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Maternal immune activation alters the sequential structure of ultrasonic communications in male rats

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ABSTRACT

Maternal immune activation (MIA) is a risk factor for schizophrenia and many of the symptoms and neurodevelopmental changes associated with this disorder have been modelled in the rodent. While several previous studies have reported that rodent ultrasonic vocalizations (USVs) are affected by MIA, no previous study has examined whether MIA affects the way that individual USVs occur over time to produce vocalisation sequences. The sequential aspect of this behaviour may be particularly important because changes in sequencing mechanisms have been proposed as a core deficit in schizophrenia. The present research generates MIA with POLY I:C administered to pregnant Sprague-Dawley rat dams at GD15. Male pairs of MIA adult offspring or pairs of their saline controls were placed into a two-chamber apparatus where they were separated from each other by a perforated plexiglass barrier. USVs were recorded for a period of 10 min and automated detection and call review were used to classify short call types in the nominal 50 kHz band of social affiliative calls. Our data show that the duration of these 50-kHz USVs is longer in MIA rat pairs and the time between calls is shorter. Furthermore, the transition probability between call pairs was different in the MIA animals compared to the control group, indicating alterations in sequential behaviour. These results provide the first evidence that USV call sequencing is altered by the MIA intervention and suggest that further investigations of these temporally extended aspects of USV production are likely to reveal useful information about the mechanisms that underlie sequence generation. This is particularly important given previous research suggesting that sequencing deficits may have a significant impact on both behaviour and cognition.

1. Introduction

Maternal immune activation (MIA) has neurodevelopmental consequences for brain development and subsequent behaviour in both human and non-human animals (Bergdolt and Dunaevsky, 2019; Brown and Meyer, 2018; Smith et al., 2007). For example, in humans MIA is a risk factor for schizophrenia (Brown and Meyer, 2018), a chronic, severe and disabling brain disease that is among the world's top ten causes of long-term disability.

The symptoms of schizophrenia are typically split into three broad categories: psychotic or positive symptoms, negative symptoms, and cognitive impairment. The overt positive symptoms consist of hallucinations and delusions, the negative symptoms include problems with motivation and social interactions and the cognitive symptoms comprise deficits in attention, memory and executive function (Keefe and Harvey, 2012; van Os and Kapur, 2009). It has been known for some time that the cognitive symptoms include wide-ranging deficits in the timing and sequencing of experience and behaviour (Ciullo et al., 2018). This includes difficulties in ascertaining stimuli sequence order and learning and reproducing motor sequences (Green et al., 1997; Pedersen et al., 2008). Recent research has indicated that these timing/sequencing deficits are not domain specific and are primary to schizophrenia (Ciullo et al., 2016), raising the possibility that they may explain some fundamental aspects of schizophrenia pathophysiology (Sterzer et al., 2016).

MIA has also been examined in animal models, which allow for a more thorough examination of the brain changes that might underlie the disorder. In rodent models, a number of schizophrenia-like changes in behaviour and brain activity, function and neurochemistry have been previously described in the adult offspring of MIA-challenged dams (Brown and Meyer, 2018; Dickerson et al., 2010; Estes and McAllister, 2016; Kleinmans and Bilkey, 2018; Wolff and Bilkey, 2015). This includes alterations in interval timing (Deane et al., 2017). However, no previous study has explicitly examined sequential behaviour in these models. To these ends, it is possible that auditory communication, which

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has previously been shown to have temporal structure (Castellucci et al., 2018; Uematsu et al., 2007), might be a useful platform from which to investigate MIA-induced changes in sequential organisation.

Much of rodent auditory communication is emitted at ultra-sonic frequencies, above the range of human hearing (Jouda et al., 2019). These ultrasonic vocalisations (USVs) have an important role in social interactions and have been linked to specific behaviours in socially engaged conspecifics (Sangiamo et al., 2020). USVs in adult rats fall into two broad categories. Those in the nominal 22-kHz frequency band are often characterised as aversive USVs as they serve an alarm/distress purpose. The duration of these calls is relatively long (1000–3000 ms) and they occur within an 18–24 kHz frequency band. In contrast, calls in the nominal 50-kHz band occur within a 30 to 90-kHz frequency range and have relatively short duration (5–90 ms), with the majority of calls being less than 50 ms in duration (Jouda et al., 2019; Wright et al., 2010). These calls are sometimes classified as appetitive and have been associated with positive affect. For example, 50-kHz call playback has been shown to initiate approach behaviour in rats, underscoring the social nature of these calls (Seffer et al., 2014). Wright et al. (2010) categorized 50-kHz USVs in terms of their acoustic characteristics, focussing on spectrographic contour in particular. By using this classification system, Wright and colleagues recognized 14 different subtypes of call. During social interactions these USV calls can often occur in a sequences (bouts) with short intervals between the individual calls (Coffey et al., 2019). These sequences are non-random with a temporal and syntactic structure (Hertz et al., 2020), that is integral to their communicative function (Castellucci et al., 2018; Uematsu et al., 2007).

USVs have previously been identified as a preclinical tool that can be used for modelling aspects of neuropsychiatric disease (Simola, 2015; Simola and Brudzynski, 2018). In this light, several previous studies have examined the effect of MIA on rodent USVs (see Jouda et al., 2019 for a review). While several of these studies have reported MIA-induced changes in call characteristics, few have investigated communication in the 50 kHz band and no previous study has investigated the effect of MIA on the sequential construction of USV bouts. The purpose of the current study is, therefore, to investigate the effect of MIA on these 50-kHz calls, with a particular focus on the transition probabilities between calls which are reflective of sequential structure.

2. Material and methods

2.1. Subjects

Forty Sprague-Dawley rats were tested in the present study. Twenty were obtained from 6 poly I:C treated litters and 20 from 6 saline control litters. To induce MIA, female Sprague-Dawley rats were mated at approximately 3-months of age. On gestational day 15, pregnant dams were anesthetised with isoflurane (5% in oxygen) and randomly selected to receive a tail vein injection of either saline dissolved poly I:C (4.0 mg/kg) or an equivalent dosage of saline as per our previous studies (Kleinmans and Bilkey, 2018; Wolff and Bilkey, 2015). Weaning of their male offspring occurred at week three after parturition, with four to six males retained from each dam. Male offspring were housed in litter-mate pairs and allowed food and water ad libitum until reaching maturity (3 months of age). Although recent research indicates there is a sexually dimorphic expression of USVs in rats (Kisko et al., 2021), only male rats were included in the present study. This was due to resource limitations, to align with previous research in our laboratory, and to limit variation in the data.

At this point animals were maintained at 85% of free-feeding weight, creating satiety conditions similar to that experienced in the wild. All animals had unlimited access to water. The housing room was temperature controlled at 21–23 °C, humidity was kept at above 40%, and lights were on for 12 h of a 24-h light/dark cycle. All testing was conducted during the light part of the cycle. The experiment was approved by the University of Otago Animal Ethics Committee following the guidelines of the NZ Animal Welfare Act 1999.

2.2. Apparatus

Testing was conducted in dimly lit room containing an open rectangular box that was separated into two regions by a plexiglass partition perforated with approximately fifty 10 mm diameter holes. In the first set of 10 recordings, each chamber measured 15 cm wide x 30 cm long with 30 cm high walls while for the remaining recordings each chamber measured 24 cm wide, 60 cm in length and with 24 cm high walls. An UltraMic 250k microphone (Dodotronic, Italy) was affixed to the rear wall of one chamber, approximately 30 cm above chamber floor height.

2.3. Procedure

Two paired (either a pair of MIA or a pair of control) rats were placed, one each, into the two chambers of the apparatus whereupon they were free to move. After a 1-min habituation period, audio from the animals was recorded for a 10-min period. Pairs of animals were always selected from home cages that were separated by an intervening cage in the home rack to control for the effect of previous interactions. Animals were monitored at regular intervals and were active throughout the recording procedure.

2.4. USV detection and analysis

The UltraMic 250k microphone sampled audio signals at 250 kHz and fed these data via USB to a computer running Audacity for Windows (www.audacityteam.org) which stored the data as WAV files. Audio data files were analysed automatically offline by the program DeepSqueak (Coffey et al., 2019) running under MATLAB R2019A. (Mathworks).

DeepSqueak uses a convolutional neural network for automatic detection and identification of USVs (Coffey et al., 2019). It was run using their supplied network which was pre-trained on short rat calls to distinguish Wright et al. (2010) classifiers. An initial benchmark for discriminating calls from background noise was set by manually screening a sample of calls. DeepSqueak was then run across this screened data set with a variety of different acceptance thresholds for the 'score' and 'tonality' parameters. The settings that obtained the best signal detection d-prime value against the benchmark (Stanislaw and Todorov, 1999) were then used for all further analyses of the full data set. Eleven sub-types of 50-kHz calls were recognized in the data. These were the downward ramp, inverted-U, short, split, complex, complex trill, flat, step down, step up, trill and upward ramp types as previously defined by Wright and colleagues (Wright et al., 2010). Spectrograms of example call types are provided in supplementary materials.

MATLAB code was written to extract and save the features of each call (such as timestamp, frequency, duration) for later analysis. From these data, the transition probabilities, that is, the likelihood that one call type would be followed by any other type, were calculated for all pairs of calls that occurred within 2 s of each other. Because call transition probabilities are dependent on the rate of occurrence of individual call types (Castellucci et al., 2016) we also generated a transition score measure from these probabilities that took differences in baseline call rates into account.

Transition scores were generated by first determining what the underlying transition probability distribution would be for randomly occurring pairs of call, given the base rate of each call type. This was achieved by repeatedly shuffling the ordinal position of each call and calculating the transition probability after each shuffle. This procedure was repeated 1000 times to generate a shuffled distribution for every call-type pairing. Importantly, the process maintained the overall temporal structure and individual call-type rate in each recording but randomised the relationship between any call and its neighbour. The actual transition probability between call pairs as recorded during the experiment was then calculated as above and converted to a z-score (the

transition score) by comparing it to the mean and variance of the random distribution. A transition score of zero indicates chance pairings between the call types. A positive transition score indicates that the actual call pairing was more likely than expected by chance, while a negative transition score indicated a pairing that was less likely to occur than chance.

Data for simple call parameters (duration and mean frequency) were coded by condition and independent sample t-tests (SPSS version 25) were performed to determine whether differences occurred between the groups. Where required, missing values were imputed from the mean of the intact values in the underlying group. A repeated measures ANOVA was used to determine whether call duration differed across groups for the different call types. Transition scores were compared by repeated measures ANOVA for the seven call types that made up more than 5% of total calls in either the MIA or control group.

3. Results

3.1. Descriptive data

A total of 4495 USV calls were detected by DeepSqueak and characterised as valid. The vast majority of these calls were in the 50-kHz range (Fig. 1), with calls in the 22-kHz range (18–24-kHz) representing only 1.4% of the total call dataset. On average control animal pairs generated 18.7 calls per minute while the MIA pairs produced 24.4, however this difference was not statistically significant ($t(18) = 0.611$, $p = 0.54$). The distribution of overall calls per minute was calculated for the two groups and is presented in Fig. 2. Although control animal pairs tended to make fewer calls than MIA pairs during the first 2 min of the recording, a Chi-Square test revealed that there was no significant between-group difference in this distribution ($X^2(9) = 10.36$, $p = 0.32$). An analysis of the characteristics of all detected calls, revealed no difference between groups for the call features of frequency, slope, sinuosity, power, tone or call quality. Calls from MIA animals were, however, substantially longer in duration than control calls ($t(12) = -3.275$, $p = 0.007$; Table 1).

The distribution of inter-call intervals was generated by placing the data into 100 ms bins out to a maximum of 2 s. A two-sample Kolmogorov-Smirnov test revealed a significant difference between the two groups ($p < 0.0001$; Fig. 3). This appeared to be due to a greater tendency for MIA animals to generate pairs of calls with short inter-call intervals of less than 300 ms.

3.2. Call sub-types

Calls were automatically separated into 11 subtypes by the DeepSqueak software (see supplementary data for examples). There was considerable variation in the rate of the different call types. Complex trill calls were the most common call type in both groups, comprising 28% and 20% of calls in MIA and control animals respectively, while downward ramp, inverted-U, short and split call types each comprised less than 2% of all calls (Fig. 4). To remove the influence of calls with low occurrence, data from the latter four subtypes were removed from further analysis. This left the seven call categories of complex, complex trill, flat, step down, step up, trill and upward ramp for additional analysis. These remaining call types each comprised more than 5% of calls in one or other of the MIA and/or control group. A mixed ANOVA that compared the proportion of these call subtypes across the MIA and control groups revealed a main effect of call type $F(6,108) = 6.616$, $p < 0.001$, but there was no effect of group ($F(1,18) = 0.18$, $p = 0.676$)and no group by call interaction ($F(6,108) = 1.33$, $p = 0.25$).

To determine whether the longer call duration observed in MIA animals was specific to particular call types a mixed ANOVA was conducted for data from the seven most common call types. This revealed a significant effect of call type ($F(6,108) = 4.668$, $p < 0.001$), a significant effect of group ($F(1,18) = 11.371$,$p = 0.003$), and a group by call type interaction ($F(1,18) = 2.969$, $p = 0.01$). These results confirm that the changes in call duration were particular to call type and post-hoc t-tests revealed significant differences (all at least $p < 0.05$) in call duration between MIA and control animals for complex, complex trill, step down, step up and trill subtypes (Table 2).

3.3. Call transition data

An analysis of call transition scores between pairs of the seven most common call types revealed both positive (preferred) and negative values (dis-preferred). These transition scores were analysed with separate mixed ANOVAs for each initial call type. Results were Bonferroni corrected for multiple testing by setting alpha at $p < 0.007$. For all seven call types there was a significant effect of call, indicating that overall, call transitions were non-random. There was a significant group by call interaction for transitions from upward ramp calls to other call types ($F(6,102) = 3.225$,$p = 0.006$) and from step down calls to other calls ($F6,102) = 3.902$,$p = 0.002$). The results of all comparisons are displayed in Tables 3a and 3b. Post-hoc t-tests for the transitions from upward ramp

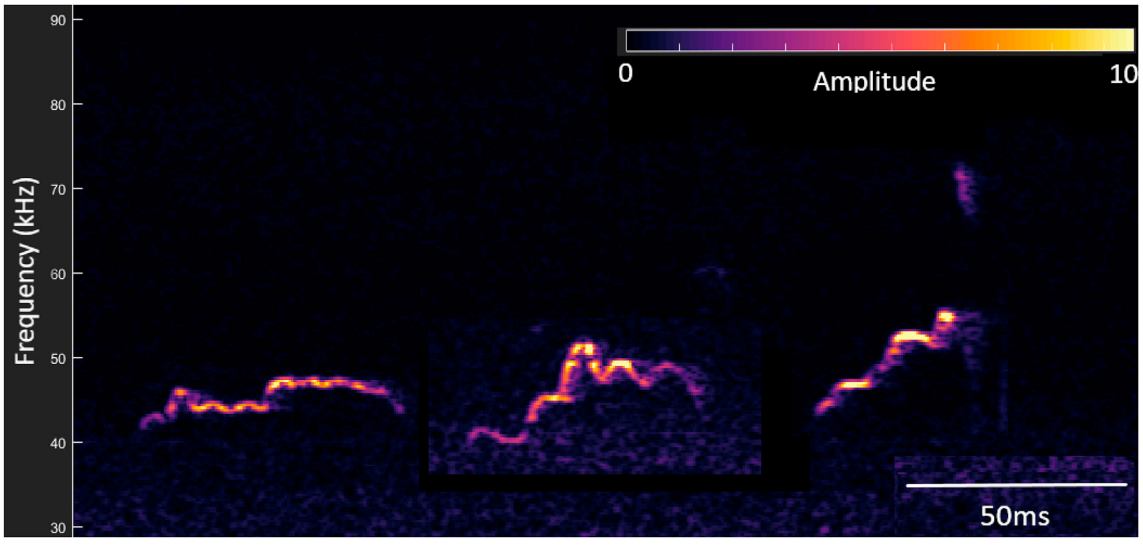

Fig. 1. Example spectrograms generated from three separate 50-kHz USVs. From left to right they were classified by DeepSqueak as complex, complex trill, and step-up calls. Note that each call is displayed in a separate time segment.

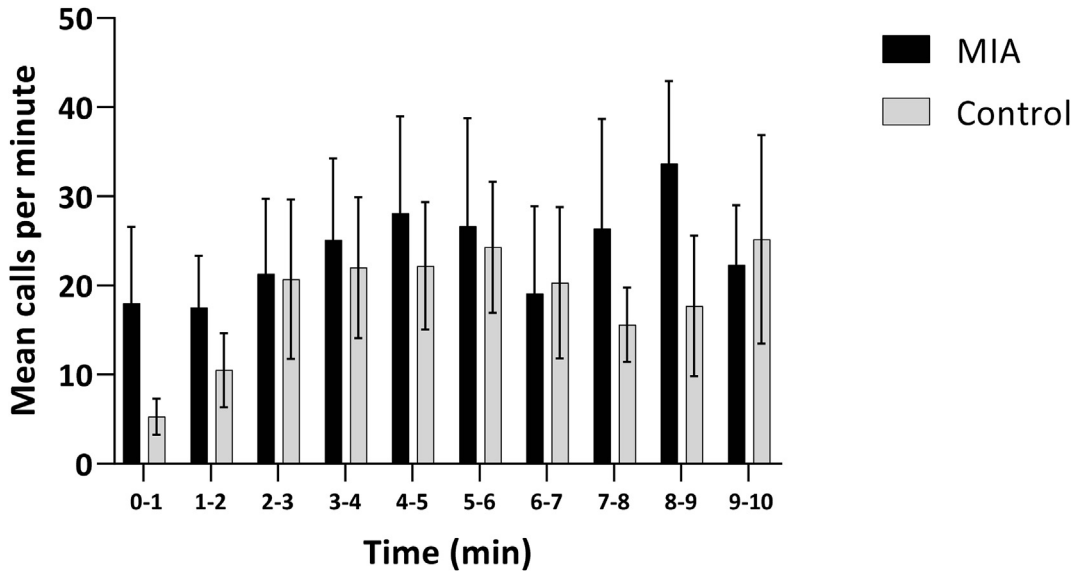

Fig. 2. The distribution of call rate, binned into 1 min blocks, for MIA and control animals across the whole 10-min recording session (mean ± sem).

Table 1
Mean (± sem) call characteristics for MIA and control animals.

Parameter	MIA	CON	p value
Frequency (kHz)	48.6 ± 2.0	45.1 ± 2.3	0.27
Duration (ms)	47.1 ± 5.3*	28.8 ± 2.2	0.004
Slope	111.1 ± 24.1	122.1 ± 47.9	0.83
Sinuosity	1.54 ± 0.06	1.58 ± 0.10	0.69
Power	−58.6 ± 2.9	−60.5 ± 2.2	0.59
Tone (dB)	0.54 ± 0.02	0.49 ± 0.07	0.07
Score	0.79 ± 0.01	0.79 ± 0.01	0.79

* = $p < 0.01$ (*t*-test).

calls revealed a significant difference between the MIA and control groups in the transition to complex trills ($t(17) = 2.195, p = 0.04$), step-down ($t(17) = 2.235, p = 0.04$) and step up ($t(17) = 3.22, p = 0.005$) calls with MIA animal pairs less likely to transition to less complex trill and step-up calls but more likely to transition to step-down calls. A similar analysis for transitions from step-down calls revealed significant between-group differences in the transition to another step-down call ($t(17) = 4.932$, $p = 0.0001$), with MIA animal pairs more likely to transition from one stepdown call to another.

4. Discussion

The present study sought to determine if the MIA manipulation

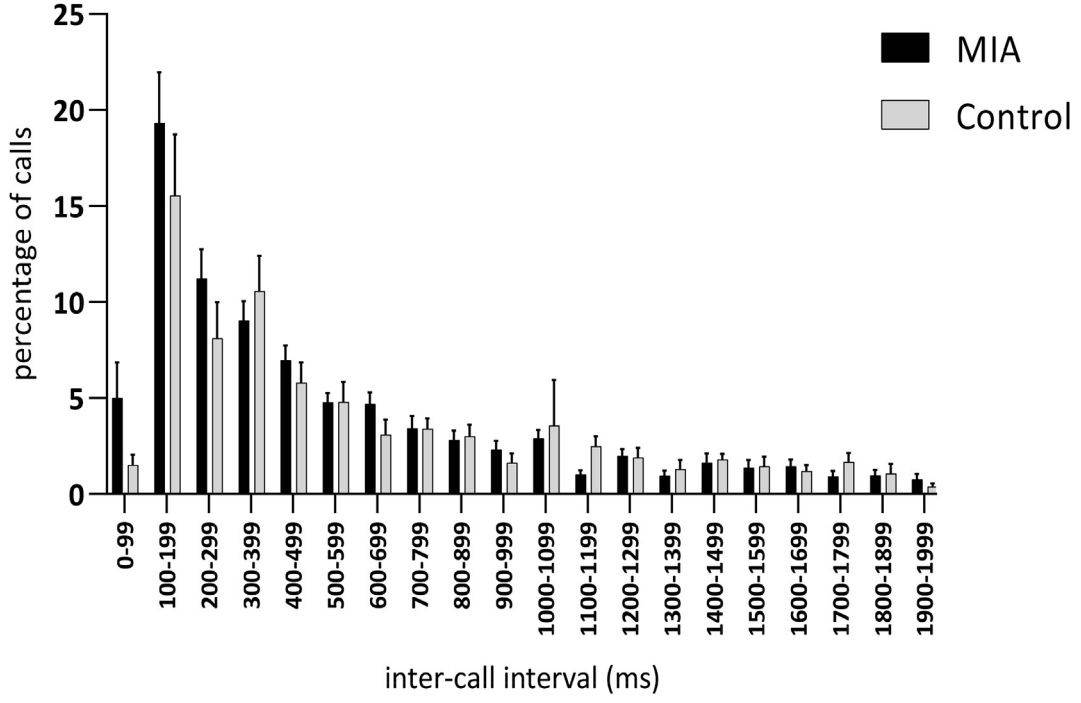

Fig. 3. Distribution of Inter-call intervals, accreted into 100 ms bins, for all call pairs occurring within 2 s of each other. MIA animals tended to generate a greater number of call pairs with inter-call intervals of less than 300 ms.

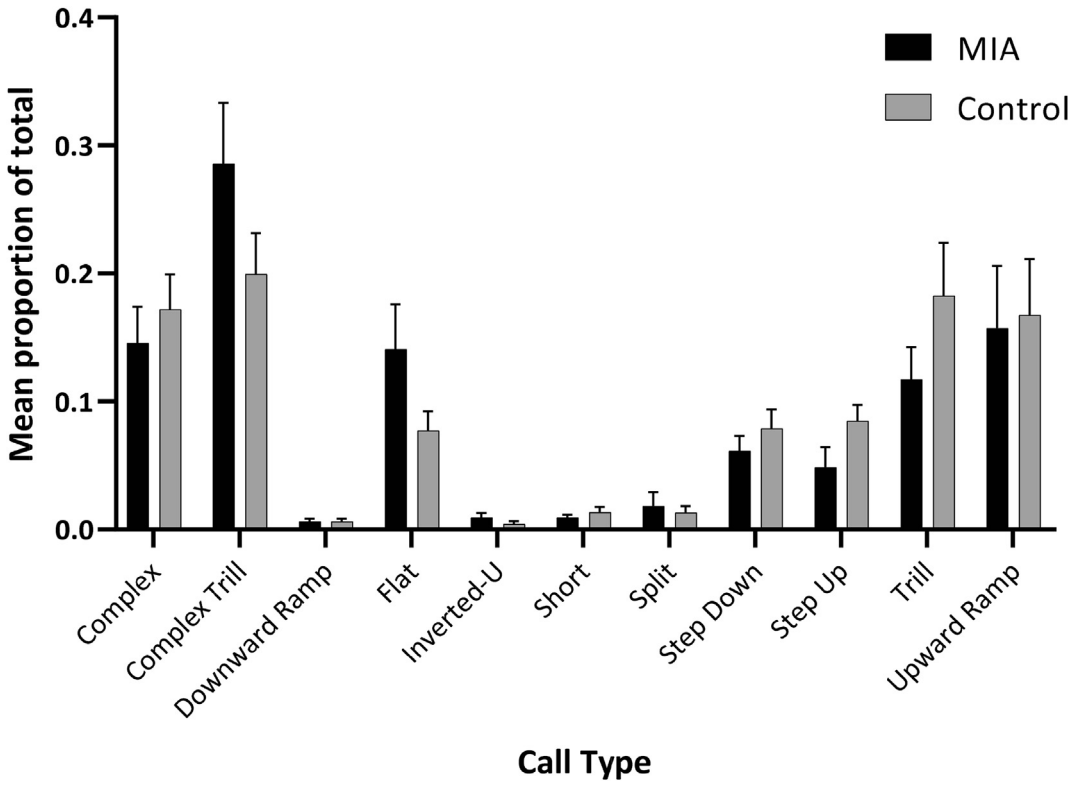

Fig. 4. Mean proportion of call types recorded from MIA and Control animals.

Table 2
Call duration in ms (mean + sem) for the seven most common call types in MIA and control animals. (* = p < 0.05, ** = p < 0.01; t-test).

	complex**	com. trill*	flat	step down**	step up*	trill*	up. ramp
MIA	33.5 ± 2.7	48.3 ± 5.3	44.3 ± 8.0	58.7 ± 7.7	40.6 ± 7.2	48.0 ± 6.5	32.8 ± 2.0
Control	23.5 ± 2.0	32.0 ± 4.8	27.1 ± 4.0	28.0 ± 4.6	19.9 ± 2.9	31.3 ± 2.1	31.1 ± 2.6

affected 50-kHz ultrasonic vocalisations recorded from pairs of adult rats located in adjacent environments. USVs occurred approximately once every 3 s in both groups over the 10-min recording period, which was consistent with that reported previously (Wright et al., 2010). The MIA manipulation did not alter the overall call rate. Furthermore, MIA did not change the distribution of calls over the recording period, nor did it alter the average spectral characteristics of the calls, such as their frequency, power or sinuosity. Mean call duration in the control group was around 30 ms, similar to that reported in previous studies (Gzielo et al., 2021; Wright et al., 2010). In contrast, there was a marked increase in call

Table 3a
Mean transition scores between the seven most common call types in control animals.

	complex	complex trill	flat	step down	step up	trill	upward ramp
complex	0.208	−0.429	−0.569	0.199	−0.346	0.070	−0.475
complex trill	−0.414	0.767	−0.820	0.035	−0.430	0.124	0.436
flat	−0.309	0.034	0.861	0.719	0.686	0.050	−0.418
step down#	−0.505	−0.032	0.421	0.275***	0.448	−0.257	−0.140
step up	−0.131	−0.517	0.486	0.013	1.267	−0.377	0.633
trill	−0.244	−0.290	−0.009	0.207	−0.466	1.087	−0.339
upward ramp#	−0.395	0.264*	−0.492	−0.471*	0.453**	−0.670	0.463

Table 3b
Mean transition scores between the seven most common call types in MIA animals.

	complex	complex trill	flat	step down	step up	trill	upward ramp
complex	1.345	−0.616	−0.467	0.313	−0.265	−0.504	−0.246
complex trill	−0.662	0.621	−0.841	−0.354	0.096	0.618	−0.347
flat	−0.751	0.259	1.732	0.253	0.180	−0.666	−0.626
step down#	−0.704	−0.095	1.353	1.951***	0.073	−0.106	−0.752
step up	−0.096	0.100	0.462	0.179	0.215	−0.603	0.277
trill	0.164	−0.300	0.360	−0.730	−0.506	1.052	0.295
upward ramp#	−0.251	−0.163*	−0.356	0.524*	−0.831**	−0.043	1.089

Key: The first call of the pair is listed in the left-most column. A positive score indicates the transition is more likely than chance, a negative score indicates the opposite. # = p < 0.01 call by group interaction in ANOVA. * = p < 0.05, ** = p < 0.01, *** = p < 0.001 in post-hoc between-group comparison.

duration in the MIA animals, with individual calls lasting almost twice as long as those from control pairs. Individual calls of MIA animals were also more likely to occur closer together in time than were those in the control animals, a change that may have been related to the extended call duration.

When calls were automatically classified using the Wright et al. (2010) categorizations, it was apparent that there was significant variation in the likelihood of animals producing a particular call-type. This variation was generally similar to that described previously, with a high proportion of trill and complex trill calls, although with a greater proportion of upward ramps in the current study (Wright et al., 2010). However, the variation in call type was not affected by the MIA manipulation as each call-type occurred in a similar proportion across both groups. Further analysis was subsequently limited to the seven call types that made up at least 5% of all calls in one or other group. An analysis of the transitions between these individual calls revealed that they were non-random in both MIA and control animals, with particular transition types being preferred, whilst others were dis-preferred. This confirms previous findings that there is a structure to these call transitions, previously referred to as syntax (Castellucci et al., 2018; Chabout et al., 2015; Hertz et al., 2020).

A comparison of the sequential relationships between calls in control and MIA rats revealed changes in the likelihood that particular call types would be followed by calls of the same or other call types. This was evident in the significant group by transition differences when upwards ramp and step down call types were followed by a second call. These sequence alterations included changes in transitions between common call types, for example, from upward ramp to complex trill. Changes in the call structure cannot be explained by MIA-induced changes in call rate, as overall this was similar between the groups. Furthermore, there was no between-group difference in the proportion of call types and the transition scores themselves were normalised to allow for the fact that calls with a high overall rate are more likely to occur after other calls. Interestingly, one of the significant changes in transition (step down to step down) involved increased repetition of the step-down call type, which suggests that this effect might result from perseveration, which has previously been described in MIA animals (Kleinmans and Bilkey, 2018).

Several previous studies have investigated the effect of MIA, as induced by Poly I:C or LPS injection, on murine USVs (reviewed in Jouda et al., 2019, and see Potasiewicz et al., 2020). Most of these studies have, however, been conducted in mice, and in juvenile animals. As far as we are aware, only one previous study has investigated USVs in adult MIA rats. Yee et al. (2012) observed a MIA-associated reduction in duration of 22-kHz aversive calls in a fear conditioning paradigm but report no difference in the duration, or any other features, of 50-kHz calls (Yee et al., 2012). It is not clear whether 50-kHz call duration was measured in this previous study, but it is possible that changes might not have been observed because the apparatus was not specifically arranged to elicit social calls. For example, the animals were recorded in isolation, within a shock chamber. Furthermore, 50-kHz calls ceased soon after the CS/US shock pairings began. Two studies have investigated the effect of MIA in older juvenile (8–10 weeks) mice. Hsiao et al. (2013) report that MIA induced by poly I:C reduced both the total number and duration of calls elicited by exposing mice to novel females. Consistent with this, Malkova et al. (2012) also found that MIA resulted in a mean reduction in call rate and call duration across numerous call types in the >30 kHz range, following exposure to a novel female. They also report a reduction in call rate, but not duration, in male-male interactions. A recent study investigating USVs in juvenile rats has also reported a reduction in calls in male animals (Gzielo et al., 2021). Clearly there is a difference between the effects described in these previous studies and our own data. However, there are a number of factors that could account for this, the most obvious being species differences, animal age and the method of eliciting calls.

As far as we are aware our data are the first showing that the sequencing of 50-kHz USVs is altered in MIA adult rats. The temporal sequencing of USVs is integral to their communicative function (Castellucci et al., 2018; Uematsu et al., 2007) suggesting that social communication between MIA animals might be altered. It is of interest to consider whether these changes in USVs might underlie some of the alterations in social affiliation that have been observed in adult MIA animals (Bergdolt and Dunaevsky, 2019; Lins et al., 2018).

Sequencing processes also underlie a range of behaviour, including the sequencing of individual movements and action sequences, and the ability to both produce and perceive sound sequences in communication (Clegg et al., 1998). This raises the possibility that MIA has a more general effect on sequencing behaviour, over and above that observed in USVs. As far as we are aware however, no prior work has examined the effects of MIA on the sequencing of any aspect of behaviour. In contrast, there is a large body of previous research showing that sequencing and timing processes are disrupted in schizophrenia, a disorder where MIA is a risk factor (Brown and Meyer, 2018). For example, patients with schizophrenia tend to perform more poorly than healthy control participants on tasks that require participants to store and retrieve sequences of conceptual information (Dreher et al., 2001; Fraser, 2004; Hill et al., 2013), and deficits in sequence learning have also been reported (Enticott et al., 2008; Green et al., 1997; Pedersen et al., 2008). These sequencing and timing disruptions are wide-ranging, affecting processes as disparate as sequenced oculomotor movements and implicit motor sequencing (Chrobak et al., 2017; Dias et al., 2021). On a broader level, an analysis of communication deficits suggests that impaired sequencing contributes to a substantial proportion of the structural communication failures observed in schizophrenia (Docherty et al., 2006).

Future work requires clarification of how the changes in USVs are expressed in MIA animals. In the current study, we were unable to determine which calls came from individual animals in each pairing. As a result, it was not possible to differentiate between a call pair where both calls originated in one animal, from a call pair where each originated from a separate animal. This aside, a large proportion of calls occurred within a few hundred milliseconds of each other, suggesting that they originated from one animal. In either case these data are of interest because they indicate that either the sequential ordering of communication between MIA rodents is impaired, or the sequential ordering of communication is impaired within the individual rodent (or both). This problem may be resolved in future studies that use sound source localization of USVs (Heckman et al., 2017).

Future studies should also track the behaviour of the individual animals as this would help determine how changes in overt social behaviour, social distance for example, might interact with USV call production. In the present study animals were separated by a divider, which prevented physical interaction of paired rats. This was advantageous as it limited the confounding effects of close behavioural interactions. However, the divider may also have limited or modified the repertoire of 50-kHz USV expression in rats. In mice, certain patterns of calls have been determined to be behaviour dependent (Sangiamo et al., 2020), and in rats there is evidence that specific 50-kHz call subtypes are associated with termination and evasion of rough and tumble play (Himmler et al., 2014). Research has also found strong associations between specific 50-kHz call types and discrete movement behaviours such as turning and jumping in the anticipatory period before play (Burke et al., 2017). It will, therefore, be important to elucidate the relationship between social behaviour and USVs in MIA animals and to explore what communicative differences exist between MIA rats tested in the present paradigm and those allowed to freely engage in more naturalistic interactions.

In summary, the present research provides the first evidence that MIA disrupts the sequencing of 50-kHz communication in pairs of adult MIA rats. The structure of these multi-element USV calls may provide a very useful signal as to the health of underlying neural timing and sequencing mechanisms. Given that MIA is a risk factor for schizophrenia, and that changes in timing and sequencing processes have been suggested as a core feature of schizophrenia, the MIA-USV sequence model may provide a novel platform from which to examine the biological bases for these changes.

Declaration of competing interest

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2021.100304.

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