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REVIEW

NEUROPROTECTIVE ROLE OF TESTOSTERONE IN THE NERVOUS SYSTEM

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Testosterone – the gonadal sex steroid hormone plays an important role in the central nervous system (CNS) development. One of the less known testosterone actions is neuroprotection. There are some evidences supporting the hypothesis that testosterone may act protectively in neurodegenerative disorders, e.g. Alzheimer's disease (AD), mild cognitive impairment (MCI) or depression. Androgens alter also the morphology, survival and axonal regeneration of motor neurons. These hormones accelerate the regeneration of hamster facial nerve and anterior tibialis sciatic nerve in rabbits following crush axotomy. Androgens exert trophic action in laryngeal motor nucleus of *Xenopus laevis*. Testosterone is linked to an increase in neuron somal size, neuritic growth, plasticity and synaptogenesis in both motoneurons of the spinal nucleus of the bulbocavernosus and several populations of pelvic autonomic neurons. The hormone reduced the extent of spinal cord damage *in vitro*. There are also evidences against the neuroprotective action of testosterone. Testosterone does not protect against methamphetamine-induced neurotoxicity of the dopaminergic system in mice and does not provide significant neuroprotection against glutamate-induced neurotoxicity. Androgens do not prevent striatal dopamine depletion induced by 1-methyl-4-phenyl--1,2,3,6-tetrahydropyridine (MPTP) in mice. Although the role of testosterone in the CNS is still poorly understood, accumulating evidence suggests that testosterone may create a future treatment for MCI and related cognitive diseases, including dementia and may influence motor neuron regeneration in adulthood. Androgen replacement therapy in selected male populations may hold therapeutic promise for the prevention and/or treatment of age-related disorders associated with neuronal injury.

Key words*: testosterone, neuroprotection, androgen receptor*

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Abbreviations: 3α-diol – *5α-androstane-3α, 17β-diol*, Aβ – *4 kDa amyloid-β peptide*, AD – *Alzheimer's disease*, ALS – *amyotrophic lateral sclerosis*, APP – *amyloid precursor protein*, AR – *androgen receptor*, CNS – *central nervous system,* DHEA – *dehydroepiandrosterone,* DHEAS – *dehydroepiandrosterone sulfate,* DHT – *dihydrotestosterone,* DHPT – *dihydrotestosterone propionate,* FMN – *facial motoneuron,* GFAP – *glial fibrillary acidic protein,* HFMN – *hamster facial motoneuron,* HRP – *horseradish peroxidase,* MCI – *mild cognitive impairment,* MPTP – *1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine,* PMN – *pudendal motoneuron,* PREG – *pregnenolone,* PREGS – *pregnenolone sulfate,* PROG – *progesterone,* sAPPa – *100 kDa nonamyloidogenic soluble APP,* SHBG – *sex hormone binding globulins,* SMN – *sciatic motoneuron,* SSRI – *selective serotonin reuptake inhibitor,* TP – *testosterone propionate*

Introduction

Testosterone, the gonadal sex steroid hormone, has various effects on numerous body tissues, including the brain. Beyond its reproductive function, this hormone is responsible for increased muscle mass, sexual function and libido, body hair and decreased risk of osteoporosis [37]. Testosterone also takes part the in nervous system development. Gender-specific morphological and behavioral patterns of the adult are determined by the presence or absence of this hormone during certain critical periods of the central nervous system (CNS) development [15]. Testosterone is physiologically secreted by testes and adrenal glands and transported by the sex hormone binding globulins (SHBG) and albumins. About 60–70% of testosterone is tightly bound to SHBG, whereas the remaining 30–40% is bound loosely to albumin. Only 0.5–2% of testosterone is free [30]. There is difference between female and male SHBG. It influences the circulating testosterone [50]. SHBG level increases with age in males, therefore, the free testosterone level is reduced and reaches its lowest levels in males above 60 years of age [48].

Testosterone acts *via* androgen receptors (ARs). Regulation of AR protein and/or AR mRNA by androgens has been observed in mammals in multiple androgen-responsive tissues, such as the brain [13], prostate [44], testes [9], ovary [76] and adrenal glands [6]. ARs are found in neurons throughout the brain [4]. The distribution of these receptors in mammals is identified using biochemical and immunocytochemical methods. There are sex-related differences in AR distribution [46]. Sexual dimorphism in total AR protein concentration in mouse brains was investigated. Males have a higher concentration of AR protein than females [51]. In male rats, neuroanatomical areas comprising nuclear AR include the medial hypothalamic area, ventromedial and dorsomedial nucleus, preoptic area, arcuate nucleus, amygdala and regions of the hippocampus, arcuate-median eminence and bed nucleus, with the greatest AR density reported in the preoptic and hypothalamic areas, septum and amygdala of these animals [68].

One of the less known testosterone action is neuroprotection. By definition, the neuroprotection is an effect that may result in salvage, recovery or regeneration of the nervous system, its cells, structure and function. Testosterone, as the endogenous agent, may in the free form cross the blood-brain barrier [30] and influence neuronal cells. Testosterone might act directly through androgen pathway or indirectly *via* conversion to estrogen. To distinguish androgen from estrogen neuroprotection, Pike [66] has examined whether testosterone protection could be inhibited by droloxifene, an estrogen receptor antagonist. He observed that neuroprotection afforded by testosterone was not reduced by droloxifene. This finding suggests that testosterone shares with estrogen the ability for neuroprotection but testosterone induces this cellular action through a separate mechanism.

The cellular effects of testosterone can be grouped into genomic and nongenomic categories. Genomic effects relate to transcription and translation of new gene products and often require many hours to fully develop. Nongenomic effect occurs very rapidly and involves ion movements and/or initiation of signal transduction cascades [19]. Pike [67] has evaluated how much time is required for testosterone exposure to affect neuroprotection. He observed that, when a genomic mechanism is involved, protection should be retained if the hormone is present only during the pretreatment. Functional levels of the regulated gene product affecting viability should remain after hormone removal. He also examined [67] whether testosterone neuroprotection is affected by the antiandrogen, flutamide. Flutamide antagonizes genomic actions resulting from activation of AR. Some evidence

suggests that flutamide may fail to block some non-genomic AR-mediated effects [5, 65]. Pike [67] has observed that flutamide failed to affect testosterone-induced neuroprotection. In addition, some observations suggest that flutamide and other antiandrogens not only failed to block AR-dependent testosterone actions but mimicked them as well [65].

Recent data suggest that testosterone may also exert neurotrophic actions. For example, Beyer et al. [7, 8] and Lustig [53] have observed neuronal differentiation and increase in neurite outgrowth after activation of androgen pathways in the cultured neural cells. Beyer et al. [7] prepared gender-specific primary cell cultures from embryonic day 15 mouse hypothalamus and cortex. Aromatase activity was higher in male compared with female tissues in the absence of sex steroids and significantly increased in males and females after testosterone treatment.

Other experiments in male rodents suggest that testosterone is linked to an increase in neuron somal size, neuritic growth, plasticity and synaptogenesis in both motoneurons of the spinal nucleus of the bulbocavernosus [21, 54] and several populations of pelvic autonomic neurons [38]. The animals tested in this experiments were prepubertally and postpubertally castrated. Ogata et al. [61] have reported that testosterone protected spinal cord neurons against neuronal damage induced by glutamate. The hormone reduced the extent of spinal cord damage *in vitro*.

There are also evidences against the neuroprotective action of testosterone. Myers et al. [58] have reported that testosterone, in contrast to estrogen, does not function as a neuroprotectant agent against methamphetamine-induced neurotoxicity of the dopaminergic system in mice. Co-infusion of testosterone and estrogen inhibited the action of the latter in gonadectomized female and male mice and failed to produce change in methamphetamine-evoked dopamine output within superfused striatal tissue fragments. Dluzen et al. [16] have observed that estrogen administration prevents reduction of dopamine concentration in corpus striatum in castrated male and female mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Ekue et al. [17] have revealed that testosterone and dihydrotestosterone (DHT), in contrast to 17β-estradiol, do not prevent mice against striatal dopamine depletion induced by MPTP. There is also evidence that testosterone did not provide significant neuroprotection against glutamate-induced neurotoxicity [70]. Glutamate-induced neurotoxicity is a model of oxidative stress, which plays an important role in Parkinson's disease. In contrast, estradiol protected mesencephalic neurons against neuronal death induced by glutamate.

The question regarding the neuroprotective activity of anabolic steroids has not yet been fully answered. It is known that e.g. epitestosterone blocks the action of testosterone and has antiandrogenic activity. Epitestosterone administration reduced the effect of testosterone propionate (TP) on body weight, weight of seminal vesicles and kidneys in castrated male mice [72]. Hammond at al. [27] have tested effect of epitestosterone on primary cultures of human neurons induced to undergo apoptosis by serum deprivation. Epitestosterone showed only a slight neuroprotective effect not related to AR.

Some studies show that also neurosteroids, which are synthesized within the nervous system by neurons and glial cells [3] may exert neurotrophic actions. Some neurosteroids show an anticonvulsant effect. Intraperitoneal administration of androsterone protects mice agains NMDA-induced seizures and mortality [12]. A similar anti-seizure effect has 5α-androstane-3α, 17β-diol (3α-Diol) in the kainic acid model of epilepsy. Frye and Reed [24] reported that subcutaneous administration of 3α-Diol prior to kainic acid injection decreases the number and duration of partial and full seizures in female ovariectomized rats.

Implication of testosterone in age-related diseases

Progressive dysfunction and death of neurons characterize neurodegenerative diseases. There are some evidences supporting the hypothesis that testosterone may act protectively in some neurodegenerative disorders: Alzheimer's disease (AD), mild cognitive impairment (MCI) or depression.

As a normal consequence of aging, men gradually face a reduction in testosterone level. Decline of the testosterone level might induce atrophy and impair compensatory responses in an insulted tissue. As a consequence, the aging brain becomes more susceptible to neurodegeneration. Testosterone exerts influence on glial fibrillary acidic protein (GFAP). GFAP is abundant astrocyte-specific intermediate filament protein [60]. Accumulating evidences in rodents and humans suggest that GFAP increases during normal aging in several re-

gions of the brain, including the hippocampus, striatum, cerebral cortex, and white fiber tracts, such as the corpus callosum, fimbria, stria terminalis, and optic tract [59]. GFAP levels in various regions of senescent rat brains are two to three times higher than those found in young adult rats [59]. Testosterone acts primarily on neuronal receptors and any increase in GFAP expression is secondary to changes in neuronal homeostasis. The increases in GFAP expression reflect reactive astrocyte hypertrophy and thus GFAP is often used as an indicator of neurodegeneration. To support the hypothesis that testosterone may exert some effect on the adult male rat brain, Day et al. [15] have checked testosterone influence on GFAP level in 24-month-old rats. They reported that testosterone replacement reversed the age-related increase in GFAP in these rats. This is conformable to the data, which showed that increased GFAP was associated with lower testosterone concentrations in older rats [59].

MCI is a state of cognitive decline associated with negligible functional loss, often preceding AD. The data suggest a conversion rate of MCI to AD of 10–15% per year. The pathophysiology of MCI comprises significant neuronal loss in the hippocampus and lowered blood flow in the subiculum [31].

Experiments on animals show that castration may cause a loss of cognitive function. For example, sexually intact male dogs were significantly less likely than neutered dogs to progress from mild to severe cognitive impairment [28]. It is connected with presence of circulating testosterone in aging sexually intact male dogs. Testosterone slows the progression of cognitive impairment. Another experiment on SAMP8 mice showed that cognitive impairment of these mice may be connected with an interaction of aging and lowered testosterone levels. The results demonstrated that testosterone replacement in aging mice alleviated age-related impairment of learning and memory [20].

There are also randomized studies on various human populations that showed the effect of androgen substitution on cognition in men. Janowsky et al. [33] have demonstrated that testosterone enhances spatial cognition of healthy men aged 60–75 years. In this group, testosterone levels were increased to the level commonly found in young men for a 3-month period. Cherrier et al. [14] have reported that testosterone enanthate supplementation during 6 weeks improves both spatial and verbal memory in healthy older men aged 50–80 years. Another study on healthy volunteers aged 61–75 years demonstrated improved working memory following testosterone enanthate [32]. It is important to note that subjects in these investigations were healthy volunteers. They did not suffer from AD and might be in the transition into andropause. Although the role of testosterone in the CNS is still poorly understood, accumulating evidence suggests that testosterone may create a future treatment for MCI and related cognitive illnesses, including dementia [74].

AD is characterized by the deposition of the senile plaques in the affected brain. The senile plaques are formed by a 4 kDa amyloid-β (Aβ) peptide. Aβ is a proteolytic product of the membrane protein, amyloid precursor protein (APP).

There are several potential mechanisms of testosterone protection against neurodegeneration connected with AD [49]. One of them is prevention of tau protein hyperphosphorylation. Normal tau proteins are microtubular binding proteins, predominantly axonal, which stabilize the neuronal cytoskeleton. Tau protein is the neuropathological hallmark of AD. Papasozomenos [62] has demonstrated that heat shock-induced hyperphosphorylation of tau protein in the brain of orchiectomized male rats can be reduced by testosterone. The second mechanism includes changes in APP metabolism to preclude Aβ formation. It has been shown that the secretion of Aβ peptides from cerebrocortical neurons of rats is decreased after treatment with testosterone. Gouras et al. [26] indicate that repeated administration of relatively high testosterone dose increases sAPPa (100 kDa nonamyloidogenic soluble APP) and decreases Aβ release from neuroblastoma cells and primary neuronal cultures 4 days after the beginning of treatment. Goodenough et al. [25] demonstrate that use of a single physiological dose of testosterone increases secretion of sAPPa from hypothalamic cells without any changes in cellular APP. Some studies reported that androgen deprivation increased plasma Aβ levels and was associated with progressive cognitive decline in humans [1]. Another mechanism includes an increase in rate of axonal regeneration *via* selective alterations of the neuronal cytoskeleton [36]. Testosterone increases expression of nerve growth factor [77] and mediates promotion of neurite growth and interneural communication through branching and arborization [41]. Next mechanism

includes synergistic stimulation of protein synthesis in combinations with other cytokines such as insulin growth factor-1 [85]. Another way of testosterone-induced protection against neurodegeneration is connected with conversion of testosterone to estrogen [84]. There is metabolic aromatase pathway in the brain, which converts testosterone to estradiol. Aromatase has been localized mainly in the hypothalamus, the preoptic area and the limbic system. This enzymatic complex uses oxygen and NADPH to convert testosterone into estrogen. Finally, testosterone may also be beneficial through its anti-apoptotic effect. Nowadays, there is no direct evidence that testosterone affects apoptosis pathways in AD, although there is preliminary evidence suggesting that testosterone withdrawal causes an acceleration of germ cell apoptosis at specific stages of the seminiferous epithelial cycle [52].

Moreover, testosterone treatment diminishes the extent of neuronal cell death following trophic factor withdrawal [27] and adrenalectomy [23]. Finally, there is *in vitro* evidence that testosterone is conductive to attenuation of Aβ-mediated apoptosis. Pike [67] has shown that the cultured hippocampal neurons of rats can be protected by testosterone and DHT from cell death induced by AD-related β-amyloid *via* an estrogen-independent mechanism.

These all reports suggest that testosterone use may be of some benefit in prevention of AD. However, the timing of the hormone replacement therapy is limited because the gene corresponding to the androgen receptor is located on the X chromosome [10] and chromosome X inactivation increases with age [78]. Therefore, less cell response to testosterone treatment is connected with the decrease in androgen receptor amount.

Some studies show that low testosterone level may be associated with depression in aging men. Seidman and Rabkin [71] have reported clinical improvement after testosterone supplementation in men who have low testosterone levels and SSRI-refractory (selective serotonin reuptake inhibitor) depression. In these men, rapid and dramatic reduction in depressive symptoms, and increase in life satisfaction was observed. Sternbach [73] has reported that testosterone replacement therapy alleviates depression as well as verbal and spatial memory in aging men. These results suggest that testosterone effect on CNS could be antidepressant, and perhaps the hormone specifically enhances SSRI efficacy.

There are evidences that neurosteroids may compensate for the age-dependent decrease in circulating hormones. Vallee et al. [79] have reported that intrahippocampal injection of pregnenolone sulfate (PREGS) corrects memory deficits in rats. The data showed that levels of conjugated pregnenolone (PREG) in the hippocampus were reduced in 2-year-old rats with memory deficits. There are also evidences, that DHT and 3α-diol may have action in the hippocampus to improve cognitive performance in male rats [22]. Weill-Engerer et al. [81] have shown the lower level of neurosteroids in six brain regions of AD patients. They observed also the correlation between two markers of AD and level of neurosteroids. Aβ peptide level was negatively correlated with PREGS level in the striatum and cerebellum. Phosphorylated tau protein levels were negatively correlated with dehydroepiandrosterone sulfate (DHEAS) concentrations in the hypothalamus. Other data demonstrated that administration of progestins reversed the age-dependent myelin abnormalities whereas administration of androgens was without effect [2]. Ibanez et al. [29] have revealed that progesterone (PROG) administration slows remyelination of axons by oligodendrocytes after toxin-induced demyelination in old male rats. Morales et al. [56, 57] have reported that dehydroepiandrosterone (DHEA) increases physical and psychological well-being in woman and men, increases lean body mass and muscle strength in men. However, most human trials demonstrating DHEA effect on cognitive performance have failed. Only one study showed antidepressant and cognition-enhancing effects of DHEA in major depression [83].

Implication of testosterone in motor neuron disease

Motor neuron diseases are characterized by progressive weakness affecting skeletal muscle groups.

It is known that steroid hormones produce permanent sexual dimorphism in neuron number, morphology, and connectivity during development. Steroids may influence also neurons in adulthood. Androgens alter the morphology, survival, and axonal regeneration of both sexually and nonsexually dimorphic motor neurons. The molecular mechanism responsible for the effect of androgens on motor neurons is poorly understood. Accumulating

evidence suggests that most motor neurons contain AR [69] and much of the effects of gonadal steroids is exerted by a receptor-mediated mechanism [18].

Brooks et al. [11] have created an *in vitro* model system to better understand the effects of androgens on lower motor neurons. Motor neuron hybrid cells in the presence of androgen develop larger cell bodies and broader neuritic processes. In addition, androgen promotes the survival of AR-expressing cells under low-serum conditions. The data showed a direct trophic effect of androgens on lower motor neurons, mediated through the AR expressed in this population of neurons.

Kurz et al. [45] have examined a group of androgen-sensitive motoneurons mediating copulatory functions in rat males. The data showed that a decrease in androgen levels after castration produced dramatic structural changes, decreasing the dendritic length and soma size of these motoneurons. Changes in dendritic morphology may influence synaptic connectivity and neuronal function. Kurz et al. [45] have reported that these changes are reversed by androgen replacement. After 4-week testosterone treatment, dendritic arbor per cell and soma size were restored to normal levels. These results indicate that androgens may regulate neuronal function in adulthood by influence on length and size of dendrites of motoneuronal somas.

Hamster facial nerve regeneration

Tanzer and Jones [75] have reported the accelerated functional recovery from facial paralysis induced by unilateral facial nerve crush axotomy in adult male hamsters after administration of TP. Systemic administration of TP resulted in an approximately 30% increase in the rate of facial nerve regeneration [41, 42]. Kujawa et al. [43] have assumed that TP exerts its accelerative effects on facial nerve regeneration through a receptor-mediated mechanism. They used nonsteroidal antiandrogen, flutamide, to test that hypothesis. The result of this study demonstrates that exposure to flutamide completely abolished the TP-induced accelerative effects on facial nerve regeneration rate. Thus, the result supports the hypothesis that mechanism by which TP acts upon hamster facial motor neuron's (HFMN) regeneration is receptor-mediated. Another study on the castrated male hamsters showed that exposure to dihydrotestosterone propionate (DHPT) (nonaromatizable androgen which cannot be converted to estrogen) at the time of nerve injury accelerated the regeneration of leading axons, following facial nerve crush axotomy. DHPT application resulted in an approximately 40% increase in the rate of regeneration, with an associated prolongation in the delay time before sprouting occurred [75]. Jones et al. [34] have examined regeneration of hamster facial motoneuron system (FMN), rat sciatic motoneurons (SMN) and rat pudendal motoneurons (PMN). They reported that exogenous administration of testosterone immediately after nerve injury impacted positively on functional recovery through actions mediated by the AR. The authors suggest that mechanism, by which steroidal enhancement of the regenerative properties of the injured motoneurons occurs, may involve pre-existing AR, modulation of the cellular stress response and heat shock proteins.

It is interesting that steroid hormones regulate ribosomal gene expression and nuclear ultrastructure in target tissues [35]. TP administration during facial nerve injury results in an increase in ribosomal levels in FMN [40]. Transcriptional activation of the rRNA gene occurs almost immediately and is maintained regardless of the presence or absence of the steroid. The rRNA transcription is rapidly activated by axotomy and processing is temporarily stalled. After TP administration, the time interval between rRNA transcription and processing is significantly shortened [39].

Testosterone and amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder, which affects upper and lower motoneurons in the motor cortex, brainstem and anterior horn of the spinal cord. The degeneration of these cells causes a progressive weakness and atrophy of skeletal muscles and finally leads to paralysis and death. There are many epidemiological risk factors, such as exposure to heavy metals, electrical or mechanical trauma, vigorous athletic activity, which have influence on occurrence of ALS. But there are also two widely accepted risk factors: age and sex [63]. ALS is a disorder of middle age with a peak of incidence between 45 and 70 years. There is an about 2-fold higher incidence of ALS in men compared to women [63]. A role of sex hormones in the ethiopathogenesis of this disorder has been suggested. Militello et al. [55] have shown that serum level of

free testosterone is significantly decreased in both men and women with ALS.

In ALS, a progressive loss of motoneurons, with the notable exception of cranial motoneurons III, IV, and VI is observed [82]. This disease may involve an AR deficiency because, what is interesting, III, IV and VI motoneurons normally lack ARs.

Laspada et al. [47] have reported that mutations in the AR gene exist in the familiar forms of ALS.

Anterior tibialis sciatic nerve regeneration

Vita et al. [80] have tested the effect of testosterone on the reinnervation of the anterior tibialis sciatic nerve following crush in rabbits. The data show that there is accelerative effect of testosterone on the regeneration process.

Hypoglossal nerve regeneration

Yu and Srinivasan [87] have reported that administration of TP at the time of hypoglossal nerve injury increases the number of horseradish peroxidase (HRP)-labeled motoneurons in the hypoglossal nucleus at postoperative time points. Yu [86] investigated whether or not administration of testosterone to male rats would promote axonal outgrowth following hypoglossectomy and determined the ability of regenerating axons to maintain or regain their original pathways. In this study, HRP axonal transport method was used. The results have shown that after regeneration of the transected hypoglossal nerve, the somatotopic representations of the hypoglossal nucleus are no longer present. The random growth of the axons into branches of the nerve in rats was irrespective of the treatment.

N. IX-X axotomy

Pérez and Kelley [64] have examined the laryngeal motor nucleus (N. IX-X) of *Xenopus laevis* to determine whether changes in AR expression are associated with trophic actions of androgens. Vocal neuromuscular system of *Xenopus laevis* is one of the most strongly androgen-regulated system in vertebrates. The data indicate that DHT-treated animals have larger number of AR mRNA-expressing cells in n. IX-X 1 month after axotomy. Androgen up-regulates expression of AR mRNA in n. IX-X. Five months after axotomy, the number of AR mRNA-expressing cells was decreased in the n. IX-X axotomized animals but DHT treatment mitigated the cell loss. These results suggest a trophic function of androgen in motor nuclei.

Data on neuroprotective activity of testosterone are shown in Table 1.

Table 1. The neuroprotective effects of testosterone in humans and animals

Men	Improvement of spatial cognition [33]
	Improvement of spatial and verbal memory [14]
	Improvement of working memory [32]
	Reduction of depressive symptoms [71]
Rats	Decrease in GFAP level in brain [15]
	Protection of hippocampal neurons from cell death [67]
	Increase in dendrtic length and soma size of motoneurons [45]
	Increase in the number of HRP-labeled motoneurons in hypoglossal nucleus [87]
	Reduction of heat shock-induced hyperphosphorylation of tau proteins in brain [62]
	Decrease in β-amyloid peptides secretion from cerebrocortical neurons [26]
Mice	Improvement of cognitive impairment [20]
Dogs	Improvement of cognitive function [28]
Hamsters	Acceleration of recovery from facial paralysis [42]
	Increase in the rate of facial nerve regeneration [75]
Rabbits	Acceleration of reinnervation of the tibialis sciatic nerve [80]
Xenopus laevis	Trophic action on laryngeal nucleus [64]

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