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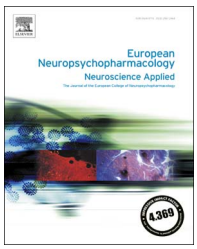

# Functional mechanism of ASP5736, a selective serotonin 5-$HT_{5A}$ receptor antagonist with potential utility for the treatment of cognitive dysfunction in schizophrenia

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**Abstract**
The 5-$HT_{5A}$ receptor is arguably the least understood 5-HT receptor. Despite widespread expression in human and rodent brains it lacks specific ligands. Our previous results suggest that 5-$HT_{5A}$ receptor antagonists may be effective against cognitive impairment in schizophrenia. In this study, using behavioral, immunohistochemical, electrophysiological and microdialysis techniques, we examined the mechanism by which ASP5736, a novel and selective 5-$HT_{5A}$ receptor antagonist, exerts a positive effect in animal models of cognitive impairment. We first confirmed the effect of ASP5736 on cognitive deficits in rats treated subchronically with phencyclidine hydrochloride (PCP) using an attentional set shifting task. Subsequently, we identified 5-$HT_{5A}$ receptors in dopaminergic (DAergic) neurons and parvalbumin (PV)-positive interneurons in the ventral tegmental area (VTA) and in PV-positive interneurons in the medial prefrontal cortex (mPFC). Burst firing of the DAergic cells in the parabrachial pigmental nucleus (PBP) in the VTA, which predominantly project to the mPFC, was significantly enhanced by treatment with ASP5736. In contrast, ASP5736 exerted no significant effect on either the firing rate or burst firing in the DA cells in the paranigral nucleus (PN), that project to the nucleus

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accumbens (N. Acc.). ASP5736 increased the release of DA and gamma-aminobutyric acid (GABA) in the mPFC of subchronically PCP-treated rats. These results support our hypothesis that ASP5736 might block the inhibitory 5-$HT_{5A}$ receptors on DAergic neurons in the VTA that project to the mPFC, and interneurons in the mPFC, and thereby improve cognitive impairment by preferentially enhancing DAergic and GABAergic neurons in the mPFC.


## 1. Introduction

Schizophrenia is a chronic, severe and disabling psychiatric disorder displaying both positive and negative as well as affective symptoms and profound cognitive deficits, including impaired working memory, attention and executive functioning (Elliott et al., 1995; Pantelis et al., 1999; Badcock et al., 2002). Executive functioning is commonly assessed using the Wisconsin Card Sort Test (WCST) or the computerized intradimensional-extradimensional (ID-ED) shift test. The ID-ED shift test has been adapted for in nonhuman primates, where selective ED shift deficits similar to those observed in first-episode schizophrenia patients can be induced by dorsolateral prefrontal cortex (PFC) lesions (Dias et al., 1996). Subsequently, also the attentional set-shifting task was found applicable to rats (Birrell and Brown, 2000), and impairment of the ability to switch attentional set by reduction of DA levels in the PFC was demonstrated (Crofts et al., 2001). Using this test, modafinil, but not olanzapine, was reported to reverse cognitive deficits in rodents induced by subchronic phencyclidine hydrochloride (PCP) treatment (Pedersen et al., 2009; Rodefer et al., 2008). Moreover, some studies (Madras et al., 2006; Hilaire et al., 2001; Dawson et al., 2012) reported that the ability of modafinil to improve cognitive flexibility in PCP-treated animals may be linked to its ability to increase DA levels in the PFC.

The 5-$HT_{5A}$ receptor was cloned in 1994 (Rees et al.), but few reports have described the function of the receptor due to a lack of specific ligands. Findings from 5-$HT_{5A}$ receptor mRNA localization (Rees et al., 1994; Pasqualetti et al., 1998) and immunolabeling studies (Oliver and Kinsey, 2000) have revealed a widespread expression of this receptor in the central nervous system, including the cerebral cortex, hippocampus, the nucleus accumbens (N. Acc.), amygdala and hypothalamus. The increased exploratory behavior in novel environments displayed by 5-$HT_{5A}$ receptor knockout mice compared to wild-type mice (Grailhe et al., 1999), together with the receptor's widespread expression, has indicated that it may be involved in mood, affective disorders, and cognitive function. Gene association studies also suggest that the 5-$HT_{5A}$ receptor may play a role in both schizophrenia and mood disorders (Thomas, 2006; Jongen-Relo et al., 2006; Rueter et al., 2006).

We recently identified ASP5736, a novel selective antagonist of the 5-$HT_{5A}$ receptor, and reported the *in vitro* and *in vivo* characterization of this compound (Yamazaki et al., 2014, 2015). Treatment with ASP5736 ameliorated cognitive impairment in animal models of schizophrenia and dementia. The compound bound to 5-$HT_{5A}$ receptors at approximately 15–50% occupancy levels in behaviorally effective doses. However, the mechanism of action of ASP5736 and its efficacy in various other animal models has not yet been clarified.

Dopaminergic (DAergic) neurons in the ventral tegmental area (VTA) project to the N. Acc and to the PFC (Erhardt et al., 2002; Svensson et al., 1995; Svensson, 2000). Whereas the neurons in the paranigral nucleus (PN) subdivision of the VTA largely project to the N. Acc, the neurons in the parabrachial pigmental nucleus (PBP), another subdivision of the VTA, predominantly project to the PFC (for review see Svensson, 2000). Considering that ASP5736 improves cognitive impairment in animal models of schizophrenia without exacerbating positive symptom-like behavior (Yamazaki et al., 2014), it would be of interest to evaluate the potential selectivity of ASP5736 for each DAergic pathway.

In this study, we examined the mechanism of action of ASP5736 with behavioral, immunohistochemical, electrophysiological and microdialysis techniques using rats. We first confirmed the effect of the compound on cognitive deficits using an attentional set-shifting task. We then examined the co-expression of 5-$HT_{5A}$ receptors in DAergic neurons and parvalbumin (PV)-positive interneurons. Subsequently we evaluated the effect of the compound on the firing rate and burst firing of DA cells in the PBP and PN in the VTA. Finally, we evaluated the effect of ASP5736 on extracellular DA and gamma-aminobutyric acid (GABA) in the mPFC.

## 2. Experimental procedures

ASP5736 and PCP were synthesized at Astellas Pharma Inc. (Tsukuba, Japan). ASP5736 was suspended in 0.5% (w/v) methylcellulose and PCP was dissolved in saline. All compounds were administered at 1 mL/kg in rats. For *in vivo* electrophysiological experiments, ASP5736 was dissolved in a mixture of 10% polyoxyethylene castor oil (cremophor EL, Sigma) and 90% saline.

### 2.1. Animals for immunohistochemistry, attentional set shifting task (ASST), and microdialysis

Male Long Evans rats (Japan SLC, Inc.) were housed in groups of three in temperature- and humidity-controlled rooms (23 ± 2 °C and 55 ± 10%) under a 12-h light/dark cycle. Food and water were available *ad libitum* in the home cages except for ASST subjects, whose food was restricted prior to the test to enhance their performance on the food-motivated task. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. (Tsukuba Research Center is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International).

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