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Activation of immediate early genes and memory formation

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Abstract. Long-term plastic changes in the brain, including those supporting memory formation, are assumed to depend on permanent functional alterations in neuronal cells that require reprogramming of gene expression. Inducible transcription factors encoded by immediate early genes such as c-*fos*, c-*jun*, *jun*-B and *zif*/268 (also known as *krox*-24, *egr*-1, *TIS* 8, *NGFI*-A or *zenk*) are supposed to act as messengers in coupling short-term neuronal activity with changes at the level of gene transcription. This review will summarize studies on the expression of transcription factor-encoding immediate early genes in the vertebrate brain during behavioral training. Special emphasis will be given to correlative or interventive experimental evidence indicative of a physiological significance of inducible transcription factors for processes underlying learning and memory formation.

Key words. Immediate early gene; transcription factor; learning; memory; brain; antisense.

Introduction

Long-term memory formation is thought to depend on long-lasting changes in synaptic efficacy involving structural rearrangements of synapses. These processes are likely to require de novo protein synthesis [1, 2]. One of the first targets of plasticity-inducing stimuli appears to be the activation of constitutively expressed transcription factors. For example, the cyclic adenosine monophosphate response element binding protein (CREB) has been shown to be critical for the formation of long-term memory in *Aplysia*, *Drosophila* and rodents (see [3], and Lamprecht, this issue). In turn, long-term consolidation of memory appears to require reprogramming of gene expression.

The first genes which undergo regulation of expression following cellular stimulation are those which do not require de novo synthesis of proteins: the immediate early genes (IEGs). Their induction occurs within minutes and is transient in nature. IEG-encoded proteins may serve diverse functions. Among the best-characterized IEG products are the Fos, Jun and Krox families of inducible transcription factors (ITFs), which are encoded by IEGs including c-*fos*, *fra*-1, *fra*-2, *fos*-B, c-*jun*, *jun*-B, *jun*-D, *krox*-20 and *zif*/268 (also known as *krox*-24, *egr*-1, *TIS* 8, *NGFI*-A or *zenk*). Once translated in the cytoplasm, these proteins enter the nucleus where they can regulate the transcription of target genes. Members of the Fos and Jun families of ITFs can dimerize either with each other to form complexes with variable transactivational abilities that bind to activating protein-1 (AP-1) consensus sites present in the promoters of many genes [4, 5], or with members of other transcription factor families, such as CREB/activating transcription factor (ATF) proteins [6]. In the nervous system, the expression of several ITFs is rapidly and transiently induced by a variety of stimuli, including growth factors, neurotransmitters, peptides, depolarization, seizures, ischemia, brain injury and sensory stimulation (for review see [7, 8]). Neurons are able to respond to bursts of activity by modulating the pattern of gene expression that may affect neuronal plasticity. Multiple signaling pathways may trigger rapid phosphorylation/dephosphorylation events which modulate the activity of constitutively expressed transcription factors, including CREB, serum response factor (SRF), and ternary complex factor (TCF) proteins, that in turn regulate the transcription of several ITF-encoding IEGs (for review see [3, 9–12]). ITFs are implicated in processes of growth and differentiation and are thought to play a pivotal, but not fully understood, role in processes supported by neuronal plasticity, such as long-

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term potentiation, kindling, regeneration and learning (for review see [7, 8, 13–17]).

This review will summarize the current literature on the expression of ITF-encoding IEGs during behavioral training in vertebrates. Special emphasis will be given to correlative or interventive experimental evidence indicative for a physiological significance of ITFs for processes underlying learning and memory formation.

Expression of ITFs during learning and memory formation

Ample experimental evidence suggests that the expression of ITF-encoding genes is induced in certain brain structures of vertebrates from bird to monkey under conditions of learning. This induction occurs rapidly and transiently during acquisition and early phases of consolidation of a memory trace, suggesting a role for ITFs in the transition from short- to long-term memory. As listed in table 1, ITF expression induced in the context of behavioral training has been used to identify brain regions activated during phases of acquisition of a new behavior (i.e. brain regions presumably involved in processes such as information processing, stimulus association and memory formation) and during testing of an already learned behavior (i.e. brain regions presumably involved in processes such as memory recall, expression of the learned behavior, relearning and extinction). A selection of these studies will be discussed below.

For associative learning, such as Pavlovian conditioning or instrumental conditioning, contingencies between a conditioned stimulus (CS) and an unconditioned stimulus (US) or between a stimulus and a behavior are critical. In studies attempting to establish a correlation between associative learning and the expression of ITF-encoding genes, a variety of experimental paradigms have been designed to discriminate between those effects caused by learning and memory formation and those caused by predisposing and concomitant factors, such as sensory stimulation, motor activity, stress, arousal and attention, in the presumed absence of learning under experimental consideration. Frequently, some learning about the experimental situation has to be considered even under control conditions.

ITF expression induced by stress and sensory stimulation

It is very difficult, at least in aversively driven learning paradigms, to exclude stress as a source of ITF induction. Acute stress can induce the expression of several ITF-encoding genes, including c-*fos*, c-*jun*, *jun*-B and *zif*/268, in a variety of brain structures. However, it has been demonstrated that repeated exposure to a stressor results in decreasing responses of these genes [18–22], whereas sensitivity to a novel stressor remains high [20, 21].

Similar observations have been reported following acute or repeated sensory stimulation without explicit interstimulus contingencies or instrumental reinforcements. For example, increased messenger RNA (mRNA) levels of *zenk* (the avian homologue of *zif*/268), observed in songbird forebrain structures following presentation of a novel species-specific song, are not evident when the same song has been presented repeatedly [23]. Exposure of chicks to complex visual environments causes induction of c-*fos*, but only if the environment is novel [24]. Distributed changes in IEG expression levels observed in rodent brain after exposure to spatial novelty [25–29] have been attenuated in repeatedly exposed animals [25, 29]. Visual presentation of novel objects to rats results in significantly higher increases in amounts of c-Fos in certain brain structures than presentation of familiar objects [30–32]. Daily application of acoustic stimuli results in a decrease of c-Fos expression in the auditory pathway of juvenile rats when compared with acute stimulation [33]. Exposure to a novel taste increases the expression of c-*fos* and of the effector IEG *arg* 3.1 in limbic and cortical brain structures of mice [34].

The studies described above indicate that application of stressors and sensory stimuli may modulate the expression of ITFs in certain brain structures. Repeated applications result in decreased neuronal responses also at the level of gene expression, implying that not a stressor or stimulus per se, but rather its novelty, is of importance for ITF induction. In other terms, ITF expression, most intensely induced by application of novel stimuli, attenuates when familiarity and experience with these stimuli increase, that is when learning about these stimuli has occurred and is thought to decrease or to disappear. Thus, ITF induction in these studies appears to correlate with simple (presumably nonassociative) types of learning, such as stimulus exposure conditioning [35], since explicit interstimulus contingencies or instrumental reinforcers are not part of these experiments. It is, however, difficult to identify every contingency present in an experiment.

ITF expression induced by behavioral training

In a number of classical and instrumental conditioning paradigms, increased ITF expression levels have been observed in distinct brain structures following a single session of training. Besides untrained animals, control groups frequently included animals subjected, for example, to CS only, to US only or to randomized CS-US (or stimulus-reinforcer) relations, that is to conditions which eliminate the contingency of interest such that no clear learning rule can be established. The predicted

Table 1. Cerebral expression of ITF-encoding genes in vertebrates in the context of behavioral training*.

Species	Behavioral paradigms	Genes	Observed changes	Brain structures	References
Zebra finch, canary	song learning	c-*fos*, c-*jun*, *zenk*	$+/-^{r,p}$	forebrain structures	[23, 80–88]
	song conditioning				
	training, *testing*	*zenk*	$+^{r}$	NCM-HVCM	[51]
Chick	imprinting				
	training/*testing*	c-*fos*	$+/-^{p}$	IMHV, HPC, HA	[54, 56]
Chick	passive avoidance				
	training/*testing*	c-*fos*, c-*jun*	$+^{r,p}$	IMHV, LPO	[24, 57]
Chick	visual discrimination				
	training, *testing*	c-*fos*, c-*jun*	$+^{r}$	forebrain	[48]
Mouse	Skinner-box (appetitive)				
	testing	c-*fos*, c-*jun*	$+^{r}$	HPC, Ctx	[62, 89]
Mouse	pheromonal learning				
	training	c-*fos*, *zif*/268	$+^{p}$	AOB	[37]
		c-*jun*	0^{p}		
Gerbil	auditory discrimination				
	CS only	c-*fos*	$+^{p}$	auditory Ctx	[90, 91]
Rat	odour conditioning				
	CS only, *testing*	c-*fos*	$+/0^{r,p}$	MOB	[92–94]
Rat	odour discrimination				
	training, *testing*	c-*fos*	$+/0^{r}$	MOB, HPC, Ctx, amygdala	[52, 95–97]
Rat	sexual learning				
	testing	c-*fos*	$+^{r}$	Ctx	[98]
Rat	Skinner-box (escape)				
	testing	c-*fos*	$+^{p}$	HPC, Ctx	[99]
Rat	motor skill learning				
	training, *testing*	c-*fos*	$+^{p}$	motor CTX	[53]
Rat	brightness discrimination				
	training	c-*fos*, c-*jun*, *jun*-B, *zif*/268	$+/0^{r,p}$	HPC, Ctx, Cbl	[38–40, 76]
Rat	spatial alternation				
	testing	c-*fos*, c-*jun*, *jun*-B, *zif*/268	$+^{r}$	HPC, Ctx	[100]
Rat	Morris water maze				
	training	c-*fos*, *zif*/268	0^{r}	dentate gyrus	[43]
Rat	active avoidance				
	training, *testing*	c-*fos*, *zif*/268	$+^{r,p}$	HPC, Ctx, Cbl, amygdala, hypothalamus, septum, nc. accumbens, tenia tecta	[49, 50, 101]
Rat	aversive conditioning			brain stem, amygdala, hypothalamus, Ctx	
	CS only	c-*fos*	$+/0^{r}$, $+^{p}$		[34, 41, 102, 103]
		fos-B, *zif*/268, *crem*	0^{r}		
	US only	c-*fos*, *zif*/268, *crem*	$+^{r,p}$		[41, 77, 78, 102–110]
		fos-B	0^{r}		
	training	c-*fos*, *zif*/268, *crem*	$+^{r}$		[41]
		fos-B	0^{r}		
	testing	c-fos	$+^{p}$		[102, 103, 106, 111–118]

Table 1. (Continued).

Species	Behavioral paradigms	Genes	Observed changes	Brain structures	References
Rat, mouse	emotional conditioning				
	training	c-*fos*, *zif*/268	$+^{r,p}$	HPC, amygdala, Ctx	[29, 42, 67, 119]
	testing	c-*fos*	$+/0^{r,p}$	widespread	[18, 29, 42, 120–127]
Rat	drug conditioning				
	training, *testing*	c-*fos*	$+/-^{p}$	limbic, brain stem	[128, 129]
Rabbit	nictit. membrane reflex				
	training	c-*fos*	$+/-^{p}$	brain stem	[36, 130]
Sheep	olfactory learning				
	training	c-*fos*, *zif*/268	$+^{r}$	Ctx	[131]
Monkey	visual pair-association vs. visual discrimination				
	testing	*zif*/268	$+^{p}$	temporal Ctx	[132]
		c-*fos*, *jun*-D	0^{p}		

*Note that only a selection of the studies is discussed in the text. AOB, accessory olfactory bulb; Cbl, cerebellum; CS, conditioned stimulus; Ctx, cortex; HA, hyperstriatum accessorium; HPC, hippocampus; IMHV, intermediate medial hyperstriatum ventrale; LPO, lobus parolfactorius; MOB, main olfactory bulb; NCM-HVCM, caudomedial neostriatum and hyperstriatum ventrale; ^{p} protein; ^{r} RNA; US, unconditioned stimulus.

outcome would be that association learning should produce changes in ITF expression that differ qualitatively and/or quantitatively from those observed under control conditions. Indeed, in rabbit brain stem nuclei, for example, conditioning and pseudoconditioning of the nictitating membrane reflex lead to quantitative differences in c-Fos expression [36]. Accordingly, in cells of the accessory olfactory bulb of female mice, an induction of c-*fos* and *zif*/268 (but not of c-*jun*) encoded proteins observed following mating requires the association of mating and pheromonal exposure, that is conditions also required for pheromonal memory formation [37]. However, a correlation between changes in ITF expression and the associative learning of interest is not always found. For example, following training of rats to acquire a footshock-motivated brightness discrimination in a Y-maze, differential spatiotemporal expression patterns of c-*fos*, c-*jun*, *jun*-B and *zif*/268 mRNAs have been reported. Similar patterns have also been observed in rats that received identical, but unpaired, stimuli in a pseudotraining procedure [38–40]. Intraperitoneal injection of LiCl, normally used as US to elicit conditioned taste aversion (CTA), results in a modulation of c-*fos*, *zif*/268 and *crem*, but not of *fos*-B, mRNA levels in nuclei of the brain stem, amygdala and hypothalamus of rats. Injection of LiCl in the context of CTA, that is after exposure to an unfamiliar taste, does not significantly augment the effect of LiCl alone [41]. Contextual fear conditioning in rodents by footshock application subsequent to exploration of a novel context results in induced c-*fos* and *zif*/268 expression levels in limbic and cortical areas. However, control experiments suggest that these changes were due to exposure either to the context or to the footshock [29, 42]. Interestingly, footshock application immediately upon context exposure, a treatment that prevents contextual fear conditioning, also considerably reduced the context-induced expression of c-*fos* in the hippocampus and parietal somatosensory cortex [29]. A few studies failed to detect modulations of ITF expression in brain structures implicated with learning and memory of the respective task. For example, Wisden et al. [43] failed to find modulations in the expression of c-*fos* and *zif*/268 mRNAs in the dentate gyrus of rats trained on a spatial task in a water maze. Similarly, in the gustatory cortex of rats, the levels of c-*fos*, *fos*-B, *zif*/268 and *crem* mRNAs were not augmented in the context of conditioned taste aversion learning [41], although protein synthesis in this structure is required for taste memory [44]. The reasons for these negative findings are presently unclear. They might point to the importance of other transcription factors, including CREB [45–47], for long-term memory formation.

Thus, training on different classical or operant conditioning paradigms leads to an increased expression of

certain ITFs in relevant brain regions. However, depending on the behavioral paradigm, control experiments performed under identical conditions but eliminating the contingency of interest may result in similar changes in ITF expression. These findings might suggest that stimulus association does not necessarily produce detectable changes in ITF expression additional to those observed following exposure to unpaired stimuli. Alternatively, associations different from those under experimental consideration might be formed in control animals and lead to changes in ITF expression similar to those observed in trained animals. In another set of experiments, training-induced ITF expression levels have been compared with those observed in animals that previously learned the respective behavior and were merely repeating it. If the induction of ITF expression was correlated to the amount of learning, it would be predicted that ITF induction is high after early training sessions and reduced after late training sessions, when only the execution of already learned behavior occurs. For example, the levels of c-*fos* and c-*jun* mRNAs were increased in the forebrain of chicks that had to learn to distinguish food grains from inedible pebbles. In chicks that were merely repeating this behavior, the increase in c-*jun* mRNA levels was significantly reduced [48]. Studies of a two-way active avoidance reaction in rats revealed that c-*fos* and *zif*/268 mRNA levels, which were strongly induced in different brain structures after a single training session, were attenuated following long-term training up to an asymptotic level of performance. As demonstrated for c-*fos*, inducibility still remained, provided that a novel, performance-elevating stimulus was given [49, 50]. In canary forebrain structures, learning the association between a song and a mild footshock led to an enhancement of *zenk* mRNA expression above the levels observed after unpaired presentation of song and shock. With repeated presentations of paired song and shock, enhancement of *zenk* expression decreased [51]. Similarly, attenuated induction of cerebral c-*fos* expression has been demonstrated following overtraining on different behavioral paradigms, for example odor discrimination [52] and motor skill learning [53].

Thus, the novelty of sensory stimuli themselves or of their association appears to be a major factor in triggering the induction of ITF expression during behavioral training. Novelty detection is certainly a necessary prerequisite of learning. Hence, strongest inductions have been observed following initial training sessions, when learning about the stimuli and their contingencies is highest. Following overtraining, that is training to an asymptotic level of performance, when novelty disappears and additional learning is thought to be absent or greatly diminished, induction of ITF expression is attenuated.

Training-induced ITF expression and memory retention

The levels of Fos protein expression have been studied in specific forebrain structures of chicks following 1 h of training with an imprinting stimulus [54]. Chicks were classified according to their preference score measured 10 min after training. As suggested by Horn [55], this score is strongly correlated with the score measured 24 h later and thus provides a reliable measure of both short- and long-term memory retention. Compared with chicks with low retention scores, those performing well in the test session showed an increased number of Fos-expressing cells in the intermediate and medial hyperstriatum ventrale (IMHV) about 1 h after the end of training [54]. The vast majority of Fos-expressing cells also express the gamma protein kinase C (PKC) isoenzyme, suggesting that a functional connection may exist between PKC activation and c-*fos* expression in IMHV neurones that are involved in learning [56].

In the same structure of chicks, increased levels of Fos and Jun proteins [57], preceded by increased c-*fos* mRNA levels [24], have been observed following one-trail passive avoidance training. Chicks were regarded as either recalling the task or as amnesic according to their behavior in a recall test 1 h after the training. Again, those chicks recalling the task showed enhanced Fos and Jun protein levels when compared with chicks that were amnesic [57]. Posttraining injection of MK-801, a noncompetitive inhibitor of NMDA-type glutamate receptors, increased the percentage of amnesic chicks and attenuated Fos and Jun protein levels in these birds, but not in those that recalled the task, suggesting that NMDA receptor activation might precede immediate early gene expression in the memory formation cascade.

Thus, individual differences of chicks to induce ITFs in the IMHV during imprinting and passive avoidance training correlate well with memory recall observed in a retention test. Pharmacological studies on passive avoidance learning suggest that retention scores observed close to the training-to-test intervals used in the above studies reflect, at least in part, protein synthesis dependent memory [58, 59].

ITF expression and pharmacological intervention in learning and memory

A number of studies have reported on correlative modulations of ITF expression following the application of drugs shown to either facilitate or impair learning and memory formation. However, the molecular and/or systemic effects of those drugs in the context of learning still remain to be elucidated.

Appetitive conditioning in a Skinner box resulted in increased levels of c-*fos* and c-*jun* mRNAs in hippocampal regions of mice. Posttraining application of

apamine, a bee venom toxin previously shown to facilitate memory processing [60, 61], additionally enhanced the training-induced increase in the expression of both genes [62].

Similarly, injection of the pentapeptide AVP(4–8), a metabolite of arginine-vasopressin facilitating the acquisition and maintenance of learning and memory, enhanced nerve growth factor gene expression in the hippocampus of rats. This effect was preceded by an increased c-*fos* expression, suggesting a role of Fos protein in the peptide-induced processes [63].

During fear conditioning, the hippocampus is critically involved in processing and transient storage of contextual information (e.g. [64–66]). Application of ethanol has been shown to severely disrupt contextual, that is hippocampal-dependent, learning whereas hippocampal-independent learning was not significantly affected. Ethanol treatment also completely blocked the context-induced hippocampal c-*fos* expression [67].

Suppression of ITF expression during learning interferes with memory formation

One of the crucial questions is whether the induced expression of distinct ITFs during learning and memory formation is of functional relevance for plastic processes and memory retention or merely correlates with changes in synaptic activity. Molecular genetic techniques, interfering with the expression of specific genes, have been used in combination with neurobiology at the systems level to analyze the significance of defined gene products for behavioral plasticity.

In mutant mice lacking c-*fos* expression, severe behavioral deficits have been observed [68]. However, these mice showed impaired sensitivities of sensory systems and had heterogeneous genetic backgrounds, making interpretation of learning deficits rather difficult. A null mutation at the c-*jun* locus causes embryonic lethality [69]. Thus, so far, knockout experiments could not contribute to elucidate the physiological significance of ITF expression for plastic changes in the central nervous system. Abilities to regulate the expression of ITF-encoding genes within restricted brain areas will further advance the study of gene function in the brain.

Antisense (AS) oligodeoxynucleotides (ODNs) are commonly designed to inhibit the synthesis of specific proteins by hybridization to the respective mRNA. Antisense approaches provide an alternative to circumvent some of the problems of current knockout techniques, such as molecular compensation, developmental defects, background genotypes and the restriction to the species mouse. As required for studies on learning and memory formation, intracerebral microinjection of AS-ODNs exerts locally and temporally restricted effects. Recent reports in vivo have demonstrated the usefulness of AS-ODNs to study the role of specific proteins in the nervous system in a behavioral context (for review see [70–72]) and specifically during learning [46, 47, 73].

The functional significance of cerebral c-Jun, Jun-B and c-Fos expression for brightness discrimination learning and memory formation in rats has been studied by use of phosphorothioate-modified AS-ODNs. As previous studies on this paradigm revealed increased protein synthesis predominantly in the hippocampus (for review see [2]), this structure was selected as target for bilateral injections of 2 nmol of the respective AS-ODNs 10 h and 2 h prior to training. Distribution studies with randomized labeled ODNs revealed strong labeling of the dorsal hippocampus, the corpus callosum and surrounding cortical areas [74]. Compared with rats pretreated with control ODN or saline, pretraining injection of c-*jun* AS-ODN influenced chiefly the performance of the task [39]. This impaired performance was not a simple reflection of an altered state of activity, since behavior in an open field test was not affected [75]. In contrast, different AS-ODNs to Jun-B impaired neither acquisition nor retention of the discrimination reaction [39]. Application of c-*fos* AS-ODN prior to brightness discrimination training drastically reduced the induction of c-Fos expression normally observed in limbic and cortical areas after the training. Acquisition of the discrimination reaction was not affected by this treatment, whereas the retention of the task in a relearning test 24 h after the initial training was specifically impaired compared with control ODN- and saline-treated rats [76]. The parameters monitored during the behavioral experiments indicate that this retention deficit did not result from influences on sensory or motor systems that may interfere with the acquisition of the task. Thus, these findings suggest that c-Jun is necessary for normal neuronal function during learning, whereas c-Fos is involved in processes underlying the formation of long-term memory.

Using phosphorothioate-modified AS-ODNs, the importance of c-Fos for acquisition and expression of conditioned taste aversion memory in rodents has been studied in nuclei of the amygdala and brain stem. Local bilateral microinjection of 5 nmol of c-*fos* AS-ODN into the amygdala of rats inhibited the basal and LiCl-induced expression of Fos protein in this structure compared with sense ODN- or saline-treated controls [77]. When applied 8 h before CTA training to saccharin, c-*fos* AS-ODN markedly reduced CTA memory tested 3–5 days after conditioning, whereas sense ODN had no effect. Injection of c-*fos* AS-ODN several days before training, before testing or into the basal ganglia was without effect on CTA memory, indicating that c-*fos* AS-ODN exerted time- and site-specific effects and caused neither residual functional or structural damage

to the amygdala nor impairments of sensory or motor faculties involved in the acquired rejection of the saccharin solution. In conclusion, the results suggest that transient expression of c-Fos in the amygdala during or immediately after CTA training is essential for encoding taste aversion memory. Swank et al. [78] injected 5 nmol of c-*fos* AS-ODN into the fourth ventricle of mice. Compared with sense ODN-treated controls, LiCl-induced c-Fos expression in brain stem nuclei, such as subdivisions of the nucleus tractus solitarius and the parabrachial nuclei, was significantly reduced 12 h after injection. Pretraining application of c-*fos* AS-ODN completely blocked the acquisition of CTA to saccharin. Infusion of antisense before testing did not impair expression, but appeared to retard extinction, of a learned taste aversion. Antisense treatment affected neither gustatory sensory functioning nor unconditioned responses to LiCl, suggesting normal sensory processing of CS and US necessary for acquisition or expression of CTA. It has been concluded that c-Fos expression in the brain stem nuclei examined is a correlate of US presentation and that c-*fos* AS-ODN blocks associative events necessary to support taste aversion learning.

The role of Fos protein expression in chick brain during acquisition and consolidation of passive avoidance memory has been elucidated by Mileusnic et al. [79] using a phosphodiester AS-ODN. Intracerebral injection of c-*fos* AS-ODN has been shown to suppress convulsant-induced c-Fos expression in the IMHV, demonstrating the biological activity of the AS-ODN used for behavioral studies. Memory retention was significantly reduced in animals intracranially injected with 40–60 nmol of c-*fos* AS-ODN 10–11 h before training compared with chicks treated with the same amounts of a scrambled ODN or with saline. Amnesia was evident by 3 h and 24 h but not by 30 min post-training, suggesting that suppression of c-Fos expression impairs the consolidation of long-term memory, whereas the initiation and maintenance of short-term memory are not affected. Injections shortly before or after training were ineffective.

Taken together, results yielded by use of different learning paradigms in different species have demonstrated that intracerebral injection of c-*fos* AS-ODN prior to training impairs the retention of a learned behavior. Several observations support the assumption that these effects were caused by impairment of memory consolidation processes and not by generalized defects in brain function. The specificity of the c-*fos* AS-ODN used has been studied in a large number of in vivo and in vitro paradigms (for review see [71, 72]). The effects of c-*fos* AS-ODN were clearly different from those of several control ODNs and of AS-ODNs to other ITFs. Neither the ODNs per se nor the suppression of c-Fos expression caused structural damage or impairment of sensory transduction that could account for learning deficits. The effects of c-*fos* AS-ODN were site-specific and transient, and no effects on performance or retrieval have been observed. The effects were confined to long- but not short-term memory.

Thus, the results obtained by antisense intervention with c-Fos expression support the hypothesis that c-Fos, certainly in combination with other transcription factors, is a link in the network of metabolic events induced by neuronal signals during learning and leading finally to the formation of long-term memory.

Conclusions

In various behavioral paradigms, increased expression levels of ITF-encoding IEGs have been observed in certain brain structures under conditions of learning and the formation of long-term memory. It is currently not possible to ascribe this IEG induction to distinct attributes of the learning process, such as sensory stimulation, motor activity, stress, arousal and attention. The novelty of presented stimuli themselves and of their association is a major factor in triggering IEG expression levels, leading to strongest inductions during early phases of training when learning is highest. IEG induction is attenuated with repeated sessions of behavioral training, when the animal gets familiar with the experimental situation and learning contributes only little to performance. As shown in the chick forebrain, training-induced IEG expression may correlate with memory retention shortly after training. The application of memory-facilitating or -inhibiting drugs is accompanied with a respective modulation of IEG expression. Specific suppression of c-Fos translation by antisense intervention impairs memory retention in different species and behavioral paradigms. Thus, although the downstream target genes and exact functions of ITFs in the nervous system remain largely elusive, a large body of experimental evidence suggests a functional significance of ITFs for processes involved in learning and consolidation of a long-term memory trace.

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1 Davis H. P. and Squire L. R. (1984) Protein synthesis and memory: a review. Psychol. Bull. **96:** 518–559
2 Matthies H. (1989) In search of cellular mechanisms of memory. Prog. Neurobiol. **32:** 277–349
3 Silva A. J., Kogan J. H., Frankland P. W. and Kida S. (1998) CREB and memory. Annu. Rev. Neurosci. **21:** 127–148
4 Chiu R., Boyle W. J., Meek J., Smeal T., Hunter T. and Karin M. (1988) The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. Cell **54:** 541–552

5 Rauscher F. J. D., Sambucetti L. C., Curran T., Distel R. J. and Spiegelman B. M. (1988) Common DNA binding site for Fos protein complexes and transcription factor AP-1. Cell **52:** 471–480

6 Hai T. and Curran T. (1991) Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc. Natl. Acad. Sci. USA **88:** 3720–3724

7 Hughes P. and Dragunow M. (1995) Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. Pharmacol. Rev. **47:** 133–178

8 Herrera D. G. and Robertson H. A. (1996) Activation of c-fos in the brain. Prog. Neurobiol. **50:** 83–107

9 Sheng M. and Greenberg M. E. (1990) The regulation and function of c-fos and other immediate early genes in the nervous system. Neuron **4:** 477–485

10 Morgan J. I. and Curran T. (1991) Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. Annu. Rev. Neurosci. **14:** 421–451

11 Ginty D. D. (1997) Calcium regulation of gene expression: isn't that spatial? Neuron **18:** 183–186

12 Chaudhuri A. (1997) Neural activity mapping with inducible transcription factors. Neuroreport **8:** iii–vii

13 Robertson H. A. (1992) Immediate-early genes, neuronal plasticity, and memory. Biochem. Cell Biol. **70:** 729–737

14 Kaczmarek L. (1993) Molecular biology of vertebrate learning: is c-fos a new beginning? J. Neurosci. Res. **34:** 377–381

15 Herdegen T. and Zimmermann M. (1994) Expression of c-Jun and JunD transcription factors represent specific changes in neuronal gene expression following axotomy. Prog. Brain Res. **103:** 153–171

16 Dragunow M. (1996) A role for immediate-early transcription factors in learning and memory. Behav. Genet. **26:** 293–299

17 Kaczmarek L. and Chaudhuri A. (1997) Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for functional mapping and neural plasticity. Brain Res. Rev. **23:** 237–256

18 Smith M. A., Banerjee S., Gold P. W. and Glowa J. (1992) Induction of c-fos mRNA in rat brain by conditioned and unconditioned stressors. Brain Res. **578:** 135–141

19 Persico A. M., Schindler C. W., O'Hara B. F., Brannock M. T. and Uhl G. R. (1993) Brain transcription factor expression: effects of acute and chronic amphetamine and injection stress. Mol. Brain Res. **20:** 91–100

20 Melia K. R., Ryabinin A. E., Schroeder R., Bloom F. E. and Wilson M. C. (1994) Induction and habituation of immediate early gene expression in rat brain by acute and repeated restraint stress. J. Neurosci. **14:** 5929–5938

21 Watanabe Y., Stone E. and McEwen B. S. (1994) Induction and habituation of c-fos and zif/268 by acute and repeated stressors. Neuroreport **5:** 1321–1324

22 Persico A. M., Schindler C. W., Zaczek R., Brannock M. T. and Uhl G. R. (1995) Brain transcription factor gene expression, neurotransmitter levels and novelty response behaviors: alterations during rat amphetamine withdrawal and following chronic injection stress. Synapse **19:** 212–227

23 Mello C., Nottebohm F. and Clayton D. (1995) Repeated exposure to one song leads to a rapid and persistent decline in an immediate early gene's response to that song in zebra finch telencephalon. J. Neurosci. **15:** 6919–6925

24 Anokhin K. V., Mileusnic R., Shamakina I. Y. and Rose S. P. (1991) Effects of early experience on c-fos gene expression in the chick forebrain. Brain Res. **544:** 101–107

25 Papa M., Pellicano M. P., Welzl H. and Sadile A. G. (1993) Distributed changes in c-Fos and c-Jun immunoreactivity in the rat brain associated with arousal and habituation to novelty. Brain Res. Bull. **32:** 509–515

26 Handa R. J., Nunley K. M. and Bollnow M. R. (1993) Induction of c-fos mRNA in the brain and anterior pituitary gland by a novel environment. Neuroreport **4:** 1079–1082

27 Kerr J. E., Beck S. G. and Handa R. J. (1996) Androgens selectively modulate c-fos messenger RNA induction in the rat hippocampus following novelty. Neuroscience **74:** 757–766

28 Montero V. M. (1997) c-fos induction in sensory pathways of rats exploring a novel complex environment: shifts of active thalamic reticular sectors by predominant sensory cues. Neuroscience **76:** 1069–1081

29 Milanovic S., Radulovic J., Laban O., Stiedl O., Henn F. and Spiess J. (1998) Production of the Fos protein after contextual fear conditioning of C57BL/6N mice. Brain Res. **784:** 37–47

30 Zhu X. O., Brown M. W., McCabe B. J. and Aggleton J. P. (1995) Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain. Neuroscience **69:** 821–829

31 Zhu X. O., McCabe B. J., Aggleton J. P. and Brown M. W. (1996) Mapping visual recognition memory through expression of the immediate early gene c-fos. Neuroreport **7:** 1871–1875

32 Zhu X. O., McCabe B. J., Aggleton J. P. and Brown M. W. (1997) Differential activation of the rat hippocampus and perirhinal cortex by novel visual stimuli and a novel environment. Neurosci. Lett. **229:** 141–143

33 Keilmann A. and Herdegen T. (1997) The c-Fos transcription factor in the auditory pathway of the juvenile rat: effects of acoustic deprivation and repetitive stimulation. Brain Res. **753:** 291–298

34 Montag-Sallaz M., Welzl H., Kuhl D., Montag D. and Schachner M. (1999) Novelty-induced increased expression of the immediate early genes c-fos and arg 3.1 in the mouse brain. J. Neurobiol. **38:** 234–246

35 Brown G. D. (1998) Operational terminology for stimulus exposure (SE) conditioning. Behav. Brain Res. **95:** 143–150

36 Irwin K. B., Craig A. D., Bracha V. and Bloedel J. R. (1992) Distribution of c-fos expression in brainstem neurons associated with conditioning and pseudo-conditioning of the rabbit nictitating membrane reflex. Neurosci. Lett. **148:** 71–75

37 Brennan P. A., Hancock D. and Keverne E. B. (1992) The expression of the immediate-early genes c-fos, egr-1 and c-jun in the accessory olfactory bulb during the formation of an olfactory memory in mice. Neuroscience **49:** 277–284

38 Tischmeyer W., Kaczmarek L., Strauss M., Jork R. and Matthies H. (1990) Accumulation of c-fos mRNA in rat hippocampus during acquisition of a brightness discrimination. Behav. Neural Biol. **54:** 165–171

39 Tischmeyer W., Grimm R., Schicknick H., Brysch W. and Schlingensiepen K.-H. (1994) Sequence-specific impairment of learning by c-jun antisense oligonucleotides. Neuroreport **5:** 1501–1504

40 Grimm R. and Tischmeyer W. (1997) Complex patterns of immediate early gene induction in rat brain following brightness discrimination training and pseudotraining. Behav. Brain Res. **84:** 109–116

41 Lamprecht R. and Dudai Y. (1995) Differential modulation of brain immediate early genes by intraperitoneal LiCl. Neuroreport **7:** 289–293

42 Rosen J. B., Fanselow M. S., Young S. L., Sitcoske M. and Maren S. (1998) Immediate-early gene expression in the amygdala following footshock stress and contextual fear conditioning. Brain Res. **796:** 132–142

43 Wisden W., Errington M. L., Williams S., Dunnett S. B., Waters C., Hitchcock D. et al. (1990) Differential expression of immediate early genes in the hippocampus and spinal cord. Neuron **4:** 603–614

44 Rosenblum K., Meiri N. and Dudai Y. (1993) Taste memory: the role of protein synthesis in gustatory cortex. Behav. Neural Biol. **59:** 49–56

45 Bourtchuladze R., Frenguelli B., Blendy J., Cioffi D., Schutz G. and Silva A. J. (1994) Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell **79:** 59–68

46 Guzowski J. F. and McGaugh J. L. (1997) Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. Proc. Natl. Acad. Sci. USA **94:** 2693–2698
47 Lamprecht R., Hazvi S. and Dudai Y. (1997) cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. J. Neurosci. **17:** 8443–8450
48 Anokhin K. V. and Rose S. P. R. (1991) Learning-induced increase of immediate early gene messenger RNA in the chick forebrain. Eur. J. Neurosci. **3:** 162–167
49 Nikolaev E., Kaminska B., Tischmeyer W., Matthies H. and Kaczmarek L. (1992) Induction of expression of genes encoding transcription factors in the rat brain elicited by behavioral training. Brain Res. Bull. **28:** 479–484
50 Nikolaev E., Werka T. and Kaczmarek L. (1992) C-fos protooncogene expression in rat brain after long-term training of two-way active avoidance reaction. Behav. Brain Res. **48:** 91–94
51 Jarvis E. D., Mello C. V. and Nottebohm F. (1995) Associative learning and stimulus novelty influence the song-induced expression of an immediate early gene in the canary forebrain. Learn. Mem. **2:** 62–80
52 Hess U. S., Lynch G. and Gall C. M. (1995) Regional patterns of c-fos mRNA expression in rat hippocampus following exploration of a novel environment versus performance of a well-learned discrimination. J. Neurosci. **15:** 7796–7809
53 Kleim J. A., Lussnig E., Schwarz E. R., Comery T. A. and Greenough W. T. (1996) Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. J. Neurosci. **16:** 4529–4535
54 McCabe B. J. and Horn G. (1994) Learning-related changes in Fos-like immunoreactivity in the chick forebrain after imprinting. Proc. Natl. Acad. Sci. USA **91:** 11417–11421
55 Horn G. (1998) Visual imprinting and the neural mechanisms of recognition memory. Trends. Neurosci. **21:** 300–305
56 Ambalavanar R., Van der Zee E. A., Bolhuis J. J., McCabe B. J. and Horn G. (1993) Co-expression of Fos immunoreactivity in protein kinase (PKC gamma)-positive neurones: quantitative analysis of a brain region involved in learning. Brain Res. **606:** 315–318
57 Freeman F. M. and Rose S. P. R. (1995) MK-801 blockade of Fos and Jun expression following passive avoidance training in the chick. Eur. J. Neurosci. **7:** 563–569
58 Patterson T. A., Alvarado M. C., Warner I. T., Bennett E. L. and Rosenzweig M. R. (1986) Memory stages and brain asymmetry in chick learning. Behav. Neurosci. **100:** 856–865
59 Crowe S. F., Zhao W. Q., Sedman G. L. and Ng K. T. (1994) 2-Deoxygalactose interferes with an intermediate processing stage of memory. Behav. Neural Biol. **61:** 206–213
60 Messier C., Mourre C., Bontempi B., Sif J., Lazdunski M. and Destrade C. (1991) Effect of apamin, a toxin that inhibits Ca(2+)-dependent K+ channels, on learning and memory processes. Brain Res. **551:** 322–326
61 Belcadi-Abbassi W. and Destrade C. (1995) Post-test apamin injection suppresses a Kamin-like effect following a learning session in mice. Neuroreport **6:** 1293–1296
62 Heurteaux C., Messier C., Destrade C. and Lazdunski M. (1993) Memory processing and apamin induce immediate early gene expression in mouse brain. Mol. Brain Res. **3:** 17–22
63 Zhou A. W., Guo J., Wang H. Y., Gu B. X. and Du Y. C. (1995) Enhancement of NGF gene expression in rat brain by the memory-enhancing peptide AVP(4–8). Peptides **16:** 581–586
64 Phillips R. G. and LeDoux J. E. (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav. Neurosci. **106:** 274–285
65 Kim J. J., Rison R. A. and Fanselow M. S. (1993) Effects of amygdala, hippocampus and periaqueductal gray lesions on short- and long-term contextual fear. Behav. Neurosci. **107:** 1093–1098
66 Abel T., Nguyen P. V., Barad M., Deuel T. A., Kandel E. R. and Bourtchouladze R. (1997) Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell **88:** 615–626
67 Melia K. R., Ryabinin A. E., Corodimas K. P., Wilson M. C. and LeDoux J. E. (1996) Hippocampal-dependent learning and experience-dependent activation of the hippocampus are preferentially disrupted by ethanol. Neuroscience **74:** 313–322
68 Paylor R., Johnson R. S., Papaioannou V., Spiegelman B. M. and Wehner J. M. (1994) Behavioral assessment of c-fos mutant mice. Brain Res. **651:** 275–282
69 Johnson R. S., van Lingen B., Papaioannou V. E. and Spiegelman B. M. (1993) A null mutation at the c-jun locus causes embryonic lethality and retarded cell growth in culture. Genes Dev. **7:** 1309–1317
70 Wahlestedt C. (1994) Antisense oligonucleotide strategies in neuropharmacology. Trends Pharmacol. Sci. **15:** 42–46
71 Chiasson B. J., Hong M. G. and Robertson H. A. (1997) Putative roles for the inducible transcription factor c-fos in the central nervous system: studies with antisense oligonucleotides. Neurochem. Int. **31:** 459–475
72 Sommer W. and Fuxe K. (1997) On the role of c-fos expression in striatal transmission. The antisense oligonucleotide approach. Neurochem. Int. **31:** 425–436
73 Schmidt R., Brysch W., Rother S. and Schlingensiepen K.-H. (1995) Inhibition of memory consolidation after active avoidance conditioning by antisense intervention with ependymin gene expression. J. Neurochem. **65:** 1465–1471
74 Brysch W., Rifai A., Tischmeyer W. and Schlingensiepen K.-H. (1996) Antisense-mediated inhibition of protein synthesis: rational drug design, pharmacokinetics, intracerebral application and organ uptake of phosphorothioate oligonucleotides. In: Methods in Molecular Medicine: Antisense Therapeutics, pp. 159–182, Agrawal S. (ed.), Humana Press, Totowa
75 Tischmeyer W., Grimm R., Lohmann K., Schicknick H. and Gundelfinger E. D. (1997) Suppression of immediate early gene expression by intracerebrally applied antisense oligonucleotides impairs mechanisms of learning and memory. In: Neurochemistry: Cellular, Molecular and Clinical Aspects, pp. 1117–1121, Teelken A. and Korf J. (eds), Plenum, New York
76 Grimm R., Schicknick H., Riede I., Gundelfinger E. D., Herdegen T., Zuschratter W. et al. (1997) Suppression of c-fos induction in rat brain impairs retention of a brightness discrimination reaction. Learn. Mem. **3:** 402–413
77 Lamprecht R. and Dudai Y. (1996) Transient expression of c-Fos in rat amygdala during training is required for encoding conditioned taste aversion memory. Learn. Mem. **3:** 31–41
78 Swank M. W., Ellis A. E. and Cochran B. N. (1996) c-Fos antisense blocks acquisition and extinction of conditioned taste aversion in mice. Neuroreport **7:** 1866–1870
79 Mileusnic R., Anokhin K. and Rose S. P. R. (1996) Antisense oligodeoxynucleotides to c-fos are amnestic for passive avoidance in the chick. Neuroreport **7:** 1269–1272
80 Mello C. V., Vicario D. S. and Clayton D. F. (1992) Song presentation induces gene expression in the songbird forebrain. Proc. Natl. Acad. Sci. USA **89:** 6818–6822
81 Mello C. V. and Clayton D. F. (1994) Song-induced ZENK gene expression in auditory pathways of songbird brain and its relation to the song control system. J. Neurosci. **14:** 6652–6666
82 Nastiuk K. L., Mello C. V., George J. M. and Clayton D. F. (1994) Immediate-early gene responses in the avian song control system: cloning and expression analysis of the canary c-jun cDNA. Mol. Brain Res. **27:** 299–309
83 Jarvis E. D., Schwabl H., Ribeiro S. and Mello C. V. (1997) Brain gene regulation by territorial singing behavior in freely ranging songbirds. Neuroreport **8:** 2073–2077

84 Jarvis E. D. and Nottebohm F. (1997) Motor-driven gene expression. Proc. Natl. Acad. Sci. USA **94:** 4097–4102
85 Jin H. and Clayton D. F. (1997) Localized changes in immediate-early gene regulation during sensory and motor learning in zebra finches. Neuron **19:** 1049–1059
86 Kimpo R. R. and Doupe A. J. (1997) FOS is induced by singing in distinct neuronal populations in a motor network. Neuron **18:** 315–325
87 Stripling R., Volman S. F. and Clayton D. F. (1997) Response modulation in the zebra finch neostriatum: relationship to nuclear gene regulation. J. Neurosci. **17:** 3883–3893
88 Mello C. V. and Ribeiro S. (1998) ZENK protein regulation by song in the brain of songbirds. J. Comp. Neurol. **393:** 426–438
89 Bertaina V. and Destrade C. (1995) Differential time courses of c-fos mRNA expression in hippocampal subfields following acquisition and recall testing in mice. Cogn. Brain Res. **2:** 269–275
90 Zuschratter W., Gass P., Herdegen T. and Scheich H. (1995) Comparison of frequency-specific c-Fos expression and fluoro-2-deoxyglucose uptake in auditory cortex of gerbils (*Meriones unguiculatus*). Eur. J. Neurosci. **7:** 1614–1626
91 Scheich H. and Zuschratter W. (1995) Mapping of stimulus features and meaning in gerbil auditory cortex with 2-deoxyglucose and c-Fos antibodies. Behav. Brain Res. **66:** 195–205
92 Johnson B. A., Woo C. C., Duong H., Nguyen V. and Leon M. (1995) A learned odor evokes an enhanced Fos-like glomerular response in the olfactory bulb of young rats. Brain Res. **699:** 192–200
93 Woo C. C., Oshita M. H. and Leon M. (1996) A learned odor decreases the number of Fos-immunopositive granule cells in the olfactory bulb of young rats. Brain Res. **716:** 149–156
94 Sallaz M. and Jourdan F. (1996) Odour-induced c-fos expression in the rat olfactory bulb: involvement of centrifugal afferents. Brain Res. **721:** 66–75
95 Hess U. S., Lynch G. and Gall C. M. (1995) Changes in c-fos mRNA expression in rat brain during odor discrimination learning: differential involvement of hippocampal subfields CA1 and CA3. J. Neurosci. **15:** 4786–4795
96 Hess U. S., Gall C. M., Granger R. and Lynch G. (1997) Differential patterns of c-fos mRNA expression in amygdala during successive stages of odor discrimination learning. Learn. Mem. **4:** 262–283
97 Gall C. M., Hess U. S. and Lynch G. (1998) Mapping brain networks engaged by, and changed by, learning. Neurobiol. Learn. Mem. **70:** 14–36
98 Bialy M., Nikolaev E., Beck J. and Kaczmarek L. (1992) Delayed c-fos expression in sensory cortex following sexual learning in male rats. Mol. Brain Res. **14:** 352–356
99 Castro-Alamancos M. A., Borrell J. and Garcia-Segura L. M. (1992) Performance in an escape task induces fos-like immunoreactivity in a specific area of the motor cortex of the rat. Neuroscience **49:** 157–162
100 Nagahara A. H. and Handa R. J. (1995) Fetal alcohol exposure alters the induction of immediate early gene mRNA in the rat prefrontal cortex after an alternation task. Alcohol Clin. Exp. Res. **19:** 1389–1397
101 Duncan G. E., Knapp D. J. and Breese G. R. (1996) Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. Brain Res. **713:** 79–91
102 Houpt T. A., Philopena J. M., Wessel T. C., Joh T. H. and Smith G. P. (1994) Increased c-fos expression in nucleus of the solitary tract correlated with conditioned taste aversion to sucrose in rats. Neurosci. Lett. **172:** 1–5
103 Yamamoto T., Sako N., Sakai N. and Iwafune A. (1997) Gustatory and visceral inputs to the amygdala of the rat: conditioned taste aversion and induction of c-Fos-like immunoreactivity. Neurosci. Lett. **226:** 127–130
104 Yamamoto T., Shimura T., Sako N., Azuma S., Bai W. Z. and Wakisaka S. (1992) C-fos expression in the rat brain after intraperitoneal injection of lithium chloride. Neuroreport **3:** 1049–1052
105 Gu Y., Gonzalez M. F., Chin D. Y. and Deutsch J. A. (1993) Expression of c-fos in brain subcortical structures in response to nauseant lithium chloride and osmotic pressure in rats. Neurosci. Lett. **157:** 49–52
106 Thiele T. E., Roitman M. F. and Bernstein I. L. (1996) c-Fos induction in rat brainstem in response to ethanol- and lithium chloride-induced conditioned taste aversions. Alcohol Clin. Exp. Res. **20:** 1023–1028
107 Van Dijk G., Thiele T. E., Donahey J. C., Campfield L. A., Smith F. J., Burn P. et al. (1996) Central infusions of leptin and GLP-1-(7-36) amide differentially stimulate c-FLI in the rat brain. Am. J. Physiol. **271:** R1096–1100
108 Sakai N. and Yamamoto T. (1997) Conditioned taste aversion and c-fos expression in the rat brainstem after administration of various USs. Neuroreport **8:** 2215–2220
109 Thiele T. E., Seeley R. J., D'Alessio D., Eng J., Bernstein I. L., Woods S. C. et al. (1998) Central infusion of glucagon-like peptide-1-(7-36) amide (GLP-1) receptor antagonist attenuates lithium chloride-induced c-Fos induction in rat brainstem. Brain Res. **801:** 164–170
110 Thiele T. E., van Dijk G., Yagaloff K. A., Fisher S. L., Schwartz M., Burn P. et al. (1998) Central infusion of melanocortin agonist MTII in rats: assessment of c-Fos expression and taste aversion. Am. J. Physiol. **274:** R248–254
111 Swank M. W. and Bernstein I. L. (1994) c-Fos induction in response to a conditioned stimulus after single trial taste aversion learning. Brain Res. **636:** 202–208
112 Schafe G. E., Seeley R. J. and Bernstein I. L. (1995) Forebrain contribution to the induction of a cellular correlate of conditioned taste aversion in the nucleus of the solitary tract. J. Neurosci. **15:** 6789–6796
113 Swank M. W., Schafe G. E. and Bernstein I. L. (1995) c-Fos induction in response to taste stimuli previously paired with amphetamine or LiCl during taste aversion learning. Brain Res. **673:** 251–261
114 Houpt T. A., Philopena J. M., Joh T. H. and Smith G. P. (1996) c-Fos induction in the rat nucleus of the solitary tract by intraoral quinine infusion depends on prior contingent pairing of quinine and lithium chloride. Physiol. Behav. **60:** 1535–1541
115 Schafe G. E. and Bernstein I. L. (1996) Forebrain contribution to the induction of a brainstem correlate of conditioned taste aversion: I. The amygdala. Brain Res. **741:** 109–116
116 Wang Y., Cummings S. L. and Gietzen D. W. (1996) Temporal-spatial pattern of c-Fos expression in the rat brain in response to indispensable amino acid deficiency. II. The learned taste aversion. Mol. Brain Res. **40:** 35–41
117 Houpt T. A., Berlin R. and Smith G. P. (1997) Subdiaphragmatic vagotomy does not attenuate c-Fos induction in the nucleus of the solitary tract after conditioned taste aversion expression. Brain Res. **747:** 85–91
118 Schafe G. E. and Bernstein I. L. (1998) Forebrain contribution to the induction of a brainstem correlate of conditioned taste aversion. II. Insular (gustatory) cortex. Brain Res. **800:** 40–47
119 Radulovic J., Kammermeier J. and Spiess J. (1998) Relationship between fos production and classical fear conditioning: effects of novelty, latent inhibition, and unconditioned stimulus preexposure. J. Neurosci. **18:** 7452–7461
120 Campeau S., Hayward M. D., Hope B. T., Rosen J. B., Nestler E. J. and Davis M. (1991) Induction of the c-fos proto-oncogene in rat amygdala during unconditioned and conditioned fear. Brain Res. **565:** 349–352
121 Pezzone M. A., Lee W. S., Hoffman G. E. and Rabin B. S. (1992) Induction of c-Fos immunoreactivity in the rat forebrain by conditioned and unconditioned aversive stimuli. Brain Res. **597:** 41–50
122 Pezzone M. A., Lee W. S., Hoffman G. E., Pezzone K. M. and Rabin B. S. (1993) Activation of brainstem catecholaminergic neurons by conditioned and unconditioned aversive stimuli as revealed by c-Fos immunoreactivity. Brain Res. **608:** 310–318
123 Beck C. H. and Fibiger H. C. (1995) Conditioned fear-induced changes in behavior and in the expression of the immediate

early gene c-fos: with and without diazepam pretreatment. J. Neurosci. **15:** 709–720
124 Sotty F., Sandner G. and Gosselin O. (1996) Latent inhibition in conditioned emotional response: c-fos immunolabelling evidence for brain areas involved in the rat. Brain Res. **737:** 243–254
125 Campeau S., Falls W. A., Cullinan W. E., Helmreich D. L., Davis M. and Watson S. J. (1997) Elicitation and reduction of fear: behavioural and neuroendocrine indices and brain induction of the immediate-early gene c-fos. Neuroscience **78:** 1087–1104
126 Carrive P., Leung P., Harris J. and Paxinos G. (1997) Conditioned fear to context is associated with increased Fos expression in the caudal ventrolateral region of the midbrain periaqueductal gray. Neuroscience **78:** 165–177
127 Hamamura T., Ichimaru Y. and Fibiger H. C. (1997) Amphetamine sensitization enhances regional c-fos expression produced by conditioned fear. Neuroscience **76:** 1097–1103
128 Brown E. E., Robertson G. S. and Fibiger H. C. (1992) Evidence for conditional neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. J. Neurosci. **12:** 4112–4121
129 Thiele T. E., Roitman M. F. and Bernstein I. L. (1998) Learned tolerance to ethanol-induced c-Fos expression in rats. Behav. Neurosci. **112:** 193–198
130 Carrive P., Kehoe E. J., Macrae M. and Paxinos G. (1997) Fos-like immunoreactivity in locus coeruleus after classical conditioning of the rabbit's nictitating membrane response. Neurosci. Lett. **223:** 33–36
131 Da Costa A. P., Broad K. D. and Kendrick K. M. (1997) Olfactory memory and maternal behaviour-induced changes in c-fos and zif/268 mRNA expression in the sheep brain. Mol. Brain Res. **46:** 63–76
132 Okuno H. and Miyashita Y. (1996) Expression of the transcription factor Zif268 in the temporal cortex of monkeys during visual paired associate learning. Eur. J. Neurosci. **8:** 2118–2128