Current Neuropharmacology
BENTHAM SCIENCE

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Journal:	*Current Neuropharmacology*
Manuscript ID	CN-2020-0012
Manuscript Type:	Invited Review
Date Submitted by the Author:	16-Jan-2020
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Keywords:	NGF, Chronic alcohol consumption, Binge drinking, Alcohol Use Disorders, Addiction, Fetal Alcohol Spectrum Disorders

Nerve Growth Factor in Alcohol Use Disorders

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Running title: NGF and alcohol

Acknowledgements
Authors thank Sapienza University of Rome and IBBC-CNR for supporting this work.

Ethical approval
Not applicable

Abstract

The nerve growth factor (NGF) belongs to the family of neurotrophic factors. Initially discovered as a signaling molecule involved in the survival, protection, differentiation and proliferation of sympathetic and peripheral sensory neurons, it also participates in the regulation of the immune system and endocrine system. NGF biological activity is due to the binding of two classes of receptors: the tropomyosin-related kinase A (TrkA), and the low-affinity NGF pan-neurotrophin receptor p75. Alcohol Use Disorders (AUD) are one of the most frequent mental disorders in developed countries, characterized by heavy drinking, despite the negative effects of alcohol on brain development and cognitive functions that cause individual's work, medical, legal, educational and social life problems. In addition, alcohol consumption during pregnancy disrupts the development of the fetal brain causing a wide range of neurobehavioral outcomes collectively known as fetal alcohol spectrum disorders (FASD). In this review, we describe crucial findings on the role of NGF in humans and animals when exposed to prenatal, chronic alcohol consumption and also on binge drinking.

Key words

NGF- Alcohol Use Disorders- Binge drinking- Chronic alcohol consumption

Nerve Growth Factor - NGF

Neurotrophic factors control cell differentiation, proliferation, growth, migration, survival, metabolism and apoptosis [1,2]. Neurotrophins belong to the family of neurotrophic factors and include polypeptide growth factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5 [3]. NGF, expressed both in the peripheral and central nervous system, is a neuropeptide that regulates the survival and proliferation of neuronal cells [2,4,5]. It was discovered in 1951 by Rita Levi-Montalcini and Victor Hamburger from a sarcoma tissue that released a soluble growth factor able to induce overgrowth of fibers from sensory or sympathetic nerve cells placed nearby [6,7]. NGF is synthesized as a 130 kD precursor, namely proNGF, that is formed by three proteins: α-NGF, β-NGF and γ-NGF. The third protein is a serine protease that cuts off the β subunit producing the 26 kD mature NGF that is biologically active [4,8,9].

NGF Receptors

NGF exerts its effects by binding two classes of receptors: the tropomyosin-receptor kinase A (TrkA), and the low-affinity NGF receptor p75 (LNGFR/$p75^{NTR}$) [2,8,10]. TrkA belongs to the family receptor of Trk, tyrosine kinases, along with TrkB and TrkC, which regulates synaptic strength and plasticity in the nervous system [11]. The receptor p75 is a low-affinity neurotrophin receptor and a member of the tumor necrosis factor receptor family [12]. The Trk subfamily of receptors is composed of immunoglobulin-C2 domains, amino acid repeats full of leucine and cysteine residues in the extracellular domain and a tyrosine kinase domain with a small cytoplasmic tail. The p75 receptor has four negatively charged cysteine-rich amino acid repeats in the extracellular domain and a single cytoplasmic domain that includes a “death” domain [13]. For the TrkA receptor, only the domain nearest to the cell membrane

is needed to bind to its ligand [14,15]. The binding of NGF to TrkA starts the homodimerization of the receptor and the autophosphorylation of certain tyrosine residues within the intracellular domains. This site of phosphorylation (Figure 1) then recruits adapter proteins that have src-homology-2 (SH-2) or phosphotyrosine-binding motifs. The adapter proteins, after phosphorylation, start several intracellular signaling cascades involved in cell survival [8,16]. A pathway activated by the binding between NGF and the receptor TrkA involves the mitogen-activated protein kinase (MAPK). This pathway induces the activation of Ras, a GTPase that phosphorylates the serine/threonine kinase Raf. This latter activates the MAPK cascade which regulates the activity of several transcription factors like the cAMP response element-binding protein (CREB), a transcription factor that translocates in the nucleus to control the expression of anti-apoptotic genes [16,17]. Although the receptor p75 does not contain a catalytic motif, it interacts with several proteins that regulate neuronal survival and differentiation as well as synaptic plasticity. The binding of NGF to p75 activates several signaling pathways. The primary signaling activated by p75 is the Jun kinase-signaling cascade. This pathway activates p53, a transcriptional factor that can initiate apoptosis. Furthermore, this cascade can activate apoptosis by increasing the expression of the Fas receptor ligand [18]. Nerve growth factor binding to the receptor p75 also stimulates the activation of NF-KB, thereby promoting neuronal survival [19]. Ligand engagement of p75 has been shown to activate acid sphingomyelinase, which results in the production of ceramide [20]. Ceramide promotes both apoptotic and survival pathways started by p75 ligation [21]. Ceramide is known to regulate many signaling pathways, such as the ERK, Jun kinase, NF-kB signaling pathways as well as the activity of TrkA phosphorylating serine residues [22,23].

NGF Functions

Nerve growth factor was initially identified as a signaling molecule involved in growth, survival and proliferation of sympathetic and sensory neurons [4,24,25]. It is also involved in the regulation of the immune system and the endocrine system including the adipoendocrine system [26–28]. Consequently, altered expression of NGF and its receptors are involved in many seemingly unrelated diseases including neuronal disorders (Alzheimer's and other neurodegenerative disease) [17,29,30], aging [31], cancer physiology [32–35], ocular diseases [36–38], growth and development [33,34,39], autoimmune diseases (rheumatic arthritis, multiple sclerosis and other autoimmune diseases) [40], oxidative stress-related diseases [41–46], neuroinflammation caused by parasitic diseases [47–54] and cardiometabolic diseases such as type 2 diabetes mellitus, obesity and metabolic syndrome [55–61]. Furthermore, pieces of evidences on humans indicate that NGF and its receptor are known to be altered in ethanol-induced toxicity, which is the inducing-cause of brain changes [62,63] and mental retardation [64–70]. A subtle role played by NGF was also hypothesized for schizophrenia development [71–77] as shown in humans and schizophrenia animal models.

In the peripheral nervous system (PNS) NGF has a central role in the development, maintenance and regeneration of mammalian sympathetic and sensory neurons [78]. During postnatal life, NGF continues to act as a survival factor for many sympathetic neurons, while sensory neurons stop to depend on this growth factor in the postnatal period [2,79]. In the central nervous system (CNS) NGF is produced by neurons and glial cells of the cerebral cortex, of the hippocampus, of the hypothalamus and acts as a protective factor of cholinergic neurons, cells that are involved in the cognitive process such as learning and memorization [4,80].

Studies carried out during the last few years found that NGF receptors are expressed in primary (thymus, bursa of Fabricius, bone marrow) and secondary (spleen, tonsils, lymph

nodes) lymphoid organs, as well as in some immunocompetent cells such as thymic epithelial cells and bone marrow stromal cells [81–91]. For this reason, both these tissues and cells are potential targets for NGF. Moreover, it has been described the role of NGF during inflammatory disorders and allergic diseases [92–98].

The fact that NGF is secreted in humans' bloodstream in response to stress and the fact that NGF cellular targets have been identified in the endocrine system, suggests that this molecule may regulate physiological homeostasis through neuroendocrine mechanisms [4,99,100]. NGF is also involved in the acquisition of male and female reproductive capacity and stimulates the hypothalamic-pituitary-adrenal axis (Figure 2) increasing the secretion of adrenocorticotrophic hormone and corticosteroids [101,102]. In addition, hormones have been shown to regulate NGF synthesis and release [103]. The exogenous administration of testosterone to female mice increases the synthesis of NGF in the submaxillary salivary glands, whereas castration in males highly reduces NGF in the glands [2].

Acetylcholine and NGF

NGF plays a key role in regulating the biochemical and morphological phenotype of basal forebrain cholinergic neurons in the fully differentiated central nervous system [104]. Cholinergic neurons are characterized by the presence of choline acetyltransferase (ChAT), the acetylcholine synthesizing enzyme, choline transporter (CHT) and vesicular acetylcholine transporter (VAChT); ChAT has been identified as the most selective marker of cholinergic cells [105,106]. In addition to its role as a trophic and survival factor for cholinergic neurons, NGF regulates the expression of CHT, ChAT and VAChT [107–109]. Disorders in NGF transport and lower processing of proNGF to mature NGF may be the cause for the selective degeneration of cholinergic neurons of the basal forebrain in the brain of patients with Alzheimer Disease (AD) [106,110]. In recent years, attention has been focused on NGF as a

potential therapeutic agent for a variety of neurodegenerative disorders [106]. Karami et al. studying the activity of ChAT in the cerebrospinal fluid (CSF) of AD patients with encapsulated cell implants releasing NGF (EC-NGF) found, after 12 months following NGF treatment, an increase in ChAT and acetylcholinesterase activity in the CSF. Moreover, CSF ChAT activity showed a high correlation with patient's performance in the cognitive test during treatment with EC-NGF. These patients remained stable in cognition long after the removal of the EC-NGF implants [106].

Alcohol Use Disorder

Alcohol use disorders (AUD) are the most frequent and untreated mental disorders in developed countries and the American Medical Association defines it as a chronic and relapsing disease [111,112]. Nearly 2 billions of people in the world consume alcohol with 76.3 million who have diagnosable alcohol use disorders [113]. According to the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) diagnostic criteria, in 2012-13, respectively 36% of males and 22.7% of females adults in the USA met the criteria for alcohol use disorders at some time in their lives [114,115]. The probability of developing alcohol use disorders raises with the frequency of binge drinking, even though most heavy drinkers do not show the criteria for alcohol dependence [116].

Alcohol abuse is associated with many different diseases. Alcohol use has been attributed to both negative and positive effects. While cardiovascular protection might be gained from very low doses, binge drinking is linked with high mortality [117,118]. The primary causes of death that depend on alcohol consumption are injury, alcoholic liver disease, heart diseases, stroke, cancer and gastrointestinal diseases [119]. Ethanol is the intoxicating agent in alcoholic drinks that leads to abuse and dependence [120]. The risk of damage increases steeply when more than 10-20 g of alcohol is consumed in a day. The transition from episodic

drinking to binge drinking increases the risk of accidents, injuries, violence and heart diseases [121].

Alcohol influences a large number of neurotransmitters in the brain that are involved in cognition, emotion and motivation [122]. Rewarding, anxiolytic and social facilitating effects are due to low doses of alcohol consumption. As the dose raises, alcohol causes cognitive and psychomotor disruptions that increases the risks of injury [123]. Alcohol crosses the blood-brain barrier and widely alters neuronal functions including phospholipid membranes, ion channels and receptors, synaptic and network functions, and intracellular signaling molecules [124]. Alcohol interacts with many neurotransmitters: it increases directly GABA, glycine, nicotinic, acetylcholine and serotonin activity; it indirectly increases dopamine, opioid and endocannabinoid activity and inhibits glutamate transmission (Figure 3). These complex effects cause acute intoxication [125].

Alcohol use disorders do not depend only on the person's moral choices, but are a result of the combined effects of many personal, social and biological factors [123]. Cultures that promote abuse in alcohol drinking as a lifestyle are responsible for the increase in AUD cases in the population [126]. Also, early initiation to alcohol consumption in adolescence is a factor that could be responsible for developing AUD in adulthood [127]. More risk factors include a family history of alcohol dependence, low parental control and little family support, childhood attitude and mood disorders, low self-control and positive association between alcohol consumption and social outcomes [128]. Twin studies have estimated that 50-70% of the risk of developing alcohol use disorders depends on genetic factors [129]. The most studied genetic correlation is with genes that reduce the risk. The liver enzymes that metabolize alcohol are alcohol dehydrogenase and the mitochondrial form of aldehyde dehydrogenase (ALDH2) [123]. People with a single copy of the allele ALDH*2 have defective alcohol metabolism and drinking alcohol causes facial flushing, sweating,

tachycardia, nausea, vomiting and headache. These reactions protect against developing alcohol use disorders [130]. To date, alleles found to be associated with alcohol dependence cause a low increase of the risk of developing alcohol use disorders [131]. These alleles alter dopaminergic, opioidergic, GABAergic, serotonergic, cholinergic and glutamatergic neurotransmission [132].

According to the DSM-5, the diagnosis of alcohol use disorders requires at least two of eleven symptoms. Three methods based on structured and short questionnaires such as the Alcohol Use Disorders Identification Test (AUDIT), the brief version AUDIT-C, and CAGE can identify patients who need further assessment [133–135]. The physical examination evaluates the symptoms due to intoxication and withdrawal. The signs of intoxication are slurred speech, ataxia and inappropriate affect. Instead, the first signs of alcohol withdrawal are restlessness, tachycardia and fine action tremor. Alcohol values are measured in the blood or in the breath [123]. Standard blood tests, liver tests and the biomarker γ-glutamyl transpeptidase (gGT) are frequently aberrant in patients with alcohol use disorders, but these investigations alone are of little value because of poor sensitivity and specificity [136,137]. Many other biomarkers for alcohol use disorders diagnosis are more sensitive and specific, but they are not widespread due to their high cost and limited availability. These biomarkers comprehend carbohydrate-deficient transferrin, ethyl glucuronide, ethyl sulphate, phosphatidyl ethanol and fatty acid ethyl esters [137–139].

NGF and Chronic Alcohol Consumption in Humans

NGF is a neurotrophic factor involved in the growth and differentiation of nerve cells and in the prevention of damage to mature neurons. NGF is also known for his beneficial effect on recovery from cognitive deficits after brain damage [140–142]. In addition, it could play an

important role in protecting neurons from cytotoxic damage induced by ethanol [143–145]. Several studies have been conducted in alcohol dependent-patients to determine the correlation between plasma NGF concentration and alcohol dependence. The next morning after admission to the Hangang Sacred Heart Hospital, Lee et al. interviewed and sampled forty-one male patients with alcohol dependence and compared them with forty-one healthy male subjects. Lee et al. found that the plasma NGF concentration was elevated in AUD patients within 24h of abstinence [146]. In the study of Kohler et al. fifteen patients with a diagnosis of alcoholism according to the DSM-IV criteria, and fifteen healthy subjects participated consecutively in a two weeks withdrawal study. Alcohol dependent subjects showed, after the acute withdrawal over two weeks of alcohol abstinence, lower NGF levels when compared to the healthy patients. In particular mean NGF concentrations increased initially, and then decreased significantly from days three to fourteen [147]. These findings are in agreement with epigenetic down-regulation of the NGF gene during alcohol withdrawal [148]. It is known that increased methylation of CpG-sites in the gene promoter reduces mRNA expression of the interested gene [149]. Heberlein investigated the correlations between alterations in NGF serum concentrations and changes in the methylation of the NGF promoter during alcohol withdrawal. Fifty-seven patients with alcohol dependence showed a significant decrease in the NGF serum levels from day seven to day fourteen of alcohol withdrawal, and a significant increase in methylation of the CpG-sites within the NGF gene promoter. These results suggest epigenetic regulation of NGF gene expression during alcohol withdrawal [148]. Alterations in proinflammatory cytokines have been associated with affective disorders which play an important role in alcohol consumption [150,151]. Recently, in alcohol dependence patients undergoing withdrawal, Heberlein and colleagues found a linear association between the methylation of the CpG-sites within the NGF gene promoter and IL-6 serum levels [152]. In a study conducted on young patients with alcohol use

disorders, Lhullier et al. found higher serum levels of NGF when compared to control [153]. Taken together these results show that NGF plasma concentration increases during intoxication to protect against the toxic effects of alcohol [154], but then decreases during the abstinence period. Nevertheless, the number of studies is not sufficient yet for consistent results.

Patients with alcohol dependence show a decline in their cognitive functions even after they quit to consume alcohol [155]. Other studies investigated the relationship between NGF plasma concentration and the decline in cognitive function of alcohol-dependent patients during the abstinence period. The trail-making test B, a test that includes motor components and visual scanning, showed an important correlation with the NGF plasma concentration. The NGF levels were higher in patients with lower trail-making test B score, indicating faster performance speed and a higher executive function. This finding may suggest a protective role of NGF in preventing neuronal damage in patients with alcohol dependence [156].

Another investigation demonstrated that withdrawal from chronic consumption of either ethanol or heroin caused a significant increase in plasma NGF, suggesting that the resulting anxiety condition may trigger the NGF release [157]. Quite interestingly, no changes were observed in the levels of bloodstream NGF of non-dependent human subjects used to drink alcoholic beverages (mean age 41 years) 30 min before and 60 min after drinking 1 pint of red wine [157]. Although the functional significance of these phenomena required further investigations, authors hypothesized that the increased levels of circulating NGF might be involved in homeostatic adaptive and/or reparative mechanisms.

NGF and Chronic Alcohol Consumption in Animals

Dependence and reward are regulated by complex neuronal circuits where the nucleus accumbens (NAc) plays a crucial role [158–160]. In the NAc there are projections of neurons

that release gamma-aminobutyric acid (GABA), and a small population of interneurons that produce either acetylcholine or GABA and different neuropeptides like neuropeptide Y (NPY) [161–163]. NPY is a neurotransmitter/neuromodulator implicated in the control of a wide range of physiological functions and behaviors, such as alcohol consumption, withdrawal and neuronal excitability [164–168]. Pereira et al. studied the effects of chronic consumption and subsequent withdrawal on the expression of NPY, acetylcholine and on the levels of ChAT in the NAc of abstinent rats that received an intracerebroventricular infusion of NGF [169]. During chronic alcohol consumption, the number of NPY-immunoreactive neurons increased and returned to control values after withdrawal, whereas the density of cholinergic varicosities was reduced by 50% during chronic consumption and by 64% during withdrawal. However, the increase in the number of NPY-immunoreactive neurons, the increase in the density of cholinergic varicosities and enlargement of cholinergic interneurons, after exogenous administration of NGF, suggests that withdrawal changes might be mediated by the withdrawal-induced disruption of NGF trophic support [169,170]. Even in the hippocampal formation, the cholinergic neurons and the GABAergic interneurons expressing NPY are vulnerable to the effects of chronic alcohol intake and abstinence [171–179]. More recently Pereira et al. studied the effects of chronic alcohol consumption and subsequent withdrawal in the dentate gyrus, a hippocampus region containing a large population of NPY-immunoreactive neurons and cholinergic innervation [162,163,180]. In this study, they show that NPY expression in the hilus of the dentate gyrus increased after withdrawal and turned back to control values after NGF intracerebroventricular infusion [181]. Differently, the levels of VAChT were reduced by 24% in chronic alcohol consumption rats and by 46% in withdrawn rats, but after the administration of NGF to withdrawn rats, the expression of VAChT increased to values above control levels [181]. These findings are in agreement with

previous studies showing that exogenous NGF protects the phenotype and prevents withdrawal-induced degeneration of the basal forebrain cholinergic neurons.

NGF and Binge Drinking in Humans

The World Health Organization (WHO) defines binge drinking as consuming at least 60 g of alcohol in one drinking episode [182]. Also, the National Institute of Alcoholism and Alcohol Abuse (NIAAA) defines binge drinking as a "pattern of drinking that brings alcohol concentration to 0.08 g/dL", a concentration reached in about two hours after five drinks (70 g of alcohol) for men and after four drinks (56 g of alcohol) for women [183]. These definitions describe binge drinking as episodic and acute alcohol intoxication. Binge drinking is widespread in adolescents and young adults and it causes neurodevelopmental impairments, violence, injuries, family, school and psychiatric problems and subsequent alcohol dependence [184–186]. Heberlain et al. studied the acute effects of alcohol intoxication in patients suffering from alcohol dependence. In this study, acute alcohol intoxication was related to an increase in NGF plasma levels, which decreased after withdrawal. These results indicate that NGF plasma levels may increase to block the toxic effects of alcohol due to acute intoxication [154].

NGF acts as a soluble mediator for different immune cells and plays a relevant role in the immune response [81]. Moreover, significant evidence indicates that ethanol abuse increases the risk of infection by impairing the ability of monocytes/macrophages to act as antigen-presenting cells and by altering the synthesis and release of cytokines [187–192]. To investigate whether or not ethanol has similar effects on NGF synthesis in blood cells as in the neurons of the CNS, Caroleo et al. studied the effects of acute ethanol exposition in blood monocyte-derived macrophages cultured in vitro [193–195]. These cells, isolated from peripheral blood of healthy donors, in basal conditions produce little NGF which increases if

they are activated by treatment in vitro with lipopolysaccharide (LPS). The acute exposure of LPS-activated cultures to ethanol alters NGF synthesis, reduces the expression of TrkA and the release of TNF-α levels [195]. Acute ethanol intoxication induces also an increase in IL-10 synthesis, an anti-inflammatory cytokine that decreases the production of proinflammatory cytokines like TNF-α and IL-1 [191].

NGF and binge drinking in animals

Adolescence represents a period in which a significant refinement of the neurotransmitter system allows the transition of an immature brain to a more mature and efficient adult brain [196]. In particular, during these modifications, cholinergic neurons are subject to maturational refinement and reinforcement of cholinergic projections [197–201]. Unfortunately, in humans, this period is also identified with a higher frequency of alcohol binge drinking which causes loss of cholinergic neurons, loss of choline acetyltransferase (ChAT), an increase in NF-κB p65 phosphorylation and an increase in its proinflammatory target genes like TNF-α [202–211]. These last findings suggest that loss of cholinergic neurons may be due to the activation of neuroimmune signaling as a result of binge drinking [212]. Recently, Vetreno et al. showed that adolescent intermittent ethanol (AIE) treatment, used as a model of human adolescent binge drinking, brought to a decrease in ChAT in neurons of the basal forebrain of adult rats and a decrease in high-affinity NGF receptor TrkA and a decrease in low-affinity receptor p75NTR, both used as markers of cholinergic neurons. Additionally, loss of ChAT after AIE treatment was associated with an increase in pNF-κB p65, a neuroimmune marker, in the basal forebrain of adult rats. These changes are blocked by the anti-inflammatory drug indomethacin, a non-steroidal molecule able to block neuroimmune signaling [212]. Together, these findings indicate that adolescent binge drinking induces neuroimmune signaling which may cause loss of cholinergic neurons in the adult basal forebrain.

The cholinergic system in the hippocampus has an essential role in spatial cognition and is a target site of EtOH neurotoxicity [213,214]. Different hypotheses have been used to explain ethanol-induced damage of cholinergic neurons, one of these is the direct toxicity of ethanol or its metabolite acetaldehyde (AcH). To verify this latter hypothesis, Jamal et al. studied in the hippocampus the effects of acute ethanol intoxication in Aldh2 knockout (Aldh2-KO) mice that lack the expression of human mitochondrial aldehyde dehydrogenase type 2 (ALDH2) [215]. Acute ethanol intoxication (2 g/kg) caused a decrease in ChAT expression in Aldh2-KO mice, an increase in acetylcholinesterase (AChE) expression and no modification in the expression of NGF in both Aldh2-KO and WT mice [215]. These findings indicate that a low level of ChAT and a high level of AChE can lead to a reduction in acetylcholine and a consequent decrease in cognitive function. Instead, an increase in the expression of NGF with consequent trophic support may only occur after chronic exposition to ethanol.

NGF and Fetal Alcohol Spectrum Disorders

The discovery of alcohol as a teratogen molecule in 1973 and the finding on long-term effects of prenatal alcohol exposure indicates that consuming alcohol during pregnancy can alter fetal development [216–218]. The effects of alcohol on fetus sphere from the absence of damage to abortion, including Fetal Alcohol Spectrum Disorders (FASD) such as Fetus alcohol syndrome (FAS), partial FAS (PFAS), associated neonatal congenital defects (Alcohol-Related Birth Defects, ARBD) and neurological development disorders (Alcohol-Related Neurodevelopmental Disorders, ARND) [219,220]. FAS is the main cause of mental retardation in the world but is also the foremost preventable cause of neurobehavioral and developmental abnormalities [221]. FAS can be suspected in neonatal age by the presence of microcephaly and typical facial dysmorphism [222–224]; during childhood, beyond the signs already described, psychomotor retardation, behavioral disorders, attention and concentration

problems can be detected [224]; during adolescence, in addition to the previous signs behavioral, scholastic and social problems can be added [224]. Even though FASD is a frequent cause of disability, the exact incidence and prevalence of FASD in the world is not clear. This underestimation of the problem leads to an incorrect diagnosis and doesn't help the possible rehabilitation of many children with mental retardation [224]. Paternal alcohol consumption may also induce changes in the newborns as shown in humans and also in animal models [225,226].

Alcohol consumption during pregnancy induces neuronal cell death in the offspring by altering the synthesis and uptake of NGF and the distribution of his receptors [227–230]. In rodents, chronic alcohol consumption reduces NGF levels in the hippocampus and reduces the ChAT activity in the septum, hippocampus and cortex [231]. Similar results were obtained when an acute administration of ethanol to pregnant rats was sufficient to change in the offspring the physiological levels of NGF in the hippocampus and the localization of p75 in the septum [194]. Alcohol consumption during pregnancy can also damage the proliferation and differentiation of neurons leading to deficits in the limbic area responsible for cognitive activity [232,233]. In the entorhinal cortex, a region of the hippocampal formation, the exposition of pregnant mice to ethanol during mouse fetal life causes neuroanatomical and neurofunctional alterations during neurogenesis of the entorhinal cortex. These morphological modifications are associated with altered levels of NGF in the entorhinal cortex of prenatal alcohol-treated mice [234]. NGF changes in the mouse brain limbic system were also disclosed following paternal alcohol consumption [3,226].

Other growth factors may regulate the survival, differentiation and maintenance of cellular phenotype [2,235,236]. NGF, hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) are the main growth factors controlling the physiopathology of the brain, liver, kidney, and are altered in mice early exposed to ethanol [3,46,229,237]. In aged

mice, the exposition to ethanol during fetal life and lactation affects these growth factors: NGF was higher in the frontal cortex and hippocampus, HGF was increased in the hippocampus and frontal cortex, and VEGF was elevated in the frontal cortex and in the hippocampus and lower in the liver [238].

During pregnancy, maternal alcohol consumption can indirectly disrupt fetal development by altering the function and interactions of maternal and fetal hormones [239] and NGF plays a crucial role in the development, maintenance and functions of the endocrine system [4,240–244]. Ceccanti et al. showed that early administration of ethanol and wine during mouse fetal life causes long-lasting changes in the thyroid, testis and adrenal glands of aged mice. In particular high levels of NGF were observed in the thyroid and testis of aged mice when exposed only to ethanol, while in the adrenal glands high levels of NGF were observed when they are exposed to both ethanol and red wine [245].

Conclusion

AUD is one of the principal cause of diseases and disabilities in the world. Furthermore, the teratogen effects of prenatal alcohol exposure are known to cause severe cognitive and behavioral deficits due to functional and anatomical changes within the brain. In the past years, different researches have described the role of NGF as a trophic and protective factor against the cytotoxic damage induced by ethanol. In particular, important findings show increased NGF plasma concentrations during alcohol intoxication and a protective role against neuronal degeneration. Future studies will help to understand these mechanisms and to develop new therapeutic strategies based on NGF trophic and protective action.

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Figure Captions

Figure 1

Binding of NGF to TrKA.

NGF binding to TrkA starts the homodimerization of the receptor and the autophosphorylation of tyrosine residues within the intracellular domains. Phosphorylation allows recruitment of adapter proteins that have src-homology-2 (SH-2) or phosphotyrosine-binding motifs. Adapter proteins, after phosphorylation, start intracellular signaling cascades involved in cell survival.

Figure 2

Main roles of NGF in physiological and pathological conditions.

NGF is involved in growth, survival, proliferation and protection of neurons in the central and peripheral nervous system. NGF is also associated with functional activities of the immune and endocrine systems and due to its neuroendocrine activity, NGF is implicated in the maintenance of the physiological homeostasis. NGF play also a key role in neuroinflammation, cardiometabolic diseases, alcoholism, autoimmunity, inflammatory disorders, allergic diseases and aging. Furthermore, NGF contributes to the acquisition of male and female reproductive capacity.

Figure 3

Effects of acute alcohol intoxication on the ventral tegmental area and nucleus accumbens.

The ventral tegmental area is involved in pleasure and reward through dopaminergic projection towards the nucleus accumbens, important brain area playing subtle roles in the cognitive processing of reward, pleasure and addiction. In the ventral tegmental area, alcohol stimulates GABAergic neurotransmission, modulates the expression and activity of acetylcholine receptors (nAChR) and activates the release of dopamine and opioid peptides which act on the neurons of the nucleus accumbens. These actions enhance dopaminergic transmission. Furthermore, alcohol inhibits the release of the excitatory neurotransmitter glutamate from nerve terminals that act on neurons in the nucleus accumbens.

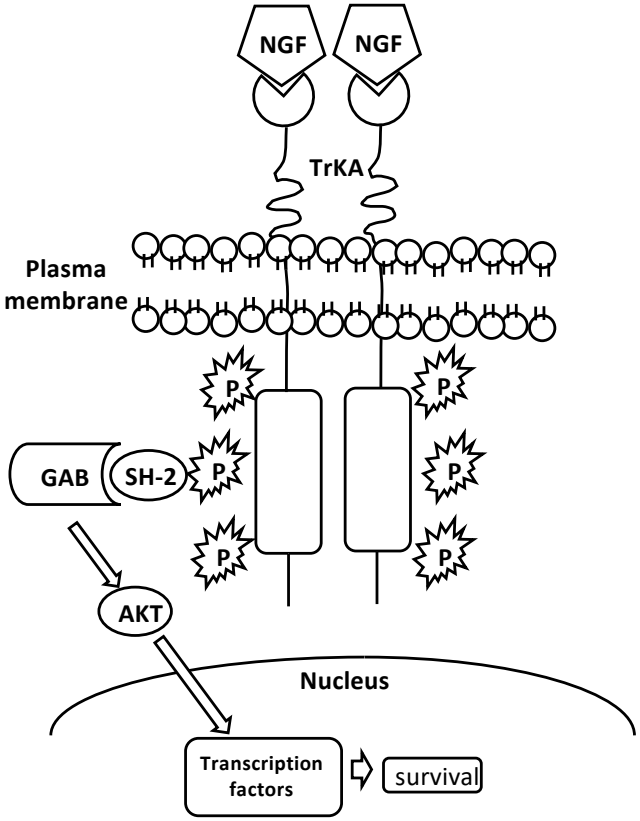
NGF
NGF
TrKA
Plasma membrane
P
P
P
P
P
P
GAB
SH-2
AKT
Nucleus
Transcription factors
survival

Figure 1

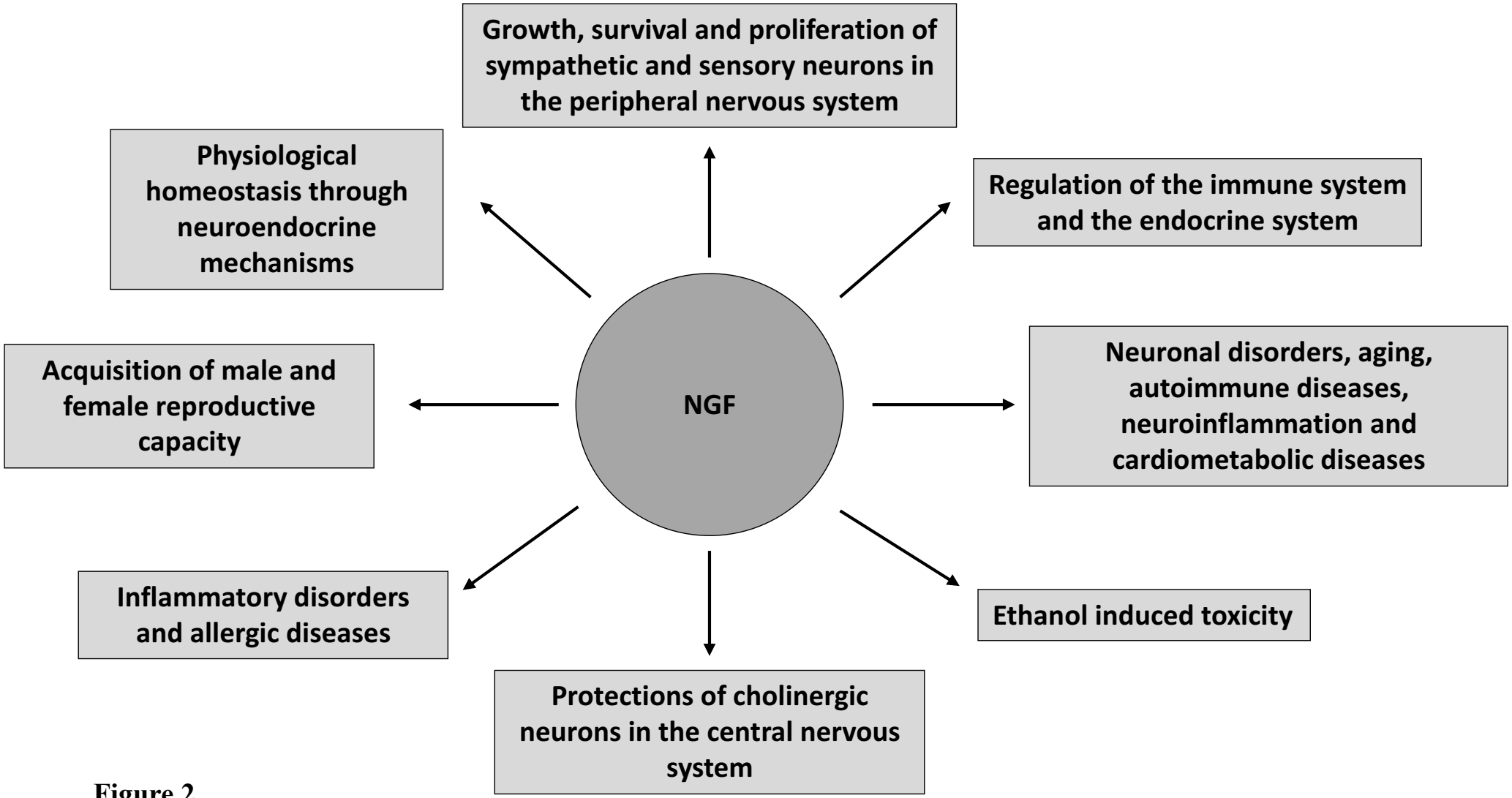
Growth, survival and proliferation of sympathetic and sensory neurons in the peripheral nervous system
Physiological homeostasis through neuroendocrine mechanisms
Regulation of the immune system and the endocrine system
Acquisition of male and female reproductive capacity
NGF
Neuronal disorders, aging, autoimmune diseases, neuroinflammation and cardiometabolic diseases
Inflammatory disorders and allergic diseases
Ethanol induced toxicity
Protections of cholinergic neurons in the central nervous system

Figure 2

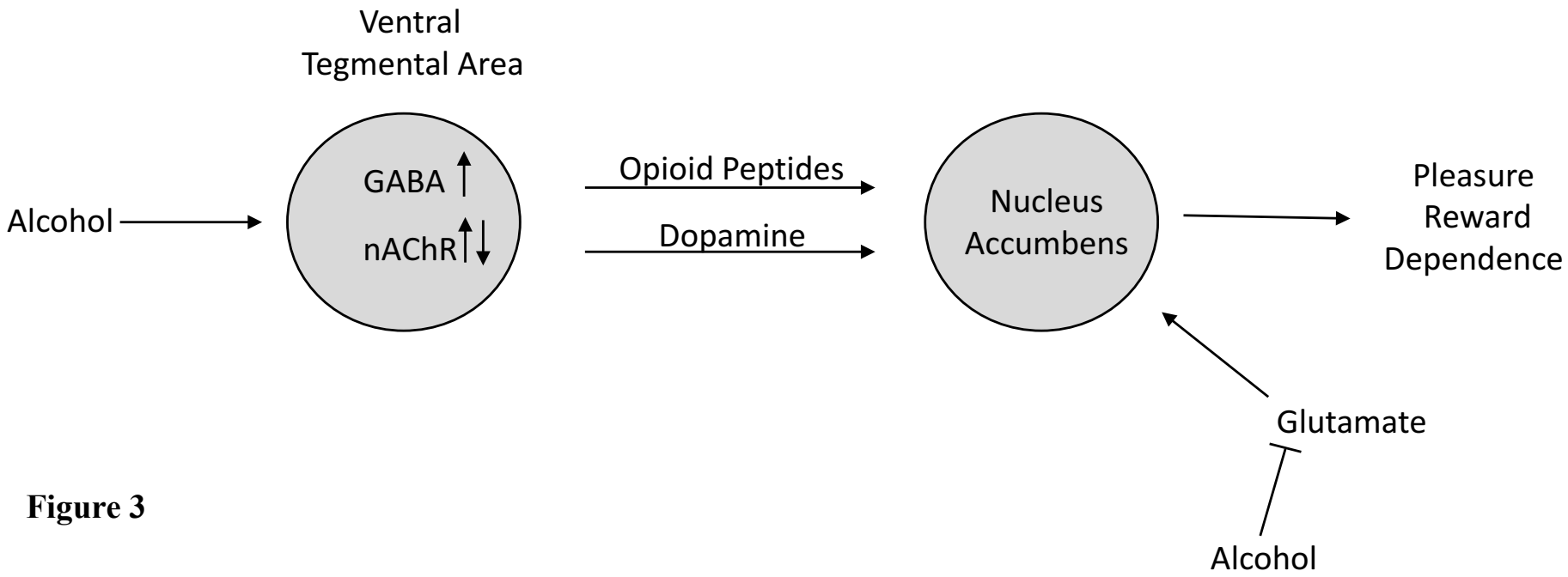
Ventral
Tegmental Area
Alcohol
GABA
nAChR
Opioid Peptides
Dopamine
Nucleus
Accumbens
Pleasure
Reward
Dependence
Glutamate
Alcohol

Figure 3