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MEMORY OF FEARFUL EVENTS: THE ROLE OF FIBROBLAST GROWTH FACTOR-2 IN FEAR ACQUISITION AND EXTINCTION

B. M. GRAHAM[a]* AND R. RICHARDSON[b]

[a]*Department of Psychiatry, Harvard Medical School and Massachusetts General Hospital, 149 13th St. Charlestown, 02129 Boston, MA, USA*

[b]*School of Psychology, UNSW, 2052, Sydney, Australia*

Abstract—Research during the past decade has led to a tremendous growth in our understanding of how fear memories are acquired and subsequently inhibited on a neural and molecular level. Such research has contributed to significant developments in the treatment of anxiety disorders, and has considerably advanced our understanding of the neurobiology of learning and memory in general. A number of recent studies have examined the role of growth factors in the formation of long-term memory for fearful events, due to their ability to cause morphological neural changes in response to environmental stimulation. In this review we first describe physiological evidence that fibroblast growth factor-2 (FGF2) receptors are highly expressed in the neural circuitry regulating fear acquisition and extinction, and that FGF2 modulates the molecular signals known to be involved in the formation of fear memories. Then we present emerging behavioral research that demonstrates that exogenous FGF2 can enhance the formation of fear conditioning and extinction memories. Finally, we briefly discuss how research into the role of FGF2 in learning and memory may be of clinical benefit, particularly in the treatment of anxiety disorders.

Key words: fibroblast growth factor-2, fear conditioning, fear extinction, anxiety, memory, long-term potentiation.

Contents

*Corresponding author. Tel: +1-617-643-1156; fax: +1-617-726-4078.
E-mail address: bmgraham@nmr.mgh.harvard.edu (B. M. Graham).
Abbreviations: BDNF, brain-derived neurotrophic factor; BLA, basolateral complex of the amygdala; BNST, bed nuclei of the stria terminalis; cAMP, cyclic adenosine monophosphate; CeA, central nucleus of the amygdala; CREB, cAMP response element binding protein; CS, conditioned stimulus; ERK, extracellular signal-regulated kinase; *Fgfr1*, FGF receptor-1; FGF2, fibroblast growth factor-2; IL, infralimbic region of the prefrontal cortex; LTP, long-term potentiation; LVGCCs, L-type voltage gated Ca^{2+} channels; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; mPFC, medial prefrontal cortex; NMDA, N-methyl-D-aspartate; PKC, calcium/phospholid-dependent protein kinase; PND, postnatal day; TrkB, tyrosine kinase B; US, unconditioned stimulus.

The neurobiology underlying fear memories, both their acquisition and inhibition (i.e., extinction), has become increasingly well characterized over the years. This has had a number of clinical applications, including the development of drugs that interfere with the consolidation/reconsolidation of fearful memories and drugs that enhance the acquisition and/or consolidation of fear extinction memories (for a recent review see Graham et al., in press). However, most of the documented neurobiological changes that characterize fear memories are relatively transient, which leads to the question of how these memories persist. One approach to this issue which has recently started being explored has focused on the role of growth factors in memory formation. Growth factors cause structural changes within the brain and therefore may be in part responsible for the persistence of fear memories. One example is brain-derived neurotrophic factor (BDNF), which has been comprehensively reviewed in a number of recent papers (Cunha et al., 2010; Lu et al., 2008). Another example is fibroblast growth factor-2 (FGF2). Although FGF2 has received much less attention than BDNF regarding its potential role in learning and memory, a vast body of research has demonstrated that FGF2 modulates, and is modulated by, the molecular processes that underlie fear memories. Further, recent research has shown that systemic FGF2 modulates both fear acquisition and extinction memories. The purpose of this review is to describe the neural, molecular, and behavioral evidence that suggests that FGF2 is involved in the regulation of fear memories.

The most common ways of modeling fear acquisition and inhibition in the laboratory are through Pavlovian fear conditioning and fear extinction, and as such, this review

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focuses on how FGF2 may be involved in the neurobiology underlying memories produced by these procedures. In Pavlovian fear conditioning a neutral conditioned stimulus (CS, e.g., a tone or a light) is paired with an aversive unconditioned stimulus (US, e.g., a mild footshock) such that subsequent presentations of the CS alone elicit fear responses such as freezing, increased heart rate, and secretion of stress hormones. This particular procedure is termed "cued fear conditioning," but it also produces fear of the context (i.e., a diffuse cue) in which the conditioning procedure took place. Contextual fear conditioning can also be achieved by merely placing the animal in a distinct context and presenting the US some time after. In extinction, the animal is exposed to the CS (i.e., the discrete cue or the diffuse context) repeatedly in the absence of any aversive outcome, and the animals' fear responses decrease as they learn that the CS no longer predicts the US. When tested with the CS the following day the animal typically exhibits long-term memory for extinction, indexed by reduced levels of conditioned fear responding.

Laboratory studies of conditioning and extinction in non-human animals have led to significant discoveries regarding the behavioral and neural processes underlying fear acquisition and fear inhibition in humans, and furthermore, have contributed to improvements in exposure-based treatments for humans with anxiety disorders (Milad et al., 2006). As such, investigations of these procedures are not only theoretically interesting, as they provide insight into basic memory processes, but they are also potentially useful clinically.

FIBROBLAST GROWTH FACTOR-2: BACKGROUND

A growth factor is any substance (usually a protein or steroid hormone) that is capable of regulating cell proliferation, differentiation, and survival. The concept that extracellular signals can cause nerve cell growth is over half a century old, yet it is only in the past two decades that knowledge about the types and diversity of growth factors, and an understanding of the intracellular signaling cascades that they initiate, has emerged (Nestler et al., 2001). FGF2 is one ligand of a large family of growth factors that are involved in many physiological processes during development through to adulthood. In vertebrates, there are 22 FGF ligands (Ornitz and Itoh, 2001) and four FGF receptors have been identified in rodents while five FGF receptors have been identified in humans (Turner et al., 2006). FGF receptors are tyrosine kinase receptors. FGF2 is a single chain polypeptide protein, and is the most widely studied ligand of the FGF family. Its extensive range of physiological functions within the CNS has been well-characterized over the past 25 years, and research into FGF2 has contributed significantly to our understanding of the development of the nervous system, and the mechanisms underlying neuronal proliferation, survival, and repair, across the lifespan.

FGF2 is expressed in most tissues, but is expressed most abundantly in both neuronal and glial cells in the CNS. While the mechanisms and conditions under which growth factors are secreted is generally not well understood, recent work has suggested that FGF2 is released during stress and upon cell damage (see below). It has been shown in rodents that upon release FGF2 can bind to all four FGF receptors, but the FGF receptor-1 (*Fgfr1*) is the main receptor to which FGF2 binds and is located on the cell surface (Klint and Claesson-Welsh, 1999). The expression of FGF2 in the CNS increases across development (Unsicker et al., 1991) and by adulthood FGF2 is expressed abundantly in most regions of the CNS.

During development, FGF2 is involved in determining brain morphology. *In vitro*, FGF2 promotes the proliferation and survival of fetal and postnatal cells cultured from many areas of the brain including the hippocampus (Ray et al., 1993; Walicke et al., 1986), the entorhinal, frontal, parietal, and occipital cortices (Walicke, 1988) and the striatum, septum, thalamus, and cerebral cortex (Matsuda et al., 1990; Morrison et al., 1986). Further, FGF2 is required for the differentiation of embryonic hippocampal cells (Vicario-Abejon et al., 1995), and the promotion of axonal branching *in vitro* (Aoyagi et al., 1994).

FGF2 is also involved in many functions of the adult brain. For example, i.c.v. infusions of FGF2 increased neurogenesis in the subventricular zone of the hippocampus (Wagner et al., 1999), and chronic posterior lateral ventricular infusions of FGF2 in middle aged rats increased neurogenesis and enhanced dendritic growth in the subventricular zone and dentate gyrus of the hippocampus (Rai et al., 2007). Furthermore, FGF2 promotes survival and regenerative plasticity in response to brain injury during adulthood. For example, lesions cause increases in FGF2 expression, and application of FGF2 in the wound site increases astrocytic density and reduces cell death, suggesting that FGF2 causes astrocytic reactivity and/or proliferation in response to injury (Anderson et al., 1988; Gomez-Pinilla et al., 1995).

FGF2 secretion also appears to be modulated by the hypothalamic-pituitary-adrenal (HPA) axis, which mediates the mammalian response to stress. For example, adrenalectomy in rats reduced the expression of FGF2 in the hippocampus, striatum, and frontal cortex, suggesting that adrenal hormones (including glucocorticoids and mineralocorticoids, which are responsible for terminating the stress response) exert control over FGF2 (see review by Molteni et al., 2001). Furthermore, restraint stress (or administration of glucocorticoids) leads to an up-regulation of FGF2 mRNA expression in various brain regions, including the hippocampus and prefrontal cortex (Molteni et al., 2001). Such studies, coupled with the fact that FGF2 increases neurogenesis, have led to the hypothesis that FGF2 plays a neuroprotective role in response to stress.

The research described above demonstrates that FGF2 has an important role in many aspects of CNS functioning, including neuronal development and regeneration, as well as the physiological stress response. In the next sections it is proposed that in addition to these physiological functions, FGF2 may also be involved in long-term memory formation. Indeed, the fact that FGF2 regu-

lates morphological changes in the brain during development and in response to external stimulation (i.e., brain injury and stress) during adulthood makes FGF2 an attractive candidate for regulating the putative structural changes that underlie the formation of long-term memory. We describe below evidence for this suggestion from research that has investigated FGF2 using neural, molecular, and behavioral levels of analysis.

NEURAL EVIDENCE FOR A ROLE FOR FGF2 IN FEAR MEMORIES

Overview of the neural circuitry involved in fear acquisition and extinction

Several neural structures have been implicated in fear acquisition. The main structure involved in all types of fear learning is the amygdala (LeDoux, 2007). Cued fear conditioning involves associating the sensory representation of the CS with that of the US, and the amygdala is widely accepted as the main neural structure where this information converges. Evidence for the role of the basolateral complex of the amygdala (BLA) in associating the CS and US comes from lesion studies, inactivation studies, and drug antagonist studies, which together have demonstrated that interfering with normal functioning of the BLA disrupts fear conditioning across different CS modalities (Campeau and Davis, 1995), without affecting shock sensitivity (Sananes and Davis, 1992) or the ability to associate a neutral cue with an appetitive stimulus (i.e., the deficit appears to be specific to fear learning, rather than a broader disruption of all associative learning; Cahill and McGaugh, 1990). More recent evidence also supports a role for the central nucleus of the amygdala (CeA) in fear conditioning, as functional inactivation of the CeA (Wilensky et al., 2006) or the lateral subdivision of the CeA (Ciocchi et al., 2010) prior to training disrupts fear conditioning.

The BLA is also involved in contextual fear conditioning, as lesions of the BLA (Goosens and Maren, 2001), inactivation of the BLA (Helmstetter and Bellgowan, 1994), and intra-amygdala infusion of an excitatory amino acid receptor inhibitor (Fanselow and Kim, 1994) all impair this type of conditioning. It is assumed that, like with cued fear conditioning, the representations of the context and the US converge within the BLA. However, it has also been hypothesized that the contextual representation becomes configured in a different neural structure prior to being projected to the amygdala. Several studies have implicated the hippocampus as the structure in which the various elements of the context become combined. For example, using a within-subjects design, Anagnostaras et al. (1999) demonstrated that rats given dorsal hippocampal lesions following fear conditioning exhibited robust fear towards a discrete cue (i.e., a tone) that had been paired with shock, but exhibited impaired memory (i.e., reduced fear) for the context in which tone conditioning took place. This suggests that the hippocampus regulates conditioning about diffuse, but not discrete, cues. Developmental studies have also contributed to the view that the hippocampus is important in contextual fear conditioning. In the rat, the hippocampus undergoes a protracted period of development in comparison to other brain structures, with substantial development occurring between the ages of postnatal day (PND) 16 and PND 23, by which time the hippocampus is generally considered to be functional, at least for contextual fear conditioning (Dumas and Rudy, 2010). Therefore, if the hippocampus is important for contextual fear conditioning, then rats younger than PND 23 should be impaired in contextual learning. Several studies by Rudy (Pugh and Rudy, 1996; Rudy, 1993; Rudy and Morledge, 1994) have confirmed that contextual fear conditioning is impaired prior to ~PND 23. Furthermore, these studies have demonstrated that the impairment in contextual fear conditioning in rats younger than PND 23 is not due to impairment in long-term memory formation per se, as young (e.g., PND 18) rats exhibit long-term memory for discrete cued fear conditioning.

The amygdala and hippocampus, along with the infralimbic region of the prefrontal cortex (IL), have also been implicated in extinction of conditioned fear. Specifically, it is thought that when an extinguished cue is presented in the extinction context the hippocampus activates the IL, which then activates inhibitory interneurons in the BLA which inhibit the output neurons in the CeA, thus preventing conditioned responding. When the CS is presented outside of the extinction context, the hippocampus does not activate the IL and so conditioned responding occurs because the amygdala is not inhibited (see Quirk and Mueller, 2008, for an excellent review of the neural circuitry mediating extinction).

FGF2 and the neural circuitry of fear acquisition and extinction

There is evidence that modulation of FGF2 leads to morphological and functional changes in the neural circuitry underlying the acquisition and extinction of conditioned fear, in particular, the hippocampus. For example, in addition to promoting the proliferation and survival of cultured fetal and postnatal hippocampal cells, FGF2 increases neurite elongation in cultured cells (Walicke et al., 1986). FGF2 is also required for the differentiation of hippocampal stem cells, as it has been demonstrated that application of FGF2 to stem cells dissociated from embryonic rats facilitated the development of these cells into neurons (Vicario-Abejon et al., 1995). Furthermore, application of FGF2 to dissociated rat embryonic hippocampal cell cultures was shown to promote the bifurcation and growth of axonal branches (Aoyagi et al., 1994). Finally, chronic application of FGF2 has been shown to increase the expression of L-type voltage gated Ca^{2+} channels (LVGCCs) in rat embryonic hippocampal cultures, leading to larger Ca^{2+} increases in response to potassium-induced depolarization (Shitaka et al., 1996). An increasing body of research has demonstrated that LVGCCs may be important to memory (e.g., Bauer et al., 2002; Weisskpof et al., 1999). Together, these studies indicate that FGF2 plays a role in determining the structure of the hippocampus by regulating cell

proliferation and morphology, as well as by increasing synaptic efficiency.

Perhaps the most compelling evidence that FGF2 modulates hippocampal morphology comes from Cheng et al. (2002), who examined the long-term neural effects of early-life exposure to FGF2 *in vivo*. They demonstrated that rats receiving a single s.c. injection of FGF2 on PND 1 exhibited increased cell proliferation in the hippocampus, resulting in a larger hippocampal volume from PND 4 throughout adulthood (PND 180 was the oldest age tested). Conversely, transgenic mice that lack *Fgfr1* (the primary receptor for FGF2) show decreased cell proliferation in the hippocampus, which results in permanent atrophy of the hippocampus. The latter finding was replicated by Ohkubo et al. (2004). Such research is noteworthy because it demonstrates that modulating early-life exposure to FGF2 has a long-lasting effect on the morphology of the hippocampus. It is unclear whether early-life FGF2 has such an effect on the morphology of neural structures other than the hippocampus, although Cheng et al. (2001) showed that a single s.c. injection of FGF2 on PND 1 facilitated neurogenesis in the cerebellum of rats. However, this effect only persisted until PND 35.

In comparison to the hippocampus, there is little research regarding the effect of FGF2 on other structures involved in fear learning (i.e., amygdala and PFC). However, recent evidence suggests that FGF2 is involved in the development of the medial prefrontal cortex (mPFC) and its projections to the limbic system. Stevens et al. (2010) demonstrated that embryonic inactivation of *Fgfr2*, or combined inactivation of *Fgfr1* and *Fgfr2*, led to a reduction in excitatory cortical neurons in the mPFC and reduced mPFC volume in mice during adulthood. *Fgfr2* transgenic mice also had fewer glutamate synaptic terminals and decreased GABAergic neurons in the bed nuclei of the stria terminalis (BNST). The BNST is part of the limbic system (often considered part of the wider amygdaloid complex) that receives projections from the mPFC and has been implicated in the anxiety response (Walker et al., 2003). The findings reported by Stevens et al. (2010) suggest that FGF2 (along with other FGF ligands that bind to *Fgfr1* and *Fgfr2*) is critically involved in the development of the mPFC, and that this has implications for the development of the BNST. We are unaware of any studies that have directly examined the consequences of FGF2 activity on amygdala morphology and/or function so this would be a promising avenue for future research.

Finally, there is some evidence that FGF2 receptors are present in high concentrations in the neural circuitry mediating fear acquisition and extinction. Gonzalez et al. (1995) conducted a comprehensive analysis of FGF2 and *Fgfr1* protein and mRNA expression, and reported that there is a widespread distribution of both throughout the adult rat brain. They noted that selected populations of neurons, including those in the CA2 field of the hippocampus and in the amygdala (and particularly the CeA), have very concentrated expression of FGF2 and *Fgfr1* protein and mRNA. FGF2 and *Fgfr1* protein and mRNA are also found in abundance in neurons and astrocytes in the human hippocampus (Ferrer and Marti, 1998; Weickert et al., 2005), and FGF2 immunopositive neurons have been found in the human frontal cortex (Cordon-Cardo et al., 1990), and more specifically, in the dorsolateral prefrontal and anterior cingulate regions (Evans et al., 2004). This suggests that FGF2 may modulate the functioning of neural structures that underlie fear acquisition and extinction; however, it should be noted that as *Fgfr1* serves as the receptor for other ligands of FGF, it is also possible that ligands other than FGF2 modulate activity in these regions.

Molecular evidence of a role for FGF2 in fear memories

It is widely accepted that the formation of long-term memory for associative learning in the mammalian brain requires long-term potentiation (LTP), an activity-dependent increase in synaptic plasticity (Bliss and Lømo, 1973). LTP, and the formation of long-term memories, requires the activation of a complex molecular signaling process. Briefly, the excitatory amino acid glutamate binds to N-methyl-D-aspartate (NMDA) receptors, which, following sufficient depolarization, open to allow calcium (Ca^{2+}) influx into the cell. The Ca^{2+} influx, together with diaglycerol, leads to activation of calcium/phospholid-dependent protein kinase (PKC) which serves to phosphorylate NMDA receptors, thereby maintaining a steady influx of Ca^{2+} to the cell. The opening of the NMDA receptor channels and the resultant influx of Ca^{2+} also activate the adenylyl cyclase pathway, which involves the activation of cyclic adenosine monophosphate (cAMP) and cyclic adenosine monophosphate-dependent protein kinase (PKA). This causes mitogen-activated protein kinase (MAPK) to phosphorylate and translocate to the cell nucleus, and the phosphorylation of MAPK, together with activation of the adenylyl cyclase pathway, cause cAMP response element binding protein (CREB) to become phosphorylated. CREB then activates downstream targets which purportedly lead to gene transcription, as well as functional and morphological changes (reviewed in Kandel, 2001). It should be noted that there are other, non-NMDA-dependent routes to LTP; however, many of the molecules involved in these other routes overlap with those described above. If FGF2 is involved in long-term memory, then it must interact with the molecules involved in this process. Indeed, several lines of evidence demonstrate that FGF2 modulates and/or is modulated by several of the molecules known to be involved in LTP and memory.

FGF2 and glutamate

The first demonstration that FGF2 may be involved in glutamate-mediated synaptic plasticity came from demonstrations that FGF2 changes glutamate receptor protein levels. Specifically, chronic application of FGF2 (over 18–48 h) to cultured embryonic hippocampal rat neurons increased AMPA receptor subunit GluR1 protein. FGF2-treated cells also exhibited greater increases in Ca^{2+} levels following AMPA receptor activation (Cheng et al., 1995). AMPA receptors are glutamate receptors that have been shown to be involved in the induction and mainte-

nance of LTP (Collingridge et al., 1983; Davies et al., 1989). It was later demonstrated that chronic FGF2 application (over 24–48 h) to cultured embryonic hippocampal rat neurons led to greater inactivation of NMDA receptors following excessive Ca^{2+} release caused by administration of an NMDA agonist (Boxer et al., 1999). This suggests that one mechanism by which FGF2 might regulate cell survival is by exerting inhibitory control over the NMDA receptor to prevent excitotoxicity.

The first demonstration that acute FGF2 could increase glutamate release came from Numakawa et al. (2002), who found that FGF2 application to cultured rat neonatal cerebral cortex neurons led to increases in glutamate release that were maintained for one-two min. This study also demonstrated that FGF2 influenced glutamate release, and subsequent synaptic plasticity, via well-known mechanisms involved in LTP, specifically, Ca^{2+} influx and MAPK phosphorylation.

FGF2 and Ca^{2+} influx

Early reports that FGF2 may be involved in Ca^{2+}-mediated synaptic transmission demonstrated that FGF2 increases Ca^{2+} mobilization *in vitro* in Swiss 3T3 cells (Kaibuchi et al., 1986; Tsuda et al., 1985). Later studies also implicated FGF2 in the transmission of Ca^{2+} via LVGCCs. For example, in addition to demonstrating that FGF2 increases glutamate release, Numakawa et al. (2002) demonstrated that this effect was blocked by nifedipine, an LVGCC blocker. This suggests that FGF2-induced increases in glutamate release are dependent, at least in part, on Ca^{2+} influx through LVGCCs. However, not all of the effects of FGF2 on glutamate are mediated through LVGCCs, as Boxer et al. (1999) found that nifedipine had no effect on the potentiation of NMDA receptor inactivation by FGF2 (see above).

FGF2 and PKC

Several studies have demonstrated that application of FGF2 to cultured embryonic Swiss 3T3 cells activates PKC (Kaibuchi et al., 1986; Nanberg et al., 1990; Tsuda et al., 1985). Additionally, activation of the PKC pathway in adult human astrocytes produced increases in FGF2 mRNA and protein levels (Moffett et al., 1998).

FGF2 and cAMP

Activation of the cAMP pathway by administration of forskolin in adult human astrocytes produced increases in FGF2 mRNA and protein levels (Moffett et al., 1998). Furthermore, FGF2 has been shown to augment increases in cAMP accumulation induced by administration of forskolin (which increases PKC) in Swiss 3T3 cells (Nanberg et al., 1990), an effect that was attenuated via down-regulation of PKC. This suggests that FGF2 might activate cAMP via recruiting PKC.

FGF2 and MAPK

Numakawa et al. (2002) demonstrated that the FGF2-induced increase in glutamate was not only dependent on Ca^{2+} influx, but also on MAPK phosphorylation. They first demonstrated that FGF2 application to cerebral cortex neuron cultures resulted in increased phosphorylation of MAPK, and then demonstrated that inhibition of MAPK via MAPK kinase (MEK) inhibitors blocked the FGF2-induced increase in glutamate release. This suggests that FGF2 recruits MAPK to enhance synaptic transmission.

The neurotrophic effects of FGF2 also appear to be mediated through increases in MAPK. Abe and Saito (2000) and Abe et al. (2001) demonstrated that *in vitro* application of FGF2 to 18-day-old rat embryonic hippocampal and cerebral cortex neurons induced phosphorylation of extracellular signal-regulated kinase (ERK) 1 and 2. The FGF2-induced phosphorylation of ERK1/2 was blocked by MEK inhibitors U0126 and PD98059, suggesting that the increase in phosphorylated ERK1/2 was due to FGF2 increasing levels of phosphorylated MEK. Abe and Saito (2000) also demonstrated that FGF2-induced prolonged survival of these cell cultures was blocked by the MEK inhibitors U0126 and PD98059, both of which had no effect on cell survival when administered without FGF2. Abe et al. (2001) then demonstrated that FGF2-induced increases in neuritic complexity (indexed by increased axonal branching) was prevented by MEK inhibitors U0126 and PD98059, both of which had no effect on neurite morphology when administered without FGF2. Furthermore, when U0126 was added 24 h after FGF2 was applied to the cultures, phosphorylated ERK 1/2 decreased to basal levels and the promotion of axonal branching terminated. Together, these studies suggest that FGF2 recruits sustained MEK signaling to regulate cell survival and neurite morphology.

FGF2 and CREB

One of the first demonstrations that FGF2 activates CREB came from Sung et al. (2001), who demonstrated that FGF2 increases phosphorylation of CREB and CRE-mediated gene transcription to regulate neuronal differentiation and outgrowth in hippocampal cell cultures. They further demonstrated that FGF2-induced neuronal outgrowth was blocked in cells that contained a dominant negative CREB construct (effectively blocking CREB activation). Intriguingly, their findings suggested that FGF2 does not phosphorylate CREB using pathways known to be involved in CREB activation, as pre-treatment with inhibitors of MAPK, PKA, and PKC did not prevent FGF2-induced CREB phosphorylation. This suggests that FGF2 activates CREB through its own distinct pathway, at least in the hippocampus.

This study was later followed by a demonstration that FGF2 regulates cell proliferation in the adult hippocampus in rats via phosphorylation of CREB (Peltier et al., 2007). Similar to Sung et al. (2001), Peltier et al. (2007) showed that FGF2-induced cell proliferation *in vitro* was completely blocked by a CREB inhibitor. They also demonstrated that cell proliferation was markedly increased in cell cultures that over-expressed CREB, but only if FGF2 was applied to these cultures. CREB over-expression did not increase cell proliferation by itself, suggesting that FGF2 recruits

CREB to increase cell proliferation in the adult nervous system. Finally, Peltier et al. (2007) also demonstrated that FGF2-induced cell proliferation was not blocked by inhibitors of MAPK. Therefore, consistent with Sung et al. (2001), these results suggest that FGF2 recruits CREB via pathways distinct from those involving MAPK. However, more recent work has demonstrated that the findings that FGF2 can phosphorylate CREB independent of MAPK activity may be region specific, as FGF2-induced phosphorylation of CREB was blocked when MAPK inhibitors were applied to cerebellar granule neurons (Ditlevsen et al., 2008).

FGF2 and long-term-potentiation

Perhaps the most compelling evidence that FGF2 is involved in regulating synaptic plasticity comes from several studies showing that FGF2 augments LTP. This has been demonstrated *in vitro* in rat hippocampal slices perfused with FGF2 (Terlau and Seifert, 1990). FGF2 produced no change in evoked responses if single pulse or paired pulse stimulation was applied to the slice, and did not change the resting membrane potential. However, it did increase the magnitude of potentiation following tetanic stimulation of the slice. Additionally, FGF2 has been shown to enhance LTP *in vivo*. Specifically, i.c.v. injections of FGF2 in anaesthetized rats led to augmented LTP when sub-threshold tetanic stimulation was applied to the dentate gyrus; however, it did not facilitate LTP when sufficient tetanic stimulation was administered to produce complete LTP (i.e., it could not further augment LTP when the synapses were fully strengthened by the above-threshold tetanic stimulation, Ishiyama et al., 1991). Finally, i.c.v. injections of FGF2 have been shown to rescue the deficits in LTP seen in fimbria-fornix-lesioned rats, animals that require greater levels of tetanic stimulation in order to induce LTP (Abe et al., 1992). Together, these studies indicate that FGF2 may not only regulate induction and maintenance of synaptic plasticity, but also that exogenous FGF2 may enhance LTP under conditions in which normal LTP is impaired.

As already noted, LTP is widely accepted to be the mechanism by which long-term memory is acquired, and therefore the finding that LTP is facilitated by FGF2 provides compelling evidence that FGF2 may be critically involved in long-term memory. Given the research outlined above demonstrating that FGF2 appears to modulate glutamate release, Ca^{2+} influx, PKC, MAPK, cAMP, and CREB, it is likely that FGF2 facilitates LTP via these same signals.

FGF2 and neurogenesis

As already mentioned, one of the well-known functions of FGF2 is to regulate adult neurogenesis. There is mixed evidence for the role of neurogenesis in learning and memory. For example, increasing neurogenesis in the dentate gyrus via exercise led to a facilitation of LTP and spatial learning in mice (Van praag et al., 1999). Furthermore, inhibiting neurogenesis impaired trace eyeblink conditioning (a hippocampus-dependent learning task in which the animal must learn to associate two stimuli that are presented separately in time), while sparing delayed eyeblink conditioning (not mediated by the hippocampus, in which an animal associates two stimuli presented overlapping in time) in rats (Shors et al., 2001). From this work, it appeared that neurogenesis was necessary for hippocampus-mediated learning and memory. However, Shors et al. (2002) later demonstrated that inhibiting neurogenesis did not always impair other types of hippocampal memory, including (in contrast to Van praag et al., 1999) spatial learning and contextual fear conditioning. Shors et al. (2002) maintained that hippocampal neurogenesis is important for some, but not all, hippocampus-mediated tasks, but others (Kempermann et al., 2004) have pointed out that the time scale of all of these studies is problematic, because the time it takes for new neurons to mature and integrate into the neuronal network makes it more plausible that the effects of disrupted neurogenesis would appear weeks after the disruption, rather than immediately after neurogenesis was inhibited. In any case, while adulthood neurogenesis appears to be involved in at least some types of hippocampus-mediated learning, the nature of its role is poorly understood at this stage.

FGF2 and the molecular biology of fear extinction

There is considerable evidence that many of the same molecules involved in fear acquisition are also involved in fear extinction. For example, NMDA receptor antagonists impair extinction (Falls et al., 1992; Lin et al., 2003), while D-cycloserine (DCS), a partial agonist of the NMDA receptor, enhances extinction (Ledgerwood et al., 2003; Walker et al., 2002). Furthermore, inhibitors of MAPK infused into the BLA block extinction (Lin et al., 2003), and other studies have shown that phosphorylated MAPK increases in the BLA following extinction (Cannich et al., 2004; Yang and Lu, 2005). Finally, Cain et al. (2002) demonstrated that systemic administration of the LVGCC blockers nifedipine and nimodipine prior to extinction training impaired within-session and long-term extinction of fear for an auditory CS and context in mice. As mentioned previously, FGF2 regulates glutamate-mediated synaptic plasticity (Numakawa et al., 2002), MAPK phosphorylation (Abe and Saito, 2000), and LVGCC expression and activation (Numakawa et al., 2002; Shitaka et al., 1996). As such, it is possible that FGF2 may also be involved in the formation and/or consolidation of extinction memories.

There is evidence that glucocorticoids are important in the formation of extinction memories. The first demonstration of this came from Barrett and Gonzalez-Lima (2004) who reported that systemic metyrapone (a corticosterone inhibitor) impaired long-term extinction of an auditory CS in mice when administered 90 min prior to extinction training, while having no effect on within-session extinction. These results have since been replicated using intra-amygdala infusions of the glucocorticoid antagonist mifepristone (Yang et al., 2006), suggesting that glucocorticoid activity in the amygdala is necessary for long-term extinction. Adrenal hormones (such as corticosterone) exert control over FGF2 activity (Molteni et al., 2001), and therefore it is possible that glucocorticoids increase FGF2 activity in or-

der to modulate extinction. Other research has demonstrated that adrenal hormones regulate FGF2 expression in the neural circuitry underlying extinction. For example, adrenalectomy in adult rats reduced FGF2 expression in the hippocampus and PFC (Riva et al., 1995a), and systemic administration of dexamethasone (a synthetic glucocorticoid) increased FGF2 mRNA expression in the hippocampus and cerebral cortex in adult rats (Riva et al., 1995b). These studies did not examine the behavioral consequences of altering FGF2 expression; however, it is possible that such manipulations of FGF2 in the neural circuitry of extinction would modulate the formation of extinction memories.

The research described above provides just four examples of molecular signals that have been implicated in extinction that have also been shown to interact with FGF2. It should be noted that there are many other molecular signals that are thought to be involved in fear extinction (for an excellent review see Myers and Davis, 2007), and several of these have been shown to be regulated by FGF2, including GABA (Antonopoloulos et al., 1997), dopamine (Fumagalli et al., 2003), acetylcholine (Belluardo et al., 1999), opioids (Ward et al., 2007), and cannabinoids (Williams et al., 2003). Hence, there is considerable evidence that FGF2 interacts with the molecular signals that underlie extinction learning.

Interim summary

The evidence reviewed above identifies two possible roles for FGF2 in memory formation. Firstly, alteration of FGF2 signaling/expression can have long-lasting "constitutive" effects which may result in a "permissive" change in memory formation. For example, an increase in FGF2 signaling has been shown to increase cell proliferation, promote dendrite formation, and enhance synaptic efficiency, all of which produce conditions that are optimal for memory formation. In this case, an enhancement in memory formation may be seen some time after the modulation of FGF2 signalling occurs. Secondly, alteration of FGF2 signaling also has acute, phasic effects, including enhancing the molecular steps in the cascade of memory (e.g., glutamate, Ca^{2+} influx, PKC, cAMP, MAPK, CREB). In this case, the alteration in FGF2 signaling must occur at the time of learning in order to detect an enhancement in memory. See Fig. 1 for a summary of FGF2's effects on neuronal functioning. Below, we review behavioral evidence that exogenous FGF2 can modulate fear memories. Where possible, we indicate whether the effects of exogenous FGF2 are likely due to permissive or phasic effects.

BEHAVIORAL EVIDENCE FOR A ROLE FOR FGF2 IN FEAR MEMORIES

FGF2 and anxiety

Despite the breadth of research examining the *physiological* consequences of endogenous and exogenous FGF2, few studies have examined the *behavioral* consequences of FGF2. However, one study has implicated FGF2 in the modulation of anxious behavior. Perez et al. (2009) bred

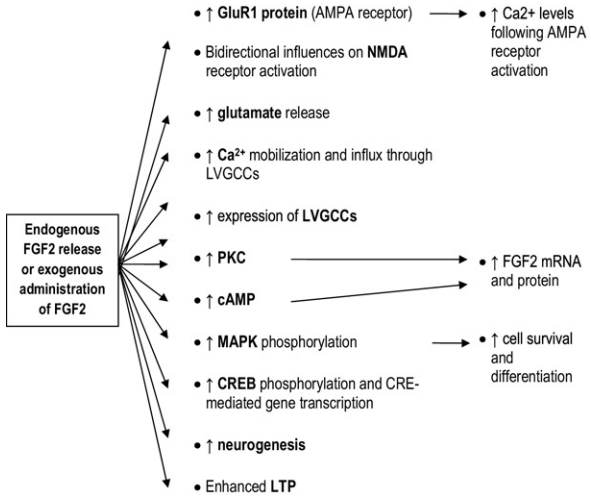

Fig. 1. Summary of the known effects of FGF2 on neural functioning.

rats that exhibited either high or low trait levels of anxiety (as measured by responses to novelty and anxiety-provoking situations), and found that highly anxious rats had lower levels of hippocampal FGF2 mRNA expression compared to low-anxious rats. They also demonstrated that placement in an enriched environment reduced anxious behavior in highly anxious rats and led to an increase of FGF2 expression in the hippocampus. Finally, they demonstrated that three weeks of treatment with exogenous FGF2 increased neurogenesis and reduced anxious behavior, an effect that was particularly pronounced in the highly anxious rats. Perez et al.'s (2009) study is noteworthy because not only does it implicate FGF2 as a modulator of state anxiety, but it also suggests that FGF2 may be a novel anxiolytic agent.

FGF2 and fear acquisition

More recent work has suggested that in addition to modulating state anxiety, systemic FGF2 can modulate the formation of fear memories. In one study, Graham and Richardson (2010a) investigated the functional implications of Cheng et al.'s (2002) finding that early-life administration of FGF2 leads to permanent structural changes in the hippocampus. They demonstrated that chronic systemic injections of FGF2 on postnatal day (PND) 1–5 led to an early emergence of contextual fear conditioning in PND 16 rats (animals this age normally show poor retention of this type of learning), and further, enhanced contextual fear conditioning in rats at an age at which they normally exhibit some learning of this type (i.e., PND 23). Graham and Richardson's (2010a) study provides support for the hypothesis that FGF2 is involved in, and can modulate, the development of the memory system. On the basis of a large body of work examining the physiological consequences of early-life FGF2, they proposed that the behavioral and cognitive consequences of early-life FGF2 were due in part to FGF2 causing long-lasting changes in the morphology and function of the hippocampus. Thus, early-

life FGF2 most likely had a permissive effect on memory formation later in life.

It was outlined previously that, in addition to causing long-term morphological neural changes, acute FGF2 also causes transient increases in many of the molecular signals known to be involved in learning and memory. As such, Graham and Richardson (2009a) investigated whether acute FGF2, administered at the time of learning, would enhance the acquisition of fear memories. They found that when FGF2 was systemically administered 15 min prior to training, it enhanced long-term memory for contextual fear conditioning in PND 16, 19, and 22 rats. This study provided support for the hypothesis that FGF2 is involved in the regulation of long-term memory formation, as it demonstrated that an acute increase in the levels of FGF2 facilitates contextual fear conditioning memory in rats across a variety of ages. Furthermore, these results were most likely due to a phasic effect on memory as acute FGF2 transiently increases the molecular cascade of memory, and any potential effects of acute FGF2 on cell proliferation and brain morphology would take time to develop. As FGF2 was administered prior to training, it is unclear whether FGF2 was modulating the acquisition or consolidation of fear conditioning. However, in both studies it was found that FGF2 (whether administered early in life or acutely at the time of learning) only facilitated contextual fear conditioning in PND 16 rats under conditions where they exhibited some basal level of long-term memory (i.e., in PND 16 rats that received more than one training trial during conditioning). FGF2 did not facilitate contextual conditioning in PND 16 rats that only received one training trial and subsequently exhibited negligible long-term memory. This latter finding suggests that FGF2 may be more involved in memory consolidation than acquisition.

FGF2 and fear extinction

The effect of systemically administered FGF2 on extinction of conditioned fear has also been examined. While chronic systemic early-life FGF2 had no effect on cued fear conditioning or extinction later in life, it did lead to precocious emergence of "adult-like" extinction in PND 16 rats (Graham and Richardson, 2010a). After ~PND 23, extinction is context-dependent in that an animal will only express low levels of fear if the extinguished CS is presented in the same context in which extinction training occurred. If the extinguished CS is presented in the original fear conditioning context, or in a completely novel context, the animal will express recovered levels of fear, a phenomenon termed "renewal" (Bouton and Bolles, 1979a). Recent research has shown that extinction is fundamentally different in rodents younger than PND 23 (Gogolla et al., 2009; Kim and Richardson, 2010). As just one example, PND 16 rats do not show renewal- they express low levels of CS-elicited fear regardless of the context in which they are tested (Kim and Richardson, 2007; Yap and Richardson, 2007). One explanation for this finding is that the hippocampus has not fully developed by PND 16 and cannot support the formation of a representation of the extinction context, and so for PND 16 rats extinction is context-independent. However, Graham and Richardson (2010a) demonstrated that PND 16 rats exhibited a precocious emergence of context-dependent extinction if they had been systemically administered FGF2 early in life. This finding is most likely due to the permissive effects of early-life FGF2 on the development of the neural circuitry mediating both fear acquisition and fear extinction.

Several recent studies have also examined the effect of acute FGF2 (i.e., its phasic effects) when it is administered at the time of extinction training in PND 23 rats (that exhibit adult-like extinction). It was found that systemic administration of acute FGF2 8 min prior to extinction training enhanced retention; that is, these rats exhibited stronger extinction memories (less fear) at test the following day (Graham and Richardson, 2009b). Interestingly, when FGF2 was administered prior to extinction training it also significantly suppressed freezing during extinction training, from the first trial of extinction. This is consistent with the finding that FGF2 may be a novel anxiolytic (Perez et al., 2009). However, FGF2 does not need to be present during extinction training to enhance extinction retention, and its effects on extinction are not due to nonspecific effects on state levels of anxiety or freezing behavior, because FGF2 enhanced extinction retention when administered immediately after, but not 4 h after, extinction training. Furthermore, when rats were given insufficient training to induce short-term extinction, FGF2 was ineffective in enhancing extinction, suggesting that FGF2 enhances the processes underlying extinction (i.e., perhaps by enhancing extinction consolidation), rather than initiating them.

The mechanisms by which FGF2 enhances extinction have also been examined. It is widely accepted that extinction, at least in rats older than PND 23, involves the formation of a context-dependent new memory that competes with the original (still intact) fear memory. This is because fear often returns after extinction when the CS is presented in a different context to that of extinction (renewal, as discussed earlier; Bouton and Bolles, 1979a), when the animal is exposed to a mild, unsignalled stressor (reinstatement, or stress-precipitated relapse; Bouton and Bolles, 1979b), or with the mere passage of time (spontaneous recovery; Quirk, 2002). If FGF2 is enhancing the formation of a context-dependent extinction memory then FGF2-treated rats should show recovery of fear under the conditions noted above. However, Graham and Richardson (2009b, 2010b) found that systemic administration of FGF2 led to significantly attenuated reinstatement and renewal, even when vehicle-treated rats were given double the amount of extinction training to equate the strength of extinction between FGF2-treated and control rats. This suggests that FGF2 may change the quality of extinction, possibly by weakening the original fear memory. Graham and Richardson (2011) examined this possibility behaviorally by exploiting recent findings regarding the molecular substrates underlying re-extinction, which refers to the process of relearning extinction following reacquisition of fear to an extinguished cue. Converging evidence strongly suggests that initial extinction and re-extinction do not depend on the same neural or molecular mechanisms. For

example, unlike initial extinction, re-extinction is not impaired by NMDA receptor (NMDAr) antagonists MK801 or DL-APV (Chan and McNally, 2009; Langton and Richardson, 2009, 2010; Laurent et al., 2008). These experiments suggest that relearning to inhibit fear does not depend on NMDAr activity. However, Graham and Richardson (2011) found that when rats were systemically injected with FGF2 immediately after extinction training, then re-trained to fear the extinguished CS, and then re-extinguished following injection with MK801, FGF2-treated rats exhibited impaired re-extinction retention. In contrast, rats that were extinguished with vehicle and then re-extinguished with MK801 did not exhibit any impairment in re-extinction retention. In other words, during re-extinction FGF2-treated rats "behaved" as if the CS was being extinguished for the first time. This effect of FGF2 was not observed when it was injected 4 h after extinction training, suggesting that the results obtained in the first experiment were due to the effects of FGF2 on extinction, rather than some nonspecific effect of prior exposure to both FGF2 and MK801. Finally, when MK801 was administered prior to reacquisition of a CS that had been extinguished followed by FGF2 or vehicle administration, reacquisition was impaired in FGF2-treated but not vehicle-treated rats. That is, FGF2-treated rats "behaved" as if they were learning the association between the CS and US for the first time. Together, these experiments suggest that FGF2 may fundamentally alter the quality of extinction, possibly by partially erasing the original fear memory. See Table 1 for a summary of FGF2's effects on fear acquisition and fear extinction.

Evidence that endogenous FGF2 regulates memory formation

The studies described above show that exogenous FGF2 is capable of modulating fear acquisition and fear extinction, suggesting that endogenous FGF2 may normally play a role in regulating fear memories. This regulatory role could be a consequence of either tonic secretion of endogenous FGF2 leading to conditions that are optimal for memory formation (e.g., increased neurogenesis, etc.; a permissive effect) or phasic secretions of FGF2 at the time of a learning experience affecting the molecular cascade underlying memory. However, it is also possible that the enhanced memory following FGF2 administration was simply a by-product of the physiological effects of FGF2. That is, it is known that in order to regulate other, non-memory related functions in the central nervous system (i.e., protection from stress, regeneration following injury), FGF2 recruits the same molecular cascades that are involved in memory. It is possible that the observed enhancement in memory is due to the effect of exogenous FGF2 on these signals, but that endogenous FGF2 is not normally involved in the regulation of memory per se. If it is the case that FGF2 activation is not necessary for long-term memory, then preventing FGF2 activation should not impair memory formation. Unfortunately, as a specific antagonist of FGF2 does not currently exist, this possibility cannot be tested. Another difficulty is that most of the data on the physiological effects of FGF2 comes from *in vitro* research, and few studies have examined the effects of FGF2 *in vivo* in behaving rats. As such, it is not clear whether FGF2 activity increases in response to learning, which would provide more compelling evidence for a potential role of endogenous FGF2 in memory.

In light of these limitations, it is reasonable to ask if there is any evidence that endogenous FGF2 is necessary for fear learning and memory. It was noted earlier that endogenous FGF2 activity and mRNA increase following stressful events (Bland et al., 2006, 2007). Furthermore, exogenous FGF2 has been shown to decrease anxious behavior (Perez et al., 2009), suggesting that one role of endogenous FGF2 may be to regulate state anxiety. Therefore it is likely that FGF2 becomes activated during fear learning (via synthesis of adrenal hormones), and may regulate the storage of such experiences via its effects on subsequent intracellular signaling cascades. The fact that FGF2 becomes activated in response to stress provides a possible mechanism by which endogenous FGF2 may modulate fear memories.

Further suggestive evidence that endogenous FGF2 may be necessary for memory is that other manipulations that improve memory also increase endogenous FGF2, suggesting that increases in FGF2 activity may be the underlying mechanism of action for memory enhancement. For example, it has been demonstrated that physical exercise enhances spatial memory (Fordyce and Farrar, 1991; Van praag et al., 1999) and memory for one-trial passive avoidance (Samorajski et al., 1985) in rats of a

Table 1. Summary of FGF2 effects on fear conditioning and extinction

Mode/timing of administration	Age trained	Effect on memory
Systemic injections from PND 1–5	PND 16 or PND 23	Enhanced memory for contextual conditioning in both ages
Systemic injections from PND 1–5	PND 16	No effect on cued conditioning recall; extinction acquisition; extinction recall; caused early emergence of renewal
Acute systemic injection 15 min prior to contextual conditioning	PND 16, 19, or 22	Enhanced memory for contextual conditioning in all ages
Acute systemic injection 8 min prior to extinction training	PND 23	Suppressed freezing throughout extinction acquisition; enhanced extinction recall
Acute systemic injection immediately after extinction training	PND 23	Enhanced extinction recall; reduced reinstatement; reduced renewal; prevented the switch from NMDA-dependent to NMDA-independent reacquisition of conditioned fear and re-extinction of fear
Acute systemic injection 4 h after extinction training	PND 23	No effect on extinction recall; did not prevent the switch from NMDA-dependent to NMDA-independent re-extinction of fear

variety of ages (including adult and aged). It is also widely recognized that increased physical activity is associated with better cognitive performance in aged humans (reviewed in Churchill et al., 2002). It is known that exercise leads to many neural changes that are regulated by FGF2, including increased neurogenesis and astrocyte reactivity, suggesting that exercise may increase endogenous FGF2. This idea is supported by Gomez-Pinilla et al. (1997), who demonstrated that voluntary wheel running in adult rats increased FGF2 mRNA in the hippocampus by the fourth night of running, suggesting that the beneficial effects of exercise on memory may be mediated partly by increases in FGF2 activity.

Another manipulation that has been shown to improve memory is administration of antidepressant medication. For example, there is evidence that some types of antidepressants (e.g., imipramine) improve memory in depressed and healthy humans as measured by standardized tests of memory (reviewed in Thompson, 1991). Additionally, it has been shown that chronic administration of the antidepressant tianeptine ameliorates spatial memory impairments in rats exposed to repeated restraint stress (Luine et al., 1994). It has been reported that chronic stress leads to dysregulation in FGF2 activity (Bland et al., 2006, 2007) which may have mediated the memory impairments reported by Luine et al. (1994). Furthermore, chronic antidepressant treatment up-regulates FGF2 activity (Mallei et al., 2002), suggesting that antidepressants may improve memory in humans and rodents by increasing endogenous FGF2. Together, research investigating the effect of exercise and antidepressants on memory and FGF2 activity provides independent evidence that endogenous FGF2 may be involved in the modulation of memory. Importantly, as exercise and antidepressants have been shown to enhance fearful memories as well as non-fear related memories (e.g., spatial learning), this suggests that endogenous FGF2 may be important in the regulation of memory in general, rather than just fear memories. However, it should be stressed that the above data are correlative, and that in order to definitively claim that FGF2 is necessary for memory it must be demonstrated that memory is impaired when FGF2 activity is blocked (see Future directions).

PRACTICAL APPLICATIONS

The research reviewed above on the role of FGF2 in memory is theoretically interesting because it provides insight into the signals that regulate the development of the adult-like memory system, as well as the signals that might regulate the persistence of long-term memories. However, an understanding of the mechanisms by which FGF2 modulates memory also has practical implications. With an aging population, the incidence of neurodegenerative disorders like dementia is increasing, and such disorders are associated with gross memory impairments. Aging is also associated with a decrease in neurotrophic factors (Rai et al., 2007), and so it is possible that a decline in FGF2 (among other neurotrophic factors) mediates the cognitive impairments seen in aging. If this is the case, then early-life exposure to FGF2 might reduce age-related declines in neurotrophic factors, and in turn, preserve memory ability. Furthermore, acute administration of FGF2 later in life may attenuate memory impairments associated with normal aging or disease processes.

In addition to possible treatments for memory impairments, research into the pharmacological enhancement of memory has also aided treatments for affective disorders, including anxiety. Therefore it is of particular clinical interest that FGF2 enhances extinction, the laboratory analogue of exposure therapy for anxiety disorders in humans, and further, reduces susceptibility to relapse. This suggests that FGF2 may be a novel pharmacological enhancer of exposure therapy in humans. While FGF2 is able to be administered to humans (it has previously been trialed in humans as a potential inducer of angiogenesis; Laham et al., 2000; Lazarous et al., 2000; Lederman et al., 2002), it has yet to be tested as a potential adjunct to exposure therapy for anxiety disorders. The studies reviewed above highlight at least two potential benefits to using FGF2 in this way. Firstly, Graham and Richardson (2009b, 2010b) demonstrated that extinction combined with FGF2 is as effective as double the amount of extinction without FGF2. As exposure therapy is time-consuming and requires extensive commitment from the patient, the finding that FGF2 produces equivalent results in half the amount of time suggests that it may improve the efficiency of exposure therapy, which may reduce treatment drop-out rates. The second benefit relates to the findings that FGF2 reduces two common types of relapse: stress-precipitated reinstatement and renewal (Graham and Richardson, 2009b, 2010b). Stress-induced relapse is a significant problem in the treatment of anxiety disorders, and renewal is a robust effect both in the laboratory and in clinical settings (e.g., Mineka et al., 1999; Mystkowski et al., 2002). Further, in a longitudinal study of the clinical course of anxiety disorders over 12 years, it was demonstrated that anxiety disorders have a largely chronic course with low recovery rates and high relapse rates (Bruce et al., 2005). This illustrates the need to target relapse rates in treatment, and thus the findings that FGF2 renders rats less susceptible to relapse may be of potential future benefit.

SUMMARY AND FUTURE DIRECTIONS

It has been 25 years since FGF2 was first identified as a neurotrophic factor (Morrison et al., 1986; Walicke et al., 1986). In that time an overwhelming amount of research has helped to define the physiological role of FGF2 in the functioning of the nervous system from conception through to adulthood. It is now known that FGF2 is critically involved in determining the structure of the brain during development, and further, that FGF2 regulates the neuronal response to injury and stress during adulthood. Despite this, very little is known about FGF2's role in behavior, and in particular, cognition and memory. However, while research examining the behavioral consequences of FGF2 is still in its infancy, results thus far suggest a promising role

for FGF2 in the regulation of fearful memories. Furthermore, the behavioral studies described are consistent with the vast body of physiological research that has demonstrated that endogenous and exogenous FGF2 modulates the molecular cascade of long-term memory.

Clearly, more research is required to investigate the nature and extent of FGF2's role in learning and memory. For example, preclinical investigations of the effect of FGF2 on fear conditioning and extinction have only been conducted in developing rats (up to PND 23). It is possible that the reported effects of FGF2 on fear memories are dependent on testing young animals. As such, future research will need to determine whether the effects of exogenous FGF2 on learning and memory vary across the lifespan, including adult and aged animals. In addition, efforts should be made to develop ways of blocking FGF2 activity at the time of learning in order to determine whether endogenous FGF2 is necessary for memory formation. This may be achieved by developing a specific antagonist or antibody to inactivate FGF2, or using genetic knockout manipulations. Indeed, it has been demonstrated that FGFR1-knockout mice exhibit impairments in a spatial memory task, along with reductions in neurogenesis and LTP (Zhao et al., 2007). However, it is difficult to interpret findings from studies that use traditional genetic knockouts (i.e., where the mutation is present from prior to birth) due to the effects of such manipulations on neuronal development. The development of methods to activate the FGFR1 mutation selectively at the time of learning (e.g., using optogenetics) would circumvent this problem.

Another area of potential interest is to determine the relationship between FGF2 and other growth factors, such as BDNF. There appears to be great overlap in the roles of the various growth factors in the central nervous system, both physiologically and behaviorally. For example, BDNF mRNA increases in the BLA following cued fear conditioning, and furthermore, blockade of the BDNF receptor via infecting neurons in the amygdala with a lentiviral vector expressing a dominant-negative tyrosine kinase B (TrkB) receptor impaired the acquisition of cued fear conditioning, without affecting fear expression (Rattiner et al., 2004). Elevated BDNF mRNA has also been reported to occur in the BLA following extinction of conditioned fear, and disruption of BNDF signaling via a lentiviral vector expressing a dominant-negative TrKB isoform disrupted long-term retention of extinction, without impairing within-session extinction (Chhatwal et al., 2006). Very recently it has been demonstrated that intra-IL infusions of BDNF reduced expression of conditioned fear the day after infusions, and this effect occurred even in the absence of extinction training (Peters et al., 2010). While it is unclear, at this stage, how the various growth factors interact, there is evidence that FGF2 application to injured retinal ganglion cells in frogs increases BDNF and TrkB mRNA levels, an effect that was blocked by an MEK inhibitor (Soto et al., 2004). This suggests that FGF2 may increase BDNF by phosphorylating MAPK. Future investigation into the signaling relationships between the various neurotrophic factors is warranted in order to better understand their regulation of synaptic plasticity underlying long-term memory.

Finally, considerable further work is required to elucidate exactly how exogenous FGF2 enhances fear acquisition and extinction. Systemic administration of FGF2 has been shown to rapidly cross the blood brain barrier (Cuevas et al., 1996; Deguchi et al., 2000) and so it is likely that, in the behavioral experiments described previously, FGF2 enhanced fear acquisition and extinction via central effects. However, it is also possible that FGF2 enhances memory, in part, via peripheral effects (e.g., increases in blood flow). Future research that compares the effects of centrally vs. systemically administered FGF2 on fear acquisition and extinction is required to address this issue. Should similar behavioral results occur when FGF2 is centrally administered, it will need to be determined which neural structures are affected by exogenous FGF2 during fear conditioning and extinction, and what molecular and cellular changes occur as a result of combining fear acquisition or extinction with FGF2. There is a dearth of literature on the effect of FGF2 on amygdala and PFC function, especially in comparison to what is known about the effect of FGF2 on hippocampal function. However, given the behavioral evidence reviewed demonstrating that systemic FGF2 modulates amygdala- and PFC-mediated fear memories, it is highly probable that FGF2 modulates amygdala and PFC function, and future research should explicitly pursue this possibility. Such research, using tools including immunohistochemistry and electrophysiology, is required to bridge the gap between the physiological research on FGF2 and the more recent behavioral research. Further investigation into the role of neurotrophic factors in behavior and cognition that combines these levels of analysis (i.e., neural, molecular, cellular, and behavioral) will inevitably lead to new insights into the mechanisms underlying the persistence of long-term memory. Furthermore, such research may potentially lead to novel therapeutic targets in the treatment of neurodegenerative and anxiety disorders.

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