Review Article

nNOS-mediated protein-protein interactions: promising targets for treating neurological and neuropsychiatric disorders

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Abstract

Neurological and neuropsychiatric disorders are one of the leading causes of disability worldwide and affect the health of billions of people. Nitric oxide (NO), a free gas with multitudinous bioactivities, is mainly produced from the oxidation of L-arginine by neuronal nitric oxide synthase (nNOS) in the brain. Inhibiting nNOS benefits a variety of neurological and neuropsychiatric disorders, including stroke, depression and anxiety disorders, post-traumatic stress disorder, Parkinson's disease, Alzheimer's disease, chronic pain, and drug addiction. Due to critical roles of nNOS in learning and memory and synaptic plasticity, direct inhibition of nNOS may cause severe side effects. Importantly, interactions of several proteins, including post-synaptic density 95 (PSD-95), carboxy-terminal PDZ ligand of nNOS (CAPON) and serotonin transporter (SERT), with the PSD/Disc-large/ZO-1 homologous (PDZ) domain of nNOS have been demonstrated to influence the subcellular distribution and activity of the enzyme in the brain. Therefore, it will be a preferable means to interfere with nNOS-mediated protein-protein interactions (PPIs), which do not lead to undesirable effects. Herein, we summarize the current literatures on nNOS-mediated PPIs involved in neurological and neuropsychiatric disorders, and the discovery of drugs targeting the PPIs, which is expected to provide potential targets for developing novel drugs and new strategy for the treatment of neurological and neuropsychiatric disorders.

Keywords: nNOS, PSD-95, CAPON, SERT, protein-protein interaction, neurological and neuropsychiatric disorder

Introduction

Neurological diseases are a group of disorders or abnormalities in the nervous system including the brain, spinal cord and neurons, commonly including stroke, epilepsy, Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and multiple sclerosis, chronic pain. Cerebral disorders that often cause psychiatric symptoms, also known as mental disorders or emotional disorders, including schizophrenia, bipolar disorder, major depressive disorder (MDD), anxiety disorder,

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attention deficit hyperactivity disorder, post-traumatic stress disorder (PTSD), addictive disorders, *etc*. Neuropsychiatric disorders mainly affect cognition, emotion and behavior. Although neurological and neuropsychiatric disorders are two different types of diseases, their pathogenesis and pathophysiology often share underlying organic dysfunction and biological signaling pathways[1], in which nNOS and nNOS -mediated protein-protein interactions (PPIs) are critical.

Nitric oxide (NO), a freely diffused gaseous molecule, has long been proven to play a critical physiological role as a second messenger, especially in the central nervous system (CNS)[2]. There are three isozymes of NO synthase (NOS), namely neuronal NOS (nNOS or NOS-I), inducible NOS (iNOS or NOS-II) and endothelial NOS (eNOS or NOS-III)[3]. Notably, the highest level of NO in the CNS is derived from nNOS that is mainly expressed in neurons[4]. The nNOS is a Ca^{2+}-dependent constitutive synthase, and its activity is strictly regulated by N-methyl-D-aspartate receptor (NMDAR)-mediated changes in the concentration of intracellular Ca^{2+}[3,5]. nNOS functions in the CNS not only through producing NO and peroxynitrite but also *via* mediating several PPIs in neurons[3,5–7]. Growing evidence indicates that nNOS activation and nNOS-mediated PPIs are substantially involved in the pathophysiology of a variety of neurological and neuropsychiatric disorders. In the past 20 years, research in our lab has been focusing on nNOS field. The basic background, research history and the role of nNOS in physiology and pathology have been detailedly discussed in our previous reviews[3,5–6]. This review is intended to present the advances of nNOS-mediated PPIs research in neurological and neuropsychiatric disorders and drug discovery over the past few years.

nNOS-mediated PPIs

The active enzyme form of nNOS is dimerization and each nNOS monomer contains a reductase domain and an oxygenase domain. Notably, nNOS contains a N-terminal post-synaptic density (PSD)/Disc-large/ZO-1 homologous (PDZ)-binding domain. Owing to the PSD domain that is structurally different from its isozymes, nNOS can be anchored to specific subcellular structures through mediating PPIs[5]. Scaffold proteins often contain several PDZ domains and are essential backbones for organization of supramolecular signaling complexes[8–9]. The PDZ domains function by binding to C-terminal residues of their protein ligands in scaffold proteins[10–11]. It has

been known that a variety of proteins bearing PDZ domains can interact with the PDZ domain of nNOS, influencing the subcellular localization and activity of nNOS in the brain[3]. Postsynaptic density protein 95 (PSD-95) is a pivotal postsynaptic scaffold protein with three PDZ domains in excitatory neurons. PDZ2 domain of PSD-95 binds directly to nNOS PDZ, and the interaction makes nNOS localize to the PSD region, which is significant for synaptic plasticity[5]. The nNOS PDZ can interact with the carboxy-terminal PDZ ligand of nNOS (CAPON), a scaffolding protein that positively regulates spine density and facilitates NO-mediated modification of synaptic plasticity[12–15]. Serotonin transporter (SERT), a protein that modulates serotoninergic signaling by uptaking serotonin (5-HT) from the synaptic cleft into presynaptic neurons, is a primary target of therapeutic drugs used in the treatment of MDD, anxiety disorder and PTSD. Recently, Chanrion and colleagues demonstrated that nNOS can interact with the C terminus of the SERT[7]. The interaction of SERT with nNOS is critical for a reciprocal modulation of serotonergic signaling. More and more evidence shows that nNOS-mediated PPIs are implicated in various neurological and neuropsychiatric disorders, offering novel therapeutic targets[12,16]. For a long time, PPIs are considered to be "undruggable", as protein interfaces with daunting large and flat interfacial areas are very different from traditional targets. However, clinical successes of drugs targeting PPIs have challenged that notion in drug discovery[17].

nNOS-mediated PPIs and neurological diseases

Stroke is the most common cause of disability, and one of the leading causes of death in the world. There have been dozens of studies reporting that variants in nNOS gene may contribute to increased ischemic stroke susceptibility[18–19]. A large amount of NO is produced within minutes after ischemic stroke, resulting in a cascade of excitotoxicity reactions[20]. The overproduction of NO is caused by overstimulation of NMDARs[3,5]. Based on this, it is possible to alleviate ischemic brain damage by blocking NMDARs and inhibiting nNOS activity[21]. However, directly inhibiting nNOS or NMDARs may cause severe side effects because of their roles in learning and memory and synaptic plasticity[16]. Moreover, selectively inhibiting nNOS may worsen neuronal injury in the late stages of stroke, as nNOS inhibitors can bring about the induction of iNOS[22–23].

PSD-95 binds both NMDARs and nNOS at

excitatory synapses through their PDZ domains, forming a tight ternary complex. Interestingly, stroke induces nNOS migrating from the cytosol to the cell membrane, facilitating its binding to PSD-95[3]. The NMDAR-dependent nNOS-PSD-95 association is crucial for neuronal death at the acute stage of stroke[16]. The key structural basis of nNOS-PSD-95 association is an intra-nNOS salt bridge between Asp62 of PDZ domain and Arg121 of β-finger domain. The disruption of salt bridge melts down the β-finger structure and prevents its interaction with PSD-95. Moreover, residues Leu107 to Phe111 on the β-finger of nNOS contribute to conformational changes induced by their binding to PSD-95 PDZ2[24]. Based on this, we designed and developed small molecule nNOS-PSD-95 inhibitor ZL006 and found that dissociating nNOS-PSD-95 with ZL006 can prevent ischemic damage after stroke without affecting NMDARs function and catalytic activity of nNOS[16]. Follow-up studies from other labs not only confirm our findings[25–28] but also show significant beneficial effects of ZL006 on neuronal atrophy and synapse loss[29]. More interestingly, our study suggests that association of nNOS with PSD-95 impairs neural repair after stroke, and blocking nNOS-PSD-95 interaction facilitates structural neuroplasticity, including neurogenesis and dendritic spine formation of mature neurons, through histone deacetylase 2 (HDAC2)-mediated epigenetic regulation[30–31]. Recently, we showed that inhibiting HDAC2 ultimately improves the prognosis of stroke in the recovery phase *via* facilitating functional and structural neuroplasticity in the brain[32–34].

Neural repair after stroke largely depends on the remodeling of existing neural networks in the peri-infarct area. The network remodeling is strictly regulated by the GABAergic system[34]. Our recent work indicates that NO production from nNOS in neurons due to nNOS-PSD-95 association is implicated in the activation of astrocyte through a NO-mediated paracrine regulation. The activated astrocytes facilitate γ-aminobutyric acid (GABA) production and the reversal of GABA transporter-3/4 (GAT-3/4) functions from GABA uptake to GABA efflux, consequently increasing immoderate tonic inhibition and impairing neuroplasticity and functional recovery from stroke[35]. Dissociating nNOS-PSD-95 inhibits astrocytes activation by reducing paracrine NO, thereby preventing the reversal of GABA transporter and promoting stroke recovery[36]. In addition, disrupting nNOS-PSD-95 interaction improves neurological and cognitive recoveries after traumatic brain injury[37] and protects spinal cord

neurons against ischemic injury[38]. Thus, the nNOS-PSD-95 interaction is a novel target for functional restoration after stroke or other neurological damage and ZL006 is a promising pharmacological lead compound.

NMDARs activation also induces the interaction of CAPON with nNOS, and reportedly, the nNOS-CAPON association leads to acute cerebral ischemic injury through p38 mitogen-activated protein kinase (p38 MAPK) pathway[39]. The specificity in CAPON binding to nNOS depends on C-terminal residues of CAPON, in which, L-Val0 is crucial[40]. Based on the molecular mechanism of nNOS-CAPON interaction[41], if D-valine is placed into the pocket of the nNOS PDZ domain, the carboxyl group of D-valine will bind to the 'GLGF' motif of nNOS, its side chain isopropyl will insert to the hydrophobic pocket, and the amino group will tend to the direction of Lys16 or Arg79 of nNOS. If a carboxyl group is attached to the amino group of D-valine, the additional COOH will form an ionic bond between the carboxylate and positive charge of Lys or Arg of nNOS. The molecule with a COOH attached to the amino group of D-valine will have a competitive advantage over L-valine for binding to nNOS PDZ because of ionic bond formation[12]. Based on this, we developed small molecule nNOS-CAPON inhibitor ZLc002[12]. Our recent study showed that ischemic stroke induces nNOS-CAPON association in the peri-infarct area at the early stage of the repair phase. More importantly, uncoupling nNOS-CAPON reverses stroke-induced spine loss and reduction in dendritic complexity, and promotes functional recovery from stroke[42].

Pain is unpleasant but necessary in preventing us from harming ourselves, and alerts us the damage to our bodies. However, too much unbearable pain can destroy our lives[43]. Maladaptive plasticity-mediated central sensitization is crucial for chronic pathological pain. NMDARs activity is responsible for the central sensitization and therefore plays a key role in the development of chronic pain[44]. Clinically used NMDARs antagonists, such as ketamine and dextromethorphan, are generally effective in patients with neuropathic pain[45]. However, direct antagonists of NMDARs can produce severe side effects, which limit their clinical use[46]. An alternative approach is to disrupt nNOS-PSD-95, the downstream of NMDARs.

Chronic pain induces nNOS-PSD-95 association in the spinal cord, and disrupting the PSD-95-nNOS interaction using ZL006 is effective in attenuating chronic pain without producing unwanted side effects associated with NMDAR[47–48]. Recent studies

demonstrate that ZL006 attenuates hemorrhage-induced thalamic pain in mice[49] and improves the negative affective component of pain[50]. Moreover, disrupting nNOS-CAPON also relieves distinct forms of chronic neuropathic pain, without unwanted motor ataxic effects[51], and ZLc002, a small molecule inhibitor of nNOS-CAPON, suppresses inflammatory and neuropathic pain[52]. Therefore, we believe that nNOS-PSD-95 and nNOS-CAPON inhibitors can be developed into a novel form of pain therapy. However, our very recent study showed that prolonged blockage of NMDARs or nNOS-PSD-95 does not prevent but aggravates nerve injury-induced central sensitization and produces analgesic tolerance, owing to that NO reduction causes GABAergic disinhibition[53]. Thus, preventing the GABAergic disinhibition is necessary for the long-term maintenance of analgesic effect of NMDARs antagonists or nNOS-PSD-95 inhibitors.

NMDAR-mediated excitotoxicity has been implicated in central mechanism of neurodegenerative diseases. AD is one of the major factors to cause cognitive impairment or dementia in old individuals[54]. The pathogenesis of AD is characterized by extracellular deposition of amyloid-β (Aβ) plaque and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein in the human brain[55]. Our study showed that NMDAR-mediated nNOS-CAPON interaction is increased in the hippocampus of APP/PS1 mice (a transgenic mouse model of AD), and blocking nNOS-CAPON interaction can prevent neuron damage, memory loss and dendritic impairments[56]. Moreover, a study by Hashimoto and colleagues demonstrated that the accumulation of CAPON in neurons induces an obviously high level of phosphorylated, insoluble tau protein and neuronal cell death, suggesting that CAPON is a novel tau-binding protein[57]. Thus, CAPON-nNOS or CAPON-tau may become a new target for developing drugs to treat AD and related diseases. nNOS-PSD-95 coupling is also implicated in AD. It is reported that ZL006 reduces Aβ$_{1-42}$-induced neuronal damage and oxidative stress through modulating Akt/Nrf2/heme oxygenase-1 signaling pathways[58]. Very interestingly, quite different from NMDAR antagonists, PSD-95-nNOS inhibitors administered at doses that are behaviorally effective in rats do not affect source and spatial memory and motor function[59], suggesting a good safety. PD is another common neurodegenerative disorder and often has variable set of symptoms, such as shaking, bradykinesia, rigidity, dementia, fatigue, pain and hyposmia[60]. It is known that 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a potent neurotoxin, can be rapidly converted into 1-methyl-4-phenylpyridinium ions (MPP$^+$) when crossing the blood brain barrier into the brain by monoamine oxidase (MAO). The MPP$^+$ selectively destroys dopaminergic (DA) neurons and causes a syndrome that simulates the core neurological symptoms of PD. Thus, exposure to MPTP has become the most commonly applied animal model of PD[61–62]. The nNOS-PSD-95 inhibitor ZL006 alleviates MPP$^+$-induced neuronal injury and apoptotic cell death in a dose-dependent manner in cultured cortical neurons, and may represent a novel class of therapeutics for PD[63].

nNOS-mediated PPIs and neuropsychiatric disorders

MDD is a major human disease, with chronic and recurrent characteristics. In recent years, hypotheses on the pathogenesis of MDD and the therapeutic targets of antidepressants have been extensively discussed. It is well known that the monoaminergic pathway plays a key role in regulating cognition and emotion[64]. Brain level of 5-HT, a vital monoamine primarily derived from the dorsal raphe nucleus (DRN), is significantly low in MDD patients. Serotonin transporter (SERT) uptakes 5-HT from the extracellular space into neurons, thereby limiting the biding of 5-HT to its receptors. Selective serotonin reuptake inhibitors (SSRIs) inhibit 5-HT reuptake *via* SERT into DRN neurons and elevate 5-HT levels throughout the brain under chronic treatment[65]. Pre-clinical and clinical studies strongly suggest the implication of the NO derived from nNOS-positive neurons in the pathology of depression[66–67]. We found that chronic mild stress (CMS) causes a substantial and long-lasting nNOS over-expression in the hippocampus. In the DRN neurons, nNOS mediates a physical combination with SERT *via* PDZ domain, decreasing 5-HT reuptake[7]. CMS-induced depression behaviors are reversed in the mice receiving nNOS inhibitor or in the null mutant mice lacking nNOS gene (nNOS$^{-/-}$)[68], implicating nNOS in the modulation of depression behaviors.

Long-term exposure to high levels of glucocorticoids is linked to depression[69]. The release of glucocorticoids is strictly regulated by hypothalamic-pituitary-adrenal (HPA) axis[70]. Our lab has investigated the molecular mechanisms underlying the behavioral effects of stress and glucocorticoids and identified hippocampal nNOS as a crucial mediator. Exposure to CMS or glucocorticoids

activates mineralocorticoid receptor (MR), and in turn, leads to NO overproduction due to nNOS overexpression in the hippocampus. The nNOS-derived NO in the hippocampus downregulates the expression of glucocorticoid receptor (GR) through both soluble guanylate cyclase (sGC)/cGMP and ONOO⁻/extracellular signal-regulated kinase (ERK) signal pathways. The downregulated GR elevates hypothalamic corticotrophin-releasing factor (CRF), a peptide that governs the activity of HPA axis[71], thereby leading to the hyperactivity of HPA axis. Differently, glucocorticoids in the hypothalamus are not involved in the regulation of HPA axis hyperactivity[72]. It is well known that the prevalence of neuropsychiatric disorders in women is approximately twice that in men. It has been demonstrated that gender difference exists in both monoamine transmitter system and HPA axis, which constitutes fundamental bases for differential susceptibility of men and women to MDD[6]. Our recent work found that the difference in the basal hippocampal NO level between male and female mice explains the sex gap of affective behaviors[73]. More interestingly, disrupting nNOS-PSD-95 using ZL006 produces antidepressant-like properties[74]. Collectively, not only nNOS but also nNOS-PSD-95 or nNOS-SERT can be exploited as novel drug targets for treating MDD.

Anxiety is a physiological reaction to stressful situations or danger. However, it may be regarded as an anxiety disorder when overwhelmingly and persistently existing[75]. Increasing evidence suggests that downregulation of serotonin 1A receptor (5-HT1AR) contributes to anxiety disorders. Our studies suggest a mechanism underlying the modulation of anxiety behaviors by 5-HT1AR: the dysfunction of 5-HT1AR upregulates nNOS expression in the hippocampus, thereby downregulates phosphorylation of cAMP-responsive element-binding protein (CREB), a nuclear transcription factor that modifies anxiety behaviors[76], and in turn, inhibits neurogenesis and synaptogenesis[77–78]. Moreover, ERK phosphorylation is implicated in the 5-HT1AR activation-induced CREB phosphorylation and plays a significant role in modifying nNOS expression and relieving anxiety-related behaviors[79]. More interestingly, we found that mice subjected to CMS display a substantial increase in nNOS-CAPON coupling in the hippocampus and a consequent anxiogenic-like phenotype, and dissociating the CMS-induced nNOS-CAPON can reverse anxiogenic-like behaviors[12]. CAPON attaches to dexamethasone-induced ras protein 1 (Dexras1) *via*

N-terminal phosphotyrosine-binding domain. Dexras1 is activated by S-nitrosylation induced by nNOS and activated Dexras1 negatively regulates the phosphorylation of ERK[12].

Nuclear factor kappa B (NF-κB) is activated by stressful events[80], and is implicated in regulating anxiety and depressive behaviors[81–82]. Our study showed that hippocampal NF-κB mediates anxiogenic behaviors through positively regulating nNOS-CAPON-Dexras1[83]. CREB-mediated brain derived neurotrophic factor (BDNF) expression is a key signaling for synaptic plasticity. Selectively blocking nNOS-CAPON interaction using ZLc-002[12]reverses impairment of structural plasticity and CMS-induced anxiogenic behaviors[84] through enhancing CREB-BDNF signaling.

Anxiety is common in patients suffering from chronic pain but the underlying mechanisms remain unclear. Our recent study indicated that chronic pain-induced anxiety is driven by excitatory neurons in the posterior subregion of paraventricular thalamic nucleus (pPVT). Chronic pain stimulates the neural circuit from pPVT excitatory neurons to nNOS-expressing neurons in the ventromedial prefrontal cortex (vmPFC), and leads to NO production in the vmPFC, thereby promoting NO-mediated α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking in vmPFC pyramidal neurons and resulting in anxiety[85].

PTSD, a pathological fear learning disease on account of previous exposure to an extremely stressful event, is characterized by inabilities to conquer fear in a safe environment[86]. Fear extinction learning under the term "exposure therapy" is first-line treatment for PTSD[87]. However, extinguished fear relapses under a number of circumstances. NMDAR-dependent synaptic plasticity is primarily involved in the consolidation of fear extinction[88]. We investigated the role of PSD-95-nNOS coupling, the downstream signaling of NMDARs activation, in fear extinction. BDNF is considered to be a key factor in the regulation of fear learning and extinction[89]. The functions of BDNF are regulated by the receptor tyrosine kinase B (TrkB), which can also be connected to PSD-95 forming the PSD-95-TrkB complex[90]. Our study showed that disassociating PSD-95-nNOS in the CA3 of dorsal hippocampus increases BDNF expression and BDNF-TrkB-PSD-95 association, and promotes contextual fear extinction[91]. In addition, we also found that the disassociating nNOS-PSD-95 promotes both neurogenesis and survival of newly-generated neurons in the dentate gyrus (DG), contributing to an enhanced retrieval of the extinction memory[92].

Enhanced fear generalization over time is a typical characteristic of the contextual fear memory, and the over-generalization of fear memory is implicated in the pathophysiology of PTSD[93–94]. We observed that retrieval of contextual fear in a novel context at a remote time point increases coupling of nNOS with PSD-95 in the anterior cingulate cortex (ACC), while disrupting nNOS-PSD-95 connection in the ACC decreases the expression of histone deacetylase 2 (HDAC2) and inhibits contextual fear generalization[95]. Interestingly, a recent study using ZL006 showed that disrupting the PSD-95-nNOS interaction selectively reduces fear memory and does not affect locomotion, social interaction, object recognition memory, and spatial memory[96]. Fear renewal is defined as return of the conditioned fear responses after extinction when a conditioned stimulus is given outside of the extinction context. Disrupting the PSD-95-nNOS interaction in the lateral amygdala using ZL006 before fear renewal inhibits fear renewal[97]. Taken together, these findings highlight PSD-95-nNOS interaction as a novel target for PTSD therapy.

Substance addiction is a neurobehavioral disorder characterized by a recurring urge to continue taking the drug regardless of harmful consequences[98]. The most common addictive drugs include opioids, cannabis, cocaine, alcohol and others. Growing evidence suggests that pharmacologically targeting the addiction-related systems is promising to control drug addiction[99]. Increased mu opioid receptor (MOR) within the nucleus accumbens (NAc) is critical for cocaine addiction[100–101]. Interestingly, nNOS inhibitors can prevent MOR overexpression and cocaine-induced conditioned place preference (CPP)[102]. Likewise, nNOS KO mice are resistant to cocaine-induced psychomotor sensitization and CPP[103]. nNOS gene is implicated in cocaine reward during adolescence of both sexes[104].

The NAc is a portal whereby cue-induced activity in cortical and limbic projections induces drug seeking[105]. nNOS is expressed in 1% of NAc neurons. Kalivas and colleagues showed that nNOS-expressing interneurons in the NAc regulate cocaine

relapse[106]. Somatostatin (SST), a growth hormone inhibitory peptide, functions as a neurotransmitter and neuromodulator in the CNS. SST+ interneurons account for <1% of NAc neurons, most of which coexpress nNOS[107]. Although rare, the activity of SST+ neurons in NAc plays a critical role in regulating behavioral responses to cocaine[108]. Moreover, our recent study demonstrated that the nNOS-PSD-95 coupling in the hippocampus plays a significant role in morphine priming-induced reinstatement, possibly through CREB dysfunction. ZL006 inhibits the reinstatement of morphine CPP[109], offering a potential target to prevent relapse of drug abuse. Together, nNOS and nNOS-PSD-95association in the CNS may be implicated in substance addiction.

Conclusions and perspectives

Under physiological conditions, nNOS can precisely regulate NO production, release, diffusion and inactivation processes in the nervous system. nNOS-mediated PPIs, including nNOS-PSD-95, nNOS-CAPON, and nNOS-SERT interactions, contribute to the development of stroke, MDD, anxiety, PTSD, AD, PD, chronic pain, drug addiction and other disorders (***Table 1***, ***Fig. 1***). Due to side effects like impairment of memory formation after direct inhibition of nNOS activity, the development of drugs targeting nNOS is limited. Instead, it will be a preferable means to interfere with specific pathway, for example, uncoupling nNOS-PSD-95, nNOS-CAPON, and nNOS-SERT interactions, which do not lead to these unwanted side effects. Based on the chemical mechanism of binding for the coupling proteins to the nNOS PDZ domain, we developed small molecule PPIs inhibitors, such as ZL006, ZLc002, *etc*. We and other follow-up studies have demonstrated that these drugs are effective for the treatment of neurological and neuropsychiatric disorders (***Table 1***, ***Fig. 1***). PPIs were commonly regarded as "undruggable" owing to protein interfaces with daunting large and flat interfacial areas. However, with clinical successes, the discovery of

Table 1 nNOS-mediated PPIs are implicated in neurological and neuropsychiatric disorders		
nNOS-mediated PPIs	Small molecule inhibitors	Neurological and neuropsychiatric disorders
nNOS-PSD-95	ZL006, IC87201	Stroke, chronic pain, AD, PD, MDD, PTSD, anxiety, addiction
nNOS-CAPON	ZLc002	Stroke, anxiety, chronic pain, AD
nNOS-SERT		MDD

nNOS: neuronal nitric oxide synthase; PSD-95: post-synaptic density 95; CAPON: carboxy-terminal PDZ ligand of nNOS; SERT: serotonin transporter; PD: Parkinson's disease; AD: Alzheimer's disease; MDD: major depressive disorder; PTSD: post-traumatic stress disorder.
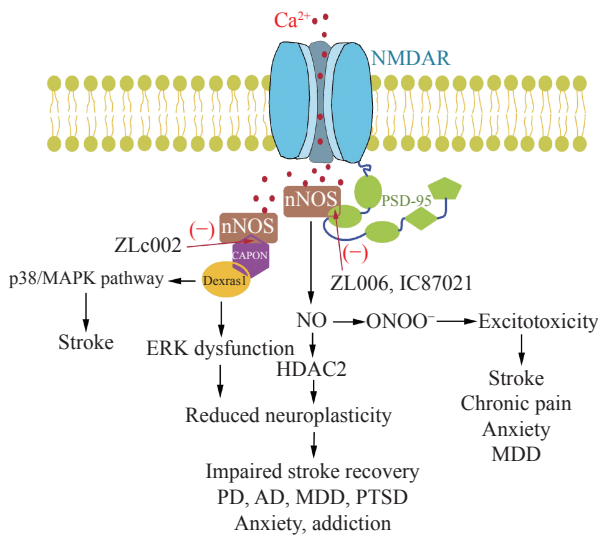
Fig. 1 **nNOS-mediated protein-protein interactions and their implications in neurological and neuropsychiatric disorders.** NMDAR: N-methyl-D-aspartate receptor; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; PSD-95: post-synaptic density 95; CAPON: carboxy terminal PDZ ligand of nNOS; Dexras1: dexamethasone-induced ras protein 1; MAPK: mitogen-activated protein kinase; ERK: extracellular signal-regulated kinase; HDAC2: histone deacetylase 2; PD: Parkinson's disease; AD: Alzheimer's disease; MDD: major depressive disorder; PTSD: post-traumatic stress disorder.

drugs targeting PPIs has gradually become a hot spot in the field of new drug research (R) and development (D). We believe that a deeper understanding of the profound pathophysiological significance of nNOS-mediated PPIs and the R/D of drugs targeting nNOS-mediated PPIs will bring hope for the clinical therapy of neurological and neuropsychiatric disorders.

Acknowledgments

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