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Expression of c-*fos* in Studies of Central Autonomic and Sensory Systems

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Introduction

One of a family of proto-oncogenes, c-*fos* is a highly regulated gene whose transcription is elevated for a short time after the application of a stimulus (Curran, 1988). It is now clear that a variety of stimuli can lead to a very similar profile of c-*fos* expression so that transcription of the gene occurs within 5 min and continues for 15–20 min (Greenberg and Ziff, 1984; Greenberg et al., 1985). Accumulation of mRNA peaks at 30–45 min (Müller et al., 1984); the synthesized protein, Fos, has a half-life of about 2 h (Curran et al., 1984; Müller et al., 1984). In cooperation with similar proteins of the Jun family, Fos acts as a transcriptional regulator by forming a protein complex

that binds to the activator-protein-1 (AP-1) binding site of DNA (Lee et al., 1988; Sheng and Greenberg, 1990). Thus, genes that contain the AP-1 binding site are, for the most part, activated by the Fos/Jun complex. It is commonly assumed that cells expressing Fos can be expected to also express the so-called late-onset genes that encode differentiated neuronal products (Sonnenberg et al., 1989a; Armstrong and Montminy, 1993).

The early expression of c-*fos* and other immediate early genes within cells has been exploited as a means to map the neurons that are responsive to specific stimuli and thus participate in functional neuroanatomical networks. Within the last four years, there has been an explosion in the number of reports in which expression of Fos has been used for these purposes; this review will primarily consider the impact that localization of the c-*fos* mRNA or the protein, Fos, has had in the areas of neuroscience dealing with autonomic and somatosensory physiology. The reader is also referred to earlier reviews (Dragunow and Faull, 1989; Morgan and Curran, 1989, 1991; Doucet et al., 1990).

The expression of c-*fos* is accompanied by the expression of Fos-related antigens that are believed to participate in transcriptional regulation (Cohen and Curran, 1988; Franza et al., 1987). Generally, c-*fos* is expressed within a few hours after the application of a stimulus whereas Fos-related antigens may be expressed for more prolonged periods of time (Morgan and Curran, 1991; Lantéri-Minet et al., 1993). Expression of the Fos-related antigens for each neural network is a complex cascade, the significance of which is not yet fully understood. Because many of the studies that will be discussed have used antibodies that recognize Fos and Fos-related antigens, the words Fos and Fos-like will be used interchangeably in this review.

Viscerosensory Stimulation

Osmotic Stimulation

Induction of Fos expression in neurons has been used to identify neurons of the forebrain that respond to osmotic challenges in the body. In support of an important role for the hypothalamus in responding to osmotic stimuli, the original observations concerning induction of Fos expression showed that dehydration for 24 h led to Fos-like immunoreactivity (FLI) in the magnocellular neurons of the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei (Sagar et al., 1988), neurons known to produce vasopressin or oxytocin. In addition, Fos was expressed in the parvocellular division of the PVN, implying that neurons in the parvocellular PVN that project to areas in the brainstem and spinal cord were also activated (Swanson and Sawchenko, 1982).

More recent studies have shown that intraperitoneal or intravenous injections of hypertonic saline led to expression of Fos in the PVN, SON, and other regions known to participate in fluid regulation: lamina terminalis, including the organum vasculosum laminae terminalis, median preoptic nucleus, and subfornical organ (Hamamura et al., 1992; Oldfield et al., 1991; Sharp et al., 1991). Appearance of Fos was maximal at 1–2 h and generally disappeared by 4–8 h (Carter and Murphy, 1990; Giovanelli et al., 1992; Sharp et al., 1991). Brattleboro rats, which are chronically dehydrated because of a deficiency in vasopressin production, show low but constant FLI in the same areas as above. When challenged further with an injection of hypertonic saline, Brattleboro rats showed increased levels of FLI in additional areas, including accessory hypothalamic nuclei and arcuate nucleus (Guldenaar et al., 1992). It is interesting to note that the 2-deoxyglucose technique, which measures glucose uptake in nervous tissues, has been used in the past to identify brain regions that respond to dehydration (Schwartz et al., 1979; Kadekaro et al., 1983). Because of the more complicated techniques required to obtain and analyze data with the 2-deoxyglucose technique, however, the results obtained by localizing Fos expression have been generally more extensive.

Because it is devoid of a blood–brain barrier, the subfornical organ (SFO) is thought to be of major importance in the transduction of osmotic stimuli to other neurons such as those of the

hypothalamus (Hatton, 1990; Johnson and Wilkins, 1983; Ramsay et al., 1983). In an attempt, therefore, to determine if the integrity of the SFO is critical for conducting the signals associated by osmotic stimulation, the SFO was lesioned 2 wk prior to administration of a hyperosmotic stimulus (Giovanelli and Bloom, 1992). Destruction of up to 75% of the SFO did not interfere with Fos expression in the PVN or SON, suggesting that the SFO is not critical for the transfer of the osmotic signal to the hypothalamus (Giovanelli and Bloom, 1992). This finding is not surprising since it has been suggested that neurons within the hypothalamus themselves act as osmosensors (Leng et al., 1992). The above studies demonstrate that visualization of Fos in response to stimulation after lesions have been made in discrete brain regions can be used effectively to study the relative importance of specific neural circuitries.

When oxytocinergic neurons of the hypothalamus were specifically studied after administration of hypertonic saline, it was found that many, but not all, of these neurons were induced to express Fos (Giovanelli et al., 1990). At peak expression, 59% of oxytocinergic neurons in the PVN and 92% in the SON expressed Fos (Giovanelli et al., 1992) indicating that osmotic stimulation leads to selective activation of subpopulations of oxytocinergic neurons in the SON. In addition, these and many studies discussed subsequently illustrate that double immunohistochemical techniques can be easily carried out to identify not only activated neurons (Fos positive), but also the neurotransmitter content of the same neurons.

Hemorrhage

After bleeding of anesthetized rats in volumes sufficient to cause the arterial pressure to drop by 50 to 60 mm Hg for 1 h, neurons expressing Fos have been found in the PVN, SON, and accessory neurosecretory nuclei such as the nucleus circularis (Shen et al., 1992a). Most of the activated cells in the PVN and SON were vasopressin-producing neurons (Shen et al., 1992b). These effects were likely owing to the drop in arterial pressure that accompanied hemorrhage since nitroprusside-induced hypotension yielded similar results (Shen et al., 1992b). The activation of vasopressingergic neurons was predictable based on the well-known pressor effects of circulating vasopressin (Harris and Loewy, 1990). In the brainstem, catecholaminergic neurons in the nucleus of the tractus solitarius (NTS) and ventrolateral medulla (VLM), some of which also projected to the spinal cord, were activated in response to hemorrhage (Dun et al., 1993), supporting the roles of these regions in cardiovascular regulation. In cat, withdrawal of 75 mL of blood over 5 min led to a drop in arterial pressure of about 55 mm Hg and expression of Fos in neurons of the VLM (nucleus subretrofacial nucleus) that had also been labeled with a retrograde tracer to identify them as spinally-projecting neurons (McAllen et al., 1992). In the latter two studies it was not determined whether the Fos expression was related to the decrease in blood volume, the drop in arterial pressure, or both.

Baroreceptor and Chemoreceptor Activation

The participation of autonomic centers in regulation of blood pressure has been demonstrated by examining Fos expression in the brain elicited by activating baroreceptor pathways. Baroreceptor activation for 1 h by means of intravenous infusions of the vasoconstrictor, phenylephrine, led to the presence of FLI in the brainstem NTS, area postrema, and VLM (Li and Dampney, 1992). Within the VLM, most of the labeled neurons were found in the so-called depressor area of the caudal VLM and not in the "pressor" rostral VLM; these data suggest that the neurons stimulated by increases in arterial pressure (caudal VLM) express FLI whereas neurons inhibited by the same stimulus (rostral VLM) do not. Furthermore, double labeling for tyrosine hydroxylase showed that, whereas specific populations of the Fos-positive VLM neurons were catecholaminergic, most of the neurons with FLI in the caudal VLM were not (Li and Dampney, 1992). In a separate study (Narváez et al., 1993), many more catecholaminergic caudal VLM neurons (up

to 75%) were found to express Fos after baroreceptor activation with phenylephrine. Since both studies used antibodies that recognized both Fos and Fos-related antigens, the reason for the differences in the two sets of results is not readily apparent.

In the forebrain, phenylephrine-induced hypertension was associated with FLI in bed nucleus of the stria terminalis, PVN, SON, and central nucleus of the amygdala although the neurotransmitter content of activated neurons was not studied (McKinley et al., 1992). On the other hand, hypotension induced with intravenous infusion of nitroprusside led, within the SON, to preferential induction of FLI within vasopressin-producing neurons (Shen et al., 1992b).

One hour of electrical stimulation of the aortic depressor nerve that, in the rat, carries mostly baroreceptor fibers caused a drop in mean arterial pressure and increases in the numbers of neurons with FLI in most of the brainstem and forebrain areas previously implicated in cardiovascular regulation, ipsilateral to the side of stimulation (McKitrick et al., 1992). Only the caudal brainstem was examined in another study in which the aortic depressor nerve was stimulated electrically for 30 min; these investigators found FLI in the ipsilateral NTS, area postrema, and paratrigeminal nucleus (Rutherfurd et al., 1992). For reasons discussed above, these results expand on those that have been obtained by measuring 2-deoxyglucose uptake in the rat brain in response to electrical stimulation of the aortic depressor nerve (Ciriello et al., 1983).

Unilateral electrical stimulation of the parabrachial nucleus of the pons (Krukoff et al., 1992), a relay center for autonomic information, led to ipsilateral increases in numbers of neurons with FLI in several regions of the brain (*see* Electrical Stimulation of Central Autonomic Centers). In addition, however, increases were seen in the NTS and VLM; the bilateral nature of the results for these two areas suggested that they were likely caused by alterations in arterial pressure that accompanied parabrachial stimulation (Krukoff et al., 1992).

A similar pattern of labeled neurons to that resulting from stimulation of the aortic depressor nerve (NTS, area postrema, and paratrigeminal nucleus) was seen after electrically stimulating the vagus nerve or after stimulating the carotid sinus nerve by stretching the common carotid artery every minute for 30 min (Rutherfurd et al., 1992). Since the carotid sinus nerve carries both baro- and chemoreceptor information, and the vagus nerve carries sensory information from other viscera, these results suggest that the three brainstem regions participate in integration of each of these types of information.

More intense electrical stimulation of the carotid sinus nerve or exposure of rats to a hypoxic environment led to similar patterns of neurons with FLI in the NTS, area postrema, VLM, raphe nuclei, and at the ventral surface of the medulla (Erickson and Millhorn, 1991), illustrating that stronger stimulation likely *recruited* larger numbers of brainstem neurons. Finally, stimulation of chemoreceptors by exposure of rats to differing amounts of CO_2 (up to 15%) led to labeled neurons in the NTS and at the ventral surface of the medulla (Sato et al., 1992).

Gastrointestinal Activation

The dorsal vagal complex in the brainstem, the hypothalamus, and the limbic system have been studied after manipulation of food intake or gastric motility (Olson et al., 1993). Whereas acute gastric distention led to FLI in the NTS, dorsal motor nucleus of the vagus, area postrema, PVN, central nucleus of the amygdala, and bed nucleus of the stria terminalis, FLI was not found in the NTS or area postrema of rats whose food intake had been potentiated by food deprivation or insulin-induced hypoglycemia. Inhibition of food intake led to Fos staining in the NTS, PVN, central nucleus of the amygdala, and bed nucleus of the stria terminalis, but only variable staining in the area postrema (Olson et al., 1993). This study indicates that related types of stimuli lead to generally similar patterns of FLI in the brain,

but that subtle differences in staining can be elicited according to the specific stimulus that is applied. In addition, the presence of Fos in areas of the forebrain classically considered to be part of the limbic system suggest that some of the results were stress-related (Olson et al., 1993).

Irritation of the abdominal viscera after an intraperitoneal injection of an acetic acid solution in unanesthetized rats resulted in significant numbers of bilaterally-located neurons with FLI in the caudal NTS (Hammond et al., 1992). Opiate neurotransmitters were likely involved the suppression of these responses since the responses could be reversed in a dose-dependent manner with morphine and other opiate agonists. In the spinal cord, the same stimulus elicited labeling of neurons in laminae I, II, VII, and X primarily in thoracic and lumbar segments (DeLeo et al., 1991; Hammond et al., 1992), patterns that were similar to neuroanatomically demonstrated terminations of visceral primary afferents using anterograde tracers (Neuhuber, 1982, 1986; Nadelhaft and Booth, 1984; Sugiura et al., 1989). On the other hand, mechanical stimulation of the viscera with distention led to greater numbers of labeled neurons as well as more intense labeling in most spinal laminae (DeLeo et al., 1991; Traub et al., 1992).

To identify neurons in the lumbar and sacral cord that participate in visceral sensory pathways from the lower viscera in a more controlled manner, the pelvic nerve was electrically stimulated in rats; labeling was found primarily in segments L6 and S1 within the superficial dorsal horn, dorsal commissure, and the region of the parasympathetic nucleus (Birder et al., 1991). Chemical irritation of the lower urinary tract with 1% acetic acid led to Fos expression in the same regions of the cord (Birder and DeGroat, 1992a). Combined chemical and mechanical stimulation of the lower urinary tract was used to show that neurons in the dorsal commissure probably respond to several types of inputs (Birder and DeGroat, 1992b). Pharmacological manipulations showed that NMDA, but not AMPA, receptors may participate in the transmission of visceral nociceptive impulses from the lower urinary tract and in mediating micturition (Birder and DeGroat, 1992a).

Electrical Stimulation of Central Autonomic Centers

The usefulness of utilizing Fos expression as a marker of functionally related neurons is becoming apparent in studies using electrical stimulation of central sites to activate mono- and multisynaptic circuits. Stimulation of the parabrachial nucleus of the pons, an autonomic relay center, in anesthetized rats led to ipsilateral increases of FLI in neurons in the PVN, SON, medial preoptic area, central nucleus of the amygdala, and cerebral cortex (Krukoff et al., 1992). Stimulation of the periaqueductal gray in conscious animals led to ipsilateral increases in neurons of the cuneiform nucleus, locus coeruleus, and hypothalamus (Sandner et al., 1992). Although several of the regions activated by electrical stimulation are known to receive direct projections from the parabrachial nucleus and periaqueductal gray, respectively, others do not, thereby supporting the concept that multineuronal circuits can be demonstrated on the basis of Fos expression (Menétrey et al., 1989; Krukoff et al., 1992; Sandner et al., 1992).

Quantitation of neurons induced to express Fos after electrical stimulation has also proved useful in understanding the functional importance of specific circuitries. Electrical stimulation of the central nucleus of the amygdala resulted in statistically significant increases in neurons with FLI in the PVN, SON, and arcuate nucleus of the hypothalamus (Petrov et al., in press). In the PVN, the largest increase was found in the parvocellular division. Since these neurons project to other autonomic centers in the brain (Swanson and Sawchenko, 1982), these results provide further evidence for the functional participation of the parvocellular PVN in autonomic responses. In the magnocellular component of the PVN and in the SON, activated neurons were identified to be both vasopressinergic and oxytocinergic (Petrov et al., in press).

The effects of electrical stimulation of the PVN on Fos expression in well known targets of the PVN have also been studied (Krukoff et al., in press). Significant increases in neurons with FLI were found in the ipsilateral arcuate nucleus, ventromedial hypothalamus, lateral parabrachial nucleus, VLM, and NTS. However, when the PVN was chemically stimulated with the excitatory amino acid, L-glutamate, in order to eliminate the effects associated with stimulation of fibers of passage during electrical stimulation, some interesting differences were observed. Namely, a substantial part of the NTS no longer contained greater numbers of neurons with FLI on the side ipsilateral to the stimulation site. These results indicate that electrical stimulation of the PVN likely activated fibers that did not originate in the PVN. In addition, although the PVN has been shown to project to the NTS using standard neuroanatomical techniques, the results obtained with L-glutamate activation suggest that these projections may not be as functionally important as expected. Alternatively, they may be recruited in response to other types of autonomic stimuli (Krukoff et al., in press).

Stress

Predictably, exposure of experimental animals to stressful stimuli leads to the expression of Fos in many of the brain areas that have been discussed above in relation to autonomic function. Considering the importance of the PVN in regulation of autonomic responses, it was not surprising that the PVN contained many neurons with FLI after immobilization stress (Chastrette et al., 1991; Imaki et al., 1992; Kononen et al., 1992; Coveñas et al., 1993; Harbuz et al., 1993) and that many of these neurons were identified as corticotropin-releasing factor-producing cells (Imaki et al., 1992; Coveñas et al., 1993; Harbuz et al., 1993) or cells that produce receptors to glucocorticoids (Kononen et al., 1992). Peripheral pain led to FLI in PVN neurons, some of which received fibers containing ACTH or enkephalin (Pretel and Piekut, 1991). Immobilization stress also led to expression of FLI in the central nucleus of the amygdala (Honkaniemi, 1992), another brain area whose role in integration of stress and autonomic responses is well known. These neurons contained one of several neuropeptides (neurotensin, enkephalin, somatostatin, or corticotropin-releasing factor) and received catecholaminergic inputs.

Other regions of the brain that have been shown to contain neurons with FLI after stressful stimuli have been applied include cortical regions, septum, medial amygdala, thalamus, basal ganglia, locus coeruleus, NTS, and VLM (Ceccatelli et al., 1989; Pezzone et al., 1992; Smith et al., 1992). In the brainstem, many of the activated neurons were found to belong to catecholaminergic cell groups (Pezzone et al., 1993). Noradrenergic transmission likely plays an important role in induction by stress of FLI in cortical neurons as well, since lesions of the locus coeruleus, a brainstem nucleus that provides the majority of noradrenergic input to other brain areas, led to decreases in the numbers of neurons with FLI in cortex owing to restraint stress (Stone et al., 1993).

The activation of autonomic centers in the brain by stressful stimuli serves to emphasize the care that must be taken to minimize stress when experiments are done to study other systems.

Circadian Activation

The suprachiasmatic nucleus (SCN) of the hypothalamus is considered to be vital to the generation of a wide variety of circadian rhythms, both physiological and behavioral (Rusak and Zucker, 1979). The SCN receives photic information either directly from the retina or multisynaptically through the ventral lateral geniculate nucleus/intergeniculate leaflet and entrains subsequent responses to the day/night cycle. The circadian system is not sensitive to entrainment by light during the daylight (Takahashi et al., 1984), but night exposure to light is highly effective in phase-shifting the pacemaker. The expression of Fos parallels this responsiveness in that it was only poorly stimulated during the daylight,

if at all, whereas brief exposure to light during night was highly effective in eliciting Fos expression in the ventrolateral SCN (Rea, 1989; Earnest et al., 1990; Kornhauser et al., 1990; Rusak et al., 1992; Mead et al., 1992). Furthermore, increased levels of light were effective in stimulating increased levels of Fos in the SCN (Kornhauser et al., 1990). The expression of Fos (and several members of the Jun family) in the dark phase has led to speculation that the biochemical machinery may be present to allow for expression of c-*fos* to regulate other transcriptional events that may be involved in phase-shifting the circadian clock (Rusak et al., 1990, 1992).

It has also been demonstrated that expression of Fos undergoes an endogenous fluctuation in the SCN with peak levels occurring during the night (Kilduff et al., 1992; Rea, 1992; Sutin and Kilduff, 1992). Most of the neurons stimulated to produce Fos during the night cycle were found in the same location (Rea, 1989) as the terminal fields of the retinohypothalamic (Johnson et al., 1988) and geniculohypothalamic (Swanson et al., 1974) tracts, supporting the concept that retinal input was responsible for stimulating Fos expression in the SCN neurons. It is not yet understood why Fos is not expressed in response to light stimulation during the daylight in view of the findings that retinal illumination during either day or night resulted in equivalent increases in firing rates of SCN neurons (Inouye, 1984). It remains to be seen whether this phenomenon can be considered as one example of c-*fos* expression that is not equivalent to neurophysiological activity (Rusak et al., 1992).

The patterns of neurons with FLI during the night cycle are apparently also dependent on when the light exposure occurs since exposure 6 h after the onset of darkness elicited larger numbers of neurons from differing populations in the SCN to express Fos than when the light exposure occurred 1 h after the onset of darkness (Rea, 1992).

Some investigators have obtained results that are contrary to the idea that FLI is inducible primarily during the dark cycle. The dorsomedial SCN has been reported to contain the largest numbers of neurons with FLI during the day (Earnest et al., 1990) and neurons with FLI in the SCN have been found to be increased in number during the daylight (Aronin et al., 1990). The discrepancies among these studies may have been caused by differing specificities of antibodies to Fos.

Neurons of the other structures important in circadian rhythm generation, the ventral lateral geniculate nucleus and the intergeniculate leaflet of the lateral geniculate complex, were also induced to express FLI with exposure to light for 30–60 min during the night cycle (Rusak et al., 1990). On the other hand, a much briefer exposure of 5 min did not induce Fos expression in either region (Kornhauser et al., 1990).

Nonphotic stimuli (e.g., running in a novel wheel, subcutaneous saline injections) also have the capacity to phase-shift circadian rhythms both during the day- or night-time components of the day/night cycle (Mrosovsky et al., 1989; Cutrera et al., 1992; Mead et al., 1992; Janik and Mrosovsky, 1992). Interestingly, when the phase-shift occurred during the daylight part of the cycle, Fos expression was not induced in the SCN (Janik and Mrosovsky, 1992; Kilduff et al., 1992; Mead et al., 1992) suggesting that expression of this immediate early gene is not an integral component of all phase-shift stimuli (Mead et al., 1992). On the other hand, the nonphotic stimulus of running in a novel wheel has been shown to cause Fos expression in the intergeniculate leaflet, suggesting that this part of the lateral geniculate complex *does* participate in mediation of nonphotic signals to the circadian clock (Janik and Mrosovsky, 1992).

Diurnal expression of Fos has also been observed in brain areas other than those discussed above. The pineal gland, hippocampus, and putamen contained increased numbers of neurons with FLI after the onset of darkness (Kononen et al., 1990, Carter, 1993) and a subset of oxytocinergic neurons of the paraventricular nucleus of the hypothalamus showed a rhythmic expression of Fos (Arey and Freeman, 1992), illustrating that the activity of many groups of neurons throughout the central nervous system may be regulated according to circadian rhythms.

Somatosensory Stimulation

Brain

The effects of somatosensory stimulation of FLI in the brain have been studied to a lesser degree than in the spinal cord (*see* Spinal Cord). Patterns of FLI that have been reported have generally corroborated the functional significance of pathways described by other means. Thus, stroking of whiskers in rats (tactile stimulation) led to FLI in the somatosensory cortex (Mack and Mack, 1992), noxious stimulation of the rat nasal mucosa led to FLI in trigeminal complex and reticular formation (Anton et al., 1991), and electrical stimulation of the trigeminal ganglion led to FLI in the nucleus caudalis of the trigeminal complex (Uhl et al., 1991). Noxious stimulation of the foot that produced tissue damage in lightly anesthetized rats led to ipsilateral increases in Fos expression in the expected thalamic areas (centrolateral, paracentral, centromedian, parafascicular, and ventrobasal nuclei) (Bullitt, 1989; Nagao et al., 1993), but also in areas less easily predicted, such as the paraventricular, submedial, and reuniens nuclei of the thalamus (Bullitt, 1989). These results showed that using the expression of Fos as a marker of activated neurons often results in the identification of a broader spectrum of functionally related neurons than might be predicted on the basis of other techniques.

Electroacupuncture, known to reduce numbers of spinal neurons with FLI induced with noxious stimulation (Lee and Beitz, 1992), also induced Fos expression in regions of the brain. These areas included the raphe nuclei, lateral parabrachial nucleus, locus coeruleus, periaqueductal gray, habenular nuclei, and hypothalamus (arcuate, lateroventral, and lateral nuclei) and suggested that these regions may participate in electroacupuncture-induced analgesia (Lee and Beitz, 1993).

Spinal Cord

Mapping of neurons that express Fos in response to application of specific noxious stimuli has broadened the understanding of which neuronal populations participate in the processing of nociceptive stimuli. Since the first study that used this approach (Hunt et al., 1987), many others have shown that noxious somatic stimulation leads to expression of FLI in discrete populations of neurons in the spinal cord. Namely, neurons with FLI were found in laminae I, II, V, and VI, with smaller numbers of neurons in laminae VII, VIII, and X. Laminae III and IV contained fewer, if any, labeled neurons (Bullitt, 1989, 1990; Draisci and Iadarola, 1989; Presley et al., 1990; Herdegen et al., 1991a,b; Naranjo et al., 1991; Pretel and Piekut, 1991; Abbadie et al., 1992; Bullitt et al., 1992). Not surprisingly, many of the neurons containing FLI have been shown to project to the thalamus (Menétrey et al., 1989).

Within the superficial laminae, large numbers of labeled neurons have been reported in the medial two-thirds of the gray matter (Bullitt, 1989; Noguchi et al., 1992). The patterns of labeled neurons in the superficial laminae correspond well with the locations of neurons previously identified, with more traditional electrophysiological and neuroanatomical methods, to participate in transmission of nociceptive information (Menétrey et al., 1977; Swett and Woolf, 1985). Neurons in deeper laminae were more likely to show FLI if stronger intensities of stimulation were maintained over a longer period of time (Bullitt et al., 1992). These results suggest once again that larger numbers of neurons, perhaps components of multisynaptic circuits, were recruited with larger stimuli.

A noxious stimulus (saline injection) to a hindpaw or ankle resulted in a wide rostrocaudal distribution of neurons with FLI and specific laminar distribution at different levels (Menétrey et al., 1989). On the other hand, more discrete rostro-caudal patterns of labeled neurons were found in the segment of entry and in adjacent segments when either the forepaw (Abbadie et al., 1992) or hindpaw (Gogas et al., 1991) was stimulated with formalin injection. These differences were likely caused by the types of stimuli applied and demonstrated the usefulness of using Fos expression as a means of identifying specific populations of neurons that are functionally related.

More long-term expression of Fos-related antigens in spinal neurons was found to be dependent on the type of stimulus. Thermal cutaneous stimulation of the hindpaw led to two "waves" of neurons with FLI, the first wave appearing in the first few hours and the second wave appearing by 8 h and lasting up to 24 h (Williams et al., 1990). Experimentally-induced arthritis led to patterns in expression of Fos-related antigens that paralleled the clinical stages of arthritis owing to injections of Freund's adjuvant so that no cells with FLI were found at 1 wk, largest numbers were found at 3 wk, and recovery occurred by 11 wk (Abbadie and Besson, 1992, 1993b). In these experiments the largest numbers of labeled neurons were found in laminae V and VI (55% of labeled cells) and the ventral horn (35%); fewer labeled neurons were seen in the superficial laminae.

A priming effect for Fos expression has been described so that larger numbers of neurons were stimulated to express Fos if a noxious stimulus was preceded by the same type of stimulus applied 1–1.5 h earlier (Leah et al., 1992). These results have been interpreted to indicate a stimulus-induced sensitization of the dorsal horn neurons (Leah et al., 1992).

Attempts have been made to identify the neurotransmitters that may be involved in nociceptive circuits as demonstrated by FLI. Double immunohistochemistry was used to demonstrate that the majority of neurons in the superficial laminae that were stimulated by application of mustard oil to the hindlimb received input from enkephalin-, substance P-, and serotonin-immunoreactive axonal varicosities (Pretel and Piekut, 1991). Nitric oxide may be involved in modulation of noxious stimuli since intrathecal administration of the nitric oxide synthase inhibitor, *N*-ω-nitro-L-arginine methyl ester, led to decreased numbers of neurons with FLI in the dorsal horn after noxious mechanical stimulation of the hindpaw (Lee et al., 1992). In addition, FLI and nitric oxide synthase immunoreactivity have been found to coexist in neurons identified as spinothalamic neurons in lamina X (Lee et al., 1993). Finally, the likely role of opiates in antagonizing nociception was demonstrated by using opiate agonists such as morphine to reversibly, and in a dose-dependent manner, reduce the numbers of spinal neurons that expressed FLI after nociceptive stimulation (Presley et al., 1990; Tölle et al., 1990; Abbadie and Besson, 1992, 1993a; Hammond et al., 1992; Yao et al., 1992). This opiate-induced analgesia may be selective for subpopulations of nociceptive neurons with greater effects demonstrated in deeper laminae (V–VIII and X) than in superficial laminae (I, II) after microliter injections of formalin into the hindpaw (Presley et al., 1990). Peripheral inflammation led to expression of Fos in a large proportion of neurons that produce dynorphin or preproenkephalin (Noguchi et al., 1991, 1992), once again suggesting that neuropeptides of the opiate family participate in analgesia. Fos expression has also been used to demonstrate that inhibitory supraspinal inputs participating in production of analgesia are likely also opioid in nature (Gogas et al., 1991).

Opiates are also likely involved in mediating electroacupuncture-induced analgesia since administration of the opiate antagonist, naloxone, blocked the suppression of spinal Fos expression that is associated with acupuncture following administration of noxious stimuli to the hindlimb (Lee and Beitz, 1992).

Attempts to block expression of Fos in spinal neurons with antagonists of NMDA receptors (Tölle et al., 1990; Wisden et al., 1990) have not been successful, indicating that blockade of calcium influx through NMDA-gated channels does not influence Fos expression in these neurons (Tölle et al., 1990).

Other

Reproductive Physiology

A single mating event induced FLI in neurons of the male rodent brain within the medial preoptic area, bed nucleus of the stria terminalis, and medial amygdala (Baum and Everitt, 1992; Kollack and Newman, 1992). Additional labeled neurons have been reported in the PVN (Kollack and Newman, 1992), midbrain central tegmen-

tal area, nucleus accumbens, and piriform cortex (Robertson et al., 1991). In the female rat, expression of Fos in noradrenergic neurons of the dorsal medulla (A2 cell group) appears to vary with the estrous cycle so that the largest numbers of these cells expressed Fos at proestrus. At estrus there was a slight reduction in noradrenergic neurons with FLI and at diestrus, only a few of these neurons contained FLI (Jennes et al., 1992). The significance of these findings is, as yet, not well understood since it is not known if the results were caused by specific reproductive events or whether underlying autonomic responses may have contributed to the results.

Interestingly, parturition has been shown to be a more effective stimulus for Fos expression in the hypothalamus than lactation even though both processes are known to activate oxytocin-producing neurons in the PVN (Fenelon et al., 1993). It has been suggested that these findings may be explained on the basis of the different firing patterns of oxytocinergic neurons that occur during each of these two types of stimulation (Fenelon et al., 1993). Alternatively, this may be an example of c-*fos* expression that is not equivalent to neurophysiological activity.

Anesthesia

The anesthetic employed in experimental animals can have important effects of Fos expression in the brain. Barbiturates such as sodium pentobarbital and the analgesic, ketamine, have been shown to block c-*fos* mRNA (Morgan et al., 1987; Dragunow and Faull, 1989; Morgan and Curran, 1989, 1991; Sonnenberg et al., 1989b; Krukoff et al., 1992; Marota et al., 1992). On the other hand, others have shown that ketamine anesthesia was associated with induction of Fos in the neocortex and thalamus (Nakao et al., 1993).

Urethane (intraperitoneal), a mixture of sodium amylobarbitone and sodium methohexitone (intraperitoneal), or methoxyfluorane (inhaled) caused selective FLI in brainstem and/or brain regions (Bullitt, 1990; Anton et al., 1991; Krukoff et al., 1992; McKitrick et al., 1992; Rutherfurd et al., 1992). These complex response patterns have been attributed to factors such as autonomic stimulation owing to secondary alterations in arterial pressure or osmotic balance in the body in the case of urethane anesthesia (Krukoff et al., 1992).

Conclusions

This survey of studies illustrates that the important role of the gene, c-*fos*, in regulating expression of subsequent genes provides the opportunity for neuroscientists to functionally map regions of the nervous system that participate in specific functions. Compared to other techniques that have been used to identify functionally related neurons, such as 2-deoxyglucose uptake or metabolic enzyme histochemistry (e.g., Krukoff et al., 1983; Krukoff and Vincent, 1989), the localization of FLI has several advantages:

1. The current techniques are generally easier to use. Relatively straightforward immunohistochemical techniques yield results quickly.
2. The results are more easily analyzed because the presence of neurons with FLI is easily discerned on the basis of stained nuclei. When a given stimulus leads to a dramatic difference in numbers of neurons with FLI, qualitative analysis may be sufficient. However, the availability of image analysis systems has made counting of labeled neurons easy and less time-consuming than ever before and as a result, quantitative analysis can be used to assess the strength of regional responses to a given stimulus. This approach is especially useful when differences in numbers of neurons with FLI are not apparent to the naked eye.
3. By combining techniques, it is now possible to simultaneously address more than one question regarding the activated neurons. As pointed out in this review, for example, one can identify the neurotransmitters found in activated neurons or the targets of these activated neurons.
4. Most commonly, results obtained for c-*fos* expression are consistent with the known anatomy and physiology of specific systems. By identifying labeled neurons in additional and perhaps unexpected areas, however, one is able to obtain an even more complete understanding of the manner in which activity in separate

regions of the nervous system is integrated to generate an appropriate response.

The advantages listed above must be tempered by a few points of caution:

1. The majority of the studies cited in this review used antibodies that recognized both Fos and Fos-related antigens. As indicated in the Introduction, there is a clear temporal difference in the appearance of these two groups of proteins in the neuron, and one must be cognizant of this fact when the data are being interpreted.
2. A small number of studies suggests that Fos is not always induced in activated neurons. Although the reasons for the absence of Fos induction in certain populations of activated neurons are not well understood at the present time, these studies point out that the absence of Fos cannot be taken as absolute proof that a group of neurons has not been stimulated.
3. The need for careful controls when studying the effects of stimulus application cannot be overstated. As pointed out in this review, underlying factors, such as anesthesia, stress associated with a particular stimulus, or even circadian rhythms may lead to expression of FLI. The influences of all of these possible contributors must be carefully considered during the interpretation of results.

To date, the image that has emerged from the large body of literature devoted to induction of c-*fos* and Fos-related antigens in neurons is that, with thoughtful experiments and careful controls, the activation of c-*fos* and other immediate early genes can be used as a powerful means to understanding the complex integrative processes of the central nervous system.

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