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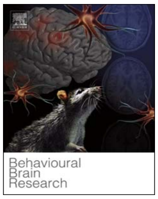

Research report

NMDA receptors in the avian amygdala and the premotor arcopallium mediate distinct aspects of appetitive extinction learning

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ABSTRACT

Extinction learning is an essential mechanism that enables constant adaptation to ever-changing environmental conditions. The underlying neural circuit is mostly studied with rodent models using auditory cued fear conditioning. In order to uncover the variant and the invariant neural properties of extinction learning, we adopted pigeons as an animal model in an appetitive sign-tracking paradigm. The animals firstly learned to respond to two conditioned stimuli in two different contexts (CS-1 in context A and CS-2 in context B), before conditioned responses to the stimuli were extinguished in the opposite contexts (CS-1 in context B and CS-2 in context A). Subsequently, responding to both stimuli was tested in both contexts. Prior to extinction training, we locally injected the *N*-methyl-D-aspartate receptor (NMDAR) antagonist 2-Amino-5-phosphonovaleric acid (APV) in either the amygdala or the (pre)motor arcopallium to investigate their involvement in extinction learning. Our findings suggest that the encoding of extinction memory required the activation of amygdala, as visible by an impairment of extinction acquisition by concurrent inactivation of local NMDARs. In contrast, consolidation and subsequent retrieval of extinction memory recruited the (pre)motor arcopallium. Also, the inactivation of arcopallial NMDARs induced a general motoric slowing during extinction training. Thus, our results reveal a double dissociation between arcopallium and amygdala with respect to acquisition and consolidation of extinction, respectively. Our study therefore provides new insights on the two key components of the avian extinction network and their resemblance to the data obtained from mammals, possibly indicating a shared neural mechanism underlying extinction learning shaped by evolution.

1. Introduction

There has been growing interest in the phenomenon of extinction learning in recent years. This is partly due to a better understanding of the neural mechanisms underlying especially fear conditioning [1,2], as well as the awareness of the clinical relevance of extinction learning in several human psychopathologies, such as anxiety disorders, substance abuse and post-traumatic stress disorder. In Pavlovian conditioning tasks, the conditioned response (CR) can be acquired after repeated pairings of an initially neutral conditioned stimulus (CS) with a biologically potent unconditioned stimulus (US). During the extinction phase, the repeated presentation of the CS without US results in a reduction of the CR. However, the CR decrement is not permanent and responding can be restored in various ways, like the passage of time (spontaneous recovery), or a context change from the extinction phase to testing (renewal). Numerous experimental investigations on the recovery of responding to an extinguished CR have given rise to the notion that extinction involves partial erasure of the original learning [3], and at the same time, the formation of a new memory trace [4].

In parallel, numerous studies were conducted to investigate the neural circuits of extinction learning. Rodent models of fear conditioning strongly suggest that amygdala, hippocampus and prefrontal cortex (PFC) constitute the core extinction network [5,6]. Current studies show that CS- and US-related signals are associated in the basolateral complex of the amygdala (BLA) during the acquisition of fear, while the central nucleus of the amygdala (CeA) initiates the fear responses [7–10]. After the extinction phase, the infralimbic area (IL) within the PFC exhibits an increased activation and acts directly on the GABAergic intercalated cells (ITC) of the amygdala [11]. Also, inputs from the BLA as the second source of activation, together with the inputs from ITC, produce a feedforward inhibition onto the CeA, resulting in an increased inhibition and thus a reduced fear output [10,12]. Besides, the increased interaction between hippocampus and PFC during extinction is believed to underlie the consolidation of extinction memory. The dependence on contextual factors during extinction memory retrieval indicates a key role of hippocampus [13].

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Pharmacological studies show that inactivation of hippocampus directly before testing prevented renewal [14–16]. Similar effects were also found for mPFC [17] suggesting that the mPFC may be an important target of hippocampal projections for contextual modulation of extinction retrieval [18,19].

Neural substrates of extinction learning in aversive conditions described above are well understood thanks to deciphering of the fear circuit in rodents. Although highly comparable results are obtained from the aversive and appetitive paradigms at the behavioral level, differences concerning the mediating neural mechanisms still exist. In our lab, we have been examining the neural structures underlying extinction using appetitive tasks with pigeons. The reason for using pigeons as a model system is that pigeons represent an excellent model for learning and memory, especially for visual stimuli. A wealth of knowledge on the mechanisms of learning was gained by studies using this model organism. Pigeons can work on large sets of visual stimuli with different reward contingencies in parallel, and adapt their behavior accordingly [20]. In addition, birds evolved in parallel to mammals since ca. 300 million years [21]. As a result, birds have a quite different brain organization that harbors some one-to-one homologies to mammals like the hippocampus but also many non-homologous, but functionally equivalent structures like the nidopallium caudolaterale (NCL) that is comparable to the mammalian PFC [22]. As a result, studying the neural basis of extinction learning in pigeons can uncover some invariant properties of extinction that is shared by evolutionary distant animals.

Recently, Lengersdorf et al. [23] showed that transient inactivation of NCL and hippocampus impairs context-specific consolidation of extinction memory in appetitive conditioning. They also indicated that the involvement of the NCL in extinction learning is specifically mediated by the *N*-methyl-D-aspartate receptors (NMDARs). Based on the observation of the high density of NMDARs in the NCL [24], injections of the NMDAR antagonist, 2-Amino-5-phosphonovalerianacid (APV), into the NCL resulted in impaired extinction learning of an appetitive task [25] without affecting consolidation of extinction memory [26]. In addition, NMDARs in pigeon's NCL are involved in contextual processing in a conditional discrimination task [27]. Taken together, these results suggest that the NCL and hippocampus in birds have comparable functions to those in mammals during extinction, shaped during 300 million years of independent evolution.

In the present study, we examined the avian amygdala and the avian arcopallium, which seems to play a role in motor behavior. According to the meanwhile outdated nomenclature of Karten and Hodos [28], this ventroposterolateral part of the bird pallium was named archistriatum and was suggested to be partly comparable to the mammalian amygdala and partly to be of motor nature [29]. Based on a large number of neuroscientific evidences, the avian nomenclature forum [21,30] accepted the dual nature of the archistriatum and its subnuclei were subsequently identified as being of amygdaloid or of somatomotor nature. While the first group is collectively called "amygdala", the second group constitutes the "arcopallium".

This view was not unanimously accepted by the entire scientific community. Especially neurogenetic studies suggested the existence of a tetrapartite pallial model that is based on field homologies of pallial divisions [31]. According to this view, neurogenetic markers make it unlikely that the arcopallium contains structures that are homologous to cerebral cortex [32]. The tetrapartite model is in flux and was recently expanded to include six divisions [33]. As important as these discoveries are, they are in conflict with various connectional, neurochemical, physiological, and even other neurogenetic studies. For example, Belgard et al. [34] compared expression patterns of more than 5000 orthologous genes and did not find evidence for the proposed homologies of the tetra- or hexapartite model. Further neurogenetic studies, some of them using thousands of genes, could also not verify these models and instead argued that the arcopallium is characterized by expression patterns that resemble neurons of cortical layer V [35–37] and/or pre/motor cortex [38]. Further neurogenetic studies confirmed the limbic nature of the amygdaloid substructures as defined by the nomenclature forum [39,40].

Also, connectional analyses show that the arcopallium shares similar connectivity patterns with the mammalian pre/motor areas, while the limbic nuclei display connections resembling the mammalian amygdala [29,30,41–47]. This is nicely shown in the connectome analyses of the avian telencephalon [48]. Using a graph theoretical analysis that is theory-free with regard to the above referred discussion, the pigeon amygdala and arcopallium turned out to constitute entirely different viscero-limbic and premotor modules, respectively. In addition, the recent study of Herold et al. [49] analyzed binding sites of 12 ligands using quantitative in vitro receptor autoradiography combined with a detailed cyto- and myeloarchitectural analysis. They revealed a clear parcellation between a limbic component that shares patterns with the mammalian amygdala and an arcopallial entity that resembled cortical systems. This separation is also true for functional, pharmacological and electrophysiological studies in various bird species that tested visual, vocal, auditory, and emotional learning, fear and reproduction behavior as well as neuroendocrine control and homeostasis. These studies also testify a functional division between a limbic (amygdaloid) and a sensorimotor (arcopallial) complex [50–59]. Based on these evidences, we depart from the assumption that amygdala and arcopallium are closely located clusters that are nevertheless differentially embedded in limbic and pre/motor circuits, respectively. It is important to note that we do not claim a homology of the arcopallium to premotor and motor cortices, but depart from a functional comparison.

The anterior and intermediate parts of this complex, incorporating the anterior (AA), dorsal (AD), and intermediate (AI) arcopallium, are considered non-limbic because of their sensory afferents and their descending motor telencephalofugal efferents [29,60]. They are seen as the premotor and motor structures that innervate pallial, diencephalic, and brainstem structures, possibly even down to cervical spinal levels [29]. On the other hand, the posterior and ventral part of the complex, mostly the posterior pallial amygdala (PoA), is regarded as visceral and limbic in its connections and is thus amygdaloid in its nature [30]. Together with the nucleus taenia of amygdala (TnA) and subpallial amygdala (SpA), PoA is currently recognized as one important part of amygdaloid complex in birds [30].

Until now, to the best of our knowledge, the involvement of arcopallium and amygdala in extinction learning in birds has not been investigated. Therefore, the aim of the present study was to investigate the role of avian arcopallium and amygdala in appetitive extinction behavioral tasks. For this purpose, we selectively blocked NMDARs in the amygdala and arcopallium [24], and adopted the within-subject sign-tracking design which has been established by Lengersdorf et al. [23]. By locally injecting APV bilaterally in the amygdala and arcopallium before extinction, we were able to demonstrate that the blockade of NMDARs in the amygdala impaired the acquisition of extinction learning, while the arcopallium plays a role in the consolidation and/or expression of extinction memory.

2. Materials and methods

2.1. Subjects

40 experimentally unsexed adult homing pigeons (*Columba livia*) from local breeders were used as subjects. The animals were housed in individual wire-mesh home cages (40 × 40 × 45 cm) in a colony room where temperature, humidity and the 12 h-light-dark circle were strictly controlled (lights on at 8 a.m.). All pigeons were maintained at 80%–90% of their normal body weight with additional free food on weekends. Water was freely available in their home cages. The experiment was approved by the national authorities of the state of North Rhine-Westphalia, Germany, and was carried out in accordance with