# PHARMACOLOGICAL AND GENETIC MODULATION OF EPISODIC-LIKE MEMORY IN THE MOUSE

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## *Zusammenfassung*

Die vorliegende Dissertation basiert auf vier Originalarbeiten, die in internationalen, begutachteten Zeitschriften veröffentlicht wurden. Die Arbeit befasst sich mit der Anwendung einer kürzlich entwickelten human-analogen episodischen Gedächtnisaufgabe in pharmakologischen und neurogenetischen Mausmodellen. Des Weiteren wurde die Rolle von NMDA-Rezeptoren, Neurokinin-3 Rezeptoren und Histamin 1 Rezeptoren als neurobiologische Grundlage für episodisches Gedächtnis untersucht. Neuropsychologische Untersuchungen von amnestischen Patienten sowie die Applikation verschiedener Lern- und Gedächtnistests bei Nagern legten schon früh die Vermutung eines existierenden, multiplen Gedächtnissystems nah. Dem episodischen Gedächtnissystem wird hierbei eine besonders wichtige Stellung eingeräumt. Episodische Gedächtnisleistungen sind höchst anfällig gegenüber Alterungsprozessen und zeigen defizitäre Veränderungen bei einer Reihe von neuropsychiatrischen Erkrankungen. Es ist daher essentiell ein human-analoges Tiermodell für episodisches Gedächtnis zu entwickeln, um auf diese Weise Erkenntnisse über die neurobiologischen Grundlagen dieses Gedächtnissystems zu gewinnen sowie mögliche therapeutische Interventionen zur Behandlung von episodischen Gedächtniseinbußen entwickeln zu können. Diese Arbeit beschreibt die exemplarische Anwendung eines kürzlich entwickelten human-analogen episodischen Gedächtnisparadigmas für Nager in pharmakologischen und neurogenetischen Studien. Die human-analoge episodische Gedächtnisaufgabe stellt

eine Kombination aus verschiedenen Versionen des Neuigkeitspräferenz-Paradigmas dar. Der Nachweis für episodische Erinnerung im Tier wird aus dessen Explorationsverhalten abgeleitet, das wiederum die Erinnerung an Inhalt („Was“ für ein Objekt wurde präsentiert), den räumlichen Kontext („wo“ wurde ein Objekt dargeboten) und den Zeitpunkt („Wann“ wurde ein Objekt dargeboten) einer Episode widerspiegelt. In Experiment 1 und 2 konnten wir demonstrieren, dass die pharmakologische Modulation des NMDA Rezeptors durch die gedächtnisfördernde Wirkung der Substanz DCS (Veröffentlichung #1), sowie die die pharmakologische Modulation des Neurokinin 3 Rezeptors durch die Substanz Senktide (Veröffentlichung #2), episodische Gedächtnisleistungen unter suboptimalen, interferenzanfälligen Lernbedingungen verbessern können. In Experiment 3 wurde die human-analoge episodische Gedächtnisaufgabe bei der Verhaltensphänotypisierung von Histamin 1 Rezeptor defizienten Mäusen angewandt. Die Ergebnisse deuten darauf hin, dass die H1R Knockoutmäuse Einbußen bei der Erinnerung an die zeitliche Sequenz der Objektpräsentation aufweisen. Weiterhin zeigen die H1R Knockoutmäusen Beeinträchtigungen bei der Erinnerung an den räumlichen Kontext der Objektpräsentation. Die Performanz der H1R Knockoutmäuse in dieser Gedächtnisaufgabe scheint eher auf familiaritätsbedingten als auf episodischen Erinnerungsprozessen zu beruhen (Veröffentlichung # 3). Im nächsten Schritt wurden die H1R Knockoutmäuse in weiteren Lern- und Gedächtnisaufgaben sowie in der neuigkeits-induzierten konditionierten Platzpräferenz untersucht, um zu überprüfen ob die

Beeinträchtigung in der episodischen Erinnerungsleistung ein selektives Defizit oder eher das Abbild einer generellen Beeinträchtigung der Gedächtnisfunktion in H1R Knockoutmäusen darstellt (Veröffentlichung #4). Zusammenfassend bestätigen die vorgestellten Arbeiten, dass die human-analoge episodische Gedächtnisaufgabe ein valides Paradigma ist, mit dem neue Substanzen gesichtet oder die genetischen Grundlagen des episodischen Gedächtnisses in der Maus untersucht werden können.

## *Abstract*

This cumulative doctorate thesis is based on 4 original research articles that have been published in international peer-reviewed journals. This thesis has two major topics. The first topic is the application and evaluation of the recently developed episodic-like memory task in pharmacological and neurogenetic research in the mouse. The second topic is the investigation of the role of NMDA-receptors, neurokinin-3 receptors and histamine-1 receptors for episodic memory function.

The neuropsychological assessment of amnesic patients as well as animal research using laboratory rats and mice has led to the notion of multiple memory systems in the brain. Here, episodic memory is especially important because it is highly sensitive to ageing and disease. Therefore, it is essential to develop a rodent animal model of episodic memory in order to investigate the neurobiology of episodic memory in health and disease as well as to evaluate possible therapeutic strategies. In this work, the exemplary application of a recently devised mouse model of human episodic memory in pharmacological and neurogenetic studies is described. The episodic-like memory task combines different versions of the novelty-preference paradigm. Episodic-like memory is inferred from exploratory behaviour which indicates the remembrance of the content ("what" kind of object was presented), place ("where" was this object placed) and temporal context ("when" was the object presented) of an episode. In experiments 1 and 2 we showed that the pharmacological modulation of NMDA-receptor function by the cognitive enhancer

d-cycloserine (Publication #1) and the stimulation of neurokinin 3-receptors by senktide (Publication #2) can both promote episodic-like memory formation under sub-optimal interference prone learning conditions. In experiment 3 we used the episodic-like memory task for the cognitive phenotyping of genetically engineered histamine H1-receptor deficient mice. The H1R-KO mice failed to show a memory for the temporal order in which objects have been presented. They also failed to remember where these objects have been encountered. The performance of the H1R-KO mice can be characterized as familiarity-based memory as opposed to episodic-like memory (Publication #3). In order to know whether this deficit reflects a general memory impairment not selective for episodic memory we next investigated several other types of learning and memory in these mice including novel-objects induced conditioned place-preference (Publication #4). In sum one can state that the episodic-like memory task for mice is indeed a suitable behavioural tool that can be used for both pharmacological and genetic studies in mice that might accelerate the discovery of therapies for episodic memory deficits in humans.

This cumulative dissertation is based on the following original research reports, which have been published in international peer-reviewed journals:

**Publication #1**

**Zlomuzica, A.**, De Souza Silva, M.A., Huston, J.P., Dere, E. (2007). NMDA-receptor modulation by D-cycloserine promotes episodic-like memory in mice. ***Psychopharmacology,*** 193, 503-509.

**Publication #2**

**Zlomuzica, A.**, Dere, E., Huston, J.P., De Souza Silva, M.A. (2008). The selective NK-3R agonist senktide promotes episodic-like memory in mice. ***Neurobiology of Learning and Memory***. In press.

**Publication #3**

Dere, E., **Zlomuzica, A.**, Viggiano, D., Watanabe, T., Sadile, A.G., Huston, J.P., De Souza-Silva, M.A. (2008). Episodic-like and procedural memory impairments in histamine H1R-KO mice coincide with changes in acetylcholine esterase activity in the hippocampus and dopamine turnover in the cerebellum. ***Neuroscience***. In revision.

**Publication #4**

**Zlomuzica, A.**, Viggiano, D., De Souza-Silva, M.A., Ishizuka, T., Gironi Carnevale, U.A., Ruocco, L.A., Watanabe, T., Sadile, A.G., Huston, J.P., Dere, E. (2008). The histamine H1-receptor mediates the motivational effects of novelty. ***European Journal of Neuroscience,*** 27, 1461-1474.

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# 1 Abbreviations

| | |
|---|---|
| 5-HIAA | 5-Hydroxyindole Acetic Acid |
| 5-HT | Serotonin |
| AchE | Acetylcholinesterase |
| CPP | Conditioned Place Preference |
| DCS | D-cycloserine |
| DOPAC | Dihydrophenylacetic Acid |
| ELM | Episodic-like Memory |
| EPSP | Excitatory Postsynaptic Potentials |
| H1R | Histamine 1 Receptor |
| H1R-KO | Histamine 1 Receptor Knockout |
| H2R | Histamine 2 Receptor |
| H3R | Histamine 3 Receptor |
| H4R | Histamine 4 Receptor |
| HDC | Histidine-Decarboxylase |
| HK-1 | Hemokinin 1 |
| NKA | Neurokinin A |
| NKB | Neurokinin B |
| NK-R | Neurokinin Receptor |
| NK-R1 | Neurokinin 1 Receptor |

| | |
|---|---|
| NK-R2 | Neurokinin 2 Receptor |
| NK-R3 | Neurokinin 3 Receptor |
| NMDA-R | N-Methyl-D-Aspartate-Receptor |
| TBS | Tris Buffered Saline |
| TH | Tyrosine Hydroxylase |
| WT | Wild Type |

# 2 Introduction

## 2.1 Animal Episodic Memory

The concept of episodic memory was formulated in the early seventies by Endel Tulving. The recollection of attended events in terms of their content, place and temporal context is known as episodic memory (Tulving, 2002). In other words episodic memory is about remembering what happened, as well as where and when it happened. In contrast, the concept of semantic memory refers to the knowledge of rules or facts which do not have a spatio-temporal connotation (Squire et al., 2004; Vargha-Khadem et al., 1997). In humans, deficits in episodic memory are observed in the course of normal aging (Burke & Mackay, 1997), after lesions to the medial temporal lobe which includes the hippocampus, and after lesions to diencephalic structures, such as the mediodorsal thalamus and the mammillary bodies, as well as the frontal cortex (Aggleton & Brown, 1999). Deficits in episodic memory are also found in a variety of neurodegenerative and psychiatric diseases such as Alzheimer`s disease (Small et al., 2003). Against this background it is desirable to have a valid animal model of episodic memory in order to evaluate possible therapeutic strategies.

It has long been held that episodic memory is unique to humans, because it was accepted that animals lack autonoetic awareness/conciousness and the ability to subjectively sense time (Tulving & Markowitsch, 1998; Tulving, 2002). These

assumptions are currently being challenged (Aggleton & Pearce, 2001; Clayton et al., 2001; Dere et al., 2006; Morris, 2001). Evidence has accumulated which indicates that various animal species, indeed, show behavioral manifestations of phenomenological and objective features of episodic memory, such as e.g. metacognition, conscious recollection, mental time travel, temporal order memory, as well as the simultaneous remembering of the what, where and when elements of unique events (for a review, see Dere et al., 2006). The initial demonstration of episodic-like memory (ELM) in food-caching birds has received much attention in the neuroscience community and was of great importance for the understanding how cognitive functions have evolved (Clayton & Dickinson, 1998). Nevertheless, some authors suspected that the behaviour of the scrub jays was merely the result of a species-specific genetic program that lacks flexibility and generalization to other information categories aside from remembering the location of food caches (Dere et al., 2006). It is possible that the capacity to remember what, where and when elements of past episodes in these food-storing birds only evolved under evolutionary pressures unique to these birds and their ecological nice, leading to the generation of memory systems that are build solely for the encoding, storage and retrieval of food caching episodes. Generally a major disadvantage of the ethological approach in developing animal models of human episodic memory is that it pretty much relies on species-specific behaviours and capacities. In this regard it is clear that only the demonstration of memory for what, where and when in rodents for stimuli, which have no natural significance for the animal and which have never been paired with a reinforcer, would allow the

definitive conclusion that ELM is a capacity of a wider variety of vertebrates. The modelling of different prerequisites and features of human episodic memory in rats and mice has led to the definition of objective behavioural criteria by which different features of episodic memory can be operationalized experimentally and assessed in both animals and humans (Aggleton & Pearce, 2001; Dere et al., 2006; Morris, 2001).

In order to test whether rodents have the capacity to recall personal experiences in terms of what happened, as well as where and when it happened to serve as an animal model for episodic memory, different versions of the novelty-preference paradigm have been combined into a single task (Kart-Teke et al., 2006, 2007). Since rodents are known to discriminate familiar and novel objects (Ennaceur & Delacour, 1988), can detect whether a familiar object is presented in a novel or familiar location (Dix & Aggleton, 1999) and are able to discriminate in which temporal order the objects have been presented (Hannesson et al., 2004), it was asked whether they are able to perform these discriminations simultaneously. It has been reported that rats and mice are able to integrate or bind what, where and when information into an unified ELM (Dere et al., 2005a, 2005b; Kart-Teke et al., 2006). Furthermore, it has been shown that acute stress impaired the animal's performance in the ELM task, which, however, could be partially reversed by the N-Methyl-D-aspartate-receptor (NMDA-R) agonist d-cycloserine (DCS) (Kart-Teke et al., 2006) and the neurokinin 1 receptor (NK-1R) antagonist SR140333 (Kart-Teke et al., 2007). In sum one can state that the different versions of the novelty-preference task can be successfully combined to measure the remembrance of the content, place and temporal context of

single experiences and that our ELM task is suitable to perform genetic, lesions and pharmacological studies. The present work examined the utility of a recently developed mouse model of human episodic memory in pharmacological (Zlomuzica et al., 2007, 2008a) and neurogenetic studies (Dere et al., 2008a) and provided the first information of the role of NMDA-Rs, neurokinin-3 receptors (NK-3Rs) and histamine 1 receptors (H1Rs) in episodic memory function. These 3 receptor classes have been selected because they are among the most promising points of attack to develop cognitive enhancers that can be used as a medication in patient populations suffering from early stages of dementia.

## 2.2 The N-Methyl-D-Aspartat Receptor

The NMDA-R is an ionotropic glutamate receptor. The activation of this receptor is regulated relatively complex. Beside other factors, receptor activation depends on both the binding of glutamate and glycine to separate binding sites, as well as on a concurrent membrane depolarisation that removes the magnesium block of the ion-channel of the receptor (Lynch, 2004). Since the activation of the receptor depends on both pre-synaptic activity and post-synaptic cell depolarisation, the NMDA-R has been proposed to function as a co-incidence detector involved in Hebbian-types of synaptic plasticity (Martin & Morris, 2002). Since episodic memory requires the association of multi-modal stimuli, the fact that NMDA-Rs are involved in associative or heterosynaptic plasticity is especially important. Associative or

heterosynaptic long-term potentiation is induced by the concurrent stimulation of a weak and a strong synaptic input to a postsynaptic neuron leading to a strengthening of the weaker one. Consequently NMDA-Rs have been implicated in the automatic encoding of one-trial event–place associations (Morris, 2006). It has been proposed that NMDA-Rs are well-suited to associate multiple features of an event to represent an episode or event. It is possible that the establishment of episodic memories is based on a mechanism similar to associative or heterosynaptic plasticity implemented by NMDA-Rs.

DCS is a partial agonist at the glycine binding site on the NMDA-R and facilitates NMDA-R mediated responses (Johnson & Ascher, 1987). DCS has promnestic effects in rodents (Land & Riccio, 1999; Pussinen & Sirvio, 1999). It reverses memory deficits after anticholinergic treatment (Ohno & Watanabe, 1996) and after septal (Riekkinen et al., 1998) and hippocampal lesions (Schuster & Schmidt, 1992). DCS also antagonizes aging-induced learning impairments (Baxter et al., 1994) and was effective in combating cognitive deficits in Alzheimers disease. In rats, DCS ameliorated a stress-induced deficit in ELM but was not able to fully reverse the deficit (Kart-Teke et al., 2006). Therefore, it was not clear whether DCS only antagonized the detrimental effects of stress on ELM or whether it acted as a true ELM enhancer. This question was addressed by using an experimental protocol designed to detect promnestic drug effects of DCS and other drugs on ELM.

## 2.3 The Neurokinin Receptor

The neuropeptide substance P is a neurotransmitter and/or neuromodulator, which has been implicated in synaptic plasticity (Langosch et al., 2005), learning and memory (Hasenöhrl et al., 2000) and the reinforcement of behaviour (Nikolaus et al., 1999). Substance P stimulates NK-R's, and has its highest affinity for the NK-1R (Hokfelt et al., 2001; Quartara & Maggi, 1998). In rats, the NK-1R has been detected in the neocortex, hippocampal formation, basal forebrain, amygdala and brainstem (Mantyh et al., 1989).

The inactivation of the NK-1R gene in mice had only modest effects on learning and memory performance (Morcuende et al., 2003). NK-1R knockout (KO) mice showed normal trace fear conditioning and a moderate improvement in the water maze task. In rats, the NK-1R antagonist SR140333 had modest facilitative effects on inhibitory avoidance performance and habituation to a novel open-field. Intraperitoneal injection of SR140333 increased extracellular acetylcholine levels in the hippocampus (Kart et al., 2004) and reinstated stress-induced deficits in ELM in rats (Kart-Teke et al., 2007).

Neurokinin A (NKA), neurokinin B (NKB), and hemokinin (HK)-1 are, as Substance P, also mammalian endogenous neuropeptides belonging to the tachykinin family, shown to be present in peripheral tissue and the brain (Helke et al., 1990). Three subtypes of the G-protein-coupled NK-Rs, the NK-1Rs, NK-2Rs and NK-3Rs mediate the effects of these endogenous neuropeptides. While Substance P predominantly

stimulates to the NK-1Rs, the NKA and NKB have higher affinities for the NK-2Rs and NK-3Rs, respectively. HK-1 has high selectivity and potency for NK-1Rs, but also binds to NK-2Rs and NK-3Rs. The NK-1- and NK-3Rs are the most common tachykinin receptors in the mammalian brain (Bellucci et al., 2002; Severini et al., 2002). In rodents, the NK-3R is strongly expressed in brain regions which have been implicated in explicit or declarative memory, such as the frontal cortices, the hippocampus and amygdala (Ding et al., 1996; Duarte et al., 2006; Shughrue et al., 1996).

Senktide is a potent and selective NK-3R agonist (Laufer et al., 1986; Massi et al., 2000) and antagonizes scopolamine-induced spatial working memory impairments in the spontaneous alternation Y-maze task when administered intracerebroventricularly to mice (Kameyama et al., 1998; Ukai et al., 1998). Senktide might have promestic effects via interactions with the cholinergic system. The brains acetylcholine system is known to be involved in basic attentional and motivational processes that are critical for normal learning and memory performance (Pepeu & Giovannini, 2004). Interestingly, the intra-septal infusion of senktide induces acetylcholine release in the hippocampus of guinea pigs (Marco et al., 1998). More than 66% of cholinergic neurons in the medial septum/diagonal band of Broca, which are known to project to the hippocampus, express NK-3Rs (Chen et al., 2001). Moreover, it has been shown that NKB, which is the major endogenous ligand of NK-3Rs, protects against NMDA-induced neurotoxcity of basal forebrain cholinergic cells (Wenk et al., 1997). These and other lines of research suggest that the activation

of the NK-3Rs might have a neuroprotective effect on cholinergic neurons. It is well known that the cholinergic neurons of the basal forebrain are an important substrate for attentional and motivational aspects of memory function, and their dysfunction has been linked to dementia due to Alzheimer's disease. One of the first cognitive symptoms of Alzheimer's disease is the loss of episodic memory (Small et al., 2003). Given that the loss of cholinergic neurons is a cardinal symptom of Alzheimer's disease and is positively correlated with the severity of cognitive symptoms (Mufson et al. 2003; Oliveira Jr. & Hodges, 2005), substances that stimulate NK-3Rs such as senktide could be considered as a potential medication for early stage Alzheimers disease.

## 2.4 The Histamine 1 Receptor

Neuronal histamine is derived from the nucleus tuberomammillaris (TM) of the posterior hypothalamus (Haas & Panula, 2003). Histamine synthesis is catalysed by the enzyme histidine-decarboxylase (HDC) converting histidine to histamine. To date two postsynaptic receptors (H1R and H2R) and one presynaptic autoreceptor (H3R) have been identified in the rodent brain. In peripheral tissues a fourth histamine receptor (H4R) was recently identified (Liu et al., 2001). These receptors differ in terms of pharmacology, localization and cellular transduction processes. Neuronal histamine has been implicated in processes subserving learning and memory; however the available data are highly controversial. Histamine facilitated

(Kamei et al., 1993) and suppressed active avoidance conditioning (Alvarez & Banzan, 1996). The HDC-blocker a-FMH both improved (Sakai et al., 1998) and impaired spatial memory in a radial-maze task (Chen et al., 1999). Furthermore, H1R antagonism improved water-maze (Hasenöhrl et al., 1999) and impaired radial-maze performance (Taga et al., 2001), whereas learning and memory in H1R knockout (H1R-KO) mice in the tests performed (inhibitory avoidance and transfer latencies in the plus-maze) was unaffected (Yanai et al., 1998a, 1998b). Contradictory results were also found with agents acting at H2R and H3R (Blandina et al., 1996; Blandina et al., 2004; Flood et al., 1998; Onodera et al., 1998). Finally, lesions and temporarily inactivation of the TM region improved habituation learning, inhibitory avoidance, discrimination and water maze learning in adult and aged rats (Frisch et al., 1998a, 1998b; Frisch et al., 1999). Research on the foundations of neuronal histamine has been hampered by the lack of specific pharmacological tools. In the past H1R-KO mice have been generated by means of homologous recombination (Inoue et al., 1996). Given the low selectivity of conventional methods including lesion studies to modulate histaminergic activity, the opportunity to test genetically modified animals such as the H1R-KO mice in learning and memory tasks might prove useful to clarify the role of brain histamine in learning and memory and especially in episodic memory. These H1R-KO mice (Inoue et al., 1996) exhibited a complex cognitive phenotype including both impairments and improvements in a variety of learning and memory tasks (Dai et al., 2007). Patients suffering from Alzheimer's disease show deficient episodic memory, while their procedural memory remains relatively

intact (Sabe et al., 1995; Small et al., 2003). Beside the most prominent brain pathologies in Alzheimer's disease, including the loss of cholinergic neurons, extracellular amyloid plaques and intracellular neurofibrillary tangles, Alzheimer's disease patients also show changes in the brains histamine system (Mazurkiewicz-Kwilecki & Prell, 1984; Mazurkiewicz-Kwilecki & Nsonwah, 1987). For example, Alzheimer's disease patients show alterations in brain histamine levels (Cacabelos et al., 1989; Mazurkiewicz-Kwilecki & Nsonwah, 1989; Panula et al., 1998) and a loss of histaminergic neurons in the nucleus tuberomammillaris (Airaksinen et al., 1991; Nakamura et al., 1993; Saper & German, 1987). Tacrine, a widely prescribed Alzheimer drug, blocks the catabolic activity of histamine-N-methyltransferase, which metabolizes histamine to telemethylhistamine, and, in consequence increases hippocampal histamine levels (Morisset et al., 1996; Nishibori et al., 1991).

# 3 Methods

The main behavioral and neurochemical methods used in the experiments for this thesis are briefly described in the following sections. For additional methods used and a full description of methods and experimental procedures please see the original publications (Dere et al., 2008; Zlomuzica et al., 2007, 2008a, 2008b).

## 3.1 The Episodic-like Memory Task

### 3.1.1 A Protocol for detecting Amnestic Effects of Experimental Manipulations

The test apparatus was a square open-field arena. Two visual cues were fixed on the east and north wall of the maze for spatial orientation. All objects were made of plastic and were available in triplicate copies and varied in terms of surface texture, colour and shape, but were of similar height. The objects were always placed in the corners of the open-field at a distance of 0.5 cm from the side walls. The mice were first subjected to three daily 10-min open-field habituation trials. On the fourth day, animals received two 10-min sample trials, followed by a test trial of 5-min duration. The inter-trial interval between the 3 trials was 50 min. The open-field was virtually divided into nine squares by 2 x 2 parallel lines. The central square was not used for object placement. For each animal, four out of eight squares were randomly chosen to position the four copies of the "old familiar" object in the first sample trial.

The second sample trial was identical to the first, except that four copies of another "recent familiar" object was present. Two copies of the "recent familiar" object were randomly placed onto positions that had been occupied in the first sample trial, and two copies were positioned in new positions, that were randomly chosen from the remaining four peripheral positions. In the test trial (trial 3), two copies of both objects were present in either stationary or displaced positions, i.e. one of the copies of each object was presented in a position encountered in the respective sample trial, i.e. sample trial 1 ("old familiar-stationary" object) or sample trial 2 ("recent familiar-stationary" object). The remaining objects were presented in new positions ("old familiar-displaced", and "recent familiar-displaced"). All four objects were placed onto positions previously encountered in the sample trials (Kart-Teke et al., 2006, 2007).

The following behaviors were scored during the sample trials and the test trial: The cumulative time spent (s) exploring the individual objects. Exploration of an object was assumed when the mouse approached an object and had physical contact with it, either with its snout and/or forepaws. Sitting next to the object or leaning against the object while exploring the wall of the open-field was not considered as object exploration. The behavioral parameters were scored by an experienced observer, who was blind with respect to the mice's genotype. Object exploration was scored semi-automatically using the EthoVision tracking system (Noldus, The Netherlands), run under the `manually record behaviors´ option.

### 3.1.2 A Protocol for detecting Promnestic Effects of Experimental Manipulations

Each mouse was subjected to a session of three trials (two learning trials and a test trial) with an inter-trial interval of 5 min. In the first sample trial (sample 1), mice were exposed to a novel object (A) located in a randomly selected corner of the open field. In the second sample trial (sample 2), mice again were exposed to a novel object (B), which was placed in a randomly selected corner different from the one used in sample trial 1. The sample trials 1 and 2 were terminated either after 15 min, or when the mouse had accumulated 40 s of object exploration. On the test trial, two identical copies of object A (A1 and A2) and two copies of object B (B1 and B2) were placed in the corners of the open field. One copy of object A and object B was placed in the same corner, in which it was already placed during the corresponding sample trial (A1 and B1), while the other copy of the objects A and B was placed in a corner in which it was not presented during the sample trials (A2 and B2). The order of object presentation, as well as the object location, was determined randomly for each mouse. Animals that did not accumulate a minimum of 20 s of object exploration during the sample trials were excluded from the data analysis.

## 3.2 Procedural Motor Learning and Long-Term Memory

Motor coordination was tested with an accelerating rotarod. The rotating rod was elevated 10 cm off the floor, had an axis diameter of 3.5 cm and a striated surface made of black rubber. During the acquisition phase, each mouse was given 3 trials (with an inter-trial interval of 10 - 25 min to control for possible effects of physical exhaustion) per day for three consecutive days. After a retention delay of 7 days the animals were given another 3 trials with the same inter-trial-interval as during the acquisition phase. The mouse was placed on the inactive drum, which thereafter was accelerated to a speed of 40 g over a period of 5 min. The mouse had to move forward on the drum, which was rotating with increasing speed along its vertical axis in order to avoid falling off. As some mice tend to passively ride around the rod, especially at higher velocities, the duration (s) of active performance until the mouse fell off the drum was registered with a cut-off after 300 s.

## 3.3 Novel-Objects induced Conditioned Place-Preference

The conditioned place-preference (CPP) apparatus was placed in a sound-attenuating cubicle and was made of white painted Plexiglas. During the conditioning phase a partition wall, without an opening, was inserted into the apparatus that divided the space into two compartments that were identical in terms of size, light intensity, floor texture, odour and colour. A prominent visual cue was

attached to the closing wall of one compartment, allowing the animals to discriminate between the two compartments. Each animal was subjected to 10 trials on 10 consecutive days, and with one trial per day. On Day 1 a baseline trial of 10 min duration was given. Here, the partition separating the two compartments was removed, allowing the animal to freely move between the compartments. During the conditioning trials on Days 2–9, the animal was placed in an alternating manner either in the center of the novel object paired compartment or in the blank compartment for 10 min. The novel object-paired compartment was randomly determined for each mouse and contained an identical pair of novel objects, which were placed in the corners opposite to the partition wall. A particular object was only presented on one of the four novel object-paired compartment trials. The sequence of the presentation of the four different objects was randomly determined for each mouse. On Day 10 a test trial was performed. The partition separating the two compartments was removed and the animal was allowed to freely move between the two compartments for 5 min. During the baseline and the test trials, the time spent in each compartment in seconds was measured. Please note that during the baseline and test trials the apparatus did not contain any objects. It is known that, in rats, repeated pairings of one compartment, but not the other, with novel objects induces a CPP for the novel object-paired compartment. Therefore, it was expected that the animals should spend more time in the former novel object-paired compartment on the test, but not on the baseline trial.

## 3.4 Neurochemical Methods

### 3.4.1 Post-mortem Acetylcholine and Monoamine Levels in the Brain

For post-mortem neurochemical analyses of brain monoamines and acetylcholine including metabolites animals are decapitated after cervical dislocation, their brains quickly removed, and the brain structures specified above are dissected out bilaterally on ice. Thereafter, the brain tissue is weighed, homogenized in ice cold 0.05N perchloric acid containing ethylhomocholine as an internal standard, centrifuged, filtered and kept at−60◦ until being analyzed. Samples are analyzed for acetylcholine and choline concentrations according to the procedure utilized by Damsma et al., 1987, except for the internal standard, and for serotonin (5-HT), 5-hydroxyindole acetic acid (5-HIAA), dopamine, homovanillic acid (HVA) and dihydrophenylacetic acid (DOPAC) levels using high-performance liquid chromatography with electrochemical detection (De Souza Silva et al., 1997).

### 3.4.2 Acetylcholinesterase Histochemistry and Tyrosine Hydroxylase Immunohistochemistry

***Acetylcholinesterase (AChE) Histochemistry***

Quantitative histochemistry for AChE activity was performed as previously described (Tien et al., 2004). Briefly, sections were washed in PBS and then incubated for 15 h at 4 °C in the following solution: S-acetylthicholine iodide (Sigma) 4mM,

ethopropazine 0.086 mM, copper sulphate 4 mM, glycine 16 mM in acetate buffer 50 mM pH5. Slides were then rinsed in PBS and immersed in 1 % sodium sulphide pH 7.5. Finally, slides were dehydrated in alcohol and coverslipped with Permount. All brains were stained at the same time with the same solutions to minimize experimental variations.

***Tyrosine Hydroxylase (TH) Immunohistochemistry***

TH-expression in the striatum was measured as follows: sections were washed in Tris Buffered Saline (TBS) and incubated with a mouse monoclonal antibody against TH (Diasorin, Stillwater, USA) at a 1:5000 dilution in 10% normal bovine serum, 0.2% triton X-100 in PBS overnight at +4 °C. After overnight incubation, sections were washed three times in PBS and then incubated with anti-mouse-biotin, conjugated (Sigma, USA) at a 1:200 dilution in 10% normal bovine serum, 0.2% Triton X-100 in PBS for 1 h at room temperature. Sections were then washed again three times in TBS and incubated in ABC (Vector Lab, USA) for 1 h. After three washes in TBS, the reaction was visualized with 0.1% Diaminobenzidine, 0.02% hydrogen peroxide in TB 0.05M at pH 7.4 for 10 min in the dark. The reaction was then stopped with cold TBS. Finally, slides were dehydrated in alcohol and coverslipped with Permount.

*Morphometric Data Acquisition*

Slides were analysed with a Zeiss Axioskop 20, equipped with a CCD high-resolution camera (Hamamatsu Photonics, Italy, C5405) and motorized XYZ stage (Proscan II, Prior). The images were captured with a 5x objective and converted by a microcomputer-assisted image analyzer (MCID Elite; Imaging Res. Inc., Canada. Tiled images over the entire filed of interest had a final resolution of 1824 x 1440 pixels.

AChE and TH expression levels were quantified over the entire sampled field according to the guidelines of Capowski (1989), and measured as relative optical density (ROD) units (ROD = log (256/ grey level). ROD units are correlated with the enzyme activity and antigen concentration (Burke et al., 1990).

# 4 Results

## 4.1 Experiment 1

Since NMDA-Rs have been implicated in associative synaptic plasticity, we asked whether the modulation of NMDA-R function by DCS, which is an agonist at the glycine-site of the NMDA-R, is able to restore or induce ELM under conditions where mice fail to show ELM. In pilot experiments, we found that an inter-trial interval of 5 min between the sample and test trials of the ELM memory task completely eliminated the exploration pattern indicative of ELM, possibly because of pro- or retroactive interference. A dose of 20 mg/kg of DCS, administered 30 min prior to the first sample trial, was effective in restoring ELM under these suboptimal interference-prone learning conditions. A dose of 0.2 mg/kg DCS was completely ineffective in promoting ELM, whereas a dose of 2.0 mg/kg DCS only rescued temporal order memory, but had no promnestic effect on the spatial component of ELM task. Compared to the saline- and 0.2 and 2.0 mg/kg DCS treated groups, mice that received the 20.0 mg/kg dose of DCS remembered the temporal order as well as the spatial position of two different objects. It has been shown that DCS improves long-term retention in a one-trial inhibitory avoidance task (Land & Riccio, 1999). In this task, the animals have to remember, after a single trial, in which of the two compartments of the apparatus (where) they received a foot-shock (what). Because this task lacks the temporal component (when) of episodic memory, it cannot be

viewed as an ELM task. Therefore, our study is the first report showing that DCS can enhance ELM in mice. The results are in line with evidence showing that DCS ameliorates cognitive deficits in Alzheimer's disease patients (Randolph et al., 1994; Schwartz et al., 1996; Tsai et al., 1999), which show deficits in episodic memory already at early stages of the disease (Small et al., 2003). In Alzheimer's disease patients, both hypo- and hyperactivity of the NMDA-R has been proposed. While the hypoactivity is addressed with glycine and DCS treatments, the hyperactivity of NMDA-R is decreased by memantine, an NMDA-R antagonist (Butterfield & Pocernich, 2003). The latter compound has already been approved for the treatment of Alzheimer's disease patients. The enhancing effect of DCS on ELM in mice might be due to facilitation of synaptic plasticity (Rouaud & Billard, 2003), e.g. in the hippocampus (Manns & Eichenbaum, 2006). In conclusion, it seems that DCS might be, indeed, a promising candidate for the treatment of deficits in episodic memory due to its modulation of NMDA-R function.

## 4.2 Experiment 2

In this experiment we examined whether the NK-3R agonist, senktide, would help mice to establish an ELM under sub-optimal interference-prone learning conditions. Dependent on the inter-trial interval used, e.g. 5 or 50 min, the ELM task can be arranged for detecting either promnestic or amnestic effects of drugs or genomic interventions. Here, we used the sub-optimal interference-prone 5-min

inter-trial interval in order to examine whether senktide would facilitate ELM. The effect of senktide was dose-dependent with the lowest dose of 0.1 mg/kg having no beneficial effect on performance. The medium (0.2 mg/kg) and high (0.4 mg/kg) doses of senktide rescued the memory for the temporal order in which two different objects had been encountered. These doses were also effective in improving memory for location of the recent familiar objects, i.e. the memory for what and where. However, only the mice which have been injected with a dose of 0.4 mg/kg showed the characteristic exploratory profile indicative of a binding of temporal order- and spatial information into an episodic-like object memory.

A recent review of the rodent one-trial object recognition literature indicates that one-trial object recognition is mediated by the perirhinal cortex, and, under some conditions, also by the hippocampus (Dere et al., 2007). The latter is also important for object–place memory (Mumby et al., 2002). Temporal order object memory seems to be mediated by the prefrontal cortex (Hanneson et al., 2004). Together these 3 brain structures have been proposed to be crucial parts of an interconnected brain system that is likely to mediate episodic-like object memory formation and recall (Dere et al., 2007). Given that the cortex and the hippocampus of the rodent brain express NK-3Rs in significant quantities, the promoting effects of senktide on temporal and spatial memory might be due to NK-3R activation or the secondary down-stream release of other neurotransmitters, e.g. acetylcholine in the hippocampus and prefrontal cortex (Marco et al., 1998; Steinberg et al., 1995). Episodic memory deficits in patient populations with mild cognitive impairment can

be rescued by AChE inhibitors and galantamine. AChE inhibitors, galantamine, *and* senktide all increase extrasynaptic hippocampal acetylcholine levels (Gron et al., 2006; Marco et al., 1998). We conclude that NK-3R stimulation by senktide can improve ELM in mice possibly via the increase of hippocampal and neocortical acetylcholine levels. Since early stages of Alzheimer`s disease is characterized by more or less selective deficits in episodic memory, it remains to be determined whether NK-3R agonists such as senktide can ameliorate such deficits in Alzheimer patients.

## 4.3 Experiment 3

In this experiment ELM and procedural memory have been investigated in H1R-KO mice. In order to relate possible behavioral deficits to neurobiological changes, we further assessed AChE activity in the hippocampus and AChE-, and TH-levels in the striatum. Furthermore, acetylcholine, 5-HT, and dopamine levels, including metabolites, were measured in the cerebellum of H1R-KO and wild type (WT) mice. The H1R-KO mice showed impaired ELM as compared to the WT mice. The performance of H1R-KO mice in the ELM task was primarily driven by familiarity-based memory processes. While the H1R-KO mice performed similar to the WT mice during the acquisition of a procedural memory as measured with an accelerating rotarod, their performance after a retention interval of seven days was significantly impaired relative to the WT mice. These findings suggest that both,

ELM and long-term procedural memory, are impaired in the H1R-KO mice. Neurochemical assays revealed that the H1R-KO mice had significantly lower levels of AChE activity in the dentate gyrus and CA1 subregions of the hippocampus as compared to the WT mice. The H1R-KO mice also displayed significantly reduced DOPAC levels and a reduced DOPAC/dopamine ratio in the cerebellum, suggesting that the dopamine turnover in the cerebellum is decelerated in H1R-KO mice. In conclusion, H1R-KO mice display severe long-term memory deficits in both ELM and procedural memory, which coincide with changes in AChE activity in the hippocampus as well as dopamine turnover in the cerebellum.

## 4.4 Experiment 4

Since the histaminergic system has been proposed to act as a reinforcement or reward-inhibiting system opposed to the mesolimbic dopamine system (Huston et al., 1997), we asked whether the memory deficits of the H1R-KO mice are the consequence of a deficient brain reinforcement system. It is well known that novelty-induced arousal can be either rewarding or aversive dependent on its intensity and the preceding internal state of arousal (Berlyne, 1969; Bronson, 1968). Neuronal histamine has been implicated in neuronal and behavioral correlates of both arousal and reinforcement processes (Huang et al., 2006; Huston et al., 1997; Lin, 2000). Therefore, we asked whether H1R-KO mice would develop a novel objects induced CPP and whether they would intact novelty-induced spatial alternation.

Although, H1R-KO mice do explore novel objects, they fail to develop a significant CPP. It seems that the reinforcing value of novel objects is diminished in the H1R-KO mice. We also found impaired novelty-induced alternation in the Y-maze as compared to controls. Furthermore, rearing activity and emotional behavior in a novel environment was also altered in H1R-KO mice, whereas object-place recognition was unaffected. In terms of brain neurochemistry, the H1R-KO mice had higher acetylcholine levels in the frontal cortex and amygdala. In the amygdala, the H1R-KO mice had also increased levels of dopamine and a lower dopamine turnover rate. Finally, the H1R-KO mice had also increased TH immunoreactivity in the several subnuclei of the amygdala.

We conclude that the H1R-KO in the mouse has a general impairing effect on learning and memory formation due to a reduction of the motivational effects of novelty and/or a dysfunctional brain reward /reinforcement system.

# 5 Discussion

## *The ELM test as a potent new tool for animal research in the field of behavioral neuroscience*

It has long been assumed that animals do not possess an episodic memory system that is comparable to the one of humans (Tulving, 2002). However, recent animal research in the field of cognitive neuroscience has provided a wealth of evidence that suggest that this might not be true (for a review, see Dere et al., 2006). Animals might be at least able to establish higher order memories for personal events that include information of the spatial and temporal context of this event. This kind of fast-acquired multi-dimensional memory has been named either what, where and when or ELM. We have demonstrated ELM in mice by combining 3 basic versions of the novelty preference paradigm into a singe task (Zlomuzica et al., 2007, 2008a). In this task ELM the mice have to remember what kind of objects they have explored and where and when they did this. After demonstration of ELM in both rats and mice, we developed two experimental protocols to gauge either amnestic (Dere et al., 2008a) or promnestic effects (Zlomuzica et al., 2007, 2008a) of experimental manipulations in mice, such as the application of drugs, brain lesions or the genetic inactivation. The novelty-preference paradigm based ELM task (Dere et al., 2005a, 2005b), with minor modifications, has been used to investigate the neuroanatomy of episodic memory in rats (Good et al., 2007a; Li & Chao, 2008), to evaluate the

cognitive phenotype of genetic mouse models of human diseases (Fernandez & Garner, 2008; Good et al., 2007b), the effects of electromagnetic radiation on memory performance (Nittby et al., 2008), and to screen for possible memory-promoting drugs at a preclinical level that can be used as a mediaction for early stages of Alzheimer`s disease or other forms of dementia (Kart-Teke et al., 2006, 2007; Zlomuzica et al., 2007, 2008a).

## *The neurobiology of ELM*

### *The systems level*

Lesion studies in rats and mice have searched for the neuroanatomical underpinnings of object recognition as well as spatial and temporal memory, and provide the groundwork to delineate the least common denominator of how the neuroanatomical circuit that underlies ELM should be organized. At the systems level it appears that the perirhinal cortex (Winters & Bussey, 2005) and in special cases the hippocampus (Broadbent et al., 2004) mediate object recognition. The hippocampus is also critical for the processing of the spatial context of an object exploration episode (Clark et al., 2001; Eacott & Norman, 2004; Ennaceur et al., 1997; Gaffan, 1994; Gilbert & Kesner, 2004; Mumby et al., 2002). The medial prefrontal cortex is known to process temporal information (Chiba et al., 1994; Mitchell and Laiacona, 1998). We have proposed that the establishment of an episodic memory requires a strong emotional activation as a basic requirement to activate the episodic

memory system (Dere et al., 2008b). The information on the emotional context of specific events is provided by the amygdale, a brain structure known to process stimuli with both aversive (Phelps & LeDoux, 2005) and rewarding value (Everitt et al., 1991; Hiroi & White, 1991; Schroeder & Packard, 2002). The perirhinal cortex, medial prefrontal cortex and amygdala maintain reciprocal connections with the hippocampus (Pitkanen et al., 2000; Swanson, 1981). Together, these four brain structures are the essential key structures of the neural circuit that mediates episodic memory (Squire et al., 2004). Among these brain structures the hippocampus is especially important because it binds the multi-modal information that comprises an event into a single memory (Sato & Yamaguchi, 2005). It has been found that rats with lesions to the CA3 region of the hippocampus are unable to bind or integrate what, where and when information into an unified ELM (Li & Chao, 2008). Both, untreated and sham operated controls, showed higher exploration of the old familiar stationary object compared to the old familiar displaced object, while the CA3-lesioned group showed the opposite preference. Thus, CA3 lesioned animals do not show the interaction between spatial and temporal factors suggestive for an integrated memory for what, where, and when. In separate experiments the authors have also shown that CA3-lesioned rats are still able to discriminate novel from familiar objects, and show intact spatial and temporal order memory. Thus, it seems that the binding of content with spatio-temporal features of a single experience into a unified ELM depends on the integrity of the CA3-subregion of the hippocampus (Li & Chao, 2008).

*The molecular level*

*The NMDA-R and ELM*

At the molecular level the establishment of episodic-like memories might require the activation of NMDA-Rs in the CA3-region of the hippocampus (Nakazawa et al., 2003). NMDA-Rs have been implicated in the association or binding of multi-modal stimuli. Hippocampal associative or heterosynaptic plasticity is mediated by NMDA-Rs (Kosub et al., 2005; Lynch, 2004). Associative or heterosynaptic long-term potentiation is induced by the concurrent stimulation of a weak and a strong synaptic input to a postsynaptic neuron, leading to a strengthening of the weaker one. For heterosynaptic long-term potentiation at hippocampal CA1 synapses to occur it needs two stimulation electrodes which concurrently activate weak and strong inputs to CA1 pyramidal cells (Dudek & Bear, 1993). A recording electrode measures population excitatory postsynaptic potentials (EPSP`s) from CA1 cells. Stimulation of the weak input alone only induces a weak population EPSP at CA1 pyramidal cells. However, after concurrent stimulation of both the weak and strong input, the stimulation of the weak input alone produces a much higher population EPSP`s at the CA1 pyramidal cells. This change in synaptic strength or efficiancy, depending on the stimulation protocol, can last for several hours (for review, see Lynch, 2004). It is possible that the establishment of episodic-like memories might be related to a similar mechanism implemented by NMDA-Rs in the CA3 region of the hippocampus. We have shown that D-cycloserine can

initiate ELM formation under sub optimal learning conditions such as short inter-trial intervals that lead to interference (Zlomuzica et al., 2007) or stress (Kart-Teke et al., 2006).

***The H1R and ELM***

As mentioned above we have proposed that episodic memory formation requires a strong emotional activation by stimuli/situations with aversive or rewarding value (Dere et al., 2008b). The histaminergic system has been proposed to act as a reinforcement or reward-inhibiting system opposed to the mesolimbic dopamine system (Huston et al., 1997). It is well known that there is a close functional relationship between brain reinforcement and memory processes. The access to novelty including novel objects is a kind of natural reward. Since the genetic inactivation of the H1R might have an detrimental impact on the function of the brain`s reward system, we tested the H1R-KO mice in terms of the integrity of different learning and memory systems, including ELM, procedural memory and a novel objects induced CPP. In support of the reinforcement theory of memory formation initially proposed by Joseph P. Huston (Huston et al., 1997), the H1R-KO mice show a general learning and memory impairment that includes explicit- and procedural memory, as well as classical conditioning (Dere et al., 2008a; Zlomuzica et al., 2008b).

*The NK-3R and ELM*

The experiment 2 of this thesis provided the first evidence that senktide, an agonist of the NK-3R, has promenstic effects in an ELM task (Zlomuzica et al., 2008a). Interestingly, in mice, the intracerebroventricular administration of senktide had an anxiolytic effect in the elevated plus-maze (Ribeiro & De Lima, 1998). Drugs that have both memory-promotiong and anti-anxiety effects are relatively rare. This is a highly desired combination of properties that might be interesting for the treatment of cognitive deficits in humans. Generally, neurokinin-related agents turn out to be a very interesting new class of drugs that have several features that makes them interesting for the treatment of early stage dementia due to Alzheimer`s or vascular disease (Chahl, 2006).

In sum the experiments of this thesis demonstrate the different versions of the novelty-preference task can be successfully combined to measure the remembrance of the content, place and temporal context of single experiences and that this task is suitable to perform genetic, lesion and pharmacological studies in the mouse.

# 6 References


Aggleton, J.P., Brown, M.W. (1999). Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. Behavioral Brain Sciences, 22, 425–444.

Aggleton, J.P., Pearce, J.M. (2001). Neural systems underlying episodic memory: insights from animal research. Philosophical Transactions of the Royal Society of London Series B—Biological Sciences 356, 1467–1482.

Airaksinen, M.S., Paetau, A., Paljarvi, L., Reinikainen, K., Riekkinen, P., Suomalainen, R., Panula, P. (1991). Histamine neurons in human hypothalamus: anatomy in normal and Alzheimer diseased brains. Neuroscience, 44, 465-481.

Alvarez, E.O., Banzan, A.M. (1986). Histamine in dorsal and ventral hippocampus. II. Effects of H1 and H2 histamine antagonists on exploratory behavior in male rats. Physiology & Behaviour, 37, 39-45.

Baxter, M.G., Lanthorn, H., Frick, K.M., Golski, S., Wan, R.Q., Olton, D.S. (1994). D-Cycloserine, a novel cognitive enhancer, improves spatial memory in aged rats. Neurobiology of Aging, 15, 207–213.

Bellucci, F., Carini, F., Catalani, C., Cucchi, P., Lecci, A., Meini, S., Patacchini, R., Quartara, L., Ricci, R., Tramontana, M., Giuliani, S., Maggi, C.A. (2002). Pharmacological profile of the novel mammalian tachykinin, hemokinin 1. British Journal of Pharmacology, 135, 266–274.

Berlyne, D.E. (1969). Arousal, reward and learning. Annals of the New York Academy of Sciences, 159, 1059-1070.

Blandina, P., Efoudebe, M., Cenni, G., Mannaioni, P., Passani, M.B. (2004). Acetylcholine, histamine, and cognition: two sides of the same coin. Learning & Memory, 11, 1-8.

Blandina, P., Giorgetti, M., Bartolini, L., Cecchi, M., Timmerman, H., Leurs, R., Pepeu, G., Giovannini, M.G. (1996). Inhibition of cortical acetylcholine release and cognitive performance by histamine H3-receptor activation in rats. British Journal of Pharmacology, 119, 1656-1664.

Broadbent, N.J., Squire, L.R., Clark, R.E. (2004). Spatial memory, recognition memory, and the hippocampus. Proceedings of the National Academy of Sciences of the United States of America, 101, 14515–14520.

Bronson, G.W. (1968). The fear of novelty. Psychological Bulletin, 69, 350-358.

Burke, D.M., Mackay, D.G. (1997). Memory, language, and ageing. Philosophical Transactions of the Royal Society of London Series B—Biological Sciences, 352, 1845-56.

Burke, R.E., Cadet, J.L., Kent, J.D., Karanas, A.L., Jackson-Lewis, V. (1990). An assessment of the validity of densitometric measures of striatal tyrosine hydroxylasepositive fibers: relationship to apomorphine-induced rotations in 6-hydroxydopamine lesioned rats. Journal of Neuroscience Methods, 35, 63-73.

Butterfield, D.A., Pocernich, C.B. (2003). The glutamatergic system and Alzheimer's disease: therapeutic implications. CNS Drugs, 17, 641–652.

Cacabelos, R., Yamatodani, A., Niigawa, H., Hariguchi, S., Tada, K., Nishimura, T., Wada, H., Brandeis, L., Pearson, J. (1989). Brain histamine in Alzheimer's disease. Methods and Findings in Experimental and Clinical Pharmacology, 11, 353-360.

Capowski, J.J. (1989). Computer techniques in neuroanatomy. New York: Plenum Press.

Chahl, L.A. (2006). Tachykinins and neuropsychiatric disorders. Current Drug Targets, 7, 993-1003.

Chen, Z., Sugimoto, Y., Kamei, C. (1999). Effects of intracerebroventricular injection of alpha-fluoromethylhistidine on radial maze performance in rats. Pharmacology, Biochemistry, and Behaviour, 64, 513-518.

Chen, L. W., Wei, L. C., Liu, H. L., Ding, Y. Q., Zhang, H., Rao, Z. R., Ju, G., Chan, Y.S. (2001). Cholinergic neurons expressing neuromedin K receptor (NK3) in the basal forebrain of the rat: A double immunofluorescence study. Neuroscience, 103, 413–422.

Chiba, A.A., Kesner, R.P., Reynolds, A.M., (1994). Memory for spatial location as a function of temporal lag in rats. Role of hippocampus and medial prefrontal cortex. Behavioral and Neural Biology, 61, 123–131.

Clark, R.E., West, A.N., Zola, S.M., Squire, L.R. (2001). Rats with lesions of the hippocampus are impaired on the delayed nonmatching-to-sample task. Hippocampus, 11, 176-186.

Clayton, N.S., Dickinson, A., (1998). Episodic-like memory during cache recovery by scrub jays. Nature, 395, 272–274.

Clayton, N.S., Griffiths, D.P., Emery, N.J., Dickinson, A. (2001b). Elements of episodic-like memory in animals. Philosophical Transactions of the Royal Society of London Series B—Biological Sciences 356, 1483–1491.

Dai, H., Kaneko, K., Kato, H., Fujii, S., Jing, Y., Xu, A., Sakurai, E., Kato, M., Okamura, N., Kuramasu, A., Yanai, K. (2007). Selective cognitive dysfunction in mice lacking histamine H1 and H2 receptors. Neuroscience Research, 57, 306-313.

Damsma, G., Lammerts van Bueren, D., Westerink, B.H., Horn, A.S. (1987). Determination of acetylcholine and choline in the femtomole range by means of HPLC, a post column enzyme reactor, and electrochemical detection. Chromotographia, 24, 827-31.

De Souza Silva, M.A., Mattern, C., Hacker, R., Nogueira, P.J., Huston J.P., Schwarting, R.K. (1997). Intranasal administration of the dopaminergic agonists L-DOPA, amphetamine, and cocaine increases dopamine activity in the neostriatum: a microdialysis study in the rat. Journal of Neurochemistry, 68, 233-239.

Dere, E., Kart-Teke, E., Huston, J.P., De Souza Silva, M.A. (2006). The case for episodic memory in animals. Neuroscience and Biobehavioral Reviews 30, 1206-1224.

Dere, E., Zlomuzica, A., Huston, J.P. (2008b). Animal episodic memory. In: Dere, E., Easton, A., Nadel, L., Huston, J.P. (Eds.) Handbook of Behavioral Neuroscience: Episodic Memory Research. Amsterdam, Elsevier Science. In press.

Dere, E., Zlomuzica, A., Viggiano, D., Watanabe, T., Sadile, A.G., Huston, J.P., De Souza-Silva, M.A. (2008a). Episodic-like and procedural memory impairments in histamine H1R-KO mice coincide with changes in acetylcholine esterase activity in the hippocampus and dopamine turnover in the cerebellum. Neuroscience. In revision

Dere, E., Huston, J.P., De Souza Silva, M.A. (2005a). Integrated memory for objects, places and temporal order: Evidence for episodic-like memory in mice. Neurobiology of Learning and Memory, 84, 214–221.

Dere, E., Huston, J.P., De Souza Silva, M.A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neuroscience and Biobehavioral Reviews, 31, 673–704.

Dere, E., Huston, J.P., De Souza Silva, M.A. (2005b). Episodic-like memory in mice: Simultaneous assessment of object, place and temporal order memory. Brain Research Protocols, 16, 10-19.

Ding, Y.Q., Shigemoto, R., Takada, M., Ohishi, H., Nakanishi, S., Mizuno, N. (1996). Localization of the neuromedin K receptor (NK3) in the central nervous system of the rat. The Journal of Comparative Neurology, 364, 290–310.

Dix, S.L., Aggleton, J.P. (1999). Extending the spontaneous preference test of recognition: Evidence of object-location and object-context recognition. Behavioural Brain Research, 99, 191–200.

Duarte, C.R., Schutz, B., Zimmer, A. (2006). Incongruent pattern of neurokinin B expression in rat and mouse brains. Cell and Tissue Research, 23, 43–51.

Dudek, S.M., Bear, M.F. (1993). Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. The Journal of Neuroscience, 13, 2910-2918.

Eacott, M.J., Norman, G. (2004). Integrated memory for object, place, and context in rats, a possible model of episodic-like memory? The Journal of Neuroscience, 24, 1948-1953.

Ennaceur, A., Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behavioural Brain Research, 31, 47–59.

Ennaceur, A., Neave, N., Aggleton, J.P. (1997). Spontaneous object recognition and object location memory in rats, the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Experimental Brain Research, 113, 509-519.

Everitt, B.J., Morris, K.A., O'Brien, A., Robbins, T.W. (1991). The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic-striatal interactions underlying reward-related processes. Neuroscience, 42, 1-18.

Flood, J.F., Uezu, K., Morley, J.E. (1998). Effect of histamine H2 and H3 receptor modulation in the septum on post-training memory processing. Psychopharmacology, 140, 279-284.

Frisch, C., Hasenöhrl, R.U., Haas, H.L., Weiler, H.A.T., Steinbusch, H.W., Huston, J.P. (1998a). Facilitation of learning after lesions of the tuberomammillary nucleus region in adult and aged rats. Experimental Brain Research, 118, 447-456.

Frisch, C., Hasenöhrl, R.U., Huston, J.P. (1999). Memory improvement by post-trial injection of lidocaine into the tuberomammillary nucleus, the source of neuronal histamine. Neurobiology of Learning and Memory, 72, 69-77.

Frisch, C., Hasenöhrl, R.U., Krauth, J., Huston, J.P. (1998b). Anxiolytic-like behavior after lesion of the tuberomammillary nucleus E2-region. Experimental Brain Research, 119, 260-264.

Gaffan, D. (1994). Scene-specific memory for objects: a model of episodic memory impairment in monkeys with fornix transection. Journal of Cognitive Neuroscience, 6, 305-320.

Gilbert, P.E., Kesner, R.P. (2004). Memory for objects and their locations, the role of the hippocampus in retention of object-place associations. Neurobiology of Learning and Memory, 81, 39-45.

Good, M.A., Barnes, P., Staal, V., McGregor, A., Honey, R.C. (2007a). Context- but not familiarity-dependent forms of object recognition are impaired following excitotoxic hippocampal lesions in rats. Behavioural Neuroscience, 121, 218-23.

Good, M.A., Hale, G., Staal, V. (2007b). Impaired "episodic-like" object memory in adult APPswe transgenic mice. Behavioural Neuroscience, 121,443-8.

Gron, G., Brandenburg, I., Wunderlich, A.P., Riepe, M.W. (2006). Inhibition of hippocampal function in mild cognitive impairment: Targeting the cholinergic hypothesis. Neurobiology of Aging, 27, 78–87.

Haas, H., Panula P. (2003). The role of histamine and the tuberomamillary nucleus in the nervous system. Nature Reviews Neuroscience, 4, 121-130.

Hannesson, D.K., Howland, J.G., Phillips, A.G. (2004). Interaction between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. The Journal of Neuroscience, 24, 4596–4604.

Hasenöhrl, R.U., Weth, K., Huston, J.P. (1999). Intraventricular infusion of the histamine H(1) receptor antagonist chlorpheniramine improves maze performance and has anxiolytic-like effects in aged hybrid Fischer 344xBrown Norway rats. Experimental Brain Research, 128, 435-440

Hasenöhrl, R.U., De Souza Silva, M.A., Nikolaus, S., Tomaz, C., Brandao, M.L., Schwarting, R.K., Huston, J.P. (2000). Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. Neuropeptides, 34, 272–280.

Helke, C.J., Krause, J.E., Mantyh, W., Couture, R., Bannon, M.J. (1990). Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. FASEB Journal, 4, 1606 –15.

Hiroi, N., White, N.M. (1991). The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. The Journal of Neuroscience, 11, 2107-2116.

Hokfelt, T., Pernow, B., Wahren, J. (2001). Substance P: a pioneer amongst neuropeptides. The Journal of Internal Medicine, 249, 27–40.

Huang, Z.L., Mochizuki, T., Qu, W.M., Hong, Z.Y., Watanabe, T., Urade, Y., Hayaishi, O. (2006). Altered sleep-wake characteristics and lack of arousal response to H3 receptor antagonist in histamine H1 receptor knockout mice. Proceedings of the National Acadademy of Science U. S. A., 103, 4687-4692.

Huston, J.P., Wagner, U., Hasenöhrl, R.U. (1997). The tuberomammillary nucleus projections in the control of learning, memory and reinforcement processes: evidence for an inhibitory role. Behavioural Brain Research, 83, 97-105.

Inoue, I., Yanai, K., Kitamura, D., Taniuchi, I., Kobayashi, T., Niimura, K., Watanabe, T., Watanabe, T. (1996). Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. Proceedings of the National Acadademy of Science U. S. A, 13316-13320.

Johnson, J.W., Ascher, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature, 325:529–531.

Kamei, C., Okumura, Y., Tasaka, K. (1993). Influence of histamine depletion on learning and memory recollection in rats. Psychopharmacology, 111, 376-382.

Kameyama, T., Ukai, M., Shinkai, N. (1998). Ameliorative effects of tachykinins on scopolamine-induced impairment of spontaneous alternation performance in mice. Methods and Findings in Experimental and Clinical Pharmacology, 20, 555–560.

Kart, E., Jocham, G., Muller, C.P., Schlömer, C., Brandao, M.L., Huston, J.P., De Souza Silva, M.A. (2004). Neurokinin-1 receptor antagonism by SR140333: enhanced in vivo ACh in the hippocampus and promnestic post-trial effects. Peptides, 25, 1959–1969.

Kart-Teke, E., De Souza-Silva, M.A., Huston, J.P., Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. Neurobiology of Learning and Memory 85, 173–182.

Kart-Teke, E., Dere, E., Brandao, M., Huston, J.P., De Souza Silva, M.A. (2007). Reinstatement of episodic-like memory in rats by NK-1 receptor antagonism. Neurobiology of Learning and Memory 87, 324–331.

Kosub, K.A., Do, V.H., Derrick, B.E. (2005). NMDA receptor antagonists block heterosynaptic long-term depression (LTD) but not long-term potentiation (LTP) in the CA3 region following lateral perforant path stimulation. Neuroscience Letters, 374, 29-34.

Land, C., Riccio, D.C. (1999). D-Cycloserine: effects on long-term retention of a conditioned response and on memory for contextual attributes. Neurobiology of Learning and Memory, 72, 158–168.

Langosch, J.M., Kupferschmid, S., Heinen, M., Walden, J., Herpfer, I., Fiebich, B. L., Lieb, K. (2005). Effects of substance P and its antagonist L- 733060 on long term potentiation in guinea pig hippocampal slices. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29, 315–319.

Laufer, R., Gilon, C., Chorev, M., Selinger, Z. (1986). Characterization of a neurokinin B receptor site in rat brain using a highly selective radioligand. The Journal of Biological Chemistry, 261, 10257–10263.

Li, J.S., Chao, Y.S. (2007). Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. Neurobiology of Learning and Memory, 89, 192-8.

Lin, J.S. (2000). Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. Sleep Medicine Reviews, 4, 471-503.

Liu, C., Wilson, S.J., Kuei, C., Lovenberg, T.W. (2001). Comparison of human, mouse, rat, and guinea pig histamine H4 receptors reveals substantial pharmacological species variation. The Journal of Pharmacology and Experimental Therapeutics, 299, 121-130.

Lynch, M.A. (2004). Long-term potentiation and memory. Physiological Reviews, 84, 87–136.

Manns, J.R., Eichenbaum, H. (2006). Evolution of declarative memory. Hippocampus, 16, 795–808.

Mantyh, P.W., Gates, T., Mantyh, C.R., Maggio, J.E. (1989). Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. The Journal of Neuroscience, 9, 258–279.

Marco, N., Thirion, A., Mons, G., Bougault, I., Le Fur, G., Soubrie, P., Steinberg, R. (1998). Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: An in vivo microdialysis approach in guinea pig. Neuropeptides, 32, 481–488.

Martin, S.J., Morris, R.G., (2002). New life in an old idea: the synaptic plasticity and memory hypothesis revisited. Hippocampus, 12, 609–636.

Massi, M., Panocka, I., de Caro, G. (2000). The psychopharmacology of tachykinin NK-3 receptors in laboratory animals. Peptides, 21, 1597–1609.

Mazurkiewicz-Kwilecki, I.M., Nsonwah, S. (1987). The influence of alphafluoromethylhistidine on the regional brain histamine and plasma corticosterone levels in aging. Canadian Journal of Physiology and Pharmacology, 65, 2154-2157.

Mazurkiewicz-Kwilecki, I.M., Nsonwah, S. (1989). Changes in the regional brain histamine and histidine levels in postmortem brains of Alzheimer patients. Canadian Journal of Physiology and Pharmacology, 67, 75-78.

Mazurkiewicz-Kwilecki, I.M., Prell, G.D. (1984). Age-related changes in brain histamine. Agents Actions, 14, 554-557.

Mitchell, J.B., Laiacona, J., (1998). The medial frontal cortex and temporal memory, tests using spontaneous exploratory behaviour in the rat. Behavioural Brain Research 97, 107–113.

Morcuende, S., Gadd, C.A., Peters, M., Moss, A., Harris, E.A., Sheasby, A., Fisher, A.S., de Felipe, C., Mantyh, P.W., Rupniak, N.M.J., Giese, K.P., Hunt, S.P. (2003). Increased neurogenesis and brain-derived neurotrophic factor in neurokinin-1 receptor gene knockout mice. European Journal of Neuroscience, 18, 1828–1836.

Morisset, S., Traiffort, E., Schwartz, J.C. (1996). Inhibition of histamine versus acetylcholine metabolism as a mechanism of tacrine activity. European Journal of Pharmacology, 315, R1-2.

Morris, R.G.M. (2006). Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. European Journal of Neuroscience, 23, 2829–2846.

Morris, R.G.M. (2001). Episodic-like memory in animals: psychological criteria, neural mechanisms and the value of episodic-like tasks to investigate animal models of neurodegenerative disease. Philosophical Transactions of the Royal Society of London Series B—Biological Sciences 356, 1453-65.

Mufson, E.J., Ginsberg, S.D., Ikonomovic, M.D., DeKosky, S.T. (2003). Human cholinergic basal forebrain: Chemoanatomy and neurologic dysfunction. Journal of Chemical Neuroanatomy, 26, 233–242.

Mumby, D.G., Gaskin, S., Glenn, M.J., Schramek, T.E., Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. Learning & Memory, 9, 49–57.

Nakamura, S., Takemura, M., Ohnishi, K., Suenaga, T., Nishimura, M., Akiguchi, I., Kimura, J., Kimura, T. (1993). Loss of large neurons and occurrence of neurofibrillary tangles in the tuberomammillary nucleus of patients with Alzheimer's disease. Neuroscience Letters, 151, 196-199.

Nikolaus, S., Huston, J.P, Hasenohrl, R.U. (1999). Reinforcing effects of neurokinin substance P in the ventral pallidum: mediation by the tachykinin NK1 receptor. European Journal of Pharmacology, 370, 93–99.

Nishibori, M., Oishi, R., Itoh, Y., Saeki, K. (1991). 9-Amino-1,2,3,4-tetrahydroacridine is a potent inhibitor of histamine N-methyltransferase. Japanese Journal of Pharmacology, 55, 539-546.

Nittby, H., Grafström, G., Tian, D.P., Malmgren, L., Brun, A., Persson, B.R., Salford, L.G., Eberhardt, J. (2008). Cognitive impairment in rats after long-term exposure to GSM-900 mobile phone radiation. Bioelectromagnetics, in press.

Ohno, M., Watanabe, S. (1996). D-Cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats. European Journal of Pharmacology, 318, 267–271.

Oliveira, A.A., Jr., Hodges, H.M. (2005). Alzheimer's disease and neural transplantation as prospective cell therapy. Current Alzheimer Research, 2, 79–95.

Onodera, K., Miyazaki, S., Imaizumi, M. (1998). Cognitive involvement by negative modulation of histamine H2 receptors in passive avoidance task in mice. Methods and Findings in Experimental and Clinical Pharmacology, 20, 307-310.

Panula, P., Rinne, J., Kuokkanen, K, Eriksson, K.S., Sallmen, T., Kalimo, H., Relja, M. (1998). Neuronal histamine deficit in Alzheimer's disease. Neuroscience, 2, 993- 997.

Pepeu, G., Giovannini, M.G. (2004). Changes in acetylcholine extracellular levels during cognitive processes. Learning & Memory, 11, 21–27.

Phelps, E.A., LeDoux, J.E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron, 48, 175-187.

Pitkanen, A., Pikkarainen, M., Nurminen, N., Ylinen, A. (2000). Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat. A review. Annals of the New York Academy of Sciences, 911, 369-391.

Pussinen, R., Sirvio, J. (1999). Effects of D-cycloserine, a positive modulator of N-methyl-D-aspartate receptors, and ST 587, a putative alpha-1 adrenergic agonist, individually and in combination, on the non-delayed and delayed foraging behaviour of rats assessed in the radial arm maze. Journal of Psychopharmacology, 13, 171–179.

Quartara, L., Maggi, C.A. (1998). The tachykinin NK1 receptor. Part II: distribution and pathophysiology. Neuropeptides, 32, 1–49.

Randolph, C., Roberts, J.W., Tierney, M.C., Bravi, D., Mouradian, M.M., Chase, T.N. (1994). D-Cycloserine treatment of Alzheimer disease. Alzheimer Disease and Associated Disorders, 8, 198–205.

Ribeiro, R.L., De Lima, T.C. (2002). Participation of GABAA receptors in the modulation of experimental anxiety by tachykinin agonists and antagonists in mice. Progress in Neuro-psychopharmacology & Biological Psychiatry, 26, 861–869.

Riekkinen, P., Ikonen, S., Riekkinen, M. (1998). D-Cycloserine, a partial NMDA receptor-associated glycine-B site agonist, enhances reversal learning, but a cholinesterase inhibitor and nicotine has no effect. Neuroreport, 9, 3647–3651.

Rouaud, E., Billard, J.M. (2003). D-Cycloserine facilitates synaptic plasticity but impairs glutamatergic neurotransmission in rat hippocampal slices. British Journal of Pharmacology, 140, 1051–1056.

Sabe, L., Jason, L., Juejati, M., Leiguarda, R., Starkstein, S.E. (1995). Dissociation between declarative and procedural learning in dementia and depression. Journal of Clinical and Experimental Neuropsychology, 17, 841-848.

Sakai, N., Sakurai, E., Sakurai, E., Yanai, K., Mirua, Y., Watanabe, T. (1998). Depletion of brain histamine induced by alpha-fluoromethylhistidine enhances radial maze performance in rats with modulation of brain amino acid levels. Life Sciences, 62, 989-994.

Saper, C.B., German, D.C. (1987). Hypothalamic pathology in Alzheimer's disease. Neuroscience Letters, 74, 364-370.

Sato, N., Yamaguchi, Y. (2005). Online formation of a hierarchical cognitive map for object-place association by theta phase coding. Hippocampus, 15, 963-978.

Schroeder, J.P., Packard, M.G. (2002). Posttraining intra-basolateral amygdala scopolamine impairs food- and amphetamine-induced conditioned place preferences. Behavioral Neuroscience, 116, 922-927.

Schuster, G.M., Schmidt, W.J. (1992). D-Cycloserine reverses the working memory impairment of hippocampal-lesioned rats in a spatial learning task. European Journal of Pharmacology, 224, 97–98.

Schwartz, B.L., Hashtroudi, S., Herting, R.L., Schwartz, P., Deutsch, S.I. (1996). D-Cycloserine enhances implicit memory in Alzheimer patients. Neurology, 46, 420–424.

Severini, C., Improta, G., Falconieri-Erspamer, G., Salvadori, S., Erspamer, V. (2002). The tachykinin peptide family. Pharmacological Reviews, 54, 285–322.

Shughrue, P.J., Lane, M.V., Merchenthaler, I. (1996). In situ hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. The Journal of Comparative Neurology, 372, 395–414.

Small, B.J., Mobly, J.L., Laukka, E.J., Jones, S., Backman, L., (2003). Cognitive deficits in preclinical Alzheimer's disease. Acta Neurologica Scandinavica, 179, 29–33.

Squire, L.R., Stark, C.E., Clark, R.E. (2004). The medial temporal lobe. Annual Review of Neuroscience, 27, 279-306.

Steinberg, R., Rodier, D., Souiclhac, J., Bougault, I., Emonds-Alt, X., Soubrie, P., L Fur, G. (1995). Pharmacological characterization of tachykinin receptors controlling acetylcholine release from rat striatum: An in vivo microdialysis study. Journal of Neurochemistry, 65, 2543–2548.

Swanson, L.W. (1981). A direct projection from Ammon's horn to prefrontal cortex in the rat. Brain Research, 217, 150-154.

Taga, C,. Sugimoto, Y., Nishiga, M., Fujii, Y., Kamei, C. (2001). Effects of vasopressin on histamine H(1) receptor antagonist-induced spatial memory deficits in rats. European Journal of Pharmacology, 423, 167-170.

Tien, L.T., Fan, L.W., Sogawa, C., Ma, T., Loh, H.H., Ho, I.K. (2004). Changes in acetylcholinesterase activity and muscarinic receptor bindings in mu-opioid receptor knockout mice. Molecular Brain Research, 126, 38-44.

Tsai, G.E., Falk, W.E., Gunther, J., Coyle, J.T. (1999). Improved cognition in Alzheimer's disease with short-term D-cycloserine treatment. The American Journal of Psychology, 156, 467–469.

Tulving, E. (2002). Episodic memory: From mind to brain. Annual Reviews in Psychology, 53, 1–25.

Tulving, E., Markowitsch, H.J., (1998). Episodic and declarative memory: role of the hippocampus. Hippocampus, 8, 198–204.

Ukai, M., Shinkai, N., Kameyama, T. (1998). Involvement of dopamine receptors in beneficial effects of tachykinins on scopolamine-induced impairment of alternation performance in mice. European Journal of Pharmacology, 350, 39–45.

Vargha-Khadem, F., Gadian, D.G., Watkins, K.E., Connelly, A., Van Paesschen, W., Mishkin, M. (1997). Differential effects of early hippocampal pathology on episodic and semantic memory. Science, 277, 376–380.

Wenk, G.L., Zajaczkowski, W., Danysz, W. (1997). Neuroprotection of acetylcholinergic basal forebrain neurons by memantine and neurokinin B. Behavioral Brain Research, 83, 129–133.

Winters, B.D., Bussey, T.J., (2005). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. The Journal of Neuroscience, 25, 52–61.

Yanai, K., Son, L.Z., Endou, M., Sakurai, E., Nakagawasai, O., Tadano, T., Kisara, K., Inoue, I., Watanabe, T. (1998a) Behavioural characterization and amounts of brain monoamines and their metabolites in mice lacking histamine H1 receptors. Neuroscience, 87, 479-487.

Yanai, K., Son, L.Z., Endou, M., Sakurai, E., Watanabe, T. (1998b). Targeting disruption of histamine H1 receptors in mice: behavioral and neurochemical characterization. Life Sciences, 62, 1607-1610.

Zlomuzica, A., Dere, E., Huston, J.P., De Souza Silva, M.A. (2008a). The selective NK-3R agonist senktide promotes episodic-like memory in mice. Neurobiology of Learning and Memory. In press.

Zlomuzica, A., Viggiano, D., De Souza-Silva, M.A., Ishizuka, T., Gironi Carnevale, U.A., Ruocco, L.A., Watanabe, T., Sadile, A.G., Huston, J.P., Dere, E. (2008b). The histamine H1-receptor mediates the motivational effects of novelty. European Journal of Neuroscience, 27, 1461-1474.

Zlomuzica, A., De Souza Silva, M.A., Huston, J.P., Dere, E. (2007). NMDA-receptor modulation by D-cycloserine promotes episodic-like memory in mice. Psychopharmacology, 193, 503-509.

# 7 Original Research Articles

**Publication #1**

**Zlomuzica, A.**, De Souza Silva, M.A., Huston, J.P., Dere, E. (2007). NMDA-receptor modulation by D-cycloserine promotes episodic-like memory in mice. ***Psychopharmacology,*** 193, 503-509.

**Publication #2**

**Zlomuzica, A.**, Dere, E., Huston, J.P., De Souza Silva, M.A. (2008). The selective NK-3R agonist senktide promotes episodic-like memory in mice. ***Neurobiology of Learning and Memory***. In press.

**Publication #3**

Dere, E., **Zlomuzica, A.**, Viggiano, D., Watanabe, T., Sadile, A.G., Huston, J.P., De Souza-Silva, M.A. (2008). Episodic-like and procedural memory impairments in histamine H1R-KO mice coincide with changes in acetylcholine esterase activity in the hippocampus and dopamine turnover in the cerebellum. ***Neuroscience***. In revision.

**Publication #4**

**Zlomuzica, A.**, Viggiano, D., De Souza-Silva, M.A., Ishizuka, T., Gironi Carnevale, U.A., Ruocco, L.A., Watanabe, T., Sadile, A.G., Huston, J.P., Dere, E. (2008). The histamine H1-receptor mediates the motivational effects of novelty. ***European Journal of Neuroscience,*** 27, 1461-1474.

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ORIGINAL INVESTIGATION

# NMDA receptor modulation by D-cycloserine promotes episodic-like memory in mice

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**Abstract**
*Rationale* NMDA-R (*N*-methyl-D-aspartate receptors) have been implicated in synaptic plasticity underlying one-trial learning of event-place associations. In rodents, episodic-like memory (ELM) of personally experienced events can be inferred from behavior that reflects the remembrance of the content (what kind of object was presented), place (where was this object placed), and temporal context (when was the object presented). We have previously shown that that D-cycloserine (DCS), an NMDA-R agonist, ameliorates stress-induced deficits in ELM.
*Objectives* In this study, we used an experimental protocol designed to detect promnestic drug effects and investigated whether DCS, which is known to enhance learning and memory, can induce ELM under conditions where mice normally do not show ELM.
*Results* Mice that have been treated i.p. with DCS (20 mg/kg) both remembered the temporal order in which two different objects had been encountered during two consecutive sample trials, as well as their spatial position during the sample trials. Most importantly, the test trial performance of these mice is compatible with ELM in terms of an integrated memory for unique experiences comprising "what", "where", and "when" information. In contrast, mice that have received either a saline injection or lower doses of DCS (0.2 and 2.0 mg/kg) did not show such an integrated ELM.
*Conclusions* To our knowledge, this is the first report showing that DCS can promote ELM in mice.

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## Introduction

NMDA-R (*N*-methyl-D-aspartate receptors) have been implicated in hippocampal synaptic plasticity and learning and memory performance. The NMDA-R functions as a coincidence receptor; that is, it senses the co-occurrence of pre-synaptic activity with post-synaptic membrane depolarization. NMDA-R are involved in both homo- and hetero-synaptic long-term potentiation and depression (Lynch 2004) and have been implicated in the automatic encoding of one-trial event–place associations (Morris 2006). Thus, the NMDA-R are well-suited to associate multiple features of an event to represent an episode or event.

DCS (D-cycloserine) is a partial agonist at the glycine-binding site on the NMDA-R and facilitates NMDA-R-mediated responses (Johnson and Ascher 1987). DCS has promnestic effects in rodents (Land and Riccio 1999; Pussinen and Sirvio 1999). It reverses memory deficits after anticholinergic treatment (Ohno and Watanabe 1996), and after septal (Riekkinen et al. 1998) and hippocampal lesions (Schuster and Schmidt 1992). DCS also antagonizes aging-induced learning impairments (Baxter et al. 1994) and was effective in combating cognitive deficits in Alzheimer patients (Tsai et al. 1999).

Episodic memory can be inferred from behavioral manifestations of the knowledge regarding the content (what happened), place (where did it happen), and temporal context (in terms of the sequence of events attended) of personally experienced events (Dere et al. 2006). Recently, we have shown that both rats and mice show episodic-like memory (ELM) in terms of *what* kind of objects they have

explored during unique sampling episodes, *where* these objects have been encountered, and most importantly, *when* these episodes took place (Dere et al. 2005a, b; Kart-Teke et al. 2006a, b). Mild acute stress, e.g., induced by an i.p. saline injection before the sampling trials, disturbed ELM in rats. DCS at a dose of 15 mg/kg, i.p., ameliorated this stress-induced deficit in ELM, but was not able to fully reverse the deficit (Kart-Teke et al. 2006a). Thus, it was not clear whether DCS just antagonized the detrimental effects of stress on ELM rather than acting as an ELM enhancer. In this study, we used an experimental protocol especially designed to detect promnestic drug effects on ELM and asked whether DCS, as a possible ELM enhancer, would induce ELM under sub-optimal learning conditions where normal mice, neither drugged nor stressed, do not show significant ELM.

## Materials and methods

### Subjects

Male C57BL/6 mice at the age of 12 weeks ($n$=38) served as experimental subjects. Each animal was group housed until 4 months of age and, thereafter, individually housed in a macrolone cage with sawdust bedding. The mice were maintained under a 12-h light/dark cycle with lights on between 7:00 A.M. to 7:00 P.M. and temperature- and humidity-controlled conditions with free access to water and standard diet (Ssniff Spezialdiäten, Soest, Germany).

The experimental procedure used in this study was designed to detect promnestic drug effects on ELM. The ELM task is a three-trial procedure with two sample trials (learning phase) and a test trial (Dere et al. 2005a, b; Kart-Teke et al. 2006a, b). Depending on the inter-trial interval (ITI) used, this task can be configured for detecting either promnestic (5-min ITI) or amnestic (50-min ITI) effects of experimental manipulations, such as drugs or stress. An ITI of 50 min, but not one of 5 min, induces significant ELM. A short ITI might impair ELM by inducing interference between the memories for sample trial 1 and 2 (Blank 2002; Bower et al. 1994; Han et al. 1998). In the present study, we used a 5-min ITI to examine whether DCS would promote ELM under such sub-optimal interference-prone learning conditions.

To compare the ELM performance of the animals before and after DCS or saline treatment, approximately half of the animals in the saline- and DCS-treated groups had been tested for ELM under baseline conditions, that is, without an injection of a drug or saline. To minimize carry-over effects, there were at least 2 weeks rest between the two tests.

### Drugs and application procedure

The mice were randomly assigned to four experimental groups as follows: The mice received either an i.p. injection of physiological saline ($n$=10) or DCS (Sigma, Steinheim, Germany), a partial NMDA-R agonist at the glycine site at doses of 0.2 ($n$=8), 2.0 ($n$=10), or 20 mg/kg ($n$=10). These doses of DCS have been reported to have promnestic actions in a variety of learning and memory tasks. All drug solutions were freshly prepared before the experiment. DCS was diluted in physiological saline and injected in a volume of 4 ml/kg. The animals received the injection 30 min before the first sample trial. To know whether DCS might improve ELM on a within-subject level, the baseline performance of six animals of the saline group and half of the animals of the DCS-treated groups had been assessed before the pharmacological study (using the same procedure described below, except that different objects were used). Because, for each mouse, the ELM test procedure lasted no longer than 43 min, one can assume that the drug was active throughout the sample and the test trials (Loscher et al. 1994).

### Apparatus and objects

The test apparatus was a square open-field arena (30× 30 cm) with 40-cm-high walls made of grey polyvinyl chloride, a metal floor, and an open roof. Two visual cues were fixed on the east and north wall of the maze, which can be used for spatial orientation. The open field was placed in a sound-attenuated cubicle. A white light bulb (60 W) was located on the ceiling of the cubicle, adjusted to provide an equal light intensity inside the open field. The experimenter observed the animal's behavior on a screen monitor, which was connected to an overhead camera suspended above the open-field arena.

All objects were available in triplicate copies and varied in terms of surface texture, color, and shape but were of similar height (18 cm). Objects used in the sample trials were substituted by identical copies for the test trial, so precluding unwanted olfactory cues. The objects were made of plastic and were of sufficient weight so that they could not be displaced by the mice. None of the objects had previously been paired with a reinforcing stimulus. The objects were always placed in the corners of the open-field at a distance of 0.5 cm from the sidewalls.

### Behavioral procedures

The mice were first subjected to three daily 10-min open-field habituation trials. The mice's performance on the ELM

task was assessed on the day after the last open-field familiarization session.

Each mouse was subjected to a session of three trials with an ITI of 5 min. In the first sample trial, mice were exposed to a novel object (A1) located in a randomly selected corner of the open field. In the second sample trial, mice again were exposed to a novel object (B1), which was placed in a randomly selected corner different to the one used in sample trial 1. To prevent over-learning due to prolonged exposure to the objects, the sample trials were terminated either after 15-min or when the mouse had accumulated 40 s of object exploration.

On the test trial, two identical copies of the object A (A2 and A3) and two copies of object B (B2 and B3) were placed in the corners of the open field. One copy of objects A and B was placed in the same corner, in which it was already placed during the corresponding sample trial (A2 and B2), while the other copy of objects A and B was placed in a corner that was not used during the sample trials (A3 and B3). Figure 1 gives an example of how objects were arranged during the sample and test trials. Between trials, the open field was cleaned with a 75% ethanol solution to remove odor cues inside the maze.

Animals that did not show at least 15 s of object exploration on the sample- or test trials were excluded from the data analysis. The time spent exploring the objects (in seconds) during the sample and test trials were recorded by an experienced observer who was blind to the experimental conditions. Object exploration was defined as a physical contact with an object either with the nose, vibrissae, or forepaws.

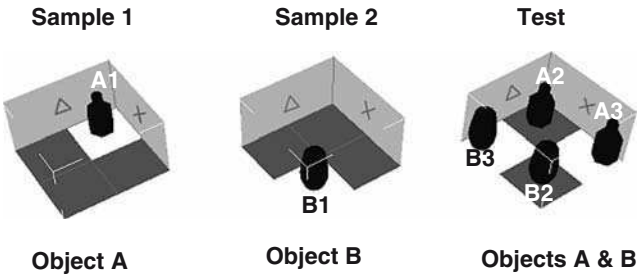


**Fig. 1** Schematic drawing of the episodic-like memory task. In the first sample trial, the mice are exposed to a novel object of type A (A1), placed in a randomly determined corner of the open field (here, the *north-east white colored corner*). The mouse is removed from the open field after it has explored the object for 40 s or after 15 min had elapsed, whatever occurs first. After an ITI of 5 min, the mouse is again placed into the open field, now containing a different novel object (*B1*) in a location different from the one used in the first sample trial (here, *south-west white colored corner*). The mouse is again removed from the open field after it has explored the novel object for 40 s or after 15 min had elapsed. After a further ITI of 5 min, the mouse is subjected to a test trial. Here, two identical copies of the objects from sample 1 and sample 2 (*A2* and *B2*) are placed in the same location already occupied during the sample trial. Two copies (*A3* and *B3*) of the sample objects are moved to a novel location not used during their respective sample trials (in this example, the *north-west* and *south-east corner colored white*). The order of object presentation as well as the object locations is determined randomly for each mouse. Episodic-like memory is characterized by the following exploration pattern: A2 > B2, B3 > B2, A2 > A3. *A2* Old familiar stationary object, *A3* old familiar displaced object, *B2* recent familiar stationary object, *B3* recent familiar displaced object

## Hypotheses

Based on previous work with rats (Kart-Teke et al. 2006a, b) and mice (Dere et al. 2005a, b), we hypothesized that dependent on the dose, the DCS- but not the saline-treated mice would spend more time exploring the "old familiar stationary" object (A2) compared to the "recent familiar stationary" object B2 (prediction 1). This would indicate that they remember both the features of the sample trial objects (what) as well as their temporal order of presentation (when). We further expected the DCS-treated mice to recognize whether a given object (what) had been displaced to a novel location (where). Specifically, the "recent familiar displaced" object (B3) should be explored for a longer time compared to the "recent familiar stationary" object B2 (prediction 2). The opposite exploration pattern was expected for the "old familiar" objects where the stationary one (A2) should be explored for a longer time relative to the displaced one, A3 (prediction 3). Because we and others previously showed that, during the test trial, rats behave as if there is an interaction between temporal and spatial information in their ELM (Kart-Teke et al. 2006a, b; Li and Chao 2007), spatial displacement of "old" vs "recent" objects was expected to have opposite effects on the animals object preference. Obviously, such an interaction cannot occur when only one of the "old familiar" objects is displaced during the test trial, while the two "recent familiar" objects are kept in place. Indeed, mice prefer the "old familiar displaced" object over the "old familiar stationary" object when none of the "recent familiar" objects is displaced (Dere et al. 2005a, b).

If the predictions 1–3 were confirmed as specified above, this would indicate that the DCS-treated mice remember the spatial position and temporal order in which the objects were encountered during the sample trials. Most importantly, it would further indicate that their memory for the temporal order somehow interacts with their spatial memory in terms of the direction of the object preference between the displaced and stationary objects. The most plausible explanation for such an interaction between these distinct types of memory (spatial vs temporal) is to assume that the mice have established an integrated memory for unique experiences comprising the "what", "where", and "when" information (Kart-Teke et al. 2006a, b). On the contrary, if this interaction were absent, one would have to assume that the mice encoded the spatial and temporal information independently. In this case, one would expect

that they would also prefer the "old familiar displaced" object over the "old familiar stationary" one, as it is normally observed in "pure" object–place memory tasks, for which temporal information is irrelevant (Mumby et al. 2002).

## Statistics

Sample and test trial data are expressed as mean±SEM and were analyzed by means of one-way analysis of variance (ANOVA) and *t* tests for dependent and independent groups. The test trial object exploration data, shown in Table 1, was used to calculate the following preference ratios (PR) for each mouse:

Prediction 1:

PR1 = Time spent exploring the old familiar stationary object / Time spent exploring the old familiar stationary + recent familiar stationary object

Prediction 2:

PR2 = Time spent exploring the recent familiar displaced object / Time spent exploring the recent familiar displaced + recent familiar stationary object

Prediction 3:

PR3 = Time spent exploring the old familiar displaced object / Time spent exploring the old familiar displaced + old familiar stationary object

Single group *t* tests (each against a comparison value of 0.5 ~ chance level) were performed on these PR for each group separately. Unless otherwise indicated, *p* values given are two-tailed (in cases where no predictions were made) and represent measures of effect.

## Results

The time the animals needed to accumulate a total of 40 s of object exploration during the sample trials and the total time spent exploring the objects during the sample and test trials were similar between groups (all $P>0.05$, one-way ANOVA, data not shown). These results suggest that all groups were equally motivated to explore the objects during the sample trials and showed comparable levels of object exploration during the test trials. There was no indication for impaired sensory-motor functions in DCS-treated animals.

As expected, the mice that had received a saline injection before the sample trial 1 failed to discriminate the temporal order in which the two objects had been presented (PR1, $P>0.05$; single group *t* test; Fig. 2). They were also unable to detect that one of the "recent familiar" objects was presented in a novel position (PR2, $P>0.05$), but instead preferred the "old familiar" object in the novel position compared to the one placed in its former location known from the sample trial one (PR3, $P>0.031$). The fact that the "old familiar displaced" object was significantly preferred over the "stationary" one suggests either intact spatial memory for sample trial 1 or that the mice directed more exploration at the object, which appeared least familiar, as it was not seen most recently, and, additionally, was placed in a novel location. However, in any case, this object exploration pattern is not compatible with ELM.

DCS given at doses of 0.2 and 2.0 mg/kg was not effective in inducing the full exploration pattern indicative of ELM. The 0.2-mg/kg group performed similar to the saline-treated group. Contrary to the hypothesis, they spent more time exploring the "old familiar displaced" object compared to the "old familiar stationary" one (PR3, $P=0.043$; one-tailed single group *t* test; Fig. 2), failed to detect the displacement of one of the "recent familiar" objects (PR2, $P>0.05$), and showed no memory for the temporal order in which the objects had been encountered (PR1, $P>0.05$). These results suggest that the 0.2 mg/kg of DCS is too low to have a beneficial effect on ELM under sub-optimal interference-inducing learning conditions.

The mice that were injected with 2.0 mg/kg DCS did not respond to the spatial displacement of the objects in the predicted way. The accordant PR were not different from chance level (PR2, $P>0.05$; PR3, $P>0.05$; Fig. 2). However, unlike the saline- and 0.2-mg/kg DCS-treated groups, the 2.0 mg/kg dose of DCS rescued temporal order memory. These mice spent more time exploring the "old

**Table 1** Mean (and ±SEM) exploration time (s) of the indicated objects during the test trial

| Treatment | Old familiar displaced | Old familiar stationary | Recent familiar displaced | Recent familiar stationary |
|---|---|---|---|---|
| Saline ($n=10$) | 11.24±1.78 | 7.56±1.44 | 8.76±1.13 | 8.09±1.56 |
| D-Cycloserine 0.2 mg /kg ($n=8$) | 8.74±0.66 | 5.87±0.87 | 7.53±1.32 | 6.53±1.84 |
| D-Cycloserine 2.0 mg/kg ($n=10$) | 11.02±2.09 | 7.96±1.18 | 8.09±1.21 | 5.27±0.65 |
| D-Cycloserine 20 mg/kg ($n=10$) | 9.27±1.27 | 11.59±1.69 | 8.97±1.03 | 6.50±1.80 |

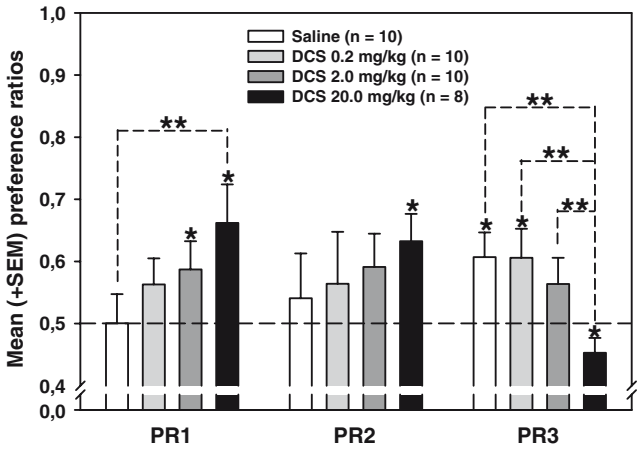


**Fig. 2** D-Cycloserine promotes episodic-like memory in mice. *Bars* represent mean (and SEM) preference ratios for the groups indicated. *$p<0.05$, single-group *t* test. The *dashed horizontal line* indicates chance level performance. **$p<0.05$, *t* test for independent groups

familiar stationary" object relative to the "recent familiar stationary" object (PR1, $P=0.044$; one-tailed single group *t* test). Because the time spent exploring the objects during sample trials 1 and 2 was similar in this group ($P>0.05$; *t* test for dependent groups; data not shown), this temporal order memory is unlikely to be due to baseline differences in the level of prior learning or familiarization with the old and recent objects. Although the 2.0-mg/kg dose of DCS was not effective in restoring full ELM, it nevertheless had a beneficial effect on temporal order memory, counteracting the short ITI-induced interference between the memories for sample trials 1 and 2.

In contrast, the 20-mg/kg DCS-treated mice showed the typical exploratory pattern indicative of ELM (Kart-Teke et al. 2006a, b; Fig. 2). This group spent more time exploring the "old familiar stationary" object than the "recent familiar stationary" object (PR1, $P=0.017$; one-tailed single group *t* test). This effect was not due to differences in the time spent exploring the objects on sample trials 1 and 2 ($P>0.05$; *t* test for dependent groups, data not shown). The mean time spent exploring the displaced copy of the recent familiar object was significantly higher than the time spent exploring the stationary copy of the recent familiar object (PR2, $P=0.009$; one-tailed single group *t* test), suggesting that these mice were able to remember where the recent familiar object had been placed during sample trial 2. They also showed significantly higher exploration times with the stationary compared to the displaced copy of the old familiar object (PR3, $P=0.048$; one-tailed single group *t* test). These results suggest, for one, that the mice remembered the spatial position in which the objects had been encountered during the sample trials and that the temporal order in which the objects were presented interacts with this spatial memory in terms of the direction of the object preference. This interaction between temporal and spatial information strongly suggests that the exploration pattern of these mice reflects an integrated memory for unique experiences comprising the "what", "where", and "when" information. If the mice would have encoded spatial and temporal information independently, one would have expected that they would also have preferred the displaced old familiar object, which had been placed in a novel location, over the stationary old familiar one. However, this was not the case, supporting the integration hypothesis. Thus, it can be assumed that a 20-mg/kg dose of DCS induces ELM for unique experiences under sub-optimal learning conditions.

Between-group comparisons revealed that the 20-mg/kg DCS group differed from the remaining groups in terms of PR3 (20 mg/kg vs saline, $P=0.0025$; 20 vs 0.2 mg/kg, $P=0.006$; 20 vs 2.0 mg/kg, $P=0.018$; one-tailed *t* test for independent groups; Fig. 2) and PR1 compared to the saline-treated group ($P=0.032$; one-tailed *t* test for independent groups).

Next, we compared the PR obtained in the baseline test with the corresponding PR of the same animals after DCS or saline injections. The PR of the saline-injected group were similar on both occasions (PR1-3, all $P>0.05$; one-tailed *t* test for dependent samples, data not shown). Thus, these mice performed worse on both baseline and vehicle-injected tests. While the PR3 0.2-mg/kg group was different from baseline ($P=0.029$), the remaining PR were not (PR1 and 2, both $P>0.05$). In line with the finding that the 2.0-mg/kg dose of DCS rescues temporal order memory after interference-inducing learning conditions, this group showed a difference in PR1 when compared to the baseline test ($P=0.005$), while the other PR were similar on both tests (PR1 and 2, both $P>0.05$). Finally, the 20-mg/kg group showed differences in both PR1 ($P=0.024$) and PR3 ($P=0.047$) but not PR2 ($P>0.05$). Thus, the 20-mg/kg dose improved the mice's ability to remember the sequence in which the objects had been presented during the sample trials and where the old familiar object had been located during the first sample trial. However, the ability to remember the position of the most recently seen object was not influenced by the 20-mg/kg dose of DCS. In sum, these findings show that DCS dose-dependently rescued ELM under sub-optimal learning conditions with the 0.2-mg/kg dose being ineffective, the 2.0-mg/kg dose promoting temporal order memory, and the 20-mg/kg dose of DCS fully restoring ELM.

## Discussion

Episodic memory can be inferred from behavioral manifestations of the knowledge regarding the content (what happened), place (where did it happened), and temporal context (in terms of the sequence of events attended) of

personally experienced events (Dere et al. 2006). Our ELM task combines different versions of the novelty-preference paradigm and subsumes one-trial object recognition memory, the memory for locations in which objects were explored, and temporal order memory for objects presented at distinct time points (reviewed in Dere et al. 2007). Recently, we showed that rodents are able to remember "where" and "when" they have explored different objects in the past (Dere et al. 2005a, b; Kart-Teke et al. 2006a, b). However, because of its all-or-nothing nature (either there is, or there is not an exploration pattern indicative of ELM), this procedure is not suited to examine whether a drug can improve ELM in mice. Therefore, we searched for a procedure that allows investigating promnestic drug effects. The simplest way to test promestic effects is to increase the difficulty of the task. To achieve this, one could either increase the ITI to induce forgetting of the sample trial information, or one could decrease the ITI to induce pro- or retroactive interference, that is a failure to remember "where" and "when" information was acquired (Blank 2002; Bower et al. 1994; Han et al. 1998). Because delay-dependent forgetting of information is a feature common to virtually all types of memory, it is not specific for episodic memory. In contrast, one of the main functions of episodic memory is to recollect the *sequence* of single episodes/events attended even if they occur back to back (Roberts 2002). Thus, it can be assumed that a drug that reduces the susceptibility to pro- or retroactive interference should also promote ELM. We found that an ITI of 5-min completely eliminates the exploration pattern indicative of ELM, possibly because of pro- or retroactive interference. In the Dere et al. (2005a, b) and Kart-Teke et al. (2006a, b) studies, an ITI of 50 min was used, which is probably less likely to produce interference.

We in this paper present evidence that DCS might be viewed as an ELM enhancer in mice. Using an experimental protocol with which C57BL/6 mice regularly fail to establish an ELM for unique experiences, we found that a dose of 20-mg/kg of DCS can restore ELM under sub-optimal learning conditions. The effect of DCS on ELM was dose dependent. A dose of 0.2 mg/kg DCS was completely ineffective in restoring ELM. A higher dose of DCS 2.0 mg/kg rescued temporal order memory, but had no promestic effect on the spatial component of ELM. Compared to the saline- and 0.2- and 2.0-mg/kg DCS-treated groups, the mice that received the highest dose of DCS both remembered the temporal order and spatial position of two different objects after a single sample trial.

It has been shown that DCS improves long-term retention in a one-trial inhibitory avoidance task (Land and Riccio 1999). In this task, the animals have to remember after a single trial in which of the two compartments of the apparatus (where) they have received a foot-shock (what). Because this task lacks the temporal component (when) of episodic memory, it cannot be viewed as an ELM task. Therefore, the present study is the first report showing that DCS can enhance ELM in mice.

Our results are in line with evidence showing that DCS ameliorates cognitive deficits in Alzheimer patients (Randolph et al. 1994; Schwartz et al. 1996; Tsai et al. 1999), which show deficits in episodic memory already at early stages of the disease (Small et al. 2003). In Alzheimer patients, both hypo- and hyperactivity of NMDA-R have been proposed. While the hypoactivity is addressed with glycine and DCS treatments, the hyperactivity of NMDA-R is decreased by memantine, an NMDA-R antagonist (Butterfield and Pocernich 2003). The latter compound has already been approved for the treatment of Alzheimer patients. The enhancing effect of DCS on ELM in mice might be due to facilitation of synaptic plasticity (Rouaud and Billard 2003), e.g., in the hippocampus (Manns and Eichenbaum 2006). In conclusion, it seems that DCS might be, indeed, a promising candidate for the treatment of deficits in episodic memory due to its modulation of NMDA-R function.

NMDA-R are composed of a NR1 and at least of one of four NR2 (A–D) and/or NR3 subunits, and their functional characteristics are determined by their subunit composition (Dingledine et al. 1999). Therefore, it remains to be determined which NMDA-R type is critical for ELM. In this regard, the evaluation of NMDA-R-related mouse mutants in terms of ELM would be promising. Mice in which the NR2B subunit was over-expressed in the forebrain displayed improved one-trial object recognition after long retention intervals and eased LTP induction in the hippocampus (Tang et al. 1999). It would be interesting to see whether these NR2B over-expression mutants would also show enhanced ELM.

Recently, using our paradigm, it has been shown that lesions to the CA3 region of the hippocampus impair ELM in rats (Li and Chao 2007). During the test trial, lesioned rats failed to show the interaction between the temporal and spatial factors of ELM. Most importantly, this deficit could not be attributed to deficits in either object–place memory, temporal order memory, or in one-trial object recognition, as the lesioned rats subsequently performed normally in the single versions of the novelty preference paradigm (Li and Chao 2007). This finding suggests that the CA3 region of the hippocampus is especially important for the integration of object, spatial, and temporal information and, thus, ELM. Because the CA3 region of the hippocampus seems to be critical for the integration of the "what", "where", and "when" aspects of ELM, it might also be interesting to know whether pharmacological blockade of CA3 NMDA-R has similar effects on ELM as a lesion of this region.

In conclusion, using an experimental protocol designed to detect the promnestic properties of drugs, we showed that NMDA receptor modulation by DCS can dose dependently promote ELM in mice. To our knowledge, this study is the first to implicate the DCS in ELM in mice.

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## References


Baxter MG, Lanthorn H, Frick KM, Golski S, Wan RQ, Olton DS (1994) D-Cycloserine, a novel cognitive enhancer, improves spatial memory in aged rats. Neurobiol Aging 15:207–213

Blank H (2002) The role of horizontal categorization in retroactive and proactive interference. Exp Psychol 49:196–207

Bower GH, Thompson-Schill S, Tulving E (1994) Reducing retroactive interference: an interference analysis. J Exp Psychol Learn Mem Cogn 20:51–66

Butterfield DA, Pocernich CB (2003) The glutamatergic system and Alzheimer's disease: therapeutic implications. CNS Drugs 17:641–652

Dere E, Huston JP, De Souza Silva MA (2005a) Integrated memory for objects, places and temporal order: evidence for episodic-like memory in mice. Neurobiol Learn Mem 84:214–221

Dere E, Huston JP, De Souza Silva MA (2005b) Protocol: episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. Brain Res Protoc 16:10–19

Dere E, Huston JP, De Souza Silva MA (2007) The pharmacology, neuroanatomy, and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev 31:673–704

Dere E, Kart-Teke E, Huston JP, De Souza Silva MA (2006) The case for episodic memory in animals. Neurosci Biobehav Rev 30:1206–1224

Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. Pharmacol Rev 51:7–61

Han JS, Gallagher M, Holland P (1998) Hippocampal lesions enhance configural learning by reducing proactive interference. Hippocampus 8:138–146

Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529–531

Kart-Teke E, De Souza Silva MA, Huston JP, Dere E (2006a) Wistar rats show episodic-like memory for unique experiences. Neurobiol Learn Mem 85:173–182

Kart-Teke E, Dere E, Huston JP, De Souza Silva MA (2006b) Reinstatement of episodic-like memory in rats by NK-1 receptor antagonism. Neurobiol Learn Mem 87:324–331

Land C, Riccio DC (1999) D-Cycloserine: effects on long-term retention of a conditioned response and on memory for contextual attributes. Neurobiol Learn Mem 72:158–168

Li JS, Chao YS (2007) Dorsal hippocampal CA3 lesions impair episodic-like memory in rats. Annual meeting of the International Behavioral Neuroscience Society (IBNS), Rio de Janeiro, 12–16 June 2007

Loscher W, Wlaz P, Rundfeldt C, Baran H, Honack D (1994) Anticonvulsant effects of the glycine/NMDA receptor ligands D-cycloserine and D-serine but not R-(+)-HA-966 in amygdala-kindled rats. Br J Pharmacol 112:97–106

Lynch MA (2004) Long-term potentiation and memory. Physiol Rev 84:87–136

Manns JR, Eichenbaum H (2006) Evolution of declarative memory. Hippocampus 16:795–808

Morris RGM (2006) Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. Eur J Neurosci 23:2829–2846

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002) Hippocampal damage and exploratory preferences in rats, memory for objects, places, and contexts. Learn Mem 9:49–57

Ohno M, Watanabe S (1996) D-Cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats. Eur J Pharmacol 318:267–271

Pussinen R, Sirvio J (1999) Effects of D-cycloserine, a positive modulator of N-methyl-D-aspartate receptors, and ST 587, a putative alpha-1 adrenergic agonist, individually and in combination, on the non-delayed and delayed foraging behaviour of rats assessed in the radial arm maze. J Psychopharmacol 13:171–179

Randolph C, Roberts JW, Tierney MC, Bravi D, Mouradian MM, Chase TN (1994) D-Cycloserine treatment of Alzheimer disease. Alzheimer Dis Assoc Disord 8:198–205

Riekkinen P, Ikonen S, Riekkinen M (1998) D-Cycloserine, a partial NMDA receptor-associated glycine-B site agonist, enhances reversal learning, but a cholinesterase inhibitor and nicotine has no effect. Neuroreport 9:3647–3651

Roberts WA (2002) Are animals stuck in time? Psych Bull 128:473–489

Rouaud E, Billard JM (2003) D-Cycloserine facilitates synaptic plasticity but impairs glutamatergic neurotransmission in rat hippocampal slices. Br J Pharmacol 140:1051–1056

Schuster GM, Schmidt WJ (1992) D-Cycloserine reverses the working memory impairment of hippocampal-lesioned rats in a spatial learning task. Eur J Pharmacol 224:97–98

Schwartz BL, Hashtroudi S, Herting RL, Schwartz P, Deutsch SI (1996) D-Cycloserine enhances implicit memory in Alzheimer patients. Neurology 46:420–424

Small BJ, Mobly JL, Laukka EJ, Jones S, Backman L (2003) Cognitive deficits in preclinical Alzheimer's disease. Acta Neurol Scand 179:29–33

Tang YP, Shimiizu E, Dube GR, Rampon C, Kerchner GA, Zhuo M, Liu G, Tsien JZ (1999) Genetic enhancement of learning and memory in mice. Nature 401:63–69

Tsai GE, Falk WE, Gunther J, Coyle JT (1999) Improved cognition in Alzheimer's disease with short-term D-cycloserine treatment. Am J Psychol 156:467–469

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# $NK_3$ receptor agonism promotes episodic-like memory in mice

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ABSTRACT

The mammalian tachykinins are a family of closely related peptides including substance P, neurokinin A, neurokinin B and, recently, also hemokinin-1. They are present in the peripheral and central nervous systems, and bind to three known neurokinin (NK) receptors, the $NK_1$-, $NK_2$- and $NK_3$ receptors. In both rodents and humans, $NK_3$ receptors are expressed in brain structures which have been associated with learning and memory. Evidence for a role of $NK_3$ receptors in learning and memory has been found in $NK_3$ receptor knockout mice. Here, we investigated the influence of the $NK_3$ receptor agonist, senktide (0.1, 0.2 and 0.4 mg/kg), on the performance of C57BL/6 mice in a recently developed episodic-like memory task. Since a promnestic effect of senktide was expected, we employed an experimental protocol that provided sub-optimal learning conditions for episodic-like memory. The results indicate that senktide promotes episodic-like memory in mice in a dose-dependent manner, providing, for the first time, evidence for an involvement of $NK_3$ receptors in episodic-like memory.



## 1. Introduction

Substance P (SP), neurokinin A (NKA), neurokinin B (NKB), and the recently identified hemokinin, (HK)-1, are mammalian endogenous neuropeptides belonging to the tachykinin family, shown to be present in peripheral tissue and the brain. They act through three subtypes of the G-protein-coupled neurokinin (NK) receptors, the $NK_1$, $NK_2$ and $NK_3$ receptors. SP preferentially binds to the $NK_1$ receptor subtype, whereas NKA and NKB have higher affinities for the $NK_2$ and $NK_3$ receptors, respectively. HK-1 has high selectivity and potency for $NK_1$ receptors, but also binds to $NK_2$ and $NK_3$ receptors. The $NK_1$- and $NK_3$ receptors are the ones most widely expressed in the mammalian brain (Bellucci et al., 2002; Severini, Improta, Falconieri-Erspamer, Salvadori, & Erspamer, 2002). SP has been implicated in the modulation of processes such as reinforcement, learning, memory, anxiety and fear, when applied centrally or systemically (Hasenohrl et al., 2000; Huston & Hasenohrl, 1995).

High to dense distributions of $NK_3$ binding sites have been identified in brain regions, which have been implicated in learning and memory, including the prefrontal, frontal and anterior cingulated cortices, the hippocampus, amygdala and medial septum of rats and mice (Ding et al., 1996; Duarte, Schutz, & Zimmer, 2006; Shughrue, Lane, & Merchenthaler, 1996). In humans, immunohistochemical analyses demonstrated $NK_3$ receptors to be widespread in the brain, including cortex, hippocampus and hypothalamus (Mileusnic et al., 1999).

Senktide is a highly potent $NK_3$ receptor agonist, showing high affinity to $NK_3$ receptors in different animal species (Laufer, Gilon, Chorev, & Selinger, 1986; Massi, Panocka, & de Caro, 2000). Senktide has also been reported to have anxiolytic effects in mice (Ribeiro & De Lima, 2002). Intracerebroventricular injections of senktide in mice antagonized scopolamine-induced spatial working memory impairment in the spontaneous alternation Y-maze task (Kameyama, Ukai, & Shinkai, 1998; Ukai, Shinkai, & Kameyama, 1998). The promnestic effects of senktide might involve the cholinergic system, which has been related to learning and memory (Pepeu & Giovannini, 2004). Infusion of senktide into the septum of guinea pigs induces acetylcholine release in the hippocampus (Marco et al., 1998), a brain region that has been implicated in spatial and episodic memory (Smith & Mizumori, 2006). Besides, at least 66% of cholinergic neurons in the medial septum/diagonal band of broca, which are known to project to the hippocampus, express $NK_3$ receptors (Chen et al., 2001).

Neurokinin B, which is the preferred endogenous ligand of $NK_3$ receptors, prevents the loss of basal forebrain cholinergic cells induced by injection of NMDA into the nucleus basalis magnocellularis of rats (Wenk, Zajaczkowski, & Danysz, 1997). Hence, it is possible that the activation of the $NK_3$ receptors has a neuroprotective effect on cholinergic neurons. In humans, the loss of cholinergic neurons, e.g., within the nucleus basalis of Meynert, has been related to the cognitive decline associated with Alzheimer's disease (Mufson, Ginsberg, Ikonomovic, & DeKosky, 2003; Oliveira Jr. & Hodges, 2005). Furthermore, the genetic inactivation of the $NK_3$ receptors specifically affected the performance of mice in learning- and memory-related tasks, while having no effect across a variety of tests assessing spontaneous activity, pain sensitivity, anxiety, and depression-like behaviour (Siuciak et al., 2007).

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We have developed an episodic-like memory task for rodents, which allows the simultaneous assessment of object, place and temporal order memory (Kart-Teke, de Souza Silva, Huston, & Dere, 2006; Kart-Teke, Dere, Brandao, Huston, & de Souza Silva, 2007; Zlomuzica, de Souza Silva, Huston, & Dere, 2007). In the present study, since we expected a memory-improving effect of senktide, we employed an experimental protocol that provides sub-optimal learning conditions for episodic-like memory (Zlomuzica et al., 2007). We evaluated the effects of subcutaneous administration of the $NK_3$ receptor agonist, senktide, on the episodic memory task in mice, with the expectation that the activation of $NK_3$ receptors improves episodic-like memory.

## 2. Materials and methods

### 2.1. Animals

Male, 4 months old, C57BL/6 mice were used. During the days of testing, the mice were individually housed, with unrestricted access to standard diet and water. The mice were kept on a 12 h light–dark cycle (lights on at 07:00 a.m.) in a temperature- and humidity-controlled room. All experiments were conducted during the light phase of the cycle, and were performed in accordance with the terms of the German Animal Protection Law.

### 2.2. Drug preparation and administration

Senktide (succinyl-(Asp$^6$,*N*-Me-Phe$^8$)-Substance P(6-11); Bachem, Germany) was initially dissolved in dimethylsulphoxide (100 μg/μl), and further diluted to the desired concentrations with phosphate-buffered physiological saline and injected subcutaneously 30 min prior to the first sample trial. The injection volume was 0.2 ml/50 g of body weight. Control animals were injected with the vehicle solution. All drug solutions were freshly prepared on the day of experiment.

### 2.3. Episodic-like memory in the object recognition task

#### 2.3.1. Apparatus

The test apparatus was a square open-field arena (30 × 30 cm), with 40 cm high walls, made of grey polyvinyl chloride, a metal floor and an open roof. For spatial orientation, two visual cues were fixed on the east and north walls of the maze. The open field was placed in a sound-attenuating chamber. A white 60 W light bulb was located on the ceiling of the chamber, adjusted to provide an equal light intensity inside the open-field arena. Inside the chamber, a video camera was suspended above the open-field arena and connected to a monitor, placed outside, allowing the animal's behaviour to be observed during the experimental procedure, and recorded for post hoc analysis.

All objects were available in triplicate copies and varied in terms of surface texture, colour and shape, but were of similar height. Objects used in the sample trials were substituted by identical copies for the test trial, thus, precluding unwanted olfactory cues. The objects were made of plastic, and their weight was sufficient to prevent displacement by the mice. They were novel to the animals and, therefore, none of them had previously been paired with any reinforcing stimulus.

#### 2.3.2. Procedure

In the week preceding habituation to the test apparatus, all animals were handled once daily. They were then habituated to the open field during three consecutive days. On each day the mouse was placed inside the empty open-field arena and was allowed to freely explore it for 10 min. On the day after habituation, the animal's performance in the episodic-like memory task was assessed. This task is a three trial procedure with two sample trials (learning phase, samples 1 and 2) and a test trial (Fig. 1). Each mouse was subjected to a session of three trials with an inter-trial interval of 5 min. The mice were randomly assigned to one of four groups: vehicle ($n = 10$), 0.1 mg/kg ($n = 12$), 0.2 mg/kg ($n = 13$), or 0.4 mg/kg senktide ($n = 11$). In the first sample trial (sample 1), mice were exposed to a novel object (A) located in a randomly selected corner of the open field. In the second sample trial (sample 2), mice again were exposed to a novel object (B), which was placed in a randomly selected corner different from the one used in sample trial 1. The sample trials 1 and 2 were terminated either after 15 min, or, when the mouse had accumulated 40 s of object exploration. On the test trial, two identical copies of object A ($A_1$ and $A_2$) and two copies of object B ($B_1$ and $B_2$) were placed in the corners of the open field. One copy of object A and object B was placed in the same corner, in which it was already placed during the corresponding sample trial ($A_1$ and $B_1$), while the other copy of the objects A and B was placed in a corner in which it was not presented during the sample trials ($A_2$ and $B_2$). Fig. 1 gives an example of how objects were arranged during the sample- and test trials. Between trials, the open field was cleaned with 75% ethanol in order to remove odour cues. Before the beginning of sample trial 1, the open field and the objects were cleaned with 75% ethanol. The order of object presentation, as well as the object location, was determined randomly for each mouse. Animals that did not accumulate a minimum of 20 s of object exploration during the sample trials were excluded from the data analysis.

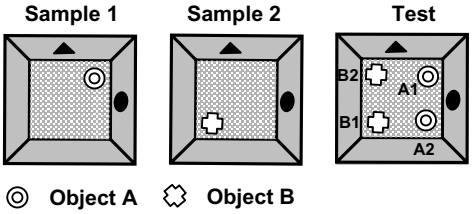


**Fig. 1.** Top-view schematic drawing of the episodic-like memory task. In the first sample trial the mouse is exposed to a novel object of type A, placed in a randomly determined corner of the open field. It is removed from the open field after the object has been explored for 40 s, or after 15 min has elapsed, whatever occurs first. After an inter-trial interval of 5 min, it is again placed into the open field, now containing a different novel object (B) in a different location from the one used in the first sample trial. It is again removed from the open field after exploration of the novel object for 40 s, or after 15 min has elapsed. After another 5 min inter-trial interval, it is subjected to the test trial. Here, two identical copies of the objects from sample 1 and sample 2 ($A_1$ and $B_1$) are placed in the same location already occupied during the sample trial. Two other copies ($A_2$ and $B_2$) of the sample objects are moved to a novel location not used during their respective sample trials. The orders of object presentation and object location are determined randomly for each animal. A performance indicative of episodic-like memory is characterized by a significant difference in the three PRs ($PR_1$, $PR_2$ and $PR_3$).

#### 2.3.3. Dependent variables

The time spent exploring the objects (in seconds) during the sample and test trials, and the time the animal needed to accumulate a total of 40 s of object exploration during the sample trials were scored by an experienced observer, who was blind to the experimental conditions. Object exploration was defined as a physical contact with an object either with the nose, vibrissae or forepaws. In the test trial, four objects were presented, and according to the sequence (old–recent), or the location (displaced–stationary) in which they were presented, they were classified as old familiar displaced (OFD), old familiar stationary (OFS), recent familiar displaced (RFD) or recent familiar stationary (RFS). The time spent exploring an object during the test trial was used to calculate the following preference ratios (PR):

$PR_1$: Indicates if the temporal order in which the objects were present can be remembered.

$$PR_1 = \text{Time spent exploring } (T_{exp})\text{OFS}/(T_{exp}\text{OFS} + T_{exp}\text{RFS})$$

$PR_2$: Indicates if the place in which the objects were presented can be remembered.

$$PR_2 = T_{exp}\text{RFD}/(T_{exp}\text{RFD} + T_{exp}\text{RFS})$$

$PR_3$: Indicates if an inverse relationship in exploration time, based on the place where the old objects are encountered, is present.

$$PR_3 = T_{exp}\text{OFD}/(T_{exp}\text{OFD} + T_{exp}\text{OFS})$$

### 2.4. Predictions

Previously, we showed that a short, 5-min, inter-trial interval impaired episodic-like memory in rodents (Zlomuzica et al., 2007). Therefore, we expected that control mice, injected with vehicle, would not show episodic-like memory. Given that senktide was expected to have promnestic effects (Kameyama et al., 1998; Ukai et al., 1998), we proposed that senktide-treated mice would discriminate both the temporal order and displacement of objects and, thus, exhibit episodic-like memory, in terms of an integrated object memory for what, where and when. In particular, during the test trial, senktide-treated mice were expected to spend significantly more time exploring the "old familiar" stationary object ($A_1$) in comparison to the "recent familiar" stationary ($B_1$) object ($A_1 > B_1$, indicated by $PR_1$). We further expected these mice to explore the "recent familiar" displaced object for a longer time relative to the "recent familiar" stationary object ($B_2 > B_1$, indicated by $PR_2$), but to spend more time exploring the "old familiar" stationary object as compared to the "old familiar" displaced object ($A_2 < A_1$, indicated by $PR_3$). The fact that the animals were expected to spend more time exploring the "old familiar" stationary in comparison to the "old familiar" displaced object can not explicitly be predicted from the "know exploration pattern" observed in object locations tests (Ennaceur, Neave, & Aggleton, 1997). However, this pattern of exploration has been consistently displayed by rats and mice in this task (Dere, Huston, & de Souza Silva, 2007; Kart-Teke, Dere et al., 2007).

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**Table 1**
Mean (±SEM) exploration time (s) and total duration (s) of sample trials 1 and 2

| Treatment | Sample trial 1 Exploration time | Sample trial 1 Duration | Sample trial 2 Exploration time | Sample trial 2 Duration |
|---|---|---|---|---|
| Vehicle ($n$ = 10) | 31.77 ± 3.74 | 626.80 ± 83.87 | 36.22 ± 2.23 | 590.50 ± 76.43 |
| Senktide 0.1 mg/kg ($n$ = 12) | 37.82 ± 1.78 | 604.91 ± 52.92 | 37.95 ± 1.77 | 610.42 ± 63.08 |
| Senktide 0.2 mg/kg ($n$ = 13) | 36.54 ± 1.81 | 684.31 ± 61.44 | 31.40 ± 3.31 | 673.00 ± 71.81 |
| Senktide 0.4 mg/kg ($n$ = 11) | 37.73 ± 1.64 | 685.73 ± 50.68 | 37.74 ± 1.72 | 553.63 ± 61.52 |

A performance indicative of episodic-like memory is characterized by a significant difference in the three PRs ($PR_1$, $PR_2$ and $PR_3$).

### 2.5. Statistics

The data obtained on the exploration time during sample trials 1 and 2 are expressed as means ± SEM. Between-groups comparisons of exploration time during sample trials 1 and 2 were performed by one-way ANOVA, followed by Bonferroni post hoc tests, where indicated. Differences in the time needed to reach the exploration criterion and the time spent exploring the objects during sample trials 1 and 2 within a group were calculated by paired $t$-tests. The data obtained on the exploration time during test trial are expressed as medians (and interquartile range). Within-group differences between the exploration times of the four objects during the test trial were calculated by the Wilcoxon test. The Mann–Whitney $U$-test was used for pair-wise group comparison against the vehicle group. The $p$ values given are one-tailed, and are considered to be significant when $p < .05$.

## 3. Results

Neither the time needed to accumulate 40 s of object exploration, nor the total time spent exploring the objects during the first and second sample trials, was significantly different between the 4 groups ($ps > .05$, one-way ANOVA followed by Bonferroni, Table 1). The results showed that all groups had comparable levels of object exploration, which excludes possible differences in the motivation to approach and explore objects during the sample trials. The total time spent exploring the objects in the test trial was also comparable between groups ($ps > .05$, one-way ANOVA, data not shown).

In line with our previous results, showing that either untreated or vehicle-treated mice do not show significant episodic-like memory when tested with an inter-trial interval of 5 min (Zlomuzica et al., 2007), we found in the present study that the vehicle-treated group also failed to show episodic-like memory. On the test trial, they were unable to discriminate the temporal order in which the objects were presented. (OFS × RFS; $p > .05$; Wilcoxon, Fig. 2). The preference in exploring the RFD object in comparison to the RFS object was also not seen in this group (RFD × RFS; $p > .05$). Additionally, the exploration pattern for OFS and OFD objects was seen in the opposite direction than the one that would be presented in a pattern indicative of episodic-like memory (OFD × OFS; $p > .037$).

Mice treated with 0.1 mg/kg, similar to the vehicle-treated group, also failed to show the exploration pattern, which is indicative of episodic-like memory. Temporal order object memory was not preserved in this group (OFS × RFS; $p > .05$). Furthermore, mice treated with 0.1 mg/kg of senktide were not able to discriminate the recent displaced from the recent stationary object, showing a deficit in memory for the spatial displacement of the objects (RFD × RFS; $p > .05$). Finally, for this group there was no difference between the time spent exploring the OFD and OFS objects ($p > .05$).

In contrast, the 0.2 and 0.4 mg/kg senktide-treated groups spent more time exploring the old stationary object as compared to the recent stationary object, suggesting that they recognized the previously present objects and remembered the order in which they appeared ($p = .003$ and $p = .002$, respectively, Wilcoxon). This suggests that both, the 0.2 and 0.4 mg/kg senktide groups, showed an intact temporal order object memory, i.e. the memory for the

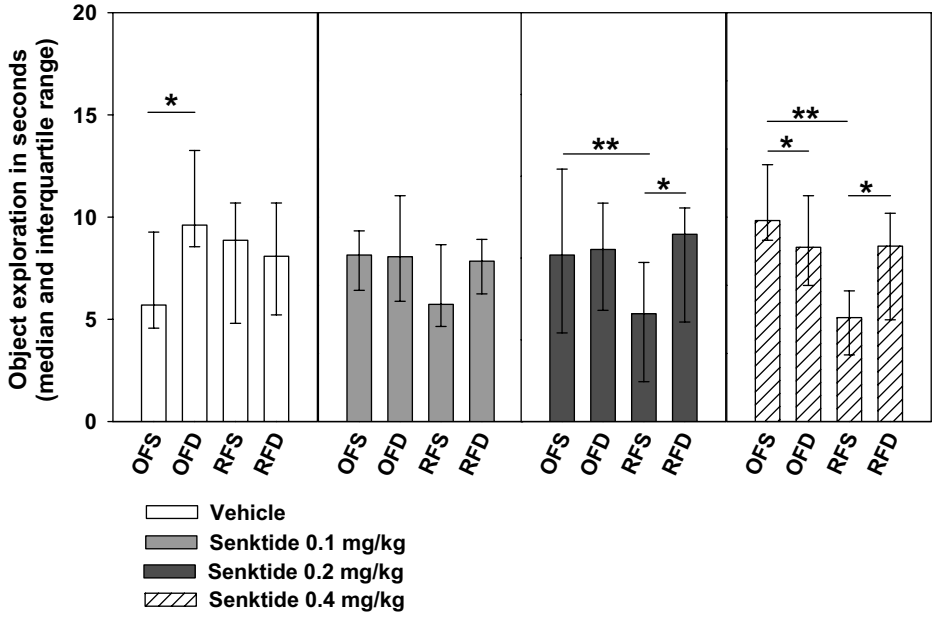


**Fig. 2.** The effects of senktide on episodic-like memory in mice. Bars represent median (interquartile range 75% and 25%) of exploration time during the test trial for the treatment groups indicated. $^{*}p < .05$ and $^{**}p \leqslant .005$, Wilcoxon, one-tailed. OFD = old familiar displaced, OFS = old familiar stationary, RFD = recent familiar displaced and RFS = recent familiar stationary.

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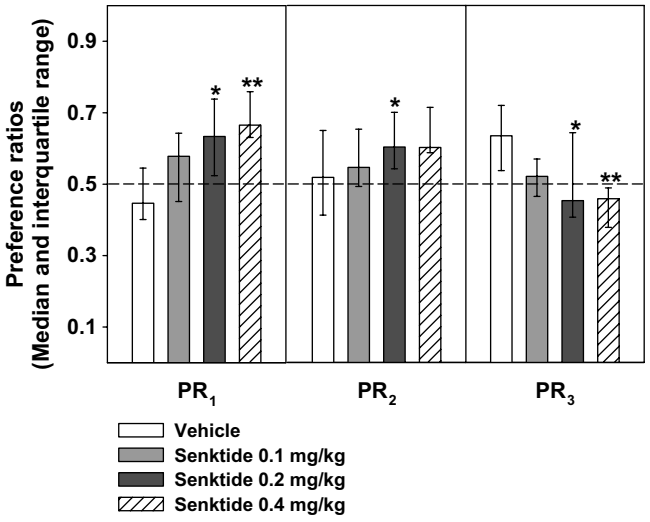


**Fig. 3.** The effects of senktide on episodic-like memory in mice. Bars represent median (interquartile range 75% and 25%) of preference ratios (PR) during the test trial for the treatment groups indicated. For details on the calculation of PR ($PR_1$, $PR_2$ and $PR_3$) see text. $^{*}(p < .05)$ and $^{**}(p \leqslant .005)$ indicate difference from vehicle group, Mann–Whitney *U*-test, one-tailed.

sequence of object presentation. Since the time needed to reach the exploration criterion, and the time spent exploring the objects, were not significantly different between sample trials 1 and 2 for both groups (*p*s > .05, paired *t*-test), the temporal order memory is unlikely due to differences in the time exposed to the objects or the time spent exploring the objects during the sample trials. In summary, a dose of 0.2 and 0.4 mg/kg of senktide rescued temporal order memory. Additionally, mice from groups, 0.2 and 0.4 mg/kg, exhibited a significant preference towards the RFD object as compared to the RFS object (0.2 mg/kg, p = .006; 0.4 mg/kg, $p = .008$). This suggests that both groups were able to remember the position in which the objects were presented during the sample trials, indicating that the senktide improved the place order memory for the objects. Besides, the 0.4 mg/kg senktide-treated group explored the OFS object significantly more than the OFD object, indicating that for the 0.4 mg/kg treated group there was an interaction between the factors, recency and spatial displacement ($p = .038$). Thus, the treatment with 0.4 mg/kg of senktide induced a pattern of object exploration that is indicative of episodic-like memory, suggesting that an integrated memory for "what", "where" and "when" was promoted (Kart-Teke et al., 2006; Kart-Teke, Dere et al., 2007; Zlomuzica et al., 2007).

Between group comparisons revealed a difference for the PR1 ratio between vehicle-treated group and both, 0.2 and 0.4 mg/kg senktide-treated groups ($p = .015$ and $p = .001$, respectively, Fig. 3). However, no effect in $PR_1$ was observed between the lowest dose of senktide (0.1 mg/kg) and the vehicle group, suggesting, once again, that only the higher doses of senktide (0.2 and 0.4 mg/kg) were effective in inducing intact temporal order memory. For $PR_2$, the 0.2 mg/kg group was different from vehicle ($p = .047$). There were differences for the $PR_3$ ratio between vehicle-treated group and both, 0.2 and 0.4 mg/kg senktide-treated groups ($p = .032$ and $p = .005$, respectively), suggesting that the 0.2 mg/kg dose of senktide effectively promoted a pattern of exploratory activity that is compatible with episodic-like memory (Kart-Teke et al., 2006; Kart-Teke, Dere et al., 2007; Zlomuzica et al., 2007).

## 4. Discussion

This study examined whether the $NK_3$ receptor agonist, senktide, administered systemically at doses of 0.1, 0.2 and 0.4 mg/kg to mice could improve episodic-like memory using an experimental design employing sub-optimal learning conditions. Dependent on the inter-trial interval used, e.g., 5 or 50 min, this task can be arranged for detecting either promnestic or amnestic effects of experimental manipulations (Zlomuzica et al., 2007). In the present study, we used a 5-min inter-trial interval, which leads to relatively meagre performance, in order to examine whether senktide would facilitate episodic-like memory under sub-optimal learning conditions. The treatment with a dose of 0.1 mg/kg had no influence on performance. In contrast, after treatment with the medium (0.2 mg/kg) and high (0.4 mg/kg) doses of senktide, the mice remembered the temporal order in which two different objects had been encountered during two consecutive sample trials, thus showing an intact memory for *what* and *when* ($PR_1$). The doses of 0.2 and 0.4 mg/kg were also effective in improving memory for location of the recent familiar objects, i.e. the memory for *what* and *where* ($PR_2$). Most important, at the dose of 0.4 mg/kg, senktide was effective in inducing an interaction between the temporal order and spatial disposition of the objects ($PR_3$).

Episodic memory can be inferred from behavioural manifestations of the knowledge regarding the content (what happened), place (where it happened) and temporal context (in terms of the sequence of events attended) of personally experienced events (Dere, Kart-Teke, Huston, & de Souza Silva, 2006). Our episodic-like memory task combines different versions of the novelty-preference paradigm and subsumes one-trial object recognition memory, the memory for locations in which objects were explored, and temporal order memory for objects presented at distinct time points (Dere et al., 2007). Previously, we elaborated our paradigm into a tool for assessing amnesic syndromes in rodents without using extensive lesion techniques or the application of amnestic drugs (Kart-Teke et al., 2006). We demonstrated that a slight modification of the paradigm abolished episodic-like memory in C57BL/6 mice under undrugged and unstressed conditions. We also showed that memory-enhancing drugs can attenuate or completely reverse this impairment in a dose-dependent manner (Zlomuzica et al., 2007).

Although peripheral effects of the systemic senktide administration cannot be ruled out, we suggest that our results can be interpreted in terms of direct central effects of the drug, since there are several studies which indicate brain penetration of systemically injected senktide (Ciccocioppo et al., 1995; Langlois, Riele, Wintmolders, Leysen, & Jurzak, 2001; Stoessl, Dourish, & Iversen, 1988).

Lesion and transient inactivation studies suggest that one-trial object recognition in rodents depends on an intact perirhinal cortex (Winters & Bussey, 2005a, 2005b), while there is evidence suggesting that the hippocampus is involved in both object–place memory (Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002) and, possibly, one-trial object recognition (Hammond, Tull, & Stackman, 2004). The medial prefrontal cortex has been implicated in the temporal order memory for objects (Hannesson, Vacca, Howland, & Phillips, 2004). Since these three brain structures are highly interconnected, they might provide the anatomical substrate mediating episodic-like memory in our paradigm. For instance, it is known that the cortex and the hippocampus of the rodent brain exhibit $NK_3$ receptors (Ding et al., 1996; Shughrue et al., 1996). Therefore, it is possible that the beneficial effects of senktide on temporal and spatial memory are mediated by $NK_3$ receptor stimulation in these brain regions. However, the exact nature of the underlying mechanisms remains speculative. Moreover, the activation of $NK_3$ receptors is accompanied by a release of several neurotransmitters, including acetylcholine (Marco et al., 1998; Steinberg et al., 1995), dopamine (Marco et al., 1998) and norepinephrine (Bert et al., 2002). In rats, novel object exploration is associated with increased hippocampal acetylcholine

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efflux (Degroot, Wolff, & Nomikos, 2005). Systemic nicotine injection improved object recognition memory in rats after 24 h retention intervals (Puma, Deschaux, Molimard, & Bizot, 1999), while systemic scopolamine injection impaired object recognition memory (Bartolini, Casamenti, & Pepeu, 1996). Episodic memory deficit in humans with mild cognitive impairment can be ameliorated by acetylcholinesterase inhibitors and galantamine treatments; both substances increase cholinergic neurotransmission in the hippocampus (Gron, Brandenburg, Wunderlich, & Riepe, 2006). Local application of senktide into the septal area was followed by an increase of acetylcholine in the hippocampus (Marco et al., 1998). In line with these results, it has been shown that senktide can attenuate the scopolamine-induced deficit in spatial working memory in mice, using the spontaneous alternation task (Kameyama et al., 1998; Ukai et al., 1998).

It is questionable whether the memory-promoting effects of senktide in this task are interrelated with its possible anxiolytic properties. Several lines of evidence indicate an intricate relationship between anxiety and memory, at the neurochemical, neuroanatomical, genetic and pharmacological levels (Kalueff, 2007). Benzodiazepine anxiolytics are known to disrupt memory (Izquierdo & Medina, 1991). Generally, brain arousal has both anxiogenic and memory-promoting effects, while brain inhibition is amnestic and anxiolytic (Kalueff, 2007). However, there are several indications that memory and anxiety do not always act synergistically (Kalueff, 2007). Intracerebroventricular injection of senktide has been reported to induce anxiolytic effects in the elevated-plus maze task (Ribeiro & De Lima, 2002; Ribeiro, Teixeira, Calixto, & De Lima, 1999). However, $NK_3$ knockout in mice had no effect in any of the elevated-plus maze behaviors, but impaired memory in different tasks (Siuciak et al., 2007). Therefore, at present, no conclusions can be made on an interrelation of possible anxiolytic and mnemonic effects of senktide.

It should be noted that the neurokinin peptide, SP, which also binds to the $NK_3$ receptor, has memory-promoting effects when injected into various parts of the brain (Hasenohrl et al., 2000; Huston & Hasenohrl, 1995). However, although SP has been shown to facilitate memory in a wide number of tasks, it has not been tested for its effects on episodic-like memory.

In conclusion, using an experimental protocol designed to detect the promnestic properties of drugs, we showed that $NK_3$ receptor stimulation by senktide can improve episodic-like memory in mice. Aging- and Alzheimer-related cognitive decline is characterized by early deficits in episodic memory. It remains to be examined whether $NK_3$ receptor agonists can ameliorate such deficits in aged rodents, Alzheimer mouse models and aged humans.

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## References

Bartolini, L., Casamenti, F., & Pepeu, G. (1996). Aniracetam restores object recognition impaired by age, scopolamine, and nucleus basalis lesions. *Pharmacology Biochemistry and Behavior, 53*, 277–283.

Bellucci, F., Carini, F., Catalani, C., Cucchi, P., Lecci, A., Meini, S., et al. (2002). Pharmacological profile of the novel mammalian tachykinin, hemokinin 1. *British Journal of Pharmacology, 135*, 266–274.

Bert, L., Rodier, D., Bougault, I., Allouard, N., Le Fur, G., Soubrie, P., et al. (2002). Permissive role of neurokinin NK(3) receptors in NK(1) receptor-mediated activation of the locus coeruleus revealed by SR 142801. *Synapse, 43*, 62–69.

Chen, L. W., Wei, L. C., Liu, H. L., Ding, Y. Q., Zhang, H., Rao, Z. R., et al. (2001). Cholinergic neurons expressing neuromedin K receptor (NK3) in the basal forebrain of the rat: A double immunofluorescence study. *Neuroscience, 103*, 413–422.

Ciccocioppo, R., Panocka, I., Pompei, P., Polidori, C., de Caro, G., & Massi, M. (1995). Subcutaneous injections of the tachykinin senktide reduce alcohol intake in alcohol-preferring rats. *Peptides, 16*, 533–537.

Degroot, A., Wolff, M. C., & Nomikos, G. G. (2005). Acute exposure to a novel object during consolidation enhances cognition. *Neuroreport, 16*, 63–67.

Dere, E., Huston, J. P., & de Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neuroscience and Biobehavioral Reviews, 31*, 673–704.

Dere, E., Kart-Teke, E., Huston, J. P., & de Souza Silva, M. A. (2006). The case for episodic memory in animals. *Neuroscience and Biobehavioral Reviews, 30*, 1206–1224.

Ding, Y. Q., Shigemoto, R., Takada, M., Ohishi, H., Nakanishi, S., & Mizuno, N. (1996). Localization of the neuromedin K receptor (NK3) in the central nervous system of the rat. *The Journal of Comparative Neurology, 364*, 290–310.

Duarte, C. R., Schutz, B., & Zimmer, A. (2006). Incongruent pattern of neurokinin B expression in rat and mouse brains. *Cell and Tissue Research, 323*, 43–51.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: The effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research, 113*(3), 509–519.

Gron, G., Brandenburg, I., Wunderlich, A. P., & Riepe, M. W. (2006). Inhibition of hippocampal function in mild cognitive impairment: Targeting the cholinergic hypothesis. *Neurobiology of Aging, 27*, 78–87.

Hammond, R. S., Tull, L. E., & Stackman, R. W. (2004). On the delay-dependent involvement of the hippocampus in object recognition memory. *Neurobiology of Learning and Memory, 82*, 26–34.

Hannesson, D. K., Vacca, G., Howland, J. G., & Phillips, A. G. (2004). Medial prefrontal cortex is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats. *Behavioral Brain Research, 153*, 273–285.

Hasenohrl, R. U., de Souza Silva, M. A., Nikolaus, S., Tomaz, C., Brandao, M. L., Schwarting, R. K., et al. (2000). Substance P and its role in neural mechanisms governing learning, anxiety and functional recovery. *Neuropeptides, 34*, 272–280.

Huston, J. P., & Hasenohrl, R. U. (1995). The role of neuropeptides in learning: Focus on the neurokinin substance P. *Behavioral Brain Research, 66*(1–2), 117–127.

Izquierdo, I., & Medina, J. H. (1991). GABAA receptor modulation of memory: The role of endogenous benzodiazepines. *Trends in Pharmacological Sciences, 12*(7), 260–265.

Kalueff, A. V. (2007). Neurobiology of memory and anxiety: From genes to behavior. *Neural Plasticity, 2007*, 78171–78182.

Kameyama, T., Ukai, M., & Shinkai, N. (1998). Ameliorative effects of tachykinins on scopolamine-induced impairment of spontaneous alternation performance in mice. *Methods and Findings in Experimental and Clinical Pharmacology, 20*, 555–560.

Kart-Teke, E., de Souza Silva, M. A., Huston, J. P., & Dere, E. (2006). Wistar rats show episodic-like memory for unique experiences. *Neurobiology of Learning and Memory, 85*, 173–182.

Kart-Teke, E., Dere, E., Brandao, M. L., Huston, J. P., & de Souza Silva, M. A. (2007). Reinstatement of episodic-like memory in rats by neurokinin-1 receptor antagonism. *Neurobiology of Learning and Memory, 87*, 324–331.

Langlois, X., Riele, P., Wintmolders, C., Leysen, J. E., & Jurzak, M. (2001). Use of the beta-imager for rapid ex vivo autoradiography exemplified with central nervous system penetrating neurokinin 3 antagonists. *The Journal of Pharmacology and Experimental Therapeutics, 299*, 712–717.

Laufer, R., Gilon, C., Chorev, M., & Selinger, Z. (1986). Characterization of a neurokinin B receptor site in rat brain using a highly selective radioligand. *The Journal of Biological Chemistry, 261*, 10257–10263.

Marco, N., Thirion, A., Mons, G., Bougault, I., Le Fur, G., Soubrie, P., et al. (1998). Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: An in vivo microdialysis approach in guinea pig. *Neuropeptides, 32*, 481–488.

Massi, M., Panocka, I., & de Caro, G. (2000). The psychopharmacology of tachykinin NK-3 receptors in laboratory animals. *Peptides, 21*, 1597–1609.

Mileusnic, D., Magnuson, D. J., Hejna, M. J., Lorens, J. B., Lorens, S. A., & Lee, J. M. (1999). Age and species-dependent differences in the neurokinin B system in rat and human brain. *Neurobiology of Aging, 20*, 19–35.

Mufson, E. J., Ginsberg, S. D., Ikonomovic, M. D., & DeKosky, S. T. (2003). Human cholinergic basal forebrain: Chemoanatomy and neurologic dysfunction. *Journal of Chemical Neuroanatomy, 26*, 233–242.

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: Memory for objects, places, and contexts. *Learning & Memory, 9*, 49–57.

Oliveira, A. A., Jr., & Hodges, H. M. (2005). Alzheimer's disease and neural transplantation as prospective cell therapy. *Current Alzheimer Research, 2*, 79–95.

Pepeu, G., & Giovannini, M. G. (2004). Changes in acetylcholine extracellular levels during cognitive processes. *Learning & Memory, 11*, 21–27.

Puma, C., Deschaux, O., Molimard, R., & Bizot, J. C. (1999). Nicotine improves memory in an object recognition task in rats. *European Neuropsychopharmacology, 9*, 323–327.

Ribeiro, R. L., & De Lima, T. C. (2002). Participation of GABAA receptors in the modulation of experimental anxiety by tachykinin agonists and antagonists in mice. *Progress in Neuro-psychopharmacology & Biological Psychiatry, 26*, 861–869.

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Ribeiro, S. J., Teixeira, R. M., Calixto, J. B., & De Lima, T. C. (1999). Tachykinin NK(3)receptor involvement in anxiety. *Neuropeptides, 33*(2), 181–188.

Severini, C., Improta, G., Falconieri-Erspamer, G., Salvadori, S., & Erspamer, V. (2002). The tachykinin peptide family. *Pharmacological Reviews, 54*, 285–322.

Shughrue, P. J., Lane, M. V., & Merchenthaler, I. (1996). In situ hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. *The Journal of Comparative Neurology, 372*, 395–414.

Siuciak, J. A., McCarthy, S. A., Martin, A. N., Chapin, D. S., Stock, J., Nadeau, D. M., et al. (2007). Disruption of the neurokinin-3 receptor (NK3) in mice leads to cognitive deficits. *Psychopharmacology, 194*, 185–195.

Smith, D. M., & Mizumori, S. J. (2006). Hippocampal place cells, context, and episodic memory. *Hippocampus, 16*, 716–729.

Steinberg, R., Rodier, D., Souiclhac, J., Bougault, I., Emonds-Alt, X., Soubrie, P., et al. (1995). Pharmacological characterization of tachykinin receptors controlling acetylcholine release from rat striatum: An in vivo microdialysis study. *Journal of Neurochemistry, 65*, 2543–2548.

Stoessl, A. J., Dourish, C. T., & Iversen, S. D. (1988). The NK-3 tachykinin agonist senktide elicits yawning and chewing mouth movements following subcutaneous administration in the rat. Evidence for cholinergic mediation. *Psychopharmacology, 95*, 502–506.

Ukai, M., Shinkai, N., & Kameyama, T. (1998). Involvement of dopamine receptors in beneficial effects of tachykinins on scopolamine-induced impairment of alternation performance in mice. *European Journal of Pharmacology, 350*, 39–45.

Wenk, G. L., Zajaczkowski, W., & Danysz, W. (1997). Neuroprotection of acetylcholinergic basal forebrain neurons by memantine and neurokinin B. *Behavioral Brain Research, 83*, 129–133.

Winters, B. D., & Bussey, T. J. (2005a). Removal of cholinergic input to perirhinal cortex disrupts object recognition but not spatial working memory in the rat. *The European Journal of Neuroscience, 21*, 2263–2270.

Winters, B. D., & Bussey, T. J. (2005b). Transient inactivation of perirhinal cortex disrupts encoding, retrieval, and consolidation of object recognition memory. *The Journal of Neuroscience, 25*, 52–61.

Zlomuzica, A., de Souza Silva, M. A., Huston, J. P., & Dere, E. (2007). NMDA receptor modulation by D-cycloserine promotes episodic-like memory in mice. *Psychopharmacology, 193*, 503–509.

Please cite this article in press as: Zlomuzica, A. et al., $NK_3$ receptor agonism promotes episodic-like memory in mice, *Neurobiology of Learning and Memory* (2008), doi:10.1016/j.nlm.2008.04.013

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# Episodic-like and procedural memory impairments in histamine H1-receptor knockout mice coincide with changes in acetylcholine esterase activity in the hippocampus and dopamine turnover in the cerebellum

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*Key words:* Alzheimer's disease, episodic memory, hippocampus, Parkinson's disease, rotarod

**ABBREVIATIONS**

ACh, acetylcholine

AChE, acetylcholine esterase

AD, Alzheimer's disease

DA, dopamine

DG, dentate gyrus

DOPAC, dihydroxyphenylacetic acid

ELM, episodic-like memory

EM, episodic memory

H1R-KO, histamine H1 receptor knockout

PD, Parkinson's disease

PM, procedural memory

PR, preference ratios

TH, tyrosine hydroxylase

WT, wild-type

5-HIAA, 5-hydroxyindole acetic acid

5-HT, serotonin

**ABSTRACT**

We investigated episodic-like (ELM) and procedural memory (PM) in histamine H1 receptor knockout (H1R-KO) mice. In order to relate possible behavioral deficits to neurobiological changes, we examined H1R-KO and wild-type (WT) mice in terms of acetylcholine esterase (AChE) activity in subregions of the hippocampus and AChE and tyrosine hydroxylase (TH) expression in the striatum. Furthermore, we analyzed acetylcholine (ACh), serotonin (5-HT) and dopamine (DA) levels, including metabolites, in the cerebellum of H1R-KO and WT mice. The H1R-KO mice showed impaired ELM as compared to the WT mice. The performance of H1R-KO mice in the ELM task was primarily driven by familiarity-based memory processes. While the H1R-KO mice performed similar to the WT mice during the acquisition of a PM as measured with an accelerating rotarod, their performance after a retention interval of 7 days was significantly impaired relative to the WT mice. These findings suggest that both ELM and long-term PM is impaired in the H1R-KO mice. Neurochemical assays revealed that the H1R-KO mice had significantly lower levels of AChE activity in the dentate gyrus (DG) and CA1 subregions of the hippocampus as compared to the WT mice. The H1R-KO mice also displayed significantly reduced dihydroxyphenylacetic acid (DOPAC) levels and a reduced DOPAC/DA ratio in the cerebellum, suggesting that the DA turnover in the cerebellum is decelerated in H1R-KO mice. In conclusion, H1R-KO mice display severe long-term memory deficits in both ELM and PM, which coincide with changes in AChE activity in the hippocampus as well as DA turnover in the cerebellum. The importance of these findings for Alzheimer's (AD) and Parkinson's disease (PD) is discussed.

## INTRODUCTION

The H1R is a metabotopic G-protein coupled receptor transcribed by an intronless gene and is expressed in the cortex, hippocampus, amygdala, hypothalamus, striatum and cerebellum (Lintunen et al., 1998). H1R-KO mice (Inoue et al., 1996) exhibited a complex cognitive phenotype including both impairments and improvements in a variety of learning and memory tasks (Dai et al., 2007, Zlomuzica et al., submitted).

Evidence from normal subjects, amnesic patients, and experimental animals has led to the notion of multiple memory systems with distinct neurobiological substrates (Squire, 2004). The remembrance of unique personal experiences in terms of their details (what), their place (where) and temporal occurrence (when) is known as episodic memory (EM) (Dere et al., 2006) and critically depends on the hippocampus. On the other hand, PM, the learning of motor skills and habits involves more the striatum (caudate/putamen) and other brain structures, such as the cerebellum. Humans suffering from hippocampal damage (Hopkins et al., 2004) or early stages of AD (Sabe et al., 1995; Small et al., 2003) exhibit impaired EM, while their PM remains intact. In contrast, early stage PD patients, with striatal DA dysfunctions, are impaired in PM, while their EM system seems preserved (Saint-Cyr et al., 1988). Interestingly, there is evidence that the cerebellum might be involved in both EM (Fliessbach et al., 2007) and PM (Molinari et al., 1997).

AD patients show changes in brain histamine levels (Panula et al., 1998) and a loss of histaminergic neurons in the nucleus tuberomammillaris, the only source of cerebral histamine (Airaksinen et al., 1991; Nakamura et al., 1993; Saper and German, 1987). Similar to AD, PD is also associated with changes in the histaminergic system. PD patients show increased brain histamine levels (Rinne et al., 2002) and changes in the activity of histidine decarboxylase (Garbarg et al.,

1983). PD patients had also higher numbers of histaminergic fibers in the substantia nigra pars. These results suggest an increase of histaminergic innervation of the substantia nigra in PD patients. We have previously demonstrated a close relationship between histaminergic and dopaminergic systems for motor performance. In rats, hemivibrissotomy induces behavioral asymmetries in the open-field test. The recovery from these behavioral asymmetries is correlated with neural plasticity in crossed and uncrossed tuberomammillary-striatal projections (Weiler et al., 1990). Behavioral asymmetries and changes in striatal DA content have also been observed after unilateral lesions of tuberomammillary nucleus or substantia nigra (Maisonnette et al., 1998).

The hippocampus has long been implicated in EM in humans (Aggleton and Brown, 1999) and ELM in animals (Ergorul and Eichenbaum, 2004). Recent human and animal studies suggest that hippocampal ACh and DA systems are involved in both the encoding of EM and PM (Gron et al., 2005; Hasselmo, 2006; Rammsayer et al., 2000; Schott et al., 2005). Given the involvement of the brain's histamine system in the brain pathology of AD and PD and their dissociative symptomatology in terms of EM and PM, we asked whether H1R-KO mice would show changes in ELM and/or PM. Furthermore, we examined H1R-KO mice in terms of AChE histochemistry in subregions of the hippocampus and AChE histochemistry and TH immunohistochemistry in the striatum. Since the cerebellum has been implicated in different types of PM, such as eye-blink classical conditioning or motor skill learning (Krakauer and Shadmehr, 2006), we analyzed ACh, 5-HT and DA levels in the cerebellum of H1R-KO and WT mice.

## EXPERIMENTAL PROCEDURES

### *Animals*

Homozygous H1R-KO mice were delivered from the Riken Research Center for Allergy and Immunology in Yokohama, Japan and were maintained at the animal breeding facilities of the University of Düsseldorf. The generation of H1R-KO mice and the absence of specific [$^3$H]pyrilamine binding (a H1R selective antagonist) in brain sections of homozygous H1R-KO mice has been described elsewhere (Inoue et al, 1996). The H1R-KO mice used in the present study have been backcrossed 8 times onto the C57BL/6 strain. Three months old male H1R-KO (n = 43) and C57BL/6 mice (n = 46) were used for behavioural or neurobiological experiments. 18 H1R-KO and 20 WT mice were tested in the ELM experiment. Thereafter, after a test pause of two weeks half of these animals (9 H1R-KO and 10 WT mice) were used for the rotarod test. Other batches of 8 H1R-KO and 8 WT mice were subjected to neurochemical tests for changes in post-mortem ACh, DA and 5-HT levels in the cerebellum. Finally, another batch of 8 H1R-KO and 8 WT mice were used for AChE and TH measurements in subregions of the hippocampus and striatum (caudate/putamen).

Animals were held in standard Makrolon cages (type 2: 22 x 16 x 13 cm) with metal covers and rodent chow (Ssniff, Spezialdiäten GMBH, Soest, Germany) and liquid available ad libitum. They were maintained on a 12 hr light/dark cycle and were tested during the light phase between 9 a.m. - 6 p.m. All experiments were performed according to the guidelines of the German Animal Protection Law and were approved by the North Rhine Westphalia State Authority.

### *Memory for what, where and when*

The test apparatus was a square open-field arena (30 x 30 cm) with 40-cm-high walls made of grey polyvinyl chloride, a metal floor and an open roof. Two visual cues were

fixed on the east and north wall of the maze, which were intended to be used for spatial orientation. The open field was placed in a sound-attenuating cubicle. A white light bulb (60 W) was located on the ceiling of the cubicle, adjusted to provide an equal light intensity inside the open-field. The experimenter observed the animal's behaviour on a screen monitor, which was connected to an overhead camera suspended above the open-field arena.

All objects were available in triplicate copies and varied in terms of surface texture, color and shape, but were of similar height (18 cm). Objects used in the sample trials were substituted by identical copies for the test trial, thus precluding unwanted olfactory cues. The objects were made of plastic and were of sufficient weight so that they could not be displaced by the mice. None of the objects had previously been paired with a reinforcing stimulus. The objects were always placed in the corners of the open-field at a distance of 0.5 cm from the side walls. Previous work ensured that the mice were able to discriminate the different objects, and there was no, per se, preference for one of these objects. For each animal two of these three objects were randomly chosen, and the order of presentation during the sample trials was randomized.

The mice were first subjected to three daily 10-min open-field habituation trials. On the fourth day, animals received two 10-min sample trials, followed by a test trial of 5-min duration. The mice were always placed in the central part of open-field. The inter-trial interval between the 3 trials was 50 min. After each trial, the objects and the open-field were thoroughly cleaned with 0.1% acetic acid solution in order to remove odor cues. The open-field was virtually divided into nine squares by 2 x 2 parallel lines. The central square was not used for object placement. For each animal, four out of eight squares were randomly chosen to position the four copies of the "old familiar" object in the first sample trial. The second sample trial was identical to the

first, except that four copies of another “recent familiar” object was present. Two copies of the “recent familiar” object were randomly placed onto positions that had been occupied in the first sample trial, and two copies were positioned in new positions, that were randomly chosen from the remaining four peripheral positions. In the test trial (trial 3), two copies of both objects were present in either stationary or displaced positions, i.e. one of the copies of each object was presented in a position encountered in the respective sample trial, i.e. sample trial 1 (“old familiar-stationary” object) or sample trial 2 (“recent familiar-stationary” object). The remaining objects were presented in new positions (“old familiar-displaced”, and “recent familiar-displaced”). All four objects were placed onto positions previously encountered in the sample trials (Kart-Teke et al., 2006; Kart-Teke et al., 2007).

The following behaviors were scored during the sample trials and the test trial: The cumulative time spent (s) exploring the individual objects. Exploration of an object was assumed when the mouse approached an object and had physical contact with it, either with its snout and/or forepaws. Sitting next to the object or leaning against the object while exploring the wall of the open-field was not considered as object exploration. The behavioral parameters were scored by an experienced observer, who was blind with respect to the mice’s genotype. Object exploration was scored semi-automatically using the EthoVision tracking system (Noldus, The Netherlands), run under the `manually record behaviors´ option.

***Motor learning and long-term memory***

Motor coordination was tested with an accelerating rotarod (TSE systems; Bad Homburg, Germany; model no.: 7650). The rotating rod was elevated 10 cm off the floor, had an axis diameter of 3.5 cm and a striated surface made of black rubber. During the acquisition phase, each mouse was given 3 trials (with an inter-trial interval of 10 - 25 min to control for possible effects of physical exhaustion) per day

for three consecutive days. After a retention delay of 7 days the animals were given another 3 trials with the same inter-trial-interval as during the acquisition phase. The mouse was placed on the inactive drum, which thereafter was accelerated to a speed of 40 g over a period of 5 min. The mouse had to move forward on the drum, which was rotating with increasing speed along its vertical axis in order to avoid falling off. As some mice tend to passively ride around the rod, especially at higher velocities, the duration (s) of active performance until the mouse fell off the drum was registered with a cut-off after 300 s.

***ACh, DA and 5-HT levels in the cerebellum***

ACh and monoamine concentrations, including metabolites and turnover ratios in the cerebellum were determined for H1R-KO and WT mice. The animals were sacrificed by cervical dislocation followed by decapitation. Their cerebellum was removed and weighed. Thereafter, the probe was homogenized with an ultrasonic device in ice cold 0.05 N perchloric acid containing ethylhomocholine and deoxyepinephrine as internal standards, centrifuged, filtered and kept at –80° until being analyzed. Samples were analyzed for ACh, DA, DOPAC, 5-HT, and 5-hydroxyindole acetic acid (5-HIAA) using high-performance liquid chromatography with electrochemical detection (for technical details, see De Souza-Silva et al., 1997; De Souza-Silva et al., 2000). Furthermore, 5-HIAA/5-HT and DOPAC/DA ratios were calculated as a measure for the rate of neurotransmitter turnover.

***Hippocampal AChE histochemistry and striatal AChE histochemistry and TH immunohistochemistry***

***Tissue preparation***

The mice were anesthetized with a mixture of ketaminhydrochloride (90.0 mg/kg, Ketavet, Pharmacia & Upjohn GmbH, Erlangen, Germany) and xylazinhydrochloride (8.0 mg/kg, Rompun, Bayer, Leverkusen, Germany). Their heart was exposed and

the left ventricle was cannulated and the right auricle was cut. The brain was first perfused with 50 ml saline and, thereafter, with 100 ml ice-cold 4 % paraformaldhehyde in PBS pH 7.4 (room temperature) under constant pressure. Then the brain was extracted and postfixed in the same fixative for 3 h at 4 C°. Thereafter, the brain was washed for 1 h in PBS at 4 °C, overnight, cryoprotected with sucrose 25 % in PBS, frozen by immersion in Isopenthane, cooled in dry ice, and stored at -80 °C. Brain coronal sections (25 μm thick) were cut with a cryostat and collected on Superfrost Plus glass slides. Adjacent sections were mounted on different slides, in order to perform different immunoreactions on alternate sections. Slides were stored at -80 °C until staining. The following brain regions were analyzed for TH and/or AChE staining: Subregions of the hippocampus (included the hilus of the DG, DG, CA1 and CA3) and the striatum (caudate/putamen).

***AChE histochemistry***

AChE cleaves released ACh from the synaptic cleft and, thus, limits or terminates its effects on postsynaptic cholinergic receptors. Quantitative histochemistry for AChE activity was performed as previously described (Tien et al., 2004). Briefly, sections were washed in PBS and then incubated for 15 h at 4 °C in the following solution: S-acetylthicholine iodide (Sigma) 4mM, ethopropazine 0.086 mM, copper sulphate 4 mM, glycine 16 mM in acetate buffer 50 mM pH5. Slides were then rinsed in PBS and immersed in 1 % sodium sulphide pH 7.5. Finally, slides were dehydrated in alcohol and coverslipped with Permount. All brains were stained at the same time with the same solutions to minimize experimental variations.

***TH immunohistochemistry***

TH is a synthesizing enzyme which converts tyrosine into L-Dopa, the precursor of DA. TH-expression in the striatum was measured as follows: sections were washed in TBS and incubated with a mouse monoclonal antibody against tyrosine

hydroxylase (Diasorin, Stillwater, USA) at a 1:5000 dilution in 10% normal bovine serum, 0.2% triton X-100 in PBS overnight at +4 °C. After overnight incubation, sections were washed three times in PBS and then incubated with anti-mouse-biotin, conjugated (Sigma, USA) at a 1:200 dilution in 10% normal bovine serum, 0.2% Triton X-100 in PBS for 1 h at room temperature. Sections were then washed again three times in TBS and incubated in ABC (Vector Lab, USA) for 1 h. After three washes in TBS, the reaction was visualized with 0.1% Diaminobenzidine, 0.02% hydrogen peroxide in TB 0.05M at pH 7.4 for 10 min in the dark. The reaction was then stopped with cold TBS. Finally, slides were dehydrated in alcohol and coverslipped with Permount.

***Morphometric data acquisition***

All morphological analyses and staining were conducted blind. Slides were analysed with a Zeiss Axioskop 20, equipped with a CCD high-resolution camera (Hamamatsu Photonics, Italy, C5405) and motorized XYZ stage (Proscan II, Prior). The images were captured with a 5x objective and converted by a microcomputer-assisted image analyzer (MCID Elite; Imaging Res. Inc., Canada. Tiled images over the entire filed of interest had a final resolution of 1824 x 1440 pixels.

AChE and TH expression levels were quantified over the entire sampled field according to the guidelines of Capowski (1989), and measured as relative optical density (ROD) units (ROD = log (256/ grey level)). ROD units are correlated with the enzyme activity and antigen concentration (Burke et al., 1990).

***Statistics***

Behavioral and neurochemical data are expressed as mean ± sem. The rotarod acquisition data were analyzed with ANOVA procedures with repeated measures on blocks of 3 daily trials. Student t-tests for unpaired data were performed on the rotarod retention test, neurotransmitter and histochemical data. The total time spent

exploring the four objects during the sample and test trials of the ELM memory experiment were analyzed by means of one-way ANOVA. The test trial object exploration data was used to calculate the following preference ratios (PR) for each mouse:

*PR1 = Temporal order memory*

$$\frac{\text{Time spent exploring the old familiar stationary object}}{\text{Time spent exploring the old familiar stationary + recent familiar stationary object}}$$

*PR2 = Object-place memory for the recent familiar objects*

$$\frac{\text{Time spent exploring the recent familiar displaced object}}{\text{Time spent exploring the recent familiar displaced + recent familiar stationary object}}$$

*PR3 = Object-place memory for the old familiar objects*

$$\frac{\text{Time spent exploring the old familiar displaced object}}{\text{Time spent exploring the old familiar displaced + old familiar stationary object}}$$

Single group t-tests (each against a comparison value of .5 ~ chance level) were performed on these PR for each group separately. Between-group differences in the above indicated PR were analyzed by means of Student t-tests for unpaired data. The p-values given are considered to be significant when lower than .05.

## RESULTS

### *ELM*

The total time spent exploring the four objects during the sample and test trials was not significantly different between the H1R-KO and WT mice ($p > .05$; repeated measures ANOVA; data not shown) suggesting that H1R-KO and WT mice were equally motivated to explore the objects during the sample trials and showed comparable levels of object exploration during the sample and test trials.

The WT mice were able to discriminate the temporal order in which the objects were presented and, accordingly, spent significantly more time exploring the “old familiar stationary” object as compared to the “recent familiar stationary” object (WT: PR1: $P = .001$; single group t-test; Fig. 1A). In contrast, the H1R-KO mice failed to discriminate the temporal order in which the two objects had been presented (H1R-KO: PR1: $P > .05$; single group t-test). Compared to the WT mice, the H1R-KO mice showed significantly lower temporal order memory scores (H1R-KO vs. WT: PR1: $p = .003$; Student t-test for unpaired data). In the group of WT mice, the mean time spent exploring the displaced copy of the recent familiar object was significantly higher than the time spent exploring the stationary copy of the recent familiar object (WT: PR2: $P = .046$; single group t-test), suggesting that the WT mice were able to remember where the recent familiar object had been placed during sample trial 2. Unlike the WT mice, the H1R-KO ones failed to detect that one “recent familiar” object had been displaced to a novel position (H1R-KO: PR2: $P > .05$, single group t-test). However, there was no significant difference between the WT and H1R-KO mice for the PR2 scores (H1R-KO vs. WT: $P = .074$; Student t-test for unpaired data). While there was no significant difference in the exploration times of the stationary compared to the displaced copy of the old familiar object in the WT mice (WT: PR3: $P > .05$; single group t-test), the H1R-KO mice preferred the “old familiar” object in the novel position

compared to the one placed in its former location known from sample trial one (H1R-KO: PR3: P > .026; single group t-test). Consequently, the PR3 ratios of the H1R-KO mice were significantly increased as compared to the WT mice (H1R-KO vs. WT: P = .028; Student t-test for unpaired data). The H1R-KO spent most of the time exploring the old familiar displaced object, which obviously appeared least familiar to them, as it was not seen most recently, and, additionally, was placed in a novel location. It seems that the object-preference of the H1R-KO mice was merely guided by familiarity-based memory.

While the WT mice were able to remember the temporal order in which the objects were presented and the spatial position in which the objects had been encountered during the second sample trial, the H1R-KO mice failed to do so and, instead, showed a preference for the old familiar displaced object as compared to the other 3 objects. The object exploration pattern of the H1R-KO is in accord with merely familiarity-based memory performance, suggesting that the absence of the histamine H1R in C57BL/6 mice impairs their object memory for what, where and when.

***Motor learning and long-term memory***

A repeated measures ANOVA revealed that the rotarod performance of both H1R-KO (H1R-KO: main effect of trials: $F(2, 10) = 14.3$, $P < .001$, Fig. 1B) and WT mice (WT: $F(2, 18) = 23.1$, $P < .001$) improved significantly across the 3 days of acquisition. However, there were no significant differences between H1R-KO and WT mice (H1R-KO vs. WT: main effect of genotype: P > .05, genotype x trials interaction: P > .05). These data suggest that, similar to a previous report (Inoue et al., 1996), neither initial motor coordination performance nor motor learning is affected in H1R-KO mice and that both groups reached a similar final performance level (P`s > .05; Students t-test for unpaired data). Next, we asked whether the H1R-KO and WT animals might differ in terms of motor long-term memory, and subjected them to 3

more trials on the rotarod after a retention interval of 7 days. Here, the H1R-KO mice were significantly impaired relative to the WT mice (H1R-KO vs. WT: P = .029; Students t-test for unpaired data).

***ACh, DA and 5-HT levels in the cerebellum***

We also analyzed whether the motor memory impairment of the H1R-KO mice might be associated with changes in ACh, DA and/or serotonin levels in the cerebellum. Compared to the controls, the H1R-KO mice had significantly lower DOPAC concentrations (H1R-KO vs. WT: P = .003; Students t-test for unpaired data; Table 1) and a lower DOPAC/DA ratio (H1R-KO vs. WT: P = .024) in the cerebellum. These results suggest that the DA turnover in the cerebellum is decelerated in H1R-KO mice. No significant differences between H1R-KO and WT were found for the other neurochemical parameters considered (all P's > .05).

***Hippocampal AChE histochemistry and striatal AChE histochemistry and TH immunohistochemistry***

Compared to the WT, the H1R-KO mice had significantly lower levels of AChE activity in the DG (H1R-KO vs. WT: P = .026; Student t-test for unpaired groups; Table 2; Fig. 2A) and CA1 subregions of the hippocampus (P = .031). Interestingly, compared to the WT mice, the H1R-KO mice had a thicker oriens layer in the CA1 region (H1R-KO: 103.71 ± 6.32 microns vs. WT: 81.29 ± 3.07 microns; P < .05; Fig. 2B), whereas no significant differences were found for the pyramidal layer, stratum radiatum or lacunosum moleculare in the CA1 region (Ps < .01). The AChE and TH expression levels in the striatum were not statistically different between groups (Ps > .05).

## DISCUSSION

### *ELM deficits in the H1R-KO mice*

The H1R-KO mice showed impaired ELM as compared to the WT mice. Unlike the WT mice, the H1R-KO mice were not able to remember the temporal order in which two different objects had been encountered during the sample trials. Compared to the WT mice, the H1R-KO mice also showed impaired spatial memory for the locations in which the recent familiar objects were placed during the second sample trial. In the WT mice the spatial displacement effect of the old familiar objects was blocked, suggesting an interaction between spatial and temporal factors in their ELM. In contrast, the H1R-KO mice preferred the old familiar displaced object in relation to the old familiar stationary object. In fact, the H1R-KO spent most of the time exploring the old familiar displaced object, which was not seen most recently, and, additionally, was placed in a novel location. This exploratory profile of the H1R-KO mice is not compatible with ELM, and, instead, indicates familiarity-based memory.

### *ELM deficits of H1R-KO mice coincide with changes in AChE activity in the DG and CA1 subregions of the hippocampus*

Neuropsychological evidence suggests that the hippocampus is of utmost importance for EM (Moscovitch et al., 2005). Within the hippocampus, the information relayed from the DG to the CA3 subregion has been proposed to be used to establish configural representations, which provide the spatiotemporal context of EM and support spatial pattern separation during the retrieval of EM (Eldridge et al., 2005; Knierim et al., 2006; Rolls and Kesner, 2006). In line with these assumptions, it has been shown that lesions to the CA3 region of the hippocampus impair ELM in rats (Li and Chao 2007). CA3-lesioned rats failed to show the interaction between the temporal and spatial factors of ELM, whereas object recognition, spatial memory, or temporal order memory was similar to controls (Li and Chao 2007). This finding

suggests that the information relayed from the DG to the CA3 subregion of the hippocampus is especially important for the integration of object, spatial, and temporal information and, thus, ELM. Alternatively, it has been proposed that temporal vs. spatial processing is based on different hippocampal subregions, which could provide the foundation of episodic memory within the hippocampus (Kesner et al., 2004). The DG and CA3 subregions of the hippocampus are thought to provide a metric spatial representation and allow spatial pattern separation, whereas the CA1 subregion is involved in temporal pattern association and temporal pattern completion. In the present study, we observed that the H1R-KO mice had a thicker oriens layer in the CA1 region of the hippocampus. It remains to be determined whether the ELM deficits of H1R-KO mice are related to this morphological alteration, possibly leading to specific impairments in temporal order memory.

The pharmacological manipulation of cholinergic neurotransmission affects EM in humans (Hasselmo et al., 1996) and modulates hippocampal synaptic plasticity (Ovsepian et al., 2004), which might subserve the encoding of new episodic memories (Hasselmo, 2006). Furthermore, it is known that AChE inhibitors improve cognitive symptoms in AD patients (Riepe, 2005) and patients with amnestic mild cognitive impairment (Gron et al., 2006). We therefore examined whether the ELM deficits of H1R-KO mice are correlated with changes in levels of AChE in the DG, CA3 and CA1 subregions of the hippocampus. The H1R-KO mice had significantly lower levels of AChE in the DG and CA1 region as compared to the WT mice. It is known that reduced AChE activity is an indicator of cholinergic impairment in AD (Eggers et al., 2006; Herholz et al., 2005). In contrast, hippocampal ACh levels, as measured via post-mortem HPLC-EC, in the H1R-KO mice were similar to WT mice (Zlomuzica et al., submitted). It is possible that the reduction in AChE expression reflects a compensatory response, which masks a cholinergic deficit in the H1R-KO

mice. Given that H1R-KO mice exhibit deficits in ELM, this compensatory response, similar to the one seen in AD patients, who also exhibit decreased AChE levels, seems not effective in terms of preventing memory impairments. It is, therefore, possible that the ELM deficits of the H1R-KO mice might be related to a dysfunctional cholinergic innervation of the hippocampus as reflected by decreased AChE activity in the DG and CA1 subregions of the hippocampus. Future studies are needed to specify how exactly the changes in cholinergic metabolism in the DG and CA1 regions of the H1R-KO mice affect information processing in the hippocampus, and how changes in hippocampal functioning translates into deficits in ELM. Furthermore, it remains to be determined whether the administration of cholinergic drugs, which are effective in ameliorating cognitive symptoms in AD, to H1R-KO mice would ameliorate their ELM deficit.

The stratum oriens is composed of the basal dendrites of pyramidal cells, few interneurons, and fibers from (i) CA3 region (ii) controlateral hippocampus (via commissure) and (iii) septum. The latter are mainly cholinergic fibers. Therefore, the change in the thickness of the stratum oriens might be related to a quantitative change in the density of the cholinergic innervation of the CA1 region.

***Long-term PM deficits of H1R-KO mice***

We also investigated whether H1R-KO mice would show changes in PM in terms of motor coordination learning and motor long-term memory using the accelerating rotarod task. The H1R-KO mice performed similar to the WT mice during the acquisition of the rotarod task. Their performance improved significantly across the 3 days of acquisition and their final performance level was similar to the WT mice. After a retention interval of 7 days, however the H1R-KO mice showed significantly lower performance scores relative to the WT mice. These findings suggest that besides ELM also motor-long term memory is impaired in the H1R-KO mice.

***PM deficits of H1R-KO mice are not related to concomitant changes in cholinergic or dopaminergic parameters in the striatum***

Since the striatum has been implicated in motor coordination performance on the rotarod (Lindgren et al., 2007), we asked whether the H1R-KO mice would exhibit changes in AChE and TH levels in the striatum. However, striatal AChE and TH levels were similar between H1R-KO and WT mice. Previously, we showed that neither ACh nor DA levels, including metabolites, are altered in the striatum of H1R-KO mice (Zlomuzica et al., submitted). Therefore, we asked wether the present results are related to neurochemical changes in the cerebellum of H1R-KO mice.

***Both EM and PM deficits of H1R-KO mice might be due to changes in DA turnover in the cerebellum***

Besides the striatum, the cerebellum has also been implicated in motor learning (Evans, 2007; Molinari et al., 1997; Schlett et al., 2004) and motor memory consolidation (Krakauer and Shadmehr, 2006). In order to know whether the long-term motor memory deficit of H1R-KO mice is due to neurochemical alternations in the cerebellum, we analyzed the levels of ACh, DA and 5-HT as well as their metabolites in H1R-KO and WT mice. ACh and 5-HT levels were similar between H1R-KO and WT mice. In contrast, DOPAC levels and the DOPAC/DA ratio were significantly reduced in the cerebellum of H1R-KO mice, suggesting that the DA turnover in the cerebellum is decelerated in H1R-KO mice. It is possible that the long-term memory deficit of the H1R-KO mice is related to these changes in cerebellar DA parameters.

There is evidence that the cerebellum might also be involved in EM (Fliessbach et al., 2007). Here, the cerebellum might contribute to the temporal component of EM. It has been proposed that the cerebellum, together with the basal ganglia, and the prefrontal cortex is involved in temporal order memory (Lalonde and Hannequin,

1999) and that DA is an important modulator of this function (Hotte et al., 2005). Therefore, it remains possible that both ELM and PM deficits of H1R-KO mice might be due to changes in DA turnover in the cerebellum.

***Are cognitive deficits in AD and PD due to pathological changes in the histamine system?***

Beside the well known brain pathologies in AD, such as degeneration of cholinergic systems, extracellular amyloid plaques, and intracellular neurofibrillary tangles, AD patients also show well documented dysfunctions in the histaminergic system (Mazurkiewicz-Kwilecki and Nsonwah, 1987; Mazurkiewicz-Kwilecki and Prell, 1984). Compared to age-matched non-demented controls, AD patients show changes in brain histamine levels (Cacabelos et al., 1989; Mazurkiewicz-Kwilecki and Nsonwah, 1989; Panula et al., 1998). AD is also associated with a loss of histaminergic neurons in the nucleus tuberomammillaris, the only source of cerebral histamine (Airaksinen et al., 1991; Nakamura et al., 1993; Saper and German, 1987). Most importantly, tacrine (a inhibitor of cholinesterase), which is prescribed to AD patients during early stages of the disease, inhibits the catabolic activity of histamine-N-methyltransferase, which normally degrades histamine to tele-methylhistamine, and, thereby, increases hippocampal histamine levels (Morisset et al., 1996; Nishibori et al., 1991). In contrast to tacrine, physostigmine is less effective in ameliorating AD symptoms and has a lower affinity to histamine-N-methyltransferase (Nishibori et al., 1991).

Similar to AD, PD is also associated with changes in the histaminergic system. PD patients show increased brain histamine levels (Rinne et al., 2002) and changes in the activity of histidine decarboxylase, the enzyme which synthetisizes histamine from L-histidine (Garbarg et al., 1983). Examination of the distribution of histaminergic fibers in the substantia nigra in post-mortem brain samples PD patients revealed increased density of histaminergic fibers in the middle portion of substantia

nigra pars compacta and reticulata. The morphology of histaminergic fibers was also altered in PD patients. They had thinner fibers and enlarged varicosities at histaminergic terminals (Anichtchik et al., 2000). These results suggest an increase of histaminergic innervation of the substantia nigra in PD patients.

***Summary and conclusions***

In the present study, we demonstrated that H1R-KO mice display severe long-term memory deficits in both ELM and PM. These memory deficits coincide with changes in AChE activity in the DG and CA1 subregions of the hippocampus as well as DA turnover in the cerebellum. Given that AD and PD patients show cognitive deficits along with changes in parameters of the histaminergic system, it is tempting to speculate that some of their cognitive symptoms might be related to changes in H1R expression, their sensitivity or function. Indeed, it has been shown that H1R levels in the frontal and temporal areas in brains of AD patients are decreased as compared to the healthy aged subjects. Moreover, the H1R binding was correlated with the severity of cognitive symptoms in AD patients within several brain areas (Higuchi et al., 2000). It would therefore be interesting to know, whether H1R-agonists can ameliorate cognitive deficits in animal models of AD or PD.

## REFERENCES

Aggleton JP, Brown MW (1999) Episodic memory, amnesia, and the hippocampal-anterior thalamic axis. Behav Brain Sci 22:425-444.

Airaksinen MS, Paetau A, Paljarvi L, Reinikainen K, Riekkinen P, Suomalainen R, Panula P (1991) Histamine neurons in human hypothalamus: anatomy in normal and Alzheimer diseased brains. Neuroscience 44:465-481.

Anichtchik OV, Rinne JO, Kalimo H, Panula P (2000) An altered histaminergic innervation of the substantia nigra in Parkinson's disease. Exp Neurol 163:20-30.

Burke RE, Cadet JL, Kent JD, Karanas AL, Jackson-Lewis V (1990) An assessment of the validity of densitometric measures of striatal tyrosine hydroxylase-positive fibers: relationship to apomorphine-induced rotations in 6-hydroxydopamine lesioned rats. J Neurosci Methods 35:63-73.

Cacabelos R, Yamatodani A, Niigawa H, Hariguchi S, Tada K, Nishimura T, Wada H, Brandeis L, Pearson J (1989) Brain histamine in Alzheimer's disease. Methods Find Exp Clin Pharmacol 11:353-360.

Capowski JJ (1989) Computer techniques in neuroanatomy. New York: Plenum Press.

Dai H, Kaneko K, Kato H, Fujii S, Jing Y, Xu A, Sakurai E, Kato M, Okamura N, Kuramasu A, Yanai K (2007) Selective cognitive dysfunction in mice lacking histamine H1 and H2 receptors. Neurosci Res 57:306-313.

De Souza Silva MA, Mattern C, Hacker R, Nogueira PJ, Huston JP, Schwarting RK (1997) Intranasal administration of the dopaminergic agonists L-DOPA, amphetamine, and cocaine increases dopamine activity in the neostriatum: a microdialysis study in the rat. J Neurochem 68:233-239.

De Souza Silva MA, Hasenohrl RU, Tomaz C, Schwarting RK, Huston JP (2000) Differential modulation of frontal cortex acetylcholine by injection of substance P into the nucleus basalis magnocellularis region in the freely-moving vs. the anesthetized preparation. Synapse 38:243-253.

Dere E, Kart-Teke E, Huston JP, De Souza Silva MA (2006) The case for episodic memory in animals. Neurosci Biobehav Rev 30:1206-1224.

Eggers C, Herholz K, Kalbe E, Heiss WD (2006) Cortical acetylcholine esterase activity and ApoE4-genotype in Alzheimer disease. Neurosci Lett 408:46-50.

Eldridge LL, Engel SA, Zeineh MM, Bookheimer SY, Knowlton BJ (2005) A dissociation of encoding and retrieval processes in the human hippocampus. J Neurosci 25:3280-3286.

Ergorul C, Eichenbaum H (2004) The hippocampus and memory for "what," "where," and "when". Learn Mem 11:397-405.

Evans GJ (2007) Synaptic signalling in cerebellar plasticity. Biol Cell 99:363-378.

Fliessbach K, Trautner P, Quesada CM, Elger CE, Weber B (2007) Cerebellar contributions to episodic memory encoding as revealed by fMRI. Neuroimage 35:1330-1337.

Garbarg M, Javoy-Agid F, Schwartz JC, Agid Y (1983) Brain histidine decarboxylase activity in Parkinson's disease. Lancet 1:74-75.

Gron G, Kirstein M, Thielscher A, Riepe MW, Spitzer M (2005) Cholinergic enhancement of episodic memory in healthy young adults. Psychopharmacology (Berl) 182:170-179.

Gron G, Brandenburg I, Wunderlich AP, Riepe MW (2006) Inhibition of hippocampal function in mild cognitive impairment: targeting the cholinergic hypothesis. Neurobiol Aging 27:78-87.

Hasselmo ME, Wyble BP, Wallenstein GV (1996) Encoding and retrieval of episodic memories: role of cholinergic and GABAergic modulation in the hippocampus. Hippocampus 6:693-708.

Hasselmo ME (2006) The role of acetylcholine in learning and memory. Curr Opin Neurobiol 16:710-715.

Herholz K, Weisenbach S, Kalbe E, Diederich NJ, Heiss WD (2005) Cerebral acetylcholine esterase activity in mild cognitive impairment. Neuroreport 16:1431-1434.

Higuchi M, Yanai K, Okamura N, Meguro K, Arai H, Itoh M, Iwata R, Ido T, Watanabe T, Sasaki H (2000) Histamine H(1) receptors in patients with Alzheimer's disease assessed by positron emission tomography. Neuroscience 99:721-729.

Hopkins RO, Waldram K, Kesner RP (2004) Sequences assessed by declarative and procedural tests of memory in amnesic patients with hippocampal damage. Neuropsychologia 42:1877-1886.

Hotte M, Naudon L, Jay TM (2005) Modulation of recognition and temporal order memory retrieval by dopamine D1 receptor in rats. Neurobiol Learn Mem 84:85-92.

Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, Niimura K, Watanabe T, Watanabe T (1996) Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. Proc Natl Acad Sci U S A 93:13316-13320.

Kart-Teke E, De Souza Silva MA, Huston JP, Dere E (2006) Wistar rats show episodic-like memory for unique experiences. Neurobiol Learn Mem 85:173-182.

Kart-Teke E, Dere E, Brandao ML, Huston JP, De Souza Silva MA (2007) Reinstatement of episodic-like memory in rats by neurokinin-1 receptor antagonism. Neurobiol Learn Mem 87:324-331.

Kesner RP, Lee I, Gilbert P (2004) A behavioral assessment of hippocampal function based on a subregional analysis. Rev Neurosci 15:333-351.

Knierim JJ, Lee I, Hargreaves EL (2006) Hippocampal place cells: parallel input streams, subregional processing, and implications for episodic memory. Hippocampus 16:755-764.

Krakauer JW, Shadmehr R (2006) Consolidation of motor memory. Trends Neurosci 29:58-64.

Lalonde R, Hannequin D (1999) The neurobiological basis of time estimation and temporal order. Rev Neurosci 10:151-173.

Li JS, Chao YS (2007) Electrolytic lesions of dorsal CA3 impair episodic-like memory in rats. Neurobiol Learn Mem, in press.

Lindgren HS, Rylander D, Ohlin KE, Lundblad M, Cenci MA (2007) The "motor complication syndrome" in rats with 6-OHDA lesions treated chronically with L-DOPA: relation to dose and route of administration. Behav Brain Res 177:150-159.

Lintunen M, Sallmen T, Karlstedt K, Fukui H, Eriksson KS, Panula P (1998) Postnatal expression of H1-receptor mRNA in the rat brain: correlation to L-histidine decarboxylase expression and local upregulation in limbic seizures. Eur J Neurosci 10:2287-2301.

Maisonnette S, Huston JP, Brandao M, Schwarting RK (1998) Behavioral asymmetries and neurochemical changes after unilateral lesions of tuberomammillary nucleus or substantia nigra. Exp Brain Res 120:273-282.

Mazurkiewicz-Kwilecki I, Nsonwah S (1987) The influence of alpha-fluoromethylhistidine on the regional brain histamine and plasma corticosterone levels in aging. Can J Physiol Pharmacol 65:2154-2157.

Mazurkiewicz-Kwilecki IM, Nsonwah S (1989) Changes in the regional brain histamine and histidine levels in postmortem brains of Alzheimer patients. Can J Physiol Pharmacol 67:75-78.

Mazurkiewicz-Kwilecki IM, Prell GD (1984) Age-related changes in brain histamine. Agents Actions 14:554-557.

Molinari M, Leggio MG, Solida A, Ciorra R, Misciagna S, Silveri MC, Petrosini L (1997) Cerebellum and procedural learning: evidence from focal cerebellar lesions. Brain 120:1753-1762.

Morisset S, Traiffort E, Schwartz JC (1996) Inhibition of histamine versus acetylcholine metabolism as a mechanism of tacrine activity. Eur J Pharmacol 315:R1-2.

Moscovitch M, Rosenbaum RS, Gilboa A, Addis DR, Westmacott R, Grady C, McAndrews MP, Levine B, Black S, Winocur G, Nadel L (2005) Functional neuroanatomy of remote episodic, semantic and spatial memory: a unified account based on multiple trace theory. J Anat 207:35-66.

Nakamura S, Takemura M, Ohnishi K, Suenaga T, Nishimura M, Akiguchi I, Kimura J, Kimura T (1993) Loss of large neurons and occurrence of neurofibrillary tangles in the tuberomammillary nucleus of patients with Alzheimer's disease. Neurosci Lett 151:196-199.

Nishibori M, Oishi R, Itoh Y, Saeki K (1991) 9-Amino-1,2,3,4-tetrahydroacridine is a potent inhibitor of histamine N-methyltransferase. Jpn J Pharmacol 55:539-546.

Ovsepian SV, Anwyl R, Rowan MJ (2004) Endogenous acetylcholine lowers the threshold for long-term potentiation induction in the CA1 area through muscarinic receptor activation: in vivo study. Eur J Neurosci 20:1267-1275.

Panula P, Rinne J, Kuokkanen K, Eriksson KS, Sallmen T, Kalimo H, Relja M (1998) Neuronal histamine deficit in Alzheimer's disease. Neuroscience 82:993-997.

Rammsayer TH, Rodewald S, Groh D (2000) Dopamine-antagonistic, anticholinergic, and GABAergic effects on declarative and procedural memory functions. Cogn Brain Res 9:61-71.

Riepe MW (2005) Cholinergic treatment: what are the early neuropathological targets? Eur J Neurol 12:3-9.

Rinne JO, Anichtchik OV, Eriksson KS, Kaslin J, Tuomisto L, Kalimo H, Roytta M, Panula P (2002) Increased brain histamine levels in Parkinson's disease but not in multiple system atrophy. J Neurochem 81:954-960.

Rolls ET, Kesner RP (2006) A computational theory of hippocampal function, and empirical tests of the theory. Prog Neurobiol 79:1-48.

Sabe L, Jason L, Juejati M, Leiguarda R, Starkstein SE (1995) Dissociation between declarative and procedural learning in dementia and depression. J Clin Exp Neuropsychol 17:841-848.

Saint-Cyr JA, Taylor AE, Lang AE (1988) Procedural learning and neostriatal dysfunction in man. Brain 111:941-959.

Saper CB, German DC (1987) Hypothalamic pathology in Alzheimer's disease. Neurosci Lett 74:364-370.

Schlett K, Pieri I, Metzger F, Marchetti L, Steigerwald F, Dere E, Kirilly D, Tarnok K, Barabas B, Varga AK, Gerspach J, Huston J, Pfizenmaier K, Kohr G, Eisel UL

(2004) Long-term NR2B expression in the cerebellum alters granule cell development and leads to NR2A down-regulation and motor deficits. Mol Cell Neurosci 27:215-226.

Schott BH, Henson RN, Richardson-Klavehn A, Becker C, Thoma V, Heinze HJ, Duzel E (2005) Redefining implicit and explicit memory: the functional neuroanatomy of priming, remembering, and control of retrieval. Proc Natl Acad Sci U S A 102:1257-1262.

Small BJ, Mobly JL, Laukka EJ, Jones S, Backman L (2003) Cognitive deficits in preclinical Alzheimer's disease. Acta Neurol Scand Suppl 179:29-33.

Squire LR (2004) Memory systems of the brain: a brief history and current perspective. Neurobiol Learn Mem 82:171-177.

Tien LT, Fan LW, Sogawa C, Ma T, Loh HH, Ho IK (2004) Changes in acetylcholinesterase activity and muscarinic receptor bindings in mu-opioid receptor knockout mice. Mol Brain Res 126:38-44.

Weiler HT, Steiner H, Huston JP (1990) Plasticity in crossed and uncrossed tuberomammillary-striatal projections in relation to recovery from behavioral asymmetries induced by hemivibrissotomy. Neuroscience 37:463-469.

Zlomuzica A, Viggiano D, De Souza-Silva MA, Ishizuka T, Gironi Carnevale UA, Ruocco LA, Watanabe T, Sadile AG, Huston JP, Dere E. The histamine H1-receptor mediates the motivational effects of novelty. Submitted.

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## FIGURE LEGENDS

**Figure 1: A. Episodic-like memory deficits in the H1R-KO mice.** Bars represent mean (and sem) preference ratios of H1R-KO and WT mice. **: $p < .05$, single-group t test; ***: $p < .05$, Student t test for unpaired data; *: $p < .1$, Student t test for unpaired data. **B. Procedural long-tem memory deficits of H1R-KO mice.** Bars represent mean (and sem) latency to fall on indicated days for H1R-KO and WT mice. *: $p < .05$, Student t test for unpaired data.

**Figure 2: A. AChE histochemistry.** Representative pictures of AChE histochemistry (upper panels) in H1R-KO mice (right panels) and WT mice (left panels). **B. Morphometric analysis of the CA1 region.** Representative pictures of the CA1 region in H1R-KO mice (right panels) and WT mice (left panels). The arrow indicates an increase in thickness of the oriens layer in the H1R-KO mice.

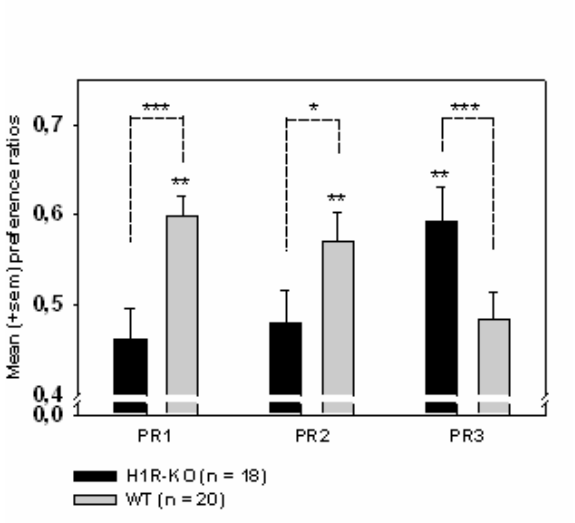
**Table 1:** Mean (± sem) levels of neurotransmitters and their metabolites, as well as metabolite/transmitter turnover ratios in the cerebellum of H1R-KO and WT mice. *: $p < .05$ (Student t-test for unpaired data). Abbreviations: ACh: acetylcholine (pmol/mg), DA: dopamine (pg/mg), DOPAC: dihydrophenylacetic acid (pg/mg), 5-HT: serotonin (pg/mg), 5-HIAA: 5-hydroxyindole acetic acid (pg/mg)

| | ACh | DA | DOPAC | DOPAC/DA | 5-HT | 5-HIAA | 5-HIAA/5-HT |
|---|---|---|---|---|---|---|---|
| H1R-KO | 5.75 ± .27 | 26.34 ± 4.87 | 11.86 ± 2.45* | .52 ± .10* | 310.7 ± 28.3 | 220.2 ± 14.1 | .73 ± .03 |
| WT | 5.38 ± .53 | 34.46 ± 7.73 | 28.21 ± 3.59 | 1.05 ± .17 | 349.8 ± 23.2 | 226.8 ± 18.7 | .65 ± .04 |

**Table 2:** Mean and SEM AChE histochemistry and TH immunohistochemistry, measured as relative optical density units in subregions of the hippocampus and the striatum of H1R-KO and WT mice. * = $p < .05$, *t*-test for independent groups.

| | AChE histochemistry in subregions of the hippocampus | | | | Striatum | |
|---|---|---|---|---|---|---|
| | Hilus | DG | CA1 | CA3 | AChE | TH |
| H1R-KO | .028 ± .004 | .092 ± .004* | .081 ± .004* | .158 ± .008 | .738 ± .018 | .056 ± .005 |
| WT | .035 ± .005 | .111 ± .006 | .102 ± .007 | .174 ± .006 | .749 ± .022 | .048 ± .004 |

A


Mean (+sem) preference ratios
0,7
0,6
0,5
0,4
0,0
***
*
***
**
**
**
PR1
PR2
PR3
H1R-KO (n = 18)
WT (n = 20)

B

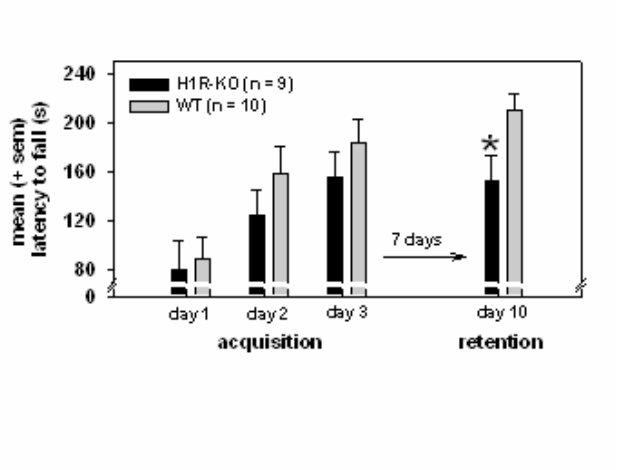

mean (+ sem) latency to fall (s)
240
200
160
120
80
0
H1R-KO (n = 9)
WT (n = 10)
*
7 days
day 1
day 2
day 3
day 10
acquisition
retention

Figure 1

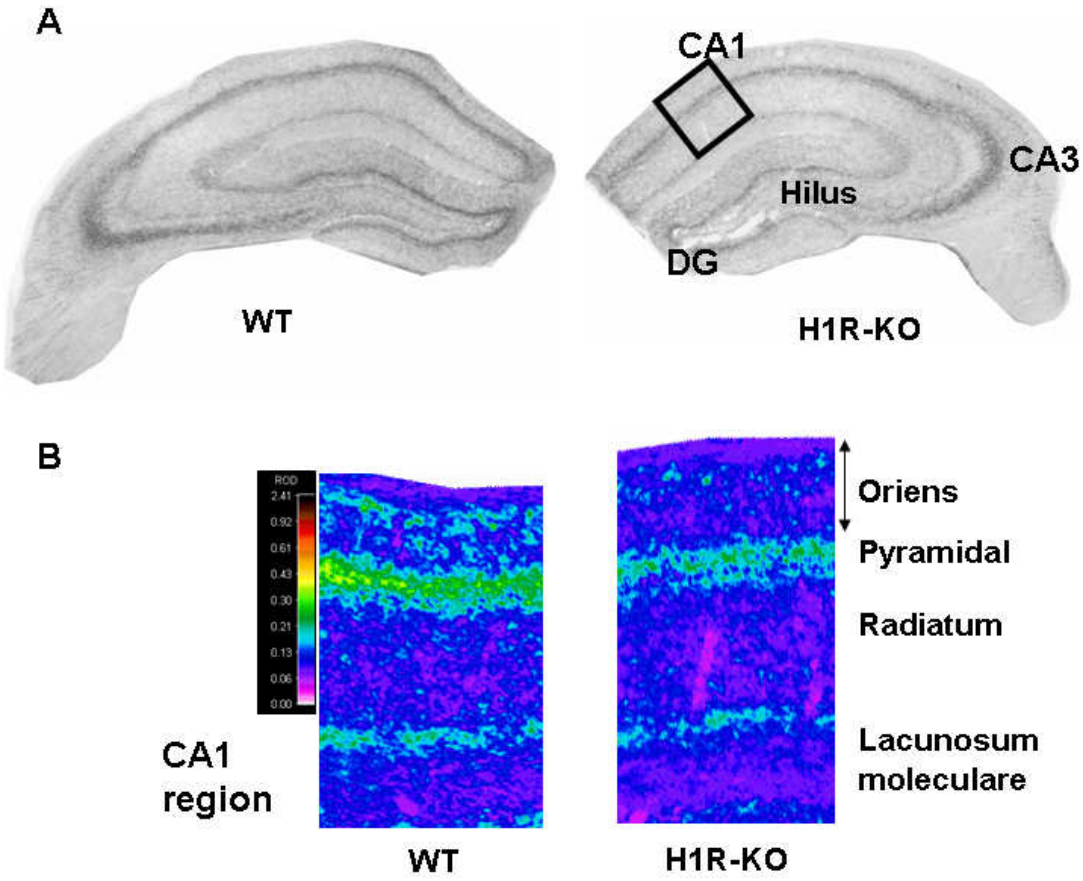

A
CA1
CA3
Hilus
DG
WT
H1R-KO
B
ROD
2.41
0.92
0.61
0.43
0.30
0.21
0.13
0.06
0.00
CA1
region
Oriens
Pyramidal
Radiatum
Lacunosum
moleculare
WT
H1R-KO

Figure 2

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# The histamine H1-receptor mediates the motivational effects of novelty

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*Keywords*: acetylcholine, amygdala, arousal, conditioned place-preference, dopamine, reinforcement

## Abstract

Novelty-induced arousal has motivational effects and can reinforce behavior. The mechanisms by which novelty acts as a reinforcer are unknown. Novelty-induced arousal can be either rewarding or aversive dependent on its intensity and the preceding state of arousal. The brain's histamine system has been implicated in both arousal and reinforcement. Histamine and histamine-1-receptor (H1R) agonists induced arousal and wakefulness in humans and rodents, e.g. by stimulating cortical acetylcholine (ACh) release. The H1R has also been implicated in processes of brain reward via interactions with the nigrostriatal- and mesolimbic dopamine (DA) systems. We asked whether the motivational effects of novelty-induced arousal are compromised in H1R knockout (KO) mice. The H1R-KO mice failed to develop a conditioned place-preference induced by novel objects. Even though they still explore novel objects, their reinforcing value is diminished. Furthermore, they showed impaired novelty-induced alternation in the Y-maze. Rearing activity and emotional behavior in a novel environment was also altered in H1R-KO mice, whereas object-place recognition was unaffected. The H1R-KO mice had higher ACh concentrations in the frontal cortex and amygdala (AMY). In the latter, the H1R-KO mice had also increased levels of DA, but a lower dihydrophenylacetic acid/DA ratio. Furthermore, the H1R-KO mice had also increased tyrosine hydroxylase immunoreactivity in the basolateral anterior, basolateral ventral and cortical AMY nuclei. We conclude that the motivational effects of novelty are diminished in H1R-KO mice, possibly due to reduced novelty-induced arousal and/or a dysfunctional brain reward system.

## Introduction

Novelty has motivational effects and can be used as a reinforcer to induce learning (Bevins & Besheer, 2005; Blatter & Schulz, 2006). However, the physiological mechanism by which novelty acts as a natural reinforcer remains elusive. Novelty induces arousal. Arousal is a state of perceptive, cognitive and behavioral activation, which promotes the gathering of exteroceptive information. Multiple brain structures, such as the frontal cortex (FC), thalamus, hypothalamus and basal forebrain, as well as multiple neurotransmitter/neuromodulators, including acetylcholine (ACh), noradrenaline, serotonin (5-HT), histamine, orexins and adenosine are involved in the induction and regulation of arousal and its behavioral consequences (Miller & O'Callaghan, 2006). Dependent on the degree of activation and the prevailing state of arousal, both rewarding and aversive effects of novelty-induced arousal have been reported (Bronson, 1968; Berlyne, 1969). Whether novelty-induced arousal is rewarding or aversive might also depend on whether the animal can choose to expose itself to or is forced to deal with novelty (Welker, 1957). In humans, interindividual differences in the behavioral and motivational responses to novelty have been taken to postulate a personality trait, termed novelty seeking (Kelley *et al.*, 2004).

Neuronal histamine, derived from the nucleus tuberomammillaris of the posterior hypothalamus, has been implicated in arousal, awakening, maintenance of wakefulness and sleep–wake cycles (Lin, 2000; Huang *et al.*, 2006). Furthermore, the brain's histamine system exerts inhibitory effects on the nigrostriatal- and mesolimbic dopamine (DA) reward systems (Huston *et al.*, 1997). In the rodent brain, two postsynaptic histamine receptors (H1R and H2R) and one presynaptic autoreceptor (H3R) have been identified. The H1R is a G-protein-coupled receptor transcribed by an intronless gene and expressed abundantly in the cerebral cortex, hippocampus, amygdala (AMY) and hypothalamus (Haas & Panula, 2003; Hasenöhrl & Huston, 2004). Pharmacological studies have implicated the H1R in brain reward via interactions with the nigrostriatal- and mesolimbic DA systems. H1R-antagonists lowered the threshold for rewarding brain stimulation, induced a conditioned place-preference (CPP; Zimmermann *et al.*, 1999), were self-administered and potentiated the rewarding effects of addictive drugs (Wang & Woolverton,

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2007). H1R-antagonists (Dringenberg *et al.*, 1998), similar to novelty (Saigusa *et al.*, 1999), increased the release of DA in the nucleus accumbens (NAc).

In humans, H1R-antagonists induce drowsiness and sedation, while H1R-agonists induce arousal and wakefulness (Lin, 2000). However, histaminergic agents also show activity at non-histamine, e.g. cholinergic, receptors (Hill *et al.*, 1997). In this regard, the H1R-knockout (KO) mice (Inoue *et al.*, 1996) are a promising alternative to study the role of the H1R in novelty-induced arousal and reward. The H1R-KO mice showed impaired locomotor activity and exploratory behavior in a novel environment (Inoue *et al.*, 1996). Orexin-hypocretin neuropeptides, the deficiency of which results in narcolepsy, infused into the lateral ventricle induced arousal in control, but not in H1R-KO, mice (Huang *et al.*, 2001). The arousal response to a H3R-antagonist, known to stimulate the release of various arousal-inducing neurotransmitters, including ACh, is impaired in the H1R-KO mice (Huang *et al.*, 2006). Based on these findings, we hypothesized that the motivational and rewarding effects of novelty should be impaired in the H1R-KO mice.

## Materials and methods

### *Experimental subjects*

Homozygous H1R-KO mice were delivered from the Riken Research Center for Allergy and Immunology, and are maintained at the animal breeding facilities of the University of Düsseldorf. The generation of H1R-KO mice and the absence of specific [$^3$H]pyrilamine binding (a H1R-selective antagonist) in brain sections of homozygous H1R-KO mice has been described elsewhere (Inoue *et al.*, 1996). In short, the H1R-KO mice were generated by homologous recombination. 129P2/OlaHsd ES cells carrying the mutated H1R-gene were injected into blastocytes from C57BL/6J mice, and the resulting male chimeras were mated with C57BL/6J mice. In order to create a homogeneous C57BL/6J background, these H1R-KO mice have been backcrossed 10 times onto the C57BL/6J strain (Sonobe *et al.*, 2004). These backcrossings lead to at least $\sim$99% of genetic homogeneity with the C57BL/6J background strain (Sigmund, 2000).

Three-month-old male H1R-KO ($n = 37$) and C57BL/6J wild-type (WT) mice ($n = 38$) were used for behavioral or neurobiological experiments. Of these animals, nine H1R-KO and 10 WT mice were tested in the open-field and for novel object-induced place-preference. Other batches of 10 H1R-KO and 10 WT mice were tested in the novelty-induced spatial alternation test, followed by the one-trial object-place recognition test. Neurochemical tests for changes in ACh and monoamine levels in the brain were performed with another 10 H1R-KO and 10 WT mice. Finally, another batch of eight H1R-KO and eight WT mice were used for acetylcholine esterase (AChE) and tyrosine hydroxylase (TH) measurements.

Animals were held in standard Makrolon cages (type 2: $22 \times 16 \times 13$ cm) with metal covers, and rodent chow (Ssniff, Spezialdiäten GMBH, Soest, Germany) and liquid available *ad libitum*. They were maintained on a 12 h light : dark cycle, and were tested during the light phase between 09.00 h and 18.00 h. All experiments were performed according to the guidelines of the German Animal Protection Law and were approved by the North Rhine Westphalia State Authority.

### *Novel object-induced CPP*

The CPP paradigm is based on Pavlovian classical conditioning, and is widely used to measure the rewarding or aversive motivational effects of drugs of abuse in rodents (Bardo & Bevins, 2000). It can further be used to document increases or decreases in the reward value of natural reinforcers, such as food, or the access to novel objects after experimental manipulations, which are assumed to affect the function of the brain's reward system (Bevins & Bardo, 1999; Bardo & Bevins, 2000). The nigrostriatal- and mesolimbic DA systems are thought to be crucial parts of the brain's reward/reinforcement system (Fibiger & Phillips, 1988; Wise, 1994). It has been shown that the acquisition and the expression of a place-preference conditioned by the access to novel objects can be blocked by the administration of DA receptor 1 (D1) or D2/D3 antagonists (Besheer *et al.*, 1999; Bevins *et al.*, 2002). Thus, it seems that under these conditions the access to novel objects operates as a natural reinforcer that stimulates dopaminergic reward circuits in the brain.

The CPP apparatus was placed in a sound-attenuating cubicle and was made of white painted Plexiglas. During the conditioning phase a partition wall, without an opening, was inserted into the apparatus that divided the space into two compartments that were identical in terms of size ($22 \times 35 \times 36$ cm), light intensity, floor texture, odor and color. A prominent visual cue (a blue circle with a diameter of 9 cm positioned at a distance of 14 cm from the floor) was attached to the closing wall of one compartment, allowing the animals to discriminate between the two compartments. Each animal was subjected to 10 trials on 10 consecutive days, and with one trial per day. On Day 1 a baseline trial of 10 min duration was given. Here, the partition separating the two compartments was removed, allowing the animal to freely move between the compartments. During the conditioning trials on Days 2–9, the animal was placed in an alternating manner either in the center of the novel object-paired compartment or in the blank compartment for 10 min. The novel object-paired compartment was randomly determined for each mouse and contained an identical pair of novel objects, which were placed in the corners opposite to the partition wall. A particular object was only presented on one of the four novel object-paired compartment trials. The sequence of the presentation of the four different objects was randomly determined for each mouse. On Day 10 a test trial was performed. The partition separating the two compartments was removed and the animal was allowed to freely move between the two compartments for 5 min. During the baseline and the test trials, the time spent in each compartment in seconds was measured. Please note that during the baseline and test trials the apparatus did not contain any objects. It is known that, in rats, repeated pairings of one compartment, but not the other, with novel objects induces a CPP for the novel object-paired compartment. Therefore, it was expected that the animals should spend more time in the former novel object-paired compartment on the test, but not on the baseline trial. In order to know whether the H1R-KO and WT mice were equally motivated to explore the novel objects, we measured the time spent in seconds exploring the objects during the conditioning trials.

### *Novelty-induced spatial alternation*

When mice are placed in a novel, Y-shaped maze, they typically show spontaneous spatial alternation behavior for some time until the Y-maze has become familiar. This task utilizes the congenital tendency of rodents to frequent relatively novel places not visited quite recently, when allowed to choose freely among respective alternatives. Spontaneous spatial alternation in a Y-maze is induced by spatial novelty and requires basic spatial working memory capacities. This alternation behavior is sensitive to both hippocampal lesions and anti-cholinergic drugs (Kokkinidis & Anisman, 1976; Hughes, 2004). The

Y-maze was made of black Plexiglas with three arms (labeled A, B and C) each 7.5 cm wide, 18 cm long, with walls of 23.5 cm height, and an open roof, radiating from a triangle-shaped central platform. One of the three arms differed from the others in respect of three rectangular gaps (2.3 × 7.5, 4.8 × 7.5 and 2.3 × 7.5 cm), which were cut into the end-wall. These gaps were separated by 1 cm, with the first one located at a height of 12 cm. The Y-maze was placed in a sound-attenuating cubicle and was illuminated by diffuse white light with an intensity of 5 lux at the center of the apparatus. The animals were placed on the central platform and were allowed to explore the Y-maze for a total of 20 arm entries. The sequence of arm entries was recorded manually (i.e. ABCBAC) for each animal. An arm entry was scored when the animal entered an arm with all four paws. Between trials the apparatus was cleaned with water, containing 75% ethanol. The following parameters were calculated from the sequence of arm entries. (i) Triplets: the number of consecutive choices of each of the three arms, without re-entries during the last three choices and irrespective of the order of the chosen arms. (ii) Alternation-ratio: as a measure of spatial working memory an alternation-ratio was computed as follows. For each mouse, the number of triplets was divided by the total number of entries minus 2.

### *One-trial object-place recognition*

It is known that mice readily approach novel objects and explore them with their vibrissae, nose and forepaws. It is assumed that a single explorative episode leaves a lasting memory trace, which also includes information about the location in which the object was encountered. It has been shown that mice can detect a mismatch between the past and present location of a familiar object after a single sample trial (Dere *et al.*, 2005, 2007).

The one-trial object-place recognition test was performed in the Y-maze described above. Each mouse was first subjected to a sample trial of 10 min duration, in which two identical copies of a novel plastic object were placed at the end of two randomly selected arms of the Y-maze. After a delay of 15 min, the animal received another trial of 10 min duration in which one of the objects was removed from its original arm and placed at the end of the arm not used for object placement during the sample trial.

For each mouse, the time spent exploring the two objects (in seconds), as well as the number of contacts with the two objects during the sample and test trials was scored off-line from video tapes, using the semiautomated EthoVision tracking system. Exploration of an object was assumed when the mouse approached an object and had physical contact with it, either with its vibrissae, snout or forepaws. Passive vicinity to an object, at a distance of more than 1.5 cm was not considered as exploratory behavior. Animals showing less then 20 s of object exploration during the sample trial were excluded from data analysis.

### *Emotional and adaptive behavior in a novel environment*

In order to examine emotional behaviors in a novel environment and adaptive responses after task repetition, the animals were given four trials in an open-field with a 24 h intertrial interval. Mice exposed to a novel environment show explorative behaviors in terms of increased locomotion and rearing activity. When mice are repeatedly placed into the same open-field a progressive reduction in these exploratory behaviors becomes evident, suggesting that the initially novel environment has become familiar (Cerbone & Sadile, 1994; Sadile, 1996). While locomotion and rearings are parameters that can be taken as measures of behavioral habituation to spatial or environmental novelty, the parameters, time spent in the corners and the center of the open-field are also measures of open space-induced anxiety. Generally, mice tend to spend significantly more time in the corners of an open-field as compared with the center, suggesting that the center of an open-field has fear- or anxiety-inducing properties, leading to strong avoidance behavior. Furthermore, it has been shown that the center of an open-field is traversed with a higher running speed, as compared with the remaining parts of the open-field, again suggesting that the center has aversive properties, inducing escape-like responses (Dere *et al.*, 2002, 2003). Repeated exposures to an open-field lead to decreases in the time spent in the center and increases in the time spent in the corners of the open-field (Dere *et al.*, 2002, 2003). This pattern reflects increases in familiarity with the open-field across the trials. Changes in these parameters in terms of relative amplitude or course would suggest altered emotional reactivity to spatial novelty.

The open-field was a rectangular chamber (30 × 30 × 40 cm) made of gray polyvinylchloride and had a gray metal floor. A video camera was mounted 50 cm above the maze. The open-field apparatus was placed in a sound-attenuating cubicle. The open-field was illuminated by diffuse white light with an intensity of 6.5 lux at the center of the apparatus. The digitized image of the animal's path was analysed with a semiautomated tracing device (EthoVision®, Noldus, the Netherlands). After each trial, the apparatus was cleaned with water containing 75% ethanol. The behavioral parameters registered during 10-min sessions were: (i) locomotion: the distance in cm an animal moved; (ii) leanings: the frequency and average duration (s) of leanings against the walls; (iii) rearings: the frequency and average duration (s) of rearings on hindlimbs (Aspide *et al.*, 1998); (iv) center time: the time spent in the central part (10 × 10 cm) of the field; (v) corner time: the time spent in the four corner squares (10 × 10 cm each); (vi) running speed: the mean running speed (cm/s) in the entire field and the central part (10 × 10 cm).

### *Brain ACh and monoamine levels in H1R-KO mice*

In order to know whether possible changes in novelty-induced CPP, novelty-induced spatial alternation and/or emotional behavior in a novel environment are associated with changes in the content and/or metabolism of brain neurotransmitters, ACh and monoamine concentrations, including metabolites, and turnover ratios were analysed in the FC, hippocampus, AMY, neo- and ventral striatum of H1R-KO and WT mice.

The animals were killed by cervical dislocation followed by decapitation; their brains were quickly removed and placed in an ice-cold brain matrix. Coronal sections were made following landmarks on the base of the brain, and the FC, hippocampus, AMY, neo- and ventral striatum were bilaterally dissected out from the coronal slices onto an ice-cold platform. Thereafter, the samples were weighed, homogenized with an ultrasonic device in ice-cold 0.05 N perchloric acid containing ethylhomocholine and deoxyepinephrine as internal standards, centrifuged, filtered and kept at −70°C until being analysed. Samples were randomly analysed for ACh, noradrenaline, DA, dihydrophenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT and 5-hydroxyindole acetic acid (5-HIAA) using high-performance liquid chromatography (HPLC) with electrochemical detection (for technical details, see De Souza-Silva *et al.*, 1997, 2000). Furthermore, 5-HIAA/5-HT, DOPAC/DA and HVA/DA ratios as a measure for the rate of neurotransmitter turnover were calculated.

### *Brain histamine levels of H1R-KO mice*

In order to know whether H1R-deficiency induces compensatory changes in the levels of histamine in various brain tissues (Treesukosol *et al.*, 2005), we also analysed H1R-KO and WT mice for changes in the concentration of histamine in the FC, AMY, neostriatum and cerebellum. The histamine levels of the brain tissue dialysates were determined by a HPLC-fluorometric method (Yamatodani *et al.*, 1985) with slight modifications (Mochizuki *et al.*, 1991). A 1/30 volume of perchloric acid (60%) was added to the brain tissue dialysate, 20 μL of this mixture was injected into the HPLC column. Histamine was separated on a cation exchanger, TSKgel SP2SW (Tosoh, Tokyo, Japan; particle size 5 μm) eluted with 0.25 M $KHz_2PO_4$ at a flow rate of 0.6 mL/min using a constant flow pump (Model CCPM, Tosoh). Histamine in the eluate was derivatized using an on-line automated Shore's *o*-phthalaldehyde method (Yamatodani *et al.*, 1985), and the fluorescence intensity was measured at 450 nm with excitation at 360 nm in a spectrofluorometer equipped with a flow cell (Model F-1050, Hitachi, Tokyo, Japan) and a chromatographic data processor (Model C-R3A, Shimadzu, Kyoto, Japan).

### *AChE histochemistry and TH immunohistochemistry in the prefrontal cortex (PFc) and AMY of H1R-KO mice*

#### *Tissue preparation*

The mice were anesthetized with a mixture of ketaminhydrochloride (90.0 mg/kg, Ketavet, Pharmacia & Upjohn GmbH, Erlangen, Germany) and xylazinhydrochloride (8.0 mg/kg, Rompun, Bayer, Leverkusen, Germany). Their heart was exposed, and the left ventricle was cannulated and the right auricle was cut. The brain was first perfused with 50 mL saline and thereafter with 100 mL ice-cold 4% paraformaldehyde in phosphate-buffered saline (PBS) pH 7.4 (room temperature) under constant pressure. Then the brain was extracted and postfixed in the same fixative for 3 h at 4 °C. Thereafter, the brain was washed for 1 h in PBS at 4 °C, overnight cryoprotected with sucrose 25% in PBS, frozen by immersion in isopenthane, cooled in dry ice, and stored at −80 °C. Brain coronal sections (25 μm thick) were cut with a cryostat and collected on Superfrost Plus glass slides. Adjacent sections were mounted on different slides, in order to perform different immunoreactions on alternate sections. Slides were stored at −80 °C until staining.

For AChE and TH staining only sections at the level of the PFc (B4.7 to B2.2 approximately) and of the AMY complex (B−2.12 to B−3.3 approximately) were used.

#### *AChE histochemistry*

Quantitative histochemistry for AChE activity was performed as previously described (Tien *et al.*, 2004). Briefly, sections were washed in PBS and then incubated for 15 h at +4 °C in the following solution: S-acetylthicholine iodide (Sigma) 4 mM, ethopropazine 0.086 mM, copper sulphate 4 mM, glycine 16 mM in acetate buffer 50 mM pH 5. Slides were then rinsed in PBS and immersed in 1% sodium sulfide pH 7.5. Finally, slides were dehydrated in alcohol and coverslipped with Permount. All brains were stained at the same time with the same solutions to minimize experimental variations.

#### *TH immunohistochemistry*

TH expression in the above-indicated amygdaloid subnuclei was measured as follows. Sections were washed in Tris-buffered saline (TBS) and incubated with a mouse monoclonal antibody against TH (Diasorin, Stillwater, USA) at a 1 : 5000 dilution in 10% normal bovine serum, 0.2% Triton X-100 in PBS overnight at +4 °C. After overnight incubation, sections were washed three times in PBS and then incubated with anti-mouse-biotin, conjugated (Sigma) at a 1 : 200 dilution in 10% normal bovine serum, 0.2% Triton X-100 in PBS for 1 h at room temperature. Sections were then washed again three times in TBS and incubated in ABC (Vector Laboratory, USA) for 1 h. After three washes in TBS, the reaction was visualized with 0.1% diaminobenzidine, 0.02% hydrogen peroxide in TB 0.05 M at pH 7.4 for 10 min in the dark. The reaction was then stopped with cold TBS. Finally, slides were dehydrated in alcohol and coverslipped with Permount.

### *Morphometric data acquisition*

All morphological analyses and staining were conducted blind. Slides were analysed with a Zeiss Axioskop 20, equipped with a CCD high-resolution camera (Hamamatsu Photonics, Italy, C5405) and motorized XYZ stage (Proscan II, Prior). The images were captured with a 5 × objective and converted by a microcomputer-assisted image analyser (MCID Elite; Imaging Res., Canada). Tiled images over the entire field of interest had a final resolution of 1824 × 1440 pixels.

The AMY complex was subdivided into the following regions based on AChE staining, using the Paxinos & Watson (1986) brain atlas as reference: lateral (La), basolateral anterior (BLA), basolateral ventral (BLV), central (Ce), medial (Me), and cortical (Co) nuclei. The same regions were analysed for TH-immunostained slides, using the adjacent section stained for AChE as reference section. PFc and subregions of the AMY (Fig. 1) were quantitatively analysed.

AChE and TH expression levels were quantified over the entire sampled field according to the guidelines of Capowski (1989), and

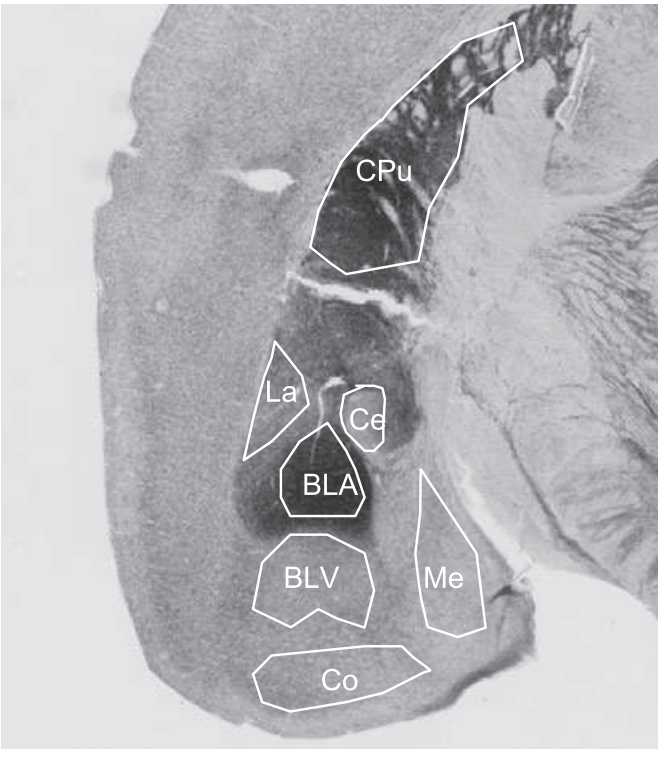


FIG. 1. The AMY complex and its subregions. The AMY complex was subdivided into the following regions using the Paxinos & Watson (1986) brain atlas as reference. Abbreviations: BLA, basolateral anterior; BLV, basolateral ventral; Ce, central; Co, cortical; Cpu, caudate putamen; La, lateral; Me, medial nuclei.

measured as relative optical density (ROD) units [ROD = log (256/gray level)]. ROD units are correlated with the enzyme activity and antigen concentration (Burke *et al.*, 1990).

### Statistics

Behavioral data have been obtained by visual/observational techniques, in part using a semiautomated recording device (EthoVision®, Noldus, Netherlands). The reliability of these measures was confirmed by determining the Spearman rank correlations between observations of the same behavioral parameters and data sets performed by the same and two different trained observers. The intraobserver Spearman correlation yielded correlation coefficients greater than 0.90. The interobserver correlations were greater than 0.85.

In the novel objects-induced CPP test a rewarding effect of exploring novel objects is reflected by the occurrence of place-preference behavior, defined as an absolute or relative increase of the time spent in the novel objects paired chamber as compared with the non-paired chamber during the test trial. The CPP is thus operationalized by a measurement of time. Obviously, this measure can only take positive values. It follows that the corresponding data distribution is positively skewed and not symmetric as in the case of normal data distributions. Consequently, a non-normal data distribution must be assumed for which, in general, the value of the variance critically depends on the value of the mean. Therefore, the CCP data were analysed by distribution-free, non-parametric statistics (Krauth, 1988). CCP data are expressed as medians with interquartile ranges and were analysed with non-parametric Mann–Whitney *U*-tests for unpaired data (conditioning trials) and Wilcoxon tests for paired data (paired vs unpaired chamber during the test trial). Spontaneous spatial alternation, object-place recognition, open-field and neurochemical data are expressed as mean ± SEM. The fulfilment of requirements (normality, variance homogeneity, etc.) for parametric analysis of data has been assessed by specific tests (Kolmogorov–Smirnov normality test, Levene test for univariate analysis, Box test of equality of covariance matrices for repeated measures analysis) included in SPSS® software. Student *t*-tests for non-paired data were performed on spontaneous spatial alternation, object-place recognition sample trial, neurochemical, AChE and TH data. Object-place recognition test trial data (novel vs familiar location) were analysed by Student's *t*-tests for paired data. In order to determine whether the Y-maze alternation ratios were above chance level, single-group *t*-tests were performed on these ratios against a comparison value of 0.5. Open-field data were analysed via repeated measures ANOVAs. Unless otherwise indicated, the *P*-values given are two-tailed and considered to be significant when lower than 0.05.

## Results

### Novel object-induced CPP

Neither the H1R-KO mice (paired vs non-paired compartment: $P \geq 0.05$; Wilcoxon test; Fig. 2B) nor the WT mice ($P \geq 0.05$; Fig. 2A) showed a preference for one of the two compartments during the initial baseline trial, when the animals had the opportunity to freely explore both compartments.

After 8 days of conditioning, a test trial identical to the baseline trial was performed, and the expression of a place preference conditioned by the access to novel objects was assessed. Comparison of the time spent in the paired vs non-paired compartment during the test trial revealed that the WT mice had developed a significant CPP ($P = 0.010$; one-tailed Wilcoxon test; Fig. 2C), whereas the H1R-KO mice failed to do so ($P \geq 0.05$; Fig. 2D). As expected, the WT mice showed a significant increase in the time spent in the paired chamber during the test trial as compared with the time spent in the same chamber during the baseline trial ($P = 0.026$), whereas no such significant change in sojourn times was observed in the H1R-KO mice ($P \geq 0.05$).

In order to know whether the deficient performance of the H1R-KO mice is due to a reduced motivation to explore novel objects, we analysed the total time spent exploring the novel objects during the conditioning trials for between-group differences. There were no significant differences between H1R-KO and WT mice in terms of the total time spent exploring the novel objects ($P > 0.05$; Mann–Whitney *U*-test), suggesting that H1R-KO mice are attracted by novel objects and show sufficient motivation to explore novel objects. These results suggest that H1R-KO mice are impaired in acquiring a novel object-induced CPP.

### Novelty-induced spatial alternation

In the novelty-induced spatial alternation task the H1R-KO mice showed a reduced number of triplets (H1R-KO: 9.2 ± 0.64; WT: 11.5 ± 0.71) and, accordingly, a significantly lower alternation ratio ($T = 2.27$, d.f. = 18, $P = 0.035$; Student's *t*-test for non-paired data; Fig. 3) as compared with the WT mice. Moreover, while the alternation performance of the WT mice was significantly above chance level ($T = 3.34$, d.f. = 9, $P = 0.009$; single-group *t*-test), the H1R-KO mice only performed at a level expected by chance ($P > 0.05$). These results suggest that the reward value of spatial novelty, which energizes/motivates spatial alternation behavior, is diminished in the H1R-KO mice.

### One-trial object-place recognition

Neither the total time spent exploring the objects, nor the number of contacts with the objects was significantly different between groups during the sample trial (both $P > 0.05$; Student's *t*-test for non-paired data, not shown). These results indicate that both groups were equally motivated to explore objects during the sample trial.

During the test trial, both WT ($T = 2.18$, d.f. = 7, $P = 0.033$; one-tailed Student's *t*-test for paired data; Fig. 4A) and H1R-KO mice ($T = 3.06$, d.f. = 9, $P = 0.007$; Fig. 4B) spent significantly more time exploring the object in the novel location relative to the object placed in the familiar location. They also showed significantly more contacts with the object placed in the novel relative to the familiar location (WT: $T = 2.79$, d.f. = 7, $P = 0.014$; Fig. 4C, H1R-KO: $T = 2.23$, d.f. = 9, $P = 0.027$; Fig. 4D). These results suggest that spatial object memory is not compromised in H1R-KO mice.

### Emotional and adaptive behavior in a novel environment

The H1R-KO and WT mice showed reductions in locomotor activity across the four trials in the open-field (main effect of trials: H1R-KO: $F_{3,24} = 5.38$, $P = 0.006$; WT: $F_{3,27} = 11.12$, $P < 0.001$; repeated measures ANOVA; Fig. 5A). There were no significant main effects of genotype or genotype–trials interaction ($P > 0.05$). These results suggest that both H1R-KO and WT mice showed similar behavioral habituation to the open-field.

Neither the H1R-KO nor the WT mice showed significant reductions in the frequency of rearings across the days of testing (both $P > 0.05$). However, compared with the WT the H1R-KO mice showed a significantly reduced number of rearings (main effect of

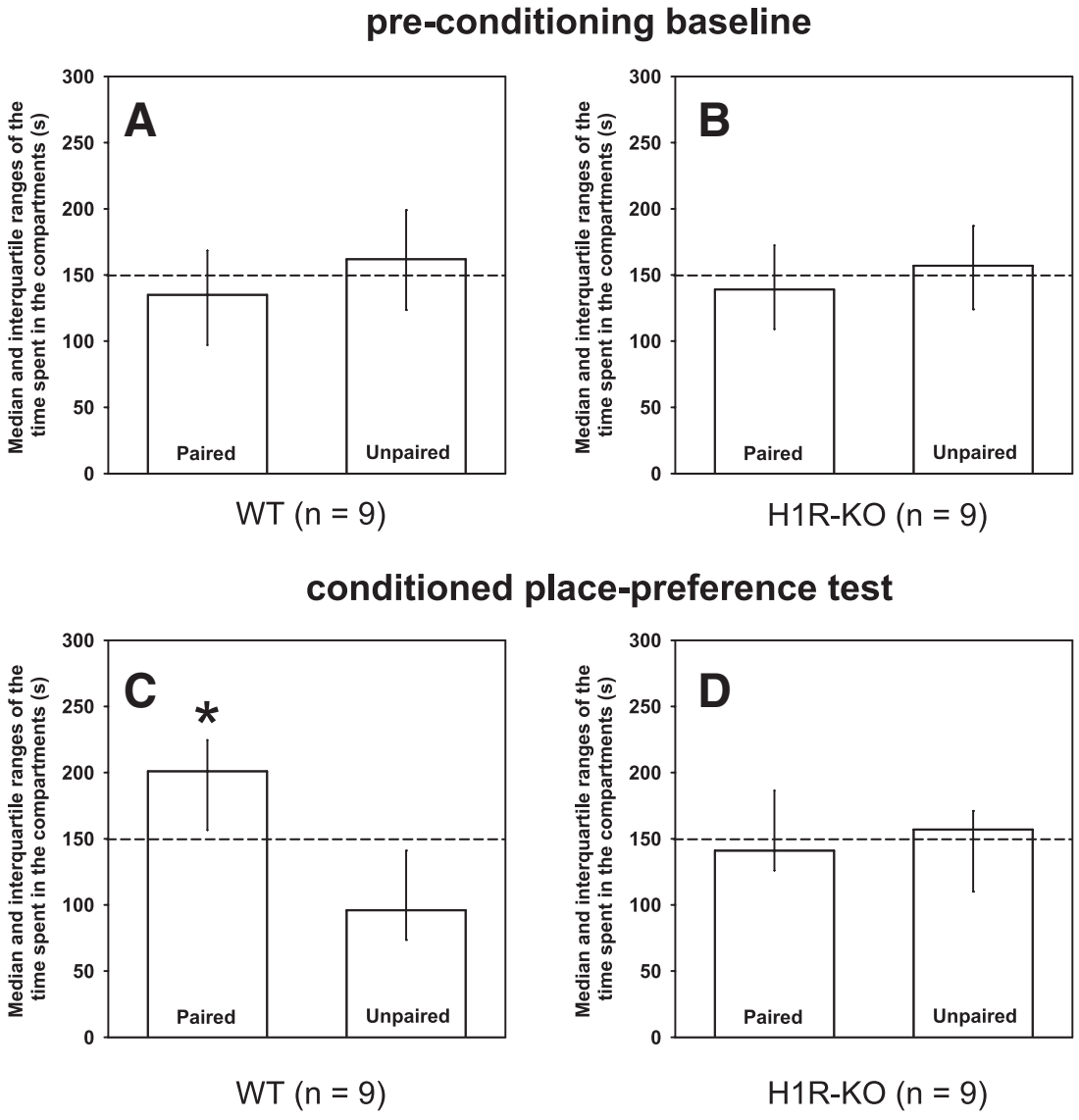


FIG. 2. H1R-knockout (KO) mice fail to develop a novel objects-induced place-preference. (A and B) Bars represent median ± interquartile ranges for the time spent in the indicated compartments during preconditioning baseline trial for wild-type (WT) and H1R-KO mice. (C and D) Bars represent median ± interquartile ranges for the time spent in the indicated compartments during the test for place-preference for WT and H1R-KO mice. *$P < 0.05$; Wilcoxon test for paired data. Dashed line indicates performance at chance level.

genotype: $F_{1,17} = 7.67$, $P < 0.013$; Fig. 5B). There was also a significant genotype–trials interaction ($F_{3,51} = 3.47$, $P < 0.023$). Whereas the WT mice showed slight reductions, the H1R-KO mice showed increases in their rearing frequency across the 4 days. There were no significant differences between the H1R-KO and WT mice regarding the average duration of neither rearings nor frequency, and average duration of leanings against the walls ($P > 0.05$). Because rearing activity is related to behavioral arousal and information gathering in novel environments, the data indicate that the motivational effect of spatial novelty is diminished in H1R-KO mice.

Across the four trials, the time spent in the corners increased (main effect of trials: H1R-KO: $F_{3,24} = 7.28$, $P = 0.001$; WT: $F_{3,27} = 25.32$, $P < 0.001$; Fig. 5C), while the time spent in the center of the open-field decreased (main effect of trials: H1R-KO: $F_{3,24} = 11.26$, $P < 0.001$; WT: $F_{3,27} = 21.27$, $P < 0.001$; Fig. 5D) in H1R-KO and WT mice. Compared with the controls, the H1R-KO mice spent significantly less time in the corners (main effect of genotype: $F_{1,17} = 15.23$, $P = 0.001$), but more time in the center of the open-field ($F_{1,17} = 9.10$, $P = 0.008$). While there was no significant genotype–trials interaction for the time spent in the corners of the open-field ($P > 0.05$), the interaction for the center time parameter was significant ($F_{3,51} = 2.90$, $P = 0.044$). Furthermore, the H1R-KO mice also showed reduced running speed in the center of the open-field on trials 2 and 3 relative to the WT mice ($F_{1,17} = 6.43$, $P = 0.021$; Fig. 5E), while the running speed in the entire open-field was not different between H1R-KO and WT mice ($P > 0.05$; Fig. 5F). These results strongly suggest that the emotional reactivity to spatial novelty is altered in the H1R-KO mice.

### *Brain ACh and monoamine levels in H1R-KO mice*

Next we analysed whether the behavioral phenotypes of the H1R-KO mice might be related to changes in ACh, DA, noradrenaline and/or 5-HT levels in the FC, hippocampus, AMY, neo- and ventral striatum.

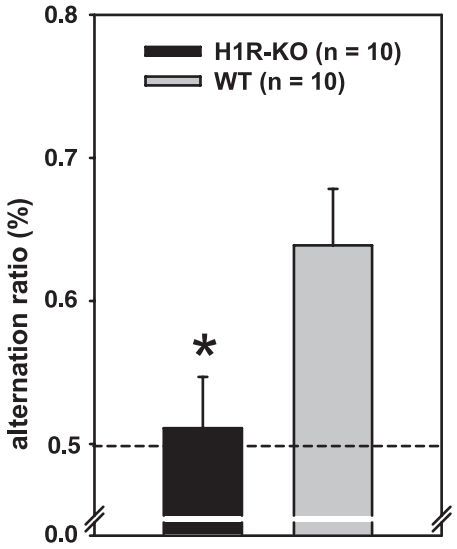


FIG. 3. H1R-knockout (KO) mice show impaired spatial novelty-induced alternation behavior in the Y-maze. Bars represent mean ± SEM percentage of alternation. *$P < 0.05$; Student's *t*-test for non-paired data. Dashed line indicates performance at chance level.

These brain regions and neurotransmitters have been implicated in novelty-induced behaviors and the modulation of learning based on reinforcement of behavior. Compared with the controls, the H1R-KO mice had significantly higher ACh concentrations in the FC ($T = -3.13$, d.f. $= 18$, $P = 0.006$; Student's *t*-test for non-paired data; Table 1) and AMY ($T = -4.14$, d.f. $= 18$, $P = 0.001$). In the AMY, the H1R-KO mice had significantly higher levels of DA ($T = -2.14$, d.f. $= 18$, $P = 0.046$; Table 3), but a lower DOPAC/DA ratio ($T = 2.31$, d.f. $= 18$, $P = 0.033$) relative to the WT mice. No significant differences between H1R-KO and WT were found for the other neurochemical parameters and/or brain structures considered (all $P > 0.05$; Tables 1–4). Therefore, it seems that H1R-deficiency selectively increases ACh levels in the FC, and ACh as well as DA levels in the AMY of H1R-KO mice.

### *Brain histamine levels in brains of H1R-KO mice*

The KO of the H1R in mice had no significant effects on histamine levels in various brain tissues, including the FC and AMY (all $P > 0.05$; Table 5), suggesting that the behavioral effects of the H1R-KO observed here are not due to compensatory changes in the levels of histamine in brain regions involved in novelty-induced behaviors.

### *AChE histochemistry*

Because we found changes in the ACh content in the PFc and AMY of H1R-KO mice, we asked whether these differences might be due to changes in AChE activity in the PFc and amygdaloid subnuclei. Representative histochemistry of AChE in coronal brain sections is shown in Fig. 6 (upper panel). However, there was no significant difference in AChE activity levels between H1R-KO and WT mice in the PFc or subregions of the AMY (all $P > 0.05$; Table 6). The increase of ACh levels in the PFc and AMY of H1R-KO mice concomitant with unchanged AChE levels in these brain regions suggest a lower utilization of ACh and thus a decreased ACh neurotransmission in these mice.

### *TH immunohistochemistry*

We further investigated whether the changes in dopaminergic parameters in the AMY of the H1R-KO mice might be related to corresponding differences in TH activity among different subnuclei of the AMY. Representative pictures of TH immunohistochemistry in both genotypes are shown in Fig. 6 (lower panel). Compared with the controls, the H1R-KO mice had a significantly higher expression of TH in the BLA ($T = -2.93$, d.f. $= 14$, $P = 0.011$), BLV ($T = -2.34$, d.f. $= 14$, $P = 0.035$) and Co ($T = -2.53$, d.f. $= 14$, $P = 0.024$) amygdaloid subnuclei. Therefore the increased DA content in the AMY of the H1R-KO mice can either be due to a reduced metabolization/turnover rate, as suggested by the lower DOPAC/DA ratio, and/or due to an increased DA synthesis by increased TH expression or higher number of TH terminals. No significant difference in TH immunoreactivity was found for the remaining AMY subnuclei or the PFc ($P > 0.05$; Table 7).

## Discussion

We examined the effects of a H1R-KO in the mouse on novelty-induced CPP, novelty-induced spatial alternation, one-trial object-place recognition and emotional behavior in a novel environment. Unlike the WT, the H1R-KO mice did not exhibit novel object-induced CPP and showed impaired novelty-induced alternation in the Y-maze. Rearing activity and emotional behavior in a novel environment was likewise altered in the H1R-KO mice, whereas one-trial object-place recognition was unchanged. The H1R-KO mice had higher ACh concentrations in the FC and AMY. However, there was no significant difference in AChE levels between H1R-KO and WT mice in the PFc or subregions of the AMY. In the latter, the H1R-KO mice had also higher levels of DA, but a lower DOPAC/DA ratio. These changes were related to corresponding differences in TH expression among different subnuclei of the AMY. The H1R-KO mice had significantly higher expression of TH in the BLA, BLV and Co amygdaloid subnuclei. No significant differences between H1R-KO and WT were found for histamine concentrations in several brain structures.

### *Novel object-induced CPP*

In H1R-KO mice the pairing of novel objects with one of two different compartments did not lead to a CCP for the compartment in which novel objects were present during the conditioning trials. It is possible that during the conditioning trials the H1R-KO mice were not interested in exploring novel objects, or avoided the novel objects due to increased neophobia. However, the H1R-KO mice showed similar levels of exploration of the novel objects as the WT mice during the conditioning trials, and did not exhibit avoidance of the novel object-paired compartment during the test trial. Thus, lack of interest or neophobia is unlikely to account for the present results. The finding that the H1R-KOs did not develop a CPP might also be related to a general learning deficit, either in terms of impairment in the encoding or retention of spatial information, an inability to associate novelty-induced reward with spatial stimuli, or failure to associate objects with spatial stimuli. However, the H1R-KO mice showed intact performance in the one-trial object-place recognition task. Therefore, it seems unlikely that the present result is due to deficits in spatial learning, or the association of objects with places. The exact role of the H1R in spatial learning is still unclear and seems to depend on task-inherent reinforcement contingencies (Dere *et al.*, 2001). Behavioral phenotyping of the H1R-KO mice in terms of learning and

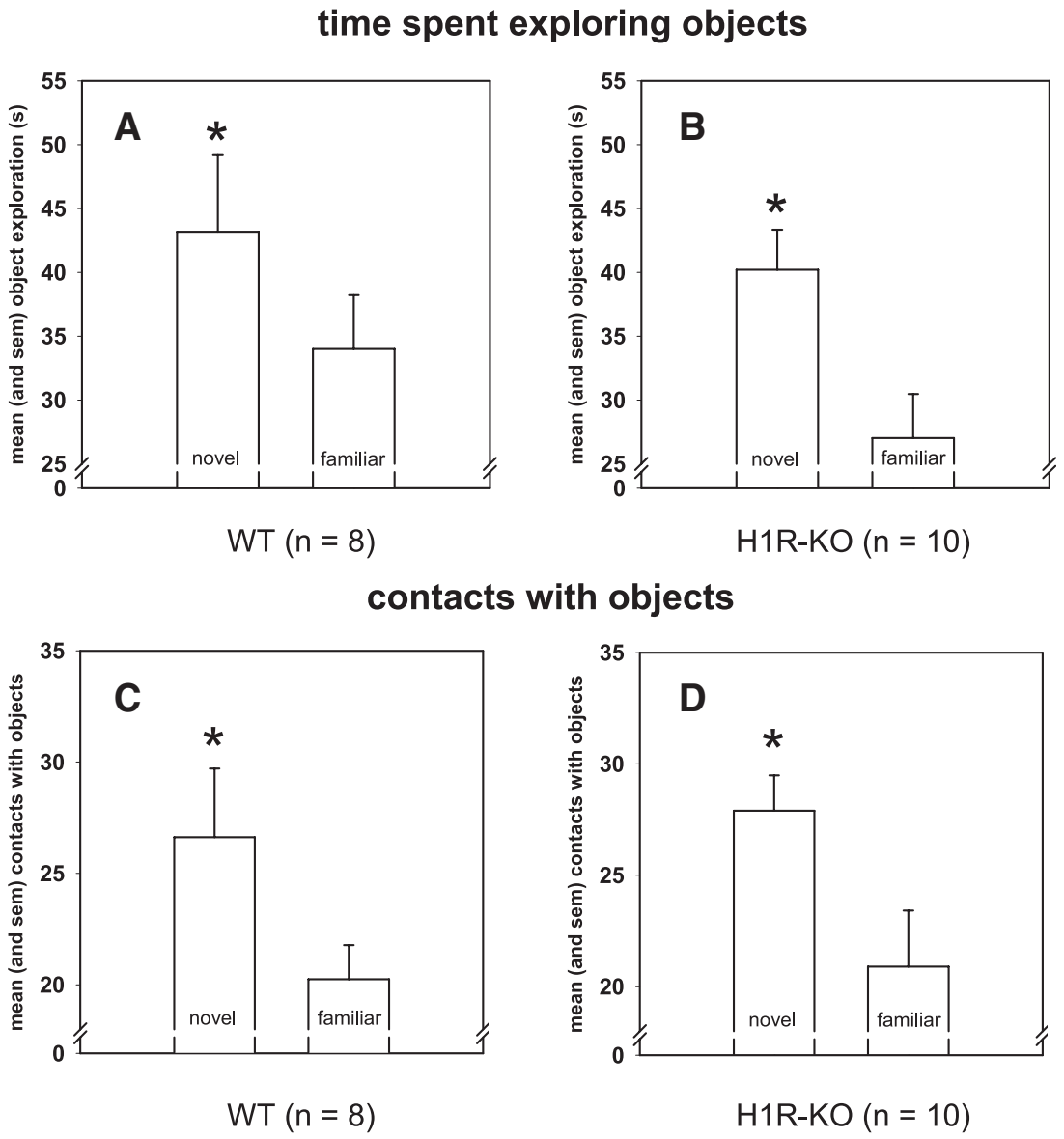


FIG. 4. One-trial object-place recognition is not impaired in the H1R-knockout (KO) mice. (A and B) Bars represent mean ± SEM time spent exploring two identical objects at indicated locations for wild-type (WT) and H1R-KO mice. (C and D) Bars represent mean ± SEM number of contacts with two identical objects at indicated locations for WT and H1R-KO mice. *$P \leq 0.05$; Student's *t*-test for paired data.

memory abilities has yielded inconsistent results. While the H1R-KO mice showed intact performance in an inhibitory avoidance task (Yanai *et al.*, 1998), they were impaired in object recognition and Barnes maze performance, but showed improved auditory and contextual fear-conditioning (Dai *et al.*, 2007). Therefore, the most parsimonious explanation for the present results is that, although the H1R-KO mice explore novel objects, novelty is not rewarding for them. It seems that H1R-KO mice approach and explore novel objects simply because they are there and because there are no other alternatives available to interact with. Should the exploration of novel objects not be rewarding for the H1R-KO mice, the novel-object paired compartment could not be associated with a rewarding brain state and, thus, could not acquire a positive valence through classical conditioning. Therefore, it is possible that H1R-mediated histaminergic neurotransmission is involved in the rewarding aspects of novelty-induced arousal, or the ability of novel objects to stimulate the brain's reward circuit.

### *Novelty-induced spatial alternation*

Rodents that are allowed to choose between a novel and familiar compartment of a place preference apparatus prefer the novel compartment over the familiar one. Pharmacological studies revealed that this innate preference reflects the rewarding effect of spatial novelty rather than a stress-related aversion to the familiar compartment acquired during the inescapable forced familiarization sessions. (Klebaur & Bardo, 1999). The mesolimbic DA pathway has been postulated as a critical link that mediates the rewarding effects of various addictive drugs. In rodents, the exploration of a novel environment stimulates the mesolimbic DA system in the same way as drugs of abuse (Bardo *et al.*, 1996; Hughes, 2007). Using a very sophisticated *in vivo* microdialysis procedure, which allows the measurement of DA release at extremely brief time intervals (5 s) in the NAc of freely moving rats, it has been demonstrated that the first entry into a novel (but not into a familiar) environment induces a transient significant increase of DA release in the shell NAc (Rebec *et al.*, 1997).

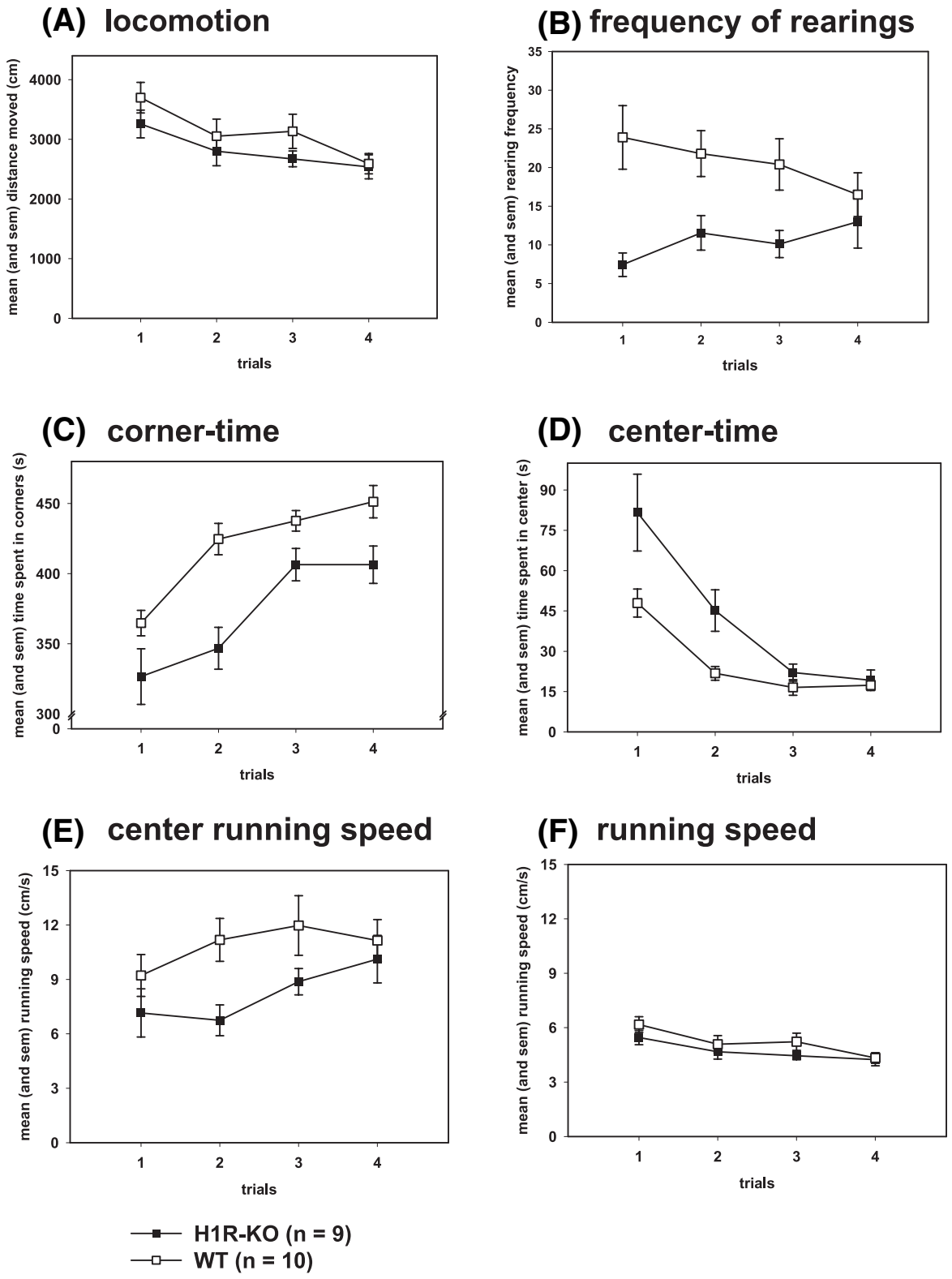


FIG. 5. H1R-knockout (KO) mice show less spatial novelty-induced emotionality in the open-field. (A) Squares represent mean ± SEM locomotion in cm on indicated trials. (B) Squares represent mean ± SEM frequency of rearings on indicated trials. (C) Squares represent mean ± SEM corner time in s on indicated trials. (D) Squares represent mean ± SEM center time in s on indicated trials. (E) Squares represent mean ± SEM running speed (cm/s) in the center of the field on indicated trials. (F) Squares represent mean ± SEM running speed (cm/s) in the entire field on indicated trials.

In the present study, we also investigated whether the exploration of a novel environment is sufficiently rewarding in the H1R-KO mice to maintain novelty-induced spatial alternation as long as in the WT mice. Here, the H1R-KO mice showed a reduced number of triplets and a lower alternation ratio, suggesting that the reward value of spatial novelty, which motivates spatial alternation behavior, is

TABLE 1. Ach concentration in the brains of H1R-KO and WT mice

| | Ach concentration (pmol/mg) | | | | |
|---|---|---|---|---|---|
| Brain area | FC | Hippocampus | AMY | Neostriatum | Ventral striatum |
| H1R-KO | 29.6 ± 3.2* | 20.2 ± 3.8 | 40.9 ± 2.3* | 74.3 ± 4.9 | 70.9 ± 8.1 |
| WT | 17.1 ± 2.4 | 17.1 ± 2.2 | 29.1 ± 1.6 | 73.6 ± 4.9 | 69.9 ± 7.5 |

Data are presenred as means ± SEM. *$P < 0.05$ (Student's *t*-test for non-paired data). AMY, amygdala; FC, frontal cortex; KO, knockout; WT, wild-type.

reduced in the H1R-KO mice. Although it is also possible that the reduced alternation performance of the H1R-KO mice in the Y-maze is due to a spatial working memory deficit, our object-place recognition data argue against such an interpretation. Thus, we conclude that the H1R mediates the rewarding effects of both spatial and object novelty.

### *Why is novelty no longer rewarding in the H1R-KO mice?*

It has been shown that histaminergic neurons have an excitatory effect via the H1R on inhibitory interneurons in the area tegmentalis ventralis and substantia nigra. These inhibitory interneurons modulate the firing frequency of dopaminergic cells projecting to the neo- and ventral striatum (Korotkova *et al.*, 2002). Therefore, it is likely that the absence of the H1R-mediated modulation of dopaminergic 'reward' cell firing might lead to abnormal motivational responses to natural reinforcers, such as spatial and object novelty. Furthermore, it has been reported that the increased DOPAC concentrations in the NAc

TABLE 2. Noradrenaline concentration in the brains of H1R-KO and WT mice

| | Noradrenaline concentration (pg/mg) | | | | |
|---|---|---|---|---|---|
| | FC | Hippocampus | AMY | Neostriatum | Ventral striatum |
| H1R-KO | 6631.9 ± 1711.3 | 26848.2 ± 9611.6 | 13103.6 ± 4253.1 | 2743.2 ± 624.4 | 40518.7 ± 7316.1 |
| WT | 6700.2 ± 2782.3 | 20724.0 ± 5130.3 | 13103.0 ± 6321.4 | 4068.3 ± 2356.6 | 43367.0 ± 11651.1 |

Data are presenred as means ± SEM. AMY, amygdala; FC, frontal cortex; KO, knockout; WT, wild-type.

TABLE 3. DA, DOPAC and HVA concentrations, and DOPAC/DA and HVA/DA turnover ratios in the brains of H1R-KO and WT mice

| Brain area | DA (pg/mg) | DOPAC (pg/mg) | HVA (pg/mg) | DOPAC/DA | HVA/DA |
|---|---|---|---|---|---|
| FC | | | | | |
| H1R-KO | 174.2 ± 25.0 | 161.8 ± 12.6 | 792.8 ± 177.4 | 1.03 ± 0.11 | 4.74 ± 1.31 |
| WT | 175.7 ± 14.5 | 152.3 ± 24.0 | 580.7 ± 126.9 | 0.87 ± 0.12 | 3.65 ± 1.04 |
| Hippocampus | | | | | |
| H1R-KO | 166.3 ± 27.8 | 141.2 ± 28.5 | 1554.3 ± 254.9 | 1.04 ± 0.17 | 12.3 ± 3.8 |
| WT | 154.5 ± 28.8 | 154.2 ± 19.1 | 1248.7 ± 168.9 | 1.17 ± 0.15 | 10.6 ± 2.3 |
| AMY | | | | | |
| H1R-KO | 1149.5 ± 91.4* | 330.5 ± 13.0 | 1923.7 ± 172.8 | 0.30 ± 0.02* | 1.76 ± 0.20 |
| WT | 884.6 ± 83.3 | 327.4 ± 22.8 | 1416.0 ± 209.2 | 0.38 ± 0.06 | 1.79 ± 0.40 |
| Neostriatum | | | | | |
| H1R-KO | 39324.2 ± 1270.3 | 3247.1 ± 177.6 | 4589.1 ± 354.6 | 0.08 ± 0.00 | 0.12 ± 0.01 |
| WT | 37011.7 ± 1236.2 | 3209.4 ± 149.6 | 6405.53 ± 2249.4 | 0.09 ± 0.00 | 0.16 ± 0.05 |
| Ventral striatum | | | | | |
| H1R-KO | 14841.5 ± 2023.8 | 2833.9 ± 168.9 | 2960.2 ± 487.8 | 0.23 ± 0.03 | 0.26 ± 0.06 |
| WT | 17383.9 ± 1854.8 | 3111.6 ± 269.0 | 3204.7 ± 272.9 | 0.19 ± 0.02 | 0. 21 ± 0.06 |

Data are presenred as means ± SEM. *$P < 0.05$ (Student's *t*-test for non-paired data). AMY, amygdala; DA, dopamine; DOPAC, dihydrophenylacetic acid; FC, frontal cortex; HVA, homovanillic acid; KO, knock-out; WT, wild-type.

TABLE 4. 5-HT and 5-HIAA concentrations, and 5-HIAA/5-HT turnover ratios in the brains of H1R-KO and WT mice

| | FC | Hippocampus | AMY | Neostriatum | Ventral striatum |
|---|---|---|---|---|---|
| 5-HT concentration (pg/mg) | | | | | |
| H1R-KO | 631.8 ± 45.4 | 535.1 ± 133.5 | 989.1 ± 56.8 | 1109.2 ± 796.9 | 1460.8 ± 711.0 |
| WT | 623.2 ± 44.4 | 420.9 ± 25.5 | 842.7 ± 61.9 | 291.7 ± 20.6 | 840.9 ± 160.1 |
| 5-HIAA concentration (pg/mg) | | | | | |
| H1R-KO | 194.0 ± 13.6 | 369.9 ± 26.5 | 871.9 ± 499.3 | 307.2 ± 26.6 | 310.6 ± 41.2 |
| WT | 211.9 ± 14.8 | 384.4 ± 31.6 | 787.7 ± 410.7 | 286.6 ± 16.7 | 351.6 ± 45.1 |
| 5-HIAA/5-HT turnover ratios | | | | | |
| H1R-KO | 0.31 ± 0.01 | 0.84 ± 0.09 | 0.95 ± 0.57 | 0.91 ± 0.10 | 0.37 ± 0.04 |
| WT | 0.31 ± 0.04 | 0.91 ± 0.07 | 0.93 ± 0.47 | 1.00 ± 0.05 | 0.46 ± 0.04 |

Data are presenred as means ± SEM. 5-HT, serotonin; 5-HIAA, 5-hydroxyindole acetic acid; AMY, amygdala; FC, frontal cortex; KO, knockout; WT, wild-type.

TABLE 5. Histamine concentrations in the brains of H1R-KO and WT mice

| | Histamine concentrations (pmol/mg) | | | |
|---|---|---|---|---|
| | FC | AMY | Neostriatum | Cerebellum |
| H1R-KO | 0.53 ± 0.03 | 0.37 ± 0.04 | 0.75 ± 0.07 | 0.10 ± 0.01 |
| WT | 0.64 ± 0.07 | 0.31 ± 0.03 | 0.73 ± 0.10 | 0.15 ± 0.04 |

Data are presenred as means ± SEM. AMY, amygdala; FC, frontal cortex; KO, knockout; WT, wild-type.

after intracerebroventricular administration of histamine can be blocked by pretreatment with an H1R-antagonist (Fleckenstein *et al.*, 1993). However, the lack of H1R-mediated effects on γ-aminobutyric acid (GABA)ergic and/or dopaminergic neurons had no effect on the DA levels in the neo- or ventral striatum. In the AMY, the H1R-KO mice had higher levels of DA, but a lower DOPAC/DA ratio relative to the WT mice, suggesting that DA turnover and transmission is attenuated in this region. We further investigated whether the changes in dopaminergic parameters in the AMY of the H1R-KO mice might be related to corresponding differences in TH

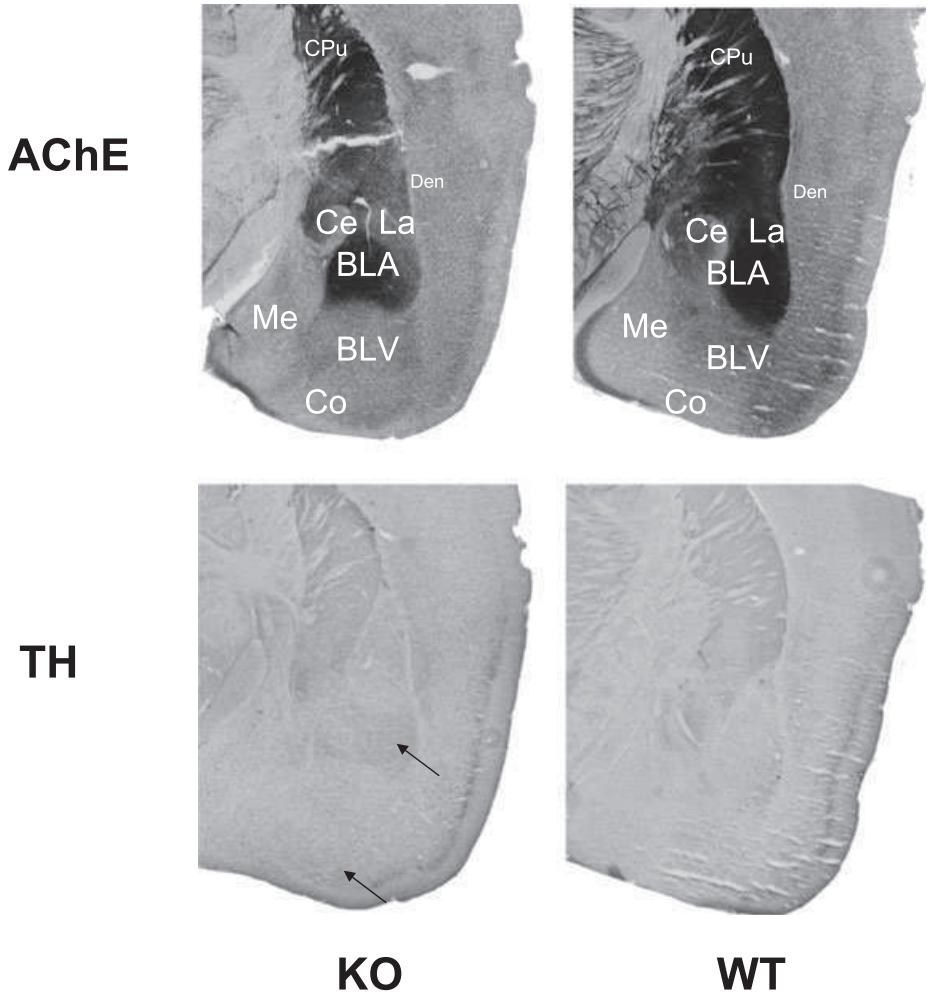


FIG. 6. Acetylcholine esterase (AChE) histochemistry and tyrosine hydroxylase (TH) immunohistochemistry. Representative pictures of AChE histochemistry (upper panels) and TH immunohistochemistry in H1R-knockout (KO) mice (left panels) and control mice (right panels). Adjacent sections were used to recognize the same AMY subnuclei. Arrows show two subnuclei with stronger TH reaction. WT, wild-type. All other abbreviations, see Fig. 1.

TABLE 6. Histochemical reaction for AChE in the FC and subnuclei of the AMY of H1R-KO and WT mice

| | Histochemical reaction for AChE (relative optical density units) | | | | | | |
|---|---|---|---|---|---|---|---|
| | PFc | La | BLA | BLV | Ce | Me | Co |
| H1R-KO | 0.070 ± 0.008 | 0.297 ± 0.039 | 0.692 ± 0.025 | 0.124 ± 0.009 | 0.145 ± 0.017 | 0.042 ± 0.009 | 0.063 ± 0.007 |
| WT | 0.064 ± 0.008 | 0.337 ± 0.041 | 0.665 ± 0.037 | 0.117 ± 0.013 | 0.163 ± 0.017 | 0.046 ± 0.009 | 0.055 ± 0.012 |

Data are presenred as means ± SEM. Amygdaloid subnuclei: BLA, basolateral anterior; BLV, basolateral ventral; Ce, central; Co, cortical; La, lateral; Me, medial; KO, knockout; PFc, prefrontal cortex; WT, wild-type.

TABLE 7. Immunohistochemical reaction for TH in different subnuclei of the amygdala of H1R-KO and WT mice

| | Immunohistochemical reaction for TH (relative optical density units) | | | | | | |
|---|---|---|---|---|---|---|---|
| | PFc | La | BLA | BLV | Ce | Me | Co |
| H1R-KO | 0.016 ± 0.001 | 0.022 ± 0.004 | 0.045 ± 0.006* | 0.030 ± 0.005* | 0.043 ± 0.005 | 0.036 ± 0.007 | 0.040 ± 0.006* |
| WT | 0.018 ± 0.002 | 0.012 ± 0.002 | 0.024 ± 0.004 | 0.016 ± 0.003 | 0.037 ± 0.005 | 0.020 ± 0.003 | 0.021 ± 0.004 |

Data are presenred as means ± SEM. *$P < 0.05$, Student's *t*-test for non-paired data. Amygdaloid subnuclei: BLA, basolateral anterior; BLV, basolateral ventral; Ce, central; Co, cortical; La, lateral; Me, medial; KO, knockout; PFc, prefrontal cortex; WT, wild-type.

activity among different subnuclei of the AMY. The H1R-KO mice showed significantly higher TH expression in the BLA, BLV and Co subnuclei of the AMY, suggesting that the increased DA content in the AMY of the H1R-KO mice might also be due to its increased synthesis by increased TH expression or higher number of TH terminals. The latter would also suggest quantitative changes in the dopaminergic projections to the AMY in brains of the H1R-KO mice.

Beside its important role in mediating fear responses and fear-induced learning (Phelps & LeDoux, 2005), the AMY has also been implicated in processes of brain reward and learning. The role of the AMY in reward-related processes might involve reciprocal connections between the BLA and the mesolimbic DA system, either with the ventral tegmental area or the NAc. The BLA provides a direct excitatory input to the NAc. BLA stimulation induces transient increases in extracellular DA in the NAc. In addition the Ce nucleus of the AMY affects the activity of dopaminergic cells in the area tegmentalis ventralis (Baxter & Murray, 2002). It is conceivable that the AMY conveys information about emotionally arousing stimuli, e.g. novelty, into the mesolimbic DA system (Johnson *et al.*, 1994). The dopaminergic innervation of the AMY might function as a feedback system signaling the AMY, e.g. whether these stimuli coincide with a rewarding brain state. It is possible that the changes in TH reactivity in several subnuclei of the AMY in the H1R-KO mice reflect changes in the dopaminergic innervation of the AMY, which in turn might be associated with an inaccurate feedback on the rewarding effects of emotionally arousing stimuli.

Interestingly, the AMY has been implicated in CPP to addictive drugs (Hiroi & White, 1991) as well as natural reinforcers such as food (Schroeder & Packard, 2002) or sweetened liquid (Everitt *et al.*, 1991). Furthermore, D1 or D2/D3 antagonists (Besheer *et al.*, 1999; Bevins *et al.*, 2002) block a novel object-induced CPP. It is possible that the changes in the DA parameters in the AMY of the H1R-KO mice are related to their impairment in the CPP task.

It is known that pharmacological blockade of the H1R has reinforcing effects and potentiates the effects of addictive drugs (Hasenöhrl & Huston, 2004). Therefore, one would have expected that the H1R-KO mice should show rather increased then decreased responses to rewarding stimuli. However, in this regard it should be noted that most H1R antagonists used in reward/addiction studies also show activity at other histamine receptors (Lim *et al.*, 2005) as well as on non-histamine, e.g. AChR and even DA-R (Du Buske, 1996). Therefore, the H1R-KO mice are a promising alternative to pharmacological studies to investigate the role of the H1R in reward-related processes. Most importantly, the H1R-KO mice can be used to test the pharmacological selectivity of H1R-related agents *in vivo*.

### *Emotional and adaptive behavior in a novel environment*

Rodents typically show increased rearing frequency in novel vs familiar environments, suggesting that rearing activity reflects behavioral arousal and information gathering in novel environments. The H1R-KO mice showed a significantly reduced number of rearings in the open-field, suggesting that the arousing effect of spatial novelty is diminished in H1R-KO mice. Reduced rearing activity in H1R-KO mice after exposure to a novel environment has been reported earlier (Inoue *et al.*, 1996). In contrast to the present results, it has also been reported that H1R-KO mice show reduced locomotor behavior in novel environments (Inoue *et al.*, 1996). This discrepancy is likely due to methodological differences between this earlier and the present study. In the Inoue *et al.* (1996) study the animals received a single trial of 30 min duration, whereas we subjected the animals to four trials with an intertrial interval of 24 h and a 10-min trial duration. If one compares the locomotion of the H1R-KO with that of the WT mice across the first 9 min in the Inoue *et al.* (1996) study (see fig. 4A in the Inoue *et al.*, 1996 study) there seems no significant difference between H1R-KO and WT mice, similar to the insignificant result between H1R-KO and WT mice that we obtained on the first day of testing. It remains to be tested whether a single 30-min session in a novel environment would lead to results in line with those of Inoue *et al.* (1996).

Novelty-induced arousal can also be aversive and can evoke fear-related responses. We assessed possible changes in the emotional reactivity to spatial novelty in H1R-KO mice by assessing the amount and course of center avoidance vs corner preference during the process of behavioral habituation. Behavioral habituation, in terms of locomotory activity, was not significantly different between H1R-KO and WT mice, suggesting intact spatial recognition in H1R-KO mice. However, the groups differed in terms of their emotional reactivity to spatial novelty. In this regard, the H1R-KO mice spent less time in the corners, but more time in the center of the open-field, and showed reduced running speed in the center of the open-field. Thus, the H1R-KO mice showed reduced emotionality when confronted with spatial novelty. We conclude that H1R-deficiency in the mouse attenuates both the rewarding as well as the aversive motivational effects of novelty.

### *Decreased motivational effects of novelty in H1R-KO mice might be due to decreased novelty-induced arousal*

Cortical ACh regulates the gating or excitability of sensory neurons during the processing of novel stimuli. In rats, exploration of a novel environment coincides with large increases of extracellular levels of cortical ACh. Both exploratory behaviors and extracellular levels of cortical ACh progressively decline when the environment becomes familiar (Inglis *et al.*, 1994; Giovannini *et al.*, 1998). The motivational effects of novelty might be due to arousal, which can be dependent on the initial state of arousal, either rewarding or aversive. The brain's ACh systems have been implicated in behavioral arousal (Sarter & Bruno, 2000). ACh release can be modulated by systemic and intracerebral application of histamine-related agents, including H1R-antagonists (Blandina *et al.*, 2004). We found increased concentrations of ACh in the FC and AMY of the H1R-KO mice. The increase of

ACh levels in the PFc and AMY of H1R-KO mice concomitant with unchanged AChE levels in these brain regions suggest a lower utilization of ACh and thus a decreased ACh neurotransmission in these mice. Therefore, it is possible that the changes in FC and AMY ACh levels have a detrimental effect on well-adjusted arousal reactions to different types of novel stimuli and their activating effect on the brain's reward and/or emotional systems.

### *Hippocampal H1R and the motivational effects of novelty*

Recently it has been shown that intrahippocampal H1R but not H2R play a significant role in the motivational effects of novelty in rats (Alvarez & Alvarez, 2008). These authors tested rats in an elevated asymmetric plus-maze either with or without novel objects, and found that rats decrease their horizontal, ambulatory and non-ambulatory activity in favor of exploration of a novel object. Rats also increased their sojourn time on the most fear-inducing arm when a novel object was present, suggesting that exploration of a novel object is indeed rewarding. This motivated exploratory response was suppressed after microinjection of histamine into the ventral hippocampus. Pretreatment with an H1R- but not an H2R-antagonist was effective to block the inhibitory effect of histamine on the object-motivated exploration (Alvarez & Alvarez, 2008). It remains to be determined whether the changes in the motivational effects of novelty in the H1R-KO mice are due to the absence of H1R-mediated effects on intrahippocampal processing of novel stimuli.

### *Conclusions*

The motivational effects of novelty are diminished or even absent in the H1R-KO mice. The changes in behavioral reactivity and adaptation to different types of novelty of the H1R-KO mice might be either due to reduced novelty-induced arousal (possibly related to the changes in ACh levels in the FC and AMY), or a dysfunctional brain reward system (possibly related to changes in the dopaminergic innervation of the AMY).

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## Abbreviations

5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; ACh, acetylcholine; AChE, acetylcholine esterase; AMY, amygdala; BLA, basolateral anterior nucleus; BLV, basolateral ventral nucleus; Ce, central nucleus; Co, cortical nucleus; CPP, conditioned place-preference; DA, dopamine; DOPAC, dihydrophenylacetic acid; FC, frontal cortex; HPLC, high-performance liquid chromatography; HR, histamine receptor; HVA, homovanillic acid; KO, knockout; La, lateral nucleus; Me, medial nucleus; NAc, nucleus accumbens; PBS, phosphate-buffered saline; ROD, relative optical density; TBS, Tris-buffered saline; TH, tyrosine hydroxylase; WT, wild-type.

## References

Alvarez, E.O. & Alvarez, P.A. (2008) Motivated exploratory behaviour in the rat: the role of hippocampus and the histaminergic neurotransmission. *Behav. Brain Res.*, **186**, 118–125.

Aspide, R., Gironi Carnevale, U.A., Sergeant, J.A. & Sadile, A.G. (1998) Non-selective attention and nitric oxide in putative animal models of attention-deficit hyperactivity disorder. *Behav. Brain Res.*, **95**, 123–133.

Bardo, M.T. & Bevins, R.A. (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology*, **153**, 31–43.

Bardo, M.T., Donohew, R.L. & Harrington, N.G. (1996) Psychobiology of novelty seeking and drug seeking behavior. *Behav. Brain Res.*, **77**, 23–43.

Baxter, M.G. & Murray, E.A. (2002) The amygdala and reward. *Nat. Rev. Neurosci.*, **3**, 563–573.

Berlyne, D.E. (1969) Arousal, reward and learning. *Ann. N.Y. Acad. Sci.*, **159**, 1059–1070.

Besheer, J., Jensen, H.C. & Bevins, R.A. (1999) Dopamine antagonism in a novel-object recognition and a novel-object place conditioning preparation with rats. *Behav. Brain Res.*, **103**, 35–44.

Bevins, R.A. & Bardo, M.T. (1999) Conditioned increase in place preference by access to novel objects: antagonism by MK-801. *Behav. Brain Res.*, **99**, 53–60.

Bevins, R.A. & Besheer, J. (2005) Novelty reward as a measure of anhedonia. *Neurosci. Biobehav. Rev.*, **29**, 707–714.

Bevins, R.A., Besheer, J., Palmatier, M.I., Jensen, H.C., Pickett, K.S. & Eurek, S. (2002) Novel-object place conditioning: behavioral and dopaminergic processes in expression of novelty reward. *Behav. Brain Res.*, **129**, 41–50.

Blandina, P., Efoudebe, M., Cenni, G., Mannaioni, P. & Passani, M.B. (2004) Acetylcholine, histamine, and cognition: two sides of the same coin. *Learn. Mem.*, **11**, 1–8.

Blatter, K. & Schulz, W. (2006) Rewarding properties of visual stimuli. *Exp. Brain Res.*, **168**, 541–546.

Bronson, G.W. (1968) The fear of novelty. *Psychol. Bull.*, **69**, 350–358.

Burke, R.E., Cadet, J.L., Kent, J.D., Karanas, A.L. & Jackson-Lewis, V. (1990) An assessment of the validity of densitometric measures of striatal tyrosine hydroxylase-positive fibers: relationship to apomorphine-induced rotations in 6-hydroxydopamine lesioned rats. *J. Neurosci. Meth.*, **35**, 63–73.

Capowski, J.J. (1989) *Computer Techniques in Neuroanatomy*. Plenum Press, New York.

Cerbone, A. & Sadile, A.G. (1994) Behavioral habituation to spatial novelty in rats: interference and noninterfernce studies. *Neurosci. Biobehav. Rev.*, **18**, 497–518.

Dai, H., Kaneko, K., Kato, H., Fujii, S., Jing, Y., Xu, A., Sakurai, E., Kato, M., Okamura, N., Kuramasu, A. & Yanai, K. (2007) Selective cognitive dysfunction in mice lacking histamine H1 and H2 receptors. *Neurosci. Res.*, **57**, 306–313.

De Souza Silva, M.A., Hasenöhrl, R.U., Tomaz, C., Schwarting, R.K. & Huston, J.P. (2000) Differential modulation of frontal cortex acetylcholine by injection of substance P into the nucleus basalis magnocellularis region in the freely-moving vs. the anesthetized preparation. *Synapse*, **38**, 243–253.

De Souza-Silva, M.A., Mattern, C., Häcker, R., Nogueira, P.J., Huston, J.P. & Schwarting, R.K. (1997) Intranasal administration of the dopaminergic agonists L-DOPA, amphetamine, and cocaine increases dopamine activity in the neostriatum: a microdialysis study in the rat. *J. Neurochem.*, **68**, 233–239.

Dere, E., De Souza Silva, M.A., Topic, B., Fiorillo, C., Li, J.S., Sadile, A.G., Frisch, C. & Huston, J.P. (2002) Aged endothelial nitric oxide synthase knockout mice exhibit higher mortality concomitant with impaired open-field habituation and alterations in forebrain neurotransmitter levels. *Genes Brain Behav.*, **1**, 204–213. [Erratum published in *Genes Brain Behav.* (2003), **2**, 64.]

Dere, E., De Souza-Silva, M.A., Frisch, C., Teubner, B., Söhl, G., Willecke, K. & Huston, J.P. (2003) Connexin30-deficient mice show increased emotionality and decreased rearing activity in the open-field along with neurochemical changes. *Eur. J. Neurosci.*, **18**, 629–638.

Dere, E., Frisch, C., De Souza Silva, M.A., Gödecke, A., Schrader, J. & Huston, J.P. (2001) Unaltered radial maze performance and brain acetylcholine of the endothelial nitric oxide synthase knockout mouse. *Neuroscience*, **107**, 561–570.

Dere, E., Huston, J.P. & De Souza Silva, M.A. (2005) Episodic-like memory in mice: simultaneous assessment of object, place and temporal order memory. *Brain Res. Protoc.*, **16**, 10–19.

Dere, E., Huston, J.P. & De Souza Silva, M.A. (2007) The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neurosci. Biobehav. Rev.*, **31**, 673–704.

Dringenberg, H.C., De Souza-Silva, M.A., Schwarting, R.K. & Huston, J.P. (1998) Increased levels of extracellular dopamine in neostriatum and nucleus accumbens after histamine H1 receptor blockade. *Naunyn Schmiedebergs Arch. Pharmacol.*, **358**, 423–429.

Du Buske, L.M. (1996) Clinical comparison of histamine H1-receptor antagonist drugs. *J. Allergy Clin. Immunol.*, **98**, S307–S318.

Everitt, B.J., Morris, K.A., O'Brien, A. & Robbins, T.W. (1991) The basolateral amygdala-ventral striatal system and conditioned place preference: further evidence of limbic–striatal interactions underlying reward-related processes. *Neuroscience*, **42**, 1–18.

Fibiger, H.C. & Phillips, A.G. (1988) Mesocorticolimbic dopamine systems and reward. *Ann. N. Y. Acad. Sci.*, **537**, 206–215.

Fleckenstein, A.E., Lookingland, K.J. & Moore, K.E. (1993) Activation of mesolimbic dopaminergic neurons following central administration of histamine is mediated by H1 receptors. *Naunyn Schmiedebergs Arch. Pharmacol.*, **347**, 50–54.

Giovannini, M.G., Bartolini, L., Kopf, S.R. & Pepeu, G. (1998) Acetylcholine release from the frontal cortex during exploratory activity. *Brain Res.*, **784**, 218–227.

Haas, H. & Panula, P. (2003) The role of histamine and the tuberomamillary nucleus in the nervous system. *Nat. Rev. Neurosci.*, **4**, 121–130.

Hasenöhrl, R.U. & Huston, J.P. (2004) Histamine. In Riedel, D.G. & Platt, B. (Eds), *Memories are Made of These: From Messengers to Molecules.* Landes Bioscience, Austin, Texas, pp. 1–23.

Hill, S.J., Ganellin, C.R., Timmerman, H., Schwartz, J.C., Shankley, N.P., Young, J.M., Schunack, W., Levi, R. & Haas, H.L. (1997) International Union of Pharmacology. XIII. Classification of histamine receptors. *Pharmacol. Rev.*, **49**, 253–278.

Hiroi, N. & White, N.M. (1991) The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. *J. Neurosci.*, **11**, 2107–2116.

Huang, Z.L., Mochizuki, T., Qu, W.M., Hong, Z.Y., Watanabe, T., Urade, Y. & Hayaishi, O. (2006) Altered sleep-wake characteristics and lack of arousal response to H3 receptor antagonist in histamine H1 receptor knockout mice. *Proc. Natl Acad. Sci. USA*, **103**, 4687–4692.

Huang, Z.L., Qu, W.M., Li, W.D., Mochizuki, T., Eguchi, N., Watanabe, T., Urade, Y. & Hayaishi, O. (2001) Arousal effect of orexin A depends on activation of the histaminergic system. *Proc. Natl Acad. Sci. USA*, **98**, 9965–9970. [Erratum published in *Proc. Natl Acad. Sci. USA*, (2002), **99**, 1098.]

Hughes, R.N. (2004) The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neurosci. Biobehav. Rev.*, **28**, 497–505.

Hughes, R.N. (2007) Neotic preferences in laboratory rodents: issues, assessment and substrates. *Neurosci. Biobehav. Rev.*, **31**, 441–464.

Huston, J.P., Wagner, U. & Hasenöhrl, R.U. (1997) The tuberomammillary nucleus projections in the control of learning, memory and reinforcement processes: evidence for an inhibitory role. *Behav. Brain Res.*, **83**, 97–105.

Inglis, F.M., Day, J.C. & Fibiger, H.C. (1994) Enhanced acetylcholine release in hippocampus and cortex during the anticipation and consumption of a palatable meal. *Neuroscience*, **62**, 1049–1056.

Inoue, I., Yanai, K., Kitamura, D., Taniuchi, I., Kobayashi, T., Niimura, K., Watanabe, T. & Watanabe, T. (1996) Impaired locomotor activity and exploratory behavior in mice lacking histamine H1 receptors. *Proc. Natl Acad. Sci. USA*, **93**, 13316–13320.

Johnson, L.R., Aylward, R.L., Hussain, Z. & Totterdell, S. (1994) Input from the amygdala to the rat nucleus accumbens: its relationship with tyrosine hydroxylase immunoreactivity and identified neurons. *Neuroscience*, **61**, 851–865.

Kelley, A.E., Schochet, T. & Landry, C.F. (2004) Risk taking and novelty seeking in adolescence: introduction to part I. *Ann. N. Y. Acad. Sci.*, **1021**, 27–32.

Klebaur, J.E. & Bardo, M.T. (1999) The effects of anxiolytic drugs on novelty-induced place preference. *Behav. Brain Res.*, **101**, 51–57.

Kokkinidis, L. & Anisman, H. (1976) Dissociation of the effects of scopolamine and d-amphetamine on a spontaneous alternation task. *Pharmacol. Biochem. Behav.*, **5**, 293–297.

Korotkova, T.M., Haas, H.L. & Brown, R.E. (2002) Histamine excites GABAergic cells in the rat substantia nigra and ventral tegmental area in vitro. *Neurosci. Lett.*, **320**, 133–136.

Krauth, J. (1988) *Distribution-free Statistics: An Application-Oriented Approach*. Elsevier, Amsterdam.

Lim, H.D., van Rijn, R.M., Ling, P., Bakker, R.A., Thurmond, R.L. & Leurs, R. (2005) Evaluation of histamine H1-, H2-, and H3-receptor ligands at the human histamine H4 receptor: identification of 4-methylhistamine as the first potent and selective H4 receptor agonist. *J. Pharmacol. Exp. Ther.*, **314**, 1310–1321.

Lin, J.S. (2000) Brain structures and mechanisms involved in the control of cortical activation and wakefulness, with emphasis on the posterior hypothalamus and histaminergic neurons. *Sleep Med. Rev.*, **4**, 471–503.

Miller, D.B. & O'Callaghan, J.P. (2006) The pharmacology of wakefulness. *Metabolism*, **55**, S13–S19.

Mochizuki, T., Yamatodani, A., Okakura, K., Takemura, M., Inagaki, N. & Wada, H. (1991) In vivo release of neuronal histamine in the hypothalamus of rats measured by microdialysis. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **343**, 190–195.

Paxinos, G. & Watson, C. (1989) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.

Phelps, E.A. & LeDoux, J.E. (2005) Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*, **48**, 175–187.

Rebec, G.V., Grabner, C.P., Johnson, M., Pierce, R.C. & Bardo, M.T. (1997) Transient increases in catecholaminergic activity in medial prefrontal cortex and nucleus accumbens shell during novelty. *Neuroscience*, **76**, 707–714.

Sadile, A.G. (1996) Long-term habituation of theta-related activity components of albino rats in the Làt-maze. In Sanberg, P.R., Ossenkopp, K.-P. & Kavaliers, M. (Eds), *Motor Activity and Movement Disorders*. Humana Press, Totowa, New Jersey.

Saigusa, T., Tuinstra, T., Koshikawa, N. & Cools, A.R. (1999) High and low responders to novelty: effects of a catecholamine synthesis inhibitor on novelty-induced changes in behaviour and release of accumbal dopamine. *Neuroscience*, **88**, 1153–1163.

Sarter, M. & Bruno, J.P. (2000) Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. *Neuroscience*, **95**, 933–952.

Schroeder, J.P. & Packard, M.G. (2002) Posttraining intra-basolateral amygdala scopolamine impairs food- and amphetamine-induced conditioned place preferences. *Behav. Neurosci.*, **116**, 922–927.

Sigmund, C.D. (2000) Viewpoint: are studies in genetically altered mice out of control? *Arterioscler. Thromb. Vasc. Biol.*, **20**, 1425–1429.

Sonobe, Y., Nakane, H., Watanabe, T. & Nakano, K. (2004) Regulation of Con A-dependent cytokine production from CD4+ and CD8+ T lymphocytes by autosecretion of histamine. *Inflamm. Res.*, **53**, 87–92.

Tien, L.-H., Fan, L.-W., Sogawa, C., Tangeng, M., Loh, H.H. & Ho, I.-K. (2004) Changes in acetylcholinesterase activity and muscarinic receptor bindings in mu-opioid knockout mice. *Mol. Brain Res.*, **126**, 38–44.

Treesukosol, Y., Ishizuka, T., Yamamoto, C., Senda, K., Tsutsumi, S., Yamatodani, A. & Yamamoto, T. (2005) Hypothalamic histamine release by taste stimuli in freely moving rats: possible implication of palatability. *Behav. Brain Res.*, **164**, 67–72.

Wang, Z. & Woolverton, W.L. (2007) Self-administration of cocaine-antihistamine combinations: super-additive reinforcing effects. *Eur. J. Pharmacol.*, **557**, 159–160.

Welker, W.I. (1957) 'Free' versus 'forced' exploration of a novel situation by rats. *Psychol. Report*, **3**, 95–108.

Wise, R.A. (1994) Cocaine reward and cocaine craving: the role of dopamine in perspective. *NIDA Res. Monogr.*, **145**, 191–206.

Yamatodani, A., Fukuda, H., Wada, H., Iwaeda, T. & Watanabe, T. (1985) High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. *J. Chromatogr.*, **344**, 115–123.

Yanai, K., Son, L.Z., Endou, M., Sakurai, E., Nakagawasai, O., Tadano, T., Kisara, K., Inoue, I., Watanabe, T. & Watanabe, T. (1998) Behavioural characterization and amounts of brain monoamines and their metabolites in mice lacking histamine H1 receptors. *Neuroscience*, **87**, 479–487.

Zimmermann, P., Privou, C. & Huston, J.P. (1999) Differential sensitivity of the caudal and rostral nucleus accumbens to the rewarding effects of a H1-histaminergic receptor blocker as measured with place-preference and self-stimulation behavior. *Neuroscience*, **94**, 93–103.

Die hier vorgelegte Dissertation habe ich eigenständig und ohne unerlaubte Hilfsmittel angefertigt. Die Dissertation wurde in der vorliegenden oder in ähnlicher Form noch bei keiner anderen Institution eingereicht. Ich habe bisher keine erfolglosen Promotionsversuche unternommen.

Düsseldorf, den 07.08.2008

(Armin Zlomuzica)