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Effects of neuronal nicotinic acetylcholine receptor allosteric modulators in animal behavior studies

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Abstract

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated cation-conducting transmembrane channels from the cys-loop receptor superfamily. The neuronal subtypes of these receptors (e.g. the $\alpha 7$ and $\alpha 4 \beta 2$ subtypes) are involved in neurobehavioral processes such as anxiety, the central processing of pain, food intake, nicotine seeking behavior, and a number of cognitive functions like learning and memory. Neuronal nAChR dysfunction is involved in the pathophysiology of many neurological disorders, and behavioral studies in animals are useful models to assess the effects of compounds that act on these receptors. Allosteric modulators are ligands that bind to the receptors at sites other than the orthosteric site where acetylcholine, the endogenous agonist for the nAChRs, binds. While conventional ligands for the neuronal nAChRs have been studied for their behavioral effects in animals, allosteric modulators for these receptors have only recently gained attention, and research on their behavioral effects is growing rapidly. Here we will discuss the behavioral effects of allosteric modulators of the neuronal nAChRs.

Keywords

Allosteric modulation; animal behavioral studies; cognition; nicotinic acetylcholine receptor; pain; positive allosteric modulators

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are cation-conducting pentameric transmembrane proteins that are activated by the endogenous neurotransmitter acetylcholine (ACh), and are located at the neuromuscular junction and in the central and peripheral nervous systems [1–3]. For the neuronal nAChRs, 12 genes have been identified thus far (nine (2–10) and three (2–4) subunits) that are widely expressed in the nervous system, on both neuronal and non-neuronal cells [1, 3–9].

The different neuronal nAChR subunits combine in various permutations to form functional receptors [1, 3]. Of the many possible subtypes of nAChRs that have previously been described, the $\alpha 7$ and the $\alpha 4 \beta 2$ receptors are the two main subtypes widely expressed in the brain, in particular in the hippocampus [10–16]. While the $\alpha 7$ subunits can form functional homopentameric receptors [1, 3], there is evidence that in both heterologous expression

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systems and in the brain, the γ subunit can also form functional receptors in combination with the δ subunit [16–18]. The γ receptors are characterized by relatively high calcium permeability and rapid desensitization upon exposure to agonists [19–21]. The heteromeric $\alpha 4 \beta 2$ receptors (composed of both the $\alpha 4$ and $\beta 2$ subunits) are expressed in at least two different stoichiometries; the $(\alpha 4)_2(\beta 2)_3$ stoichiometry which binds to ACh and nicotine with higher affinity, and the $(\alpha 4)_3(\beta 2)_2$ stoichiometry which binds with lower affinity [22–26]. The γ and $\alpha 4 \beta 2$ nAChRs are found at pre- and postsynaptic sites, as well as non-synaptic sites in various regions of the brain [27–33]. Due to the widespread distribution of nAChRs in the central nervous system, it is not surprising that they are involved in the pathophysiology of many neurological diseases and conditions [1, 3, 34], including (but not limited to) nicotine addiction [35–37], Alzheimer's disease [33, 38, 39], human epilepsy syndrome [40–43], schizophrenia [44–46] and autism [47–49]. Experimental evidence also suggests that nAChRs are involved in cognitive processes like attention [50–53] and learning and memory [54, 55], in the central processing of pain [56–65], and in psychological behaviors such as anxiety [54, 66–69] and depression [68, 70, 71].

The neuronal nAChRs behave as allosteric proteins with multiple, inter-convertible conformations [72–77]. Many different pharmacological ligands preferentially bind to and stabilize the nAChR in a specific conformation [12, 77, 78]. While agonists bind to the orthosteric site, there are many ligands which are known to bind to the receptor at sites separate and distinct from the orthosteric site; these are referred to as allosteric sites [12, 77–84], and the ligands that bind to the nAChRs at these sites are referred to as allosteric modulators [12, 78, 85]. These allosteric modulators can be classified as positive (PAMs) or negative allosteric modulators (NAMs) [12, 78, 85, 86]; PAMs are compounds that increase the receptor response induced by an agonist [12, 78], while NAMs on the other hand are compounds which decrease the receptor response [12, 83]. PAMs do not have any agonist activity on their own, but they change the ability of the orthosteric ligand (agonist) to affect channel opening [12, 78, 85]. Therefore, allosteric modulators provide an alternative approach to manipulate nAChR function [39, 87–89]. For example, PAMs can potentially increase the effectiveness of endogenous ACh that is released into the synapse, and strengthen the cholinergic tone without directly activating nAChRs [12, 85]. It is known that partial and full agonists cause desensitization of nAChRs and an upregulation of receptor expression levels [90–93]. Since the PAMs do not activate the nAChRs directly, they do not induce either desensitization or upregulation. In the presence of PAMs, the nAChR-driven cholinergic synapse will remain under the control of the released endogenous ACh, and only the magnitude of the nAChR response will increase due to the PAM. Thus the nAChR PAMs provide a distinct advantage over agonists. Since the γ and $\alpha 4 \beta 2$ nAChRs are widely distributed in the brain and are involved in the pathophysiology of a variety of neurological diseases and conditions, allosteric modulators for these receptors hold immense therapeutic potential.

Agonists for nAChRs have been under investigation for a variety of neurological disorders for some time [94–96]. Animal behavioral studies, including animal models with nAChR gene knockouts, provide a suitable and useful tool to assess the effects of pharmacological agents that target these receptors [96–99]. Behavioral studies with nAChR agonists have primarily focused on animal models of cognition, depression and neuropathic pain [50–55, 59–61, 68, 70, 71]. However to date, there are few studies that identify the effects of nAChR PAMs in different paradigms of animal behavior, with little to no published clinical data. Here we will discuss what has been reported thus far on the behavioral studies using different allosteric modulators for the neuronal nAChRs.

2. Behavioral studies with allosteric modulators of the $\alpha 7$ receptors

Behavioral studies with allosteric modulators of the $\alpha 7$ receptors have mostly focused on PAMs since there is evidence that increasing (rather than decreasing) cholinergic output through these receptors causes an enhancement of cognitive performance and alleviation of pain [78, 100]. Table 1 provides a summary of the effects of $\alpha 7$ receptor PAMs in different animal behavioral studies. Many PAMs for $\alpha 7$ receptors have been discovered, and they are divided into two categories; a) type I PAMs which increase the receptor response evoked by agonists and maintains the response kinetics (e.g. desensitization), and b) type II PAMs which enhance the receptor response induced by agonists and dramatically reduces desensitization [78]. The structures of several $\alpha 7$ receptor PAMs are shown in Figure 1.

2.1 Effects of $\alpha 7$ receptor PAMs in cognition

The first PAM to be tested on animals was the type II PAM PNU-120596 (1-(5-chloro-2, 4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea) which is selective for the $\alpha 7$ receptors [101]. Systemic administration of PNU-120596 to rats improved the auditory gating deficit caused by amphetamine in a model reflecting the circuit level disturbance associated with schizophrenia [101]. In Long-Evans rats, PNU-120596 (3–30 mg/kg dose) was able to ameliorate the MK-801-induced auditory gating deficits in the pre-pulse inhibition test [102]. Another type II PAM for $\alpha 7$ receptors, JNJ-1930942, was shown to improve sensory gating in DBA/2 mice [103].

Apart from animal models of schizophrenia, behavioral studies with $\alpha 7$ receptor PAMs have evaluated their ability to influence cognitive function directly. Accordingly in a social discrimination test used to measure short-term memory, PNU-120596 produced sustained pro-cognitive effects after chronic administration [104]. In an attentional set-shifting test performed on adult female hooded Lister rats, PNU-120596 (10 mg/kg) significantly improved performance in phencyclidine (PCP)-treated female rats [105]. Another behavioral study with PNU-120596 has looked at its ability to improve spatial-learning memory assessed by the radial arm maze test and episodic memory assessed by novel object recognition test in Sprague-Dawley rats [54]. In this study, an acute dose of 1, 3 or 10 mg/kg of PNU-120596 failed to improve spatial-learning or episodic memory by itself [54]. However when cognitive impairment was pharmacologically induced with scopolamine (1 mg/kg), PNU-120596 was able to reverse both forms of memory impairment and restore it back to normal levels [54] (Figure 2). In both of these studies [54, 105], PNU-120596 alone was unable to induce cognitive improvement in acute doses. This may be due to the fact that normal animals may already be performing at the peak of their cognitive abilities; such a ceiling effect for cognitive performance has previously been observed [106, 107]. Since PNU-120596 is able to restore cognitive performance in cases where there is cognitive impairment, this indicates that $\alpha 7$ receptor PAMs may be useful in clinical situations where there is impairment of cognitive function (e.g. stroke-induced reduction in cognitive ability or Alzheimer's disease).

Recently PNU-120596 was shown to enhance the effects of donepezil (an acetylcholinesterase inhibitor) on learning and memory in aged rodents and non-human primates [108]. While PNU-120596 alone did not improve cognition, the data from this study indicated that it does increase the effective dose range of donepezil in learning/memory-related tasks in young and age-impaired animal models [108]. In addition, PNU-120596 has also been shown to improve short-term memory in the rat social recognition test [109]. Another type II $\alpha 7$ receptor PAM, A-867744, was shown to normalize gating deficits in a rodent model of sensory gating [110].

A selective type I PAM for the γ_7 receptors, NS-1738 (1-(5-chloro-2-hydroxy-phenyl)-3-(2-chloro-5-trifluoromethyl-phenyl)-urea), was shown to relieve the (–)-scopolamine-induced deficit in acquisition of a water-maze learning task, a model of spatial long-term memory in rats [111]. NS-1738 also improved performance in a social recognition test (a model of short-term memory in rats) similar to nicotine [111]. While it was not reported whether NS-1738 by itself in acute or chronic doses improved cognition, NS-1738 was able to restore cognition in cases of impairment [111]. Another type I γ_7 receptor PAM, SB-206553, which also acts as an antagonist of the serotonin 5-HT_{2B/C} receptor, was shown to relieve the MK-801-induced impairment in pre-pulse inhibition in an γ_7 receptor-dependent manner [102].

Another type I γ_7 nAChR PAM, ABT-779, has been shown to be effective in improving pre-attention, working memory, short-term recognition memory, and in inducing memory consolidation [112]. XY-4083, a compound initially referred to as compound 6 (N-(4-chlorophenyl)- α -[(4-chloro-phenyl)amino]methylene]-3-methyl-5-isoxazoleacet-amide), was generated from a library of GABA_A receptor PAMs, but was found to be a selective γ_7 receptor PAM [113]. In rodent models of behavior, XY-4083 corrected the sensory-gating deficits and improved working memory tested in an eight-arm radial maze [113]. AVL-3288, which is yet another name for compound 6, failed to increase short-term memory after repeated administration, but not with a single acute dose [104]. Another compound, 522-054, which is both an α_5 GABA_A receptor NAM and an γ_7 nAChR PAM, was able to restore the scopolamine-disrupted learning and memory when tested with the 8-arm radial maze test [114]. Similarly, it also restored scopolamine impairments of attention in the five-choice serial reaction time test [114].

2.2 Effects of α_7 receptor PAMs in pain

Agonists for γ_7 receptors are known to have antinociceptive and anti-inflammatory properties in rodent models of pain and inflammation [115, 116]. In a study conducted in rats, PNU-120596 (30 mg/kg) significantly reduced mechanical hyperalgesia and weight-bearing deficits similar to that seen with diclofenac, a non-steroidal anti-inflammatory drug [56]. Additionally, PNU-120596 was also able to attenuate carrageenan-induced increases in levels of TNF- α and IL-6 within the hind paw edema in rats [56]. PNU-120596 relieved nociception in a dose-dependent manner by itself [59], while also decreasing formalin-induced pain in mice in combination with choline, nicotine and PHA-543613 [59]. Therefore, isobolographic analysis suggests that PNU-120596 acts synergistically with γ_7 receptor agonists to elicit antinociceptive effects in the formalin pain model in mice [59].

A similar study carried out in mice compared the type I γ_7 receptor PAM NS-1738 and type II PAM PNU-120596 for their effects in the carrageenan-induced inflammatory pain and chronic constriction injury models for neuropathic pain [61]. Both NS-1738 and PNU-120596 were able to reduce the heat-induced hyperalgesia [61]. PNU-120596 also reduced edema induced by a hind paw infusion of carrageenan in mice [61]. In chronic constriction injury, a dose-dependent anti-hyperalgesic and anti-allodynic effect of PNU-120596 for up to 6 hours was observed with a single acute dose [61]. However NS-1738 did not have any effect in mice with chronic constriction injury [61].

The effects of both NS-1738 and PNU-120596 have also been studied in acute and chronic pain in mice [60]. Neither of these γ_7 receptor PAMs was able to reduce acute thermal pain, however PNU-120596 did reduce paw-licking behavior in mice in the formalin test [60]. Furthermore, there was no development of tolerance due to the treatment with PNU-120596 at sub-chronic doses [60]. Additionally, this study also found that the activation of extracellular signal-regulated kinase-1/2 pathways is the likely mechanism of the

antinociceptive effect of PNU-120596 [60]. Therefore PNU-120596 appeared to be better at relieving inflammation and reducing neuropathic pain than NS-1738.

As discussed above, both type I and type II PAMs for the α_7 receptors have been used to assess their effects on cognitive enhancement and pain reduction [54, 59–61, 101–105, 108–114]. NS-1738 (type I PAM) and PNU-120596 (type II PAM) both show a positive effect on cognition, in particular when it is impaired with pharmacological treatment [54, 101, 102, 104, 105, 108, 109, 111]. Both types of PAMs (type I & II) show improvement in auditory and sensory gating as well as pre-pulse inhibition [101–103, 110, 112, 113]. This indicates that both types of PAMs for the α_7 receptors have the potential for developing into treatments for schizophrenia. Regarding their ability in relieving pain, apart from the carrageenan-induced thermal hyperalgesia, the type I PAM NS-1738 does not show positive effects in any other pain model [60, 61]. However, the type II PAM PNU-120596 had a positive effect in the carrageenan-induced thermal hyperalgesia, inflammation, edema, formalin test and in chronic constriction injury for mechanical allodynia [56, 59–61]. With the studies conducted thus far, it appears that both type I and type II PAMs have a beneficial effect in cognitive behavioral tests, and in tests which relate to the symptoms of schizophrenia. Further studies will surely enhance our understanding of the similarities and differences in the behavioral actions of type I and type II α_7 receptor PAMs.

3. Behavioral studies with allosteric modulators of the $\alpha_4\beta_2$ receptors

Many ligands are known to act as allosteric modulators for the $\alpha_4\beta_2$ subtype of nAChRs; these include 17 β -estradiol [79], NS-9283 [62–64], desformylflustrabromine [80, 81], and LY-2087101(a (2-amino-5-keto) thiazole compound) [117] which act as PAMs, and KAB-18 [83], UCI-30002 [118] and progesterone [119] which act as NAMs. To date, most of the behavioral research in animals with allosteric modulators for $\alpha_4\beta_2$ receptors has focused on PAMs, similar to that seen with the α_7 receptors. In addition only one $\alpha_4\beta_2$ receptor PAM, NS-9283 (Figure 1), has been tested for its behavioral effects in rodents. Behavioral studies with $\alpha_4\beta_2$ receptor PAMs have mainly focused on their ability to reduce pain and improve cognitive function (Table 2).

3.1 Effects of $\alpha_4\beta_2$ receptor PAMs in pain

Functional evaluation of NS-9283 on $\alpha_4\beta_2$ receptors expressed in *Xenopus* oocytes indicated that NS-9283 is a PAM for the $(\alpha_4)_3(\beta_2)_2$ nAChR (which binds ACh with lower affinity), but not for the $(\alpha_4)_2(\beta_2)_3$ receptor (which binds ACh with higher affinity) [65]. Studies using NS-9283 (also known as A-969933) to test its effect on pain have been done in combination with agonists (full or partial) [64]. Acute administration of NS-9283 alone *in vivo* did not affect mechanical allodynia in the spinal nerve ligation test, a model of neuropathic pain in rats [64]. However co-administration of NS-9283 with ABT-594, an agonist for nAChRs, increased the anti-allodynic effects of ABT-594 [64]. The dose at which ABT-594 produces an analgesic effect also causes nausea and emesis via the activation of the $\alpha_3\alpha_4$ receptors [64]. NS-9283 selectively enhances the potency of ABT-594 at the $\alpha_4\beta_2$, but not at the $\alpha_3\alpha_4$ nAChRs [64].

NS-9283 by itself had no significant effect in nociception and inflammation in various rodent models of pain [63]. The effect of co-administration of ABT-594 with NS-9283 has been studied on locomotor activity, rotarod performance, edge-balance and on body temperature in naïve rats [63]. NS-9283 alone had no effect on edge performance or body temperature [63], and NS-9283 along with ABT-594 does not cause significant edge performance deficit. While ABT-594 alone does reduce body temperature [63], NS-9283 does not worsen the decrease in the body temperature as compared to animals that received treatment with vehicle alone [63]. NS-9283 by itself induced no effect on locomotor activity,

and in combination with ABT-594, it does not produce significant changes in the spontaneous horizontal exploratory activities in rats [63]. There is an 18% decrease in the latency to fall in the rotating rod test in rats dosed with NS-9283 and ABT-594 when compared to vehicle-treated rats [63]. In another study, NS-9283 was used along with the partial agonist NS-3965 to assess their effects in the formalin test used to test for pain in rats, and NS-9283 was able to produce a strong potentiation of the antinociceptive actions of NS-3965 [62]. These studies with NS-9283 show that combining a PAM for $\alpha_4\beta_2$ receptors, along with an agonist, can be a viable strategy in therapeutic drug development for treatment of neuropathic pain that would lack any major adverse effects that may be seen with an agonist.

3.2 Effects of $\alpha_4\beta_2$ receptor PAMs in cognition

NS-9283 has been studied for its effects on cognitive performance in behavioral tests performed in rodents. In oxidopamine-lesioned rats, a model of Parkinson's disease, NS-9283 alone did not induce rotational behavior [62]. However in combination with the partial agonist NS-3956, NS-9283 was able to block rotational behaviors in the rats [62]. NS-9283 was found to reverse the PCP-induced impairment in rats, thus NS-9283 has a positive effect on pre-attentional sensory information processing. In addition, NS-9283 was tested for its effects on short-term episodic memory in Sprague–Dawley rats with the social recognition test [65]. NS-9283 showed an improvement in social recognition memory by the rats at doses of 0.075, 0.75 and 7.5 mg/kg administered via intraperitoneal injection [65]. A five-choice serial reaction time test was used to access the effect of NS-9283 on sustained attention in male Lister hooded rats [65]; NS-9283 induced an improvement in attention performance in this task [65]. Additional tests with NS-9283 also demonstrated its cognitive-enhancing properties [65]. The Morris water maze test was used to evaluate the action of NS-9283 on reference memory [65]. It was found that NS-9283 caused a significant reduction in the PCP-induced deficits and improved task acquisition in rats comparable to treatment with vehicle alone [65]. Moreover, mecamylamine (a nAChR antagonist) was able to reverse these effects of NS-9283 in the Morris water maze test [65].

4. Pharmacokinetic/Pharmacodynamic (PK/PD) relationship of nAChR PAMs

The PAMs for the α_7 (PNU-120596 and NS-1738) and $\alpha_4\beta_2$ (NS-9283) receptors have been tested for their effects on cognition and nociception, and it has been observed that the dose of these compounds needed to induce a positive effect may be different; for example the dose required to induce a beneficial effect in pain models is different from the dose required to induce pro-cognitive effects [54, 56, 59–61, 111]. This indicates the importance of considering pharmacokinetic parameters such as bioavailability of these compounds while assessing their pharmacodynamic effects. The inconsistency in pharmacokinetics and pharmacodynamics of nAChR ligands in cognition are well established, and brief exposure is sufficient to induce long lasting cognitive effects [120]. However the pharmacokinetic/pharmacodynamic (PK/PD) relationship of PNU-120596 is not well understood [105]. It is possible that pharmacokinetic parameters like the maximum plasma concentration of the PAMs can drive their pharmacodynamic response [105]. Additional studies will be needed to determine the full PK/PD profile of all known PAMs for nAChRs.

5. PAMs with dual actions

In addition to the PAMs for $\alpha_4\beta_2$ and α_7 receptors already discussed here, other PAMs like galantamine and physostigmine have also been studied in different behavioral paradigms in animals [121, 122]. However in addition to being PAMs for nAChRs, galantamine and

physostigmine are also acetylcholinesterase inhibitors [39]. While the beneficial effects of these two compounds in restoring cholinergic tone in the synapse is expected to be enhanced by these dual actions [12, 39], it can be difficult to determine whether the neuropsychiatric effects of these compounds are due to their effects on acetylcholinesterase or allosteric modulation of the neuronal nAChRs.

6. PAMs for other nAChR subtypes

In functional studies, the anthelmintic compounds levamisole [123] and morantel [124] have been identified as PAMs for human $\alpha 3 \beta 2$ and $\alpha 3 \beta 4$ nAChR subtypes, however no behavioral studies with either of these compounds have been reported thus far.

Hypothalamic $\alpha 3 \beta 4$ receptors have been identified as the nAChR subtype that is involved in the regulation of food intake in mice [125], therefore nAChR PAMs for subtypes other than the $\alpha 4 \beta 2$ and $\alpha 7$ receptors may have important clinical applications.

7. Conclusion

The recent focus on the discovery and characterization of nAChR PAMs has created new opportunities for targeting these receptors and developing drug treatments in diseases and conditions where they may be involved. It is known that nAChRs are involved in neuropsychological process in the brain like cognition, central processing of pain, anxiety and depression [4, 33, 34, 38, 39, 47, 59–61, 126]. Allosteric modulation provides an alternative mechanism of nAChR manipulation; as such, it is imperative that allosteric modulators (PAMs or NAMs) for these receptors be further tested for their effects on behavior in animals. Behavioral studies that measure the effects of allosteric modulators for nAChRs will certainly be a necessary part of pre-clinical studies of these compounds, and such studies will generate important information about these compounds that can influence their further clinical development.

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Abbreviations

ACh	acetylcholine
nAChRs	neuronal nicotinic acetylcholine receptors
PAM	positive allosteric modulator
NAM	negative allosteric modulator

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PNU-120596

NS-1738

Compound-6 (XY-4083 / AVL-3288)

SB-206553

JNJ-1930942

A-867744

NS-9283

Figure 1.

Structure of α_7 and $\alpha_4\beta_2$ receptor PAMs that have been used for behavioral studies in animals. For the α_7 receptor compounds, NS-1738, Compound-6 (XY-4083/AVL-3288), and SB-206553 are type I PAMs, while compounds JNJ-1930942 PNU-120596, and A-867744 are type II PAMs. NS-9283 is a PAM for the $\alpha_4\beta_2$ receptor.

Figure 2.

The α_7 receptor PAM PNU-120596 is not able to improve (a) working or (b) reference memory by itself, but it can reverse both forms of memory impairment and restore it back to normal when impairment is induced with scopolamine [54].

Table 1

Effects of 7 receptor PAMs in animal behavioral studies

Compound (Type of PAM)	Test	Behavioral Paradigm	Effect	Species	Note	Reference
PNU-120596 (Type - II)	Auditory gating	Pre-attention	+	Sprague Dawley rats	amphetamine treated	[101]
	Pre-pulse Inhibition	Pre-attention	+	Long-Evans rats	MK-801 treated	[102]
	Social discrimination	Short-term memory	+	Wistar rats		[104]
	Attentional set-shifting task	Attention	+	hooded Lister rats	phencyclidine treated	[105]
	12-arm radial maze	Spatial learning memory	+	Sprague Dawley rats	scopolamine treated	[54]
	Novel object recognition	Episodic memory	+	Sprague Dawley rats	scopolamine treated	[54]
	Social recognition	Short term memory	+	Rats		[109]
	Object recognition	Episodic memory	No effect	Sprague Dawley rats		[108]
	Carrageenan injection	Hyperalgesia	+	Rats		[56]
	Carrageenan injection	Inflammation	+	Rats		[56]
	Formalin test	Noiception	+	Mice		[59]
	Carrageenan injection	Thermal Hyperalgesia	+	Mice		[61]
	Carrageenan injection	Edema	+	Mice		[61]
	Chronic constriction injury	Thermal hyperalgesia	+	Mice		[61]
	Chronic constriction injury	Mechanical allodynia	+	Mice		[61]
NS-1738 (Type - I)	Tail-Flick test	Mechanical pain	No effect	Mice		[60]
	Hot-Plate test	Thermal pain	No effect	Mice		[60]
	Mechanical Sensitivity test	Noiception	No effect	Mice		[60]
	Formalin test	Tonic pain	+	Mice		[60]
	Locomotor activity	Locomotion/Anxiety	No effect	Mice		[60]
	Rotarod test	Motor Coordination	No effect	Mice		[60]
	Morris water maze	Spatial learning	+	Rats	scopolamine treated	[111]
	Social recognition	Short-term memory	+	Rats		[111]
	Carrageenan injection	Thermal Hyperalgesia	+	Mice		[61]
	Carrageenan injection	Edema	No effect	Mice		[61]
	Chronic constriction injury	Thermal hyperalgesia	No effect	Mice		[61]
	Chronic constriction injury	Mechanical allodynia	No effect	Mice		[61]
	Tail-Flick test	Mechanical pain	No effect	Mice		[60]
	Hot-Plate test	Thermal pain	No effect	Mice		[60]

Compound (Type of PAM)	Test	Behavioral Paradigm	Effect	Species	Note	Reference
A-867744 (Type - II) ABT-779 (Type - I)	Mechanical Sensitivity test	Nociception	No effect	Mice		[60]
	Formalin test	Tonic pain	No effect	Mice		[60]
	Auditory gating	Pre-attention	+	DBA/2 mouse		[110]
	Auditory gating	Pre-attention	+	DBA/2 mice		[112]
SB-206553 (Type - I) Compound-6 (XY-4083) (AVL-3288) (Type - I)	Inhibitory avoidance	Memory consolidation	+	CD-1 mice		[112]
	Social recognition	Short-term memory	+	Rat		[112]
	Delayed-matching to-sample task	Working memory	+	Rhesus monkey		[112]
	Pre-pulse Inhibition	Pre-attention	+	Long-Evans rats	MK-801 treated	[102]
JNJ-1930942 (Type - II) 522-054 (Type - II)	Sensory gating	Pre-attention	+	DBA/2 mice		[113]
	8-Arm radial maze	Spatial learning memory	+	Sprague Dawley rats		[113]
	Social discrimination	Short term memory	+	Wistar rats		[104]
	Sensory gating	Pre-attention	+	DBA/2 mice		[103]
Five-Choice Serial Reaction Time Test	8-Arm radial maze	Learning and memory	+	Adult male Sprague-Dawley rats	Scopolamine treated	[114]
		Attention	+	Adult male Sprague-Dawley rats	Scopolamine treated	[114]

+ = Positive effect

Table 2

Effects of 4 2 receptor PAM (NS-9283) in animal behavioral studies

Compound	Test	Behavioral Paradigm	Effect	Species	Note	Reference
NS-9283	Spinal nerve ligation	Neuropathic pain	No effect	Sprague Dawley rats		[64]
	Arterial pressure	Cardiovascular effects	No effect	anesthetized dog		[64]
	Carrageenan injection	Thermal Hyperalgesia	No effect	Sprague Dawley rats		[63]
	Skin incision test	Post-operative pain	No effect	Sprague Dawley rats		[63]
	Monoiodoacetate-induced osteoarthritis	Joint Pain	No effect	Sprague Dawley rats		[63]
	Rotarod test	Motor Coordination	No effect	Sprague Dawley rats		[63]
	Open field exploration	Locomotion	No effect	Sprague Dawley rats		[63]
	Edge balance test	Motor coordination	No effect	Sprague Dawley rats		[63]
	Body temperature	Metabolism	No effect	Sprague Dawley rats		[63]
	Rotational behavior	Model for arkinson's disease	+	Sprague Dawley rats		[62]
	Formalin test	Nociception	No effect	Sprague Dawley rats		[62]
	Social recognition	Short-term memory	+	Sprague Dawley rats		[65]
	Five-choice serial reaction time task	Attention	+	Lister hooded rats		[65]
	Morris water maze	Spatial learning	+	Wistar rats	phencyclidine treated	[65]
	Core body temperature	Metabolism	No effect	NMRI mice		[65]

+ = Positive effect