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Linking the genomic signatures of human beat synchronization and learned song in birds

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The development of rhythmicity is foundational to communicative and social behaviours in humans and many other species, and mechanisms of synchrony could be conserved across species. The goal of the current paper is to explore evolutionary hypotheses linking vocal learning and beat synchronization through genomic approaches, testing the prediction that genetic underpinnings of birdsong also contribute to the aetiology of human interactions with musical beat structure. We combined state-of-the-art-genomic datasets that account for underlying polygenicity of these traits: birdsong genome-wide transcriptomics linked to singing in zebra finches, and a human genome-wide association study of beat synchronization. Results of competitive gene set analysis revealed that the genetic architecture of human beat synchronization is significantly enriched for birdsong genes expressed in songbird Area X (a key nucleus for vocal learning, and homologous to human basal ganglia). These findings complement ethological and neural evidence of the relationship between vocal learning and beat synchronization, supporting a framework of some degree of common genomic substrates underlying rhythm-related behaviours in two clades, humans and songbirds (the largest evolutionary radiation of vocal learners). Future cross-species approaches investigating the genetic underpinnings of beat synchronization in a broad evolutionary context are discussed.

This article is part of the theme issue 'Synchrony and rhythm interaction: from the brain to behavioural ecology'.

1. Introduction

(a) Beat synchronization in humans: brain and behaviour

The development of rhythmicity is foundational to communicative and social behaviours in many species, including humans. In music, rhythmic patterns are anchored by a percept of a steady quasi-periodic 'beat' or pulse, which facilitates synchrony through neural mechanisms of prediction [1]. The ability to synchronize to a musical beat is a core feature of *musicality* [2]. Timing networks in the brain involve many areas including cortical and subcortical motor regions [3], and musical beat perception and synchronization are particularly supported by basal-ganglia-thalamo-cortical networks [4]. Within these brain networks, large populations of neurons fire in synchrony when humans listen to—and coordinate movement with—auditory *rhythms* in music; the temporal

precision of this neural activity is scaffolded by the predictability of the hierarchical beat structure of music. Understanding the aetiology of *beat synchronization* is not only of intrinsic basic science interest, but also highly relevant for understanding the evolutionary origins of music, as well as speech/language [5].

(b) Harnessing cross-species and evolutionary approaches for studying the relationship between songbird vocal learning and human rhythm

Humans constitute only one species-level data point for traits of interest for our species. Comparative animal work can help formulate, test and refine evolutionary explanations for behaviours seen in humans. Probing which other animal species show a particular human-relevant behaviour and examining the contexts in which it may have evolved across taxa can function as a testing ground for hypotheses about human evolution. As an analogy, to understand the evolution of a physical trait like flippers in seals [6], studying seals alone would not suffice. Evidence from close mammalian relatives (e.g. dogs) would highlight *homologous* structures: why seals have flippers where dogs have legs. (In the case of deep homology, the genetic architectures of many traits are deeply conserved within the tree of life [7].) In addition, studying *analogous* structures such as sharks' fins and penguins' wings would also be important to pinpoint similar evolutionary pressures, because they are all appendages adapted independently for swimming.

Why do humans have beat synchronization capabilities, and how did they evolve? One can probe for their presence in either close relatives to humans or other species, which may have convergently evolved aspects of synchrony [8]. The capacity for the beat has only been found in a few non-human animals; for most species, there is the absence of evidence rather than dispositive negative evidence [9,10]. There is overlap between the few mammal and bird species (e.g. [11]) found to be capable of beat perception and synchronization and vocal learner species: species that acquire or imitate vocalizations from other individuals. This overlap between the rare capabilities of beat synchronization and *vocal learning* may reflect a cross-species mechanistic link rooted in features shared in the evolution of musical and language-related traits [12].

As both humans and songbirds possess vocal learning and rhythmic capabilities, they are excellent joint candidate taxa to investigate the genetic bases of these abilities. There are similarities in the functional anatomy of avian and mammalian forebrains, though the structure of the cortex is layered in mammals and nuclear in birds [13,14]. According to a cross-species hypothesis, the neural circuitry responsible for beat perception and synchronization could piggyback on the circuitry involved in vocal learning [12]. This hypothesis states that vocal learning is a necessary but not sufficient condition for beat perception and synchronization, i.e. species with beat perception and synchronization are likely to also be vocal learners, although the reverse is not necessarily the case (not all vocal learners are capable of beat perception and synchronization). Of the intriguing recent findings from comparative work examining rhythm and vocal learning traits in non-human species [15], a highlight is that zebra finches have some degree of rhythmic capabilities [16] that may share underlying biological aetiology with some aspects of beat perception and synchronization [17]. Multiple other avian vocal learners demonstrate the capacity to align their movements to a beat, such as parrots dancing to the music of varying tempos [11,18]. While this type of beat synchronization has not been explicitly tested to date in zebra finches, they have been shown to anticipate rhythmic noises and adjust the timing of their vocalizations accordingly, which carries similarities to motor entrainment to an external beat [19–25].

Even in the absence of beat synchrony behaviours, some avian vocal learners still exceptionally possess some of the foundational features of beat processing, including aspects of motor circuitry specialized for predicting the timing of events, and even more specifically, temporal prediction of auditory periodicities. These observations form the basis of Patel's *revised vocal learning and rhythmic synchronization hypothesis*, which posits that these sophisticated temporal sensitivities and motor circuitry in vocal learning birds constitute an evolutionary *pre-adaptation* of human beat synchronization [26].

Our work is situated within a larger literature about zebra finches, as the dominant model organism for vocal learning studies [27]; they have been thoroughly studied for the hierarchical properties of their songs [28]. Crucially, the sensorimotor brain areas involved in song learning and production have recently been found to mediate the timing of vocal interactions [25,29]. For these reasons, zebra finches constitute a promising model species for comparative studies of rhythmicity [19–22,24]. In particular, they are an interesting avenue for comparative genetics of rhythm because: (i) they have a developed vocal communication system with rhythmic characteristics and hierarchically structured features with parallels to human communication traits, (ii) their brains and genomes have been thoroughly studied, and (iii) songbird vocal learning is a promising model of the genetic architecture of human communication, hence offering the promise of analogous or even deep homologous features to human rhythm [19–24]. The specialized auditory-motor circuitry underlying complex vocal learning in songbirds shares notable neural processes with beat synchronization [26], even when typically human levels of synchronization are not directly observed. These shared resources open the possibility of the similar genetic architecture underlying such neural processes across these two related traits and species.

(c) Probing the evolutionary basis of beat synchronization with genomic methods

We thus propose that musical beat synchronization in humans may have evolved by building upon biological features that already existed for other forms of rhythmic communication [1,30] and that this overlap can be detected with genetic methods. In particular, relevant genetic variation for vocal learning could have furnished the biological precursors of the signature of beat synchronization in the modern human genome. Prior work has attempted to link *candidate genes* for music-related phenotypes across species but has been hindered by the limited between-species overlap in small candidate gene samples [31]; such studies are also vulnerable to issues with lack of replication specifically affecting candidate gene results (see [32,33]). As there is increasing evidence that human cognitive traits (including communication and musicality) are subserved not by one or a few single genes but by

polygenic architecture, it is crucial to use large scale, well-powered population studies for genome-wide discovery [34] and to inform further biological investigations.

Therefore, a robust approach to investigating connections between the genetics of rhythmic behaviour in humans and non-human vocal learners should make use of well-powered polygenic approaches. In the present study, we deploy summary statistics from a recent *genome-wide association study* (GWAS) of beat synchronization [35] that included 606 825 research participants. The genetic architecture of beat synchronization was found to be highly polygenic; there were 67 genome-wide significant independent loci in the GWAS, thus cataloguing alleles at each (autosomal) locus that were differentiated by the beat synchronization phenotypes. Moreover, *gene-based GWAS* (a complementary analysis method) revealed 125 genes that passed the significance threshold (this method provides gene-based *p*-values from multiple single nucleotide polymorphisms (SNPs) mapped to each gene). In both analysis methods, weights are generated for all genes and SNPs tested, including those that are sub-threshold, allowing for heritability enrichment analyses that take the polygenic genetic architecture into account. Post-GWAS analyses demonstrated that the heritability of beat synchronization was enriched for genes expressed in brain tissue and several other markers of central nervous system function.

In parallel, the discovery of the molecular mechanisms underlying vocal learning has recently accelerated with rich genomic approaches such as weighted gene expression network analysis [36] applied to custom avian *microarray* data. Findings of thousands of genes whose expression is differentially linked to singing learned songs also demonstrate that polygenicity is characteristic of the genetic architecture of vocal learning in songbirds [36–39], similarly to other *complex traits* [34].

Taken together, musical beat synchronization in humans could have evolved using pre-existing features that existed for other forms of communication in other species, with relevant genetic architecture of rhythmic interactions convergent (or conserved) across species. While prior work exploring candidate genes for musicality phenotypes across species was limited by methodological issues that did not account for polygenicity of complex communication traits, recent technical advances in genomics of birdsong and human beat traits have now made it possible to use higher quality genome-wide data from these separate lines of research. In this paper, we take a first step to exploring the prediction that pre-existing genetic architecture for communication in vocal learning birds exhibits common genomic substrates with human musical beat synchronization ability. In particular, we predict that human beat-associated genes will be enriched for genes involved in singing in zebra finches. For the first time, we use recently generated genome-wide data for both human rhythm and songbird vocal learning in order to test the relationship between these traits, using *gene set analysis* (GSA) [40].

2. Methods

(a) Overview

Briefly, our approach (figure 1) involves the generation and testing of zebra finch gene sets consisting of genes differentially expressed in association with singing behaviour, followed by examining these gene sets in association with human GWAS data of a beat synchronization phenotype. We first aggregated the state of the art of the genetic basis of songbird vocal learning through two routes: (i) a literature review of candidate gene expression studies in zebra finches in association with song production and song learning, and (ii) recent genomic evidence (from a custom avian microarray [46,47]) in eight gene sets from experiments testing multiple facets of zebra finch singing behaviour. We then used competitive GSA to test the enrichment of these gene sets in human GWAS results for musical beat synchronization. Finally, we examined the subset of genes overlapping among these phenotypes and performed additional analyses (imputed gene expression in human brain tissue) to generate hypotheses for further functional studies. Given the interdisciplinary nature of this work, key terms are provided in the Glossary in the electronic supplementary material, File, Part I.

(b) Birdsong gene sets

(i) Birdsong gene sets derived from literature review of candidate gene expression studies

Sets 1 and 2: overview. To capture the state of the candidate gene literature on vocal learning in zebra finches, we conducted a literature review of candidate gene studies for song behaviour and song learning in zebra finches (details in the electronic supplementary material, File, Part II). In songbirds, hearing or producing song can induce increased neuronal firing and immediate changes in gene expression in the brain [48]. One method of measuring these genetic markers of neuronal activity is through *in situ* hybridization studies, which hybridize a labelled RNA probe directly onto brain slices to detect the amount of target RNA that has been transcribed in different brain regions. This allows for the expression of a gene of interest to be compared between birds that had recently sung versus not sung (or had recently heard song versus a control condition). Briefly, we categorized each of the findings from 16 studies in the candidate gene literature search into two categories: 'behaviour', indicating that the act of singing or listening modified gene expression, or 'learning', indicating that gene expression differences were observed between developmental stages of song learning (see the electronic supplementary material, File, Part II, for details). The categorized findings from the 16 studies were compiled into two candidate gene sets based on their results (set 1: candidate genes: song behaviour and set 2: candidate genes: song learning; electronic supplementary material, tables S1 and S2).

(ii) Birdsong gene sets derived from whole-genome microarray studies

Sets 3–6. To conduct a more genome-wide assessment of gene expression differences, we compiled data from microarray studies comparing song-related phenotypes. Using an Agilent microarray chip constructed to study genome-wide *transcriptomics* in zebra finches [46], Whitney *et al.* [38] compared microarray data on birds that had been silent versus those that had sung for up to 7 h (grouped in 1 h increments); from each bird, tissue was collected after either silence or singing from four nuclei in the song system: HVC, Area X, the lateral magnocellular nucleus of the anterior nidopallium (LMAN) and the robust nucleus of the arcopallium (RA). To

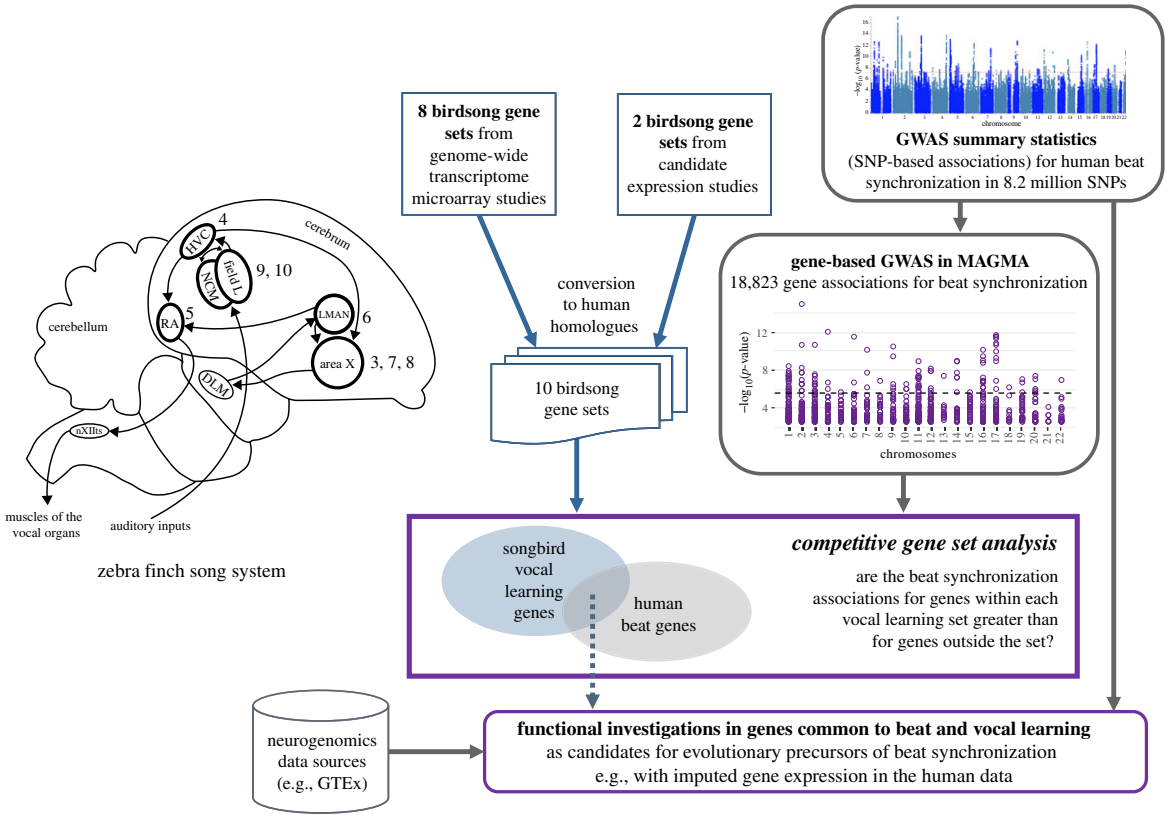

Figure 1. Overview of approach. On the left, a simplified schematic of the song system in the zebra finch brain is shown, focusing on the regions profiled in microarray studies. The *song system* consists of two pathways from a key brain region, HVC, to neurons that project to vocal muscles [41,42]. In the vocal motor pathway, which is related to both song learning and production, HVC projects directly to RA. In the anterior forebrain pathway, which is related to song learning, HVC is indirectly linked to RA via Area X, DLM and LMAN. Auditory information from the hair cells in the ear connects to HVC via Field L and NCM. The region of origin of each of the numbered gene sets in the current paper (sets 3–10) are indicated in the brain diagram. Starting from top middle and top right: flowchart showing components of analyses in the present paper. Birdsong sets are derived from extant data (microarray and candidate gene studies). The genetic architecture of human beat synchronization [35] is derived from a genome-wide association study (GWAS results are shown in a SNP-based Manhattan plot and in gene-based GWAS from MAGMA). SNPs or genes (respectively) appear ordered by chromosome on the *x*-axes of each plot, with the inverse log of *p*-values on the *y*-axes, to indicate the strength of association with the beat synchronization phenotype. Dotted horizontal lines are the cut-off for genome-wide significance. Then, competitive GSA [43] tests whether the GWAS is enriched for each birdsong set. Follow-up analyses bring in other data sources and methods (such as GTEx and S-PrediXcan [44,45]) to explore genes common to both traits (vocal learning and beat synchronization). DLM, dorsal lateral nucleus of the medial thalamus; HVC (proper name); LMAN, the lateral magnocellular nucleus of the anterior nidopallium; NCM, caudal medial nidopallium; nXIIts, nucleus XII, tracheosyringeal part; RA, robust nucleus of the arcopallium; GSA, gene set analysis. (Online version in colour.)

find transcripts that were significantly up- or down-regulated across these timepoints, for each brain region we compared the gene expression between the silent birds and the singing birds (pooling the birds that had sung for any duration) using the GEO2R analysis pipeline on NCBI, where the raw microarray data are hosted. The resulting transcripts that were significantly up- or downregulated (false discovery rate (FDR)-corrected $p < 0.05$) in each brain area formed the basis of four gene sets: singing versus silence—Area X (set 3), singing versus silence—HVC (set 4), singing versus silence—RA (set 5) and singing versus silence—LMAN (set 6) (electronic supplementary material, tables S3–S6).

Sets 7–8. Hilliard *et al.* [37] collected microarray data on birds that had either sung or been silent, collecting tissue from Area X as well as a non-song-related brain region, the ventral striato-pallidum (VSP; a brain region involved in non-vocal motor function). Using the same microarray as [38], they compared gene expression in Area X between birds that had been silent versus those that had sung for 2 h, controlling for the *differential expression* of genes in VSP. They then looked for an association between gene expression differences and song behaviour, both related to the presence or absence of singing and related to the number of motifs sung by the bird. The transcripts that were significantly up- or down-regulated in the singing birds (FDR-corrected $p < 0.05$) form two gene sets: singing versus silence—Area X (controlling for VSP) (set 7) and number of motifs sung—Area X (controlling for VSP) (set 8), reported in the electronic supplementary material, tables S7 and S8.

Sets 9–10. Finally, Drnevich *et al.* [49] compiled data from multiple microarray experiments performed across multiple laboratories, including an experiment that tested for gene expression differences in auditory brain regions (caudal medial nidopallium and the L2a portion of Field L) between zebra finches that had heard (versus not heard) song playbacks, in female and male birds separately. The genes that were significantly up- or down-regulated in these experiments form two gene sets: listening/playback—female (set 9) and listening/playback—male (set 10), reported in the electronic supplementary material, tables S9 and S10.

In preparation for GSA in the human data (described below), we converted the microarray identifiers (Clone IDs) to human Ensembl IDs. First, we used the gene names provided with a subset of Clone IDs in the microarray data

and converted these gene names to Ensembl IDs using the gConvert function in gProfiler [50]. For microarray transcripts that could not be identified using this method, we converted the zebra finch Ensembl IDs (ENSTG IDs) provided with the microarray data to human v. 92 Ensembl IDs (ENSG IDs) using the gOrth function in gProfiler.

(c) Beat synchronization genome-wide association study dataset (humans)

We used the human beat synchronization GWAS dataset from Niarchou *et al*. [35]. Briefly, the study population was $n = 606\,285$ individuals (58% female, mean age 52 years, s.d. = 18.5) participating in research with personal genetics company, 23andMe, Inc. Participants provided informed consent and participated in the research online, under a protocol approved by the external AAHRPP-accredited Institutional Review Board (IRB), Ethical & Independent Review Services (E&I Review). Participants were included in the analysis on the basis of consent status as checked at the time data analyses were initiated. The phenotype was self-reported beat synchronization, i.e. responses to the question 'Can you clap in time with a musical beat?' (yes versus no). The self-report phenotype was validated in a series of experiments; most relevant here, this single item was shown in a separate sample of $n = 542$ to correlate with measured beat synchronization task performance to musical stimuli (such that individuals who answered 'yes' were more likely to have lower standard deviation of the asynchrony, i.e. they were more accurate synchronizers). The GWAS was restricted to unrelated individuals of European ancestry. The authors conducted GWAS using logistic regression under an additive genetic model, while adjusting for age, sex, the top five principal components and genotype platform. SNPs were excluded when characterized by minor allele frequency less than 0.01, low imputation quality ($R^2 < 0.3$), or indels, resulting in 8 288 850 SNPs. Full demographic, IRB and quality control details are reported in [35].

Niarchou *et al*. [35] used MAGMA v. 1.08 [43] within web-based FUMA v. 1.34 [51] for genome-wide gene-based analysis of SNPs on autosomal chromosomes (here we use the version with genes on chromosome X excluded because sex determination in zebra finches is not directly comparable to humans). MAGMA annotates SNPs to protein-coding genes and conducts association tests. The analysis used the SNP-wise mean model in MAGMA, which takes *p*-values from the GWAS summary statistics to test the joint signal of all SNPs in a gene in association with a phenotype, simultaneously taking the linkage disequilibrium (LD) between those SNPs into account. MAGMA results (mapped to Ensembl v. 92; reported in [35]) showed that 125 genes surpassed the genome-wide gene-based threshold for association with beat synchronization ($p < 2.6 \times 10^{-6}$), shown in figure 1. The full model statistics for the test statistics of 18 823 genes, as output from MAGMA, were used in the current paper for hypothesis-driven gene set analysis (below). It is important to note that there is sub-threshold signal that contributes to the heritability of the trait, and that the relative weights of the entire range of *p*-values become input to the enrichment analyses; to show this, we also report the top 1000 genes associated with beat synchronization in the electronic supplementary material, table S11.

(d) Competitive gene set analysis

(i) Gene set analysis of the test sets

We performed competitive GSA with MAGMA (v. 1.08) [43] to test the human beat GWAS data for enrichment of each of the 10 birdsong gene sets. Competitive GSA, described and validated in [40,43], compares the gene-based associations of genes inside the set to genes outside the set, while controlling for gene size, gene density and size of the gene set. Bonferroni threshold of $p = 0.005$ was used to determine the significance of the enrichment results. We followed up on significant gene set associations with conditional GSA, conditioning on average gene expression in brain tissue as a (continuous) gene property (using methods described in [52]). Given that the source birdsong gene expression data certainly includes genes linked to motor function, we also performed conditional GSA on the birdsong sets, conditioning on another existing gene set from the Molecular Signatures Database [53]: locomotory behaviour. See the electronic supplementary material, File, section III.a for details.

(ii) Additional control analyses

We performed a series of control analyses using GSA in additional datasets to rule out confounds potentially arising from the polygenic nature of both data sources. First, we tested whether the beat synchronization GWAS was enriched for another large gene set, derived from gene expression for an unrelated trait in a songbird (*seasonal migration behaviour*). Separately, as hypothesized negative controls, we also tested whether two other human complex motor/neurological traits (*usual walking pace* and *headache pain*) in GWASs that were similarly well-powered to the beat GWAS, would be enriched for the birdsong gene sets. Details and results of these analyses are reported in the electronic supplementary material, File, sections III.b and III.c.

(e) Gene expression analysis on the top overlapping genes in select brain tissues

We then performed preliminary genetic analyses to explore possible evolutionary precursors of human beat ability, potentially providing insight on the vocal learning—beat perception and synchronization hypothesis [1,12]. Out of the birdsong genes that were also top-associated with human beat synchronization, we asked: Which have (imputed) gene expression[1] associations with beat synchronization phenotypes in brain tissues?

For this analysis, we took a conservative approach, narrowed only to the list of 477 genes common across the three significant sets (*overlap_birdset*). We also characterized the statistical enrichment of genes belonging to *overlap_birdset* using PANTHER (Protein Analysis THrough Evolutionary Relationships classification system; Analysis Type: PANTHER Overrepresentation Test (Fisher's exact test); Reference List: *Homo sapiens* genes; Annotation dataset: gene ontology (GO) biological process complete, GO database released 9 October 2020; FDR-corrected *p*-value < 0.05; see the electronic supplementary material, table S12). Gene-based (MAGMA) associations with beat synchronization for each gene of *overlap_birdset* also present in the GWAS set are reported in the electronic supplementary material, table S13.

We selected four brain tissue types (for which predictor models are available in GTEx v. 8; [44]) from regions both:

Table 1. Competitive GSA results for beat synchronization. (MAGMA-based competitive GSA results for the enrichment of each of the songbird vocal learning sets in relation to the human GWAS beat synchronization associations. Statistically significant sets are in italics. See figure 2 legend for details.)

gene set	NGENES	BETA	BETA_STD	SE	*p*	source study
set 1: candidate genes—song behaviour	10	0.392	0.009	0.325	0.114	literature review
set 2: candidate genes—song learning	9	0.061	0.001	0.354	0.432	literature review
set 3: singing versus silence—Area X	*3102*	*0.076*	*0.028*	*0.019*	2.36×10^{-5}	*Whitney et al.* [38]
set 4: singing versus silence—HVC	8	0.081	0.002	0.358	0.411	Whitney *et al.* [38]
set 5: singing versus silence—RA	26	0.273	0.010	0.183	0.068	Whitney *et al.* [38]
set 6: singing versus silence—LMAN	24	0.314	0.011	0.184	0.044	Whitney *et al.* [38]
set 7: singing versus silence—Area X (controlling for VSP)	*1205*	*0.101*	*0.025*	*0.029*	2.33×10^{-4}	*Hilliard et al.* [37]
set 8: number motifs sung—Area X (controlling for VSP)	*1669*	*0.117*	*0.033*	*0.025*	1.25×10^{-6}	*Hilliard et al.* [37]
set 9: listening/playback—female	9	0.114	0.002	0.367	0.378	Drnevich *et al.* [49]
set 10: listening/playback—male	260	0.085	0.010	0.057	0.069	Drnevich *et al.* [49]
overlap_birdset	*462*	*0.172*	*0.027*	*0.045*	7.42×10^{-5}	*genes common to sets 3, 7 and 8*

(i) known from the neuroimaging literature to be active during beat synchronization in humans (e.g. [4]), and (ii) characterized as homologues to vocal learning areas in zebra finch based on transcriptional findings [39]: (i) cortex, (ii) basal ganglia (BG) caudate, (iii) BG putamen, and (iv) BG nucleus accumbens. We generated *query_set* from the top-ranked 25 beat synchronization genes that were common to *overlap_birdset*.

Imputed gene expression analysis was performed with S-PrediXcan v. 0.6.4 (also known as MetaXcan [45]), using GTEx v. 8, on each of the available genes of *query_set* for each of the four tissues. S-PrediXcan is an extension of PrediXcan [54] for use in genomic summary statistics; both versions allow the user to test associations between imputed expression and a phenotype (here, beat synchronization), taking LD into account. Twenty-four gene-tissue pairs were tested for their associations with beat synchronization; not all tissue-gene pairs are available in GTEx (e.g. a *FOXP2* model was not available in these tissues), resulting in 13 unique genes tested. A Bonferroni threshold of 2.08×10^{-3} was applied. While these results are not independent from MAGMA data, they are a first step at exploring the genomic data with a different tool that allows us to connect the genetic and neural architecture.

3. Results

(a) Competitive gene set analysis

Competitive GSA implemented in MAGMA showed no significant enrichment of the two candidate gene sets (set 1: song behaviour $p = 0.11$, set 2: song learning $p = 0.43$) in the human beat synchronization GWAS. Set 3 (singing versus silence—Area X) was enriched ($p < 0.001$). Gene sets originating from data reported in [38], derived from the same experimental contrasts of singing versus silence in other zebra finch brain regions, were not significant (sets 4 and 5: HVC and RA, respectively, $p > 0.05$), and set 6 (LMAN) was suggestive ($p = 0.04$) but did not reach the Bonferroni statistical threshold ($p = 0.005$). Beat synchronization was enriched ($p < 0.001$) for both sets derived from data reported in [37]: singing versus silence (set 7) and number of motifs sung (set 8), in which gene expression had been measured in Area X controlling for gene expression in another non-song related area (VSP). Finally, the two sets that correspond to genes activated by song listening/playback [49] were not significant. Additionally, the gene set consisting of 477 genes common to the three significantly enriched sets (*overlap_birdset)* was also significantly enriched in the beat GWAS ($p = 7.4 \times 10^{-5}$). GSA results are reported in table 1 and figure 2; note that the number of genes in the set reflects the number available for GSA in the human MAGMA set (a small number of genes from each birdsong set was not available in the human dataset). Importantly, results of conditional GSA (method described in [52]), show that the beat GWAS is still enriched for sets 3, 7, 8 and *overlap_birdset* when controlling for average brain expression levels as a gene property, or for a locomotory behaviour gene set (see the electronic supplementary material, File, Part III.a). Thus, the enrichment of birdsong sets in the beat GWAS does not appear to be solely linked to brain expression as a gene property, or to genes previously linked to motor behaviour.

Additional control analyses and results are described in the electronic supplementary material, File, Parts III.b–d. Briefly, the beat GWAS was not enriched for a seasonal migration behaviour gene set ($p = 0.298$). In addition, the four birdsong sets were not enriched either in the two control GWASs (usual walking pace and headache pain), thus further ruling out general effects of motor function and brain expression as alternative explanations for the primary results.

(b) Gene expression results: exploration of potential evolutionary precursors of beat synchronization

In our gene expression exploration of the human data for specific vocal learning genes that could be candidates for evolutionary precursors of beat synchronization, we identified 10 gene-tissue pairs that show differential imputed expression (see endnote 1) in relation to beat synchronization, in one or more of the selected brain tissues tested. A summary of the results is presented in table 2, with the

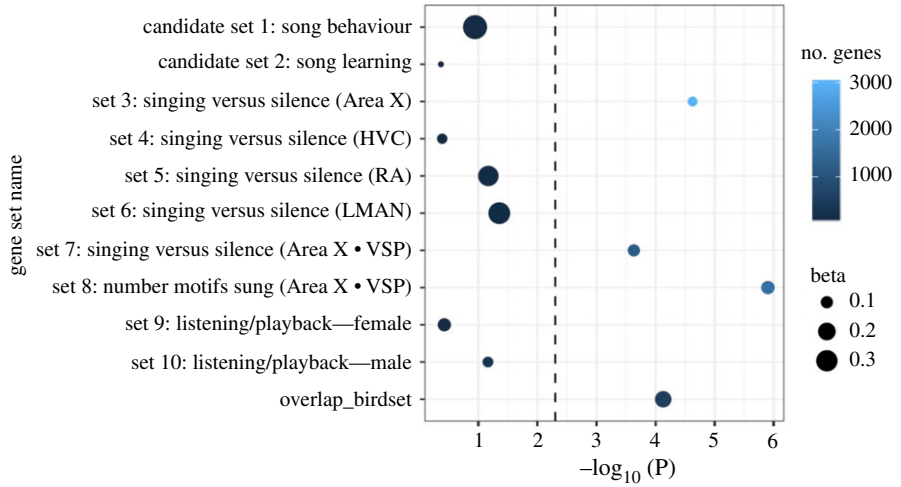

Figure 2. Gene set analysis results for beat synchronization. MAGMA-based competitive GSA results for the enrichment of each of the birdsong sets in relation to the human GWAS beat synchronization associations. Gene sets are plotted on the *y*-axis, with uncorrected *p*-values on the *x*-axis in $-\log_{10}$; number of genes in the set is represented by the colour scale, and the enrichment beta value for the set is shown by circle size. The Bonferroni threshold is indicated by the vertical dashed line. (Online version in colour.)

Table 2. Summary of imputed gene expression associated with beat synchronization in top hits overlapping with birdsong sets, for each of four brain tissues tested, with S-PrediXcan. (BG, basal ganglia. Uncorrected *p*-values for the association between gene expression and human beat synchronization are presented here, with significant *p*-values in italics after Bonferroni thresholding. Full analysis output is in the electronic supplementary material, table S14. n.a. indicates that the predictor model was not available for that particular gene-tissue pair.)

Ensembl ID (ENSG)	gene name	BG nucleus accumbens	BG caudate	BG putamen	cortex
ENSG00000155511	*GRIA1*	1.26×10^{-7}	n.a.	n.a.	n.a.
ENSG00000157111	*TMEM171*	0.005	0.004	0.005	0.028
ENSG00000138750	*NUP54*	0.013	*0.001*	*0.002*	7.50×10^{-4}
ENSG00000048740	*CELF2*	0.080	n.a.	n.a.	n.a.
ENSG00000081189	*MEF2C*	0.442	n.a.	n.a.	n.a.
ENSG00000121904	*CSMD2*	0.501	n.a.	0.459	0.808
ENSG00000003056	*M6PR*	n.a.	5.48×10^{-4}	n.a.	1.05×10^{-4}
ENSG00000025039	*RRAGD*	n.a.	n.a.	n.a.	0.092
ENSG00000088448	*ANKRD10*	n.a.	0.006	n.a.	7.39×10^{-4}
ENSG00000117505	*DR1*	n.a.	2.64×10^{-4}	n.a.	1.80×10^{-3}
ENSG00000126091	*ST3GAL3*	n.a.	n.a.	n.a.	0.672
ENSG00000150456	*EEF1AKMT1*	n.a.	n.a.	n.a.	0.275
ENSG00000169925	*BRD3*	n.a.	n.a.	n.a.	9.79×10^{-4}

full output statistics from S-PrediXcan for gene-tissue pairs in the electronic supplementary material, table S14. Notably, beat synchronization is highly associated with the expression of *GRIA1* (in the basal ganglia-nucleus accumbens), a glutamate receptor family gene that codes for Glutamate Ionotropic Receptor AMPA Type Subunit 1. Significant associations were also found for *M6PR*, *ANKRD10*, *DR1*, *NUP54* and *BRD3* (table 2).

4. Discussion

In this study, we examine the genetic basis for the evolution of synchronous rhythmic behaviour by combining, to our knowledge for the first time, state-of-the-art-genomic evidence from two independent lines of research: birdsong genome-wide transcriptomics, and a human GWAS of musical beat synchronization abilities. This was accomplished by using genome-wide microarray-derived lists of genes whose expression occurs in association with multiple facets of singing behaviour in zebra finches [37,38,49]. Upon conversion to human homologues, we tested enrichment for each of eight birdsong gene lists, and two sets of candidate genes, with competitive GSA. This analysis revealed that the human beat synchronization GWAS is significantly enriched for several sets of genes expressed in zebra finch Area X (homologous to human basal ganglia) in association with singing behaviour. We then further explored potential evolutionary precursors of beat synchronization, by identifying a subset of top-associated human beat genes that were also common across the three significant birdsong sets, and then imputing gene expression (per [45]) in the cortex and basal ganglia tissues. Taken together,

these findings provide novel molecular genetic support for specific predictions of evolutionary hypotheses linking vocal learning to beat perception and synchronization [1,12,26,30,55].

It is crucial to note that the genetic architecture of both of these traits (vocal learning in zebra finches and beat synchronization in humans) is highly polygenic, revealed by the data sources we drew from for each species, which had used methods that provide excellent genome-wide coverage. The findings of enrichment in the human beat GWAS of gene sets consisting of a large number of genes related to zebra finches' production of the learned song thus revealed that the relationship between these traits is also polygenic, consistent with theoretical accounts of the evolution of complex traits [34,56]. Gene set enrichment approaches are useful for detecting biological functions associated with the polygenic genetic architecture of complex traits [40], where the heritability results from an accumulation of small effects [57]; GSA has been robustly used for phenotypes such as cognition and brain morphology [58,59], obsessive-compulsive disorder [60] and musicality [35]. In contrast to the microarray-derived gene sets, the two gene sets we tested from the candidate gene literature for song behaviour and song learning did not reach statistical significance for enrichment in the beat synchronization GWAS. Cross-species approaches to communication traits (i.e. [31]) have by necessity focused on candidate genes, as most existing studies linking gene expression to communication behaviours test only a small number of genes. In addition, many of these genes are immediate early genes, which are genes whose mRNA levels change rapidly in response to a stimulus [61–63]. Immediate early gene studies help localize neural activity and have led to fascinating cross-species results regarding the neural architecture of communication behaviours. To study the genetic architecture of such complex behaviours, however, candidate gene studies (especially when those candidates did not originate from genome-wide approaches) are probably missing most of the true polygenic signal and are subject to false positives and other statistical problems [33].

On the other hand, polygenic and genome-wide approaches fit with the robustness of evidence that co-regulation and co-expression of gene networks at a large scale drive individual differences in avian vocal learning [37,38] and complex (i.e. non-Mendelian) behavioural phenotypes in humans [64]; these results are also consistent, for example, with extensive polygenic pleiotropy driving associations between related neurological phenotypes [65,66]. A highly overlapping genetic basis among correlated traits (i.e. [67]) highlights the validity of genome-wide approaches of complex traits even when the phenotype is difficult to define discretely such as in the case of vocal learning [23,68,69].

Each of the three birdsong sets (sets 3, 7 and 8) that was significantly associated with beat synchronization originated from gene expression studies of Area X, an epicenter of neural mechanisms of song learning in zebra finches. The experimental manipulation used in the primary data sources [37,38] included binary contrasts of gene expression after singing compared to silence (sets 3 and 7) as well as a linear relationship between expression levels and number of motifs sung (set 8). The latter set, in particular, speaks to genes linked to *individual differences* in song behaviour, akin to the type of individual differences measured in GWAS [64]. GSA did not reveal enrichment for listening/playback in zebra finch auditory areas (sets 9 and 10; though it should be noted that these gene sets were smaller and therefore less powered to detect small effects), thus suggesting that the relationship between vocal learning in songbirds and human beat synchronization need not be mediated by perceptual-only mechanisms. Rather, the three significantly associated sets correspond to song production, achieved through sensorimotor integration. Area X is also homologous to human basal ganglia, a set of subcortical nuclei key to musical rhythm processing [4,70–72]; the similarities in the genetic and neural architecture of cortico-basal ganglia-cortical circuitry has prompted systematic cross-species investigation of specialized transcriptional characteristics [39], as well as evolutionary accounts of vocal learning in relation to beat synchronization [1].

Our initial exploration of the overlap between beat synchronization and vocal learning genes led to additional evidence linking the neural and genetic architecture of beat synchronization in several homologous genes also specialized in oscine vocal learning. Notably, imputed gene expression analyses (applying S-PrediXcan [45] to the human genomic data, limited to homologues of several genes common to the three significantly enriched birdsong sets) revealed a significant association between beat synchronization and expression in BG nucleus accumbens of *GRIA1*, a glutamate receptor gene located on chromosome 5q33.2. *GRIA1* is associated with human neurological phenotypes (i.e. [73]), and belongs to the family of glutamate receptor genes that regulate neural transmission; prior work has also highlighted the role of glutamate receptor subunit gene regulation in avian vocal learning [38,74,75]. Our analysis also uncovered several other candidates for evolutionary precursors of beat synchronization in the cortex and basal ganglia tissue: *M6PR* expression in cortex and BG caudate; *ANKRD10* expression in cortex; *DR1* in cortex and BG caudate; *NUP54* in the cortex, BG caudate and BG putamen; and *BRD3* in the cortex. It is important to note that this approach was just one of many ways that gene expression data in humans can be harnessed in relation to GWAS and gene set results, and that our analysis made use of GTEx v. 8 gene-tissue predictor models [44], which are not available on all gene-tissue pairs (see Methods) and have a rather coarse spatial resolution. Future work is needed to integrate additional neurogenomics resources that span the neural architecture of human and avian data at a higher resolution to investigate the molecular dimension of evolutionary relationships between rhythm and vocal learning and specific auditory-motor networks that may converge across species.

Several hypotheses have been put forward to explain why humans and other animals have rhythm capacities in general, and a propensity for beat perception and synchronization in particular. Some hypotheses highlight the importance of phylogenetic continuity [76] in developing rhythm capacities, while others its functional [15,77], ecological [78] or interactive [79,80] contexts. The most discussed and tested hypothesis to date is mechanistic: it proposes that the neural circuitry for vocal learning is foundational for the evolution of beat synchronization [12]. A recent update to the vocal learning hypothesis ([26] in this issue) emphasizes the role of specific facets of sophisticated auditory-motor circuitry (i.e. enhanced detection of periodicities, processing of temporal predictions, and strong connections between motor planning regions and auditory areas) in species that demonstrate learning of complex vocal sequences, and describes how these facets are also foundational to beat perception and synchronization in

humans (see also [81]). These foundational auditory-motor processes as observed in vocal learning birds are proposed to have a fortuitous pre-adaptive relationship with human beat traits, thus suggesting convergent evolution [26].

In the light of this framework, our results provide, to our knowledge, the first molecular support of the prediction about shared genetic architecture generated from the revised vocal learning and rhythmic synchronization hypothesis [26]. As pointed out by Patel, although it is not yet known if zebra finches possess the totality of auditory, motor and social components (e.g. capacity to imitate non-vocal body movements) needed to spontaneously move in synchrony to a musical beat analogously to humans, their neural systems for the auditory-motor components of vocal learning used to learn and produce complex vocal songs are indeed enriched for elaborate temporal precision and motor coordination. Human beat capacities are thus potentially able to piggyback on the underlying neural and genetic architecture of these existing components. The present findings suggest this might be the case, distinct from and complementary to recent findings of rhythmic processing in zebra finches [17,28].

Taken together, these novel findings of enrichment of birdsong gene sets among the polygenic genomic signature of beat synchronization are promising as a proof of concept of a particular aspect of the evolution of rhythm ability, and pave the way for avenues of further exploration with more granular analytic approaches for exploring the nature of the underlying mechanisms. A limitation of the current work is that we cannot yet distinguish between the effects of convergent evolution versus homologous genes (with a common origin) that link the two traits. Additionally, the human GWAS data on beat synchronization [35] is based on a single self-report of beat synchronization ability and in individuals of European ancestry only; GWAS results can vary subtly depending on the ancestry of the population [82] (though the methods for gene-based GWAS and gene set enrichment analyses used here assess influences at the gene level and may already compensate somewhat for ancestry-related differences in allele frequencies at the SNP level). Cross-species approaches should thus be revisited in the future when other rhythm traits (i.e. beat perception ability) become available in population-wide samples from multiple ancestries. Our findings suggest that interdisciplinary integration of methods from computational genetics, neuroscience and evolution used to address the aetiology of rhythm traits may also lead to exciting enhancements of models of vocal learning as precursors to speech. Such investigations may particularly shed light on phenotypic correlations in humans between rhythm and speech–language traits (see [83,84]) potentially driven by pleiotropy (shared genetic architecture: [85]). We foresee new genetics work taking into account similarities in the functional organization of neural underpinnings achieved through transcriptomics of vocal learning in songbirds and human speech [39], along with beat synchronization and perception traits in humans [81] as potential opportunities for exploration.

Moreover, as comprehensive genome-wide gene expression (and gene set) data become available in relation to vocal learning phenotypes in other species, similar cross-species gene set enrichment analyses can be explored. The choice of species should be aimed at testing evolutionary hypotheses against each other. For instance, testing avian species with limited vocal learning capacities will allow a comparison between the phylogenetic continuity and vocal learning hypotheses concerning the evolution of rhythmicity. Suboscines are the most closely related group of avian species to songbirds with no attested vocal learning capacities [86], so phylogenetic continuity would predict similar results to zebra finches, while vocal learning would not. Likewise, testing non-human primates could provide the best case of continuity to humans with limited vocal learning capacities [87]. Some new-world monkeys, such as marmosets, perform turn-taking and spontaneous rhythm interaction; their inclusion, therefore, could provide potential support for hypotheses in which ecology and interaction play central roles [88]. Finally, non-human mammals with developed vocal learning capacities and different degrees of functional and ecological specializations for rhythm—such as pinnipeds and bats—could provide a useful testbench to contrast multiple alternative hypotheses [89–91]. For instance, once genome-wide transcriptome data linked to vocal learning is available from bats or pinnipeds (and perhaps even for gene expression changes associated with *unlearned* vocalizations, for comparison), one could envision using such data as the basis for enrichment analyses within human GWAS data, and assessing to what degree the genetic signature of species' vocal behaviours exert independent or common/shared genetic effects on human beat perception and synchronization.

In conclusion, our findings of common genomic substrates between birdsong in zebra finches and beat synchronization in humans complement ethological and neural evidence of a cross-species relationship between vocal learning and beat processing. With the comparative genetics of rhythm and vocal learning still in its infancy, our results provide a limited though encouraging proof of concept of this novel approach for investigating the evolution of rhythm traits.

Ethics. We used the human beat synchronization GWAS dataset from Niarchou *et al*. [35]. The study population was $n = 606\,285$ individuals (58% female, mean age 52 years, s.d.= 18.5) participating in research with personal genetics company, 23andMe, Inc. Participants provided informed consent and participated in the research online, under a protocol approved by the external AAHRPP-accredited IRB, Ethical & Independent Review Services (E&I Review). Participants were included in the analysis on the basis of consent status as checked at the time data analyses were initiated.

Data accessibility. Analysis scripts and variables used in the Gene Set Analyses are available at https://osf.io/tvh35/, with the exception of the full gene-based GWAS file. The full GWAS summary statistics for the 23andMe dataset are available through 23andMe to qualified researchers under an agreement that protects the privacy of the 23andMe participants. Please visit https://research.23andme.com/collaborate/#dataset-access for more information and to apply to access the data.

Authors' contributions. R.L.G., N.J.C. and N.C. conceived of the project, designed experiments and conducted analyses. C.M.R. and R.E. compiled studies for analysis and constructed candidate gene sets. R.L.G., N.C., A.R., J.H.B., M.N. and N.J.C. refined experimental design and interpreted results. The 23andMe Research Team collected the human beat synchronization data, conducted GWAS and reviewed the manuscript. M.N. and A.S. conducted gene-based GWAS analysis. R.L.G., N.C., A.R., A.S. and J.H.B. drafted the manuscript and figures, with input and approval from all authors.

Competing interests. Members of the 23andMe Research Team are employees of 23andMe, Inc., and hold stock or stock options in 23andMe. All other authors declare no competing interests.

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Endnote

[1]Imputed gene expression here refers to a human transcriptome analysis method called PrediXcan [54], involving statistically inferring gene expression levels based on associations between genotypes and a phenotype, using a predictor model from separate data that have linked genetic variation to gene expression levels in various tissues via RNAseq methods. The analyses yield associations between gene expression in a given tissue and a phenotype, without having to assay tissues directly in the population of interest.

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