Muscarinic M1 receptor modulation of cognition using a translational approach : relevance for dementia and schizophrenia

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Muscarinic M₁ receptor modulation of cognition using a translational approach

Relevance for dementia and schizophrenia

COLOFON

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Relevance for dementia and schizophrenia

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
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CHAPTER 1

Introduction

ACETYLCHOLINE AND COGNITION

Cognitive (dys)function can be viewed as one of the main research themes within the field of psychology and neuroscience. Impairments in cognition usually occur as an epiphenomenon of brain diseases and/or neuropsychiatric disorders, yet these are not limited to one specific neurological affliction and can occur at any age. For instance, in the Netherlands approximately 156,726 people suffered from psychotic disorders, whereas about 210,366 people were afflicted with dementia in the year 2010. The annual cost for these disorders has been estimated to lie at 3972 million for psychosis, and 2958 million for dementia (Gustavsson et al., 2011). Neurological diseases are hence associated with significant reductions in quality of life for patients and their families, and place a heavy economical burden on society. In the clinical population, impairments are rarely restricted to only one or few cognitive domains (e.g., attention, memory, language, orientation, praxis, executive function, and problem solving: Sadock et al., 2009). Moreover, patient samples can be very heterogeneous in terms of etiology, treatment and disease history, cognitive deficits, clinical symptoms, disease progression, and socioeconomic factors (e.g., Cummings, 2000). This heterogeneity makes it difficult to correlate cognitive dysfunctions and/or clinical symptoms with specific neuropathological or neurochemical findings. Therefore, a lot of effort is dedicated to modeling cognitive deficits with pharmacological tools that temporarily and reversibly impact neurotransmission in healthy animals and young volunteers (e.g., Gilles & Luthringer, 2007). These pharmacological models are essential for unraveling the neurobiological basis of cognition, and are used as methods to screen potential cognition-enhancing medications (Levin & Buccafusco, 2006).

For instance, the non-selective cholinergic (i.e., muscarinic) antagonist scopolamine has been employed extensively to model age- and dementia-related impairments in cognitive functions, especially memory (see **Chapter 2**). The guiding principle for the use of scopolamine as a pharmacological method to investigate cholinergic impairment has been the cholinergic hypothesis of geriatric memory dysfunction, which was formulated by Bartus et al. (1982) 30 years ago: *Stated in its most simple and direct terms, the cholinergic hypothesis asserts that significant, functional disturbances in cholinergic activity occur in the brains of aged and especially demented patients, these disturbances play an important role in the memory loss and related cognitive problems associated with old age and dementia, and proper enhancement or restoration of cholinergic function may significantly reduce the severity of the cognitive loss* (Bartus et al., 1985, p. 332).

The specific role of acetylcholine in cognition – i.e. learning/memory and attention – has been the subject of much debate during the last couple of decades, although initial evidence was promising. For instance, findings of reduced cholinergic enzyme activity in post-mortem brain tissue of Alzheimer patients (Bowen et al., 1976; Perry et al., 1977; Whitehouse et al., 1982), learning and memory impairments after cholinergic lesioning of basal forebrain neurons with neurotoxins in animals and after administration of the muscarinic antagonist scopolamine (for an extensive overview, see **Chapter 2** and Baxter & Murg, 2002) corroborated the assumptions of the cholinergic hypothesis. However, early neurotoxins used for this purpose were not fully selective for cholinergic neurons, and subsequent animal experiments using the more selective immunotoxin 192 IgG-saporin to lesion basal forebrain projections yielded attentional instead of learning/memory impairments (e.g., Baxter & Gallagher, 1996; Baxter et al., 1997; Chiba et al., 1999; McGaughy et

al., 2000; Sarter et al., 2003; Torres et al., 1994; Voytko, 1996). Moreover, behavioral paradigms which assess stimulus discrimination and/or (sustained) attention processes were shown to be relatively more sensitive to impairments induced by the muscarinic antagonist scopolamine compared to tasks which measure learning/memory functions (i.e., effects at relatively lower scopolamine doses; see **Chapter 2**). Hence, a dissociation has emerged between researchers emphasizing the role of acetylcholine in attention (Sarter et al., 2003; 2005; 2009) vs. those still adhering to views which emphasize the involvement of the cholinergic system in learning/memory functions (Hasselmo, 1999; 2006; Hasselmo et al., 2002; Hasselmo & McGaughy, 2004). However, by generalizing the effects of cholinergic neurotransmission on cognitive functions to learning/memory vs. attention, these hypotheses fail to recognize that cholinergic projections can be found diffusely throughout the brain and hence acetylcholine might have differential effects dependent on receptor subtype and brain region targeted.

In addition, an exclusive role for acetylcholine in geriatric memory dysfunction is too restrictive. For example, cholinergic impairments have also been noted in Parkinson's disease (e.g., Calabresi et al., 2006), mood disorders (e.g., Drevets et al., 2008), and schizophrenia (e.g., Sarter et al., 2012). Despite the strong research emphasis on dopaminergic and glutamatergic neurotransmitter changes in schizophrenia, several lines of evidence also suggest a role for cholinergic (i.e., muscarinic) signaling (e.g., Brooks et al., 2011; Sarter et al., 2012; Tandon et al., 1991; 1999). For example, postmortem and in-vivo imaging studies have shown a reduction of muscarinic receptor density or binding in the caudate-putamen (Dean et al., 1996; but see Dean et al., 2000), the hippocampal formation (Crook et al., 2000; but see Scarr et al., 2007), prefrontal cortex (Crook et al., 2001; Scarr et al., 2008), superior temporal gyrus (Deng & Huang, 2005), and cingulate cortex (Newell et al., 2007; Zavitsanou et al., 2004). Furthermore, cognitive impairments associated with schizophrenia have been suggested to be the most severe in the domains of memory and attention (amongst others; see Sadock et al., 2009). Thus, the question arises whether aberrant cholinergic signaling is a common neuropathological pathway underlying both dementia and schizophrenia. Conversely, administration of cholinergic (i.e., muscarinic) antagonists might hence serve as a model for cognitive impairments associated with aging, Alzheimer's disease, and schizophrenia.

Interestingly, findings of studies using muscarinic drugs are in line with a role for aberrant cholinergic signaling in schizophrenia. For instance, high doses of the non-selective muscarinic antagonists scopolamine and atropine have both been shown to induce an *anti-muscarinic psychosis* consisting of visual hallucinations, delusions, hyperactive and stereotypical behavior, loss of attention and memory, disorganized thinking and confusion (Barak & Weiner, 2009). Whereas amphetamine-induced psychosis mainly mimics the positive symptoms of schizophrenia and is thought to reflect the acute stage of the disorder, anti-muscarinic psychosis also includes some cognitive symptoms and is seen as a model for chronic schizophrenia (Barak & Weiner, 2009). Moreover, administration of muscarinic (M_1) antagonists biperiden or procyclidine has been shown to increase positive symptomatology in schizophrenia patients (Johnstone et al., 1983; Tandon et al., 1990; 1992), yet to reduce negative symptoms (Tandon et al., 1992).

In sum, although Alzheimer's disease can be characterized as a neurodegenerative illness, and schizophrenia as a neurodevelopmental disorder, they appear to share a common denominator: cholinergic impairment. However, as noted previously, cholinergic neu-

rons and projections are found throughout the central nervous system (e.g., Mesulam, 1990; Mesulam et al., 1983). Hence, for the purpose of modeling cholinergic impairments associated with Alzheimer's disease and/or schizophrenia, it might be preferable to use drugs which bind to cholinergic receptor subtypes in a relatively selective manner. In addition, non-selective cholinergic (i.e., muscarinic) antagonists such as scopolamine are capable of inducing a wide range of peripheral side-effects¹ (**Chapter 2**). By using drugs which target cholinergic receptors more selectively, it is expected that correlating blockade of cholinergic neurotransmission with specific cognitive dysfunctions will be relatively more straightforward.

ACETYLCHOLINE IN THE BRAIN AND BODY

Acetylcholine exerts its effects on brain function via binding to and interacting with two main classes of cholinergic receptors; i.e., nicotinic and muscarinic. Nicotinic receptors are ionotropic receptors or ligand-gated ion channels, of which the actions can be characterized by a fast onset and a short duration of postsynaptic effects (Kandel et al., 2000). The second class of cholinergic receptors is made up by the muscarinic receptors, which are the main focus of the current dissertation. All muscarinic receptors are metabotropic or G-protein-coupled receptors which actions have a slower onset and longer duration relative to those of nicotinic receptors (Caulfield, 1993). In Table 1 an overview is given of the signaling cascades, localization and disease relevance of muscarinic receptor subtypes. As is evident in this Table, the most predominant receptor subtype found in the brain is the muscarinic M₁ receptor, which is mainly expressed in the cerebral cortex, hippocampus, amygdala and striatum (Caulfield, 1993; Flynn et al., 1997). Interestingly, in the hippocampus – a site which is heavily implicated in learning and memory processes – the majority of muscarinic receptors are of the M₁ subtype (Volpicelli & Levey, 2004). Furthermore, density of the M₁ receptor in the periphery is relatively sparse compared to muscarinic receptors M₂-M₅ (Caulfield, 1993). Thus, drugs which selectively block the effects of acetylcholine at muscarinic M₁ receptor sites might be preferable as a pharmacological model for cognitive deficits associated with aging, dementia, and schizophrenia.

AIM OF THE DISSERTATION

The main goal of the current thesis is to determine the role of the muscarinic M₁ receptor in cognition. Based on the predominance of the muscarinic M₁ receptor in the hippocampus (e.g., Volpicelli & Levey, 2004), it is hypothesized that drugs targeting the muscarinic M₁ receptor will serve as a model for learning/memory rather than attentional impairment. Secondly, given the relatively low density of the muscarinic M₁ receptor in peripheral tissues, it is expected that drugs targeting this receptor subtype will display less severe side-effects compared to non-selective muscarinic ligands. Finally, results will be interpreted in a psychopathological framework; i.e., they will be related to findings in patient groups which

¹ Since the current thesis focuses on the central nervous system, actions of cholinergic drugs at peripheral sites are interpreted as side-effects which might hamper interpretation of central effects on cognition and behavior.

are known to exhibit cholinergic dysfunction, such as Alzheimer's disease and schizophrenia (Bartus et al., 1982; Sarter et al., 2012).

METHODOLOGY

The majority of the pharmacological studies described in the current dissertation are conducted with a placebo-controlled crossover design which, in the case of human experiments, is performed double-blind. We use several drugs to examine the effects of cholinergic manipulations on cognition and behavior, which generally fall into two categories: 1) muscarinic antagonists, i.e., scopolamine and biperiden, and 2) cholinesterase inhibitors, i.e., donepezil and rivastigmine. The cholinergic hypothesis postulated by Bartus et al. (1982) has provided the rationale for the use of the non-selective muscarinic antagonist scopolamine as a standard/reference drug for inducing age- and dementia-related cognitive deficits in healthy humans and animals (Ebert & Kirch, 1998; Flood & Cherkin, 1986). However, as will be described in further detail in **Chapter 2**, the tendency of scopolamine to bind non-selectively to muscarinic receptors hampers interpretation of systemic applications of the drug; i.e., widespread blockade of muscarinic receptors in the brain and body makes it difficult to dissociate central and peripheral drug effects from one-another.

Therefore, all studies described in this dissertation use the muscarinic M_1 antagonist biperiden (often in comparison to and/or in combination with scopolamine, donepezil or rivastigmine), to explore the role of the M_1 receptor subtype in cognition. It has about 10-fold higher affinity for M_1 as compared to M_2 - M_5 receptors (Bolden et al., 1992; Katayama et al., 1990). Although its selectivity for muscarinic receptors can be characterized as relative or *functional* rather than absolute (Eglen, 2006), it is nevertheless currently the most selective M_1 antagonist available for use in human participants. Moreover, muscarinic M_1 agonists are not yet approved for use in humans (Bradley et al., 2010). Therefore we chose to employ cholinesterase inhibitors donepezil and rivastigmine to examine their interactions with biperiden on cognitive function in rats and humans, respectively.

In addition, the studies described in this dissertation can be characterized as translational, as the cognitive effects of cholinergic manipulations are explored in both normal volunteers and healthy rats. Since animal models of cognitive dysfunction are used extensively as a tool for screening potential cognition-enhancing medications, it is pertinent that the usability of muscarinic drugs for this purpose is explored. Furthermore, we chose to test young, healthy participants instead of patients groups in order to be able to assess whether muscarinic drugs would be capable of inducing cognitive deficits comparable to those in aging, Alzheimer's disease and/or schizophrenia.

Lastly, we employed EEG paradigms in several of our experiments in order to enhance translatability of results; particularly, we chose to use a learned irrelevance (LIrr), paired-click, and novelty oddball task. Schizophrenia patients have been shown to display deficits in these paradigms (e.g., Baldeweg et al., 2004; Boutros et al., 2004; Orosz et al., 2008), and we sought to determine to what extent aberrant cholinergic signaling might contribute to this phenomenon. Central to schizophrenia is the idea that individuals suffering from this disorder have difficulties inhibiting irrelevant input and/or display deficits in the gating of repetitive stimuli (Patterson et al., 2008). These processes can be measured with LIrr and paired-click paradigms, respectively. It is assumed that the lack in the ability to suppress

redundant information causes it to gain access to conscious processing, ultimately leading to sensory overload and attentional dysfunction in schizophrenia patients. Like the paired-click task, the novelty oddball task also makes use of the automatic processing of auditory stimuli in the absence of effortful attention (e.g., Javitt et al., 2008; Leiser et al., 2011; Lijffijt et al., 2009). It generates a so-called mismatch negativity (MMN) ERP potential which provides an index of context-dependent change detection and/or sensory memory (e.g., Toyomaki et al., 2008). Schizophrenia patients generally show deficits in MMN generation (e.g., Light & Braff, 2005).

OUTLINE OF THE DISSERTATION

Chapter 2 attempts to answer the following question: what cognitive and physiological processes are affected by the non-selective muscarinic antagonist scopolamine? Papers are reviewed in which scopolamine is used as a standard/reference drug to induce cognitive impairment in animals. At the end of **Chapter 2**, muscarinic M_1 antagonists are put forward as a more selective way of inducing cholinergic-induced cognitive deficits.

Chapters 3 and 4 try to provide an answer to the question whether muscarinic M_1 antagonists indeed have relatively more specific effects on cognition (i.e., on learning/memory rather than other central and/or peripheral processes) in healthy rats and healthy volunteers. In **Chapter 3**, a battery of operant tests is used which assess sensorimotor responding (fixed ratio schedule of reinforcement; FR5), food motivation (progressive ratio schedule of reinforcement; PR10), attention and working memory (delayed non-matching to position: DNMTPT) in order to determine whether the muscarinic M_1 antagonist biperiden induces relatively more selective effects on memory compared to the non-selective muscarinic antagonist scopolamine. **Chapter 4** explores the effects of biperiden on verbal memory, psychomotor behavior and self-reports of side-effects in young, healthy participants, again to determine the specificity of biperiden as a cognition-impairing drug.

Chapters 5, 6 and 7 assess whether the muscarinic M_1 receptor is involved in several cognitive impairments and event-related potential (ERP) abnormalities seen in schizophrenia. In **Chapter 5**, we expect that biperiden will disrupt learned irrelevance (LIrr) and ERPs in healthy volunteers. **Chapter 6** describes the interaction between muscarinic antagonists scopolamine and biperiden, and the cholinesterase inhibitor donepezil on sensory gating in the rat. We anticipate a disruption of sensory gating after both scopolamine and biperiden, and reversal by donepezil. However, in line with their respective binding affinities we hypothesize that scopolamine will affect auditory processing more severely than biperiden (i.e., beyond a deficit in sensory gating). **Chapter 7** explores the effects of biperiden and rivastigmine on sensory gating and mismatch negativity in young, healthy participants. Again, we expect that biperiden will disrupt auditory processing, sensory gating and mismatch negativity (MMN), and that rivastigmine will reverse these effects. Finally, **Chapter 8** gives an overview and general of the main findings of this dissertation; suggestions for future research are provided.

Table 1. Muscarinic receptor subtypes: signaling, expression, and disease relevance (modified from: Caulfield, 1993; Flynn et al., 1997; Langmead et al., 2008).

Characteristics	Muscarinic receptor subtype				
	M ₁	M ₂	M ₃	M ₄	M ₅
Signaling cascade	G _q (excitatory) Post-synaptic, presynaptic in striatum	G _{i/o} (inhibitory) Presynaptic	G _q (excitatory) Pre- and postsy- naptic	G _{i/o} (inhibitory) Pre- and postsynaptic	G _q (excitatory) Postsynaptic
Central expres- sion	Cortex, hippo- campus, amygdala, striatum	Basal fore- brain/septum, cortex, striatum, thalamus, hypothalamus, pons, cerebel- lum	Relatively low abundance but widely distributed in the brain (e.g., cortex, hippo- campus, thala- mus)	Striatum, also low presence in cortex and hippocampus	Low abun- dance, particu- larly, substantia nigra
Peripheral ex- pression	Endothelial cells in vascu- lar tissue	Smooth muscle tissue at respira- tory, vascular, and gastrointes- tinal sites, heart	Salivary glands, smooth muscle tissue at pupil- lary, respiratory, vascular, and gastrointestinal sites	Spinal cord	Little data, likely low expression but widely distrib- uted
Disease relevance	Alzheimer’s disease, schizophrenia, cognitive dysfunction	Alzheimer’s disease, cogni- tive dysfunction, pain	Urinary inconti- nence, chronic obstructive pulmonary disorder, irrita- ble bowel syndrome	Parkinson’s disease, schizophrenia, pain	Drug depend- ence, Parkin- son’s disease, schizophrenia

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CHAPTER 2

The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioral studies

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INTRODUCTION

Short historic overview

In ancient times people already knew that certain plant extracts possess the capability to influence the mental state of an individual (Schultes & Hofmann, 1979). The extracts of several herbs and plants like Belladonna, Mandrake, Datura and Henbane were used to make “magic potions” which could induce delusional states of the mind. After ingestion, most individuals would lose all sense of reality and afterwards could not recall what occurred during the time of intoxication. Furthermore, Belladonna extract was used in the Middle Ages by women who wished to increase their attractiveness by pupil dilation. Much later it was discovered that these herbs and plants contain a relatively high concentration of tropan alkaloids such as atropine, hyoscyamine and scopolamine hydrobromide (hereafter abbreviated as scopolamine), which are the active ingredients responsible for inducing hallucinations.

Of these tropan alkaloids, the non-selective muscarinic antagonist scopolamine has frequently been employed in the clinic, mainly to inhibit the effects of parasympathic activation (Brown & Taylor, 1996; see also Safer & Allen, 1971). However, there exists a multitude of other clinical applications of this compound. For instance, the amnesic properties of scopolamine were already noticed at the beginning of this century (Gaus, 1906; Thompson & Cotterill, 1909) and therefore the substance was employed for obstetrical analgesia. Moreover, in 1874 the use of belladonna to antagonize Parkinsonism tremor was reported. A fourth well-known application of scopolamine is the alleviation of motion sickness (Klöcker et al., 2001; Parrott, 1989). Besides being employed in a clinical setting, scopolamine has also been used to facilitate interrogation (Geis, 1961).

Traditionally, scopolamine has been used in the field of neuropsychopharmacology as a standard/reference drug for inducing age- and dementia-related cognitive deficits in healthy humans and animals (Ebert & Kirch, 1998; Flood & Cherkin, 1986). Interestingly, the clinical observation that scopolamine treatment as a premedication for anesthesia frequently gave rise to amnesia stimulated research on the effects of cholinergic blockade on human memory (see Hardy & Wakely, 1962; Kopelman, 1986). The use of scopolamine as a pharmacological model of *cholinergic amnesia* became very popular after the cholinergic hypothesis of geriatric memory dysfunction was postulated (Bartus et al., 1982). This hypothesis assumed that the age-related decline in cognitive functions was predominantly related to a decrease in the integrity of cholinergic neurotransmission. Since scopolamine-induced amnesia was most likely caused by a blockade of cholinergic signaling, it was used to model cognitive deficits associated with aging and (Alzheimer’s) dementia (Flood & Cherkin, 1986). The first experimental studies that evaluated the effects of scopolamine on cognitive functions in man were published in the early seventies (e.g., Crow & Grove-White, 1973; Safer & Allen, 1971). Drachman and Leavitt (1974) were the first who claimed that, when given to healthy volunteers, scopolamine mimicked many of the cognitive dysfunctions that could be observed in aging and dementia.

The significance of scopolamine use in humans also gave rise to studies in the field of animal research. The first experiments that investigated the central effects of scopolamine in animals were reported in the 1950’s (e.g., Boren & Navarro, 1959; Hearst, 1959). Scopolamine was employed to assess the role of the cholinergic system in non-specific types of behavior such as the rate of responding in operant tasks. Muscarinic antagonists such as

scopolamine were presumed to induce behavioral disinhibition² (Carlton, 1963). Later studies have employed scopolamine as a golden standard drug to validate new test models of cognitive function (Ennaceur & Meliani, 1992), and to investigate the role of the cholinergic system in cognition - for instance by testing its interaction with other drugs (Levin, 1988) and specific brain lesions (Chudasama et al., 2004). These experiments supported the notion that scopolamine induced impairments in a wide variety of cognitive-behavioral tasks (Chintoh et al., 2003; Estapé, 2002; Flood & Cherkin, 1986; Hodges Jr. et al., 2009; Hughes et al., 2004; Ison & Bowen, 2000; Nomura et al., 1994; Robinson et al., 2004; Sambeth et al., 2007). Another important objective in which scopolamine has often been used is the preclinical testing of new substances that are designed to treat cognitive dysfunction (Snyder et al., 2005). Thus, if a compound is effective in reversing scopolamine-induced cognitive deficits in animals, it might also improve cognitive function in healthy participants or people diagnosed with a neuropsychiatric disorder. One of the advantages of the scopolamine model is that it provides a simple and quick way for testing cognition-enhancing properties of new drugs. However, this type of approach in preclinical screening of novel substances is associated with limited predictive validity, as it tends to yield a high number of false positives (i.e., drugs that were found to counteract scopolamine-induced deficits in preclinical research often do not have the same efficacy in clinical studies: Sarter, 2006).

The cholinergic hypothesis

A more recent hypothesis regarding the role of acetylcholine in learning and memory processes has been put forward (Hasselmo, 1999, 2006; Hasselmo et al., 2002; Hasselmo & McGaughy, 2004; Meeter et al., 2004). It has been suggested that acetylcholine plays an important role in hippocampal mode shifting between encoding and retrieval. Specifically, a network model has been proposed which features the entorhinal cortex, dentate gyrus, CA3, CA1 and the medial septum (Meeter et al., 2004). In this model, high acetylcholine would allow acquisition of new information to take place, while low acetylcholine would enable recall of previously stored memories (Hasselmo & McGaughy, 2004). Hence, encoding should be impaired by decreases in acetylcholine and consolidation, and retrieval is likely to be affected by increases in acetylcholine. Support in favor of this hypothesis is the finding that scopolamine predominantly impairs acquisition processes and can even facilitate consolidation (e.g., Winters et al., 2006; 2007; 2008; Young et al., 1995). Moreover, in a delayed non-matching to sample task intraseptal infusion of the cholinergic agonist carbachol 30 min. prior to the sample was found to have no effect on performance. In contrast, infusion immediately or 90 min. after the sample produced amnesia (Bunce et al., 2004).

However, the cholinergic hypothesis of memory function as originally put forward by Bartus et al. (1982) has undergone a revision after several lesion studies were performed which used the highly specific cholinergic toxin 192 IgG-saporin (Wiley et al., 1995). This immunotoxin is a combination of a ribosome inactivating protein (saporin, which is derived from the plant *Saponaria officinalis*) and a monoclonal antibody (192 IgG) which acts on the low-affinity nerve growth factor (NGF) receptor p75 (Wiley et al., 1991). Saporin is a toxin

² At the time it was believed that the cholinergic system served to inhibit an activating system. Hence, reducing cholinergic neurotransmission by administration of a muscarinic antagonist would disinhibit this activating system and cause behavioral activation/disinhibition.

which enzymatically shuts down ribosome protein synthesis, leading to cell death (Wrenn & Wiley, 1998). The addition of 192 IgG to saporin enables the induction of selective cholinergic basal forebrain lesions in rats, as only the cholinergic neurons in this brain area express the p75 receptor (Wenk, 1997). However, animals that were lesioned with this toxin did not show clear learning and memory impairments and hence it appeared that the influence of acetylcholine was more subtle than assumed (e.g., Baxter & Gallagher, 1996; Baxter et al., 1997; Chiba et al., 1999; McGaughy et al., 2000; Torres et al., 1994; Voytko, 1996). Furthermore, it was found that attention tests tended to be relatively more sensitive to treatment by scopolamine as compared to learning and memory tasks (Hodges Jr. et al., 2009; Humby et al., 1999; Jones et al., 1995; Jones & Higgins, 1995; Mirza, 2000; Shannon & Eberle, 2006). Indeed, scopolamine disrupted performance in these tasks at much lower doses than those which were effective in learning and memory tests. It was concluded that acetylcholine is mainly involved in attentional processes (Blokland, 1995; Everitt & Robbins, 1997; Fibiger, 1991; Sarter & Bruno, 1997).

Apart from the role of acetylcholine in cognition, this neurotransmitter has also been strongly implicated in optimization of the signal-to-noise ratio in the parietal and visual striate cortex (Murphy & Sillito, 1991; Sato et al., 1987), a feature which is deemed essential for stimulus processing. For instance, in rats that were performing a sustained attention task, cholinergic deafferentation was found to diminish the neuronal activation of detected cues and enhance neuronal activity to distractor stimuli in neurons in the posterior parietal cortex (Broussard et al., 2009). According to the authors, this implicated an important modulatory role of acetylcholine in the detection and processing of relevant stimuli and the inhibitory suppression of irrelevant cues under attentionally challenging conditions. Moreover, it has been reported that increasing acetylcholine levels with donepezil reduces the spatial spread of excitatory visual responses in human visual cortex, which was suggested to be caused by a facilitation of thalamocortical synaptic transmission and/or suppression of intracortical connections (Silver et al., 2008). These effects were interpreted by the authors to be consistent with a role of acetylcholine in reducing excitatory receptive field size of cortical neurons in animals. Furthermore, it was reported that attending to the particular receptive field of a V1 neuron caused an increase in firing rate, which was enhanced by low doses of acetylcholine and modulated by the muscarinic antagonist scopolamine, but not by the nicotinic antagonist mecamylamine (Herrero et al., 2008). These results thus demonstrated an important role for muscarinic but not nicotinic receptor mechanisms in cholinergic attentional modulation.

These recent notions concerning the function of acetylcholine have, of course, direct consequences for interpreting effects of scopolamine on cognition. Scopolamine is a relatively specific antagonist of muscarinic receptors, i.e. not nicotinic at low doses (Schmeller et al., 1995; Weiner, 1980), and its effects should most likely be interpreted within a cholinergic framework. In conclusion, acetylcholine seems to be primarily involved in attentional processes (Blokland, 1995; Everitt & Robbins, 1997; Fibiger, 1991; Sarter & Bruno, 1997) and plasticity of sensory neurons (Rasmusson, 2000). This constitutes a shift from the claims of the cholinergic hypothesis of learning and memory in the early eighties (Bartus et al., 1982; 1985) and from the relatively more restrictive hypothesis which was put forward more recently (Hasselmo, 1999; 2006; Hasselmo & McGaughy, 2004; Parikh et al., 2007).

AIM AND SCOPE OF THIS REVIEW

The current review provides a summary of the effects of scopolamine on animal behavior. First, the general properties of scopolamine and effects of this drug on non-behavioral measures will be discussed. Next, an overview will be given of behavioral studies which have assessed the effects of peripheral and central administration of scopolamine. Finally, the validity of scopolamine as a pharmacological model for inducing cognitive impairment will be debated and muscarinic M_1 antagonists will be introduced as a more selective and hence, more effective way of inducing cognitive deficits due to cholinergic blockade. Since scopolamine can be viewed as the golden standard for inducing cholinergic impairments, it was decided to put the focus of the review on this compound. Discussion of non-selective muscarinic drugs other than scopolamine is beyond the scope of this review (with the exception of selective muscarinic M_1 antagonists which will be put forward as an alternative for scopolamine). The specific characteristics of various drugs (e.g., blood brain barrier penetration, central versus peripheral effects, duration of action) may lead to differences in the effects of these ligands on cognition, which may therefore complicate interpretation of the outcome of different studies. In short, in the field of neuropsychopharmacology several additional non-selective muscarinic antagonists have been used besides scopolamine. For instance, atropine has also been frequently employed in behavioral studies, although less often than scopolamine. Atropine is less potent than scopolamine (about a factor 10, depending on assay), but in general effects on task performance are highly comparable between both compounds.

An additional restriction was made with respect to the type of experiments which were included in this review. Reference is made to studies which primary aim was to investigate the effects of scopolamine (or selective muscarinic M_1 antagonists) on cognition and behavior. Consequently, studies which have employed non-behavioral parameters (such as in-vitro and/or imaging experiments) will only be discussed very superficially. Moreover, no extensive review is given of experiments in which pharmacological reversal of scopolamine-induced impairment is investigated. Since a vast amount of studies on scopolamine has accumulated over the past 40 years, it would be impossible to refer to all of them. Nevertheless, it is our opinion that the most important and influential articles have been included in the present review.

GENERAL PROPERTIES OF SCOPOLAMINE

Biochemical characteristics of scopolamine

Scopolamine has generally been regarded as a non-selective muscarinic receptor antagonist (Bolden et al., 1992). The affinity of scopolamine to bind to the muscarinic receptor is about 1 nM in the brain of rats (Lee & El Fakahany, 1985). Furthermore, it has been demonstrated that scopolamine has a high selectivity for the muscarinic receptor (Hulme et al., 1978), although high doses of scopolamine can also block nicotinic receptors (Schmeller et al., 1995; Weiner, 1980). Currently, five different subtypes of muscarinic receptors have been characterized (Caulfield, 1993). This differentiation in muscarinic receptors was discovered after finding discrepancies in the binding characteristics of several ligands (e.g., scopolamine, pirenzepine: Messer et al., 1987). Pharmacological methods provided evi-

dence for the existence of three receptor subtypes (M_1 , M_2 and M_3 , see Waelbroeck et al., 1986), whereas the fourth and fifth muscarinic receptor subtypes were discovered only after molecular tools became available (Yasuda et al., 1992). All muscarinic receptors are G-protein coupled receptors: M_1 , M_3 and M_5 are linked to G_q which activates phospholipase-C, whereas M_2 and M_4 are coupled to $G_{i/o}$ and can affect adenylyl cyclase or various ion channels (Conn et al., 2009). The M_2 and M_3 receptors are located presynaptically (Caulfield, 1993), where they act as autoreceptors to regulate neurotransmitter release (Bymaster et al., 2003). The orthosteric binding sites, to which acetylcholine and several muscarinic compounds bind, have been well characterized (Caulfield, 1993; Caulfield & Birdsall, 1998). However, allosteric muscarinic receptor sites are not as extensively defined; these sites could be interesting targets for the development of more selective muscarinic compounds which can modulate the activation of orthosteric muscarinic receptors by acetylcholine (Wess, 2005).

Table 1. Distribution of the different muscarinic subtypes in the brain and body (adapted from: Caulfield, 1993). Of note, no distinction was made between findings from studies using mRNA and anti-body techniques.

Brain area	Central distribution of muscarinic receptor subtypes				
	M_1	M_2	M_3	M_4	M_5
Basal forebrain/ septum		XX			
Cortex	XX		X	X	
Hippocampus	XX	X	X	X	X
Amygdala	X				
Striatum	X	X	X	XX	
Peripheral tissues	Peripheral distribution of muscarinic receptor subtypes				
	M_1	M_2	M_3	M_4	M_5
Heart		XX			
Lung		X	X	XX	
Ileum		X	XX		
Exocrine Glands	X		XX		

X : low/modest mRNA expression or immunoprecipitation.

XX : high/strong mRNA expression or immunoprecipitation.

Molecular biological studies revealed that there are five different genes responsible for the expression of muscarinic receptors, denoted M_1 - M_5 (Bonner et al., 1987; 1988). Furthermore, each receptor subtype likely has its own specific functional role reflected by differences in physiological distribution. In Table 1 an overview is given of the localization of muscarinic receptors in the brain and body. Localization and distribution of these subtypes was determined using mRNA techniques and receptor subtype-specific antibodies. It appears that M_1 (and M_5) receptors are predominantly located in the brain, although a high immunoprecipitation of M_1 receptors has also been observed in the vas deferens (Caulfield, 1993). On the other hand, the M_2 , M_3 and M_4 receptors seem to be evenly distributed throughout the body and the brain. Since scopolamine has no preference for any muscarinic subtype (Bolden et al., 1992), it non-selectively blocks the effects of acetylcholine at muscarinic receptors and thus affects several physiological functions. However, it appears

that the central effects of scopolamine might be mediated by the M_1 and (possibly) M_5 receptors because of their specific distribution (Caulfield, 1993).

PERIPHERAL VS. CENTRAL EFFECTS OF SCOPOLAMINE

Most studies have used systemic injections (IV, IM, IP, SC) to investigate the effects of scopolamine, as this is a relatively easy manner to apply the drug. Unfortunately, systemic administration makes it difficult to differentiate between central and peripheral effects of a compound. In the case of scopolamine, one way to deal with this problem is to include an experimental group that is given methylscopolamine at an equivalent dose (Harvey et al., 1983). Methylscopolamine, or scopolamine methylbromide, is a quaternary form of scopolamine that has the same receptor binding characteristics but does not readily cross the blood-brain barrier (Pradhan & Roth, 1968). The substance has been used as a control condition for the peripheral side effects of scopolamine hydrobromide treatment (Evans, 1975). Thus, if scopolamine hydrobromide impairs cognitive performance at a given dose and an equivalent dose of methylscopolamine does not, it can be assumed that the effects of the former are mediated by a central mechanism. In animal studies the use of methylscopolamine has been questioned, as it has been found to have an influence on measures of cognitive performance (e.g., Andrews et al., 1994; Herremans et al., 1995; Pakarinen & Moerschbaecher, 1993; van Haaren & van Hest, 1989). This would suggest that methylscopolamine can also have central actions, especially when high doses are used. A microdialysis study showed that methylscopolamine indeed increased cortical acetylcholine release, although it should be mentioned that this effect occurred at relatively higher doses (factor 2-4) than those which are commonly used for administration of scopolamine hydrobromide (Moore et al., 1992). Although there exist a lot of studies that show no central/cognitive effects of methylscopolamine, abovementioned data caution against simply assuming that this compound can be used a tool to dissociate central and peripheral effects of scopolamine hydrobromide.

NON-BEHAVIORAL EFFECTS OF SCOPOLAMINE

Pupil diameter, salivation and smooth muscle function

It is well-known that scopolamine dilates the pupil and impairs the lens accommodation (Leopold & Comroe, 1948), an effect likely to be mediated by the muscarinic M_3 receptor (Shiraishi & Takayanagi, 1993). In a study by Jones and Higgins (1995), a dose-dependent increase in pupil size was found after scopolamine injections (0.01-0.1 mg/kg, SC), which was already observed at a dose of 0.01 mg/kg. Interestingly, methylscopolamine was approximately twice more effective in dilating the pupil than scopolamine hydrobromide, suggesting that the peripheral effects of the former are relatively more pronounced than those of the latter. Scopolamine-induced mydriasis could lead to a disruption of performance in visual discrimination tasks. For instance, due to an increased sensitivity to light, visual stimuli of relatively high intensity might be experienced as aversive (Leaton & Kreindler, 1972). Alternatively, effects of scopolamine on pupil diameter could also improve

performance on visual discrimination tasks: stimuli might be more easily perceived if they are presented very briefly or at a low illuminance.

Moreover, scopolamine reduces salivation which is probably mediated by cholinergic blockade at muscarinic M_3 receptors, but M_1 and M_5 receptors could also play a minor role (Dai et al., 1991; Flynn et al., 1997; Shannon et al., 1994; Shida et al., 1993; Tobin et al., 2002). Because of this dry mouth effect of scopolamine solid food rewards are likely to become more difficult to consume. This could decrease the palatability of dry food, which could in turn interfere with performance on behavioral tasks that rely on this type of reinforcer in order to motivate the animals. Indeed, scopolamine was reported to already decrease dry food consumption at a dose of 0.01 mg/kg, but it was suggested that using a liquid reward could counteract this side-effect (Hodges Jr. et al., 2009).

In the respiratory, vascular and gastrointestinal system, M_2 and M_3 receptors appear to mediate contraction of smooth muscle (Eglen et al., 1996). In what way non-selective effects of scopolamine on peripheral smooth muscle functions disrupt behavioral performance has not been directly assessed. Nevertheless, high doses of scopolamine might induce gastrointestinal distress (e.g., constipation) or give rise to changes in cerebral blood flow and glucose consumption due to vasodilation/vasoconstriction, which in turn could interfere with behavioral performance.

ELECTROENCEPHALOGRAM

A great deal of research has been dedicated to the neurobiological mechanisms underlying the cortical electroencephalogram (EEG). One of the pacemakers of the cortical EEG appears to be the cholinergic system (see Brimblecombe, 1974; Dringenberg & Vanderwolf, 1998). Muscarinic agonists have been found to activate the EEG in rats (i.e., low voltage fast activity; LVFA). On the other hand, scopolamine decreased the LVFA after a relative high dose (5 mg/kg, IP) in anesthetized rats (Dringenberg & Vanderwolf, 1997). However, a decrease in LVFA in rats has also been observed after administration of relatively lower doses of scopolamine (0.8 mg/kg IP: Riekkinen et al., 1990) or after injecting it iontophoretically (applied 5×10^{-8} A) into the cortex (Spehlmann & Norcross, 1982). These effects seemed to be mediated by the ascending cholinergic fibers from the basal forebrain (Vanderwolf, 1988) and indicated that scopolamine gives rise to a cortical deactivating state. In addition, it appeared that scopolamine can also induce disturbances in gamma oscillations (Rodriguez et al., 2004). The effects of scopolamine (0.1, 0.2, 0.5, 1 mg/kg, SC) on event-related potentials (ERPs) have also been assessed. For instance, scopolamine reduced the amplitude of auditory evoked potentials (i.e., P18 and N40 components) in a dose-dependent manner (Campbell et al., 1995), which again suggested that it tends to lower EEG activity. These results were replicated by a more recent study (Sambeth et al., 2007).

It appears to be quite a challenging task to relate animal EEG measures to behavioral data. For instance, a study by Sambeth et al. (2007) reported an impairment of object recognition, an increase of the theta frequency in the EEG and a change in the N1 and N2 components of the AEP closer to baseline after scopolamine treatment (0.1 mg/kg, IP). Both the acetylcholinesterase inhibitor donepezil (3 mg/kg, PO) and nicotine (0.1 mg/kg, IP) reversed the impairment in object recognition. However, combination treatment of scopolamine (0.1 mg/kg, IP) with donepezil (3 mg/kg, PO) increased theta even more, whereas

nicotine (0.1 mg/kg, IP) reversed the scopolamine-induced increment of theta to placebo level. The clear dissociation between effects of drug combination treatments on memory and EEG suggests that the relation between these two measures is not straightforward.

In human participants scopolamine has been reported to increase delta and theta activity whilst decreasing alpha and beta frequency bands (Ebert & Kirch, 1998), indicative of a general slowing of EEG activity. Scopolamine (0.25-0.75 mg, IM) was also found to reduce the amplitude and increase the latency of flash-VEP P2-N3 ERPs with no effect on pattern-VEPs (Bajalan et al., 1986; Sannita et al., 1993). Moreover, scopolamine decreased P3 amplitude of auditory evoked potentials (Meador et al., 1995). A few studies have examined the relation between effects of scopolamine on EEG power and memory performance. For instance, Sloan et al. (1992) found no correlation between slowing of EEG power and decreased word recall on a verbal memory task after scopolamine. In contrast, Kikuchi et al. (1999) reported a negative correlation between memory score and absolute delta and alpha-1 power, whereas relative alpha-1 power was negatively correlated and relative alpha-2 power positively correlated with mnemonic performance after scopolamine administration. Overall, large alpha and small theta power seems indicative of good cognitive performance (Klimesch, 1999), which is in concurrence with scopolamine-induced slowing of EEG activity and concomitant disruption of performance.

In conclusion, in both animals and humans scopolamine appears to decrease the power of higher frequency bands – i.e., LVFA in animals, alpha and beta frequencies in humans (Dringenberg & Vanderwolf, 1997; Ebert & Kirch, 1998). Moreover, scopolamine reduced the amplitude of auditory evoked potentials in rats and humans (Campbell et al., 1995; Meador et al., 1995), although it should be mentioned that ERPs are not directly comparable between the two species (Sambeth et al., 2003; 2004). Scopolamine is also found to disrupt cognitive performance in rats as well as in humans (Kikuchi et al., 1999; Sambeth et al., 2007; Sloan et al., 1992).

BEHAVIORAL EFFECTS OF SCOPOLAMINE

In Table 2 an overview is given of the effects of systemic administration of scopolamine in various behavioral paradigms. Studies on the effects of scopolamine on behavior and cognition have been sorted in reverse chronological order per behavioral test paradigm. Doses were peripherally administered in rats, mice, rabbits, Mongolian gerbils, cats, pigeons or monkeys. Table 3 provides an overview of the effects of scopolamine in different behavioral tasks after central administration. Studies on the effect of scopolamine on behavior and cognition have been sorted in reverse chronological order per targeted brain region and behavioral paradigm.

The discussion of the behavioral effects of scopolamine will be divided into five parts. The first part will deal with the effects of scopolamine on locomotor behavior and motor learning. In the following three sections the effects of scopolamine on anxiety, sensory/stimulus discrimination and attention will be discussed. Of note, although drug effects on the latter two functions cannot always be dissociated from one another, discussion of effects of scopolamine on sensory/stimulus discrimination vs. attention will be separated on basis of the claims made in the respective reports. Furthermore, effects on sensory or stimulus discrimination tests are usually strictly related to presentation of the stimulus,

whereas effects on attentional tasks can also be attributed to other (non-)cognitive factors. The fifth section will discuss the effects of scopolamine on learning and memory.

Locomotor activity and motor learning

Scopolamine has been generally found to increase locomotor activity already at systemic doses of 0.056 mg/kg and higher (Chintoh et al., 2003; Day et al., 1991; Gholamreza et al., 2002; Nomura et al., 1994; Pradhan & Roth, 1968; Renfro et al., 1972; Sipos et al., 1999; Watanabe & Shimizu, 1989). Cholinergic signaling in the hippocampus, striatum and/or frontal cortex appeared to be correlated positively with scopolamine-induced hyperactivity (Day et al., 1991) and central infusion of scopolamine (1, 10 μ g in 0.5 μ L/site) in the amygdala was found to increase locomotor activity (Nomura et al., 1994). In contrast, several experiments which used memory and/or operant tasks have reported a decrease in locomotor activity after systemic administration of scopolamine (Anisman & Kokkinidis, 1975; Besheer et al., 2001; Hodges Jr. et al., 2009; Masuoka et al., 2006; Myhrer et al., 2004; Pradhan & Roth, 1968) or no effect at all (Feigley, 1974; Humby et al., 1999). Thus, the role of scopolamine in locomotor behaviour – i.e., an increase or a decrease in activity, appears to be dependent on various experimental factors (such as strain, test, type of reinforcement, drug doses, etc.). For instance, a recent study showed that the effect of scopolamine on general activity in light-dark boxes was dependent on room novelty and gender of the rats (Hughes et al., 2004). For the scope of the current review it is sufficient to note that possible effects of scopolamine on locomotor activity should be taken into account when evaluating the role of scopolamine in cognition, especially in tests in which locomotor activity is closely related to the main dependent variable (see also Anisman, 1975).

A recent study examined the role of scopolamine on motor skills and motor learning (Thouvarecq et al., 2001). It was shown that methylscopolamine (0.6, 1.2 mg/kg, SC) impaired the motor skills of mice whereas the same dose of scopolamine hydrobromide (0.6, 1.2 mg/kg, SC) did not. Moreover, motor learning was found to be affected by scopolamine hydrobromide but not by methylscopolamine. It was reported that motor skills were independent of motor learning, as no correlation between the scores was found. In conclusion, the dissociation between motor skills and motor learning on the one hand, and the dissociation between the effects of scopolamine hydrobromide and methylscopolamine on the other, would strongly suggest that scopolamine hydrobromide mainly affects motor learning abilities rather than motor skills.

Anxiety

The effects of scopolamine on anxiety-related behaviors have been investigated in a limited number of papers (Hughes et al., 2004; Smythe et al., 1996). Of note, the distinction between tasks assessing locomotor activity (as discussed above) and those measuring anxiety can be rather small, since these paradigms often use similar experimental setups (such as the open field). Therefore, when interpreting effects of scopolamine on performance in these paradigms, one should keep in mind that an underlying effect on locomotor behavior could be present. Nevertheless, special changes to the apparatus allow for making inferences with respect to anxiety. For instance, by dividing an open field in a dark and a bright compartment it is possible to measure the time animals spend in each of these sections. In a study that assessed light-dark preferences, it was reported that scopolamine (2 mg/kg, IP)

lowered the number of transitions to the light side (Hughes et al., 2004). Furthermore, in the black-white box, scopolamine (0.05, 0.1 mg/kg, IP) was found to increase anxiety-related behavior on both activity-dependent as well as activity-independent parameters (Smythe et al., 1996). Thus, possible behavioral activating effects of the substance could be excluded. Moreover, the doses used in this study were much lower than those used in studies that reported behavioral activation. In the same experiment, infusion of scopolamine in the hippocampus (15, 30 μ g in 3 μ l/side) increased anxiogenic behavior in the black-white box, while locomotor activity was unaffected. In conclusion, scopolamine generally has an anxiogenic effect on behavior which should be taken into account when interpreting effects of this ligand in other behavioral tasks.

Stimulus discrimination

The manner in which stimulus discrimination can be studied and drug effects can be assessed in animal models has been thoroughly described in the review by Heise and Milar (1984). In this paper, great attention was devoted to the different ways in which stimulus discrimination could be investigated in animals and also to possible confounding factors that could lead to erroneous conclusions. For instance, it was suggested that assessments of drug effects on stimulus discrimination can be made more sensitive by interposing a delay between stimulus presentation and response phase. Although this procedure has mainly been used to examine memory functions (see section on learning and memory below), it can also be employed for the evaluation of drug effects on stimulus discrimination, i.e., discrimination is expected to be the strongest at the zero-delay and gradually declines as a function of the length of the delay (Heise & Milar, 1984). Moreover, signal detection theory-derived measures would provide a valuable tool for the evaluation of drug effects on stimulus discrimination. Although the abovementioned arguments strongly support the use of this method, only few animal studies have actually done so.

Several studies have indicated that scopolamine can have differential effects on discrimination performance, as it has been reported to influence accuracy, response latency and missed trials (Drinkenburg et al., 1995). For instance, a study by Bushnell et al. (1997) showed that scopolamine (0.03, 0.056, 0.1 mg/kg, SC) decreased $p(\text{hit})$ – an effect which was dependent on stimulus intensity, increased $p(\text{false alarm})$, number of omissions and reaction time. An additional problem with interpreting effects of scopolamine on discrimination performance is the fact that the dose at which performance was impaired varied a lot between studies: effects on discrimination accuracy have been reported for doses between 0.005 (Kirk et al., 1988) and 2.0 mg/kg (Robinson et al., 2004). It has been suggested that this could be due to differences in salience between stimuli (Andrews et al., 1992), since discrimination of relatively non-salient stimuli appears to be more easily disrupted by scopolamine (Evans, 1975). However, task difficulty cannot fully account for all discrepancies that exist between experiments. Of note, scopolamine appears to not only influence visual discrimination (probably due to its peripheral effects on pupil diameter and lens accommodation), but also auditory discrimination (Ison & Bowen, 2000; Jones, & Shannon, 2000; Sipos et al., 2001; van Haaren & van Hest, 1989), yet not olfactory discrimination (Doty et al., 2003). The second finding suggests that scopolamine can also influence central sensory processes. Indeed, studies which used central infusion of scopolamine have implicated the (dorsal) hippocampus (Brito et al., 1983; Carli et al., 1995; 1999) and periform,

dorsomedial prefrontal and ventromedial prefrontal cortex (Broersen et al., 1995; Herremans et al., 1997; Wilson, 2001) in discrimination processes.

In summary, stimulus discrimination is very sensitive to scopolamine treatment, especially when delayed response tasks and/or signal detection theory-derived measures are used to analyze performance. However, it should be noted that not all studies have reported a scopolamine-induced discrimination deficit (Harder et al., 1998). Effects of scopolamine on missed trials and increased response latencies tend to be relatively more consistent. In particular, doses as low as 0.005 and 0.025 mg/kg were able to decrease the sensitivity parameter (Herremans et al., 1995; Kirk et al., 1988). Furthermore, effects of scopolamine on visual discrimination could be (partly) caused by pupil dilation (Leopold & Comroe, 1948), and hence may not be necessarily caused by central cholinergic blockade only.

Attention

Several studies have specifically evaluated the effects of scopolamine on tasks that were developed to study attentional processes in rodents. A relatively older study by Cheal (1981) assessed the role of acetylcholine in memory vs. behavioral disinhibition vs. stimulus selection in gerbils. By using an object recognition test, it was determined that scopolamine treatment (0.1-10 mg/kg, SC) did not affect memory performance, response inhibition or stimulus selection. However, based on the duration that the gerbils inspected the objects it was concluded that scopolamine probably impaired the maintenance of attention.

In the 5-choice serial reaction time task scopolamine (0.03, 0.075, 0.1 mg/kg, SC) was found to impair performance accuracy under normal conditions (i.e., standard light intensity and no interfering stimuli) only at the highest dose tested: Jones & Higgins, 1995). However, these effects became more apparent when a burst of white noise was introduced during the inter-trial interval. It was suggested that these data underscored the notion that the cholinergic system is involved in selective attention. Similar to the aforementioned studies, scopolamine (0.075 and 0.1 mg/kg) also increased the number of missed trials. Subsequently, a revised version of the 5-choice serial reaction time task was developed which supposedly could dissociate drug effects on attentional vs. motivational or motor processes (Higgs et al., 2000). It was found that scopolamine (0.03, 0.075, 0.1 mg/kg, IP) did not affect accuracy performance in this modified version. On the other hand, at a dose of 0.075 mg/kg a clear increase in response latency and number of omissions was observed. A detailed analysis of signaled vs. non-signaled trials indicated that the effects of scopolamine could not solely be due to attentional dysfunctions. These findings (i.e., relatively reliable effects of scopolamine on general performance measures but no consistent effects on discrimination accuracy parameters) have been corroborated by other experiments using the 5-choice serial reaction time task (Callahan et al., 1993; Higgs et al., 2000; Hodges Jr. et al., 2009; McGaughy et al., 1994; Pattij et al., 2007; Shannon & Eberle, 2006; Spinelli et al., 2006). A study by Davidson and Marocco (2000) using central infusion of scopolamine (4, 7 mg in 1 mL Ringer solution) has implicated the intraparietal cortex in attentional shifting (i.e., covert orienting).

In summary, it appears that the effects of scopolamine on attention tasks are not unequivocal. Although different test paradigms have been utilized that presumably assess sustained attention, scopolamine did not consistently impair accuracy performance (Andrews et al., 1992; Doty et al., 2003; Leaton & Kreindler, 1972). In addition, scopolamine

consistently increased general non-cognitive performance measures, such as response latency and the number of missed trials (Andrews et al., 1992; Bushnell et al., 1997; Drinkenburg et al., 1995). Although the use of signal detection theory-derived measures would probably make attention task performance more sensitive to drug treatments, most studies have used standard parameters, such as percentage correct and number of omissions (Ksir & Slifer, 1982). Nevertheless, the consensus appears to be that scopolamine mainly affects visuospatial sustained attention at doses of 0.02 mg/kg and higher (Callahan et al., 1993; Cheal, 1981; Hodges Jr. et al., 2009; Hoff et al., 2007; Humby et al., 1999; Leblond et al., 2002; Spinelli et al., 2006). In addition, a few experiments have reported effects of scopolamine on cross-modal (visual and auditory) divided attention (McGaughy et al., 1994) and covert orienting of visuospatial attention (Davidson & Marrocco, 2000; Phillips et al., 2000).

Learning and memory

Several papers have given an overview of the effects of cholinergic drugs on learning and memory performance, but not specifically related to scopolamine (Blokland, 1995; Hagan & Morris, 1988; Izquierdo, 1989; McDonald & Overmier, 1998; McNamara & Skelton, 1993; Smith, 1988; Spencer & Lal, 1983). Since the amount of studies in which scopolamine was used to induce performance deficits is tremendous, only a short summary of the effects of scopolamine on learning and memory will be provided in this section. A more comprehensive review of the effects of anticholinergic drugs per behavioral task can be found in the papers of Hagan and Morris (1988), and Spencer and Lal (1983).

First of all, object recognition and spatial alternation tasks have been reasonably consistent in detecting drug effects in the sense that peripherally administered scopolamine generally tends to interfere with short-term memory (Baron et al., 1998; Lieben et al., 2005; Means et al., 1996; Sambeth et al., 2007). However, the effects of scopolamine in operant (delayed) alternation have been assumed to also be related with discrimination processes (Heise et al., 1976). Experiments using central infusion of scopolamine have implicated the infralimbic cortex (Wall et al., 2001), medial septal area (Givens & Olton, 1995; Markowska et al., 1995), caudate nucleus (Prado-Alcala et al., 1978) and dorsal hippocampus as brain regions involved in spatial alternation (Brito et al., 1983). Infusion of scopolamine into the perirhinal cortex (Abe et al., 2004; Warburton et al., 2003; Winters et al., 2006; 2007) impairs object recognition.

In the radial maze, clear performance deficits were generally apparent after systemic scopolamine administration (Masuoka et al., 2006), although it must be noted that in some studies relative high doses of scopolamine (≥ 0.5 mg/kg) were used (Okaichi & Jarrard, 1982). In general, doses of 0.1 mg/kg and higher are required to induce impairments in this task (Buresová & Bures, 1982; Peele & Baron, 1988b; Wirsching et al., 1984). Apart from the effects on acquisition, scopolamine especially disrupted performance when delay procedures were used, which suggests that scopolamine specifically impairs (spatial) working memory (Buresová & Bures, 1982; Peele & Baron, 1988b; Stevens, 1981). Moreover, when utilizing the radial maze procedure in which both working and reference memory can be assessed simultaneously, studies have provided support for a specific effect of scopolamine on working memory (Beatty & Bierley, 1985; Hodges Jr. et al., 2009; Wirsching et al., 1984). However, the selective disruption of working memory after scopolamine has been questioned by other studies (Eckerman et al., 1980; Godding et al., 1982; Okaichi & Jarrard,

1982; Okaichi et al., 1989; Peele & Baron, 1988a). Central infusion of scopolamine in the basal forebrain, dorsomedial thalamus nucleus, medial raphe nucleus (Mishima et al., 2000) and hippocampus (Kim & Levin, 1996) has been found to disrupt radial maze performance. In the Morris water maze, systemic administration of scopolamine has been reported to be more effective in disrupting acquisition than impairing retention (Cozzolino et al., 1994).

Moreover, it was reported that the effects of scopolamine were dependent on age and pool wall brightness (Paylor & Rudy, 1990) and pretraining (Saucier et al., 1996), which suggests that the effects of scopolamine in the Morris task may not only be related with spatial learning. Furthermore, some studies have reported that a dose of at least 0.1 mg/kg was needed to impair place learning (Buresová et al., 1986). In contrast, other experiments showed that higher doses (i.e., 0.8–1.0 mg/kg, IP) did not impair the acquisition of the Morris task (Decker et al., 1990; Riekkinen et al., 1990). Studies using central infusion of scopolamine have reported a role for the posterior cingulate (Riekkinen et al., 1995), septum (Elvander et al., 2004) and dorsal hippocampus (Blokland et al., 1992; Herrera-Morales et al., 2007) in Morris water maze performance.

The majority of studies using delayed (non-)matching procedures have indicated that systemical administration of scopolamine induces a delay-independent impairment at relatively low doses (0.1 mg/kg and lower, see also Steckler et al., 1998), although other studies have challenged this finding (Estepé & Steckler, 2002; Ruotsalainen et al., 1998; Santi & Weise, 1995; Stanhope et al., 1995). Furthermore, in most experiments scopolamine also affected measures of responding, i.e., increased number of omissions, decrease in number of completed trials and increase in response latency (Estepé & Steckler, 2002; Kirkby et al., 1995). Experiments using central administration of scopolamine have implicated the (dorso)medial prefrontal cortex (Broersen et al., 1994; Dunnett et al., 1990; Herremans et al., 1996), prelimbic cortex (Granon & Poucet, 1995) and hippocampus (Dunnett et al., 1990; Robinson & Mao, 1997) in delayed (non-)matching performance.

There exist a multitude of studies that have used scopolamine to assess the role of acetylcholine in anxiety-related memory (Anagnostaras et al., 1999; Roldán, 1997; Rudy & Pugh, 1996; Spangler et al., 1989). For instance, in an active avoidance study by Flood and Cherkin (1986), relatively low doses of scopolamine (0.01 mg/kg 1h before training or 0.1 mg/kg immediately after training, SC) improved retention – probably due to a behavioral activating effect of scopolamine, whereas higher doses of scopolamine (0.1 mg/kg 1h before training or 1.0 mg/kg immediately after training, SC) disrupted performance. Moreover, these effects were dependent on time of administration: injection of scopolamine 1h before the learning phase was relatively more effective in altering retention performance as compared to administration immediately after the learning phase (i.e., effects at lower doses). A study by Oliverio (1968) indicated an interaction effect between the amount of active avoidance pretraining and scopolamine treatment: a dose of 2 mg/kg (IP) ameliorated performance in naïve mice, whereas a similar dose disrupted performance in mice which were pretrained. Using central infusion of scopolamine (10–20 µg), Hamilton and Grossman (1969) have reported a role for the septum in active avoidance in cats.

In several passive avoidance studies (Nomura et al., 1994; Quirarte et al., 1994; Rush, 1988), systemic administration of scopolamine just before training appeared relatively most effective in impairing retention measures (as compared to administration at least 1h before training, after avoidance training or before avoidance testing). However, it seems that age of the animals is also a mediating factor in determining the sensitivity of passive

avoidance performance to disruption by scopolamine; an article of Feigley (1974) reported effects of scopolamine in 20-28 day old rats at a dose of 0.5 mg/kg (IP) whereas in 70-84 day old animals, a dose of 4 mg/kg (IP) disrupted passive avoidance retention. Moreover, amount of pretraining influenced the effect of scopolamine on passive avoidance performance as well, as a dose of 0.5 mg/kg (IP) was found to impair retention in moderately trained rats whereas a similar dose had no effect on retention in over-trained animals (Pazzagli & Pepeu, 1964).

Lastly, level of shock intensity also played a mediating role in the effect of scopolamine on passive avoidance retention; a study by Quirarte et al. (1994) showed that after a dose of 8 mg/kg (IP) using low foot shock conditions, no state-dependency was found, whilst the same dose did have a state-dependent effect when a high level of foot shock was employed. Thus, in avoidance tests effects of systemically administered scopolamine appear to be dependent on the type of avoidance task (i.e., passive vs. active avoidance), time of injection (Flood & Cherkin, 1986; Quirarte et al., 1994), training experience (Oliverio, 1968; Pazzagli & Pepeu, 1964), age of the animals (Feigley, 1974), level of shock intensity (Quirarte et al., 1994), and dose conditions (Flood & Cherkin, 1986). Moreover, central administration of scopolamine in frontal cortex (Santucci & Shaw, 2003), anterior but not posterior cingulate (Riekkinen et al., 1995), hippocampus (Wiener & Messer, 1973), striatum (Diaz del Guante et al., 1991) and amygdala (Bianchin et al., 1999; Nomura et al., 1994) tends to disrupt passive avoidance retention.

Results of experiments in which the effects of scopolamine on fear conditioning were investigated tend to be quite variable, which could again be related to modest differences in test circumstances such as time of administration (Anagnostaras et al., 1999). Nevertheless, systemic injection of scopolamine was most often found to impair both context and cued conditioning (Anagnostaras et al., 1999; Rudy, 1996), an effect which might be mediated by the amygdala (Phillips & LeDoux, 1992). However, no effects (Rudy & Pugh, 1996), effects on only context (Anagnostaras et al., 1995) or cued conditioning (Young et al., 1995), and even a facilitation of cued conditioning have also been reported (Young et al., 1995). In contrast, central infusion of scopolamine in the (dorsal) hippocampus has been reported to impair contextual but not cued conditioning (Rogers & Kesner, 2004; Wallenstein & Vago, 2001). In summary, effects of scopolamine on tasks assessing learning and memory were observed at systemic doses higher than 0.03 mg/kg (dependent on the particular paradigm used). It appears that scopolamine is particularly effective in impairing acquisition/learning and short-term and working memory.

DISCUSSION

Summary and interpretation of the behavioral effects of scopolamine

A review of the literature on scopolamine (see Tables 2 and 3) clearly shows that this drug is capable of inducing various behavioral changes in several animal species (Bohdanecky et al., 1967; Cheal, 1981; Hamilton & Grossman, 1969; Harvey et al., 1983; Pakarinen & Moerschbaeche, 1993; Robinson et al., 2004; Santi & Weise, 1995; Savage et al., 1996; Spinelli et al., 2006), and that scopolamine can cause very potent performance impairment on tests of learning and memory (Anagnostaras et al., 1999; Hodges Jr. et al., 2009; Means et al., 1996; Roldán, 1997; Sambeth et al., 2007; Spangler et al., 1989; Spinelli et al., 2006). This

would corroborate the view that scopolamine is an amnesic drug that disrupts central cholinergic neurotransmission. Initially, much effort has been dedicated to examining whether scopolamine disrupts consolidation vs. retrieval, learning vs. memory, short-term vs. long-term memory, and working vs. reference memory. Since the most consistent effects are found in tests in which a delay interval was interposed between the sample and recognition/choice phase (Estepé & Steckler, 2002; Givens & Olton, 1995; Savage et al., 1996; Stanhope et al., 1995), it has been argued that scopolamine predominantly affects short-term memory processes. However, it has become increasingly clear that tasks that assess stimulus discrimination and/or (sustained) attention are much more sensitive to pick up impairment after scopolamine treatment (i.e., effects at relatively lower doses, see Fig. 1), especially when signal detection-derived measures and/or delay procedures are used. These findings would suggest a role of acetylcholine in sensory processing and attention rather than short-term memory (Sarter et al., 2003). This conclusion is strengthened by the fact that cholinergic lesion studies have yielded comparable results – i.e., on discrimination/attention rather than learning/memory, see above (e.g., Baxter & Gallagher, 1996; Baxter et al., 1997; Chiba et al., 1999; McGaughy et al., 2000; Torres et al., 1994; Voytko, 1996).

Unfortunately, due to the non-specific binding characteristics of scopolamine (Bolden et al., 1992), cognitive-behavioral effects of systemic administration are not very selective. In Figure 1 a schematic overview is made of effects of peripheral administration of scopolamine as a function of behavioral paradigm. As is shown, with increasingly higher doses scopolamine can also impair performance in behavioral tasks which assess non-mnemonic and/or non-attentional processes, such as anxiety (Hughes et al., 2004; Smythe et al., 1996). Furthermore, scopolamine can influence non-cognitive aspects of behavior: it has a rather robust effect on locomotor activity (decrease or increase), response rate (decrease), omissions (increase), and response latency (increase). Scopolamine is also known to affect peripheral, physiological processes such as pupil size (Leopold & Comroe, 1948) and salivation (Dai et al., 1991; Flynn et al., 1997; Shannon et al., 1994; Shida et al., 1993; Tobin et al., 2002). These peripheral effects were found in dose ranges that also disrupt measures of cognitive behavior. Thus, use of (high doses of) scopolamine will affect additional cognitive and non-cognitive aspects of behavior that could directly interfere with performance in tasks assessing stimulus discrimination, attention or memory. As is the case for all drugs, effects of scopolamine on the abovementioned functions are found to be dose-dependent albeit that the dose response curves can differ between behavioral tasks (see Fig. 1).

Systemic administration of scopolamine thus tends to influence both peripheral and central mechanisms and therefore effects on these functions cannot always be dissociated from one another. Hence, systemic scopolamine injections can only have a limited contribution in understanding the role of the central cholinergic system in cognition. It is assumed that central infusion of compounds in animals offers information with respect to the function of a neurotransmitter in a specific brain region. Such an approach would enable an assessment of the influence of muscarinic receptors in different brain structures on cognitive performance. As receptor subtypes are not evenly distributed in the brain, central administration will also lead to differences in blockade between muscarinic receptor subtypes. Thus, studies that investigate the effects of intracerebral injections may provide a manner to dissociate effects of scopolamine on performance in (non-)cognitive tasks, especially those in which scopolamine is injected into more than one brain region (e.g., striatum

and hippocampus). Furthermore, it has been suggested that specific projections of cholinergic cells in the nucleus basalis diagonal band complex might provide brain regions with some degree of selectivity in terms of cognition (Price & Stern, 1983; van der Zee & Luiten, 1999).

However, experiments that have used central administration of scopolamine do not provide a clear-cut picture of the role of the cholinergic system for each brain region (see Table 3): cognitive deficits seem diverse and only to some extent related to brain structure. For example, short-term memory impairments have been found after injections in several brain regions, such as the hippocampus (Brito et al., 1983; Dunnett et al., 1990; Herrera-Morales et al., 2007; Mishima et al., 2000), septum (Givens & Olton, 1995; Markowska et al., 1995), and amygdala (Ingles et al., 1993; Ohno et al., 1993). Moreover, centrally applied drugs are also capable of exerting widespread effects (e.g., Jolas et al., 1995).

Nevertheless, if a rough differentiation is made, it seems that the cortical regions are involved in attentional processes (Davidson & Marrocco, 2000), short-term memory functions (Abe et al., 2004; Granon et al., 1995; Wall et al., 2001; Warburton et al., 2003; Winters et al., 2006), (conditional) discrimination (Broersen et al., 1995; Herremans et al., 1997; Wilson, 2001), and conditioned taste aversion (Gutiérrez et al., 2003; Naor & Dudai, 1996; Ramírez-Lugo et al., 2003). The amygdala and septohippocampal system appear necessary for anxiety-related behavior (e.g., Smythe et al., 1998), and working and short-term memory (Brito et al., 1983; Dunnett et al., 1990; Givens & Olton, 1995; Herrera-Morales et al., 2007; Ingles et al., 1993; Markowska et al., 1995; Mishima et al., 2000; Ohno et al., 1993), although one study did not support the latter conclusion (Robinson & Mao, 1997). The striatal cholinergic system appears to modulate cognitive functions such as behavioral flexibility (Ragozzino et al., 2002), but also affects the execution of motor functions (Blokland, 1998; Chambers & van Hartesveldt, 1984; Vrijmoed de Vries & Cools, 1986).

These findings would implicate that the cognitive effects of systemic administration of scopolamine are not mediated by cholinergic blockade in a number of distinct brain areas but are rather caused by a non-selective, extensive inhibition of central cholinergic signaling. It was shown that *in vivo* administration of [³H]-scopolamine (IV) over a period of 240 min. with a plasma concentration of about 20 nM could be traced in many brain regions of the rat (Frey et al., 1985). There was a good correlation between [³H]-scopolamine binding and receptor density, indicating that this ligand does not preferentially bind to a specific brain area. Thus, effects of systemic scopolamine treatment on cognitive function should be regarded as the end result of widespread blockade of muscarinic receptors throughout the brain. Moreover, studies using central administration of scopolamine have indicated that the dose range required to affect different behaviors was comparable among different brain structures (i.e., 5-15 µg). In contrast, the effective dose range of systemically administered scopolamine appears to be greatly dependent on the particular behavioral task that is employed. In other words, experiments which have investigated the effect of central administration of scopolamine on cognition cannot easily explain the finding that this drug more potently affects performance in sensory/stimulus discrimination and attentional tasks rather than learning and memory paradigms.

In conclusion, the notion that scopolamine is an amnesic drug cannot be refuted, but as discussed in the present review, the mnemonic effects of scopolamine may not result from a specific effect on learning and memory processes. Thus, it is likely that effects on learning and memory performance which are observed after higher doses are mediated by

1) primary effects on attention and sensory/stimulus discrimination, 2) non-specific effects on behavior (e.g., locomotor activity, anxiety), and 3) peripheral side-effects (e.g., pupil dilation, salivation). At the very least, a detailed account should be given in order to rule out side-effects as an underlying cause of memory impairments observed at higher doses. For each specific cognitive test, multi-parametric studies are needed to assess which dose (or dose range) of scopolamine induces cognitive deficits without causing behavioral side effects. In line with a paper in which a low dose of amphetamine was discussed (Grilly & Loveland, 2001), scopolamine doses higher than 0.1 mg/kg should be considered as high doses in rats and mice.

Figure 1. Schematic overview of the dose-response effects of scopolamine in several behavioral tasks. Doses were peripherally administered in rats and mice, as dose ranges used in monkeys cannot be directly compared with those in rodents. The left side of the box corresponds with the minimal dose at which effects have been reported in a particular behavioral task. The brighter boxes on top indicate the occurrence and onset of other (non-cognitive) effects of scopolamine. Table 2 provides an overview of studies which have assessed the effect of peripheral administration of scopolamine on behavior.

SCOPOLAMINE AS A MODEL FOR COGNITIVE IMPAIRMENT

The use of scopolamine in humans may be a useful tool to investigate the role of acetylcholine in cognition. However, animal studies have shown that this type of research, i.e., pe-

ripheral administration of scopolamine, suffers important limitations because of the reasons that have been delineated in this paper. Scopolamine blocks acetylcholine at muscarinic receptors in a non-selective manner (Bolden et al., 1992); because of this widespread mode of action it may not be the optimal choice as a model of (muscarinic and/or cholinergic) cognitive impairment. On basis of the localization of different muscarinic receptors subtypes (see Table 1), it may be more useful to use ligands that block muscarinic receptors more selectively and thus are likely to affect cognitive function in a relatively more specific manner.

In this respect it might be interesting to explore the usability of selective muscarinic M_1 antagonists as a pharmacological model of cognitive deficits. As the M_1 receptor is predominantly located centrally and in brain regions such as cortex and hippocampus which are thought to be important for learning and memory (Caulfield, 1993), it is expected that M_1 antagonists will exert less peripheral side effects as compared to scopolamine and will impair cognition (especially memory) more selectively. This would supposedly make interpretation of the effects of selective M_1 antagonists on cognition relatively more straightforward. Moreover, muscarinic M_1 receptor agonists have been pursued as a treatment option for alleviation of cognitive impairments seen in disorders such as (Alzheimer's) dementia (Bodick et al., 1997) or schizophrenia (Shekhar et al., 2008): unfortunately, with limited success due to a lack of ligand selectivity to the M_1 receptor (Heinrich et al., 2009). Thus, selective M_1 antagonists might constitute a relatively more valid pharmacological model of cognitive impairment as compared to the non-selective muscarinic antagonist scopolamine.

Muscarinic M_1 antagonists

Several studies have used selective M_1 antagonists to assess the role of the muscarinic M_1 receptor in cognition and behavior. Deficits have been reported in passive avoidance tasks (Fornari et al., 2000; Kimura et al., 1999; Kramer-Soares et al., 2006; Roldán, 1997), contextual fear conditioning (Kramer-Soares et al., 2006) and object recognition (Myhrer et al., 2004; 2008) after administration of M_1 antagonists. To the best of our knowledge, only the muscarinic M_1 antagonist pirenzepine has been infused into distinct brain areas to determine its effects on cognition. When administered in the insular cortex, pirenzepine (100 mM in 1.0 μ L/site) was reported to affect acquisition of conditioned taste aversion, but did not affect retrieval (Ramírez-Lugo et al., 2003). Moreover, hippocampal or intracerebroventricular injection of pirenzepine (0.32 - 34.55 μ g) affected spontaneous alternation and caused performance deficits in the Morris water maze (Hagan et al., 1987; Herrera-Morales et al., 2007; Hunter & Roberts, 1988), T-maze (Messer et al., 1987a; 1987b; 1990), radial maze (Mishima et al., 2000; Sala et al., 1991), spontaneous alternation (Ukai et al., 1995a, 1995b), delayed matching to position (Andrews et al., 1994; Aura et al., 1997) and the three-panel runway test (Ohno et al., 1994a; 1994b), indicative of an effect of this compound on memory functions. However, there are some indications that the M_1 receptor might be implicated in processes beyond memory, such as reversal learning (McCool et al., 2008; Tzavos et al., 2004) or anxiety (Wall et al., 2001).

In humans, the M_1 antagonist biperiden (2 mg) impaired verbal episodic memory, locomotor behavior, motor learning and/or attentional processes without causing sedation (Wezenberg et al., 2005). Moreover, in human participants scopolamine was generally found to have a much more widespread effect on cognitive performance as compared to

biperiden (Ebert et al., 1998; Wesnes, 1988). In these studies, scopolamine was also associated with relatively more side effects, such as sedation, accommodation disturbances and dry mouth. Of note, because of the highly conserved nature of the orthosteric binding site amongst muscarinic receptors, selectivity of muscarinic ligands for a particular receptor subtype might be limited and subject to improvement (Bolden et al., 1992; Heinrich et al., 2009). In other words, behavioral effects of “selective” muscarinic antagonists that target the orthosteric M_1 receptor site could also be caused by blockade of muscarinic receptors other than M_1 .

Conclusions

Scopolamine has widespread effects on physiological functioning and hence it also has a broad side-effect profile, which can be explained by the non-selective muscarinic binding characteristics of this ligand (Bolden et al., 1992). Moreover, the pharmacodynamic properties of scopolamine might give rise to non-selective effects of this drug on behavioral performance. In other words, this compound does not possess the appropriate pharmacological qualities in order to be able to affect just one aspect of cognition (such as mnemonic or attentional processes). Ideally, relating cognitive impairments to the impact of a model ligand on a particular receptor type should be relatively straightforward. Although scopolamine has a strong history as a model for cholinergic impairment, its pharmacological unspecificity renders it unfit for use as a golden standard drug. Thus, the validity of scopolamine as a drug of choice for inducing cognitive impairments should be questioned.

Ligands which antagonize the muscarinic M_1 receptor can be regarded as a good alternative for scopolamine because of their ability to induce more selective cholinergic receptor blockade. Based on the relatively central distribution of the muscarinic M_1 receptor (Caulfield, 1993), the relatively more specific cognitive effects of M_1 antagonists (Andrews et al., 1994; Aura et al., 1997; Hagan et al., 1987; Herrera-Morales et al., 2007; Hunter & Roberts, 1988; Messer et al., 1987a; 1987b; 1990; Mishima et al., 2000; Ohno et al., 1994a; 1994b; Sala et al., 1991; Ukai et al., 1995a; 1995b), and the relatively more favorable side-effect profile of M_1 antagonists (Ebert et al., 1998; Wesnes, 1988; Wezenberg et al., 2005), these compounds should be investigated in terms of their usability as a pharmacological model of cognitive impairment. However, at this point not enough effort has been put in determining the effects of M_1 antagonists on memory vs. attention vs. other cognitive and non-cognitive processes (side-effects), partly because of a lack of highly selective muscarinic compounds (Bolden et al., 1992; Heinrich et al., 2009). More research is needed to develop M_1 ligands with a more selective binding profile (for instance by acting at allosteric sites for a particular muscarinic receptor type) and to assess whether M_1 antagonists can really be regarded as a valid pharmacological model for inducing cognitive (particularly mnemonic) deficits.

Table 2a. Effects of peripheral administration of scopolamine on stimulus discrimination in animals. All doses are in mg/kg.

Stimulus discrimina- tion paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Simple response dis- crimination task	Rats (F344)	0.05, 0.1, 0.39	IP	0.05	0.39	P(hit) ↓	(Moore et al., 1992)
				0.1		P(false alarm) =	
				-		Response latency ↑	
				-		Sensitivity ↓	
Choice response dis- crimination task	Rats (F344)	0.025, 0.05, 0.1, 0.39	IP	0.025		Response bias ↓	(Moore et al., 1992)
				0.05		P(hit) ↓	
				0.05		P(false alarm) ↓	
				0.39		P(false alarm) ↑	
				0.39		Accuracy ↓	
				-		Sensitivity ↓ (age-dependent)	
Two-choice, discrete-trial Rats (LE) signal-detection task		0.03, 0.056, 0.1	SC	-		Response bias ↓	(Bushnell et al., 1997)
				0.1		Response latency ↑	
				0.1		P(hit) ↓ (dependent on stimulus intensity)	
				0.1		P(false alarm) ↑	
				0.03		Omissions ↑	
				0.03		Response latency ↑	
Signal detection	Mice (NMRI)	0.156, 0.625	IP	0.156		Signal detection ↓	(Jensen et al., 1987)
				-		Response bias ↑	
Cued target detection (Posner task)	Rhesus monkeys	0.0001, 0.00025, 0.0005	IM	0.0001		Accuracy ↓	(Davidson et al., 1999)
				0.0005		Response latency ↑ (for orienting, but not alerting component)	
Visual discrimination	Rats (SD)	0.01, 0.04, 0.16, 0.63, 2.5, 10	IP	0.16		Responses ↓	(Colpaert et al., 2001)
				0.63		Choice accuracy ↓	
				0.63		Response latency ↑	
				0.63		Percentage responses on selected lever ↓	
	Marmoset monkeys	0.06	IM	0.06	0.06	Simple visual discrimination =	(Harder et al., 1998)
						Complex visual discrimination ↓	
	Rats (SD)	0.03125, 0.0625, 0.125, 0.25, 0.5, 1.0	IP	1.0		Accuracy ↓	(Dencoff & Hodge, 1995)
				0.03125		Responding ↓	
	Rats (LE)	0.0125, 0.05, 0.2, 0.8	SC	0.05		Accuracy ↓	(Drinkenburg et al., 1995)
				0.0125		Omissions ↑	
				0.05		Response latency ↑	
	Rats (W)	0.02, 0.078, 0.313, 0.46, 0.625	IP	0.078	0.625	Accuracy =	(Andrews et al., 1992)
				0.078		Omissions ↑	
						Response latency ↑	

Stimulus discrimination paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rhesus monkeys	0.03, 0.04, 0.05	IM	0.03		Visual discrimination ↓	(Bartus & Johnson, 1976)
	Stumptail monkeys	0.0075, 0.015, 0.03, 0.06	IM	0.015 0.03 0.015		Low luminance Discrimination accuracy ↓ Response bias ↑ Omissions ↑ Response latency =	(Evans, 1975)
					0.06	High luminance Discrimination accuracy ↓ Response bias = Omissions ↑ Response latency ↑	
				0.06 0.06 0.03	0.06		
	Rats (HM)	0.5	IP	0.5	0.5	Discrimination accuracy = Omissions ↑	(Leaton & Kreindler, 1972)
Visual discrimination test Rats (LH), mice (C57BL6/J)		0.2, 2.0	IP			Rats Spatial reference memory ↓ Visual discrimination ↓	(Robinson et al., 2004)
				0.1 2.0		Mice Visual discrimination ↓	
	Mice (C57BL6/J)	2.0	IP	2.0		Visual acuity ↓	(Robinson et al., 2004)
Self-ordered spatial search	Rhesus monkeys	0.003, 0.01, 0.014, 0.017, 0.024	IM	0.003		Proportion of correct trials ↓ (dependent on task difficulty)	(Taffe et al., 1999)
FR discrimination	Squirrel monkeys	0.001-0.18	IM	0.01 0.001		Accuracy ↓ Response rate ↓	(Pakarinen & Moerschbaecher, 1993)
Conditional visuospatial discrimination	Marmoset monkeys	0.06	IM	0.06		Visuospatial discrimination ↓	(Harder et al., 1998)
Paired discrimination task	Rats (F344/BNNia)	0.025, 0.1	IP	0.1 0.025		Accuracy ↓ (independent of age and stimulus length) Omissions ↑ (independent of age and stimulus length) Response latency =	(McGaughy & Sarter, 1995)
					0.1		
	Rats (SD)	0.02, 0.05, 0.1, 0.2	SC		0.2 0.2 0.1	Completed trials = Errors on visual trials = Errors on spatial trials ↑	(Grauer & Kapon, 1996)

Stimulus discrimination paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Brightness discrimination task	Rats (SD)	0.0625, 0.125, 0.25, 0.5	IP	0.05	0.5	Small difference condition Brightness discrimination ↓ Perceptual bias =	(Miliar, 1981)
						Large difference condition Brightness discrimination = Perceptual bias ↑	
						Easy version Percentage correct ↓ Omissions ↑	
						Difficult version Percentage correct ↓ Omissions ↑	
Two-key simultaneous brightness discrimination	Rats (LE)	0.12, 0.25, 0.50, 1.0	IP	0.0625	0.5	Discrimination performance ↓ (dependent on task difficulty)	(Ksir & Slifer, 1982)
						Omissions ↑	
						Intertrial interval responses =	
						Accuracy ↓ Response latency ↑ (delay-dependent)	
Delayed visual discrimination	Rats (W)	0.02, 0.078, 0.313, 0.46, 0.625	IP	0.313	0.313	Discrimination ↓	(Andrews et al., 1992)
						Accuracy ↓	
						Response latency ↑ (delay-dependent)	
						Discrimination ↓	
Delayed conditional discrimination	Rats (W)	0.0056 – 0.032 (titrated doses)	IP	0.013	0.0056	Acquisition Accuracy ↓ Response rate ↓	(Weisman et al., 1987)
						60 min. delay Accuracy ↓ Response rate ↓	
						24h delay Accuracy = Response rate =	
						Performance Accuracy ↓ Response rate ↓	
						0.032 0.032	
						0.018 0.018	
						Sensitivity ↓ Response bias =	
						1.0	
						Sensitivity ↓ Response bias =	
						0.375	
Delayed conditional discrimination	Rats (SD)	0.005, 0.014, 0.042, 0.125, 0.375	IP	0.005	0.375	Sensitivity ↓ Response bias =	(Kirk et al., 1988)
						Discrimination ↓	
Delayed conditional discrimination	Rats (W)	0.1, 0.5, 1.0	IP	0.1	0.1	Discrimination ↓	(Weisman et al., 1987)

Stimulus discrimination paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Visual and auditory discrimination	Rats (W)	0.125, 0.25, 0.50, 1.0	IP	0.5		Visual stimuli (independent of sex) Discrimination ↓ Omissions ↑	(van Haaren & van Hest, 1989)
				0.5		Auditory stimuli (independent of sex) Discrimination ↓ Omissions ↑	
	Rats (SD)	0.125, 0.25, 0.50, 0.75	IP	0.125		Errors ↑ (dependent on modality and pretraining)	(Warburton, 1974)
Acoustic startle response	Rats (SD)	0.056–1.0	IP			100 dB noise pulses Peak startle amplitude ↑ Latency peak startle amplitude ↓ Prepulse inhibition =	(Sipos et al., 2001)
				0.1 0.1	1.0	120 dB noise pulses Peak startle amplitude = Latency peak startle amplitude = Prepulse inhibition ↓	
Auditory gap detection assessed by startle reflex	Rats (LE)	0.5, 1.0, 2.0	IP	1.0 2.0		Inhibition of startle by gap ↓ Duration gap threshold ↑	(Ison & Bowen, 2000)
				0.3	1.0	Prepulse inhibition ↓ Startle amplitude =	
Appetitive negative patterning successive discrimination	Rats (DA)	0.025, 0.05, 0.1	IP	0.025 0.025 0.025		Choice accuracy ↓ Latency ↑ Completed trials ↓	(Richmond et al., 1997)
					0.2 0.2 0.2	Percentage correct = Sensitivity index = Responsivity index = Session duration =	
Olfactory discrimination	Rats (LE)	0.1, 0.125, 0.15, 0.2	IP		0.2 0.2 0.2 0.2		(Doty et al., 2003)

Symbols: = : no change, ↑ : increase, ↓ : decrease, - : not mentioned in article. Abbreviations: A: Albino, DA: dark agouti strain, F344: Fischer-derived F344 strain, FR: fixed ratio, HM: Holtzman strain, IM: intramuscular, IP: intraperitoneal, KW: Kuo-Wistar strain, LE: Long-Evans (Hooded) strain, LH: Lister Hooded strain, SC: subcutaneous, SD: Sprague-Dawley strain, W: Wistar strain.

Table 2b. Effects of peripheral administration of scopolamine on attention in animals. All doses are in mg/kg.

Attention paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Object exploration (sustained attention)	Mongolian gerbils	0.01, 0.05, 0.1, 1.0, 10	SC	10		Contact duration ↓	(Cheal, 1981)
				10		Habituation of contact duration ↓	
				0.1		Contact frequency ↑	
				0.05		Habituation of contact frequency ↓	
				0.01		Locomotor activity ↑	
5-choice serial reaction time task	Rats (LE)	0.05, 0.1, 0.2, 0.4	IP	0.1	0.4	Percentage correct =	(Hodges Jr. et al., 2009)
						Percentage omissions ↑	
	Mice (C57BL/6, DBA/2, 129/Sv)	0.003, 0.01, 0.03, 0.1, 0.3, 0.5, 1.0	IP		0.4	Response latency =	(Pattij et al., 2007)
						C57BL/6	
				0.3		Accuracy ↓	
				0.5		Response latency ↑	
				0.1		Premature responses ↑	
				0.5		Omissions ↑	
						DBA/2	
				0.1		Accuracy ↓	
				0.5		Response latency ↑	
				0.5		Premature responses ↑	
Rats (SD)		0.003, 0.01, 0.03, 0.1, 0.3	SC		1.0	Omissions =	(Shannon & Eberle, 2006)
						129/Sv	
				0.5		Accuracy ↓	
					1.0	Response latency =	
				0.5		Premature responses ↑	
				0.1		Omissions ↑	
						Percentage correct ↓ (dependent on stimulus duration)	
				0.3		Percentage incorrect responses ↓ (dependent on stimulus duration)	
				0.1	0.3	Percentage omissions ↑	
				0.1		Hit rate =	
Marmoset monkeys		0.02	SC		0.02	Anticipatory responses ↑	(Spinelli et al., 2006)
						Accuracy =	
				0.02		Correct responses ↓ (dependent on stimulus duration)	
				0.02		Omissions ↑ (dependent on stimulus duration)	
				0.02		Lever release latency ↓ (dependent on stimulus duration)	

Attention paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (LH)	0.03, 0.075, 0.1	IP		0.1	Lever-then-light trials No effects on accuracy, omissions or correct response latency	(Higgs et al., 2000)
					0.1	Lever-then-no-light trials Accuracy =	
				0.075	0.1	Omissions = Correct response latency ↑	
					0.1	Light-alone trials Accuracy =	
	Rats (LH)	0.01, 0.03, 0.1	SC	0.075		Omissions ↑	(Mirza, 2000)
				0.075		Correct response latency ↑	
				0.1		Correct responses ↓	
				0.01		Anticipatory responses ↓	
	Mice (F1 of C57BL/6 x DBA/2, F1 of C57BL/6 x 129Sv)	0.01, 0.03, 0.02, 0.2, 2.0	IP		0.1	Perseverative responses =	(Humby et al., 1999)
					0.1	Omissions ↑	
					0.1	Correct response latency =	
					0.1	Completed trials ↓	
						F1 of C57BL/6 x DBA/2	
				0.02		Accuracy ↓	
				0.02		Omissions ↑	
				0.1		Correct response latency ↑	
				0.2		Locomotor activity ↑	
						F1 of C57BL/6 x 129Sv	
	Rats (LH)	0.01, 0.03, 0.075	SC	1.0		Accuracy ↓	(Jones et al., 1995)
				0.2		Omissions ↑	
				0.2		Correct response latency ↑	
				0.2		Locomotor activity ↑	
						Young rats	
					0.075	No effects on percentage correct, correct response latency or omissions	
						Middle-aged rats	
				0.03		Percentage correct ↓	
				0.03		Latency of correct responses ↑	
				0.03		Percentage omissions ↑	

Attention paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (LH)	0.03, 0.075, 0.1	SC	0.1		Standard conditions	(Jones & Higgins, 1995)
				0.075		Percentage correct ↓	
					0.1	Omissions ↑	
				0.075		Premature responses =	
	Rats (LH)	0.075	SC		0.1	Perseverative responses ↑	(Jones & Higgins, 1995)
						Latency of correct responses =	
					0.075	Variable stimulus intensity	
				0.075		Percentage correct =	
	Rats (LH)	0.075	SC	0.075		Omissions ↑	(Jones & Higgins, 1995)
				0.075		Magazine latency ↑	
					0.075	Variable stimulus duration	
				0.075		Percentage correct =	
	Rats (LH)	0.075	SC	0.075		Omissions ↑	(Jones & Higgins, 1995)
				0.075		Magazine latency ↑	
					0.075	Variable inter-trial interval	
				0.075		Percentage correct =	
	Rats (KW)	0.05, 0.1, 0.15, 0.2	IP		0.2	Omissions =	(Jääkäia et al., 1992)
						Magazine latency ↑	
						White noise distracter	
				0.075		Percentage correct ↓	
	Rats (LH)	0.03, 0.06	IP	0.05		Omissions ↑	(Phillips et al., 2000)
				0.05		Correct response latency ↑	
				0.03		Percentage correct ↓	
				0.03		Response latency ↑	
	Rats (L)	0.1, 0.3	IP	0.03		Validity effect ↑	(Hoff et al., 2007)
				0.3		Percentage correct ↓	
				0.3		Omissions ↑	
				0.3		Side bias ↑	
	Rats (LE)	0.05, 0.1, 0.3, 0.5, 1.0	IP	0.3		Hits ↓ (signal duration of 500 ms)	(McQuail & Burk, 2006)
					1.0	Misses =	
				1.0		Omissions ↑	
	Mice (C57BL/6)	0.8	IP	0.8		Percentage correct ↓ (signal duration of 1, 2s)	(Leblond et al., 2002)
				0.8		Response latency ↑ (signal duration of 0.5, 1, 2s)	

Attention paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rhesus monkeys	0.01	IM	0.01	0.01	Response accuracy = Reinforced responses ↓ (dependent on stimulus duration and number of trials completed) Omissions ↓	(Callahan et al., 1993)
				0.01			
Crossmodal (visual and auditory) divided attention task	Rats (LE)	0.03, 0.06, 0.1	IP	0.06 0.1	0.1	Response accuracy = Response latency ↑ (modality-dependent) Completed trials ↓	(McGaughy et al., 1994)

Symbols: = : no change, ↑ : increase, ↓ : decrease. Abbreviations: IM: intramuscular, IP: intraperitoneal, KW: Kuo:Wistar strain, L: Lewis strain, LE: Long-Evans (Hooded) strain, LH: Lister Hooded strain, SC: subcutaneous, Sprague-Dawley strain.

Table 2c. Effects of peripheral administration of scopolamine on memory in animals. All doses are in mg/kg.

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Spontaneous alternation	Mice (AJJ, DBA/2J, C57BL/6J)	10	IP	10	10	Alternation ↓ Locomotor activity ↑	(Anisman, 1975)
	Mice (AJJ, DBA/2J, C57BL/6J)	1.0, 3.0, 5.0, 10	IP	3.0		All strains Alternation ↓ AJJ Locomotor activity ↑ DBA/2J Locomotor activity ↑ C57BL/6 Locomotor activity ↓	(Anisman & Kokkinidis, 1975)
	Rats (HBS)	0.1, 1.0	IP	0.1 1.0		Alternation ↓ Responses ↓	(Drew et al., 1973)
	Rats (HM)	1.0	IP	1.0		Administration 15 min. before or after first trial (Squire, 1969) Alternation ↓	
Spatial alternation	Rats (SD)	0.5, 1.0, 2.0	SC	0.5		Alternation ↓	(Means et al., 1996)
Double spatial alternation	Rats (W, SD)	0.25, 0.5, 0.75, 1.0	IP	0.25 0.25 0.25		Errors ↑ Perseverative errors ↑ Switching errors ↑ Response bias = 1.0	(Warburton & Heise, 1972)
Delayed spatial alternation	Rats (LE)	0.01, 0.03, 0.1, 0.3, 1.0	IP	1.0 0.3		Alternation ↓ Response latency ↑	(Baron et al., 1998)
	Rats (LE)	0.2, 0.4	IP	0.2		Alternation ↓ (delay-dependent) Performance time = Completed trials = 0.4 0.4	(Givens & Olton, 1995)
	Rats (F344)	0.01, 0.03, 0.1	SC	0.03 0.01		Alternation ↓ (delay-dependent) Response rate ↓	(Bymaster et al., 1993)
	Rats (SD)	0.1, 0.3, 0.6	IP	0.1		No delay Alternation ↓ 30s delay Alternation = 0.6	(Moran, 1993)
	Rats (SD)	0.01, 0.03, 0.1, 0.3	IP	0.03	0.3	Correct responses ↓ (delay-dependent) Correct rehearsal operations =	(Dudchenko & Sarter, 1992)
	Rats (SD)	0.03, 0.1	IP	0.03		Alternation ↓ (delay-dependent)	(Holley et al., 1992)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (F344)	0.1	SC	0.1 0.1		Alternation ↓ Response rate ↓	(Shannon et al., 1990)
	Rats (SD)	0.125, 0.25	IP	0.25	0.25	Alternating discrimination Accuracy = Number of responses ↓	(Heise et al., 1976)
	Rats (SD)	0.125, 0.25, 0.5, 1.0	IP	0.25 0.5		Variable inter-trial interval spatial alternation Accuracy ↓ Number of responses ↓	(Heise et al., 1976)
	Mice (C57BL/6)	0.5, 2.0	IP	0.5		Accuracy ↓	(Alperm & Marriott, 1973)
	Rats (H)	0.4, 1.2	IP	1.2		Alternation ↓	(Douglas & Isaacson, 1966)
Reinforced alternation	Rats (LE)	0.05, 0.2	SC	0.05 0.05		Alternation ↓ Response latency ↑	(Clarke & Fibiger, 1990)
	Rats (F344)	0.01, 0.03, 0.1	SC	0.1 0.03		Alternation ↓ Response rate ↓, (dependent on retention interval)	(Shannon & Love, 2004)
Object recognition task	Rats (W)	0.1	IP	0.1 0.1		Exploration during T1 ↓ Object recognition ↓	(Sambeth et al., 2007)
	Rats (W)	0.03, 0.1, 0.3	SC, IP	0.1		Object recognition ↓	(Lieben et al., 2005)
	Rats (W)	0.15	IP	0.15 0.15 0.15 0.15		Object recognition ↓ Total exploration ↓ Locomotor activity ↓ Rearing ↓ or ↑	(Myhrer et al., 2004)
	Rats (PDA)	0.05	IP	0.05	0.05	Administration before T1 Object recognition ↓ Administration after T1 Object recognition =	(Warburton et al., 2003)
	Rats (SD)	0.1, 0.3, 1.0	SC	0.1 1.0	1.0	Object recognition ↓ Exploration time = Locomotor activity ↓	(Besheer et al., 2001)
	Mice (Swiss)	0.3, 1.0, 3.0	SC	0.3 1.0 0.3		Object recognition ↓ Locomotor activity ↑ Rearing ↓	(Dodart et al., 1997)
	Rats (W)	0.2	IP	0.2		Object recognition ↓	(Vannucchi et al., 1997)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Cone field	Rats (W)	0.125, 0.25, 0.5, 1.0, 2.0	IP	0.5		Object recognition ↓ (dependent on inter-trial interval) Exploration time ↓	(Ennaceur & Meliani, 1992)
				0.125			
	Rats (W)	0.3, 0.5, 0.7	IP	0.5		Working memory ↓ Reference memory ↓ Inter-visit interval ↑ Collected rewards ↓	(Bouger & van der Staay, 2005)
Three panel runway task	Rats (W)	0.1, 0.18, 0.32	IP	0.32	0.32	Working and reference memory Errors ↑ Latency =	(Kobayashi et al., 1995)
T-maze	Rats (SD)	0.5, 0.8	IP	0.5		Novelty habituation ↓	(Leaton, 1968)
	Rats (SD)	0.1, 0.3, 0.6	IP	0.6	0.6	Errors ↑ Trial latency ↑	(Moran, 1993)
Double Y-maze	Rats (W)	0.1, 0.4, 0.8	IP	0.1		8-month old rats Working memory ↓ (delay-dependent) Reference memory ↓	(Biggan et al., 1996)
				0.4			
	Rats (W)	0.05, 0.1, 0.4	IP	0.05		16-month old rats Working memory ↓ (delay-dependent) Reference memory ↓	(Biggan et al., 1996)
				0.4			
Radial maze	Rats (LE)	0.01, 0.03, 0.05, 0.1, 0.3	IP	0.3	0.3 0.3	Working memory errors ↑ Reference memory errors = Total arm entries = Locomotor activity ↓	(Hodges Jr. et al., 2009)
				0.1			
	Rats (W)	0.2, 0.5, 1.0	IP	0.5		Working memory errors ↑ Reference memory errors ↑ Locomotor activity ↓	(Masuoka et al., 2006)
				0.5 0.2			
	Rats (W)	0.5	IP	0.5		Correct choices ↓ Errors ↑	(Mishima et al., 2000)
				0.5			
	Rats (SD)	0.0625, 0.125, 0.25, 0.5	IP	0.25 0.5 0.5		No delay, administration 20 min. before testing Working memory errors ↑ Pellets retrieved ↓ Run time ↑	(Pilcher et al., 1997)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (SD)	0.25, 0.5, 1.0	IP			3h delay, administration 20 min. before testing (Plicher et al., 1997) Working memory errors = Reference memory errors ↑ Pellets retrieved = Run time =	
				0.5	1.0 1.0 1.0		
	Rats (SD)	0.125, 0.5, 1.0	IP			3h delay, administration after completion of half of the maze Working memory errors = Reference memory errors ↑ Pellets retrieved = Run time =	(Plicher et al., 1997)
	Rats (W)	0.125, 0.25, 0.5, 1.0, 2.0	IP	0.25 0.25 0.25		Errors ↑ Correct choices before first error ↓ Run time ↑	(Ennaceur & Meliani, 1992)
	Rats (LE)	0.5	IP	0.5		Low learning criterion Working memory errors ↑ Reference memory errors = High learning criterion Working memory errors ↑ Reference memory errors ↑	(Lydon & Nakajima, 1992)
				0.5 0.5	0.5		
	Rats (W)	0.1, 0.4, 0.8	IP	0.4 0.4 0.4 0.4 0.4		Accuracy ↓ Working memory ↓ Reference memory ↓ Omissions ↑ Locomotor activity ↓	(Okaichi et al., 1989)
				0.1 0.1 0.1 0.1			
	Rats (LE)	0.03, 0.056, 0.1, 0.177, 0.3	IP	0.1 0.1 0.1 0.1		Accuracy ↓ Working memory errors ↑ Reference memory errors ↑ Latency ↑	(Peele & Baron, 1988a)
	Rats (LE)	0.03, 0.1, 0.3, 0.56, 1.0	IP	0.3 0.3		Accuracy ↓ Run time ↑	(Peele & Baron, 1988b)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (F344, SD, W)	0.125, 0.25, 0.5, 1.0	IP	0.5 0.5 1.0	F344 Choice accuracy ↓ Correct choices ↓ Errors = Run time ↑ Sprague Dawley Choice accuracy ↓ Correct choices ↓ Errors ↑ Run time ↑ Wistar Choice accuracy ↓ Correct choices ↓ Errors ↑ Run time ↑	(Higashida & Ogawa, 1987)	
	Rats (H)	0.2	IP	0.2		Errors ↑ (delay-dependent)	(Buresová et al., 1986)
	Rats (A)	0.125, 0.25, 0.5	IP	0.25	0.5	Working memory errors ↑ Reference memory errors =	(Beatty & Bierley, 1985)
	Rats (W)	0.1, 0.4, 0.8	IP	0.1	0.8	Working memory ↓ Reference memory =	(Wirsching et al., 1984)
	Rats (D)	0.1	IP		0.1	No break Continuous choice performance = 5 min. break Working memory errors ↑	(Buresová & Bures, 1982)
	Rats (A)	1.0, 2.0, 5.0	IP		5.0 5.0	Administration 0h and 2h after 4th choice Accuracy = Errors = Administration 3h before the first 4 choices Accuracy ↓ Errors =	(Goddling et al., 1982)
	Rats (SD)	0.5, 1.0, 1.5	IP	0.5 0.5 0.5 0.5	5.0	Choice accuracy ↓ Working memory ↓ Reference memory ↓ Run time ↑	(Okaichi & Jarrard, 1982)
	Rats (W)	0.3	SC	0.3		Acquisition performance ↓	(Stevens, 1981)
	Rats (W)	0.5	IP	0.5		Acquisition ↓ (dependent on use of spatial strategy)	(Watts et al., 1981)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Water maze	Rats (SD)	0.1, 0.17, 0.3, 0.56, 1.0	IP	0.17 0.17		Choice accuracy ↓ Rate of arm selection ↓	(Eckerman et al., 1980)
	Rats (LE)	0.01, 0.03, 0.05, 0.1, 0.3	IP	0.1	0.3 0.3 0.3	Acquisition ↓ Information trial (platform in new location) = Cued navigation = Swim speed =	(Hodges Jr. et al., 2009)
	Mice (KM)	0.5, 1.0	IP		1.0	Swim speed = Hidden platform test Escape latency ↑ Path length ↑ Probe trial Time spent in training quadrant ↓ Path length in target quadrant ↓	(Zhang et al., 2008)
	Rats (LH)	0.25	IP		0.25	Escape latency =	(Harder et al., 1996)
	Rats (LE)	1.0	IP	1.0 1.0		Search time ↑ (counteracted by pretraining) Time spent in training quadrant ↓ (counteracted by pretraining) Thigmotaxic swimming ↑ Locomotor activity ↑	(Saucier et al., 1996)
	Mice (C57BL/6)	0.1, 1.0	IP		1.0 1.0	Cued navigation No effects in both male and female mice Spatial navigation <i>Male mice</i> No effects <i>Female mice</i> Path length ↑ Escape latency ↑ Thigmotaxic swimming ↑	(Berger-Sweeney et al., 1995)
	Mice (C57BL/6)	0.1, 1.0	IP		1.0 1.0 1.0	Cued navigation No effects in both male and female mice Spatial navigation Path length = Escape latency = Thigmotaxic swimming ↑ (independent of sex)	(Berger-Sweeney et al., 1995)
	Rats (F344)	0.05, 0.1, 0.15, 0.3, 0.6, 1.0	SC	0.1	1.0	Acquisition escape latency ↑ Retention escape latency =	(Cozzolino et al., 1994)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (LH)	0.5	IP	0.5 0.5		Escape latency ↑ Time spent in platform vicinity ↓	(Smith et al., 1994)
	Rats (SD)	0.3	IP		0.3	Escape latency =	(Decker et al., 1990)
	Rats (KW)	0.15, 0.8	IP	0.8 0.8 0.8		Escape latency ↑ Swim speed ↑ Distance in training quadrant ↓	(Riekkinen et al., 1990)
	Rats (H)	0.1, 0.2	IP	0.1 0.1		Acquisition escape latency ↑ Retention escape latency ↑	(Buresová et al., 1986)
	Rats (H)	0.2, 1.0	IP	1.0		Escape latency after overtraining ↓	(Buresová et al., 1986)
	Rats (H)	0.2, 0.4, 1.0	IP	1.0		Working memory ↓	(Buresová et al., 1986)
Water maze (visible platform, varied location)	Rats (LE)	1.0	SC	1.0		Escape latency ↑	(Paylor & Rudy, 1990)
Water maze (visible platform, fixed location)	Rats (LE)	1.0	SC	1.0 1.0		Search time in training quadrant ↓ Platform crossings in training quadrant ↓	(Paylor & Rudy, 1990)
	Rats (LE)	1.0	SC	1.0		Escape latency ↑ (dependent on age and tank brightness) Search time in training quadrant ↓ Platform crossings in training quadrant ↓ Thigmotaxic swimming ↑	(Paylor & Rudy, 1990)
	Rats (LE)	1.0	SC	1.0		Escape latency ↑ (independent of platform discriminability)	(Paylor & Rudy, 1990)
Delayed matching	Marmoset monkeys	0.005, 0.01, 0.015	SC	0.015		Percentage correct ↓	(Spinelli et al., 2006)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Mice (C57Bl/6 J)(Co)	0.8	IP			4h interval between presentation and retention (Maviel & Durkin, 2003)	
						<i>Injection 15 min. before presentation</i>	
						Percentage correct ↓	
						Response latency ↑	
						<i>Injection 30s after presentation</i>	
						Percentage correct ↓	
						Response latency ↑	
						<i>Injection 15 min. after presentation</i>	
						Percentage correct ↓	
						Response latency ↑	
	Rats (SD)	0.1, 0.4, 0.8	IP	0.8	0.8	<i>Injection 3 h and 45 min. after presentation</i>	(Ormerod & Beninger, 2002)
						Percentage correct ↓	
						Response latency =	
						20 min. interval between presentation and retention, injection 30s after presentation	
						Percentage correct ↓	
						Response latency =	
						Radial maze	
						Sample latency ↑	
						Choice latency ↑	
						Water maze	
	Rats (LE)	0.01, 0.03, 0.1, 0.3, 1.0	IP	0.8	0.8	Sample latency =	(Baron et al., 1998)
						Choice latency ↑	
						Accuracy =	
						Response latency ↓	
						Radial maze	
						Percentage correct ↓	
						Omissions ↑	
						Response bias ↑	
						Sample latency ↑	
						Water maze	
	Rats (LH)	0.03, 0.075, 0.1	SC	0.03 0.075 0.075 0.075	0.03 0.075 0.075 0.075	Percentage correct ↓	(Kirkby et al., 1995)
						Omissions ↑	
						Response bias ↑	
						Sample latency ↑	
						Radial maze	
						Percentage correct ↓	
						Omissions ↑	
						Response bias ↑	
						Sample latency ↑	
						Water maze	
	Pigeons (WC)	0.02	IM	0.02	0.02	Accuracy ↓ (delay-dependent)	(Santi & Weise, 1995)
						Accuracy ↓ (delay-dependent)	
						Radial maze	
						Percentage correct ↓	
						Omissions ↑	
						Response bias ↑	
						Sample latency ↑	
						Water maze	
						Percentage correct ↓ (delay-dependent)	
						Discriminability ↓ (delay-dependent)	
	Rats (SD)	0.025, 0.05, 0.1	SC	0.025 0.025	0.025 0.025	Percentage correct ↓ (delay-dependent)	(Stanhope et al., 1995)
						Discriminability ↓ (delay-dependent)	
						Trial latency =	
						Response bias ↑ (delay-dependent)	
						Radial maze	
						Percentage correct ↓	
						Omissions ↑	
						Response bias ↑	
						Sample latency ↑	
						Water maze	

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (SD)	0.03, 0.1, 0.3	IP	0.3	0.3	Completed trials ↓ Zero delay Accuracy = Longer delay (titrated to 75 percent correct) Accuracy ↓	(Buxton et al., 1994)
	Rats (SD)	0.125, 0.25, 0.5, 1.0	IP	0.25 0.125		Accuracy ↓ (delay-dependent) Response rate ↓	(Dunnett, 1985)
	Rhesus monkeys	0.01, 0.012, 0.015, 0.02, 0.025	IM	0.01		Accuracy ↓ (delay-dependent)	(Bartus & Johnson, 1976)
	Rhesus monkeys	0.05	IM	0.05 0.05		Accuracy ↓ Relative response frequency ↓	(Glick & Jarvik, 1989)
	Rhesus monkeys, stump-tailed monkeys	0.2, 0.4	IM	0.4 0.2		Accuracy ↓ Response rate ↓	(Bohdanecy et al., 1967)
Delayed non-matching	Rats (LE)	0.01, 0.03, 0.05, 0.1, 0.3	IP	0.1 0.05 0.05		Percentage correct ↓ Sample latency ↑ Locomotor activity ↓	(Hodges Jr. et al., 2009)
	Rats (LE)	0.025, 0.2	IP	0.1 0.2 0.2		Dry reward Correct choices ↓ Completed trials ↓ Sample latency ↑	(Hodges Jr. et al., 2009)
	Rats (LE)	0.1, 0.2	IP	0.1 0.1 0.2		Liquid reward Correct choices ↓ Completed trials ↓ Sample latency ↑	(Hodges Jr. et al., 2009)
	Mice (DBA/2, C57BL/6)	0.1, 0.5, 1.0	IP	0.3 0.5 1.0 1.0		Percentage correct ↓ (delay- and strain-dependent) Response bias ↑ (delay-dependent) Omissions ↑ (strain-dependent) Sample latency ↑ (strain-dependent)	(Estape & Steckler, 2002)
	Mice (DBA/2, C57BL/6)	0.1, 0.5, 1.0	IP	1.0	1.0 1.0 1.0	Reinforcers ↓ Percentage correct = Sensitivity index = Response bias (index Y) = Sample latency ↑	(Estape & Steckler, 2001)
	Rhesus monkeys	0.003, 0.01, 0.014, 0.017	IM	0.014		Choice accuracy ↓	(Taffe et al., 1999)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (W)	0.075, 0.15	IP	0.075 0.075 0.075 0.075		Choice accuracy ↓ (delay-dependent) Completed trials ↓ Sample omissions ↑ Sample latency ↑	(Ruotsalainen et al., 1998)
	Rats (LH)	0.065, 0.125, 0.25	IP	0.125 0.25 0.125 0.25		Percentage correct ↓ Correct response latency ↑ Magazine latency ↑ Omissions ↑	(Chudasama & Muir, 1997)
	Rats (LE)	0.05, 0.2, 0.8	SC	0.05 0.1 0.2 0.05		Percentage correct ↓ Response latency ↑ Completed trials ↓ Nosepoke entries ↓	(Andrews et al., 1994)
	Rats (SD)	0.03, 0.1, 0.3	IP			Zero delay and longer delay (titrated to 75 percent correct) Accuracy ↓ Completed trials ↓	(Buxton et al., 1994)
	Rhesus monkeys	0.01, 0.0178	IM	0.01		Administration before acquisition Percentage correct ↓ Administration after acquisition Percentage correct =	(Aigner et al., 1991)
	Rats (W)	0.125, 0.25, 0.50, 1.0	IP	0.25 0.25	0.0178	Male rats Accuracy ↓ (delay-dependent) Percentage non-initiated trials ↑ Female rats Accuracy ↓ (delay-dependent) Percentage non-initiated trials ↑	(van Hest et al., 1990)
	Rats (PDA)	0.01, 0.03, 0.05, 0.1	IP	0.05 0.1		Percentage correct ↓ Correction trials after error ↑	(Huston & Aggleton, 1987)
	Rats (PDA)	0.01, 0.03, 0.06	IP	0.06		Percentage correct ↓ (delay-dependent)	(Huston & Aggleton, 1987)
	Rhesus monkeys	0.001, 0.0032, 0.0056, 0.01, 0.0178, 0.032	IM	0.0056		Percentage correct ↓	(Aigner & Mishkin, 1986)
Olfactory delayed (non-matching)	Rats (LE)	0.03, 0.1, 0.3	IP	0.3		Percentage correct ↓	(Winters et al., 2000)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Delayed conditional discrimination	Rats (LE)	0.05, 0.075, 0.1, 0.15	IP	-	0.15	Choice accuracy ↓ (delay-dependent)	(Wan et al., 1997)
					0.15	Response bias =	
				0.15		Response probability = Delay interval responses ↑	
Olfactory memory	Rats (LE)	0.5	IP	0.5		Odor cross-habituation/generalization ↑	(Wilson, 2001)
						Non-overlapping odor pairs	
						Discrimination ↓ Overlapping odor pairs Discrimination ↓	
Latent inhibition	Rats (SD)	0.125, 0.25, 0.5	IP	0.5			(De Rosa & Hasselmo, 2000)
					0.25		
	Rats (W)	0.15, 0.5, 1.0	IP	0.15		Administration before pre-exposure and conditioning	(Barak & Weiner, 2007)
						Latent inhibition ↓	
					1.0	Latent inhibition =	
	Rats (W)	0.15	IP	0.15		Administration before pre-exposure	(Barak & Weiner, 2007)
						Latent inhibition ↓	
					0.15	Administration before conditioning Latent inhibition =	
Fear conditioning	Rats (LE)	0.01, 0.1, 1.0, 10, 100	IP	1.0 100 1.0		Pre-training administration	(Anagnostaras et al., 1999)
						Context conditioning ↓	
						Cued conditioning ↓ Locomotor activity ↑	
	Rats (LE)	10	IP	10		Post-training administration	(Anagnostaras et al., 1999)
						No effects on context or cued conditioning	
	Rats (LE)	1.0, 50	IP	50		Post-training administration in 23-day old or 70-day old rats	(Anagnostaras et al., 1999)
						No effects on context or cued conditioning	
	Rats (LE)	1.0, 100	IP	100		Behavioral reactivity to shock =	(Anagnostaras et al., 1999)
	Rats (LE)	1.0	SC	1.0 1.0		Administration immediately before acquisition	(Rudy, 1996)
						Context conditioning ↓	
						Cued conditioning ↓	
	Rats (LE)	0.2, 0.6, 1.0	SC	0.2 0.6		Administration immediately before acquisition	(Rudy, 1996)
						Context conditioning ↓	
						Cued conditioning ↓	

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (LE)	1.0	SC			Administration immediately before, 10 min. after, or 30 min. after acquisition Context conditioning ↓ Cued conditioning ↓	(Rudy, 1996)
	Rats (LE)	1.0	SC	1.0 1.0		Administration 3h after acquisition Context conditioning ↓ Cued conditioning ↓ Administration 24h after acquisition Context conditioning = Cued conditioning =	(Rudy, 1996)
	Rats (LE)	1.0	SC		1.0 1.0	Context conditioning = Cued conditioning =	(Rudy & Pugh, 1996)
	Rats (LE)	1.0	IP	1.0		Context conditioning ↓ Cued conditioning =	(Anagnostaras et al., 1995)
	Rats (LE)	0.1, 1.0	IP		1.0	Administration before training Context conditioning = Cued conditioning ↓	(Young et al., 1995)
	Rats (LE)	1.0	IP	1.0		Administration after training Context conditioning = Cued conditioning ↑	(Young et al., 1995)
	Rabbits (NZ)	0.0005, 0.025, 0.1, 0.4, 1.6	IV	0.025 0.1		Percentage conditioned responses ↓ Nictitating membrane response latency ↓ Latent inhibition =	(Harvey et al., 1983)
	Rabbits (NZ)	1.6	IV		1.6 1.6 1.6 1.6	Percentage unconditioned responses = Shock-intensity threshold = Amplitude unconditioned responses = Nictitating membrane latency =	(Harvey et al., 1983)
	Rabbits (NZ)	0.4, 1.6	IV	0.4 0.4		Conditioned responses ↓ Nictitating membrane latency ↑	(Harvey et al., 1983)
	Rabbits (NZ)	1.5	SC		1.5 1.5	Amplitude nictitating membrane response = Habituation nictitating membrane response =	(Moore et al., 1976)
	Rabbits (NZ)	1.5	SC	1.5		Acquisition of nictitating membrane response ↓ Latent inhibition =	(Moore et al., 1976)
	Rabbits (NZ)	1.5	SC	1.5		Conditioned auditory threshold for nictitating membrane response ↑	(Moore et al., 1976)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Fear conditioned re-sponse inhibition	Mice (A/J, DBA/2J, C57BL/6J)	1.0, 10	IP	1.0		AJ and DBA/2J Response inhibition ↓	(Anisman, 1975)
				10		C57BL/6J Response inhibition ↑	
Active avoidance	Rats (SD)	1.0	IP	1.0		Conditioned suppression of drinking behavior ↓	(Berger & Stein, 1969)
	Rats (F344)	0.5, 0.75	IP	0.5		Errors ↑ (independent of age)	(Spangler et al., 1989)
				0.5	0.75	Alternation errors ↑ (independent of age) Runtimes =	
	Mice (SW)	1.0	SC	1.0		Avoidance response retention ↓ (1, 3 and ≥ 14 days after training)	(Quartermain & Leo, 1988)
	Mice (CD-1)	0.01, 0.1, 1.0	SC			Administration 1h before training	(Flood & Cherkin, 1986)
0.01					Active avoidance retention ↑		
0.1					Active avoidance retention ↓		
					Administration immediately after training		
				0.1		Active avoidance retention ↑	
				1.0		Active avoidance retention ↓	
	Rats (A)	2.0	IP	2.0		Avoidance responses ↓	(Renfro et al., 1972)
				2.0		Crossovers to safe chamber ↑	
	Cats	0.05	IP	0.05		Avoidance acquisition ↓ Returns to food ↓	(Hamilton & Grossman, 1969)
	Mice (DBA/2J)	2.0	IP	2.0		Latent inhibition ↓	(Oliverio, 1968)
					Naive mice		
				2.0		Avoidance responses ↑	
						Pretrained mice	
				2.0		Avoidance responses ↓	
	Rats (WR)	2.0	IP		2.0	Shocks =	(Pradhan & Roth, 1968)
					2.0	Avoidance responses =	
					2.0	Time out responses =	

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Incomplete avoidance learning (Kamin effect)	Rhesus monkeys	0.00316, 0.01, 0.0316, 0.1, 0.316	IV	0.0316 0.1 0.0316		Visual discrimination test (position) Missed avoidance trials ↑ Missed escape trials ↑ Incorrect responses ↑	(Samuel et al., 1965)
						Visual discrimination test (form) Missed avoidance trials ↑ Missed escape trials ↑ Incorrect responses ↑	
						Discriminated continuous avoidance test Response rates ↑	
						1h retention delay Avoidance responses ↑	
						24h retention delay Avoidance responses =	
Passive avoidance	Rats (SD)	0.5	IP	0.5	0.5	Retention ↓	(Thompson & Nielsen, 1972)
	Rats (W)	4.0, 8.0, 16	IP	8.0		Retention ↓	(Roldan et al., 1997)
	Rats (W)	0.2	IP	0.2		Retention ↓	(Vannucchi et al., 1997)
		0.1, 0.3, 1.0	IP	1.0		Latency to enter dark compartment ↓	(Ohno & Watanabe, 1996)
	Rats (W)	8.0	IP	8.0		Administration 30 or 5 min. before training Retention ↓ Administration 120, 60 min. before training or 30 min. after training Retention =	(Quirarte et al., 1994)
	Rats (W)	4.0, 8.0, 12	IP	8.0 8.0 8.0	8.0	Low footshock intensity <i>Administration 5 min. before training</i> Retention ↓ <i>Administration both 5 min. before training and 5 min. before retention testing</i> Retention ↓	(Quirarte et al., 1994)
						High footshock intensity <i>Administration 5 min. before training</i> Retention ↓ <i>Administration both 5 min. before training and 5 min. before retention testing</i> Retention = (state-dependency)	
	Rats (W)	8.0	IP	8.0	8.0	Training latency = Escape latency = Retention score ↓	(Cruz-Morales et al., 1992)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
	Rats (SD)	0.3, 1.0	IP		1.0 1.0	Physical reaction to shock = Latency to enter dark compartment =	(Decker et al., 1990)
	Mice (NMRI)	0.3, 1.0, 3.0	IP	0.3		Administration 5 min. before training Retention ↓	(Rush, 1988)
	Mice (NMRI)	0.3, 3.0	IP	0.3		Administration 5 min. before training Retention ↓ Administration immediately after training Retention =	(Rush, 1988)
	Mice (NMRI)	10, 30	IP	30		Administration immediately after training Retention ↓	(Rush, 1988)
	Mice (NMRI)	3.0	IP	3.0 3.0		Administration 5 min. before training Retention ↓ Administration 5 min. before retention test Retention ↓ Administration 5 min. before training and 5 min. before retention test Retention ↓	(Rush, 1988)
	Mice (NMRI)	0.3, 1.0, 3.0	IP	1.0 1.0		Acquisition ↓ Retention ↓	(Jensen et al., 1987)
	Rats (LB)	0.5	IP	0.5		Acquisition ↓	(Blozovski & Hennock, 1982)
	Rats (SD)	0.5, 1.0, 2.0	IP	0.5		20 - 28 day old rats Retention ↓ Locomotor activity = 16 or 70-85 day old rats Retention = Locomotor activity =	(Feigley, 1974)
	Rats (SD)	4.0, 8.0, 16	IP		16	16 -17 day old rats Retention = Locomotor activity ↑ 70 - 84 day old rats Retention ↓ Locomotor activity =	(Feigley, 1974)
	Rats (W)	0.1, 0.2, 0.4, 0.8	SC	0.2		Administration during acquisition or retention Passive avoidance responses ↓	(Meyers, 1965)

Memory paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Passive avoidance (step-through)	Rats (W)	0.5	IP	0.5		Moderately trained rats Retention ↓ (dependent on decrease in central acetylcholine) Overtreated rats Retention =	(Pazzagli & Pepeu, 1964)
	Mice (Std-ddY)	0.5, 1.0, 2.0	IP	0.5	0.5	Administration 30 min. before learning trial Latency to enter dark compartment ↓	(Nomura et al., 1994)
					2.0	Administration immediately after learning trial Latency to enter dark compartment =	
					2.0	Administration 30 min. before testing trial Latency to enter dark compartment =	
Passive avoidance (step-down)	Mice (Std-ddY)	0.5, 1.0, 2.0	IP	0.5		Administration 30 min. before learning trial Errors ↑	(Nomura et al., 1994)
						Administration immediately after learning trial Errors =	
					2.0	Administration 30 min. before testing trial Errors =	
					2.0		
Passive avoidance extinction	Rats (W)	1.0, 2.0, 4.0, 8.0	IP	2.0		Passive avoidance responses ↑	(Roldan et al., 2001)
	Rats (W)	1.0, 2.0, 4.0, 8.0	IP	2.0		Passive avoidance responses ↑ (dependent on retention interval)	(Roldan et al., 2001)
Sidman avoidance	Rats (F344, W)	0.063, 0.13, 0.25, 0.5	SC	0.063 0.13 0.063 0.063		F344 Response rate ↑ Shock rate ↓	(Kuribara, 1982)
						Wistar Response rate ↑ Shock rate ↓	
Passive avoidance extinction	Rats (WR)	1.0	IP	1.0	1.0	Shocks = Avoidance responses ↓	(Pradhan & Roth, 1968)

Symbols: = : no change, ↑ : increase, ↓ : decrease, - : not mentioned in article. Abbreviations: A: Albino, D: Druckray strain; F344: Fischer-derived F344 strain, H: Hooded strain, HBS: Hooded Blue Spruce strain, HM: Holtzman strain, IM: intramuscular, IP: intraperitoneal, KM: Kunming strain, KW: Kuo:Wistar strain, LB: London Black strain, LE: Long-Evans (Hooded) strain, LH: Lister Hooded strain, NZ: New Zealand white albino rabbits, PDA: pigmented dark agouti, SC: subcutaneous, SD: Sprague-Dawley strain, SW: Swiss Webster strain, W: Wistar strain, WC: White Carneaux pigeons, WR: Wistar-derived Walter Reed strain.

Table 2d. Effects of peripheral administration of scopolamine on operant conditioning in animals. All doses are in mg/kg.

Operant conditioning paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
FR schedule	Rats (LE)	0.005, 0.01, 0.03, 0.05, 0.1	IP	0.005		Lever presses ↓	(Hodges Jr. et al., 2009)
	Rats (LE)	0.1, 0.2, 0.4	IP	0.1		Dry reward	(Hodges Jr. et al., 2009)
						Lever presses ↓	
						Liquid reward	
				0.1		Lever presses ↓	
	Rats (WR)	0.2, 1.0, 2.0	IP	1.0		FR responses ↓	(Pradhan & Roth, 1968)
				1.0		Time-out responses ↓	
FR delayed alternation	Rats (F344)	0.01, 0.03, 0.1, 0.3	SC			FR1 FR1 schedule	(Shannon et al., 1990)
				0.1		Percentage correct ↓ (dependent on retention interval)	
				0.1		Response rate ↓	
						FR1 FR10 schedule	
				0.1		Percentage correct ↓ (dependent on retention interval)	
						Response rate ↓	
				0.03		FR10 FR1 schedule	
				0.3		Percentage correct ↓	
				0.03		Response rate ↓	
						FR10 FR10 schedule	
FR and FI schedule	Patas monkeys	0.0024, 0.0042, 0.01, 0.042, 0.13, 0.24	IM			Percentage correct ↓ (dependent on retention interval)	(Moersbacher et al., 1979)
				0.1		Response rate ↓	
				0.3		FR10 FR10 schedule	
				0.3		Response rate ↓	
	Rats (SD)	0.1, 0.2, 0.8, 1.6	IP	0.2		Response rate ↓	(Boren & Navarro, 1959)
PR schedule	Marmoset monkeys	0.01 – 0.06	SC	0.03		Obtained reinforcers ↓	(Spinelli et al., 2006)
	Rhesus monkeys	0.003, 0.01, 0.014, 0.017, 0.024	IM	0.014		Obtained reinforcers ↓	(Taffe et al., 1999)
Two-bar ratio	Rats (W)	0.5, 0.75, 1.0	IP			Interceptive stimulus condition	(Wagman & Maxey, 1969)
				0.75		Work bar responses before reward bar response ↓	
						Exteroceptive stimulus condition	
				0.75		Work bar responses before reward bar response ↑	

Operant conditioning paradigm	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
DRL	Rhesus monkeys	0.01, 0.018, 0.032, 0.056	IM	0.032 0.032		DRL responses ↓ Earned rewards ↓	(McDonough, 1982)
	Rats (SD)	0.5	IP	0.5		Percentage reinforcements ↓	(Meyer et al., 1976)
	Rats (W)	0.0625, 0.125, 0.25, 0.5, 0.75	IP	0.0625		Stimulus sensitivity ↓	(Brown & Warburton, 1971)
Operant sequence acquisition	Rats (F344)	0.01, 0.03, 0.1, 0.3	SC	0.1		Completed rules ↓	(Shannon & Love, 2004)
				0.03		Response rate ↓	
				0.3	0.3	Percentage correct chains = Completed sequences ↓	
Repeated sequence acquisition procedure	Rats (LE)	0.5, 1.0, 2.0, 3.0	IP	0.5		Accuracy ↓	(Cohn et al., 1992)
					3.0	Response rate =	
				2.0 1.0		Skipping responses ↑ Perseverative responses ↑	
Repeated transition procedure	Rats (LE)	0.5, 1.0, 2.0, 3.0	IP	0.5 2.0		Accuracy ↓ Response rate ↓	(Cohn et al., 1992)

Symbols: = : no change, ↑ : increase, ↓ : decrease. Abbreviations: DRL: differential reinforcement of low rate, F344: Fischer-derived F344 strain, FI: fixed interval, FR: fixed ratio, IM: intramuscular, IP: intraperitoneal, LE: Long-Evans (Hooded) strain, PR: progressive ratio, SC: subcutaneous, SD: Sprague-Dawley strain, W: Wistar strain, WR: Wistar-derived Walter Reed strain.

Table 2e. Effects of peripheral administration of scopolamine on other behavioral tasks and side-effects in animals. All doses are in mg/kg.

Other behavioral paradigms	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Pupil dilation	Rats (LH)	0.01, 0.03, 0.075, 0.1	SC	0.01		Pupil diameter ↑	(Jones & Higgins, 1995)
Food and water intake	Rats (WR)	1.5, 2.0	IP	1.5		Food and water intake ↓	(Pradhan & Roth, 1968)
Dry food intake	Rats (LE)	0.005, 0.01, 0.03, 0.05, 0.1	IP	0.01		Dry food consumption ↓	(Hodges Jr. et al., 2009)
Locomotor activity	Rats (SD)	0.056–1.0 (ED ₅₀ = 0.12)	IP	0.056		Locomotor activity ↑	(Sipos et al., 1999)
	Mice (Std-ddY)	0.5, 1.0, 2.0	IP			Administration 30 min. before learning trial Locomotor activity ↑	(Nomura et al., 1994)
				2.0		Administration immediately after learning trial Locomotor activity ↑	
				2.0		Locomotor activity ↑	
				1.0		Administration 30 min. before testing trial Locomotor activity ↑	
	Rats (W)	4.0	SC	4.0		Locomotor activity ↑	(Day et al., 1991)
	Rats (W)	2.5, 5.0, 10	IP	2.5		Locomotor activity ↑	(Watanabe & Shimizu, 1989)
	Rats (A)	2.0	IP	2.0		Locomotor activity ↑	(Renfro et al., 1972)
	Rats (WR)	1.0, 2.0	IP	1.0		Locomotor activity ↑	(Pradhan & Roth, 1968)
				2.0		Locomotor activity ↓	
Open field	Rats (W)	3.0	IP	3.0		Locomotor activity ↑	(Chintoh et al., 2003)
	Rats (SD)	0.1	SC	0.1		Locomotor activity ↑	(Gholamreza et al., 2002)
Reaction time task	Rats (L)	0.1, 0.3, 1.0	IP	1.0		Response latency ↓	(Blokland et al., 2001)
				1.0		Response latency per hold duration ↓	
				0.1		Motor time ↑	
				1.0		Premature responses ↑	
	Rhesus monkeys	0.003, 0.01, 0.014, 0.017	IM	0.014		Movement time ↑	(Taffe et al., 1999)
					0.017	Release latency =	
					0.017	Touch-miss errors =	
Swim to platform task	Rats (strain not specified)	0.2, 0.5, 2.0, 5.0, 15 (ED ₅₀ = 1.0)	SC, IP	0.5		Errors to locate platform ↑ (independent of route of administration)	(Vanderwolf, 1991)
Motor skill tasks	Mice (C57BL/6)	0.6, 1.2	SC		1.2	Motor skill performance =	(Thouvaracq et al., 2001)
Bimanual motor task	Rhesus monkeys	0.003, 0.01, 0.014, 0.017, 0.024	IM	0.014		Time to complete motor task ↑	(Taffe et al., 1999)
Rotarod	Mice (C57BL/6)	0.6, 1.2	SC	1.2		Motor learning ↓ (age-dependent)	(Thouvaracq et al., 2001)

Other behavioral paradigms	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Novelty exploration	Rats (H)	0.1, 0.25, 0.5, 0.75, 1.0	IP	0.1 0.1 0.1 0.5		Reactions to novelty ↓ Rearing ↓ Cells entered ↓ Cells entered ↑	(Hughes et al., 1975)
	Mice (C57BL/6, DBA/2, 1.25, 2.5, 5.0, 10 F1 of C57BL/6 x DBA/2)		IV	1.25 1.25 2.5		C57BL/6 Object sniffing ↓ Leaning ↓ Rearing ↓ DBA/2 Object sniffing = Leaning ↑ Rearing = F1 hybrid of C57BL/6 and DBA/2 Object sniffing = Leaning ↓ Rearing ↓	(van Abeelen & Strijbosch, 1969)
				2.5	10		
					10		
				1.25 2.5	10		
1-trial stimulus-reward association task	Rhesus monkeys	0.01, 0.0178	IM	0.01		Recall of stimulus-reward associations ↓	(Aigner & Mishkin, 1993)
Serial-probe recognition	Rhesus monkeys	0.0056, 0.008, 0.01, 0.017, 0.03	IM	0.01 0.03		Accuracy ↓ Completed trials ↓	(Myers et al., 2002)
1-choice serial reaction time task	Mice (F1 of C57BL/6 x DBA/2, F1 of C57BL/6 x 129Sv)	0.02, 0.2, 2.0	IP		2.0 2.0 2.0 2.0	Accuracy = Omissions = Correct response latency = Locomotor activity =	(Humby et al., 1999)
Delayed stimulus-comparison task	Rats (SD)	0.01, 0.03, 0.1	SC	0.1 0.1 0.1		Percentage correct ↓ Response latency ↑ Discriminability ↓ Bias =	(Shannon & Yang, 2007)
Social-olfactory investigation	Mice (CD-1)	0.1625, 0.325, 0.65, 1.0	IP	0.1625 0.1625 -	0.1	Habituation to unfamiliar intruder ↓ Aggressive behavior ↓ Locomotor activity ↓	(Winslow & Camacho, 1995)
Perceptual bisection	Rats (SD)	0.133, 0.075, 0.10, 0.237, 0.422	IP	0.133 0.237		Bisection point ↓ Discriminability ↓	(Shurtleff et al., 1992)

Other behavioral paradigms	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Activity and light-dark preferences	Rats (W)	1.0, 2.0	IP	1.0	2.0	Male rats Locomotor activity ↑ (in novel room) Latency to enter light side = Transitions to light side ↓ Time spent in light side =	(Hughes et al., 2004)
						Female rats Locomotor activity ↑ (in familiar room) Latency to enter light side = Transitions to light side ↓ Time spent in light side ↓	
Black-white box	Rats (LH)	0.05, 0.1	IP	0.1		Time to exit white chamber ↓	(Smythe et al., 1996)
				0.05 0.05 0.05		Time spent in black chamber ↑ Crossings ↓ Locomotor activity ↑ (in white chamber)	
Affective and attentional set-shifting	Rats (LE)	0.25	IP	0.25	0.25	Administration before IDS IDS = IDS reversal ↓	(Chen et al., 2004)
Spatial delayed response task	Rats (LE)	0.1, 0.25	IP	0.1		Administration before EDS EDS ↓ EDS reversal ↓	(Chen et al., 2004)
				0.25			
				0.03		Standard conditions Accuracy ↓ (delay-dependent)	
				0.01		Reduced stimulus brightness Accuracy ↓ (delay-dependent)	
Go/no-go task	Rats (SD)	0.125, 0.25, 0.50	IP	0.25		Go responses ↓	(Milar et al., 1978)
				0.25		No-go responses ↑	
				0.25		Go/go discrimination ↓	
Go/no-go task	Rats (SD)	0.125, 0.25, 0.50	IP	0.5 0.25 0.5 0.5 0.5 0.5		Light Inhibitory Go responses ↓ No-go responses ↑ Go/go discrimination ↓ Tone Excitatory Go responses ↓ No-go responses ↑ Go/go discrimination ↓	(Milar et al., 1978)
Go/no-go task	Rats (W)	0.0625, 0.125, 0.25	IP	0.0625		Sensitivity ↓	(Warburton & Brown, 1971)

Other behavioral paradigms	Species	Dose conditions	Route	Min. effective dose	Max. ineffective dose	Effect	References
Delayed go/no-go task	Rats (SD)	0.125, 0.25, 0.5	IP	0.125 0.125 0.125		Hit accuracy ↓ (delay-dependent) Trial initiations ↓ Sensitivity ↓ (delay-dependent) Response bias =	(Viscardi & Heise, 1986)
Go/no-go acoustic delayed matching to sample	Rhesus monkeys	0.003, 0.005, 0.01	IM	0.005	0.01 0.01 0.01	Performance accuracy ↓ Motor responses = Food motivation = Responses to rewarded sound =	(Plakke et al., 2008)
Object reaching with detour task	Marmoset monkeys	0.01 – 0.06	SC	0.06 0.03		Percentage successful responses ↓ Percentage trials completed ↓	(Spinelli et al., 2006)

Symbols: = : no change, ↑ : increase, ↓ : decrease, - : not mentioned in article. Abbreviations: A: Albino, EDS: extradimensional shift, H: Hooded strain, IDS: intradimensional shift, IM: intramuscular, IP: intraperitoneal, L: Lewis strain, LE: Long-Evans (Hooded) strain, LH: Lister Hooded strain, SC: subcutaneous, SD: Sprague-Dawley strain, W: Wistar strain, WR: Wistar-derived Walter Reed strain.

Table 3a. Effects of central administration of scopolamine on stimulus discrimination and attention in animals.

Stimulus discrimination paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Cued target detection (Posner) task	Intraparietal cortex	Rhesus monkeys	4, 7 mg in 1 mL Ringer solution	7 mg	7 mg	Performance accuracy ↓ Response latency ↑ (for peripheral orienting, but not alerting components)	(Davidson & Marrocco, 2000)
Discrimination task	Dorsal hippocampus	Rats (LE)	4, 12, 35 µg in 1 µl/side	35 µg		Discrimination ↓	(Brito et al., 1983)
	Dorsal hippocampus	Rats (Crt:CD (SD)BR)	4 µg in 1 µl/side	4 µg	4 µg 4 µg	Proportion of correct choices ↓ Choice latency = Omissions =	(Cari et al., 1999)
	Dorsal hippocampus	Rats (CD-COBS)	3.75 µg in 1 µl/side	3.75 µg	3.75 µg 3.75 µg	Proportion of correct choices ↓ Choice latency = Omissions =	(Cari et al., 1995)
Odor discrimination task	Periform cortex	Rats (LE)	500 µM	500 µM		Odor cross-habituation/generalization ↑	(Wilson, 2001)
Spatial discrimination task	Dorsal hippocampus	Rats (CD-COBS)	1.87, 3.75, 7.5, 15, 30 µg in 1 µl/side	3.75 µg	30 µg	Proportion of correct choices ↓ Choice latency = Omissions ↑	(Cari et al., 1997)
Non-spatial discrimination task	Dorsal hippocampus	Rats (CD-COBS)	1.87, 3.75, 7.5, 15, 30 µg in 1 µl/side		30 µg 30 µg	Proportion of correct choices = Choice latency =	(Cari et al., 1997)
Successive spatial discrimination task	Prelimbic/infralimbic cortex	Rats (LE)	1, 5, 10 µg		10 µg	Latency =	(Ragozzino & Kesner, 1998)
Delayed conditional discrimination task	Dorsomedial prefrontal cortex	Rats (W)	10, 30 µg in 1 µL	10 µg 30 µg		Discrimination ↓ Response bias ↓	(Herremans et al., 1997)
	Ventromedial prefrontal cortex	Rats (W)	5, 15 µg in 0.5 µl	15 µg 15 µg 15 µg		Percentage correct trials ↓ (delay-dependent) Trials ↓ (delay-dependent) Response latency ↑ (delay-dependent)	(Broersen et al., 1995)
Temporal discrimination test (FI schedule)	Striatum	Rats (L)	3, 10 µg/side		10 µg	Completed trials = Response rate ↓	(Blokland, 1998)
Two-choice discrimination and reversal	Dorsomedial striatum	Rats (LE)	1, 8 µg in 0.5 µl/side	8 µg	8 µg	Acquisition = Reversal learning ↓ Perseverative errors during reversal = Regressive errors during reversal ↑	(Ragozzino et al., 2002)
Sustained attention task	Lateral ventricle	Rhesus monkeys	0.004, 0.12, 12.5, 40.0 µg/kg/h	12.5 µg/kg/h		Responses ↓ (dependent on stimulus duration and number of trials completed)	(Callahan et al., 1993)

Symbols: = : no change, ↑ : increase, ↓ : decrease. Abbreviations: FI: fixed interval, L: Lewis strain, LE: Long-Evans (Hooded) strain, W: Wistar strain.

Table 3b. Effects of central administration of scopolamine on memory in animals.

Memory paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Spontaneous alternation	Infralimbic cortex	Mice (CD-1)	5, 10, 20 nmol in 0.5 µl	20 nmol		Alternation ↓	(Wall et al., 2001)
Spatial alternation	Parietal cortex	Rats (W)	30 µg in 3 µl/side		30 µg	Spatial alternation =	(Prado-Alcala et al., 1978)
	Medial septal area	Rats (F344)	2, 5, 15 µg	5 µg		Young rats Choice accuracy ↓ Aged rats Choice accuracy ↓	(Markowska et al., 1995)
	Anterior caudate nucleus	Rats (W)	30 µg in 3 µl/side	30 µg		Low level of training <i>First and second administration</i> Spatial alternation ↓ High level of training <i>First administration</i> Spatial alternation ↓ <i>Second administration</i> Spatial alternation =	(Prado-Alcala et al., 1978)
	Posterior caudate nucleus	Rats (W)	30 µg in 3 µl/side		30 µg	Spatial alternation =	(Prado-Alcala et al., 1978)
Delayed spatial alternation	Medial septal area	Rats (LE)	15 µg in 0.5 µL	15 µg		Choice accuracy ↓ (delay-dependent)	(Givens & Olton, 1995)
Reinforced alternation	Dorsal hippocampus	Rats (LE)	4, 12, 35 µg in 1 µl/side	35 µg		Delayed alternation ↓	(Brito et al., 1983)
Object recognition task	Perirhinal cortex	Rats (LH)	10 µg in 1 µl/side	10 µg		Infusion immediately before T1 Object recognition ↓ Infusion immediately after T1 Object recognition ↑ Infusion immediately before T2 Object recognition =	(Winters et al., 2006)
	Perirhinal cortex	Rats (LH)	10 µg in 1 µl/side	10 µg	10 µg	Infusion immediately after T1, 20 min. or 40 min. after T1 Object recognition ↑	(Winters et al., 2006)
	Perirhinal cortex	Rats (LH)	10 µg in 1 µl/side	10 µg		Infusion immediately after T1, 8, 16 or 20h after T1 Object recognition ↑	(Winters et al., 2006)
	Perirhinal cortex	Rats (LH)	10 µg in 1 µl/side	10 µg		Infusion 3h before T1 Object recognition ↑	(Winters et al., 2006)

Memory paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
	Perirhinal cortex	Rats (LE)	20, 80 mM, in 1 µl/side	20 mM		Object recognition ↓	(Abe et al., 2004)
	Perirhinal cortex	Rats (PDA)	10 µg in 1 µl	10 µg		Object recognition ↓	(Warburton et al., 2003)
Object recognition task (proactive interference)	Perirhinal cortex	Rats (LH)	10 µg in 1 µl/side	10 µg		Proactive interference by a preposed object ↓	(Winters et al., 2007)
Object recognition task (retroactive interference)	Perirhinal cortex	Rats (LH)	10 µg in 1 µl/side	10 µg		Retroactive interference by an interposed object ↓	(Winters et al., 2007)
Three-panel runway task	Amygdala	Rats (W)	1, 3.2, 5.6 µg/side			Working memory task Errors ↑ Latency ↑ Reference memory task Errors = Latency =	(Ohno et al., 1993)
Y-maze	Hippocampus	Rats (SD)	0.58% solution in 20 µl	5.6 µg 5.6 µg	0.58% solution in 20 µl	Administration 1 or 3 days after acquisition Appetitive discrimination ↓ Administration 30 min, 7, 14 or 21 days after acquisition Appetitive discrimination =	(Wiener & Deutsch, 1968)
Double Y-maze	Amygdala	Rats (SD)	8, 24, 72 µg in 0.5 µL	24 µg 72 µg		Working memory ↓ Reference memory ↓	(Ingles et al., 1993)
Radial maze	Basal forebrain	Rats (W)	2 µg in 2 µl/side	2 µg 2 µg		Administration 30 min. before testing Correct choices ↓ Errors ↑ Administration 10 min. before testing Correct choices = Errors =	(Mishima et al., 2000)
	Dorsal hippocampus	Rats (W)	2 µg in 2 µl/side	2 µg 2 µg		Administration 30 min. before testing Correct choices ↓ Errors ↑ Administration 10 min. before testing Correct choices = Errors =	(Mishima et al., 2000)
	Ventral hippocampus	Rats (W)	2 µg in 2 µl/side	2 µg 2 µg		Correct choices ↓ Errors ↑	(Mishima et al., 2000)

Memory paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
	Ventral hippocampus	Rats (SD)	1, 3.3, 10 µg/side	10.0 µg	10.0 µg	Choice accuracy ↓ Choice latency =	(Kim & Levin, 1996)
	Dorsomedial thalamus nucleus	Rats (W)	2 µg in 2 µl/side			Administration 30 min. before testing Correct choices = Errors = Administration 10 min. before testing Correct choices ↓ Errors ↑	(Mishima et al., 2000)
	Medial raphe nucleus	Rats (W)	2 µg in 2 µl/side	2 µg 2 µg		Administration 30 min. before testing Correct choices ↓ Errors ↑ Administration 10 min. before testing Correct choices = Errors =	(Mishima et al., 2000)
	Lateral ventricle	Rats (W)	20 µg in 2 µl	20 µg 20 µg		Correct choices ↓ Errors ↑	(Mishima et al., 2000)
Spatial radial maze task	Amygdala	Rat (SD)	5 µg in 0.5 µL		5 µg 5 µg	Working memory errors = Reference memory errors =	(McIntyre et al., 1998)
Water maze	Anterior cingulate	Rats (KW)	3, 10, 30 µg in 0.5 µl		30 µg	Pretraining infusion Escape distance =	(Riekkinen et al., 1995)
	Posterior cingulate	Rats (KW)	3, 10, 30 µg in 0.5 µl	10 µg		Pretraining infusion Escape latency ↑ Posttraining or pretesting infusion Escape latency =	(Riekkinen et al., 1995)
	Septum	Rats (SD)	10, 15 µg in 0.5 µL	15 µg 10 µg	15 µg	Escape latency = Swim distance ↑ Swim speed ↑ Time spent in training quadrant =	(Elvander et al., 2004)
	Dorsal hippocampus	Rats (W)	30, 60 µg in 0.5 µL	30 µg 30 µg 30 µg		Escape latency during training ↑ Target latency ↑ Target crossings ↑	(Herrera-Morales et al., 2007)
	Dorsal hippocampus	Rats (L)	35 µg in 1 µl/side	35 µg		Repeated acquisition performance ↓	(Bjokland et al., 1992)

Memory paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Delayed matching	Olfactory bulb	Rats (W)	0.625, 1.3, 2.6, 13, 26, 52, 130 nM in 1 µl (titrated minimal effective doses)	2.6 nM	130 nM	Granular location Immediate memory (4s delay) = Short-term memory (30s delay) ↓	(Ravel et al., 1994)
						Glomerular location Immediate memory (4s delay) = Short-term memory (30s delay) =	
						Percentage correct trials ↓ (delay-dependent) Response bias ↑ (delay-dependent) Nosepoke frequency ↓ (delay-dependent) Sample latency ↑	
	Dorsomedial prefrontal cortex	Rats (W)	5, 15 µg in 0.5 µl	15 µg 15 µg 15 µg 5 µg	30 µg	Percentage correct trials ↓ (delay-dependent) Response bias ↑ (delay-dependent) Nosepoke frequency ↓ (delay-dependent) Sample latency ↑	(Broersen et al., 1994)
	Medial prefrontal cortex	Rats (W)	10, 30 µg in 1 µL	30 µg - 8.5 µg	30 µg	Discrimination accuracy ↓ Response bias = Response latency ↑	(Heremans et al., 1996)
	Prelimbic cortex	Rats (LE)	0.85, 8.5, 85 µg in 0.5 µL	8.5 µg	85 µg	Working memory ↓ Reference memory =	(Granon et al., 1995)
Non-matching	Hippocampus	Rats (LE)	30 µg in 0.5µL/side	30 µg 30 µg		Response time ↑ Percentage correct choices ↓	(Messer, Thomas, & Hoss, 1987)
Delayed non-matching	Medial prefrontal cortex	Rats (SD)	4, 12 µg in 1µl	12 µg	12 µg	Percentage correct ↓	(Dunnett et al., 1990)
	Prelimbic cortex	Rats (LE)	0.85, 8.5, 85 µg in 0.5 µL	8.5 µg	85 µg	Working memory ↓ Reference memory =	(Granon et al., 1995)
	Dorsal hippocampus	Rats (SD)	0.65, 1.3, 2.6, 4, 8, 12 µg in 0.5 µl/side	4 µg 4 µg	12 µg	Number of trials ↓ Sample omissions ↑ Response bias =	(Robinson & Mao, 1997)
	Dorsal hippocampus	Rats (SD)	4, 12, 35 µg in 1µl	12 µg	12 µg	Percentage correct ↓ (delay-dependent)	(Dunnett et al., 1990)
	Ventral hippocampus	Rats (SD)	1.3, 4, 12, 36 µg in 0.5 µl/side	4 µg 4 µg 4 µg	36 µg 36 µg	Trials ↓ Sample omissions ↑ Sample response accuracy ↓ Response bias = Anticipatory responses =	(Robinson & Mao, 1997)
Latent inhibition	Insular cortex	Rats (W)	30 µg in 0.5 µL	30 µg		Latent inhibition ↓	(Naor & Dudai, 1996)
Conditioned place preference task	Amygdala	Rats (SD)	5 µg in 0.5 µL	5 µg		Memory for place previously paired with food reward ↓	(McIntyre et al., 1998)

Memory paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Taste recognition memory	Perirhinal cortex	Rats (W)	30 µg in 0.5 µl/side	30 µg		Saccharin intake ↓ (interpreted as increased neophobia)	(Gutiérrez et al., 2004)
	Insular cortex	Rats (W)	30 µg in 0.5 µl/side	30 µg		Administration before and after taste presentation Saccharin intake ↓ (interpreted as increased neophobia)	(Gutiérrez et al., 2003)
Odor aversion	Dorsal hippocampus	Rats (SD)	30 µg in 3 µl/side		30 µg	Odor aversion =	(Bermúdez-Rattoni et al., 1987)
Odor-taste aversion	Frontoparietal cortex	Rats (SD)	30 µg in 3 µl/side		30 µg 30 µg	Odor aversion = Taste aversion =	(Bermúdez-Rattoni et al., 1987)
	Dorsal hippocampus	Rats (SD)	30 µg in 3 µl/side	30 µg	30 µg	Odor aversion ↑ Taste aversion =	(Bermúdez-Rattoni et al., 1987)
Conditioned taste aversion	Parietal cortex	Rats (W)	30 µg in 0.5 µl		30 µg	CTA memory =	(Naor & Dudai, 1996)
	Perirhinal cortex	Rats (W)	30 µg in 0.5 µl/side	30 µg 30 µg		Short-term memory CTA memory ↓ Long-term memory CTA memory ↓	(Gutiérrez et al., 2004)
	Insular cortex	Rats (W)	30 µg in 0.5 µl/side	30 µg	30 µg	Administration before taste presentation CTA memory ↓ Administration after taste presentation CTA memory =	(Gutiérrez et al., 2003)
	Insular cortex	Rats (W)	136 mM in 1.0 µl/side	136 mM		Administration before acquisition CTA memory ↓ Administration before retention CTA memory =	(Ramírez-Lugo et al., 2003)
	Insular cortex	Rats (W)	30 µg in 0.5 µl	30 µg		Administration 20 min. before CS CTA memory ↓ Administration 5 min. after CS CTA memory = Administration 20 min. before US CTA memory = Administration 20 min. before retrieval test CTA memory =	(Naor & Dudai, 1996)
Odor-shock conditioning	Dorsal hippocampus	Rats (SD)	30 µg in 3 µl/side		30 µg	Odor aversion =	(Bermúdez-Rattoni et al., 1987)

Memory paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Fear conditioning	Hippocampus	Rats (LE)	35 µg in 1 µl/side	35 µg	35 µg	Acquisition of fear conditioning ↓	(Rogers & Kesner, 2004)
				35 µg		Contextual conditioning ↓ Tone conditioning =	
Trace conditioning	Dorsal hippocampus	Rats (SD)	5, 25 µg in 0.25 µl	25 µg	25 µg	Pre-and posttraining infusion Contextual conditioning ↓ Tone conditioning =	(Wallenstein & Vago, 2001)
				10 µg		Trace conditioning ↓ Delay conditioning = Context conditioning =	
Active avoidance	Septum	Cats	10-20 µg	-	-	Avoidance acquisition ↓ Returns to food =	(Hamilton & Grossman, 1969)
				1.25, 2.5, 5, 10, 20 µg in 1 µl		Forgetting of avoidance response ↑	
Passive avoidance	Frontal cortex	Rats (SD)	30 µg/µl/rat	1.25 µg	30 µg	Retention ↓	(Flood et al., 1981) (Santucci & Shaw, 2003)
				10 µg			
	Anterior cingulate	Rats (KW)	3, 10, 30 µg in 0.5 µl	10 µg	10 µg	Pretraining infusion Retention ↓	(Riekkinen et al., 1995)
				10 µg		Posttraining infusion Retention ↓	
	Posterior cingulate	Rats (KW)	3, 10, 30 µg in 0.5 µl	0.095 mg	30 µg	Pretesting infusion Retention =	(Riekkinen et al., 1995)
	Hippocampus	Rats (W)	0.095 mg in 3µl/side	0.095 mg	0.095 mg	Pretraining, posttraining or pretesting infusion Retention =	(Wiener & Messer, 1973)
	Antero-dorsal striatum	Rats (W)	15, 30 µg in 3 µl	30 µg	30 µg	Retention test 1 or 3 days after acquisition Retention =	(Diaz del Guante et al., 1991)
	Amygdala	Rats (W)	2 µg/side	2 µg	2 µg	Retention test 5, 7 or 10 days after acquisition Retention ↓	(Blanchin et al., 1999)
						Injection 2 or 8 min. after training Retention ↓	
						Injection 15 min. after training Retention =	
						Long term memory ↓ Working memory =	

Memory paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Passive avoidance (step-through)	Amygdala	Mice (Std-ddY)	1, 10 µg in 0.5 µL/side	10 µg		Administration 30 min. before learning trial Latency to enter dark compartment ↓	(Nomura et al., 1994)
				1 µg		Administration immediately after learning trial Latency to enter dark compartment ↓	
					10 µg	Administration 30 min. before testing trial Latency to enter dark compartment =	
Passive avoidance (step-down)	Amygdala	Mice (Std-ddY)	1, 10 µg in 0.5 µL/side	10 µg		Administration 30 min. before learning trial Errors ↑	(Nomura et al., 1994)
					10 µg	Administration immediately after learning trial Errors =	
					10 µg	Administration 30 min. before testing trial Errors =	

Symbols: = : no change, ↑ : increase, ↓ : decrease, - : not mentioned in article. Abbreviations: CTA: conditioned taste aversion, F344: Fischer-derived F344 strain, KW: Kuo:Wistar strain, L: Lewis strain, LE: Long-Evans (Hooded) strain, LH: Lister Hooded strain, PDA: pigmented dark agouti, SD: Sprague-Dawley strain, W: Wistar strain.

Table 3c. Effects of central administration of scopolamine on operant conditioning in animals.

Operant conditioning paradigm	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Reaction time task	Striatum	Rats (L)	3, 10 µg/side		10 µg	Response latency =	(Blokland & Honig, 1999)
					10 µg	Locomotor performance =	
				3 µg		Completed trials ↓	
				3 µg		Anticipatory responses ↑	
	Striatum	Rats (L)	3, 10 µg/side		10 µg	Response latency =	(Blokland, 1998)
				10 µg		Completed trials ↓	
				10 µg		Anticipatory responses ↓	
					10 µg	Response distribution =	
Auto-shaping	Caudate putamen	Rats (W)	30 µg in 3 µl/side	30 µg		Acquisition of a bar press response ↓	(Bermúdez-Rattoni et al., 1986)
CRF schedule	Parietal cortex	Rats (W)	20 µg in 3 µl/side		20 µg	Level press rate =	(Prado-Alcala et al., 1980)
	Anterior caudate nucleus	Rats (W)	20 µg in 3 µl/side	20 µg		Low or medium level of training	(Prado-Alcala et al., 1980)
						Level press rate ↓	
					20 µg	High level of training	
						Level press rate =	
Conditioned reward responding	Ventral tegmental area	Rats (LE)	5, 10 µg/0.5 µL	5 µg		Pellet consumption ↓	(Sharf & Ranaldi, 2006)
PR schedule	Ventral tegmental area	Rats (LE)	5 µg/0.5 µL	5 µg		Conditioned reward responses =	(Sharf & Ranaldi, 2006)
						Break point ↓	
Lever-press extinction	Medial prefrontal cortex	Rats (W)	3, 9 µg in 0.5 µL	9 µg		Lever press extinction ↓	(Manuki et al., 2003)

Symbols: = : no change, ↑ : increase, ↓ : decrease. Abbreviations: CRF: continuous reinforcement, L: Lewis strain, LE: Long-Evans (Hooded) strain, PR: progressive ratio, W: Wistar strain.

Table 3d. Effects of central administration of scopolamine on other behavioral tasks and side-effects in animals.

Other behavioral paradigms	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Free feeding	Ventral tegmental area	Rats (LE)	5 µg/0.5 µL	5 µg		Pellet consumption ↓	(Sharf & Ranaldi, 2006)
Locomotor activity	Amygdala	Mice (Std-ddY)	1, 10 µg in 0.5 µL/side		10 µg	Administration 30 min. before learning trial Locomotor activity = Administration immediately after learning trial Locomotor activity ↑ Administration 30 min. before testing trial Locomotor activity =	(Nomura et al., 1994)
Postural deviation	Anterior neocortex	Rats (LE)	20, 30, 40 µg in 0.25 µL/side		40 µg	Contralateral deviation = Rearing ↑	(Chambers & van Hartesveldt, 1984)
	Striatum	Rats (LE)	20, 30, 40 µg in 0.25 µL/side	30 µg 20 µg 30 µg		Contralateral deviation ↑ Rearing ↑	(Chambers & van Hartesveldt, 1984)
Swimming task	Dorsal striatum	Rats (W)	0.5 µg in 0.5 µL/side	0.5 µg		Continuous sinking behavior ↓	(Vrijmoed de Vries & Cools, 1986)
	Ventral striatum	Rats (W)	0.5 µg in 0.5 µL/side	0.5 µg		Continuous sinking behavior ↑	(Vrijmoed de Vries & Cools, 1986)
Black-white box	Hippocampus	Rats (LH)	15, 30 µg in 3 µL/side	30 µg 15 µg	30 µg 30 µg	Time to exit white chamber ↓ Time to re-enter white chamber ↑ Time spent in white chamber = Locomotor activity =	(Smythe et al., 1998)
Elevated plus maze	Infralimbic cortex	Mice (CD-1)	5, 10, 20 nmol in 0.5 µL	20 nmol 5 nmol 20 nmol 20 nmol		Open arm time ratio ↓ Unprotected head dips ↓ Unprotected stretch attends ↓ Closed arm entries ↑	(Wall et al., 2001)
Social transmission of food preference	Prelimbic cortex	Rats (W)	20 µg/side	20 µg	20 µg 20 µg 20 µg 20 µg 20 µg	Socially transmitted food preference ↓ Odor detection = Food motivation = Locomotor activity and exploration = Social interaction = Antineophobic reaction =	(Boix-Trelis et al., 2007)
	Basolateral amygdala	Rats (W)	20 µg in 0.5 µL/side	20 µg	20 µg 20 µg 20 µg	Socially transmitted food preference ↓ Odor detection = Food motivation = Locomotor activity =	(Carballo-Márquez et al., 2009)

Other behavioral paradigms	Target region	Species	Dose conditions	Min. effective dose	Max. ineffective dose	Effect	References
Go/no-go task	Prelimbic/ infralimbic cortex	Rats (LE)	1, 5, 10 µg	5 µg		Spatial working memory ↓ (lag-dependent)	(Ragozzino & Kesner, 1998)
	Anterior cingulate	Rats (LE)	1, 5, 10 µg		10 µg	Spatial working memory =	(Ragozzino & Kesner, 1998)

Symbols: = : no change, ↑ : increase, ↓ : decrease. Abbreviations: LE: Long-Evans (Hooded) strain, LH: Lister Hooded strain, W: Wistar strain.

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CHAPTER 3

A comparison of scopolamine and biperiden as a rodent model for cholinergic cognitive impairment

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INTRODUCTION

The muscarinic antagonist scopolamine hydrobromide is used as the gold standard for inducing deficits in human and animal models of memory dysfunction. Justification for this purpose has been provided by the cholinergic hypothesis of geriatric memory dysfunction proposed in the early eighties by Bartus et al. (1982). The scopolamine model is still used extensively for preclinical testing of new substances designed to treat cognitive impairment (e.g., Barak & Weiner, 2009; Buccafusco et al., 2008; Cunha et al., 2008; Loiseau et al., 2008; Vaisman & Pelled, 2009). However, its use in cognition research is surrounded by controversy (see **Chapter 2** and Hodges Jr. et al., 2009). Scopolamine is non-selective in terms of binding affinity and, depending on its dose, has the capability to block cholinergic neurotransmission at all muscarinic receptor subtypes M_1 - M_5 (Bolden et al., 1992; Bymaster et al., 2003). As muscarinic receptors are found throughout the brain and body (Caulfield, 1993), scopolamine is able to induce widespread effects.

Systemic injections of scopolamine are capable of disrupting several autonomic nervous system functions. At doses of 0.01 mg/kg and higher, scopolamine can reduce salivation (dry mouth side-effect: Dai et al., 1991; Hodges Jr. et al., 2009; Shiraishi & Takayanagi, 1993), which may lower responding in tasks which employ solid food rewards in order to motivate the animals. Although this problem can be dealt with by using liquid reinforcers (Hodges Jr. et al., 2009), the majority of studies still favor dry food pellets (see **Chapter 2**). Further, most behavioral tasks also have a strong non-cognitive component which can be influenced by scopolamine treatment (e.g., increases in locomotor activity, response latency, and omissions at doses lower than 0.03 mg/kg, see **Chapter 2** and Bushnell et al., 1997; Mirza, 2000; Phillips et al., 2000; Sipos et al., 1999). Performance on behavioral tasks assessing sensory/stimulus discrimination and/or attentional processes appears to be most susceptible to scopolamine treatment (Hodges Jr. et al., 2009). Only if doses higher than 0.1 mg/kg are administered systemically, robust performance deficits on a variety of learning and memory tasks are reported (see **Chapter 2**). Therefore it has been argued that acetylcholine is predominantly involved in mediating discriminatory and attentional processes (Blokland, 1995; Everitt & Robbins, 1997; Fibiger, 1991; Sarter & Bruno, 1997) rather than learning and memory functions (Bartus et al., 1982). In sum, the validity of scopolamine as a tool for inducing cognitive dysfunction is questionable.

Of note, systemic administration makes it difficult to dissociate central and peripheral effects. One way to address this issue is to include an experimental group that is given methylscopolamine, a quaternary form of scopolamine that has the same receptor binding characteristics but supposedly does not cross the blood-brain barrier when given at an equivalent dose (Evans, 1975; Harvey et al., 1983; Pradhan & Roth, 1968). However, several animal studies have shown that methylscopolamine can influence measures of cognitive performance (e.g., Andrews et al., 1994; Herremans et al., 1995; Moore et al., 1992; Pakarinen & Moerschbaeher, 1993; van Haaren & van Hest, 1989). In addition, methylscopolamine cannot control for the widespread blockade of central muscarinic receptors after systemic administration of scopolamine (Frey et al., 1985).

Several of the muscarinic receptor subtypes M_1 - M_5 might underlie the cognitive effects of scopolamine. Muscarinic M_2 presynaptic autoreceptors have an inhibitory effect on acetylcholine efflux (Bymaster et al., 2003). Hence, M_2 antagonists might act as cognitive enhancers by elevating central cholinergic tone, but behavioral data are mixed (Carey et al.,

2001; Daniel & Dohanich, 2001; Messer & Miller, 1988; Quirion et al., 1995). The role of M_3 receptors in cognition is as of yet quite obscure (Bymaster et al., 2003), although one study showed a potential role of M_3 receptors in cognition (Poulin et al., 2010). Information on the involvement of M_4 presynaptic autoreceptors and M_5 postsynaptic in cognitive function is also relatively limited (Wess, 2004); there are some indications that the M_5 receptor is implicated in central cerebral blood flow and memory processes (Araya et al., 2006).

Evidence for a role in mnemonic processes in both rodents and humans is strongest for the postsynaptic muscarinic M_1 receptor (e.g., Conn et al., 2009; Fornari et al., 2000; Kimura et al., 1999; Kramer-Soares et al., 2006; Roldán, 1997; Wezenberg et al., 2005). This receptor is predominantly located in brain regions thought to be important for learning and memory such as cortex and hippocampus; presence of the M_1 receptor in the periphery is relatively limited (Caulfield, 1993; Volpicelli & Levey, 2004). Hence, M_1 antagonists are considered an interesting option with regards to finding novel pharmacological alternatives to induce cognitive impairment which are not so much hampered by issues of non-selectivity or peripheral side-effects (Conn et al., 2009).

This is the first study to compare the effects of scopolamine versus the relatively more selective muscarinic M_1 antagonist biperiden (Bolden et al., 1992) on various facets of behavior. Specifically, we wanted to dissociate behavioral effects of these two drugs on a battery of four operant tasks: fixed ratio (FR5) and progressive ratio (PR10) schedules of reinforcement (assessing sensorimotor responding and food motivation, respectively) versus performance on an attention task and a delayed non-matching to position task (DNMTP; assessing short-term memory). On basis of the direct comparison between both drugs we wanted to determine whether biperiden would be preferable over scopolamine as a cholinergic memory deficit model.

METHODS

Subjects

All experimental procedures were approved by the local ethical committee for animal experiments at Maastricht University and met governmental guidelines. Twenty male 3-month old Wistar rats (Harlan, NL) served as subjects in this study. To ensure consistency, the same animals were used in all behavioral tasks. They were housed in pairs in standard type III Makrolon™ cages on sawdust bedding in an air-conditioned room (21°C, 45-55% humidity) under a reversed light/dark cycle (lights on from 7 PM to 7 AM). Rats were housed in the room in which they were tested. All testing was performed between 12 and 6 PM. Rats had free access to water, but were subjected to a food deprivation regime from Monday through Friday, in order to reduce their weight to about 90% of their free feeding weight. Food was given ad libitum from Friday afternoon to Sunday afternoon. Food was taken away at Sunday afternoon which caused a sufficient appetite at the morning session on Monday.

Apparatus

Rats were trained and tested in 10 identical Skinner boxes (40 x 30 x 33 cm). The ceiling of these conditioning chambers contained a light that illuminated the conditioning chamber

during experiments. The left and right sidewalls served as control panels. A recess (5 x 5 cm), built into the left side panel 2.5 cm above the grid floor, contained a food tray with a hinged panel into which a pellet dispenser delivered 45-mg food pellets (Bioserve TestDiet AIN-76A rodent tablets, Frenchtown, NJ, USA). Two retractable stainless steel levers (4 cm wide) projected 2 cm into the conditioning chamber and were located 6 cm from both sides of the recess, 12 cm above the grid floor. The conditioning chambers were enclosed in sound-attenuating housing. Background noise was produced by a radio and an exhaust fan. A personal computer controlled the experimental equipment and collected the data.

Fixed ratio (5) task

Rats first underwent five magazine training sessions and were then subjected five times to continuous reinforcement (CRF). Next the rats were trained on a fixed ratio schedule of reinforcement, in which they had to press a lever for five times (FR5) in order to obtain a 45 mg food reward. Reinforcement was continuous; i.e., each set of five lever presses was rewarded. A session was terminated after 60 trials or 30 min., whichever came first. Rats were trained once a day, Monday to Friday, and were given eight FR5 sessions before drug testing started. The measure used to evaluate performance on the FR5 schedule was inter-response time (i.e., time between consecutive lever presses which was averaged for each animal).

Progressive ratio (10) task

After finishing drug testing in the FR5 task, rats immediately started training on a progressive ratio (PR10) schedule of reinforcement (Hodos, 1961). PR tasks are generally used to assess the reinforcing efficacy of a particular type of reward. The rats had to progressively increase the response requirement (steps of 10 lever presses) to obtain a food reward. For the first food pellet they were required to press 10 times, for the next reinforcement they had to press the lever 20 times, and so on. A session was terminated if a rat did not press the lever for 3 min. or after 1050 lever presses, whichever came first. Rats were trained once a day, Monday to Friday, and were given eight PR10 sessions before drug testing started. The measure used to evaluate performance in the PR10 task was breakpoint (i.e., number of lever presses made during a session).

Attention task

After the PR10 task, the rats were subjected to one CRF session before they started training in the attention task. During this task, a light stimulus was presented either on the left side or on the right side of the food reward tray. The duration of the light stimulus varied randomly between 3, 1 and 0.3s. One second after the light stimulus was extinguished, the two levers were inserted simultaneously. When the rat hit the lever on the side of the prior light stimulus (*correct response*), the rat was rewarded with a food pellet followed by an inter-trial interval of 5-10s. When the rat hit the lever on the opposite side of the previous light stimulus (*incorrect response*), the rat was not rewarded and a time-out period of 5s was followed by an inter-trial interval. When the rat did not hit a lever within 3s (*omission*), the rat was not rewarded and both levers were retracted followed by a time-out period of 5s and an inter-trial interval. A session was terminated after 80 trials or 40 min., whichever came first. A more detailed description of this task is provided by Hoff et al. (2007). Rats

were trained once a day, Monday to Friday, and were given eight attention task sessions before drug testing started. The derived behavioral measures were percentage correct, percentage omissions, response time (averaged across all stimulus durations) and two signal detection theory derived measures:

- 1) **Sensitivity index (SI)**: a signal detection measure for discriminability which was calculated as follows:

$$SI = \frac{h - f}{2(h + f) - (h + f)^2}$$

where

$$h = \frac{\text{correct left}}{\text{correct left} + \text{incorrect right}}$$

and

$$f = \frac{\text{incorrect left}}{\text{incorrect left} + \text{correct right}}$$

A value of zero reflects no discrimination whereas a value of 1 reflects perfect discrimination.

- 2) **Index Y**: a signal detection derived variable for evaluating a response bias. This parameter is calculated as follows:

$$\text{Index Y} = \frac{\text{percentage correct left} - \text{percentage correct right}}{\text{percentage correct left} + \text{percentage correct right}}$$

A more detailed description of SI, index Y and other signal detection measures can be found elsewhere (Steckler, 2001).

Delayed non-matching to position

After the rats had finished drug testing on the attention task, they immediately started training in a non-matching to position task (NMTP), to which subsequently delays were added. This paradigm consisted of two stages: a sample and a choice phase. In the sample phase, one of the two levers was inserted into the operant chamber. After the rat had pressed the sample lever, it was retracted and the rat was required to poke its nose against the hinged panel which gave access to the pellet magazine (positioned equidistantly between the two levers). This was done in order to prevent the rats from using a behavioral strategy (i.e. mediating behavior, Herremans & Hijzen, 1997) to perform the DNMT task (e.g., after pressing a lever they can move to the other side and wait for the lever to come out). More than one panel press or keeping the nose in the food tray was without consequence.

After the rat had pushed the panel at least once and had pulled its nose out of the food hopper, both levers were inserted (the choice phase) and the rat was required to press the lever opposite to the one in the sample phase. It was physically not possible for an animal to keep its nose in the pellet magazine and press the levers, as the food hopper and response levers were placed too far apart. A non-matching lever press was continuously reinforced with a food reward. There was a 5s time-out period (and no food reward) when the lever pressed in the choice phase was the same one as in the sample phase (i.e., when the response was incorrect). The inter-trial interval was always 8s (also in subsequent testing in the DNMT task). A session was terminated after 80 trials or 60 min., whichever came first. No limited hold period was used for the sample or choice phase, which means that no omission errors were recorded. A more elaborate description of the NMTP training can be found elsewhere (Blokland et al., 2004; Prickaerts et al., 1999).

Rats were trained once a day, Monday to Friday and received five NMTP training sessions and a single forced choice NMTP session (in which the task was continued only after a correct response was given) before delay intervals were being introduced in between the sample and choice phase. The duration of the delays was gradually increased over successive training sessions over a period of about two and a half weeks. In order to speed up DNMT training rats now received two daily sessions. The delay interval was randomly chosen from the following five alternatives: 0, 2, 4, 8 or 16s. The animals were able to keep their nose in the food hopper during the delays or press the food panel repeatedly without any consequence, but could not press the levers as these were retracted during the delay. In previous studies, we have not observed the animals developing a mediating strategy while performing the DNMT task (e.g., Blokland et al., 2004), as they were required to press the panel of the food well before the choice phase was presented. The measures used to evaluate performance in the DNMT were percentage correct, response time (averaged across all delays), SI and index Y (see section *Attention task* for more information on these parameters).

Drug treatment

Dose range and pretreatment time were chosen based on previous scopolamine and biperiden data (e.g., Hodges Jr. et al., 2009; Jones, & Shannon, 2000). Dose conditions were determined according to their position on a logarithmic scale. For example, biperiden doses were 1, 3 and 10 mg/kg. When converted to logarithms, these values are approximately equally spaced: 0.0, 0.5 and 1.0, respectively. Doses were titrated on basis of behavioral effects found in our essay. Scopolamine hydrobromide trihydrate 99% (obtained from Acros Organics) was dissolved in isotonic saline in doses 0, 0.1, 0.3 and 1 mg/kg (mg salt per kg body weight), whereas biperiden lactate (Akineton® 5mg/mL, obtained from Laboratorio Farmaceutico S.I.T.) was dissolved in Milli-Q purified water in doses 0, 1, 3 and 10 mg/kg (mg salt per kg body weight). We used quite high doses of scopolamine and biperiden (1 and 10 mg/kg, respectively) as an upper limit at which – certainly in case of scopolamine – serious behavioral side-effects were expected. All drug solutions were prepared freshly each day prior to testing. Scopolamine and biperiden were both injected in a volume of 2 ml/kg (IP) with a pretreatment time of 30 min. Each drug dose was tested once per rat per test. On each testing day, only one scopolamine and one biperiden dose was given, with half of the rats receiving scopolamine and the other half receiving biperiden. The order of doses was semi-randomized over testing days.

Repeated testing

Repeated testing of drugs in the same group of animals offers several advantages over between-group studies (e.g., better statistical power). However, this particular type of design can be associated with tolerance, drug sensitivity and carry-over effects. To ensure sufficient wash-out of the drug, testing days were always separated by at least one drug-free day on which the animals received FR5, PR10, attention task or DNMT training. Frequency of administration and dose level were kept as low as possible (i.e., no higher doses were tested than those yielding a significant behavioral effect). This procedure minimized the number of injections each rat received. In order to further minimize group differences due to receptor changes, the drug that was given (scopolamine or biperiden) alternated between groups for the different behavioral tasks; i.e., ten rats received only scopolamine doses and the other ten only received biperiden doses during testing of one paradigm. When testing of the next behavioral test started this order was reversed: rats which had previously received scopolamine, now received biperiden and vice versa.

Statistical analysis

Data were analyzed by parametric analysis of variance (mixed-model ANOVA; SPSS 15.0) with Dose as within-subject variable and Drug as between-subject variable. In case an interaction with Drug and/or a main effect of Drug was found, a repeated measures ANOVA was performed for each Drug separately, with Dose as within-subject variable (and possibly Stimulus Duration or Delay). Hence, drug effects of scopolamine and biperiden were compared with their own vehicle condition: i.e., scopolamine with saline and biperiden with Milli-Q. For the analysis of the attention task and the DNMT, Stimulus Duration and Delay were added as additional within-subject variables, respectively. In case a significant Dose x Stimulus Duration or Dose x Delay interaction was reported, several repeated measure ANOVAs were run separately for Stimulus Duration or Delay, respectively. One exception was the measure response time; here, data was averaged for each animal and collapsed across Stimulus Duration or Delay. Differences from vehicle conditions were always examined with a Least Significant Difference (LSD) post-hoc test. Due to some mechanical issues, occasionally data of nine rats were used for analysis.

RESULTS

Fixed ratio (5) task

Three rats failed to complete 60 trials within 30 min. after a dose of 1 mg/kg scopolamine. Figure 1 shows the effects of scopolamine and biperiden on inter-response time in a FR5 schedule of reinforcement. In the mixed model ANOVA, the within-subject effect of Dose on inter-response time varied per level of Drug (Dose x Drug interaction effect; $F(3,48) = 10.77$, $P < 0.001$). Therefore two separate repeated measure ANOVAs for the different levels of Drug were performed. For the group treated with scopolamine, inter-response time in the FR5 task was increased (main effect of Dose; $F(3,24) = 12.82$, $P < 0.001$; see Fig. 1a). Post-hoc analysis showed that the 0.3 mg/kg ($P < 0.05$) and 1 mg/kg ($P < 0.01$) doses slowed responding. In the group treated with biperiden, there was an increase in FR5 inter-response time (main effect of Dose; $F(3,24) = 7.80$, $P < 0.01$; see Fig. 1b). Post-hoc analysis indicated only an effect of the high 10 mg/kg dose ($P < 0.01$).

Figure 1. The effects of scopolamine (0.1, 0.3, 1 mg/kg, IP) and biperiden (1, 3, 10 mg/kg, IP) on a FR5 schedule of reinforcement. (a) Inter-response time. Scopolamine slowed sensorimotor responding at a dose of 0.3 and 1 mg/kg. (b) Inter-response time. Biperiden slowed sensorimotor responding at a dose of 10 mg/kg. Data represent mean (+SEM). Asterisks indicate differences from vehicle condition (*: $P < 0.05$, **: $P < 0.01$).

Progressive ratio (10) task

Figure 2 shows the effects of scopolamine and biperiden on breakpoint and inter-response time on a PR10 schedule of reinforcement. In the mixed model ANOVA, the within-subject effect of Dose on breakpoint did not vary per level of Drug (no Dose x Drug interaction effect; $F(2,36) = 0.77$, n.s.). Further, breakpoint was differentially affected by Dose (main effect of Dose; $F(2,36) = 5.57$, $P < 0.01$). The between-subject analysis of Drug showed that scopolamine and biperiden differentially affected breakpoint (main effect of Drug; $F(1,18) = 5.93$, $P < 0.05$). Therefore, two separate repeated measure ANOVAs for the different levels of Drug were performed. For the group treated with scopolamine, breakpoint in the PR10 task was reduced (main effect of Dose; $F(2,18) = 3.91$, $P < 0.05$; see Fig. 2a). Post-hoc analysis showed an effect of the 1 mg/kg dose ($P < 0.05$). For the group treated with biperiden, no change in breakpoint (no main effect of Dose; $F(2,18) = 2.10$, n.s.; see Fig. 2b) was found.

In the mixed model ANOVA, the within-subject effect of Dose on inter-response time did not vary per level of Drug (no Dose x Drug interaction effect; $F(2,36) = 0.38$, n.s.; see Fig. 2c and Fig. 2d). The within-subject analysis of Dose was not significant (no main effect of Dose; $F(2,36) = 3.04$, n.s.), which means that the different dose conditions also did not change inter-response time. The between-subject analysis of Drug showed that scopolamine and biperiden did not differentially affect inter-response time (no main effect of Drug; $F(1,18) = 0.32$, n.s.).

Figure 2. The effects of scopolamine (0.3, 1 mg/kg, IP) and biperiden (3, 10 mg/kg, IP) on a PR10 schedule of reinforcement. (a) Breakpoint. Scopolamine decreased food motivation at a dose of 1 mg/kg. (b) Breakpoint. Biperiden did not have an effect on food motivation. (c) Inter-response time. Scopolamine slowed sensorimotor responding at a dose of 1 mg/kg. (d) Inter-response time. Biperiden did not have an effect on sensorimotor responding. Data represent mean ($\pm$ SEM). Asterisks indicate differences from vehicle condition (*: $P < 0.05$).

Attention task

Figures 3a and 3b show the effects of scopolamine and biperiden on percentage correct in the attention task. In the mixed model ANOVA, the within-subject effect of Dose on percentage correct did not vary per level of Drug and Stimulus Duration (no Dose $\times$ Drug $\times$ Stimulus Duration interaction effect; $F(4,68) = 0.75$, n.s.). However, the within-subject effect of Dose on percentage correct did vary per level of Drug (Dose $\times$ Drug interaction effect; $F(2,34) = 7.96$, $P < 0.01$). The within-subject effect of Stimulus Duration on percentage correct was also different per level of Drug (Stimulus Duration $\times$ Drug interaction effect; $F(2,34) = 3.82$, $P < 0.05$). Therefore separate repeated measures ANOVAs for the two levels of Drug were performed. In the group treated with scopolamine, the within-subject effect of Stimulus Duration on percentage correct was not different per level of Dose (no Stimulus

Duration x Dose interaction effect; $F(4,36) = 0.83$, n.s.; see Fig. 3a). There was a reduction in percentage correct responses with shorter stimulus durations (main effect of Stimulus Duration; $F(2,18) = 40.06$, $P < 0.001$). Scopolamine decreased percentage correct responses in the attention task (main effect of Dose; $F(2,18) = 20.55$, $P < 0.001$). Post-hoc analysis showed that at a dose of 0.3 mg/kg scopolamine lowered percentage correct score as compared to the vehicle condition ($P < 0.01$). In the group treated with biperiden, the within-subject effect of Stimulus Duration on percentage correct was not different per level of Dose (no Stimulus Duration x Dose interaction effect; $F(4,32) = 0.36$, n.s.; see Fig. 3b). Moreover, shorter stimulus durations reduced percentage correct (main effect of Stimulus Duration; $F(2,16) = 149.29$, $P < 0.001$). Biperiden treatment did not affect the measure percentage correct (no main effect of Dose; $F(2,16) = 2.69$, n.s.).

Figures 3c and 3d show the effects of scopolamine and biperiden on percentage omissions in the attention task. In the mixed model ANOVA, the within-subject effect of Dose on percentage omissions did not vary per level of Drug and Stimulus Duration (no Dose x Drug x Stimulus Duration interaction effect; $F(4,68) = 0.98$, n.s.). The within-subject effect of Stimulus Duration on percentage omissions was also not different per level of Drug (no Stimulus Duration x Drug interaction effect; $F(2,34) = 1.16$, n.s.). In contrast, the within-subject effect of Dose on percentage omissions did vary per level of Drug (Dose x Drug interaction effect; $F(2,34) = 6.98$, $P < 0.01$). Therefore, separate repeated measures ANOVAs for the two levels of Drug were performed. In the group treated with scopolamine, the within-subject effect of Stimulus Duration on percentage omissions was not different per level of Dose (no Stimulus Duration x Dose interaction effect; $F(4,36) = 0.95$, n.s.; see Fig. 3c). There was no change in percentage omissions with shorter stimulus durations (no main effect of Stimulus Duration; $F(2,18) = 1.70$, n.s.). Scopolamine enhanced percentage omissions in the attention task (main effect of Dose; $F(2,18) = 15.66$, $P < 0.001$). Post-hoc analysis showed that at a dose of 0.3 mg/kg scopolamine augmented percentage omissions as compared to the vehicle condition ($P < 0.01$). In the group treated with biperiden, the within-subject effect of Stimulus Duration on percentage omissions was not different per level of Dose (no Stimulus Duration x Dose interaction effect; $F(4,32) = 0.61$, n.s.; see Fig. 3d). Moreover, shorter stimulus durations had no effect on percentage omissions (no main effect of Stimulus Duration; $F(2,16) = 0.01$, n.s.). Biperiden treatment did not affect the measure percentage omissions (no main effect of Dose; $F(2,16) = 3.31$, n.s.).

Figures 3e and 3f show the effects of scopolamine and biperiden on response time in the attention task. The within-subject effect of Dose on response time was found to vary per level of Drug (Dose x Drug interaction effect; $F(2,34) = 5.59$, $P < 0.01$). Therefore, separate repeated measures ANOVAs for the two levels of Drug were performed. In the group treated with scopolamine, response time was slowed (main effect of Dose; $F(2,18) = 27.74$, $P < 0.001$; see Fig. 3e). Post-hoc analysis showed an effect of the 0.3 mg/kg dose ($P < 0.001$). In the biperiden group, response time was also significantly changed (main effect of Dose; $F(2,16) = 4.28$, $P < 0.05$; see Fig. 3f). However, post-hoc analysis revealed no differences between vehicle and dose conditions.

Figure 3. The effects of scopolamine (0.1, 0.3 mg/kg, IP) and biperiden (3, 10 mg/kg, IP) on performance measures in the attention task. (a) Percentage correct responses. Scopolamine decreased accuracy independent of stimulus duration at a dose of 0.3 mg/kg. (b) Percentage correct responses. Biperiden did not have an effect on accuracy. (c) Percentage omissions. Scopolamine increased response omissions independent of stimulus duration at a dose of 0.3 mg/kg. (d) Percentage omissions. Biperiden did not have an effect on response omissions. (e) Response time. Scopolamine slowed sensorimotor responding at a dose of 0.3 mg/kg. (f) Response time. Biperiden did not have an effect on sensorimotor responding. Data represent mean (+SEM). Asterisks indicate differences from vehicle condition (**: $P < 0.01$, ***: $P < 0.001$).

Figures 4a and 4b show the effects of scopolamine and biperiden on sensitivity index (SI) in the attention task. In the mixed model ANOVA, the within-subject effect of Dose on SI did not vary per level of Drug and Stimulus Duration (no Dose x Drug x Stimulus Duration interaction effect; $F(4,68) = 1.34$, n.s.). However, the within-subject effect of Dose on SI did vary per level of Drug (Dose x Drug interaction effect; $F(2,34) = 6.30$, $P < 0.01$). The within-subject effect of Stimulus Duration on SI was also different per level of Drug (Stimulus Duration x Drug interaction effect; $F(2,34) = 4.63$, $P < 0.05$). Therefore, separate repeated measures ANOVAs for the two levels of Drug were performed. In the group treated with scopolamine, the within-subject effect of Stimulus Duration on SI varied per level of Dose (Stimulus Duration x Dose interaction effect; $F(4,36) = 2.82$, $P < 0.05$; see Fig. 4a). Hence, separate repeated measures ANOVAs were performed per level of Stimulus Duration. Post-hoc analyses showed that scopolamine reduced SI at all stimulus duration conditions. In the group treated with biperiden, the within-subject effect of Stimulus Duration on SI was not different per level of Dose (no Stimulus Duration x Dose interaction effect; $F(4,32) = 0.12$, n.s.; see Fig. 4b). Shorter stimulus durations reduced SI (main effect of Stimulus Duration; $F(2,16) = 151.65$, $P < 0.001$). Biperiden treatment did not affect the measure SI (no main effect of Dose; $F(2,16) = 1.15$, n.s.).

Figures 4c and 4d show the effects of scopolamine and biperiden on index Y in the attention task. In the mixed model ANOVA, the within-subject effect of Dose on response bias did not vary per level of Drug and Stimulus Duration (no Dose x Drug x Stimulus Duration interaction effect; $F(4,68) = 0.68$, n.s.). The within-subject effect of Stimulus Duration on index Y was also not different per level of Drug (no Stimulus Duration x Drug interaction effect; $F(2,34) = 0.11$, n.s.). The within-subject effect of Dose on response bias did not vary per level of Drug (no Dose x Drug interaction effect; $F(2,34) = 1.53$, n.s.). Index Y was differentially affected by Dose (main effect of Dose; $F(2,34) = 4.00$, $P < 0.05$). Post-hoc analysis showed that the highest dose conditions of scopolamine and biperiden augmented index Y as compared to the vehicle condition ($P < 0.05$). However, the between-subject analysis of Drug showed that scopolamine and biperiden did not differentially affect index Y (no main effect of Drug; $F(1,17) = 3.13$, n.s.). Because we sought to determine which Drug was responsible for the main effect of Dose in the mixed-model analysis, we did separate repeated measures ANOVAs per level of Drug. In the group treated with scopolamine, the within-subject effect of Stimulus Duration on index Y was not different per level of Dose (no Stimulus Duration x Dose interaction effect; $F(4,36) = 0.32$, n.s.; see Fig. 4c). There was a change in index Y with shorter stimulus durations (main effect of Stimulus Duration; $F(2,18) = 12.40$, $P < 0.001$). Scopolamine did not affect the index Y measure (no main effect of Dose; $F(2,18) = 3.35$, n.s.). In the group treated with biperiden, the within-subject effect of Stimulus Duration on index Y was not different per level of Dose (no Stimulus Duration x Dose interaction effect; $F(4,32) = 1.79$, n.s.; see Fig. 4d). Shorter stimulus durations reduced index Y (main effect of Stimulus Duration; $F(2,16) = 15.22$, $P < 0.001$). Biperiden treatment did not affect index Y (no main effect of Dose; $F(2,16) = 1.48$, n.s.).

Figure 4. The effects of scopolamine (0.1, 0.3 mg/kg, IP) and biperiden (3, 10 mg/kg, IP) on signal detection theory measures in the attention task. (a) Sensitivity Index (SI). Scopolamine decreased discriminability dependent of stimulus duration at a dose of 0.3 mg/kg. (b) Sensitivity Index (SI). Biperiden did not have an effect on discriminability. (c) Index Y. Scopolamine did not have an effect on response bias. (d) Index Y. Biperiden did not have an effect on response bias. Data represent mean (+SEM). Asterisks indicate differences from vehicle condition (*: $P < 0.05$, **: $P < 0.01$).

Delayed non-matching to position

Figures 5a and 5b show the effects of scopolamine and biperiden on percentage correct in the DNMTTP task. In the mixed model ANOVA on percentage correct, the Dose effect varied per level of Delay and Drug (Dose $\times$ Delay $\times$ Drug interaction effect; $F(8,144) = 3.69$, $P < 0.01$). Therefore, separate repeated measures ANOVAs for the two levels of Drug were performed. In the group treated with scopolamine, the within-subject effect of Delay on percentage correct was different per level of Dose (Delay $\times$ Dose interaction effect; $F(8,72) = 3.84$, $P < 0.01$; see Fig. 5a). Hence, additional repeated measures ANOVAs were performed per level of Delay. Scopolamine affected percentage correct at the 0-, 2-, 4- and 8-s delay conditions (main effect of Dose; $F_s(2,18) > 9.23$, $P_s < 0.01$). Post-hoc analyses showed that a dose of 0.3 mg/kg was different from the vehicle condition. In addition, a dose of 0.1 mg/kg also affected accuracy performance at the 8-s delay condition. Scopolamine had no

effect on percentage correct at 16-s delay condition (no main effect of Dose; $F(2,18) = 0.31$, n.s.).

In the group treated with biperiden, the within-subject effect of Delay on percentage correct was different per level of Dose (Delay $\times$ Dose interaction effect; $F(8,72) = 2.30$, $P < 0.05$; see Fig. 5b). Hence, separate repeated measures ANOVAs were performed per level of Delay. Biperiden did not affect percentage correct at the 0- and 16-s delay condition (no main effect of Dose; $F_s(2,18) < 2.95$, n.s.). However, biperiden impaired percentage correct in trials with a 2-, 4-, and 8-s delay (main effect of Dose; $F_s(2,18) > 5.84$, $P_s < 0.05$); Separate post-hoc analyses demonstrated that the 3 mg/kg dose decreased percentage correct as compared to the vehicle condition.

Figure 5. The effects of scopolamine (0.1, 0.3 mg/kg, IP) and biperiden (1, 3 mg/kg, IP) on performance measures in the DNMT task. (a) Percentage correct responses. Scopolamine decreased accuracy delay-dependently at a dose of 0.1 and 0.3 mg/kg. (b) Percentage correct responses. Biperiden decreased delay-dependently accuracy at a dose of 3 mg/kg. (c) Response time. Scopolamine had no effect on sensorimotor responding. (d) Response time. Biperiden had no effect on sensorimotor responding. Data represent mean (+SEM). Asterisks indicate differences from vehicle condition (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

Figures 5c and 5d show the effects of scopolamine and biperiden on response time in the DNMT task. The within-subject effect of Dose on response time did not vary per level of Drug (no Dose x Drug interaction effect; $F(2,36) = 2.06$, n.s.). There was no effect of Dose (no main effect of Dose; $F(2,36) = 1.06$, n.s.); scopolamine and biperiden did not differentially affect response time (no main effect of Drug; $F(1,18) = 1.44$, n.s.).

Figure 6. The effects of scopolamine (0.1, 0.3 mg/kg, IP) and biperiden (1, 3 mg/kg, IP) on signal detection theory measures in the DNMT task. (a) Sensitivity Index (SI). Scopolamine decreased discriminability delay-dependently at a dose of 0.1 and 0.3 mg/kg. (b) Sensitivity Index (SI). Biperiden decreased discriminability delay-dependently at a dose of 3 mg/kg. (c) Index Y. Scopolamine decreased response bias delay-independently at a dose of 0.3 mg/kg. (d) Index Y. Biperiden decreased response bias delay-independently at a dose of 3 mg/kg. Data represent mean (+SEM). Asterisks indicate differences from vehicle condition (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

Figures 6a and 6b show the effects of scopolamine and biperiden on SI in the DNMT task. In the mixed model ANOVA on SI, the Dose effect varied per level of Delay and Drug (Dose x Delay x Drug interaction effect; $F(8,144) = 3.68$, $P < 0.01$). Therefore, separate repeated measures ANOVAs for the two levels of Drug were performed. In the group treated with scopolamine, the within-subject effect of Delay on SI was different per level of Dose (Delay x Dose interaction effect; $F(8,72) = 3.84$, $P < 0.01$; see Fig. 6a). Hence, additional repeated

measures ANOVAs were performed per level of Delay. In the 0-, 2-, 4- and 8-s delay trials a main effect of Dose was found ($F_s(2,18) > 9.23$, $P_s < 0.01$). Separate post-hoc analyses showed that at a dose of 0.3 mg/kg scopolamine reduced SI as compared to the vehicle condition. In addition a dose of 0.1 mg/kg also affected performance at the 8-s delay condition. Scopolamine had no effect on SI in trials which used a 16-s delay (no main effect of Dose; $F(2,18) = 0.31$, n.s.).

In the group treated with biperiden, the within-subject effect of Delay on SI was different per level of Dose (Delay x Dose interaction effect; $F(8,72) = 2.30$, $P < 0.05$; see Fig. 6b). Hence, separate repeated measures ANOVAs were performed per level of Delay. In trials with a 0- or 16-s delay biperiden did not affect SI (no main effect of Dose; $F_s(2,18) < 2.95$, n.s.). Biperiden did influence SI in trials with a 2-, 4- and 8-s delay (main effect of Dose; $F_s(2,18) > 5.84$, $P_s < 0.05$); post-hoc analysis revealed that the 3 mg/kg dose decreased SI as compared to the vehicle condition ($P < 0.01$).

Figures 6c and 6d show the effects of scopolamine and biperiden on index Y in the DNMT task. In the mixed model ANOVA, the within-subject effect of Dose on response bias did not vary per level of Drug and Delay (no Dose x Drug x Delay interaction effect; $F(8,136) = 1.13$, n.s.). The within-subject effect of Delay on index Y was also not different per level of Drug (no Delay x Drug interaction effect; $F(4,68) = 0.43$, n.s.). Moreover, the within-subject effect of Dose on response bias did not vary per level of Drug (no Dose x Drug interaction effect; $F(2,34) = 3.06$, n.s.). The within-subject effect of Dose on index Y was also not different per level of Delay (no Dose x Delay interaction effect; $F(8,136) = 1.78$, n.s.). Index Y was differentially affected by Dose (main effect of Dose; $F(2,34) = 22.24$, $P < 0.001$). The between-subject analysis of Drug showed that scopolamine and biperiden differentially affected index Y (main effect of Drug; $F(1,17) = 4.49$, $P < 0.05$). Therefore, separate repeated measures ANOVAs for the two levels of Drug were performed. In the group treated with SCOP, the within-subject effect of Delay on response bias was not different per level of Dose (no Delay x Dose interaction effect; $F(8,64) = 1.67$, n.s.; see Fig. 6c). Index Y increased with longer delays (main effect of Delay; $F(4,32) = 12.28$, $P < 0.001$). Scopolamine enhanced response bias in the DNMT task (main effect of Dose; $F(2,16) = 11.62$, $P < 0.01$). Post-hoc analysis showed that at a dose of 0.3 mg/kg scopolamine augmented index Y as compared to the vehicle condition ($P < 0.01$). In the group treated with biperiden, the within-subject effect of Delay on index Y was not different per level of Dose (no Delay x Dose interaction effect; $F(8,72) = 1.29$, n.s.; see Fig. 6d). Moreover, with longer delays response bias was increased (main effect of Delay; $F(4,36) = 18.92$, $P < 0.001$). Biperiden was shown to increase response bias in the DNMT task (main effect of Dose; $F(2,18) = 12.55$, $P < 0.001$). Post-hoc analysis showed that at a dose of 3 mg/kg biperiden augmented index Y as compared to the vehicle condition ($P < 0.01$).

DISCUSSION

The main objective of the current study was to compare the effects of the non-selective muscarinic antagonist scopolamine and the M_1 antagonist biperiden after systemic injections on different aspects of operant behavior: sensorimotor responding (FR5), food motivation (PR10), attention and short-term memory (DNMT). The direct comparison of both drugs allowed evaluation with respect to the usability of biperiden, as opposed to scopola-

mine, as a suitable model of cholinergic memory dysfunction. Since biperiden is relatively selective for M_1 receptors which can be found predominantly in brain areas involved in learning and memory (Caulfield, 1993; Volpicelli & Levey, 2004), we expected also a more selective effect of biperiden on cognition and behavioral performance. In Table 1 an overview is given of the effects of both drugs on the various behavioral measures. A wide range of behavioral impairments were found after scopolamine; cognitive and peripheral effects were not dissociable on basis of dose conditions. Moreover, performance deficits in the short-term memory task were of a non-mnemonic nature. In contrast, biperiden more selectively impaired DNMT performance at a dose of 3 mg/kg, at which no peripheral effects were found; sensorimotor responding was slowed after the 10 mg/kg dose.

Of note, some caution should be taken with respect to the selectivity of muscarinic (ant)agonists. Due to the highly conserved nature of the orthosteric binding site amongst muscarinic receptors, drugs that target these can be characterized as possessing relative rather than absolute receptor subtype selectivity. These issues should be borne in mind when trying to attribute effects of these drugs to specific muscarinic receptor subtypes. For instance, biperiden has about 10-fold higher affinity for M_1 as compared to M_2 - M_5 receptors (see Bolden et al., 1992; Katayama et al., 1990). The pharmacokinetic characteristics of biperiden are quite favorable; cerebellar levels after a dose of 3.2 mg/kg (IV) have been reported to be about 10 times higher than plasma levels (see Nakashima et al., 1993; Sävälähti et al., 1988; Yokogawa et al., 1990; 1992). Regardless, biperiden is currently the drug of choice for making a direct comparison with SCOP, particularly as it is approved for use in humans and therefore suitable for translational research. Some other drugs that target the muscarinic M_1 receptor cannot be used in systemic injections because they do not cross the blood-brain barrier (e.g., pirenzepine: Guthrie et al., 2000).

Table 1. An overview of the effects of scopolamine and biperiden on sensorimotor responding, food motivation, attention and short-term memory. Doses are given in mg/kg, IP.

Paradigm	Drug	
	Scopolamine	Biperiden
FR5	Inter-response time ↑ (0.3, 1)	Inter-response time ↑ (10)
PR10	Breakpoint ↓ (1) Inter-response time =	Breakpoint = Inter-response time =
AT	Percentage correct ↓ (0.3, DI) Percentage omissions ↑ (0.3, DI) SI ↓ (0.3, DD) Index Y = Response time ↑ (0.3)	Percentage correct = Percentage omissions = SI = Index Y = Response time =
DNMT	Percentage correct ↓ (0.1, 0.3, DD) SI ↓ (0.1, 0.3, DD) Index Y ↑ (0.3, DI) Response time =	Percentage correct ↓ (3, DD) SI ↓ (3, DD) Index Y ↑ (3, DI) Response time =

Symbols: = : no change; ↑ : increase; ↓ : decrease

Abbreviations: FR5: fixed ratio 5, PR10: progressive ratio 10, AT: attention task, DNMT: delayed non-matching to position, DD: delay-dependent effect, DI: delay-independent effect, SI: Sensitivity Index.

Sensorimotor responding

Both scopolamine and biperiden were found to slow sensorimotor responding on a FR5 schedule of reinforcement, although at a different dose (0.3 and 1 mg/kg for scopolamine and 10 mg/kg for biperiden, see Fig.1). Although both scopolamine and biperiden increased inter-response time in the FR5 task it should be noted that the effects of biperiden were smaller as compared to scopolamine. Biperiden, at a dose of 10 mg/kg, slowed FR5 responses by 26% as compared to the vehicle condition. Scopolamine, at a dose of 0.3 and 1 mg/kg, increased inter-response time by about 32% and 298%, respectively. In FR tasks, scopolamine has generally been found to decrease lever presses independent of reward type (dry vs. wet, Hodges Jr. et al., 2009), which has been ascribed to central muscarinic blockade. However, the minimal effective dose reported in these studies does show quite some variability (0.005-1.0 mg/kg IP, Hodges Jr. et al., 2009; Pradhan & Roth, 1968). In a brightness discrimination task, biperiden (0.25-2mg/kg, SC) was found to reduce the rate of reinforcement (Liu, 1996). Furthermore, intracerebroventricular infusion of the M₁ antagonist pirenzepine (10, 30 µg in 2.5 µL) increased the sample latency in a DNMTTP task (Aura et al., 1997), although a reduction in response latency has also been reported (Andrews et al., 1994).

These results suggest that the effect of systemic administration of scopolamine and biperiden on sensorimotor responding is at least partially mediated by the M₁ receptor. However, from the present data it cannot be deduced whether this change in sensorimotor responding is caused by central or peripheral M₁ blockade, or both. For instance, in the periphery M₁ receptors have been found in abundance in rat sympathetic ganglia such as the superior cervical ganglion (Caulfield & Birdsall, 1998). However, the effects of scopolamine and biperiden on sensorimotor responding could also result from an interaction between M₁ and striatal dopaminergic signaling (De Klippel et al., 1993; Gerber et al., 2001).

Food motivation

Scopolamine (1 mg/kg) was found to decrease food motivation and slow sensorimotor responding on a PR10 schedule, whereas biperiden (3, 10 mg/kg) did not have an effect on these measures (see Fig. 2). This is in accordance with studies on monkeys performing a PR schedule where scopolamine reduced the number of obtained reinforcements (Spinelli et al., 2006; Taffe et al., 1999). Food and water intake in rats was found to be diminished after scopolamine administration (Hodges Jr. et al., 2009; Pradhan & Roth, 1968). To the best of our knowledge, neither biperiden, nor any other M₁ antagonists have been tested in paradigms assessing food motivation and/or free feeding behavior. Although it is possible that a higher dose of biperiden would have yielded a reduction in motivation, our dose range is comparable to those used in other behavioral studies (Jones, & Shannon, 2000; Kimura et al., 1999; Liu, 1996; Myers et al., 2002; Myhrer et al., 2008; Roldán, 1997; Sipos et al., 1999; 2001).

Again, it cannot be decisively determined whether the decrement in motivation for food after administration of scopolamine is a central or a peripheral effect. Scopolamine has been known to induce dry mouth (Hodges Jr. et al., 2009), which might affect the palatability and thus the hedonic impact of dry food rewards. Of note, when using a liquid reward a central but not peripheral effect of scopolamine has been found in FR5 and DNMTTP paradigms (Hodges Jr. et al., 2009). Nevertheless, a decrease in “liking” dry food

rewards after systemic administration of scopolamine could interfere with behavioral performance. Particularly the M_3 (Dai et al., 1991; Shida et al., 1993) but also the M_1 and M_5 receptor have been implicated in rat salivary responses (Flynn et al., 1997; Shannon et al., 1994; Tobin et al., 2002). Thus, according to the literature biperiden is capable of interfering with salivation to some extent. However, the current data suggests that any reductions in salivation after biperiden doses of 10 mg/kg and lower are not sufficient to interfere with food motivation. These findings are in contrast with those of scopolamine, which is likely to more fully block muscarinic receptor subtypes in rat salivary glands and to profoundly affect food motivation. A central effect of scopolamine might also interfere with incentive-driven behaviors (such as PR performance). Centrally infused scopolamine in rat nucleus accumbens has been found to reduce sucrose consumption (1 μ g/side or 10 μ g/side) and breakpoint (5.0 μ g/side) in a PR paradigm (Pratt & Kelley, 2004). Furthermore, muscarinic receptors appear to be implicated in reward-driven motivational behaviors via excitatory interactions with dopamine in the nucleus accumbens and ventral tegmental area (Forster et al., 2001; Yeomans & Baptista, 1997).

Attention

At a dose of 0.3 mg/kg, scopolamine decreased percentage correct, increased percentage omissions and response time independent of the duration of the stimulus (see Fig. 3). Discriminability (SI) was also reduced after the 0.3 mg/kg dose; however the effect of scopolamine was dependent on stimulus duration (see Fig. 4). Response bias (index Y) was unaffected after scopolamine (0.1, 0.3 mg/kg) or biperiden (3, 10 mg/kg). Biperiden also did not affect any of the other measures in the attention task. In attentional paradigms such as the 5-choice serial reaction time task, scopolamine has been reported to disrupt visuospatial sustained attention at doses of 0.02 mg/kg and higher (Callahan et al., 1993; Cheal, 1981; Hodges Jr. et al., 2009; Hoff et al., 2007; Humby et al., 1999; Leblond et al., 2002; Spinelli et al., 2006); however, behavioral effects of scopolamine on attentional accuracy are not reported consistently (Andrews et al., 1992; Doty et al., 2003; Leaton & Kreindler, 1972). Moreover, scopolamine has been shown to influence general non-cognitive performance measures, such as response latency and number of missed trials (Andrews et al., 1992; Bushnell et al., 1997; Drinkenburg et al., 1995). As scopolamine also disrupted performance in the FR5 and PR10 tasks, its effect on attention could (partially) be caused by deficits in sensorimotor responding and/or food motivation. Moreover, it is unlikely that M_1 receptor blockade underlies the attentional impairment, as biperiden had no effect on this task. To the best of our knowledge, effects of M_1 antagonists have not been assessed in attentional paradigms before. Further studies are required in order to provide more support for the lack of a role of M_1 and potentially other muscarinic receptors in attention.

Short-term memory

Similar to its effects on the attention task, scopolamine impaired various performance measures in the DNMT task (see Fig. 5 and 6). Scopolamine decreased percentage correct and discriminability (SI) in a delay-dependent manner, and increased response bias (index Y) delay-independently at a dose of 0.3 mg/kg. Scopolamine already affected DNMT performance at the shortest delay(s), whereas it failed to disrupt performance in trials with the longest delay. The latter is likely due to a floor effect; i.e., accuracy of the animals in the 16-

s delay trials was already at around chance level (50% correct) even in the vehicle condition, and therefore further impairment due to cholinergic blockade could not be induced. The different delay intervals between sample and choice phase are presumed to produce a temporal performance gradient with longer retention intervals yielding poorer DNMT performance as short-term memory functions are taxed in a progressively increasing manner. Thus, the disruption of DNMT performance at the zero delay indicates an effect of scopolamine on other (non-)cognitive processes rather than just short-term memory. As the current study demonstrates, the effects of scopolamine (0.3 mg/kg) on sensorimotor responding, food motivation and/or attention could (at least partially) underlie deficits in DNMT performance.

The majority of studies using delayed (non-)matching procedures have reported a delay-independent impairment after relatively low doses which again suggests that scopolamine does not specifically affect short-term memory functions (e.g., 0.05, 0.075 and 0.1 mg/kg IP, Herremans et al., 1995; 0.1 mg/kg IP, Hodges Jr. et al., 2009), although some articles have challenged this finding (Estepé & Steckler, 2002; Ruotsalainen et al., 1998; Santi & Weise, 1995; Stanhope et al., 1995). Furthermore, in most studies using systemic injections scopolamine also affected measures of responding; it increased number of omissions, decreased number of completed trials and increased response latency (Estepé & Steckler, 2002; Kirkby et al., 1995). Central administration of scopolamine in the medial prefrontal cortex (Dunnett et al., 1990; Herremans et al., 1996; 1997), prelimbic cortex (Granon & Poucet, 1995) and hippocampus (Robinson & Mao, 1997) has been shown to yield a delay-independent reduction of DNMT response accuracy (but see Broersen et al., 1994; 1995; Dunnett et al., 1990; Granon et al., 1995), and increases in number of omissions (Robinson & Mao, 1997).

It is likely that DNMT deficits produced by scopolamine can be partially attributed to M_1 blockade, which is in line with the results reported by Bymaster et al. (1993). Biperiden (3 mg/kg) was found to decrease percentage correct and discriminability (SI) in a delay-dependent manner, and increase response bias (index Y) delay-independently. Biperiden did not influence response time at the doses used in the DNMT (1, 3 mg/kg, IP) which is in line with our findings in the FR5 task. As is shown in Figures 5 and 6, biperiden did not affect DNMT performance at the zero delay. However, as the delay interval increased, biperiden increasingly impaired accuracy performance as compared to the vehicle condition. Thus, the disruption of DNMT performance at longer delays but not the shortest delay indicates a genuine effect of biperiden on short-term memory functions rather than other (non-)cognitive processes. These effects are unlikely to be caused by deficits in sensorimotor responding, food motivation or attention, as biperiden (at a dose of 3 mg/kg) did not affect performance on the FR5 or PR10 schedule of reinforcement or in the attention task. Of note, the lack of an effect of biperiden at the 16-s delay is again likely due to a floor effect. Taken together, these findings suggest a role for the M_1 receptor in mediating short-term memory functions. This would implicate selective M_1 antagonists such as biperiden as a promising alternative instead of the gold standard drug scopolamine as a tool for inducing cholinergic mnemonic impairments in animals.

Effects of systemic administration of biperiden on DNMT performance have not been assessed previously; however, M_1 antagonists have been found to affect performance in a variety of other behavioral tasks which measure (short-term) memory. For instance, deficits have been reported in passive avoidance tasks (Fornari et al., 2000; Kimura et al., 1999;

Kramer-Soares et al., 2006; Roldán, 1997), contextual fear conditioning (Kramer-Soares et al., 2006; but see Sheffler et al., 2009), spatial alternation (Bymaster et al., 1993) and object recognition (Myhrer et al., 2004; 2008) after systemic injections. Furthermore, M_1 agonists have been shown to improve DNMTF performance in animals which were cognitively impaired after cholinergic lesioning (McDonald et al., 1998), scopolamine administration or aging (Bartholomeo et al., 2000), which suggests that enhanced M_1 signaling can be sufficient in order to reverse memory deficits. Infusion of the muscarinic M_1 antagonist pirenzepine (35 μ g in 0.5 μ L/site) in the dorsal hippocampus impaired accuracy performance on a DNMTF task (Messer et al., 1987; 1990); this implicates the importance of M_1 receptor signaling in the hippocampus for accurate DNMTF responding.

Of note, there are some indications that the M_1 receptor might also be involved in reversal learning (McCool et al., 2008; Tzavos et al., 2004) and anxiety (Wall et al., 2001). In addition, muscarinic (M_1) blockade in non-hippocampal brain regions might also be responsible for short-term memory effects; for instance, intact performance on non-matching tasks seems to also require the prefrontal, entorhinal and perirhinal cortices (Otto & Eichenbaum, 1992). Thus, our study does not exclude the involvement of muscarinic receptor subtypes other than M_1 and brain regions other than the septo-hippocampal system in memory functions, nor the engagement of the M_1 receptor in other cognitive processes besides memory (e.g., Araya et al., 2006; Carey et al., 2001; Daniel & Dohanich, 2001; McCool et al., 2008; Messer & Miller, 1988; Poulin et al., 2010; Power et al., 2003; Quirion et al., 1995; Tzavos et al., 2004; Wall et al., 2001; Wess, 2004).

In fact, the manner in which M_1 receptors affect memory processes is still under investigation; one possibility is the modulation of glutamatergic neurotransmission and/or synaptic plasticity (see Caulfield, 1993; Hasselmo, 1999; 2006). Muscarinic M_1 receptors couple to Gq-proteins which activate several signaling cascades via phospholipase (PLC) (Caulfield, 1993; Jones, 1993; Liu et al., 2006), which can ultimately influence Ca^{2+} and K^+ currents (Liu et al., 2006), raise cyclic AMP levels (Jones, 1993), and can stimulate other receptor systems such as glutamatergic N-methyl-D-aspartate (NMDA) receptor currents produced by hippocampal CA1 pyramidal neurons (Calabresi et al., 1998; Ma et al., 2009; Marino et al., 1998). Moreover, M_1 receptors and NR1a NMDA receptor subunits were found to be colocalized at glutamatergic synapses, suggestive of a direct interaction between the two receptor systems. A link between M_1 receptor signaling and long-term potentiation (LTP), a mechanism which is thought to underlie learning and memory processes, has also been put forward (Boddeke et al., 1992; Burgard & Sarvey, 1990; Calabresi et al., 1999; Doralp & Leung, 2008; Kamsler et al., 2010; Ovsepian et al., 2004; Shinoo et al., 2005). For instance, it has been shown that muscarinic agonists and antagonists which act preferentially on the M_1 receptor are able to facilitate or prevent the induction of LTP in rat dentate gyrus (Burgard & Sarvey, 1990), CA1 (Boddeke et al., 1992; Doralp & Leung, 2008; Ovsepian et al., 2004) and striatum (Calabresi et al., 1999), respectively.

Future studies on the role of muscarinic receptors should focus on determining whether there exists some degree of dissociation between muscarinic receptor subtypes in terms of their involvement in memory (or other cognitive functions) as is reflected by their differential distribution in the brain (e.g., Rouse & Levey, 1996). It is likely that particular muscarinic subtypes are particularly important for a restricted (set of) cognitive subdomain(s); e.g., hippocampal M_1 receptors are important for working but not reference memory (Ohno et al., 1994). Furthermore, it is imperative that the manner in which M_1 recep-

tors influence memory processes is more extensively investigated. Information on the exact signaling cascade(s) downstream of the muscarinic M_1 receptor that are responsible for its effects on memory could lead to interesting implications for the development of novel treatments for disorders in which memory is impaired, such as Alzheimer's disease or schizophrenia. Lastly, additional behavioral validation is required to firmly establish the usability of M_1 antagonists instead of the gold standard scopolamine for producing cholinergic amnesia in healthy animals and human participants.

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CHAPTER 4

The muscarinic M₁ antagonist biperiden induces verbal memory impairment in young, healthy participants

Implications for Alzheimer's disease

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INTRODUCTION

The cholinergic hypothesis of geriatric memory dysfunction proposed in the early eighties by Bartus et al. (1982) has been highly influential, leading to a wealth of research into the involvement of acetylcholine in mnemonic processes. One of the tools used for this purpose is the non-selective muscarinic antagonist scopolamine. Experiments in animals and humans have determined that scopolamine interferes with the encoding of new information, yet leaves the retrieval of previously stored information intact – i.e., it induces anterograde but not retrograde amnesia (see **Chapter 2** and Atri et al., 2004; Bishop et al., 1996; Broks et al., 1988; Green et al., 2005; Kamboj & Curran, 2006; Mintzer & Griffiths, 2001, 2005; 2007; Nakra et al., 1992; Pomara et al., 2004; Rasch et al., 2006; Robbins et al., 1997; Sherman et al., 2003; Snyder et al., 2005; Sperling et al., 2002; but see van Ruitenbeek et al., 2008). Furthermore, scopolamine tends to particularly affect declarative memory (e.g., episodic and working memory), while leaving procedural memory intact (e.g., Green et al., 2005; Sperling et al., 2002; but see Mintzer & Griffiths, 2003).

However, several studies have suggested that scopolamine actually impairs attentional rather than mnemonic processes, and evidence is the strongest for sustained attention (see **Chapter 2** and Brandeis et al., 1992; Broks et al., 1988; Duka et al., 1996; Dunne & Hartley, 1986; Ellis et al., 2006; Wesnes & Revell, 1984; Wesnes & Warburton, 1984; Witte et al., 1997). Given the fact that scopolamine is a non-selective muscarinic antagonist which induces quite a range of side-effects (e.g., dry mouth, pupil dilation, sedation; see **Chapter 2**), it is unclear whether these attentional effects of the drug reflect central or peripheral actions. Drugs that block muscarinic receptors subtypes M_1 - M_5 more selectively might be preferable over scopolamine as a means to induce specific memory impairments. In this respect, the muscarinic M_1 receptor subtype can be seen as a viable target, since this receptor is located mostly centrally instead of peripherally and in brain regions thought to be involved in learning and memory (Caulfield, 1993).

Recently we have shown that the muscarinic M_1 antagonist biperiden – at a dose of 3 mg/kg – is capable of disrupting short-term memory performance, but not sensorimotor responding, food motivation or attention in rats (see **Chapter 3**). A human study by Wezenberg et al. (2005) demonstrated impairments in verbal memory, visuospatial processes and motor learning, but not picture memory after 2 mg of biperiden. Effects of biperiden on working memory were equivocal, and there were no indications of sedation. In contrast, Nakra et al. (1992) reported disruptive effects of the muscarinic M_1 antagonist trihexyphenidyl (2 mg) on immediate and delayed recall of verbal and visual material in healthy elderly participants. General orientation, attention-concentration and learning of word associations were shown to be unaffected. Pomara et al. (2004) demonstrated an association between memory impairments induced by trihexyphenidyl (1, 2 mg) and the APOE- $\epsilon 4$ allele, which is a major genetic risk factor for Alzheimer's disease. Specifically, total recall scores of both the $\epsilon 4$ and non- $\epsilon 4$ carriers were affected by trihexyphenidyl, yet the non- $\epsilon 4$ improved significantly on delay recall scores several hours later, whereas the $\epsilon 4$ group did not. The authors concluded that $\epsilon 4$ carriers are more vulnerable to events which impact the cholinergic system.

The current study attempts to replicate the findings of Wezenberg et al. (2005) with regards to the disruptive effects of biperiden on verbal memory. We expected that biperiden would induce lower immediate and delayed recall scores, and lower recognition scores

on a verbal memory test. In addition, we anticipated that biperiden would not affect psychomotor performance in a choice reaction time task and would not induce side-effects on a self-report questionnaire.

METHODS

Participants

Sixteen (7 male, 9 female; mean age of 23.4 years (SD = 3.2, range = 19-31)) healthy volunteers were recruited from the University of Helsinki through email advertisements. Participants had a body mass index of 18.5 to 30. They filled out a medical questionnaire before participation. Exclusion criteria were past or current psychiatric, neurological, cardiac, gastrointestinal, haematological, hepatic, pulmonary, or renal illness, pregnancy, lactation, excessive alcohol consumption (intake of more than 20 glasses per week), use of any medication other than oral contraceptives, having a first-degree relative with a current or past psychiatric disorder, and presence of other deficits that could be expected to influence performance. All subjects gave a signed informed consent before inclusion and were financially rewarded for their participation. The study was approved by the National Research Ethics Council of Finland, based in Helsinki.

Study design and procedures

The study was conducted according to a double-blind, placebo-controlled crossover design. In the course of the week before the actual test sessions, the participants received a training session to minimize any possible learning effects. Subjects were not allowed to use any psychoactive medication within 5 days before drug intake. Participants were asked to abstain from alcohol on a testing day and 24h before testing. They were also not allowed to smoke and were requested not to consume any caffeine-, teaine- or aspartame-containing beverages on a testing day. One hour before testing the participants were asked to fill out a questionnaire on physical complaints (see below) and were given a capsule containing either a placebo, or 2 mg biperiden hydrochloride (Akineton®, instant release). They were provided lunch immediately afterwards: this was done in order to reduce the chances of participants developing any side-effects due to biperiden intake. Lunch consisted of a can of caffeine-free soda, 1-3 slices of bread and cheese, ham, or marmelade. The duration of testing was around 1.5 hrs, after which the participants were asked to fill out the questionnaire again. The test session finished about 3 hours after drug intake. We aimed to separate test sessions by about 7 days to ensure sufficient washout of biperiden.

Drug treatment

Biperiden is a muscarinic M₁ antagonist approved for the treatment of Parkinson symptoms which develop due to use of first-generation antipsychotics (e.g., Ogino et al., 2011). It has about 10-fold higher affinity for M₁ as compared to M₂-M₅ receptors and it is thus the most selective M₁ antagonist available for use in human participants (Bolden et al., 1992; Katayama et al., 1990). Peak plasma concentrations are reached around 1-2 hours after a single dose administration followed by a rapid initial decline to values around 12% of the peak values at 6 hours after intake. This is subsequently followed by a slow terminal elimination

phase at 48h (Hollmann et al., 1984; 1987). The most common side effects of biperiden on the central nervous system are drowsiness, vertigo, headache, and dizziness. Peripheral side effects consist of blurred vision, mydriasis, dry mouth, impaired sweating, abdominal discomfort, and obstipation (Mintzer & Burns, 2000; Peters, 1989; Tune et al., 1992). We chose for a dose of 2 mg as this lies well within the range of the therapeutically recommended doses for biperiden (1-4 mg). Moreover, oral treatment with 2 mg biperiden has been shown to impair cognitive performance in healthy elderly (Wezenberg et al., 2005). Biperiden was purchased and labelled by Yliopiston Pharmacy in Helsinki according to local guidelines.

Verbal learning task (VLT)

The VLT is an adapted version of the Rey auditory verbal learning test (Lezak, 1995), which assesses short- and long-term memory function for verbal information. The word list was extended by Riedel et al. (1999) in order to avoid ceiling effects of cognition-enhancing drugs. The adapted version of the test consists of a list of 30 monosyllabic words (18 nouns and 12 adjectives, balanced for positive, negative and neutral words) in English. The words were shown on a computer screen for 1s. Three trials with the same item sequence were presented and each trial ended with a free recall of the words (immediate recall). Thirty min. after the third trial, the participant was asked to recall as many words as possible (delayed recall). Subsequently, a verbal recognition test (VRT) was presented, consisting of all former words and 30 new but comparable words (distracters). The words were shown on a computer screen for 2s and participants were asked to rate whether they were presented in the learning trials by a yes/no response. The inter-word interval was 2s.

The VLT yielded several behavioral measures. Firstly, the number of words correctly recalled in the three learning trials is a measure of short-term verbal memory learning and retrieval. Furthermore, the number of recalled words 30 min. after learning constitutes the delayed recall score, which represents long-term verbal memory retrieval. Next to correctly recalled words, we also scored doubly and incorrectly recalled words for both the immediate and the delayed recall. For the VRT, the percentage correctly recognized old and new items were calculated. Reaction times (in ms) were also recorded, which was used as a measure of speed of retrieval from long-term memory and also provides an indication of sedative side-effects of biperiden.

Choice reaction time task (CRT)

This task measures general alertness and motor speed; in the current context it was used to assess whether biperiden would impair psychomotor performance. Participants were presented with an arrow that is either shown on the left, or on the right side of the screen. There were two kinds of arrows, one pointing to the left (left arrow), the other pointing to the right (right arrow). If the participant saw the left arrow, he/she was required to press the left button. If the right arrow was presented, the participant pressed the right button. Both arrows could be presented either on the left, or on the right side of the screen. This left 4 possible stimulus sets: 1) left arrow presented on the left side (congruent left), 2) left arrow presented on the right side (incongruent left), 3) right arrow presented on the right side (congruent right), and 4) right arrow presented on the left side (incongruent right). Forty stimuli of each category were presented, so in total there were 160 trials. The interval

between the presentations of two arrows was random between 2-4s and the duration of the test was around 8 min. Dependent variables were accuracy (i.e., the average correct responses to the congruent and incongruent trials) and reaction time (i.e., for the congruent and incongruent trials, in ms).

Questionnaires

Physical or mental complaints were assessed with a self-report questionnaire consisting of 31 possible complaints to be rated on a 4-point scale; a score of zero indicated that the participant was not experiencing the complaint, and a score of three meant he or she was experiencing it a great deal. A difference score of mean score_{T1} (1 hour after drug intake) – mean score_{T0} (baseline, before drug intake) was calculated per subscale for each participant and used for analysis.

Statistical analysis

Statistical data were analysed using SPSS. For all analyses, we used $\alpha = 0.05$. Post-hoc testing was performed with a Least Significant Difference (LSD) test. To determine possible treatment effects on immediate recall in the VLT, behavioral data were analyzed by parametric analysis of variance (repeated measures ANOVA) with Drug (2 levels: placebo and biperiden) and Trial (3 levels for the 3 trials of the immediate recall) as within-subject variables. The data of the delayed recall were analyzed with paired samples t-tests with Drug (placebo vs. biperiden) as independent variable. In the case of the VRT and CRT, we performed repeated measures ANOVAs with Drug (2 levels: placebo and biperiden) and Stimulus (VRT: 2 levels: old vs. new items; CRT: 2 levels: congruent vs. incongruent). Analyses were performed separately for the dependent variables: i.e., in the case of the VLT: correctly, doubly and incorrectly recalled words; for the VRT: correctly recognized items and reaction time; for the CRT: accuracy and reaction time.

The questionnaire data were analyzed by paired samples t-tests with Drug (placebo vs. biperiden) as independent variable. We only analyzed those side-effects relevant for biperiden intake: sleepiness, dizziness, nausea, restlessness, heart palpitations, stomach ache, bloated stomach, decreased appetite, dry mouth, tiredness, blurred vision, drowsiness, loss of concentration, nervousness, apathy, and inability to tolerate bright light. T-tests were done separately for each subscale.

RESULTS

Verbal learning task (VLT)

Figure 1 shows the effects of biperiden on immediate and delayed recall of words in the VLT. In the repeated measures analysis of the immediate recall data, the effect of Treatment on correctly recalled words did not vary per level of Trial (no Treatment * Trial interaction effect; $F(2,30) = 0.83$, n.s.). Treatment was found to affect word recall (main effect of Treatment; $F(2,30) = 11.79$, $P < 0.01$); post-hoc analysis showed that biperiden decreased the immediate recall score by an average of about 4 words compared to placebo. Furthermore, participants were able to recall increasingly more words across the three trials of immediate recall (main effect of Trial; $F(2,30) = 233.30$, $P < 0.001$). There were no effects of

Treatment and Trial on doubly or incorrectly recalled words ($F_s < 1.44$, n.s.). In the paired samples t-tests of the delayed recall data, there was a significant difference in correctly recalled words after placebo ($M = 20.31$, $SD = 6.29$) and biperiden conditions ($M = 15.19$, $SD = 3.64$; $t(15) = 3.42$, $P < 0.01$). There were no effects of biperiden on doubly ($t(15) = 0.00$, n.s.) or incorrectly recalled words ($t(15) = -1.43$, n.s.)

Figure 1. Effects of biperiden on immediate and delayed recall of words in the VLT. Participants were able to increasingly recall more words across the immediate trials. Biperiden decreased the immediate and delayed recall score by an average of about 4 words compared to placebo.

Verbal Recognition Task (VRT)

Figure 2 shows the effects of biperiden on percentage correctly recognized words in the VRT. The effect of Treatment on percentage correctly recognized old vs. new items did not vary per level of Stimulus (no Treatment * Stimulus interaction effect; $F(1,13) = 0.42$, n.s.). Treatment was found to affect percentage correct in the recognition task (main effect of Treatment; $F(1,13) = 9.87$, $P < 0.01$); post-hoc analysis showed that after biperiden, the correct recognition of old and new items was reduced with approximately 8% relative to placebo. There was no main effect of Stimulus on percentage correct ($F(1,13) = 0.42$, n.s.). For reaction time, there was no Treat*Stimulus interaction ($F(1,13) = 0.44$, n.s.), nor a main effect of Treatment on reaction time ($F(1,13) = 1.68$, n.s.). Participants reacted significantly faster to old vs. new items (main effect of Stimulus; $F(1,13) = 22.91$, $P < 0.001$).

Choice Reaction Time Task (CRT)

There were no interactions or main effects with Treatment or Stimulus on accuracy in the CRT ($F_s < 3.90$, n.s.). As for the reaction time data, there was no Treatment * Stimulus interaction ($F(1,14) = 0.17$, n.s.) nor a main effect of Treatment ($F(1,14) = 0.48$, n.s.). Stimulus was shown to affect reaction time; post-hoc analysis showed that participants reacted relatively slower to the incongruent trials.

Figure 2. Effects of biperiden on percentage correctly recognized words in the VRT. After biperiden, the correct recognition of old and new items was reduced with approximately 8% relative to placebo.

Complaints

Participants did not report experiencing side-effects after intake of biperiden ($T_s > -1.96$, n.s.).

DISCUSSION

The goal of the current study was to assess whether biperiden would disrupt verbal memory, affect psychomotor performance and induce side-effects in young, healthy participants. After biperiden, participants recalled on average about 4 words less on the immediate recall and 5 words less on the delayed recall of the VLT. Word recognition scores in the VRT were reduced by 8% after biperiden. Biperiden did not influence reaction times in the VRT or the CRT, which would be indicative of a lack of psychomotor effects. There was also no effect of biperiden on accuracy in the CRT, nor did participants report experiencing any side-effects.

Our findings are in line with those found in the literature on memory-impairing effects of muscarinic antagonists in humans (Atri et al., 2004; Bishop et al., 1996; Broks et al., 1988; Green et al., 2005; Kamboj & Curran, 2006; Mintzer & Griffiths, 2001; 2005; 2007; Nakra et al., 1992; Pomara et al., 2004; Rasch et al., 2006; Robbins et al., 1997; Sherman et al., 2003; Snyder et al., 2005; Sperling et al., 2002; but see van Ruitenbeek et al., 2008). Specifically, the current results successfully replicated the findings of Wezenberg et al. (2005) who also showed impairments in immediate and delayed recall and recognition of verbal material. However, cognition-impairing effects of muscarinic M₁ antagonists generally do not seem to be specific for any particular memory system or sensory modality, as

they have been found to influence not only verbal, but also visual, short-term, and working memory (Nakra et al., 1992; Wezenberg et al., 2005).

Furthermore, scopolamine has been shown to disrupt attentional processes, which might underlie its deleterious effects on memory (see **Chapter 2** and Brandeis et al., 1992; Broks et al., 1988; Duka et al., 1996; Dunne & Hartley, 1986; Ellis et al., 2006; Wesnes & Revell, 1984; Wesnes & Warburton, 1984; Witte et al., 1997). However, given the fact that scopolamine tends to have a high affinity for blocking muscarinic receptors regardless of receptor subtype, it might be interesting to explore whether the same holds for muscarinic antagonists which are relatively more selective for the M_1 receptor. Evidence in favor of a role for the muscarinic M_1 receptor in attention is so far quite equivocal. For instance, Wezenberg et al. (2005) reported that 2 mg of biperiden impaired movement time in a motor learning task, which was interpreted as an attentional effect rather than a slowing of psychomotor performance. In addition, biperiden also appeared to disrupt visuospatial processes, but effects were not as strong and robust relative to those in the memory tasks. In contrast, Nakra et al. (1992) reported no effect of the muscarinic M_1 antagonist trihexyphenidyl (2 mg) on attention-concentration measures in the Wechsler Memory Scale. In line with the latter finding, we recently reported a lack of effects of biperiden on accuracy performance in a visual target detection task assessing learned irrelevance (Llrr) processes (see **Chapter 5**). In sum, muscarinic M_1 antagonists such as biperiden do seem to affect cognition in a relatively more selective manner than scopolamine; i.e., when examining effects on mnemonic vs. psychomotor and attentional processes.

In the current study we found no evidence of psychomotor slowing after biperiden; i.e., there were not effects on reaction time in the VRT or the CRT. Wezenberg et al. (2005) reported increased reaction time in a short-term memory test, yet it is unclear whether this findings represents a central (i.e., mnemonic) rather than a peripheral (i.e., psychomotor) effect. Nakra et al. (1992) showed no increase in side-effects (i.e., no blurred vision or sedation) on a self-rating scale after biperiden. A recent study by our lab which investigated the effects of biperiden on Llrr showed that reaction time was increased after biperiden relative to placebo (see **Chapter 5**). Again, it is hard to determine whether this effect is due to blockade of central or peripheral muscarinic M_1 receptors; i.e., whether biperiden induced a global decrease in task performance and/or psychomotor slowing. Of note, the Llrr task was set up as a visual target detection paradigm; these types of tests might be relatively more sensitive to deleterious psychomotor effects of muscarinic M_1 antagonists as compared to recognition or CRT paradigms. In addition, participants did not report any side-effects of biperiden on our complaints questionnaire, which is in line with previous findings by our lab (see **Chapter 5**).

In this light, muscarinic M_1 antagonists might serve as a pharmacological model for inducing mnemonic deficits present in Alzheimer's disease. Indeed, several lines of evidence point towards an involvement of aberrant muscarinic M_1 signaling in this disorder (but see Liu et al., 2005). For example, a post-mortem study by Ladner et al. (1995) demonstrated a loss of binding of the cholinergic agonist carbachol to the M_1 receptor in superior frontal and primary visual cortices, but not in the dorsal striatum. These findings would imply that in frontal and visual regions, the M_1 receptor is uncoupled from its G-protein, thereby impairing cholinergic signal transduction. These findings were replicated and extended by Tsang et al. (2006), who showed that M_1 receptor/G-protein uncoupling in the frontal but not temporal cortex was positively correlated with the rate of cognitive decline;

i.e., both mild/ moderate and severe Alzheimer patients showed uncoupling in temporal regions, but only the severely affected dementia patients displayed frontal cortical uncoupling.

In sum, biperiden is capable of impairing verbal memory rather selectively, i.e., without inducing clear peripheral side-effects which could adversely affect performance. There are a few indications that muscarinic M₁ antagonists could induce psychomotor slowing; however, evidence is far from conclusive. Muscarinic M₁ antagonists might serve as a translational model for inducing selective mnemonic deficits as seen in neuropsychiatric disorders, particularly Alzheimer's disease. Future studies should determine whether there exists some degree of dissociation in the effects of muscarinic receptor subtypes M₁-M₅ on cognition. Moreover, it is imperative that the mnemonic effects of drugs targeting the M₁ receptor subtype are further explored for dissociations in terms of stimulus modality and memory systems affected (i.e., working, verbal, visual, or spatial).

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CHAPTER 5

Human electrophysiological correlates of learned irrelevance: Effects of the muscarinic M₁ antagonist biperiden

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INTRODUCTION

If a conditioned stimulus (CS) and an unconditioned stimulus (US) are repeatedly presented in a random, uncorrelated manner, it is believed that an animal or human learns that the occurrence of one stimulus is irrelevant to the occurrence of the other one (e.g., Baker, 1976; Mackintosh, 1973). Next, if the former inconsequential CS becomes paired with the US, the subsequent acquisition of the CS-US association is delayed compared to stimuli which are novel. This phenomenon, the retardation of associative CS-US learning or classical conditioning after prior non-contingent exposure to the conditioning stimuli, is called learned irrelevance (Llrr; Gal et al., 2005). Of note, presenting only the CS prior to subsequent conditioning also induces a delay in CS-US learning called latent inhibition, which is viewed as closely related to Llrr (Allen et al., 2002).

In acute, first-episode schizophrenia Llrr has been found to be reduced or fully disrupted; the acquisition of associations related to preexposed stimuli occurs faster in this group of patients compared to normal controls (Gal et al., 2005; Orosz et al., 2011; Young et al., 2005). This means that Llrr is attenuated or even absent and acute schizophrenics actually outperform healthy individuals on these tasks. Furthermore, in chronic schizophrenia patients a failure to learn the CS-US association has been reported (Gal et al., 2005; Young et al., 2005), indicative of a global learning deficit rather than impaired Llrr. High-risk individuals (i.e., showing prodromal signs of psychosis) and schizotypic people also show Llrr deficits, whereas asymptomatic first-degree relatives of schizophrenics are seemingly unaffected. Combined, these findings are indicative of Llrr being a state marker for psychosis but not a trait marker for a potential schizophrenia endophenotype (Orosz et al., 2011).

There is a great lack of electrophysiological (EEG) data on human Llrr, although there are a few studies on event-related potentials (ERPs) of latent inhibition (Guterman et al., 1996). Therefore, the present study measured EEG along with Llrr in healthy individuals; the main focus was on the N1 and P3 ERPs. The N1 peak is a negative component which occurs around 70-140 ms post-stimulus at frontal and central electrode channels. This component seems to be related to the visual properties of a stimulus but can be modulated by selective attention. More specifically, its amplitude has been shown to be larger (i.e., more negative) for attended than for unattended or divided-attention stimuli (García-Larrea et al., 1992; Golob et al., 2002; Jerger et al., 1992; Kho et al., 2003; Näätänen, 1990; Rockstroh et al., 1996; White & Yee, 1997). The N1 is also important for discrimination processes, as it is absent if participants merely have to detect the presence of a stimulus (Mangun & Hillyard, 1991). As Llrr might be interpreted as an attentional phenomenon (Lubow, 2005), we decided to focus on the N1 component in our analysis of the ERP data. The P3 peak is a large positive-going waveform occurring about 250-500 ms post-stimulus. The P3 can be divided into two subcomponents: an early occurring peak in response to novel or alerting stimuli – called the P3a – and a later component – dubbed the P3b – which occurs in most tasks requiring a decision process contingent on stimulus discrimination (Donchin, 1981).

There is a vast amount of literature on the role of the dopaminergic and glutaminergic neurotransmitter systems in schizophrenia. However, alterations in muscarinic signaling also appear to underlie the disorder (Brooks et al., 2011; Sarter et al., 2012; Tandon et al., 1991; 1999). Postmortem and in-vivo imaging studies have shown a reduction of muscarinic receptors density or binding in certain brain regions of schizophrenia patients (but see

Garcia-Rill et al., 1995; Owen et al., 1981; Watanabe et al., 1983) and data seems most consistent for the muscarinic M₁ receptor subtype (Crook et al., 2000; 2001; Dean et al., 1996; Deng & Huang, 2005; Newell et al., 2007; Scarr et al., 2008; Zavitsanou et al., 2004; but see Scarr et al., 2007; Dean et al., 2000). However, most of these binding studies have used [3H]pirenzepine as a radioligand, which also binds to the muscarinic M₄ receptor subtype (Barak, 2009). Thus, differentiating between the muscarinic M₁ and M₄ receptor is not possible on the basis of these studies.

The current study is the first to assess the effects of the muscarinic M₁ antagonist biperiden in healthy, young volunteers using a within-subject Lirr paradigm suitable for ERP measurement. We expected that our Lirr paradigm would be successful in inducing Lirr in the placebo condition. We expected that the Lirr effect would be disrupted after biperiden treatment. With respect to the ERPs, we hypothesized that the Lirr conditions would be most notably reflected in the N1 and P3a/P3b components. After biperiden, Lirr would be disrupted as indicated by similar reaction times to PE-cued and NPE-cued targets (i.e., no increase in reaction time due to pre-exposure). We expected that biperiden would particularly affect the N1 and P3a/P3b peaks; i.e., biperiden is expected to reduce the amplitudes and/or increase the latencies of these components.

METHODS

Participants

Seventeen (7 male, 10 female; mean age of 22.4 years (SD = 3.0, range = 19-29)) healthy volunteers were recruited from Maastricht University through poster advertisements. Participants were required to be between 18-35 years of age. We decided on a restricted age range because EEG and ERPs can change with age and can be differentially sensitive to cholinergic modulation (Bennett et al., 2004; Fjell & Walhovd, 2004; Pekkonen et al., 2005). Participants were also required to have a body mass index of 18.5 to 30. They received an extensive medical screening before testing, consisting of a medical questionnaire, physical examination, measurement of blood pressure and pulse rate, blood samples for haematology and biochemistry, urine samples for drug screen and pregnancy test, and a resting electrocardiogram. Exclusion criteria were past or current psychiatric, neurological, cardiac, gastrointestinal, haematological, hepatic, pulmonary, or renal illness, pregnancy, lactation, excessive alcohol consumption (intake of more than 20 glasses per week), use of any medication other than oral contraceptives, having a first-degree relative with a current or past psychiatric disorder, and presence of other deficits that could be expected to influence performance. All subjects gave a signed informed consent before inclusion and were financially rewarded for their participation. The study was approved by the Medical Ethics Committee of Maastricht University.

Study design and procedures

The study was conducted according to a double-blind, placebo-controlled crossover design. In the course of the week before the actual test sessions, the participants received a training session to minimize any possible learning effects. Subjects were not allowed to use any psychoactive medication within 5 days before drug intake. Participants were asked to

abstain from alcohol on a testing day and 24h before testing. They were also not allowed to smoke and were requested not to consume any caffeine-, teaine- or aspartame-containing beverages on a testing day. One hour before testing the participants were asked to fill out some questionnaires on mood and physical complaints (see below) and were given a capsule containing either a placebo, or 2 mg biperiden hydrochloride (Akineton®, instant release). They were provided lunch immediately afterwards: this was done in order to reduce the chances of participants developing any side-effects due to biperiden intake. Lunch consisted of a can of caffeine-free soda, gluten-free bread and sweet bread toppings. After drug intake EEG electrodes and a cap were placed. After about one hour of testing, the participant had a short break, in which he or she was asked to fill out the same questionnaires again. The test session finished about 3 hours after drug intake. We aimed to separate test sessions by about 7 days to ensure sufficient washout of biperiden (average number of washout days was 9.3).

Drug treatment

Biperiden is a muscarinic M_1 antagonist approved for the treatment of Parkinson symptoms which develop due to use of first-generation antipsychotics (e.g., Ogino et al., 2011). It has about 10-fold higher affinity for M_1 as compared to M_2 - M_5 receptors and it is thus the most selective M_1 antagonist available for use in human participants (Bolden et al., 1992; Katayama et al., 1990). Peak plasma concentrations are reached around 1-2 hours after a single dose administration followed by a rapid initial decline to values around 12% of the peak values at 6 hours after intake. This is subsequently followed by a slow terminal elimination phase at 48h (Hollmann et al., 1984; 1987). The most common side effects of biperiden on the central nervous system are drowsiness, vertigo, headache, and dizziness. Peripheral side effects consist of blurred vision, mydriasis, dry mouth, impaired sweating, abdominal discomfort, and obstipation (e.g., Mintzer & Burns, 2000; Peters, 1989; Tune et al., 1992). We chose for a dose of 2 mg as this lies well within the range of the therapeutically recommended doses for biperiden (1-4 mg). Moreover, oral treatment with 2 mg biperiden has been shown to impair cognitive performance in healthy elderly (Wezenberg et al., 2005). Biperiden was purchased, blinded, and labelled by the pharmacy of the University Hospital Maastricht according to the relevant GMP guidelines.

Llrr task

The within-subject Llrr paradigm was based on the one developed by Young et al. (2005), which was further modified by Gal et al. (2005) and Orosz et al. (2007; 2008; 2011). The paradigm was presented as a visual target detection task using letter characters (see Fig. 1). Capital Latin letters were shown successively on the computer screen for 1s each, with an inter-stimulus interval of 1s. The letters were font size 250, coloured white (RGB: 255-255-255) on a black background (RGB: 0-0-0) and appeared in the center of the monitor. The participants were instructed to press the spacebar as soon as the target letter X appeared on screen. In addition to the target, there were 10 other consonants and vowels presented during a single version of the task. These letters could either be preceding the X – called predictor letters – or fill in the spaces between the predictor-target combinations – called filler letters. During one test session, 375 non-target and 75 target letters were presented, which means that the test duration was approximately 15 min. The subjects were pre-

sented a series of fifteen blocks of 30 letters each. The blocks were divided into 3 different conditions (with each block/condition presented 5 times):

- 1) non-preexposed (NPE) blocks - In NPE blocks the target X was always predicted by the same letter, which was not presented before during the task. Each NPE letter acted as a target predictor in one particular NPE block and was not presented before or in later test sessions. Thus, NPE cues reliably predicted the target and enabled full prediction of it.
- 2) preexposed (PE) blocks - In a PE block the target was preceded five times by the same letter. However, this PE predictor letter had already been shown in previous blocks as a filler letter (i.e., uncorrelated to the target letter). In other words, whereas NPE predictors were completely novel, PE predictors were not. Therefore, prediction of the occurrence of the letter X was partial in this condition.
- 3) random (RAN) blocks - Targets appeared randomly after different consonants or vowels which also served as PE and filler letters. Prediction of the target letter was therefore zero in the random blocks.

According to the degree of prediction, reaction time to target was expected to be the lowest for the NPE-, somewhat higher for the PE- and the highest for the RAN-cued targets. In case of Lirr, the average reaction time in PE is supposed to be significantly higher than in NPE ($RT_{PE} > RT_{NPE}$, see Orosz et al., 2008). NPE letters were always completely new; participants had not seen them before in the Lirr task at all. Our main outcome variables were reaction time (in ms) to the target and a Lirr-index, which was calculated as follows:

$$Lirr - index = \frac{RT_{PE}}{RT_{RAN}} - \frac{RT_{NPE}}{RT_{RAN}}$$

A score greater than zero would be indicative of intact Lirr (Gal et al., 2005). In order to monitor whether participants had understood the instructions and were well motivated to perform the Lirr task, we also measured number of hits (actual target response), misses (failure of target response), false alarms (response to filler letters) and premature responses (response to predictors).

Questionnaires

To assess subjective mood changes, the Profile of Mood States (POMS) was used (McNair et al., 1971). This self-report questionnaire has 32 visual analogue scales which measure the mood states tension-anxiety, depression-dejection, anger-hostility, fatigue-inertia and vigor-activity. We also used the Bond and Lader mood scale (Bond & Lader, 1974), which has 16 visual analogue scales that yield three mood factors; contented-discontented, alert-drowsy, and calm-excited. Physical or mental complaints were assessed with a self-report questionnaire consisting of 31 possible complaints to be rated on a 4-point scale. For all three questionnaires, a difference score of mean score_{T1} (1 hour after drug intake) – mean score_{T0} (baseline, before drug intake) was calculated and used for analysis.

Figure 1. A schematic presentation of the Lirr test set-up and the letter sequences of a test session. The order of blocks is the same for all test sessions and is shown on the first column. The horizontal lines show the letter sequence of the corresponding blocks in detail. Regardless of condition, each block contains 30 letters: 5 targets (in bold), 5 target predictors (in bold, preceding the target letter X) and 20 filler letters (PE letters: B, E, J, Q, Y). Adapted from Orosz et al. (2008).

EEG and ERPs

For the EEG recording, the Neuroscan Synamps system was used with sample rate set at 1000 Hz. Data were filtered between 0.05-100 Hz. EEG was recorded from 32 electrodes placed on the scalp using an elastic cap (Electro-Cap International, United States) and positioned according to the 10-20 system (Jasper, 1958). The horizontal electro-oculogram (HEOG) was measured with two electrodes placed on the outer canthus of the left and right eye, and two electrodes placed below and above the centre of the left eye recorded the vertical electrooculogram (VEOG). Two electrodes behind the ears served as reference electrodes. Before placing the electrodes, all locations were cleaned with alcohol and gently scrubbed with a gel, to ensure good conduction of the signal. Impedance was kept below 5 Ω. During the EEG recordings, the participants were sitting in an electrically shielded and sound-attenuated room with the lights dimmed.

All EEG data were analyzed with Vision Analyzer 2.0. Before data analysis, the EEG data were visually inspected offline, to check for artefacts. The EEG signal was filtered with a

high pass filter set at 1 Hz (12 dB slope) and a low pass filter set at 30 Hz (12 dB slope). The ERP epochs were set from 100 ms prior to stimulus onset to 900 ms after onset, using the 100 ms pre-stimulus as baseline. Eye movement artefacts were filtered out of the EEG using the data of the VEOG channel and the method developed by Semlitsch et al. (1986). This way, every participant had about the same number of artifact-free trials which were combined into the ERP averages. Separate averages were calculated for the predictors and the targets, as well as for the NPE, PE, and RAN stimuli. For the predictor stimuli, N1 and P3a peaks were noted on the Fz, FCz and Cz channels. In case of the targets, N1 and P3b peaks could be distinguished on the CPz, CP3, CP4, Pz, P3, and P4 electrodes. Please refer to Table 1 for the time windows chosen for ERP peak detection analysis. Peak windows were determined based on the grand averages. Peak amplitudes were calculated and latencies were computed based on when the peak was reached.

Table 1. Time windows (in ms) used for ERP analyses.

Stimulus type	Peaks		
	N1	P3a	P3b
Predictors	45-140	320-500	N/A
Targets	35-125	N/A	185-500

Abbreviations: N/A: not applicable

Statistical analysis

Only participants who had at least 80% target hits, i.e., 60 of the total of 75, were included in the behavioral and ERP analyses. This criterion was used to ensure that all participants understood and were able to follow the instructions. Statistical data were analysed using SPSS. For all analyses, significant interactions were examined in more detail by doing additional ANOVA analyses split for each level of one of the interaction variables. Post-hoc testing was performed with a Least Significant Difference (LSD) post-hoc test. For both the behavioral and ERP data, the first trial of a block was always excluded from analysis under the assumption that implicit associative learning will need at least one CS-US or predictor-target pairing in order to be expressed. Subsequently, behavioral and ERP data were collapsed across Blocks.

To determine possible treatment effects on the Lirr index, behavioral data were analyzed by parametric analysis of variance (repeated measures ANOVA) with Drug (2 levels: placebo and biperiden) as within-subject variable. For the analyses of the reaction time data, Lirr Condition (3 levels: RAN, PE and NPE) was added as an additional within-subject variable. We then evaluated whether the Lirr conditions had a differential effect on the ERPs. Therefore, the placebo data were analyzed using repeated measures ANOVAs with Lirr Condition (3 levels: RAN, PE and NPE) and Electrode (3 levels for predictors: Fz, Fcz and FCz, 2 levels for targets: CPz, Pz). We determined the effects of biperiden on the processing of predictor and target letters by doing the same analyses with Drug (2 levels: placebo and biperiden) as an extra within-subject variable. ERP analyses were done separately for amplitude and latency, stimulus type (i.e., predictors and targets) and each of the ERP peaks.

Questionnaire data were analyzed with paired samples T-tests separately for each subscale. The POMS had 5 subscales, whereas for the Bond & Lader we only analyzed the alert-drowsy subscale, as this one is the best validated. The self-report questionnaire con-

sisted of 31 questions, but we only analyzed those side-effects relevant for biperiden intake: sleepiness, dizziness, nausea, restlessness, heart palpitations, stomach ache, bloated stomach, decreased appetite, dry mouth, tiredness, blurred vision, drowsiness, loss of concentration, nervousness, apathy, and inability to tolerate bright light.

RESULTS

One participant did not meet the requirement of at least 80% target hits and was therefore excluded from behavioral and ERP analysis. Part of the behavioral data was missing for another subject; this person was also not included, leaving a total of 15 participants.

Behavioral data: Llrr effects

Figure 2 shows the effects of the Llrr conditons on reaction time to the target letters. In the analysis of the reaction time data, there was a main effect of Llrr Condition on reaction time ($F(2,28) = 21.73, P < 0.001$). Post-hoc analysis showed that reaction times to the target letters were faster for the NPE compared to the RAN ($P < 0.001$) and PE condition ($P < 0.001$).

Figure 2. Effects of the Llrr conditions and biperiden on reaction time to the target letters (means + SEM, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). In the placebo condition intact Llrr is present, reflected by larger reaction times for PE- than for NPE-cued target letters. Biperiden prolonged reaction time compared to placebo, but did not affect Llrr.

ERP data: Lirr effects on predictor and target letters

In the placebo analyses of the predictor letters, there were no relevant task effects on N1 amplitude and latency ($F_s < 1.69$, n.s.) or P3a amplitude and latency ($F_s < 2.50$, n.s.). In the placebo analyses of the target letters, there were no relevant task effects on N1 amplitude and latency ($F_s < 1.58$, n.s.) or on P3b amplitude ($F_s < 1.17$, n.s.). The effect of Lirr Condition on P3b latency did not vary per level of Electrode (no Lirr Condition * Electrode interaction effects; $F(10,130) = 0.44$, n.s.), yet P3b latency was found to differ between Lirr conditions (main effect of Lirr Condition; $F(2,26) = 11.99$, $P < 0.001$); post-hoc analysis showed that the P3b latency of NPE-cued targets occurred earlier compared to the P3b latency of RAN-cued ($P < 0.001$) and PE-cued targets ($P < 0.05$). Figure 3 shows the effects of the Lirr conditions on the P3b component of the target letters in the placebo condition.

Figure 3. Effects of the Lirr conditions on the P3b component of the target letters in the placebo condition. Shown here: Pz electrode channel. P3b latency is shortest in the NPE condition, intermediate for PE-cued targets and longest for targets belonging to the RAN condition (***: $P < 0.001$).

Behavioral data: effect of biperiden

Figures 2 and 4 show the effects of biperiden on reaction time for the target letters and the Lirr-index. The effect of Drug did not vary per level of Lirr Condition (no Drug * Lirr Condition interaction effect; $F(2,28) = 2.46$, n.s.). Reaction times were increased after biperiden treatment (main effect of Drug; $F(1,14) = 12.43$, $P < 0.01$). Biperiden had no effect on the Lirr-index (no main effect of Drug; $F(1,14) = 0.06$, n.s.).

Figure 4. Effects of biperiden on the Lirr-index (means + SEM). In the placebo condition intact Lirr is present, reflected by a Lirr-index larger than zero. The same holds for the biperiden condition; in other words, biperiden failed to affect Lirr.

ERP data: effects of biperiden on predictor and target letters

Figure 5 shows the effects of biperiden on the N1 component of the PE predictor letters. The analysis of the amplitude of the N1 peak showed that the effect of biperiden varied per level of Electrode (Treatment * Electrode interaction effect; $F(2,26) = 7.54, P < 0.01$) and per level of Lirr Condition (Treatment * Lirr Condition interaction effect; $F(2,26) = 3.94, P < 0.05$). Therefore we decided to do separate repeated measures ANOVAs per Lirr condition. In the analyses of RANP and NPE predictors, there were no interaction or main effects of biperiden on N1 amplitude ($F_s < 1.98, n.s.$). For the PE predictors, the effect of Treatment on N1 amplitude varied per Electrode (Treatment * Electrode interaction effect; $F(2,26) = 7.42, P < 0.01$); therefore we performed separate repeated measures ANOVAs per Electrode. Biperiden was found to increase the N1 amplitude at Fz and FCz ($F_s > 5.77, P_s < 0.05$). There were no relevant interaction effects or main effects of biperiden on N1 latency ($F_s < 2.40, n.s.$), or on P3a amplitude or latency ($F_s < 2.25, n.s.$) of the predictor letters. Furthermore, for the targets there were no effects of biperiden on N1 amplitude and latency ($F_s < 3.25, n.s.$), or on P3b amplitude and latency ($F_s < 4.18, n.s.$).

Questionnaire data

There were no effects of biperiden on the subscales of the self-report questionnaire ($T_s > -1.87, n.s.$), the POMS ($T_s > -0.92, n.s.$) or the Bond & Lader ($T_s > -1.52, n.s.$).

Figure 5. Effects of biperiden on the N1 component of the PE predictor letters. Shown here: Fz electrode channel. Biperiden increased N1 amplitude compared to placebo (*: $P < 0.05$).

DISCUSSION

The goal of the current study was to develop a Lirr task suitable for ERP measurement and to examine the effects of the muscarinic M₁ antagonist biperiden on behavioral performance and ERP correlates of Lirr in healthy young volunteers. The Lirr paradigm used in our current study was successful in inducing Lirr in healthy, young volunteers who received placebo medication (see Fig. 2 and Fig. 4). For the behavioral results, Lirr was reflected in shorter reaction times in relation to the degree of target predictability: fastest to NPE-cued targets which were fully predictable, slower to PE-cued targets which were partially predictable and slowest for RAN-cued targets that yielded zero prediction. These findings are in accordance with earlier reports using within-subject Lirr-paradigms in humans (Gal et al., 2005; Orosz et al., 2007; 2008; 2011; Young et al., 2005). Moreover, our results are also in line with studies investigating the facilitating effect of stimulus predictability on reaction time (Barcelo & Knight, 2007; Fogelson et al., 2008; Suwazono et al., 2000). The Lirr-index after placebo was 0.2 in our experiment, which is well over zero and therefore indicative of a robust Lirr effect of our task. In comparison, the Lirr-index reported by Gal et al. (2005) in his group of healthy normal controls was about 0.1.

The three Lirr conditions (RAN, PE and NPE) affected P3b latency (but not amplitude) of the target letters as a function of the participant's degree of uncertainty about the occurrence of the target letter X (see Fig. 3). The P3b occurred earliest after presentation of NPE-cued targets (averaged over all electrode channels), relatively later for PE-cued targets, and was the slowest to occur for the RAN-cued targets. P3b latency is usually interpreted as an indication of stimulus evaluation speed, with shorter latencies indicative of superior cognitive performance (Polich & Criado, 2006). The traditional view states that the P3b starts to occur when stimulus evaluation processes are completed (Sutton et al., 1965; 1967). The finding that the P3b latency of a target stimulus is shortened by a predictor stimulus signaling subsequent target presentation is supported by evidence of earlier studies (Duncan-Johnson & Donchin, 1977; 1980; Fogelson et al., 2008), which showed that P3b latency is decreased for targets which are considered highly probable than those which are less probable. Furthermore, as already suggested by Fogelson et al. (2008), the enhancement of stimulus evaluation speed of predictable targets appears to be cognitive rather than per-

ceptual; our target stimuli were always similar (i.e., throughout the task the target stimulus was the letter X) and there was no influence of Lrr conditions on early perceptual ERP components (e.g., N1). In line with our findings, previous studies utilizing discrimination tasks in which participants are instructed to favour accuracy over speed have shown a positive correlation between P3b latency and reaction time performance (Kutas et al., 1977; but see Verleger, 1997). Taken together, the effects of the Lrr conditions on P3b but not N1 latency suggest that Lrr is more related to stimulus evaluation processes rather than early perceptual/attentional mechanisms.

A behavioral disruption of Lrr after biperiden treatment was expected to be reflected by similar reaction times to NPE-cued and PE-cued targets (i.e., no increase in reaction time due to preexposure). However, biperiden did not affect Lrr as participants still showed faster reaction times to NPE-cued as opposed to PE-cued target letters (see Fig. 2). Similarly, the Lrr-index was not reduced (see Fig. 4) and participants did not report any side-effects on our questionnaires after biperiden. The only discernible effect of biperiden was an overall increase in reaction time. In line with our results, Wezenberg et al. (2005) reported a dose of 2 mg biperiden to increase movement time in a motor learning task. However, the authors interpreted this effect as being due to attentional deficits, rather than a general slowing of psychomotor performance.

Several studies have reported a detrimental effect of biperiden on memory processes. In the study of Guthrie et al. (2000), impairments were found in backward but not forward digit span. Both of these tasks are thought to tap into several cognitive processes, among which memory and attention (Larrabee & Kane, 1986). In the same study biperiden also affected verbal memory; on a selective reminding task participants recalled fewer words at both immediate and delayed recall. These results were replicated and extended by Wezenberg et al. (2005). These studies thus provide support for the view that the muscarinic M₁ receptor is important for memory functions. Theories about Lrr have focused on behavioral switching (Weiner, 2003), attention (Lubow, 2005; Schmajuk, 2005) and inhibition of redundant stimuli (Gluck & Myers, 1993). Hence, Lrr is thought of as a paradigm that measures attentional rather than mnemonic abilities, which might explain why a memory-impairing drug such as biperiden failed to have an effect on our Lrr task.

Biperiden also increased the amplitude of the N1 component of the PE predictor letters – which were previously irrelevant but later on became partial predictors of the target stimulus (see Fig. 5). This was an unsuspected finding given our hypothesis of reduced amplitudes and/or increased latencies of the N1, P3a or P3b peaks after biperiden. It is unclear whether the biperiden-induced increment in N1 amplitude reflects cognitive/central or non-cognitive/peripheral processes. Biperiden has been known to cause blurred vision in healthy volunteers (e.g., Fleischhacker et al., 1987); however, impaired vision would be expected to reduce N1 amplitude rather than increase it. Moreover, our participants did not report visual impairments after biperiden compared to placebo. Thirdly, blurred vision would be likely to globally influence N1 amplitude: that is, also the N1 amplitude of RAN and NPE predictor letters would be affected, and not only that of the PE predictors. The only non-cognitive effect of biperiden we did find was a general slowing of psychomotor performance (see above). However, responding to a target stimulus takes place much later after presentation of that stimulus, whereas the N1 is an early perceptual component which is regarded as separate from response behavior (Näätänen et al., 1988).

A cognitive explanation of the increment in N1 amplitude of PE predictor letters after biperiden intake is also not very straightforward. Given the finding that N1 amplitudes are larger for attended than for unattended stimuli (e.g., Haider et al., 1964), a drug which is known to disrupt cognition would be expected to reduce N1 amplitude rather than to increase it. It might be the case that after biperiden, there is an overcompensation of the irrelevant-to-relevant switch or, in other words, participants needed to allocate more attentional resources in order to successfully link the presentation of the previously irrelevant PE predictor with the subsequent occurrence of the target letter. Taken together, our results would argue for an attentional effect of biperiden.

Interestingly, a study examining latent inhibition and ERPs in acute and stable, partially remitted schizophrenia patients and healthy controls reported results which were comparable to our own (Kathmann et al., 2000). All participants exhibited robust latent inhibition; in other words, schizophrenics were not impaired in this regard. Schizophrenic patients did show slower reaction times compared to the normal, healthy controls which is in accordance with our biperiden data. In contrast to the behavioral results, the electrophysiological data did differentiate between diagnostic groups. Pre-exposure affected the N1 amplitudes to CS+ stimuli, which were irrelevant during pre-exposure but subsequently served as predictors during acquisition. Thus, these stimuli were conceptually similar to our PE predictor letters. Specifically, N1 amplitudes of CS+ stimuli were decreased in healthy controls, increased in acute schizophrenics and unchanged in partially remitted schizophrenics after pre-exposure. The authors interpreted this finding as an enhancement of allocation of attention to previously irrelevant stimuli or alternatively, as a failure to inhibit previously irrelevant stimuli from gaining access to attentional processing in the acute schizophrenic patient group. Of note, all patients received neuroleptic medication, which means that an effect of these drugs on ERPs cannot be excluded.

In sum, our Lirr paradigm induced robust Lirr in healthy young volunteers. As for the ERP results, Lirr was reflected in shorter P3b latency (i.e., faster stimulus evaluation processing) in relation to the degree of target predictability. Lirr was not affected after biperiden, but the N1 amplitude of the PE predictor letters was increased (suggestive of increased allocation of attentional/behavioral switching resources). The present data are inconclusive as to the attentional or mnemonic effects of biperiden, although the ERP data suggest a role in early information processing. Changes in Lirr after drug intake might ultimately serve as a psychopharmacological model for neuropsychiatric disorders, such as schizophrenia. For translational purposes, it would be pertinent to also determine drug-induced alterations in ERP correlates of Lirr.

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CHAPTER 6

Cholinergic gating of hippocampal auditory evoked potentials in freely moving rats

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INTRODUCTION

Sensory gating is thought to be essential for filtering out background information in order to allocate sufficient processing capacity for salient and/or more important stimuli (Miyazato et al., 1999). In humans, intact sensory gating is defined as a reduction in amplitude of the P50 peak to the second of two identical, consecutive auditory clicks presented 500ms apart (S1: first click or conditioning stimulus, S2: second click or test stimulus: Boutros et al., 2004; Brockhaus-Dumke et al., 2008; Oranje et al., 2006). Perturbations in auditory filtering, such as disrupted sensory gating of the P50 and N100 components, appear to be a candidate trait marker of schizophrenia (Cadenhead et al., 2000; Olincy et al., 2010; Simons et al., 2011; but see De Wilde et al., 2007). Hence, there has been considerable interest in the development of translational rat models to elucidate the underlying neural and neurochemical mechanisms involved in sensory gating.

However, there is some debate as to which component(s) in the rat auditory evoked potential (AEP) is the analogue of the human P50 (de Bruin et al., 2001). The human P50 peak displays gating at an inter-stimulus interval of 500 ms and has been shown to be influenced by stimulus repetition; i.e., there is a gradual decrease in P50 amplitude with increasingly more paired-click trials, especially for S1 (e.g., Lamberti et al., 1993). Moreover, the P50 is dependent on the inter-stimulus interval; i.e., there is a progressive recovery of P50 amplitude of S2 with ISIs longer than 1 s, thereby reducing sensory gating (e.g., Adler et al., 1982). The P50 is mediated by cholinergic signaling, as administration of the non-selective muscarinic antagonist scopolamine in human participants has been shown to reduce P50 amplitude or delay the occurrence of the P50 (Buchwald et al., 1991; Pekkonen et al., 2005). Therefore, these characteristics have been used as criteria for evaluating rat equivalents of the human P50. Several early positive and later-occurring negative peaks have been put forward, such as the P13 or P17, and the N22, N40 or N50, respectively (Adler et al., 1986; Boutros et al., 1997; de Bruin et al., 2001; Miyazato et al., 1996). The source of these discrepancies in peak latencies between labs remains elusive, but can likely be attributed to methodological differences between studies.

Several pharmacological studies in rats have indicated a role for muscarinic neurotransmission in auditory processing. For instance, Miyazato et al. (1995) have reported a reduction in P13 amplitude after systemic injections of the muscarinic antagonist scopolamine at doses of 0.2, 1 and 5 mg/kg (IP). Campbell et al. (1995) revealed that the amplitudes of P18 and N40 components to auditory stimuli were markedly decreased after systemic administration of scopolamine (0.1-10 mg/kg, SC). These findings were extended by the study of Sambeth et al. (2007) which showed that the amplitudes of the N1 and N2 peaks became more negative but closer to baseline after scopolamine (0.1 mg/kg, IP), which was not reversed by concurrent administration of donepezil (3 mg/kg, IP). The authors concluded that this was due to a decrement in arousal induced by scopolamine. Two experiments have employed central infusion of scopolamine into discrete brain areas to determine effects on sensory gating. Teneud et al. (2000) showed that injections of the cholinergic agonist carbachol (0.2, 1 or 5 mM bilaterally) in the pedunculo-pontine nucleus reduced P13 amplitude in a dose- and time-dependent manner, which was reversed by concurrent administration of scopolamine (20 mM bilaterally). Luntz-Leybman et al. (1992) demonstrated decrements in N40 amplitude in the CA3 layer of the hippocampus after

cerebroventricular infusion of nicotinic antagonists, but not the muscarinic antagonist scopolamine.

It is currently unclear which muscarinic receptor subtype(s) might underlie the effects of scopolamine on auditory processing. One of the brain regions showing inhibition of response to recurrent information is the hippocampus, which unsurprisingly has been strongly implicated in rat sensory gating (e.g., Bickford-Wimer et al., 1990; Bickford et al., 1993; Krause et al., 2003). It receives cholinergic innervations from the medial septal nucleus (Mesulam et al., 1983; Woolf et al., 1984) and shows an abundance of muscarinic receptors, especially of the M_1 subtype (see **Chapter 2**). Hence, blockade of the muscarinic M_1 receptor subtype might underlie the effects of scopolamine on auditory processing.

Therefore, the present study investigated the effects of the non-selective muscarinic antagonist scopolamine, the more selective muscarinic M_1 antagonist biperiden and the cholinesterase inhibitor donepezil (also in combination with scopolamine and biperiden) on AEPs and sensory gating. Based on previous studies we expected that after scopolamine, all AEP components would show reduced amplitudes and/or delayed latencies. Since biperiden is a relative specific muscarinic M_1 antagonist we anticipated a more selective effect on auditory processing. Thus, not all AEP peaks will be affected after biperiden. Effects of scopolamine and biperiden would be reversible by concurrent administration of donepezil.

METHODS

Subjects

All experimental procedures were approved by the local ethical committee for animal experiments at Maastricht University and met governmental guidelines. Fourteen 3-month old male Wistar rats (Harlan, NL) served as subjects in this study. They were housed in pairs in standard type III Makrolon™ cages on sawdust bedding in an air-conditioned room (21°C, 45–55% humidity) under a reversed light/dark cycle (lights on from 5 PM to 5 AM). After surgery, animals were singly housed in cages with a flat top which are normally used for individually ventilated conditions. This was done in order to ensure that the rats would not damage their electrodes. Rats were housed in the room in which they were tested. All testing was performed between 9 AM and 5 PM. The animals had unlimited access to food and water.

Surgical procedures and EEG recording

Rats were injected subcutaneously with the analgesic drug Temgesic (0.1 mg/kg). Next, rats were anesthetized with inhalation of isoflurane and placed into a stereotaxic frame. After making a vertical incision and exposing the skull, lidocaine was used as a local anesthetic before removing the periosteum. Subsequently, the bregma intersection was used as a landmark in order to determine the electrode position corresponding to the dorsal hippocampus (anterior-posterior -2.8, medial-lateral -1.8, dorsal-ventral -2.6). Small holes were drilled in the skull to allow the insertion of electrodes. The reference and ground electrodes were placed in the cerebellum. In order to fixate the electrode on the skull surface three screws and dental acrylic cement were used. The animals were allowed three weeks to recover from surgery.

During the next two weeks, the rats were habituated to the experimental procedures – i.e., the test chamber, the EEG acquisition system, the paired-click task and drug administration – before actual drug testing took place. During those habituation sessions the EEG signal was visually monitored for any abnormalities. EEG was always recorded from unrestrained, alert rats. The EEG was filtered between 1 and 133 Hz and sampled at 1000 Hz. The animals underwent a total of nine testing sessions; we tested saline, scopolamine and biperiden twice, whereas donepezil, scopolamine-donepezil and biperiden-donepezil were tested once. Each session started with the injection of the drugs. Thirty min. later, the rats were attached to the EEG equipment and EEG was visually checked. Two test chambers with EEG equipment were at our disposal; therefore two rats could be tested simultaneously.

Paired-click task

The paired-click task was used as an electrophysiological technique to assess sensory gating mechanisms (Adler et al., 1982). Two identical 2500 Hz clicks (S1 and S2) were binaurally presented via loudspeakers. The duration of each click was 4 ms with a sound intensity of 80 dB. A short inter-stimulus interval of 500 ms was used, as this inter-stimulus interval has been shown to provide maximum differentiation between the sensory gating capacities of healthy participants and schizophrenia patients (Nagamoto et al., 1989; Oranje et al., 2006). The pairs were presented in a random intertrial interval of 6-10 s, in order to allow neuronal recovery (Zouridakis & Boutros, 1992). During each session 70 pairs of clicks were presented. No behavioral measures were recorded during this task.

Drug administration

Please refer to Table 1 for information on drug administration. Doses and pretreatment time were chosen based on previous scopolamine and biperiden data (see **Chapter 3** and Sambeth et al., 2007). The order of doses was semi-randomized over testing days. All animals received the same treatment condition in a test session. Saline, scopolamine and biperiden were tested twice on separate testing days, whereas donepezil and the combination treatments were tested only once. Drug solutions were prepared freshly each day prior to testing. Test leaders were not blinded for the treatment conditions, as they could not influence the data (i.e., only EEG recording, no behavioral data). To ensure sufficient wash-out of the drug, testing days were always separated by at least one drug-free day (for half-lives please refer to Legault et al., 2004; Yokogawa et al., 1992).

EEG data analysis

All EEG data was analyzed with Vision Analyzer 2.0. Before data analysis, the EEG data were visually inspected offline, to check for artefacts. The EEG signal was filtered offline with a high pass filter set at 1 Hz (12 dB slope) and a low pass filter set at 30 Hz (12 dB slope). AEP epochs were set from 100 ms prior to stimulus onset to 500 ms after onset. After manual artefact rejection, separate averages were calculated for each rat, stimulus, and treatment condition. Based on the grand averages we determined the position of the P1, N1 and P2 peaks for S1 and S2. Peak amplitudes and latencies were calculated based on when the peak was reached. Please see Table 2 for the time windows chosen for peak detection.

Table 1. Drug administration.

Drug	Supplier	Vehicle	Pretreatment time	Administration route	Volume (mL/kg)	Dose conditions (mg/kg)
Scopolamine hydrobromide trihydrate 99%	Acros Organics	Isotonic saline	30 min.	IP	1	0.1
Biperiden lactate (Akineton®)	Laboratorio Farmaceutico S.I.T.	Milli-Q purified water	30 min.	IP	1	1
Donepezil (Aricept®)	Apin chemicals	Milli-Q purified water	30 min.	PO	2	3

Table 2. Time windows (in ms) used for analysis of the AEP data.

Peaks	Drug condition					
	Saline	Scopolamine	Biperiden	Donepezil	Scopolamine-Donepezil	Biperiden-Donepezil
P1	10-50	10-50	10-50	10-50	10-40	10-50
N1	20-70	20-70	30-80	30-80	30-80	20-70
P2	50-100	60-100	50-110	50-100	50-100	50-100

Statistics

In some cases a particular peak was missing in the AEP data. In order to be able to do repeated measures ANOVAs, we replaced missing values with the average of rats that did show that peak. In the case of two testing days (i.e., for saline, scopolamine and biperiden) the data were averaged before statistical analysis. All data were analyzed using SPSS.

In order to determine whether there was intact sensory gating, AEP data of the saline condition were analyzed with repeated measures ANOVAs with Stimulus (2 levels: S1 and S2) as within-subject variable. Next, we wanted to assess the effects of scopolamine and donepezil on sensory gating. Therefore we performed several repeated measures ANOVAs with Drug (4 levels: saline, scopolamine, donepezil, and scopolamine-donepezil) and Stimulus (2 levels: S1 and S2) as within-subject variables. Finally, we wanted to assess the effects of biperiden and donepezil on sensory gating. Therefore we did several repeated measures ANOVAs with Drug (4 levels: saline, biperiden, donepezil, and biperiden-donepezil) and Stimulus (2 levels: S1 and S2) as within-subject variables.

All analyses were done separately for amplitude and latency, and for each of the AEP components (i.e., P1, N1 and P2). Significant interactions between Treatment and Stimulus would be reflective of a Treatment effect on sensory gating. In this case we performed additional ANOVAs split for each level of Stimulus (i.e., S1 vs. S2) in order to examine simple effects. For reasons of brevity, we decided to not discuss main effects of Stimulus in the 4x2 Treatment analyses, as these effects were already covered by analyses on the saline data with respect to the effect of Stimulus. Post-hoc analyses were performed with a Least Significant Difference (LSD) test; we decided to only focus on treatment effects relative to the saline condition.

RESULTS

Five rats lost their electrodes prematurely and/or did not have a clear EEG signal. These were eliminated from the final analysis, leaving a total of 9 rats.

Stimulus effects in the saline condition on AEPs and sensory gating

Figure 1 shows an overview of the effects of scopolamine, biperiden, donepezil and the combined treatments on hippocampal processing of auditory stimuli. In the analyses of the saline data, there was no main effect of Stimulus on P1 or P2 amplitude (no main effect of Stimulus; $F_s < 2.59$, n.s.). N1 amplitude was larger for S1 compared to S2 (main effect of Stimulus; $F(1,8) = 5.78$, $P < 0.05$). As for the latency data, there was no main effect of Stimulus on P1 or P2 latency (no main effect of Stimulus; $F_s < 2.78$, n.s.). N1 latency was slowed for S1 compared to S2 (main effect of Stimulus; $F(1,8) = 6.88$, $P < 0.05$).

Effects of scopolamine, donepezil and the combination treatment on AEPs and sensory gating

The treatment analyses of P1 or P2 amplitude did not yield any relevant effects ($F_s < 2.98$, n.s.). An overview of the effects of scopolamine, biperiden, donepezil and the combination treatments on N1 amplitude is shown in Figure 2. The effect of Treatment on N1 amplitude varied per level of Stimulus (Treatment * Stimulus interaction effect; $F(3,24) = 11.88$, $P < 0.001$). Therefore we performed simple effects analysis per level of Stimulus. In the analysis of S1, N1 amplitude was shown to be different between treatment conditions (main effect of Treatment; $F(3,24) = 5.77$, $P < 0.01$); post-hoc analysis indicated that the N1 amplitude of the S1 stimulus was larger after donepezil ($P < 0.01$) and scopolamine-donepezil ($P < 0.01$) compared to the saline condition. In the analysis of S2, N1 amplitude was also different between treatment conditions (main effect of Treatment; $F(3,24) = 5.26$, $P < 0.01$); post-hoc analysis indicated that the N1 amplitude of the S2 stimulus was larger after scopolamine ($P < 0.001$) and smaller after donepezil ($P < 0.01$) compared to saline.

The effect of Treatment on P1 latency did not vary per level of Stimulus (no Treatment * Stimulus interaction effect; $F(3,24) = 2.39$, n.s.). P1 latency was shown to be different between treatment conditions (main effect of Treatment; $F(3,24) = 4.40$, $P < 0.05$); post-hoc analysis indicated that P1 latency was smaller after scopolamine compared to saline ($P < 0.05$). In the analysis of N1 latency, the effect of Treatment varied per level of Stimulus (Treatment * Stimulus interaction effect; $F(3,24) = 4.49$, $P < 0.05$). Therefore we performed simple effects analyses per level of Stimulus. In the analysis of S1, there was no main effect of Treatment on N1 latency ($F(3,24) = 1.98$, n.s.). In the analysis of S2, N1 latency was shown to be different between treatment conditions (main effect of Treatment; $F(3,24) = 3.58$, $P < 0.05$); post-hoc analysis indicated that the N1 latency of the S2 stimulus was larger after scopolamine compared to saline ($P < 0.05$). The effect of Treatment on P2 latency did not differ per level of Stimulus (no Treatment * Stimulus interaction effect; $F(3,24) = 1.09$, n.s.). P2 latency was shown to be different between treatment conditions (main effect of Treatment; $F(3,24) = 5.37$, $P < 0.01$); post-hoc analysis indicated that P2 latency was larger after scopolamine ($P < 0.05$) and after scopolamine-donepezil ($P < 0.01$) compared to saline.

Figure 1. The effects of the treatment conditions on hippocampal processing of auditory stimuli. Amplitude effects: (a) After saline, N1 amplitude was larger for the S1 compared to the S2 stimulus. (b) There was a Treatment*Stimulus interaction in the analysis of the N1 amplitude data. N1 amplitude of the S2 stimulus was increased after scopolamine, which reflects a reduction in sensory gating. (c) Biperiden increased the N1 amplitude of the S2 stimulus, suggestive of a reduction in sensory gating. (d) After donepezil, N1 amplitude of the S1 stimulus was increased, whereas N1 amplitude of the S2 stimulus was reduced. This finding would suggest that donepezil increases sensory gating at the N1 peak. (e) Scopolamine-donepezil induced an increase in N1 amplitude of the S1 stimulus, which reflects an increase in sensory gating. (f) Biperiden-donepezil did not affect auditory processing. Latency effects: (a) After saline, N1 latency was larger for the S1 compared to the S2 stimulus. (b) After scopolamine, the occurrence of the P1 peak was earlier compared to the saline condition. Scopolamine also increased the N1 latency of the S2 stimulus and P2 latency of both stimulus types. (c, d, e, f) Biperiden, donepezil, scopolamine-donepezil and biperiden-donepezil did not affect peak latencies.

Effects of biperiden, donepezil and the combination treatment on AEPs and sensory gating

The effect of Treatment on P1 amplitude did not differ per level of Stimulus (no Treatment * Stimulus interaction effect; $F(3,24) = 0.27$, n.s.). There was a main effect of Treatment ($F(3,24) = 4.87$, $P < 0.01$); however, post-hoc analysis did not yield any significant effects. In the analysis of N1 amplitude, the effect of Treatment varied per level of Stimulus (Treatment * Stimulus interaction effect; $F(3,24) = 9.90$, $P < 0.001$). Therefore we performed simple effects analysis per level of Stimulus. In the analysis of S1, N1 amplitude was shown to be different between treatment conditions (main effect of Treatment; post-hoc analysis indicated that the N1 amplitude of the S1 stimulus was larger after donepezil compared to saline ($P < 0.01$)). In the analysis of S2, N1 amplitude was shown to be different between treatment conditions (main effect of Treatment; $F(3,24) = 13.44$, $P < 0.001$); post-hoc analysis indicated that the N1 amplitude of the S2 stimulus was larger after biperiden ($P < 0.001$)).

and smaller after donepezil ($P < 0.05$) compared to saline. The treatment analysis of P2 amplitude, P1 latency or P2 latency did not yield any relevant interaction or main effects ($F_s < 1.72$, n.s.). In the analysis of N1 latency there was a main effect of Treatment ($F(3,24) = 3.30$, $P < 0.05$); however, post-hoc analysis did not yield any significant effects.

Figure 2. Effects of the treatment and stimulus conditions on N1 amplitude. Both scopolamine and biperiden disrupted sensory gating by increasing N1 amplitude of the S2 stimulus. Scopolamine-donepezil increased N1 amplitude of S1. This likely reflects an underlying effect of donepezil, also given the fact that donepezil by itself improved sensory gating by enhancing N1 amplitude of S1, and reducing N1 amplitude of the S2 stimulus. Moreover, after scopolamine-donepezil N1 amplitude of the S2 stimulus was similar to the saline condition, reflecting a reversal of the effects of scopolamine by donepezil. After the biperiden-donepezil combination treatment, N1 amplitude was statistically similar to the saline condition, reflecting a reversal of the effects of biperiden by donepezil.

DISCUSSION

The goal of the current study was to assess the effects of the muscarinic antagonists scopolamine and biperiden, and the cholinesterase inhibitor donepezil on auditory processing and sensory gating. In Table 3 an overview is given of the effects of our treatment conditions.

Summary of results

In the present study we saw a clear sensory gating effect at the N1 component in the saline condition. Specifically, there was a relative decrease of N1 amplitude to the second click (S2) compared with the first (S1), which is a measure of sensory gating. The P1 and P2 peaks did not show any sensory gating effects. This would make the N1 component a probably candidate as the rat equivalent of human sensory gating.

Scopolamine disrupted sensory gating of the N1 component (i.e., there was a Treatment * Stimulus interaction). A closer look at the data revealed that an increase in N1 am-

plitude of the S2 stimulus relative to the saline condition was responsible for this effect. Scopolamine combined with donepezil enhanced sensory gating by increasing the N1 amplitude of S1. N1 amplitude of the S2 stimulus was unchanged relative to saline after scopolamine-donepezil. The former finding is likely due to an underlying effect of donepezil, also given the fact that donepezil by itself (i.e., without add-on treatment with a muscarinic antagonist) improved sensory gating by enhancing N1 amplitude to S1, and by decreasing N1 amplitude to the S2 stimulus. The lack of effect on the S2 stimulus after the combination treatment is likely reflective of a reversal of the scopolamine-induced increment in N1 amplitude of the S2 stimulus by donepezil. Scopolamine did not affect P1 or P2 amplitude.

As expected, biperiden had much more selective effects on auditory processing. It impaired sensory gating by increasing the N1 amplitude of the S2 stimulus (i.e., we found a Treatment * Stimulus interaction), which was fully reversed by concurrent administration of donepezil. The biperiden-donepezil combination treatment also did not influence N1 amplitude of S1. Biperiden did not influence P1 or P2 amplitude.

There were also several latency effects. In the saline condition, N1 latency was faster for S2 relative to the S1 stimulus. After scopolamine, P1 latency was decreased, which was reversed by concurrent administration of donepezil. Scopolamine also slowed N1 latency of the S2 stimulus relative to the saline condition, which was reversed by concurrent administration of donepezil. Lastly, P2 latency was increased after scopolamine. This was also the case for the combination treatment, which implies that donepezil was unable to reverse the effects of scopolamine on P2 latency. Biperiden did not influence peak latencies.

In sum, scopolamine induced widespread effects on auditory processing besides affecting sensory gating at the N1 component. Donepezil was also not able to completely counteract all these effects. In contrast, biperiden only influenced sensory gating at the N1 peak, which was fully reversed by concurrent donepezil administration. These findings are in line with the binding affinities of scopolamine and biperiden for muscarinic receptors (i.e., non-selective versus relatively selective for the muscarinic M₁ receptor subtype, Bolden et al., 1992; Katayama et al., 1990). Furthermore, our results strongly suggest that biperiden is to be preferred over scopolamine as a means for pharmacologically inducing cholinergic impairments in auditory processing in healthy rats.

Effects of cholinergic drugs on AEPs in rats

The findings of the current study are in contrast with those reported in the literature. Several articles have demonstrated a reduction in peak amplitude of early positive, but not negative components after scopolamine (Miyazato et al., 1995; Teneud et al., 2000). However, Campbell et al. (1995) found that scopolamine induced a decrement in amplitudes of both P18 and N40 peaks, which was more pronounced for the latter than the former component. Moreover, Luntz-Leybman et al. (1992) reported no effect of scopolamine on N40 gating. There are several methodological differences which might explain the discrepancies in findings between these experiments.

Table 3. Treatment effects on AEPs and sensory gating. In case of the saline condition, a comparison is made between S1 and S2. Drug effects are always compared to the saline condition. Treat*Stim reflects an interaction effect, which implies that a particular drug influences sensory gating. In this case, drug effects are presented as simple effects (i.e., per level of Stimulus).

Treatment conditions	Peaks					
	P1		N1		P2	
	Amplitude	Latency	Amplitude	Latency	Amplitude	Latency
Saline	n.s.	n.s.	S1 > S2 (<i>P</i> < 0.05)	S1 > S2 (<i>P</i> < 0.05)	n.s.	n.s.
Scopolamine	n.s.	↓ (<i>P</i> < 0.05)	SG ↓ (Treat*Stim) S1: n.s. S2: ↑ (<i>P</i> < 0.001)	(Treat*Stim) S1: n.s. S2: ↑ (<i>P</i> < 0.05)	n.s.	↑ (<i>P</i> < 0.05)
Scopolamine- donepezil	n.s.	n.s.	SG ↑ (Treat*Stim) S1: ↑ (<i>P</i> < 0.01) S2: n.s.	n.s.	n.s.	↑ (<i>P</i> < 0.01)
Biperiden	n.s.	n.s.	SG ↓ (Treat*Stim) S1: n.s. S2: ↑ (<i>P</i> < 0.001)	n.s.	n.s.	n.s.
Biperiden- donepezil	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Donepezil	n.s.	n.s.	SG ↑ (Treat*Stim) S1: ↑ (<i>Ps</i> < 0.01) S2: ↓ (<i>Ps</i> < 0.05)	n.s.	n.s.	n.s.

Abbreviations: Amp: Amplitude, Lat: Latency, SG: sensory gating, Stim: Stimulus, Treat: Treatment, ↑: increased, ↓: decreased, n.s.: not significant.

Firstly, we recorded EEG from freely moving rats receiving systemic injections of cholinergic drugs, whereas Luntz-Leybman et al. (1992) and Miyazato et al. (1995) used recordings from anesthetized animals in which scopolamine was injected centrally (i.e., in the lateral ventricle and in the pedunculopontine nucleus, respectively). Anesthetic drugs are likely to interact with the effects of muscarinic antagonists on auditory processing. In addition, it has been shown previously that motor behavior increases acetylcholine levels in the hippocampus (Dudar et al., 1979). Hence, hippocampal AEP recordings in freely moving animals might differ from those of immobile, anesthetized animals in terms of baseline cholinergic tone and might therefore also respond differently to disruption by anti-muscarinic agents. Moreover, systemic and central administration of drugs is not fully comparable in terms of effects on behavior and EEG. Systemic injections also induce peripheral side-effects, caused by binding of a drug outside of the central nervous system (see **Chapter 2**).

Secondly, different recording sites were used for our study and those previously reported in the literature (Campbell et al., 1995; Luntz-Leybman et al., 1992; Miyazato et al., 1995; Teneud et al., 2000). We measured EEG in the dorsal hippocampus, whereas previous studies have used area CA3 in the ventral hippocampus (Luntz-Leybman et al., 1992), the pedunculopontine nucleus (Teneud et al., 2000) or the vertex (Campbell et al., 1995; Miyazato et al., 1995) as recording sites. These brain areas comprise distinct parts of the cholinergic system (Mesulam et al., 1983; Woolf & Butcher, 1986; Woolf et al., 1984) and might therefore be differentially sensitive to effects of (anti-)muscarinic drugs on auditory processing due to regional diversity in cholinergic innervation. In addition, within the hippocampus the muscarinic M_1 receptor has been shown to be enriched in dorsal versus ventral sites (i.e., area CA1 compared to CA3: Levey et al., 1995). The fact that in the current study we have used a muscarinic M_1 receptor antagonist and have measured AEPs in a brain region especially dense in muscarinic M_1 receptors might have contributed to the discrepancies between the current study and those reported in the literature (Campbell et al., 1995; Luntz-Leybman et al., 1992; Miyazato et al., 1995; Teneud et al., 2000). That is, blockade of muscarinic M_2 – M_5 receptors might be partly responsible for the effects of scopolamine on auditory processing in previous studies. This is also the reason why a more selective muscarinic antagonist such as biperiden is preferable over scopolamine in order to be able to attribute changes in AEP components to blockade of particular muscarinic receptor subtype(s).

Of note, the study by Sambeth et al. (2007) was comparable to the current one in terms of experimental setup. They also recorded AEPs from the dorsal hippocampus in free-moving Wistar rats and used the same doses for scopolamine and donepezil. Scopolamine (0.1 mg/kg, IP) made the N1 amplitude more negative, which was in line with our own findings. However, the authors concluded that the N1 became closer to baseline after scopolamine. Concurrent administration of donepezil (3 mg/kg, IP) failed to reverse these effects.

Effects of cholinergic drugs on AEPs in humans

Human studies on sensory gating have generally reported quite contradicting findings. For instance, Buchwald et al. (1991) and Pekkonen et al. (2005) demonstrated reduced amplitudes and/or delayed latencies of the P50 and N100 components after scopolamine. Buchwald et al. (1991) also demonstrated a reversal of the scopolamine-induced decrease in P50 amplitude by concurrent administration of physostigmine. Another study from our lab

assessed whether biperiden and the cholinesterase inhibitor rivastigmine would influence sensory gating and mismatch negativity (MMN: see **Chapter 7**). Biperiden and a combination treatment of biperiden and rivastigmine both increased P50 amplitude relative to the saline condition. The latter was likely caused by an underlying effect of biperiden, also given the fact that rivastigmine alone left the P50 unchanged. There was no effect of biperiden or rivastigmine on sensory gating. Furthermore, in the same study N100 and P200 latency was delayed after biperiden, which was reversed by rivastigmine. In contrast to the current findings, biperiden did not affect the amplitude of early negative peaks.

Cholinergic drugs as a model for auditory processing and sensory gating deficits in schizophrenia

In sum, data on the effects of cholinergic drugs on auditory processing and sensory gating in rats and healthy participants are not very straightforward, which can be partly explained by species and/or methodological differences between experiments. Overall, muscarinic antagonists and cholinesterase inhibitors appear to mainly affect early positive and negative peaks in the AEP. Moreover, they influence sensory gating in the rat, but not in healthy human participants. However, the question remains whether cholinergic drugs would be useful as a translational model for aberrant auditory processing in schizophrenia. Although impairments in sensory gating are not reported consistently in schizophrenia (e.g., Jin et al., 1997; 1998; Kathmann & Engel, 1990), the main finding is that suppression deficits can be attributed to a reduction of the P50 and N100 response to S1 (e.g., Brockhaus-Dumke et al., 2008; but see Jin et al., 1997; Patterson et al., 2000). These reports are hard to relate to our own results of disrupted sensory gating due to increased N1 amplitude of the S2 stimulus after scopolamine and biperiden.

However, the validity of auditory perturbations as an endophenotype for schizophrenia has been questioned. For instance, there are indications that auditory processing deficits might be a state instead of a trait marker (e.g., Bramon et al., 2004; Nagamoto et al., 1996). In addition, the specificity of auditory processing deficits in schizophrenia is not very high. In aging and age-related disorders such as mild cognitive impairment and Alzheimer's disease, an increase in amplitudes of early AEP components (Golob et al., 2001; Irimajiri et al., 2005) and reduced sensory gating due to an increase in P50 amplitude of the S2 click has been reported (Cancelli et al., 2006; Jessen et al., 2001; Thomas et al., 2010; but see Buchwald et al., 1989; Fein et al., 1994). These findings are thus much more in line with our current results of increased N1 amplitudes induced by scopolamine and biperiden. Therefore, we would like to suggest that changes in auditory processing and sensory gating induced by cholinergic drugs may serve as a translational model for aging instead of schizophrenia. For future research, it would be interesting to determine possible dissociations between drugs that target different muscarinic receptor subtypes in terms of their effects on auditory processing and the relation with cognitive functions. The present study suggests that this may be relevant for aging and/or Alzheimer's dementia but probably not for schizophrenia.

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CHAPTER 7

Cholinergic modulation of auditory processing and novelty detection in human participants

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INTRODUCTION

Suppression of redundant auditory information and facilitation of deviant, novel or salient sounds can be assessed with electrophysiological paradigms such as paired-click and oddball tasks, respectively. Both of these make use of the automatic processing of auditory stimuli in the absence of effortful attention, yielding so-called auditory evoked potentials (AEPs) typically consisting of a P50-N100-P200 complex (e.g., Javitt et al., 2008; Leiser et al., 2011; Lijffijt et al., 2009). It has been suggested by Boutros et al. (2004) that these three components reflect distinct stages of information processing – i.e., P50; pre-attentive, N100; early-attentive, and P200; late-attentive mechanisms. In paired-click paradigms, participants are presented with two identical auditory stimuli temporally spaced 500 ms apart (Boutros et al., 2004). The amplitude of the P50 peak to the first click – also termed S1 or conditioning stimulus – is usually larger than that to the second click – called S2 or the test stimulus (Oranje et al., 2006); this is indicative of inhibition of redundant input and intact sensory gating (Brockhaus-Dumke et al., 2008). Oddball paradigms can elicit several AEP components in addition to the P50-N100-P200 complex, such as the mismatch negativity (MMN) and P3a. In these tasks, infrequent deviant and/or novel sounds are embedded within a string of repetitive standard tones (Garrido et al., 2009; Näätänen et al., 2004; Pekkonen et al., 2005).

In schizophrenia, mechanisms of redundancy suppression and change detection are disrupted, resulting in an overflow of sensory input and associated symptomatology such as cognitive fragmentation and thought disorder (Ford, 1999; McGhie & Chapman, 1961). Impaired sensory filtering mechanisms in schizophrenia patients have been linked to several electrophysiological findings, such as deficient sensory gating reflected by attenuated N100 and P50 amplitude suppression in the paired-click paradigm (Boutros et al., 2004; Brockhaus-Dumke et al., 2008; Erwin, 1998; Olincy et al., 2010), decreased MMN during a novelty oddball task, and reduced amplitudes and delayed latencies of N100, P200 and P300 components (Bramon et al., 2004a; 2004b; Jeon & Polich, 2001; Patterson et al., 2008; Simons et al., 2011; Souza et al., 1995; Umbricht & Krljes, 2005; but see De Wilde et al., 2007).³ Studies of unaffected family members of schizophrenia patients, schizotypal and high-risk individuals have indicated that electrophysiological correlates of perturbed auditory processing are likely to be a trait marker, or candidate endophenotype for schizophrenia (Cadenhead et al., 2000; Olincy et al., 2010; Price et al., 2006; Simons et al., 2011; but see Bramon et al., 2004a; De Wilde et al., 2007; Umbricht et al., 2003).

Besides the strong involvement of the dopaminergic and glutaminergic neurotransmitter systems in schizophrenia, alterations in muscarinic M_1 signaling also appear to underlie the disorder (e.g., Sarter et al., 2012). For example, administration of the muscarinic M_1 antagonists biperiden or procyclidine has been shown to increase positive symptoms (Johnstone et al., 1983; Tandon et al., 1990), yet reduce negative symptoms in schizophrenia patients (Tandon et al., 1992). In addition, cholinesterase inhibitors and muscarinic M_1 agonists have been found to modestly improve various measures of cognitive function, particularly attention and short-term memory (Bora et al., 2005; Buchanan et al., 2002;

³ Of note, the existing literature on P300 abnormalities in schizophrenia has focused mostly on the P3b subcomponent, although a limited number of studies have also reported reduced P3a amplitudes (Grillon et al., 1990; Turetsky et al., 2000).

2008; Friedman, 2004; Lee et al., 2007; Risch et al., 2007; Schubert et al., 2006; Shekhar et al., 2008; but see Friedman et al., 2002;).

Despite a vast amount of research on the role of nicotinic receptors (e.g., Adler et al., 1998; Pritchard et al., 2004), data on the role of muscarinic neurotransmission in human AEPs is relatively limited. The non-selective muscarinic antagonist scopolamine has been found to reduce the amplitudes of the P50, N100, P200 and P300 components (Buchwald et al., 1991; Curran et al., 1998). A MEG study by Pekkonen et al. (2001) also reported a reduction of MMNm amplitude after scopolamine. Moreover, delayed P50, N100 and P300, but not P200 or MMN latencies have been demonstrated after scopolamine administration (Knott et al., 1999; Pekkonen et al., 2005). A few studies have looked into the effects of cholinesterase inhibitors on AEPs and sensory gating. For instance, a reduction in P50 amplitude by scopolamine was shown to be reversible by administration of physostigmine (Buchwald et al., 1991). Furthermore, in a study by Buchanan et al. (2002), donepezil was shown to modestly improve P50 suppression in schizophrenia patients. Lastly, physostigmine was able to partially counteract the effects of scopolamine on the P300 component (i.e., reduced amplitude and delayed latency: Meador et al., 1987; 1988). Given the involvement of the muscarinic M_1 receptor in schizophrenia, it would be interesting to find out whether drugs that target this receptor would affect AEP components.

The current study is the first to assess the effects of the muscarinic M_1 antagonist biperiden and the cholinesterase inhibitor rivastigmine on AEPs, sensory gating and MMN in young, healthy volunteers. We expected that after biperiden, AEPs would be affected in a manner similar to scopolamine, i.e., reduced amplitudes of the P50, N100, P200, P3a and MMN peaks, and increased latencies of the P50, N100 and P3a components. Effects on the amplitudes and latencies of the P50 and P3a peaks will be reversible by administration of rivastigmine. Moreover, given the cognition-deteriorating effects of biperiden (e.g., Wezenberg et al., 2005), we anticipated that biperiden would disrupt P50 suppression and that rivastigmine would be able to counteract this. Lastly, paired-click and novelty oddball tasks measure discrete sensory filtering processes (i.e., redundancy suppression and change detection, respectively); hence, we did expect to find some dissociations between these paradigms in terms of drug effects on AEPs.

METHODS

Participants

Nineteen (7 male, 12 female; mean age of 21.89 years (SD = 2.8, range = 18-28)) healthy volunteers were recruited from Maastricht University through poster advertisements. Participants had a body mass index between 18.5 and 30. They received a medical screening before testing, consisting of a medical questionnaire, physical examination, measurement of blood pressure and pulse rate, blood samples for haematology and biochemistry, urine samples for drug screen and pregnancy test, and a resting electrocardiogram. Exclusion criteria were past or current psychiatric, neurological, cardiac, gastrointestinal, haematological, hepatic, pulmonary, or renal illness, pregnancy, lactation, excessive alcohol consumption (intake of more than 20 glasses per week), use of any medication other than oral contraceptives, having a first-degree relative with a current or past psychiatric disorder, and presence of other deficits that could be expected to influence performance. All sub-

jects gave a signed informed consent before inclusion and were financially rewarded for their participation. The study was approved by the Medical Ethics Committee of Maastricht University and therefore performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

Study design and procedures

The study was conducted according to a double-blind, placebo-controlled cross-over design. Subjects were not allowed to use any psychoactive medication within 5 days before drug intake. One hour before testing, participants were asked to fill out questionnaires on mood and physical complaints (see below). Immediately afterwards, they were given two capsules for oral intake. These included either: 1) placebo; i.e., two placebo capsules; 2) biperiden; i.e., one biperiden hydrochloride capsule of 2 mg (Akineton®, instant release) and one placebo capsule; 3) rivastigmine; i.e., one rivastigmine tartrate capsule of 3 mg (Exelon®) and one placebo capsule; or 4) a combination treatment of biperiden and rivastigmine; i.e., one biperiden hydrochloride capsule of 2 mg (Akineton®, instant release) and one rivastigmine tartrate capsule of 3 mg (Exelon®).

Participants were provided lunch immediately afterwards: this was done in order to reduce the chances of participants developing any side-effects due to biperiden and rivastigmine intake. Lunch consisted of a theine-free tea, and bread with savory or sweet bread toppings. Next, EEG electrodes and a cap were placed. Exactly 60 min. after drug intake behavioral testing and EEG recording started. After about one hour of testing, the participant had a short break, in which he or she was asked to fill out the same questionnaires again. The test session finished about 3h after drug intake. Participants underwent four testing days and on each testing day one of the four treatments was administered. To prevent order effects participants were randomly assigned to one of twenty balanced treatment sequences. All treatments were administered with a washout period of seven days.

Drug treatment

Biperiden is a muscarinic M_1 antagonist approved for the adjunctive treatment of Parkinson's disease and for extrapyramidal symptoms related to antipsychotic drug therapy (e.g., Ogino et al., 2011). It has about 10-fold higher affinity for M_1 as compared to M_2 - M_5 receptors and it is thus the most selective M_1 antagonist available for use in human participants (Bolden et al., 1992; Katayama et al., 1990). In humans, peak plasma concentrations are reached around 1-2h after a single dose administration followed by a rapid initial decline to values around 12% of the peak values at 6h after intake. This is subsequently followed by a slow terminal elimination phase at 48h (Hollmann et al., 1984; 1987). The most common side effects of biperiden on the central nervous system are drowsiness, vertigo, headache, and dizziness. Peripheral side effects consist of blurred vision, dry mouth, impaired sweating, abdominal discomfort, and obstipation. Biperiden also causes some degree of mydriasis (e.g., Mintzer & Burns, 2000).

Rivastigmine is an intermediate-acting cholinesterase inhibitor approved for symptomatic treatment of mild to moderate Alzheimer's disease (Jann et al., 2002; Lefevre et al., 2008). It stimulates cholinergic neurotransmission by preventing the breakdown of acetylcholine in the synaptic cleft. Specifically, it inhibits the enzymes acetylcholinesterase and butyrylcholinesterase which metabolize acetylcholine (Jann et al., 2002). Peak plasma con-

centrations are reached around 50-70 min. and the half-life is around 60-90 min. (Farlow, 2003; Jann et al., 2002). The duration of cholinesterase inhibition is suggested to be around 8-10 hours after drug intake. The most common side effects seen in association with the use of rivastigmine occur in the gastrointestinal tract and central nervous system; these include abdominal pain, nausea, vomiting, diarrhea, headache and dizziness. Dose conditions of biperiden and rivastigmine were chosen based on the study by Wezenberg et al. (2005). Both drugs were purchased, blinded, and labelled by the pharmacy of the University Hospital Maastricht according to relevant GMP guidelines.

Questionnaires

To assess subjective mood changes, we used the Profile of Mood States (POMS: McNair et al., 1971) and the Bond and Lader mood scale (Bond & Lader, 1974). Physical or mental complaints were assessed with a self-report questionnaire consisting of 31 possible complaints to be rated on a 4-point scale; a score of zero indicates that the participant is not experiencing the complaint, and a score of three means he or she is experiencing it a great deal. For all three questionnaires, a difference score of mean score_{T1} (1 hour after drug intake) – mean score_{T0} (baseline, before drug intake) was calculated per subscale for each participant and used for analysis.

Paired-click paradigm

The paired-click task was used as an electrophysiological technique to assess sensory gating mechanisms (Adler et al., 1982). Two identical 1000 Hz clicks (S1 and S2) were binaurally presented via loudspeakers. The duration of each click was 4ms with a sound intensity of 60 dB. A short inter-stimulus interval of 500 ms was used, as this inter-stimulus interval has been shown to provide maximum differentiation between the sensory gating capacities of healthy participants and schizophrenia patients (Nagamoto et al., 1989; Oranje et al., 2006). The pairs were presented in a random intertrial interval of 6-10s, in order to allow neuronal recovery (Zouridakis & Boutros, 1992). During each session 75 pairs of clicks were presented. Participants viewed a silent animated movie whilst listening to the paired-clicks. No behavioral measures were recorded during this task.

Novelty oddball paradigm

The novelty oddball task was used as an assessment of involuntary attention processes such as the MMN. Three types of auditory stimuli were presented, consisting of frequent standard, infrequent deviant and infrequent novel stimuli. The standard and deviant stimuli were 750 Hz and 500 Hz tones, each with two upper harmonic components (1500 and 2250; 1000 and 1500 Hz, respectively). The intensity of the first and second harmonic components was decreased compared to the fundamental by 3 and 6 dB, respectively. Novel stimuli consisted of three stimulus categories of 20 different auditory stimuli, namely animal, human, and mechanical sounds. The deviant and novel stimuli were each presented in 12.5% of the trials. All sounds had a duration of 300 ms with 10ms rise and fall times. The tones were presented with a 1000 ms stimulus-onset asynchrony and equal intensities. Participants viewed a silent animated movie whilst listening to the auditory stimuli. No behavioral measures were recorded during this task, making this a passive novelty oddball task.

EEG acquisition

For the EEG recording, the Neuroscan Synamps system was used, with sample rate set at 1000 Hz. Data were filtered between 0.05-100 Hz. EEG was recorded from 32 electrodes placed on the scalp using an elastic cap (Electro-Cap International, United States) and positioned according to the 10-20 system (Jasper, 1958). The horizontal electro-oculogram (HEOG) was measured with two electrodes placed on the outer canthus of the left and right eye, and two electrodes placed below and above the centre of the left eye recorded the vertical electro-oculogram (VEOG). Two electrodes behind the ears served as reference electrodes. Before placing the electrodes, all locations were cleaned with alcohol and gently scrubbed with a gel, to ensure good conduction of the signal. Impedance was kept below 5 Ω . During the EEG recordings, the participants were sitting in an electrically shielded and sound-attenuated room with the lights dimmed.

EEG analysis

All EEG data was analyzed with Vision Analyzer 2.0. Before data analysis, the EEG data were visually inspected offline, to check for artefacts. The EEG signal was filtered offline with a high pass filter set at 1 Hz (12 dB slope) and a low pass filter set at 30 Hz (12 dB slope). Eye movement artefacts were filtered out of the EEG using the data of the VEOG channel and the method developed by Semlitsch et al. (1986). This way, every participant had about the same number of artifact-free trials which were combined into the AEP averages.

In case of the paired-click task, we decided to segment the EEG according to click-pairs instead of separately for each stimulus. Thus, the EEG epochs were set from 100 ms prior to S1 onset to 1000 ms after onset. One segment included both S1 and S2, using the 100 ms preceding the first click in the stimulus-pair as baseline. Segmenting the data for S1 and S2 separately would have implied using the 100 ms preceding S2 as a baseline for this stimulus. However, at this point the EEG signal had not yet returned to its default value, which would have skewed the baseline correction used for S2. After automatic artefact rejection, averages were calculated. Based on the grand averages we determined the position of the P50, N100 and P200 peaks for S1 and S2. Peak amplitudes were calculated and latencies were computed based on when the peak was reached. Of note, since we chose to analyze S1 and S2 within the same segment, the peaks for S2 were actually P550, N600 and P700. Please see Table 1 for the time windows chosen for the paired-click peak detection.

For the novelty oddball data, the EEG epochs were set from 100 ms prior to stimulus onset to 900 ms after onset, using the 100 ms pre-stimulus as baseline. After automatic artefact rejection, averages were calculated for each stimulus separately (standards, deviants and novels). Based on the grand averages we determined the position of the P50, N100 and P200 peaks for the standard, deviant and novel stimuli. Moreover, two difference waves were calculated by subtracting the response to the standard from the deviant and novel stimuli. For these difference waves, we determined the position of the MMN and P3a peaks. Peak amplitudes were calculated and latencies were computed based on when the peak was reached. Please see Table 2 for the time windows chosen for novelty oddball peak detection.

Table 1. Time windows (in ms) used for AEP analyses of the paired-click data.

Stimulus type	Peaks		
	Pre-attentive: P50	Early-attentive: N100	Late-attentive: P200
S1	20 – 90	70 – 150	150 – 240
S2	520 – 590	570 – 650	650 – 740

Table 2. Time windows (in ms) used for AEP analyses of the novelty oddball data.

Stimulus type	Peaks		
	Pre-attentive: P50	Early-attentive: N100	Late-attentive: P200
Standards	20 – 120	90 – 150	140 – 240
Deviants	20 – 120	90 – 150	180 – 260
Novels	20 – 120	90 – 150	180 – 260
Stimulus type	Change detection: MMN	Novelty processing: P3a	
Dev – Stand	110 – 170	170 – 300	
Nov – Stand	110 – 170	170 – 300	

Abbreviations: Dev – Stand: difference wave of the deviant versus the standard stimuli, MMN: mismatch negativity, Nov – Stand: difference wave of the novel versus the standard stimuli.

Rationale for electrode selection

In total we recorded and analyzed EEG from 32 electrodes, but we only included the data of the Fz, FCz and Cz electrode channels in our final statistical analysis. In the literature, the Cz electrode is usually taken for measurement of sensory gating (e.g., Boutros et al., 2004). However, since the prefrontal cortex is also involved in sensory gating (Grunwald et al., 2003; Williams et al., 2011), we chose to also include the Fz and FCz electrode channels. As for the novelty oddball task, we were mainly interested in the novelty P3a, which is a frontocentral component (Comerchero & Polich, 1999; Donchin, 1981). Hence, the Fz, FCz and Cz electrode channels were the obvious choice and in accordance with other studies evaluating the same ERP components in these paradigms. Of note, the participants were presented with a test battery containing additional paradigms besides the paired-click and novelty oddball task. We chose to measure EEG from a total of 32 electrodes, because different paradigms would elicit different ERPs, each with a different scalp distribution. Thus, electrode channels other than the Fz, FCz and Cz would be employed for the ERP analysis of these additional paradigms.

Statistics

All data were analyzed using SPSS. To determine possible treatment effects on the processing of the paired-click task, AEP data were analyzed by several repeated measures ANOVAs with Drug (4 levels: placebo, biperiden, rivastigmine and the combination treatment), Stimulus (2 levels: S1 and S2) and Electrode (3 levels: Fz, FCz and Cz) as within-subject variables. Effects on P50 suppression would be reflected by a significant Treatment * Stimulus interaction. Next, we wanted to see if our treatment conditions would affect overall stimulus processing in the novelty oddball task. Therefore, we ran repeated measures ANOVAs on the AEP data of the standard stimuli, with Drug (4 levels: placebo, biperiden, rivastigmine and the combination) and Electrode (3 levels: Fz, FCz and Cz) as within-subject variables. Next we sought to determine if our treatment conditions influenced the difference scores we calculated (also see previous section). Therefore we performed several repeated

measures ANOVAs with Drug (4 levels: placebo, biperiden, rivastigmine and the combination), Stimulus (2 levels: Diff_{deviant} and Diff_{novel}) and Electrode (3 levels: Fz, FCz and Cz) as within-subject variables.

For the AEP data, all analyses were done separately for amplitude and latency, and for each of the AEP components. Most of the repeated measures analyses yielded significant interactions with the variables Electrode and Stimulus. In these cases, we performed additional ANOVAs split for each level of Electrode in order to examine simple effects. For reasons of brevity, we decided to only discuss these simple effects analyses in the results section, and to leave out the overall analysis. If one of the overall repeated measures ANOVA did not yield a significant interaction term, or if a Treatment * Stimulus interaction was present, this is stated clearly in the results section. For all analyses, we used $\alpha = 0.05$. Post-hoc analysis was performed with a Least Significant Difference (LSD) test. We chose to only compare the data of the treatment conditions with placebo, so per analysis a total of 3 post-hoc comparisons were made.

The questionnaire data were analyzed by repeated measures ANOVAs with Drug (4 levels: placebo, biperiden, rivastigmine and the combination treatment), as within-subject variable. These analyses were done separately for each subscale. Post-hoc analysis was performed with a Least Significant Difference (LSD) test. We chose to only compare the data of the treatment conditions with placebo, so per analysis a total of 3 post-hoc comparisons were made. The POMS had 5 subscales, whereas for the Bond and Lader we only analyzed the best validated subscale: alert-drowsy. For the self-report questionnaire we only analyzed those side-effects which would be expected after biperiden and/or rivastigmine intake.

RESULTS

Four participants dropped out prematurely due to side-effects of the drugs, leaving a total of 15 participants. Two participants withdrew immediately after the first testing session. They had received biperiden and rivastigmine, respectively. The other two participants resigned after three testing sessions. One person got first the combination treatment, then biperiden, and lastly rivastigmine, whereas the other had received the combination treatment, rivastigmine and placebo, respectively. In sum, this indicates that especially rivastigmine was prone to induce participant dropout due to side-effects.

Treatment effects on auditory processing and redundancy suppression in the paired-click task

Figure 1 shows the S1 and S2 AEP responses of the placebo condition, whilst Figures 2 and 3 display the effects of the treatment and stimulus conditions on P50 amplitude and N100 latency in the paired-click task, respectively.

In the simple effects analyses of all three electrode channels (i.e., Fz, FCz and Cz), the effect of Treatment on P50 amplitude did not vary per level of Stimulus (no Treatment * Stimulus interaction effect; $F_s < 1.21$, n.s.). The P50 amplitude of S2 was smaller compared to that of S1 (main effect of Stimulus; $F_s > 28.24$, $P_s < 0.001$). Furthermore, P50 amplitude was shown to be different between treatment conditions (main effect of Treatment; $F_s >$

4.75, $P_s < 0.01$); post-hoc analysis indicated that P50 amplitude was larger after biperiden ($P_s < 0.01$) and the combination treatment ($P_s < 0.05$) compared to placebo.

In the simple effects analysis of the Fz electrode data, there were no interactions or main effects on P50 latency ($F_s < 0.68$, n.s.). For the analyses of the FCz and Cz electrodes, the effect of Treatment did not vary per level of Stimulus (no Treatment * Stimulus interaction effect; $F_s < 1.90$, n.s.). The P50 latency of S2 occurred earlier compared to the P50 latency of S1 (main effect of Stimulus; $F_s > 4.67$, $P_s < 0.05$), yet Treatment did not influence P50 latency on FCz and Cz (no main effect of Treatment; $F_s < 2.61$, n.s.).

Figure 1. AEP responses to S1 and S2 of the paired-click task (shown here: Cz electrode). The amplitude and latency of the P50, N100 and the P200 peaks were smaller for S2 compared to S1 ($P_s < 0.05$).

Figure 2. Effects of the treatment and stimulus conditions on P50 amplitude of the paired-click task (shown here: Fz electrode, means + SEM, *: $P_s < 0.05$, ***: $P_s < 0.001$). P50 amplitude was larger after biperiden ($P_s < 0.01$) and the combination treatment ($P_s < 0.05$) compared to placebo. There was no Treatment * Stimulus interaction, and therefore no effect on sensory gating.

In the simple effects analyses of all three electrode channels (i.e., Fz, FCz and Cz), the effect of Treatment on N100 amplitude did not vary per level of Stimulus (no Treatment * Stimulus interaction effect; $F_s < 0.50$, n.s.). The N100 amplitude of S2 was smaller compared to that of S1 (main effect of Stimulus; $F_s > 89.76$, $P_s < 0.001$), yet Treatment did not influence N100 amplitude (no main effect of Treatment; $F_s < 2.59$, n.s.).

In the simple effects analyses of all three electrode channels (i.e., Fz, FCz and Cz), the effect of Treatment on N100 latency did not vary per level of Stimulus (no Treatment * Stimulus interaction effect; $F_s < 1.79$, n.s.). The N100 peak of S2 occurred earlier compared to the N100 of S1 for Fz, FCz and Cz (main effect of Stimulus; $F(1,18) = 5.48$, $P < 0.05$). Moreover, in the simple effects analysis of the Fz electrode, N100 latency was shown to be different between treatment conditions (main effect of Treatment; $F_s > 3.16$, $P_s < 0.05$); post-hoc analysis showed that the N100 occurred later after biperiden compared to placebo ($P < 0.05$). For the FCz and Cz electrodes, Treatment did not influence N100 latency (no main effect of Treatment; $F_s < 2.70$, n.s.).

Figure 3. Effects of the treatment and stimulus conditions on N100 latency of the paired-click task (shown here: Fz electrode, means + SEM, *: $P < 0.05$). N100 latency was larger after biperiden compared to placebo ($P_s < 0.05$); this effect was reversed by concurrent administration of rivastigmine (i.e., after the combination treatment N100 latency was statistically similar to placebo).

In the simple effects analyses of all three electrode channels (i.e., Fz, FCz and Cz), the effect of Treatment on P200 amplitude did not vary per level of Stimulus (no Treatment * Stimulus interaction effect; $F_s < 1.47$, n.s.). The P200 amplitude of S2 was smaller compared to that of S1 (main effect of Stimulus; $F_s > 100.40$, $P_s < 0.001$), yet Treatment did not influence P200 amplitude for Fz, FCz and Cz (no main effect of Treatment; $F_s < 1.35$, n.s.).

In the simple effects analyses of the Fz and FCz electrode channels, the effect of Treatment on P200 latency did not vary per level of Stimulus (no Treatment * Stimulus interaction effect; $F_s < 1.39$, n.s.). The P200 latency of S2 was shorter compared to that of S1 (main effect of Stimulus; $F_s > 7.71$, $P_s < 0.05$), yet Treatment did not influence P200 latency at Fz and FCz (no main effect of Treatment; $F_s < 1.16$, n.s.). For the simple effects

analysis of the Cz electrode, the effect of Treatment varied per level of Stimulus (Treatment * Stimulus interaction effect; $F(3,54) = 3.09$, $P < 0.05$). Hence we did separate repeated measures analyses per level of Stimulus. For the data of the S1 stimulus, P200 latency was shown to be different between treatment conditions (main effect of Treatment; $F(3,54) = 4.23$, $P < 0.01$); post-hoc analysis showed that the P200 occurred later after biperiden ($P < 0.05$) and after the combination treatment ($P < 0.05$) compared to placebo. There were no effects in the analysis of the S2 data ($F(3,54) = 0.50$, n.s.).

Treatment effects on auditory processing of the standard stimuli in the novelty oddball task

Figure 4 shows the AEP responses to the standard, deviant and novel stimuli of the placebo condition, whereas Figure 5 displays the effects of the four treatment conditions on N100 latency of the standard stimuli in the novelty oddball task.

Figure 4. AEP responses to the standard, deviant and novel stimuli of the novelty oddball task (shown here: Cz electrode). Generally, deviant and novel sounds elicited larger amplitudes than standards ($P_s < 0.05$).

In the overall analysis of P50 amplitude of the standard stimuli, the effect of Treatment did not vary per level of Electrode (no Electrode * Treatment interaction effect; $F(6,108) = 0.32$, n.s.). P50 amplitude was shown to differ according to treatment conditions (main effect of Treatment; $F(3,54) = 5.43$, $P < 0.01$); however, post-hoc analysis did not yield any significant results.

The overall analysis of P50 latency demonstrated that the effect of Treatment did not vary per level of Electrode (no Electrode * Treatment interaction effect; $F(6,108) = 0.43$, n.s.). P50 latency was shown to be different between treatment conditions (main effect of Treatment; $F(3,54) = 3.02$, $P < 0.05$); post-hoc analysis showed that the P50 occurred later after biperiden compared to placebo ($P < 0.05$).

The repeated measures ANOVA of N100 amplitude of the standard stimuli did not yield any relevant effects ($F_s < 2.38$, n.s.). With regards to the overall analysis of N100 latency, the effect of Treatment did not vary per level of Electrode (no Electrode * Treatment interaction effect; $F(6,108) = 1.34$, n.s.). N100 latency was shown to differ according to treatment conditions (main effect of Treatment; $F(3,54) = 6.83$, $P < 0.01$); post-hoc analysis showed that the N100 occurred earlier after rivastigmine compared to placebo ($P < 0.05$).

The repeated measures analysis of P200 amplitude of the standard stimuli did not yield any relevant effects ($F_s < 1.43$, n.s.). The overall analysis of P200 latency demonstrated that

the effect of Treatment did not vary per level of Electrode (no Electrode * Treatment interaction effect; $F(6,108) = 1.20$, n.s.). P200 latency was shown to be different between treatment conditions (main effect of Treatment; $F(3,54) = 3.45$, $P < 0.05$); however, post-hoc analysis did not yield any Treatment effects compared to placebo.

Figure 5. Effects of the treatment conditions on N100 latency of the novelty oddball task (shown here: Fz electrode, means + SEM, *: $P < 0.05$). N100 latency was shorter after rivastigmine compared to placebo ($P_s < 0.05$); this effect was reversed by concurrent administration of biperiden (i.e., after the combination treatment N100 latency was statistically similar to placebo).

Treatment effects on change detection and novelty processing in the novelty oddball task

Figure 6 visualizes the deviant-standard and novel-standard difference waves of the novelty oddball task, whereas Figure 7 shows the effects of the treatment and stimulus difference scores on P3a amplitude in the novelty oddball task. In the simple effects analysis of the Fz electrode data, the effect of Treatment did not vary per level of Stimulus (no Stimulus * Treatment interaction effect; $F(3,54) = 0.14$, n.s.). The MMN amplitude of the Diff_{novel} wave was smaller compared to that of the Diff_{deviant} wave (main effect of Stimulus; $F(1,18) = 6.84$, $P < 0.05$), yet Treatment did not influence MMN amplitude (no main effect of Treatment; $F(3,54) = 1.20$, n.s.). The simple effects analyses of the FCz and Cz electrode channels did not yield any relevant effects on MMN amplitude ($F_s < 1.52$, n.s.).

There were no interaction effects in the overall repeated measures analysis of MMN latency ($F_s < 1.50$, n.s.). The MMN of the Diff_{novel} wave occurred later compared to that of the Diff_{deviant} wave (main effect of Stimulus; $F(1,18) = 30.51$, $P < 0.001$), yet Treatment did not influence MMN amplitude (no main effect of Treatment; $F(3,54) = 0.69$, n.s.).

The simple effects analyses of all three electrode channels (i.e., Fz, FCz and Cz) did not yield any interaction effects on P3a amplitude ($F_s < 0.22$, n.s.). The P3a amplitude of the Diff_{novel} wave was larger compared to that of the Diff_{deviant} wave (main effect of Stimulus; $F_s > 10.68$, $P_s < 0.01$). Furthermore, Treatment influenced P3a amplitude for Fz, FCz and Cz (main effect of Treatment; $F_s > 3.42$, $P_s < 0.05$); however, post-hoc analysis of the Fz and FCz electrodes did not yield any significant results. The post-hoc analysis of the Cz electrode demonstrated that P3a amplitude was increased after rivastigmine ($P < 0.05$).

Figure 6. Deviant-standard and novel-standard difference waves of the novelty oddball task (shown here: FCz electrode). The MMN amplitude of the novel-standard was smaller compared to that of the deviant-standard difference score (only at the Fz electrode; $P < 0.05$). Moreover, the MMN occurred later for the novel-standard compared to that of the deviant-standard difference score ($P < 0.001$). The P3a amplitude of the novel-standard was larger compared to that of the deviant-standard difference score ($P_s < 0.01$). There were no effects of the difference scores on P3a latency.

In the simple effects analyses of the Fz and Cz electrode channels, there were no interactions or main effects on P3a latency ($F_s < 2.36$, n.s.). For the FCz electrode, the effect of treatment on P3a latency did not vary per level of Stimulus (no Stimulus * Treatment interaction effect; $F(3,54) = 1.25$, n.s.). Furthermore, P3a latency was statistically similar between the Diff_{deviant} and Diff_{novel} wave (no main effect of Stimulus; $F(1,18) = 2.01$, n.s.). Treatment was found to affect P3a latency (main effect of Treatment; $F(3,54) = 3.00$, $P < 0.05$); however, post-hoc analysis did not yield any significant results.

Figure 7. Effects of the treatment conditions on P3a amplitude of the difference scores in the novelty oddball task (shown here: Cz electrode, means + SEM, *: $P < 0.05$). P3a amplitude was larger after rivastigmine compared to placebo ($P_s < 0.05$); this effect was reversed by concurrent administration of biperiden (i.e., after the combination treatment P3a amplitude was statistically similar to placebo).

Treatment effects on questionnaires

Table 3 shows the average difference scores (T_1-T_0) for the four treatment conditions on the POMS subscales. The analysis of the POMS data revealed that Treatment had an effect on the depression-dejection scale (main effect of Treatment; $F(3,54) = 7.36, P < 0.001$); post-hoc analysis showed that after biperiden, participants felt happier compared to placebo ($P < 0.01$). Moreover, Treatment also influenced the fatigue-inertia scale (main effect of Treatment; $F(3,54) = 20.59, P < 0.001$); post-hoc analysis showed that participants felt less tired after biperiden ($P < 0.001$) and after rivastigmine ($P < 0.01$) compared to the placebo condition. Furthermore, Treatment also affected the vigor-activity scale (main effect of Treatment); post-hoc analysis showed that participants felt less energetic after biperiden ($P < 0.01$) and after rivastigmine ($P < 0.01$) compared to the placebo condition. There were no Treatment effects on the POMS scales anger-hostility and tension-anxiety ($F_s < 2.09$, n.s.). Treatment also did not influence the Bond and Lader Alertness scale ($F(3,54) = 2.42$, n.s.) nor any of the self-report scales ($F_s < 2.71$, n.s.).

Table 3. Treatment effects on the difference scores (T_1-T_0) of the POMS subscales; means (+ SEMs). Significance asterisks reflect Treatment effects compared to the placebo condition.

POMS subscales	Treatment conditions			
	Placebo	Biperiden	Rivastigmine	Combination
Depression-Dejection	20.26 (12.77)	-34.37 ** (10.61)	-14.05 (10.34)	32.68 (9.30)
Anger-Hostility	51.37 (34.91)	-14.16 (11.06)	-15.47 (11.18)	-19.58 (26.50)
Fatigue-Inertia	47.84 (13.80)	-54.42 *** (12.85)	-32.53 ** (12.07)	71.11 (12.74)
Vigor-Activity	43.11 (11.83)	-49.47 ** (14.97)	-28.37 ** (12.94)	69.37 (17.30)
Tension-Anxiety	3.37 (8.82)	0.95 (11.04)	-1.95 (10.61)	0.68 (5.71)

Abbreviations and symbols: POMS: Profile of Mood States, **: $P < 0.01$, ***: $P < 0.001$

DISCUSSION

The goal of the current study was to assess the effects of biperiden and rivastigmine on sensory gating and MMN in young, healthy participants. In Table 4 an overview is given of the effects of both drugs on the various AEPs.

Paired-click task

For the paired-click task, both biperiden and the combination treatment enlarged the amplitude of the P50 peak (see Fig. 2). The latter is likely due to an underlying effect of biperiden, also given the fact that rivastigmine did not change the P50 relative to placebo. This would argue for a role of the muscarinic M_1 receptor in pre-attentive processing. Further-

more, P50 gating was unaffected by any of the treatments (i.e., no interaction between Treatment and Stimulus), which suggests that the effects of biperiden and the combination treatment on P50 amplitude were statistically similar for S1 and S2. These results were quite unexpected in light of our hypothesis stating reduced AEP peak amplitudes after biperiden and reversal by rivastigmine. Moreover, they are in contrast with studies on the muscarinic antagonist scopolamine which have reported a disappearance of the P50 and reversal by concomitant administration of a cholinesterase inhibitor (Buchwald et al., 1991), or a delay of the P50 (Pekkonen et al., 2005). However, a study by Pekkonen et al. (2001) which investigated the effects of scopolamine on a novelty oddball paradigm using MEG, also reported an enhancement of P50m amplitude. Furthermore, very modest effects of donepezil on P50 gating were reported by Buchanan et al. (2002).

Relating our findings to the P50 suppression deficits reported in schizophrenia is not very straightforward. Indeed, impairments of P50 gating have not been found consistently in the schizophrenia literature, which has been ascribed to differences in methodology and low test-retest reliability between studies (e.g., Jin et al., 1997; 1998; Kathmann & Engel, 1990). Furthermore, in contrast to the effects of biperiden, impaired P50 suppression (i.e., inflated S2/S1 ratio) in schizophrenia has generally been attributed to a reduction of the initial P50 response to S1. Some authors have argued that this decrement might be due to higher P50 latency variability (e.g., Jin et al., 1997; Patterson et al., 2000). However, this cannot explain our results of larger P50 amplitude after biperiden, as high temporal variability in P50 latency would cause a reduction in P50 amplitude. Arguably, effects of schizophrenia status and (anti-)cholinergic drugs on the P50 as reported in the literature are likely mediated by a muscarinic receptor subtype other than M_1 (i.e., M_2 - M_5), or alternatively, by nicotinic receptors (Adler et al., 1998). Of note, in aging, mild cognitive impairment and Alzheimer's disease, P50 amplitude is also progressively enhanced (Golob et al., 2001a; 2001b; Irimajiri et al., 2005; Thomas et al., 2010; but see Buchwald et al., 1989; Fein et al., 1994), which is in line with our current findings. This would suggest that the increment in P50 amplitude seen in healthy aging and age-related neurodegenerative disorders might mirror those seen after biperiden and hence, might be muscarinic M_1 -mediated.

An additional effect we found in the paired-click task was a delay in N100 latency after biperiden, which was reversed by concurrent treatment with rivastigmine (see Fig. 3). Rivastigmine by itself did not affect N100 latency in the paired-click task. These findings would suggest that the effect of biperiden on N100 latency is M_1 -mediated. Furthermore, it is likely that changes in neurotransmission at M_2 - M_5 receptors can interact with the effects of biperiden at the M_1 receptor, given the fact that rivastigmine was able to reverse the biperiden-induced delay in N100 latency. Our findings are in accordance with those reported on scopolamine (Pekkonen et al., 2001; 2005). Furthermore, a study by Simons et al. (2011) also demonstrated increased N100 latency in schizophrenia patients and their siblings, which the authors concluded might be regarded as a manifestation of familial liability for psychosis. Of note, delays in N100 latency are not specific for schizophrenia, as these have also been reported in aged participants (Iragui et al., 1993; but see Amenedo & Diaz, 1998). In contrast, in a previous study using a learned irrelevance (Lirr) task we found an enhancement of N100 amplitude after biperiden, and no effect on peak latency (see **Chapter 5**). We concluded that this effect was due to an increased allocation of attentional/behavioral switching resources. However, this involved a visual N100 which might partly explain the discrepancy in effects of biperiden between studies.

Table 4. Treatment effects on AEPs in the paired-click paradigm and novelty oddball task. A significance definition of $\alpha = 0.05$ was used for all analyses. Effects at specific electrode channels are stated in the Table; if no such statement is added, the particular effect was found at all electrode channels. In case of the AEP analysis of the standard stimuli in the novelty oddball task, we did not examine Stimulus effects (i.e., only one Stimulus type was included in the analysis). As for Treatment, there was a Treatment * Stimulus interaction in the analysis of P200 latency in the paired-click task. All other table entries reflect main effects of Treatment.

Paradigm	ERPs	Stimulus effects	Treatment effects		
		Saline	Biperiden	Rivastigmine	Combination
Paired-click	P50	Amplitude: S1 > S2 Latency: S1 > S2 (FcZ, Cz)	Amplitude ↑ (Fz)	=	Amplitude ↑
	N100	Amplitude: S1 > S2 Latency: S1 > S2	Latency ↑	=	=
	P200	Amplitude: S1 > S2 Latency: S1 > S2 (Fz, FCz)	Treat * Stim (Cz): S1: Latency ↑ S2: Latency =	=	=
Novelty oddball: standards	P50	N/A	Latency ↑	=	=
	N100	N/A	=	Latency ↓	=
	P200	N/A	=	=	=
Novelty oddball: difference scores	MMN	Amplitude: Diff _{deviant} > Diff _{novel} (Fz) Latency: Diff _{novel} > Diff _{deviant}	=	=	=
	P3a	Amplitude: Diff _{novel} > Diff _{deviant} Latency: =	=	Amplitude ↑ (Cz)	=

Abbreviations and symbols: Treat * Stim: Treatment * Stimulus interaction, MMN: mismatch negativity, N/A: not applicable, ↑: increase, ↓: decrease, =: not significant.

In the paired-click task biperiden induced a delay in P200 latency of the S1, but not S2 stimulus, which was reversed by concurrent administration of rivastigmine. Rivastigmine by itself did not affect P200 latency in the paired-click task. Based on our hypothesis which focused on P200 amplitude, we did not expect to find an effect of biperiden on latency. Thus, our finding is in contrast with studies reported no change in P200 latency after scopolamine (Pekkonen et al., 2005) or decreased P200 amplitude after scopolamine (Curran et al., 1998). However, as Simons et al. (2011) indicated, slowing of initial auditory processing might have downstream consequences for later peaks; i.e., delayed latency of the N100 component might also retard the occurrence of the P200. Again, the psychopathological

specificity for delayed P200 latency is low; besides schizophrenia and healthy siblings of schizophrenia patients (Karoumi et al., 2000; Ogura et al., 1991), the same finding has also been reported in aged volunteers (e.g., Bertoli et al., 2002; Iragui et al., 1993; but see Amedo & Diaz, 1998). Also, some studies have reported decreased P200 amplitudes rather than a latency effect in schizophrenia (e.g., Boutros et al., 2004; Shenton et al., 1989) or even no effects on the P200 component in schizophrenia patients (e.g., Karoumi et al., 2000).

Novelty oddball task

Biperiden induced a slowing of pre-attentive auditory processing, reflected by a delay in P50 latency which was reversed by concurrent treatment with rivastigmine. Rivastigmine by itself did not affect P50 latency in the novelty oddball task. These findings would suggest that the effect of biperiden on P50 latency is M_1 -mediated. Furthermore, it is likely that changes in neurotransmission at M_2 - M_5 receptors can interact with the effects of biperiden at the M_1 receptor, given the fact that rivastigmine was able to reverse the biperiden-induced delay in P50 latency. Pekkonen et al. (2005) also found a delayed auditory P50 after administration of scopolamine (but see Buchwald et al., 1991). Moreover, clinical studies usually focus on P50 amplitude, as P50 latency of schizophrenia patients is generally unchanged relative to normal controls (e.g., Bramon et al., 2004b). However, Jin et al. (1997) and Patterson et al. (2000) demonstrated a high temporal variability in P50 latency of the S1, but not S2 stimulus in schizophrenia patients. This variability contributed significantly to the averaged P50 amplitude and to the S2/S1 ratio used as a measure for sensory gating, and hence might partly underlie the usually reported finding of disrupted P50 gating in schizophrenia patients. Our results of delayed P50 latency after biperiden in the novelty oddball task suggest that the muscarinic M_1 receptor might underlie findings of high temporal variability in P50 latency in schizophrenia patients.

After rivastigmine the N100 peak to standard stimuli occurred earlier compared to the placebo condition (see Fig. 5). This finding was unexpected, as we anticipated that rivastigmine would mainly affect the P50 and P3a peaks. Studies on cholinesterase inhibitors have generally reported effects on components other than the N100, such as enhanced P50 suppression (e.g., Buchanan et al., 2002). Concurrent treatment with biperiden reversed the rivastigmine-induced effects, whereas biperiden by itself did not affect N100 latency in the novelty oddball paradigm. The fact that biperiden failed to influence N100 latency suggests an effect of rivastigmine which is mediated by a cholinergic receptor different than the M_1 ; i.e., M_2 - M_5 or nicotinic receptors. Rivastigmine enhances cholinergic signalling non-selectively, which means that nicotinic and/or muscarinic receptors other than M_1 might also instigate changes in the N100 component (Kadir et al., 2007; but see Inami et al., 2005). However, it is likely that M_1 -mediated neurotransmission can interact with the effects of rivastigmine, given the fact that biperiden was able to reverse the rivastigmine-induced decrease in N100 latency. Findings of delayed N100 latency in schizophrenia patients and their siblings (e.g., Simons et al., 2011) suggest that rivastigmine might be beneficial for alleviating deficits in attentional processing. This is in accordance with studies reporting beneficial effects of cholinesterase inhibitors on tests of attention and memory (Friedman, 2004; Schubert et al., 2006; but see Dyer et al., 2008; Friedman et al., 2002; Lee et al., 2007). There were no effects of biperiden, rivastigmine or the combination treatment on the P200 component.

Unexpectedly, we did not find an effect of biperiden or rivastigmine on the MMN, which suggests that muscarinic M_1 receptors are not involved in the frontal MMN component. Our findings are in contrast with pharmacological studies on cholinergic signalling and MMN. For instance, a MEG study by Pekkonen et al. (2001) reported a reduction of MMNm amplitude by the non-selective muscarinic antagonist scopolamine. However, this discrepancy might be partly explained by differences in sensitivity between MEG and EEG (e.g., Hämäläinen et al., 1993). Specifically, the MMN seen in MEG originates in the auditory cortex and occurs relatively earlier than the EEG-based MMN, which is mainly generated by the frontal cortex (Rinne et al., 2000).

In the novelty oddball task rivastigmine increased novelty processing, reflected by an enhancement of P3a amplitude of the difference wave at the Cz electrode (see Fig. 7). Concurrent treatment with biperiden reversed the rivastigmine-induced effects, whereas biperiden by itself did not affect P3a amplitude in the novelty oddball paradigm. The fact that biperiden failed to influence the P3a suggests an effect of rivastigmine which is mediated by a cholinergic receptor different than the M_1 , i.e., M_2 - M_5 or nicotinic receptors. However, it is likely that M_1 -mediated neurotransmission can interact with the effects of rivastigmine, given the fact that biperiden was able to reverse the rivastigmine-induced increase in P3a amplitude. Since this is the first study reporting effects of cholinergic drugs on P3a amplitude using a passive novelty oddball task, it is difficult to relate our findings to those found in the literature. There do exist a few studies on the effects of scopolamine which have reported a smaller P300 amplitude (Curran et al., 1998; Meador et al., 1989; but see Knott et al., 1999; Potter et al., 2000) and subsequent reversal by the cholinesterase inhibitor physostigmine (Meador et al., 1987). Others have demonstrated an effect of scopolamine on P300 latency (Meador et al., 1988; 1989; but see Knott et al., 1999), which could be (partially) counteracted by physostigmine administration (Meador et al., 1987; 1988). However, all of these studies used an active oddball paradigm, in which participants are instructed to give a behavioral response to target stimuli. Due to the higher attentional effort which must be paid, active oddball paradigms generally induce a parietal P3b reflecting context updating/working memory function, instead of a frontocentral P3a peak indicative of novelty/attentional processes (Comerchero & Polich, 1999; Donchin, 1981).

In schizophrenia, decreased P3a amplitude has been reported, which is generally interpreted as a reduction in attentional capacity, or alternatively, as a change in allocation of processing resources (Grillon et al., 1990; Turetsky et al., 2000). Again, the clinical specificity for P3a abnormalities is low; the P3a is also smaller and delayed in aged subjects (Fjell & Walhovd, 2004; Fjell et al., 2007; Knight, 1987; Pontifex et al., 2009). Findings of P3a abnormalities in schizophrenia and aging could be indicative of a common underlying frontal impairment sensitive to treatment by cholinesterase inhibitors.

Paired-click vs. novelty oddball task

We found several dissociations in terms of treatment effects between the two auditory paradigms used in the current study. Overall, it appears that the paired-click paradigm was relatively more sensitive to changes in muscarinic neurotransmission, especially those induced by biperiden. It is difficult to account for the discrepancies in treatment effects between the tasks. Paired-click paradigms present participants with a repetitive sequence consisting of uniform sounds and thus assess the sensory filtering of incoming stimuli which

are deemed uninformative. Novelty oddball tasks provide participants with stimuli which differ in terms of frequency of occurrence (i.e., deviants and novels) embedded in a string of familiar sounds (i.e., standards) and hence measure auditory deviation from familiarity. Although we chose to analyze treatment effects on the P50, N100 and P200 AEPs of just the standard stimuli in the novelty oddball task, the presence of infrequently occurring deviant or novel sounds might already change the auditory processing of the standards compared to a task which uses uniform stimuli (as was the case for the paired-click task). For instance, the presence of infrequent sounds in the novelty oddball task might have reduced the impact of sensory filtering mechanisms on the frequent stimuli, simply because participants show less habituation to these sounds. In short, although both paired-click paradigms and novelty oddball tasks employ auditory stimuli, they assess distinct sensory processes. Biperiden and rivastigmine have differential effects on the AEPs generated by these paradigms.

Questionnaires

With regards to the questionnaires, participants were found to be less energetic on the POMS after biperiden and rivastigmine (see Table 3). However, the POMS questionnaire also indicated that biperiden and rivastigmine made the volunteers feel less tired, and that biperiden made them even feel happier. This latter finding is in line with previous studies reporting an antidepressant effect of scopolamine (Furey & Drevets, 2006; Furey et al., 2010). In contrast, the Bond & Lader subscale we used, alert-drowsy, did not yield any treatment effects. Furthermore, our self-report questionnaire also included some questions pertaining to energy level of the participants (i.e., sleepiness, tiredness, drowsiness, listlessness). However, no changes in self-report complaints were noted after our treatment conditions. On basis of the questionnaire data it is therefore unclear whether the participants experienced any significant sedative side-effects. However, four participants dropped out of the study prematurely, so this cannot be excluded.

Conclusions

Biperiden increased P50 amplitude and prolonged N100 and P200 latency in the paired-click task. However, we presume that the delayed latency of later-occurring peaks (i.e., P200) is likely due to slowing of initial auditory processing after biperiden (i.e., N100). Rivastigmine was able to reverse the biperiden-induced delay in N100 and P200 latency in the paired-click task, but was unable to do so for the increment in P50 amplitude after biperiden. As for the novelty oddball task, biperiden increased P50 latency which was reversed by concurrent administration of rivastigmine. Rivastigmine shortened N100 latency and enhanced P3a amplitude, both of which were reversed by biperiden.

Overall, attributing specific treatment effects on AEPs to the muscarinic M_1 receptor is not very straightforward. The only exception is the effect of biperiden on pre-attentive processing (i.e., P50 amplitude) in the paired-click task, which was truly independent from that of rivastigmine. There also seems to be a role for the M_1 receptor in early-attentive mechanisms (i.e., N100 latency) in the paired-click task, and in pre-attentive processing (i.e., P50 latency) in the novelty oddball paradigm. However, reversal of effects of biperiden by concurrent rivastigmine treatment makes the evidence in favor of M_1 -mediation much more equivocal. Muscarinic receptors other than M_1 (i.e., M_2 - M_5) and nicotinic receptors

appear to be important for early-attentive (N100 latency) and novelty processing (i.e., P3a amplitude) in the novelty oddball paradigm. Yet again, reversal of effects of rivastigmine by concurrent biperiden treatment is not fully reflective of a role for M_2 - M_5 and/or nicotinic, but not M_1 receptors.

It remains to be seen whether the effects of cholinergic drugs on AEPs are specifically related to the abnormalities found in schizophrenia. Indeed, the usefulness of some of these markers as endophenotypes for schizophrenia has been questioned (e.g., Simons et al., 2011). Alternatively, aberrant auditory processing could also be indicative of a general disturbance in frontal functioning shared by several neuropsychiatric disorders and/or neurodegenerative changes seen in aging. Future experimental research should focus on determining whether there exists some degree of dissociation between muscarinic receptor subtypes in terms of their involvement in normal and pathological auditory processing. This would be beneficial for discovering specific trait markers of different neuropsychiatric disorders. Furthermore, new studies investigating the role of muscarinic receptors in auditory processing should look into dissociations between effects of muscarinic M_1 antagonists and cholinesterase inhibitors on redundancy suppression versus change detection. This can be done by experimentally varying the task parameters of paired-click and/or novelty oddball paradigms (e.g., stimulus intensity, duration, and/or pitch, inter-stimulus or inter-pair interval, stimulus frequency) to determine which aspects of these tasks are most sensitive to changes in muscarinic signaling.

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CHAPTER 8

General Discussion

AIM OF THE DISSERTATION

The main aim of the current dissertation was to investigate the role of the muscarinic M_1 receptor in cognition. To summarize and interpret the findings, the following questions were addressed: 1) Does biperiden induce selective effects on cognition? 2) Are effects of cholinergic drugs (i.e., biperiden and cholinesterase inhibitors donepezil and rivastigmine) comparable with respect to behavior vs. EEG? 3) Are effects of cholinergic drugs on cognition and EEG comparable in humans vs. rats? 4) How do effects of cholinergic drugs on cognition and EEG relate to findings in patient groups which are presumed to have cholinergic dysfunction (i.e., Alzheimer's disease and schizophrenia)? Of note, we chose to only refer to studies using scopolamine and/or biperiden. Discussion of muscarinic drugs other than these is considered beyond the scope of this dissertation, as differences in pharmacodynamic and pharmacokinetic properties might complicate interpretation of the outcome of different studies.⁴

SELECTIVITY OF BIPERIDEN

Behavior and cognition

In Tables 1 and 2, an overview is provided of the effects of biperiden in rats and humans, respectively. As delineated in **Chapter 2**, besides affecting learning and memory processes scopolamine can induce a wide range of effects on cognition and behavior. This is in line with the assumption that scopolamine induces non-selective, extensive blockade of muscarinic signaling throughout the brain and body – i.e., without much regard for muscarinic receptor subtype or brain region. In **Chapter 3** we have demonstrated that at a dose of 3 mg/kg (IP) biperiden can induce selective effects on working memory but not psychomotor responding, food motivation or attention in rats. In contrast, scopolamine had widespread effects on all of these measures.

In humans, it seems that biperiden displayed the most robust effects on memory (**Chapter 4**), but did not influence attentional parameters (i.e., LIRR; **Chapter 5**). Effects on self-report questionnaires and psychomotor performance were equivocal (**Chapters 4, 5 and 7**), but are in line with the view that biperiden induces only minor side-effects. Thus, findings so far indicate that biperiden has relatively more selective effects on cognition compared to scopolamine (i.e., on learning/memory but not attention, see **Chapters 4, 5 and Atri et al., 2004; Bahro et al., 1995; Bishop et al., 1996; Blin et al., 1994; Broks et al., 1988; Duka et al., 1996; Ellis et al., 2006; Fredrickson et al., 2008; Ghoneim & Mewaldt, 1975; Grasby et al., 1995; Green et al., 2005; Kamboj & Curran, 2006; Leopold & Comroe, 1948; Little et al., 1998; Mintzer & Griffiths, 2003; Thienel et al., 2009**).

⁴ In short, biperiden is the only selective M_1 antagonist approved for treatment of anti-psychotic-induced extrapyramidal symptoms in the Netherlands (e.g., Wezenberg et al., 2005). Besides biperiden, trihexyphenidyl, benztropine, and procyclidine also are available for anticholinergic treatment in the USA and are hence also the most commonly used drugs in the research literature. Of these four, biperiden exhibits the greatest relative specificity for M_1 compared to M_2 receptors, whereas affinity for M_3 and M_4 receptors is comparable between these drugs. Of note, the muscarinic antagonist pirenzepine is considered the most selective for muscarinic M_1 receptors. However, due to the fact that it does not readily cross the blood-brain barrier, its use is limited to central drug infusion in animal studies (Guthrie et al., 2000).

Taken together, effects of biperiden on cognition in rats and humans can be characterized as follows: 1) primary effects on learning/memory— which are likely mediated by blockade of postsynaptic muscarinic M_1 receptors in hippocampus (**Chapters 2, 3 and 4**), 2) non-specific central effects on psychomotor slowing – which are likely mediated via muscarinic M_1 -dopamine interactions in striatum (see **Chapters 2-5** and De Klippel et al., 1993; Gerber et al., 2001; Thomsen et al., 2010). Peripheral side-effects are limited compared to scopolamine – i.e., at low doses there are minor to no effects on salivation or sedation (**Chapters 2-5 and 7**, but see: Doods et al., 1987; Liang et al., 2010). Although these effects appear dissociable in our studies based on dose conditions, a review of the literature shows that this is not always the case (see Tables 1 and 2), and more effort is needed to corroborate our results. Findings of effects of biperiden on other central (e.g., attention, visuospatial processing, motor learning, prepulse inhibition, mood, anxiety, and sedation) or peripheral processes (e.g., pupil dilation, pain perception, body temperature, smooth muscle tissue) need replication and/or are equivocal (see Tables 1 and 2).

EEG

In **Chapter 6**, the effects of scopolamine, biperiden and the acetylcholinesterase inhibitor donepezil on hippocampal auditory evoked potentials (AEPs) and sensory gating in rats were investigated.⁵ Scopolamine and biperiden both disrupted sensory gating by enhancing N1 amplitude of the S2 stimulus. In contrast to biperiden, scopolamine also had several latency effects. Thus, scopolamine globally affected auditory processing, whereas effects of biperiden were relatively more selective (i.e., restricted to N1 sensory gating). Studies on effects of scopolamine on rat AEPs in the literature have generally reported reductions in peak amplitude of early positive, but not negative components (Luntz-Leybman et al., 1992; Miyazato et al., 1995; Teneud et al., 2000; but see Campbell et al., 1995), which might be due to differences in muscarinic binding affinity between scopolamine vs. biperiden (see **Chapters 2, 3 and 6**). Unfortunately, biperiden has only been tested quantitatively (i.e., on EEG frequency bands), which makes the study in **Chapter 6** the very first to describe effects of biperiden on AEPs in rats.

The studies described in **Chapters 5 and 7** are the very first to report effects of biperiden on ERPs in humans. In **Chapter 5** we have assessed the effects of biperiden on a Lrr task. Biperiden affected early perceptual processing, as it increased the N1 amplitude of preexposed (PE) predictor letters. In **Chapter 7**, we tested the effects of biperiden in a paired-click and novelty oddball paradigm. Again, biperiden affected early processing components.⁶ In contrast, scopolamine has been shown to affect both early and late ERP components in visual (Brandeis et al., 1992; Callaway et al., 1991; Donchin, 1981; but see Knott et al., 1999) and auditory paradigms. (Buchwald et al., 1991; Curran et al., 1998; Meador et al., 1987; but see Pekkonen et al., 2005).

Effects of biperiden on latencies of pre- and early attentional components (i.e., P50, N1) suggest that similar findings after scopolamine might be mediated by muscarinic M_1 receptor blockade (e.g., Pekkonen et al., 2005). However, amplitude effects of biperiden

⁵ For scopolamine vs. biperiden comparison purposes, we will put relatively less focus on interpretation of the effects of acetylcholinesterase inhibitors donepezil and rivastigmine. These will be discussed in the parts of the discussion pertaining to the behavior vs. EEG and human vs. rat comparisons.

⁶ The effect on P200 latency in the paired-click task in **Chapter 7** was interpreted as reflective of a downstream effect on the N100 (cf. Simons et al., 2011).

appear to be characterized by increments, whereas scopolamine generally induces decrements in ERP amplitudes in humans (Buchwald et al., 1991; Curran et al., 1998; but see Meador et al., 1987; Pekkonen et al., 2005). Lastly, effects of scopolamine on late ERP components (e.g., P200, P300) are likely due to blockade of muscarinic M_2 - M_5 receptors. A cognitive interpretation of these effects (i.e., reflective of memory vs. attention) will be provided in the sections below.

CHOLINERGIC DRUG EFFECTS: BEHAVIOR VS. EEG

In **Chapters 3 and 4**, we have seen that biperiden induced deficits in working memory in rats, and verbal learning and memory in human volunteers, respectively. Attentional processes – i.e., visual attention and Llrr; see **Chapters 3 and 5**, respectively – were shown to be unaffected in both species after biperiden. In contrast to the behavioral data, the EEG data suggests an effect of biperiden on pre- and early attentional processing (see **Chapters 5, 6 and 7**). This discrepancy in effects of biperiden on behavior vs. EEG could be explained by 1) exclusive effects of biperiden on attention, which underlie those on memory performance, 2) exclusive effects of biperiden on memory, whereas attentional effects on ERPs can be explained by an additional central or peripheral process which is affected, or 3) primary effects of biperiden on memory, and secondary on attention.

Findings of muscarinic M_1 receptors in a wide range of cortical areas (Caulfield, 1993) could serve as evidence corroborating the first hypothesis – i.e., mnemonic effects of biperiden on behavior can be explained by underlying effects on attention. However, the first hypothesis cannot account for the fact that we failed to find an effect of biperiden on attentional paradigms in **Chapters 3 and 5**, whereas disruptions of mnemonic functions after biperiden were relatively robust in both rats and humans in **Chapters 3 and 4**. Furthermore, in **Chapter 3** we have shown that the impairment in working memory after biperiden in rats could be characterized as delay-dependent, and that performance at the zero-delay was statistically similar to saline. Intact performance at the zero-delay but not at longer delays would indicate an exclusive effect of biperiden on memory, whereas an underlying deficit in attention after biperiden would likely give rise to a working memory impairment across all delays.

However, the possibility remains that our paradigms in **Chapters 3 and 5** were not sensitive enough to pick up attentional deficits after biperiden. As for the visual attention paradigm used for the rat study in **Chapter 3**, it was able to detect scopolamine-induced impairments, which would contradict this line of reasoning. However, underlying effects of scopolamine on psychomotor responding and/or food motivation could also have been responsible for the disruption in performance on the visual attention paradigm in **Chapter 3**. The Llrr task used in the human study described in **Chapter 5** was set up as a visual target detection paradigm. Theories about Llrr have focused on behavioral switching (Weiner, 2003), attention (Lubow, 2005; Schmajuk, 2005) and inhibition of redundant stimuli (Gluck & Myers, 1993). Hence, Llrr is thought of as a paradigm that measures attentional (or implicit associative learning) rather than mnemonic abilities. In sum, on basis of the findings in the current dissertation it is unlikely that the muscarinic M_1 receptor has a strong involvement in attention, but further studies are warranted.

Alternatively, the second hypothesis on the discrepancy of effects of biperiden on behavior vs. EEG emphasizes an exclusive effect of biperiden on memory, whereas attentional effects on ERP data are caused by an additional central or peripheral process. Scopolamine has been shown to induce blurry vision due to pupil dilation, and some studies have indicated that muscarinic M_1 blockade by biperiden might also affect ocular function (Almasieh et al., 2010; Gupta et al., 1994; but see Shiraishi & Takayanagi, 1993). Indeed, at 5 mg (IV) biperiden has been shown to cause blurred vision in healthy volunteers (e.g., Fleischhacker et al., 1987). However, our participants did not report visual impairments on the self-report questionnaires after biperiden in the studies described in **Chapters 4, 5, and 7**. Secondly, blurred vision would be likely to globally influence ERP peaks, and not just those belonging to one particular stimulus type (i.e., not only preexposed predictor letters, see **Chapter 5**). In addition, changes in ocular function after biperiden cannot explain why in **Chapters 6 and 7** we have also found changes in pre- and early attentional ERP components using auditory paradigms.

Since biperiden influences attentional ERPs across modalities, this would argue for the possibility of a central process which is affected. For instance, these could be caused by sedative effects of biperiden. However, this is also not very plausible, as participants usually did not report feeling tired or drowsy on our self-report questionnaires in **Chapters 4 and 5** (but see **Chapter 7**). Furthermore, reductions in arousal are associated with a decrease in N1 amplitude (Hillyard et al., 1973), which is in contrast with our findings in **Chapter 5**.

This line of reasoning leads us to the third hypothesis regarding the discrepancy between mnemonic effects of biperiden on behavior vs. attentional effects on ERP components: i.e., primary effects of biperiden on memory, and secondary on attention. This hypothesis would implicate that memory processes are more sensitive to disruption by biperiden and are hence already apparent at low doses at a behavioral level, whereas attentional functions are less easily affected, i.e., at low doses only effects on ERP components but not behavior. This argumentation would be consistent with the central distribution of the muscarinic M_1 receptor, which is present not only in the hippocampus, but also in cortical areas. Of note, our findings described in **Chapter 6**, i.e., deficits in hippocampal sensory gating after biperiden would argue against this line of reasoning. That is, muscarinic M_1 receptors in the hippocampus do not appear to be specifically involved in mnemonic processes.

CHOLINERGIC DRUG EFFECTS: HUMAN VS. RAT

With regards to our behavioral data, biperiden had fairly consistent effects in humans and rats, i.e., effects on memory but not attention (**Chapters 3 and 4**). However, our ERP findings were much less straightforward in terms of translational comparability. In human participants, biperiden enhanced the amplitudes and latencies of early perceptual peaks across modalities (**Chapter 5 and 7**), whereas in rats it only had amplitude effects in an auditory paradigm (**Chapter 6**). Moreover, in human volunteers biperiden did not influence sensory gating (**Chapter 7**), yet in rats we have shown a disruption of sensory gating after biperiden (**Chapter 6**). In the human study described in **Chapter 7**, concurrent intake of the cholinesterase inhibitor rivastigmine was able to undo only some of the effects of biperiden. In contrast, in the rat study in **Chapter 6** the cholinesterase inhibitor donepezil was

able to fully reverse the effects of biperiden on sensory gating. In humans, rivastigmine by itself affected both early and late AEP components (i.e., N100 and P3a; see **Chapter 7**), whereas in rats donepezil only affected early perceptual processing (i.e., N1; see **Chapter 6**).

Several methodological differences between these studies could account for the discrepancy in effects of biperiden on ERP components in humans vs. rats. First of all, EEG is measured in a distinctively different way between species. In humans, intermediate tissues – i.e., blood vessels, cerebrospinal fluid, brain meninges, skull and skin – all distort the EEG signal recorded at the scalp. Moreover, the EEG signal which is picked up by a single electrode likely reflects the sum of electrical field activity occurring synchronously in neurons which are located in cortical layers close to the scalp (Cacioppo et al., 2000). Given the relative thickness of the cortex in humans relative to that of rats, this implies that neuronal activity occurring in deeper regions such as the hippocampus is not readily picked up by scalp electrodes. That is, source localization with EEG would require a higher number of electrodes than the 32 channels we have used in our human studies (**Chapters 5 and 7**). In the rat study described in **Chapter 6**, we inserted electrodes locally in the dorsal hippocampus. Since the hippocampus is an important generator of sensory gating in both humans and rats (e.g., Bickford-Wimer et al., 1990; Bickford et al., 1993; Boutros et al., 2005; Grunwald et al., 2003; Krause et al., 2003; Williams et al., 2011), it might have been the case that the scalp electrodes used in our human studies were not sensitive enough to pick up biperiden-induced impairment of sensory gating and possible reversal of this deficit by acetylcholinesterase inhibitors (i.e., statistical type II error).

Of note, the doses we used were also very different between species; i.e., 3 mg/kg and 10 mg/kg induced behavioral impairments in rats (**Chapter 3**), and changes in AEP components were present after 1 mg/kg of biperiden (**Chapter 6**). In contrast, for our human studies we used a biperiden dose of 2 mg (**Chapters 4, 5, and 7**). Formulas used for the translation of drug doses from animals to humans are usually based on body surface area (see Fig. 1 and Reagan-Shaw et al., 2008). For instance, a human dose of 2 mg would be equivalent to 0.03 mg/kg (using an average human weight of 60 kg, also see Reagan-Shaw et al., 2008). Although this number is much lower than the 1 and 3 mg/kg biperiden doses we have used in rats, it does correspond well with the scopolamine doses of 0.01 and 0.03 mg/kg (**Chapters 3 and 6**).

In order to calculate the human doses which are equivalent to 1 and 3 mg/kg biperiden in rats (**Chapters 3 and 6**), we have used the conversion data for guinea pigs (see Table 1 in Reagan-Shaw et al., 2008). Namely, the weights of the rats in our study were more akin to those of guinea pigs (i.e., 400 grams for guinea pigs vs. 150 grams for rats, as listed in Reagan-Shaw et al., 2008). Using these data and the formula given below (formula for dose translation based on body surface area, taken from Reagan-Shaw et al., 2008), a rat dose of 1 mg/kg biperiden would be similar to a human dose of 0.21 mg/kg, which equals about 13 mg for an average human being (using an average weight of 60 kg, see Table 1 in Reagan-Shaw et al., 2008). A rat dose of 3 mg/kg biperiden would be equivalent to a dose of 0.65 mg/kg and hence approximately 39 mg for a human participant. These human equivalent doses of 13 and 39 mg are obviously much higher than the 2 mg of biperiden we have used in our human study, which would suggest that human participants, or alternatively, that human behavioral and EEG paradigms are relatively much more sensitive to the disruptive effects of biperiden.

$$HED(mg/kg) = animal\ dose\ (mg/kg) * \frac{Animal\ Km}{Human\ Km}$$

However, this formula does not take into account any species differences between humans and rats in terms of pharmacokinetics or pharmacodynamics. A variety of papers have assessed pharmacokinetic properties of scopolamine and biperiden (in rats: Yokogawa et al., 1990; 1992; Lenz et al., 2012; Nakashima et al., 1987; 1993; in humans: Ebert et al., 2001; Grimaldi et al., 1986; Guthrie et al., 2000; Hollmann et al., 1984; 1987). For example, in normal control rats (9-21 weeks old), serum concentrations of 1 mg/kg scopolamine (1 mg/kg, IV, half an hour after injection) have been shown to be between 80 - 100 ng/mL, whereas those of biperiden (3.2 mg/kg, IV, half an hour after injection) were found to be 400 - 600 ng/mL (Nakashima et al., 1993, and personal communication with J. Ishizaki, June 19th, 2012). Unfortunately, given the differences in dose conditions (in our studies we used 0.1, 0.3, and 1 mg/kg scopolamine and 1, 3, and 10 mg/kg biperiden) and route of administration (i.e., IP instead of IV injections), it is hard to relate these findings to those of our experiments (see **Chapters 3 and 6**).

The same holds for human studies on the pharmacokinetics of biperiden. For instance, in a study by Hollmann et al. (1987) participants who received a light breakfast and 4 mg instant-release tablets of biperiden, plasma concentrations were 3.48 ng/mL (biperiden base) 1h after intake (i.e., this is also the time at which we started testing in our human studies; see **Chapters 4, 5 and 7**). However, given that we have used a much lower dose of 2 mg, these plasma concentrations are not representative for our human experiments.

With respect to pharmacodynamics, several studies have assessed the binding of scopolamine and biperiden to muscarinic receptors in the rat and human brain (e.g., in rats: Ishizaki et al., 1998; Kimura et al., 1999; Nakashima et al., 1993; in humans: Sudo et al., 1999). For example, Nakashima et al. (1993) has shown that inhibition of [³H]-QNB binding by scopolamine (1 mg/kg, IV) and biperiden (3.2 mg/kg, IV) in the rat brain is highly comparable (albeit at lower doses; about a factor 3). Moreover, Ishizaki et al. (1998) demonstrated that drug concentrations in various brain regions in the rat decreased parallel to those in plasma after biperiden administration (3.2 mg/kg, IV). In a human study by Sudo et al. (1999) it has been found that after intake of biperiden (4 mg, PO), there was a good relationship between plasma concentration and muscarinic receptor occupancy in frontal cortex and striatum. Thus, in both species concentrations of biperiden in the periphery (i.e., blood plasma) appear to be reflective of those in the brain. However, there exist several differences between studies in the literature and our current experiments with respect to dose conditions (i.e., doses used in our studies are lower) and route of administration (PO instead of IV), which complicates interpretation.

In sum, biperiden had relatively consistent effects with respect to behavior in humans and rats, as it disrupted memory but not attentional processes. Regarding our EEG data, biperiden seemed to particularly affect early ERP components. We presume that differences in methodology, especially with respect to the sensitivity of the EEG equipment to pick up on hippocampal activity, underlie the discrepancies between human and rat studies. Furthermore, some progress has been made with respect to determining the pharmacodynamic and pharmacokinetic properties of scopolamine and biperiden in humans vs. rats. However, a direct comparison of plasma and brain concentrations at various doses of

scopolamine and biperiden and in several species is currently lacking, which is pertinent in order to be able to compare effective dose ranges used in translational research.

ROLE MUSCARINIC M_1 RECEPTOR IN NEUROPSYCHIATRIC DISORDERS

One of the aims of the current dissertation was to relate findings of cognitive dysfunction after biperiden to neuropsychiatric disorders known to exhibit aberrant cholinergic signaling, such as Alzheimer's disease and schizophrenia (Bartus et al., 1982; Sarter et al., 2012). This discussion will be split into three sections: memory, Lrrr, and auditory processing.

Memory

Memory deficits are common to both dementia and schizophrenia. Specifically, Alzheimer patients generally display lower scores on immediate and delayed recall trials, and higher forgetting rates (Estevez-Gonzalez et al., 2003; Greenaway et al., 2006). However, in Alzheimer patients an increase in only false positive errors has been reported with respect to word recognition (Greenaway et al., 2006). This is in contrast with our findings in **Chapter 4**, where we showed reduced recognition scores after biperiden regardless for old or new items— i.e., participants reported both more false positives (incorrectly identifying a new word as old) and false negatives (incorrectly labeling an old word as new).

Schizophrenia patients have also been shown to have impairments in word learning and recall (e.g., Hill et al., 2004). Evidence with respect to word recognition in schizophrenia patients is inconclusive, as both a higher rate of false positives (Weiss et al., 2004) but also false negatives (Jessen et al., 2003) has been reported. In sum, the reduction in recall scores after biperiden appears to be in line with findings of verbal memory impairment in both Alzheimer and schizophrenia patients. However, decreases in word recognition regardless for stimulus type (old vs. new) after biperiden does not reflect findings in the clinical literature.

In **Chapter 3** we demonstrated an impairment in working memory after biperiden in rats. Working memory deficits appear to be one of the core features of schizophrenia (e.g., Abi-Dargham et al., 2002; Manoach, 2003). However, a quite recent meta-analysis showed that delay periods beyond 1s did not worsen working memory deficits in schizophrenia patients (Lee & Park, 2005). This is in disagreement with our findings in **Chapter 3** where we have demonstrated a deterioration of working memory after biperiden which got progressively worse as an increasingly longer delay was interspersed between the sample and choice phase of the delayed non-matching to position (DNMTP) task. While this line of reasoning does not exclude the possibility of a role for the cholinergic system in working memory problems in schizophrenia, it does suggest a lack of involvement of the muscarinic M_1 receptor. In contrast, deficits in working memory in Alzheimer's disease have been reported previously (e.g., Baddeley et al., 1999), especially in early-onset Alzheimer patients (e.g., Kalpouzos et al., 2005). In sum, our result of a delay-dependent deficit in working memory performance after biperiden in rats is more in line with the findings in Alzheimer's disease rather than schizophrenia.

Learned irrelevance (Llrr)

In **Chapter 5** we have demonstrated that after biperiden human Llrr performance was still intact, showing that the muscarinic M_1 receptor is likely not involved in implicit learning. The only behavioral effect of biperiden was an overall increase in reaction time to the targets, regardless of the type of predictor letter preceding the target (RAN, PE, or NPE). With respect to the ERP data, biperiden increased the amplitude of the N1 component of the PE predictor letters, which we interpreted as an overcompensation of the irrelevant-to-relevant switch. Our findings of a lack of effect of biperiden on Llrr are in strong contrast with those in the literature reporting Llrr deficits in acute schizophrenics and a failure to learn the cue-target association in chronic schizophrenics (Gal et al., 2005; Orosz et al., 2008; Young et al., 2005).

Interestingly, one report by Kathmann et al. (2000) found results comparable to our own; i.e., in acute and stable, partially remitted schizophrenic patients, there was no impairment of latent inhibition – a phenomenon closely related to Llrr. Moreover, schizophrenic patients did show slower RTs compared to the normal, healthy controls which is in accordance with our biperiden data. In addition, N1 amplitudes of CS+ stimuli – which were conceptually similar to our PE predictor letters – were decreased in healthy controls, increased in acute schizophrenics and unchanged in partially remitted schizophrenics after pre-exposure. In sum, the findings of **Chapter 5** are only partially in line with those reported in the schizophrenia literature on Llrr and visual ERPs. Deficits in Llrr in schizophrenia are likely related to changes in dopaminergic (e.g., Slabosz et al., 2006), rather than cholinergic signaling.

Auditory processing

Most studies investigating sensory gating in schizophrenia have reported impaired P50 suppression due to a reduction of the initial P50 response to S1 (e.g., Jin & Potkin, 1996; but see Jin et al., 1997; Patterson et al., 2000). These reports are thus in contrast with our findings of an enhancement of P50 amplitude but no effects on sensory gating after biperiden in human volunteers (**Chapter 7**), or with our results of disrupted sensory gating due to an enhancement of N1 amplitude at the S2 click after biperiden in rats (**Chapter 6**). However, in aging and age-related disorders such as mild cognitive impairment and Alzheimer's disease, an increase in amplitudes of early AEP components (Golob et al. 2001a; 2001b; Irimajiri et al., 2005) and reduced sensory gating due to an increase in P50 amplitude of the S2 click have also been reported (Cancelli et al., 2006; Jessen et al., 2001; Thomas et al., 2010; but see Buchwald et al., 1989; Fein et al., 1994).

In the human study in **Chapter 7**, we have shown that biperiden delayed the latencies of both early positive and negative peaks (i.e., N100 latency in the paired-click task and P50 latency in the novelty oddball paradigm). In schizophrenia, it seems that only early negative components are slowed (i.e., delayed latencies of N100, but not P50: Bramon et al., 2004; Karoumi et al., 2000; Ogura et al., 1991; Pekkonen et al., 2001; 2005), whereas aged participants and people with mild cognitive impairment display delayed latencies of early processing peaks regardless for polarity (Bertoli et al., 2002; Golob et al., 2001a; Iragui et al., 1993; but see Amenedo & Diaz, 1998). In sum, our data are in line with a role for muscarinic M_1 signaling in cognitive impairments seen in aging and age-related disorders rather than schizophrenia. Moreover, neurodegenerative changes in muscarinic M_1 signaling giv-

ing rise to early perceptual/attentional processing deficits may be early indicators of dementia.

Directions for future research

Future research should try to scrutinize the selective effects of biperiden compared to scopolamine, especially with respect to findings of mnemonic but not attentional deficits after biperiden. Given that low doses of biperiden are capable of affecting early perceptual/attentional ERP components across modalities, it would be interesting to determine whether higher doses of biperiden might be able to also induce attentional impairments at a behavioral level. However, at higher dose levels biperiden might have the tendency to also induce more peripheral side-effects, thereby complicating the interpretation of behavioral data. Moreover, it is pertinent that biperiden is tested in memory paradigms along with recording of EEG, to assesses whether it would influence mnemonic ERP components such as the P300 (particularly the P3b: Polich & Criado, 2006).

In this respect, the development of ligands which are more selective for a particular muscarinic receptor subtype would be an important step in determining muscarinic receptor modulation of cognition. However, this has been challenging due to the highly conserved orthosteric acetylcholine binding site of muscarinic receptors (Bolden et al., 1992; Heinrich et al., 2009). Drugs that act at allosteric sites might provide a better means to selectively disrupt or enhance muscarinic M_1 signaling (e.g., Digby et al., 2012; Mohr et al., 2004; Ragozzino et al., 2012). However, these might activate or inhibit different regulatory pathways compared to drugs binding to orthosteric receptor sites (Davis et al., 2009) and have generally not yet been approved for use in human research. The question remains how we can link cognitive effects of partially selective muscarinic M_1 antagonists to blockade of a particular muscarinic receptor subtype in a particular brain region. An additional way to tackle this issue is to examine effects of selective muscarinic M_2 - M_5 antagonists, and to combine animal and human psychopharmacological studies with central drug infusion and/or imaging methods (EEG, fMRI). For instance, the effects of scopolamine on attentional processes are likely mediated via muscarinic receptors other than M_1 . Hence, selective muscarinic M_1 - M_5 antagonists need to be perceived as additional pharmacological tools besides the golden standard scopolamine to elucidate muscarinic receptor modulation of cognition and behavior.

Table 1. Effects of peripheral administration of biperiden on behavior and cognition in animals. All doses are in mg/kg.

Experimental paradigm	Species	Doses	Route	Min. effective dose	Max. ineffective dose	Effect	References
Serial-probe recognition	Rhesus monkeys	0.001, 0.01, 0.03, 0.1, 0.3, 0.56, 1.0	IM	0.3	1.0	Accuracy ↓ Completed trials =	(Myers et al., 2002)
Passive avoidance	Rats (W)	0.1, 1.0, 10	SC	0.1		Training Step-down latency ↓ Retention test (1, 3, 5, or 7 days after training) Step-down latency ↓ Response to footshock =	(Kimura et al., 1999)
	Rats (W)	2.0, 4.0, 8.0, 16	IP	4		Passive avoidance retention ↓	(Roldan et al., 1997)
Object exploration	Rats (W)	0.11	IP	0.11 0.11		Preference for novel object = Preference for novel smell = Locomotor activity ↓ Rearing ↓	(Myhrer et al., 2008)
Operant responding	Rats (W)	1.0, 3.0, 10	IP	10		FR5 Inter-response time ↑ PR10 Breakpoint = Inter-response time = AT Percentage correct = Percentage omissions = Response time = Sensitivity Index = Index Y = DNMTP Percentage correct ↓ Response time = Sensitivity Index ↓ Index Y ↑	Chapter 3
Multiple FR 10-response, DRL 10s schedule	Rats (SD)	0.125, 0.25, 0.5, 1.0, 2.0	SC	0.25 0.25		Reinforcement rate ↓ Non-reinforced responses ↑	(Liu, 1996)
Conditioned place preference	Mice (C57BL6/J)	0.1, 1.0, 10	IP	10		Time spent in saline-paired compartment = Time spent in cocaine-paired compartment ↓ Locomotion =	(Ramos et al., 2012)
	Mice (C57BL6/J)	10	IP	10		Time spent in cocaine-paired compartment ↓ Time spent in non-paired compartment =	(Zacarias et al., 2012)

Experimental paradigm	Species	Doses	Route	Min. effective dose	Max. ineffective dose	Effect	References
Cocaine-induced behavioral sensitization	Rats (W)	0.125, 0.5	IP	0.125	0.5	Time spent in biped-paired compartment = Time spent in biped+morphine-paired compartment ↑ Time spent in biped+haloperidol-paired compartment =	(Allahverdiyev et al., 2011)
	Mice (C57BL6/J)	10	IP	10	10	Saline-paired locomotion = Cocaine-paired locomotion ↑	(Ramos et al., 2012)
	Rats (SD)	0.1–10	IP			100 dB noise pulses Peak startle amplitude = Latency to peak startle amplitude ↓ Prepulse inhibition = 120 dB noise pulses Peak startle amplitude = Latency to peak startle amplitude = Prepulse inhibition ↓	(Sipos et al., 2001)
Prepulse inhibition	Rats (SD)	0.3, 1.0, 3.0, 10	SC	1.0	10.0	Prepulse inhibition = Startle amplitude ↓	(Jones, 2000)
Circling behavior	Pig tailed macaque	0.1, 0.32	IM	0.1 0.1		Contraversive circling ↑ Ipsiversve circling ↑	(Domino & Ni, 2008)
Locomotor activity	Rats (SD)	0.1–10	IP	- (ED ₅₀ = 1.18) - (ED ₅₀ = 1.30)		Locomotor activity ↑ Fine motor activity ↑	(Sipos et al., 1999)

Symbols: = : no change, ↑ : increase, ↓ : decrease, - : not mentioned in article. Abbreviations: AT: attention task, DNMTP: delayed non-matching to position, FR: fixed ratio, IM: intramuscular, IP: intraperitoneal, PO: per os (oral), PR: progressive ratio, SC: subcutaneous, SD: Sprague-Dawley strain, W: Wistar strain.

Table 2. Effects of biperiden on behavior and cognition in humans. All doses are in mg.

Experimental paradigm	Doses	Route	Effect	References
Selective reminding task	4	PO	Number of words recalled ↓ Consistent long-term retrieval ↓ Delayed recall ↓	(Guthrie et al., 2000)
Episodic memory	2	PO	Word list Immediate recall ↓ Delayed recall ↓ Recognition ↓ Picture memory Simple visual memory = Self-ordered retrieval =	(Wezenberg et al., 2005)
Chapter 4				
Verbal Learning Task				
	2	PO	Immediate recall ↓ Delayed recall ↓ Recognition ↓ Reaction time =	
Working memory tasks	2	PO	1-back task Movement time to reach target = Distance covered to reach target ↑ 2-back task Movement time to reach target = Distance covered to reach target = SDRT STM number correct = STM response time ↑ LTM number correct = LTM response time ↑	(Wezenberg et al., 2005)
Forward and backward digit span	4	PO	Forward digit span = Backward digit span ↓	(Guthrie et al., 2000)
MMSE	2	PO	MMSE score ↓	(Liang et al., 2010)
Llrr	2	PO	Llrr =Reaction time ↑	Chapter 5
Visuospatial tasks	2	PO	Tangled lines Number correct = SDST Number correct = Matching time ↑ Writing time =	(Wezenberg et al., 2005)

Experimental paradigm	Doses	Route	Effect	References
Bech-Rafaelson Mania Scale	5	IV	15min. after injection Mood ↑ 6h after injection Mood ↑	(Fleischhacker et al., 1987)
Motor learning	2	PO	Maze task Number of loops = Pursuit task Number of rotations = Time per loop ↑	(Wezenberg et al., 2005)
Finger-tapping speed	4	PO	Speed =	(Guthrie et al., 2000)
Cardiac response to standing	4	PO	Ratio of longest to shortest R-R interval ↑ (vagotonic effect)	(Guthrie et al., 2000)
Pupil size	4	PO	Instant release tablet Fasting overnight Pupil size ↑ Breakfast 1h before administration Pupil size ↑ Slow release tablet Fasting overnight Pupil size ↑ Breakfast 1h before administration Pupil size =	(Hollmann et al., 1987)
	4	PO	Pupil size ↑ (2, 4, and 8h after administration)	(Grimaldi et al., 1986)
	4	IV	Pupil size ↑ (0.5, 1, and 2h after administration)	(Grimaldi et al., 1986)
	4	PO	Pupil size ↑	(Hollmann et al., 1984)
Visual accommodation	4	PO	Instant release tablet + breakfast 1h before administration Visual accommodation ↑ Slow release tablet + breakfast 1h before administration Visual accommodation =	(Hollmann et al., 1987)
	4	PO	Visual accommodation ↑	(Hollmann et al., 1984)
Salivation	2	PO	Drooling Rating Scale Score ↓	(Liang et al., 2010)
	4	PO	Salivation ↓	(Guthrie et al., 2000)
	4	PO	Instant release tablet + fasting overnight Salivation ↓ Slow release tablet + fasting overnight Salivation =	(Hollmann et al., 1987)
	4	PO	Salivation =	(Grimaldi et al., 1986)

Experimental paradigm	Doses	Route	Effect	References
	4	IV	Salivation ↓ (0.5 and 1h after administration)	(Grimaldi et al., 1986)
Neurophysiological sedation	2	PO	Peak velocity of saccadic eye movements =	(Wezenberg et al., 2005)
Behavioral sedation	2	PO	Simple reaction time = Choice reaction time =	(Wezenberg et al., 2005)
Subjective sedation	2	PO	Bond & Lader Scale Alertness = Calmness = Contentedness =	(Wezenberg et al., 2005)
Visual analog scales	4	PO	Sedation ↑ Dizziness ↑ Dry mouth =	(Guthrie et al., 2000)
Complaints questionnaires	4	PO	Instant release tablet + breakfast 1h before administration Fatigue ↑ Drowsiness ↑ Dizziness ↑ Dry mouth = Headache =	(Hollmann et al., 1987)
			Weariness ↑ Fatigue ↑ Dizziness ↑	(Hollmann et al., 1984)
	2	PO	Complaints =	Chapters 4, 5, 7
POMS	2	PO	Tension-anxiety = Depression-dejection = Anger-hostility = Fatigue-inertia = Vigor-activity =	Chapter 5
	2	PO	Tension-anxiety = Depression-dejection ↓ Anger-hostility = Fatigue-inertia ↓ Vigor-activity ↓	Chapter 7
Bond & Lader	2	PO	Alert-drowsy =	Chapter 5
	2	PO	Alert-drowsy =	Chapter 7

Symbols: = : no change, ↑ : increase, ↓ : decrease. Abbreviations: IV: intravenous, Lirr: learned irrelevance LTM: long-term memory, MMSE: Mini Mental State Examination, PO: per os (oral), SDRT: Symbol Digit Recall Test, SDST: Symbol Digit Substitution Test, STM: short-term memory

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SUMMARY

One of the main research themes in psychology and neuroscience is cognitive dysfunction, which usually occurs as an epiphenomenon of brain diseases and/or neuropsychiatric disorders. Unfortunately, patient heterogeneity makes it difficult to correlate cognitive deficits with specific neurobiological findings. Therefore, a lot of effort is dedicated to mimicking cognitive impairments with pharmacological tools that temporarily impact neurotransmission in healthy animals and young volunteers (Gilles & Luthringer, 2007).

The non-selective cholinergic (i.e., muscarinic) antagonist scopolamine has been employed extensively as a standard/reference drug to induce age- and dementia-related impairments in cognitive function. The guiding principle for its use has been the cholinergic hypothesis of geriatric memory dysfunction, which assumes that the age-related decline in cognitive functions is predominantly related to a decrease in the integrity of cholinergic neurotransmission (Bartus et al., 1982). However, an exclusive role for acetylcholine in geriatric memory dysfunction can be viewed as too restrictive, as cholinergic impairments have also been noted in schizophrenia (Sarter et al., 2012). Thus, the question arises whether aberrant cholinergic signaling might be a common neuropathological pathway underlying aging, dementia and schizophrenia, and whether administration of cholinergic (i.e., muscarinic) antagonists might hence serve as a model for cognitive impairments associated with these disorders.

Chapter 2 has provided an overview of the effects of scopolamine on animal behavior. It appears that behavioral paradigms which assess stimulus discrimination and/or (sustained) attention processes are relatively more sensitive to impairments induced by the muscarinic antagonist scopolamine compared to tasks which measure learning/memory functions (i.e., effects at relatively lower doses). These findings have spurred a lot of debate on the specific role of acetylcholine in cognition – i.e. on learning/memory vs. attention (Hasselmo, 1999; 2006; Hasselmo et al., 2002; Hasselmo & McGaughy, 2004; Sarter et al., 2003; 2005; 2009). It is concluded that effects on learning and memory performance which are observed after higher doses of scopolamine are mediated by 1) primary effects on attention and sensory/stimulus discrimination, 2) non-specific effects on behavior (locomotor activity, anxiety), and 3) peripheral side-effects (pupil dilation, salivation). Finally, the validity of scopolamine as a pharmacological model for cognitive impairment is questionable, as its non-selectivity with regards to muscarinic receptors complicates interpretation of behavioral findings. On the basis of the distribution of the muscarinic M_1 receptor in the brain and body (Caulfield, 1993), muscarinic M_1 antagonists are put forward as a more selective and effective way of inducing cholinergic deficits.

The study in **Chapter 3** investigated in rats whether the muscarinic M_1 receptor antagonist biperiden would induce more selective effects on a battery of operant tasks: i.e., fixed ratio and progressive ratio schedules of reinforcement, an attention paradigm and delayed non-matching to position task. Scopolamine was found to disrupt performance in all behavioral paradigms. Biperiden induced relatively more selective deficits; it had no effect on food motivation or attention, and effects on psychomotor performance and working memory were dissociable based on dose conditions (i.e., 10 mg/kg and 3 mg/kg, respectively). It was concluded that muscarinic M_1 antagonists such as biperiden should be considered as a pharmacological model for cholinergic mnemonic deficits in animals, due to their selectivity with regards to mnemonic effects.

The goal of the study in **Chapter 4** was to assess the selectivity of the muscarinic M_1 antagonist biperiden with regards to verbal memory, psychomotor performance and self-reports of side-effects in young, healthy human volunteers. After biperiden, participants recalled on average about 4 words less on the immediate and delayed recall of the verbal learning task. Word recognition scores in the verbal recognition task were reduced by 8% after biperiden. Biperiden did not influence reaction times in a verbal recognition task or a choice reaction time task, which is indicative of a lack of psychomotor effects. There was also no effect of biperiden on accuracy in the choice reaction time task, nor did participants report experiencing any side-effects. It was concluded that biperiden is capable of impairing verbal memory rather selectively, i.e., without inducing clear peripheral side-effects which could adversely affect performance.

The study in **Chapter 5** investigated the effects of the muscarinic M_1 antagonist biperiden on a within-subject learned irrelevance (Llrr) paradigm and event-related potentials (ERPs) in humans. Llrr refers to a reduction in associative learning after pre-exposure of the conditioned (CS) and unconditioned stimulus (US) in a non-contingent fashion (Baker, 1976; Mackintosh, 1973). This paradigm might serve as a translational model for (pre)attentive information processing deficits in schizophrenia controls (Gal et al., 2005; Orosz et al., 2011; Young et al., 2005). Given the involvement of the cholinergic system in the pathophysiology of schizophrenia (Sarter et al., 2012), it was hypothesized that biperiden would disrupt Llrr performance. Unexpectedly, biperiden had no effect on the behavioral Llrr measures, although prolonged reaction times were evident. The N1 amplitude of the pre-exposed (PE) predictor letters was increased after biperiden, suggesting an effect of this drug on early perceptual processing. In conclusion, the within-subject paradigm used in the current study in combination with ERPs can reveal brain mechanisms involved in Llrr. M_1 antagonism does not appear to be involved in Llrr deficits as reported in schizophrenia.

The study described in **Chapter 6** assessed the effects of the non-selective muscarinic antagonist scopolamine, the muscarinic M_1 antagonist biperiden, the cholinesterase inhibitor donepezil and their combination on auditory evoked potentials (AEPs) and sensory gating in rats. As perturbations in auditory filtering appear to be a candidate trait marker of schizophrenia (Cadenhead et al., 2000; Olincy et al., 2010; Simons et al., 2011), there has been considerable interest in the development of translational rat models to elucidate the underlying neural and neurochemical mechanisms involved. Scopolamine and biperiden both disrupted sensory gating by increasing N1 amplitude for the S2 click. Scopolamine also affected the latencies of the P1, N1 and P2 peaks. Donepezil was able to fully reverse the effects of biperiden on N1 sensory gating, but had residual effects when combined with scopolamine (i.e., enhancement of sensory gating). Donepezil by itself improved sensory gating by increasing N1 amplitude of S1, and reducing N1 amplitude of the S2 click. In conclusion, due to its relatively more selective effects, biperiden is to be preferred over scopolamine as a means for pharmacologically inducing impairments in auditory processing in healthy rats.

The goal of the study in **Chapter 7** was to investigate the effects of the muscarinic M_1 antagonist biperiden and the cholinesterase inhibitor rivastigmine on AEPs, sensory gating and mismatch negativity (MMN) in young, healthy volunteers. Suppression of redundant auditory information and facilitation of deviant, novel or salient sounds can be assessed with paired-click and oddball tasks, respectively (Boutros et al., 2004; Garrido et al., 2009; Näätänen et al., 2004; Pekkonen et al., 2005). Electrophysiological correlates of perturbed

auditory processing found in these paradigms are likely to be a trait marker for schizophrenia (Cadenhead et al., 2000; Olincy et al., 2010; Price et al., 2006; Simons et al., 2011). Biperiden increased P50 amplitude and prolonged N100 and P200 latency in the paired-click task.⁷ Rivastigmine was able to reverse the effects of biperiden on N100 and P200 latency. Biperiden increased P50 latency in the novelty oddball task, which was reversed by concurrent administration of rivastigmine. Rivastigmine shortened N100 latency and enhanced P3a amplitude in the novelty oddball paradigm, both of which were reversed by biperiden. It was concluded that the muscarinic M₁ receptor appears to be involved in pre-attentive processing of auditory information.

Taken together, effects of biperiden on behavior in rats and humans can be characterized as follows: 1) primary effects on learning/memory— which are likely mediated by blockade of postsynaptic muscarinic M₁ receptors in the hippocampus (**Chapters 2, 3 and 4**) and 2) non-specific central effects on psychomotor slowing – which are likely mediated via muscarinic M₁-dopamine interactions in striatum (see **Chapters 2-5** and De Klippel et al., 1993; Gerber et al., 2001; Thomsen et al., 2010). Peripheral side-effects are limited compared to scopolamine – i.e., at low doses there are minor to no effects on salivation or sedation (**Chapters 2-5 and 7**).

With respect to ERPs, biperiden generally induces increments in the amplitudes of early perceptual components (i.e., N1 in rats, and P50 and N100 in humans, see **Chapters 6 and 7**). Thus, effects of biperiden on behavior appear to be focused on mnemonic functions, whereas those on ERPs seem to be more related to attentional processes. This discrepancy is possibly due to primary effects of biperiden on memory, and secondary on attention. It appears that muscarinic M₁ modulation of cognition is complex and dependent on brain region.

We have also found some different findings between species with respect to our EEG studies – i.e., disruption of hippocampal sensory gating in rats vs. a lack of effect of biperiden on human sensory gating. We presume that differences in methodology, especially with respect to the sensitivity of the EEG equipment to pick up on hippocampal activity, underlie the discrepancies between species. Furthermore, some progress has been made with respect to determining the pharmacodynamic and pharmacokinetic properties of scopolamine and biperiden in humans vs. rats. However, a direct comparison of plasma and brain concentrations at various doses of scopolamine and biperiden and in several species is currently lacking, which is pertinent in order to be able to compare effective dose ranges used in translational research.

In contrast to our findings in **Chapters 6 and 7**, most studies investigating sensory gating in schizophrenia have reported impaired P50 suppression due to a reduction of the initial P50 response to S1 (Jin & Potkin, 1996). However, in aging and age-related disorders such as mild cognitive impairment and Alzheimer's disease, an increase in amplitudes of early AEP components (Golob et al., 2001a; 2001b; Irimajiri et al., 2005) and reduced sensory gating due to an increase in P50 amplitude of the S2 click has also been reported (Cancelli et al., 2006; Jessen et al., 2001; Thomas et al., 2010). Therefore, changes in auditory processing and sensory gating induced by muscarinic drugs may serve as a translational model for aging rather than schizophrenia.

⁷ The effect on P200 latency in the paired-click task in **Chapter 7** was interpreted as reflective of a downstream effect on the N100 (cf. Simons et al., 2011).

Future research should replicate/confirm the selective effects of biperiden compared to scopolamine, especially with respect to findings of mnemonic but not attentional deficits after biperiden. Moreover, it is pertinent that biperiden is tested in memory paradigms along with recording of EEG, to assess whether it would influence mnemonic ERP components such as the P300 (particularly the P3b: Polich & Criado, 2006). In this respect, the development of ligands which are more selective for a particular muscarinic receptor subtype would be an important step in determining muscarinic receptor modulation of cognition. However, this has been challenging due to the highly conserved orthosteric acetylcholine binding site of muscarinic receptors (Bolden et al., 1992; Heinrich et al., 2009). Nevertheless, selective muscarinic M_1 - M_5 antagonists need to be perceived as additional pharmacological tools besides the golden standard scopolamine to elucidate muscarinic receptor modulation of cognition and behavior.

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ABBREVIATIONS

A:	albino	Llrr:	learned irrelevance
AEP:	auditory-evoked potential	LSD:	least significant difference
AT:	attention task	LTM:	long-term memory
Bip:	biperiden	LTP:	long-term potentiation
CRF:	continuous reinforcement	MMN:	mismatch negativity
CRT:	choice reaction time task	MMSE:	Mini Mental State Examination
CTA:	conditioned taste aversion	NMTP:	non-matching to position
CS:	conditioned stimulus	Nov:	novel stimuli
D:	Druckray strain	NPE:	non-preexposed
Dev:	deviant stimuli	NZ:	New Zealand white albino rabbits
DNMTP:	delayed non-matching to position	PDA:	pigmented dark agouti
Dpz:	donepezil	PE:	preexposed
DRL:	differential reinforcement of low rate	PO:	per os, oral
EDS:	extradimensional shift	PR:	progressive ratio
ERP:	event-related potential	RAN:	random
F344:	Fischer-derived F344 strain	RT:	reaction time
FI:	fixed interval	Sal:	saline
Fig.:	Figure	SC:	subcutaneous
FR:	fixed ratio	Scop:	scopolamine
H:	Hooded strain	SD:	Sprague-Dawley strain
HBS:	Hooded Blue Spruce strain	SDRT:	Symbol Digit Recall Test
HM:	Holtzman strain	SDST:	Symbol Digit Substitution Test
IDS:	intradimensional shift	SI:	sensitivity index
IM:	intramuscular	Stand:	standard stimuli
IP:	intraperitoneal	STM:	short-term memory
IV:	intravenous	SW:	Swiss Webster strain
KM:	Kunming strain	US:	unconditioned stimulus
KW:	Kuo:Wistar strain	VLT:	verbal learning task
L:	Lewis strain	VRT:	verbal recognition task
LB:	London Black strain	W:	Wistar strain
LE:	Long-Evans (Hooded) strain	WC:	White Carneaux pigeons
LH:	Lister Hooded strain	WR:	Wistar-derived Walter Reed strain

CURRICULUM VITAE

Inge Klinkenberg was born on December 18, 1983 in Kerkrade. In 2002, she graduated from secondary school (VWO) at College Rolduc in Kerkrade. In September 2002, she started studying General Health Sciences at Maastricht University, but after a few months switched to Psychology. After obtaining her Bachelor's degree in Biological Psychology in 2005, she was admitted to the Research Master track Neuropsychology. During the completion of her Master thesis she worked as a student assistant at Maastricht University. In March 2008, she received her Master's degree in Neuropsychology with honors and started working as a PhD student in the department of Neuropsychology and Psychopharmacology at Maastricht University. During her PhD she had the opportunity to visit the preclinical CNS Research lab at F.Hoffmann-La Roche where she attained additional experience with behavioral testing.

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