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Out of Context: NMDA Receptor Antagonism in the Avian "Prefrontal Cortex" Impairs Context Processing in a Conditional Discrimination Task

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Processing of context information is implicated in prefrontal functions as response selection or attention. *N*-methyl-D-aspartate (NMDA) receptors in the mammalian prefrontal cortex (PFC) and in the nidopallium caudolaterale (NCL) of birds, the avian functional equivalent of the PFC, are involved in learning, which also requires processing of context. The authors investigated the role of NMDA receptors in the pigeon (*Columba livia*) NCL for context processing and response selection in a simultaneous-matching-to-sample task with 2 trial types, requiring either processing of context information, delivered by a conditional stimulus (context dependent), or only recall of a stimulus–response association (fixed response). The competitive NMDA antagonist DL-2-amino-5-phosphonovaleric acid impaired performance only in context-dependent trials. Therefore, NMDA receptors in the avian PFC participate in response selection requiring context processing rather than in response selection per se.

Keywords: NMDA receptor, context processing, avian, prefrontal, DL-AP5

A primary function of the prefrontal cortex (PFC) is integration of information from the external environment and internal states of the organism to initiate appropriate behavior in a given situation. Integration of information requires processing of the context delivered by the actual situation, whereas initiation of appropriate behavior requires response selection. The function *context processing* therefore refers to the ability to actively hold relevant context information in mind in such a form that it can be used to mediate task-appropriate behavior (Cohen, Barch, Carter, & Servan-Schreiber, 1999). The function *response selection* refers to choosing an adequate response from two or more available alternatives. There is evidence that the PFC is implicated in both functions.

Deficits in PFC-related tasks exhibited by patients with schizophrenia or frontal lesions as well as by healthy older adults demonstrate a causal link between PFC hypofunction and impairments in context processing (Barch et al., 2001; Barch, Carter, MacDonald, Braver, & Cohen, 2003; Braver et al., 2001; Cohen et al., 1999; Kerns & Berenbaum, 2003; Metzler, 2001). Lesion studies in rats (Morgan & LeDoux, 1999) and single cell recordings in monkeys (Watanabe, Hikosaka, Sakagami, & Shirakawa, 2002) also demonstrate the participation of the lateral PFC in integration of contextual information. Consequently, a model of PFC function (Cohen & Servan-Schreiber, 1992; Servan-Schreiber, Cohen, & Steingard, 1996) proposes a comprehensive context-processing function located in the PFC that is supposed to subserve various prefrontal functions that are usually treated and investigated independently, such as active memory, attention, and response inhibition.

In many instances, the choice of an adequate behavioral response, that is, response selection, requires processing of relevant contextual information—for example, in a conditional discrimination (Winocur & Eskes, 1998). Therefore, conditional discrimination tasks are a useful behavioral paradigm for the investigation of context processing. In other instances, however, context information is negligible for response selection—for example, in unambiguous stimulus–response associations that always require the same response to the same stimulus (Delatour & Gisquet-Verrier, 1996).

Ventrolateral PFC lesions in rats and monkeys also cause deficits in response selection, in particular during conditional associative learning (Bussey, Wise, & Murray, 2001; Petrides, 1982, 1987; Winocur & Eskes, 1998); thus, the ventral PFC is considered essential for conditional associative learning and response selection, as information about stimulus, response, and response outcome is available only in this region (Passingham, Toni, & Rushworth, 2000).

N-methyl-D-aspartate (NMDA) receptors in prefrontal regions participate in reversal and extinction learning in rats (Bohn, Giertler, & Hauber, 2003) and pigeons (Lissek, Diekamp, & Güntürkün, 2002; Lissek & Güntürkün, 2003), situations in which relevant external context must be considered to alter existing stimulus–response associations. These results hint at prefrontal NMDA receptor involvement in context processing during learning. However, because NMDA receptor blockade in various brain regions does not impair performance of previously learned tasks

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(Bohn et al., 2003; Kelley, Smith-Roe, & Holahan, 1997; Smith-Roe, Sadeghian, & Kelley, 1999), it remains unclear whether prefrontal NMDA receptors are also involved in context processing and response selection during performance of a well-trained task. In a previous study (Lissek & Güntürkün, 2004), we found impaired performance in matching tasks with and without short-term memory load, pointing at deficits in response selection, after NMDA receptor blockade in the pigeon PFC, the nidopallium caudolaterale (NCL).

The NCL is an area in the avian forebrain considered functionally equivalent to the mammalian PFC. This conclusion rests on a large data set involving neuroanatomical, physiological, neurochemical, and behavioral results. Neuroanatomical studies showed the NCL to receive multimodal input from all secondary sensory areas of the forebrain (Leutgeb, Husband, Riters, Shimizu, & Bingman, 1996), to project to telencephalic motor output structures as well as to the basal ganglia (Kröner and Güntürkün, 1999), to be innervated by dopaminergic fibers from midbrain cell groups A8–A10 (Metzger, Jiang, Wang, & Braun, 1996), and to be characterized by a high density of dopamine D_1 receptors (Schnabel et al., 1997). Physiological studies demonstrated that single units in the NCL code for the upcoming reward (Kalt, Diekamp, & Güntürkün, 1999), bridge the delay between stimulus and response by high sustained activity levels (Diekamp, Kalt, & Güntürkün, 2002), and code for the subjective reward value of a reinforcer (Kalenscher et al., 2005). Neurochemical analyses showed that relative (Divac, Mogensen, & Björklund, 1985) and absolute concentrations of catecholamines (Karakuyu, Diekamp, & Güntürkün, 2003) as well as the relation of different dopamine metabolites (Bast, Diekamp, Thiel, Schwarting, & Güntürkün, 2002) matched the data from the mammalian PFC. Behavioral experiments revealed an involvement of the NCL in tasks testing working memory (Diekamp, Gagliardo, & Güntürkün, 2002; Mogensen & Divac, 1993), reversal learning (Lissek et al., 2002), response selection (Lissek & Güntürkün, 2004), and choice behavior (Kalenscher, Diekamp, & Güntürkün, 2003). All these findings, gathered with diverse techniques, closely match the conditions of the PFC.

In this study we investigate the role of NMDA receptors in the pigeon NCL for response selection and context processing. Because in most standard tasks these two functions cannot be disambiguated, we developed a novel adaptation of a simultaneous-matching-to-sample (SMTS) task that enabled us to differentiate between instances of response selection with and without the additional requirement of context processing. By presenting two different task types, we were able to compare—within each single session—the animals' performance in a conditional discrimination task that required context processing and a task requiring only response selection from unambiguous alternatives, without a conditional component. In line with the evidence on the crucial role of NMDA receptors in learning tasks, we hypothesized NMDA receptor involvement predominantly for response selection in the conditional discrimination task requiring context processing.

Method

Subjects

Subjects were 9 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 cycle. During experiments, they were maintained at 80% of their free-feeding weight and received water and grit ad libitum.

Apparatus

A conventional Skinner box (36.0 cm long × 34.0 cm high × 36.0 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 Operant Learning Conditioning Unit System software (Version 1.2.01, Frank Buschmann International, Bochum, Germany). The three pecking keys (2.5 cm in diameter) were arranged in a horizontal row on the back wall 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, and blue, yellow, red, and green lights were displayed during training and experimental sessions in the SMTS task. The Skinner box was illuminated by a houselight.

Matching Task With Context-Dependent and Fixed-Response Trials

On the basis of 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 also lit, 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, but 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, responding to one color of each pair was always wrong, whereas responding to the other was always correct. Because the fixed-response trials constituted basically a simple stimulus–response association, the pigeon could select the correct response without processing the context information delivered by the sample color. Because, 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) and trials consisting of simple stimulus–response associations. If NMDA receptors in the NCL were involved in response selection per se, we would expect deficits to occur in both trial types. If NMDA receptors, however, were involved in context-dependent response selection only, 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 for 120 s or until the animal responded to the key. Ten responses to this key led to the additional presentation of the matching stimuli on the lateral keys for a maximum of 5 s. Responding to the lateral key showing the color matching the sample key gave 3-s access to the feeder. Responding to the nonmatching color resulted in a time out of 15 s, during which all lights, including the houselight, were switched off. The intertrial interval was 10 s. Trials were

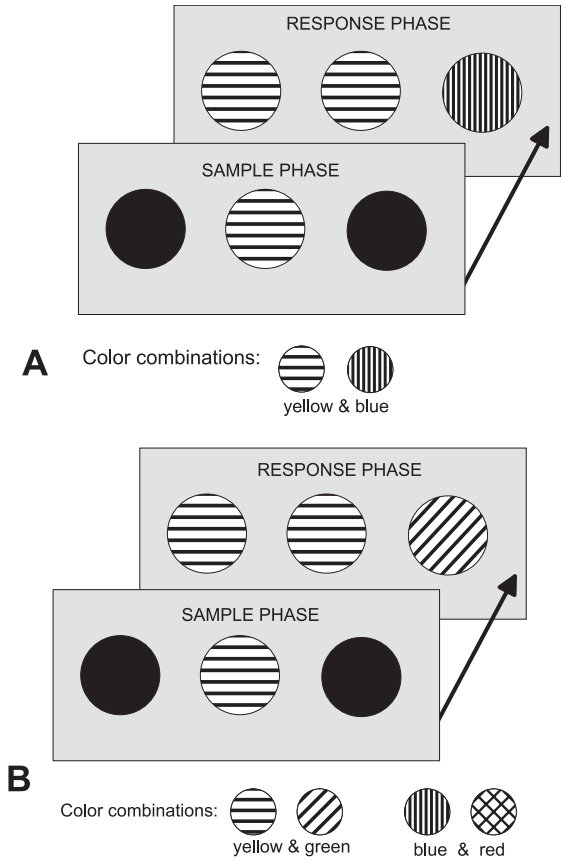

Figure 1. Examples for context-dependent (A) and fixed-response (B) trials in the matching task. In context-dependent trials, a combination of two colors (yellow–blue) was used, with the correct response depending on the sample key color (yellow or blue) in a given trial. In fixed-response trials, these 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+, whereas green and red were always the S−. Thus, although 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. S = stimulus.

repeated only when there was either an untimely response to the lateral keys during the presentation time of the sample stimulus or no response to the lateral keys during the response phase—that is, the presentation of the matching stimuli.

Although the matching stimuli were counterbalanced for side of presentation across trials in both trial types, the frequency of color pairs appearing on the matching keys was not balanced, as 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. However, because there was no way to achieve balancing of color combinations without compromising the balancing of trial types, 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 animal until the learning criterion of at least 90% correct responses, calculated separately for each trial type, was reached in five subsequent training sessions. Animals required, on average, five sessions to achieve this criterion; there were no differences between context-dependent and fixed-response trials in the number of sessions required. The percentage of correct responses during the last five sessions of training was 96.28% (*SEM* = 0.57) for the context-dependent trials and 99.42% (*SEM* = 0.16) for the fixed-response trials.

Surgery

For surgery, pigeons were anesthetized with Ketamine–Rompun (40 mg/kg im and 8 mg/kg im, respectively). Aiming at the NCL, we vertically inserted two stainless steel cannulas per hemisphere under stereotaxical guidance to reach the following coordinates: A 5.25, L 5.00, and A 5.25, L 7.50 (Karten & Hodos, 1967). 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 90% correct responses.

Experimental Sessions

We used a within-subject design in that each pigeon was alternately tested under both treatment conditions: blockade of NCL using the competitive NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (DL-AP5; Sigma-Aldrich, St. Louis, MO) or infusion of vehicle (saline solution, 0.9% [wt/vol] sodium chloride [NaCl]). 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 DL-AP5 or vehicle locally into the NCL. DL-AP5 was dissolved in saline solution (total volume = 2.0 μl, containing 10.0 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 that, 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 1.0-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. Immediately after the infusion procedure, which took about 12–15 min, the pigeons had to perform the task. One session per day was conducted. To prevent sequence effects, we infused pigeons on successive days alternately with either DL-AP5 or vehicle, with the first infusion being DL-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, we reconstructed the positions of the cannula tips 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 × 5 experimental sessions (NMDA receptor blockade and vehicle infusion) with the performance in the last five training sessions by means of analysis of variance (ANOVA) with repeated measures. By means of a test for matched samples, we compared the percentage of error increase in both trial types. 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—Location 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]-DL-2-amino-7-phosphonoheptanoic acid, which has diffusional characteristics supposedly identical to those of DL-AP5, in the rat hippocampus (Morris, Halliwell, & Bowery, 1989), found that with an infusion volume of 1.0 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 A 5.25 and L 5.00 and L 7.50, was largely restricted to the NCL, which has an anterior–posterior extent of 3.5 mm (AP 3.75 to AP 7.25) and a lateral–medial extent of 5.0 mm (L 3.50 to L 8.50; Karten & Hodos, 1967; Waldmann & Güntürkün, 1993). Also, diffusion into areas ventral to the NCL can be largely excluded, as cannula tips were located at a distance of about 1 mm from the ventral border between the NCL and neighboring structures.

Retention Session

In the retention session performed 5–6 days after surgery, all animals reached the required performance criterion of 90% correct responses for each trial type.

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 the 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 the 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 with NMDA receptor blockade in

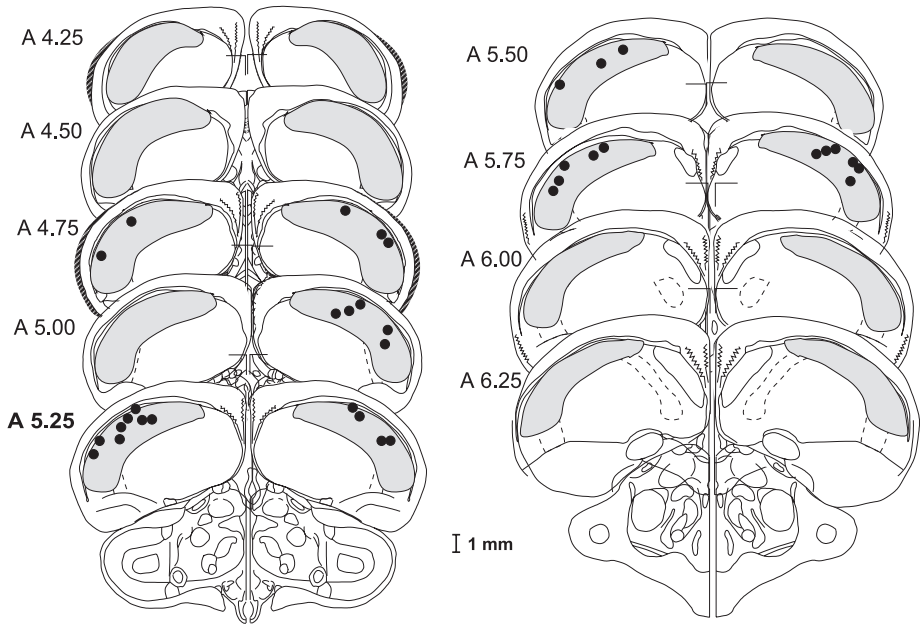

Figure 2. Schematic frontal sections of the pigeon brain showing the injection sites for DL-2-amino-5-phosphonovaleric acid or vehicle. Dots represent the lower tips of the cannulas, numbers represent the distance (anterior) to the center of the ear bars, and boldface type indicates the frontal plane level at which cannulas were aimed. The nidopallium caudolaterale according to Waldmann and Güntürkün (1993) is depicted in light gray. Reprinted from the *Stereotaxic Atlas of the Brain of the Pigeon (Columba livia)*, by H. J. Karten and W. Hodos, pp. 88–104. Copyright 1967, with permission of Johns Hopkins University Press.

the NCL and following vehicle infusion in context-dependent and fixed-response trials, respectively. Mean values of context-dependent errors were as follows: DL-AP5, 14.89% (*SEM* = 2.55); vehicle, 6.16% (*SEM* = 1.60); training, 3.72% (*SEM* = 0.57). Mean values of fixed-response errors were as follows: DL-AP5, 0.28% (*SEM* = 0.12); vehicle, 0.16% (*SEM* = 0.12); training, 0.16% (*SEM* = 0.16). An ANOVA with repeated measures and the two within-subject factors treatment and trial type gave significant main effects of treatment, $F(2, 16) = 16.434$, $p < .001$, and trial type, $F(1, 8) = 37.163$, $p < .001$. The Treatment × Trial Type interaction was also significant, $F(2, 16) = 17.043$, $p \leq .001$, indicating that the DL-AP5 treatment impaired performance only in the context-dependent trials, not in the fixed-response trials, whereas performance following the vehicle treatment was unimpaired in both conditions. Planned contrasts showed significant differences between DL-AP5 and vehicle performance, $F(1, 8) = 31.920$, $p < .001$, and between DL-AP5 and training performance, $F(1, 8) = 19.156$, $p < .01$, but not between vehicle and training performance, $F(1, 8) = 1.586$, $p = .243$ (see Figure 3). Thus, there was no impairment in any trial type following the infusion of saline, whereas NMDA receptor antagonism in the NCL led to deficits only in context-dependent trials, not in fixed-response trials.

Percentage of errors in context-dependent trials in each training and experimental session. To check for possible changes in performance over the course of the experimental sessions, we calculated an ANOVA with repeated measures and the two within-subject factors treatment and session for the percentage of errors in context-dependent trials. Besides the highly significant main effect for treatment, $F(2, 16) = 16.872$, $p < .001$, we found a significant main effect for session, $F(4, 32) = 2.795$, $p < .05$, and a significant Treatment × Session interaction, $F(8, 64) = 2.306$, $p < .05$ (see Figure 4). These results appear to reflect a slight reduction in errors over the course of the sessions that can be observed in the DL-AP5 as well as in the vehicle condition. With DL-AP5 treatment, mean errors in the first session amounted to 22.50% (*SEM* = 5.07), and in the fifth session they amounted to 9.72% (*SEM* = 3.32). With vehicle treatment, errors in the first session were 8.61% (*SEM* = 1.51), and in the fifth session they were 5.55% (*SEM* = 1.30).

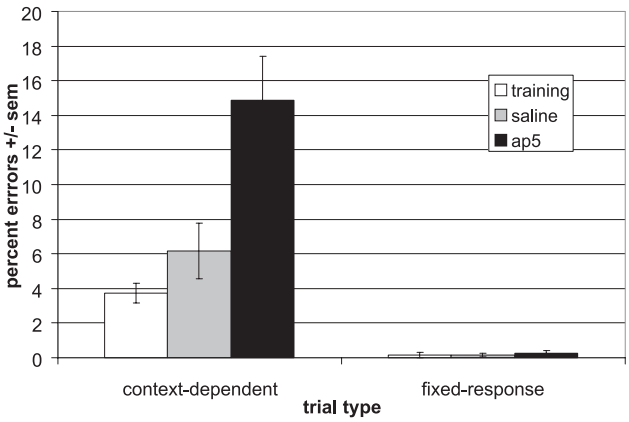

Figure 3. Percentage of errors plus or minus *SEM* in three different treatment conditions: training, after saline infusion, and *N*-methyl-D-aspartate receptor antagonism (ap5) in the different trial types. There was a significant main effect of treatment, $F(2, 16) = 16.434$, $p = .000$; a significant main effect of trial type, $F(1, 8) = 37.163$, $p = .000$; and a significant Treatment × Trial Type interaction, $F(2, 16) = 17.043$, $p = .000$, indicating that DL-2-amino-5-phosphonovaleric acid treatment impaired performance only in the context-dependent trials, not in the fixed-response trials, whereas the saline condition did not impair performance at all.

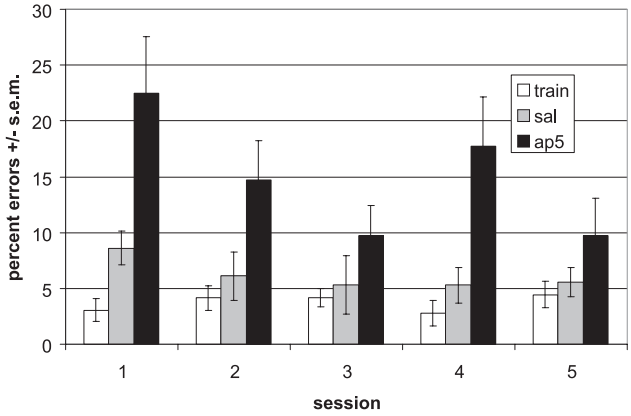

Figure 4. Percentage of errors plus or minus *SEM* for each of the five sessions of each experimental condition: *N*-methyl-D-aspartate receptor blockade in the nidopallium caudolaterale (ap5) and vehicle infusion (sal) as well as each of the five criterion training (train) sessions. An analysis of variance with repeated measures yielded significant effects for treatment, $F(2, 16) = 16.872$, $p < .01$, and session, $F(4, 32) = 2.795$, $p < .05$, as well as a significant Treatment × Session interaction, $F(8, 64) = 2.306$, $p < .05$.

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 s, and the matching phase lasted 5 s. 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 significantly in both vehicle and DL-AP5 conditions compared with the training level, as indicated by a significant treatment effect, $F(2, 16) = 9.633$, $p < .01$. However, a Bonferroni post hoc test ($p = 1.0$) demonstrated that there was no statistical difference between DL-AP5 and vehicle treatments with regard to the number of timeouts, 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 5).

Timeouts in the matching phase in both treatment conditions did not change significantly compared with training. Neither in fixed-response trials, $F(2, 16) = 0.068$, *ns*, nor in context-dependent trials, $F(2, 16) = 0.550$, *ns*, was there a significant difference among the three treatment conditions. Comparable to the results regarding the sample timeouts, matching timeouts indicated that there was no deficit in DL-AP5–treated animals with regard to motivation and distractibility, as compared with performance with vehicle treatment.

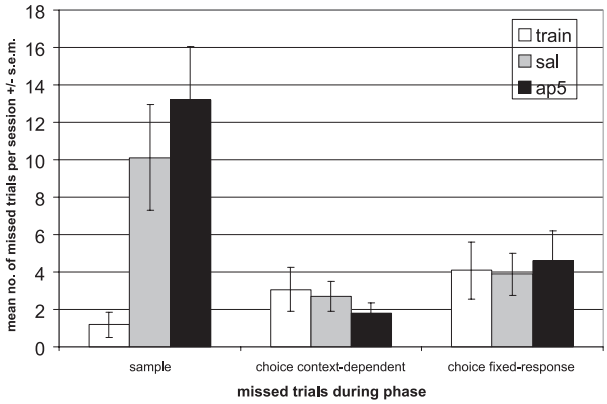

Figure 5. Number of missed trials plus or minus *SEM* during training sessions (train) and in the two experimental conditions: *N*-methyl-D-aspartate receptor blockade in the nidopallium caudolaterale (ap5) and vehicle infusion (sal). Missed trials during the sample phase and the response phase of context-dependent and fixed-response trials are shown separately. There was a significant treatment effect in the number of timeouts during the sample phase, $F(2, 16) = 9.633$, $p < .01$. However, in the matching phases there were no statistical differences among training, DL-2-amino-5-phosphonovaleric acid, and saline.

Discussion

The main results of our experiment are as follows:

1. NMDA receptor antagonism in the NCL significantly impaired performance in the context-dependent trials but not in the 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 stimulus–response associations.

2. The observed impairments are not attributable to increased distractibility or to deficits in motivation.

3. Response selection in the SMTS task was not based on fixed color pattern–response associations.

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 the 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, whereas the deficits in the context-dependent task are at odds with these findings. Although there are a few exceptions (Lee, Choi, Brown, & Kim, 2001; Roesler, Kuyven, Kruel, Quevedo, & Ferreira, 1998), unimpaired recall has been demonstrated for various conditioning scenarios (Baron & Moerschbaecher, 1996; Bohn et al., 2003; Churchill et al., 2001; Di Ciano, Cardinal, Cowell, Little, & Everitt, 2001; Kelley et al., 1997; Smith-Roe et al., 1999; Xu, Bazner, Qi, Johnson, & Freidhoff, 2003). However, in most of these studies, the requirement was to recall 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 can be the S+, with the actual S+ in a given trial being indicated only by the sample color. The higher demands of this conditional discrimination might be particularly sensitive to NMDA receptor antagonism in the NCL. Our results also correspond to PFC lesion studies reporting deficits in conditional discrimination in rats and monkeys (Petrides, 1982, 1991; Winocur & Eskes, 1998).

Reversal and extinction learning, requiring permanent changes in stimulus–response associations, were found to be impaired after NMDA receptor blockade in the rat orbitofrontal cortex (Bohn et al., 2003) and in the pigeon NCL (Lissek et al., 2002; Lissek & Güntürkün, 2003). Set shifting, requiring temporary switches between response alternatives that are correct in principle, also was found to be impaired with NMDA receptor blockade in the rat PFC (Stefani, Groth, & Moghaddam, 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. If we combine these results with the present findings, it is conceivable that NMDA receptors in the NCL are involved not only in permanently altering previously established stimulus–response associations but also in temporarily 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 stimulus–response associations from reference memory.

NMDA Antagonism-Induced Impairments in the Context-Dependent Task Occur Because of Deficits in Using Contextual Information Required by the Conditional Rule

The error increase in context-dependent trials might be related to deficits representing the conditional rule, as was argued in a study with PFC-lesioned subjects (Winocur & Eskes, 1998). In the present study, performance of a task requiring recall of a conditional rule was impaired also with NMDA receptor blockade in the NCL. Nevertheless, we did not observe a drop to chance level performance, as would be expected if the rule was completely inaccessible. Thus, it appears that the conditional rule requiring the use of contextual information 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 (Passingham, Myers, Rawlins, Lightfoot, & Fearn, 1988; Winocur, 1991), monkeys (Petrides, 1982, 1991), and humans (Petrides, 1985, 1991). Deficits in rule learning and response selection can coexist during conditional associative learning, and the PFC may participate in both functions (Stuss, Eskes, & Foster, 1994). It was suggested that, in general, a PFC-based deficit in

conditional associative learning performance might be reflected by impaired application of learned stimulus–response associations and impaired use of trial-specific information in the process of selecting correct responses (Winocur & Eskes, 1998). Our findings extend these results by demonstrating that NMDA receptors in prefrontal areas of the pigeon are presumably involved in the use of response-relevant context information.

NMDA Receptor Antagonism-Induced Impairments Are Not Attributable to Deficits in Motivation or to Increased Distractibility

During the sample phase, the number of missed trials increased significantly in both DL-AP5 and vehicle treatments compared with training. However, there was no significant difference between these experimental conditions. During the response phase, there was no significant difference in the number of missed trials among training, DL-AP5, and vehicle treatment both for context-dependent and for fixed-response trials. Taken together, these results indicate that there was no specific motivational deficit or increased distractibility following NMDA receptor blockade in the NCL, compared with the vehicle infusion, that might account for higher error rates.

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 stimulus–response 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 and instead turn the context-dependent task into another instance of a fixed-response task, in which unambiguous color patterns (instead of individual colors) are associated with certain responses. However, if pigeons had adopted such a pattern-based strategy, error rates should not have differed between context-dependent and fixed-response trials, as task difficulty would have been identical. Furthermore, performance in context-dependent trials should not have deteriorated in the DL-AP5 condition compared with the vehicle condition, as responding based on established and unambiguous associations tends not to be impaired by NMDA receptor antagonism. Therefore, the observed difference in error rates renders it extremely unlikely 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 DL-AP5 and vehicle conditions lends additional support to an assumption that the deficits observed in the DL-AP5 condition during the context-dependent trials cannot be attributed to deficits in rule recall, as long as recall pertains only to previously acquired, unambiguous stimulus–response associations.

Functional Equivalency of the NCL and the PFC

As outlined in detail in the introduction, there is evidence from a large variety of experiments that the NCL is a functional equivalent of the PFC. This study, too, underlines that the NCL apparently performs functions similar to those of the PFC with regard to the integration and usage of task-relevant information in an appetitive conditioning paradigm. However, it is important to note that even such an impressive number of similarities across taxa as reported in the introduction does not necessarily imply a homology between the NCL and the PFC in terms of their phyletic continuity. Indeed, there are strong topographical and genetic arguments that make it likely that the NCL and the PFC are not homologous but represent a remarkable case of evolutionary convergence (homoplasy; Medina & Reiner, 2000; Puelles et al., 2000).

In fact, even within the class of mammals there is an ongoing dispute regarding whether rats possess a prefrontal region that is comparable to the dorsolateral PFC of primates (Uylings, Groenwegen, & Kolb, 2003) or not (Preuss, 1995). Against this background, it seems to be premature to speculate about equivalencies of subfields of the NCL and the PFC, although there is some evidence for a parcellation of the NCL (Diekamp et al., 2002; Kröner & Güntürkün, 1999; Riters, Erichsen, Krebs, & Bingman, 1999). We therefore have refrained from comparing the NCL with specific PFC subfields and instead have discussed the context-processing function of the NCL as a whole.

Conclusion

In extension of previous results demonstrating the involvement of NCL-based NMDA receptors in acquiring correct responding during various learning processes, we have shown here for the first time that NMDA receptors in the pigeon equivalent of the PFC 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 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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