Electrophysiological mismatch response recorded in awake pigeons from the avian functional equivalent of the primary auditory cortex

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The neural response to occasional variations in acoustic stimuli in a regular sequence of sounds generates an \textit{N}-methyl-\textit{D}-aspartate receptor-modulated event-related potential in primates and rodents in the primary auditory cortex known as mismatch negativity (MMN). The current study investigated MMN in pigeons (\textit{Columba livia} L) through intracranial recordings from Field L of the caudomedial nidopallium, the avian functional equivalent of the mammalian primary auditory cortex. Auditory evoked field potentials were recorded from awake birds using a low-frequency (800 Hz) and high-frequency (1400 Hz) deviant auditory oddball procedure with deviant-as-standard (flip-flop design) and multiple-standard control conditions. An MMN-like field potential was recorded and blocked with systemic 5 mg/kg ketamine administration. Our results are similar to human and rodent findings of an MMN-like event-related potential in birds suggestive of similar auditory sensory memory mechanisms in birds and mammals that are homologue from a common ancestor 300 million years ago or resulted from convergent evolution. \textit{NeuroReport} 26:239–244 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

\textbf{NeuroReport} 2015, 26:239–244

Keywords: caudomedial nidopallium, \textit{Columba livia} L, field L, ketamine, mismatch negativity, \textit{N}-methyl-\textit{D}-aspartate receptor, schizophrenia, telencephalon

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Received 17 November 2014 accepted 2 January 2015

\textbf{Introduction}

The neural response to occasional variations in acoustic stimuli in a regular sequence of sounds generates an event-related potential (ERP) in the human brain known as mismatch negativity (MMN). MMN is extracted as a difference waveform by subtracting the ERP to frequent or standard sounds from the ERP to infrequent deviant sounds. Because of the lower probability of deviant stimuli, however, the difference waveform is also likely to include some changes in obligatory ERP components, which reflect a physical stimulus difference and neural refractoriness in afferent mechanisms [1].

The auditory cortex is the main source of MMN, with a distributed network of secondary sources in temporal, frontal and parietal regions [2]. MMN generation is not reliant on active attention to the sound sequence and is therefore often described as preattentive; however, it is reliant on a memory record of the immediate history of auditory information, a record that appears to have similar sensory resolution to auditory perception [3].

A substantial body of research [4] indicates that the memory system underlying MMN enables the brain to process sounds with respect to a relevant acoustic context and to automatically identify events that might be behaviourally relevant, thereby prompting an attention switch for further processing. This memory system is considered as evidence of a ‘primitive intelligence’ in the auditory system, which incorporates a model of the acoustic context used to make perceptual inferences about the nature of future sound events.

Although most MMN research to date has focussed on humans when investigating the properties of the underlying auditory information processes, animal studies have recently attracted increased attention [5–7]. Research on animal models of human MMN appears to be largely motivated by the robust and well-replicated finding of an MMN reduction in schizophrenia [8], which is correlated with a disease-specific brain pathology (i.e. grey matter loss in the superior temporal, prefrontal and parietal cortices and its progression during the course of illness) and clinical outcomes [9].

Consistent with the ‘neurodevelopmental glutamate hypofunction model’ of schizophrenia, pharmacological studies have demonstrated that MMN is critically dependent on the functional state of \textit{N}-methyl-\textit{D}-aspartate (NMDA) glutamate receptors [10–13]. For instance, NMDA receptor antagonists induce symptoms and cognitive impairments in healthy individuals that resemble...
those of schizophrenia [12], whereas healthy individuals with the smallest MMN develop more severe ‘psychotic’ reactions following administration of the glutamate antagonist ketamine [13]. Conversely, agents that enhance NMDA receptor function increase MMN amplitude in schizophrenia [14].

Hence, MMN appears to be a unique psychophysiological endophenotype of schizophrenia [15] that links some of the defining psychopathologies and neuro-pathologies of the disorder with NMDA receptor dysfunction [16]. To date, MMN animal models have mostly include rodents. However, the developmental phases of synapse formation and maturation in birds and humans are relatively distinct, whereas in rats the maturation phase occurs largely simultaneously with the synapse formation phase [17]. In this respect, rodents are not the best models when investigating neurodevelopmental disorders such as schizophrenia. Birds, however, provide a unique opportunity of experimental access to prenatal aspects of brain development – that is, before hatching – as opposed to accessing foetal rodent brains in utero.

The current study aimed to identify intracranial MMN-like ERPs from Field L of the caudomedial nidopallium in the avian telencephalon [18], a brain structure considered highly comparable to the mammalian primary auditory cortex with respect to afferent and efferent connections, neurotransmitters, cytoarchitecture and tonotopic organization [19]. To test the face validity, a subanaesthetic ketamine challenge was used to attenuate MMN-like field potentials [10].

**Methods**

Experiments were conducted with approval from the Ruhr-University of Bochum animal research ethics committee. Five adult male pigeons (*Columbia livia* L.) of local stock were used (430–510 g body weight). Each bird was housed separately. Food and water were provided *ad libitum*.

Surgery was performed under general urethane anaesthesia. Recording electrodes were implanted stereotactically into Field L of the caudomedial nidopallium, with the coordinates AP: +6.0 mm, ML: +2.0 mm and DV: −4 mm according to Karten and Hodos [20]. The electrodes consisted of a pair of insulated stainless steel wires (0.08 mm diameter) with bare tips (0.5 mm) staggered 3 mm apart (for details Schall et al. [21]). A loop of bare stainless steel wire placed posterior under the scalp served as the indifferent electrode. Postoperative care included treatment with antibiotics and analgesics.

The recordings in awake birds commenced 3 weeks after the surgery. The animals were restrained in a cloth sack, their heads protruding through an opening. Leads with plugs established the link between the chronically implanted electrodes and the EEG recording system (Brain Amp DC; Brain Products Inc., Brain Products GmbH, Gilching, Germany). The auditory evoked potentials were digitized at 1000 Hz, band pass filtered between 30 Hz (24 dB/oct) and 0.01 Hz and amplified with a resolution of 0.1 μV and a maximum range of 3.3 mV. EEG data were analysed offline for 500 ms epochs (100 ms before and 400 ms after stimulus onset) using baseline correction, artefact rejection and averaging. Epochs with movement or eye-blink artefacts were excluded when the EEG signal exceeded a difference of 200 μV within 200 ms in each segment.

Pure 800 and 1400 Hz tones of 60 ms duration with 10 ms onset and offset ramps, respectively, were delivered at about 60–70 dB Sound Pressure Level through a loudspeaker placed near the pigeon’s head. Two randomized sequences of 10 min were presented at 500 ms inter-stimulus intervals (i.e. stimulus offset to onset), with the low-pitch tone serving as the frequent standard stimulus (STD) and the high-pitch tone as the rare (P = 0.125) deviant stimulus (DEV) and vice versa as a means to control for obligatory sound frequency effects (i.e. standard-as-deviant or ‘flip-flop design’). Further recordings were performed about 5 min after intramuscular injections of 5 mg/kg ketamine.

As a control procedure for potential stimulus probability confounds, DEV and STD recorded in three birds while embedded with 275, 350, 500, 2600, 5000 and 9600 Hz pure tones presented at equal probability of P = 0.125 in a randomized sequence (multistandard control condition [7]; Fig. 1).

Animals were killed following completion of the experiments using a lethal dose of pentobarbital. Their brains were extracted from the skull, fixed in formalin, sectioned with a freezing microtome and Nissl-stained to verify electrode tracks and tip positions.

Raw recording data were normalized to the scale 0–1 by assigning the value of 0 to the largest negative recording value of the ERP and 1 to the largest positive recording value of the ERP across all recording conditions for each individual bird to account for between-subject differences in scaling. Normalized data of individual ERP peaks and MMN mean amplitudes were parametrically tested after the procedures for small samples [22]. An α-value less than 0.05 (two-sided) was accepted as statistically significant.

**Results**

Data from one bird were excluded from group analysis due to poor signal-to-noise quality and electrode misplacement just outside the target area.

Data presented here are based on greater than 85% usable recordings following artefact rejection. The averaged auditory evoked potentials consisted of an early positive peak (P1), with a poststimulus onset latency of 30 ms, followed by a prominent negative peak (N1) at
about 55 ms, a positive peak (P2) at 90 ms, a smaller negative peak (N2) at about 125 ms, and two late positive peaks at 205 ms (P3) and 265 ms (P4; Fig. 2). However, the P4 component was only present in response to deviant stimuli (Fig. 3a).

An MMN-like field potential peaking at N2 was confirmed for the high-pitch deviant versus low-pitch standard contrast ($t = 7.8, d.f. = 3, P = 0.004$; Fig. 2b) but not for the reversed contrast ($t = 0.7, d.f. = 3, P = 0.53$; Fig. 2a). The former MMN-like ERP was further confirmed for the high-pitch deviant versus high-pitch standard contrast ($t = 5.2, d.f. = 3, P = 0.014$) but not for the low-pitch deviant versus low-pitch standard contrast ($t = 2.3, d.f. = 3, P = 0.10$; Fig. 2c). Finally, contrasting the combined low-pitch and high-pitch deviant ERPs with the combined low-pitch and high-pitch standard ERPs recorded in the separate multistandard recording procedure (Fig. 1) resulted in an MMN-like response ranging from 50 to 250 ms after stimulus onset ($t = 4.3, d.f. = 2, P < 0.05$; Fig. 2c).

Systemic intramuscular 5 mg/kg ketamine injections differentially affected standard and deviant stimulus processing (Fig. 3). P2 peak amplitudes in response to standard stimuli were reduced with ketamine ($t = 3.4, d.f. = 3, P = 0.04$; Fig. 3a), whereas predominantly larger P1 and P3, and smaller N2 peak amplitudes were recorded in response to deviant stimuli (Fig. 3b), thereby resulting in a significant reduction in the MMN-like ERP in the poststimulus onset period of 100–230 ms ($t = 4.6, d.f. = 3, P = 0.02$; Fig. 3c and d).

### Discussion

We found intracranial MMN-like ERPs in Field L of pigeons in response to pitch deviance ranging from 50 to 250 ms after stimulus onset. This MMN-like response at around the N2 peak appears to be more pronounced in response to rare high-pitch deviants than in response to low-pitch deviants, even when controlling for tone pitch (‘flip-flop’ control) and stimulus habituation (multistandard control). This finding is consistent with findings of MMN-like ERPs in rodents [7,23–26]. Obligatory frequency mechanisms are unlikely to account for the mismatch response in our study as auditory evoked field potentials in response to low-pitch and high-pitch STD did not significantly differ and, furthermore, the magnitude of Field L auditory field potentials in pigeons does not significantly differ between 800 and 1400 Hz, which is close to the optimum frequency range of this brain area [18]. This frequency range is also substantially lower than that of rodents and corresponds more closely to the human optimal frequency range. However, minor differences in ERP amplitude may be present depending on electrode positioning relative to tonotopic optima [18].

Consistent with human and animal literature [6,7,10–13], the MMN-like ERP in pigeons was diminished by systemic low-dose (nonanaesthetic) ketamine administration by differentially affecting the respective ERPs in response to STD and DEV tones. In particular, the late positive peak (P2) was smaller with ketamine, without affecting any other component of the STD ERP. By contrast, all positive components (P1, P2 and P3) increased in magnitude with ketamine for the DEV ERP, whereas N2 became smaller. The net effect was a reduction in the mismatch response 100–230 ms after stimulus onset (equivalent to P2, N2 and P3), whereas the early MMN in the latency range of the N1 component remained unchanged with ketamine.

We also found a late positive ERP component (P4) with a latency of 250–350 ms that was only present for the DEV
response, thus resembling a human P300 ERP component that is usually elicited in oddball paradigms. Notably, this late P4 component, like the early N1 component, was not significantly attenuated by ketamine. These findings are consistent with the anatomical literature showing that Field L is surrounded by a high NMDA-receptor-dense shell [27].

Taking these findings together, our study revealed an MMN-like ERP in birds. This finding suggests that a mammalian cortex is not a prerequisite for auditory mismatch processing. The apparent communality of the psychophysiological and pharmacological characteristics spanning across species like birds, rodents and nonhuman and human primates seems to point towards a phylogenetically ancient mechanism that may be common to all vertebrates that have evolved hearing. As the latter has evolved from the vibration senses, it is not surprising that an electrophysiological mismatch response can also be recorded in response to somatosensory oddball stimuli in humans [28].

 Nonetheless, some caution needs to be exerted when interpreting our data. Although the findings are robust, our study is limited by sample size and requires replication. Future studies should also use a drug challenge procedure testing drug effects dose-dependently, while more detailed psychophysiological characterization of the...
MMN-like ERP in birds is indicated, for instance, by testing other deviant features (e.g. stimulus duration or intensity, or using more complex sounds) and varying oddball probabilities.

Conclusion
The current study has provided some evidence for auditory sensory memory processing that appears to be homologous to that in mammals and suggestive of a common ancestry 300 million years ago or of convergent evolution. Subsequent research, however, should focus on the optimization and standardization of this procedure, thus establishing a tool to test the avian auditory system comparatively. This research may also lead to new insights into important aspects of the pathophysiology underlying schizophrenia by investigating an avian animal model, which is more closely aligned to the time course of neurodevelopmental synapse formation and maturation in primates than to that in rodents [17].

Acknowledgements
The authors thank Qian Xiao, Sabine Kesch, Arianne Schwarz, Tobias Otto, Maik Stüttgen, Eva-Lotte Schall, Gabi Salzmann and Juliane Brenscheidt for their assistance. They are grateful for Pat Michies’ advice on study design, and they thank Ross Fulham for his valuable comments on their manuscript. Ulrich Schall was supported by Deutscher Akademischer Austauschdienst (Reference A/09/11785). He also received infrastructure support from the Hunter Medical & Schizophrenia Research Institutes and the New South Wales Ministries of Health and Trade & Investment (Australia) while conducting the study. Onur Güntürkün was supported by the Deutsche Forschungsgemeinschaft (SFB 874).
authors thank Qian Xiao, Sabine Kesch, Arianne Schwarz, Tobias Otto, Maik Stüttgen, Eva-Lotte Schall, Gabi Salzmann and Juliane Brenscheidt for their assistance.

Conflicts of interest
There are no conflicts of interest.

References