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Regulation of neuronal plasticity by the DNA repair associated Gadd45 proteins

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ABSTRACT

Neurons respond rapidly to extracellular stimuli by activating signaling pathways that modulate the function of already synthetized proteins. Alternatively, signal transduction to the cell nucleus induces *de novo* synthesis of proteins required for long-lasting adaptations. These complementary strategies are necessary for neuronal plasticity processes that underlie, among other functions, the formation of memories. Nonetheless, it is still not fully understood how the coupling between different stimuli and the activity of constitutively and/or *de novo* expressed proteins gate neuronal plasticity. Here, we discuss the molecular functions of the Growth Arrest and DNA Damage 45 (Gadd45) family of proteins in neuronal adaptation. We highlight recent findings that indicate that Gadd45 family members regulate this function through multiple cellular processes (e.g., DNA demethylation, gene expression, RNA stability, MAPK signaling). We then summarize the regulation of Gadd45 expression in neurons and put forward the hypothesis that the constitutive and neuronal activity-induced pools of Gadd45 proteins have distinct and complementary roles in modulating neuronal plasticity. Therefore, we propose that Gadd45 proteins are essential for brain function and their dysfunction might underlie pathophysiological conditions such as neuropsychiatric disorders.

1. Introduction

The ability to accurately respond to extracellular stimuli is essential for short- and long-term adaptations and to store information in the brain. In response to a stimulus, neurons activate signal transduction pathways in the scale of milliseconds to minutes. This fast-acting response modulates the activity of already synthetized proteins through posttranslational modifications, inducing short-lasting neuronal plasticity changes. In a complementary strategy, activation of transduction pathways conveys synaptic activity to the nucleus that lead to gene transcription activation (Alberini and Kandel, 2014) as well as *de novo* synthesis of proteins locally at active synapses for long-term storage of information (Santini et al., 2014). In this review, we will focus on the role of the Growth Arrest and DNA Damage 45 (Gadd45) family in these processes. Gadd45 family members have multifactorial functions in the brain; they regulate signal transduction pathways and are targets for transcriptional regulation in response to neuronal activity (Sultan and Sweatt, 2013) (Figs. 1–3). Through both mechanisms Gadd45 proteins are involved in the regulation of short- and long-lasting forms of neuronal plasticity.

The Gadd45 family is comprised of three members: Gadd45α, Gadd45β and Gadd45γ, which are small proteins (~18 KDa) with both nuclear and cytoplasmic expression (Tamura et al., 2012). The genes coding each family member were first identified in cell lines following irradiation stress and interleukin treatment (Fornace et al., 1988; Beadling et al., 1993). Research from the last years has started to uncover the functions of this family in the brain. These include regulation of signaling pathways, RNA metabolism, DNA demethylation and gene expression associated with memory formation, visual cortex plasticity, adult neurogenesis, or neurological disorders. The three family members are expressed in most brain regions in baseline conditions (Matsunaga et al., 2015) and the expression of *Gadd45β/γ* is regulated by neuronal activity (Benito et al., 2011; Brito et al., 2020a). Although, prior studies suggested that *Gadd45β/γ* cellular functions require their *de novo*

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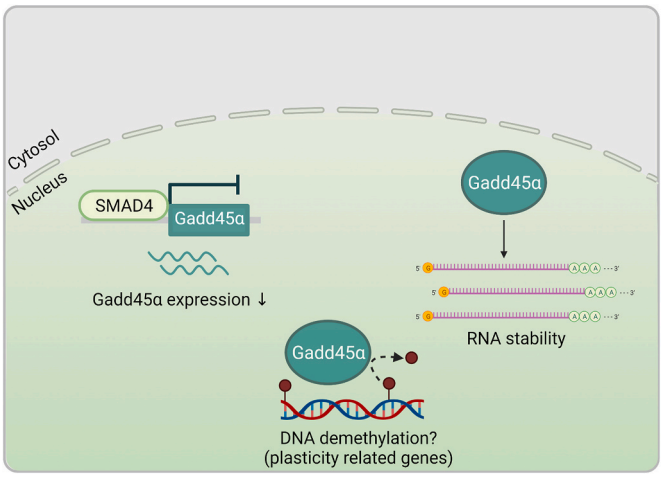

Fig. 1. Regulation of *Gadd45α* expression and its neuronal molecular functions. Gadd45α expression depends on mothers against decapentaplegic homolog 4 (SMAD4)-mediated transcription, which represses Gadd45α expression in baseline conditions (Grassi et al., 2017). Gadd45α has been recently shown to bind to memory-associated transcripts and possibly regulate their stability (Aparisi Rey et al., 2019). It is to note that Gadd45α has been shown in other cell types to promote DNA demethylation, although this role has not been explored in the brain. Created with BioRender.com.

synthesis in response to stimuli (Sultan and Sweatt, 2013), recent findings have shown that their baseline pool also plays a role in the regulation of signaling pathways (Apulei et al., 2019; Brito et al., 2020a, 2020b). This evidence suggests that both the baseline and de *novo* synthetized pools of Gadd45 proteins are required for neuronal function.

To facilitate discussion and interpretation of recent findings, we will dissociate the functions of baseline from activity-induced expression of Gadd45 proteins. First, we will discuss the molecular mechanisms that regulate the constitutive expression of Gadd45 proteins and its functions (Figs. 1–3 and Table 1). Next, we will describe mechanisms that control the transcription of Gadd45 proteins in response to neuronal activity and its contribution to neuronal function (Figs. 2 and 3 and Table 2).

1.1. Role of basal Gadd45 expression in neuronal plasticity

As above mentioned, Gadd45α, in contrast to Gadd45β/γ, is not induced by neuronal activation. Therefore, the functions of this protein are likely attributed to its constitutive expression (Fig. 1). A recent study showed that Gadd45α gene expression depends on the transcription factor mothers against decapentaplegic homolog 4 (Smad4), which represses Gadd45α expression in dissociated hippocampal neurons (Grassi et al., 2017). More specifically, in one set of experiments the authors showed that knocking-down SMAD4 increased Gadd45α levels, indicating a repressive role for this transcription factor on Gadd45α expression. When hippocampal neurons were depolarized upon KCl treatment, SMAD4 knock-down led to higher levels of Gadd45α compared to baseline conditions. This data indicates that SMAD4 is actively repressing Gadd45α expression in baseline conditions and upon KCl-induced neuronal activity (Grassi et al., 2017). The functions of Gadd45α have not been extensively characterized in the brain. Aparisi Rey et al. (2019) evaluated a possible function for Gadd45α in memory formation. They found that Gadd45α knock-out mice (Gadd45α-KO) display impaired hippocampal-dependent memory consolidation and long-term potentiation (LTP) (Aparisi Rey et al., 2019). The authors showed that virus-mediated hippocampal expression of full length Gadd45α rescued LTP and memory deficits present in Gadd45α-KO mice. These observations indicate that Gadd45α in the mouse hippocampus is necessary for proper cognitive function. In a later set of experiments, the authors investigated a link between Gadd45α function as

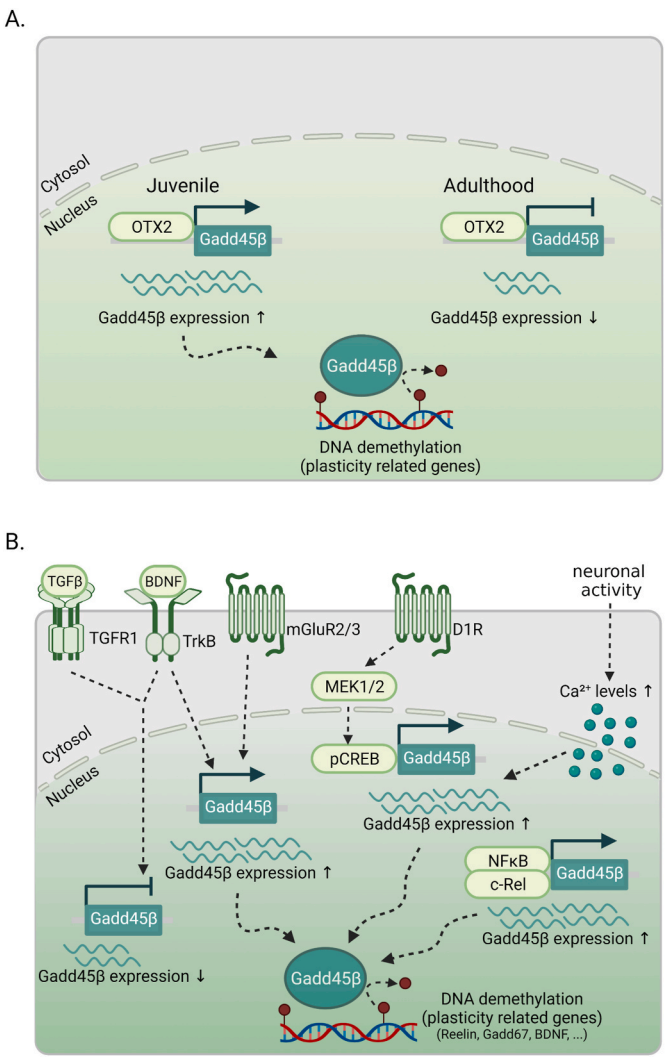

Fig. 2. Regulation of *Gadd45β* expression and its neuronal molecular functions. **A)** Baseline *Gadd45β* expression is dependent on the transcription factor orthodenticle homeobox 2 homeoprotein (OTX2) in the mouse visual cortex (Apulei et al., 2019). In the juvenile brain, OTX2 induces the expression of Gadd45β, while after this period of cortical plasticity, OTX2 has a repressive role on Gadd45β transcription. Functionally, Gadd45β reduces DNA methylation in the promoters of several plasticity-associated genes (Apulei et al., 2019). **B)** Activity-dependent *Gadd45β* expression is triggered by nuclear calcium increase (Zhang et al., 2009), activation of group II metabotropic glutamate receptors (mGluR2/3) (Matrisciano et al., 2011), tropomyosin receptor kinase B (TRKB) receptor (Grassi et al., 2017) and dopamine receptor type 1 (DRD1) receptor (Zipperly et al., 2021). On the contrary coinciding activation of TRKB receptors and TGF beta receptor 1 (TGFBR1) repress activity-dependent Gadd45β expression (Grassi et al., 2017). Gadd45β expression is also dependent on the transcriptional activity of c-Rel/NF-κB (Jarome et al., 2015). The activity-dependent Gadd45β protein pool has been shown to promote DNA demethylation in plasticity-related genes (Ma et al., 2009; Matrisciano et al., 2011; Jarome et al., 2015; Zipperly et al., 2021). Created with BioRender.com.

an RNA binding protein (Sytnikova et al., 2011) and mRNA metabolism in the hippocampus. They found that Gadd45α binds to RNAs that contain extended 3′UTRs and they suggested that this binding enables Gadd45α to regulate the mRNA stability of approximately 400 genes, many of which are crucial for memory and synaptic function (e.g., *Grin2a*, *Grin2b*, *Kcnq3*, *Grm5*). Furthermore, the expression of a mutant form of Gadd45α incapable of RNA binding did not rescue Gadd45α-KO-induced memory deficits. This elegant set of experiments

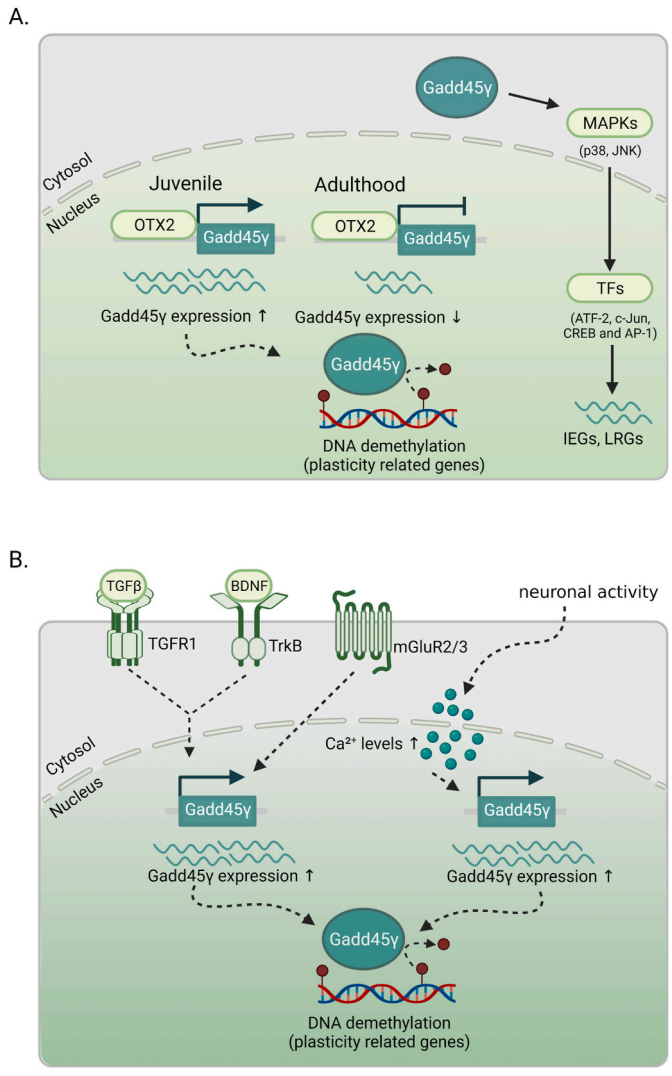

Fig. 3. Regulation of *Gadd45γ* expression and its neuronal molecular functions. A) Baseline Gadd45γ expression is dependent on orthodenticle homeobox 2 homeoprotein (OTX2)-mediated transcription in the mouse visual cortex (Apulei et al., 2019). Similarly, to regulation of Gadd45β expression, in the juvenile mouse brain OTX2 induces the expression of Gadd45γ, although during adulthood OTX2 represses Gadd45γ transcription. After neuronal activity, activation of the mitogen-activated protein kinase (MAPK) pathway (p38 and JNK), transcription factors (ATF-2, c-Jun, CREB and AP-1) and activity-dependent gene transcription depends on Gadd45γ expression in hippocampal neurons (Brito et al., 2020a). **B)** Neuronal activity rises nuclear calcium levels that potentiate Gadd45γ expression (Zhang et al., 2009). Moreover activation of mGluR2/3 (Matrisciano et al., 2011) or joint action of TRKB and TGFBR1 receptors (Grassi et al., 2017) also activates Gadd45γ transcription. The activity-dependent Gadd45γ protein pool has only been shown to promote DNA demethylation in memory-related genes, several hours after a learning stimulus (Li et al., 2019). Created with BioRender.com.

therefore implicates the RNA-binding capacity of Gadd45α in the stabilization of memory-related transcripts with extended 3′UTRs (Aparisi Rey et al., 2019). In addition, Gadd45α might also modulate gene expression by alternative mechanisms such as DNA demethylation. Particularly a recent report suggests that upon neuronal activity, Gadd45α binds to the promoter region of the brain-derived neurotrophic factor (*Bndf)* exon I, possibly regulating the DNA methylation status (Feng et al., 2021).

Only a handful of studies investigated the molecular mechanisms that regulate *Gadd45β/γ* baseline expression. Recent evidence showed that *Gadd45β/γ* baseline expression is regulated by the transcription

Table 1
Basal pool of Gadd45 proteins in synaptic plasticity and memory formation.

Gadd45 member	Organism/ system	Manipulation	Outcome	Reference (s)
Gadd45α	C57BL/6N mice	Gadd45α-KO	LTP and hippocampus-dependent memory impairments Reduced expression of memory-related genes	Aparisi Rey et al. (2019)
		Gadd45α overexpression	Tendency for LTP increase and enhanced hippocampus-dependent memory	Aparisi Rey et al. (2019)
	Cortical neurons	KCl treatment	Gadd45α occupancy at the *BDNF* exon I promoter and reduced DNA methylation	Feng et al. (2021)
	Hippocampal neurons	SMAD4-shRNA	Increased Gadd45α expression (baseline) Increased Gadd45α expression (KCl 4h)	Grassi et al. (2017)
	BALB/c mice/ hippocampus and prefrontal cortex	Unpredictable chronic mild stress	Reduced Gadd45α expression	
Gadd45β	C57Bl/6J mice/visual cortex	Cortex injection of OTX2 recombinant protein	Increased Gadd45β expression	Apulei et al. (2019)
		Gadd45β overexpression	Increased expression of memory-related genes Reduced DNA methylation	
	Psychotic patients/ prefrontal cortical layers II, III, and V	–	Increased Gadd45β expression Less Gadd45β promoter binding at the *BDNF* promoter Increased DNA methylation at the *BDNF* promoter	Gavin et al. (2012)
	BALB/c mice/ hippocampus and prefrontal cortex	Unpredictable chronic mild stress	Decreased Gadd45β expression	Grassi et al. (2017)
	BALB/c mice/ hypothalamus		Increased Gadd45β expression	
	Aged Humans/ hippocampus	–	Increased Gadd45β expression	Brito et al. (2020b)
Gadd45γ	C57Bl/6J mice/visual cortex	Cortex injection of OTX2 recombinant protein	Increased Gadd45γ expression	Apulei et al. (2019)
	Aged C57Bl/6J mice/ hippocampus	–	Reduced Gadd45γ expression	Brito et al. (2020a)
	Hippocampal neurons	Gadd45γ-shRNA		

(*continued on next page*)

Table 1 (*continued*)

Gadd45 member	Organism/ system	Manipulation	Outcome	Reference (s)
			Impaired MAPK signaling (p38, JNK) Impaired transcription factor activation (ATF-1, CREB, c-Jun) Reduced expression of memory-related genes	
	C57Bl/6N mice/ hippocampus	Gadd45γ-shRNA	hippocampus-dependent memory impairments	
	Aged Humans/ hippocampus	–	Increased Gadd45γ expression	Brito et al. (2020b)
	Hippocampal neurons	Gadd45γ overexpression	Increased CREB activation Reduced expression of memory-related genes	
	C57Bl/6N mice/ hippocampus		hippocampus-dependent memory impairments	
	BALB/c mice/ hippocampus and prefrontal cortex	Unpredictable chronic mild stress	Decreased Gadd45β expression	Grassi et al. (2017)

factor orthodenticle homeobox 2 homeoprotein (Otx2) in the mouse visual cortex (Apulei et al., 2019). Otx2 is an established regulator of GABAergic parvalbumin interneuron maturation, required for cortical visual plasticity in mice. In this study, the authors identified five and three putative Otx2 binding sequences in the *Gadd45β/γ* genes, respectively. Interestingly, Otx2 regulates Gadd45β/γ transcription in opposite directions in juvenile and adult mice, consistent with a role for Gadd45β/γ in defining the critical period of visual cortical plasticity. Specifically, in juvenile visual cortex Otx2 activates Gadd45β/γ expression. This is associated with increased DNA demethylation and expression of plasticity-related genes thought to promote cortical plasticity. In contrast, in the adult, Otx2 downregulates Gadd45β/γ expression, a mechanism possibly associated with the closure of the plasticity critical period.

Besides active DNA demethylation and regulation of RNA stability, other mechanisms have been recently attributed to Gadd45 proteins. In tissues outside of the nervous system Gadd45 proteins have been shown to activate mitogen-activated protein kinases (MAPKs) signaling, although it was unclear if such was the case in the brain (Takekawa and Saito, 1998; Tornatore et al., 2008). A recent study showed that upon neuronal activity Gadd45γ activates the c-Jun N-terminal kinase (JNK) and p38 MAPKs in hippocampal neurons (Brito et al., 2020a). This activation occurs within minutes after neuronal activity, likely recruiting baseline Gadd45γ. Using a virus-mediated knock-down strategy, the authors demonstrated that Gadd45γ is required for the activation of the transcription factors cAMP response element (CRE) binding protein (CREB) and activator protein 1 (AP-1) in response to neuronal activity. This resulted in impaired levels of immediate early (IEG) and late response (LRG) genes expression associated with these transcription factors (Brito et al., 2020a). Moreover, *in vivo* knock-down of Gadd45γ impaired long-term memory and forms of short-term memory. Interestingly, Gadd45β knock-down did not impact CREB or AP-1 activity or memory performance, indicating that Gadd45γ, but not Gadd45β, is required for memory formation. In a follow-up study, the same group found that raising the levels of Gadd45γ above physiological levels increased phosphorylated CREB (Brito et al., 2020b). Nonetheless a sustained CREB activation was associated with reduced levels of IEG expression and impaired long-term memory. Collectively, these findings suggest that both a prolonged increase and decrease of baseline Gadd45γ levels is detrimental for neuronal plasticity. In summary, Gadd45 basal expression is required for active DNA demethylation, RNA stability and MAPK activation during periods of cortical or hippocampal plasticity and memory formation (Figs. 1, 2A and 3A).

1.2. Role of the activity-induced Gadd45 expression in neuronal plasticity

Neuronal activation triggers glutamate receptor activation and rises in intracellular calcium levels which induce Gadd45β/γ transcription in neurons (Ma et al., 2009; Zhang et al., 2009; Matrisciano et al., 2011; Leach et al., 2012; Sultan et al., 2012; Jarome et al., 2015; Brito et al., 2020a) (Table 2). Notably the regulatory regions of *Gadd45β/γ* genes contain CRE sites (Zhang et al., 2009), suggesting that CREB is a likely regulator of their expression. Using *in vitro* models, two studies showed that KCl- or bicuculline-induced depolarization of mature hippocampal neurons induce the transcription of Gadd45β/γ (Zhang et al., 2007; Grassi et al., 2017). Interestingly, despite being both induced by neuronal activity, the signaling pathways that regulate the expression of each gene are distinct (Grassi et al., 2017). KCl-dependent neuronal depolarization leads to the release of Bdnf and transforming growth factor beta (Tgf-β) and the activation of the downstream signaling pathways exerts opposing effects in Gadd45β/γ expression (Figs. 2B and 3B). Whereas Bdnf- and Tgf-β-signaling repress the expression of Gadd45β, these signaling pathways exert a positive effect on Gadd45γ expression. This data indicates that transcriptional regulation of Gadd45β/γ is controlled by multiple stimuli, and that the balance between the different stimuli determines the transcriptional outcome (Table 2). Another study investigated the regulation of Gadd45β expression in the CA1 region of the hippocampus in response to learning (Jarome et al., 2015). Using *in silico* analysis, the authors identified consensus binding sites for the transcription factor c-Rel in the Gadd45β promoter, suggesting that c-Rel is involved in the regulation of Gadd45β expression. The authors found increased occupancy of Gadd45β and reduced DNA methylation in the promoter region of *Bdnf* exon IV after contextual-fear learning, indicating that Gadd45β occupancy decreased DNA methylation in this region. Importantly, there was no detectable increase in Gadd45β nor a decrease in methylation levels of the *Bdnf* exon IV after learning in c-Rel-KO mice. These findings indicate that c-Rel is required for *de novo* Gadd45β expression in response to associative learning, that modulates the methylation of *Bdnf*. Recently a study showed that acute cocaine administration and dopamine stimulation induces *de novo* Gadd45β expression in the rat nucleus accumbens and primary striatal cultures, respectively (Zipperly et al., 2021). In striatal cultures, the authors showed that the dopamine-triggered increase in *Gadd45β* expression is regulated by dopamine receptor type 1 activation, MEK 1/2 and CREB activities (Zipperly et al., 2021) (Fig. 2B).

Other studies have shown that Gadd45β is necessary for activity-induced DNA demethylation of additional genes – including *Bdnf* exon IX and *Fgf-1B* in the dentate gyrus of the hippocampus (Ma et al., 2009) and *Bdnf* exon IX, *Reelin* and glutamate decarboxylase-67 (*Gad67*) in the mouse frontal cortex (Matrisciano et al., 2011). Using genome wide approaches, Zipperly et al. (2021) showed that Gadd45β is required for dopamine-induced changes in DNA methylation and gene expression. Surprisingly, this study suggested that not only dopamine-associated DNA demethylation, but also methylation requires Gadd45β expression. Whether the gain in methylation is a direct consequence of Gadd45β activity or results from prolonged Gadd45β knockdown and possible altered expression of additional epigenetic regulators remains to be investigated. Taken together these studies suggest that activity induced Gadd45β plays a role in the regulation of the methylation status

Table 2
Activity-induced pool of Gadd45 proteins in synaptic plasticity and memory formation.

Gadd45 member	Stimulus	Organism/system	Manipulation	Outcome	Reference(s)
Gadd45β	mGluR2/3 activation	Swiss Albino mice/frontal cortex and hippocampus	Systemic injection of mGlu2/3 receptor agonist	Increased Gadd45β expression Reduced methylation of reelin, BDNF and GADD67 promoters	Matrisciano et al. (2011)
			Systemic injection of mGlu2/3 receptor agonist	Increased Gadd45β binding to reelin, BDNF and GADD67 promoters	
			Systemic injection of mGlu2/3 receptor antagonist	Abolishment of Gadd45β expression	
	Electro-convulsive treatment	C57BL/6 mice/dentate gyrus	–	Increased Gadd45β expression	Ma et al. (2009)
			Gadd45β knockout	Impaired proliferation of neural progenitors and dendritic growth Reduced activity-dependent DNA demethylation of BDNF and FGF-1B Reduced activity-dependent expression BDNF and FGF-1B	
	Fear conditioning	Sprague-Dawley rats/CA1 of the hippocampus	–	Increased Gadd45γ expression	Jarome et al. (2015)
			Pharmacological NF-kB inhibition	Impaired Gadd45γ expression	
		C57BL/6J mice or Sprague-Dawley rats/CA1 of the hippocampus	c-rel$^{-/-}$ knockout mice or siRNA- knock-down of c-rel	Impaired Gadd45γ expression Gadd45β binding at the BDNF gene Impaired BDNF DNA demethylation	
	Object exploration	C57Bl/6J mice/hippocampus	–	Increased Gadd45β expression	Brito et al. (2020a)
	Bicuculline treatment	Hippocampal neurons	–	Increased Gadd45β expression	
	Passive avoidance	C57BL/6N mice	–	Increased Gadd45β expression	Aparisi Rey et al. (2019)
	KCl treatment	Hippocampal neurons	–	Increased Gadd45β expression	Grassi et al. (2017)
			Receptor, type II-like 1 (ALK1)/TGFBR1 inhibitor	Increased Gadd45β expression	
			TRKB-inhibiting antibody	Decreased Gadd45β expression	
			Receptor, type II-like 1 (ALK1)/TGFBR1 inhibitor and TRKB-inhibiting antibody	Decreased Gadd45β expression	
	Dopamine treatment	Striatal neurons	-	Increased Gadd45β expression	Zipperly et al. (2021)
			Dopamine receptor type 1 antagonist	Decreased Gadd45β expression	
			MEK 1/2 inhibition	Decreased Gadd45β expression	
			CREB inhibition	Decreased Gadd45β expression	
	Dopamine receptor type 1 agonist		Gadd45β-shRNA	Reduced activity-dependent changes in DNA methylation Reduced expression of IEGs Reduced action potential burst duration	
	Cocaine administration	Sprague–Dawley rats/Nucleus accumbens	–	Increased Gadd45β expression	
		Gadd45β knockout mice	Gadd45β knockout	Reduced cocaine-paired place preference	
		Nucleus accumbens	CRISPR/Cas9	Reduced cocaine-paired place preference	
Gadd45γ	Bicuculline treatment	Hippocampal neurons	–	Increased Gadd45γ expression	(Brito et al., 2020a)
	Object exploration	C57Bl/6J mice/hippocampus			
	KCl treatment	Hippocampal neurons	–		Grassi et al. (2017)
			Receptor, type II-like 1 (ALK1)/TGFBR1 inhibitor and TRKB-inhibiting antibody	Decreased Gadd45γ expression	
	Fear conditioning	C57BL/6J mice/Prelimbic prefrontal cortex	–	Increased Gadd45γ expression	Li et al. (2019)
			Gadd45γ-shRNA	Reduced Gadd45γ occupancy at IEG promotors Increase in promoter DNA methylation of IEGs Reduced expression of IEGs Impaired fear- memory	

of several genes upon stimuli (Fig. 2B).

Besides Gadd45β, activity-induced Gadd45γ expression is also necessary for DNA demethylation and gene expression associated with memory formation. In 2018, a study found that Gadd45γ is expressed 5 h after cued fear learning in the mouse prelimbic cortex (Li et al., 2019). This expression was associated with an increase in Gadd45γ occupancy at the promoters of memory-related genes (*Arc, c-Fos, Npas4, and Cyr61*) (Fig. 3B). Reducing Gadd45γ expression at 5 h led to increased promoter DNA methylation and reduced mRNA levels of these genes and impaired cued fear learning. The authors proposed a model where hours after the first induction of memory-related genes, Gadd45γ is recruited to the promoter of the same genes. This recruitment supposedly promotes activity-dependent DNA demethylation, triggering a second period of gene expression required for memory consolidation.

Altogether, these findings indicate that *de novo* expression of Gadd45β/γ is required for memory formation by processes of DNA demethylation in plasticity-associated genes.

1.3. Ending remarks

In this review, we highlighted different roles of constitutive and activity-induced Gadd45 cellular pools in neuronal plasticity. These

findings stress the importance of these proteins in brain function and the necessity to dissociate the two protein pools. However, the study of this protein family presents additional challenges. First, it has become evident that Gadd45 proteins regulate DNA demethylation in neurons. Although only recent work started to evaluate a role for Gadd45α as a neuronal regulator of DNA demethylation (Feng et al., 2021). Moreover, Gadd45 proteins might have different functions in different cell types and brain regions (Tables 1 and 2). This might help to explain current seemingly contradictory findings. Specifically, whereas one study showed that Gadd45β-KO mice promoted hippocampal-dependent memory deficits (Leach et al., 2012), the other study observed memory enhancements (Sultan et al., 2012). Moreover, a more recent study demonstrated that acute reduction of Gadd45β in the hippocampus did not induce changes in hippocampal-dependent memory (Brito et al., 2020a). These apparent conflicting results might be attributed to different functions of Gadd45β throughout the brain. Additionally, the strategies used to manipulate Gadd45 expression in the abovementioned loss-of-function studies may differently affect the constitutively expressed and *de novo* expressed Gadd45 proteins. In summary, we are just starting to uncover the complex role of these proteins. Thus, future studies are required to understand how baseline or activity-induced Gadd45 pools regulate brain function. Furthermore, it remains to be understood how the distinct family members are specifically recruited to the various cellular functions and whether they target similar or distinct gene pools for demethylation. Elucidating these mechanisms will not only advance our understanding of the mechanisms underlying long-lasting brain adaptions, but also identify possible therapeutic targets for neurological disorders.

CRediT authorship contribution statement

David V.C. Brito: conceptualized the manuscript, wrote the first draft of the manuscript. **Janina Kupke:** designed and created the figures. **Kubra Gulmez Karaca:** designed and created the figures. **Ana M. M. Oliveira:** conceptualized the manuscript. All authors revised and edited the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crneur.2022.100031.

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