Sontheimer and Ritchie, 1995; Sontheimer et al., 1996) that are not capable of generating action potentials. Na^+ channels in the mammalian nervous system are trimeric complexes comprising an α-subunit associated with the smaller subunits β1 and β2, depending on the type of tissue and developmental stage (Hartshorne et al., 1982; Agnew, 1984; Catterall, 1984). The α-subunit contains the molecular elements that determine ion selectivity and voltage sensitivity of the channel. Molecular cloning has identified several distinct cDNAs derived from a multigene family expressed in the CNS and encoding functional Na^+ channels: rat brain I, II, IIA, III, rat sympathetic nervous system (SNS), and rat Na6 (Noda et al., 1986; Auld et al., 1988; Kayano et al., 1988; Schaller et al., 1995; Akopian et al., 1996). α-Subunits also exist as different splice isoforms (Sarao et al., 1991; Yarowsky et al., 1991; Schaller et al., 1992; Gustafson et al., 1993). Additionally, a distinct peripheral Na^+ channel selectively

Table 1
Regulation of Voltage-Gated Ion Channels[a]

Channel	Modulator	Biological effect
Na^+	Thyroid hormone (hippocampal neurons)	↑ Function
	NGF (PC 12 cells)	↑ Function type II/IIA and PN1 α-subunit mRNA
	NGF (DRG neurons)	↑ α-subunit I and β1-subunit mRNA; ↓ α-subunit III mRNA
	NGF (cutaneous afferent neurons after axotomy)	↑ TTX-resistant function α-SNS mRNA
K^+	NGF, NT-3, NT-4/5, CNTF (SK-N-SH neuroblastoma cells)	↑ Delayed rectifier function
	NGF (PC 12 cells)	↑ Kv2.1 protein, function
	aFGF (ciliary ganglion neurons)	↑ I_A function
	NGF (ciliary ganglion neurons)	↑ I_K (Ca)
	PDGF, bFGF (Xenopus oocytes)	↓ Kv1.5 function
	Glucocorticoid (GH3 pituitary cells)	↑ Kv1.5 mRNA, protein, function
	Thyrotropin-releasing hormone	↓ Kv1.5 and Kv 2.1 mRNA, protein, function
Ca^{2+}	Progesterone, estradiol (hippocampal neurons)	↑ Sustained/transient function
	Glucocorticoids, mineralocorticoids (CA1 hippocampal neurons)	↑ I_{Ca} function
	Mineralocorticoids (CA1 pyramidal neurons)	↓ P/Q- and L-type mRNA, protein
	Glucocorticoids (CA1 pyramidal neurons)	↑ P/Q- and L-type mRNA, protein
	bFGF (fetal hippocampal neurons)	↑ L-type function
	NGF (basal forebrain neurons)	↑ L- and N-type function
	NGF (PC12 cells)	↑ Low-threshold T-type, N-type function
	NGF (DRG neurons)	↑ Function
	Isoproterenol, VIP, forskolin, 8Br-cAMP (astrocytes)	↑ L-type function
	bFGF (human retinal glia)	↑ L-type function

[a]Summary of effects of hormones and growth factors on the expression of voltage-gated ion channels, as described in the text. For each modulator listed, the cell system under study is indicated in parentheses. The symbols ↑ and ↓ indicate up- and downregulation, respectively. The nature of regulation by each modulator is specified in the last column. Function = channel activity, as measured by biophysical methods (e.g. electrophysiology, calcium imaging).

expressed in neurons, PN1, has been identified by Northern blot analysis (Toledo-Aral et al., 1997). Gautron et al. (1992) have also reported the cloning of the NaG Na^+ channels from cultured cortical astrocytes, whose partial sequence is distinct from the other cloned subunits. More recent work, however has demonstrated that transcripts of this gene are also expressed in dorsal root ganglion (DRG) neurons and, at lower levels, in hippocampus, cerebellum, and spinal cord soon after birth (Felts et al., 1997).

The expression of Na^+ channel RNA transcripts is developmentally regulated in the central and peripheral nervous system. Distinct spatial and temporal patterns of subunit mRNA expression are observed during maturation of the rat nervous system (Felts et al., 1997). For example, among the α-subunits, Na^+ channel I is expressed at higher levels at post-

natal stages and in caudal regions of the brain. Conversely, Na^+ channel II was found at similar levels in pre- and postnatal brain, but at higher levels in rostral regions. Finally, Na^+ channel III is predominantly expressed at early developmental stages and downregulated in the adult. In parallel to these molecular changes, modifications in Na^+ channel function also occur during maturation of the nervous system. Na^+ current densities largely increase during pre- and postnatal development in both chick and rat (Gottmann et al., 1988; Huguenard et al., 1988; Black and Waxman, 1996).

These molecular and electrophysiological data indicate that transcription of genes encoding Na^+ channel subunits and expression of functional channels in distinct cell types is either regulated by an intrinsic developmental program or results from cell-to-cell interactions during development. These interactions are likely to be at least in part mediated by soluble cellular factors (*see* Table 1).

Regulation of Na^+ Channels by Thyroid Hormone

Among the various cellular factors that influence brain development, thyroid hormone was shown to regulate the expression of a variety of membrane channels, receptors, and transporters (Honegger and Lenoir, 1980; Patel et al., 1980; Dussault and Ruel, 1987; Tejani-Butt et al., 1993). It is, therefore, possible that some of the severe effects of hypothyroidism in the brain are caused by defects in the expression of genes encoding key membrane proteins that are involved in neuronal excitability and synaptic transmission. In a recent study, Potthoff and Dietzel (1997) demonstrated that 3,5,3'-triiodo-L-thyronine (T3) selectively increased the density of voltage-activated Na^+ currents in neonatal cultured hippocampal neurons from newborn rats, but had no effects on K^+ currents (Potthoff and Dietzel, 1997). This finding could have important implications, because the increase in Na^+ current density and conduction velocity observed during neuronal maturation could be partially under the control of T3, and the absence of these developmental events could contribute to the neurological symptoms typical of hypothyroidism.

Regulation of Na^+ Channels by Neurotrophins

Neurotrophins, such as NGF, are found in the nervous system from early developmental stages (Thoenen, 1995; Henderson, 1996). NGF influences the survival, growth, and differentiation of sympathetic and sensory neurons of the peripheral nervous system (PNS), and of selective populations of cholinergic neurons in the CNS (Levi-Montalcini and Angeletti, 1968; Levi-Montalcini, 1987; Hefti et al., 1989; Crowley et al., 1994). Perhaps among the best studied regulation of a voltage-dependent ionic channel by an identified cellular factor is the effect of NGF on Na^+ channel expression and function in PC12 cells. This clonal cell line isolated from a rat adrenal chromaffin tumor has been widely used as a model of neuronal differentiation and function (Greene and Tischler, 1982). PC12 cells acquire a sympathetic neuron-like phenotype after NGF treatment, including arrest in proliferation, neurite extension, and upregulation of functional Na^+ channels (Greene and Tischler, 1982) and acetylcholine receptors (*see* Regulation of Ligand-Gated Channels). Rudy et al. (1987) studied the phenomenon of functional induction of Na^+ channels in PC12 cells after a 2 wk treatment with NGF. The neurotrophin had two distinct effects: it increased the overall number of functional channels and induced a population of TTX-resistant Na^+ channels. The molecular basis of these effects was analyzed in subsequent experiments (Mandel et al., 1988; D'Arcangelo et al., 1993). These studies showed that undifferentiated PC12 cells expressed type II/IIA but not type I Na^+ channel genes, and that NGF treatment increased the relative levels of transcripts encoding type II/IIA and peripheral nerve type 1 (PN1) Na^+ channel α subunits. Type I gene expression was not modified by NGF.

The intracellular pathway that links NGF receptors with Na^+ channel induction involves activation of cAMP-dependent protein kinase (PKA). Using patch-clamp recordings, Kalman et al. (1990) demonstrated that:

1. Agents that increased cAMP in PC12 cells also upregulated Na^+ channel expression to the same extent as NGF;
2. Selective protein inhibitors of the PKA catalytic subunit prevented the effects of NGF and cAMP on Na^+ channel expression; and
3. Protein kinase C (PKC) activators did not modify Na^+ channel number.

Molecular analysis at the RNA level revealed that the induction of type II gene expression by NGF was PKA-dependent, whereas PN1 upregulation was not (Ginty et al., 1992; D'Arcangelo and Halegoua, 1993). Furthermore, the effects of NGF on type II mRNA and on functional Na^+ channels were independent of Ras activity (D'Arcangelo and Halegoua, 1993), implying that the intracellular signaling pathway involved in the regulation of distinct Na^+ channel types by NGF was branched. Finally, the effects of NGF on type II Na^+ channels were reproduced by basic FGF (bFGF) (Ginty et al., 1991).

PN1 induction by NGF occurs with a time-course different from type II and through a distinct intracellular pathway. PN1 mRNA and functional voltage-gated Na^+ channels were significantly and selectively increased by a brief (1 min) pulse of NGF (Toledo-Aral et al., 1995). PN1 induction by NGF was found to be transcriptional and required immediate early gene induction. Similarly brief exposures of PC12 cells to interferon γ (IFNγ), epidermal growth factor (EGF), and bFGF also caused a significant increase in PN1 mRNA (Toledo-Aral et al., 1995), suggesting that these factors may share some pathways of transcriptional induction of the PN1 gene. PN1 transcripts increased 3 h after removal of NGF and the earliest increase in voltage-activated Na^+ current was observed after 8 h, indicating that only a few hours are necessary for Na^+ channel protein translation and insertion into the plasma membrane.

Expression and Regulation of Na^+ Channels in DRG Neurons

A second example of a well studied cell type displaying Na^+ channel plasticity is provided by DRG neurons. These cells express a variety of Na^+ channel genes and different Na^+ currents that can be distinguished based on their kinetic properties, voltage-dependence of activation and inactivation, and TTX-sensitivity (Caffrey et al., 1992; Honmou et al., 1994; Rizzo et al., 1994). In DRG cells, expression of Na^+ channel RNAs is developmentally regulated (Felts et al., 1997; Schwartz et al., 1990; Roy and Narahashi, 1992). *In situ* hybridization analysis in rat tissue demonstrated that, at embryonic d 17 (E17), among the α-subunits Na^+ channel mRNA I is expressed at low levels in the majority of DRG neurons (Felts et al., 1997). On the other hand, Na^+ channel II/IIA and III mRNAs show similar patterns of expression, i.e., significantly higher in some subsets of DRG neurons (Felts et al., 1997). The NaG and β1 mRNAs exhibit low levels of expression in all cells (Felts et al., 1997).

In adult tissue, the pattern of expression of Na^+ channel mRNAs is distinct from that in the embryo. Na^+ channel mRNA I is upregulated in the majority of DRG neurons, whereas Na^+ channel II/IIA mRNAs are significantly downregulated. Furthermore, Na^+ channel III mRNA is not detectable in any DRG cells (Felts et al., 1997). Finally, both NaG and β1 mRNAs display significant upregulation, especially in a subpopulation of DRG neurons (Felts et al., 1997).

The difference in Na^+ channel RNA expression between early developmental stages and adult indicates that changes occur either at the transcriptional level (e.g., in response to cellular factors that activate transcription in a developmentally regulated fashion) or as part of an intrinsic developmental program. Studies performed in DRG neurons treated with NGF suggest that the developmental changes in Na^+ channel gene expression are at least in part caused by the action of an exogenous factor. In fact, exposure of DRG neurons to NGF some-

what resembles the dynamic changes in Na^+ channel RNA expression that occur during development. In particular, expression of both α-subunit I and β1-subunit mRNAs is increased by NGF, whereas α-subunit III mRNA is decreased by the growth factor (Zur et al., 1995).

Regulation of Na^+ Channels After Axotomy and by Target Cells

Trophic factors released by target cells may participate in regulating and maintaining the morphological and electrophysiological properties of afferent cells during development and in the adult animal. This is exemplified by the changes that occur in sensory neurons after peripheral nerve transection, in particular at the level of expression of membrane ion channels (Wall and Devor, 1981; Titmus and Faber, 1990). In axotomized adult rat cutaneous afferent neurons, Oyelese et al. (1997) found a reduction in kinetically slow TTX-resistant Na^+ current, accompanied by a decrease in the number of neurons expressing action potentials with an inflection in the falling phase. Kinetically slow Na^+ currents most likely underlie this component of the action potential. In the same cells, an increase in fast TTX-sensitive Na^+ currents and in GABA-triggered responses was observed.

In situ hybridization analysis in adult DRG neurons a few days after axotomy demonstrated that drastic alterations in Na^+ channel subunit expression occur at the RNA level (Waxman et al., 1994; Black and Waxman, 1996). Upregulation of mRNA I and II/IIA was observed, and most importantly *de novo* transcription of type II Na^+ channel gene was induced in many injured DRG neurons. Conversely, NaG mRNA levels remained unchanged. These findings require further biochemical analysis on Na^+ channel protein expression, but demonstrate that axotomy triggers important retrograde changes in injured neurons at the level of Na^+ channel gene expression.

Neurotrophins acting at TrkA and TrkB receptors play an important and rather selective role in modulating the electrophysiological properties of adult neurons after nerve transection. NGF applied in vivo after sciatic nerve transection caused a significant increase in the number of cutaneous afferent neurons displaying TTX-resistant Na^+ currents and action potentials with inflections on the falling phase (Oyelese et al., 1997). NGF did not affect the increase in GABA-mediated currents observed after axotomy (Oyelese et al., 1997). Differently from NGF, brain-derived neurotrophic factor (BDNF) did not modify action potential waveform, but partially prevented the effects of axotomy on GABA currents (Oyelese et al., 1997).

The molecular basis of the changes in Na^+ currents resuting from NGF treatment were further analyzed in axotomized C-type DRG neurons (Dib-Hajj et al., 1998). In vivo administration of the neurotrophin prevented the decline in TTX-resistant Na^+ current and α-SNS mRNA levels caused by axotomy, as determined by patch-clamp recording, RT-PCR, and *in situ* hybridization. These findings indicate that α-SNS Na^+ channels underlie at least partially this TTX-resistant current, and further molecular analysis will determine whether the effects of NGF are transcriptional or rather affect α-SNS mRNA stability.

Regulation of Na^+ Expression in Neurons by Glia

The question of how distinct cell types of the nervous system can reciprocally influence each other to modulate expression of voltage-dependent ionic channels has been addressed in different cell culture systems. Hinson et al. (1997) studied regulation of Na^+ channel mRNA and currents in E15 DRG neurons cultured for 2–3 wk in the presence or absence of Schwann cells (SCs) or SC-conditioned media. *In situ* hybridization analysis revealed that SCs significantly increased expression of NaG, Na6, SNS, and β2 mRNAs. SC-conditioned medium only increased expression of SNS mRNA. This upregulation at the RNA level was matched by an increase in Na^+ channel immunoreactivity and in functional channel

density. Although the SC factor responsible for this upregulation has not yet been identified, these results clearly indicate that SCs drastically modify Na^+ channel expression in DRG neurons. Interestingly, the Na^+ channel phenotype displayed by DRG neurons cocultured with SCs is similar to that at P15 *in situ*, indicating that partnership with glia triggers changes in Na^+ channel expression similar to those that occur during development in vivo. Since SCs are a rich source of neurotophic factors, including NGF, brain-derived neurotrophic factor (BDNF), and ciliary neurotrophic factor (CNTF) (Bandtlow et al., 1987; Acheson et al., 1991; Friedman et al., 1992), future analysis with selective antibodies for these factors and their receptors will help in identifying the suspect.

Another example of glial-mediated regulation of neuronal Na^+ channels is provided by the effects of astrocyte-conditioned medium on NB69 neuroblastoma cells (Urbano et al., 1997). In this system, an unidentified factor released by astrocytes causes a switch of a TTX-insensitive slow current to a TTX-sensitive fast current. The underlying molecular basis of this switch is unknown.

Expression and Regulation of Na^+ Channels in Glia

Na^+ channels are also expressed at a lower density in astrocytes and oligodendrocytes (Sontheimer et al., 1989, 1996; Barres et al., 1990a). The physiological role of Na^+ channels in glia has not yet been elucidated, but astrocytes express a variety of Na^+ channel RNAs and display functional channels in vivo (Sontheimer et al., 1996). Evidence that expression of Na^+ channels in glia is under neuronal control is provided by experiments of optic nerve transection or removal of the retina (Barres et al., 1990a; Minturn et al., 1992). In both cases, Na^+ channel expression was drastically downregulated in astrocytes. If after transection astrocytes were cocultured with retinal ganglion cells, Na^+ channel expression was restored (Barres et al., 1990a). In spinal cord astrocytes, neurons had the opposite effect on Na^+ channel expression, because either DRG cells or their conditioned medium caused downregulation of Na^+ channels (Thio et al., 1993). Altogether, these studies indicate that neurons modulate Na^+ channel expression in astrocytes, and they also show that signals with opposite biological effects on channel expression may be released from distinct neuronal cell types.

K^+ Channels

Classification and Topography of K^+ Channels

Like Na^+ channels, K^+ channels control excitability of neurons and muscles, although they are also abundantly expressed in nonexcitable cells of the central and peripheral nervous systems, astrocytes, oligodendrocytes, and Schwann cells (Duffy et al., 1995). By using molecular cloning, two multimember families of K^+ channels have been identified; voltage-gated and inwardly rectifying (Jan and Jan, 1997a,b). These two families are structurally distinct. Voltage-gated K^+ channels contain six putative transmembrane domains in each α subunit and their β subunits lack transmembrane segments. The β subunits of Ca^{2+}-activated channels comprise two transmembrane domains (Jan and Jan, 1997a,b). Inwardly rectifying channels are formed by two putative transmembrane segments in the α subunits, whereas multiple transmembrane domains have been found in one of the β subunits, the ATP-sensitive K^+ channel (Jan and Jan, 1997a). The α subunits of the voltage-dependent channels belong to four major subfamilies, Shaker, Shab, Shaw, and Shal, or Kv.1, 2, 3, and 4 (Jan and Jan, 1997a).

Despite their enormous variety (e.g., 18 distinct genes encoding voltage-gated K^+ channel α subunits and belonging to six subfamilies have been found in the mammalian nervous system) and difference in gating mechanisms, all K^+ channels contain homologous structural domains essential for K^+ permeation. In particular the S4–S5 loop and the S6 segments in the voltage-gated channel α subunits, as well as the M2 segment of the inwardly rectifying α

subunits, display high homology. Furthermore, a loop (called H5) P region between the transmembrane segments 5 and 6 in the voltage-dependent, and 1 and 2 in the inwardly rectifying channels contains the conserved sequence GYG, which plays an important role in K^+ permeation (Jan and Jan, 1997a).

In the nervous system, expression and function of K^+ channels can be regulated by a variety of signals, including neurotrophic factors, hormones, and neurotransmitters (*see below* and Table 1). Because of the enormous molecular and functional heterogeneity of K^+ channels, as well as their ubiquitous expression, cellular models that allow study of the effects of identified signals on specific K^+ channel subtypes are of fundamental importance. The understanding of these signals and their K^+ channel subunit target(s), combined with the availability of deletion mutants for neurotrophic factors (Crowley et al., 1994; Henderson, 1996), will identify the major pathways of regulation of K^+ channel expression during development.

In view of the multiplicity and extreme complexity of K^+ channel subtypes, the reader is referred to more specific literature describing regulation of K^+ channel gene expression during development (Beckh and Pongs, 1990; Ribera, 1990; Swanson et al., 1990; Wilson and Chiu, 1990; Behrens and Latorre, 1991; Drewe et al., 1992; Perney et al., 1992; Ribera and Spitzer, 1992; Perney and Kaczmarek, 1993; Trimmer, 1993). As a general rule, the patterns of K^+ channel gene expression display marked temporal and spatial differences during development of the vertebrate nervous system. In some cases expression patterns are distinct and specific for each member of a certain family, such as RCK genes (Beckh and Pongs, 1990). In other instances, expression of members of a specific subfamily appears to be coordinated (Ribera and Spitzer, 1992). Additionally, the mRNA levels for specific K^+ channel splice variants are selectively regulated during development, such as for the two splice isoforms of the Kv3.1 gene (Liu and Kaczmarek, 1998; *see below*). Cellular factors, cell–cell interactions, neuronal activity, and hormones contribute to generate the final mosaic of K^+ channel expression in the mature nervous system. Some key examples and the relative cellular models are discussed here.

Regulation of K^+ Channels by Neurotrophins

In SK-N-SH neuroblastoma cells, distinct neurotrophins produced similar effects on functional expression of K^+-channels. NGF, NT-3, and NT-4/5, but not BDNF, all significantly increased K^+-channel activity (Lesser et al., 1997). A delayed rectifier current was the predominant type found before and after neurotrophin treatment, with unaltered functional properties (Lesser et al., 1997). Therefore, neurotrophins most likely regulate the levels of constitutively expressed K^+ channel subunits underlying these currents, rather than trigger expression of new subunits. Interestingly, the overall effects of NGF on K^+ current expression were mimicked by CNTF, which also increased delayed-rectifier currents (Sheng et al., 1992). However, an additional TEA-insensitive current found in NGF-treated cells was not induced by CNTF (Sheng et al., 1992); therefore, the pathways of regulation of K^+ channels by distinct neurotrophins may converge on certain subtypes and diverge on others. It is tempting to speculate that each neurotrophin could play either a permissive role on subunit expression and alter the density of channel, or an instructive role and trigger expression of a new channel subtype.

In many neuronal subpopulations, K^+ channel expression is polarized either in the axonal or in the dendritic compartment. Regulation of potassium channel expression by target-released factors, such as NGF, could therefore modulate K^+ channel distribution during neuronal development, as demonstrated by studies performed in PC12 cells. Sharma et al. (1993) demonstrated that expression of the neuronal delayed rectifier K^+ channel Kv2.1 was increased by NGF. The effect of NGF appeared to be mainly translational, since it occurred at the protein level without a parallel increase in Kv2.1 mRNA. Importantly, in differentiated

PC12 cells, expression of Kv2.1 protein was preferentially found at growth cones, where electrophysiological analysis demonstrated the existence of low conductance K^+ channels with functional properties similar to recombinant Kv2.1 (Sharma et al., 1993). The marked increase of Kv2.1 expression triggered by NGF in the growth cone region was also consistent with the presence of macroscopic delayed rectifier currents, as detected by whole-cone recordings (Sharma et al., 1993). These findings indicate that localized effects of neurotrophins and interactions with specific cytoskeletal proteins may play an important role in segregating membrane channel expression, thereby determining neuronal polarity during development.

Regulation of K^+ Channels by Target Tissue and Growth Factors

The role played by target tissue in the modulation of neuronal K^+ channel expression was analyzed electrophysiologically in developing chick neurons by Dryer and his colleagues (Dourado and Dryer, 1992, 1994; Raucher and Dryer, 1995). This study was facilitated by the developmentally regulated and sequential expression of functionally distinct K^+ currents in chick ciliary ganglion cells. The first K^+ current to appear is a slowly inactivating voltage-dependent, I_{DR}, that is followed by a transient (I_A) and a Ca^{2+}-activated [I_K(Ca)] current only at the time of synapse formation between ciliary ganglion neurons and target tissue in the eye (Dourado and Dryer, 1992). In support of the hypothesis that this sequential expression of K^+ currents is target tissue-dependent, Dourado and Dryer (1992) found that, in ciliary ganglion neurons isolated before synaptogenesis (E9) and maintained in culture for a few days, I_{DR} was expressed at normal functional levels, whereas both I_A and I_K(Ca) were greatly reduced. Normal levels of I_A expression were restored when:

1. Ciliary ganglion neurons were cocultured with striated muscle cells, but not with fibroblasts;
2. Cells were treated with acidic fibroblast growth factor (aFGF); and
3. An aqueous extract of chick brain was added to the cell culture medium.

CNTF or bFGF did not affect I_A expression, indicating that aFGF (known to be present in the chick eye) (Hill et al., 1991) or an aFGF-like factor is likely to mediate the effect of target tissue on expression of this K^+ current.

In a conceptually similar set of experiments, Raucher and Dryer (1995) demonstrated that expression of I_K(Ca) is also regulated by environmental cues (Raucher and Dryer, 1995). This current is functionally similar to I_K (Ca) in chick ciliary ganglion, as demonstrated by single channel subtype characterization (Raucher and Dryer, 1995), and is significantly upregulated by target tissue and target-derived soluble factors, among which is NGF (Raucher and Dryer, 1995). By analogy with mammalian lymphocytes, induction of I_K(Ca) channels may involve activation of *ras* oncogenes (Raucher and Dryer, 1995).

A different strategy was used by Timpe and Fantl (1994) to analyze the effects of identified growth factors on a specific K^+ channel subtype. Kv1.5 and PDGF or bFGF receptors were coexpressed in Xenopus oocytes, and the effects of receptor activation on K^+ current were analyzed. The advantage of this approach is represented by the possibility of identifying the modulatory pathway(s) of channel regulation by the growth factors and the relevant sites in the channel protein by using mutant forms of receptors and channels. Activation of either PDGF or bFGF receptors triggered a decrease in Kv1.5 amplitude within a few minutes after treatment. Parallel biochemical experiments involving the use of a mutant FGF receptor that does not activate phospholipase C and injection of IP3 in oocytes demonstrated that activation of this enzyme is required for growth factor-induced modulation (Timpe and Fantl, 1994).

Regulation of K^+ Channels by Hormones

The physiological studies described above indicate that functional expression of K^+ channels is regulated by factors that are known to affect nerve cell development, however, they

do not identify the level of regulation through a detailed molecular analysis. This has been successfully accomplished in studies that examined K^+ channel expression in pituitary cells and their regulation by hormones. In a clonal rat pituitary cell line, GH_3, the mRNA of the K^+ channel Kv1.5 was significantly increased within a few hours after treatment with the synthetic glucocorticoid dexamethasone (Takimoto et al., 1993). The sex steroids progesterone, estradiol, and testosterone were ineffective, and the inductive effect of dexamethasone was not observed on the K^+ channel Kv2.1 (Attardi et al., 1993; Takimoto et al., 1993). Nuclear run-on assays demonstrated that the effect of the steroid was caused by an increase in the rate of Kv1.5 gene transcription, and parallel experiments with the transcription inhibitor actinomycin D indicated that Kv1.5 mRNA turnover was not altered by dexamethasone (Takimoto et al., 1993). The levels of Kv1.5 protein were also increased by the steroid with a time-course that followed that of the mRNA by a few hours.

In a recombinant expression system (Xenopus oocytes), Kv1.5 encoded a delayed rectifier K^+ current (Duffy et al., 1995). Consistent with the induction of Kv1.5 at the mRNA and protein levels, dexamethasone increased non-inactivating, delayed rectifier K^+ currents in GH_3 cells (Takimoto et al., 1993). These findings in a pituitary cell line are physiologically important, because in the CNS Kv1.5 gene is preferentially expressed in the hypothalamus, a brain area that also displays a high density of glucocorticoid receptors (GRs) (De Kloet, 1991). An increase in Kv1.5 functional expression may be a mechanism by which glucocorticoids increase inhibition in the hypothalamus/pituitary system. The selective effects of dexamethasone on Kv1.5 vs Kv2.1 indicate that this might be accomplished by rapidly increasing the transcription rate of a K^+ channel gene.

Opposite effects on K^+ channel transcription and function were observed with the neuropeptide thyrotropin-releasing hormone (TRH) (Takimoto et al., 1995). In GH_3 cells, TRH caused a significant decrease in Kv1.5 and Kv2.1 mRNAs resulting from a decrease in gene transcription rate. TRH also reduced Kv1.5 and Kv2.1 protein expression to a similar extent. Concomitant with a decrease in these subunits at the RNA and protein levels, voltage-gated potassium current density was also decreased. In particular, both an inactivating and a non-inactivating component of the current were reduced after long-term treatment with TRH.

The experiments in GH_3 cells indicate that changes in K^+ channel gene transcription rate have a direct impact on the expression of functional channels in the membrane, and suggest that gene transcription may be a primary level of regulation for these channels in the mammalian brain. Further studies are now necessary to establish whether the results obtained in the cell line GH_3 can be extended to pituitary cells *in situ* as well as to neural cells. Electrophysiological analysis in CA1 hippocampal neurons demonstrated that K^+ channel function was not modified by estradiol or progesterone, whereas Ca^{2+} channel activity was enhanced (*see below*). Further studies that analyze gene expression in the brain throughout development will be necessary to determine whether the hormones that are active in GH_3 cells also regulate K^+ channel expression in the brain.

Regulation of K^+ Channel Splice Variant Expression

A further level of complexity is represented by the fact that K^+ channel genes exist in several splice isoforms and that expression of distinct splice variants of a specific gene can be regulated by different cellular factors. For example, two splice isoforms of the Kv3.1 gene, a and b, are differentially regulated by bFGF and K^+-induced depolarization in rat cerebellar slice cultures (Liu and Kaczmarek, 1998). Expression of Kv3.1a and Kv3.1b is differentially regulated during cerebellar development. Kv3.1a is abundantly expressed at earlier developmental stages, whereas Kv3.1b is upregulated between postnatal d 8 (P8) and 14, and predominates in adult neurons. Com-

bined treatment of the slices with both bFGF and depolarizing K^+ concentrations selectively increases Kv3.1b transcript levels, whereas bFGF alone triggers a similar increase in both Kv3.1a and Kv3.1b. Interestingly, the effects of the growth factor alone are PKC-mediated, whereas bFGF + depolarization act through a PKC-independent pathway. These findings are physiologically relevant for a number of reasons. First, they contribute to our understanding of how the molecular and functional diversity of K^+ channels is generated in the nervous system (Luneau et al., 1991). Second, they identify some of the cellular signals that determine the temporal expression of Kv3.1a and Kv3.1b during cerebellar development. Third, they indicate that distinct but interacting intracellular pathways may positively or negatively regulate K^+ channel gene expression within the same cell population. Finally, they suggest that changes in K^+ channel expression may occur during critical phases of neuronal development to modulate excitability and frequency of action potentials (Liu and Kaczmarek, 1998).

Regulation of K^+ Channel Expression in Neurons by Glia

Heterologous cellular interactions may also affect K^+ channel expression in the brain, as demonstrated by the significant modulation of K^+ currents in hippocampal neurons by astrocytes. In mixed primary cultures, Wu and Barish (1994) found that in neurons in direct contact with astrocytes, a transient current (named A-current) was selectively upregulated, whereas a second type of transient current (D-current) was significantly reduced. A delayed rectifier current, on the other hand, was larger in neurons grown in isolation. The K^+ channel phenotype of hippocampal neurons was modified by astrocytes, because a direct comparison of current densities demonstrated that A-currents were predominant in neurons grown either on or directly in contact with astroglia, whereas D-currents were expressed at much higher levels in neurons distant from glia. Further analysis demonstrated that the astrocytic effects on neuronal K^+ currents were:

1. Not mediated by a soluble factor;
2. Required contact with *living* glia; and
3. Were RNA-synthesis dependent (Wu and Barish, 1994).

These findings further indicate that glial cells could directly or indirectly regulate the voltage-dependent channel phenotype in neurons, either by soluble factors or by cell–cell contact. Therefore, neuronal function is strongly dependent on glial elements, which not only buffer extracellular K^+ and amino acids (Kettenmann and Ransom, 1995), but also regulate the synthesis of membrane channels that ultimately determine the characteristics of action potentials in neurons.

Expression and Regulation of K^+ Channels in Glia

K^+ channel expression is also regulated in nonneuronal cells, astrocytes, oligodendrocytes, and Schwann cells. In glia, these channels are involved not only in K^+ spatial buffering, but also in the control of cell proliferation (Chiu and Wilson, 1989; Knutson et al., 1997). Macroglial cells display distinct K^+ channel phenotypes depending on developmental stage and proliferative state (Sontheimer et al., 1989; Barres et al., 1990b; Gallo et al., 1996; Knutson et al., 1997). For example, in cells of the oligodendrocyte lineage, dividing oligodendrocyte progenitors display large outward delayed rectifier and transient currents, but almost undetectable inward currents. In quiescent cells or differentiated oligodendrocytes, on the other hand, inward currents are the only K^+ conductances expressed (Sontheimer et al., 1989; Barres et al., 1990b; Gallo et al., 1996; Knutson et al., 1997).

Mitogenic factors stimulate functional expression of outward delayed rectifier K^+ currents in all types of glia, and agents that block these currents inhibit proliferation of astrocytes, oligodendrocyte progenitors, and Schwann cells (Chiu and Wilson, 1989; Pappas et al., 1994; Gallo et al., 1996; Knutson et al., 1997). It is not

yet established whether mitogenic factors induce outward K^+ current in glia by modifying subunit gene expression or by posttranslational mechanisms. Additionally, the identity of the K^+ channel subunits being regulated has not been elucidated. The finding that a variety of distinct factors that stimulate cell proliferation also trigger K^+ current expression suggests that common or converging intracellular pathways are being activated. Importantly, the involvement of K^+ channels in the regulation of G1-S transition in eukaryotic cell cycle has also been demonstrated for cells other than glia, such as lymphocytes (DeCoursey et al., 1984).

Ca^{2+} Channels

Ca^{2+} Channels Subunits and Subtypes

Voltage-dependent Ca^{2+} channels control a multitude of physiological processes in the central and peripheral nervous systems. These range from cell excitability to transmitter release, cell migration and differentiation, and stimulus-induced gene expression (Hess, 1990; Ghosh and Greenberg, 1995). Based on electrophysiological and pharmacological properties, Ca^{2+} channels have been classified into five distinct subtypes: T, L, N, P/Q, and R (Tsien et al., 1991; Walker and De Waard, 1998). These channels are differentially distributed and associated with diverse physiological functions. For example, L-type channels are ubiquitously expressed not only in the brain, but also in skeletal and cardiac muscle, in which they play a pivotal role in excitation–contraction coupling (Tsien et al., 1991). Conversely, N and P/Q channels are directly involved in exocytosis and neurotransmission, as demonstrated by functional experiments with specific channel blockers and by their localization at docked vesicles within presynaptic terminals (Nooney and Lodge, 1986; Takahashi and Momiyama, 1993; Wheeler et al., 1994).

Protein purification and molecular cloning have identified the presence of three distinct subunit types as structural units of voltage-dependent Ca^{2+} channels in the brain. Both L- and N-type channels contain a high-mol-w α1 subunit, which constitutes the ion pore of the channel and contains the voltage sensor (Tanabe et al., 1987; Witcher et al., 1993). The α1 subunit comprises a total of 24 transmembrane segments, organized in four highly homologous transmembrane domains, similar to the structure of sodium channels (Tanabe et al., 1987). The intermediate molecular weight α2-δ subunit, on the other hand, is formed by two polypeptides derived from the same gene that are linked by a disulfide bridge. The α2-δ subunit is anchored to the membrane by a highly hydrophobic region in the δ peptide. The extracellular domain of the α2-δ subunit peptide is heavily glycosylated (De Jongh et al., 1990). The low-mol-w β subunit is hydrophilic and cytoplasmic, directly interacts with α1, and is likely to be involved in anchoring of the channel (Hullin et al., 1992). An additional γ subunit has been identified in skeletal L-type channels. This comprises four transmembrane domains with cytoplasmic N- and C-termini (Wissenbach et al., 1998). So far, six α1 genes (α1A–E and α1S) have been identified, together with four β (β1–4) and one α2-δ (Snutch et al., 1991; Birnbauer et al., 1994). Ca^{2+} channel subunits exist in different splice variants, increasing enormously the structural and functional diversity of this class of channels (Ellis et al., 1988; Snutch et al., 1991; Birnbauer et al., 1994).

Expression During Development

Ca^{2+} current expression and regulation during development is rather complex; however, it appears that, as a general rule, upregulation of different Ca^{2+} channel currents occurs in parallel with cell differentiation in the central and peripheral nervous system. Also, the properties of the currents that first appear during nerve cell embryogenesis are essentially the same as those in terminally differentiated cells. Voltage-activated Ca^{2+} currents are found in embryonic neural cells (e.g., chick DRG and rat tectal progenitors) (Grantyn et al., 1989; Gottmann et al., 1991) and in stem cell lines (Strübing et al., 1997). In a variety of neurons, low voltage-activated (LVA) Ca^{2+} currents are

first expressed and soon downregulated in embryogenesis. For example, in chick ciliary ganglion neurons these currents are present between E4.5 and E8, but absent at E11 (Dryer, 1994). A similar downregulation has been observed in neurons of the rat inferior olive and hippocampus (Pettigrew et al., 1988; Thompson and Wong, 1991), as well as in chick motoneurons (McCobb et al., 1989) and in glia (Puro and Mano, 1991). It has been speculated that a physiological function of this current is to provide transmembrane Ca^{2+} influx at low resting potentials to regulate plasticity and modulate gene expression in embryonic neural cells during development (Ghosh and Greenberg, 1995; Hardingham et al., 1997). High voltage-activated currents, on the other hand, are upregulated in parallel with downregulation of the LVA currents in many cell types (Puro and Mano, 1991; Kostyuk et al., 1993; Lewis et al., 1993; Kobrinsky et al., 1994; Randall and Tsien, 1995).

In retinal ganglion cells, small Ca^{2+} currents were detected at E17/E18, and increased in amplitude until P16 (Schmid and Guenther, 1996). This result was obtained by patch-clamp recordings in retinal slices, indicating that in this neuronal population Ca^{2+} channel expression is developmentally regulated *in situ*. In embryonic stem cell-derived neurons, four distinct Ca^{2+} channel subtypes were found to be expressed, including L-, N-, P/Q-, and R-type, whose density increases during neuronal maturation (Strübing et al., 1995).

Regulation of Ca^{2+} Channels by Hormones

Ca^{2+} currents have been characterized in a large variety of neurons, including the different cell types of the hippocampus. In a particular subset of neurons, CA1 pyramidal cells, expression of distinct Ca^{2+} currents is developmentally regulated and is at least in part modulated by different hormones. Pyramidal neurons are a likely target of progesterone and estradiol, since they express intracellular receptors to these hormones (Pellettier et al., 1988; Hagihara et al., 1992). Joëls and Karst (1995) demonstrated that in hippocampal slices from ovariectomized rats the amplitude of both sustained and transient Ca^{2+} currents was enhanced by progesterone and estradiol. On the other hand, treatment with the same hormones did not modify voltage-gated potassium conductances. In CA1 pyramidal neurons, estradiol and progesterone cause significant changes in distal dendritic spine density (Gould et al., 1990). The changes in Ca^{2+} currents observed could therefore reflect enhanced Ca^{2+} influx through newly formed channels in dendritic spines developed after steroid treatment. The timecourse of the hormone effects on Ca^{2+} channel expression indicates that intracellular steroid receptors are most likely to be involved, rather than short-term effects on the cell membrane.

A genetic approach was used to demonstrate that corticosteroid hormones regulate the membrane properties of hippocampal CA1 neurons through the activation of mineralocorticoid (MR) and glucocorticoid (GR) receptors. Hesen et al. (1996) performed electrophysiological recordings in tissue slices prepared from mice carrying a targeted GR gene disruption. The voltage properties of the activation and inactivation of the two inward Ca^{2+} currents (I_{Ca}, non-inactivating ($I_{Ca,ni}$) and I_{Ca}, inactivating ($I_{Ca,i}$)) were similar in homozygous, heterozygous, and wild-type animals. In homozygous mice, i.e., in animals in which MRs were exclusively occupied, the amplitude of the Ca^{2+} currents in hippocampal neurons was significantly larger than in wild-type mice. Additionally, in adrenalectomized heterozygous or wild-type animals, where neither MR nor GR were activated, the amplitude of Ca^{2+} currents was also larger than in their respective controls. These data suggest that both receptor systems are necessary to regulate Ca^{2+} channel function in CA1 hippocampal neurons, because in the absence of GR activation, or of both MRs and GRs, similar effects were observed. Possible mechanisms include synergism or heterodimerization between the two receptors, as shown in recombinant in vitro systems (Trapp et al., 1994).

The role of corticosteroid hormones in the regulation of neuronal Ca^{2+} current expression may change during development (Karst et al., 1997). Adrenalectomy of 1–6-mo-old rats demonstrated that the resulting effects on distinct Ca^{2+} currents were developmentally regulated. In 1-mo-old rats, adrenalectomy only increased the high threshold $I_{Ca,ni}$, whereas in 6-mo-old rats a selective increase of the low threshold $I_{Ca,i}$ was observed. Both currents were significantly larger in adrenalectomized 3-mo-old rats. *In situ* hybridization analysis revealed that mRNA expression of the α1D L-type Ca^{2+} channel subunit was not modified after adrenalectomy, indicating that the effects of corticosteroids my be either at the protein level or on channel function.

Using a more sensitive technique, however, Nair et al. (1998) revealed significant changes in Ca^{2+} channel mRNA levels after adrenalectomy and corticosteroid treatment. These authors studied Ca^{2+} currents and Ca^{2+} channel mRNA expression in single hippocampal CA1 pyramidal neurons by combining electrophysiological and single cell reverse transcriptase-polymerase chain reaction (RT-PCR) analyses. Adrenalectomized rats were compared to sham-operated controls, as well as to adrenalectomized rats treated with different doses of corticosterone that would predominantly activate either MRs or GRs. MR occupation significantly decreased P/Q- and L-type Ca^{2+} channel mRNAs and proteins. On the other hand, additional occupation of GRs generated opposite effects on the same subunits. N-type subunits were not modified by any treatment. These changes at the mRNA level were not matched by similar alterations in Ca^{2+} currents, as determined electrophysiologically (Nair et al., 1998). These changes may have accured for several reasons, including the fact that electrophysiological whole-cell recordings were performed on acutely isolated neurons lacking part of their dendritic tree, where the changes in Ca^{2+} channel expression should have occurred after steroid treatment. Alternatively, the selective downregulation of a small population of Ca^{2+} channels may have not been apparent in whole-cell Ca^{2+} current measurements. Finally, the action of corticosteroid may be very complex and involve changes in other membrane conductances or properties.

Regulation of Ca^{2+} Channels by Growth Factors and Neurotrophins

The complexity of Ca^{2+} channel gene regulation is exemplified by a detailed study of the rat brain α_{1D} gene (RBα_{1D}), encoding an L-type calcium channel subunit (Kamp et al., 1995). This gene is transcriptionally regulated in NG108-15 neuroblastoma–glioma cells after differentiation with prostaglandin E_1 or retinoic acid, and mRNA encoding the RBα_{1D} subunit are detectable by Northern blot only after differentiation. Kamp et al. (1995) isolated a 15.2 kb clone that comprised part of the RBα_{1D} gene, including the 5′ upstream sequence. Using deletion analysis of RBα_{1D}-CAT constructs in transfected NG108-15 neuroblastoma–glioma cells, in combination with DNase footprinting and electrophoretic mobility shift assays, two distinct regions of exon 1 of the gene were found to be involved in transcriptional regulation of this subunit. These included an $(ATG)_7$ trinucleotide repeat that functions as an enhancer (Kamp et al., 1995). The molecular cloning of the RBα_{1D} subunit 5′ upstream sequence will facilitate not only the understanding of how Ca^{2+} channels can be transcriptionally regulated, but will also allow the identification of the DNA elements involved.

At least two distinct growth factors, NGF and bFGF, have been identified as modulators of L-type channel expression in the nervous system. Both factors have potent effects in the regulation of nerve cell development, and in particular in the acquisition of a differentiated neuronal phenotype, which includes functional expression of voltage-dependent Ca^{2+} channels. Shitaka et al. (1996) reported that in fetal hippocampal neurons chronic treatment with bFGF increased Ca^{2+} channel function. In Ca^{2+}-imaging experiments, high-K^+ stimulated calcium responses were significantly larger in bFGF-treated neurons than in control cells, and pharmacological analysis indicated that L-type

channels were mostly involved. Moreover, whole-cell electrophysiological experiments showed that high-voltage Ca^{2+} currents were increased in hippocampal neurons after treatment with bFGF. Interestingly, the newly formed L-type Ca^{2+} channels were found on both the cell body and neurites. Although the subunits regulated by the growth factor were not identified, treatment with RNA and protein synthesis inhibitors indicated that they were both required for the effect of bFGF.

The effects of NGF on Ca^{2+} channel expression were studied in two distinct systems, embryonic basal forebrain neurons and PC12 cells. In basal forebrain neurons, NGF treatment for a few days caused a significant increase in both the L- and the N-type components of Ca^{2+} currents, as determined electrophysiologically (Levine et al., 1995). This effect appeared to be rather selective, because: BDNF did not affect Ca^{2+} current expression and the total cell capacitance was not modified after growth factor treatment, indicating that the enhancement of Ca^{2+} currents was not caused by a general trophic effect of NGF on these cells.

Usowicz et al. (1990) demonstrated that PC12 cells differentiated with NGF express both L- and N-type Ca^{2+} channels, however, a preferential increase in N-type channels was observed after differentiation. Analysis of the changes in Ca^{2+} current expression triggered by NGF in PC12 cells was also performed by Lewis et al. (1993). Whole-cell patch-clamp recordings combined with a pharmacological analysis indicated that NGF increased functional expression of ω-conotoxin GVIA (ω-CgTX)-sensitive N-type Ca^{2+} channels. In the same study, neuronal differentiation of PC12 cells was induced by activation of the temperature-sensitive tyrosine kinase $pp60^{v\text{-}src}$. Genetically modified PC12 cells (PC12/v-*src*) differentiated morphologically by extending neurites in a fashion similar to NGF-treated cells. Importantly, activation of the tyrosine kinase caused changes in Ca^{2+} channel expression very similar to those triggered by NGF, including an increase in current density and enhanced expression of ω-CgTX-sensitive N-type Ca^{2+} channels. These findings further indicate that upregulation of Ca^{2+} channel expression is one of the obligatory steps that occur during neuronal differentiation, because the product of the v-*src* oncogene mimics several features of NGF-induced differentiation (Alema' et al., 1985).

The important question raised by these studies is whether the intracellular pathways that lead to morphological changes of nerve cells are also involved in their physiological differentiation, i.e., in the expression of voltage-dependent ion channels. Both bFGF and NGF activate receptor tyrosine kinases, which trigger a cascade of molecular events, including the activation of the membrane-associated GTP-binding protein $p21^{ras}$, B-Raf kinase, Mek-1 kinase, and the extracellular signal-regulated kinases ERK1 and ERK2 (Barbacid et al., 1991; Gomez and Cohen, 1991; Howe et al., 1992; Kyriakis et al., 1992; Thomas et al., 1992; Wood et al., 1992; Jaiswall et al., 1994; Marshall, 1995). Cell-cycle arrest and morphological differentiation of PC12 cells require sustained activation of the $p21^{ras}$/ERK pathway (Traverse et al., 1992, 1994; Cowley et al., 1994; Marshall, 1995), and NGF- and bFGF-mediated induction of ω-CgTX-sensitive Ca^{2+} channels also requires $p21^{ras}$ signaling (Pollock and Rane, 1996). However, sustained activation of this pathway *per se* induced morphological differentiation, but failed to increase Ca^{2+} current density (Pollock and Rane, 1996), indicating that additional signals are involved in the acquisition of a differentiated physiological phenotype in nerve cells.

Similar to PC12 cells, voltage-dependent Ca^{2+} channels are also upregulated by exposure to NGF in DRG neurons (Fitzgerald and Dolphin, 1997). The effects of NGF involve tyrosine kinase activation of $p21^{ras}$, but also the *c-src* tyrosine kinase $pp60^{c\text{-}src}$, suggesting that multiple and parallel pathways of regulation involving distinct kinases regulate Ca^{2+} channel expression during differentiation (Fitzgerald and Dolphin, 1997).

In addition to their role in membrane excitability and neurotransmitter release, Ca^{2+} channels play an important role in the modula-

tion of gene expression, plasticity during development, and survival of neural cells (Tsien et al., 1988; Koike et al., 1989; Johnson et al., 1992; Murrell and Tolkovsky, 1993; Ghosh and Greenberg, 1995). Furthermore, NGF and neurotransmitters can interact to modulate Ca^{2+} influx through the neuronal cell membrane, as shown by the effects of NGF and excitatory amino acids on survival and neurite outgrowth of cultured Purkinje cells (Cohen-Cory et al., 1991). These findings indicate that expression of Ca^{2+} channels is not only a landmark of neuronal differentiation, but may also be an obligatory step upstream of other processes that greatly affect nerve cell physiology and function.

Expression and Regulation of Ca^{2+} Channels in Glia

Like the other families of voltage-activated ionic channels, Ca^{2+} channels are also expressed in glial cells. In astrocytes, MacVicar (1984) first demonstrated that voltage-dependent Ca^{2+} channels are expressed at high density. In subsequent studies, low voltage-activated and high voltage-activated Ca^{2+} currents, presumably of the T- and L-type, respectively, have been found in astrocytes, oligodendrocytes, and Schwann cells both in culture and in vivo (Verkhratsky and Kettenmann, 1996). Voltage-dependent Ca^{2+} channels have also been identified in retinal glial cells (Newman, 1985).

Neuron–glia interactions modulate expression of voltage-dependent Ca^{2+} channels in astrocytes. In purified cortical astrocytes, L-type Ca^{2+} currents were induced by agents that enhanced intracellular cAMP levels, such as the β-receptor agonist isoproterenol, vasoactive intestinal polypeptide (VIP), and forskolin (Barres et al., 1989). The effects of these agents were mimicked by the cAMP analog 8Br-cAMP. In the same type of astrocytes, Corvalan et al. (1990) demonstrated that two types of voltage-dependent Ca^{2+} currents, otherwise not detected in purified astrocytes, could be found in cocultures with rat embryonic neurons. Both currents were blocked by Cd^{2+} and Co^{2+} and were not detected in astrocyte cocultured with oligodendrocytes (Corvalan et al., 1990).

The mechanism underlying Ca^{2+} channel induction by neurons or cAMP-elevating agents is not yet defined. Induction could occur at the transcriptional, translational, or posttranslational (protein phosphorylation) level. Channel subunits could be recruited from intracellular compartments to the membrane and assembled in new functional channels. Alternatively, nonfunctional channels may be present in the membrane and formation of functional Ca^{2+} channels may be triggered by the cytoskeletal rearrangements and morphological changes that occur in astrocytes upon cAMP elevation or interactions with neurons (Opas et al., 1986). The identification of the regulated subunits will facilitate a detailed analysis of the mechanism of induction of voltage-dependent Ca^{2+} channels in astrocytes and will also provide the basis for studying changes in Ca^{2+} channel expression in astrocytes during development in vivo.

Other endogenous factors can drastically modulate Ca^{2+} channel function in glia. For example, bFGF has been identified as a key regulatory factor of human retinal glial cells (Puro and Mano, 1991). Two types of voltage-dependent Ca^{2+} channels were identified in human retinal glia based on their functional features (Puro and Mano, 1991). Patch-clamp analysis revealed expression of both T- and L-type currents, displaying distinct threshold of activation and sensitivity to the L-type channel blocker nifedipine. bFGF selectively increased L-type Ca^{2+} current amplitudes within minutes after treatment of the cells (Puro and Mano, 1991). Retinal glial cells express bFGF receptors (Mascarelli et al., 1990). bFGF is present in the mammalian retina (Gospodarowicz et al., 1986) and is a potent mitogen for retinal glial cells (Puro and Mano, 1991). A direct link between the effects of bFGF on Ca^{2+} channels and on cell proliferation is presently lacking. However, the finding that nifedipine not only blocks L-type channels, but also the bFGF-induced mitogenic response, would strongly suggest that dihydroperidine-sensitive Ca^{2+}

channels play an important role in retinal glial proliferation.

Regulation of Ligand-Gated Ion Channels

Introduction

The group of ligand-gated neurotransmitter receptor ion channels is a receptor family comprising the nicotinic acetylcholine (nACh), glutamate, GABA, 5HT3, and glycine receptors. These receptors are multimeric structures often formed from different subunit families. Since subunit assembly plays an important role in generating ion-channel heterogeneity, modulating receptor subunit composition may be predicted to have important functional consequences. For instance, the adult muscle ε nAChR subunit, which replaces the fetal γ subunit, results in enhanced calcium permeability (Mishina et al., 1986). This was confirmed using recombinant receptors in Xenopus oocytes (Cens et al., 1997). In addition, ligand-binding properties are also determined by subunit composition. Molecular cloning of cDNAs for ligand-gated ion channel subunits has made it possible to study the pharmacology of recombinant receptors with defined subunit composition. Of notable clinical importance is the regulation of $GABA_A$ receptors by benzodiazepine receptor ligands, which depends on the type of α subunit variant in the receptor complex (Rabow et al., 1995). The sensitivity of the NMDA receptor to agonists, antagonists, glycine, histamine, spermine, and Mg^{2+} block is also conferred by subunit composition (Nutter and Adams, 1991; Buller et al., 1994; Williams, 1994; Williams et al., 1994; Zhang et al., 1994). Furthermore, the relationship between specific channel subunits and the effects of anesthetics on $GABA_A$ and glutamate receptors has been examined in detail (Harris et al., 1995). These examples clearly attest to the importance of understanding the molecular mechanisms that govern the levels of available subunit forms for the individual ion channels. Changes in receptor subunit stoichiometry can alter ligand sensitivity and hence gating properties of the ion channel. Although numerous pharmacological agents have been shown to regulate the expression of ligand-gated ion channel subunits, we have chosen instead to focus on the roles of endogenous effectors of development and homeostasis: peptide growth factors and steroids. A summary of these studies is given in Table 2.

Nicotinic Acetylcholine Receptors (nAChR)

nAChR Subunits and Subtypes

The neurotransmitter acetylcholine acts on two classes of receptors: nicotinic and muscarinic. Nicotinic receptors are ligand-gated ion channels, whereas muscarinic receptors are coupled to G proteins. The latter belong to a different superfamily that activates diverse second messenger pathways. Most nicotinic AChRs (nAChRs) on neurons are cation-specific, but do not distinguish readily among monovalent cations, although they have a significant permeability to Ca^{2+} (Nutter and Adams, 1991). Neuronal nAChRs are pharmacologically distinct from muscle nAChRs with regard to agonist potency. Some CNS nAChRs bind α-bungarotoxin (α-Bgtx); others do not (Sargent, 1993). In fact, the acetylcholine receptor family tree may be thought of as having three branches:

1. Muscle receptors, which bind the snake venom α-Bgtx;
2. Neuronal receptors that do not bind α-Bgtx; and
3. Neuronal receptors that do bind α-Bgtx.

Ganglionic nAChRs are also pharmacologically distinct from those in the CNS (Sargent, 1993). The best characterized nAChRs to date remain the muscle receptors present at the neuromuscular junction (NMJ). Although the primary focus of this article is neural systems, examination of gene expression in the well-established NMJ may provide useful models from which parallels in neural cells can be drawn.

Table 2
Regulation of Ligand-Gated Ion Channels[a]

Receptor channel	Modulator	Biological effect
nACh	NGF (superior cervical ganglion cells)	↑ α3, α4, α5, α7 subunit mRNA
	NGF (PC12 cells)	↑ Function, β4 subunit mRNA
		↑ α3 subunit mRNA
		↓ α3, α5, β3, β4 subunit mRNA
	CGRP (myotubes)[b]	↑ α subunit mRNA, binding
	Neuregulins (muscle cells)[b]	↑ α subunit mRNA
		↑ δ subunit transcription
		↑ ε subunit transcription
	Retinoic acid (P19 embryonal carcinoma)	↑ α3, α4, β2 mRNA, binding
$GABA_A$	NT3 (cortical neurons)	↑ Function
	PDGF-BB (hippocampal neurons)	↑ Function
	Insulin (hippocampal neurons)	↑ Function
	Neurosteroid (cortical neurons)	↓ Function
		↓ α3, α2, β2, β3 subunit mRNA
	Estrogen	↑ α2, γ1 subunit mRNA
	Progesterone (hippocampus)	↓ α1, α2 subunit mRNA
		↑ γ2 subunit mRNA
	Glucocorticoids (hippocampus)	↓ α1 subunit mRNA
		↓ γ2 subunit mRNA
		↓ β2 subunit RNA
Glutamate	bFGF (hippocampal neurons)	↓ NMDAR P-71 mRNA and protein
		↑ GluR1 subunit protein
		↑ AMPAR function
		↓ NMDAR function
	bFGF (oligodendrocyte progenitors)	↑ GluR1 subunit mRNA, protein
		↑ GluR3 mRNA
	PDGF + bFGF (oligodendrocyte progenitors)	↑ GluR1 mRNA, protein
		↑ AMPAR function
	Neuregulins (cerebellar slices)	↑ NR2C subunit mRNA
	NGF (PC12 cells)	↑ NR1 transcription
	Glucocorticoids (hippocampus)	↑ NR2A, NR2B subunit mRNA
		↑ NMDAR binding
		↑ KA1, KA2, GluR7 subunit mRNA
	Mineralocorticoids (hippocampus)	↑ GluR2 vs GluR1 subunit mRNA
		↑ NR2A vs NR2B subunit mRNA, NMDAR function
		↑ KA1, KA2, GluR6 subunit mRNA
	Estradiol + progesterone (hypothalamus)	↑ Binding
		↑ GluR1 subunit protein
	Estradiol (hypthalamus)	↑ GluR1, GluR2/3 subunit protein
	Testosterone (hypothalamus)	↑ GluR1, GluR2/3 subunit protein
	Estradiol (hippocampus)	↑ NMDAR1 subunit protein
		↑ NMDAR binding
	Anabolic androgens (hypothalamus and hippocampus)	↓ NR1, NR2A, and NR2B subunit mRNA

[a]Summary of the effects of hormones and growth factors on the expression of ligand-gated ion channels, as described in the text. For each modulator listed, the cell system or the brain region under study is indicated in parentheses. The symbols ↑ and ↓ indicate up- and downregulation, respectively. The nature of regulation by each modulator is specified in the last column. Function = channel activity, as measured by biophysical methods (e.g., electrophysiology, calcium imaging).

[b]Regulation of nACh receptors by CGRP and neuregulins in muscle cells was included (*see also* text), because the available detailed analysis in these cells may provide a model for similar studies in neural cells.

The subunit stoichiometry for the embryonic muscle receptors is $\alpha 2\beta 1\gamma\delta$ and the genes encoding all four subunit types have been cloned and sequenced. A fifth or 'adult' ε gene has also been cloned whose product substitutes for the fetal γ form later in development (Schuetze and Role, 1987; Witzeman et al., 1990). This change is correlated with an increase in the channel conductance and a decrease in the mean open time, leading to accelerated decay of synaptic currents (Mishina et al., 1986). Although the basic structure and function of neuronal nAChRs resemble those of the NMJ nAChR, many neuronal AChRs do not appear to function in the classic postsynaptic, directly excitatory process characteristic of NMJ. The pharmacology and electrophysiological properties of the NMJ have been described in detail (Galzi and Changeaux, 1995). The functional properties and regulation of neuronal nAChRs, which possess a higher level of subunit diversity and are expressed at lower levels and in more heterogeneous tissues, are, however, less well characterized.

The branch of neuronal, non-Bgtx-binding nAChRs is formed from combinations of $\alpha 2$, $\alpha 3$, $\alpha 4$, or $\alpha 6$ subunits with $\beta 2$ or $\beta 4$ subunits, sometimes including $\alpha 5$ or $\beta 3$ in addition (Sargent, 1993; McGehee and Role, 1995; Lindstrom, 1996, 1997). A very large number of permutations and combinations of subtypes are possible, but a few predominate. $\alpha 4\beta 2$ AChRs alone account for more than 90% of the high-affinity nicotine-binding sites in brain (Whiting and Lindstrom, 1988; Flores et al., 1992). The third branch includes AChRs that are antagonized by α-Bgtx. These are composed of $\alpha 7$, $\alpha 8$, or $\alpha 9$ subunits (Couturier et al., 1990; Schoepfer et al., 1990; Elgoyhen et al., 1994), which can function as homomeric receptors (Couturier et al., 1990; Seguela et al., 1993; Gerzanich et al., 1994). Schoepfer et al. (1990) have shown that brain structures that react with α-Bgtx contain the $\alpha 7$ subunit of the neuronal nAChR. Thus, eight neuronal nAChRα genes ($\alpha 2$–9) and three β genes ($\beta 2$–4) have been cloned (Boulter et al., 1986, 1990) and pleiotropy of the neuronal nAChR displaying a $2\alpha + 3\beta$ subunit composition in the mammalian CNS is caused by the multitude of different subtypes of subunits. Similar to the muscle α genes, neuronal α genes encode a ligand-binding subunit. Amino acid residues from β subunits also contribute to agonist and antagonist binding sites of neuronal nAChRs (Sargent, 1993). The distribution of these receptors as studied by *in situ* hybridization as well as with monoclonal antibodies revealed that individual genes display highly restricted, distinct, albeit overlapping patterns of expression (Deneris et al., 1989).

Regulation of nAChR Following Axotomy

Neuronal nAChRs mediate synaptic transmission in many parts of the vertebrate CNS, retina, and autonomic ganglia. Neuronal nAChRs are also expressed by PC12 cells (Greene and Tischler, 1976) and adrenal chromaffin cells (Maconochie and Knight, 1992; Gu et al., 1996), as well as by nonneural cells, such as the developing chick muscle (Corriveau et al., 1995). The expression of several nAChR transcripts has been shown to be regulated during rat brain development (*see* Zoli et al., 1995) and as the result of denervation or axotomy. The levels of $\alpha 3$ and $\alpha 7$ mRNA increase during the first 2 wk of postnatal development in the rat superior cervical ganglion (SCG), a period coinciding with formation of pre- and postsynaptic connections, whereas the levels of $\alpha 5$, $\beta 2$, and $\beta 4$ mRNAs remain unchanged.

It is thought that at least some developmental increases in nAChR subunit protein and transcript levels are caused by the process of innervation. Using chick ciliary ganglion neurons that have developed in the absence of innervation, Levey and coworkers (1995) showed that neuronal $\alpha 3$ and $\beta 4$ transcript levels were increased by innervation and target tissue interactions, but $\alpha 5$ transcripts were increased only by innervation. In another paradigm supporting a role for innervation, axotomy of adult rat SCG results in dramatic decreases in $\alpha 3$, $\alpha 5$, $\alpha 7$, and $\beta 4$ subunit transcripts, but an increase in $\beta 2$ (Zhou et al., 1998).

These two examples of segregated regulation of nAChR gene expression suggest that transcriptional mechanisms lead to heterogeneous expression within the nervous system. At least two kinds of retrograde signals have been postulated to regulate changes occurring in axotomized peripheral neurons. NGF, for one, has been shown to prevent the axotomy-induced decrease in tyrosine hydroxylase activity (Kessler and Black, 1979; Federoff et al., 1992). A second signal, leukemia inhibitory factor (LIF), has been shown to be produced by nonneuronal cells after axotomy (Banner and Patterson, 1994; Curtis et al., 1994) and to mediate increases in galanin, VIP, and substance P content in SCG organ cultures (Sun et al., 1994; Sun and Zigmond, 1996). Zhou et al. (1998) found that NGF, when applied in vitro to rat SCG explants, partially rescued, in a dose-dependent manner, the levels of the α3, α5, α7, and β4 subunits, but had no effect on β2. Although similar in vitro studies were not reported for LIF, the finding that the decrease in expression of nAChR transcripts was not significantly different between $LIF^{+/+}$ and $LIF^{-/-}$ mice following axotomy led the same authors to conclude that LIF is not involved in axotomy-induced changes in AChR expression. However, given that there were several notable differences in nAChR expression between rat and mouse, namely in transcript characteristics, it remains possible that subtle species differences may extend to include factors that are involved in regulating gene expression following exotomy or injury.

Although functional changes are often observed to be associated with changes in subunit gene expression at the RNA level, there are numerous instances where this does not occur. A large reduction in receptor mRNA in cultured cells, compared with levels in vivo, need not result in functional changes, such as ligand binding, because posttranscriptional events, such as increased efficiency of translation, subunit assembly, or receptor stabilization, may regulate functional properties of receptors to compensate for low transcript levels (Corriveau and Berg, 1994). Conversely, ACh sensitivity can be increased by cAMP analogs or phosphodiesterase inhibitors without the need for new protein synthesis (Margiotta et al., 1987), because of an apparent conversion of nonfunctional receptors to a functional state rather than a change in single-channel properties. The neuropeptide VIP, a potent stimulator of intracellular cAMP levels, can regulate ACh receptors in chick ciliary ganglion neurons (Gurantz et al., 1994). As a result, an increase in ACh-induced conductance response was observed, which may be attributed to a posttranslational response. There is also evidence that phosphorylation mechanisms can operate to increase the surface appearance of nAChRs posttranslationally (Rothhut et al., 1996).

Regulation of nAChR Expression by NGF and Other Peptide Factors

It is known that NGF influences the growth and differentiation of selective populations of cholinergic neurons in the CNS (Halegoua et al., 1991). PC12 cells express a cluster of neuronal nAChR genes (β4, α3, α5) (Boulter et al., 1990) and respond to NGF treatment with a broad spectrum of changes that lead to the acquisition of a sympathetic neuron-like phenotype *(see above)*, including an increase in the number of functional AChRs (Amy and Bennett, 1983). NGF treatment for 7 d has been shown to increase the steady-state RNA levels of α3, α5, α7, β2, and β4 subunits and consequently, the occurrence of ACh-induced single-channel activity (Henderson et al., 1994) in PC12 cells. NGF is known to initiate a signaling cascade that involves a wide variety of second messenger pathways that may be protein kinase A (PKA)-dependent (Cremins, 1986; Ginty et al., 1992) or PKA-independent (Damon et al., 1990; Ginty et al., 1991). Henderson et al., (1994) have found that the NGF-mediated induction of nAChR mRNAs occurs in both wild-type and PKA-deficient PC12 cells, providing evidence that, unlike its effect on sodium channel expression, the response of nAChRs is PKA-independent. PC12 cells are also amenable to genetic approaches,

which have been used to isolate mutant sublines (Bothwell et al., 1980; Burnstein and Greene, 1982), including NGF nonresponsive variants (nnr). These variants, which express p75 but undetectable levels of Trk (Loeb et al., 1991), were used to study the mechanisms underlying the actions of NGF on nAChR expression (Fanger et al., 1995). In nnr cells, there is a visible decrease in the basal expression of β2 and β4 subunit mRNAs accompanied by an increase in α3 mRNA levels compared to wild-type PC12 cells (Fanger et al., 1995). Since coexpression of β subunits with α subunits is often required to produce functional nAChR channels, the decreased levels of β subunit mRNAs in nnr cells could result in a dramatic decrease in the expression of functional nAChR, although this was not reported. Neither treatment with NGF nor transfection with a cDNA encoding a Trk A neurotrophin receptor was able to restore the response to NGF, suggesting that there are influences on nAChR expression that override normal trk receptor activation.

The NGF-stimulated increase in nAChR expression in wild-type PC12 cells has been examined at the DNA level with respect to β4 expression, and it has been shown to be at least partly the result of enhanced transcriptional activity mediated by sequences located within a 226-bp region spanning –89 to +137 (Hu et al., 1994). Whiting et al. (1987) reported contrasting results—a decrease in α3 mRNA after a 3-d treatment with NGF. Rogers et al. (1992) also described a decrease in levels of α3, α5, β3, and β4 mRNAs following 48 h treatment with NGF, whereas β2 levels were increased. These changes occurred in the absence of any clear changes in ACh-induced current. Thus, even though NGF affects the expression and function of nAChRs, the nature of its effects may well depend on the PC12 subclone under study and treatment paradigm employed.

Although transcription initiation sites of the α2, α3, β2, and α7 subunits have been reported (Matter-Sadzinski et al., 1992; Bessis et al., 1993; Yang et al., 1994, 1995), identification of DNA sequences mediating growth factor-induced responses have lagged behind studies of *cis*-acting determinants of neuron-specific expression (Bigger et al., 1996, 1997; Milton et al., 1996; Bessis et al., 1997; Du et al., 1997; McDonough and Deneris, 1997; Yang et al., 1997). The transcription of the chicken α7 gene is known to be regulated by DNA elements that control neuronal specificity of the promoter (Matter-Sadzinski et al., 1992). Promoter elements have also been identified in the chick β3 gene that confer cell-specific expression in the developing retina (Matter et al., 1995).

Interestingly, a novel 19-bp motif in the rat β4 gene that specifically binds a brain-enriched nuclear protein (Hu et al., 1995) lies within the same 226-bp 5′ flanking region that was shown to mediate NGF-stimulated transcriptional activation in transiently transfected PC12 cells (Hu et al., 1994). The DNA-binding factors were identified as NARPs, or neuronal nAChR receptor promoter-binding proteins, a homolog of Purα, which stimulates transcription of the myelin basic protein gene in oligodendrocyte cells (Haas et al., 1995). It is, however, still unclear which *trans*-acting factors regulate the NGF response of the β4 gene.

Another peptide factor, calcitonin gene-related peptide (CGRP), shown to be present in the NMJ, was found to increase the expression of α subunits of muscle nAChR (Changeux et al., 1992). In fact, CGRP has been shown to increase α-subunit mRNA levels and the number of α-bungarotoxin binding sites in chick myotubes (Fontaine et al., 1987). This suggests that an interaction between CGRP and nAChR might also operate in the CNS. Indeed, in the primate forebrain, localization of nAChR and CGRP was demonstrated in the basal nucleus of Meynert (BNM) by immunohistochemistry (Csillik et al., 1998). The origin of CGRP is thought to be extrinsic since no CGRP-immunopositive perikarya in BNM were found, indicating that CGRP-containing axons establish contacts with cholinergic cells. Although direct evidence of a role for CGRP in neuronal nAChR maintenance is still lacking, this study provides some microstructural support for the notion that, in a manner similar to that in NMJ,

CGRP can potentially contribute to neuronal nAChR expression or function.

There is now growing interest in the regulation of nAChR expression by neuregulins, a group of motor-derived trophic factors that comprise neu differentiation factor, heregulin, glial growth factor, and acetylcholine-receptor-inducing activity (Carraway and Burden, 1995). These factors function as ligands for epidermal growth factor receptor-related (erbB) tyrosine kinases and stimulate nAChR synthesis at the NMJ (Falls et al., 1993; Marchionni et al., 1993; Altiok et al., 1995; Chu et al., 1995; Jo et al., 1995; Zhu et al., 1995). Muscle innervation induces expression of adult-type nAChRs at the endplate, and the synaptic induction of nAChR genes by neuregulins has been shown to require activation of a Ras/MAP kinase pathway (Si et al., 1996; Tansey et al., 1996). Protein-tyrosine phosphatase (PTPase) activity also selectively suppresses the adult-type genes and contributes to synapse-specific expression of nAChRs (Sapru et al., 1994). The molecular mechanism underlying the neuregulin response involves the activation of MAP-kinase responsive proteins, such as the Ets-2 transcription factor, a member of a family of factors bearing an ETS DNA-binding domain (Donaldson et al., 1996). ETS domain proteins are a class of sequence-specific transcription factors that have been implicated in regulating vertebrate cell phenotypes, based on evidence from Drosophila *ets* genes *Pointed* and *Yan*, which act antagonistically in R7 photoreceptor neuron induction and differentiation (O'Neill et al., 1994; Treier et al., 1995).

A role for the Ets-2 transcription factor is indicated in the expression of the adult-type muscle nAChR. A 15-bp promoter sequence in the rat muscle ε subunit gene, which bears a putative Ets binding site, is critical not only for neuregulin- and Ras-dependent induction of this gene, but also its suppression of transcriptional activity in response to PTPase. Furthermore, neuregulin-dependent induction of the ε gene is abrogated by overexpression of an Ets-2-dominant negative mutant (Sapru et al., 1998). Similar mechanisms have not been revealed for the neuronal nAChRs, but interestingly, a novel ETS-domain factor, Pet-1, was isolated in PC12 cells. In cotransfection assays, Pet-1 activates the transcription of reporter constructs bearing the 3′ untranslated exon of the neuronal β4 gene that contains two consensus ETS binding sites (Fyodorov et al., 1998). The expression of Pet-1 was shown to be enriched in neural cells and adrenal gland, and its activity as a transactivator observed to be cell type-specific. Pet-1 is also expressed in rat brain, and although both the adrenal gland and SCG arise from the sympathoadrenal sublineage of the neural crest, Pet-1 gene expression was absent in the SCG, raising the possibility that it may act as a regulator of terminal differentiation of the chromaffin cell phenotype (Fyodorov et al., 1998). It remains to be seen, however, whether the restricted expression and activity of Pet-1 may arise from an intrinsic program of differentiation or result from the convergence of trophic signals upon neural cells during development.

Regulation of nAChR Expression by Retinoic Acid

In addition to PC12 cells, another system worthy of further study is P19 mouse embryonal carcinoma cells. These cells differentiate into cell types reminiscent of those in the mammalian nervous system, including neuronal-like cells, following treatment with retinoic acid (RA) (Bain et al., 1994). P19 cells differentiated by RA express neuronal markers, including glutamate receptors (Ray and Gottlieb, 1993), acetylcholinesterase, and neurofilament proteins (Jones-Villeneuve et al., 1982). P19 cells coexpress nAChR α4 and β2, as demonstrated by dual-label immunocytochemistry and single-cell PCR. In contrast, NGF-differentiated PC12 cells do not express α4 and consequently lack the high-affinity nicotine binding sites that are found in nervous tissue. Nonetheless, in this embryonal cell system that expresses the α3, α4, α5, β2, β3, and β4 transcripts, α3, α4, and β2 subunits are selectively upregulated by retinoic acid (Cauley et al., 1996). Retinoic acid treatment and aggregation

of P19 cells induces them to express nAChR subunit RNAs and proteins as well as high-affinity nicotine binding sites similar to those in the adult mammalian brain. Neither retinoic acid nor cell adhesion to plastic alone was sufficient to induce this increase, suggesting that some event in the process of cell differentiation, other than, or in addition to, a direct genomic action of RA on the upregulated genes, such as cell–cell contact, may be involved in modulating gene expression.

Although significant progress has been made in the identification of distinct acetylcholine receptor subunits and their pharmacological characteristics, it is apparent that the mechanisms controlling the expression of neuronal nicotinic receptor gene combinations exhibiting ligand binding comparable to that in vivo are still unclear. Unraveling such regulatory pathways will contribute greatly to understanding the role and mode of function of these receptors in the developing nervous systems.

GABA Receptors

$GABA_A$ Receptor Subunits

GABA is the major inhibitory neurotransmitter in the CNS. GABA receptors can be classified according to their respective transduction mechanisms following activation as the $GABA_A$ and $GABA_B$ receptor, respectively. The predominant type, termed $GABA_A$, and a recently identified type, $GABA_C$, have integral chloride-selective channels, whereas metabotropic $GABA_B$ receptors are coupled to Ca^{2+} or K^+ ion channels via GTP-binding proteins. Molecular cloning has revealed a large $GABA_A$ receptor gene family comprising 6 α, 4 β, 3 γ, 1 δ, 2 ρ, and 1 π subunits, which are believed to form pharmacologically distinct pentameric receptor isoforms. Heterogeneity is further increased by alternative exon splicing of pre-mRNA, which generates, from one gene, two differently distributed forms of γ2 and β2 subunits, with short and long peptides (Glencorse et al., 1991; Harvey et al., 1994). In addition to GABA, the $GABA_A$ receptor also binds several allosteric modulators, including benzodiazepines, steroids, and barbiturates (Macdonald and Olsen, 1994). Along with the α subunit, studies show that γ subunits are particularly important in determining the pharmacodynamic properties of $GABA_A$ receptors and their affinity to benzodiazepine compounds, which in turn increases the potency of GABA without altering maximal conductance of the channel.

Although the exact composition and stoichiometry of native $GABA_A$ receptors remains a challenge to study, reports of ligand-binding patterns (Bureau and Olsen, 1993) and receptor subunit expression (Wisden et al., 1992) hint at the presence of a wide variety of receptor isoforms. Native $GABA_A$ receptors appear to be heterooligomeric complexes and numerous permutations for the assembly of $GABA_A$ receptors are possible, although some combinations of receptor subunits may preferentially aggregate (Burt and Kamatchi, 1991). The distribution of mRNAs in the CNS determined by *in situ* hybridization is very different for each subtype. For instance, the ρ subunit is expressed predominantly in retina (Cutting et al., 1991) and the α6-subunit mRNA is localized almost exclusively in cerebellar granule cells, whereas various α, β, and γ subtypes and one δ subunit show very different regional and developmental distribution (Richards et al., 1991; Poulter et al., 1992; Wisden et al., 1992).

Developmental Regulation of $GABA_A$ Receptor Expression in Cerebellum

$GABA_A$ receptors develop in spatiotemporal synchrony with GABAergic pathways in the rat (Schlumpf et al., 1989; Cobas et al., 1991) and these receptors appear to be functional, based on electrophysiological criteria in acutely dissociated cells (Fiszman et al., 1990; Serafini et al., 1995). Insight into the rules governing coordinated expression of receptor subunits was provided by the observation that δ subunit expression was repressed in cerebellar granule cells of $\alpha6^{-/-}$ animals (Jones et al., 1997), although this inhibition was reported to

occur at the posttranslational level. Ablation of α6 expression resulted in dramatic reductions in cerebellar muscimol binding and benzodiazepine-insentive GABA-induced currents whereas α1 protein and binding properties were not significantly affected (Jones et al., 1997). Thus, the translation of one subunit appears to be dependent on the stable expression of another.

The mechanisms that underlie developmental regulation of $GABA_A$ subunit expression may involve both intrinsic mechanisms and local extrinsic factors. The spatiotemporal association of GABAergic innervation with the expression of functional $GABA_A$ receptors suggests that afferent innervation may stimulate expression of the receptors. In fact, Meinecke and Rakic (1990) demonstrated that GABAergic axons innervate recently migrated granule cells prior to the appearance of $GABA_A$ receptors, suggesting that GABA released from these afferents could modulate expression of its own receptor. β_2 and γ_2 may be similarly regulated by granule cell afferents. Cerebellar granule neurons follow an intricate and well-characterized pathway of postnatal development. Early postnatal rat cerebellar cells cultured under defined conditions yield a nearly homogeneous neuronal population, making them a model system for studying cell-specific developmental processes. Morphological and biochemical differentiation in cerebellar granule cells cultured from 8-d-old rats has been extensively characterized (Gallo et al., 1982). Studies have demonstrated that receptor gene expression in the developing cerebellar cortex undergoes striking changes. mRNAs encoding α1, β2, and γ2-subunits are low in the first postnatal week, but they rapidly rise two- to fourfold between P10 and P17 in the granule and Purkinje cell layers (Gambarana et al., 1990, 1991). This period of development temporally coincides with cerebellar maturation and synaptogenesis and events occurring during this time may modulate receptor expression. To determine whether these postnatal increases in vivo were preprogrammed or dependent on extrinsic factors, Beattie and Siegel (1993) followed $GABA_A$ receptor subunit expression in cultured cerebellar granule neurons prepared from rats during various stages of development. Cultures taken from embryonic d 19 and postnatal d 2 rats showed constant levels of subunit mRNAs over 20 and 16 d in culture, respectively, whereas those from P10 showed a dramatic rise in β2 and γ2 subunit RNAs by 7 d in culture. The magnitude and time-course of these increases were similar to the developmental changes that occurred in vivo, indicating that granule neurons received extrinsic developmental cues prior to P10. α1, α6 mRNAs and diazepam-insensitive binding, which is characteristic of α1α6-containing $GABA_A$ receptors, are also upregulated in P2 and P10-derived cultures (Thompson et al., 1996a). Despite the observation that chronic *N*-methyl-D-aspartate (NMDA) receptor agonists promote α1 and α5 subunit expression (Memo et al., 1991), the hypothesis of afferent activation of ionotropic glutamate receptors was excluded since antagonists of non-NMDA and NMDA receptors were without effect (Thompson et al., 1996a). Thus, the factor(s) responsible for mediating these changes has yet to be identified, although a study showing an induction of α1 subunit expression with forskolin (Thompson et al., 1996b) hints at a regulatory role for metabotropic receptor activation.

Regulation of $GABA_A$ Receptor Expression by Growth Factors

To date, there has been little direct evidence to link peptide growth factors with GABA receptor synthesis. Although the effects of neurotrophins on survival and differentiation of various neuronal populations have been well studied, much less is known about their role in synaptic function. Members of the NGF family of neurotrophic factors and PDGF modulate synaptic transmission in cortical and hippocampal neurons (Kim et al., 1994; Kang and Schuman, 1995; Valenzuela et al., 1997). Neurotrophin-3 (NT-3) was shown to increase the frequency of spontaneous action potentials in

developing cortical neurons by reducing GABAergic synaptic transmission (Kim et al., 1994). This effect was, however, transient, and represents a novel mode of action of neurotrophins, in addition to their well-established long-lasting effects on neuronal survival and differentiation. Reports of modulatory actions on hippocampal GABA receptors also describe rapid inhibitory responses elicited by other growth factors like PDGF. Brief treatment with nanomolar concentrations with PDGF-BB produced long-lasting reversible inhibition of $GABA_A$ receptor-mediated inhibitory synaptic transmission in hippocampal CA1 neurons and mouse brain membrane vesicles. Further examination in Xenopus oocytes, using recombinant $GABA_A$ receptors and PDGF receptors, revealed that PLCγ was involved in the PDGF-induced increase in intracellular Ca^{2+} levels and that this was independent of subunit composition of $GABA_A$ receptors (Valenzuela et al., 1995). This latter result indicates that the process is unlikely to affect subunit synthesis and hence points to a posttranslational mode of functional modulation.

The pancreatic hormone insulin was reported to promote the surface expression of functional $GABA_A$ receptors (Wan et al., 1997). It was found that the β2 subunit was required for insulin-induced increase in surface expression of the receptor, since HEK293 cells transfected with subunit combinations that excluded β2 subunit showed little surface labeling. The translocation was further confirmed in hippocampal neurons, in which the amplitude of inhibitory currents was enhanced through increasing the number of functional postsynaptic $GABA_A$ receptors. This example of insulin-induced synaptic plasticity in neural cells, again, did not involve *de novo* receptor synthesis, but rather the rapid translocation of subunits to postsynaptic membranes.

Regulation of $GABA_A$ Receptor Expression by Steroid Hormones

There is now substantial evidence to indicate that steroid hormones are important regulators of $GABA_A$ receptor function as well. Despite early observations of rapid steroid effects that were incompatible with modulation of nuclear transcription via genomic events, nongenomic steroid action has been widely recognized only recently. Steroids, known to originate primarily from the gonads and adrenal glands, can also be synthesized in the brain (Jung-Testas et al., 1989; Akwa et al., 1993; Koenig et al., 1995), through the actions of 5α-reductase and 3α-hydroxysteroid dehydrogenase activities present in neural cells. Considerable interest has focused on the actions of progesterone metabolites on $GABA_A$ receptors (Majewska, 1992; Paul and Purdy, 1992). Two major metabolites of progesterone 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP) and 3α,21-dihydroxy-5α-pregnan-20-one (THDOC) augment GABA receptor-mediated chloride ion conductance in cultured rat hippocampal neurons (Majewska et al., 1986). These neurosteroids compete for high-affinity binding to the $GABA_A$ receptor complex and were found to be modulators of GABA responses in native membranes as well as in reconstituted $GABA_A$ receptors (Majewska et al., 1986; Puia et al., 1990).

Although many studies have focused on the acute nongenomic effects of neurosteroids on neural transmission (for reviews, *see* Joëls and de Kloet, 1992; Wehling, 1997; Alonso and Lopez-Coviella, 1998), few have examined their more long-term genomic effects of neurosteroids. Recently, treatment for 5 d with the progesterone derivative 5α-pregnan-3α-ol-20-one, or 5α3α, was found to produce downregulation of $GABA_A$ binding sites and $^{36}Cl^-$ influx in isolated cortical neurons (Yu and Ticku, 1995a,b). These effects were also associated with decreased efficacy of GABA-induced chloride ion influx and pentobarbitol potentiation of GABA currents (Yu and Ticku, 1995b; Yu et al., 1996a). The molecular basis was shown to involve a decrease in $GABA_A$ receptor α3 and α2 subunit as well as β2 and β3 subunit mRNA levels (Yu et al., 1996b). Since α- and β-subunits are needed to bind GABA agonists and α variants determine the degree of coupling between GABA and benzodiazepine

sites, the decrease in α3 and α2 mRNA could result in functionally different $GABA_A$ receptor isoforms, thus explaining the heterologous uncoupling with neurosteroid treatment. By the same token, decreased β2 and β3-subunit mRNA levels following 5α3α treatment could result not only in downregulation of GABA binding, but also decreased efficacy of GABA to elicit GABA responses.

Gonadal steroids have also been demonstrated to affect GABA receptor function. Although there is as yet no evidence to suggest that estrogen is a direct allosteric modulator of GABA receptor function, studies have implicated a role for estrogen in the regulation of ligand binding to the $GABA_A$ receptor (Maggi and Perez, 1986; Lasaga et al., 1988; O'Connor et al., 1988). Of all the neuronal phenotypes shown to express estrogen receptors in the preoptic area (POA), GABA neurons are the most abundant. It was found that the POA and bed nucleus of the stria terminalis (BNST) of the rat, regions known to be rich in estrogen receptor-containing cells (Cintra et al., 1986; Simerly et al., 1990), coincide with areas of the brain where estrogen altered the binding of the $GABA_A$ channel blocker ^{35}S-butylbicyclophosphorothiorate but not of agonist ^{3}H-muscimol (O'Connor et al., 1988; Canonaco et al., 1993). This functional restriction indicated that transcriptional regulation by estrogen of GABA transmission was plausible. Experiments have shown that estrogen has a stimulatory influence on preoptic GABA concentrations, and there is evidence that estrogen upregulates transcription of the GAT-1 GABA transporter gene (Herbison, 1997). In agreement with increased sensitivity of GABA transmission with estrogen, Herbison and Fenelon (1995) have mapped by *in situ* hybridization estrogen-responsive GABA receptor subunit transcripts in the adult female rat. There may be some discrepancy over $GABA_A$ receptor subunit distribution in the POA—α1,2,3,5, β1–3, and γ1,2 have been reported (Araki and Tohyama, 1992; Wisden et al., 1992; Peterson et al., 1993a), but Wisden et al. (1992) showed using *in situ* autoradiograms that only three subunits, α2, β3, and γ1, were expressed at reasonably high levels in the POA and BNST. Herbison and Fenelon (1995) determined the effects of estrogen replacement in ovariectomized rats with respect to GABA receptor subunit expression and found significant increases in α2 and γ1 mRNA in the medial preoptic nucleus (MPN) and principle encapsulated nucleus of the BNST following estrogen treatment for 7 d, but not after 24 h. No changes at all were observed in the β3 subunit. Dual-lable immunocytochemistry demonstrated that about two-thirds of the α2 subunit-immunoreactive cells in the MPN also possessed the estrogen receptor. This, coupled with the time-course of induction, indirectly hints at a genomic effect of estrogen on subunit expression. The fact that β3 subunit is not altered by estrogen treatment was supported by studies of Peterson et al. (1993b), suggesting that the number of $GABA_A$ receptors utilizing the β3 subunit remains stable and that the α and γ subunit content within a β3-containing $GABA_A$ receptor may occur, although these issues still need to be addressed.

In defined regions within the hippocampus, progesterone was found to suppress the levels of α1 and α2 RNA while stimulating expression of γ2 (Weiland and Orchinik, 1995). Its suppressive effects were dependent on estradiol priming, which is consistent with the induction of progesterone receptors by estrogens (Parsons et al., 1982), but the stimulatory effect cannot be explained by a simple mechanism because combined treatment with estradiol and progesterone was without significant effect, as was estradiol alone. The implications of such steroid responses remain unclear but may be expected to involve enhanced benzodiazepine sensitivity of hippocampal $GABA_A$ receptors, which is a γ2-dependent event.

In addition to gonadal steroids, adrenocorticosteroids also modulate the activity of the GABA receptor system. In the hippocampus, short-term adrenalectomy results in increased levels of α1, α2, β2, and γ2 mRNA, whereas β1

levels remained unchanged. These changes were region-specific: α1 and γ2 increased in CA3, β2 in the dentate gyrus and CA2, but no modulation was observed in CA1 or cingulate cortex. These effects were reversed by cortisol replacement (Orchinik et al., 1994), showing that corticosterone was responsible for the observed changes in subunit expression. The suppression of α1 mRNA levels in corticosterone-treated animals is somewhat consistent with the decrease in α1 subunit mRNA levels in whole mouse hippocampus following chronic exposure to swim stress (Montpied et al., 1993), although the two paradigms are clearly distinct. If changes in mRNA levels reflect corresponding changes in functional proteins, then corticosterone-induced changes in subunit expression might potentially modulate GABAergic synaptic inhibition by altering the subunit composition and hence the pharmacological properties of the assembled receptors. Indeed, expression studies have found that $GABA_A$ receptor subunit composition alters the efficacy of GABA to stimulate chloride ion conductance through changes in the gating properties of recombinant channels (Macdonald and Angelotti, 1993). Clearly, further studies at the genomic level will be needed to address the molecular pathways mediating steroid-induced changes in $GABA_A$ receptor subunit gene expression.

Glutamate Receptors (GluRs)

GluR Subunits and Subtypes

Glutamate is the major mediator of fast excitatory transmission in the mammalian CNS. GluRs are found throughout both the central and peripheral nervous systems, in neurons and glia, as well as in some other cell types, including endocrine cells of the pituitary and pineal glands. Two broad categories of GluRs have been identified: ion channel-forming or "ionotropic" and the "metabotropic" receptors, coupled to GTP-binding proteins and linked to phospholipase C or adenylate cyclase activity. The ionotropic receptors are further subdivided into three distinct, cation-selective groups based on their preferred agonists, *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole acid (AMPA), and kainate (KA). Each type of GluR includes a variety of subunits and their variants. To date, 22 individual ionotropic GluR genes have been identified, which are classified into subfamilies according to amino-acid homology. It is possible that still more genes remain to be discovered. Receptor diversity is increased even further by differential splicing of multiexon genes or by mRNA editing, a recently discovered mechanism that specifically alters a single codon, usually at a position critical for a functional property of the protein (for reviews, *see* Hollmann and Heinemann, 1994; Seeburg, 1996). The NMDA receptors consist of NR1 subunits and NR2 subunits, of which there are four distinct genes, NR2A–D. GluR1–4 have high-binding affinity for AMPA, whereas GluR5–7 and KA1–2 have higher affinity for kainate. AMPA- and kainate-type subunits do not readily coassemble (Wenthold et al., 1992; Puchalski et al., 1994), and it has been shown that AMPA- and kainate-preferring receptors can exist simultaneously within single cells as functionally independent entities and are unlikely to crossassemble between groups (Partin et al., 1993). GluRs 1–4 form functional heteromeric assemblies with each other and there is electrophysiological evidence that within the kainate family, KA2 can form heteromeric channels with GluR5, GluR6, and GluR7 (Herb et al., 1992; Howe, 1996; Swanson et al., 1996; Schiffer et al., 1997). Initially, AMPA receptor ion channels in neurons were thought to be formed by a combination of five subunits of the GluR1–4 proteins (Wenthold et al., 1992) just as neuronal NMDA receptors have been assumed to have a pentameric structure (Chazot et al., 1994). However, recent reports using electrophysiological and biochemical approaches have since provided evidence that GluRs may in fact share tetrameric structures with the voltage-gated potassium

channels (Mano and Teichberg, 1998; Rosemund et al., 1998).

Regulation of GluR Subunit Expression Following Axotomy

Recent studies have indicated that the NMDA receptor may be involved in neuron death after axotomy. Blockade of NMDAR rescues spinal motoneurons after peripheral nerve section in the neonatal rat (Sanner et al., 1994). NMDA receptor subunits NR1, NR2B, and NR2D were identified in spinal motoneurons (Monyer et al., 1992; Tolle et al., 1994). Piehl et al. (1995) have examined the levels of NR1, NR2B protein and NR2B RNA in two lesion models leading to partial motoneuron death: neonatal sciatic nerve transection and ventral root avulsion in the adult. A clear downregulation of subunit expression was observed. This is similar to the expression of other mRNAs encoding proteins involved in neurotransmission (Piehl et al., 1993). It is known that application of NMDAR antagonist MK-801 increases survival of severed neurons (Mentis et al., 1993) and that administration of NMDA 1 wk after crushing sciatic nerve in postnatal rats induces neuronal death (Greensmith et al., 1994), suggesting that neuronal death following axonal injury may be a consequence of NMDA excitotoxicity. The downregulation of NMDAR following axotomy may thus appear inconsistent with its neurotoxic role, but several explanations may exist. Glutamate-induced excitotoxicity may occur before downregulation of receptor expression, or susceptibility to glutamate may be the result of changes in the chemical environment. Indeed, as previously mentioned, several target-derived trophic factors, such as BDNF (Sendtner et al., 1992; Yan et al., 1992), NT-3 (Sendtner et al., 1992), NT-4, IGF-1, LIF (Hughes et al., 1993), and CNTF (Sendtner et al., 1990) were previously shown to rescue axotomized neonatal motoneurons from death. More recently, synergistic effects of BDNF and glial-derived neurotrophic factor (GDNF) have also been demonstrated (Vejsada et al., 1998). It is therefore possible that loss of target-derived growth factors, in addition to NMDAR-mediated effects, contribute to induce death in lesioned motoneurons, although little is known about the effects of growth factors on NMDAR expression under these conditions.

Regulation of GluR Expression by Growth Factors

Sensitivity to glutamate excitotoxicity *per se* can be modulated by neurotrophins. For example, pretreatment with bFGF protected cultured hippocampal neurons from toxic effects of high glutamate concentrations (Mattson et al., 1989). This may involve regulating the expression of GluRs, since bFGF suppressed levels of NMDAR protein in these neurons (Mattson et al., 1993). In a more recent study, the expression of GluR1–4 in bFGF-treated cultures was also examined (Cheng et al., 1995). bFGF selectively elevated GluR1 protein levels while attenuating Ca^{2+} responses to NMDA receptor activation, consistent with the earlier observation of NMDA receptor protein downregulation. Thus, neurotrophic factors may modulate vulnerability to excitotoxicity and neuronal plasticity by differentially regulating the expression of specific GluR subunits. A role for bFGF is also implicated in developmental control of GluR function in glial cells. GluR1 expression, as well as that for GluR3, KA1, KA2, and GluR7, in cortical oligodendrocyte progenitor cells has been shown to be upregulated by bFGF, but the selective elevation of GluR1 was effected through a synergistic action of PDGF and bFGF (Chew et al., 1997). These changes are mediated by an increase in the rate of transcription of the GluR1 gene, leading to increased protein levels and is ultimately manifested in alterations in conductance properties of treated cells in culture. Specifically, this involved increased AMPA-activated current densities along with an increase in rectification of AMPA receptor responses, which would be consistent with a preferential increase in expression of GluR1 relative to GluR2 (Chew et al., 1997). It is interesting that the maintenance of progeni-

tors in a proliferative precursor state by PDGF and bFGF should be associated with changes in GluR expression and function. These observations are somewhat consistent with the developmental profile of GluR1 expression in the neocortex, where mRNA levels decline dramatically toward adulthood (Pellegrini-Giampietro et al., 1991), although the GluR1-positive cell types were not identified in those experiments. Future studies will be needed to address GluR1 gene expression in different glial populations. AMPA receptor activation has previously been shown to inhibit proliferation and differentiation of these oligodendrocyte progenitor cells (Gallo et al., 1996), providing functional evidence for the importance of GluR activity in glial cell growth and maturation.

Regulation of GluR Expression by Neuregulins and NGF

Developmental changes in NMDAR expression have been well studied in the cerebellum. Cerebellar granule cells express both NR2A and NR2B in the external granule cell layer. After migration into the internal granule cell layer where granule cells are innervated by mossy fibers, NR2B mRNA levels are downregulated, whereas that of NR2C is enhanced (Watanabe et al., 1992). The expression of the NR2C subunit in the internal granule cell layer of the maturing cerebellum was recently shown to be dramatically stimulated by neuregulin-β, a member of the neuregulins, which stimulate acetylcholine receptors at the NMJ. When examined in cerebellar slice cultures grown in the absence of mossy fiber input, the stimulation by neuregulin was specifically dependent on synaptic activity by NMDA receptors, because the NMDA-selective channel blocker D-2-amino-5-phosphonovalerate (AP5) prevented upregulation of NR2C by neuregulin, whereas AMPA/KA receptor antagonists did not (Ozaki et al., 1997). It was therefore proposed that neuregulins may promote expression of lower-conducting NMDA receptors by preferentially increasing NR2C levels during granule cell maturation. At this time, little is known, however, of the molecular mechanism underlying the upregulation of NR2C by neuregulins.

In stark contrast, remarkable progress has been made in elucidating the molecular events mediating the stimulatory effect of NGF on NR1 gene expression in PC12 cells. The promoter of the essential subunit of the NMDAR heteromer, NR1, was shown to contain a GSG motif (Bai and Kusiak, 1993), the cognate DNA-binding site for NGF-inducible/early growth reaction factor (NGFI/Egr) family of transcription factors that are rapidly induced by neurotrophins, including NGF (Joseph et al., 1988; Crosby et al., 1991; Patwardhan et al., 1991). In both transient and stable transfections, NGF consistently upregulated the activity of NR1 promoter reporter constructs, at doses that paralleled those for neurite outgrowth and differentiation of PC12 cells. This effect of NGF on promoter activity was mediated by its high affinity receptor, Trk A, and could not be mimicked by related neurotrophins, e.g., BDNF, NT3, and NT4 (Bai and Kusiak, 1997). Deletion and mutational analyses revealed that both GSG and Sp1 sites were involved in basal and NGF-stimulated transcription. This region of the promoter bound Sp1 protein from PC12 and HeLa cell extracts, but only PC12 cells expressed the promoter construct, suggesting that an additional, as yet uncharacterized, interaction with GSG-binding proteins may account for cell-specific promoter activity (Bai and Kusiak, 1995). In a more recent study, single-stranded DNA-binding proteins were indicated in binding the sense strand of this region (Bai et al., 1998) that may act in concert with a neuron-restrictive silencer element (NRSE) to govern gene expression. Thus, a complex mechanism appears to be emerging: multiple transcription factors may be involved in the regulation of NR1 promoter activity, not only with regard to NGF-inducibility, but also tissue-specific basal expression, since deletion of the NRSE alone was insufficient to completely restore transcriptional activity in nonneuronal cells to levels comparable to that in neuronal cells (Bai et

al., 1998). Interestingly, in spite of a clear stimulation of NR1 promoter activity by NGF, it was shown that NGF induced no changes in either the levels of endogenous NR1 RNA (Bai and Kusiak, 1997), or the appearance of NMDA-evoked channel activity in PC12 cells (Sucher et al., 1993). In the latter study, NR1 protein remained undetectable in both undifferentiated and NGF-differentiated PC12 cells. This suggests that either negative regulatory elements are missing from the reporter constructs or that there are additional mechanisms acting at the posttranscriptional level to regulate the functional expression of NMDA receptor proteins in these cells (Sucher et al., 1993).

Regulation of GluR Expression by Adrenal Steroid Hormones

Adrenal corticosteroid hormones, such as corticosterone, mediate a wide range of metabolic responses to stress. A role for glutamate in the control of the hypothalamo-pituitary-adrenal axis was implicated by the stimulation of pituitary ACTH secretion following direct injection of glutamate into the PVN (Darlington et al., 1989). Intraventricular (Makara and Stark, 1975) or systemic administration of glutamate or GluR agonists (Farah et al., 1991; Jezova et al., 1991) produced similar effects, although attempts at demonstrating an effect, in vitro, of GluR agonists on corticotrophin releasing factor (CRF) release have thus far been unsuccessful (Costa et al., 1992; Patchev et al., 1994). In further support of glutamate involvement, it was found that more than 70% of paraventricular CRF-positive neurons in the rat hypothalamus contain NR1 mRNA, whereas NR2A and NR2B were undetectable. A significant proportion of CRF cells express AMPA receptor subunit GluR1, GluR B, and KA2 mRNAs as well (Aubry et al., 1996). NR1 mRNA levels have been reported to be increased in hypothalamic paraventricular and supraoptic nuclei following a single exposure to immobilization stress (Bartanusz et al., 1995). In addition, significant upregulation of NR1 mRNA and a selective decrease in GluR1 mRNA was also observed in hippocampal CA1 and CA3 pyramidal cells, along with increased NR2B mRNA in CA3 (Bartanusz et al., 1995).

Experimental paradigms chronically modifying plasma corticosterone levels have been shown to elicit parallel increases in RNA levels of NR2A and NR2B, but not NR1 in the rat hippocampus (Weiland et al., 1997). Binding to MK-801 was also increased without any change in binding affinity, indicating an increase in receptor channel protein and number of binding sites (Weiland et al., 1997). In another study, chronic corticosterone treatment was shown to affect proteins responsible for Ca^{2+} influx in hippocampal neurons, e.g., Ca^{2+} channels *(see above)* and GluRs (Nair et al., 1998). In this latter study, two doses of corticosterone were used—a lower, which results primarily in the occupation of high affinity MRs, and a higher dose, in which both MRs and 10-fold lower affinity GRs are occupied. Predominant mineralocorticoid receptor occupation resulted in increased GluR2/GluR1 and NR2A/NR2B mRNA ratios in CA1 neurons (Nair et al., 1998). Concomitant restriction of Ca^{2+} influx was also observed, which may be explained by an alteration in relative subunit levels and receptor subunit composition. In contrast, additional glucocorticoid receptor occupation at high doses of corticosterone modulated receptor subunit expression in a manner consistent with enhanced Ca^{2+} influx. This enhancement of Ca^{2+} influx was not observed in preparations of acutely dissociated neurons (Weiland et al., 1997), suggesting that the corticosteroid-induced current was probably generated in dendrites. Interestingly and surprisingly, the Ca^{2+} conductance at these high doses was similar to adrenalectomized subjects (Nair et al., 1998). This effect may well be related to a feedback response to elevated plasma corticosteroid content, since the increase in GluR2 mRNA seen at low steroid doses was reversed at high doses. The highest NR2A to NR2B mRNA ratios occurred at low corticosterone doses compared with adrenalectomized animals and those treated with high

steroid doses. Although the significance of these changes remains to be clarified, they nonetheless mimic, at the molecular level, previous observations of the opposing effects of MR activation vs both MR and GR activation (Joëls and de Kloet, 1994).

Adrenalectomy did not affect the expression pattern for the kainate receptor subunits GluR6, GluR7, KA1, and KA2, but MR occupation resulted in an enhanced expression of KA1, KA2, and GluR6 subunit RNAs in the CA1 region when compared with adrenalectomized or sham controls, whereas MR+GR occupation, similar to NMDARs, suppressed the expression of KA1, KA2, and GluR7 subunits compared with MR occupation alone (Joëls et al., 1996). Although kainate-evoked responses were not examined in this study, these observations are likely to have functional consequences since kainate-induced excitotoxicity in the hippocampus may be exacerbated by elevated levels of corticosterone during prolonged exposure to chronic stress (McIntosh et al., 1998).

Regulation of GluRs by Gonadal Steroids

A cascade of events within the hypothalamic-pituitary-gonadal system determines the onset of puberty. Excitatory amino acids, such as glutamate, influence the hypothalamic gonadotropin-releasing hormone (GnRH) neuronal system. Kainate has been shown to cause an increase in plasma luteinizing hormone (LH) levels (Abbud and Smith, 1991) and both kainate and NMDA cause large increases in GnRH secretion from the mediobasal hypothalamus (Carbona et al., 1996), which can in turn elicit pituitary LH release. Gonadal steroids may upregulate GluR concentrations as a potential mechanism for enhancing glutamate neurotransmission. This may occur in the hypothalamus, septum, and amygdala, where these receptors coexist with gonadal steroid binding sites. Surprisingly, neither acute nor chronic treatment with estradiol or progesterone affected NMDA receptor binding or NR1 mRNA levels in the hypothalamus of adult female rats (Brann et al., 1993). Testosterone treatment was also without effect in the male rat hypothalamus, as was castration (Kus et al., 1995). The combined treatment of estradiol and progesterone caused a significant increase in ^{3}H-glutamate binding in the preoptic nucleus of the hypothalamus, which was not displaced by NMDA (Weiland, 1992), suggesting an elevation of non-NMDA receptors. Recent studies showed that estradiol treatment, alone or in combination with progesterone, increased AMPA receptor GluR1-immunoreactivity in the POA and ARC of immature female rats (Brann and Mahesh, 1994). Diano et al. (1997) also described an upregulation of GluR1, GluR2/3 levels after estradiol and testosterone treatment specifically in the hypothalamus, whereas no changes in the septum, BNST, and amygdala could be detected. To date, comparative studies examining the effect of steroids on kainate receptor expression have not been reported. Thus, it appears that hypothalamic AMPA receptor levels are regulated by both estradiol and progesterone, whereas hypothalamic NMDA receptors are not.

Some evidence has suggested that estrogens may influence GluR function in the hippocampus, a region not directly associated with reproductive processes, but rather with reproductive and nonreproductive behaviors related to learning and memory. Estradiol has an important role in cognitive function in experimental animals (Luine, 1994; Singh et al., 1994) and was shown to depress the threshold for seizure activity in kindled animals, an experimental model for epilepsy (Gevorkyan et al., 1989), which is a condition mediated by the hippocampus. The treatment of ovariectomized rats with estradiol for 2 d increased glutamate binding to NMDA receptors in the CA1 region (Weiland, 1992), whereas the density of binding to KA or AMPA receptors remained unaffected. Using both *in situ* hybridization and immunocytochemistry, Gazzaley et al. (1996) concluded that the estradiol-induced NMDAR1 expression in CA1 was

regulated by a posttranscriptional mechanism, because increases in immunofluorescence intensity occurred without concomitant changes in mRNA levels. In support of this observation, Kohama et al. (1998) also reported a lack of effect of estradiol on NMDAR1, GluR1, and GluR2 mRNAs in the primate hippocampus and temporal cortex. This lends support to a previous report describing NMDAR1 transcripts in PC12 cells that were not translated (Sucher et al., 1993), suggesting the importance of posttranscriptional mechanisms in the regulation of NR1 gene expression. Besides estrogens, testosterone has been suggested to act on memory functions (Flood et al., 1992) and has also been known to exert an anticonvulsant effect (Schwartz-Giblin et al., 1989). Anabolic androgens have been shown to affect the expression of NR2A subunit in both the hypothalamus and hippocampus of the male rat (Le Greves et al., 1997). Intramuscular injections of nandrolone decanoate resulted in significant decrease in the NR2A and NR2B subunit mRNA levels in the hypothalamus and a decreased expression of NR1 mRNA in nucleus accumbens. The physiological significance of this effect is unclear, but is speculated to be related to the expression of aggressive behavior and impairment of cognitive function mediated by the hypothalamus and hippocampus, as well as stimulation of the brain reward system via the nucleus accumbens (Koob, 1992).

Concluding Remarks

Neural cells acquire their final identity through a complex series of events that culminate in the acquisition of a differentiated phenotype. The progressive expression of functional ionic channels in the cell membrane is part of this process for both neurons and glia. Several distinct and possibly overlapping mechanisms may act in parallel to direct and modulate expression of ligand- and voltage-gated ion channels in neural cells. In the present review, we have discussed the evidence that some hormones and growth factors play a pivotal role in changing expression of membrane channels in cells of the CNS and PNS during development. These physiological changes, together with other major cellular modifications, ultimately direct different cell populations to acquire the functional properties of mature neurons and glia.

One of the future goals of the research in this field is to determine whether the action of these hormones and factors is simply permissive, or if they act instructively in determining a specific channel phenotype. Additionally, it will be extremely important to determine the sequence in which these signals act during development. For example, we need to discover whether hormones and/or growth factors primarily trigger expression of the first detectable membrane channels in embryonic neural precursor cells, voltage-dependent Na^+-, and GABA-activated channels (Ben-Ari et al., 1997).

This analysis will also help to solve a number of other issues that are of primary importance for our understanding of brain physiology. Is there a general rule that determines the sequence of membrane channel expression? To what extent are growth factors and hormones involved in this sequential expression? Since voltage- and ligand-gated channels are assemblies of several subunits, can hormones and growth factors change the composition and the functional properties of membrane channels in the mature brain? Finally, are there common rules of channel regulation across different families of voltage- and ligand-gated channels and distinct channel subtypes? Answers to these questions will finally clarify the fundamental issue of how the ontogeny of key membrane proteins of the nervous system unfolds, and how the complex physiological interactions between neurons and glia are established in the brain.

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References

Abbud R. and Smith M. S. (1991) Differences in the luteinizing hormone and prolactin responses to multiple injections of kainate as compared to N-methyl-D,L-aspartate, in cycling rats. *Endocrinology* **129,** 3254–3258.

Acheson A., Barker P. A., Anderson R. F., Miller F. D., and Murphy R. A. (1991) Detection of brain-derived neurotrophic factor-like activity in fibroblasts and Schwann cells: inhibition by antibody to NGF. *Neuron* **7,** 265–273.

Agnew W. S. (1984) Voltage-regulated sodium channel molecules. *Ann. Rev. Physiol.* **46,** 517–530.

Akopian A. N., Sivilotti L., and Wood J. N. (1996) A tetrodoxin-resistant voltage-gated sodium channel expressed by sensory neurons. *Nature* **379,** 258–262.

Akwa Y., Sawawes N., Robel P., Baulieu E. E., and Le Goascogne C. (1993) Astrocytes and neurosteroids: metabolism of prenenolone and dehydroepiandrosterone. Regulation by cell density. *J. Cell Biol.* **121,** 135–143.

Alema' S., Casalbore P., Agostini E., and Tato' F. (1985) Differentiation of PC12 phaeochromocytoma cells induced by v-*src* oncogene. *Nature* **316,** 557–559.

Alonso R. and Lopez-Coviella I. (1998) Gonadal steroids and neuronal function. *Neurochem. Res.* **23,** 675–688.

Altiok N., Bessereau J. L., and Changeux J. P. (1995) *ERBB3* and *ERBB2/NEU* mediate the effect of heregulin on acetylcholine-receptor gene expression in muscle-differential expression at the endplate. *EMBO J.* **14,** 4258–4266.

Amy C. M. and Bennett E. L. (1983) Increased sodium ion conductance through nicotinic acetylcholine receptor channels in PC12 cells exposed to nerve growth factors. *J. Neurosci.* **3,** 1547–1553.

Araki T. and Tohyama M. (1992) Region-specific expression of $GABA_A$ receptor α_3 and α_4 subunit mRNAs in the rat brain. *Mol. Brain Res.* **12,** 293–314.

Attardi B., Takimoto K., Gealy R., Severns C., and Levitan E. S. (1993) Glucocorticoid induced up-regulation of a pituitary K^+ channel mRNA *in vitro* and *in vivo*. *Rec. Channels* **1,** 287–293.

Aubry J-M., Bartanusz V., Pagliusi S., Schulz P., and Kiss J. Z. (1996) Expression of ionotropic glutamate receptor subunit mRNAs by paraventricular corticotropin-releasing factor (CRF) neurons. *Neurosci. Lett.* **205,** 95–98.

Auld V. J., Goldin A. L., Krafte D. S., Marshall J., Dunn J. M., Catterall W. A., Lester H. A., Davidson N., and Dunn R. J. (1988) A rat brain Na^+ channel α-subunit with novel gating properties. *Neuron* **1,** 449–461.

Bai G. and Kusiak J. W. (1993) Cloning and analysis of the 5′ flanking sequence of the rat N-methyl-D-aspartate receptor 1 (NMDAR1) gene. *Biochim Biophys. Acta* **1152,** 197–200.

Bai G. and Kusiak J. W. (1995) Functional analysis of the proximal 5′-flanking region of the N-methyl-D-aspartate receptor subunit gene. *J. Biol. Chem.* **270,** 7737–7744.

Bai G. and Kusiak J. W. (1997) Nerve growth factor up-regulates the N-methyl-D-aspartate receptor subunit 1 promoter in PC12 cells. *J. Biol. Chem.* **272,** 5936–5942.

Bai G., Norton D. D., Prenger M. S., and Kusiak J. W. (1998) Single-stranded DNA-binding proteins and neuron-restrictive silencer factor participate in cell-specific transcriptional control of the NMDAR1 gene. *J. Biol. Chem.* **273,** 1086–1091.

Bain G., Ray W. J., Yao M., and Gottlieb D. I. (1994) From embryonal carcinoma cells to neurons: the p19 pathway. *Bioassays* **16,** 343–348.

Bandtlow C. E., Heumann R., Schwab M. E., and Thoenen H. (1987) Cellular localization of nerve growth factor synthesis by in situ hybridization. *EMBO J.* **6,** 891–899.

Banner L. R. and Patterson P. H. (1994) Major changes in the expression of the mRNAs for cholinergic differentiation factor/leukemia inhibitory factor and its receptor after injury to adult peripheral nerves and ganglia. *Proc. Natl. Acad. Sci. USA* **91,** 7109–7113.

Barbacid M., Lamballe F., Pulido D., and Klein R. (1991) The trk family of tyrosine protein kinase receptors. *Biochim. Biophys. Acta* **1072,** 115–127.

Barres B. A., Chun L. L. Y., and Corey D. P. (1989) Calcium current in cortical astrocytes: induction by cAMP and neurotransmitters and permissive effect of serum factors. *J. Neurosci.* **9,** 3169–3175.

Barres B. A., Koroshetz W. J., Chun L. L. Y., and Corey D. P. (1990a) Ion channel expression by white matter glia: the type-1 astrocyte. *Neuron* **5,** 527–544.

Barres B. A., Koroshetz W. J., Swartz K. J., Chun L. L. Y., and Corey D. P. (1990b) Ion channel expression by white matter glia: the O-2A glial progenitor cell. *Neuron* **4,** 507–524.

Bartanusz V., Aubry J-M., Pagliusi S., Jezova D., Baffi J., and Kiss J. Z. (1995) Stress-induced changes in messenger RNA levels of N-methyl-

D-aspartate and AMPA receptor subunits in selected regions of the rat hippocampus and hypothalamus. *Neuroscience* **66,** 247–252.

Baulieu E. E. and Robel P. (1995) Non-genomic mechanisms of action of steroid hormones, in *Non-Reproductive actions of Sex Steroids,* (Bock G. R. and Goode J. A., eds.) Ciba Foundation Symposium 191, Wiley, Chichuster, UK, pp. 24–42.

Beattie C. E. and Siegel R. E. (1993) Developmental cues modulate $GABA_A$ receptor subunit mRNA expression in cultured cerebellar granule neurons. *J. Neurosci.* **13,** 1784–1792.

Beckh S. and Pongs O. (1990) Members of the RCK potassium channel family are differentially expressed in the rat nervous system. *EMBO J.* **7,** 2457–2463.

Behrens M. I. and Latorre R. (1991) Potassium channels in developing excitable cells. *Curr. Topic Membr.* **39,** 325–355.

Ben-Ari Y., Khazipov R., Leinekugel X., Caillard O., and Gaiarsa J.-L. (1997) $GABA_A$, NMDA and AMPA receptors: a developmentally regulated "menage a trois". *Trends Neurosci.* **20,** 523–529.

Bernal J. and Nunez J. (1995) Thyroid hormones and brain development. *Eur. J. Endocrinol.* **133,** 390–398.

Bessis A., Champtiaux N., Chatelin L., and Changeux J.-P. (1997) The neuron-restrictive silencer element: a dual enhancer/silencer crucial for patterned expression of a nicotinic receptor gene in the brain. *Proc. Natl. Acad. Sci. USA* **94,** 5906–5911.

Bessis A., Savatier N., Devillers-Thiery A., Bejanin S., and Changeux J.-P. (1993) Negative regulatory elements upstream of a novel exon of the neuronal nicotinic acetylcholine receptor alpha-2 subunit gene. *Nucleic Acids Res.* **21,** 2185–2192.

Bigger C. B., Casanova E. A., and Gardner P. D. (1996) Transcriptional regulation of neuronal nicotinic acetylcholine receptor genes: functional interactions between Sp1 and the rat β4 subunit gene promoter. *J. Biol. Chem.* **271,** 32,842–32,848.

Bigger C. B., Melnikova I. N., and Gardner P. D. (1997) Sp1 and Sp3 regulate expression of the neuronal nicotinic acetylcholine receptor beta 4 subunit gene. *J. Biol. Chem.* **272,** 25,976–25,982.

Birnbauer L., Campbell K. P., Catterall W. A., Harpold M. M., Hofmann F., Horne W. A., Mori Y., Schwartz A., Snutch T. P., Tanabe T., and Tsien R. W. (1994) The naming of voltage-gated calcium channels. *Neuron* **13,** 505,506.

Black J. A. and Waxman S. G. (1996) Sodium channel expression: a dynamic process in neurons and non-neuronal cells. *Dev. Neurosci.* **18,** 139–152.

Bothwell M. A., Schechter A. L., and Vaughn K. M. (1980) Clonal variants of PC12 pheochromocytoma cells with altered response to nerve growth factor. *Cell* **21,** 857–866.

Boulter J., Evans J., Goldman D., Martin G., Treco D., and Heinemann S. (1986) Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor alpha-subunit. *Nature* **319,** 368–374.

Boulter J., O'Shea-Greenfield A., Duvosin R. M., Connolly J. G., Wada E., Jensen A., Gardner P. D., Ballivet M., Deneris E. S., McKinnon D., Heinemann S., and Patrick J. (1990) α3, α5 and β4: three members of the rat neuronal nicotinic acetycholine receptor-related gene family form a gene cluster. *J. Biol. Chem.* **265,** 4472–4482.

Brann D. W. and Mahesh V. B. (1994) Excitatory amino acids: function and significance in reproduction and neuroendocrine regulation. *Front. Neuroendocrinol.* **15,** 3–49.

Brann D. W., Zamorano P. L., Chorich L. P., and Mahesh V. B. (1993) Steroid hormone effects on NMDA receptor binding and NMDA receptor mRNA levels in the hypothalamus and cerebral cortex of the adult rat. *Neuroendocrinology* **58,** 666–672.

Buller A. L., Larson H. C., Schneider B. E., Beaton J. A., Morrisett R. A., and Monaghan D. T. (1994) The molecular basis of NMDA receptor subtypes: native receptor diversity is predicted by subunit composition. *J. Neurosci.* **14,** 5471–5484.

Bureau M. H., and Olsen R. W. (1993) $GABA_A$ receptor subtypes—ligand binding heterogeneity demonstrated by photoaffinity labeling and autoradiography. *J. Neurochem.* **61,** 1479–1491.

Burnstein D. E. and Greene L. A. (1982) Nerve growth factor has both mitogenic and anti-mitogenic activity. *Dev. Biol.* **94,** 477–482.

Burt D. R. and Kamatchi G. L. (1991) $GABA_A$ receptor subtypes—from pharmacology to molecular biology. *FASEB J.* **5,** 2916–2923.

Caffrey J. M., Eng D. L., Black J. A., Waxman S. G., and Kocsis J. D. (1992) Three types of sodium channels in adult dorsal root ganglion neurons. *Brain Res.* **592,** 283–297.

Canonaco M., Tavolaro R., and Maggi A. (1993) Steroid hormones and receptors of the $GABA_A$ supramolecular complex. *Neuroendocrinology* **57,** 974–984.

Carbona S., Szwarcfarb B., Rondina D., Feleder C., and Moguilevsky J. A. (1996) Differential effects of the N-methyl-D-aspartate and non-N-methyl-D-aspartate receptors of the excitatory amino acids system on LH and FSH secretion. Its effects on the hypothalamic luteinizing hormone releasing hormone during maturation in male rats. *Brain Res.* **707,** 139–145.

Carraway K. L. and Burden S. J. (1995) Neuregulins and their receptors. *Curr. Opin. Neurobiol.* **5,** 606–612.

Catterall W. A. (1984) The molecular basis of neuronal excitability. *Science* **223,** 653–661.

Cauley K., Marks M., Gahring L. C., and Rogers S. W. (1996) Nicotinic receptor subunits α3, α4 and β2 and high affinity nicotine binding sites are expressed by P19 embryonal cells. *J. Neurobiol.* **30,** 303–314.

Cens T., Nargeot J., and Charnet P. (1997) Ca^{2+}-permeability of muscle nicotinic acetylcholine receptor is increased by expression of the ε subunit. *Rec. Channels* **5,** 29–40.

Changeux J. P., Duclert A., and Sekine S. (1992) Calcitonin gene-related peptides and neuromuscular interactions. *Ann. NY Acad. Sci.* **657,** 361–378.

Chazot P. L., Coleman S. K., Cik M., and Stephenson F. A. (1994) Molecular characterization of N-methyl-D-aspartate receptors expressed in mammalian cells yields evidence for the co-existence of three subunit types within a discrete receptor molecule. *J. Biol. Chem.* **269,** 24,403–24,409.

Cheng B., Furukawa K., O'Keefe J. A., Goodman Y., Kihiko M., Fabian T., and Mattson M. P. (1995) Basic fibroblast growth factor selectively increases AMPA-receptor subunit GluR1 protein level and differentially modulates Ca^{2+} responses to AMPA and NMDA in hippocampal neurons. *J. Neurochem.* **6,** 2525–2536.

Chew L.-J., Fleck M. W., Wright P., Scherer S. E., Mayer M. L., and Gallo V. (1997) Growth factor-induced transcription of GluR1 increases functional AMPA receptor density in glial progenitor cells. *J. Neurosci.* **17,** 227–240.

Chiu S. Y. and Wilson G. F. (1989) The role of potassium channels in Schwann cell proliferation in Wallerian degeneration of explant rabbit sciatic nerves. *J. Physiol. (Lond.)* **408,** 199–222.

Chu G. C., Moscoso L. M., Sliwkowski M. X., and Merlie J. P. (1995) Regulation of the acetylcholine receptor epsilon subunit gene by recombinant ARIA—an in vitro model for transynaptic gene regulation. Neuron **14,** 329–339.

Cintra A., Fuxe K., Harfstrand A., Agnati L. F., Miller L. S., Greene J. L., and Gustafsson J. (1986) On the cellular localisation and distribution of estrogen receptors in the tel- and diencephalon using monoclonal antibodies to human estrogen receptor. *Neurochem. Int.* **8,** 587–595.

Cobas A., Fairen A., Alvarez-Bolado G., and Sanchez M. P. (1991) Prenatal development of the intrinsic neurons of the rat neocortex: a comparative study of the distribution of GABA-immunoreactive cells and the $GABA_A$ receptor. *Neuroscience* **40,** 375–397.

Cohen-Cory S., Dreyfus C. F., and Black I. B. (1991) NGF and excitatory neurotransmitters regulate survival and morphogenesis of cultured cerebellar Purkinje cells. *J. Neurosci.* **11,** 462–471.

Corriveau R. A. and Berg D. K. (1994) Neurons in culture maintain acetylcholine receptor levels with far fewer transcripts than *in vivo*. *J. Neurobiol.* **25,** 1579–1592.

Corriveau R. A., Romano S. J., Conroy W. G., Oliva L., and Berg D. K. (1995) Expression of neuronal acetylcholine receptor genes in vertebrate skeletal muscle during development. *J. Neurosci.* **15,** 1372–1383.

Corvalan V., Cole R., deVellis J., and Hagiwara S. (1990) Neuronal modulation of calcium channel activity in cultured rat astrocytes. *Proc. Natl. Acad. Sci. USA* **87,** 4345–4348.

Costa A., Yasin S. A., Hucks D., Forsling M. L., Besser G. M., and Grossman A. (1992) Differential effects of neuroexcitatory amino acids on corticotropin-releasing hormone-41 and vasopressin release from rat hypothalamic explants. *Endocrinology* **131,** 2595–2602.

Couturier S., Bertrand D., Matter J., Hernandez M., Bertrand S., Millar N., Valera S., Barkas T., and Ballivet M. (1990) A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homomeric channel blocked by α-bungarotoxin. *Neuron* **5,** 847–856.

Cowley S., Paterson H., Kemp P., and Marshall C. J. (1994) Activation of MAP kinase is necessary and sufficient for PC12 differentiation and transformation of NIH 3T3 cells. *Cell* **77,** 841–852.

Cremins J., Wagner J. A., and Haleguoa S. (1986) Nerve growth factor action is mediated by cAMP- and Ca^{2+}/phospholipid-dependent protein kinases. *J. Cell. Biol.* **103,** 887–893.

Crosby S. D., Puetz J. J., Simburger K. S., Fahrner T. J., and Milbrandt J. (1991) The early response gene NGFI-C encodes a zinc finger transcriptional activator and is a member of the GCGGGGGCG

(GSG) element-binding protein family. *Mol. Cell Biol.* **11,** 3835–3841.

Crowley C., Spencer S. D., Nishimura N. C., Chen K. S., Pitts-Meek S., Armanini M. P., Ling, L. H., MacMahon S. B., Shelyton D. L., Levinson A. D., and Phillips H. S. (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. *Cell* **76,** 1001–1011.

Csillik B., Rakic P., and Knyihar-Csillik E. (1998) Peptidergic innervation and the nicotinic acetylchline receptor in the primate basal nucleus. *Eur. J. Neurosci.* **10,** 573–585.

Curtis R., Scherer S. S., Somogyi R., Adryan K. M., lp N. Y., Zhu Y., Lindsay R. M., and DiStefano P. S. (1994) Retrograde axonal transport of LIF is increased by peripheral nerve injury: correlation with increased LIF expression in distal nerve. *Neuron* **12,** 191–204.

Cutting G. R., Lu L., O'Hara B. F., Kasch L. M., Montrose-Rafizadeh C., Donovan D. M., Shimada S., Antonavakis S. E., Guggino W. B., and Uhl G. R. (1991) Cloning of the gamma-aminobutyric acid (GABA) rho I cDNA: a GABA receptor subunit highly expressed in the retina. *Proc. Natl. Acad. Sci. USA* **88,** 2673–2677.

D'Arcangelo G. and Halegoua S. (1993) A branched signaling pathway for nerve growth factor is revealed by Src-, Ras-, and Raf-mediated gene inductions. *Mol. Cell. Biol.* **13,** 3146–3155.

D'Arcangelo G., Paradiso K., Shepherd D., Brehm P., Halegoua S., and Mandel G. (1993) Neuronal growth factor regulation of two different sodium channel types through distinct signal transduction pathways. *J. Cell Biol.* **4,** 915–921.

Damon D., D'Amore P. A., and Wagner J. A. (1990) Nerve growth factor and fibroblast growth factor regulate neurite outgrowth and gene expression in PC12 cells via both protein kinase C- and cAMP-independent mechanisms. *J. Cell. Biol.* **110,** 1333–1339.

Darlington D. N., Miyamoto M., Keil L., and Dallman M. (1989) Paraventricular stimulation with glutamate elicits bradycardia and pituitary responses. *Am. J. Physiol.* **256,** R112–R119.

DeCoursey T. E., Chandy K. G., Gupta S., and Cahalan M. D. (1984) Voltage-gated K^+ channels in human T-lymphocytes: a role in mitogenesis? *Nature* **307,** 465–468.

De Jongh K. S., Warner C., and Catterall W. A. (1990) Subunits of purified calcium channels. Alpha 2 and delta are encoded by the same gene. *J. Biol. Chem.* **265,** 14,738–14,741.

De Kloet E. R. (1991) Brain corticosteroid receptor balance and homeostatic control. *Frontiers Neuroendocrinol.* **12,** 95–164.

De Vries G. J., De Bruin J. P. C., Uylings H. B. M., and Corner M. A., eds. (1984) *Prog. Brain Res.,* vol. 61.

Deneris E. S., Boulter J., Swanson L. W., Patrick J., and Heinemann S. (1989) β3: a new member of nicotinic acetylcholine receptor gene family is expressed in brain. *J. Biol. Chem.* **264,** 6268–6272.

Diano S., Naftolin F., and Horvath T. L. (1997) Gonadal steroids target AMPA glutamate receptor-containing neurons in the rat hypothalamus, septum and amygdala: a morphological and biochemical study. *Endocrinology* **138,** 778–789.

Dib-Hajj S. D., Black J. A., Cummins T. R., Kenney A. M., Kocsis J. D., and Waxman S. G. (1998) Rescue of α-SNS sodium channel expression in small dorsal root ganglion neurons following axotomy by in vivo administration of nerve growth factor. *J. Neurophysiol.* **79,** 2668–2678.

Dietzell. (1995) Voltage-gated ion currents in embryogenesis. *Persp. Dev. Neurobiol.* **2,** 293–308.

Donaldson L. W., Petersen J. M., Graves B. J., and Mclntosh L. P. (1996) Solution structure of the ETS domain from murine Ets-1: a winged helix-turn-helix DNA binding motif. *EMBO J.* **15,** 125–134.

Dourado M. M. and Dryer S. E. (1992) Changes in the electrical properties of chick ciliary ganglion neurones during embryonic development. *J. Physiol. (Lond.)* **449,** 411–428.

Dourado M. M. and Dryer S. E. (1994) Regulation of A-currents by cell-cell interactions and neurotrophic factors in developing chick parasympathetic neurones. *J. Physiol. (Lond.)* **474,** 367–377.

Drewe J. A., Verma S., Frech G., and Joho R. H. (1992) Distinct spatial and temporal expression patterns of K^+ channel mRNAs from different subfamilies. *J. Neurosci.* **12,** 538–548.

Dryer S. E. (1994) Functional development of the parasympathetic neurons of the avian ciliary ganglion: a classic model system for the study of neuronal differentiation and development. *Progr. Neurobiol.* **43,** 281–322.

Du Q., Tomkinson A. E., and Gardner P. D. (1997) Transcriptional regulation of neuronal nicotinic acetylcholine receptor genes: a possible role for the DNA binding protein Purα. *J. Biol. Chem.* **272,** 14990–14995.

Duffy S., Fraser D. D., and MacVicar B. A. (1995) Potassium channels, in *Neuroglia* (Kettenmann H. and Ransom B. R.), eds. Oxford University Press, New York, pp. 185–201.

Dussault J. H. and Ruel J. (1987) Thyroid hormones and brain development. *Ann. Rev. Physiol.* **49,** 321–334.

Elgoyhen A., Johnson D., Boulter J., Velter D., and Heinemann S. (1994) α9, an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. *Cell* **79,** 705–715.

Ellis S. B., Williams M. E., Ways N. R., Brenner R., Sharp A. H., Leung A. T., Campbell K. P., McKenna E., Koch W. J., Hui A., Schwartz A., and Harpold M. M. (1988) Sequence and expression of mRNAs encoding the α1 and the α2 subunits of a DHP-sensitive calcium channel. *Science* **241,** 1661–1664.

Falls D. L., Rosen K. M., Corfas G., Lane W. S., and Fischbach G. D. (1993) ARIA, a protein that stimulates acetylcholine receptor synthesis, is a member of the NEU ligand family. *Cell* **72,** 801–815.

Fanger G. R., Brennan C., Henderson L. P., Gardner P. D., and Maue R. A. (1995) Differential expression of sodium channels and nicotinic acetylcholine receptor channels in nnr variants of the PC12 pheochromocytoma cell line. *J. Memb. Biol.* **144,** 71–80.

Farah J. M., Rao T. S., Mick S. J., Coyne K. E., and lyegenar S. (1991) N-methyl-D-aspartate treatment increases circulating adrenocorticotropin and luteinizing hormone in the rat. *Endocrinology* **128,** 1875–1880.

Federoff H. J., Geschwind M. D., Geller A. I., and Kessler J. A. (1992) Expression of nerve growth factor in vivo from a defective Herpes simplex virus I vector prevents effects of axotomy on sympathetic ganglia. *Proc. Natl. Acad. Sci. USA* **89,** 1636–1640.

Felts P. A., Yokoyama S., Dib-Hajj S., Black I. J. A., and Waxman S. G. (1997) Sodium channel α-subunit mRNAs I, II, III, NaG, Na6 and hNE (PN1): different expression patterns in developing rat nervous system. *Mol. Brain Res.* **45,** 71–82.

Fiszman M. L., Novotny E. A., Lange G. D., and Barker J. L. (1990). Embryonic and early postnatal hippocampal cells respond to nanomolar concentrations of muscimol. *Dev. Brain. Res.* **53,** 186–193.

Fitzgerald E. M. and Dolphin A. C. (1997) Regulation of rat neuronal voltage-dependent calcium channels by endogenous p21-ras. *Eur. J. Neurosci.* **9,** 1252–1261.

Flood J. F., Morley J. E., and Roberts E. (1992) Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proc. Natl. Acad. Sci. USA* **89,** 1567–1571.

Flores C. M., Rogers S. W., Pabreza L. A., Wolfe B. B., and Kellar K. J. (1992) A subtype of nicotinic cholinergic receptor in rat brain is comprised of α4 and β2 subunits and is up-regulated by chronic nicotine treatment. *Mol. Pharmacol.* **41,** 31–37.

Fontaine B., Klarsfeld A., and Changeux J-P. (1987) Calcitonin gene-related peptide and muscle activity regulate acetylcholine receptor alpha-subunit mRNA levels by distinct intracellular pathways. *J. Cell. Biol.* **5,** 1337–1342.

Friedman B., Scherer S. S., Rudge J. S., Helgren M., Morrisey D., McClain J., Wang D., Weigand S. J., Lindsay R. M., and Ip N. Y. (1992) Regulation of ciliary neurotrophic factor expression in myelin-related Schwann cells in vivo. *Neuron* **9,** 295–305.

Fyodorov D., Nelson T., and Deneris E. (1998) Pet-1, a novel ETS domain factor that can activate neuronal nAChR gene transcription. *J. Neurobiol.* **34,** 151–163.

Gallin W. J. and Greenberg M. E. (1995) Calcium regulation of gene expression in neurons: the mode of entry matters. *Curr. Opin. Neurobiol.* **5,** 367–374.

Gallo V., Ciotti M. T., Coletti A., Aloisi F., and Levi G. (1982) Selective release of glutamate from cerebellar granule cells differentiating in culture. *Proc. Natl. Acad. Sci. USA* **79,** 7919–7923.

Gallo V., Zhou J. M., McBain C. J., Wright P. W., Knutson P. L., and Armstrong R. C. (1996) Oligodendrocyte progenitor cell proliferation and lineage progression are regulated by glutamate receptor-mediated K^+ channel block. *J. Neurosci.* **16,** 2659–2670.

Galzi J. L. and Changeux J. P. (1995) Neuronal nicotinic receptors: molecular organization and regulation. *Neuropharmacology* **34,** 563–582.

Gambarana C., Beattie C. E., Rodriguez Z. R., and Siegel R. E. (1991) Region-specific expression of messenger RNAs encoding $GABA_A$ receptor subunits in the developing rat brain. *Neuroscience* **45,** 423–432.

Gambarana C., Pittman R., and Siegel R. E. (1990) Developmental expression of the $GABA_A$ receptor α1 subunit mRNA in the rat brain. *J. Neurobiol.* **21,** 1169–1179.

Gautron S., Dos Santos G., Pinto-Henrique D., Koulakoff A., Gros F., and Berwald-Netter Y. (1992) The glial voltage-gated sodium channel: cell-and tissue-specific mRNA expression. *Proc. Natl. Acad. Sci. USA* **89,** 7272–7276.

Gazzaley A. H., Weiland N. G., McEwen B. S., and Morrison J. H. (1996) Differential regulation of NMDAR1 mRNA and protein by estradiol in the rat hippocampus. *J. Neurosci.* **16,** 6830–6838.

George A. L. (1995) Molecular genetics of ion channels diseases. *Kidney Int.* **48,** 1180–1190.

Gerzanich V., Anand R., and Lindstrom J. (1994) Homomoers of α8 subunits of nicotinic receptors functionally expressed in Xenopus oocytes exhibit similar channel but contrasting binding site properties compared to α7 homomers. *Mol. Pharmacol.* **45,** 212–220.

Gevorkyan E. S., Nazaryan K. B., and Kostanyan A. A. (1989) Modifying effect of estradiol and progesterone on epileptic activity of the rat brain. *Neurosci. Behav. Physiol.* **19,** 412–415.

Ghosh A. and Greenberg M. E. (1995) Calcium signaling in neurons: molecular mechanisms and cellular consequences. *Science* **268,** 239–247.

Ginty D. D., Fanger G. R., Wagner J. A., and Maue R. A. (1992) The activity of cAMP-dependent protein kinase is required at a posttranslational level for induction of voltage-dependent sodium channels by peptide growth factors in PC12 cells. *J. Cell Biol.* **116,** 1465–1473.

Ginty D. D., Glowacka D., DeFranco C., and Wagner J. A. (1991) Nerve growth factor-induced neuronal differentiation after dominant repression of both type I and type II cAMP-dependent protein kinase activities. *J. Biol. Chem.* **266,** 15,325–15,333.

Glencorse T. A., Bateson A. N., and Darlison M. G. (1991) Sequence of the chicken GABA-A receptor gamma2 subunit cDNA. *Nucleic Acids Res.* **10,** 7157.

Gomez N. and Cohen P. (1991) Dissection of the protein kinase cascade by which nerve growth factor activates MAP kinases. *Nature* **353,** 170–173.

Gospodarowicz D., Neufeld G., and Schwigerer L. (1986) Molecular and biological characterization of fibroblast growth factor, an angiogenic factor which also controls proliferation and differentiation of mesoderm and neuroectoderm derived cells. *Cell Differ.* **19,** 1–17.

Gottmann K., Dietzel I. D., Lux H. D., Huck S., and Rohrer H. (1988) Development of inward currents in chick sensory and autonomic neuronal precursor cells in culture. *J. Neurosci.* **8,** 3722–3732.

Gottmann K., Rohrer H., and Lux H. D. (1991) Distribution of Ca^{2+} and Na^{+} conductances during neuronal differentiation of chick DRG precursor cells. *J. Neurosci.* **11,** 3371–3378.

Gould E., Woolley C. S., Frankfurt M., and McEwen B. S. (1990) Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J. Neurosci.* **10,** 1286–1291.

Grantyn R., Perouansky M., Rodriguez-Tebar A., and Lux H. D. (1989) Expression of depolarizing voltage-and transmitter-activated currents in neuronal precursor cells from the rat brain is preceded by a proton-activated sodium current. *Dev. Brain Res.* **49,** 150–155.

Greene L. A. and Kaplan D. R. (1995) Early events in neurotrophin signalling via Trk and p75 receptors. *Curr. Opin. Neurobiol.* **5,** 579–587.

Greene L. A. and Tischler A. S. (1982) PC12 pheochromocytoma cells in neurobiology research. *Adv. Cell. Neurobiol.* **3,** 373–414.

Greene L. A. and Tischler A. S. (1976) Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc. Natl. Acad. Sci. USA* **73,** 2424–2428.

Greensmith L., Hasen H. I., and Vrbova G. (1994) Nerve injury increases the susceptibility of motoneurons to N-methyl-D-aspartate-induced neurotoxicity in the developing rat. *Neuroscience* **58,** 727–733.

Gu H. J., Wenger B. W., Lopez I., McKay D. B., and Boyd R. T. (1996) Characterization and localization of adrenal nicotinic acetylcholine receptors: evidence that mAb35—nicotinic receptors are the principal receptors mediating adrenal catecholamine secretion. *J. Neurochem.* **66,** 1454–1461.

Gurantz D., Harootunian A. T., Tsien R. Y., Dionne V. E., and Margiotta J. F. (1994) VIP modulates neuronal nicotinic acetylcholine receptor function by a cyclic AMP-dependent mechanism. *J. Neurosci.* **14,** 3540–3547.

Gustafson T. A., Clevinger E. C., O'Neill T. J., Yarowsky P. J., and Krueger B. K. (1993) Mutually exclusive exon splicing of type III brain sodium channel alpha subunit RNA generates developmentally regulated isoforms in rat brain. *J. Biol. Chem.* **268,** 18,648–18,653.

Haas S., Thatikunta P., Steplewski A., Johnson E. M., Khaliki K., and Amini S. (1995) A 39-kD DNA-binding protein from mouse brain stimulates transcription of myelin basic protein gene in oligodendrocytic cells. *J. Cell Biol.* **130,** 1171–1179.

Hagihara K., Hirata S., Osada T., Hirai M., and Kato J. (1992) Distribution of cells containing progesteron receptor mRNA in the female rat di- and telencephalon: an *in situ* hybridization study. *Mol. Brain Res.* **14,** 239–249.

Halegoua S., Armstrong R. C., and Kremer N. E. (1991) Dissecting the mode of action of a neuronal growth factor, in *Current Topics in Microbiology and Immunology,* vol. 165 (Bothwell M., ed.) Springer-Verlag, Berlin, pp. 119–170.

Hardingham G. E., Chawla S., Johnson C. M., and Bading H. (1997) Distinct functions of nuclear and cytoplasmic calcium in the control of gene expression. *Nature* **385,** 260–265.

Harris R. A., Mihic S. J., Dildymayfield J. E., and Machu T. K. (1995) Actions of anaesthetics on ligand-gated ion channels—role of receptor subunit composition. *FASEB J.* **9,** 1454–1462.

Hartshorne R. P., Messner D. J., Coppersmith J. C., and Catterall W. A. (1982) The saxitoxin-receptor of the sodium channel from rat brain: evidence for two non-identical β-subunits. *J. Biol. Chem.* **257,** 13888–13891.

Harvey R. J., Chinchetru M. A., and Darlison M. G. (1994) Alternative splicing of a 51-nucleotide exon that encodes a putative protein kinase C phosphorylation site generates two forms of the chicken γ-aminobutyric $acid_A$ receptor β2 subunit. *J. Neurochem.* **62,** 10–16.

Hefti F., Hartikka J., and Knusel B. (1989) Function of neurotrophic factors in the adult and aging brain and their possible use in the treatment of neurodegenerative diseases. *Neurobiol. Aging* **10,** 515–533.

Henderson C. E. (1996) Role of neurotrophic factors in neuronal development. *Curr. Opin. Neurobiol.* **6,** 64–70.

Henderson L. P., Gdovin M. J., Liu C. L., Gardner P. D., and Maue R. A. (1994) Nerve growth factor increases nicotinic acetylcholine receptor gene expression and current density in wild type and protein kinase A-deficient PC12 cells. *J. Neurosci.* **14,** 1153–1163.

Herb A., Burnashev N., Werner P., Sakmann B., Wisden W., and Seeburg P. H. (1992) The KA2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. *Neuron* **8,** 775–785.

Herbison A. E. (1997) Estrogen regulation of GABA transmission in rat preoptic area. *Brain Res. Bull.* **44,** 321–326.

Herbison A. E. and Fenelon V. S. (1995) Estrogen regulation of $GABA_A$ receptor subunit mRNA expression in preoptic area and bed nucleus of the stria terminalis of female rat brain. *J. Neurosci.* **15,** 2328–2337.

Hesen W., Karst H., Meijer O., Cole T. J., Schmid W., de Kloet E. R., Schütz G., and Joëls M. (1996) Hippocampal cell responses in mice with a targeted glucocorticoid receptor gene disruption. *J. Neurosci.* **16,** 6766–6774.

Hess P. (1990) Calcium channels in vertebrate cells. *Annu. Rev. Neurosci.* **13,** 337–356.

Hill C. E., Belford D. A., Godovac-Zimmerman D. A., and Hendry I. A. (1991) Class 1 heparin binding growth factor promotes the differentiation but not survival of ciliary neurones in vivo. *Dev. Brain Res.* **63,** 13–19.

Hille B. (1992) *Ionic Channels of Excitable Membranes.* Sinauer Associates. Sunderland, MA.

Hinson A. W., Gu X. Q., Dib-Hajj S., Black J. A., and Waxman S. G. (1997) Schwann cells modulate sodium channel expression in spinal sensory neurons in vitro. Glia **21,** 339–349.

Hollmann M. and Heinemann S. (1994) Cloned glutamate receptors. *Ann. Rev. Neurosci.* **17,** 31–108.

Honegger P. and Lenoir D. (1980) Triiodothyronine enhancement of neuronal differentiation in aggregating fetal rat brain cells cultured in a chemically-defined medium. *Brain Res.* **199,** 425–434.

Honmou O., Utzschneider D. A., Rizzo M. A., Bowe C. M., Waxman S. G., and Kocsis J. D. (1994) Delayed depolarization and slow sodium currents in cutaneous afferents. *J. Neurophysiol.* **71,** 1627–1637.

Howe J. R. (1996) Homomeric and heteromeric channels formed from the kainate-type subunits GluR6 and KA2 have very small, but different, unitary conductances. *J. Neurophysiol.* **76,** 510–519.

Howe L. R., Leevers S. J., Gomez N., Nakielny S., Cohen P., and Marshall C. J. (1992) Activation of the MAP kinase pathway by the protein kinase raf. *Cell* **71,** 335–342.

Hu M., Bigger C. B., and Gardner P. D. (1995) A novel regulatory element of a nicotinic acetylcholine receptor gene interacts with a DNA binding activity enriched in rat brain. *J. Biol. Chem.* **270,** 4497–4502.

Hu M., Theobald N. L. W., and Gardner P. D. (1994) Nerve growth factor increases the transcriptional activity of the rat neuronal nicotinic acetylcholine receptor β4 subunit promoter in transfected PC12 cells. *J. Neurochem.* **62,** 392–395.

Hughes R. A., Sendtner M., and Thoenen H. (1993) Members of several gene families influence survival of rat motoneurons in vitro and in vivo. *J. Neurosci. Res.* **36,** 663–671.

Huguenard J. R., Hamill, O. P., and Prince D. A. (1988) Developmental changes in Na^+ conductances in rat neocortical neurons: appearance of a slowly inactivating component. *J. Neurophysiol.* **59,** 778–795.

Hullin R., Singer-Lahat D., and Freichel M. (1992) Calcium channel β subunit heterogeneity. *EMBO J.* **11,** 885–890.

Jaiswall R. K., Moodie S. A., Wolfman A., and Landreth G. A. (1994) The mitogen-activated protein kinase cascade is activated by B-Raf in response to nerve growth factor through interaction with p21ras. *Mol. Cell. Biol.* **14,** 6944–6953.

Jan L. Y. and Jan Y. N. (1997a) Voltage-gated and inwardly rectifying potassium channels. *J. Physiol. (Lond.)* **505,** 267–282.

Jan L. Y. and Jan Y. N. (1997b) Cloned potassium channels from eukaryotes and prokaryotes. *Ann. Rev. Neurosci.* **20,** 91–123.

Jezova D., Oliver C., and Jurcovicova J. (1991) Stimulation of adrenocorticotropin but not prolactin and catecholamine release by N-methyl-aspartic acid. *Neuroendocrinology* **54,** 488–492.

Jo S. A., Zhu X., Marchionni M. A., and Burden S. J. (1995) Neuregulins are concentrated at nerve-muscle synapse and activate acetylcholine receptor gene expression. *Nature* **373,** 158–161.

Joëls M. and de Kloet E. R. (1992) Control of neuronal excitability by corticosteroid hormones. *Trends Neurosci.* **15,** 25–30.

Joëls M. and de Kloet E. R. (1994) Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. *Prog. Neurobiol.* **43,** 1–36.

Joëls M. and Karst H. (1995) Effects of estradiol and progesterone on voltage-gated calcium and potassium conductances in rat CA1 hippocampal neurons. *J. Neurosci.* **15,** 4289–4297.

Joëls M., Bosma A., Hendriksen H., Diegenbach P., and Kamphuis W. (1996) Corticosteroid actions on the expression of kainate receptor subunit mRNAs in rat hippocampus. *Mol. Brain Res.* **37,** 15–20.

Johnson E. M. Jr, Koike T., and Franklin J. L. (1992) A calcium set point hypothesis of neuronal dependence on neurotrophic factors. *Exp. Neurol.* **115,** 163–166.

Jones A., Korpi E. R., McKernan R. M., Pelz R., Nusser Z., Makela R., Mellor J. R., Pollard S., Bahn S., Stephenson F. A., Randall A. D., Sieghart W., Somogyi P., Smith A. J. H., and Wisden W. (1997) Ligand-gated ion channel subunit partnerships: $GABA_A$ receptor α_6 subunit gene inactivation inhibits δ subunit expression. *J. Neurosci.* **17,** 1350–1362.

Jones-Villeneuve E. M. V., McBurney M. W., Rogers K. A., and Kalnins V. I. (1982) Retinoic acid induces embryonal carcinoma cells to differentiate into neurons and glial cells. *J. Cell Biol.* **94,** 253–262.

Joseph L. J., Le Beau M. M., Jamieson G. J., Acharya S., Shows T. B., Rowley J. D., and Sukhatme V. P. (1988) Molecular cloning, sequencing, and mapping of *EGR2*, a human early growth response gene encoding a protein with "zinc-binding finger" structure. *Proc. Natl. Acad. Sci. USA* **85,** 7164–7168.

Jung-Testas I., Hu Z., Robel P., and Baulieu E. E. (1989) Biosynthesis of prenenolone and progesterone in primary cultures of rat glial cells. *Endocrinology* **125,** 2083–2091.

Kalman D., Wong B., Horvai A. E., Cline M. J., and O'Lague P. H. (1990) Nerve growth factor acts through a cAMP-dependent protein kinase to increase the number of sodium channels in PC12 cells. *Neuron* **2,** 355–366.

Kamp T. J., Mitas M., Fields K. L., Asoh S., Chin H., Marban E., and Nirenberg M. (1995) Transcriptional regulation of the neuronal L-type calcium channel α_{1D} subunit gene. *Cell. Mol. Neurobiol.* **15,** 307–326.

Kang H. J. and Schuman E. M. (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science* **267,** 1658–1662.

Karst H., Werkman T. R., Struik M., Bosma A., and Joëls M. (1997) Effects of adrenalectomy on Ca^{2+} currents and Ca^{2+} channel subunit mRNA expression in hippocampal CA1 neurons of young rats. *Synapse* **26,** 155–164.

Kayano T., Noda M., Flockerzi V., Takahashi H., and Numa S. (1988) Primary structure of the rat brain sodium channel III deduced from the cDNA sequence. *FEBS Lett.* **228,** 187–194.

Kessler J. A. and Black I. B. (1979) The role of axonal transport in the regulation of enzyme activity in sympathetic ganglia of adult rats. *Brain Res.* **171,** 415–424.

Kettenmann H. and Ransom B. (1995) *Neuroglia.* Oxford University Press, New York.

Kim H. G., Wang T., Olafsson P., and Lu B. (1994) Neurotrophin 3 potentiates neuronal activity and inhibits γ-aminobutyratergic synaptic transmission in cortical neurons. *Proc. Natl. Acad. Sci. USA* **91,** 12,341–12,345.

Knutson P., Ghiani C. A., Zhou J.-M., Gallo V., and McBain C. J. (1997) K^+ channel expression and

cell proliferation are regulated by intracellular sodium and membrance depolarization in oligodendrocyte progenitor cells. *J. Neurosci.* **17,** 2669–2682.

Kobrinsky E. M., Pearson H. A., and Dolphin A. C. (1994) Low- and high-voltage-activated calcium channel currents and their modulation in the dorsal root ganglion cell line ND7-23. *Neuroscience* **58,** 539–552.

Koenig H. L., Schumacher M., Ferzaz B., Thi A. N., Ressouches A., Guennoun R., Jung-Testas I., Robel P., Akwa Y., and Baulieu E. E. (1995) Progesterone synthesis and myelin formation by Schwann cells. *Science* **268,** 1500–1503.

Kohama S. G., Garyfallou V. T., and Urbanski H. F. (1998) Regional distribution of glutamate receptor mRNA in the monkey hippocampus and temporal cortex: influence of estradiol. *Mol. Brain Res.* **53,** 328–332.

Koike T., Martin D. P., and Johnson E. M. Jr. (1989) Role of Ca^{2+} channels in the ability of membrane depolarization to prevent neuronal death induced by trophic factor deprivation: evidence that levels of internal Ca^{2+} determine nerve growth factor dependence of sympathetic ganglion cells. *Proc. Natl. Acad. Sci. USA* **86,** 6421–6425.

Koob G. F. (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathway. *Trends Pharmacol. Sci.* **13,** 177–184.

Kostyuk P., Pronchuck N., Savchenko A., and Verkhratsky A. (1993) Calcium currents in aged rat dorsal root ganglion neurones. *J. Physiol. (Lond.)* **461,** 467–483.

Kubo, Y. (1989) Development of ion channels and neurofilaments during neuronal differentiation of mouse embryonic carcinoma cell lines. *J. Physiol. (Lond.)* **409,** 497–523.

Kus L., Handa R., Sanderson J., Kerr J., and Beitz A. (1995) Distribution of NMDAR1 receptor subunit mRNA and [I^{125}]MK-801 binding in the hypothalamus of intact, castrate and castrate-DHTP treated male rats. *Mol. Brain Res.* **28,** 55–60.

Kyriakis J. M., App H., Zhang X. F., Banerjee P., Brautigan D. L., Rapp U. R., and Avruch J. (1992) Raf-1 activates MAP kinase-kinase. *Nature* **358,** 417–421.

Lasaga M., Duvilauski B. H., Seilicovich A., Afione S., and Debuljuk L (1988) Effect of sex steroids on GABA receptors in the rat hypothalamus and anterior pituitary gland. *Eur. J. Pharmacol.* **155,** 163–166.

Le Greves P., Huang W., Johansson P., Thornwall M., Zhou Q., and Nyberg F. (1997) Effects of an anabolic-androgenic steroid on the regulation of the NMDA receptor NR1, NR2A and NR2B subunit mRNAs in brain regions of the male rat. *Neurosci. Lett.* **226,** 61–64.

Lesser S. S. and Lo D. C. (1995) Regulation of voltage-gated ion channels by NGF and ciliary neurotrophic factor in SK-N-SH neuroblastoma cells. *J. Neurosci.* **15,** 253–261.

Lesser S. S., Sherwood N. T., and Lo D. C. (1997) Neurotrophins differentially regulate voltage-gated ion channels. *Mol. Cell. Neurosci.* **10,** 173–183.

Levey M. S., Brumwell C. L., Dryer S. E., and Jacob M. H. (1995) Innervation and target tissue interactions differentially regulate acetylcholine receptor subunit mRNA levels in developing neurons in situ. *Neuron* **14,** 153–162.

Levi-Montalcini R. (1987) The nerve growth factor: thirty-five years later. *EMBO J.* **6,** 1145–1154.

Levi-Montalcini R. and Angeletti P. U. (1968) Nerve growth factor. *Physiol. Rev.* **48,** 534–569.

Levine E. S., Dreyfus C. F., Black I. B., and Plummer M. R. (1995) Differential effects of NGF and BDNF on voltage-gated calcium currents in embryonic basal forebrain neurons. *J. Neurosci.* **15,** 3084–3091.

Lewis D. L., de Aizpurua H. J., and Rausch D. M. (1993) Enhanced expression of Ca^{2+} channels by nerve growth factor and the v-src oncogene in rat phaeochromocytoma cells. *J. Physiol. (Lond.)* **465,** 325–342.

Lindstrom J. (1996) Neuronal nicotinic acetylcholine receptors, in *Ion Channels,* vol. 4 (Toshio N., ed.), Plenum, New York, pp. 377–450.

Lindstrom J. (1997) Nicotinic acetylcholine receptors in health and disease. *Mol. Neurobiol.* **15,** 193–222.

Liu S. J. and Kaczmarek L. K. (1998) The expression of two splice variants of the Kv3.1 potassium channel gene is regulated by different signaling pathways. *J. Neurosci.* **18,** 2881–2890.

Loeb D. M., Maragos J., Martin-Zanca D., Chao M. V., Parada L. F., and Greene L. A. (1991) The trk proto-oncogene resuces NGF responsiveness in mutant NGF-nonresponsive PC12 cell lines. *Cell* **66,** 961–966.

Luine V. N. (1994) Steroid hormone influences on spatial memory. *Ann. NY Acad. Sci.* **14,** 201–211.

Luneau C. J., Williams J. B., Marshall J., Levitan E. S., Oliva C., Smith J. S., Antanavage J., Folander K., Stein R. B., Swanson R., Kaczmarek L. K., and Buhrow S. A. (1991) Alternative splicing contributes to K^+ channel diversity in the mam-

malian central nervous system. *Proc. Natl. Acad. Sci. USA* **88,** 3932–3936.

Macdonald R. L., and Angelotti T. P. (1993) Native and recombinant $GABA_A$ receptor channels. *Cell Physiol. Biochem.* **3,** 352–373.

Macdonald R. L., and Olsen R. W. (1994) $GABA_A$ receptor channels. *Ann. Rev. Neurosci.* **17,** 569–602.

Maconochie D. J. and Knight D. E. (1992) A study of the bovine adrenal chromaffin nicotinic receptor using patch clamp and concentration-jump techniques. *J. Physiol. (Lond.)* **454,** 124–153.

MacVicar B. A. (1984) Voltage-dependent calcium channels in glial cells. *Science* **226,** 1345–1347.

Maggi A. and Perez J. (1986) Estrogen-induced upregulation of γ-aminobutyric acid receptors in the CNS of rodents. *J. Neurochem.* **47,** 1793–1797.

Majewska M. D. (1992) Neurosteroids: endogenous bimodal modulators of the $GABA_A$ receptor. Mechanism of action and physiological significance. *Prog. Neurobiol.* **38,** 379–395.

Majewska M. D., Harrison N. L., Schwartz R. D., Barker J. L., and Paul S. M. (1986) Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science* **232,** 1004–1007.

Makara G. B. and Stark E. (1975) Effect of intraventricular glutamate on ACTH release. *Neuroendocrinology* **18,** 213–216.

Mandel G., Cooperman S. S., Maue R. A., Goodman R. H., and Brehm P. (1988) Selective induction of brain type II Na^+ channels by nerve growth factor. *Proc. Natl. Acad. Sci. USA* **85,** 924–928.

Mano I. and Teichberg V. I. (1998) A tetrameric subunit stoichiometry for a glutamate receptor-channel complex. *NeuroReport* **9,** 327–331.

Marchionni M. A., Goodearl A. D. J., Chen M. S., Berminghammcdonogh O., Kirk C., Hendricks M., Danehy F., Misumi D., Sudhalter J., Kobayashi K., Wroblewski D., Lynch C., Baldassare M., Hiles I., Davis J. B., Hsuan J. J., Totty N. F., Otsu M., McBurney R. N., Waterfield M. D., Stroobant P., and Gwynne D. (1993) Glial growth factors are alternatively spliced *ERBB2* ligands expressed in the nervous system. *Nature* **362,** 312–318.

Margiotta J. F., Berg D. K., and Dionne V. E. (1987) Cyclic AMP regulates the proportion of functional acetylcholine receptors on chicken ciliary ganglion neurons. *Proc. Natl. Acad. Sci. USA* **84,** 8155–8159.

Marshall C. J. (1995) Specificity of receptor tyrosine kinase signalling: transient versus sustained extracellular signal-regulated kinase activation. *Cell* **80,** 179–185.

Mascarelli J., Tassin J., and Courtois Y. (1990) Fibroblast growth factor phosphorylation and receptors in rod outer segments. Interaction with glial cells: intracrine and paracrine role in retina. *Invest. Ophthalmol. Vis. Sci.* **31,** 227 (Suppl.).

Matter J. M., Mattersadzinski L., and Ballivet M. (1995) Activity of the beta-3 nicotinic receptor promoter is a marker of neuron fate determination during retina development. *J. Neurosci.* **15,** 5919–5928.

Matter-Sadzinski L., Hernandez M., Roztocil T., Ballivet M., and Matter J. (1992) Neuronal specificity of the α7 nicotinic acetylcholine receptor promoter develops during morphogenesis of the central nervous system. *EMBO J.* **11,** 4529–4538.

Mattson M. P., Kumar K., Cheng B., Wang H., and Michaelis E. K. (1993) Basic FGF regulates the expression of a functional 71 kDa NMDA receptor protein that mediates calcium influx and neurotoxicity in cultured hippocampal neurons. *J. Neurosci.* **13,** 4575–4588.

Mattson M. P., Murrain M., Guthrie P. B., and Kater S. B. (1989) Fibroblast growth factor and glutamate: opposing actions in the generation and degeneration of hippocampal neuroarchitecture. *J. Neurosci.* **9,** 3728–3740.

McCobb D. P., Best P. M., and Beam K. G. (1989) Development alters the expression of calcium currents in chick limb motoneurons. *Neuron* **2,** 1633–1643.

McDonough J. and Deneris E. (1997) β43′: an enhancer displaying neural-restricted activity is located in the 3′-ultranslated exon of the rat nicotinic acetylcholine receptor β4 gene. *J. Neurosci.* **17,** 2273–2283.

McEwen B. S. (1991) Steroid hormones are multifunctional messengers to the brain. *Trends Endocrinol. Metab.* **6,** 62–67.

McGehee D. S. and Role L. W. (1995) Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Ann. Rev. Physiol.* **57,** 521–546.

McIntosh L. J., Cortopassi K. M., and Sapolsky R. M. (1998) Glucocorticoids may alter antioxidant enzyme capacity in the brain: kainic acid studies. *Brain Res.* **791,** 215–222.

McKinnon R. D., Matsui T., Dubois-Dalcq M., and Aaronson S. A. (1990) FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* **5,** 603–614.

Meinecke D. L. and Rakic P. (1990) Developmental expression of GABA and subunits of the $GABA_A$ receptor complex in an inhibitory synaptic circuit in the rat cerebellum. *Dev. Brain Res.* **55,** 73–86.

Memo M., Bovolin P., Costa E., and Grayson D. R. (1991) Regulation of γ-aminobutyric $acid_A$ receptor subunit expression by activation of N-methyl-D-aspartate-selective glutamate receptors. *Mol. Pharmacol.* **39,** 599–603.

Mentis G. Z., Greensmith L., and Vrbova G. (1993) Motoneurons destined to die are rescued by blocking NMDA receptors by MK801. *Neuroscience* **54,** 283–285.

Milton N. G. N., Bessis A., Changeux J-P., and Latchman D. S. (1996) Differential regulation of neuronal nicotinic acetylcholine receptor subunit gene promoters by Brn-3 POU family transcription factors. *Biochem. J.* **317,** 419–423.

Minturn J. E., Sontheimer H., Black J. A., Ransom B. R., and Waxman S. G. (1992) Sodium channel expression in optic nerve astrocytes chronically-deprived of axonal contact. *Glia* **6,** 19–29.

Mishina M., Takai T., Imoto K., Noda M., Takahashi T., Numa S., Methfessel C., and Sakmann B. (1986) Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. *Nature* **321,** 406–411.

Montpied P., Weizman A., Weizman R., Kook K. A., Morrow A. L., and Paul S. M. (1993) Repeated swim stress reduces $GABA_A$ receptor α subunit mRNAs in the mouse hippocampus. *Mol. Brain Res.* **18,** 267–272.

Monyer H., Sprengel R., Schoepfer R., Herb A., Higuchi M., Lomeli H., Burnashev N., Sakmann B., and Seeburg P. (1992) Heterodimeric NMDA receptors: molecular and functional distinction of subtypes. *Science* **256,** 1217–1221.

Murrell R. D. and Tolkovsky A. M. (1993) Role of voltage-gated Ca^{2+} channels and intracellular Ca^{2+} in rat sympathetic neuron survival and function promoted by high K^+ and cyclic AMP in the presence or in the absence of NGF. *Eur. J. Neurosci.* **5,** 1261–1272.

Nair S. M., Werkman T. R., Craig J., Finnell R., Joëls M., and Eberwine J. H. (1998) Corticosteroid regulation of ion channel conductances and mRNA levels in individual hippocampal CA1 neurons. *J. Neurosci.* **18,** 2685–2696.

Newman E. A. (1985) Voltage-dependent calcium and potassium channels in retinal glial cells. *Nature* **317,** 809–811.

Noda M., Ikeda T., Kayano T., Suzuki H., Takeshima H., Kurasaki M., Takahashi H., and Numa S. (1986) Existence of distinct sodium channel messenger RNAs in rat brain. *Nature* **320,** 188–192.

Nooney J. and Lodge D. (1986) The use of invertebrate peptide toxins to establish Ca^{2+} channel identity of CA3-CA1 neurotransmission in rat hippocampal slices. *Eur. J. Pharmacol.* **306,** 41–50.

Nutter T. J. and Adams D. J. (1991) The permeability of neuronal nicotinic receptor channels to monovalent and divalent inorganic cations. *Biophys. J.* **59,** A34.

O'Connor L. H., Nock B., and McEwen B. S. (1988) Regional specificity of gamma-aminobutyric acid receptor regulation by estradiol. *Neuroendocrinology* **47,** 473–481.

O'Neill E. M., Rebay I., Tjian R., and Rubin G. M. (1994) The activities of two Ets-related transcription factors required for Drosophila eye development are modulated by the Ras/MAPK pathway. *Cell* **78,** 137–147.

Opas M., Kalnins V. I., and Fedoroff S. (1986) Spectrin does not redistribute with actin during dbcAMP-induced changes in astrocytes in vitro. *Dev. Brain Res.* **25,** 314–317.

Orchinik M., Weiland N. G., and McEwen B. S. (1994) Adrenalectomy selectively regulates $GABA_A$ receptor subunit expression in the hippocampus. *Mol. Cell Neurosci.* **5,** 451–458.

Oyelese A. A., Rizzo M. A., Waxman S. G., and Kocsis J. D. (1997) Differential effects of NGF and BDNF on axotomy-induced changes in $GABA_A$-receptor-mediated conductance and sodium currents in cutaneous afferent neurons. *J. Neurophysiol.* **78,** 31–42.

Ozaki M., Sasner M., Yano R., Lu H. S., and Buonanno A. (1997) Neuregulin-β induces expression of an NMDA-receptor subunit. *Nature* **390,** 691–694.

Pappas C. A., Ulrich N., and Sontheimer H. (1994) Reduction of glial proliferation by K^+ channel blockers is mediated by changes in pH_i. *NeuroReport* **6,** 193–196.

Parsons B., Rainbow T. C., MacLusky N. J., and McEwen B. S. (1982) Progestin receptor levels in rat hypothatlamic and limbic nuclei. *J. Neurosci.* **2,** 1446–1452.

Partin K. M., Patneau D. K., Winters C. A., Mayer M. L., and Buonanno A. (1993) Selective modulation of desensitization at AMPA versus kainate receptors by cyclothiazide and concanavalin A. *Neuron* **11,** 1069–1082.

Patchev V. K., Karalis K., and Chrousos G. P (1994) Effects of excitatory amino acid transmitters on

hypothalamic corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) release in vitro: implications in pituitary-adrenal regulation. *Brain Res.* **633,** 312–316.

Patel A. J., Smith R. M., Kingsbury A. E., Hunt A., and Balazs R. (1980) Effects of thyroid state on brain development: muscarinic acetylcholine and GABA receptors. *Brain Res.* **198,** 389–402.

Patwardhan S., Gashler A., Siegel M. G., Chang L. C., Joseph L. J., Shows T. B., Le B. M., and Sukhatme V. P. (1991) EGR3, a novel member of the Egr family of genes encoding immediate-early transcription factors. *Oncogene* **6,** 917–928.

Paul S. M. and Purdy R. H. (1992) Neuroactive steroids. *FASEB J.* **6,** 2311–2322.

Pelletier G., Liao N., Follea N., and Govindan M. V. (1988) Mapping of estrogen receptor-producing cells in rat brain by in situ hybridization. *Neurosci. Lett.* **94,** 23–28.

Pelligrini-Giampietro D. E., Bennett M. V., and Zukin R. S. (1991) Differential expression of three glutamate receptor genes in developing rat brain: an in situ hybridisation study. *Proc. Natl. Acad. Sci. USA* **88,** 4157–4161.

Perney T. M. and Kaczmarek L. K. (1993) Expression and regulation of mammalian K^+ channel genes. *Neurosciences* **5,** 135–145.

Perney T. M., Marshall J., Martin K. A., Hockfield S., and Kaczmarek L. K. (1992) Expression of the mRNAs for the Kv3.1 potassium channel gene in the adult and developing brain. *J. Neurophys.* **68,** 756–766.

Peterson S. L., McCrone S., Coy D., Adelman J. P., and Mahon L. C. (1993a) $GABA_A$ receptor subunit mRNAs in cells of the preoptic area: colocalisation with LHRH mRNA using dual-label in situ hybridisation histochemistry. *Endocr. J.* **1,** 29–34.

Peterson S. L., Reeves A., Keller M., Gardoner E., Mahan L. C., and McCrone S. (1993b) Effects of estradiol (E2) and progesterone (P4) on expression of mRNAs encoding $GABA_A$ receptor subunits. *Soc. Neurosci. Abstr.* **19,** 489.14

Pettigrew A. G., Crepel F., and Krupa M. (1988) Development of ionic conductances in neurons of the inferior olive in the rat: an in vitro study. *Proc. R. Soc. Lond. B* **234,** 199–218.

Piehl F., Arvidsson U., Johnson H., Cullheim S., Dagerlind A., Ulfhake B., Cao Y., Elde R., Pettersson R. F., Terenius L., and Hökfelt T. (1993) GAP-43, aFGF, CCK and α- and β-CGRP, in rat spinal motoneurons subjected to axotomy and/or dorsal root severance. *Eur. J. Neurosci.* **5,** 1321–1333.

Piehl F., Tabar G., and Cullheim S. (1995) Expression of NMDA receptor mRNAs in rat motoneurons is down-regulated after axotomy. *Eur. J. Neurosci.* **7,** 2101–2110.

Pollock J. D. and Rane S. G. (1996) $p21^{ras}$ signaling is necessary but not sufficient to mediate neurotrophin induction of calcium channels in PC12 cells. *J. Biol. Chem.* **271,** 8008–8014.

Potthoff O. and Dietzel I. D. (1997) Thyroid hormone regulates Na^+ currents in cultured hippocampal neurons from postnatal rats. *Proc. R. Soc. Lond.* **264,** 367–373.

Poulter M. O., Barker J. L., O'Carroll A. M., Lolait S. J., and Mahan L. C. (1992) Differential and transient expression of $GABA_A$ receptor α-subunit mRNAs in the developing rat CNS. *J. Neurosci.* **12,** 2888–2900.

Puchalski R. B., Louis J-C., Brose N., Traynelis S. F., Egebjerg J., Kukekov V., Wenthold R. J., Rogers S. W., Lin F., Moran T., Morrison J. H., and Heinemann S. F. (1994) Selective RNA editing and subunit assembly of native glutamate receptors. *Neuron* **13,** 131–147.

Puia G., Santi M. R., Vicini S., Pritchett D., Purdy R. H., Paul S. M., Seeburg P., and Costa E. (1990) Neurosteroids act on recombinant human $GABA_A$ receptors. *Neuron* **4,** 759–765.

Puro D. G. and Mano T. (1991) Modulation of calcium channels in human retinal glial cells by basic fibroblast growth factor: a possible role in retinal pathobiology. *J. Neurosci.* **11,** 1873–1880.

Rabow L. E., Russek S. J., and Farb D. H. (1995) From ion currents to genomic analysis—recent advances in $GABA_A$ receptor research. *Synapse* **21,** 189–274.

Raff M. C. (1989) Glial cell diversification in the rat optic nerve. *Science* **243,** 1450–1455.

Randall A. and Tsien R. W. (1995) Pharmacological dissection of multiple types of Ca^{2+} channel currents in rat cerebellar granule neurons. *J. Neurosci.* **15,** 2995–3012.

Raucher S. and Dryer S. E. (1995) Target-derived factors regulate the expression of Ca^{2+}-activated K^+ currents in developing chick sympathetic neurones. *J. Physiol. (Lond.)* **486,** 605–614.

Ray W. J. and Gottlieb D. I. (1993) Expression of ionotropic glutamate receptor genes by P19 embryonal carcinoma cells. *Biochem. Biophys. Res. Comm.* **197,** 1475–1482.

Ribera A. B. (1990) A potassium channel gene is expressed at neural induction. *Neuron* **5,** 691–701.

Ribera A. B. and Spitzer N. C. (1992) Developmental regulation of potassium channels and the

impact on neuronal differentiation, in *Ion Channels* (Narahashi T., ed.), Plenum, New York, pp. 1–38.

Richards J. G., Schoch P., and Haefely W. (1991) Benzodiazepine receptors: new vistas. *Sem. Neurosci.* **3,** 191–203.

Rizzo M. A., Kocsis J. D., and Waxman S. G. (1994) Slow sodium conductances of dorsal root ganglion neurons: intraneuronal homogeneity and interneuronal heterogeneity. *J. Neurophysiol.* **72,** 2796–2815.

Rogers S. W., Mandelzys A., Deneris E. S., Cooper E., and Heinemann S. (1992) The expression of nicotinic acetylcholine receptors by PC12 cells treated with NGF. *J. Neurosci.* **12,** 4611–4623.

Role L. and Berg D. K. (1996) Nicotinic receptors in the development and modulation of CNS synapses. *Neuron* **16,** 1077–1085.

Rosemund C., Stern-Bach Y., and Stevens C. F. (1998) The tetrameric structure of a glutamate receptor channel. *Science* **280,** 1596–1599.

Rosen L. B., Ginty D. D., and Greenberg M. E. (1995) Calcium regulation of gene expression. *Adv. Second Messenger Phosphoprotein Res.* **30,** 225–253.

Rothhut B, Romano S. J., Vijayaraghavan S., and Berg D. K. (1996) Post-translational regulation of neuronal acetylcholine receptors stably expressed in a mouse fibroblast cell line. *J. Neurobiol.* **29,** 115–125.

Roy M. L. and Narahashi T. (1992) Differential properties of tetrodotoxin-sensitive and terodotoxin-resistant sodium channels in rat dorsal root ganglion neurons. *J. Neurosci.* **12,** 2104–2111.

Rudy B., Kirschenbaum B., Rukenstein A., and Greene L. A. (1987) Nerve growth factor increases the number of functional Na channels and induces TTX-resistant Na channels in PC12 pheochromocytoma cells. *J. Neurosci.* **7,** 1613–1625.

Sah D. W. Y., Ray J., and Gage F. H. (1997) Regulation of voltage- and ligand-gated currents in rat hippocampal progenitor cells in vitro. *J. Neurobiol.* **32,** 95–110.

Sanner C. A., Cunningham T. J., and Goldberger M. E. (1994) NMDA receptor blockade rescues Clarke's and red nucleus neurons after spinal hemisection. *J. Neurosci.* **14,** 6472–6480.

Sapru M. K., Florance S. K., Kirk C., and Goldman D. (1998) Identification of a neuregulin and protein-tyrosine phophatase response element in the nicotinic acetylcholine receptor epsilon subunit gene: regulatory role of an Ets transcription factor. *Proc. Natl. Acad. Sci. USA* **95,** 1289–1294.

Sapru M. K., Zhou G., and Goldman D. (1994) Protein-tyrosine phosphatases specifically regulate muscle adult-type nicotinic acetylcholine receptor gene expression. *J. Biol. Chem.* **269,** 27,811–27,814.

Sarao R., Gupta S. K., Auld V. J., and Dunn R. J. (1991) Developmentally regulated alternative RNA splicing of rat brain sodium channel mRNAs. *Nucleic Acid Res.* **19,** 5673–5679.

Sargent P. B. (1993) The diversity of neuronal nicotinic acetylcholine receptors. *Ann. Rev. Neurosci.* **16,** 403–443.

Schaller K. L., Krzemien D. M., Yarowsky P. J., Krueger B. K., and Caldwell J. H. (1995) A novel abundant sodium channel expressed in neurons and glia. *J. Neurosci.* **15,** 3231–3242.

Schaller K. L., Krzemien, D. M., McKenna N. M., and Caldwell J. H. (1992) Alternatively spliced sodium channel transcripts in brain and muscle. *J. Neurosci.* **12,** 1370–1381.

Schiffer H. H., Swanson G. T., and Heinemann S. F. (1997) Rat GluR7 and a carboxyterminal splice variant, GluR7b, are functional kainate receptor subunits with a low sensitivity to glutamate. *Neuron* **19,** 1141–1146.

Schlumpf M., Ramseier H., Abriel H., Youmbi M., Baumann J. B., and Lichtensteiger W. (1989) Diazepam effects on the fetus. *Neurotoxicology* **10,** 501–516.

Schmid S. and Guenther E. (1996) Developmental regulation of voltage-activated Na^+ and Ca^{2+} currents in rat retinal ganglion cells. *NeuroReport* **7,** 677–681.

Schoepfer R., Conroy W. G., Whiting P., Gore M., and Lindstrom J. (1990) Brain α-bungarotoxin-binding protein cDNAs and mAbs reveal subtypes of this branch of the ligand-gated ion channel superfamily. *Neuron* **5,** 35–48.

Schuetze S. M. and Role L. W. (1987) Developmental regulation of nicotinic acetylcholine receptors. *Ann. Rev. Neurosci.* **10,** 403–457.

Schwartz A., Palti Y., and Meiri H. (1990) Structural and developmental differences between three types of sodium channels in dorsal root ganglion cells of newborn rats. *J. Membr. Biol.* **116,** 117–128.

Schwartz-Giblin S., Korotzer A., and Pfaff D. W. (1989) Steroid hormone effects on picrotoxin-induced seizures in female and male rats. *Brain Res.* **476,** 240–247.

Seeburg P. H. (1996) The role of RNA editing in controlling glutamate receptor channel properties. *J. Neurochem.* **66,** 1–5.

Seguela P., Wadiche J., Dinnelly-Millet K., Dani J., and Patrick J. (1993) Molecular cloning, functional properties, and distribution of rat brain α7, a nicotinic cation channel highly permeable to calcium. *J. Neurosci.* **13,** 596–604.

Sendtner M., Holtmann B., Kolbeck R., Thoenen H., and Barde Y. A. (1992) Brain-derived neurotrophic factor prevents the death of motoneurons in newborn rats after nerve section. *Nature* **360,** 757–759.

Sendtner M., Kreutzberg G. W., and Thoenen H. (1990) Ciliary neurotrophic factor prevents the degeneration of motoneurons after axotomy. *Nature* **345,** 440,441.

Serafini R., Valeyev A. V., Barker J. L., and Poulter M. O. (1995) Depolarizing GABA-activated Cl^- channels in embryonic rat spinal and olfactory bulb cells. *J. Physiol. (Lond.)* **488,** 371–386.

Sharma N., D'Arcangelo G., Kleinklaus A., Halegoua S., and Trimmer J. S. (1993) Nerve growth factor regulates the abundance and distribution of K^+ channels in PC12 cells. *J. Cell Biol.* **123,** 1835–1843.

Sheng M., Tsaur M.-L., Jan Y. N., and Jan L. Y. (1992) Subcellular segregation of two A-type K^+ channel protein in rat central neurons. *Neuron* **9,** 271–284.

Shitaka Y., Matsuki N., Saito H., and Katsuki H. (1996) Basic fibroblast growth factor increases functional L-type Ca^{2+} channels in fetal rat hippocampal neurons: implications for neurite morphogenesis *in vitro*. *J. Neurosci.* **16,** 6476–6489.

Si J., Luo Z., and Mei L. (1996) Induction of acetylcholine receptor gene expression by ARIA requires activation of mitogen-activated protein kinase. *J. Biol. Chem.* **271,** 19,752–19,759.

Simerly R. B., Chang C., Muramatsu M., and Swanson L. W. (1990) Distribution of adrogen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridisation study. *J. Comp. Neurol.* **294,** 76–95.

Singh M., Meyer E. M., Millard W. J., and Simpkins J. W. (1994) Ovarian steroid deprivation results in reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. *Brain Res.* **644,** 305–312.

Snider W. D. (1994) Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* **77,** 627–638.

Snutch T. P., Tomlison W. J., Leonard J. P., and Gilbert S. (1991) Distinct calcium channels are generated by alternative splicing and are differentially expressed in the mammalian CNS. *Neuron* **7,** 45–57.

Sontheimer H. and Ritchie J. M. (1995) Voltage-gated sodium and calcium channels, in *Neuroglia* (Kettenmann H. and Ransom B. R., eds.), Oxford University Press, New York, pp. 202–220.

Sontheimer H., Black I., and Waxman S. G. (1996) Voltage-gated Na^+ channels in glia: properties and possible functions. *Trends Neurosci.* **19,** 325–331.

Sontheimer H., Trotter J., Schachner M., and Kettenmann H. (1989) Channel expression correlates with differentiation stage during the development of oligodendrocytes from their precursor cells in culture. *Neuron* **2,** 1135–1145.

Strübing C., Ahnert-Hilger G., Shan J., Wiedenmann B., Hescheler J., and Wobus A. M. (1995) Differentiation of pluripotent embryonic stem cells into the neuronal lineage *in vitro* gives rise to mature inhibitory and excitatory neurons. *Mech. Dev.* **53,** 275–287.

Strübing C., Rohwedel J., Ahnert-Hilger G., Wiedenmann B., Hescheler J., and Wobus A. M. (1997) Development of G protein-mediated Ca^{2+} channel regulation in mouse embryonic stem cell-derived neurons. *Eur. J. Neurosci.* **9,** 824–832.

Sucher N. J., Brose N., Dietcher D. L., Awobuluyi M., Gassic G. P., Bading H., Cepko C. L., Greenberg M. E., Jahn R., Heinemann S. F., and Lipton S. A. (1993) Expression of endogenous NMDAR1 transcripts without receptor protein suggests post-transcriptional control in PC12 cells. *J. Biol. Chem.* **30,** 22,299–22,304.

Sun Y. and Zigmond R. (1996) Involvement of leukemia inhibitory factor in the increases in galanin and vasoactive intestinal peptide mRNA and the decreases in neuropeptide Y and tyrosine hydroxylase mRNA after axotomy of sympathetic neurons. *J. Neurochem.* **67,** 1751–1760.

Sun Y., Rao M. S., Zigmond R. E., and Landis S. C. (1994) Regulation of vasoactive intestinal peptide expression in sympathetic neurons in culture and after axotomy: the role of cholinergic diferentiation factor/leukemia inhibitory factor. *J. Neurosci.* **25,** 415–430.

Swanson G. T., Feldmeyer D., Kaneda M., and Cull-Candy S. G. (1996) Effect of editing and subunit co-assembly on single-channel properties of recombinant kainate receptors. *J. Physiol. (Lond.)* **492,** 129–142.

Swanson R., Marshall J., Smith J. S., Williams J. B., Boyle M. B., Folander K., Luneau C. J., Antanavage J., Oliva C., Buhrow S. A., Bennett D., Stein R. B., and Kaczmarek L. K. (1990) Cloning and expression of cDNA and genomic clones encod-

ing three delayed rectifier potassium channels in rat brain. *Neuron* **4,** 929–939.

Takahashi T. and Momiyama A. (1993) Different types of calcium channels mediate central synaptic transmission. *Nature* **366,** 156–158.

Takimoto K., Fomina A. F., Gealy R., Trimmer J. S., and Levitan E. S. (1993) Dexamethasone rapidly induces Kv1.5 K^+ channel gene transcription and expression in clonal pituitary cells. *Neuron* **11,** 359–369.

Takimoto K., Gealy R., Fomina A. F., Trimmer J. S., and Levitan E. S. (1995) Inhibition of voltage-gated K^+ channel gene expression by the neuropeptide thyrotropin-releasing hormone. *J. Neurosci.* **15,** 449–457.

Tanabe T., Takeshima H., Mikami A., Flockerzi V., Takahashi H., Kangawa K., Kojima M., Matsuo H., Hirose T., and Numa S. (1987) Primary structure of the receptor for calcium channel blockers from skeletal muscle. *Nature* **328,** 313–318.

Tansey M. G., Chu G. C., and Merlie J. P. (1996) ARIA/HRG regulates AChR epsilon subunit gene expression at the neuromuscular synapse via activation of phophatidylinositol 3-kinase and Ras/MAPK pathway. *J. Cell Biol.* **134,** 465–476.

Tejani-Butt S. M., Yang J., and Kaviani A. (1993) Time course of altered thyroid states on 5-HT_{1A} receptors and 5-HT uptake sites in rat brain: an autoradiographic analysis. *Neuroendocrinology* **57,** 1011–1018.

Thio C. L., Waxman S. G., and Sontheimer H. (1993) Ion channels in spinal cord astrocytes in vitro. III. Modulation of channel expression by co-culture with neurons and neuron-conditioned medium. *J. Neurophysiol.* **69,** 819–831.

Thoenen H. (1995) Neurotrophins and neuronal plasticity. *Science* **270,** 593–598.

Thomas S. M., DeMarco M., D'Arcangelo G., Halegoua S., and Brugge J. S. (1992) Ras is essential for nerve growth factor and phorbol ester-induced tyrosine phosphorylation of MAP kinases. *Cell* **68,** 1031–1040.

Thompson S. M. and Wong R. K. S. (1991) Development of calcium current subtypes in isolated rat hippocampal pyramidal cells. *J. Physiol. (Lond.)* **439,** 671–689.

Thompson C. L., Pollard S., and Stephenson F. A. (1996a) Developmental regulation of expression of $GABA_A$ receptor α1 and α6 subunits in cultured rat cerebellar granule cells. *Neuropharmacology* **35,** 1337–1346.

Thompson C. L., Pollard S., and Stephenson F. A. (1996b) Bidirectional regulation of $GABA_A$ receptor α1 and α6 subunit expression by a cyclic AMP-mediated signalling mechanism in cerebellar granule cells in primary culture. *J. Neurochem.* **67,** 434–437.

Timpe L. C. and Fantl W. J. (1994) Modulation of a voltage-activated potassium channel by peptide growth factor receptors. *J. Neurosci.* **14,** 1195–1201.

Titmus M. J. and Faber D. S. (1990) Axotomy-induced alterations in the electrophysiological characteristics of neurons. *Prog. Neurobiol.* **35,** 1–51.

Toledo-Aral J. J., Brehm P., Halegoua S., and Mandel G. (1995) A single pulse of nerve growth factor triggers long-term neuronal excitability through sodium channel gene induction. *Neuron* **14,** 607–611.

Toledo-Aral J. J., Moss B. L., He Z.-J., Koszowski A. G., Whisenand T., Levinson S. R., Wolf J. J., Silos-Santiago I., Halegoua S., and Mandel G. (1997) Identification of PN1, a predominant voltage-dependent sodium channel expressed principally in peripheral neurons. *Proc. Natl. Acad. Sci. USA* **94,** 1527–1532.

Tolle T. R., Berthele A., Zieglgansberger W., Seeburg P. H., and Wisden W. (1994) The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. *J. Neurosci.* **13,** 5009–5028.

Trapp T., Rupprecht R., Castren M., Reul J. M. H. M., and Holsboer F. (1994) Heterodimerization between mineralocorticoid and glucocorticoid receptor: a new principle of glucocorticoid action in the CNS. *Neuron* **13,** 1457–1462.

Traverse S., Gomez N., Paterson H., Marshall C., and Cohen P. (1992) Sustained activation of the mitogen-activated protein (MAP) kinase cascade may be required for differentiation of PC12 cells. *Biochem. J.* **288,** 351–355.

Traverse S., Seedorf K., Paterson H., Marshall C., Cohen P., and Ulrich A. (1994) EGF triggers neuronal differentiation of PC12 cells that overexpress the EGF receptor. *Curr. Biol.* **4,** 694–701.

Treier M., Bohmann D., and Mlodzik M. (1995) Jun cooperates with the ETS domain protein *POINTED* to induce photoreceptor R7 fate in the Drosophila eye. *Cell* **83,** 753–760.

Trimmer J. S. (1993) Expression of Kv2.1 delayed rectifier K^+ channel isoforms in the developing rat brain. *FEBS Lett.* **324,** 205–210.

Tsien R. W., Ellinor P. T., and Horne W. A. (1991) Molecular diversity of voltage-dependent Ca^{2+} channels. *Trends Pharmacol. Sci.* **12,** 349–354.

Tsien R. W., Lipscombe D., Madison D. V., Bley K. R., and Fox A. P. (1988) Multiple types of neuronal calcium channels and their selective modulation. *Trends Neurosci.* **11,** 431–438.

Urbano F. J., Sierra F., Colombo J. A., Velasco J. M., and Buno W. (1997) Different voltage-gated sodium currents are expressed by human neuroblastoma NB69 cells when cultured in defined serum-free and in astroglial-conditioned media. *Glia* **19,** 161–170.

Usowicz M. M., Porzig H., Becker C., and Reuter H. (1990) Differential expression by nerve growth factor of two types of Ca^{2+} channels in rat phaeochromocytoma cell lines. *J. Physiol.* **426,** 95–116.

Valenzuela C. F., Kazlaukas A., Brozowski S. J., Weiner J. L., Demali K. A., McDonald B. J., Moss S. J., Dunwiddie T. V., and Harris R. A. (1995) Platelet-derived growth factor receptor is a novel modulator of type A γ-aminobutyric acid-gated ion channels. *Mol. Pharmacol.* **48,** 1099–1107.

Valenzuela C. F., Kazlauskas A., and Weiner J. L. (1997) Roles of platelet-derived growth factor in the developing and mature nervous systems. *Brain Res. Rev.* **24,** 77–89.

Van der Greer P. and Hunter T. (1994) Receptor protein tyrosine kinases and their signal transduction pathways. *Ann. Rev. Cell Biol.* **10,** 251–337.

Vejsada R., Tseng J. L., Lindsay R. M., Acheson A., Aebischer P., and Kato A. C. (1998) Synergistic but transient rescue effects of BDNF and GDNF on axotomized neonatal motoneurons. *Neuroscience* **84,** 129–139.

Verkhratsky A. and Kettenmann H. (1996) Calcium signaling in glial cells. *Trends Neurosci.* **19,** 346–352.

Vescovi A. L., Reynolds B. A., Fraser D. D., and Weiss S. (1993) bFGF regulates the proliferative fate of unipotent (neuronal) and bipotent (neuronal/astroglial) EGF-generated CNS progenitor cells. *Neuron* **11,** 951–966.

Walker D. and De Waard M. (1998) Subunit interaction sites in voltage-dependent Ca^{2+} channels: role in channel function. *Trends Neurosci.* **21,** 148–154.

Wall P. D. and Devor M. (1981) The effect of peripheral nerve injury on dorsal root potentials and on transmission of afferent signals into the spinal cord. *Brain Res.* **209,** 95–111.

Wan Q., Xiong Z. Q., Man H. Y., Ackerley C. A., Braunton J., Lu W. Y., Becker L. E., MacDonald J. F., and Wang Y. T. (1997) Recruitment of functional $GABA_A$ receptors to postsynaptic domains by insulin. *Nature* **388,** 686–690.

Watanabe M., Inoue Y., Sakimura K., and Mishina M. (1992) Developmental changes in distribution of NMDA receptor channel subunit mRNAs. *NeuroReport* **3,** 1138–1140.

Waxman S. G., Kocsis J. D., and Black J. A. (1994) Type III sodium channel mRNA is expressed in embryonic but not in adult spinal sensory neurons, and is reexpressed following axotomy. *J. Neurophysiol.* **72,** 466–470.

Wehling M. (1997) Specific, nongenomic actions of steroid hormones. *Ann. Rev. Physiol.* **59,** 365–393.

Weiland N. G. (1992) Estradiol selectively regulates agonist binding sites on the N-methyl-D-aspartate receptor complex in the CA1 region of the hippocampus. *Endocrinology* **131,** 662–668.

Weiland N. G., Orchinik M., and Tanapat P. (1997) Chronic corticosterone treatment induces parallel changes in N-methyl-D-aspartate receptor subunit messenger RNA levels and antagonist binding sites in the hippocampus. *Neuroscience* **78,** 653–662.

Weiland N. G. and Orchinik M. (1995) Specific subunit mRNAs of the $GABA_A$ receptor are regulated by progesterone in subfields of the hippocampus. *Mol. Brain Res.* **32,** 271–278.

Wenthold R. J., Yokotani N., Doi K., and Wada K. (1992) Immunochemical characterization of the non-NMDA glutamate receptor using subunit-specific antibodies: evidence for a hetero-oligomeric structure in rat brain. *J. Biol. Chem.* **267,** 501–507.

Wheeler D., Randall A., and Tsien R. (1994) Roles of N-type and Q-type Ca^{2+} channels in supporting synaptic transmission. *Science* **264,** 107–111.

Whiting P. and Lindstrom J. (1988) Characterization of bovine and human neuronal nicotinic acetylcholine receptors using monoclonal antibodies. *J. Neurosci.* **8,** 3395–3404.

Whiting P. J., Schoepfer R., Swanson L. W., Simmons D. M., and Lindstrom J. M. (1987) Functional acetylcholine receptor in PC12 cells reacts with a monoclonal antibody to brain nicotinic receptors. *Nature* **327,** 515–518.

Wiedehage J., Schmidtmayer J., and Buse E. (1992) Development of membrane currents in mammalian neuroventricular cells from the early neural tube stage *in vitro:* aspects of the neuronal lineage. *Int. J. Dev. Neurosci.* **10,** 375–385.

Williams K. (1994) Subunit-specific potentiation of recombinant N-methyl-D-aspartate receptors by histamine. *Mol. Pharmacol.* **46,** 531–541.

Williams K., Zappia A. M., Pritchett D. B., Shen Y. M., and Molinoff P. B. (1994) Sensitivity of the N-

methyl-D-aspartate receptor to polyamines is controlled by NR2 subunits. *Mol. Pharmacol.* **45,** 803–809.

Wilson G. F. and Chiu S. Y. (1990) Regulation of potassium channels in Schwann cells during early developmental myelinogenesis. *J. Neurosci.* **10,** 1615–1625.

Wisden W., Laurie D. J., Monyer H., and Seeburg P. H. (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain I. Telencephalon, diencephalon, mesencephalon. *J. Neurosci.* **12,** 1040–1062.

Wissenbach U., Bosse-Doenecke E., Freise D., Ludwig A., Murakami M., Hofmann F., and Flockerzi V. (1998) The structure of the murine calcium channel gamma-subunit gene and protein. *J. Biol. Chem.* **379,** 45–50.

Witcher D. R., Dewaard M., Sakamoto J., Franzini-Armstrong C., Pragnell M., Kahl S. D., and Campbell K. P. (1993) Subunit identification and reconstitution of the N-type Ca^{2+} channel complex purified from brain. *Science* **261,** 486–489.

Witzemann V., Stein E., Barg B., Konno T., Koenen M., Kues W., Criado M., Hofmann M., and Sakmann B. (1990) Primary structure and functional expression of the α, β, γ, δ and ε subunits of the acetylcholine receptor from rat muscle. *Eur. J. Biochem.* **194,** 347–448.

Wood K. W., Sarnecki C., Roberts T. M., and Blenis J. (1992) Ras mediates nerve growth factor receptor modulation of three signal-transducing protein kinases: MAP kinases, Raf-1, and RSK. *Cell* **68,** 1041–1050.

Wu R.-L. and Barish M. E. (1994) Astroglial modulation of transient potassium current development in cultured mouse hippocampal neurons. *J. Neurosci.* **14,** 1677–1687.

Yan Q., Elliott J., and Snider W. D. (1992) Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature* **360,** 753–755.

Yang X., Fyodorov D., and Deneris E. S. (1995) Transcriptional analysis of acetylcholine receptor alpha 3 gene promoter motifs that bind Sp1 and AP2. *J. Biol. Chem.* **270,** 8514–8520.

Yang X., McDonough J., Fyodorov D., Morris M., Wang F., and Deneris E. S. (1994) Characterization of an acetylcholine receptor alpha 3 gene promoter and its activation by the POU domain factor SCIP/Tst-1. *J. Biol. Chem.* **269,** 10,252–10,264.

Yang X., Yang F., Fyodorov D., Wang F., McDonough J., Herrup K., and Deneris E. (1997) Elements between the protein-coding regions of the adjacent β4 and α3 acetylcholine receptor genes direct neuron-specific expression in the central nervous system. *J. Neurobiol.* **32,** 311–324.

Yarowsky P. J., Krueger B. K., Olson C. E., Clevinger E. C., and Koos R. D. (1991) Brain and heart sodium channel subtype mRNA expression in rat cerebral cortex. *Proc. Natl. Acad. Sci. USA* **88,** 9453–9457.

Yu R. and Ticku M. K. (1995a) Chronic neurosteroid treatment produces functional heterologous uncoupling at the $GABA_A$-benzodiazepine receptor complex in mammalian cortical neurons. *Mol. Pharmacol.* **47,** 603–610.

Yu R. and Ticku M. K. (1995b) Chronic neurosteroid treatment decreases the efficacy of benzodiazepine ligands and neurosteroids at the $GABA_A$ receptor complex in mammalian cortical neurons. *J. Pharmacol. Exp. Ther.* **275,** 784–789.

Yu R. Hay M., and Ticku M. K. (1996a) Chronic neurosteroid treatment attenuates single cell GABA(A) response and its potentiation by modulators in cortical neurons. *Brain Res.* **706,** 160–162.

Yu R., Follesa P., and Ticku M. K. (1996b) Down-regulation of the GABA receptor subunits mRNA levels in mammalian cultured cortical neurons following chronic neurosteroid treatment. *Mol. Brain. Res.* **41,** 163–168.

Zhang L., Zheng X., Paupard M.-C., Wang A. P., Santchi L., Friedman L. K., Zukin R. S., and Bennett M. V. L. (1994) Spermine potentiation of recombinant N-methyl-D-aspartate receptors is affected by subunit composition. *Proc. Natl. Acad. Sci. USA* **91,** 10,883–10,887.

Zhou Y., Deneris E., and Zigmond R. E. (1998) Differential regulation of levels of nicotinic receptor subunit transcripts in adult sympathetic neurons after axotomy. *J. Neurobiol.* **34,** 164–178.

Zhu X. J., Lai C., Thomas S., and Burden S. J. (1995) Neuregulin receptors, *ERBB3* and *ERBB4,* are localized at neuromuscular synapses. *EMBO J.* **14,** 5842–5848.

Zoli M., LeNovere N., Hill J. A. Jr., and Changeux J.-P. (1995) Developmental regulation of nicotinic acetylcholine receptor subunit mRNAs in the rat central and peripheral nervous systems. *J. Neurosci.* **15,** 1912–1939.

Zur K. B., Oh Y., Waxman S. G., and Black J. A. (1995) Differential up-regulation of sodium channel α- and β1-subunit mRNAs in cultured embryonic DRG neurons following exposure to NGF. *Mol. Brain Res.* **30,** 97–103.