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Integration of contextual cues into memory depends on "prefrontal" N-methyl-D-aspartate receptors

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ABSTRACT

Every learning event is embedded in a context, but not always does the context become an integral part of the memory; however, for extinction learning it usually does, resulting in context-specific conditioned responding. The neuronal mechanisms underlying contextual control have been mainly investigated for Pavlovian fear extinction with a focus on hippocampal structures. However, the initial acquisition of novel responses can be subject to contextual control as well, although the neuronal mechanisms are mostly unknown. Here, we tested the hypothesis that contextual control of acquisition depends on glutamatergic transmission underlying executive functions in forebrain areas, e.g. by shifting attention to critical cues. Thus, we antagonized N-methyl-D-aspartate (NMDA) receptors with 2-amino-5-phosphonovaleric acid (AP5) in the pigeon nidopallium caudolaterale, the functional analogue of mammalian prefrontal cortex, during the concomitant acquisition and extinction of conditioned responding to two different stimuli. This paradigm has previously been shown to lead to contextual control over extinguished as well as non-extinguished responding. NMDA receptor blockade resulted in an impairment of extinction learning, but left the acquisition of responses to a novel stimulus unaffected. Critically, when responses were tested in a different context in the retrieval phase, we observed that NMDA receptor blockade led to the abolishment of contextual control over acquisition performance. This result is predicted by a model describing response inclination as the product of associative strength and contextual gain. In this model, learning under AP5 leads to a change in the contextual gain on the learned association, possibly via the modulation of attentional mechanisms.

1. Introduction

Decades of research have shown that the extinction of conditioned responses after initial acquisition is mostly based on a new inhibitory learning process which is context-dependent (Bouton, 2004). Since extinguished behavior re-occurs outside the extinction context, it is assumed that extinction is more context-specific than excitatory conditioning (see Todd, Vurbic, & Bouton, 2014 for a recent review). Neuronal mechanisms of contextual control of behavior are mainly investigated in regard to extinction learning. In addition, researchers have largely focused on the hippocampus because of its role in processing visual-spatial information (Maren, Phan, & Liberzon, 2013). However, despite demonstrations of contextual control of non-extinguished behavior (Bernal-Gamboa, Nieto, & Rosas, 2015; Bernal-Gamboa, Rosas, & Callejas-Aguilera, 2014; Harris, Jones, Bailey, & Westbrook, 2000; Leon, Matias, & Rosas, 2012; Rosas & Callejas-Aguilera, 2006; Starosta et al., 2016), investigations into the neuronal mechanisms of this phenomenon are rare. Sharpe and Killcross showed for example, that lesioning the prelimbic part of the prefrontal cortex (PFC) prevents learning about contextual cues (Sharpe & Killcross, 2014b, 2015a) by modulating the direction of attention (Sharpe & Killcross, 2014a, 2015b). However, knowledge about specific neurotransmitter systems involved within the PFC is lacking. N-methyl-D-aspartate (NMDA) receptors present a prime locus of neuronal plasticity and are critically involved in learning and memory (Sweatt, 2016). Accordingly, this study aimed to investigate the role of NMDA receptors in contextual learning that accompanies acquisition of conditioned behavior.

We chose pigeons as experimental animals because of their inherent ability to work on multiple visual conditioned stimuli in parallel (Colombo & Scarf, 2012; Güntürkün, Stüttgen, & Manns, 2014; Starosta, Güntürkün, & Stüttgen, 2013; Starosta, Stüttgen, & Güntürkün, 2014; Starosta et al., 2016). The avian brain lacks a

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laminated cerebral cortex, but there is strong evidence that one pallial area (nidopallium caudolaterale, NCL) serves as a functional equivalent to mammalian PFC in general (Güntürkün, 2005; Güntürkün & Bugnyar, 2016), and in regard to function and distribution of NMDA receptors (Herold et al., 2011; Lissek & Güntürkün, 2003).

To study the role of NMDA receptors in contextual learning, we subjected pigeons to a modified ABA renewal paradigm. In a first experimental phase, acquisition of responses to one stimulus (CS1) took place in context A. Then, extinction of response to this stimulus took place in context B. In addition, animals acquired responses to a second stimulus (CS2) in B. This procedure has been shown to induce contextual control of extinguished as well as non-extinguished behavior (Starosta et al., 2016). During learning in context B (acquisition of CS2 along with extinction of CS1), we either locally antagonized NMDA receptors in the NCL with AP5 or administered saline. In a third experimental phase, responding to both CSs was tested drug-free in both contexts. Critically, we used a within-subject design and thereby equated the learning histories of the contexts. We hypothesized that a blockade of NMDA receptors in the avian analogue of PFC during learning leads to an abolishment of contextual control of behavior by disrupting the integration of contextual information into the memory trace.

2. Materials and methods

2.1. Subjects

Subjects were eighteen unsexed, experimentally-naive homing pigeons (Columbia *livia*). Birds were obtained from local breeders and raised in the institute's own aviary. Animals were housed in groups of eight in small aviaries with a 12 h dark-light cycle (lights off at 8 p.m.), and food-deprived to 85–90% of their free-feeding weight. Water was available at all times; on weekends, food was freely available; on weekdays, food was restricted to the period of daily testing. All subjects were treated according to the German guidelines for the care and use of animals in science. All procedures were approved by a national ethics committee of the State of North Rhine-Westphalia, Germany.

2.2. Behavioral apparatus

Experimental testing took place in two operant champers, each measuring 34 cm × 34 cm × 50 cm. Colored patterns similar to those depicted in Starosta et al. (2014) served as conditioned stimuli (CS). CSs were presented on an LCD touch screen mounted against the back wall of the chamber. Key pecks were registered from the touch screen and produced audible feedback clicks. A food hopper was placed below the monitor and permitted intermittent access to food (grain). The chambers were housed in sound-attenuating shells. In one chamber (context A), the walls were lined with yellow wallpaper and loudspeakers provided white noise, whereas in the other chamber (context B), the walls were painted red, and brown noise was audible. The designation of physical contexts as acquisition or extinction contexts as well as which context was used for testing after drug application was counterbalanced across animals (see description of phase 2 below). All hardware was controlled by custom-written MATLAB code (The MathWorks, Natick, MA) using the Biopsychology Toolbox (Rose, Otto, & Dittrich, 2008).

2.3. Surgery

Before the training started, animals underwent surgery for cannula implantation to allow for the local application of the NMDA receptor antagonist AP5 during the second experimental phase. The targeted region was the center of the nidopallium caudolaterale as defined by the following coordinates: AP +5.25 mm, ML ±7.5 mm, DV +1.1 mm (Karten & Hodos, 1967). For initial anesthesia, animals were injected with a combination of ketamine and xylazine (7:3 units, 0.15 ml/100 g body weight, Ketavet, Zoetis, Berlin, Germany; Rompun, Bayer GmbH, Leverkusen, Germany); anesthesia was maintained with isoflurane (Forane 100% (V/V); Abbvie, Ludwigshafen, Germany). After induction of anesthesia, the feathers on the head were cut. The head was placed in a stereotaxic apparatus and the scalp was cut and pulled sideways. Above the NCL, a small hole was drilled into the skull and the dura was removed. Then, the cannula was lowered into the brain. The hole was covered with vaseline and above that light-curing dental cement was applied. Six to eight micro-screws were placed into the skull to anchor the cannulas (C315G; 8 mm, Plastics One) to the head with dental cement. The wound was sutured and local antibiotics (tyrosur powder, Engelhard Arzneimittel, Niederdorfleben, Germany) were applied. Animals received analgesics (0.3 ml, 10 mg/ml, Rimaldyl, Pfizer GmbH, Münster, Germany) for a minimum of two consecutive days after surgery and were allowed to recover for two weeks before testing started.

2.4. Behavioral training

We used a modified ABA-renewal paradigm based on previous designs (Lengersdorf, Marks, Uengoer, Stüttgen, & Güntürkün, 2015; Lengersdorf, Stüttgen, Uengoer, & Güntürkün, 2014; Rescorla, 2008). Please see Fig. 1 for an illustration of the paradigm and Table 1 for an overview of the stimuli and reinforcement conditions in each phase. Every animal served as its own control by being tested both under saline and drug (AP5) on separate days (within-subject design; sequence counterbalanced across animals). We used a sign-tracking paradigm; i.e. presentation of the unconditioned stimulus (US) was not dependent on the animals' response. That way, contexts were equated for their learning histories. The stimulus presentation sequence was randomized during all experimental phases.

2.4.1. Pre-training

Animals were habituated to the conditioning chambers and confronted with two control stimuli (one CS + (Target, consistently followed by food); one CS − (NonTarget, never followed by food)). Target and NonTarget were presented throughout the experiment, and their reinforcement contingencies did not change. We used these two control stimuli to assess the upper and lower boundaries of response strength in the various experimental conditions. During the Pre-Training phase, animals were tested daily in each context for four weeks. First, an autoshaping procedure for the Target stimulus was performed (Pre-Training I in Table 1, 5 sessions à 60 trials in both contexts). In the second week, the NonTarget stimulus was introduced (Pre-Training II in Table 1, 15 sessions à 62 trials in both contexts). There were two warm-up Target-presentations at the very beginning of each session. These warm-up trials were included in every session of the experiment but not analyzed. Table 1 gives an overview of the stimuli and reinforcement conditions in each phase. The inter-stimulus-interval (ITI) lasted 120 s during the first two weeks of Pre-Training, and was decreased stepwise down to 30 s in the last two weeks of the Pre-training phase. In all following experimental phases the ITI lasted 30 s. Stimulus presentation time was 5 s for all stimuli in all phases.

2.4.2. Phase 1

In this phase, animals were confronted with a new stimulus in each context (henceforth, CS1-A and CS1-B). CS1-A was only

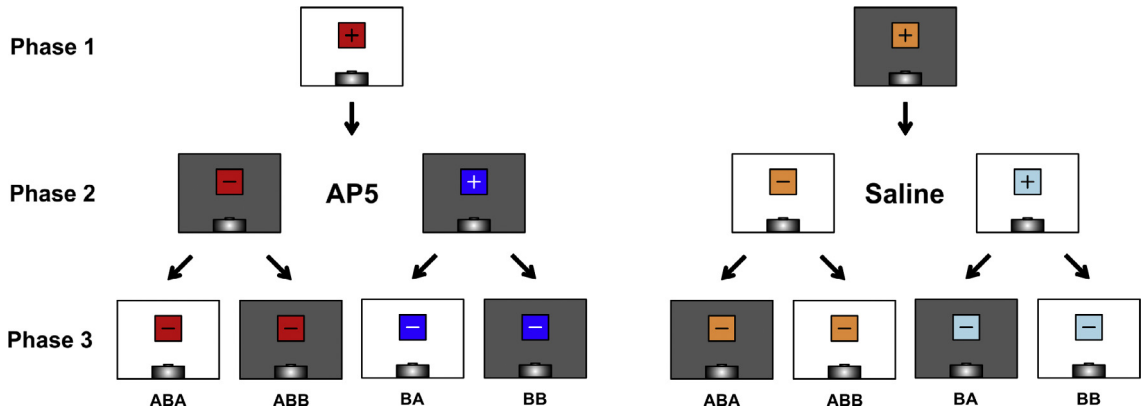

Fig. 1. Depiction of the modified within-subject ABA renewal design. Single pictures illustrate physical contexts. Red and orange squares with a plus indicate the two different conditioned stimuli in the first phase where stimulus presentation was followed by food. Before the second phase started saline or AP5 was microinjected locally to the NCL. Then, stimuli from phase 1 were presented in a different context and no longer followed by food (minus) and a novel stimulus followed by food was introduced (blue and cyan squares with a plus). In the retrieval phase, all stimuli were tested in extinction in both contexts. The Target stimulus was present and followed by food in all sessions, and the NonTarget stimulus was present but not followed by food in all sessions (not shown here). Contexts and drug application were balanced across subjects. This figure shows one possible example. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Overview of the training procedure. For every phase of the experiment the number of trials for each stimulus in each context is shown. Plus (+) indicates stimulus presentation followed by food; minus (−) a presentation without food presentation.

Phase	Context	Target	Non-target	CS1a or CS1b	CS2a or CS2b	Trials per session
Pretraining I	A	60 (+)				60
	B	60 (+)				60
Pretraining II	A	32 (+)	30 (−)			62
	B	32 (+)	30 (−)			62
Phase1	A	18 (+)	16 (−)	16 (+)		50
5 sessions each	B	18 (+)	16 (−)	16 (+)		50
Phase2	A	26 (+)	24 (−)	52 (−)	52 (+)	154
1 session each	B	26 (+)	24 (−)	52 (−)	52 (+)	154
Phase3	A	44 (+)	12 (−)	12x CS1-A (−) and 12x CS1-B (−)	12x CS2-A (−) and 12x CS2-B (−)	104
1 session each	B	44 (+)	12 (−)	12x CS1-A (−) and 12x CS1-B (−)	12x CS2-A (−) and 12x CS2-B (−)	104

presented in context A, while CS1-B was only presented in context B. Both stimuli were followed by food. CS1-A or CS1-B as well as the NonTarget stimulus were presented 16 times in each session while the Target stimulus was presented 18 times (50 trials per session in total). As in Pre-Training, animals were tested twice per day (one session in each context; sequence counterbalanced across animals) for five consecutive days resulting in a total of 5 sessions in each context in phase 1.

2.4.3. Phase 2

This phase consisted of two sessions for each animal: one with drug infusion, one with saline treatment. The two sessions were spaced 48 h apart to allow for complete washout of the drug. In each session, either the CS1-A or CS1-B was presented 52 times. They were presented in a different context than in Phase 1 (i.e. CS1-A in context B, CS1-B in context A), and both were no longer followed by food. Two new stimuli (CS2-A; CS2-B; one in each context) were also presented in this phase (52 times each). Their presentation was consistently followed by food. Thus, extinction of responding to one CS1 and acquisition of responding to one CS2 took place simultaneously and either under AP5 or saline treatment. In addition, Target and NonTarget were presented 26 or 24 times which adds up to 154 trials in each session of phase 2. The sequence of drug/saline administration was counterbalanced across animals, i.e. seven out of the 15 animals (three animals had to be excluded due to insufficient performance during Pretraining; see results) were first tested under saline while eight were tested under AP5 first. In addition, we counterbalanced the assignment of stimuli and contexts to drug conditions, yielding four groups of animals: four animals were tested first in context A with drug application while another four were tested first in Context B with drug. In addition, three animal were tested with saline first in context A and another four with saline in context B. The conditions were reversed in the second session of the extinction phase.

2.4.4. Phase 3

In the last phase of the experiment, all four experimental CSs as well as Target and NonTarget were presented in each context on the same day (one session in each context, sessions spaced 1 h apart). The Target was presented 44 times and followed by food while NonTarget, CS1 and CS2 were presented 12 times each and not followed by food. Thus, each session had 104 trials. The sequence of contexts tested was counterbalanced across animals. Testing took place under drug-free conditions, 48 h after the last extinction session.

2.5. Drug application

Drug application took place during phase 2 of the experiment. Each animal was tested in each context either after saline or AP5 application. Thus, each animal served as its own control (within-subject-design). 10–15 min before each extinction session, AP5 (Sigma–Aldrich Co., St. Louis, USA) or physiological saline was microinjected bilaterally in the NCL. We injected 0.5 μl of the respective fluids per cannula (AP5: total volume 1 μl, containing

5 mg of AP5; 0.5 μl per cannula, i.e., 2.5 μg of AP5 per cannula, saline: total volume 1 μl; 0.5 μl per cannula). Sequence and contexts in which drug application took place were counterbalanced across animals (see Section 2.4.3).

2.6. Analyses

The absolute number of conditioned responses (pecks directed onto the conditioned stimuli) during stimulus presentation served as the main dependent variable in this experiment. Consistent with the experimental design, only within-subject comparisons were performed, including repeated-measures analysis of variance (rmANOVA) with one or two within-subject factors (blocks of trials or context and stimuli), and paired t-tests. In addition, we computed effect sizes (η^2 for rmANOVAs and Hedges' g for pairwise comparisons). All analyses were conducted in MATLAB (The Mathworks, Natick, MA) using the Measures of Effect Size Toolbox (Hentschke & Stüttgen, 2011).

3. Results

Three out of 18 animals showed no differential responses in any of the experimental phases between rewarded and non-rewarded stimuli (<80% correct). The data of these animals were discarded, resulting in a final data set of 15 animals.

3.1. Histology

After completion of the experiment, cannula positions were verified with immunohistochemical techniques as described in Lengersdorf et al. (2015). Fig. 2 shows the histological analyses of cannula position of the 15 animals included in this study. All cannula positions were centered well within the boundaries of the NCL as defined by Kröner and Güntürkün (1999).

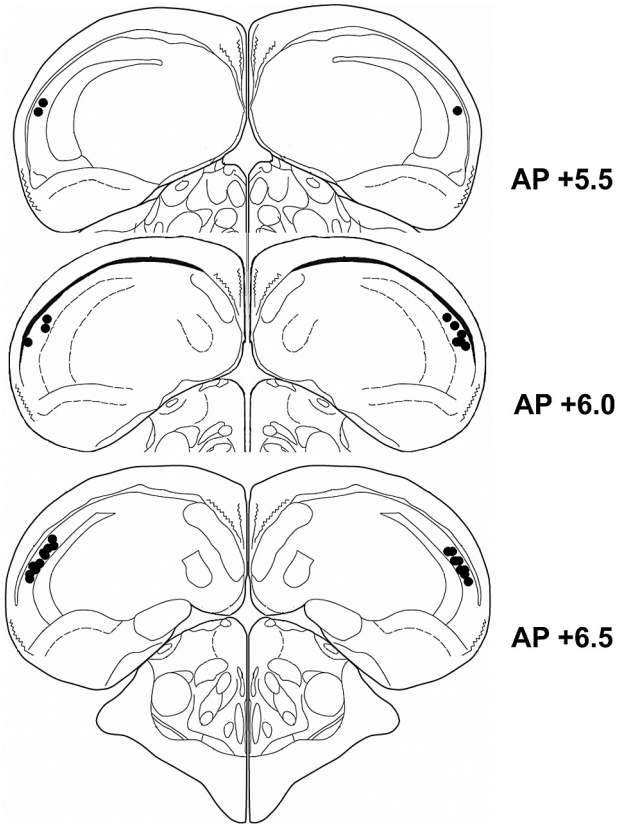

Fig. 2. Histological data. Schematic slices of the pigeon brain. Dots represent the tips of the injection cannulas (AP5 injection sites). Pictures are based on the brain atlas by Karten and Hodos (1967).

3.2. Behavioral data

Pre-Training was uneventful (data not shown). Fig. 3 shows the mean number of responses to different stimuli of all animals in the three experimental phases.

3.2.1. Phase 1

In the first phase of the experiment, animals were confronted with one new CS in each context (CS1-A in A and CS1-B in B) in addition to the control stimuli. Presentation of these new CS was always followed by food. The data from this phase are shown in Fig. 3A. To test for successful acquisition of responding and to exclude any systematic influences of the physical contexts or stimuli, we analyzed the mean number of responses to the control stimuli and CS1s in both contexts. We defined successful acquisition as a difference in responses between stimuli followed or not followed by food. Thus, we computed a two-way-repeated measures ANOVA with the within-subject factors stimulus (NonTarget, CS1) and context (A, B). This yielded a significant effect of stimulus ($F(1,14) = 32.04$, $p < 0.001$, $\eta^2 = 0.52$), no effect of context ($F(1,14) = 1.85$, $p = 0.20$, $\eta^2 = 0.002$) and no stimulus-context interaction ($F(1,14) = 2.22$, $p = 0.16$, $\eta^2 = 0.002$). Thus, animals responded more to the CS1 than to the NonTarget and their responses did not differ between contexts.

3.2.2. Phase 2

In the second phase of the experiment, CS1 presentations were no longer followed by food and they took place in a different context, respectively (CS1-A in B and CS1-B in A). In addition, animals were confronted with a novel stimulus in each context (CS2-A, CS2-B) which was consistently followed by food. This phase consisted of two experimental sessions. One took place after drug (AP5), the other after saline application (sequence counterbalanced across animals).

Fig. 3B shows mean response counts to CS1 and CS2 in drug and saline conditions. For the CS1, an rmANOVA with factors time (block of four trials) and treatment (AP5, saline) revealed a main effect of time ($F(12,168) = 19.20$, $p < 0.001$, $\eta^2 = 0.21$), reflecting a decrease in responding. In addition, we observed a main effect of treatment ($F(1,14) = 6.14$, $p = 0.03$, $\eta^2 = 0.09$) and a block-treatment interaction ($F(12,168) = 3.00$, $p < 0.001$, $\eta^2 = 0.04$), indicating more responses under AP5 than under saline, which was more pronounced at the end of the session.

In the same way, we computed an rmANOVA with factors time and treatment for CS2. This yielded again a significant effect of time ($F(12,168) = 3.48$, $p < 0.001$, $\eta^2 = 0.02$), reflecting an increase in responding over the time course of the session. However, we observed no main effect of treatment ($F(1,14) = 0.41$, $p = 0.53$, $\eta^2 = 0.01$) and no block-treatment interaction ($F(12,168) = 0.60$, $p = 0.84$, $\eta^2 = 0.004$). Thus, different from the extinction of a conditioned response, AP5 application to the NCL had no measurable effect on the acquisition of the conditioned response.

Similar to our previous study (Lengersdorf et al., 2015), we observed a nonspecific increase in responding to the Target under AP5 compared to the saline condition in the second half of the experimental session (blocks 5–12; data not shown). An rmANOVA with factors time and treatment for responses to the Target revealed a significant interaction ($F(11,154) = 2.61$, $p = 0.005$, $\eta^2 = 0.02$) reflecting the nonspecific increase of responses at the end of the session. This nonspecific increase of conditioned responding could, in principle, mask successful extinction of responding to CS1. We corrected for this effect by normalizing

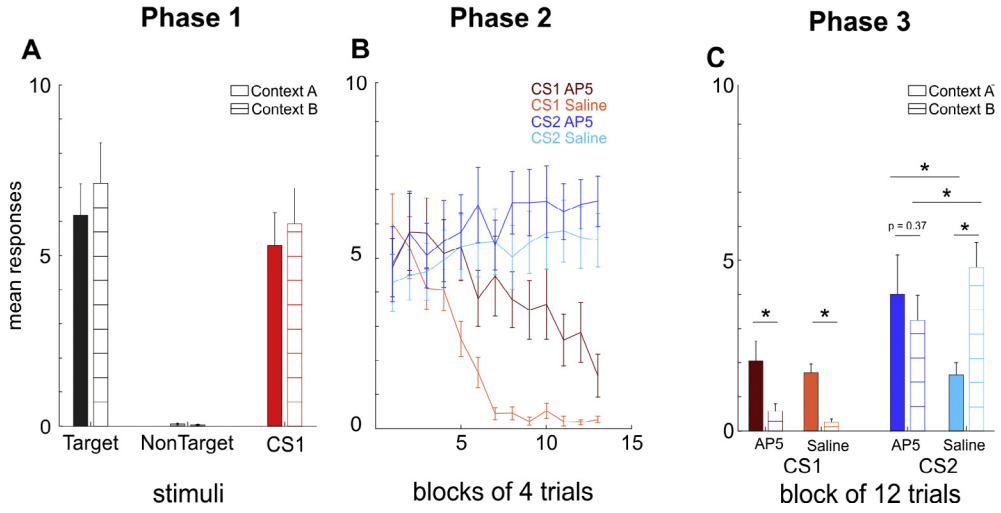

Fig. 3. Absolute response counts (±SEM) during all three experimental phases. (A) Mean absolute responses (±SEM) to the Target, NonTarget and CS1 in the last session of phase 1 are shown. Solid bars show responses in physical context A while striped bars indicate responses in context B. Animals responded more to the CS1 then to the NonTarget, and the amount of responding did not differ between contexts. (B) Mean absolute responses (±SEM) to the CS1 and CS2 under saline or AP5 (in blocks of four trials) during the second phase. Responses to different stimuli are color-coded. In this phase responding to the CS1 decreased while responding to the CS2 increased. (C) Mean absolute responses (±SEM) averaged over the twelve trials in the retrieval phase and split up by context as well as treatment. Asterisks indicate $p < 0.05$. Under saline, a clear context effect could be observed, i.e. stronger responding in the respective acquisition context (A for CS1 and B for CS2). This effect was not affected by AP5 for the CS1. However, for the CS2 no context-specific responding was observed when responding was acquired under AP5.

response rates. We multiplied the average number of CS1-responses under AP5 in a given bin of four consecutive trials by the ratio of Target responses under saline and drug in the same bin of four trials, separately for each animal. After that, we repeated the preceding analyses (see Lengersdorf et al., 2015 for a detailed rationale and details of the procedure). Importantly, this normalization had no qualitative effect on the results: extinction of conditioned responding was still attenuated under AP5 as indicated by a main effect of treatment ($F(1,14) = 8.24$, $p = 0.01$, $\eta^2 = 0.07$), time: $F(12,168) = 10.74$, $p < 0.001$, $\eta^2 = 0.18$; interaction ($F(12,168) = 1.21$, $p = 0.28$, $\eta^2 = 0.02$). In the same manner, normalization of the CS2 responses still failed to yield a drug effect (treatment ($F(1,14) = 1.47$, $p = 0.25$, $\eta^2 = 0.016$), time: $F(12,168) = 1.01$, $p = 0.44$, $\eta^2 = 0.008$; interaction ($F(12,168) = 1.31$, $p = 0.22$, $\eta^2 = 0.01$). We conclude that elevated response levels to CS1 reflect an impairment of extinction learning or the expression of extinction learning rather than nonspecific motor effects.

3.2.3. Phase 3

Two days after the last extinction session, response strength to all stimuli were tested in both contexts. Fig. 3C shows the average number of responses to CS1 and CS2 in both contexts when responding was previously extinguished/acquired under AP5 or saline. To investigate possible influences of the drug treatment on the context-specificity of responding, we computed for both stimuli an rmANOVA with the within subject factors treatment and context. For the CS1 this resulted in a main effect of context ($F(1,14) = 12.66$, $p < 0.001$, $\eta^2 = 0.24$), but neither a treatment nor a treatment-context interaction effect reached statistical significance (treatment: $F(1,14) = 0.73$, $p = 0.4$, $\eta^2 = 0.01$; interaction ($F(1,14) = 0.004$, $p = 0.95$, $\eta^2 = 0.00$). Thus, we observed renewal (more responses in the context of acquisition (A) than the context of extinction (B)) under AP5 as well as under saline conditions which was confirmed by post hoc paired t-tests (AP5: $t(14) = 3.57$, $p = 0.003$, $g = 0.84$; saline: $t(14) = 6.59$, $p < 0.001$, $g = 1.84$). Critically, when we computed the same rmANOVA for the CS2 as for the CS1, we observed context-specific responding in the saline but not in the AP5 condition as indicated by a significant context by treatment interaction ($F(1,14) = 16.31$, $p = 0.001$, $\eta^2 = 0.09$). In addition, we observed a main effect of context ($F(1,14) = 5.52$, $p = 0.03$, $\eta^2 = 0.05$), but no treatment effect ($F(1,14) = 0.32$, $p = 0.58$, $\eta^2 = 0.004$). However, the significant context by treatment interaction confirmed the described context-specificity of responses under saline but not under AP5. To further investigate the origin of the attenuated context effect, we performed post hoc paired t-tests. These indicated that, under AP5, responses in the same context (B) were decreased compared to responses under saline ($t(14) = 2.48$, $p = 0.03$, $g = 0.69$), suggesting an impaired consolidation of the association since acquisition learning in phase 2 was unaffected. In addition, responding in a new context (A) was increased compared to saline ($t(14) = 2.48$, $p = 0.03$, $g = 0.53$). This effect hints at a failed integration of contextual cues into the memory, leading to an abolishment of contextual control of behavior.

In summary, we observed that blocking NMDA receptors slowed down the process of extinction learning, but not the acquisition of a conditioned response. However, we observed less expression of the recently acquired memory in a test phase. In regard to the context-specificity of responses, contextual control of acquisition, but not extinction memory, was affected when learning took place under AP5.

4. Discussion

We sought to elucidate the neuronal mechanisms of contextual control over acquisition of a conditioned response and started out with the hypothesis that NMDA receptors in executive brain areas mediate contextual control of behavior, e.g. by shifting attention to critical cues. To test our assumption, we subjected pigeons to a within-subject sign-tracking paradigm where animals acquired conditioned responses to one stimulus, while responses to another stimulus were simultaneously extinguished. This has been shown to lead to contextual control over extinguished as well as non-extinguished responding before (Starosta et al., 2016). In addition, we blocked NMDA receptors in the avian prefrontal analogue with AP5 while these learning processes simultaneously took place. We observed an impairment in extinguishing but not in acquiring a conditioned response under AP5 (the former has been shown before by us: Lengersdorf et al. (2015). In addition, we found that

NMDA receptor blockade impaired consolidation of acquisition memory and, most importantly, we observed that context-specificity of acquisition memory vanished when learning took place under AP5. Together, these findings confirm our starting hypothesis about prefrontal mechanisms underlying the contextual control of behavior.

Regarding the effect of AP5 application during acquisition and extinction of a conditioned response, the present results resemble previous ones. Like Lengersdorf et al. (2015), we observed attenuated extinction learning under the influence of AP5. This also matches observations from Lissek, Diekamp, and Güntürkün (2002) who observed an impairment of reversal learning, and a detailed analysis suggested that this impaired reversal learning resulted exclusively from impaired extinction of the previously reinforced response. A discrepancy with one earlier study (Lengersdorf et al., 2014) is that impaired extinction was not observed after silencing neurons in the nidopallium caudolaterale (NCL) with tetrodotoxin (TTX) – a blocker of voltage-gated sodium channels. As we have argued before (Lengersdorf et al., 2015) this difference likely derives from the different drugs that were used. While TTX silences neuronal activity, NMDA receptor antagonists actually enhance neuronal activity in prefrontal areas. For example, the NMDA receptor antagonist MK-801 was shown to decrease the activity of inhibitory interneurons in PFC and thereby indirectly increase the firing rate of pyramidal neurons (Homayoun & Moghaddam, 2007). Similarly, the magnitudes of working memory impairment as well as motor activity increase correlate with increased activity of PFC neurons after systemic injections of MK-801 in rats (Jackson, Homayoun, & Moghaddam, 2004). Critically, in the pigeon NCL, fast-spiking neurons which resemble GABAergic interneurons and project onto principal neurons have been described (Kröner, Gottmann, Hatt, & Güntürkün, 2002). Because the NCL is interconnected with a large number of sensory-associative, limbic and motoric areas and therefore is considered an important hub of the bird forebrain (Shanahan, Bingman, Shimizu, Wild, & Güntürkün, 2013), it is reasonable that increased activity due to an NMDA receptor blockade in this region also interferes with extinction learning. However, one limitation of the present study as well as the other mentioned studies is that we cannot dissociate if increased responding observed under AP5 during extinction reflects an impairment of extinction learning per se or merely its expression. This issue might be addressed by future research testing responding in the retrieval phase under AP5.

In the third phase of the experiment, we did not observe an increase of spontaneous recovery of responses to CS1 when extinction took place under either AP5 or saline. We assign this mismatch to our previous work (Lengersdorf et al., 2015) in which spontaneous recovery was elevated after AP5 but not saline application to different response levels under AP5 at the end of the extinction session. The increased spontaneous recovery in that previous study was interpreted as the consequence of increased responding at the end of the extinction session. In the present study, animals underwent twice as many extinction trials which led to a non-significant effect of drug in the last block of the extinction session for the CS1 ($t(14) = 1.95$, $p = 0.07$). The same is true for the test session: on a descriptive level, one observes higher responding to CS1 under AP5. However, this effect does not reach statistical significance ($t(14) = 1.34$, $p = 0.16$). We conclude that extinction learning is somewhat attenuated under AP5, and with more extinction trials the effect of AP5 is not observable (does not reach statistical significance) at the end of extinction and in the test session.

At first glance, our result of attenuated extinction but unimpaired consolidation upon administration of NMDA receptor antagonists is at odds with others reporting rather faster extinction and a consolidation deficit of extinction learning (Lee, Milton, & Everitt, 2006; Santini, Muller, & Quirk, 2001). We see two reason for this discrepancy. First, the mentioned studies in rodents antagonized NMDA receptors systemically while here we injected AP5 locally in the NCL. This can explain the difference, because manipulation of NMDA receptors with systemic injections affect extinction via affecting the amygdala (Walker, Ressler, Lu, & Davis, 2002), among all other brain structures expressing NMDA receptors. In addition, as we argue above, systemic injection of an NMDA receptors antagonist increased neuronal firing in prefrontal structures as well and motor activity in rats (Jackson et al., 2004). While in the above mentioned studies extinction would manifest in more motor activity (less freezing), in our appetitive design, less motor activity (less pecking) is the behavioral readout of extinction. While we used a control stimulus to account for unsystematic motor effects and see impaired extinction nonetheless, we argue that the observed difference stems from the different application methods (systemic or local) or how unsystematic motor effects influence the behavioral readout of extinction. Importantly, a study in rodents which applied NMDA receptors antagonists locally to prefrontal structures also report an impairment of extinction learning in a conditioned place preference paradigm (Hsu & Packard, 2008).

Our results emphasize the specific involvement of executive regions in extinction learning, while these regions seem to play a minor role in the process of acquiring a conditioned response. Similar conclusions were drawn from imaging and lesion studies in humans and rodents (Milad et al., 2007; Peters, Kalivas, & Quirk, 2009; Quirk, Russo, Barron, & Lebron, 2000), as well from the before-mentioned study in pigeons in which NMDA receptors were blocked during a reversal task (Lissek et al., 2002). However, acquiring a conditioned response under AP5 in our study induced an impairment in the consolidation of the association (decrease of responding to the CS2 in the same context as acquisition in the retrieval phase). We interpret decreased responding in the same context as a consolidation deficit, because acquisition learning in phase 2 was unaffected. While the consolidation of acquisition memory was never explicitly tested in the before-mentioned studies in pigeons, impaired consolidation is in line with experiments supporting the general role of NMDA receptors in synaptic plasticity and consolidation processes (Sweatt, 2016).

The main result of this study is that context-specificity for acquisition memory (assessed with CS2) vanished when learning took place under AP5. More precisely, we observed a decrease in responding to CS2 when retrieval testing took place in the context of acquisition, and an increase in responding to CS2 when testing took place in a different context (comparing saline and AP5 conditions). We interpret the decrease in responses in the same context as a distortion of the consolidation but not the encoding of acquisition learning, because acquiring the association was unaffected in phase 2. We propose in addition that the increased response rate in the new context is a consequence of impaired contextual learning under AP5. Previously, we put forward the "contextual gain model", which treats contextual cues as a modulating factor of associative strength by attenuating its gain (Starosta et al., 2016; see Delamater & Westbrook, 2014, and Urcelay & Miller, 2014, for similar ideas). In this model, associative strength (AS) is multiplied by a factor (contextual gain, CG) between 1 (context of learning) and 0 (maximally distinct context), and this product defines the overall response inclination (RI) of the animals ($RI = AS * CG$). The more different the context of testing from that of learning, the lower the response inclination, given a fixed level of associative strength. The contextual gain model and the influences of AP5 on responding are exemplified in Fig. 4. Under saline, the gain and associative strength and therefore also the response inclination, was maximal when responses to the CS2 was tested in the original learning context (bar D; AS = high; gain = 1). However, when responding to the

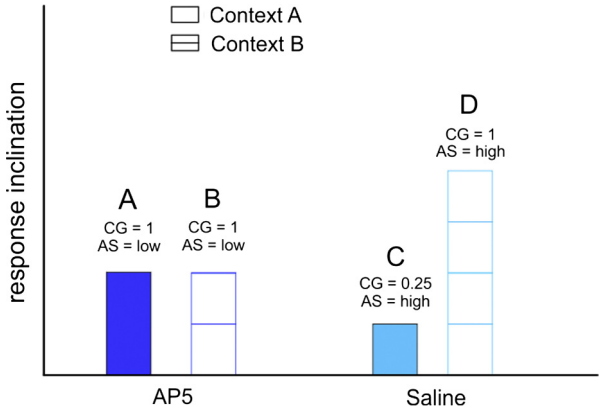

Fig. 4. Contextual gain model. Response inclination (RI) is shown for different conditions. RI is defined as the product of associative strength (AS) and contextual gain (CG): RI = AS * CG. Color-coded bars correspond to differential modulatory influence by the context, AS and drug application. A: AS = low; CG = 1; B: AS = low; CG = 1; C: AS = high; CG = 0.25; D: AS = high; CG = 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CS2 was tested within a different context, response inclination was reduced due to a small contextual gain (bar C; AS = high; gain = 0.25). As a result, one observes less responding in a new context (C compared to D). Learning under AP5 led to an abolishment of contextual influences and thus no difference in the contextual gain between the contexts (gain = 1). It follows that responding in a different context is increased when acquired under AP5 (A compared to C). This is exactly what we observed. In addition, we observed a decrease in responding in the original learning context when learning took place under AP5. We interpret this as a consolidation deficit which decreases the associative strength under AP5. As a result, one observes an overall decrease in response inclination in the same context (D compared to B) as well as in the different context (A compared to D). In summary, we propose that contextual cues are integrated into the memory about a stimulus, and thereby modulate associative strength. However, when glutamate transmission within prefrontal areas is altered by AP5 during learning, this contextual modulation vanishes.

These results match with previous studies investigating the role of PFC in using informative contextual cues. Sharpe and Killcross showed in a series of experiments that the prelimbic part of the PFC is critical for contextual bi-conditional discrimination and fear renewal (Sharpe & Killcross, 2014b, 2015a) by acting on the regulation of attention to contextual cues (Sharpe & Killcross, 2014a, 2015b). The data presented here expands on this in two ways: First, they provide evidence that prefrontal areas guide the integration of contextual cues for association even though these cues do not provide information for the specific association. In addition, we pinpoint a specific neurotransmitter receptor involved in contextual learning.

At first glance, unimpaired contextual control of extinguished behavior under AP5 is not in line with the explanation of vanished contextual control under AP5 presented above. If the integration of contextual cues is impaired under AP5, this should hold for extinguished as well as non-extinguished behavior. There are several explanations for this apparent contradiction. First, contextual control of extinguished behavior could be independent of prefrontal glutamate transmission but depend on other neuronal mechanisms. In this framework, the hippocampus as well as the amygdala were indicated as key structures. For example, Maren and Hobin (2007) reported that context-dependent neuronal activity in the amygdala was mediated by the hippocampus and inactivation of the hippocampus led to an impairment in context-specific memory retrieval (Corcoran & Maren, 2001). In general, the hippocampus seems to regulate context-specific fear after extinction (Bouton, Westbrook, Corcoran, & Maren, 2006; Maren et al., 2013; Sotres-Bayon, Sierra-Mercado, Pardilla-Delgado, & Quirk, 2012). However, this neuronal mechanism is rather seen as an interaction between the amygdala, hippocampus and prefrontal structures und would predict an involvement of the PFC in context specific associations. Therefore, we hold a second, mechanistic explanation for more likely. There is strong evidence that in the current paradigm contextual cues for the first association (CS1) were already integrated before the extinction phase: Rescorla (2008) compared in a very similar design response strength after conditioning either in the same or in another context (AA vs. AB) and found greater responding in the same context. Thus, conditioned responding was context-specific after the acquisition phase already. Based on this result, we argue that alteration of glutamatergic transmission does not affect contextual control of the extinction or does so only to a limited amount. When comparing effect sizes for the context effect under saline and AP5 ($g = 1.84$ and $g = 0.84$, respectively; both are significant), it becomes obvious that the context effect is also reduced for the CS1, suggesting that the integration of contextual information for the CS1 possible takes place during both acquisition and extinction. Thus, blocking glutamate transmission in PFC during extinction is not sufficient to fully block contextual control of extinguished behavior.

To explain why context has a bigger impact on extinguished behavior, some have argued that context serves to disentangle the ambiguous meaning of a stimulus (Bouton, 2004). Others have proposed that increased attention induced by experiencing extinction builds the basis of the high context-specificity of extinction memory (Rosas, Callejas-Aguilera, Aalvarez, & Fernandet Abad, 2006; Rosas, Todd, & Bouton, 2013). In our opinion, the present data indirectly support the latter view, because we show context-specific responding to non-ambiguous stimuli. Importantly, this specificity can be reduced when learning takes place under modified glutamate transmission within executive structures – a system strongly implicated in the allocation of attention (Carli & Invernizzi, 2014; Chang, Lane, & Tsai, 2014).

To conclude, we show that the establishment of contextual control as well as the consolidation of first-learned behavior is critically dependent on the functioning of NMDA receptors in prefrontal regions. We suggest that the decreased contextual control is a result of attenuated attention to external cues due to the manipulation of the glutamate system.

Conflict of interest

The authors declare no competing financial interests.

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