The role of NMDA receptors in the pigeon prefrontal cortex

(Nidopallium caudolaterale)

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TABLE OF CONTENTS

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

1. INTRODUCTION

The prefrontal cortex (PFC) is a forebrain area in mammals crucial for integrating information from other brain regions in order to flexibly select and initiate behavior appropriate to the actual situation. It mediates input from higher order sensory and limbic areas, which describe and evaluate the situation in its relevant and irrelevant aspects, and motor output delivering the response appropriate to the actual situation. In order to be able to do this, the PFC subserves the functions of short term memory to maintain information relevant to the present task, and of working memory to manipulate this information. PFC is involved in learning processes, delivering flexible control over the connections between stimulus and response. Moreover, it is implicated in response selection, i.e. choosing of an adequate response with regard to the environmental conditions. For choosing a correct response, context processing is indispensable, meaning that PFC has to represent and consider all relevant information in the environment. Furthermore, PFC is involved in response inhibition, i.e. in suppression of inappropriate responses.
Thus short term memory, working memory, associative learning, response selection, context processing and response inhibition are prerequisites of a functional PFC that altogether enable flexible adaptation of the organism to changing environmental conditions.

NMDA receptors in various brain areas have been demonstrated to play a prominent role for learning and memory processes. As voltage- and ligand-dependent ion channels that open only if the presynaptic and postsynaptic neurons are activated simultaneously, their activation is a crucial step in the induction of long term potentiation (LTP) (Collingridge et al., 1983), which facilitates synaptic transmission and is thus considered the neural correlate of learning. A role for NMDA receptors in working memory is also discussed in neurocomputational studies. The mammalian brain possesses high densities of NMDA receptors, they are also abundant in PFC. As already demonstrated by several studies, they are presumably also involved in functions performed by PFC.

The Nidopallium caudolaterale (NCL) is an associative area in the avian forebrain which is considered functionally equivalent to PFC in mammals based on neuroanatomical, behavioral, electrophysiological and microdialysis data. NMDA receptors are also found abundantly in NCL (Bock et al., 1997).

A pilot study using blockade of NMDA receptors in the pigeon NCL (Lissek et al., 2002) demonstrated significant impairments in learning of a color reversal task. Due to the perseverative responding to the previously rewarded stimulus exhibited by the experimental group, the antagonist-treated animals needed significantly more trials than controls to learn the reversal. This deficit was particularly prominent during the first 2 reversal sessions, but performance differences remained also during the following 4 reversal sessions. The precise cause for the perseveration could not be detected in the scope of this task. Possible causes are deficits in extinction (refraining from responding to a no longer rewarded stimulus), impaired short term memory (no memory for the previous result of one's own actions), impaired response inhibition (of a response detected as being inappropriate), deficits in response selection (from a repertoire of potentially correct responses) or a lack in context processing (insufficient consideration of the relevant information available in the task situation). Therefore, in this thesis, these prefrontal functions which might have contributed to the perseverative impairment shall be investigated separately in individual behavioral experiments using local blockade of NMDA receptors in the NCL.

Experiment 1 (chapter 2) investigates the role of NMDA receptors in NCL for extinction learning and response inhibition.
Experiment 2 (chapter 3) deals with the possible functions of NCL-based NMDA receptors for short term memory and response selection.
Experiment 3 (chapter 4) studies the role of NMDA receptors in NCL for response selection and context processing.

In the following sections, evidence for the abovementioned PFC-based functions, the role of NMDA receptors within the brain in general and within PFC in particular, as well as data on the NCL will be discussed in more detail. Afterwards, a rationale and an overview of the experiments conducted in the scope of this thesis will be presented.

1.1 Anatomy of the prefrontal cortex

The human frontal lobes occupy almost a third of the cortical area in the human cerebral hemispheres. They can be subdivided into three main areas: motor-premotor areas (BA 4, 6, 8 and 44 according to the Brodmann nomenclature, 1909), paralimbic areas (BA 12, 24,

25 and 32 located on the medial surface of the hemispheres), and heteromodal association cortex (BA 8, 9, 10, 11, 12, 32, 45, 46 and 47). The term prefrontal cortex refers almost exclusively to the paralimbic and heteromodal components of the frontal lobe (Mesulam, 2002). In humans, prefrontal cortex thus comprises all neocortical areas of the frontal lobe rostral to the premotor cortex up to the frontal pole. The heteromodal component is characterized by an isocortical architecture with high neuronal density, organized in six layers, while the paralimbic areas are characterized by a gradual transition from allocortex to isocortex, tending to have a lower neuronal density and less than six layers. The heteromodal component is commonly further subdivided into two main areas termed dorsolateral PFC (dlPFC) (BA 8, 9, 10, 44, 45, 46) and orbitofrontal cortex (OFC) (BA 11, 12 and 47) (Birbaumer, 1996). However, some researchers use a further distinction between dorsolateral PFC and ventrolateral PFC (vlPFC), the latter comprising the area of the inferior frontal gyrus and corresponding loosely to BA areas 44, 45 and 47 (Fletcher & Henson, 2001), while the former is assumed to correspond to BA 9 and 46.

The PFC receives projections of the N. mediodorsalis of the thalamus (MD) (Rose & Woolsey, 1948; Divac et al., 1978), and from other thalamic nuclei and direct subcortical and limbic afferents from the pons, the tegmentum, the hypothalamus, and the amygdala (reviews in Fuster, 1989; Groenewegen, 1990). Moreover, there are afferents from hippocampus, cingulate cortex, and from adjacent areas of the limbic cortex (Goldman-Rakic et al., 1984; Pandya & Yeterian, 1990; Condé et al., 1995). The PFC further maintains connections with sensory and para-sensory association areas processing visual, auditory and somatosensory input in parietal and temporal cortex, respectively (Jones & Powell, 1970; Pandya & Yeterian, 1990) and with premotor, supplementary motor and motor cortex.

In nonhuman primates, PFC is subdivided into dorsolateral PFC (Walker's areas 8, 9, 10, 45, 46; Walker, 1940) and orbitofrontal / ventromedial PFC (Walker's areas 11, 12, 13).

In rats, PFC is assumed to be subdivided into orbitofrontal PFC and medial PFC, and it was proposed that medial PFC, in particular the prelimbic/infralimbic areas, could be functionally analogous to the dorsolateral PFC in primates (Rose & Wolsey, 1948; Akert, 1964). In contrast, it has been argued that rats may not possess a region homologous to dorsolateral PFC in primates (Preuss, 1995), and that instead medial PFC in rats might be homologous to orbitofrontal and medial PFC in primates (Uylings & van Eden, 1990; Preuss, 1995), thus dorsolateral PFC might be unique to primates.

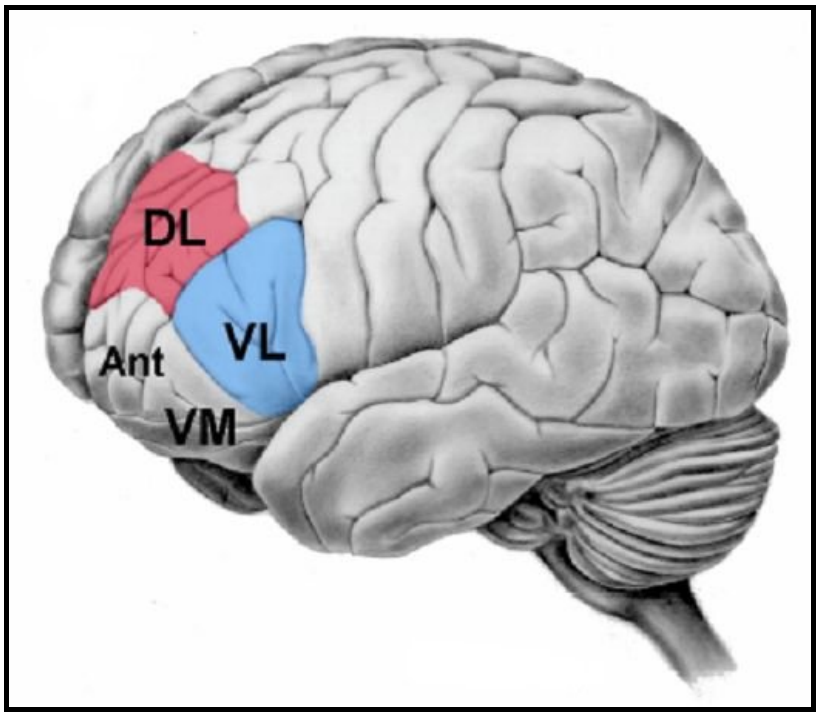

Fig. 1: The human PFC (dorsolateral and ventrolateral areas colored)

1.2 Pathophysiology of the frontal cortex / PFC in humans

Frontal lobe syndrome

The term frontal lobe syndrome refers to a set of symptoms most commonly encountered after prefrontal lesions in humans. Although the syndrome is mostly considered a unitary entity, in fact frontal patients can exhibit various patterns of deficits, allowing to identify two canonical subtypes. One is the frontal abulic syndrome, characterized by a loss of initiative and concentration, a propensity for apathy and lack of emotion. The second subtype, which may be termed the frontal disinhibition syndrome, displays a lack of judgment, insight and foresight, and deficits in learning from experience (Mesulam, 2002). The umbrella term ‘executive functions’ is widely used to describe functions subserved by human frontal cortex, comprising attentional control (selective and sustained attention, response inhibition), goal setting (initiating, planning, problem solving, strategic behavior), and cognitive flexibility (working memory, shifts in attention, self-monitoring and –regulation). Accordingly, executive dysfunction exhibited by frontal patients is reflected by poor planning and organization, difficulties in generating and implementing strategies,

perseveration and inability to correct errors or use feedback, and rigid or concrete thought processes (Stuss & Benson, 1986).

Thus neuropsychological tests of frontal patients reveal impairments in response inhibition (Perret, 1974), associative learning (Petrides, 1991; Petrides & Milner, 1982) and working / short term memory (Ferreira et al., 1998). Patients exhibit perseverative behavior in tests requiring flexible adaptation of responding, such as the Wisconsin Card Sorting Test (Milner, 1964), do not adhere to rules and are exceedingly prepared to take risks. This latter fact has been extensively studied in a line of research proposing that a considerable portion of the deficits typically observed in frontal patients with ventromedial prefrontal cortex (vmPFC) damage might be due to impaired processing of emotions to guide decision making, a proposal called the somatic marker hypothesis (Damasio, 1994, 1996, 1999). Tests using a specially developed gambling task demonstrated that patients with vmPFC damage, in contrast to controls, did not learn to choose from the advantageous card decks, which yielded lower immediate reward, but a higher overall reward. This deficit was accompanied by a failure to generate anticipatory skin conductance responses to the disadvantageous card decks (Bechara et al., 1994, 2000). These results are assumed to explain some of the real-life problems in decision making of frontal patients, which are sometimes not revealed by standard neuropsychological tests. A further test reflecting everyday requirements, in which frontal patients typically show deficits (Shallice & Burgess, 1991), is the Multiple Errands Test (ME). Typical errors include: ineffective planning, infringement of rules, misunderstandings regarding instructions, errands were not or insufficiently carried out. Together, these typical errors reflect the deficit of frontal patients in planning and strategic thinking.

Many symptoms found in frontal patients are also typical for patients suffering from psychiatric or neurological disorders, particularly from schizophrenia or Parkinson's disease. For each of these disorders there is evidence of hypofunction of the frontal lobes.

Schizophrenia

The severe mental disorder of schizophrenia is characterized by two sets of symptoms: positive symptoms including disordered thoughts, hallucinations, delusions, and negative symptoms including lack of affect, social withdrawal and reduced motivation. (Carlson, 1998). Evidence suggests that these two sets of symptoms arise from different malfunctions. While positive symptoms appear to involve excessive dopaminergic activity, especially in the PFC, negative symptoms seem to be related to brain damage.

The original dopamine hypothesis of schizophrenia assumed that the symptoms of schizophrenia were caused by hyperactivity of the dopaminergic system (Snyder, 1976). Later on, this hypothesis was reconceptualized and extended by Davis et al. (1991), stating that in schizophrenic patients functional DA activity was higher only in the mesolimbic system, which projects from the ventral tegmental area (VTA) to nucleus accumbens and amygdala, while functional DA activity in the mesocortical system, projecting from VTA to neocortical areas, was lower. The authors hypothesize that schizophrenia is characterized by abnormally low prefrontal DA activity (causing the negative symptoms) leading to excessive DA activity in mesolimbic DA neurons (causing the positive symptoms).

There is a lot of evidence indicating that schizophrenic patients exhibit decreased activity in PFC in tasks challenging prefrontal functions, in contrast to the increased PFC activity found in normal subjects (Taylor, 1996). These deficits in prefrontal function, so-called "hypofrontality" or prefrontal hypometabolism, were proposed to be primarily caused by subcortical abnormalities that reduce dopaminergic input to the PFC (Weinberger et al., 1988). However, hypofrontality itself might cause excitation of the VTA dopaminergic system. Excitatory glutamatergic neurons in PFC send efferents to the VTA where they synapse on DA neurons projecting to NAc (Sesack & Pickel, 1992). In particular, AMPA and NMDA receptors in the PFC are assumed to regulate DA release in the VTA (Takahata & Moghaddam, 1998) and thus in turn influence DA efflux in PFC. Normally, PFC-based NMDA receptors are assumed to provide tonic inhibitory control on DA release in PFC (Takahata & Moghaddam, 1998). In schizophrenia, however, NMDA receptor hypofunction in PFC might be related to the pathological processes/cognitive deficits in combination with DA system dysfunctions. In the NMDA receptor hypofunction (NRH) hypothesis of schizophrenia, a unified hypothesis formulated by Olney & Farber (1995) which considers both NMDA and DA contributions, NMDA receptor hypofunction is considered the key mechanism which may account for major clinical and pathophysiological aspects of the disorder, and which can supposedly explain both positive and negative symptoms.

Parkinson's Disease

Even in early stages of the disease, the motor disturbances in Parkinson's disease (PD) are accompanied by intellectual impairments (Taylor et al., 1986; Owen et al., 1992), and research suggests that frontal lobe dysfunction may underlie these deficits (Gotham et al.,

1988; Owen et al., 1992, 1993, 1995). In Parkinson patients, deficits in cognitive planning and spatial working memory are often associated with prefrontal damage (Morris et al., 1988; Owen et al., 1992, 1993, 1996; Postle et al., 1997), but they may be exarcerbated by depressed mood in early Parkinson's disease (Uekermann et al., 2003). However, dopamine depletion, the key feature of PD, is more prominent in the striatum than in the frontal cortex (Agid et al., 1987, Kish et al., 1988), thus it is considered unlikely that the 'frontal' deficits found in PD are caused by frontal damage alone. Imaging studies demonstrated that during cognitive planning and spatial working memory tasks, regional cerebral blood flow in PFC of PD patients is normal, however, it is abnormal in the basal ganglia (Owen et al., 1996, Dagher et al., 2001). Thus, impaired performance might be due to interruption of frontostriatal circuitry (Alexander et al., 1986; Owen et al., 1992; Zgaljardic et al., 2003; Lewis et al., 2003).

Attention Deficit Hyperactivity Disorder (ADHD)

The clinical symptoms of ADHD comprise inattentiveness, distractability, hyperactivity and impulsivity. Moreover, ADHD patients exhibit deficits in working memory and response inhibition (Levy & Swanson, 2001).

It is assumed that the disease is associated with dysfunction of the PFC (Rubia et al., 1999; Benson, 1991) respectively with damages to the frontostriatal circuitry (Bradshaw & Sheppard, 2000; Casey et al., 1997) causing deficits in the inhibitory mechanisms mediated by PFC. Especially right dlPFC is assumed to have a role in response inhibition (Casey et al., 1997), although an involvement of orbitofrontal cortex has also been suggested (Itami & Uno, 2002). A morphological correlate of the disorder is reduced size of prefrontal brain regions (Sowell et al., 2003; Hill et al., 2003) affecting both white and gray matter (Mostofsky et al., 2002). Clinical and experimental evidence suggests an dysfunction of DA systems, mainly in the mesocortical /mesocorticolimbic DA system, to be involved in ADHD. This dopamine theory of ADHD is supported by genetic, neuroimaging, and stimulant medication studies, confirming an inhibitory dopaminergic effect at striatal/prefrontal level (for a review, see Levy & Swanson, 2001) which is decreased in ADHD patients (Russell, 2002).

1.3 Functional organization of PFC

In spite of the extensive research on (human) PFC, there is no consensus on its functional organization, especially with regard to its role in working / short term memory (Goldman-Rakic, 2000; Miller, 2000; Duncan & Owen, 2000). There is evidence that PFC is organized with respect to processing distinctions such as maintenance (ventral PFC) versus manipulation (dorsal PFC) (D'Esposito et al., 1998; Smith & Jonides, 1999; Haxby et al., 2000; Curtis et al., 2000) or maintenance / retrieval (ventral) versus monitoring (middorsal) (Petrides, 2000; Kessels et al., 2000). Another distinction posits differential involvement of the left and right hemispheres of PFC in the functions of encoding (left PFC) and retrieval (right PFC) (Tulving et al., 1994). On the other hand, there is evidence that PFC is organized according to distinctions between materials or information domains, i.e. spatial (dorsal) versus nonspatial (ventral) PFC (Goldman-Rakic 1987, 1995; Levy & Goldman-Rakic, 2000) or verbal (left) versus non-verbal (right) PFC (Smith & Jonides, 1997, Kelley et al., 1998; Raye et al., 2000). Moreover, some findings suggested that PFC may not exhibit such specificity (Nystrom et al., 2000). It is thus possible that PFC is organized according to criteria that do not fall into these relatively global distinctions. One reason for the lack of consensus about the functional organization of PFC could be that the typical tasks used for assessing its short term memory functions are rather complex, requiring multiple processes. In imaging studies, it is therefore difficult to determine the degree to which PFC activity reflects the type of information operated upon rather than differences in the processing strategies used for different materials.

An alternative idea for the functional organization of frontal cortex does not predominantly emphasize working memory functions, but instead coined the term "working with memory" as the predominant function of frontal cortex (for a review, see Moscovitch & Winocur, 2002), meaning that strategic contributions of frontal cortex refer to working and short-term memory as well as to long-term memory. The model proposes that frontal cortex uses established memories to direct other activities, such as new learning, problem solving and behavioral planning. Regions of the frontal cortex are assumed to strategically operate upon medial temporal lobe information encoding and retrieval, by organizing input at encoding, initiating and directing search at retrieval, and monitoring and verifying information to check whether they fit with the goals of tasks and to place them in proper temporal-spatial context. In this framework, dorsolateral PFC is involved in monitoring and evaluation of information under uncertainty, while the function of ventrolateral PFC is

cue specification and/or maintenance at retrieval and encoding, and premotor cortex is likely involved in memory-based response selection and inhibition.
Thus, although ideas regarding its functional organization vary, there is broad evidence regarding the general involvement of PFC in the functions described in more detail in the following sections.

1.4 PFC and associative / extinction learning

By associative learning, previously independent external stimuli and responses become associated, by extinction this association is abolished, respectively a different association is superimposed upon it. Associative learning and its complement, extinction, are basic functions enabling organisms to adapt their behavior to altered environmental conditions. Due to its connectivity with posterior cortical and subcortical areas PFC is a promising candidate for the wiring of such associations. Especially ventral PFC is assumed to have a role in associative learning, since PET and fMRI demonstrates learning-related increases when subjects learn visual associations (Passingham et al., 2000). A number of animal lesion studies too demonstrates involvement of PFC in such learning processes for ventral (see Passingham et al., 2000), and dorsolateral PFC (Petrides, 1982 etc.). Behavioral studies using electrolytic lesions show mPFC lesions in rats to cause impairments in learning for example maze tasks (Joel et al., 1997a; Fritts et al., 1998, Winocur & Moscovitch, 1990) or in reversal learning while acquiring a Skinner box analogue of the WCST (Joel et al., 1997b).
Excitotoxic lesions (for example by NMDA or glutmate), have the advantage over electrolytic lesions to leave fibers of passage intact. Such excitotoxic lesions of mPFC or oPFC in rats were found to impair reversal learning of S-R associations for odors (Ferry et al., 2000) due to perseveration on the previously rewarded stimulus, to lead to deficits in reversal of associating stimuli with different reward magnitudes (Bohn et al., 2003), and to slow reversal learning in the water maze (Lacroix et al., 2002). Unimpaired by these lesions, however, was acquisition of an S-R association (Ferry et al., 2000, Bohn et al., 2003), of a two-way active avoidance (Lacroix et al., 1998) , of a visual discrimination task (Chudasama & Robbins, 2993), and of a spatial Y-maze reference memory task (Deacon et al., 2003). Further unimpaired is recall of a previously acquired association (Bohn et al.,

2003). Thus, excitotoxic prefrontal lesions in general result in impaired reversal learning, while leaving acquisition and performance intact.

Human frontal patients were found impaired in a variety of learning tasks associated with prefrontal functions: they exhibit deficits in sequence learning in visuomotor tasks (Gomez Beldarrain et al., 2002), in learning of go-no go tasks (Drewe, 1975), but also in encoding during word list learning (Stuss et al., 2004). Moreover, frontal patients experience significant deficits in acquiring the conditional association between two different stimuli (Petrides, 1982b) or in learning the WCST (Milner, 1964). They are also severy impaired in paired word-associate learning using the AB-AC paradigm, in which one cue word associated with a target word in the AB condition has to be associated with a different target word in the AC condition, a task requiring to ignore the first association and learn a new one (Shimamura et al., 1995). Taken together, the learning deficits in frontally lesioned subjects are often related to an inability to alter previously established associations, however, acquisition of some types of task can also be impaired, which might be due to deficits in short term memory, response inhibition or response selection.

1.5 PFC and short term / working memory

Lesion and imaging studies show the participation of PFC in working memory and short term memory. The term working memory (WM) is generally used to refer to the ability to maintain information on-line, often in the service of a particular goal or task. In research on humans, a distinction betweeen working memory and short term memory (STM) is clearly stated by defining the term short term memory as comprising mere maintenance of stimuli, whereas working memory means additional manipulation of stimuli (such as placing stimuli in a different order, by arithmetics performed on numerical stimuli, etc.). In animal research literature, however, these terms are often used synonomously, thus in many cases the term working memory is employed even when the task requires only maintenance of stimuli.

Early behavioral evidence for implication of PFC in working memory stems from an experiment by Jacobsen (1936), who found that PFC lesions in monkeys caused deficits in delayed responding. Many results on PFC involvement in WM come from lesion studies in rats, demonstrating impairments of spatial and non-spatial WM after mPFC lesions

(Kesner et al., 1987; Freeman & Stanton, 1992) particularly after prelimbic/infralimbic lesions (Ragozzino et al., 1998; Delatour & Gisquet-Verrier, 1996, 1999) while other research did not find medial PFC specifically involved in WM (Delatour & Gisquet-Verrier, 2000).

On the other hand, medial PFC lesions in rats were found to cause delay-independent impairments in a DNMTS task (Porter et al., 2000). Further experiments in which excitotoxic NMDA lesions were placed in the dorsal mPFC of rats also demonstrated delay-independent increased perseveration in a delayed alternation task in a t-maze. (Sanchez-Santed et al., 1997) respectively impairments in WM for egocentric responses in a DMTS task (Ragozzino & Kesner, 2001), indicating that not only WM, but other functions might have been compromised. Thus, while there is converging evidence on general PFC involvement in working and short term memory, in some tasks additional prefrontal functions required for performance might contribute to observed deficits, therefore they cannot be exclusively attributed to impaired maintenance of stimuli on-line.

Human research on working memory is done mainly with frontal patients or by imaging studies evaluating brain activation in healthy subjects. Large frontal lesions involving both ventral and dorsal lateral PFC areas were found to impair both maintenance and monitoring of object and spatial information (Muller et al., 2002), while lesions of only one or the other area had no effect. Frontal patients were found impaired in delayed responding and delayed alternation tasks due to a tendency to persevere on previous behavior (Verin et al., 1993).

A comprehensive meta-analysis (D'Esposito & Postle, 1999) of performance of frontal patients in task requiring STM or WM did not find impairments in tasks requiring mere maintenance, such as span tasks. Deficits, however, occurred as soon as delayed responding to stimuli was required. Moreover, in frontal patients, impairments often occur only in so-called self-ordered WM tasks (Owen et al., 1990; Petrides & Milner, 1982), a type of task in which the subjects are free to choose a sequence of responding to stimuli presented, the only instruction being not to respond to the same stimulus twice. Findings in human patients were corroborated by replication studies using such self-ordered WM tasks with PFC-lesioned monkeys (Petrides, 1991, 1995; Passingham, 1985). Further converging evidence comes from an fMRI study in normal subjects, reporting dlPFC activation during delays only for tasks requiring the preparation of a response, but not for mere maintenance of a visuospatial stimulus in STM (Pochon et al., 2001). It is conceivable that damages to

PFC induce deficits in WM / STM particularly in such instances in which remembering or preparation of one's own actions is required.
Imaging studies consistently find activation in dorsolateral and ventrolateral PFC during working memory, however, there are different opinions regarding the functions subserved by these areas (see chapter 1.3). Neuroimaging provides evidence of a differentiation with regard to processes, with ventrolateral PFC activation during maintenance of verbal, object and spatial material, while dorsolateral PFC is often found engaged only in manipulation processes, with a tendency towards a hemispheric left – right distinction for verbal/object and spatial information, respectively, in both areas (for a review, see Fletcher & Henson, 2001).

Electrophysiological evidence for participation of PFC in maintaining information on-line in working memory comes from single-cell recordings of neurons in PFC of monkeys and rats exhibiting delay-related activity. This delay activity has been demonstrated to be content- or object-specific in that individual neurons code the spatial location of an object (Funahashi et al., 1989; Rao et al., 1997), or the identity of an object (Quintana et al., 1988; Rao et al., 1997; Rainer et al., 1998). The activity does not depend on mere physical properties of the stimuli, but on their behavioral significance (Yamatani, 1990), also across different sensory modalities (Watanabe, 1996). However, neuronal activity in PFC is not restricted to maintaining stimulus information during the delay period of working / short term memory tasks, as will be outlined in the following sections.

1.6 PFC and response inhibition

Inhibition of a prepotent or inadequate response is another function in which PFC is implicated. In go/no-go tasks, where responding to one stimulus is correct, while responding to a different stimulus must be inhibited, or in stop-signal tasks, that require inhibition of a response that has already been initiated, a deficit in response inhibition can become particularly prominent. Ample evidence from imaging, electrophysiological and lesion studies shows involvement of PFC in response inhibition.
Event-related fMRI studies in humans demonstrate that brain activation related to the response inhibition component in no-go tasks and stop-signal tasks is located in ventrolateral and dorsolateral PFC (Liddle et al., 2001), particularly in right PFC (Garavan

et al., 2002; Rubia et al., 2003, Hazeltine et al., 2000, Konishi et al., 1998). These results are corroborated by a PET study comparing activation in response inhibition to response selection (Kawashima et al., 1996). This work reports increased activation of right PFC in response inhibition relative to response selection, indicating that areas mediating these two functions do not entirely overlap. Inhibition of responses to salient, but irrelevant events also is mediated by right PFC, as a study with ADHD patients and normals controls suggests (Casey et al., 1997). Another event-related fMRI study reports right dlPFC involvement in response inhibition only for go/no-go tasks containing working memory load (Mostofsky et al., 2003). A context effect for response inhibition was reported by a study which found comparatively more activation in ventral PFC, cingulate and superior parietal cortex in no-go trials preceded by a large number of go-trials relative to less preceding go-trials (Durston et al., 2002). A further area generally found activated during response inhibition in go/no-go tasks is parietal cortex (Garavan et al., 2002, Durston et al., 2002, Watanabe et al., 2002).

Electrophysiological multiple unit recordings revealed differences between PFC activity in go and no-go trials in the rat (Sakurai & Sugimoto, 1986), single unit recordings found no-go activity in monkey dorsolateral PFC related to response inhibition (Iwabuchi & Kubota, 1998), as well as different types exhibiting activity selectively in go, no-go or both trial types (Watanabe, 1986).

PFC lesions too cause deficits in the inhibition of inadequate responses. Lesions of mPFC in two different rat strains impaired response inhibition in a visual timing task (Broersen & Uylings, 1999). In particular prelimbic PFC lesions in rats also impaired acquisition of a go/no-go task (Delatour & Gisquet-Verrier, 1996). OFC lesions in rats caused deficits in reversal, but not in acquisition of a go/no-go odor discrimination task (Schoenbaum et al., 2002), although OFC has been implicated in go-nogo performance in monkeys (Iversen & Mishkin, 1970). Temporary inactivations of the dorsolateral PFC (Oishi et al., 1995) and local blockade of alpha-2-adrenoceptors in PFC (Ma et al., 2003) in monkeys impaired performance and response inhibition in a go/no-go task. On the other hand, lesions of mPFC in rats did not impair response inhibition in a stop-signal task (Eagle & Robbins, 2003). Frontal patients with lateral PFC lesions also exhibit inhibitory deficits in cognitive tasks requiring suppression of previously learned material (Shimamura et al., 1995, Mangels et al, 1996).

1.7 PFC and response selection

PFC is also involved in selection of an adequate response in the absence of impaired response inhibition, as demonstrated by a variety of studies in animals and frontal patients. However, there is some disagreement in human and animal studies with regard to the specific prefrontal region involved. Animal lesion studies in rats and monkeys propose participation of ventrolateral PFC (Petrides, 1982, 1987, Winocur & Eskes, 1998), but not medial PFC (Delatour & Gisquet-Verrier, 1999) in response selection. FMRI and rTMS studies with human participants, however, rather demonstrate involvement of dlPFC in response selection: Response selection in humans is assumed to activate dorsal PFC and superior parietal cortices, but not ventral PFC (Schumacher & D'Esposito, 2002). DlPFC was found, together with mPFC, required for response selection even without working memory load (Hadland et al, 2001). Another distinction was made between response selection to spatial vs. non-spatial stimuli: spatial response selection activated right PFC and right parietal regions, whereas non-spatial response selection activated left PFC and left parietal areas (Schumacher et al., 2003).
Electrophysiological studies show neuronal activity in PFC of monkeys specifically related to particular response-reward combinations (Matsumoto et al., 2003), a prefrontal activity likely to underlie goal-based response selection. In a go/no-go task featuring two distinct task conditions on which responses were to be based, e.g. either "color" or "motion", it was found that neurons in the PFC of monkeys that code for a stimulus feature, e.g. "color", which is relevant in one task type, and therefore can discriminate between go and no-go trials in this task, still show activity in the other task, where this stimulus is irrelevant since the correct response has to be chosen according to "motion". These results indicate that relevant and irrelevant S-R features run in parallel up to the stage of response selection (Lauwereyns et al., 2001).

The term response selection can refer to a selection of behavior from an array of previously learned, unchanging S-R associations, but also to the selection of an appropriate response from several competing responses. An event-related fMRI study with healthy human participants showed a double dissociation of brain areas involved in response selection on the basis of previously learned S-R associations and in response selection from competing responses. The former type was found to involve parietal cortex, while only the latter activated lateral PFC (Bunge et al., 2002).

Competing responses are present if for example both responses are on principle reinforced, however, a further stimulus indicating the correct response for a given trial has to be observed in order to enable correct responding. Conditional association tasks are a typical example for this requirement. Noncompeting responses, on the other hand, are responses which are unequivocally associated with a certain stimulus, such as in go/no-go tasks.
It is conceivable that in case of competing responses the consideration of the actual context of a situation, and thus possibly the role of PFC, is more important than in case of a selection from an array of responses which are each unequivocally associated with only one stimulus.

1.8 PFC and context processing

Therefore, PFC has been implicated also in a function termed context processing, meaning the processing of information actively held in mind in such a form that it can be used to mediate task appropriate behavior (Cohen et al., 1999). Such information may contain specific prior stimuli, results of processing a sequence of prior stimuli, or task instructions. Context processing is supposed to be distinguishable from short-term memory since the latter refers to processes involved in the temporary storage of recently presented information, which may or may not have relevance for later behavior (Cohen & Servan-Schreiber, 1992). Context processing, however, refers to information that always has relevance for later behavior, although context representations may or may not correspond to the identity of previously presented information (Cohen et al., 1999).
In schizophrenia patients and healthy older adults, impairments of context processing are found which might be attributable to PFC hypofunction and malfunctions of the DA system (Braver et al., 2001 ; Barch et al., 2001, 2003; Cohen et al., 1999). Volunteers had to perform an AX continuous performance task (CPT), in which a sequence of letters is presented and responding to the letter X is required, but only in case it was preceded by the letter A. Possible error types are: AY-errors, meaning premature responding to a Y because it was preceded by an A, since AX combinations have a much higher frequency than AY combinations. BX-errors, on the other hand, mean responding to an X even though it was not preceded by an A, but some other letter. AY-errors and BX-errors thus can be considered context-induced and context-failure errors, respectively. In contrast to healthy young adults, elderly people and schizophrenia patients tend to make more BX-errors than AY-errors (Barch et al., 2001a, b; Braver et al., 2001, Javitt et al., 2000), indicating that

they respond to the salient X stimulus and disregard the preceding context, in spite of having learned the rules of the task.

The corresponding connectionist model of PFC function (Cohen & Servan-Schreiber, 1992, Braver et al., 1995) assumes that context processing is a basic function of PFC which, by mediating both storage and control, subserves several functions that are often considered to be independent of each other, such as active memory, attention and inhibition. It is proposed that errors in tasks challenging these functions can be explained altogether from the malfunction of this context processing module, which is assumed to be located in dorsolateral PFC.

Apart from this line of research, there are further studies implicating PFC in context processing. An ERP study found context retrieval associated with PFC function - in contrast to item retrieval, which appeared predominantly associated with medial temporal lobe function (Graham & Cabeza, 2001). Animal studies too show a participation of PFC in context processing: Ventrolateral PFC lesions impair processing of contextual information in fear conditioning and extinction (Morgan & LeDoux, 1999). Neurons in PFC of monkeys were found to code differentially for specific cues (Ito et al., 2001) both during presentation and choice phases (Hoshi et al., 2000), for motivational context information about the type of reward (Leon & Shadlen, 1999; Watanabe et al., 2002), selectively only for stimulus-reward (Kobayashi et al., 2002), or specific action-reward combinations (Funahashi & Takeda, 2002; Matsumoto et al., 2003), or for the incentive value of the reward, when it is relevant to performance (Schoenbaum & Setlow, 2001). Moreover, activity of individual PFC neurons in monkeys was found to represent both the cues and the associated responses, demonstrating a role for PFC in learning arbitrary cue-response associations (Asaad et al., 1998). Thus, neuronal activity in PFC appears to pertain not only to mere maintenance of information about a stimulus, but rather represents a variety of additional information, presumably enabling PFC to represent the complete set of context variables relevant to a task, ranging from cue-response associations to response-reward associations.

1.9 Properties of the NMDA receptor

The N-methyl-D-aspartate (NMDA) receptor is a specialized ionotropic glutamate receptor controlling a calcium channel that is normally blocked by magnesium (Mg2+) ions. It is

named after the articificial ligand that was found to activate it. NMDA receptors contain – at least - six different binding sites with partially opposing effects upon receptor activation. Four sites are located on the exterior of the receptor: there is a binding site for glutamate as the primary endogenous excitatory agonist, a binding site for glycine which acts as an obligatory co-agonist and is normally present in the extracellular fluid of the brain, a binding site for polyamine with facilitatory effects and a binding site for zinc (Zn2+) which has inhibitory effects. Within the ion channel, there are two more binding sites: for Mg2+ and phencyclidine (PCP). PCP acts as an inverse agonist: when it is attached to its binding sites, Ca2+ ions cannot pass through the ion channel. The natural ligand of the PCP site and its functions are not yet known.

The NMDA receptor is considered a voltage- and ligand-gated ion channel, since for channel opening, both glutamate and glycine must be attached to their binding sites. Moreover, the magnesium ion must be displaced from its binding site, which happens if the postsynaptic membrane is depolarized, presumably by action of co-localized AMPA receptors. When it is open, the ion channel controlled by the NMDA receptor permits entrance of sodium and calcium ions into the cell, which causes further depolarization and initiates a cascade of calcium-dependent processes (Carlson, 1998).

NMDA receptors are found in most areas of cerebral cortex (of humans, monkeys), high densities occur in hippocampus (Bockers et al., 1994), especially in field CA1, moreover in inferotemporal and prefrontal cortex of humans and monkeys (Huntley et al., 1997, Bockers et al., 1994) and rats (Takita et al., 1997).

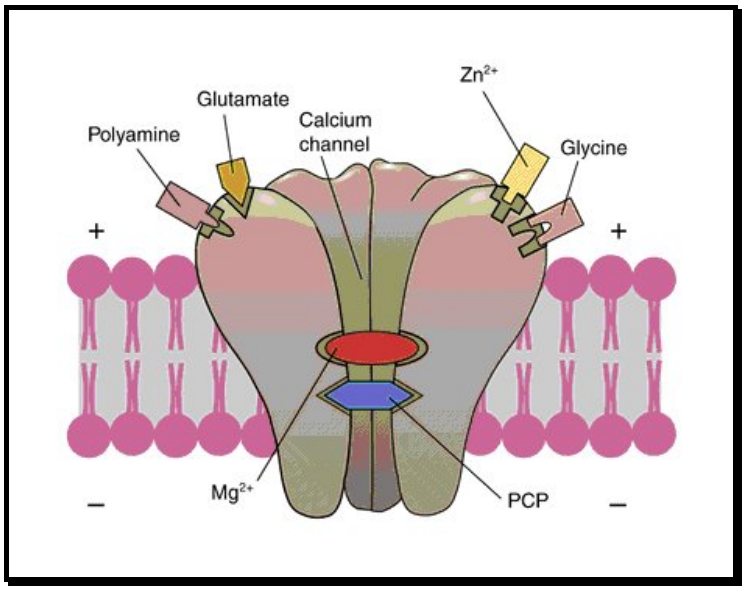

Fig. 2: Schematic illustration of the NMDA receptor with its binding sites

Subtypes of NMDA receptors

The current model of the composition of NMDA receptors suggests that these receptors assemble from two subunits, the NR1 subunit (Moriyoshi et al., 1991), of which seven subtypes (NR1A to 1G) were identified (Sugihara et al., 1992), and an NR 2 subunit of which four types (NR2A to 2D) have been characterized (Ikeda et al, 1992; Ishii et al., 1993). Recently, a third subunit termed NR3 (with two subtypes NR3A-B) has been described (Das et al., 1998; Eriksson et al., 2002; Nishi et al., 2001). The NR1 subunit is the principal constituent of the NMDA receptor, being expressed at substantial levels in virtually all central neurons (Moriyoshi et al., 1991). The NR2 subtypes (NR2A to NR2D) are highly related to each other, but only distantly related to the NR1 subunit. Some of the properties of NMDA receptors differ depending on which of the four modulatory NR2 subunits assembles with the principal NR1 subunit. (Seeburg et al., 1994). The various subunits are differentially distributed in the CNS (Goebel & Poosch, 1999). The NR1 subunits are distributed ubiquitary in the CNS, with a particular high density in cortex and hippocampus of adult mammals (Ozawa et al., 1998; Whiting & Priestly, 1998). The NR2C subunits are preferentially localized in spinal cord and cerebellum, while the NR2D subunits are rarest in the CNS of adults, localized mostly in brain stem and diencephalon (Mori & Mishina, 1995).

NMDA receptor antagonists

Due to the various binding sites present on the NMDA receptor, different types of antagonists can affect NMDA receptor functioning. Competitive NMDA antagonists attach to the glutamate or to the glycine binding site, and thus displace the endogenous ligand and prevent opening of the ion channel. Widely used competitive NMDA antagonists for the glutamate binding site are AP5, CPP, CPPene, LY 233053, for the glycine binding site: 7-chlorkynurenic acid, and (+)-HA-966. Non-competitive antagonists attach to a different binding site than that of the endogenous ligand. Widely used are non-competitive NMDA antagonists of the open channel blocker type, such as MK-801, PCP, ketamine and memantine. These antagonists act by blocking the already opened ion channel, meaning that - in contrast to other antagonists - an open channel blocker can only inactivate a previously activated NMDA receptor (Stark et al., 2000).

Antagonists can also differ regarding their sensitivity for the different NMDA receptor subtypes. While AP5, for example, has a similarly high affinity for all NR2 subtypes, CPP

tends to have a higher affinity for NR2A/B than for NR2C/D subtypes (Hrabetowa et al., 2000).

NMDA receptors and LTP

Due to their specific properties described above, NMDA receptors can detect simultaneous pre- and postsynaptic activity, therefore they may be considered the neuronal correlate of the Hebbian principle (Hebb, 1949) which describes that by simultaneous activation of pre- and postsynaptic neurons a synaptic connection can be strengthened. Research on the possible role of NMDA receptors for learning and memory began following the discovery by Collingridge et al. (1983) that NMDA receptor activation is a crucial step in the induction of associative long term potentiation (LTP) in the hippocampus, which had been described first by Bliss & Lomo in 1973. Since then, LTP has been demonstrated elsewhere in the brain, also in prefrontal cortex (Baranyi et al., 1991). For piriform and entorhinal cortex, for amygdala, and for PFC, NMDA receptor involvement in LTP has been shown (Clugnet & LeDoux, 1990; Lynch et al., 1991; Jay et al., 1995; Hirsch & Crepel, 1991). However, there are NMDA receptor-independent forms of LTP, for example in rat visual cortex (Aroniadou & Teyler, 1991), in mammalian hippocampus, field CA1 and CA3 (Cavus & Teyler, 1998; Stricker et al., 1999; Johnston et al., 1992), and also in the pigeon hippocampus (Wieraszko & Ball, 1993).

The induction of NMDA receptor-dependent LTP comprises structural alterations at the postsynaptic level, by increasing the number of AMPA receptors on the postsynaptic membrane (Tocco et al., 1992; Liao et al., 1995, Lu et al., 2001) and by formation of perforated synapses (Geinisman et al., 1991, 1996). It was suggested that the increase in AMPA receptors is caused by the development of perforated synapses (Edwards, 1995). Moreover, there are changes on the presynaptic level indicated by increased emission of glutamate from the presynaptic neuron. Both structural changes and presynaptic changes are supposedly induced by the intracellular increase of Ca2+ which activates calcium-dependent enzymes. These enzymes either participate in the structural changes (Ca2+/calmodulin-kinase, protein kinase C) or may act as retrograde messengers (nitric oxide synthase) causing increased glutamate release from the presynapse (Gustafsson & Wigström, 1988).

NMDA receptors appear to be implicated not only in induction of LTP, but might also be involved in decay of LTP which normally occurs within week after its induction, as

demonstrated by a study in which NMDA receptor antagonism by CPP blocked decay of perforant path-dentate LTP over a one-week period when administered daily. Moreover it blocked decay of the protein-synthesis dependent phase of LTP when administered two days after LTP induction (Villareal et al., 2002). The authors suggest that LTP is normally a persistent process that is actively reversed by NMDA receptor activation. Moreover, NMDA receptors can also participate in long term depression (LTD) in hippocampus and prefrontal cortex (Hirsch & Crepel, 1991), in a way that LTP and LTD presumably are induced by distinct subpopulations of NMDA receptors, as antagonists with higher affinity for NR2A/B than for NR2C/D subtypes demonstrated higher potency for inhibition of LTP than of LTD (Hrabetowa et al., 2000).

1.10 NMDA receptors (in PFC) and learning

The crucial role of NMDA receptors in various brain areas for learning in general has been demonstrated in a large number of studies investigating the function of NMDA receptors in different brain regions of mammals and birds. In mammals, research until recently focussed on nucleus accumbens, hippocampus, and the amygdala, and on the functions of spatial learning, fear conditioning and adaptive avoidance. In avian species, the involvement of NMDA receptors in imprinting, song learning and homing was predominantly investigated.

The seminal work of Morris et al. (1986, 1989) for the first time demonstrated that NMDA receptor antagonism by DL-AP5, supposedly acting on hippocampus, causes deficits in spatial learning of the platform location in a water maze, while leaving retention of previously acquired spatial information unaffected. Further studies (Butcher et al., 1990, Davis et al., 1992) proved that the NMDA receptor antagonist D-AP5 did not only disrupt spatial learning, but also the formation of long-term potentiation (LTP) in hippocampus in vivo. Since then, many studies confirmed these effects of NMDA receptor blockade upon spatial learning also by means of uncompetitive NMDA antagonists. Thus both AP5 and MK801, intracerebroventricularly applied, were found to impair acquisition, but not retention of spatial learning of mice in a Morris milk maze (Heale & Harley, 1990). Systemic MK-801 caused deficits in acquisition, but not retention of a radial maze task (Malenfant et al., 1991) and a water maze task (Kant et al., 1991).

Blockade of NMDA receptors in the amygdala impairs acquisition (Miserendino et al., 1990; Rodrigues et al., 2001; Lee & Kim, 1998) as well as extinction (Falls et al., 1992; Davis, 2002) of fear conditioning. In most cases, recall or expression of previously acquired fear conditioning remains unaffected by NMDA receptor antagonism (Campeau et al., 1992; Miserendino et al., 1990), but some studies also report deficits in expression of conditioned fear (Lee et al., 2001, Fendt, 2001). In avoidance learning, amygdaloid NMDA receptor antagonism yields a similar pattern of results: acquisition, but not performance of inhibitory avoidance learning is negatively affected (Roesler et al., 2000; Savonenko et al., 2003).

NMDA receptor antagonism in the mammalian Nucleus accumbens (NAc) leads to deficits in a multitude of learning tasks, possibly reflecting NAc involvement in the rewarding aspects of learning. NMDA receptor blockade was found to impair acquisition of Pavlovian conditioning (Di Ciano et al., 2001) and passive avoidance learning in rats (Martinez et al., 2002, Gargiulo et al., 1999), of spatial learning of a water maze task in mice (Sargolini et al., 2003), or a radial maze task in rats (Smith-Roe et al., 1999). Again, performance is not affected, neither in passive avoidance learning (Gargiulo et al., 1999) nor in spatial learning (Smith-Roe et al., 1999).

NMDA receptors in the chick neostriatum dorsocaudale (Ndc) (= NCL in pigeons) play a critical role during auditory and visual imprinting learning (Braun et al., 1999). Blockade in another region relevant to imprinting, the mediorostral neostriatum/hyperstriatum ventrale (MNH) too impaired imprinting (Bock et al., 1996). Systemic NMDA receptor blockade in homing pigeons disrupted navigational learning, but had no effect on associative learning in an operant chamber (Riters & Bingman, 1994). Another study comparing the effects of NMDA receptor blockade in homing and non-homing pigeon breeds found impairments in spatial reference memory only in nonhoming pigeons, while homing birds were unaffected (Meehan et al. 1996). In zebra finches, systemic NMDA antagonism during song model presentation impaired normal song development in adult animals (Aamodt et al., 1996).

There are not many studies exploring the effect of NMDA receptor antagonists upon human learning and memory. Healthy human volunteers treated with ketamine were impaired in episodic and procedural memory (Morgan et al., 2004), and in performance of the Wisconsin Card Sorting Test (WCST), where total errors increased due to a high amount of perseverative errors (Krystal et al., 2000). Moreover, differential effects of NMDA receptor antagonism on memory for object drawings and face photographs were

found in a study administering memantine to human subjects before learning, and testing recall 1,5 hours later: while object recognition was impaired, face recognition was not (Rammsayer, 2001). NMDA receptor blockade was found to cause deficits in acquisition, but not in recall of motor memories (Donchin et al., 2002), comparable to animal studies.
In summary, evidence from such studies on the role of NMDA receptors for learning points to an involvement of NMDA receptors in acquisition of associations between a stimulus and a response rather than in recall or performance of previously acquired associations.

In comparison, until now only very few studies investigated the participation of PFC-based NMDA receptors in learning by using local NMDA antagonism. They found that NMDA receptor inactivation in rat OFC impaired reversal learning of an association between stimuli and reward magnitudes (Bohn et al., 2003). NMDA receptor blockade in rat mPFC caused deficits in set-shifting in a maze task due to increased perseverative tendencies (Stefani et al., 2003) and deficits in acquisition of an instrumental lever-press task (Baldwin et al, 2002). These results in general are parelleled by those found in PFC lesion studies and further extend these findings by demonstrating the involvement of NMDA receptors in PFC-mediated behavioral flexibility.

1.11 NMDA receptors (in PFC) and working / short term memory

The involvement of NMDA receptors in working / short term memory is being investigated by two lines of research: behavioral animal studies and neurocomputational modelling.
A large number of studies investigated spatial working memory by systemically blocking NMDA receptors by means of noncompetitive NMDA antagonists, the evidence for their involvement is mixed: Deficits of spatial short term memory were found in mice and rats (Gutnikow & Rawlins, 1996; Shapiro & O‘Connor, 1992; Wilcott & Qu, 1990). Spatial delayed alternation was found to be impaired too by MK 801 (Verma & Moghaddam, 1996). Other researchers reported no impairment in short term memory in rats and primates performing a DMTP and a DMTS task, respectively (Popke et al., 2001; Ballard & McAllister, 2000). The competitive NMDA antagonist D-AP5 infused intraventricularly, presumably affecting primarily hippocampal function in rats, too had no effects on any

retention interval in a DMTS task (Lyford et al., 1993). A further experiment compared performance in the DMTP task following systemic injection of various NMDA receptor antagonists (MK 801, CPP, +HA966) in rats and found that each led to delay-independent performance impairments (Doyle et al., 1998). Another study, however, reported dissociations between competitive and non-competitive NMDA receptor antagonists upon performance in a DMTP task in rats (Cole et al., 1993), in which intraperitoneal infusions of CPP and MK 801 caused delay-dependent and delay-independent impairments, respectively. A further study also reported reduced accuracy of matching at all delays in the same task after MK 801 injections (Stephens & Cole, 1996).

Another study comparing the effects of D1 receptor blockade with those of NMDA receptor blockade in rats, using intraperitoneal injections of the antagonists, demonstrated dose- and delay-dependent impairments only for the D1 receptor antagonism, while NMDA receptor blockade led to impairments which were insensitive to the memory load, instead caused chance-level performance in all delays (Aultman & Moghaddam, 2001).

Studies with healthy human volunteers using systemic NMDA receptor blockade by ketamine report deficits in short term memory as measured by free recall and recognition memory (Malhotra et al., 1996), in working and episodic memory (Adler et al., 1998; Ahn et al., 2003; Morgan et al., 2004), and dose-dependent impairing effects upon performance in a spatial DMTS task with unimpaired attentional function (Newcomer et al., 1999). A dissociation between NMDA receptor antagonism effects upon maintenance and manipulation in working memory was found by Honey et al. (2003), who reported selective impairments for the manipulation component. A further study reports impairments in both immediate and late recall of verbal and non-verbal memory, but not of spatial memory after systemic NMDA receptor blockade with SDZ EAA 494 (Rockstroh et al., 1996).

Taken together, several, but not all, behavioral studies using systemic NMDA receptor antagonism in animals and humans report participation of NMDA receptors in short term memory, without, however, being able to unambiguously state which brain areas were responsible for the deficits. One single study using local blockade of NMDA receptors in the PFC of rats (Aura & Riekkinen, 1999) performing a DMTS task reported no decrease in the overall number of correct responses, but merely non-cognitive impairments, such as changes in response latency.

Recordings from PFC neurons in monkeys during a delayed visual discrimination demonstrated on the behavioral level that NMDA receptor blockade by AP5 reduced the

duration of short-term information retention and increased the delay before the motor response was made. Significant desynchronization in the activity of the groups of neurons studied accompanied these deficits (Dudkin et al., 1997).
Thus evidence from behavioral and electrophysiological studies regarding the role of NMDA receptors, in particular in PFC, for working and short term memory is quite controversial, comparable to the evidence for a general involvement of PFC in working memory processes.

A possible reason for these differences in results might be the type of task which was used. As mentioned above in the section regarding the general role of PFC in working and short term memory, it is conceivable that PFC is involved in delayed responding rather than in mere maintenance of presented stimuli (D'Esposito & Postle, 1999). In animal studies that reported deficits in spatial working memory after NMDA receptor blockade, responding, not only maintenance of stimuli was required. Delayed alternation or delayed matching to sample requires animals to remember their own previous actions or presented stimuli, respectively, to guide their response. Therefore such tasks contain the requirement to use the remembered information instead of merely reproducing it, as is the case in - for example - span tasks, which appear to measure pure maintenance.

Another reason for discrepancies regarding NMDA receptor involvement in working memory performance might be site-selective effects of NMDA antagonists, which were reported as results of a comparison of noncompetitive (MK801, PCP and memantine) and competitive (SDZ EAA 494, NPC17742) NMDA antagonists in rats performing a working memory task (DNMTS). This study found that only the noncompetitive NMDA antagonists significantly impaired accuracy and discriminability, particularly at brief delays, while none of the competitive antagonists led to any impairment (Willmore et al., 2001). However, results from many studies do not correspond to this distinction between the effects of competitive and non-competitive NMDA antagonists, as several experiments using non-competitive NMDA blockers nevertheless did not find working memory impairments, while some studies using competitive antagonists reported such deficits.

Neurocomputational studies, on the other hand, propose an involvement of NMDA receptors in working memory. Various specific properties of NMDA receptors are assumed to provide characteristics needed for persistent activity underlying WM in PFC. For

example, NMDA receptor mediated excitatory postsynaptic potentials (EPSPs) are considered critical for WM (Lisman et al., 1998), synaptic reverberation is viewed as a likely mechanism for active maintenance of working memory in PFC, thus NMDA receptors are considered as participating in the stabilization of persistent delay activity (Tegner et al., 2002). The recurrent synaptic excitation mediated primarily by NMDA receptors is assumed to provide stable spontaneous and persistent activity underlying spatial WM in the PFC (Compte et al., 2000). In another model, the slow NMDA receptor-mediated synaptic transmission is likely required for sustaining network activity at low firing rates and thus NMDA receptors are assumed to have a critical role for normal WM function of the PFC (Wang, 1999). However, a further study using intracellular recordings from deep layer PFC neurons showed that the persistent activity required for working memory was driven by non-NMDA glutamate receptors (Seamans et al., 2003).

Other models highlight the interaction of NMDA receptors and dopamine, as does the report by Tanaka (2002), where the NMDA to AMPA-channel transmission ratio, assumed to be controlled and changed by DA, is proposed to be responsible for operations with multiple items in spatial WM. A low ratio causes “replacement” of previously loaded targets to new ones. Intermediate ratios cause “addition” of new items to old ones. High ratios cause “rejections” of new target stimuli. A further model tested possible functional implications of the dopaminergic modulation of NMDA synaptic conductances and found that DA-induced increase in NMDA conductances contributed to an increase in stability of the target pattern. (Durstewitz et al., 2000).

In the next section, evidence regarding the interaction between DA and NMDA will be discussed in more detail.

1.12 Interaction of glutamate/NMDA and DA in the PFC

Working memory functions subserved by the PFC are highly dependent on mesocortical dopamine (DA) transmission. Antagonism of DA D1 receptors in PFC impairs working memory in primates and rats (Sawaguchi et al., 1990; Seamans et al., 1998). During working memory tasks, DA efflux in primate PFC was found to increase (Watanabe et al., 1997) However, there seems to be an inverted U-shaped functional relation between working memory performance and DA levels in PFC, indicating that DA levels that are too low or too high will impair working memory, while a medium level of DA will ensure

good working memory performance (Zahrt et al., 1997; Floresco & Phillips, 2001). Especially D1 receptor activation via DA is involved in this scenario (Sawaguchi & Goldman-Rakic, 1991; Goldman-Rakic et al., 2000). A dynamic model for working memory-related neuronal activity posits an increase in errors at elevated DA levels (Deco & Rolls, 2003).

Dopaminergic projections from midbrain to striatum and frontal cortex play a role in processing of rewards. DA neurons demonstrate short, phasic activation in response to unexpected rewards in various behavioral situations. This activation might encode a prediction error, e.g. the discrepancy between an actual and predicted reward, and thus might deliver a teaching signal for behavior and learning (Schultz, 2000, 2001). However, a study measuring DA during a spatial delayed response task in a radial maze found that DA efflux in rat mPFC was increased in a phasic manner, independent of reward, but dependent on retrieval of specific trial-unique memories (Phillips et al., 2004).

A large body of evidence shows that both systemic NMDA receptor blockade and local PFC NMDA receptor antagonism increase DA levels in PFC (Feenstra et al., 2002; Lorrain et al., 2003, Takahata & Moghaddam, 1998), while stimulation of NMDA receptors in PFC reduces the basal release of DA (Hata et al., 1990), decreases the extracellular concentrations of DA and DA metabolites in PFC (Feenstra et al., 1995), or has no effect on DA release in PFC (Jedema & Moghaddam, 1996), suggesting that PFC-based NMDA receptors normally exert a tonic inhibitory control on DA release in PFC (Takahata & Moghaddam, 1998, Kashiwa et al., 1995). Thus, excessively high levels of prefrontal DA might be a secondary effect of NMDA receptor hypofunction in PFC (Takahata & Moghaddam, 1998). Another study found that increasing endogenous extracellular glutamate led to a decrease of extracellular DA metabolites DOPAC and HVA, an effect which could be blocked by NMDA antagonism. The authors conclude that endogenous glutamate acts preferentially through NMDA receptors to decrease DA metabolism (Del Arco & Mora, 1999). In the chick MNH, the infusion of NMDA also led to a decrease in HVA (Gruss et al., 1999). Comparably, it was found that both systemic and local PFC NMDA receptor antagonism in the rat caused an increase of DOPAC levels and DA utilization in PFC (Umino et al., 1998). However, a study observed opposing effects of noncompetitive and competitive NMDA antagonists upon DA metabolism in rat PFC as measured by the ratio of DA and DOPAC, demonstrating that DA metabolism increased in response to non-competitive NMDA antagonists (dizolcipine and memantine), but

decreased in response to the competitive NMDA antagonist CGP 39551 (Bubser et al., 1992). Thus it appears that, depending on the antagonist used, NMDA receptor blockade might have a twofold effect on dopaminergic processes in PFC by increasing DA levels and potentially also by increasing DA metabolism in PFC.

However, activation of PFC NMDA receptors by the agonist NMDA can have concentration-dependent opposite effects on DA release in PFC: low concentrations may lead to a decrease, high concentrations to an increase of DA in PFC (Jedema & Moghaddam, 1996; Feenstra et al., 1995). Moreover, an interaction between NMDA NR 1 and D1 receptors was found to provide a mechanism by means of which the activation of NMDA receptors can upregulate D1 receptor function by increasing the plasma membrane insertion of D1 receptors (Pei et al., 2004).

Taken together, these results regarding the interaction of NMDA and DA in PFC render it likely that NMDA receptor antagonism in PFC will entail elevated DA and additionally have effects on DA metabolism in PFC and that both factors, dysfunctional NMDA receptors and altered dopaminergic processes, may contribute to impairments in learning and working memory.

DA also modulates glutamate transmission in the PFC and thus presumably has effects on NMDA receptor function. Dopamine agonists such as amphetamine (Del Arco et al., 1998) or apomorphine (Porras et al., 1997) were found to increase extracellular concentration of glutamate in the rat PFC. In contrast to this, a selective D1 DA agonist reduced the glutamate concentration in the rat PFC (Abekawa et al., 2000). Accordingly, DA via the D1 receptor increases NMDA-like synaptic currents in the PFC (Kita et al., 1999; Moore et al., 1998; Seamans et al., 1999).

There are various possibilities how glutamate and dopamine may interact in the PFC (Kodama et al., 2002). One possibility is direct interaction. Axonal terminals of dopaminergic neurons from the VTA synapse on the spines of PFC pyramidal neurons together with the terminals of glutamatergic inputs (Smiley & Goldman-Rakic, 1993), thus it is possible that DA controls the excitability of these glutamatergic pyramidal neurons (Goldman-Rakic, 1999). A second possibility is the interaction through GABA interneurons. Glutamate possesses facilitatory effects on GABA, as NMDA antagonists decrease the extracellular concentrations of GABA (Yonezawa et al., 1998), while increased extracellular glutamate increases GABA (Del Arco & Mora, 1999). GABA

agonists inhibit and GABA antagonists enhance the DA release (Santiago et al., 1993). Thus, stimulation or attenuation of GABA release via NMDA agonists or antagonists could decrease or increase DA release in the PFC, respectively (Jedema & Moghaddam, 1996). A third mechanism for the control of glutamate and DA release in PFC might be feedback circuits from PFC to the thalamus and the VTA. Although up to now there is no direct evidence for such a mechanism, reciprocal connectivities exist between PFC and VTA and between PFC and the limbic system. (Goldman-Rakic, 1987), moreover there exists a closed loop of connections from PFC to the basal ganglia, to the thalamus and back to the PFC (Alexander et al., 1986). There are efferents from VTA to the striatum and limbic system, which in turn connect with the PFC (Oades & Halliday, 1987).

1.13 The pigeon Nidopallium caudolaterale (NCL)

Anatomy of the NCL

The nidopallium caudolaterale (NCL) is a semilunar brain region located in the posterior forebrain of birds, with an extent of about A 3.75 mm to 7.5 mm in the anterior-posterior dimension and L 3.50 mm to 8.50 mm in the lateral-medial dimension (Karten & Hodos, 1967; Waldmann & Güntürkün, 1993). Both caudally and dorsally it borders on the lateral ventricle in the posterior part of the nidopallium. The NCL, as the avian telencephalon in general, is organized in homogenous cell groups and not in laminae, as is the case in the mammalian cortex.

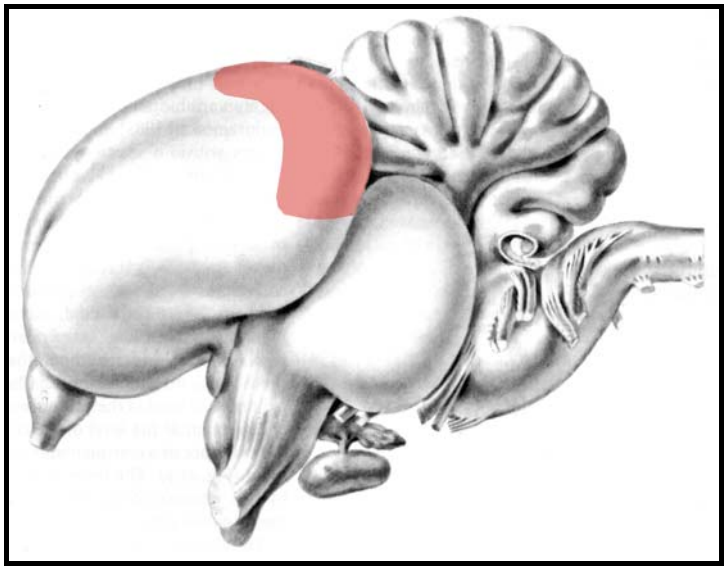

Fig. 3: The pigeon brain with the NCL area (depicted in pink)

Connectivity

The NCL receives afferents from and sends efferents to secondary sensory areas of all sensory modalities (Kröner & Güntürkün, 1999; Leutgeb et al., 1996). These afferents possess highly overlapping projection areas within the NCL (Kröner & Güntürkün, 1999). It receives thalamic input from N. mediodorsalis posterior thalami (DLP) (Waldmann & Güntürkün, 1993), which based on its connectivities, differs from the mammalian N. mediodorsalis (MD), the thalamic afferent nucleus of the PFC. It is however possible that both nuclei have similar functions in interaction with the forebrain (Güntürkün, 1997). A further afferent projection stems from the viscerolimbic part of the arcopallium, which is considered equivalent to the mammalian amygdala (Zeier & Karten, 1971; Davies et al., 1997).

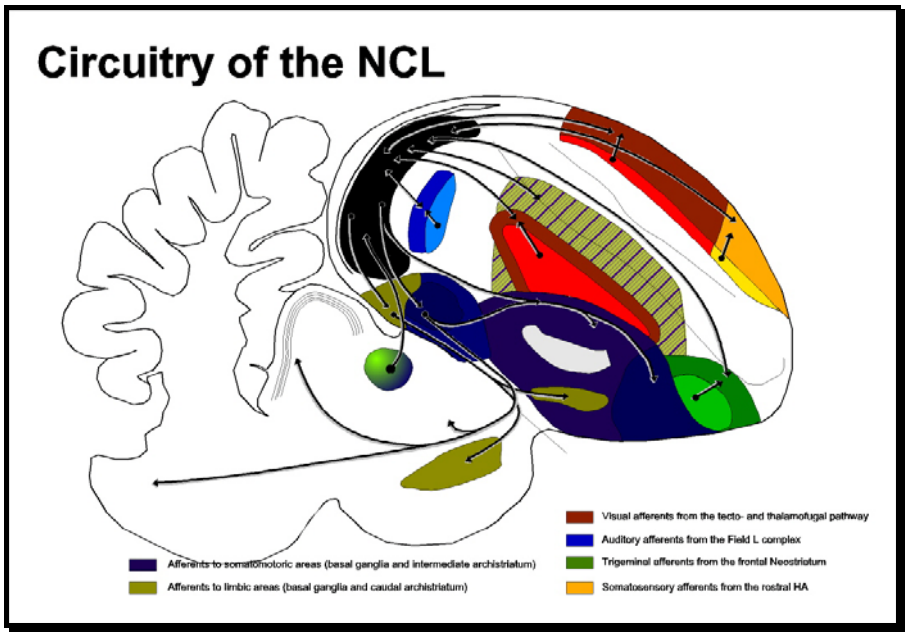

Fig. 4: schematic description of the NCL connectivity (Kröner & Güntürkün, 1999)

Dopaminergic innervation

The NCL shows a high dopamine (DA) content (Divac & Mogensen, 1985, Divac et al., 1994) and a dense innervation by dopaminergic fibers (Waldmann & Güntürkün, 1993, Divac et al., 1994, Wynne & Güntürkün, 1995) and is thus distinguishable from the surrounding caudal neostriatum which exhibits lower dopaminergic innervation.

Moreover, NCL displays a high density of dopamine D1 receptors. In the nidopallium, an increase of D1 receptors can be observed from rostral to caudal as well as from medial to lateral extent, thus the highest density of D1 receptors in the avian telencephalon is found in the NCL (and in caudal HV) (Durstewitz et al., 1999).

Functional equivalency with the mammalian PFC

Based on converging evidence from neuroanatomical, electrophysiological and behavioral studies, the avian NCL is considered functionally equivalent to mammalian PFC.

Behavioral studies demonstrate that NCL lesions lead to deficits in various tasks challenging prefrontal functions, e.g. go/no-go tasks (Güntürkün, 1997; Aldavert-Vera et al., 1999), and reversal learning (Hartmann & Güntürkün, 1998) requiring response inhibition and behavioral flexibility, respectively. With regard to the prominent prefrontal function of working memory, some studies do not find any spatial working memory impairments following NCL lesions (Gagliardo et al., 1997), while others report such deficits in working memory (Güntürkün, 1997) for example in delayed alternation (Gagliardo et al., 1996; Mogensen & Divac, 1982, 1993). Hints for an involvement of NCL in working memory tasks also comes from electrophysiological recordings which found NCL neurons exhibiting - comparable to PFC neurons - delay- and reward-expectancy-related activity (Kalt et al., 1999, Diekamp et al., 2002).

Further parallels to mammalian PFC were revealed by studies using local NCL D1 receptor antagonism which led to performance deficits in a reversal task (Diekamp et al., 2001) and a spatial working memory task, here only WM performance was impaired, but not reference memory (Güntürkün & Durstewitz, 2000). A combined behavioral / microdialysis study measuring concentrations of DA and its metabolites in NCL during both a non-spatial WM task (DMTS) using a 4 seconds delay and a task with minimal memory load of 0 seconds delay (MTS) found a significant increase - compared to baseline - in DA release during performance of the WM task, but not during the non-WM task. The concentration of the DA metabolite DOPAC, however, increased in both tasks, while HVA (homovanillic acid) increased in neither task (Karakuyu, 2003).

A functional segregation of NCL subareas was suggested in a lesion study comparing the effects of dorsal and ventral NCL lesions on a working memory task: dorsal NCL might be involved in active working memory, while ventral NCL may mediate perseverative behavior (Diekamp et al., 2002). Still, both lesions groups exhibited delay-independent performance deficits with an increase of errors by about 10%.

In summary, NCL was found involved in the prefrontal functions of working memory, response inhibition and behavioral flexibility, providing evidence for a functional equivalency to the mammalian PFC.

1.14 NMDA receptors in the avian brain and their role in learning

Evidence for the presence of NMDA receptors in the telencephalon of avian species is provided by an autoradiographic study that revealed high densities of NMDA receptors in the dorso-caudal neostriatum (Ndc), the brain area in the chick equivalent to NCL in pigeons, and in the MNH of domestic chicks (Bock et al., 1997). Dense populations of NMDA receptors have also been found in the avian hippocampal formation (Sakurai, 1991).

A study investigating the effects of systemic NMDA receptor blockade in homing and non-homing pigeon breeds on performance in the radial arm maze found no deficits in spatial working memory in neither group (Meehan, 1996). A further study focussing on homing pigeons found associative learning, as measured by a conditional discrimination task, unimpaired by systemic NMDA receptor blockade (Riters & Bingman, 1994). NMDA receptors in the chicken NCL or intermediate and medial hyperstriatum ventrale (IMHV) have already been demonstrated to be involved in imprinting (Bock et al., 1996, 1997; McCabe et al., 1992) and in passive avoidance learning (Stewart et al, 1992; Burchuladze & Rose, 1992), while NMDA receptor antagonism in the pigeon NCL impaired color reversal learning, but not retention of an acquired reversal (Lissek et al., 2002).

1.15 Aims of the present thesis

In summary, the above review of the substantial literature on PFC and NCL function for learning and memory shows that prefrontal regions participate in learning phenomena by functions subserving learning, such as response inhibition, response selection, context processing and working memory. The role of NMDA receptors in various brain areas for learning is also very well documented, moreover, neurocomputational models implicate them in working memory. In contrast to this, there is very little behavioral evidence about the specific role of NMDA receptors in PFC and NCL for functions related to learning and memory.

The aim of this thesis thus was to extend the existing knowledge about the role of NMDA receptors in the avian NCL by investigating their possible involvement in the prefrontal functions of extinction, response inhibition, working memory, response selection and context processing. By local antagonism of NCL-based NMDA receptors during learning

or performance of different tasks that challenge prefrontal functions, the NMDA receptor-specific contribution to the functions mentioned above was to be elucidated.

Experiment 1 : Extinction and Response Inhibition

In the experiment described in chapter 2, an investigation was undertaken to determine whether NMDA receptor antagonism in or lesions of the NCL have an effect upon extinction learning and/or behavioral disinhibition. Extinction learning requires to refrain from responding to a previously rewarded stimulus (S+) due to non-continued reinforcement of this behavior. An impairment in extinction of an instrumental response, visible in continued responding to the former S+, however, may also result from a general deficit in behavioral inhibition. To control for such a deficit, a special task was devised enabling disambiguation of the possible contributions of a genuine "unlearning" deficit versus behavioral disinhibition to an hypothesized extinction deficit following NMDA receptor blockade.

Pigeons were trained in a color discrimination task, in which responding to the first of two successively presented colors (red) on a pecking key had to be inhibited while responding to the second color (green) was food-reinforced. During the experimental extinction sessions, also responding to the green color was no longer reinforced. Thus the response rate to the green color constituted extinction performance, while the response rate to the red color served as a measure for behavioral inhibition or disinhibition.

A between-subjects-design was applied, in which performance in the extinction session and the two extinction-recall sessions of two experimental groups, one receiving lesions of NCL (n=6), the other being treated with local NCL NMDA receptor antagonism (n=10) was compared with a control group (n=8), receiving vehicle infusions into the NCL.

Experiment 2: Short term memory and Response Selection

The second experiment described in chapter 3 dealt with the functions of NMDA receptors for working / short term memory and response selection, respectively. In most studies investigating PFC contribution to spatial or non-spatial working/short term memory performance, there is no control condition in which the same task has to be performed without an interposed delay or even without the necessity to remember the sample stimulus. Such a control condition, however, would allow for assessment of the separate contributions of the components 'memory load' and 'response selection' to performance.

In this study, we therefore evaluated the contribution of NMDA receptors in NCL to these components by comparing animals' performance in two tasks requiring both or only one component. Two groups of pigeons (n=16 and n=8) were trained in either a delayed-matching-to-sample task (DMTS) requiring maintenance in working memory and response selection, or a simultaneous-matching-to-sample task (SMTS), requiring only response selection. During experimental sessions, animals had to perform these tasks either following NMDA receptor antagonism in the NCL or after infusion of vehicle. Thus we applied a combined between-subjects-design, comparing performance of the two groups in the two tasks, and a within-subjects-design, comparing performance of the animals under the two experimental conditions within the same task.

Experiment 3: Context processing

In the third experiment, detailed description in chapter 4, the focus was on implication of NCL-based NMDA receptors in context processing during response selection in visual discrimination. A special task, constituting an extension of a normal SMTS-task, was developed. In this task, pigeons were confronted with two types of trials, each requiring merely response selection between two simultaneously presented color stimuli, either based on stable S-R associations which they could retrieve from reference memory, or based on a conditional association, which necessitated to consider the sample stimulus as contextual indicator for correct responding, as is usually the case in any SMTS task. One group of pigeons (n=9) was trained in this special SMTS task. In the 10 experimental sessions, each animal was tested alternately in two conditions: NMDA receptor blockade and vehicle infusion.

All three experiments were conducted in skinner boxes for pigeons, equipped with pecking keys and a solenoid-operated food hopper. In all three behavioral studies performed, pharmacological blockade of NMDA receptors was accomplished by infusing the competitive NMDA antagonist DL-AP5 (D,L-2-amino-5-phosponovaleric acid) through four previously implanted cannulas aiming at the NCL (coordinates A 5.25, L 5.00 and L 7.50 according to the pigeon brain atlas by Karten & Hodos, 1967) immediately before the experimental sessions. The total infusion volume was 2 µl containing 10 µg DL-AP5, i.e. 0.5 µl (2.5 µg DL-AP5) per cannula.

In the following chapters, these three studies will be described in detail.

CHAPTER 2:

Dissociation of extinction and behavioral disinhibition: the role of NMDA receptors in the pigeon associative forebrain during extinction

Behavioral/Systems/Cognitive

Dissociation of Extinction and Behavioral Disinhibition: The Role of NMDA Receptors in the Pigeon Associative Forebrain during Extinction

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Extinction is a unique learning process that requires the alteration of stimulus–response associations such that the organism ceases to respond to a previously rewarded stimulus. Extinction is mostly studied with fear conditioning and is impaired by lesions of the prefrontal cortex as well as by blockade of NMDA receptors in the amygdala. Because previous tasks could not clearly disambiguate extinction from behavioral disinhibition, the underlying process was difficult to define. In this study, we examined the possible role of NMDA receptors and the pigeon "prefrontal cortex," the neostriatum caudolaterale (NCL), for extinction of appetitive instrumental conditioning. We used a new design that discerns extinction from behavioral disinhibition. Our results demonstrate that NCL lesions cause deficits neither in extinction learning nor in extinction recall. However, blockade of NMDA receptors in the pigeon NCL by DL-AP-5 drastically impairs extinction learning without producing behavioral disinhibition or deficits in extinction recall. We suggest that NMDA receptors in the NCL contribute to the establishment of a learning process that selectively signals the change in value of the instrumental stimulus. Although NCL plays a key role for extinction learning, other structures can subsume similar functions after postlesional regeneration.

Key words: **NMDA receptor; prefrontal cortex; learning; extinction; avian; behavioral disinhibition; DL-AP-5**

Introduction

Learning pertains not only to the acquisition of new associations, but also to rearrangements of existing ones. Altering previously acquired associations usually involves extinction learning. Results from lesion experiments in mammals point to the involvement of the prefrontal cortex (PFC) and amygdala in the extinction of conditioned responses: in macaques, the extinction of appetitive instrumental conditioning is retarded by frontal cortex lesions (Butter, 1969; Jones and Mishkin, 1972). In rats, the maturation of PFC regions is necessary for instrumental extinction learning (Nair et al., 2001). Extinction of classically conditioned fear responses is sometimes found to be impaired (Morgan et al., 1993; Quirk et al., 1998) or unimpaired (Gewirtz et al., 1997; Morgan & LeDoux, 1999; Quirk et al., 2000) by PFC lesions in rats, but in any case seems to require amygdalar processes. The role of the amygdala in extinction was investigated mainly with regard to NMDA receptors, using local or systemic injections of a NMDA antagonist in classical fear conditioning paradigms. Local NMDA receptor blockade in the amygdala blocks extinction learning in rats (Falls et al., 1992; Davis, 2002;Walker & Davis, 2002). The systemic injection of a NMDA receptor antagonist can have the same effect (Baker & Azorlosa, 1996) or specifically impairs only extinction recall (Santini et al., 2001).

To our knowledge, the specific role of NMDA receptors in PFC for extinction learning has not been examined, and our question was whether the results of these fear-conditioning experiments are specific for aversive stimulation or whether NMDA-dependent processes are also implicated in the extinction of appetitive instrumental conditioning. However, in a fear extinction paradigm, two variables are confounded: resumption of a previously suppressed instrumental behavior after fear extinction may be attributable to mere behavioral disinhibition and not necessarily to associative changes regarding the conditioned stimulus. Therefore, impaired extinction of instrumental responses in a non-aversive extinction paradigm might as well be attributable either to a general behavioral disinhibition or to deficits in the acquisition of extinction learning. Therefore, we devised a special extinction task that enabled us to measure and analyze each of these two aspects independently.

Our animal model is the "prefrontal cortex" of the pigeon: the neostriatum caudolaterale (NCL). The NCL is a forebrain area in birds that is considered a functional equivalent to mammalian PFC because of multiple converging evidence from behavioral (Mogensen and Divac, 1982, 1993; Gagliardo et al., 1996, 1997; Güntürkün, 1997; Diekamp et al., 2001, 2002), electrophysiological (Kalt et al., 1999), and neuroanatomical (Divac et al., 1994; Wynne & Güntürkün, 1995; Leutgeb et al., 1996; Metzger et al., 1998; Kröner & Güntürkün, 1999) data.

In a previous study, local injections of the NMDA receptor antagonist DL-AP-5 into the NCL during color reversal learning

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had revealed a perseveration on the old *S+* combined with an unimpaired first-time acquisition of the new *S+* (Lissek et al., 2002). These data suggested that not acquisition of the new instrumental stimulus, but the extinction of the old, previously rewarded response, was adversely affected by the NMDA receptor blockade. However, this study was also unable to clarify if the deficits were attributable to impaired acquisition of extinction learning or to behavioral disinhibition. Therefore, the aim of the present study was to investigate the role of NMDA receptors in the NCL during the extinction of an instrumental response, using a paradigm enabling the dissociation of these two processes.

Materials and Methods

Subjects. Subjects were 24 unsexed and experimentally naive pigeons (*Columba livia*), obtained from local breeders. All animals were individually caged in a temperature- and humidity-controlled room on a 12 hr light/dark schedule. During experiments, they were maintained at 80% of their free-feeding weight and received water and grit *ad libitum*.

Apparatus. A conventional Skinner box (36 cm long × 34 cm high × 36 cm wide) was used for training and experiments. The Skinner box was equipped with one pecking key and a solenoid-operated food hopper in the back wall and was computer-controlled by means of a digital input/output board. On the pecking key (2.5 cm in diameter and situated in the center of the wall), white light was displayed during autoshaping, and red or green light was displayed for training and experimental sessions of the extinction task. The Skinner box was illuminated by a house light.

Pretraining and color discrimination task. After an autoshaping procedure, in which pigeons acquired the association between responding to a single pecking key illuminated by white light and subsequent food reward, pigeons were trained in a color discrimination task, which we designed to separate the effects of disinhibition and extinction in the experimental sessions: In each training trial, pigeons were confronted with a single pecking key displaying first the color red for 30 sec, then the color green for 5 sec. Pecking on the green key yielded 2 sec access to the food tray after a delay of 1 sec, whereas pecking on the red key had no effect at all. So there was one stimulus for which responding was never rewarded (red), and a second stimulus for which responding was always rewarded (green). The rewarded green stimulus was later to become the extinction stimulus, whereas the red light served as a measure for behavioral disinhibition during extinction. A training session lasted 80 trials; the learning criterion was set to 80% correct responses in each of three subsequent sessions. After reaching a learning criterion, pigeons were randomly assigned to three treatment groups: a NMDA antagonist group (AP-5, $n = 10$), an NCL lesion group (LES, $n = 6$), and a saline control group (SAL, $n = 8$).

Surgery. For surgery, pigeons were anesthetized with ketamine-rompun (40 mg/kg and 8 mg/kg, respectively, i.m.).

Implantation of guide cannulas: AP-5 and SAL group. Aiming at the NCL, two stainless steel cannulas per hemisphere were vertically inserted under stereotaxical guidance (Karten and Hodos, 1967) to reach the following coordinates: anterior (A), 5.25; lateral (L), 5.00; and A 5.25; L 7.50. Cannulas were inserted to 1 mm below the brain surface and were secured with dental acrylic.

Electrolytic lesions: LES group. Tungsten electrodes (0.2 mm in diameter) insulated to within 0.5 mm of the tip were lowered to the following coordinates (Karten and Hodos, 1967): A 4.00, L 5.00 and 6.50; A 5.00, L 4.50, 6.00, and 7.50; A 6.00, L 4.50, 6.00, and 7.50; and A 7.00, L 5.00, 6.50, and 7.75. Each lesion was made by lowering the tip of the electrode 1.5 mm below the brain surface and passing 25 mA of anodal current (positive electrode in brain) for 10 sec.

After 5–6 d of recovery for the AP-5 and SAL groups and 7–10 d for the LES group, pigeons were tested for retention of the color-discrimination task (criterion: a minimum of 80% correct responses in the retention session).

Extinction procedure. Three extinction sessions were conducted: one initial and two recall sessions. The last ones were used to control the memory for the previously acquired extinction. These three sessions were performed on three successive days, with each session lasting 220 trials. The trials were identical to the color-discrimination task, with the one exception that responding to the green key did not result in reinforcement or in any other consequences.

Immediately before each of the extinction sessions, subjects belonging to the AP-5 group received bilateral infusions of the competitive NMDA receptor antagonist DL-AP-5 locally into the NCL. AP-5 was dissolved in saline solution (total volume, 2 μl, containing 10 μg of DL-AP-5; 0.5 μl, i.e., 2.5 μg of DL-AP-5 per cannula). Infusions were made through interior cannulas protruding 1 mm from the tip of the guide cannulas into the brain tissue. We used a microinfusion pump equipped with two 1 μl Hamilton (Reno, NV) syringes to deliver the volume at a flow rate of 0.2 μl/min. Afterward, the infusion cannulas remained in place for another 2 min to allow for diffusion of the infused volume. To infuse through all four cannulas, we performed this procedure twice. Subjects belonging to the SAL group were submitted to the same procedure, receiving vehicle (saline solution) only. Immediately after the infusion procedure, which took ~12–15 min, the pigeons had to perform the task. All pigeons of these two groups received three infusions of either AP-5 or vehicle during this study. Subjects belonging to the LES group did not receive any additional treatment before being submitted to the extinction sessions.

Histology. To enable reconstruction of the locations of the guide cannulas as well as of the lesion volume, we perfused the pigeons intracardially with 0.9% (w/v) saline (40°C) and a 4% (w/v) paraformaldehyde solution (4°C). The brains were removed, postfixed, and cut into 40 μm frontal slices on a freezing microtome. After staining the slices with cresyl violet, the positions of the cannula tips as well as the lesions were reconstructed at intervals of 500 μm from A 4.00 to A 9.00 and transferred onto standard sections from the pigeon brain atlas (Karten and Hodos, 1967).

Statistical analyses. During training and extinction sessions, we collected the following behavioral data: responses to the green key and responses to the red key. Responses to the green key (i.e., extinction performance) of the three groups were compared by means of ANOVA, followed by a Bonferroni *post hoc* test. From responses to the green and red keys during training compared with extinction we calculated pre/post response ratios for both keys and each subject. Those ratios were again compared by means of ANOVA followed by a Bonferroni *post hoc* test, if applicable.

Results

Histology

All cannula injection sites were located within the NCL. Eighty percent of the sites were located within a range of ±0.5 mm from A 5.25. The remaining 20% were situated in a range of ±1 mm from A 5.25 (Fig. 1*A*). Bilateral lesions were located within the NCL, in a range from A 4.5 up to A 8.5. Figure 1*B,C* shows the lesioned brain areas for minimum lesions [i.e., areas where lesions in at least two subjects overlap (*B*)] and maximum lesions [i.e., areas covered by lesions from all subjects together (*C*)].

Retention session

In the retention session, all animals reached the criterion of a minimum of 80% correct responses and participated in the following experimental sessions.

Extinction learning (session 1)

Responses to the green key

Decreased responding to the green key was the measure for successful extinction: responding to this formerly rewarded stimulus is supposed to cease after a few trials of non-reinforcement. Both the SAL and LES groups showed rapid extinction of the instrumental response with a mean of 37.16 (LES) responses and 37.12 (SAL) responses during the complete session lasting 220 trials, whereas the AP-5 group showed a mean of 93.3 responses.

The univariate ANOVA showed a highly significant effect of group ($F_{(2)} = 11.771$; $p < 0.001$) (Fig. 2*A*). A Bonferroni *post hoc* test revealed a significant difference between the AP-5 group and

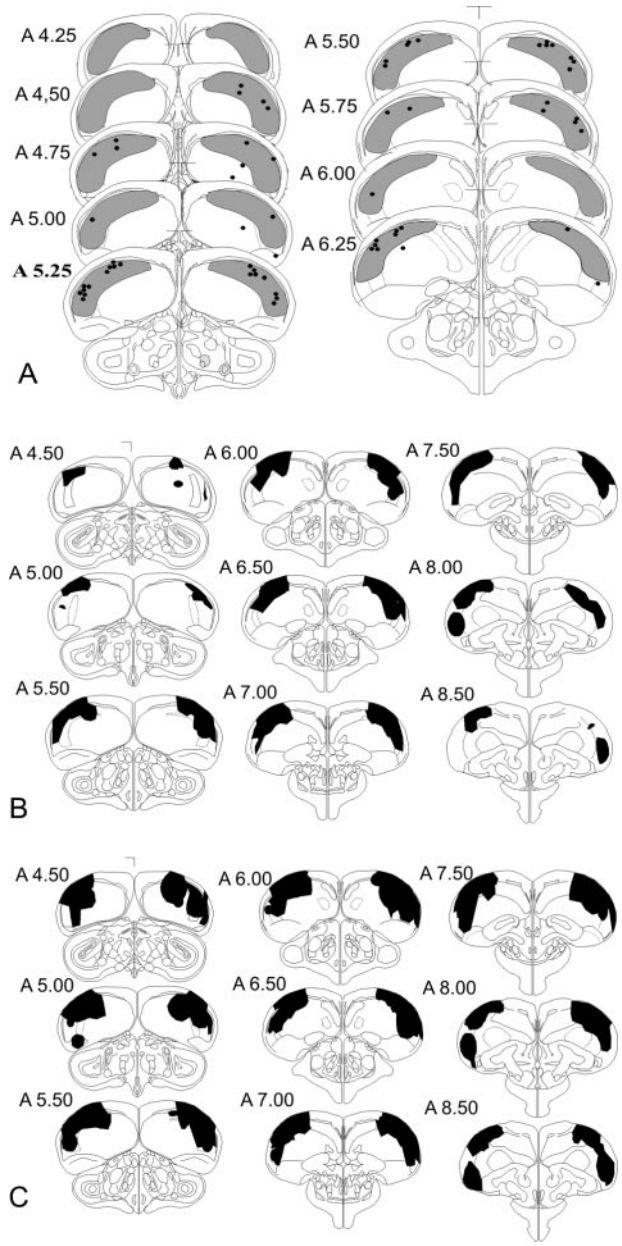

Figure 1. Injection sites and lesion locations. *A,* Schematic frontal sections of the pigeon brain showing the injection sites for AP-5 and saline solution. Dots represent the lower tips of the cannulas; numbers represent the distance (in millimeters) anterior to the center of the ear bars; boldface indicates the frontal plane level at which cannulas were aimed. The NCL area according to Waldmann and Güntürkün (1993) is depicted in light gray. *B,* Schematic frontal sections of the pigeon brain showing in black the minimum lesion volumes (i.e., areas where lesions in at least two subjects overlap). *C,* Schematic frontal sections of the pigeon brain showing in black the maximum lesion volumes (i.e., areas covered by lesions from all subjects together). This figure was adapted from graphs in *Stereotaxic Atlas of the Brain of the Pigeon* (Karten and Hodos, 1967).

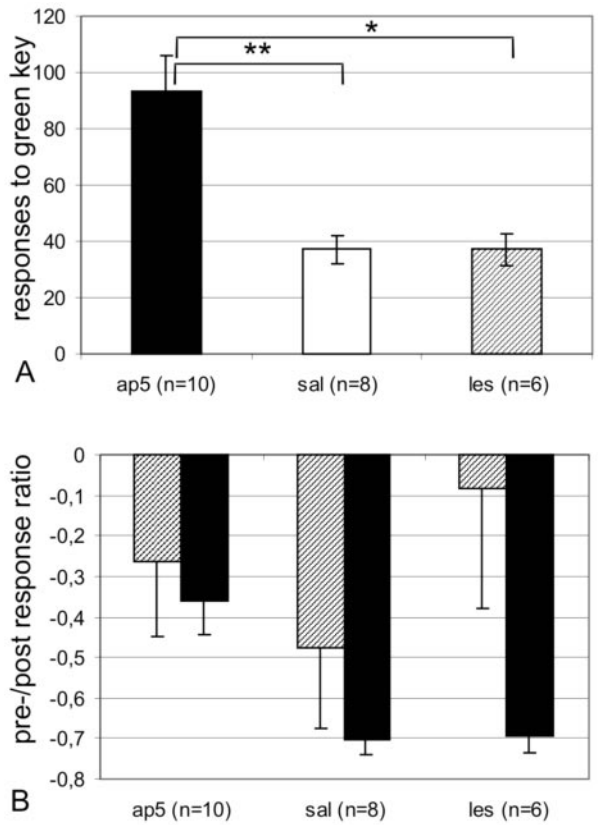

Figure 2. Responses during extinction. *A,* Mean ± SEM responses to the green key during the extinction session made by AP-5-treated (solid columns), lesioned (hatched columns), and vehicle-treated (open columns) pigeons. *B,* Means ± SEM values for the pre/post response ratio to the green (solid bars) and red (hatched bars) keys, respectively.

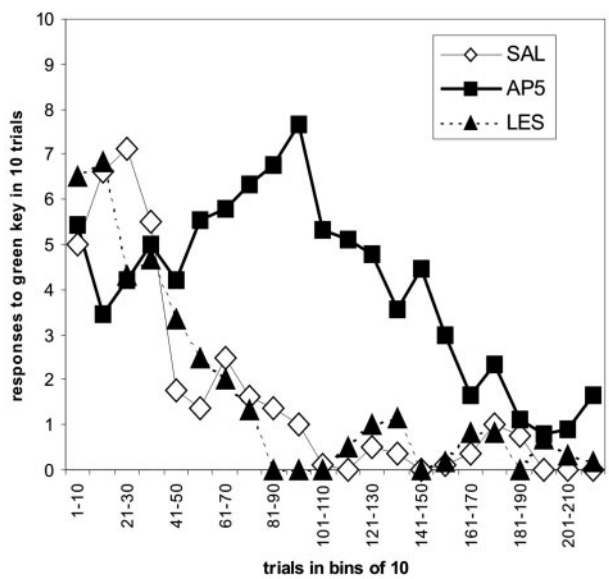

Figure 3. Extinction curves from all three groups. Mean response values to the green key per bin of 10 trials each.

both the SAL group ($p < .001$) and the LES group ($p < 0.01$). The SAL and LES groups did not differ in their responding to the green key during extinction.

Responses to the red key: comparison with presurgery level

Increased responding to the red key (compared with the training level) was the measure for disinhibition, for which we calculated a pre/post pecking ratio. The "pre" value was constituted by the sum of all pecks onto the red key during the last five training sessions ($5 \times 80 = 400$ trials), the post value was constituted by the sum of all pecks onto the red key during the extinction session of 220 trials. These values were recalculated to responses per 100 trials, and the ratio was calculated according to the formula:

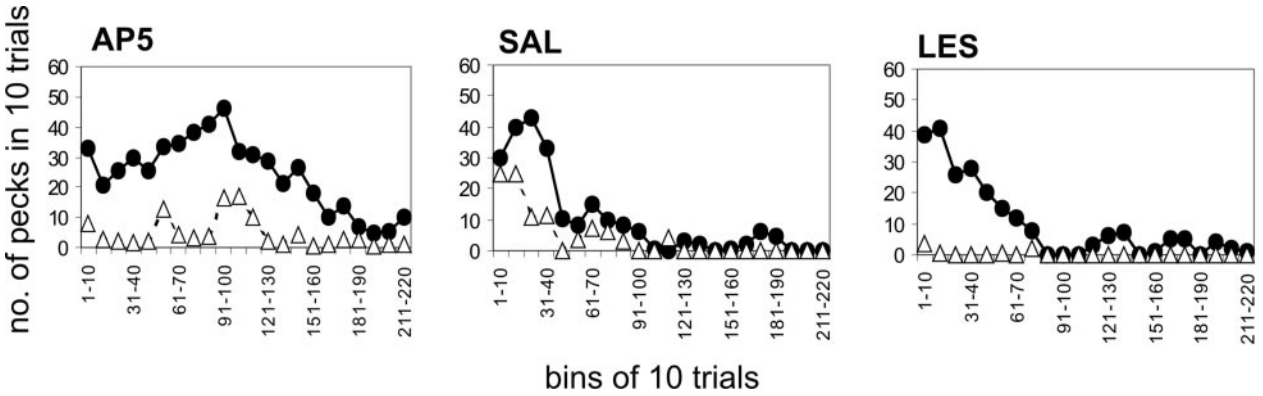

Figure 4. Pecking behavior. Mean pecking responses onto the red and the green key during the extinction session in bins of 10 trials each, shown separately for each experimental group: *A,* AP-5 group. *B,* SAL group. *C,* LES group. Filled circles, Pecks onto the green key; open triangles, pecks onto the red key.

post − pre/post + pre. No change in pecking behavior would yield a result of 0, increased responses in the extinction session results in positive values, decreased responding during extinction in negative values. All three groups showed only a slight reduction of pecking behavior on the red key (Fig. 2*B*). ANOVA did not reveal significant differences in pre/post ratios between groups ($F_{(2)} = 0.730$; p not significant). This result shows that there was no general behavioral disinhibition in any of the groups that could have caused extinction deficits.

Pre/post Ratio for the Green Key

According to the same formula, we calculated a pre/post ratio for the green key, which gave a significant group effect ($F_{(2)} = 9.614$; $p < 0.001$). Although the SAL and LES groups both showed a considerable reduction in responding to the green key, AP-5 animals exhibited the smallest reduction, reflecting their perseverative responding to the green key (Fig. 2*B*).

A Bonferroni *post hoc* test showed a significant difference only between the AP-5 and LES groups ($p < 0.01$) and the AP-5 and SAL groups ($p < 0.01$), but not between the SAL and LES groups.

Extinction curves

Figure 3 shows combined extinction curves for the AP-5, saline, and lesion groups. We calculated these extinction curves by pooling response data from 10 trials each during the extinction session for each group, resulting in 22 data points in time for the complete session with a maximum value of 10 and a minimum of 0 responses during 10 trials. The figure illustrates the differences in the course of extinction between the SAL and LES groups on the one hand and the AP-5 group on the other hand. The individual extinction curves, from which we derived the pooled data (data not shown), demonstrate that all subjects started their responding at a very high level of 8–10 responses in 10 trials. But only in the SAL and LES groups did the continuous experience of non-reinforcement result in a fast drop in their responding to 1–2 responses in 10 trials after ~40 trials, whereas the AP-5 group showed this decrease only after ~160 trials.

Although the combined curve may give the impression that the AP-5 group started at a lower response level than the remaining two groups, this is because some of the subjects started responding only after some trials had passed unattended. This effect, although occurring in all groups, was most prominent in the AP-5 group.

Pecking behavior onto the red key

Figure 4 shows the pecking behavior onto the red key over the extinction session separately for all three groups; for comparison purposes the pecking behavior onto the green key is also plotted. Response data from 10 trials were pooled, every individual peck during these 10 trials was counted, resulting in a total of 22 data points in time for each color for the complete session. An univariate ANOVA showed that the pecking behavior onto the red key did not differ between groups ($F_{(2)} = 0.708$; p not significant). Furthermore, there was no systematic relation between lesion extent and red key pecking behavior.

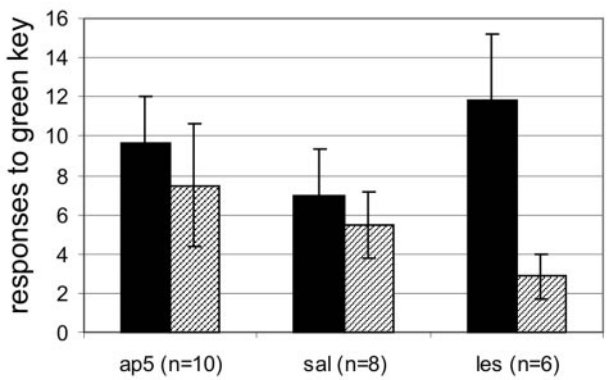

Figure 5. Responses during recall of extinction. Mean ± SEM responses of the three groups to the green key during the recall of extinction sessions. Session 2, Solid bars; session 3, hatched bars.

Recall of extinction (sessions 2 and 3)

Responses to the green key

An ANOVA with repeated measures for the extinction recall sessions 2 and 3 revealed no significant differences between the performance of the three groups ($F_{(2)} = 0.567$; p not significant). The effect of session was also not significant ($F_{(1)} = 3.112$; $p = 0.092$, although there was a tendency toward fewer responses in session 3 compared with session 2. This trend was particularly clear in the LES group. In general, NMDA receptor blockade did not impair the recall of a previously acquired extinction. This implies that the delayed acquisition in extinction session 1 did not impair consolidation (Fig. 5).

Discussion

The main results of this study are: (1) Blocking of NMDA receptors in NCL results in severe deficits in extinction of an instrumental response, whereas recall of a learned extinction remains unimpaired. (2) The extinction learning deficit in AP-5 subjects is not caused by a general behavioral disinhibition. (3) On the

other hand, lesioning the NCL causes deficits neither in extinction learning nor in extinction recall.

Dissociating disinhibition and deficits of extinction

The present results show that the extinction of an instrumental response was impaired after NMDA receptor blockade because AP-5 subjects continued to respond to the previously rewarded stimulus despite the fact that no reinforcement was obtained anymore. This perseverative behavior has been observed in experimental animals after PFC lesions (Butter, 1969, Jones and Mishkin, 1972, Dias et al., 1996; Collins et al., 1998), and after dopamine D_1 and NMDA receptor blockade in the pigeon NCL (Diekamp et al., 2002; Lissek et al., 2002). It is also a typical symptom of prefrontal dysfunctions in humans (Milner, 1964; Fuster, 1989; Vilki, 1989; Rolls et al., 1994). Although perseveration is often considered to result from a lack of behavioral inhibition (Rolls et al., 1994; Hauser, 1999), in most tasks a disambiguation of disinhibition and impaired extinction learning is not possible. However, this was permitted by the experimental design of our study.

Therefore, our results enable us to show that the extinction learning deficit in AP-5 subjects is not caused by behavioral disinhibition: although responding to the previously rewarded stimulus (green) was continued, there was even a slight reduction in responding to the never-rewarded stimulus (red). The collapsed extinction curves of the three groups (Fig. 3) clearly illustrate the differences: saline and lesion groups reduced their responding to the no-longer-rewarded stimulus after only a few trials, whereas AP-5 animals continued to respond at a very high level for much longer, until finally they too decreased their responding. In addition, the differential responses to the green and the red stimulus reveal that the blockade of NMDA receptors did not impair long-term memory retrieval of the properties of the red key. Instead, the impairment was selective for uncoupling either the association between the formerly rewarded stimulus and the learned response or for altering a representation of the incentive value of the green key (Schoenbaum et al., 2002). Indeed, single unit properties of the NCL reveal response patterns tuned to the representation of relevant stimuli and responses as well as to the evaluation and anticipation of reward (Kalt et al., 1999; Diekamp et al., 2002).

This is consistent with studies showing that the establishment of long-term memory for extinction involves an activation of NMDA receptors (Santini et al., 2001). A recent study demonstrated that the constitution of extinction memory results in cellular prefrontal activations correlated with the recall of extinction (Milad and Quirk, 2002). This result strongly argues for the notion that extinction is not caused by the erasure of an association, but instead it forms a new engram. Milad and Quirk (2002) assume that the neurons constituting this memory trace might indirectly inhibit amygdalar processes that modulate freezing behavior. Our results possibly extend this interpretation because they make it likely that a selective NMDA-dependent acquisition of extinction is also able to alter an appetitive instrumental paradigm, in which extinction is accompanied by a reduction of an instrumental response and not, as in fear conditioning, by an increase.

AP-5 injections did not interfere with the recall of a finally acquired extinction. Thus, once NMDA-dependent synaptic rearrangements accompanying extinction are established, an ignition of the altered assemblies does not depend on an activation of NMDA receptors. A similar dissociation between learning and recall with respect to the effects of NMDA receptor antagonists in various brain regions has been observed in a number of studies using a variety of learning paradigms (Morris, 1989; Miserendino et al., 1990; Shapiro and Caramanos, 1990; Campeau et al., 1992; Xu & Davis, 1992).

Unimpaired extinction and recall after NCL lesions

Lesioning the NCL caused deficits neither in extinction learning nor in extinction recall. Subjects whose NCL had virtually vanished showed extinction at a rate comparable to unimpaired controls and were also statistically indistinguishable in terms of extinction recall. One reason for the differential effects of lesions and receptor blockades could be that the area covered by the lesions was smaller than the effective range of the receptor antagonist. In a pilot study, the spread of AP-5 was evaluated by injecting into the NCL 0.5 μl of the fluorescent tracer rhodamine isothiocyanate, known for its wide diffusion area, resulting in an average spread of 1 mm in diameter around the tip of the cannula. Therefore, infusions of this volume through guide cannulas on positions L 5.00 and L 7.50 should cover the lateral–medial range of the NCL, but diffusion should extend neither anteriorly and posteriorly beyond the NCL area nor into the adjacent ventricle (for a detailed description, see Lissek et al., 2002). Thus, the presumed spread of the NMDA antagonist AP-5 is not likely to extend beyond the range covered by the lesions in our subjects, rendering the above explanation for differential effects unlikely.

A possible solution for the lack of postlesional deficits is the assumption that although extinction learning importantly encompasses NCL processes, it can in principle also be mediated by other structures. This is consistent with speculations that prefrontal functions are subsumed by other structures with practice and suggests that these systems operate in parallel with NCL (Miller, 2000; Schoenbaum et al., 2002). In the case of NCL lesions, brain regeneration processes that are launched in the recovery period of 7–10 d after the lesion would enable subjects to solve the task despite a lesioned NCL. However, a blockade of NMDA receptors within the NCL during extinction learning represents an experimental intervention that takes place before the forebrain areas outside NCL had the possibility to overtake prefrontal functions. As a result, extinction learning is seriously perturbed during the effective time period of the antagonist. Thus, we assume a shift of functions to other areas in the postlesional days to be the main reason for the counterintuitive result that NCL lesions produce fewer deficits than NMDA receptor blockades. This view is consistent with literature showing seemingly contrasting effects of prefrontal lesions on extinction: Although a study with primates finds extinction deficits of an instrumental response after lesioning the PFC (Butter, 1969), other experiments report unimpaired extinction of conditioned emotional responses after PFC lesions (Gewirtz et al., 1997; Morgan and LeDoux, 1999). Moreover, some reports even reveal dissociations between the effects of lesions and receptor blockades in the same area (Wolf et al., 1995). Thus, prefrontal areas might be critical to learn the extinction of previously rewarded associations. However, their function is replaceable by other systems if time for neuronal reorganization is provided.

In summary, the findings of this study demonstrate for the first time that NMDA receptors in the NCL, an associative area in the avian forebrain that is functionally equivalent to the mammalian PFC, are involved in extinction learning of an instrumental response. The perseveration behavior that occurred after NMDA receptor blockade was not caused by a general behavioral disinhibition, but rather by a deficit in acquiring extinction learning. We conclude that the activation of NMDA receptors in the avian

"prefrontal cortex" is a necessary prerequisite for the establishment of a memory trace that enables extinction behavior.

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CHAPTER 3:

Maintenance in working memory or response selection? Functions of NMDA receptors in the pigeon "prefrontal cortex"

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Research report

Maintenance in working memory or response selection? Functions of NMDA receptors in the pigeon "prefrontal cortex"

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Abstract

The prefrontal cortex is involved in various aspects of working memory like stimulus maintenance and response selection functions. Neurobehavioral studies and neurocomputational models assume a role for NMDA receptors in prefrontal cortex for maintenance processes, while our previous studies on NMDA receptors in the avian prefrontal cortex-analogue, the nidopallium caudolaterale (NCL), showed them to be involved in response selection functions. Various tasks used in PFC-related research address in fact both functions, so they cannot disambiguate their separate contributions to performance. In order to investigate the role of NMDA receptors in avian NCL for stimulus maintenance and response selection, we trained pigeons in a delayed matching-to sample (DMTS) task, requiring both functions, and a simultaneous matching to sample (SMTS) task, requiring only response selection. After reaching criterion, pigeons had to perform the tasks alternately under local NMDA receptor blockade in NCL (DL-AP5) and after infusion of vehicle (saline solution). Blockade of NCL-based NMDA receptors led to significant increases in error rates in both DMTS and SMTS—compared with the same subjects' performance during training and in the control condition. However, there was no additional increase in errors due to the additional maintenance component, so the impairment appears to be due to deficits in adequate selection of responses, the function necessary for both tasks. We conclude that NMDA receptors in the pigeon NCL participate in response selection rather than stimulus maintenance in tasks requiring the processing of context information.

Keywords: NMDA receptor; Prefrontal cortex; Avian; Working memory; Response selection; DL-AP5

1. Introduction

Working memory is a system of cognitive mechanisms for the temporary storage and manipulation of information. Temporary storage refers to the ability to maintain items for a limited period of time and thus coincides with the classic definition of short term memory. Manipulation, on the other side, includes operations like monitoring of self generated behavior and decisions among alternatives. The prefrontal cortex (PFC) in mammals has a pivotal role in the organization of complex behavior and in doing so recruits numerous cognitive functions that are subsumed under the definition of working memory, among them maintenance of information and response selection. Many behavioral paradigms commonly used in PFC-related research, like delayed matching to sample, in fact make demands on both functions and are thus unable to disambiguate between individual cognitive components.

PFC lesions can lead to a collapse of working memory functions [7,17,20,27,40], also temporary blockade of various receptor types situated in PFC, for example the Dopamine D1-receptors, can have the same effect [51,52]. Dopamine release within PFC enhances persistent Na(+) and NMDA conductances, thus increasing stability of activated neural representations due to long-lasting NMDA-dependent EPSPs that could enable recurrent exitatory synapses to achieve a stable persistent state [36,57]. These effects could reflect parameters of a neural system tuned to maintain cellular assemblies during delay periods [16], representing the short term memory component of the system. Consequently, a number of studies also report deficits in spatial working memory after NMDA receptor blockade in mice and rats [24,55,61]. However, some other studies do not report any impairments [4,46], find working memory deficits only in unfamiliar, but not in familiar environments [53], or observe delay-independent deficits [15]. A major drawback to these studies, however, is the systemic application of the NMDA antagonist, rendering it impossible to conclude which brain area generates the deficit. The only study using local blockade of prefrontal NMDA receptors

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in rats reports no decrease in the percentage of correct responses in a spatial working memory task [2]. These results cast doubt on the assumption of an NMDA receptor mediated prefrontal mechanism to maintain memory traces for short periods of time.

Regarding the role of PFC for response selection, much evidence comes from lesion studies in animals, showing that ventrolateral PFC in particular is involved in this function [44,45,62]. Imaging studies with human participants corroborate these findings. A recent fMRI-study showed a double dissociation of prefrontal areas participating in response selection (area 46) and in maintenance (area 8) [49]. Another fMRI-study evaluated the contributions of PFC and parietal cortex to response selection, concluding that the role of PFC is selecting between competing responses, whereas parietal cortex activates possible responses on the basis of learned S–R associations [8]. A study using repetitive transcranial magnetic stimulation (rTMS) in humans found that performance in a response selection task, even without short term memory load, depends on activation of dorsolateral PFC [25]. While these studies clearly reveal a contribution of the PFC for the response selection aspect of working memory, they are unable to show if prefrontal NMDA receptors are involved in this function.

In previous studies we could show NMDA receptors in the pigeon nidopallium caudolaterale (NCL) (formerly neostriatum caudolaterale; new nomenclature according to [47]) to play an important role in different learning processes that require continuous adaptation of responses to changing environmental conditions [37,38]. The NCL is an avian brain area considered functionally equivalent to PFC in mammals based on behavioral [13,18,19,22,23,26,41,42], electrophysiological [29,58] and neuroanatomical [32,35,39] data. The NCL thus constitutes a brain structure in birds which, like PFC in mammals, is designed for adapting behavior to changing environmental conditions. Therefore, research on the avian equivalent of PFC can provide additional insight into general principles of prefrontal processing, which might apply to all organisms requiring these adaptive functions.

To our knowledge, no study ever evaluated prefrontal NMDA receptor function in PFC in a mere response selection task, without a possibly confounding short term memory element. In this study, we therefore investigated the role of NMDA receptors in the avian "prefrontal cortex" for maintenance and response selection processes separately, by comparing pigeons' performance under local blockade of NCL-based NMDA receptors in two stimulus discrimination tasks: a delayed matching to sample (DMTS) and a simultaneous matching to sample (SMTS) task.

The SMTS task contains only the component of response selection, without any short term memory load, because the indicator for the correct response is visible during the response phase. The DMTS task requires—in addition to the response selection component—a requirement for maintenance in working memory, since here the sample stimulus indicating the correct choice is not available during the response phase. The contribution of these two components to task performance can be dissociated by the method of cognitive subtraction: when NMDA receptors in NCL participate only in the maintenance of stimuli over a delay, an NMDA receptor blockade should cause deficits in the DMTS, but not in the SMTS task. When NMDA receptors participate only in the response selection component, deficits in both tasks should occur. When NMDA receptors participate in both functions, deficits in the DMTS task should be more severe, compared to the SMTS task, due to additive effects of response selection and memory load requirements.

2. Materials and methods

2.1. Subjects

Subjects were 24 unsexed and experimentally naïve pigeons (*Columba livia*), obtained from local breeders. All animals were individually caged in a temperature- and humidity-controlled room on a 12-h light-dark schedule. During experiments, they were maintained at 80% of their free-feeding weight and received water and grit ad libitum.

2.2. Apparatus

A conventional Skinner box (36 cm long × 34 cm high × 36 cm wide) was used for training and experiments. The Skinner box was equipped with three pecking keys and a solenoid-operated food hopper and was computer-controlled by means of a digital input/output board. The three pecking keys (2.5 cm in diameter) were arranged in a horizontal row on the backwall of the Skinner box (18.5 cm above the floor). The food hopper was located beneath the center key. On the pecking keys white light was displayed during pretraining sessions, blue and yellow light was displayed during training and experimental sessions in the delayed and simultaneous matching to sample tasks. The Skinner box was illuminated by a houselight.

2.3. Pretraining in the matching to sample tasks

After an autoshaping procedure, in which pigeons acquired the association between responding to a single pecking key illuminated by white light and subsequent food reward, pigeons were trained in the delayed matching to sample task (DMTS) and the simultaneous matching to sample task (SMTS), respectively.

2.3.1. Delayed matching to sample task

Each trial started with the presentation of the sample stimulus, i.e., yellow or blue light, on the center key. Pigeons had to peck the center key 15 times to switch it off and to start the delay phase, which lasted 0, 1 or 2 s. After the delay, the lateral keys were lit with the matching stimuli, one with the blue, the other with the yellow light. Responding to the same

color as shown on the sample key yielded 3 s access to the feeder and was counted as a correct response. Responding to the non-matching colour resulted in a 15 s timeout and was counted as an error. Each training session lasted 48 trials, that is 16 trials per delay duration. Trials were repeated only when there was either a response to the lateral keys during the presentation time of the sample stimulus, or when there was no response to the lateral keys during the presentation of the matching stimuli. In addition to the delays of 1 and 2 s, we introduced a 0 s delay in order to present trials with a minimal memory load. These trials however, do not provide the sample key as an indication for the correct response during the response phase, as is the case in the SMTS task.

The delays of 0, 1 and 2 s were used since in these delays pigeons reached and maintained a performance accuracy of about 85% correct responses after a reasonable amount of training. In longer delays (4 and 8 s) which were used during training too, pigeons did not acquire the training criterion (i.e., an accuracy of above 80%), but remained at a performance level of about 60% correct responses. Since this baseline was too low to allow for meaningful comparisons, we excluded these delays from the experimental analysis.

2.3.2. Simultaneous matching to sample task

Like in DMTS, each trial started with the presentation of the sample stimulus on the center key. Here, however, 15 responses to this key led to the additional presentation of the matching stimuli on the lateral keys. Again, responding to the lateral key showing the matching color to the center key gave 3 s access to the feeder and responding to the non-matching color resulted in a timeout of 15 s. Each session lasted 80 trials. Trials were repeated only when there was either a response to the lateral keys during the presentation time of the sample stimulus, or when there was no response to the lateral keys during the presentation of the matching stimuli.

Pigeons were randomly assigned to either the DMTS-task ($n = 16$) or the SMTS-task ($n = 8$).

2.4. Surgery

For surgery, pigeons were anesthetized with Ketamine-Rompun (40 mg/kg and 8 mg/kg, respectively, i.m.). Aiming at the NCL, two stainless steel cannulas per hemisphere were vertically inserted under stereotaxical guidance [30] to reach the following coordinates: A 5.25, L 5.00 and A 5.25, L 7.50. Cannulas were inserted to 1 mm below the brain surface and were secured with dental acrylic. After 5–6 days of recovery, pigeons were tested for retention of the matching task, the criterion was 80% correct responses.

2.5. Experimental sessions

For both groups, we applied a within-subjects-design for the treatment: each pigeon was tested under both treatment conditions: blockade of NCL using the competitive NMDA receptor antagonist DL-AP5 (Sigma-Aldrich) or: infusion of only vehicle (0.9% NaCl–saline solution). We conducted six experimental sessions each in the DMTS task and in the SMTS task.

Immediately before each of the experimental sessions, pigeons received bilateral infusions of either the competitive NMDA receptor antagonist DL-AP5 or vehicle (saline solution) locally into the NCL. AP5 was dissolved in saline solution (total volume = 2 μl, containing 10 μg DL-AP5, 0.5 μl, i.e., 2.5 μg DL-AP5 per cannula). We aimed at producing only localized diffusion by using small volumes of fluid and applying a concentration which in previous studies with pigeons had proved effective but did not produce motor or motivational deficits [37,38]. Moreover, in studies on birds [6] and rats [9,34,54] similar concentrations and infusion volumes were also used successfully. Infusions were made through interior cannulas protruding 1 mm from the tip of the guide cannulas into the brain tissue. We used a microinfusion pump equipped with two 1 μl-Hamilton (Reno, NV) syringes to deliver the volume at a flow rate of 0.2 μl/min. Afterwards, the infusion cannulas remained in place for another 2 min to allow for diffusion of the infused volume. To infuse through all four cannulas, we performed this procedure twice. Immediately after the infusion procedure, which took about 12–15 min, the pigeons had to perform the task. Pigeons of both the DMTS group and the SMTS group each received a total of six infusions (3× AP5, 3× vehicle). To prevent sequence effects, pigeons were infused alternately with either AP5 or vehicle, with the first infusion being AP5 in half of the subjects, and vehicle in the remaining half.

2.6. Histology

To enable reconstruction of the locations of the guide cannulas, we perfused the pigeons intracardially with 0.9% (w/v) saline (40 °C) and a 4% (w/v) paraformaldehyde solution (4 °C). The brains were removed, postfixed, and cut into 40 μm frontal slices on a freezing microtome. After staining the slices with cresyl violet, the positions of the cannula tips were reconstructed at intervals of 500 μm from A 4.00 to A 8.00 and transferred onto standard sections from the pigeon brain atlas [30].

2.7. Statistical analyses

During the experimental sessions, we registered the number of correct responses and errors made in the SMTS task and total number of correct responses and errors, as well as correct responses and errors in the individual delay phases for the DMTS task. We compared correct responses during the experiment with the performance during the last three training sessions by means of an ANOVA and Bonferroni post hoc tests. We calculated the error increase compared to the training level (last three presurgery training sessions) for each individual subject and experimental condition and compared the resulting error increase rates for the two ex-

perimental conditions by means of a *t*-test for matched samples. We compared errors in the individual sessions under the two experimental conditions by means of an ANOVA. Further we compared the performance of the groups in the two tasks using an ANOVA with repeated measures.

3. Results

3.1. Histology

All cannula injection sites were located within the NCL. Seventy-five percent of the sites (72 out of 96) were located within a range of ±0.5 mm from the target location A 5.25. The remaining 25% (24 out of 96) were situated in a range of ±1 mm from A 5.25 (see Fig. 1). Diffusion of a fluid in brain tissue depends on both the volume and the concentration of the substance. A volume of 0.5 μl produces a droplet of 0.8 mm diameter around the tip of the infusion cannula. The spread of such a volume from the site of infusion depends on the characteristics of the substance used. In order to restrict diffusion, in any case it is advisable not to infuse volumes exceeding 0.5 μl [59]. In a pilot study, the spread of a AP5 was evaluated by injecting into the NCL 0.5 μl of the fluorescent tracer rhodamine isothyioncyanate, known for its wide diffusion area, resulting in an average spread of 1 mm in diameter around the tip of the cannula. A study considering diffusion of [^{3}H]-AP7, which has diffusional

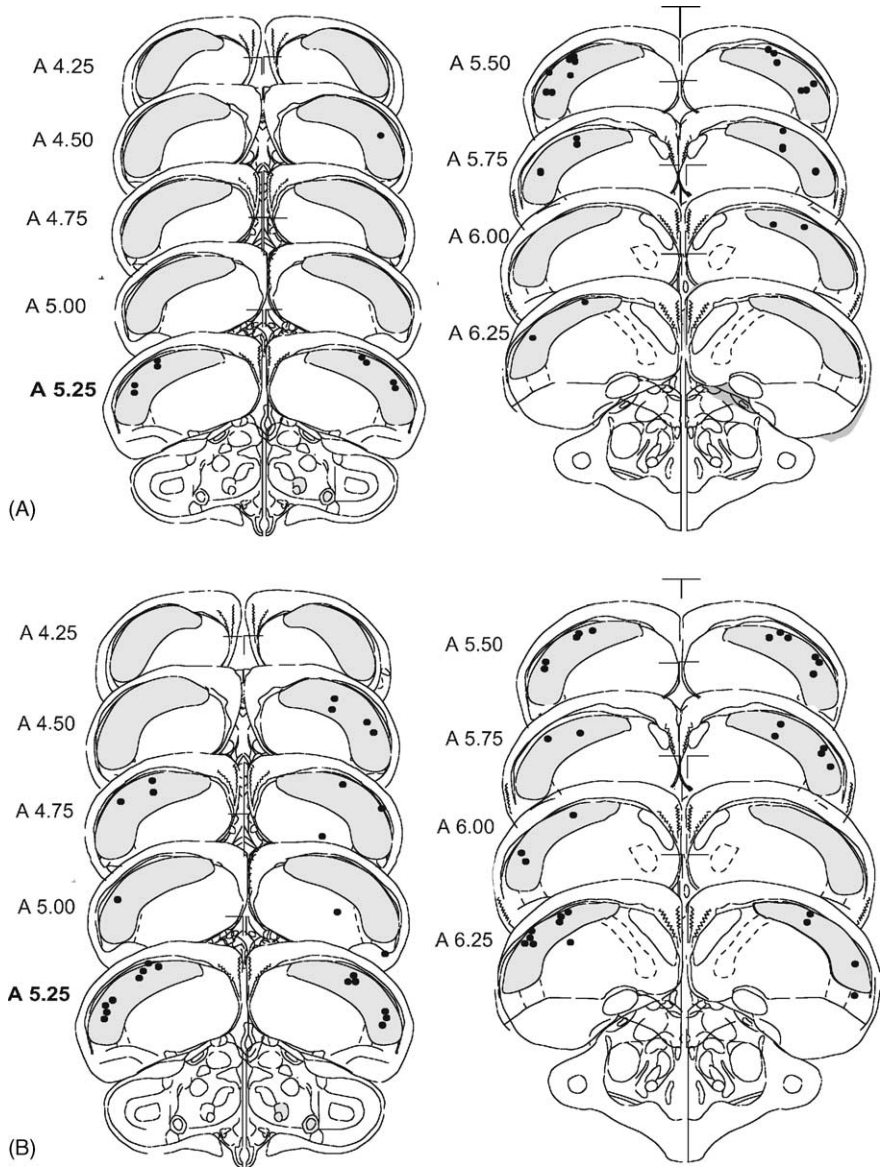

Fig. 1. Schematic frontal sections of the pigeon brain showing the injection sites for AP5 and or vehicle for (A) SMTS group and (B) DMTS group. Dots represent the lower tips of the cannulas, numbers represent the distance (anterior) to the center of the ear bars, boldface indicates the frontal plane level at which cannulas were aimed. The NCL area according to Waldmann and Güntürkün [56] is depicted in light grey. Figure adapted from graphs in Stereotaxic Atlas of the Brain of the Pigeon [30].

characteristics supposedly identical to DL-AP5, in the rat hippocampus [43], found that with an infusion volume of 1 μl (twice the volume we infused per cannula) and a concentration of 10 mM, radiation values had dropped to about 50% at 1.5 mm around the actual infusion site. 3 mm around the infusion site, values had dropped further to almost 0%. These results support our assumption that the spread of the infusion volume of 0.5 μl per cannula, placed at coordinates anterior A 5.25 and lateral L 5.00 and 7.50, was largely restricted to the NCL, which has an anterior–posterior extent of 3.5 mm (A 3.75–7.25) and a lateral–medial extent of 5 mm (L 3.50–8.50) [30,56].

3.2. Retention

All pigeons reached the criterion of 80% correct responses in the retention test after surgery and participated in the following experimental sessions.

3.3. DMTS task

3.3.1. Percent correct responses in training and experiment

All animals mastered all six experimental sessions in the DMTS task, which were conducted alternately under the two treatment conditions. A comparison of correct responses in training (TRAIN), following saline infusion (SAL) and under NMDA receptor blockade (AP5) by means of an ANOVA gave a significant main effect of treatment $F(2) = 12.451$ $P < 0.001$ (see Fig. 2). A Bonferroni post hoc test demonstrated significant differences between AP5 and SAL ($P = 0.025$) and between AP5 and TRAIN ($P = 0.000$), but not between SAL and TRAIN ($P = 0.096$). Even under NMDA receptor blockade, performance remained well above chance level, indicating that information about the task was not completely unavailable in this experimental condition. Mean percentages of correct responses were: TRAIN: 85.85%, SAL 79.56%, AP5 71.7%.

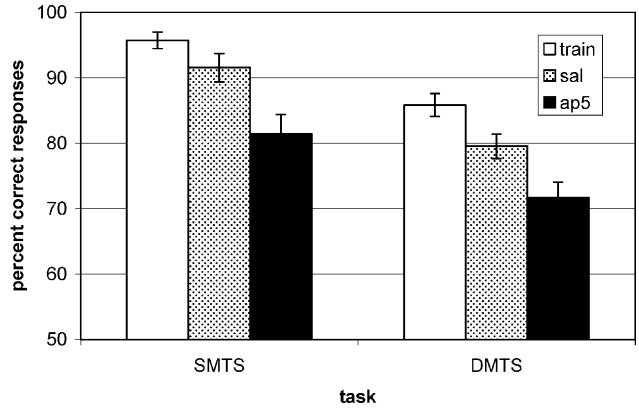

Fig. 2. Percent correct responses in three different treatment conditions: training (TRAIN), after saline infusion (SAL) and NMDA receptor antagonism (AP5) in the SMTS task and the DMTS task. In both tasks there was a significant main effect of treatment (DMTS: $F(2) = 12.451$ $P \leq 0.001$; SMTS: $F(2) = 10.659$ $P < 0.001$). In both tasks Bonferroni post hoc tests showed significant differences between AP5 and SAL (DMTS: $P = 0.025$, SMTS: $P = 0.014$) and between AP5 and TRAIN (DMTS: $P = 0.000$, SMTS: $P = 0.001$). Differences between SAL and TRAIN were not significant in either task. In spite of the impairment due to the NMDA receptor blockade, subjects' performance remained well above chance level (50%).

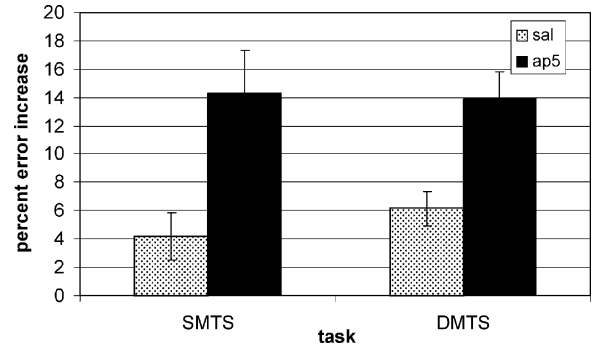

Fig. 3. Error increase in percent compared to the training level in the SMTS task and the DMTS task after saline infusion (SAL) and NMDA receptor antagonism (AP5). *t*-tests for matched samples showed for both tasks significant differences between the treatment conditions (DMTS: $t(15) = 4.136$ $P = 0.001$; SMTS: $t(7) = 5.241$ $P = 0.001$).

3.3.2. Percent overall error increase in the experimental conditions compared to training

The error increase in percent compared to training of both experimental conditions was demonstrated to be significantly different between treatments by a *t*-test for matched samples: $t(15) = 4.136$ $P = 0.001$. (Fig. 3).

3.3.3. Percent error increase in the individual delays compared to training

The percentages of error increase in the individual delays differed significantly between the two treatments (Fig. 4). An ANOVA with repeated measures revealed only this main effect of treatment to be significant ($F(1) = 11.968$ $P < 0.01$. There was no additional significant effect of delay

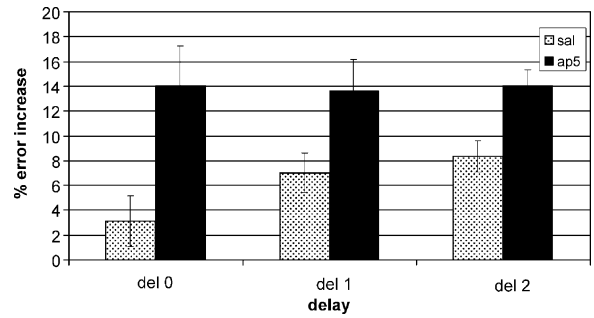

Fig. 4. Error increase in the individual delays of 0, 1 and 2 s (del 0, del 1, del 2) of the DMTS task relative to training under both treatment conditions (SAL and AP5). An ANOVA with repeated measures shows only a significant main effect of the treatment: $F(1) = 11.968$ $P < 0.01$: subjects made significantly more errors when treated with AP5 than when treated with SAL, regardless of the delay duration. There was no significant effect of delay ($F(2) = 1.132$ n.s.) nor of the interaction ($F(2) = 1.243$ n.s.).

duration upon performance ($F(2) = 1.132$ $P = 0.329$), neither was there a significant interaction ($F(2) = 1.243$ $P = 0.296$). Percentages of error increase remained constant in the AP5 group over all delays (means: delay 0 s: 14.06%, delay 1 s: 13.67%, delay 2 s: 14.06%) while there was a slight tendency in the SAL group to higher error increases in longer delays (means: delay 0 s: 3.12%, delay 1 s: 7.03%, delay 2 s: 8.33%).

3.4. SMTS task

3.4.1. Percentage correct responses in training and experiment

All animals mastered all six experimental sessions in the SMTS task, which were conducted alternately under the two treatment conditions. A comparison of correct responses in training (TRAIN), following saline infusion (SAL) and under NMDA receptor blockade (AP5) by means of an ANOVA showed a significant main effect of treatment $F(2) = 10.659$ $P < 0.001$ (see Fig. 2). The Bonferroni post hoc test yielded significant differences between AP5 and SAL ($P = 0.014$) and between AP5 and TRAIN ($P = 0.001$), but not between SAL and TRAIN ($P = 0.612$). Again, in spite of the NMDA receptor blockade, performance remained well above chance level (50%), suggesting that information about the task was not completely unavailable. Mean percentages of correct responses were: TRAIN: 95.73%, SAL 91.56%, AP5 81.46%.

3.4.2. Percent error increase in the experimental conditions compared to training

The error increase in percent compared to training of both experimental conditions was demonstrated to be significantly different between treatments by a *t*-test for matched samples: $t(7) = 5.241$ $P = 0.001$. (Fig. 3).

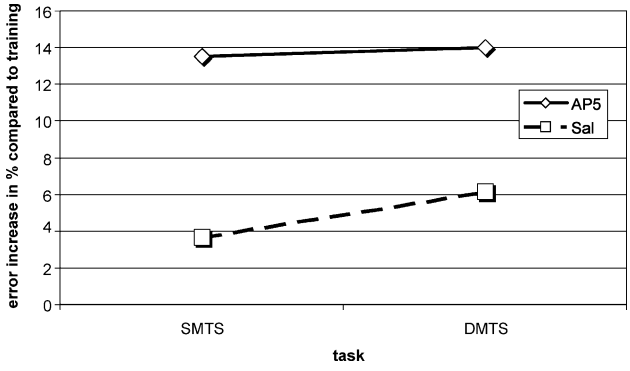

Fig. 5. Error increase in percent compared to training—comparison of both tasks (SMTS and DMTS) and both treatment conditions (SAL and AP5). A comparison by ANOVA with repeated measures gave a highly significant effect of the within-subjects factor "treatment" ($F(1) = 31.964$ $P < 0.001$), while the between-subjects factor "task" was not significant ($F(1) = 0.977$ n.s.). There was no significant interaction either ($F(1) = 0.466$ n.s.).

3.5. Comparison of the DMTS and the SMTS task

The error increase in percent for both tasks and treatment conditions was compared by means of an ANOVA with repeated measures, which demonstrated a highly significant main effect of the within-subjects factor "treatment" $F(1) = 31.964$ $P < 0.001$ but no significant effect of the between-subjects factor "task" $F(1) = 0.977$ n.s. and a non-significant interaction $F(1) = 0.446$ n.s. See Fig. 5: the slight increase in errors from SMTS task to DMTS task observed under both treatment conditions is statistically not significant.

4. Discussion

The main results of this study are:

- NMDA receptor blockade in NCL impairs performance in both tasks, in the SMTS task requiring only response selection, and in the DMTS task requiring response selection plus maintenance of a stimulus over a delay.
- Increased task difficulty by introduction of an additional maintenance component does not lead to an increase in error rates from SMTS to DMTS, neither under AP5 nor under SAL conditions.

In conclusion, NMDA receptors in NCL seem to have a function in response selection, rather than in maintenance of a stimulus over a delay.

4.1. NMDA receptor blockade in NCL impairs performance in both SMTS and DMTS task

With NMDA receptor blockade in the NCL, we found significantly higher error rates in both tasks, compared to the respective training level, while the performance following vehicle infusion remained statistically undistinguishable from training. This means that only the AP5 treatment had an adverse effect on performance. A comparison of the error increase under both treatment conditions relative to the training level showed significant differences between AP5 and SAL conditions in both tasks. Thus, NMDA receptor blockade already leads to impairments in the SMTS task which requires response selection only. It also causes performance deficits in the DMTS task which requires response selection plus maintenance, but DMTS does not additionally decrease performance. Although performance in both tasks deteriorated following NMDA receptor blockade, it did not decrease to 50% chance level (81.46% correct in SMTS, 71.7% correct in DMTS). Chance level performance could be expected if all stored information about the task became temporarily unavailable due to the NMDA receptor antagonism. However, pigeons to some extent seemed able to remember the basic S–R associations of the tasks. Presumably our infusions did not affect all NMDA receptors in all NCL areas, thus overall NMDA receptor activity was not com-

pletely stopped, but instead reduced, leading to the observed impairment.

4.2. Performance in the working memory task

AP5 treatment during the DMTS task, which requires the working memory components maintenance and selection, resulted in similar deficits as NCL lesions [14,22] This is also true for nonspatial DMTS tasks, in which NCL-lesioned pigeons showed deficits similar to those shown by the AP5 group, in all delay durations (1–2 s), and also in a delay of 0 s [14]. An SMTS task without any short term memory load, however, was not performed with these animals. In many studies, lesions of mammalian PFC are also reported to lead to deficits in spatial [21,60] and non-spatial [50] working memory tasks, although there are exceptions where performance in spatial tasks after excitotoxic NMDA PFC lesions in rats [33] and following electrolytic lesions [28] is not impaired. A comprehensive review of studies on working memory in frontal patients with lesions of dlPFC found that performance in simple span tasks, requiring only short term memory, was never impaired whereas performance in delayed responding was significantly reduced in most cases [11]. A possible interpretation of these results might be that perhaps it was not the maintenance component, but the inability to select responses which caused the observed impairment.

In our study, a separate analysis of the performance deficits in the individual delays (Fig. 4) shows that following AP5 infusion, the error increase was delay-independent, i.e., statistically indistinguishable in all delays, regardless of a low or high short term memory load. Only in the control condition there was a non-significant tendency towards higher error rates in longer delays. These results resemble those found in a study comparing the effects of D1 and NMDA receptor blockades on spatial working memory, resulting in dose- and delay-dependent impairments only after D1 blockade, while the NMDA receptor blockade only caused delay-independent, chance level performance at all delays [1].

4.3. Increasing task difficulty by introduction of delay periods does not lead to an increase in error rates

When comparing error increases in both tasks under both treatment conditions, we do not find a significant effect of task difficulty on performance changes. Given that the DMTS task is more demanding, as it requires not only response selection, due to its additional requirement of stimulus maintenance, error rates should have increased compared to SMTS, provided NMDA receptors in NCL were needed for short term memory. Moreover, if NMDA receptors in NCL were implicated in both functions, we should have found a more prominent error increase in AP5 than in SAL. However, in both groups there was no statistically significant error increase difference between SMTS and DMTS. So neither availability nor unavailability of NMDA receptors during the task seem to have any effect upon performance with regard to the additional memory load. Consequently, we only find a highly significant main effect of treatment, indicating that the NMDA receptor blockade by AP5 impaired performance in both tasks to a similar extent (Fig. 5).

The only study providing evidence for effects of local NMDA receptor blockade in rat PFC [2] on working memory in a spatial task (delayed nonmatching to place), using a different NMDA antagonist (CPP), reports mixed results. There was no decrease in the percentage of correct responses after infusion of different doses into dmPFC and dlPFC, respectively, compared to vehicle infusion. However, non-cognitive deficits such as increase in the percentage of omissions and latency of sample presses occurred after NMDA receptor blockade in dmPFC, but not in dlPFC. So while PFC lesions and D1-receptor blockades obviously have an impact on working memory performance, NMDA antagonists do not. Consequently, NMDA receptors are either not implicated in stabilizing cellular assemblies that maintain information during delays, or this function is swiftly compensated for by other means.

So there is converging evidence from lesion and receptor blockade studies in PFC and NCL showing a participation of mammalian and avian prefrontal areas for delayed matching and responding tasks; these results are in general compatible with our findings. Our findings, however, extend these results by demonstrating that NMDA receptor based prefrontal functions are required also in matching tasks which do not make demands on short term memory.

4.4. Performance in response selection

To our knowledge, up to now no experiment specifically studied the importance of NMDA receptors in PFC for response selection. A number of studies demonstrated that NMDA receptor antagonists in various brain regions cause acquisition deficits for different types of tasks [5,12,31,48], among them simple stimulus discrimination tasks [3,46], but usually do not lead to performance deficits in a previously acquired stimulus discrimination [10,31,46]. Thus, the observed performance deficits in a previously trained SMTS task supposedly hint at a role of NMDA receptors in NCL which goes beyond a function required for acquisition of a stimulus discrimination.

4.5. NMDA receptors in the avian NCL have a function for response selection rather than for maintenance in working memory

In summary, we found that with NMDA receptor blockade in NCL, performance is impaired to a similar extent in two tasks, one of which requires short term memory, while the other does not. It appears that NMDA receptor blockade impairs the component common to both tasks, i.e., response selection.

Response selection is found impaired after PFC lesions in rats and monkeys, in particular after ventrolateral PFC lesions [44,62]. However, there appear to be at least two different types of response selection, only one of which can be attributed to PFC, as fMRI data in humans indicate that PFC selects responses only between competing alternatives, while parietal cortex activates responses on the basis of learned S–R associations [8]. A common feature of the two tasks used in our study is that in the response selection component, a choice has to be made between two responses which both are—on principle—correct, and can therefore be considered 'competing responses'. In order to choose the correct one in a given trial, consideration of the context information delivered by the sample stimulus is indispensable. In the SMTS task, on principle, it could be possible to learn patterns composed of the three pecking keys and the associations with the subsequent respective responses by rote, instead of using the sample stimulus as a contextual indicator. This would transform the task into a simple set of S–R associations. Two reasons make it unlikely that the pigeons used such a strategy.

First, performance in a simple discriminative S–R association task is mostly unimpaired by temporary inactivation of NMDA receptors [10,46] in various brain regions. In a previous study, we too found that NMDA receptor blockade in NCL did not impair correct responding with regard to an established, constant S–R association [38].

Second, it is not possible to use a similar strategy for the DMTS task: there are no patterns which can be unambiguously associated to a response. So, if we proposed such a learning strategy for the SMTS task, we would have to assume two different, and differentially impaired, processes for the SMTS and the DMTS task, respectively, which nevertheless produced similar deficits in performance and which were both dependent on NMDA receptor activation in NCL. Such an explanation would be much less parsimonious than assuming that the deficits arose from an NMDA receptor dependent task requirement present in both tasks, the selection of a contextually adequate response.

Thus, it seems that NMDA receptor antagonists in NCL produce deficits in performance of a well-trained task only if this task contains the requirement of response selection between competing alternatives and, in order to do so, the necessity to consider actual context information.

5. Conclusion

In summary, the results of the present study demonstrate for the first time that inactivation of NMDA receptors in the avian NCL impairs response selection in tasks requiring processing of context information, rather than impairing maintenance in working memory, since an additional working memory load does not deteriorate performance any further. Thus, NMDA receptors in the avian prefrontal cortex seem to participate in response selection, a function previously found to be mediated by ventrolateral PFC in mammals. Our results therefore provide further evidence for the functional equivalency between avian NCL and mammalian PFC.

Acknowledgements

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CHAPTER 4:

Out of context - NMDA receptor antagonism in the avian “prefrontal cortex” impairs context processing in a conditional discrimination task

Out of context - NMDA receptor antagonism in the avian "prefrontal cortex" impairs context processing in a conditional discrimination task

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Abstract

Processing of relevant context information is implicated in many prefrontally based functions such as response selection, behavioral inhibition or attention. Prefrontal cortex (PFC) lesions lead to deficits in context processing and in response selection. NMDA receptors in the mammalian PFC as well as in the NCL (Nidopallium caudolaterale) of birds, the avian functional equivalent to PFC, are involved in learning processes that also require context processing. In this study we investigated the function of NMDA receptors in the pigeon NCL for response selection and context processing in a previously trained simultaneous matching task consisting of two trial types: one requiring context processing of a conditional stimulus for correct response selection (context-dependent trials), while the other required only recall of a previously acquired stimulus-response association (fixed-response trials). Results demonstrated that NMDA receptor antagonism in NCL impaired response selection performance only in the context-dependent trials, but not in the fixed-response trials, due to increased error rates. We conclude that NMDA receptors in the avian "prefrontal cortex" are involved in response selection requiring processing of relevant context information involving conditional stimuli rather than in response selection per se.

Introduction

A primary function of the prefrontal cortex (PFC) is integration of context information to initiate appropriate behavior in a given situation. Context processing refers to the ability to integrate relevant information from the external environment and from internal states in such a form that it can be used to mediate task appropriate behavior. Response selection means choosing an adequate response from an array of alternatives. There is evidence that PFC is implicated in both functions.

Deficits in PFC-related tasks in patients with schizophrenia or frontal lesions as well as in healthy elderly people demonstrate a causal link between PFC hypofunction and context processing impairments (Braver et al., 2001; Kerns & Berenbaum, 2003; Barch et al., 2001, 2003; Cohen et al., 1999, Metzler, 2001). Lesion studies in rats (Morgan & LeDoux, 1999) and single cell recordings in monkeys (Watanabe et al., 2002) also demonstrate the involvement of the PFC in context processing. Consequently, a model of PFC function (Servan-Schreiber et al., 1996; Cohen & Servan-Schreiber, 1992) proposes a prefrontal context-processing function subserving various PFC-related functions which are usually treated and investigated independently.

In many instances, response selection requires processing of relevant context information, e.g. in conditional discrimination tasks (Winocur & Eskes, 1998). In other instances, however, context information might be negligable for response selection, as in stimulus-response associations that always require the same response to the same stimulus (Delatour & Gisquet-Verrier, 1996). Ventrolateral PFC lesions in rats and monkeys cause deficits in response selection, especially in conditional associative learning tasks (Petrides, 1982, 1987; Winocur & Eskes, 1998; Bussey et al., 2001). Ventral PFC is considered essential for both conditional associative learning and response selection, since information about stimulus, response, and response outcome is available only in this region (Passingham et al., 2000). In humans, an fMRI-study found dlPFC to mediate response selection (Schumacher & D'Esposito, 2002), while an rTMS study reported dlPFC involvement in response selection even without short term memory load (Hadland et al., 2001). Another fMRI study found human PFC to be involved only in response selection between competing responses, while parietal cortex activated responses based on learned S-R associations (Bunge et al. 2002).

In rats and pigeons, prefrontal NMDA receptors participate in reversal and extinction learning (Bohn et al., 2003; Lissek et al., 2002, Lissek & Güntürkün, 2003). Since these learning procedures require the context to be considered in order to alter existing S-R

associations, prefrontal NMDA receptors seem to participate in context processing during learning. However, it is unclear whether prefrontal NMDA receptors are also involved in context processing and response selection during well-trained tasks, as NMDA receptor blockade in other brain regions did not impair performance of previously learned tasks (Kelley et al., 1997, Smith-Roe et al., 1999; Bohn et al, 2003). In a previous study (Lissek & Güntürkün, 2004, in press), we found impaired performance in matching tasks that also points at deficits in response selection after NMDA receptor blockade in the pigeon "prefrontal cortex", the Nidopallium caudolaterale (NCL). The NCL is an area in the avian forebrain considered functionally equivalent to mammalian PFC based on neuroanatomical (Kröner & Güntürkün, 1999; Leutgeb et al., 1996; Waldmann & Güntürkün, 1993, Metzger et al., 1998), electrophysiological (Kalt et al., 1999, Watanabe, 1996) and behavioral (Mogensen & Divac, 1982, 1993; Gagliardo et al., 1996, 1997; Güntürkün, 1997; Hartmann & Güntürkün, 1998; Diekamp et al., 2001, 2002; Güntürkün & Durstewitz, 2000) data.

Unfortunately, it is very difficult to disambiguate context processing and response selection and some evidence cited above might involve more than one of these processes. To investigate the role of NCL-based NMDA receptors for response selection during performance of a previously acquired task, we trained pigeons in a novel adaptation of an SMTS task that enabled us to differentiate between response selection processes with or without context processing.

Materials and Methods

Subjects

Subjects were 9 unsexed and experimentally naïve pigeons (Columba livia), obtained from local breeders. All animals were individually caged in a temperature- and humidity-controlled room on a 12-hr light-dark schedule. During experiments, they were maintained at 80 % of their free-feeding weight and received water and grit ad libitum.

Apparatus

A conventional Skinner box (36 cm long x 34 cm high x 36 cm wide) was used for training and experiments. The Skinner box was equipped with three pecking keys and a solenoid-operated food hopper and was computer-controlled by means of a digital input/output board controlled by the special OLCUS (Operant Learning Conditioning Unit System)

software. The three pecking keys (2.5 cm in diameter) were arranged in a horizontal row on the backwall of the Skinner box (18.5 cm above the floor). The food hopper was located beneath the center key. On the pecking keys white light was displayed during pretraining sessions, blue, yellow, red and green lights were displayed during training and experimental sessions in the simultaneous matching to sample (SMTS) task. The Skinner box was illuminated by a houselight.

Matching task with context-dependent and fixed-response trials

Based on an SMTS task, we devised a novel matching task enabling us to differentiate two forms of response selection by using two different trial types: context-dependent trials and fixed-response trials (see illustration in figure 1). The context-dependent trials were canonical SMTS trials, in which the contextual indicator delivered by the sample color must be considered for correct response selection. For the context-dependent trials, we used a combination of two colors (yellow and blue). At the beginning of each trial, one of these colors appeared on the sample key, after the pigeon's response to this sample key the two matching keys were lit additionally, and the pigeon's task was to respond to the matching key displaying the same color as the sample key. In the fixed-response trials, this sequence was the same, however, color combinations were different: each of the two colors used in the context-dependent trials was paired with a different color, resulting in two color combinations: yellow and green, blue and red. In these pairings, yellow and blue were always correct.

In context-dependent trials, both colors presented in a trial could in principle be correct. Therefore, for correct response selection in a given trial, processing of the conditional context, delivered by the sample color, was indispensable. In contrast to this, in fixed-response trials the response to one color of each pair was always wrong, while responding to the other was always correct. Since the fixed-response trials constituted basically a simple S-R association, the correct response could be selected without processing the context information delivered by the sample color. Since for both trial types, only the color hinting at the correct response could be presented on the sample key, pigeons could not anticipate during the sample phase which trial type was to follow.

Thus we combined within one task trials containing conditional associations (Iversen, 1997) with trials consisting of simple S-R associations. If NMDA receptors in NCL were involved in response selection per se, we would expect deficits to occur in both trial types. If NDMA receptors, however, were involved in context-dependent response selection only,

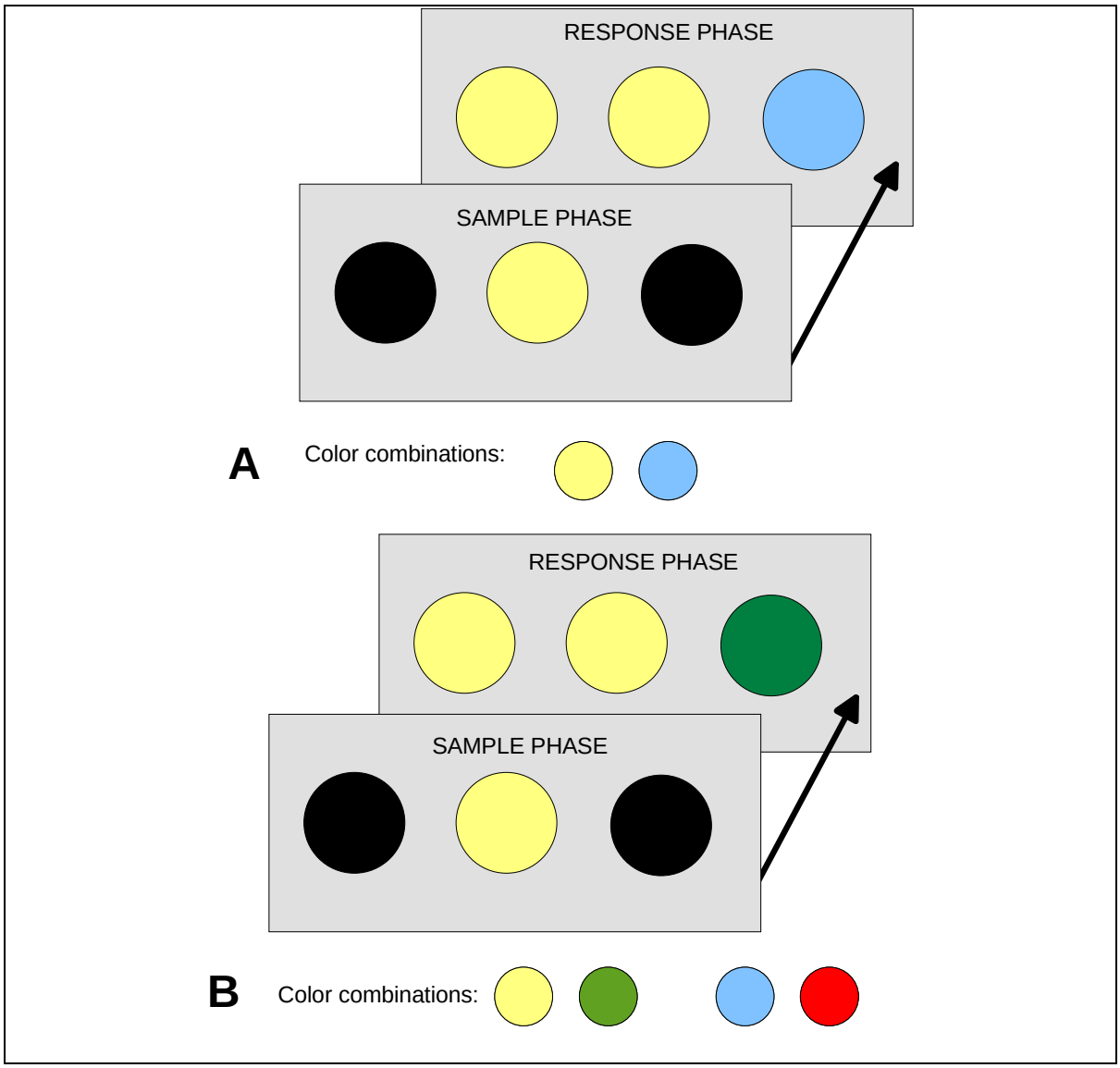

Fig. 1. Examples for context-dependent (A) and fixed-response (B) trials in the matching task. In context-dependent trials, a combination of two colors (yellow-blue) was used, with the correct response depending on the sample key color (yellow or blue) in a given trial. In fixed-response trials, these two colors were each combined with a different color, resulting in two color combinations: yellow-green and blue-red. In these combinations, however, yellow and blue were always the S+, while green and red were always the S-. Thus while in both trial types the sample key delivered the context information indicating the correct response, this information was necessary for correct responding only in the context-dependent trials, whereas in fixed-response trials it could be ignored.

we would expect deficits only for the context-dependent, but not for the fixed-response trials.

Each session consisted of a total of 80 trials, i.e. 40 trials of each trial type, presented in randomized order. Each trial of the task started with the presentation of the sample stimulus on the center key. 10 responses to this key led to the additional presentation of the matching stimuli on the lateral keys. Responding to the lateral key showing the color matching the sample key gave 3 sec access to the feeder. Responding to the nonmatching

color resulted in a timeout of 15 seconds, during which all lights, including the houselight, were switched off. Trials were repeated only when there was either an untimely response to the lateral keys during the presentation time of the sample stimulus, or when there was no response to the lateral keys during the response phase, i.e. the presentation of the matching stimuli.

The frequency of color pairs appearing on the matching keys thus was not balanced, since the combination blue-yellow was presented 40 times in each session, whereas the combinations blue-red and yellow-green appeared 20 times each in each session. But since there was no way to achieve balancing of color combinations without compromising the balancing of trial types, and since we hypothesized that the conditional association was harder to learn than the simple S-R association, we decided to prefer unbalanced color combinations to unbalanced trial types. By balancing the frequency of trial types, in any case, we gave the animals equal opportunities to acquire both associations.

Pretraining in the Matching Task

After an autoshaping procedure, in which pigeons acquired the association between responding to a single pecking key illuminated by white light and subsequent food reward, pigeons were trained in the matching task. Training was continued for each individual animal until the learning criterion of at least 90 % correct responses for each trial type was reached in five subsequent training sessions. The percentage of correct responses during the last 5 sessions of training was 96.28 % (0.572 s.e.m.) for the context-dependent and 99.83 % (0.167 s.e.m.) for the fixed-response trials.

Surgery

For surgery, pigeons were anesthetized with Ketamine-Rompun (40 mg/kg and 8 mg/kg, respectively, im). Aiming at the NCL, two stainless steel cannulas per hemisphere were vertically inserted under stereotaxical guidance (Karten & Hodos, 1967) to reach the following coordinates: A 5.25, L 5.00 and A 5.25, L 7.50. Cannulas were inserted to 1 mm below the brain surface and were secured with dental acrylic. After 5-6 days of recovery, pigeons were tested for retention of the matching task, the criterion was 80 % correct responses.

Experimental Sessions

We used a within-subjects-design: each pigeon was alternately tested under both treatment conditions: blockade of NCL using the competitive NMDA receptor antagonist DL-AP5 (Sigma-Aldrich) or infusion of vehicle (0.9 % NaCl - saline solution). In total, 10 experimental sessions were conducted, 5 sessions for each condition.

Immediately before each of the experimental sessions, pigeons received bilateral infusions of either the competitive NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (DL-AP5) or vehicle (saline solution, 0.9 % NaCl) locally into the NCL. AP5 was dissolved in saline solution (total volume = 2 µl, containing 10 µg DL-AP5, 0.5 µl, i.e. 2.5 µg DL-AP5 per cannula). We aimed at producing only localized diffusion by using small volumes of fluid and applying a concentration which in previous studies with pigeons had proved effective but did not produce motor or motivational deficits (Lissek et al., 2002; Lissek & Güntürkün, 2003, 2004). Infusions were made through interior cannulas protruding 1 mm from the tip of the guide cannulas into the brain tissue. We used a microinfusion pump equipped with two 1 µl-Hamilton (Reno, NV) syringes to deliver the volume at a flow rate of 0.2 µl/min. Afterwards, the infusion cannulas remained in place for another 2 min to allow for diffusion of the infused volume. To infuse through all four cannulas, we performed this procedure twice. Immediately after the infusion procedure, which took about 12-15 min, the pigeons had to perform the task. One session per day was conducted. In order to prevent sequence effects, pigeons were infused on successive days alternately with either AP5 or vehicle, with the first infusion being AP5 in half of the subjects, and vehicle in the remaining half.

Histology

To enable reconstruction of the locations of the guide cannulas, we perfused the pigeons intracardially with 0.9% (wt/vol) saline (40°C) and a 4% (wt/vol) paraformaldehyde solution (4°C). The brains were removed, postfixed, and cut into 40 µm frontal slices on a freezing microtome. After staining the slices with cresyl violet, the positions of the cannula tips were reconstructed at intervals of 500 µm from A 4.00 to A 8.00 and transferred onto standard sections from the pigeon brain atlas [Karten & Hodos, 1967].

Statistical Analyses

During the experimental sessions, we registered the number of correct responses and errors made separately for the two trial types of the matching task. We compared the errors during the 2 x 5 experimental sessions (NMDA receptor blockade and vehicle infusion) with the performance in the last 5 training sessions by means of ANOVA with repeated measures. By means of a T-test for matched samples, we compared the percentual error increase in both trial types. The different types of errors (spatial, color, spatial+color and other errors) which animals could make were determined, counted and compared between treatments and over sessions by means of ANOVA with repeated measures. Moreover, we compared the number of missed trials (trials passed without a response of the pigeon) in training and experimental sessions and between the two trial types.

Results

Histology – Locations of the cannulas

All cannula injection sites were located within the NCL, within a range of +/- 0.5 mm from the target location A 5.25 according to the pigeon brain atlas (Karten & Hodos, 1967) (see figure 2). Results from a pilot study evaluating the spread of a 0.5 µl volume by injecting the fluorescent tracer rhodamine isothyioncyanate, known for its wide diffusion area, demonstrated an average spread of 1 mm in diameter around the tip of the cannula. A study considering diffusion of [^{3}H]-AP7, which has diffusional characteristics supposedly identical to DL-AP5, in the rat hippocampus (Morris et al., 1989), found that with an infusion volume of 1 µl (twice the volume we infused per cannula) and a concentration of 10 mM, radiation values had dropped to about 50 % at 1.5 mm around the actual infusion site, and to almost 0 % at 3 mm around the infusion site. These results support our assumption that the spread of an infusion volume of 0.5 µl per cannula, placed at coordinates anterior A 5.25 and lateral L 5.00 and 7.50, was largely restricted to the NCL, which has an anterior-posterior extent of 3.5 mm (A 3.75 to A 7.25) and a lateral-medial extent of 5 mm (L 3.50 to L 8.50) (Karten & Hodos, 1967; Waldmann & Güntürkün, 1993).

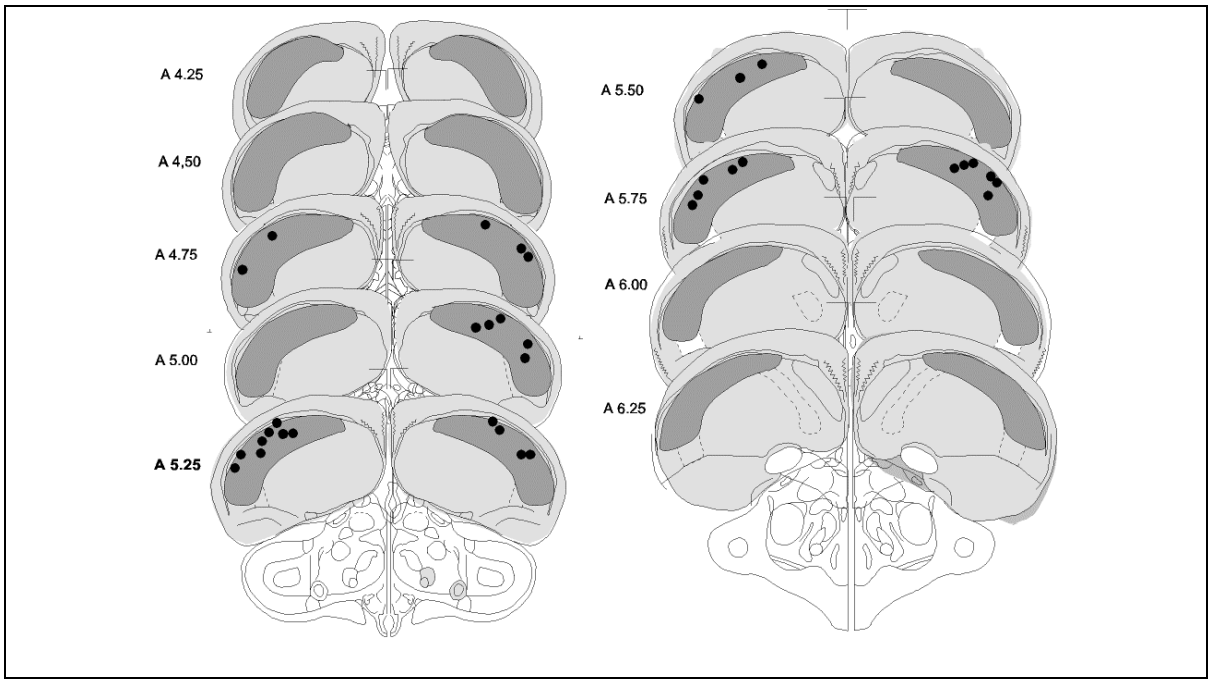

Fig 2. Schematic frontal sections of the pigeon brain showing the injection sites for AP5 and or SAL. Dots represent the lower tips of the cannulas, numbers represent the distance (anterior) to the center of the ear bars, boldface indicates the frontal plane level at which cannulas were aimed. The NCL according to Waldmann & Güntürkün (1993) is depicted in dark grey. Figure adapted from graphs in the Stereotaxic Atlas of the Brain of the Pigeon (Karten & Hodos, 1967).

Retention session

In the retention session performed 5-6 days after surgery, all animals reached the required performance criterion of min. 90 % correct responses.

Performance in the experimental sessions

After the successful retention session, pigeons were tested for their performance in a total of 10 experimental sessions, 5 for each of two experimental conditions: NMDA receptor antagonism in NCL and vehicle infusion. Although all subjects completed all sessions well above chance level (chance level: 50 % correct responses), demonstrating that they were still able to perform the task, there were obvious impairments under NMDA receptor blockade revealed by an increase in errors in the context-dependent, but not in fixed-response trials.

Percentage of errors in training, after NMDA receptor antagonism and vehicle infusion

We evaluated the performance of pigeons in the task by comparing the percentage of errors the animals made during training (TRAIN), with NMDA receptor blockade in NCL (AP5), and following vehicle infusion (SAL) in context-dependent and fixed-response trials, respectively. Mean values of context-dependent errors were: AP5 14.89 % (+/- 2.54 s.e.m), SAL 6.16 % (+/- 3.72 s.e.m), TRAIN 3.72 % (+/- 0.16 s.e.m). Mean values of fixed-response errors were: AP5 0.28 % (+/- 0.12 s.e.m.), SAL 0.16 % (+/- 0.16 s.e.m.), TRAIN 0.16 % (+/- 0.16 s.e.m.). An Anova with repeated measures and the two within-subjects-factors treatment and trialtype gave a significant main effects of treatment ($F(2) = 16.434$ $p<.001$) and trialtype ($F(1)= 37.163$ $p<.001$. The treatment*trialtype interaction was also significant ($F(2)= 17.043$ $p=<.001$), indicating that the AP5 treatment impaired performance only in the context-dependent trials, but not in the fixed-response trials, while the SAL condition did not impair performance at all. (see figure 3)

Percentage of error increases in the two experimental conditions compared to training

A direct comparison of the error increase in percent in the two experimental conditions of AP5 and SAL by means of T-tests for matched samples showed a significant difference only for the context-dependent ($T(8)= 5.832$ $p<.001$), but not for the fixed-response trials ($T(8)= 1.242$ $p=.249$). Thus while NMDA receptor blockade impaired performance in the context-dependent trials significantly, it had no deteriorating impact on the fixed-response trials (see figure 4).

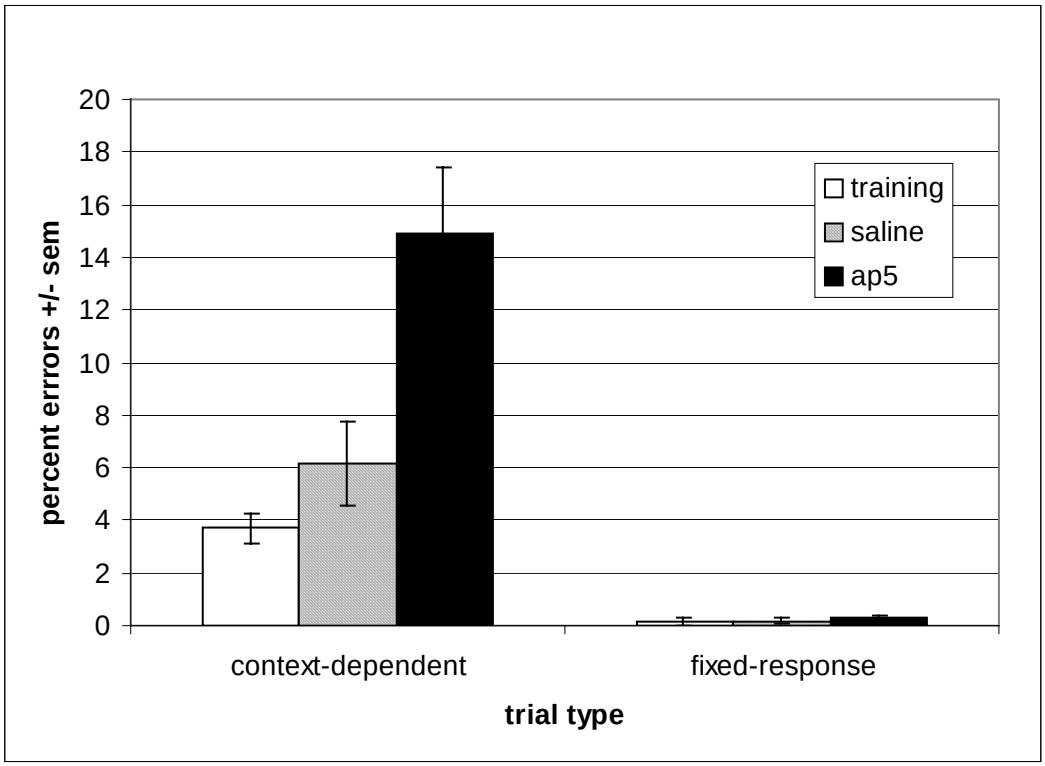

Fig 3. Percent errors in three different treatment conditions: training (TRAIN), after saline infusion (SAL), and NMDA receptor antagonism (AP5) in the different trial types. There was a significant main effect of treatment (F(2)= 16.434 p=.000), of trial type (F(19=37.163 p=.000) and a significant treatment*trialtype interaction (F(2)=17.043 p=.000), indicating that AP5 treatment impaired performance only in the context-dependent, but not in the fixed-response trials, while the SAL condition did not impair performance at all.

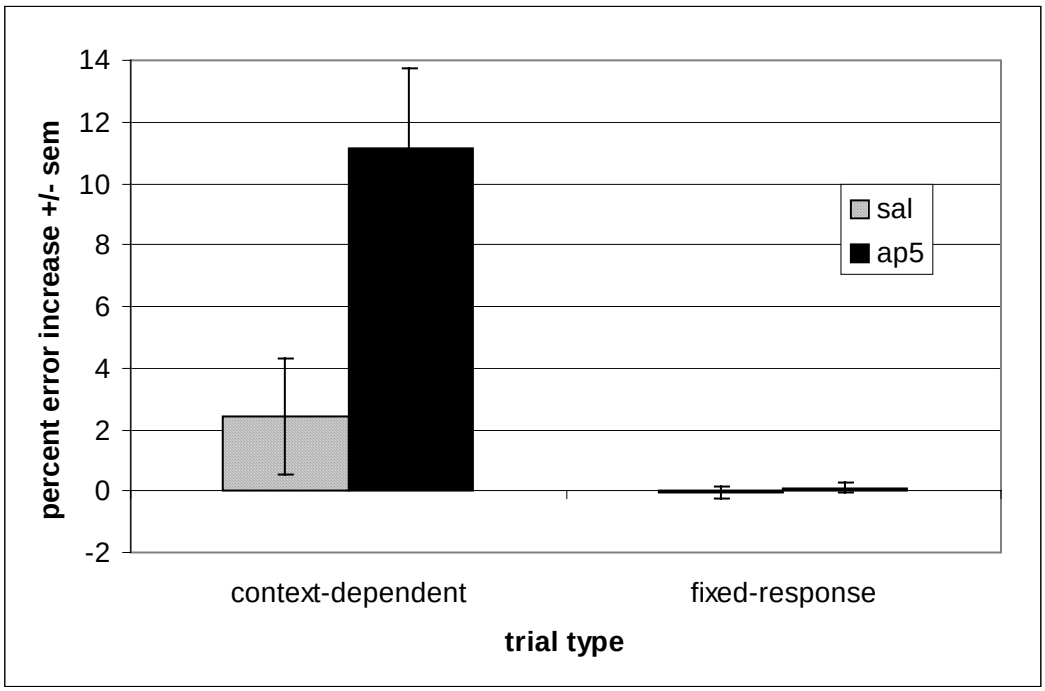

Fig. 4. Percentage of error increases in the two experimental conditions NMDA receptor blockade in NCL (AP5) and vehicle infusion (SAL) as compared to training. T-Tests for matched samples demonstrated a significant difference only for context-dependent (T(8)=5.832 p=.000), but not for fixed-response trials (T(8)= 1.242 p=.249).

Comparison of different error types

By analyzing the different error types we aimed at identifying behavioural strategies which might contribute to the overall error increase observed in the AP5 condition. We identified four types of errors that pigeons could commit during the task: spatial, color, combined spatial+color, and random errors. A color error was defined as an error occurring due to the fact that the pigeon responded wrongly to the same color as in the previous trial, regardless of its location. A spatial error was defined as an error occurring due to the animal responding wrongly to the same spatial location, regardless of its color, as in the previous trial. A combined spatial+color error was defined as an error occurring because the animal responded wrongly to the same spatial-color combination as in the previous trial. Under the term random errors we subsumed all errors that did not fit into one of the above categories, i.e. errors that occurred because animals choose a different color-location combination than in the previous trial for their next response. In almost all cases, the response that was made before an error occurred was correct.

Within these error types, strategic tendencies towards perseveration are reflected by an increase in color and spatial errors that is higher than an increase of random errors; whereas a strategic tendency to adhere to the "color" rule is reflected in more color than spatial errors. If both strategic tendencies add up in behaviour, most errors should be of the color+spatial type.

A comparison of the mean values of the four error types made under different treatment condition, by means of ANOVA with repeated measures, showed highly significant main effects of treatment ($F(2)= 16.064$ $p<.001$), of errortype ($F(3)= 14.210$ $p<.001$) and a significant treatment*errortype interaction ($F(6)=2.529$ $p=.033$) (see figure 5). Planned comparisons of the three treatment conditions within each error type showed significant differences of AP5 vs. TRAIN in color errors ($F(1)=38.227$ $p<.001$), color+spatial errors ($F(1)=17.067$ $p=.003$) and random errors ($F(1)= 6.916$ $p=.030$). Spatial errors in the AP5 treatment did not increase relative to TRAIN ($F(1)= 4.360$, $p=.070$). Following SAL treatment, no errortype showed significant changes relative to training (spatial $F(1)= 1.164$ $p=.312$; color $F(1)=.626$ $p=.452$; color+spatial $F(1)=3.651$ $p=.092$; random $F(1)=.016$ $p=.902$). Moreover, differences between SAL and AP5 were statistically significant for the following error types: color ($F(1)= 48.167$ $p<.001$), color+spatial ($F(1)= 11.405$ $p<.01$) and random errors ($F(1)= 8.475$ $p<.05$). Spatial errors did not differ significantly between experimental groups ($F(1)= 4.414$ $p=.69$).

Taken together, the results demonstrate that, with the exception of spatial errors, absolute values of all error types increased following NMDA receptor blockade compared to SAL and TRAIN. Moreover, in the AP5 condition, a tendency towards perseveration and adherence to the "color" rule is visible in the increase of color+spatial errors.

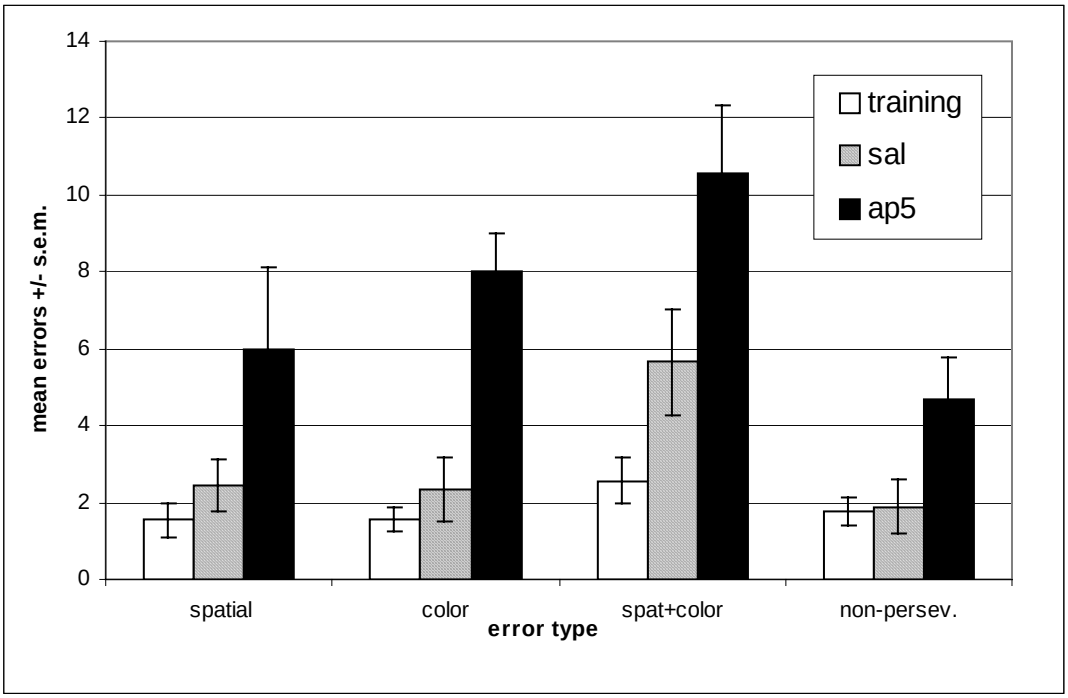

Fig 5. Comparison of the amount of four different types of error committed with NMDA receptor blockade (AP5), vehicle infusion (SAL) and training (TRAIN). Spatial errors did not differ significantly neither between TRAIN and SAL nor between TRAIN and AP5. All three other error types also did not differ significantly between TRAIN and SAL; however, these error types all differed significantly both between TRAIN and AP5 and SAL and AP5 (see results section).

Missed trials in training and experimental sessions

We counted the number of missed trials (timeouts) in two phases of each trial: during presentation of the sample color (sample phase) and during additional presentation of the matching colors (matching phase). The sample phase in each trial lasted 120 seconds, the matching phase 5 seconds. If pigeons did not respond to the sample key or one of the matching keys, respectively, during these phases, either a sample timeout or a matching timeout was registered. We further differentiated between matching timeouts in context-dependent and fixed-response trials.

Timeouts in the sample phase increased in both SAL and AP5 conditions significantly, compared to the training level as indicated by a significant treatment effect ($F(2)= 9.633$ $p<.01$). However, planned comparisons demonstrated that there was no statistical difference between the increases in each of these experimental conditions. ($F(8)= .944$

p=.360), indicating that if there was a motivational deficit in responding to the sample key, it was related to both experimental treatments and not due to the NMDA receptor blockade. (see figure 6).

Timeouts in the matching phase did not change as much as timeouts in the sample phase did. In fixed-response trials, there was no significant difference between the three treatment conditions (F(2)=.236 p=.793). In context-dependent trials, again there was no significant treatment effect (F(2)=.948 p=.408), however, planned comparisons demonstrated a significant difference between the SAL and AP5 condition (F(8)=9.689 p=.014), due to the fact that timeouts decreased slightly less in the SAL condition than in the AP5 condition, but not between the TRAIN and AP5 conditions (F(8)= 1.227 p=.300). Comparable to the results regarding the sample timeouts, matching timeouts too indicated that there was no deficit in AP5-treated animals with regard to motivation and distractability.

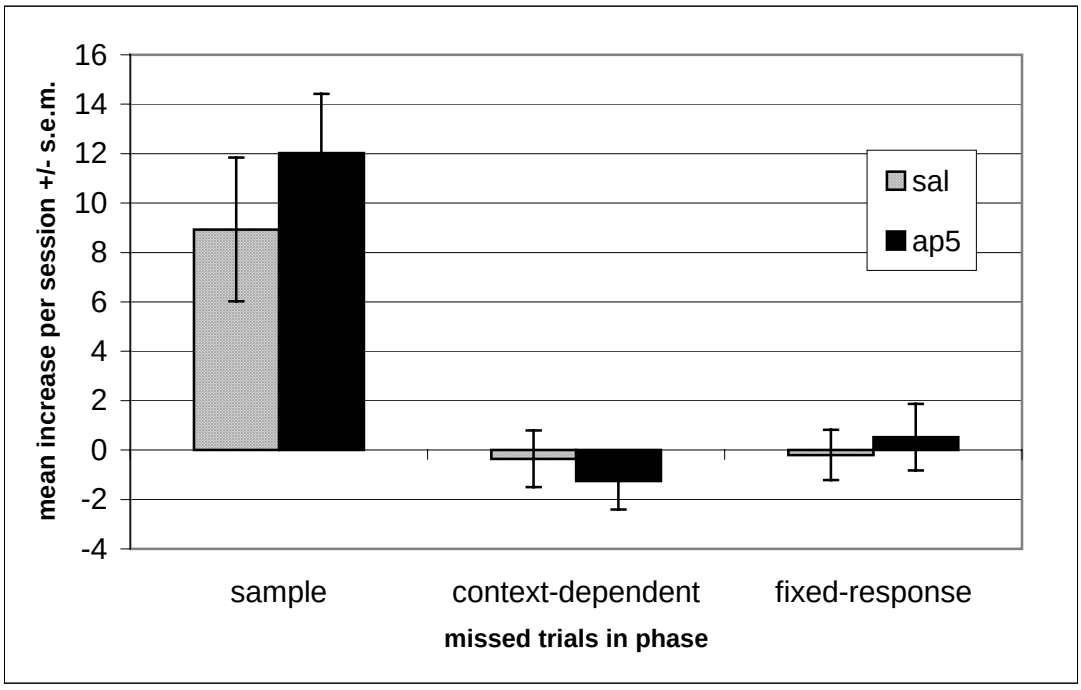

Fig 6. Increase in missed trials compared to training in the two experimental conditions NMDA receptor blockade in NCL (AP5) and vehicle infusion (SAL) during the sample phase and the response phase of context-dependent and fixed-response trials.

Discussion

The main result of our experiment is: NMDA receptor antagonism in NCL significantly impairs performance in the context-dependent trials, but not in fixed-response trials. Thus, NMDA receptor activation appears necessary for response selection based on contextual information, but not for response selection requiring recall of fixed S-R associations. The observed impairments were not attributable to increased distractability or to deficits in motivation, and response selection in the SMTS task was not based on fixed color pattern-response associations. Together these data show that prefrontal NMDA receptors participate in response selection based on integration of contextual information.

Prefrontal NMDA receptors mediate context-dependent response selection

Blockade of NMDA receptors in the pigeon NCL led to significantly more errors in context-dependent trials than during training and after infusion of vehicle. In contrast, NMDA receptor blockade in NCL did not have any deteriorating impact on performance in the fixed-response trials compared with both the vehicle infusion and the prior training.

The unimpaired performance in the fixed-response task corresponds to the general notion that NMDA receptor antagonism in various brain areas does not impair the recall of a previously acquired association, while the deficits in the context-dependent task are at odds with these findings. Although there are a few exceptions (Roesler et al., 1998; Lee et al., 2001), unimpaired recall has been demonstrated for various conditioning scenarios (Xu et al., 2003; Di Ciano et al., 2001; Churchill et al., 2001; Bohn et al., 2003; Baron & Moerschbaecher, 1996; Kelley et al., 1997, Smith-Roe et al., 1999). However, in most of these studies, the requirement was to recall previously acquired, unambiguous stimulus-response associations, with one stimulus being the S+ and the other being the S-. This requirement applied also to the fixed-response trials of our study. In contrast to this, our context-dependent trials involved conditional stimulus-response associations, in which both stimuli potentially could be the S+, with the actual S+ in a given trial being indicated only by the sample color. The context-dependency of this conditional discrimination might be particularly sensitive to NMDA receptor antagonism in NCL. Our results also correspond to PFC lesion studies reporting deficits in conditional discrimination in rats and monkeys (Winocur & Eskes, 1998; Petrides, 1982, 1991).

It is even possible that unambiguous stimulus-response associations do not implicate prefrontal areas at all: an imaging study with human participants demonstrated that only response selection for competing responses, requiring a choice between potentially correct response alternatives, involves activation of PFC, while response selection based on previously acquired S-R associations only involves parietal cortex (Bunge et al., 2002). In our study, competing responses were present only in the context-dependent trials, but not in the fixed-response trials. In line with this findings, it is conceivable that neither NCL in general, nor NMDA receptors in NCL in particular, might contribute to response selection required in the fixed-response trials, explaining why our NMDA-blockades had no effect upon this task. Thus, NMDA receptors in NCL are implicated in response selection only during the context-dependent task.

There is evidence that tasks requiring either permanent changes of previously acquired stimulus-response associations during the course of a session, or temporary within-session switches between competing response alternatives, are both impaired by prefrontal NMDA- receptor antagonism. Accordingly, reversal and extinction learning, requiring permanent changes in stimulus-response associations, were found impaired after NMDA receptor blockade in the rat PFC (Bohn et al., 2003) and in the pigeon NCL (Lissek et al., 2002; Lissek & Güntürkün, 2003). Set-shifting, requiring temporary switching between in principle correct response alternatives, also was found impaired with NMDA receptor blockade in the rat PFC (Stefani et al., 2003). Common to these different tasks is the necessity to consider actual contextual information for selection or acquisition of the correct response in the presence of response alternatives. It is therefore possible that the underlying processes could both be dependent on NMDA receptor activation in prefrontal areas.

Combining these results with the present findings, it it conceivable that NMDA receptors in NCL are involved not only in permanently altering previously established stimulus-response associations, but also in temporary switching between two competing stimulus-response associations. In summary, NCL-based NMDA receptors appear to be involved in response selection requiring the processing of context, but not in response selection requiring only recall of learned S-R associations from reference memory.

NMDA-antagonism induced impairments in the context-dependent task occur due to deficits in using contextual information necessary to apply a conditional rule

The error increase in context-dependent trials might be related to deficits representing the conditional rule contained in this type of task, as was argued in a study with PFC-lesioned subjects (Winocur & Eskes, 1998). Recall of the conditional rule, however, did not appear to be completely lost due to NMDA receptor blockade in the NCL, but rather temporarily compromised. We did not observe a drop to chance level performance which would be expected if the rule was completely unaccessible. Thus it appears that the contextual information guiding the response based on the conditional rule, rather than the conditional rule itself, was often disregarded, leading to increased errors.

Conditional associative learning appears to be highly sensitive to damages to the PFC, as demonstrated by a number of studies in rats (Winocur, 1991; Passingham et al., 1988), monkeys (Petrides, 1982, 1991) and humans (Petrides, 1985, 1991). Deficits in rule learning and response selection can coexist during conditional associative learning and PFC may participate in both functions (Stuss et al., 1994). It was suggested that in general, a PFC-based deficit in conditional associative learning performance might be reflected by impaired application of learned S-R associations and impaired use of trial-specific information in the process of selecting correct responses (Winocur & Eskes, 1998). Our results, however, indicate that – with regard to NMDA receptors in prefrontal areas of the pigeon - the observed deficits rather pertain to the latter, namely to the impaired use of response-relevant context information.

NMDA receptor-antagonism induced impairments are not attributable to deficits in motivation or to increased distractability, but are associated with a tendency towards perseveration

During the sample phase, the number of missed trials increased significantly in both AP5 and SAL treatments compared to training with no significant difference being present between these two conditions. During the response phase, there was no increase in the number of missed trials in the AP5 treatment for both trial types compared to training. The same result applied to fixed-response trials in the SAL treatment. Taken together, these results indicate that there was no specific motivational deficit or increased distractability following NMDA receptor blockade in NCL, compared to vehicle infusion, which might account for higher error rates.

The comparison of the different error types that occurred during performance of the context-dependent task demonstrated significant increases in color, color+spatial and random errors, while spatial errors were only mildly increased. The absolute number of spatial+color combination errors was highest, followed by color errors, spatial errors and random errors. These results reveal a perseverational tendency, i.e. the tendency to repeat a response that proved successful in the preceding trial, while at the same time disregarding contextual information present in the actual trial. This outcome corresponds to the generally observed perseverational tendency in subjects with frontal lesions.

Response selection in the SMTS-task was not based on fixed color pattern - response associations

It could be argued that pigeons acquired the SMTS task by forming S-R associations between the displayed color patterns (i.e. blue-blue-yellow or blue-yellow-yellow) and subsequent responses (i.e. responding to the left key or right key, respectively). During performance, they merely would have to recall these associations. Such a learning strategy would enable animals to disregard the conditional discrimination implemented in the context-dependent trials. Then, however, performance in context-dependent and fixed-response trials should remain on the same level in the AP5 and SAL conditions, which was not the case. This result excludes the possibility that animals based their responding on a recall of stimulus pattern - response associations.

Moreover, the unimpaired recall of the unambiguous stimulus-response associations present in the fixed-response trials in both AP5 and SAL conditions lends additional support to an assumption that the deficits observed in the AP5 condition during the context-dependent trials cannot be attributed to deficits in recall, as long as recall pertains only to previously acquired, unambiguous stimulus-response associations.

Conclusion

In extension of previous results demonstrating the involvement of NCL-based NMDA receptors in acquiring correct responding during various learning processes, we showed here that NMDA receptors in the pigeon ‘prefrontal cortex’ also participate in response selection during the performance of a well-trained task. Their participation appears to be confined to tasks containing a conditional rule that requires context processing for correct

response selection. In general, the findings from this study support the notion of prefrontal involvement in conditional discriminations, context processing and response selection, and in addition deliver evidence that NMDA- receptors in prefrontal areas play a key role in these functions.

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CHAPTER 5: GENERAL DISCUSSION

5. GENERAL DISCUSSION

In this thesis, the role of NMDA receptors in the NCL, the avian functional equivalent of the PFC, was investigated for various functions assumed to rely on the integrity of prefrontal areas. From the three experiments performed, a general picture emerged which hints at involvement of NMDA receptors in several functions that were already shown to be impaired by prefrontal lesions, indicating that inactivation of NMDA receptors is sufficient to cause similar deficits, and thus demonstrating their key role in these functions. In contrast, other functions, for which prefrontal participation was previously demonstrated by lesion and receptor blockade studies, were not impaired by NMDA antagonism alone, indicating that those functions may not require NMDA receptor activation, but that they may instead recruit other systems within the PFC or brain areas outside PFC.

While NMDA receptor activation, in general, seems to be necessary for processes involving alteration of existing associations and switching between such associations, NMDA receptors are obviously not involved in recall of established and unambiguous assocations, nor do they appear to participate in maintenance in working memory.

5.1 Extinction and behavioral inhibition

NMDA receptor blockade leads to deficits in extinction

Under NMDA receptor blockade in NCL, extinction of a previously learned instrumental response is impaired. The deficits result from significantly continued, perseverative responding to the formerly rewarded stimulus, despite the fact that reinforcement is not obtained any longer. In comparison to the vehicle-treated control group (SAL) and the lesion group (LES), NMDA antagonist-treated pigeons (AP5) show a significantly higher number of trials during which they continuously respond to the now nonrewarded stimulus until they finally acquire the new association.

NMDA receptor blockade does not impair behavioral inhibition

In contrast to the above findings, behavioral inhibition as measured by responding to the never-rewarded stimulus during extinction is not negatively affected. Comparable to the SAL and LES groups, the AP5 group does not display a higher ratio of responding to this stimulus than during training.

NMDA receptor blockade does not impair recall of extinction

Recall of a finally acquired extinction is also not impaired. After AP5 subjects had finally learned to refrain from responding to the no longer rewarded stimulus during the extinction sessions, they were able to recall this altered association - despite NMDA receptor antagonism - during the following two sessions testing retention of the extinction. In these sessions, AP5 animals did not respond more often to the obsolete S+ than the SAL and LES groups did.

Taken together, the results from this experiment demonstrate that the extinction deficit exhibited by the AP5 subjects results from a particular impairment in altering an existing S-R association, but not from generally impaired response inhibition. In contrast to this, retrieval of previously stored associations from reference memory, which do not need to be altered, is not negatively affected. This is shown by unimpaired performance with regard to the never-rewarded stimulus as well as during extinction recall.

Behavioral inhibition in the extinction task and in go/no-go tasks

The extinction task can be considered as a special form of a go/no-go task, with the exception that there are no separate go and no-go trials, but the go/no-go requirement is incorporated within each single trial: first there is a no-go phase (red), in which pigeons have to refrain from responding, followed by a go phase (green), during which responding is obligatory in order to receive the reinforcement. Go/no-go tasks are typically used as a measure of response inhibition, and many studies show that PFC-lesioned subjects (human or animal) are impaired in withholding responding during no-go trials. Thus, the results presented here, both for the AP5 group and in particular for the LES group, appear contradictory to these findings in mammals and to previous studies on birds. However, those studies often reported deficits particularly for acquisition and reversal (Schoenbaum et al., 2002; Delatour & Gisquet-Verrier, 1996; Aldavert-Vera et al., 1999) of a go/no-go task, but did not test performance of a preoperatively acquired go/no-go association, on which this study focussed, at least with regard to responding to the red color. Although impairments in performance of a go/no-go task were sometimes reported (Güntürkün, 1997), other researchers did not find response inhibition impairments in performance of a stop signal task (Eagle & Robbins, 2003), which corresponds to the present results. Thus it might be possible that as far as response inhibition is concerned, deficits occur mostly

during learning or re-learning, which again might reflect a deficit in acquiring a new or altered S-R association.

For patients with frontal dysfunction, however, it was found that correct responding could be impaired even though patients were cognitively aware of the correct response (Goldberg et al, 1987; Rolls et al., 1994). This finding corroborates an assumption that the observed deficits might refer to impaired inhibition of a response realized as being inadequate, which in turn could mean that learning of the new association took place but was not implemented in behavior. On the other hand, a study testing healthy patients treated with ketamine found that performance in the WCST was only impaired during the first test session, but not during the second (Krystal et al., 2000). This finding might lend support to an assumption that during the first session the learning of new associations was negatively affected, whereas in the second session ketamine did not influence correct response selection from now-established associations, indicating that response inhibition was not impaired by NMDA receptor antagonism.

Learning as observed in animals, however, can only refer to overt behavior. Therefore the possibility cannot be ruled out completely that during extinction animals were merely impaired in inhibiting the inadequate response with regard to a new association. In any case, however, the study demonstrates that behavioral inhibition, with regard to associations that can be retrieved from reference memory, is not defective following NCL lesions and NMDA receptor antagonism. This is visible in the AP5 and LES subjects' correct performance during recall of extinction as well as concerning the never rewarded 'no-go' stimulus.

Extinction learning after NCL lesions

Unimpaired performance of the NCL-lesioned animals in the extinction session corresponds to reports of unimpaired extinction of conditioned emotional responses after PFC lesions (Gewirtz et al., 1997; Morgan & LeDoux, 1999). The result is also comparable to a study testing acquisition and reversal of a radial maze task, in which mPFC lesioned rats, although they were impaired in acquisition, showed no deficits in the reversal condition (Joel et al., 1997). Still there is quite a number of studies reporting deficits in altering S-R associations after PFC and NCL lesions (Joel et al., 1997b; Ferry et al., 2000; Hartmann & Güntürkün, 1998), therefore the results for the LES group appear to be at odds with the typical findings. However, sometimes performance recovery after PFC

lesions was reported for performance in working memory tasks (Dunnett et al., 1999; Diekamp et al., 2002), and for reversal learning (Schoenbaum et al., 2002). Such recovery may be causally related to both the time interval between surgery and experimental sessions and to additional training provided by the experimental sessions, presumably enabling other structures to take over functions that are performed by PFC in initial learning (Miller, 2000b).

In summary, the results of the extinction experiment indicate that the performance deficit found during reversal learning following NMDA receptor blockade in NCL (Lissek et al., 2002) apparently was not due to impaired behavioral inhibition in general, but rather to a selective impairment in refraining from responding to a previously rewarded stimulus. These results thus demonstrate, on the one hand, that NMDA receptor antagonism in NCL causes severe impairments in altering established S-R associations. On the other hand, they indicate that NMDA receptors are not involved in recall of previously established S-R associations, both regarding to the never-rewarded stimulus (in the extinction session) as well as concerning the previously rewarded stimulus (in the extinction recall sessions).

5.2 Maintenance in working memory and response selection

In the second experiment, we therefore tested pigeons' performance in a task requiring only recall of previously trained S-R associations, which should be unimpaired in view of the above findings, and introduced a challenge to other prefrontal functions, namely maintenance in working memory (short term memory), and response selection between two simultaneously presented alternatives, respectively.

NMDA receptor blockade impairs response selection in delayed and simulteneous matching

In the DMTS and SMTS task, response selection between two alternatives was negatively affected only under NMDA receptor blockade, compared to the performance of the same subjects during the training and SAL sessions. In spite of the error increase in the AP5 treatment condition, performance did not deteriorate to chance level, therefore information about the basic rules of the task presumably was not completely lost, but merely attenuated by NMDA antagonism in NCL.

NMDA receptor blockade does not impair maintenance in working memory

Maintenance in working memory obviously was unaffected by the pharmacological intervention, since the performance deficit was not aggravated by introducing an additional memory load: Errors in the delay of 0 seconds were as high as errors in the delays of 1 and 2 seconds. Moreover, the total percentage of errors compared to training increased to a similar amount in both the DMTS and the SMTS task.

Thus these results hint at involvement of NMDA receptors in the pigeon NCL in response selection rather than in short term memory.

Comparison of the results to previous research on NMDA receptor antagonism and working memory

With regard to working / short term memory, these results correspond to the study of Aura & Riekkinen (1999) who tested rats under local NMDA receptor antagonism in dlPFC and dmPFC in a DMTS task and did not find impairments in performance as measured by the number of correct responses. This is the only study allowing a real comparison with the present work due to the local application of the NMDA antagonist.

Further studies using systemic NMDA receptor blockade yielded controversial results, as outlined in the introduction. Some studies report delay-independent performance deficits in the spatial and non-spatial working memory tasks DMTP and DMTS (Doyle et al., 1998; Aultman & Moghaddam, 2001), causing chance-level performance in both tasks. Doyle et al. observed these delay-independent impairments for three different NMDA antagonists: for CPP, dizolcipine and (+)-HA-966, thus the effects of NMDA antagonists acting on different recognition sites were similar. Other work found no impairments at all (Popke et al., 2001; Ballard & McAllister, 2000), while again other researchers found deficits in spatial working memory (Gutnikow & Rawlins, 1996; Shapiro & O'Connor, 1992; Wilcott & Qu, 1990).

A combined behavioral and microdialysis study tested rats in spatial delayed alternation following systemic NMDA receptor blockade by ketamine and simultaneously measured DA levels in PFC. It was found that at a dose that impaired STM performance, the DA release in PFC was increased (Verma & Moghaddam, 1996).

The major problem of studies using systemic application of the NMDA antagonist, however, is that they cannot disambiguate contributions from different brain areas, i.e. PFC

and hippocampus, to working memory performance. A study combining lesions and pharmacological inactivations proposed that medial PFC and dorsal hippocampus process spatial short-term memory in parallel and can serve as a compensatory mechanism for each other. In particular PFC is more likely involved in spatial short-term memory in the range of seconds (10 seconds), while the hippocampal role might be more in the range of a longer period, from more than 10 seconds to minutes (5 minutes) (Lee & Kesner, 2003). Discrepancies between the results of the systemic NMDA antagonism studies thus could be related to the delay duration parameter.

A further idea put forward in a recent study suggests that there might be differential effects of competitive and non-competitive NMDA receptor antagonists upon working memory performance, producing impairments in a delayed nonmatch-to-sample (DNMTS) task only after intraperitoneal infusions of non-competitive, but not after infusions of competitive antagonists (Willmore et al., 2001). The site-selective effects observed in this experiment, however, are not completely consistent with results presented by other researchers, who found working memory to be impaired also following NMDA receptor antagonism by competitive antagonists (Cole et al., 1993, Doyle et al., 1998, Tan et al., 1989). However, at least one of these studies applied a conditional discrimination task (Tan et al., 1989), which is - as will be outlined in the following paragraph - a type of task potentially susceptible to disruption by NMDA receptor antagonism even if no delay is introduced. In any case, it remains doubtful whether the occurrence or non-occurrence of impairments in working memory can be attributed to the use of non-competitive or competitive NMDA antagonists, respectively.

In any case, local receptor blockades can provide better evidence for the involvement of NMDA receptors in NCL or PFC than systemic administration of the antagonist can. Based on the results of the few experiments using local prefrontal NMDA receptor antagonism, at present it appears that NMDA receptor activation is not required for maintenance in short term memory.

Combined requirements on maintenance in working memory and response selection in working memory tasks

While NMDA receptor contribution to working memory remains controversial, even when results from systemic administration are considered, PFC involvement in working memory

and short term memory has been demonstrated by a large body of evidence, both by lesion studies and D1 receptor antagonism studies.

However, at least part of these deficits might be attributable to the hardly separable requirements on maintenance and response selection present in typical delay tasks. An fMRI study found activity in dlPFC area 46 related to selection rather than to maintenance in working memory (Rowe & Passingham, 2001). Moreover, there is evidence from a large meta-analysis showing that in human frontal patients, mere maintenance of items in working memory during span tasks was never impaired, in contrast to significant impairments found in delayed responding (D'Esposito & Postle, 1999). Self-ordered responding incorporated in a WM task in particular seems to be impaired in frontal patients and non-human primates with prefrontal lesions (Petrides, 1991, 1995, Collins et al., 1998). DA depletion in PFC, however, does not have any detrimental effect on performance in self-ordered sequencing tasks (Collins et al., 1998), rendering it likely that such a task, even though containing a working memory component, is not exclusively dependent on DA-mediated processes in PFC.

A microdialysis study comparing changes in concentrations of glutamate and DA in the primate PFC during performance of two tasks differing only in their recruitment of working memory, i.e. a spatial delayed alternation task and a task with comparable motor requirements, but without a working memory component (Kodama et al., 2002), found a double dissociation with increase in DA only during the working memory task, but not during the sensory-guided task. Glutamate increased only during the sensory-guided task, but not during the working memory task. The authors conclude that the increase in DA might be beneficial for working memory only, whereas the increase in glutamate is relevant for the task in which the requirement consists of selecting the correct response according to a visual signal, i.e. associating a response with a stimulus.

Thus indirect evidence points at involvement of PFC in working memory rather insofar as the working memory requirement contains an element of response selection. Within this scenario, the involvement of DA in working memory function might pertain mainly to the maintenance aspect, while activation of glutamate receptors might be crucial for selection of responses. Given the increase in PFC DA observed after NMDA receptor blockade in PFC (Feenstra et al., 2002; Lorrain et al., 2003; Takahata & Moghaddam, 1998), and the fact that excessive DA impairs WM performance (Zahrt et al., 1997; Floresco & Phillips, 2001), it might be considered surprising that NMDA receptor antagonism would not cause

impairments in the maintenance component. Possible explanations will be discussed in a following section.

Other research comparing performance in delayed and simultaneous matching

Apart from the present work, to my knowledge there are no studies using NMDA receptor antagonism in PFC for comparisons of performance in matching tasks with and without delay or even in comparisons of delayed and simultaneous matching. However, PFC lesions in monkeys were shown to yield similar effects on DMTS and SMTS as presented here. A lesion study which investigated the effects of combined orbital and ventral PFC lesions in macaques on short term memory in a well-trained DMTS task also had animals perform the same task in an SMTS setting and is thus comparable to the present study (Bussey et al., 2001). Their findings corroborate the results presented here, as animals were severely impaired in both tasks, even performing at chance level in all postoperative sessions. A further lesion study (Rushworth et al., 1997) has reported a similar deficit on MTS tasks. In this study, animals with lesions of ventral PFC were postoperatively impaired in the DMTS task, with a delay-independent increase in errors of approximately 14% in the 2 sec delay and approximately 10% in the 4 sec delay, compared to the preoperative level. The 0 sec delay performance was unimpaired, however, which is at odds with the result of the present study.

Electrophysiological studies show PFC participation in response selection and rule representation

Electrophysiological evidence demonstrates neuronal activity in PFC during various phases of performance of tasks requiring working memory. Early investigations into this matter highlighted involvement of PFC particularly in working memory, by demonstrating the existence of 'delay neurons' in PFC, i.e. neurons exhibiting activity during delays in working / short term memory tasks (e.g. Fuster & Alexander, 1971; Watanabe, 1981; Miller et al., 1996). However, for example in a DMTS task, a response must be selected based on the integration of memorized and current sensory information. Therefore, maintaining a stimulus on-line to bridge a delay is not sufficient to solve the task. Consequently, later research found neuronal activity in lateral PFC of monkeys to pertain to cue, delay, choice and response phases during a combined delayed matching to sample or place task (Hoshi et al., 2000). During the choice or response selection phase, activity reflecting past sensory information, activity sensitive for the configuration of the choice

cue, and activity reflecting the properties of the target to be responded to was found. Moreover, delay activity was found to link object information and spatial information needed to guide behavior (Rao et al., 1997).

PFC activity during delay tasks could also be observed related to reward, reward expectancy, and current emotional value of rewards (Watanabe, 1989; Tremblay & Schultz, 2000; Rolls et al., 1986, 1989). Moreover, it could be shown that reward-related activity occurs in PFC even in tasks without a working memory component (Hikosaka & Watanabe, 2000) and that most of those dlPFC neurons that are active during a delay phase in remembering a stimulus also exhibit activity when the same stimulus remains visible (Tsujimoto & Sawaguchi, 2004). Taken together, the findings suggest that neurons in PFC are involved in sensory-motor integration by representing the task requirements as well as the relevance of external events for the organism. Thus also electrophysiological evidence supports a role of PFC in rule representation and response selection that goes beyond maintaining stimuli on-line over a delay.

Performance in well-trained tasks

In any case the present study found that, with NMDA receptor antagonism, subjects perform worse in a well-learned stimulus discrimination task even if there is no maintenance of items in working memory required. In contrast to this finding, many studies report that performance in well-trained tasks, in which an association was acquired before, is unaffected by NMDA receptor blockade in various brain regions (Smith-Roe et al., 1999; Roesler et al., 2000; Churchill et al., 2001). How can these results be reconciled with the present findings? A possible argumentation could be based on the substantial difference between the tasks used in those studies, compared to the tasks used in the present work. In those studies, mostly the S-R associations in question were unambiguous in a way that there was always one S+ and one S-. In matching tasks, this is not the case. Here both matching stimuli are – in principle – S+. Which one is the S+ in a given trial is determined by the sample stimulus displayed previously or simultaneously. Thus, in order to perform correctly in this task, it is not sufficient to associate responding to each of these stimuli with reinforcement, it is moreover necessary to learn under which condition, namely the color on the sample key, a certain matching stimulus will be the S+ in a given trial. Thus a matching task can be considered a special case of a conditional discrimination task (Iversen, 1997). It has been demonstrated that PFC is involved in conditional associative learning (Milner & Petrides, 1984; Petrides 1990; 1995). Therefore, the

discrepancies between this and other studies regarding performance in well-learned tasks might reflect a difference in the type of task. Performance in a task in which unambiguous S-R information can be retrieved from reference memory might be more resistant against effects of NMDA receptor antagonism in NCL than performance in a task which requires consideration of conditional rules and actual context for correct responding.

5.3 Response selection from context or from reference memory - Context processing

In the third experiment, we tested this idea by comparing pigeons' performance under NCL NMDA antagonism in two different, previously well-trained, trial types in the framework of a SMTS task. Context-dependent trials corresponded to canonical SMTS trials, as used in the previous experiment, while fixed-response trials required only recall of a previously acquired S-R association.

NMDA receptor antagonism impairs context-dependent response selection, but not response selection from reference memory

Comparison of performance in the two variants of response selection demonstrates that antagonism of NCL-based NMDA receptors in the pigeon caused significant impairments only in trials requiring response selection indicated by context, while response selection based on recall of fixed S-R associations from reference memory was left completely unaffected.

This behavior pattern suggests that while subjects, in spite of NMDA receptor antagonism, are still able to select a response unambiguously associated with a stimulus, they are impaired in selecting such a response if a conditional rule is interposed between stimulus and response.

The impairment is caused by perseverative errors

The errors during performance of the context-dependent trials under NMDA receptor blockade occurred predominantly because pigeons repeated the same response that proved successful in the previous trial; thus errors tended to be perseverative rather than random. Interestingly, spatial errors were rare and did not differ significantly from performance after infusion of vehicle and during training, in contrast to color errors and color-spatial combination errors, which increased significantly. It is therefore reasonable to assume that

animals retained fragments of the basic rule that color is of importance somehow, although they were unable to apply this rule correctly in all trials. The fact that overall performance remained well above chance level lends support to this assumption.

Matching tasks as special instances of conditioned discriminations

As outlined in the previous paragraph, performance in DMTS and SMTS tasks can be considered special instances of a conditional discrimination (Iversen, 1997). In conditional discrimination tasks, different stimuli are associated with different responses, in the special case of MTS tasks, different combinations of stimuli, presented simultaneously or sequentially, are associated with different responses. In each trial the subject must select, from among two or more alternatives, the response that is appropriate to the most recently presented stimulus combination. A consistent finding for this type of task was that it is impaired by frontal lesions in rats (Passingham et al., 1988; Winocur, 1991) in monkeys (Petrides, 1982, 1991b), in particular of areas 6 and 8 (Milner & Petrides, 1984; Petrides 1990; 1995) and in humans (Petrides 1985, 1991b). Impairments of conditional associative learning following frontal lesions have been characterized as a WM deficit resulting from lesion-induced inability to retain trial-specific information over the delay period. The results from the present study, however, point to a different conclusion and are consistent with findings of other studies comparing effects of hippocampal and frontal lesions upon a conditional task (Winocur, 1991; Winocur & Eskes, 1998). In contrast to hippocampal lesioned animals, rats with frontal lesions were impaired at learning the conditional rule. Since there was no S-R delay during training, the frontal lesion deficit was not linked to the WM component of the task. The authors concluded that the effects of frontal lesions pertained to conditional rule learning or to the process of response selection. However, when modifiying the task to reduce demands on response selection, frontally lesioned rats improved significantly (Winocur & Eskes, 1998). Thus it was suggested that frontal lesions interfere with the ability to use critical information in the context of a learned rule for accurate response selection.

Performance in a conditional associative task requires processing of the context indicating the correct response. i.e. context-dependent behavior, which can be defined as the adaptive ability to produce the appropriate response to a given stimulus, dependent upon the context in which it appears (Dominey & Boussaoud, 1997). In a fixed S-R association task, however, such context can be ignored since it does not deliver any additional information

for response selection. Context information appears to be represented and integrated in prefrontal areas, as revealed by electrophysiological studies in monkeys (Asaad et al., 1998; Watanabe et al., 2002) and studies in schizophrenic patients (Barch et al., 2001b, 2003). A study recording neuronal activity in monkey PFC during a conditional task (Boussaoud & Wise, 1993), found a large number of neurons exhibiting context-dependent activity, as indicated by increased activity to a cue with the same physical properties appearing as a contextual indicator as opposed to its appearance as a response indicator.

In line with this evidence, deficits in performance in the context-dependent trials after NMDA receptor blockade in NCL could be caused by impairments in either recall / representation of the conditional rule or in processing of the context necessary for responding according to the remembered rule. If NMDA receptor-antagonism had blocked recall of the conditional rule, performance rather should have dropped to chance level due to mostly random errors. However, performance remained well above chance and the errors committed tended to be perseverative rather than random, indicating that some information about the task rules was represented during performance, in particular that spatial position+color or color was important, but not spatial position alone. It thus appears that rule representation was not blocked completely, but that rather application of the rule was impaired due to a lack in context processing.

Performance in fixed-response trials does not recruit (NMDA receptors in) NCL

In contrast to the context-dependent trials, performance in the fixed-response trials under NMDA receptor blockade was comparable to the same subjects' behavior during training and vehicle infusion. The results indicate that this type of task might either not recruit NMDA receptors in NCL or does not require NCL intervention at all. These results for the fixed-response trial type are consistent with the general finding that NMDA receptor blockade in various brain areas hardly ever impairs performance of previously learned tasks (Morris et al., 1986; Campeau et al., 1992; Smith-Roe et al., 1999). Moreover, there is evidence that in humans, parietal cortex, and not PFC, is involved in retrieving previously learned S-R associations (Bunge et al., 2002). Although these results cannot readily be transferred to avian species, it is at least possible that in pigeons too, distinct brain areas might mediate these different task types with their different requirements on prefrontal functions. A PFC lesion study, in which rats were confronted with a task quite similar to the one used in the present experiment (Delatour & Gisquet-Verrier, 1996),

however, reports a similar pattern of results: PFC was not required for performance in trials where the response remained constant from trial to trial, but was necessary only in those trials where the accurate response changed from trial to trial, requiring the processing of previous information. Therefore it is possible that in pigeons too, neither NCL nor NMDA receptors participate in the fixed-response task type.
If, however, in birds both tasks were mediated by the NCL or NMDA receptors in NCL, then the comparison of results from the context-dependent and fixed-response tasks would lend additional support to the above assumption that not deficient rule representation, but impaired context processing led to the exclusive deficits in the context-dependent task type, since rule representation was obviously completely unimpaired in fixed-response trials.

Taken together, the results demonstrate that NMDA receptors in NCL are not involved in response selection from unambiguous S-R associations per se, but rather in response selection in certain instances where, in addition to retrieving S-R associations from reference memory, a conditional rule has to be applied and, in order to do so, contextual indicators have to be observed and processed.

5.4 How could NMDA receptor antagonism in the NCL influence learning and working memory?

Possible mechanisms by means of which NMDA receptor antagonism in NCL could influence learning and memory processes pertain to NMDA-receptor dependent synaptic plasticity, and to the modulation of DA mechanisms and glutamate release via NMDA receptors.

NMDA receptor blockade in NCL might influence synaptic plasticity and thus have effects on learning and memory

NMDA receptor activation is required for most forms of LTP. For both mammals and birds it could be shown that LTP can be blocked by, among others, the competitive NMDA receptor antagonist AP5 (e.g. Morris et al., 1986; Wang et al., 1994). Moreover, it has been demonstrated that the impairments in learning observed after NMDA receptor blockade occur by virtue of the disruption of LTP and are not only incidentally linked to it (Morris et

al., 1986, 1989; Davis et al., 1992). Therefore, LTP is often considered the neuronal correlate of learning due to its ability to mediate synaptic facilitation and thus to participate in the establishment of new assocations. On the behavioral level, prominent deficits after NMDA receptor blockade particularly in prefrontal regions, besides influencing the establishment of associations, also relate to alterations of pre-established associations. It is conceivable that the same cell assemblies that take part in establishing an association are also involved in reversal and extinction of this association, as in the nucleus accumbens it has been demonstrated that the same neurons that are active during performance attenuate their firing during extinction (Hollander et al., 2002). The mechanism by means of which changes in existing associations are brought about is not very well investigated. Depotentiation of LTP has been considered a possible mechanism of memory loss (Huang & Hsu, 2001); however, the physiological relevance of depotentiation is still unclear. By depotentiation reversal of synaptic strength to pre-LTP levels can be accomplished; thus it differs from LTD in that it is dependent on pre-established LTP. Depotentiation also requires activation of NMDA receptors, as it can be blocked by AP5 (Fujii et al., 1991; O'Dell & Kandel, 1994), although there appear to exist also NMDA receptor-independent forms of depotentiation (Bashir & Collingridge, 1994; Stäubli & Chun, 1996). However, depotentiation seemingly is achieved optimally within a limited time interval following LTP induction, which has led to the belief that LTP requires a period of consolidation which is vulnerable to depotentiation.

Also LTD can be blocked by AP5, since this NMDA antagonist has a similarly high affinity for both the NR2 subtypes NR2A/B and NR2 C/D, which appear to be differentially involved in inducing LTP and LTD, respectively (Hrabetowa et al., 2000). However, since LTD occurs on synapses which have not previously undergone LTP (Huang & Hsu, 2001), it is unclear whether it could be involved in changing pre-existing associations.

Thus deficits related to establishing new associations appear to be due to impaired LTP. By what mechanism, however, an alteration of existing, obsolete associations is brought about could depend on whether the same or different neuronal assemblies are involved in establishing the altered association. If the same neuronal assemblies were involved, a depotentiation mechanism might be required, if different neuronal assemblies were involved, newly established LTP might be sufficient for the new association, however, it

still remains unclear how the obsolete association is uncoupled, if by means of LTD or depotentiation.

NMDA receptor blockade in NCL might influence DA levels and thus have effects on learning and memory

In the mammalian PFC as well as in the avian NCL, DA ist strongly involved in working memory, particularly through its action on D1 receptors. An optimal DA level, respectively optimal D1 receptor activation, is indispensable for fully functional working memory, whereas supranormal D1 receptor stimulation in the PFC of rats impairs working memory performance (Zahrt et al, 1997). The relation between performance and DA / D1 receptor activation is represented by an inverted-U-shape function (Goldman-Rakic, 2000). An optimum DA level in PFC is supposed to stabilize memory traces adequately against interference, whereas excessive DA levels in PFC are assumed to cause extreme stabilization of memory traces rendering them completely resistant against interference and changes, resulting in the overt behavior of perseveration (Yang & Seamans, 1996, Zahrt et al., 1997).

Moreover, for learning processes, DA has been proposed to act as an error detection signal, meaning DA might encode a so-called ‘reward prediction error’, which is considered essential for conditioning to occur. Behavioral studies show that learning is related to the predictability of the reinforcement or reward (Rescorla & Wagner, 1972; Mackintosh, 1975). The Rescorla-Wagner model, devised for instances of classical conditioning, assumes that learning occurs only if the reward is surprising or unpredicted, relative to the expectations of the organism. The degree to which a reward is unpredicted is indicated by the prediction error, i.e. by the discrepancy between the reward obtained and the reward predicted. If an unpredicted reward occurs after a response, then the prediction error is positive, and learning occurs: the response and its consequence become associated. If the reward has become predictable after learning has occurred, the prediction error falls to zero and no more learning takes place. However, if the expected reward is not received after repeating a learned action, as is the case in extinction learning, then the prediction error is assumed to fall to a negative value and the behavior is extinguished.

It was proposed that the phasic DA response delivered by the DA neurons in the VTA, projecting to NAc. and the frontal cortex, which occurs for example in instrumental

conditioning after the presentation of rewards and after stimuli predicting reward (Schultz, 1986; Ljungberg et al., 1992) might encode a reward prediction error. This prediction error might constitute a teaching signal for learning processes (Hollermann & Schultz, 1998; Schultz et al., 1997).

Therefore, for studies investigating the role of NMDA receptors on PFC function in learning and working memory, it might well be important to consider the effects an NMDA receptor blockade has on DA in PFC. Research demonstrated that both systemic and local PFC NMDA receptor antagonism cause an increase of DA in the PFC (Feenstra et al., 2002, Lorrain et al., 2003, Takahata & Moghaddam, 1998), leading to the proposal that NMDA receptors normally exert a tonic inhibitory function controlling DA release in PFC (Kashiwa et al., 1995, Takahata & Moghaddam, 1998).

Thus the functional interaction between NMDA receptors and DA in PFC could be crucial for efficient learning and possibly working memory. Since the inhibitory function of NMDA receptors on DA appears to fail in case of NMDA receptor antagonism, PFC DA could rise to excessive levels and in this way prolongate learning processes, for example reversal learning, because of perseveration (Yang & Seamans, 1996).

On the other hand, an increase of DA levels caused by NMDA receptor antagonism might have differential, less detrimental effects on performance components in working memory tasks, since the optimum DA levels for different processes may differ. It has been proposed that in PFC, reversal and other learning processes are mediated by OFC, while working memory is mediated by dorsolateral PFC, and that these two areas might require different DA levels for optimal functioning (Arnsten & Robbins, 2002). In PD patients, it was demonstrated that an L-Dopa treatment that proved beneficial for spatial WM and task set-switching presumably depending on dlPFC (Cools et al., 2001), disrupted a different form of reversal learning dependent on OFC (Swainson et al., 2000), as L-Dopa medication might produce excessive levels of DA receptor stimulation in this area. Thus, in principle, it is conceivable that also in other organisms an NMDA receptor blockade at a dose that impairs learning and response selection via increased DA levels would not have negative effects upon WM processes.

Moreover, it has to be considered that in certain situations, NMDA receptor antagonism may not have immediate effects upon DA increase, as seen in the study by Takahata and Moghaddam (1998): A significant increase in PFC DA was observed only about 40 minutes after the beginning of AP5 application via the microdialysis probe, and returned to

baseline level about 40 minutes after cessation of the application. Although it is unclear whether the delay before a DA increase can be observed will be similar with a single microinfusion of an NMDA antagonist, as applied in the present experiment, it is at least possible that in experimental sessions with a duration shorter than about 30-40 minutes, detrimental effects of a NMDA antagonism-induced DA increase will not occur.

In pigeons, it could be shown that recruitment of short term memory during a DMTS task causes an increase of DA release in the NCL (Karakuyu, 2003) associated with correct performance in this task. D1 receptor blockades in NCL also caused impairments in spatial short term memory in pigeons (Güntürkün & Durstewitz, 2000). Thus, comparable to its role in the mammalian PFC, DA in the avian NCL seems to be beneficial for short term memory processes. However, it has not yet been investigated whether the effects of NMDA receptor antagonism upon DA release in the NCL match those observed in the PFC.

NMDA receptor blockade might increase glutamate levels and thus have effects on learning and memory

In addition to the effects on DA, a further possible consequence of blocking NMDA receptors is the increased release of glutamate in PFC and other brain regions (Moghaddam et al., 1997; Adams & Moghaddam, 1998). Given this evidence, it has been proposed that an excessive release of glutamate and the subsequent hyperstimulation of postsynaptic neurons might in part explain cognitive and behavioral disturbances associated with NMDA receptor hypofunction (Moghaddam et al., 1997; Adams & Moghaddam, 1998). However, effects of NMDA antagonists might have dose-dependent opposing effects upon glutamate release, as was observed in the case of ketamine, where only low doses increased glutamate in the PFC, while high anaesthetic doses decreased glutamate and intermediate doses had no effect (Moghaddam et al., 1997). The same dose of ketamine that increased glutamate also increased DA levels in PFC and caused deficits in a spatial WM task. The authors considered the DA increase to be secondary to the increase of glutamate and the subsequent stimulation of non-NMDA glutamate receptors, as blockade of AMPA/kainate receptors ameliorated the behavioral deficit. A further study using the NMDA antagonist PCP (Adams & Moghaddam, 1998), found that the NMDA antagonist-induced increase in DA release might not primarily account for the WM deficits observed,

since these deficits did not persist after presumed cessation of the antagonist action, despite continuously elevated DA levels.
Thus ist is possible that NMDA receptor antagonist actions both on glutamate and DA release might contribute to the deficits observed following NMDA receptor blockade.

In summary, all three NMDA receptor-mediated processes discussed here might in some way of another influence the learning and memory requirements present in the tasks.

5.5 Comparison of lesions and D1 receptor blockades with NMDA antagonism in NCL

When comparing the results presented here with prior NCL lesion and D1 receptor blockade studies in pigeons, similarities as well as differences can be observed.
While the present experiments did not find NMDA receptor involvement in short term memory, prior studies reported deficits in both spatial and non-spatial short term memory tasks following NCL lesions (Güntürkün, 1997; Gagliardo et al., 1996; Mogensen & Divac, 1993) and D1 receptor blockades (Güntürkün & Durstewitz, 2000). Dorsal NCL lesions caused more severe deficits in non-spatial short term memory in a DMTS task than ventral and total NCL lesions (Diekamp et al., 2002), based on the observation that recovery from the lesion-induced deficits occurred only in ventral and total NCL lesions, but not in dorsal NCL lesions, while initial post-surgery performance was impaired in all lesion groups. However, comparable to the NMDA receptor blockade results, the deficits in this lesion study were delay-independent, moreover, increased perseverative behavior was observed in all lesion groups. Taken together, these findings might support the assumption that the observed deficits could have been at least partially due to impaired response selection. In the experiments of this thesis, perseveration occurred following NMDA receptor blockade of the complete NCL, therefore they are most readily comparable to the total NCL lesions. Interestingly, comparing these two groups with regard to perseverative errors reveals that while with NMDA receptor blockade, color errors increased more than spatial errors did, with total NCL lesions both error types increased in a similar manner. Since in both tasks correct response selection had to be based on color, and not on spatial cues, it can be speculated that with NMDA receptor blockade, the rules of the tasks were better preserved than with NCL lesions.

Deficits in reversal learning were previously reported following diverse experimental manipulations in pigeon NCL: after lesions (Hartmann &Güntürkün, 1998), and D1 receptor blockade (Diekamp et al., 2001), as well as NMDA receptor blockade (Lissek et al., 2002). A potentially contributing factor, behavioral disinhibition, was left unimpaired in the present extinction task by both NMDA receptor blockades and NCL lesions. Previous studies found different results with regard to response inhibition in go/no-go tasks: while one study reports no deficits in acquisition (Hartmann & Güntürkün, 1998), others found impairments in acquisition (Aldavert-Vera et al., 1999) or performance (Güntürkün, 1997). With few exceptions (Aldavert-Vera et al., 1999), response selection based on previously established S-R associations as measured by visual discrimination tasks is completely unimpaired (Hartmann & Güntürkün, 1998; Gagliardo et al., 1996) or only mildly impaired (Mogensen & Divac, 1993) after NCL lesions, corresponding to the present results from the fixed-response trials in the third experiment.

In summary, NMDA receptor blockade in NCL was sufficient to cause deficits in a subset of those functions that are impaired following NCL lesions, namely in re-learning and in the response selection component of conditional tasks, indicating that NMDA receptor activation in NCL is crucially involved in these functions.

Response selection based on previously established S-R associations was unaffected both after NCL lesions and NMDA receptor blockades, thus it is conceivable that NCL does not participate in this function.

Some functions in which deficits were observed after NCL lesions, however, remained unimpaired after NMDA receptor blockades: i.e. short term memory and response inhibition, indicating that NCL NMDA receptor blockade may not affect structures in or beyond NCL that underlie these functions.

5.6 Summary discussion of all results

Taken together, the results of the experiments performed in the scope of this thesis can be summarized as follows: Under NMDA receptor blockade in the avian NCL, the following functions are impaired:

- extinction learning
- selection of the adequate response in DMTS and SMTS matching tasks
- response selection based on contextual information

On the other hand, the following functions remain unaffected by NCL NMDA receptor antagonism:

- behavioral inhibition
- recall of extinction
- maintenance in working memory
- response selection based on retrieval from reference memory

In the first two experiments, this thesis demonstrated involvement of NMDA receptors within NCL in extinction and response selection, i.e. in adapting a previously learned association and in choosing a correct response from competing alternatives. The third experiment extended these findings by showing a dissociation between response selection based on unambiguous S-R associations, and response selection requiring context processing, based on a conditional rule linking S to R, revealing deficits only in the latter condition.

Response inhibition and response selection based on unambiguous S-R associations were both unaffected by the NCL NMDA receptor blockade, the same applies for recall of once established, unambiguous S-R associations. Moreover, a previous study (Lissek et al., 2002) demonstrated that first time acquisition of a S-R association was unimpeded by NMDA receptor antagonism in NCL.

All tasks in which deficits were observed presumably require the processing of the (external and internal) context in order to choose the adequate response. External context here means stimuli indicating the correct response based on a conditional rule more complex than the rule applied in an unambigous S-R association, or based on feedback the subject receives after its response. In the extinction task, requiring alteration of an established S-R association, the feedback received by the subject after its responses has to be taken into consideration in order to adapt the association and subsequently the behavioral response. In the matching task, requiring consideration of an additional stimulus indicating the correct response, the context delivered by this sample stimulus has to be taken into consideration in order to choose the correct response in a given trial.

However, there is a difference between the extinction and matching tasks. In extinction and in such reversal tasks in which the S-R contingency is altered only once per session, a permanent change from the now obsolete to the newly valid S-R association is required. In

matching tasks, a temporary switch between two valid response alternatives is required between trials within a session. As can be seen from the experimental results in this thesis, both the permanent alteration of an S-R association and the temporary switches between S-R associations seem to require NMDA receptors in the NCL, even though these processes operate on different time scales.
Taken together, these results render it likely that NMDA receptors in the NCL are predominantly involved in behavioral flexibility by adapting responses according to constantly altered reinforcement conditions as well as according to conditional rules linking a stimulus to a response.

There is evidence that PFC participates in shifts between in principle valid response alternatives as well as in permanent changes of S-R associations. The WCST for example requires shifting between different sorting criteria which are all – in principle – valid, and frontal patients (Milner, 1964) as well as non-human primates with prefrontal lesions (Dias et al., 1996) are impaired in performance of this task. A recent study even demonstrated participation of NMDA receptors in the PFC of rats for set-shifting in a radial maze (Stefani et al., 2003). In this task, rats had to choose the alleys in the radial maze according to brightness and texture criteria, and it could be shown that non-competitive local NMDA receptor antagonism in the PFC by MK-801 impaired intra-dimensional shifts as well as extra-dimensional shifts. Reversal learning and extinction learning, requiring permanent changes of S-R associations, are impaired too after PFC lesions (Bohn et al., 2003). Also NCL lesions have been previously found to impair reversal learning (Hartmann & Güntürkün, 1998). The findings for the mammalian PFC are paralleled by the results regarding the avian NCL presented here, both with regard to permanent changes, as demonstrated in the extinction task and in the reversal task of a previous study (Lissek et al., 2002), and with regard to shifts between valid response alternatives, as demonstrated in the matching tasks.

The presence of competing response alternatives can be considered an element common to both types of tasks, regardless of whether they require a permanent change of response behavior or a temporary selection of the correct response from possible alternatives. In humans, response selection from competing alternatives was found to rely on PFC only, whereas response selection from reference memory recruited parietal cortex and not PFC (Bunge et al., 2002). This again corresponds to our findings that response selection with

regard to an unambiguous S-R association, as well as response recall after learning had taken place, was unimpaired following NMDA receptor antagonism in NCL. In these two situations, which do not require any alteration of, or selection between, existing S-R associations, NMDA receptors in NCL do not seem to have a role. This finding is in line with other research on the functions of NMDA receptors in various brain areas, such as amygdala (Campeau et al., 1992; Miserendino et al., 1990) and hippocampus (Morris et al., 1986, 1989).

Summarizing the results of the present thesis, it appears that NMDA receptors in NCL are predominantly involved in adapting behavior according to the requirements of the actual situation, not only by mediating long-term changes in S-R associations, but also by mediating short-term switching between in principle valid response alternatives. NMDA receptor dysfunction presumably disrupts the synaptic plasticity required for these alterations, and/or leads to excessively high DA levels in PFC causing inflexibility, which is behaviorally reflected in perseveration and impaired processing of relevant context information.
However, NMDA receptors in the NCL do not appear to participate in recall of unambiguous, established S-R associations which can be retrieved from reference memory without any competition of responses, nor did NMDA receptor antagonism at the dose used in these experiment exert a negative influence on working / short term memory.

While the present thesis described the involvement of NMDA receptors in NCL for several prominent prefrontal functions, thereby extending the existing knowledge about the avian functional equivalent of mammalian PFC, there remain many open questions regarding the mechanisms in which NCL-based NMDA receptors are involved, as well as regarding further functions they might perform. Future research might investigate the role of NMDA receptors in NCL e.g. for self-ordered working memory tasks, for response inhibition based on conditional rules, or on the effect NMDA receptor antagonism in NCL has for dopamine efflux.

6. REFERENCES Chapters 1 & 5 (Introduction & General Discussion)

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7. ATTACHMENTS:

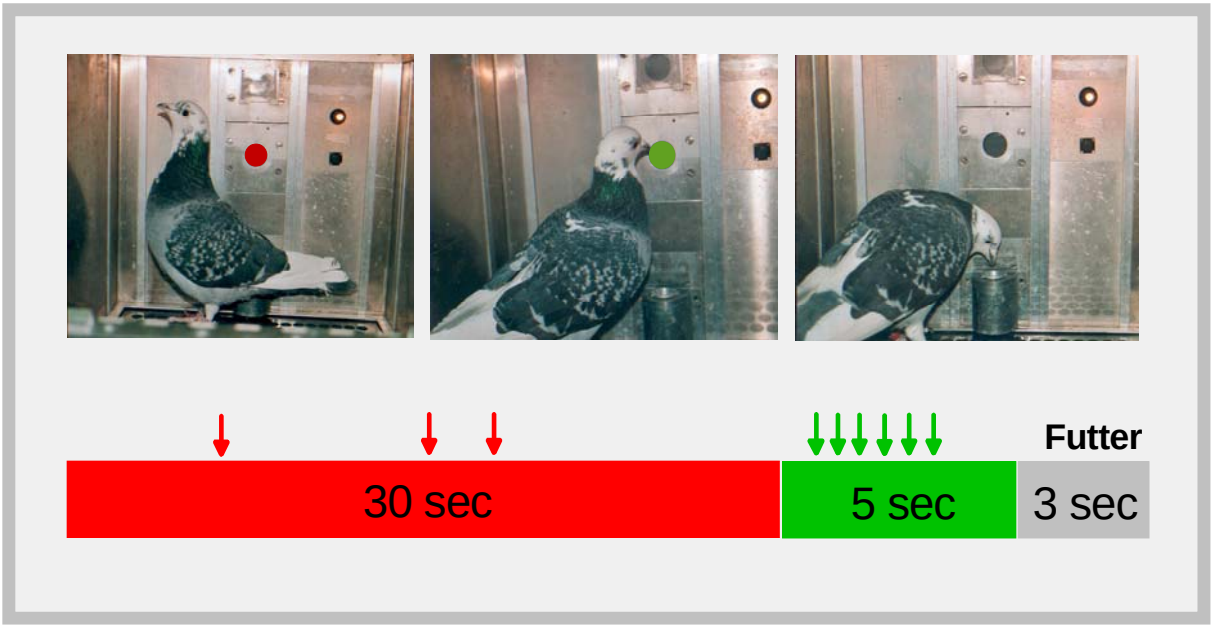
1. List of abbreviations
2. Illustration of the task used in Experiment 1 (Chapter 2)
3. Illustration of the task used in Experiment 2 (Chapter 3)
4. Positions of the cannulas for microinfusion into the NCL
5. Copy of the publication: Lissek S, Diekamp B & Güntürkün O (2002): Impaired learning of a color reversal task after NMDA receptor blockade in the pigeon (Columba livia) associative forebrain (Neostriatum Caudolaterale)

7. 1 List of Abbreviations:

ADHD	Attention deficit hyperactivity disorder
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	Analysis of Variance
AP5	2-amino-5-phosphonovaleric acid, 2-amino-5-phosphonovalerate
BA	Brodmann's area
Ca2+	Calcium
CGP 39551	carboxyethyl ester of DL-(E)-2-amino-4-methyl-phosphono-3-pentonoic acid
CNS	central nervous system
CPP	3-[2-carboxypiperazin-4-yl]-propanephosphonic acid
CPPene	3-((+/-)-2-carboxypiperazin-4-yl)-1-phosphonic acid
CPT	continuous performance task
D1	Dopamine D1 receptor
DA	Dopamine
D-AP5	D-2-Amino-5-Phosphonovaleric Acid, D-2-Amino-5-Phosphonovalerate
DL-AP5	D,L-2-Amino-5-Phosphonovaleric Acid, D,L-2-Amino-5-Phosphonovalerate
dlPFC	dorsolateral prefrontal cortex
dmPFC	dorsomedial prefrontal cortex
DMTP	Delayed matching to place
DMTS	Delayed matching to sample
DNMTP	Delayed non-matching to place
DNMTS	Delayed non-matching to sample
DOPAC	Dihydroxyphenylacetic acid
EPSP	excitatory post-synaptic potential
ERP	event-related potential
fMRI	functional magnetic resonance imaging
(+)-HA-966	3-amino-1-hydroxy-2-pyrrolidinone
HV	Hyperstriatum ventrale
HVA	Homovanillic Acid
IMHV	intermediate and medial Hyperstriatum ventrale
LES	lesion
LTD	Long term depression
LTP	Long term potentiation
LY 233053	4-(1H-tetrazol-5-ylmethyl)-piperidine-2-carboxylic acid
K+	Potassium

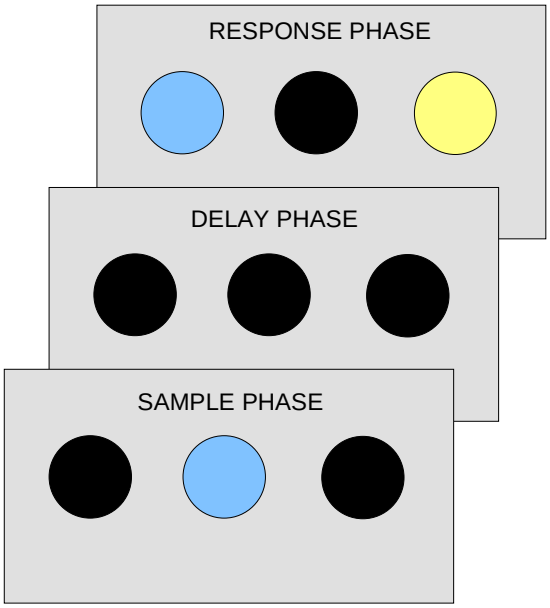
MD	Nucleus mediodorsalis
ME	Multiple errands test
Mg2+	magnesium
MK-801	dizocilpine, (+)-5-methyl-10,11-dihydro-5H-dibenz(a,d)cycloheptene-5,10-imine hydrogen maleate
mPFC	medial prefrontal cortex
Na2+	Sodium
NAc	Nucleus accumbens
NCL	Nidopallium caudolaterale, previously: Neostriatum caudolaterale
Ndc	dorsocaudal Nidopallium
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NPC 17742	2-amino-3-[2-(2-phosphono-ethyl)-cyclohexyl]-propionic acid
NR1	NMDA receptor subunit 1
NR2	NMDA receptor subunit 2
NR3	NMDA receptor subunit 3
OFC	orbitofrontal cortex
PCP	Phencyclidine
PD	Parkinson's disease
PFC	Prefrontal cortex
rTMS	repetitive transcranial magnetic stimulation
S+	discriminative stimulus signalling reinforcement of the instrumental response
S-	discriminative stimulus signalling non-reinforcement of the instrumental response
SAL	saline, NaCl 0.9 %
SDZ EAA 494	= D-CPPene: (S)-(E)-4-(3-phosphonoprop-2-enyl)-piperazine-2-carboxylic acid)
SEM	Standard error of means
SMTS	Simultaneous matching to sample
S-R	stimulus – response
STM	short term memory
VTA	Ventral tegmental area
WCST	Wisconsin Card Sorting Test
WM	Working memory
Zn2+	Zinc

7.2 Illustration of the task used in Experiment 1 (CHAPTER 2)

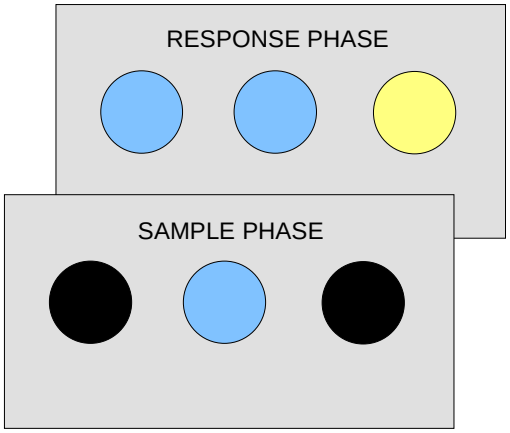


Each trial starts with the color RED displayed on the pecking key for 30 seconds, during which the pigeon has to refrain from responding to the key. Afterwards, the color GREEN is displayed for 5 seconds on the pecking key, here the pigeon has to respond three times to the key in order to receive the food reward.

7.3 Illustration of the task used in Experiment 2 (Chapter 3)



Delayed Matching to sample task

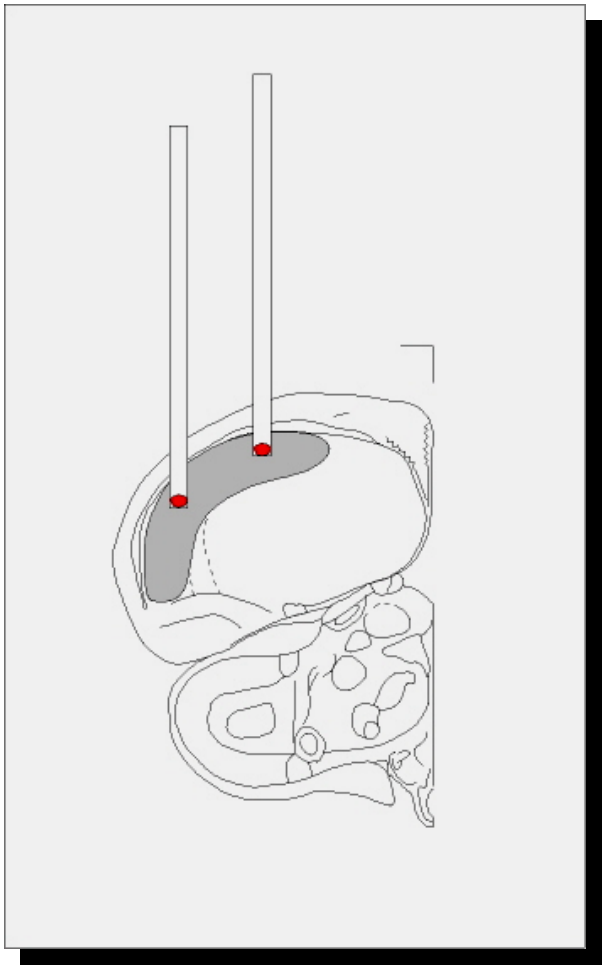


Simultaneous matching to sample task

In the DMTS task, 15 pecks onto the sample key during the sample phase start the delay phase (duration 0 to 2 seconds), followed by the response phase, during which the animal has to respond within 5 seconds display time to the matching key showing the same color as the sample key in order to receive the food reward.

In the SMTS task, the sample phase is followed immediately by the response phase after the animal has responded 15 times to the sample key.

7.4 Positions of the cannulas for microinfusion into the NCL



Cannulas were located at A 5.25 and L 5.0 and 7.5 according to the coordinates of the Stereotaxic atlas of the brain of the pigeon (*Columba livia*) by Karten & Hodos (1967).

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Impaired Learning of a Color Reversal Task After NMDA Receptor Blockade in the Pigeon (*Columba livia*) Associative Forebrain (Neostriatum Caudolaterale)

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The neostriatum caudolaterale (NCL) in the pigeon (*Columba livia*) forebrain is a multisensory associative area and a functional equivalent to the mammalian prefrontal cortex (PFC). To investigate the role of *N*-methyl-D-aspartate (NMDA) receptors in the NCL for learning flexibility, the authors trained pigeons in a color reversal task while locally blocking NMDA receptors with D,L-2-2-amino-5-phosphonovalerate (AP-5). Controls received saline injections. AP-5-treated pigeons made significantly more errors and showed significantly stronger perseveration in a learning strategy applied by both groups but were unimpaired in initial learning. Results indicate that NMDA receptors in the NCL are necessary for efficient performance in this PFC-sensitive task, and that they are involved in extinction of obsolete information rather than in acquiring new information.

The aim of the present study was to examine the role of *N*-methyl-D-aspartate (NMDA) receptors in the neostriatum caudolaterale (NCL) of the pigeon in a task sensitive for prefrontal functions. A large body of evidence suggests the NCL to be a functional equivalent to the mammalian prefrontal cortex (PFC); this was first pointed out by Divac, who coined the term posterodorso-lateral neostriatum to describe this brain area (Mogensen & Divac, 1982). Comparable to the PFC, the NCL maintains reciprocal connections to secondary sensory areas of all modalities and projections to somatomotor and limbic zones (Kröner & Güntürkün, 1999; Leutgeb, Husband, Riters, Shimizu, & Bingman, 1996; Metzger, Jiang, & Braun, 1998; Pandya & Yeterian, 1996). Furthermore, it receives a dense dopaminergic innervation from midbrain structures (Divac, Thibault, Skageberg, Palacios, & Dietl, 1994; Metzger et al., 1998; Waldmann & Güntürkün, 1993; Wynne & Güntürkün, 1995). Behavioral evidence shows that lesions of the NCL lead to performance deficits in working memory tasks (Gagliardo, Bonadonna, & Divac, 1996; Gagliardo, Mazzotto, & Divac, 1997; Güntürkün, 1997; Mogensen & Divac, 1982, 1993), pattern reversal (Hartmann & Güntürkün, 1998), delayed alternation (Gagliardo & Divac, 1993), and go/no-go-tasks (Aldavert-Vera, Costa-Miserachs, Divac, & Delius, 1999; Güntürkün, 1997). Moreover, temporary blockade of dopamine D_1 receptors in the NCL has been found to cause impairments in a discrimination reversal task (Diekamp, Kalt, Ruhm, Koch, & Güntürkün, 2001) and a working memory task (Güntürkün & Durstewitz, 2000). Electrophysiological studies identified NCL neurons in that—comparable to PFC neurons—show delay- and reward expectancy-related activity (Fuster, 1989; Kalt, Diekamp, & Güntürkün, 1999; Watanabe, 1996). Thus, multiple evidence points to a functional equivalency between the avian NCL and the mammalian PFC. High densities of NMDA receptors have been identified within the avian NCL, and it is conceivable that they play a prominent role in some aspects of functions subserved by the NCL. Indeed, NMDA receptors in the NCL of young chickens have been shown to be involved in one-trial passive-avoidance learning (Stewart, Bourne, & Steele, 1992) and imprinting (Bock, Schnabel, & Braun, 1997; Bock, Wolf, & Braun, 1996). These learning processes are characterized by their rapid onset and, once established, by their long-lasting stability. However, because high densities of NMDA receptors also occur in adult pigeons, it is likely that they also play a role in other learning functions.

Reversal learning is a typical task that probes behavioral flexibility, a feature attributed to the PFC. In a reversal task, animals or human subjects have to learn that the previously rewarded stimulus (S+) is now incorrect, and that the former S− is the new S+. Lesions to the PFC drastically impair performance in reversal tasks (Daum, Schugens, Channon, Polkey, & Gray, 1991; Rosenkilde, 1979). To our knowledge, no study has ever analyzed whether prefrontal NMDA receptors mediate the ability for reversal learning. Therefore, the first aim of the present study was to investigate whether these receptors within the NCL are involved in reversal learning. A detailed analysis of the reversal learning process shows that subjects have to acquire two different sets of information: first, learning to cease responding to the former S+, and second, learning that the previous S− has to be selected (Macphail, 1976). Given the differential ability of NMDA receptors to modulate long-term depression (LTD) and long-term potentiation (LTP; Castro-Alamancos, Donoghue, & Connors, 1995; Gean & Lin, 1993; Hrabetova & Sacktor, 1997), the second aim of the present study was to establish a detailed behavioral analysis to reveal the specific role of these receptors in a "frontal" area, in a task probing behavioral flexibility.

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Method

Subjects

Subjects were 15 unsexed and experimentally naive pigeons (*Columba livia*), age 1–7 years, obtained from local breeders. They were individually housed in cages in a temperature- and humidity-controlled room on a 12-hr light–dark schedule. During experiments, they were maintained at 80% of their free-feeding weight and received water and grit ad libitum.

Apparatus

Two conventional and functionally identical Skinner boxes (36 cm long × 34 cm high × 36 cm wide) were used. Each was equipped with two pecking keys and a solenoid-operated food hopper in the back wall and was computer-controlled by means of a digital input/output board. On the pecking keys (2.5 cm in diameter), white light was displayed for pretraining, and red or green light was displayed for experimental sessions. The food hopper was situated in the center of the wall, below the two keys. Above the food hopper, a reinforcement light signaled the availability of food. The Skinner box was illuminated by a houselight.

Pretraining

The pigeons first received an autoshaping procedure in which they acquired the association between responding to a single pecking key illuminated by white light and subsequent food reward. This was followed by pretraining, during which pigeons learned to discriminate between two pecking keys and to respond only to the key displaying white light. Pretraining lasted until pigeons reached learning criterion (at least 80% correct responses in each of three consecutive sessions).

Surgery

For surgery, pigeons were anesthetized with Ketamine–Rompun (40 mg/kg and 8 mg/kg, respectively, im). Stainless steel guide cannulas were implanted stereotaxically (Karten & Hodos, 1967), aiming at the NCL. Two cannulas per hemisphere were vertically inserted to reach the following coordinates: A 5.25, L 5.00; and A 5.25, L 7.50. Cannulas were inserted to 1 mm below the brain surface and were secured with dental acrylic. After 3–4 days of recovery, pigeons were tested for retention of the pretraining task (criterion: 80% correct responses in the retention session).

Color Reversal Learning Procedure

Experimental training consisted of seven sessions: the first one for the acquisition of a color discrimination (red vs. green), and six subsequent sessions for color reversal learning. Each of the seven sessions was followed by a retention session approximately 2 days later. The interval between the retention session and the next reversal session was about 24 hr. All acquisition, reversal, and retention sessions lasted until learning criterion (15 correct responses in a row) was reached, with session duration not to exceed 3 hr.

In the acquisition session, the color that the pigeon chose first was considered the S+ (positive stimulus) for the session. In each of the following color reversal sessions, the contingencies of the colors were reversed: If in acquisition the S+ was red, in Reversal 1 it was green, in Reversal 2 it was red again, and so on. The stimulus colors were displayed on the pecking keys according to a quasirandomized sequence (Fellows, 1967). We applied a fixed ratio-3 schedule, allowing 3 s access to food after a correct response and delivering a 5-s time-out after a response to the incorrect color.

Immediately before the acquisition and reversal sessions, experimental pigeons received infusions of the competitive NMDA receptor antagonist D,L-2-2-amino-5-phosphonovalerate (AP-5) locally into the NCL (AP-5 dissolved in saline solution; total volume = 2 μl, containing 10 μg AP-5, 0.5 μl [2.5 μg AP-5] per cannula). Infusions were made through interior cannulas protruding 1 mm from the tip of the implanted cannulas that guided them into the brain tissue. We used a microinfusion pump equipped with two 1-μl Hamilton (Reno, NV) syringes to deliver the volume at a flow rate of 0.2 μl/min. Afterward, the infusion cannulas remained in place for another 2 min to allow for diffusion of the infused volume. To infuse through all four cannulas, we performed this procedure twice. Control pigeons were submitted to the same procedure, receiving saline solution only. Immediately after the infusion procedure, which took about 12–15 min, the pigeons had to perform the task. All pigeons received a total of seven infusions of either AP-5 or vehicle.

Histology

To reconstruct the locations of the guide cannulas, we perfused the pigeons intracardially with 0.9% (wt/vol) saline (40 °C) and a 4% (wt/vol) paraformaldehyde solution (4 °C). The brains were removed, postfixed, and cut into 40-μm frontal slices on a freezing microtome. The slices were stained with cresyl violet. The lowest point of the lesion left by the cannulas was considered the injection site.

Results

All injection sites were located within the NCL. Seventy-eight percent of the sites were located within a range of ±0.5 mm from A 5.25. Twenty-two percent were situated anteriorly up to A 6.25 (see Figure 1).

During training sessions, the total number of errors and percentage of errors were recorded for each session for each pigeon. The color acquisition performance of the AP-5 ($n = 7$) and saline ($n = 8$) groups was compared by means of a one-factor analysis of variance (ANOVA, one-tailed). The color reversal performance of AP-5 and saline groups was compared by means of a 2 (groups) × 6 (sessions) repeated measures ANOVA (one-tailed) for all of the above scores. The tests for total number of errors and percentage of errors were one-tailed due to our directed hypothesis: Considering the role of NMDA receptors for various learning phenomena, we expected the AP-5 group to show poorer performance, as revealed by a larger number of errors. Additional tests examining the learning strategies were two-tailed.

In the acquisition of the color discrimination, there were no significant differences in the total number of errors between groups (see Figure 2 and Figure 3).

In color reversal learning, the AP-5 group made more errors than controls until reaching criterion. This difference was significant for the absolute number of errors, $F(1) = 5.94$, $p < .05$ (one-tailed), as well as for relative error rates, $F(1) = 7.02$, $p < .01$ (one-tailed). There was a significant effect of the sequence of reversal sessions, both for absolute errors, $F(5) = 19.92$, $p < .01$, and for relative errors, $F(5) = 25.49$, $p < .01$. No significant interactions were found in either total errors or percentage of errors. In both groups, error rates were lower in later reversal sessions (see Figure 3 and Figure 4).

The pigeons' behavior during a reversal session could be compartmentalized into distinct types that might represent the use of diverse strategies to solve the task. These were first described by Macphail (1976), who distinguished between three successive measures: color perseveration, side perseveration, and correct strategy. *Color perseveration* represents the number of trials in which the subject responds continuously to the wrong color despite negative feedback. *Side perseveration* is a measure for those trials in which the subject responds to one pecking key only, irrespective

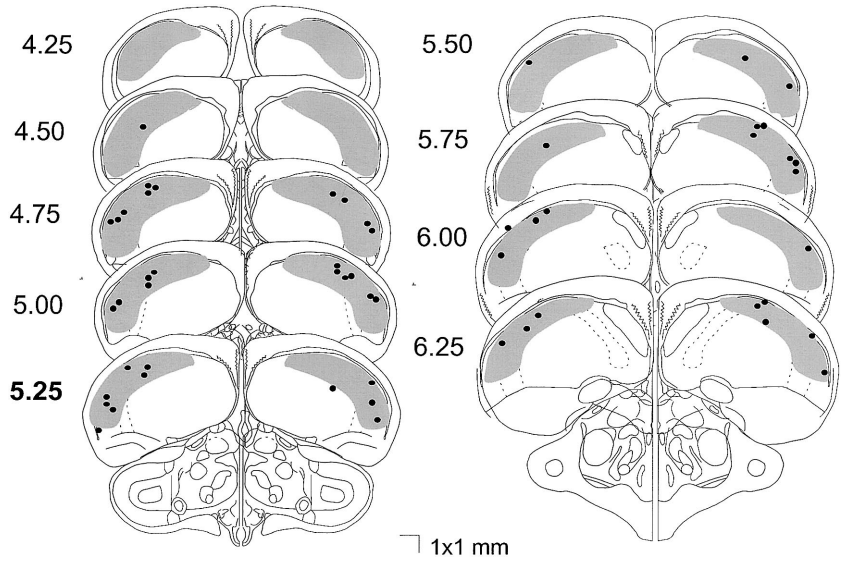

Figure 1. Schematic frontal sections of the pigeon brain showing the injection sites for the AP-5 and/or saline solutions. Dots represent the lower tips of the cannulas; numbers represent the distance (in millimeters) anterior to the center of the ear bars; and boldface indicates the frontal plane level at which injections were aimed. The neostriatum caudolaterale area according to Waldmann and Güntürkün (1993) is depicted in light gray. From *Stereotaxic Atlas of the Brain of the Pigeon (Columba livia)*, by H. J. Karten and W. Hodos (pp. 88, 90, 92, 94, 96, 98, 100, 102, and 104), 1967, Baltimore: Johns Hopkins Press. Copyright 1967 by Johns Hopkins Press. Adapted with permission.

of the color it displayed. Finally, *correct strategy* represents the number of trials in which the subject responds to the now-correct color, alternating between pecking keys if necessary. To separate different strategic phases, the complete sequence of trials was transformed into distinct bins of 12 trials each, as the quasirandomized stimulus presentation sequence provided for equal distribution of colors to both pecking keys only within a 12-trial sequence. For each of these bins, the percentage of each strategy was calculated. This procedure enables separation of color perseveration from side perseveration and correct strategy. Finally, the percentages of the individual bins were summed up separately for each strategy, resulting in three strategy measures for each reversal

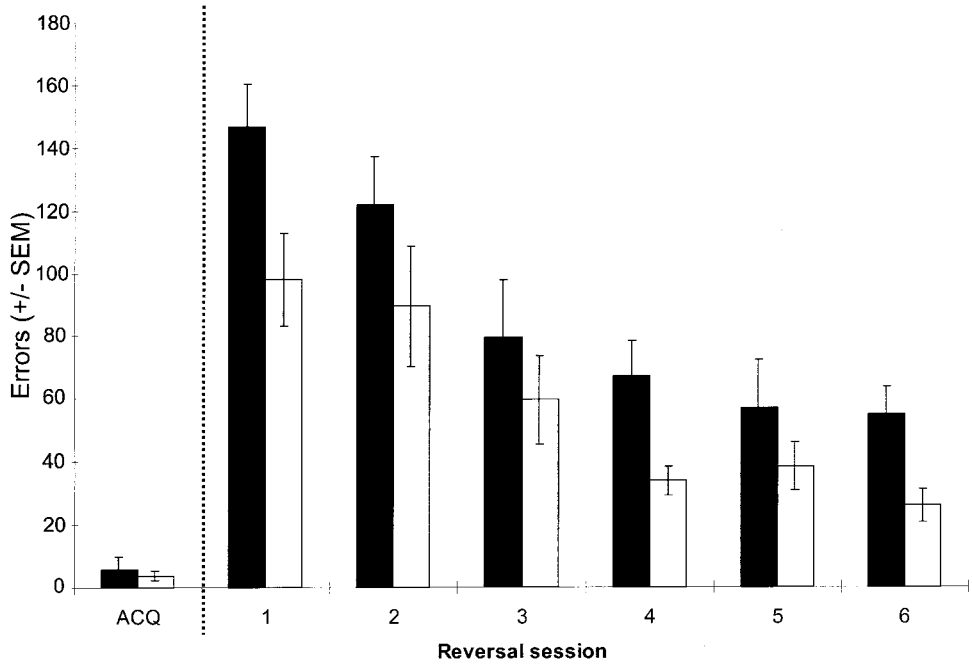

Figure 2. Mean (± *SEM*) total number of errors to criterion made by AP-5-treated (solid bars) and control (open bars) pigeons during first-time acquisition (ACQ) of the color reversal task and in the six reversal sessions.

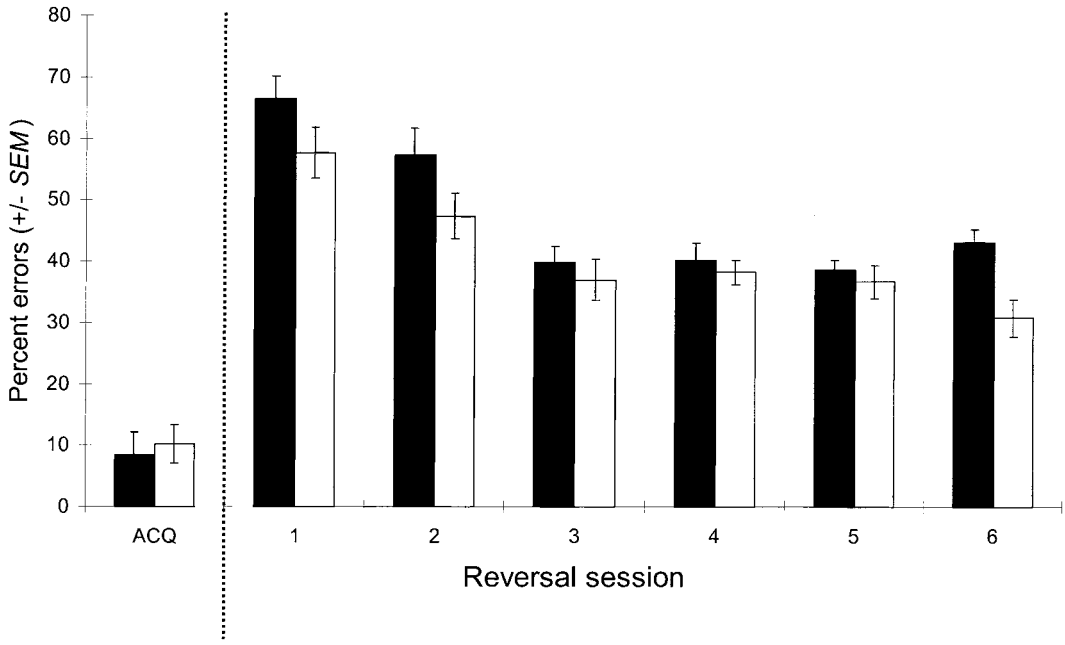

Figure 3. Mean (± *SEM*) percentage of errors relative to total number of trials made by AP-5-treated (solid bars) and control (open bars) pigeons, calculated for each session during first-time acquisition (ACQ) of the color reversal task and in six reversal sessions.

session. In the reversal sessions, all pigeons started with color perseveration. The number of trials on which this strategy was used differed significantly between groups, $F(1) = 8.09$, $p < .05$ (two-tailed), with AP-5-treated pigeons showing a considerably stronger color perseveration tendency than controls. In addition, there was a significant main effect of session, $F(5) = 31.06$, $p < .01$ (see Figure 4). Side perseveration superseded color perseveration, and for this second strategy, there was again a significant main effect of session, $F(5) = 3.30$, $p \leq .01$. Differences between groups were not significant, $F(1) = 2.04$, although the AP-5 group lingered in this phase longer than did controls (see Figure 4).

With regard to correct strategy, no significant differences between groups could be detected, nor was there a main effect of session or significant interaction (see Figure 4).

Discussion

The results of this study demonstrate similarities as well as distinct differences between the learning performance of AP-5- and vehicle-treated pigeons. First, in acquisition of the color discrimination, we observed only a slight and nonsignificant impairment of AP-5-treated pigeons compared with controls. Second, although both groups were capable of learning the color discrimination as well as the color reversal task, pigeons with temporary blockade of NMDA receptors in the NCL were significantly impaired during the color reversal learning process. Third, both groups seemed to gradually acquire a higher order strategy, enabling them to speed up their learning in later reversal learning sessions.

Impaired Color Reversal Learning

In color reversal learning, the AP-5 group displayed a significantly higher error score, which was due to their significantly increased color perseveration tendency on the S−, particularly during early reversals (1 and 2). At the same time, neither side perseveration nor correct response strategy behavior differed significantly between groups. Thus it was mainly on the previously acquired behavior that AP-5-treated pigeons showed increased perseveration despite negative feedback. It is conceivable that they were unable to use the feedback as efficiently as saline-treated pigeons did and therefore needed more examples of the altered stimulus–response–consequence configuration to learn the new association. However, after finally adopting the side strategy, AP-5-treated pigeons were undistinguishable from controls with regard to their ability to give it up again for the sake of the correct strategy. In summary, the deficit of the AP-5 group in strategy usage was not in reacting to a novel S+, but in ceasing to react to the previously learned S+. Once they "unlearned" the obsolete S+, they were as quick as controls in acquiring the novel S+.

On the other hand, NMDA receptor blockade in the NCL obviously did not influence the pigeons' performance in the first-time acquisition of a color discrimination. This dissociation in the performance of the AP-5-treated pigeons might be due to an important difference between acquisition of a color discrimination and color reversal learning: During acquisition, a completely new stimulus–response association is being formed. In color reversal, however, the new, reversed stimulus–response association has to compete with the stimulus–response association established previously. In parallel to learning something new, something previously learned must be unlearned to allow the new stimulus–response association to guide behavior. This might not merely constitute an additional learning load but might involve another type of process: extinction of a previously acquired association. Learning and unlearning, or extinction, are probably related to the neuronal mechanisms of LTP and LTD, respectively. Induction of LTP and LTD can be blocked by AP-5 in vitro (Castro-Alamancos

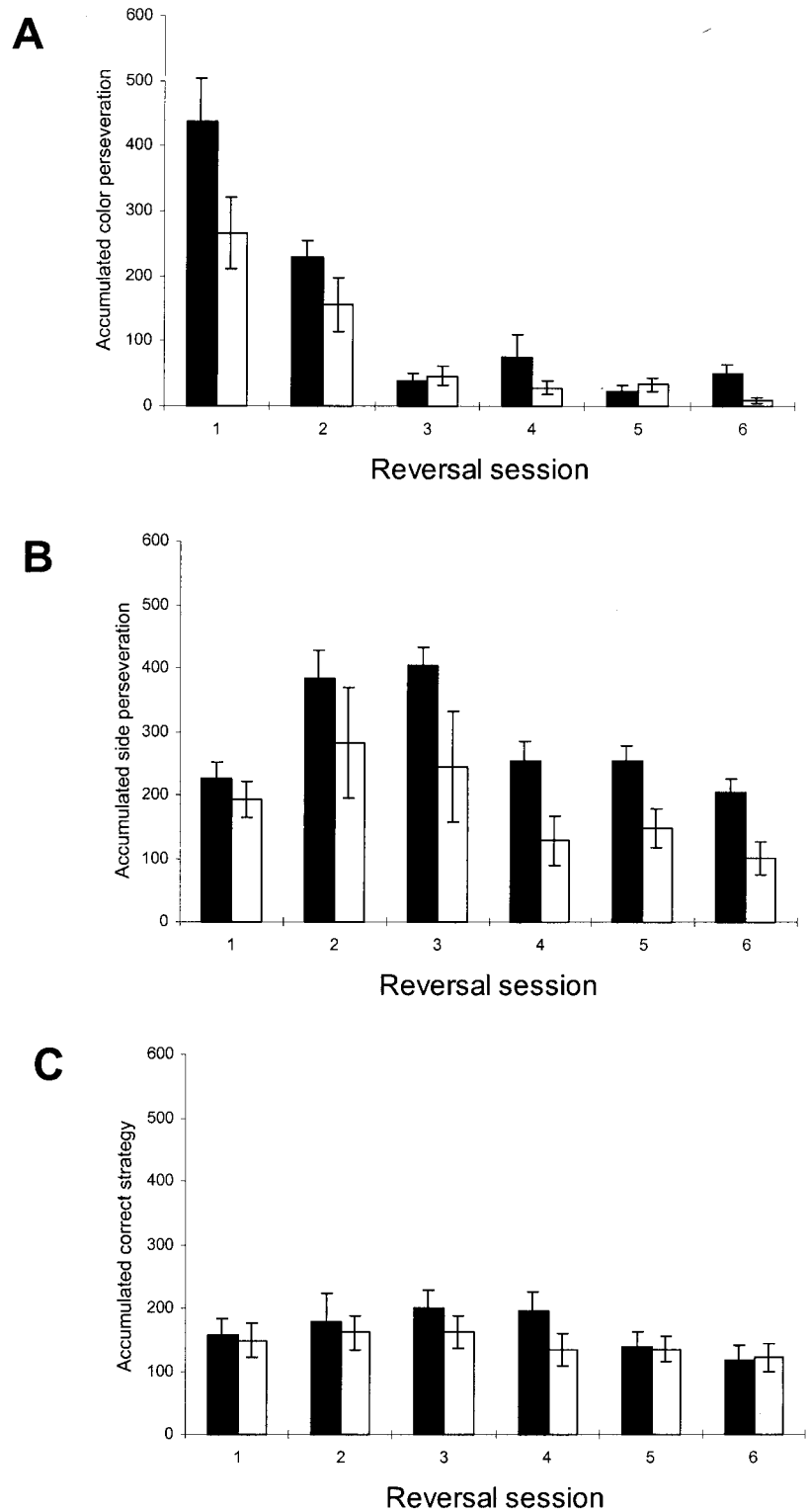

Figure 4. Mean (± *SEM*) performance in color reversal tasks by AP-5-treated (solid bars) and control (open bars) pigeons using different learning strategies. The first strategy, color perseveration (A), shows significant differences between groups, whereas side perseveration (B) and correct strategy (C) do not.

et al., 1995; Gean & Lin, 1993; Hrabetova & Sacktor, 1997), and LTP alone was shown to be blocked by AP-5 in vivo (Morris, 1989). Because there is evidence of LTP and LTD being induced by activation of distinct subpopulations of NMDA receptors (Hrabetova & Sacktor, 1997), it is conceivable that NMDA receptors take part in learning as well as in unlearning. An involvement of NMDA receptors in extinction procedures has already been demonstrated in extinction of conditioned fear, which could be blocked by local AP-5 infusions into the amygdala (Falls, Miserendino, & Davis, 1992).

The results of the present study suggest that extinction of an established response to the S− (previously S+), rather than acquisition of a response to the new S+, might be mediated by NMDA receptors in the NCL. Impaired extinction caused by NMDA receptor blockade might lead to the observed increase in perseverative behavior during reversals, but not during acquisition, in which no extinction is required. In principle, however, it is possible that initial acquisition was not affected because the synaptic rearrangements necessary took place outside the NCL, or independently of NMDA receptor functions in the NCL. Our results do not permit us to rule out this possibility. Another possibility is that the acquisition of a color discrimination is too easy to be impaired by the treatment. However, results from other studies show the NCL to also be involved in acquisition, as lesions of the NCL cause impairments in the reacquisition of a visual discrimination (Aldavert-Vera et al., 1999). In our study, AP-5-treated pigeons showed a small, nonsignificant acquisition deficit of the color discrimination task. Therefore, it is conceivable that NMDA receptors might also participate in acquisition and require LTP, although to a lesser extent than in reversal learning.

Unimpaired Learning of a Higher Order Strategy

In our study, AP-5-treated pigeons were especially impaired during the first reversal sessions, although the effect leveled out in later reversals. In an identical task, under blockade of D_1 receptors, no deficits were found during the first 25 reversal sessions; however, impairments appeared in later reversals. Thus, blockade of NMDA receptors impaired the onset of learning, whereas blockade of D_1 receptors caused deficits in later learning phases. These differential effects might reflect two different cognitive strategies (Diekamp, Prior, & Güntürkün, 1999): Whereas pigeons seemed to treat the color stimuli in the first reversal sessions as prototypical S+ or S−, signaling food or no food, they later learned that a certain color was related to food only temporarily. Therefore, the first reversal sessions seem to represent true reversals, in which the new S+ is learned and stored in long-term memory, presumably by NMDA receptor-mediated synaptic rearrangements. In later reversals, however, the pigeons seemed to keep the temporary S+ in working memory for the current session, conceivably by mechanisms involving the participation of D_1 receptors. (Güntürkün & Durstewitz, 2000; Izquierdo et al., 1998; Sawaguchi & Goldman-Rakic, 1991, 1994). Not only did the AP-5 group show this performance improvement during later reversals, but the saline group did as well. Thus, this learning-to-learn effect seems to work independently of NMDA receptor participation. To determine the correct color for the current session, pigeons used a side strategy, which presumably is a means to this end (Diekamp et al., 1999). Thus a higher order strategy, namely switching between always-present alternatives, might in later reversals replace the prior strategy of erasing one stimulus–response association and establishing another. Although erasing and establishing may require activity of NMDA receptors in the NCL, switching might work without them.

Area Specificity

To evaluate the spread of AP-5 during a pilot study, we injected 0.5 μl of the fluorescent tracer rhodamine isothiocyanate, known for its wide diffusion area, into the NCL. These cases revealed an average spread of 1 mm diameter around the tip of the cannula, ranging from 0.49 mm to 1.68 mm diameter. Therefore, injections through guide cannulas on positions L 5.00 and L 7.50 (separated by 2.50 mm) should cover the lateral–medial range of the NCL but should not extend anteriorly and posteriorly to areas outside the NCL. Similarly, diffusions into the ventricle are unlikely. Thus, the behavioral effects observed were probably not due to the spread of AP-5 to adjacent brain regions via the ventricle or the brain tissue. A study considering diffusion of AP-5 in the rat hippocampus used [3H]-AP7, which has diffusional characteristics supposedly identical to those of AP-5. It was found that with an injection volume of 1 μl (twice the volume we used per cannula), diffusion had dropped to about 50% at 1.5 mm around the actual injection site. Three millimeters around the injection site, values had dropped further, to almost 0% (Morris, Halliwell, & Bowery, 1989). These considerations are important against the background of studies showing that lesions of the Wulst result in reversal deficits (Macphail, 1971; Shimizu & Hodos, 1989). Because the posterior border of the Wulst is more than 4 mm distant from the most anteriorly situated cannulas of the present study, it is highly unlikely that AP-5 injections into the NCL affected processes within the Wulst.

For both mammals and birds, it is well known that there are various forebrain areas that, when lesioned, lead to impaired performance in reversal tasks. Thus far, our data do not imply that reversal deficits will occur only if the NCL is temporarily blocked or lesioned. They demonstrate, however, that NMDA receptors in the NCL play a key role in mediating reversal learning processes.

Summary

Taken together, the results of the present study demonstrate for the first time that NMDA receptors in the avian NCL are necessary for learning processes. It is conceivable that their effect on reversal learning involves participating in extinction of previously learned associations. In this task, impairment of their functioning is visible as perseveration on a suboptimal strategy. In a broader context, their normal functioning might be comparable to the PFC-related ability to adjust behavior to changing environmental conditions.

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ERKLÄRUNG

Die hier vorgelegte Dissertation wurde von mir selbst und ohne unerlaubte fremde Hilfe angefertigt. Ausser den im Quellen- und Literaturverzeichnis und in Anmerkungen im Text genannten Hilfsmitteln wurden keine weiteren benutzt.

Neben der mit „Sehr Gut“ bestandenen Diplom-Hauptprüfung im Fach Psychologie an der Ruhr-Universität Bochum habe ich bislang keine weiteren Prüfungen abgelegt.
Ich habe diese Dissertation weder in dieser noch in irgendeiner anderen Fassung bereits einer anderen Fakultät vorgelegt. Ich habe darüberhinaus bislang auch keine andere Dissertation vorgelegt.

Bochum, den 07. Mai 2004

Silke Lissek

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