Behavioural Brain Research 265 (2014) 93–100

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Transient inactivation of the pigeon hippocampus or the nidopallium caudolaterale during extinction learning impairs extinction retrieval in an appetitive conditioning paradigm

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HIGHLIGHTS

- We present a within-subject ABA renewal task suitable for pharmacological interventions.
- We inactivate the nidopallium caudolaterale or the hippocampus during extinction.
- Inactivation of either structure reduces conditioned responding.
- Inactivation results in enhanced spontaneous recovery without affecting renewal.

ARTICLE INFO

Article history:
Received 15 September 2013
Received in revised form 14 February 2014
Accepted 17 February 2014
Available online 22 February 2014

Keywords:
Contextual learning
Tetrodotoxin
Renewal
Sign tracking

ABSTRACT

The majority of experiments exploring context-dependent extinction learning employ Pavlovian fear conditioning in rodents. Since mechanisms of appetitive and aversive learning are known to differ at the neuronal level, we sought to investigate extinction learning in an appetitive setting. Working with pigeons, we established a within-subject ABA renewal paradigm based on Rescorla (Q J Exp Psychol 61:1793) and combined it with pharmacological interventions during extinction. From the fear conditioning literature, it is known that both prefrontal cortex and the hippocampus are core structures for context-specific extinction learning. Accordingly, we transiently inactivated the nidopallium caudolaterale (NCL, a functional analogue of mammalian prefrontal cortex) and the hippocampus in separate experiments by intracranial infusion of the sodium-channel blocker tetrodotoxin immediately before extinction training. We find that TTX in both structures non-specifically suppresses conditioned responding, as revealed by a reduction of response rate to both the extinguished conditioned stimulus and a control stimulus which remained reinforced throughout the experiment. Furthermore, TTX during extinction training impaired later extinction retrieval assessed under drug-free conditions. This was true when responding to the extinguished stimulus was assessed in the context of extinction but not when tested in the context of acquisition, although both contexts were matched with respect to their history of conditioning. These results indicate that both NCL and hippocampus are involved in extinction learning under appetitive conditions or, more specifically, in the consolidation of extinction memory, and that their contribution to extinction is context-specific.

1. Introduction

Animals are confronted with an ever-changing environment and have thus evolved the ability to continuously learn and remember new information. However, an acquired association that was valid in the past might not hold anymore today. Therefore, learning also involves the ability to extinguish memorized associations. Extinction is a novel learning event that does not erase the old trace in its entirety [1]. Also, extinction is known to be highly context-specific, and therefore extinguished responses can reappear if the conditioned stimulus is presented in a context that is different from the one during extinction learning [2]. This phenomenon is known as renewal and has guided research on context-specific learning during the last decades [2–7].

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http://dx.doi.org/10.1016/j.bbr.2014.02.025
0166-4328/

The understanding of the behavioral and the neural basis of extinction learning rely mostly on rodent data obtained in fear conditioning procedures [8]. Three structures appear to play key roles: the prefrontal cortex (PFC), the hippocampus, and the amygdala [9]. The hippocampus consolidates the memory trace and interacts with amygdalar substructures, and transmits contextual information to the PFC [10,11]. In rats, different PFC subregions subserve either excitatory (prelimbic subregion) or inhibitory (infralimbic subregion) functions for extinction learning [9]. Lesions of hippocampus and PFC seem not to interfere with extinction learning per se but with context coding during renewal or extinction retrieval, respectively [12,13].

The overwhelming majority of studies on the neural basis of extinction were conducted with aversive conditioning procedures. However, it is well known that aversive and appetitive events are in part differently processed. For example, inhibition of the endocannabinoid CB1 receptor leads to a disruption of extinction for aversive memories, while leaving extinction under appetitive conditions intact [14,15]. Part of the endocannabinoid effects on extinction is probably mediated by hippocampal and amygdalar mechanisms [16–18].

Pigeons are popular model animals in experimental psychology [19–21], and many details of extinction learning were described using these animals [4,22–24]. In addition, the last decade has witnessed a major shift in our understanding of the bird brain, and it is now well established that the pallia of birds and mammals are homologous [25,26]. While the avian hippocampus is one-to-one homologous to its mammalian counterpart, the nidopallium caudolaterale (NCL) in birds and the mammalian PFC are functionally similar but constitute a case of evolutionary convergence without being homologous as a pallial field [27,28]. Consequently, one of our aims is to reveal if the NCL and the hippocampus of the pigeon play similar roles as in rodents for extinction learning.

Rescorla [4] demonstrated context-specific extinction learning in pigeons under appetitive Pavlovian conditioning terms in a within-subject design. In his study, the birds acquired a response to conditioned stimulus (CS) A in context A and to a CS B in context B. Subsequently, responses to CS A and CS B were no longer reinforced when they appeared in their opposed context (CS A in context B; CS B in context A). The final test then occurred in both contexts for both stimuli. We adopted Rescorla's paradigm [4] and extended it by temporarily inactivating the hippocampus or the NCL in a within-group design by means of the sodium-channel blocker tetrodotoxin (TTX). Studies from our laboratory already showed that NMDA receptors in the NCL are required for reversal learning [22,29] and that NMDA blockade results in an inability to adjust responses to changing contextual circumstances [23]. Thus, our aim is to further our understanding on the neural fundaments of extinction with appetitive tasks in birds and to compare them with those known from fear conditioning in rodents.

2. Materials and methods

2.1. Subjects

Sixteen adult and experimentally naïve pigeons (*Columba livia*) served in the NCL experiment and twenty-four animals served in the hippocampus experiment. Birds were obtained from local breeders and housed in individual wire-mesh cages ($30 \times 30 \times 45$ cm) inside a colony room. Temperature and humidity as well as the 12-hr light–dark schedule were strictly controlled (lights on at 8 am). During the experiment animals were maintained close to 85% of their free feeding weight with additional free food on weekends. Water was available ad libitum. All experiments were approved by the national authorities of the state of North Rhine-Westphalia, Germany and carried out in accordance with the National Institute of Health Guide for Care for Laboratory Animals.

2.2. Surgery

The birds were chronically implanted with 26-gauge (8 mm) stainless steel guide cannulas (Plastics One Inc., Roanoke, USA). NCL animals were implanted bilaterally with a single cannula in each hemisphere, and hippocampus animals were implanted with two cannulas per hemisphere. For surgery, birds were premedicated with Dolorex (0.3 ml, 10 mg/ml, Butorphanol, Intervet, MSD Animal Health, Unterschleißheim, Germany) as painkiller and anesthetized with Isoflorane (Forane 100% (V/V), Mark 5, Medical Developments International, Abbott GmbH & Co KG, Wiesbaden, Germany). Small craniotomies were performed above the target areas, the dura mater was removed and cannulas were inserted slowly into the brain under visual control and targeted to the following coordinates: NCL: AP +6.5 mm, L ±7.2 mm, V +1.8 mm; hippocampus: A +5.7 mm and +7.7 mm, L immediately lateral from the superior sagittal sinus, V +0.5 mm [30]. Hippocampal cannulas were inserted at an angle of 30° relative to the coronal plane. Up to eight stainless steel microscrews (Small Parts, Logansports, USA) were attached to the bone to anchor the dental cement encasing the cannulas. Postoperatively, Carpofen (0.3 ml, 10 mg/ml, Rimaldyl, Pfizer GmbH, Münster, Germany) was applied twice daily as an analgetic. Pigeons were allowed to recover 7–10 days before initial training started.

2.3. Behavioral apparatus

All subjects were trained in two skinner boxes of similar shape ($36\,\text{cm} \times 34\,\text{cm} \times 36\,\text{cm}$). The rear and side walls of the chambers were covered with colored wallpaper either by 2.5 cm wide vertical tan stripes spaced 5 cm apart on red background (context A) or by yellow marbling pattern on white background (context B) [4]). White noise was provided in context A and brown noise in context B (approximately 80 dB SPL) to increase distinctness of the two environments. Both chambers were housed in sound-attenuating cubicles to mask extraneous sounds. The rear wall of context A featured two translucent rectangular pecking keys ($2\,\text{cm} \times 2\,\text{cm}$; 12 cm above the floor), but only the right key was used in this experiment. In context B, a single pecking key ($2\,\text{cm} \times 2\,\text{cm}$; 12 cm above the floor) was situated at the center of the rear wall. Both boxes were illuminated with 6 W light bulbs placed either in the center of the ceiling (context A) or on the upper edge of the side wall (context B). Stimuli were presented on LCD flat screen monitors (context A: Belinea Model No.: 10 15 36; context B: Philips Model: Brilliance 17S1/00) mounted against the back walls of the chambers. Overall, four different visual stimuli were used: a black rectangle on a green background (*CS 1*), yellow and orange circles on a blue background (*CS 2*), white marbling on rose background (*non-target*) and small diagonally oriented white rectangles on an orange background (*target*). Each effective key peck produced an audible feedback click. Otherwise, key pecks were inconsequential throughout the experiment. During acquisition, the target and the CS were followed by food reward delivered immediately at stimulus offset (5 s stimulus presentation time); the non-target was never followed by food. Food (grain) was delivered by a food hopper positioned at the lower middle of the rear wall. Whenever food was available, a feeder light 2 cm above the food hopper was illuminated. The hardware was controlled by custom-written Matlab code (The Mathworks, Natick, MA; [31]).

2.4. Procedure

The experimental procedure consisted of five separate phases, to be detailed below and illustrated in Fig. 1 and Table 1.

2.4.1. Pretraining I

Initially, animals were submitted to a simple sign tracking procedure with a single stimulus (*target*). In each session in Pretraining I, the target stimulus was presented 48 times for 5 s and immediately followed by 3 s access to grain.

The intertrial interval was fixed at 45 s. Animals received two training sessions on each workday, one session in each of the two contexts, spaced 2 h apart and conducted in alternating succession. Once the learning criterion of 80% responses in both contexts on three consecutive days was reached, the animals entered the next phase of training, Pretraining II.

2.4.2. Pretraining II

Conditions were identical to those in Pretraining I except that the target stimulus was presented only 24 times per session, and a non-rewarded stimulus (*non-target*) was introduced (12 presentations per session). The non-target was shown for up to 5 s and was never followed by reward. In case the animal responded to the non-target, the house light was turned off immediately for 3 s and a clearly audible tone (sawtooth wave at 1000 Hz) was presented. The order of stimulus presentation was randomized with the exception that each session started with two target presentations. A minimum of 80% correct responses for both stimuli in both contexts was required to enter the next phase of training.

The inclusion of the target and the non-target stimuli was done to control for any non-systematic effects triggered by injecting a pharmacological substance (unspecific up- or downregulation of responding). Additionally, was reasoned that the occasional presentation of a non-rewarded stimulus would enhance the birds' attention towards the stimuli. Target and non-target stimuli were presented in both contexts.

2.4.3. Acquisition

In addition to the target and the non-target stimuli, CS 1 was introduced in context A and CS 2 was introduced in context B; both CSs were followed by food reinforcement after 5 s fixed stimulus appearance. Each stimulus was presented 12 times per session. Acquisition training was conducted for a minimum of six days with two sessions per day – one in context A for CS 1 and one in context B for CS 2. Overall, pigeons had to reach a performance criterion of 80% correct responses for both CSs across three consecutive days to be transferred to extinction training.

2.4.4. Extinction

Extinction took place on two days with one day without training in between to allow for complete washout of TTX. On days without training, animals were provided with 10 g of grain. During extinction training, non-reinforced CS 1 trials were conducted in context B on one day and non-reinforced CS 2 trials were conducted in context A on the other day, with half of the animals being exposed first to extinction in context A and the other half first to extinction in context B. All other conditions were identical to acquisition training, with the exception that the target and the CSs, were presented 24 rather than 12 times, respectively, whereby the number of non-target presentations (12 times) remained unchanged.

Approximately 30 min before extinction training commenced, either 1 μl TTX (10 ng/μl, tetrodotoxin citrate, Tocris, see [32] for a more detailed description) or 1 μl saline was infused bilaterally into the NCL or the hippocampus. Hence a single animal received TTX in one extinction session and saline in the other in a within-subject design. TTX is completely washed out 48 h after injection [33].

2.4.5. Test

Finally, responding to CS 1 and CS 2 as well as target and non-target stimuli was observed in both contexts in balanced succession 48 h after the second extinction session. Each stimulus was

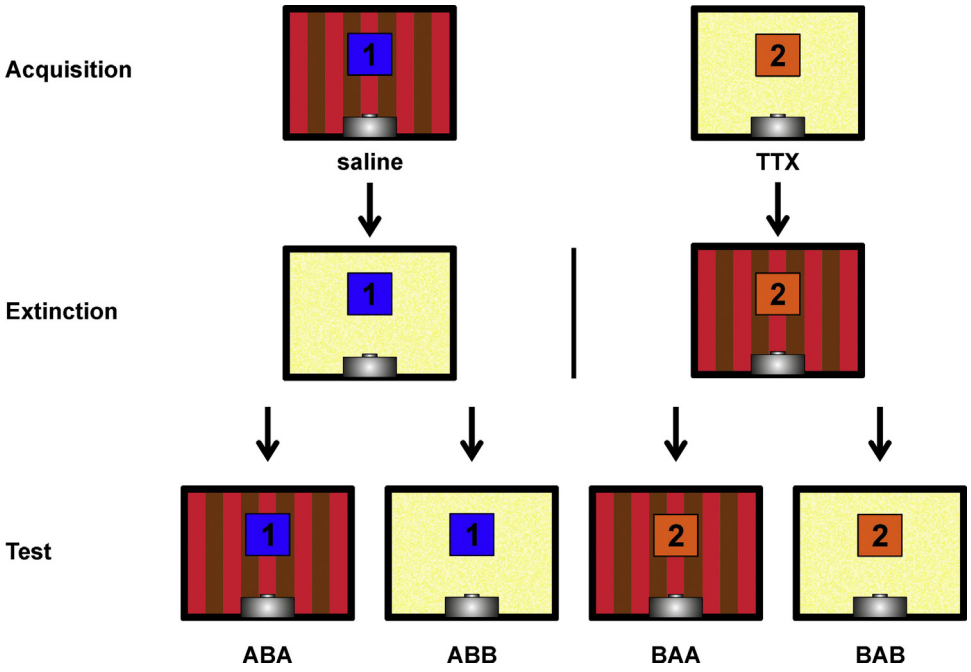

Fig. 1. Depiction of the within-subject ABA renewal design. Single pictures show rear walls of the two different conditioning chambers A and B. The blue and orange squares with numbers 1 and 2 indicate the two different conditioned stimuli. Not shown are the target stimulus (present and reinforced in all sessions) and the non-target stimulus (present and non-reinforced in all sessions). Contexts, stimuli and injection sequences were balanced across subjects, hence this figures shows a single possible example. For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.

Table 1
Overview of the experimental procedure ((+) = rewarded stimulus; (−) = non-rewarded stimulus; CSA = conditioned stimulus A; CSB = conditioned stimulus B; — = no stimulus presentation).

Phase	Context	No. target	No. non-target	No. CSA or CSB
Pretraining I	A	48x (+)	—	—
	B	48x (+)	—	—
Pretraining II	A	24x (+)	12x (−)	—
	B	24x (+)	12x (−)	—
Acquisition	A	12x (+)	12x (−)	12x CSA (+)
	B	12x (+)	12x (−)	12x CSB (+)
Extinction	A	24x (+)	12x (−)	24x CSB (−)
	B	24x (+)	12x (−)	24x CSA (−)
Test	A	12x (+)	12x (−)	12x CSA (−) & 12x CSB (−)
	B	12x (+)	12x (−)	12x CSA (−) & 12x CSB (−)

presented 12 times, but only target presentations were followed by reinforcement.

2.5. Histology

All histological analyses were done similarly to Helduser et al. [32] with the focus on the current regions of interest.

2.6. Data analysis

The absolute number of responses during stimulus presentation was the main dependent variable in this study. In addition, we computed the percentage of trials on which a conditioned response was observed (i.e. >1 key peck). We used two-way repeated measures analysis of variance (ANOVA) and paired-samples *t*-tests to analyze the response data.

CS responses during extinction training under TTX were normalized by multiplying the average number of responses in a given bin of four consecutive trials by the ratio of target responses under saline and TTX in the same bin of four trials.

Responding to the non-target was near or at zero throughout the entire experiment and was not affected by any experimental manipulation; therefore, we decided to omit this information from the results figures.

Analyses were conducted employing the Statistics Toolbox of Matlab R2012a (The Mathworks, Natick, USA).

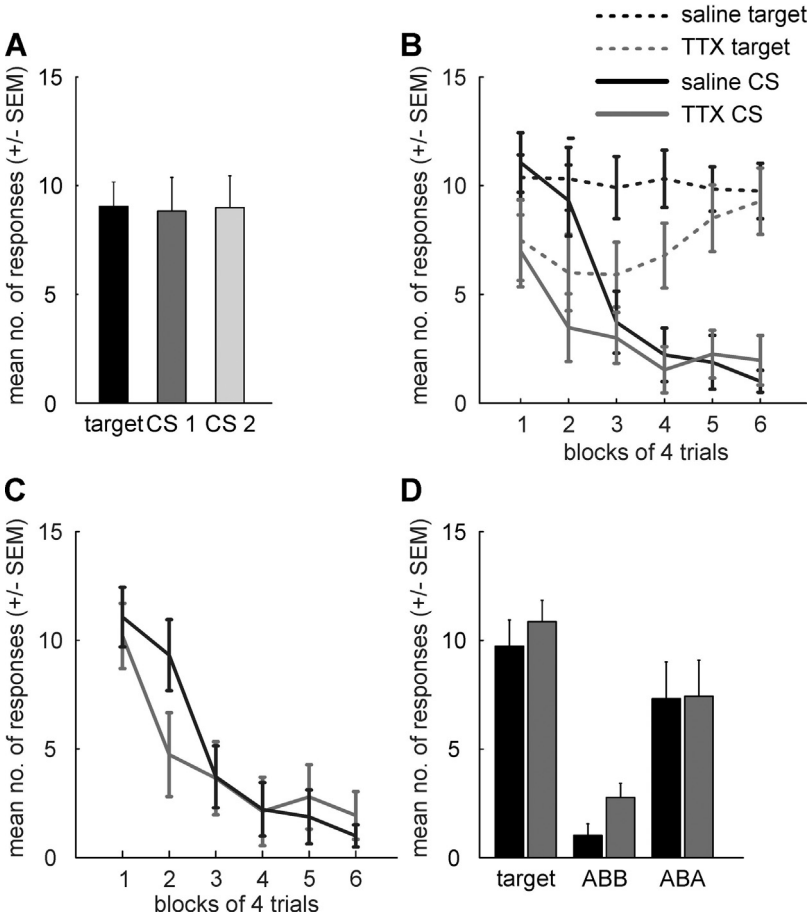

Fig. 2. Results of NCL inactivation. (A) Mean response rates (+/–SEM) in the last four acquisition sessions for target and conditioned stimuli. (B) Mean response rates (+/–SEM) during extinction training, shown separately for target and conditioned stimuli and in TTX and saline conditions. (C) Normalized response rates for the conditioned stimulus reveals comparable extinction dynamics. (D) Mean response rates (+/–SEM) in the retrieval sessions in contexts A and B. For simplicity, we collectively refer to ABA/BAB and ABB/BAA as ABA and ABB, respectively.

3. Results

3.1. Inactivation of NCL during extinction learning

3.1.1. Histology

Overall, eight animals were included for analysis. Histological analysis (Fig. 4A) showed bilateral well-centered cannulas within the NCL. One of the subjects was included even though one cannula was at the very anterior border within the NCL. The remaining subjects were excluded due to improper cannula positions ($n = 1$), failing to reach the learning criteria ($n = 2$) or complete behavioral suppression during extinction under TTX ($n = 5$).

3.1.2. Acquisition

Conditioning went uneventful. Response rates for all reinforced stimuli in the last four sessions were similar (Fig. 2A; target: 9 ± 1; CS A: 8.8 ± 1.3; CS B: 9 ± 1.3 (means $\pm$ SEM)).

3.1.3. Extinction

Fig. 2B depicts the time course of responding to each of the stimuli during extinction training. Obviously, responding to the CSs decreased over the course of the session both under TTX and under saline. Responding to the target was fairly stable, but TTX resulted in an initially decreased response rate early in the session. A two-way repeated measures ANOVA revealed a significant main effect of treatment ($F(1,7) = 5.7$, $p = 0.049$) but not of block ($F(5,35) = 1.2$, $p = 0.308$) along with a significant treatment $\times$ block interaction ($F(5,35) = 3.5$, $p = 0.012$), reflecting reduced response rates during target presentation under TTX early in the session.

Regarding the CS, a two-way repeated measures ANOVA yielded a significant main effect of block ($F(5,35) = 16.4$, $p < 0.001$) but not of treatment ($F(1,7) = 3.2$, $p = 0.119$) and a significant interaction of treatment and block ($F(5,35) = 4.9$, $p = 0.002$), again reflecting decreased response rates under TTX relative to the saline control, and this difference was again most pronounced in the first two blocks of the session.

At first glance, inactivation of the NCL seems to facilitate extinction learning: responding to the CS ceases earlier in the session under TTX than under saline. However, TTX treatment yielded reduced response rates to the target stimulus as well, so the reduced response rates to the CS under TTX cannot simply be taken to imply enhanced extinction. Indeed, when normalizing response rates relative to the target stimulus, the difference between TTX and saline disappears, suggesting a similar time course of extinction in both conditions (Fig. 2C; repeated measures ANOVA: block: $F(5,30) = 18.1$, $p < 10^{-7}$; treatment: $F(1,6) = 0.2$, $p = 0.646$; interaction: $F(5,30) = 1.9$, $p = 0.124$).

3.1.4. Retrieval

Fig. 2D shows response rates for the CSs and the target in the test sessions in both contexts. A two-way repeated measures ANOVA revealed no main effects for either context or treatment ($F(1,7) = 0.5$, $p = 0.501$ and $F(1,7) = 1.8$, $p = 0.222$, respectively), but a significant interaction of the two factors $F(1,7) = 22.3$, $p = 0.002$). Pairwise comparisons showed no significant difference in ABA responding between treatment conditions ($t(7) = 0.1$, $p = 0.947$) but a trend towards a statistically significant difference in ABB responding ($t(7) = 2$, $p = 0.092$). Importantly, the latter effects were

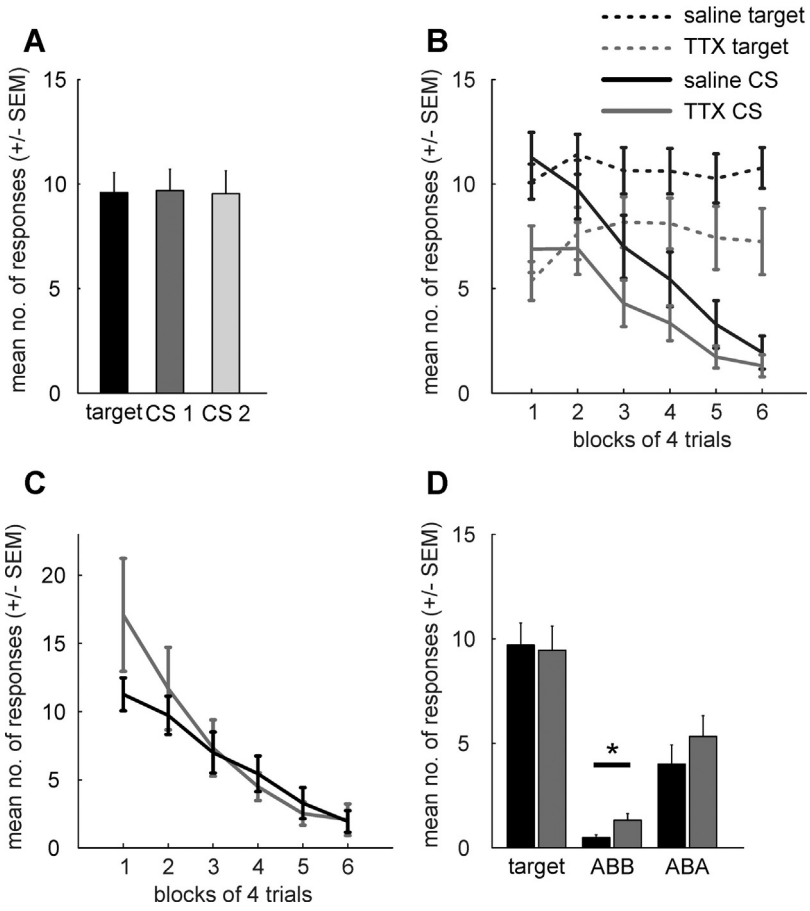

Fig. 3. Results of hippocampus inactivation. (A) Mean response rates (+/–SEM) in the last four acquisition sessions for target and conditioned stimuli. (B) Mean response rates (+/–SEM) during extinction training, shown separately for target and conditioned stimuli and in TTX and saline conditions. (C) Normalized response rates for the conditioned stimulus reveals comparable extinction dynamics. (D) Mean response rates (+/–SEM) in the retrieval sessions in contexts A and B.

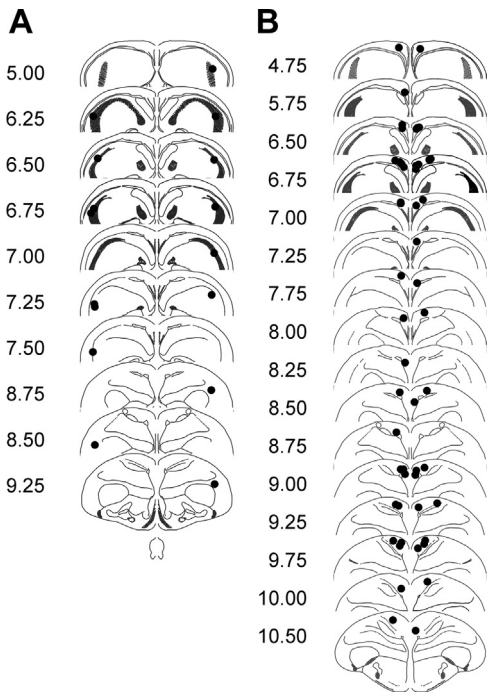

Fig. 4. Histological data. Schematic slices of the pigeon brain, highlighting NCL (A) and hippocampus (B) injection sites. Dots represent the tips of the injection cannulas. Pictures are based on the brain atlas by [30].

significant when taking the percentage of trials with conditioned responses as dependent variable (ABA: $t(7) = 0.36$, $p = 0.732$; ABB: $t(7) = 2.5$, $p = 0.042$), constituting evidence that ABB responding was indeed affected by NCL inactivation.

As response rates were quite high in the ABA condition after both treatments, it could be that differences in responding went undetected because of a potential ceiling effect. However, responses to the CS extinguished under saline during ABA retrieval were still significantly reduced compared to target responding ($t(7) = 2.7$, $p = 0.032$).

To sum up, NCL inactivation during extinction unspecifically suppressed conditioned responding without clear evidence of affecting the speed of learning. However, there was some evidence that extinction learning was actually impaired by NCL inactivation, signified by enhanced conditioned responding to the extinguished CS in the context of extinction (ABB) after TTX as compared to saline treatment.

3.2. Inactivation of hippocampus during extinction learning

3.2.1. Histology

Overall, response data of 13 animals was deemed suitable for analysis (see Fig. 3B for histology). In all remaining cases, the positions of the cannula were either not within the hippocampus ($n = 5$), subjects did not respond during extinction training ($n = 5$) or did not satisfy our learning criterion ($n = 1$).

3.2.2. Acquisition

Conditioning went uneventful. Response rates for all reinforced stimuli in the last four sessions were similar (Fig. 3A; target: 9.6 ± 1; CS A: 9.7 ± 1.2; CS B: 9.5 ± 1 (means $\pm$ SEM)).

3.2.3. Extinction

Fig. 3B shows response rates during extinction training separately for each stimulus type and treatment condition. Response rates to the target remain on a relatively stable level and CS responses decrease during extinction training. Similar to the NCL data, responding to all conditioned stimuli was reduced under the influence of TTX. There was a significant effect of treatment for target responses ($F(1,12) = 13.8$; $p = 0.003$), along with a marginally significant effect of block ($F(5,60) = 2.3$, $p = 0.06$) but no interaction ($F(5,60) = 1.1$, $p = 0.352$).

Responding to the CS decreased over the course of the session, as evidenced by a significant effect of block ($F(5,60) = 24$, $p < 10^{-12}$). Additionally, there was a main effect of treatment ($F(1,12) = 5.6$, $p = 0.035$) and a significant interaction of the two factors ($F(5,60) = 2.6$, $p = 0.034$).

Again, normalized response rates did not differ significantly across treatment groups (Fig. 3C), as shown by a two-way repeated measures ANOVA (block: $F(5,50) = 10.9$, $p < 10^{-6}$; treatment: $F(1,10) = 0.7$, $p = 0.425$; interaction: $F(5,50) = 1.8$, $p = 0.129$).

3.2.4. Retrieval

A two-way repeated measures ANOVA did not show significant effects for context or treatment ($F(1,12) = 0.1$, $p = 0.709$ and $F(1,12) = 2.9$, $p = 0.116$) but a significant interaction of the two factors ($F(1,12) = 28.1$, $p < 10^{-3}$). Once more mirroring the results after NCL inactivation, there was no significant difference between treatment groups when testing retrieval of extinction in the acquisition context (ABA, $t(12) = 1.1$, $p = 0.3$) but when testing in the context of extinction (ABB, $t(12) = 2.5$, $p = 0.028$).

To exclude the possibility that the absence of a difference in ABA was due to a ceiling effect (see above), we tested whether responding to the CS extinguished under saline was reduced compared to responding to the target, and that was indeed the case ($t(12) = 1.5$, $p = 0.001$), speaking against a ceiling effect masking differences in extinction retrieval when testing in the context of acquisition.

In conclusion, transient inactivation of the hippocampus unspecifically reduced overall response rate during extinction, similar to inactivation of the NCL. During retrieval of extinction, responses to the CS extinguished under TTX were increased when testing in the context of extinction (ABB) but not the context of acquisition (ABA).

4. Discussion

Our experiments show that pharmacological inactivation of the pigeon NCL or hippocampus during extinction training impaired subsequent retrieval of extinction memory when tested in the context of extinction (spontaneous recovery, ABB) but not in the context of acquisition (renewal, ABA). Additionally, we observed that TTX injection in both areas resulted in a general suppression of conditioned responding, while the principal extinction dynamics were not affected. Thus, our data show that both the 'prefrontal' NCL as well as the avian hippocampus play an important role in the encoding of extinction memory.

Multiple sources of evidence point to an involvement of the PFC in recall of extinction memory. Here, especially the infralimbic prefrontal cortex of rats seems to be relevant. Lesions or pharmacological inactivations of this area do not affect extinction learning per se but subsequent extinction memory retrieval [8,9,34,35]. Additionally, more specific neural manipulations with e.g. NMDA- [12,36] or mGlu5-receptor blockers [37] before or subsequent to extinction learning in the infralimbic prefrontal cortex impair extinction retrieval on the next day. Sepulveda-Orengo et al. [38] observed that prefrontal mGluR5 activation promotes consolidation of fear extinction by regulating the intrinsic excitability of infralimbic neurons. Electrophysiological data revealed that the degree of infralimbic burst firing is correlated with extinction retrieval [39]. Human studies revealed ventromedial prefrontal activity in a predictive learning task during extinction memory

retrieval [40]. Thus, prefrontal neurons seem to be active during extinction learning, readjust their synaptic weights in the hours subsequent to extinction and then play a critical role when extinction memory is retrieved [9]. Such an interpretation is in line with our findings of TTX-injections into the pigeon NCL. Transient inactivation of the NCL during extinction did not alter extinction learning dynamics but seemed to prevent proper extinction memory consolidation at the 'prefrontal' level. Consequently, in subsequent sessions an increased response rate to the previously extinguished CS was observed. Taken together, the avian NCL assumes a similar function in the expression of extinction learning as subcomponents of the PFC (infralimbic PFC in rats, ventromedial PFC in humans). This interpretation is in line with previous studies showing that antagonizing NMDA-receptors in the pigeons NCL affect extinction and context integration [22,23].

As for the NCL, also TTX infusions into the hippocampus did not affect the principal dynamics of extinction learning but compromised extinction retrieval in the ABB test. Indeed, several strands of evidence show that the mammalian hippocampus plays a major role in extinction memory retrieval. Diverse studies documented that alteration in hippocampal activity alters extinction learning [41–43]. Extinction learning is also accompanied by changes of theta oscillation coupling between the hippocampus, the amygdala and the prefrontal cortex in mice [44]. Contextual extinction learning is represented by hippocampal activity as well and here the activity level correlates with the predicted impact of the renewal effect [40]. Overall, the hippocampus is involved in all kinds of context-dependent conditioning tasks [9,45–47]. Transient hippocampal inactivation during extinction learning results in poor extinction retrieval [42,48]. The results of Bast et al. [49] indicate that rats injected with TTX into ventral hippocampus immediately before extinction were able to decode neither contextual nor tone cues in a retrieval test. Enhanced spontaneous recovery for TTX-treated pigeons in our study therefore indicates that only memory of extinction learning is disrupted. Hence we conclude that the hippocampus is involved in building context-specific extinction memory. Beyond extinction learning as a specific learning procedure, the hippocampus is responsible for the transfer and consolidation of short-term memory to long-term memory stored in the pallium. Thus, hippocampal TTX-injections could prevent the consolidation of extinction memory, thus increasing spontaneous recovery in the ABB design. Indeed, TTX concentrations remain high within the tissue for at least 4 h after injection [33] and could therefore affect the consolidation process after extinction training.

For both NCL and hippocampus ABA retrieval remains unaffected and a ceiling effect could be excluded. This underlines the assumption that impaired extinction learning was entirely linked to the context of extinction.

During extinction learning we observed a reduction of conditioned responses for animals with TTX-injections both into NCL and hippocampus. Due to our within-subject design and the addition of the target stimulus, it was possible to show that this response reduction was not specific for the conditioned stimulus but reflected a general behavioral inhibition. Presently we can only speculate about the reasons for this effect. The NCL is critical for response selection [32,50,51] and response timing [52]. Helduser et al. [32] reported that NCL-inactivated pigeons frequently fail to initiate trials and explained these failures by attentional deficits. Single unit recordings show that some NCL-neurons are associated with learned response patterns [53–55]. This is similar to studies in the mammalian PFC that describe prefrontal neurons which code for learned targets, conditioned movements and the selection of responses depending on the history of reward within the task [56,57]. Consequently, prelimbic PFC-lesions in rats also reduce responding in appetitive tasks during extinction learning, possibly by affecting a differential outcome-mediated initiation and selection of learned responses [58].

Alternatively, the reduction in response rate could be due to a reduction in the hedonic value of the rewards. The NCL is heavily implicated in reward processing [54,55], and therefore its inactivation might affect the subject's incentive motivation to perform the sign-tracking response. Furthermore, appetitive extinction is used as a model for depressive disorders, and the response decline in appetitive extinction is slowed down by the administration of antidepressant drugs [59,60]. However, it is difficult to reconcile this interpretation with the context-specificity of extinction retrieval impairment. Thus, while the overall response reduction might be attributed to decreased incentive motivation, an additional interpretation in terms of context-specific extinction memory consolidation is required.

Regarding TTX-injections into the hippocampus, we also observed an overall reduction in response rate. Again, this is similar to the mammalian hippocampus in which infusions of TTX into the ventral hippocampus before fear extinction conditioning also results in hypoactivity [49]. Similarly, hippocampal ablation in pigeons before acquisition training also results in reduced conditioned responding [61]. While the latter authors did not find impaired contextual transfer in hippocampus-ablated pigeons, this apparent discrepancy could be readily explained by a crucial difference in procedure: they tested for transfer of responding of a 'first-learned' association (the sign-tracking response), while we tested for transfer of a 'second-learned' association (extinction memory), which is well known to be much more context-specific (e.g. [62]).

Importantly, the overall response strength during extinction could affect subsequent retrieval due to a reduction of response-outcome pairings during extinction and a concomitant reduction of the efficacy of extinction training [63,64]. Indeed, Krupa and Thomson [65] found that preventing conditioned responding during extinction results in an impairment of extinction learning. However, this interpretation is at odds with our finding that extinction retrieval is unaffected when tested in the context of acquisition. Although we could not find statistical support for this interpretation (see results), we are still inclined to believe that such accounts of associative learning could explain at least a smaller part of the result pattern.

To summarize, the current experiment showed that the NCL and the hippocampus of pigeons are involved in context-dependent encoding of extinction memory in an appetitive conditioning paradigm, demonstrating the involvement of 'prefrontal' and hippocampal areas in appetitive extinction learning. Subsequent studies using selective manipulations of NMDA-receptors could elucidate more specific mechanisms of context-specific extinction learning in birds and provide further insights into its neural network.

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