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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 appli-

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cation 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 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 nonmatching 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 experimental 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

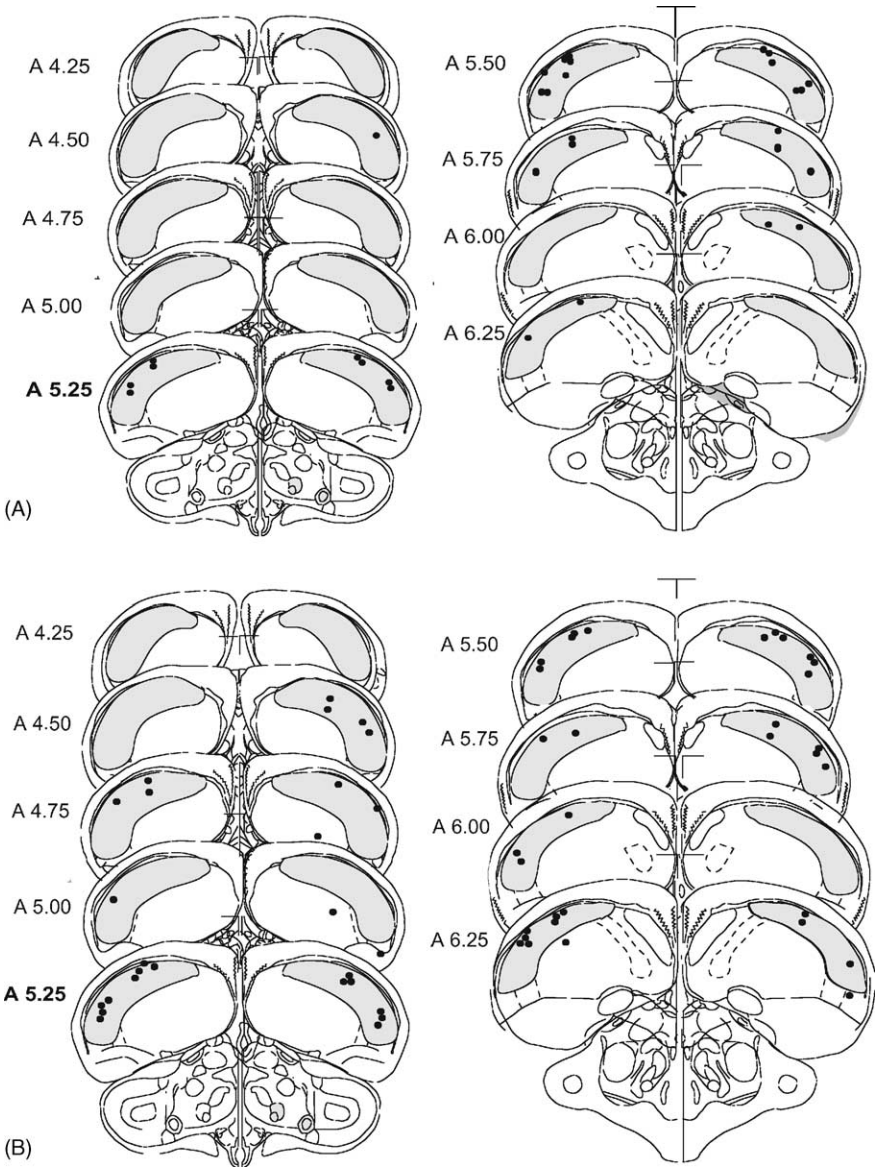

Fig. 1. Schematic frontal sections of the pigeon brain showing the injection sites for AP5 and or vehicle for (A) SMTS group and (B) DMTS group. Dots represent the lower tips of the cannulas, numbers represent the distance (anterior) to the center of the ear bars, boldface indicates the frontal plane 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%.

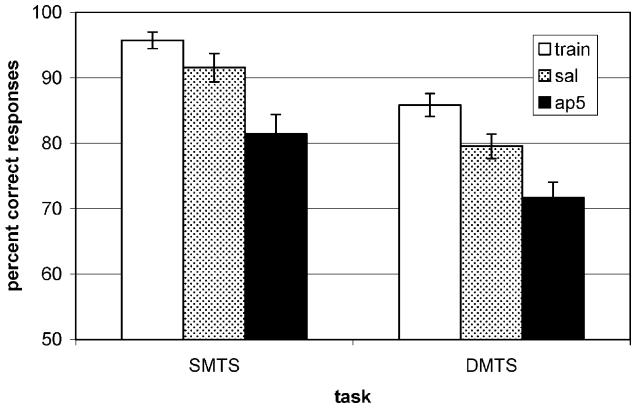

Fig. 2. Percent correct responses in three different treatment conditions: training (TRAIN), after saline infusion (SAL) and NMDA receptor antagonism (AP5) in the SMTS task and the DMTS task. In both tasks there was a significant main effect of treatment (DMTS: $F(2) = 12.451$ $P \leq 0.001$; SMTS: $F(2) = 10.659$ $P < 0.001$). 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%).

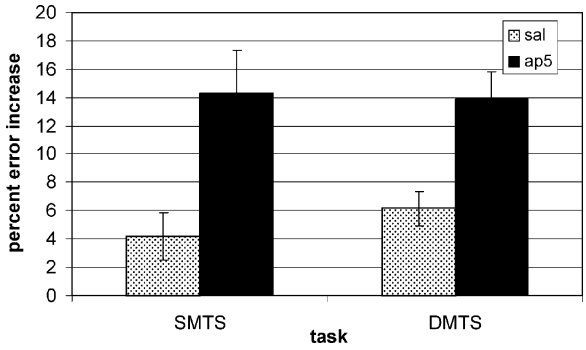

Fig. 3. Error increase in percent compared to the training level in the SMTS task and the DMTS task after saline infusion (SAL) and NMDA receptor antagonism (AP5). *t*-tests for matched samples showed for both tasks significant differences between the treatment conditions (DMTS: $t(15) = 4.136$ $P =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

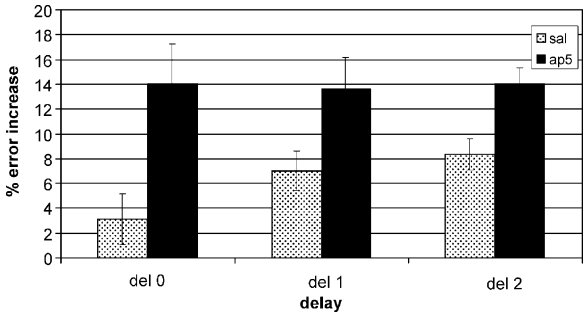

Fig. 4. Error increase in the individual delays of 0, 1 and 2 s (del 0, del 1, del 2) of the DMTS task relative to training under both treatment conditions (SAL and AP5). An ANOVA with repeated measures shows only a significant main effect of the treatment: $F(1) = 11.968$ $P < 0.01$: subjects made significantly 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).

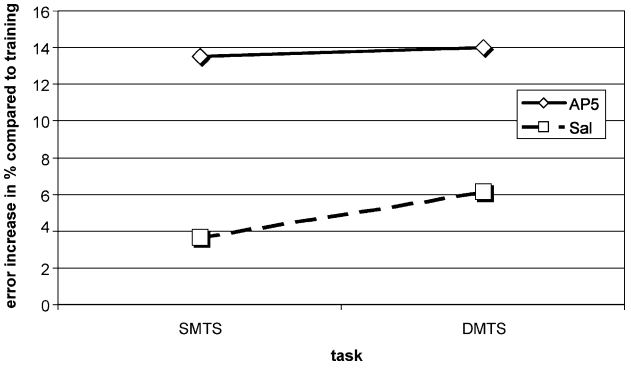

Fig. 5. Error increase in percent compared to training—comparison of both tasks (SMTS and DMTS) and both treatment conditions (SAL and AP5). A comparison by ANOVA with repeated measures gave a highly significant effect of the within-subjects factor "treatment" ($F(1) = 31.964$ $P < 0.001$), while the 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.

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