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

Ulrich Schall[a,b,c], Bernhard W. Müller[d,e,f], Christian Kärgel[f] and Onur Güntürkün[g]

The neural response to occasional variations in acoustic stimuli in a regular sequence of sounds generates an *N*-methyl-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 (*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. *NeuroReport* **26:239–244**

NeuroReport 2015, **26:**239–244

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

[a]Priority Centre for Translational Neuroscience & Mental Health Research, The University of Newcastle, Callaghan, [b]Schizophrenia Research Institute, Sydney, [c]Hunter Medical Research Institute, New Lambton, Australia, [d]Department of Psychology, University of Wuppertal, Wuppertal, [e]Department of Psychiatry and Psychotherapy, Faculty of Medicine, LVR-Hospital Essen, [f]Institute of Forensic Psychiatry, Faculty of Medicine, University of Duisburg-Essen, Essen and [g]Institute of Cognitive Neuroscience, Division of Biological Psychology, Ruhr-University of Bochum, Bochum, Germany

Correspondence to Ulrich Schall, Priority Centre for Translational Neuroscience & Mental Health Research, The University of Newcastle, Mater Hospital, McAuley Centre, Waratah New South Wales 2298, Australia
Tel: +61 2 40335727; fax: +61 2 40335692;
e-mail: ulrich.schall@newcastle.edu.au

Received 17 November 2014 **accepted** 2 January 2015

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 regular 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 *N*-methyl-D-aspartate (NMDA) glutamate receptors [10–13]. For instance, NMDA receptor antagonists induce symptoms and cognitive impairments in healthy individuals that resemble

 DOI: 10.1097/WNR.0000000000000323

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 neuropathologies 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 uteri*.

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 (*Columba 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 interstimulus 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.

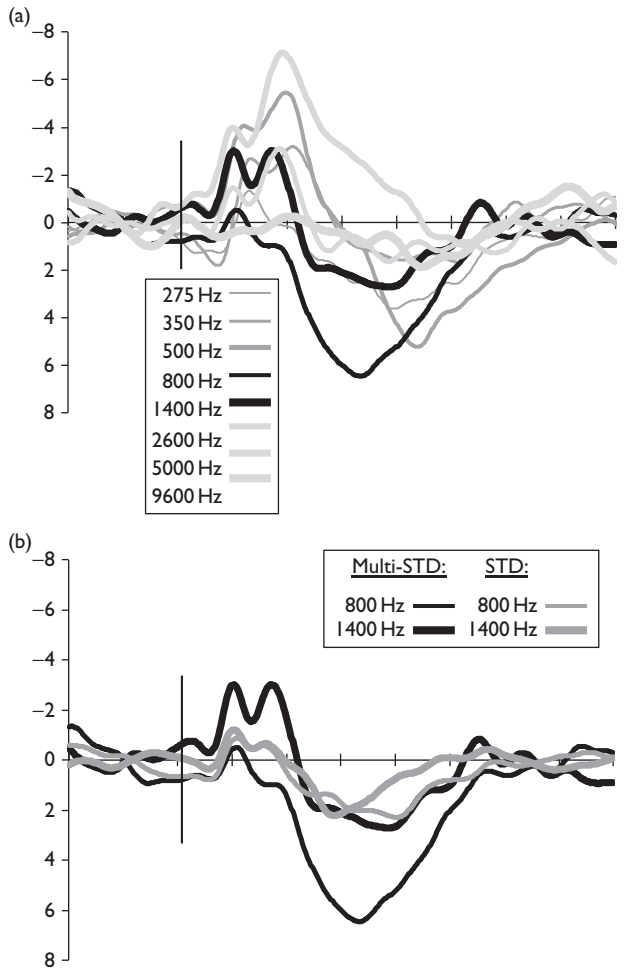
Raw recording data were normalized to the scale 0–1 by assigning the value of 0 to the largest negative recording value of the ERP and 1 to the largest positive recording value of the ERP across all recording conditions for each individual bird to account for between-subject differences in scaling. Normalized 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% useable 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

Fig. 1



Group-averaged event-related potentials (ERPs) with the horizontal x-axis representing 500 ms and the vertical line marking stimulus onset at 100 ms. The vertical y-axis represents μV units with negative up. (a) ERPs in response to a random sequence of 275, 350, 500, 800, 1400, 2600, 5000, and 9600 Hz presented at an equal probability of $P = 0.125$ in a multistandard procedure (Multi-STD). (b) Comparison of ERPs in response to 800 and 1400 Hz recorded as Multi-STD versus STD ERPs recorded in the respective mismatch negativity procedures.

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).

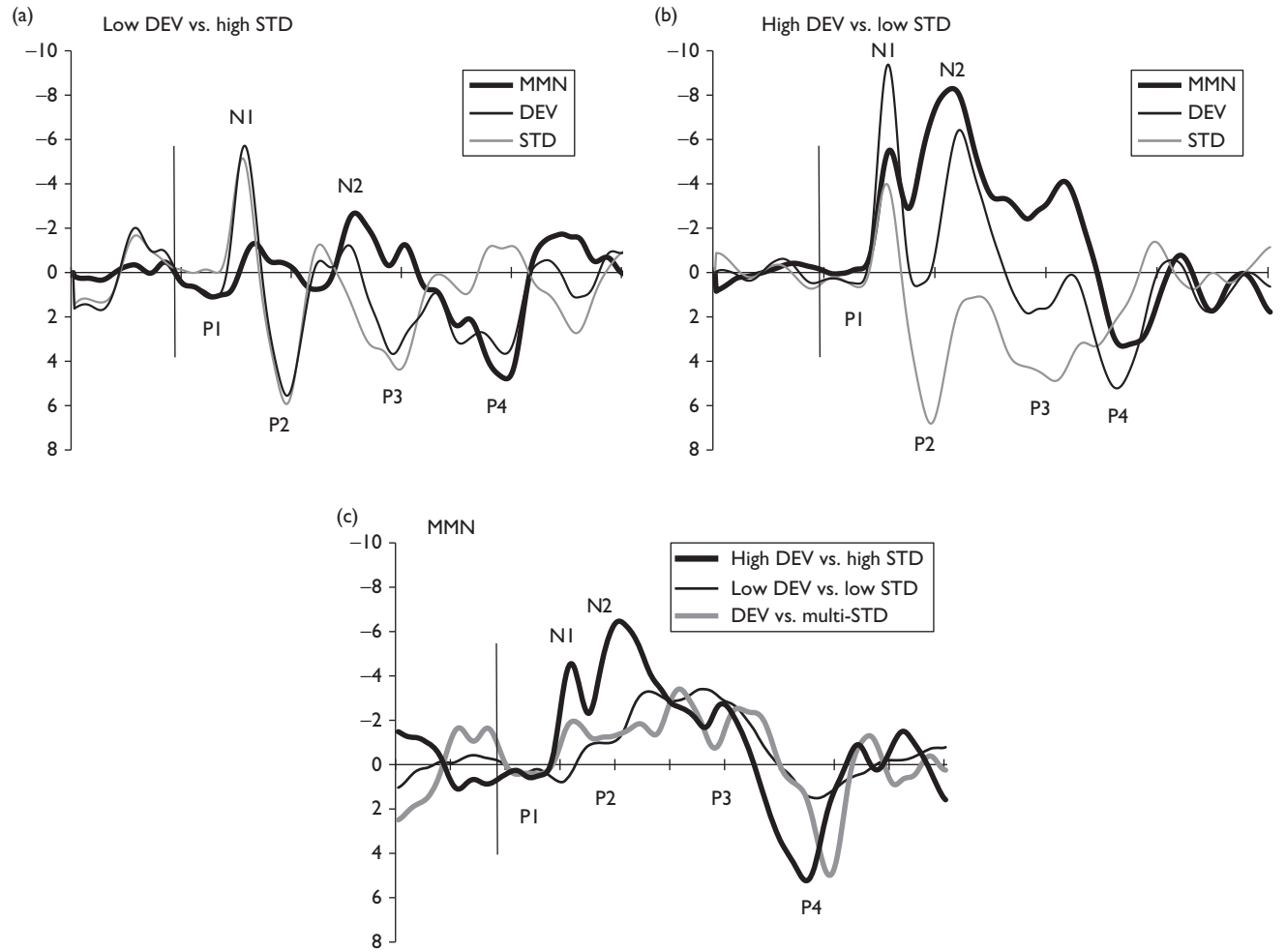
Discussion

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

Fig. 2



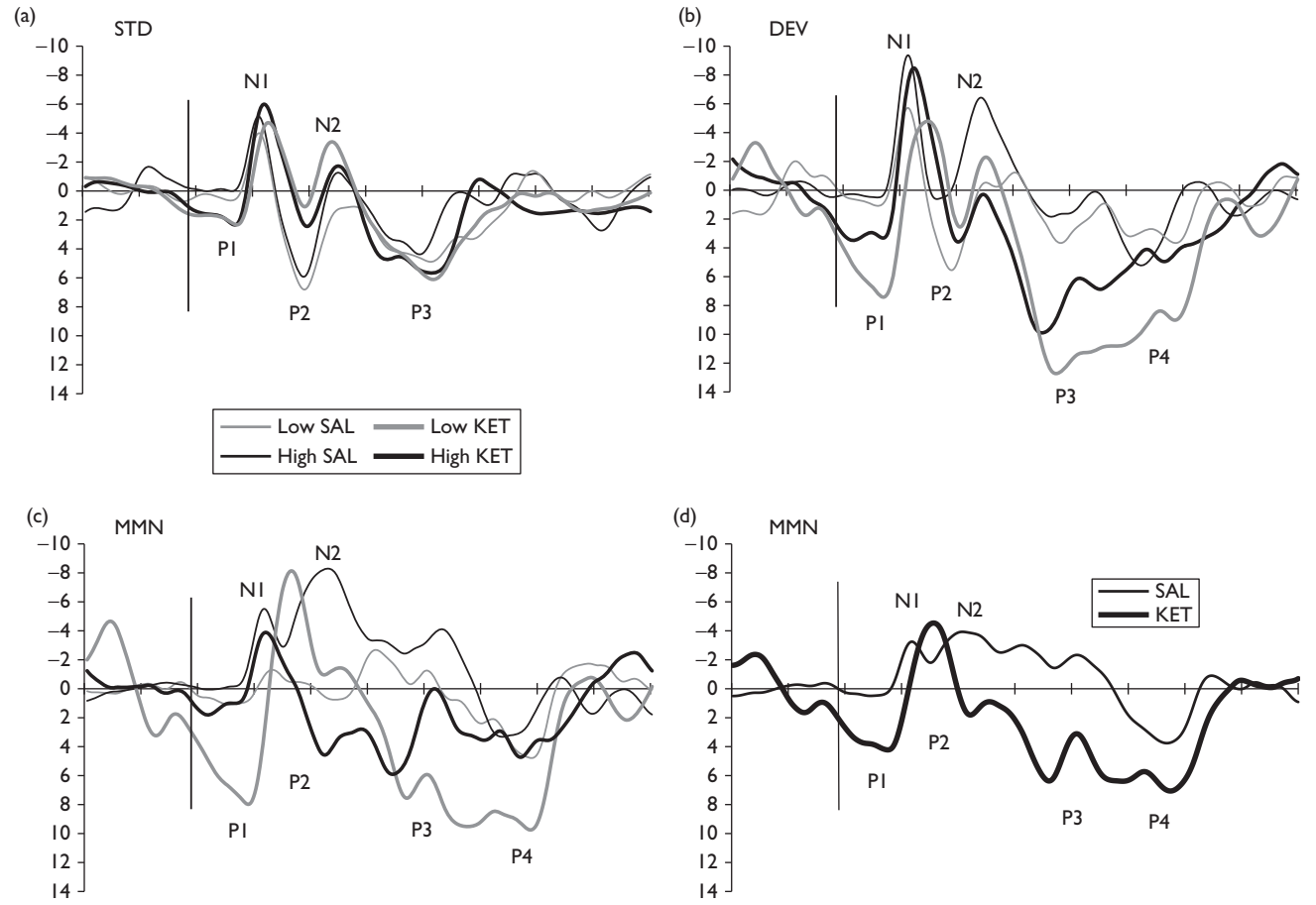
Group-averaged event-related potentials (ERPs) in response to low (800 Hz) and high (1400 Hz) pitch tones presented as frequent standard (STD) or rare ($P = 0.125$) deviant (DEV) oddball stimuli and corresponding mismatch negativity (MMN) subtraction waveform (DEV−STD). The horizontal x-axis represents 500 ms and the vertical line marks stimulus onset at 100 ms. The vertical y-axis represents μV units with negative up. Successive positive (P1, P2, P3 and P4) and negative (N1 and N2) peaks are labelled. (a) Low-pitch DEV versus high-pitch STD stimuli and (b) high-pitch DEV versus low-pitch STD presented within the same recording series resulted in an MMN-like response predominantly in the high-DEV versus low-STD contrast condition. (c) DEV minus STD-subtraction waveforms (MMN) of low-pitch DEV versus low-pitch STD ERPs and high-pitch DEV versus high-pitch STD ERPs (flip-flop design), as well as DEV versus STD recorded separately using the multistandard procedure and averaging across high-pitch and low-pitch conditions suggests an MMN-like field potential ranging from 50 to 250 ms after stimulus onset when controlling for potential stimulus frequency and probability confounds.

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

Fig. 3



Group-averaged event-related potentials (ERPs) in response to low-pitch (800 Hz) and high-pitch (1400 Hz) tones presented as frequent standard (STD) or rare ($P = 0.125$) deviant (DEV) oddball stimuli recorded in response to saline (SAL) versus systemic 5 mg/kg ketamine (KET) injections. Successive positive (P1, P2, P3 and P4) and negative (N1 and N2) peaks are labelled. (a) KET predominantly results in an amplitude reduction of the P2 ERP component recorded for the low-pitch and high-pitch STD stimuli. (b) By contrast, KET application reduces N2 in response to high-frequency DEV stimuli but increases the amplitudes of the P1 and P3 ERP components in response to DEV stimuli. (c) As a result, the MMN-like response is reduced for the late P2, N2 and P3 ERP components, with little effect on the early N1 ERP component when combining the low-pitch and high-pitch contrast conditions (d).

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). The

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

1 Evstigneeva MD, Aleksandrov AA. Mismatch negativity: the contribution of differences in the refractoriness of stimulus-specific neuron populations. *Neurosci Behav Physiol* 2009; **39**:833–840.

2 Fulham WR, Michie PT, Ward PB, Rasser PE, Todd J, Johnston PJ*, et al*. Mismatch negativity in recent-onset and chronic schizophrenia: a current source density analysis. *PLoS One* 2014; **9**:e100221.

3 Näätänen R. Mismatch negativity: clinical research and possible applications. *Int J Psychophysiol* 2003; **48**:179–188.

4 Näätänen R, Tervaniemi M, Sussman E, Paavilainen P, Winkler I. "Primitive intelligence" in the auditory cortex. *Trends Neurosci* 2001; **24**:283–288.

5 Amann LC, Gandal MJ, Halene TB, Ehrlichman RS, White SL, McCarren HS, Siegel SJ. Mouse behavioral endophenotypes for schizophrenia. *Brain Res Bull* 2010; **83**:147–161.

6 Nakamura T, Michie PT, Fulham WR, Todd J, Budd TW, Schall U*, et al*. Epidural auditory event-related potentials in the rat to frequency and duration deviants: evidence of mismatch negativity? *Front Psychol* 2011; **2**: 367.

7 Harms L, Fulham WR, Todd J, Budd TW, Hunter M, Meehan C*, et al*. Mismatch negativity (MMN) in freely-moving rats with several experimental controls. *PLoS One* 2014; **9**:e110892.

8 Umbricht D, Krljes S. Mismatch negativity in schizophrenia: a meta-analysis. *Schizophr Res* 2005; **76**:1–23.

9 Rasser PE, Schall U, Todd J, Michie PT, Ward PB, Johnston P*, et al*. Gray matter deficits, mismatch negativity, and outcomes in schizophrenia. *Schizophr Bull* 2011; **37**:131–140.

10 Javitt DC, Steinschneider M, Schroeder CE, Arezzo JC. Role of cortical *N*-methyl-D-aspartate receptors in auditory sensory memory and mismatch negativity generation: implications for schizophrenia. *Proc Natl Acad Sci USA* 1996; **93**:11962–11967.

11 Javitt DC. Intracortical mechanisms of mismatch negativity dysfunction in schizophrenia. *Audiol Neurootol* 2000; **5**:207–215.

12 Umbricht D, Schmid L, Koller R, Vollenweider FX, Hell D, Javitt DC. Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. *Arch Gen Psychiatry* 2000; **57**:1139–1147.

13 Umbricht D, Koller R, Vollenweider FX, Schmid L. Mismatch negativity predicts psychotic experiences induced by NMDA receptor antagonist in healthy volunteers. *Biol Psychiatry* 2002; **51**:400–406.

14 Lavoie S, Murray MM, Deppen P, Knyazeva MG, Berk M, Boulat O*, et al*. Glutathione precursor, *N*-acetyl-cysteine, improves mismatch negativity in schizophrenia patients. *Neuropsychopharmacology* 2008; **33**:2187–2199.

15 Todd J, Michie PT, Schall U, Ward PB, Catts SV. Mismatch negativity (MMN) reduction in schizophrenia-impaired prediction – error generation, estimation or salience? *Int J Psychophysiol* 2012; **83**:222–231.

16 Todd J, Harms L, Schall U, Michie PT. Mismatch negativity: translating the potential. *Front Psychiatry* 2013; **4**:171.

17 Atkinson R, Migues PV, Cammarota M, Kavanagh JM, Hunter M, Rostas JA. Biochemical, behavioural and electrophysiological investigations of brain maturation in chickens. *Brain Res Bull* 2008; **76**:217–223.

18 Wang Y, Brzozowska-Prechtl A, Karten HJ. Laminar and columnar auditory cortex in avian brain. *Proc Nat Acad of Sci USA* 2010; **107**:12676–12681.

19 Jarvis ED, Güntürkün O, Bruce L, Csillag A, Karten H, Kuenzel W*, et al*. Avian brains and a new understanding of vertebrate brain evolution. *Nat Rev Neurosci* 2005; **6**:151–159.

20 Karten HJ, Hodos W. *A stereotactic atlas of the brain of the pigeon*. Baltimore, Maryland: Johns Hopkins Press; 1967.

21 Schall U, Keysers C, Kast B. Pharmacology of sensory gating in the ascending auditory system of the pigeon (*Columba livia*). *Psychopharmacology (Berl)* 1999; **145**:273–282.

22 De Winter JCF. Using the Student's *t*-test with extremely small sample sizes. *Pract Assess Res Eval* 2013; **18**:1–12.

23 Ruusuvirta T, Penttonen M, Korhonen T. Auditory cortical event-related potentials to pitch deviances in rats. *Neurosci Lett* 1998; **248**:45–48.

24 Tikhonravov D, Neuvonen T, Pertovaara A, Savioja K, Ruusuvirta T, Näätänen R, Carlson S. Effects of an NMDA-receptor antagonist MK-801 on an MMN-like response recorded in anesthetized rats. *Brain Res* 2008; **1203**: 97–102.

25 Roger C, Hasbroucq T, Rabat A, Vidal F, Burle B. Neurophysics of temporal discrimination in the rat: a mismatch negativity study. *Psychophysiology* 2009; **46**:1028–1032.

26 Tikhonravov D, Neuvonen T, Pertovaara A, Savioja K, Ruusuvirta T, Näätänen R, Carlson S. Dose-related effects of memantine on a mismatch negativity-like response in anesthetized rats. *Neuroscience* 2010; **167**:1175–1182.

27 Herold C, Palomero-Gallagher N, Hellmann B, Kröner S, Theiss C, Güntürkün O, Zilles K. The receptor architecture of the pigeons' nidopallium caudolaterale – an avian analogue to the prefrontal cortex. *Brain Struct Funct* 2011; **216**:239–254.

28 Shinozaki N, Yabe H, Sutoh T, Hiruma T, Kaneko S. Somatosensory automatic responses to deviant stimuli. *Brain Res Cogn Brain Res* 1998; **7**:165–171.