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Physiology

Near absence of differential gene expression in the retina of rainbow trout after exposure to a magnetic pulse: implications for magnetoreception

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The ability to perceive the Earth's magnetic field, or magnetoreception, exists in numerous animals. Although the mechanism underlying magnetoreception has not been clearly established in any species, in salmonid fish, it is hypothesized to occur by means of crystals of magnetite associated with nervous tissue such as the brain, olfactory organ or retina. In this study, rainbow trout (*Oncorhynchus mykiss*) were exposed to a brief magnetic pulse known to disrupt magnetic orientation behaviour in several animals. Changes in gene expression induced by the pulse were then examined in the retina. Analyses indicated that the pulse elicited differential expression of only a single gene, *gamma-crystallin M3-like* (*crygm3*). The near absence of an effect of the magnetic pulse on gene expression in the retina stands in sharp contrast to a recent study in which 181 genes were differentially expressed in brain tissue of *O. mykiss* after exposure to the same pulse. Overall, our results suggest either that magnetite-based magnetoreceptors in trout are not located in the retina, or else that they are unaffected by magnetic pulses that can disrupt magnetic orientation behaviour in animals.

1. Introduction

Diverse animals detect the Earth's magnetic field and use it in orientation and navigation [1]. Despite evidence that magnetoreception is widespread phylogenetically, little is known about the neural and molecular mechanisms that underlie it. One hypothesis is that magnetoreception involves interactions between magnetic minerals (e.g. magnetite) and the ambient geomagnetic field [1]. Salmonids such as rainbow trout (*Oncorhynchus mykiss*) have proved useful as a model species for studies of magnetite-based magnetoreception. Trout spontaneously orient to the geomagnetic field under some conditions [2,3] and can be conditioned to respond to magnetic stimuli [4]. Moreover, candidate magnetoreceptor cells [5] and genes [6] putatively involved in magnetoreception have been identified. Nevertheless, despite a search spanning several decades, magnetite-based magnetoreceptors have yet to be unambiguously identified in any animal, and the role of magnetite in magnetoreception remains unknown.

One manipulation known to disrupt magnetic-orientation behaviour in several animals is exposure to a powerful magnetic pulse [1]. Recently, the transcriptomic response of the rainbow trout's brain to a magnetic pulse was assessed, and the pulse was found to alter expression of 181 genes, including *ferritin*, the primary intracellular iron binding and storage protein [6]. This finding is consistent with the interpretation that magnetoreception in trout is mediated by iron-containing

Table 1. Summary of the RNA-seq data. The mean number of sequencing reads are shown in millions with standard deviation within parentheses. Accession numbers for sequences are deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra).

group	retina	treatment	*n*	raw reads	trimmed reads	mapped reads	SRA accessions
RC	right	control	6	51.9 (14.8)	38.4 (10.8)	35.9 (10.1)	SRR5738246
							SRR5738250
							SRR5738254
							SRR5738266
							SRR5738268
							SRR5738257
LC	left	control	6	50.4 (18.6)	37.3 (14.1)	34.9 (13.4)	SRR5738247
							SRR5738251
							SRR5738255
							SRR5738267
							SRR5738269
							SRR5738256
RP	right	pulsed	6	41.9 (4.1)	31.2 (2.8)	29.3 (2.7)	SRR5738248
							SRR5738252
							SRR5738260
							SRR5738262
							SRR5738264
							SRR5738259
LP	left	pulsed	6	47.9 (7.0)	35.1 (5.6)	32.9 (5.2)	SRR5738249
							SRR5738253
							SRR5738261
							SRR5738263
							SRR5738265
							SRR5738258
overall			24	48.0 (12.3)	35.5 (9.2)	33.3 (8.7)	

magnetoreceptors, possibly composed of magnetite, and that such receptors must be repaired or replaced after disturbance by a magnetic pulse. An additional finding was that the pulse affected expression of several genes encoding proteins mediating rod opsin-based pigment sensitivity in the retina, chromophore metabolism, and the development and repair of the optic nerve. These results suggested that photoreceptive pathways or structures (e.g. retina and pineal body) might also play a role in trout magnetoreception.

As a first step towards determining if photoreceptive pathways are involved in magnetoreception in trout, we investigated whether a magnetic pulse affects gene expression in the retina. Contrary to expectations, the pulse had little to no effect on retinal gene expression. These results stand in sharp contrast to the effect on gene expression in the brain after exposure to the same pulse [6]. The findings are consistent with the hypothesis that magnetite-based magnetoreceptors exist in or near the brain of trout, but probably not in the retina.

2. Material and methods

A summary of the methods is available below; see the electronic supplementary material for a complete description of all procedures.

Twelve juvenile rainbow trout were exposed to either a magnetic pulse (0.085 T magnetic field for 5 ms; $n = 6$) or a control sham pulse ($n = 6$) as described previously [6]. A total of 15 min elapsed between treatment and preservation in liquid nitrogen. We separately dissected the retinas from both left and right eyes (24 in total; table 1) and purified total RNA. Separate cDNA libraries were generated for each RNA sample, barcoded and sequenced across four lanes of an Illumina NextSeq 500 (75 bp, paired-end reads).

Sequence reads were trimmed and mapped to the trout reference nuclear (GenBank accession CCAF010000000) [7] and mitochondrial (GenBank accession NC_001717.1) genomes as reported in [6]. A reference-guided approach was used to reconstruct the transcriptome and detect potentially novel transcripts and/or splice variants [8]. Novel genes were assigned as putative long non-coding RNAs (lncRNAs) by comparison with a lncRNA database for trout [9].

Two different methods were used to assess differential gene expression, measured as the binary log of the fold change ($\log_2$FC). First, we quantified transcript abundance as the fragments per kilobase of transcript per million mapped reads (FPKM) with [10] and compared expression, promoter usage and splicing between three groups of retinas: (i) right control (RC) versus left control (LC); (ii) right pulsed (RP) versus RC and (iii) left pulsed (LP) versus LC. Second, we fit a generalized linear model via a negative binomial distribution to each annotated

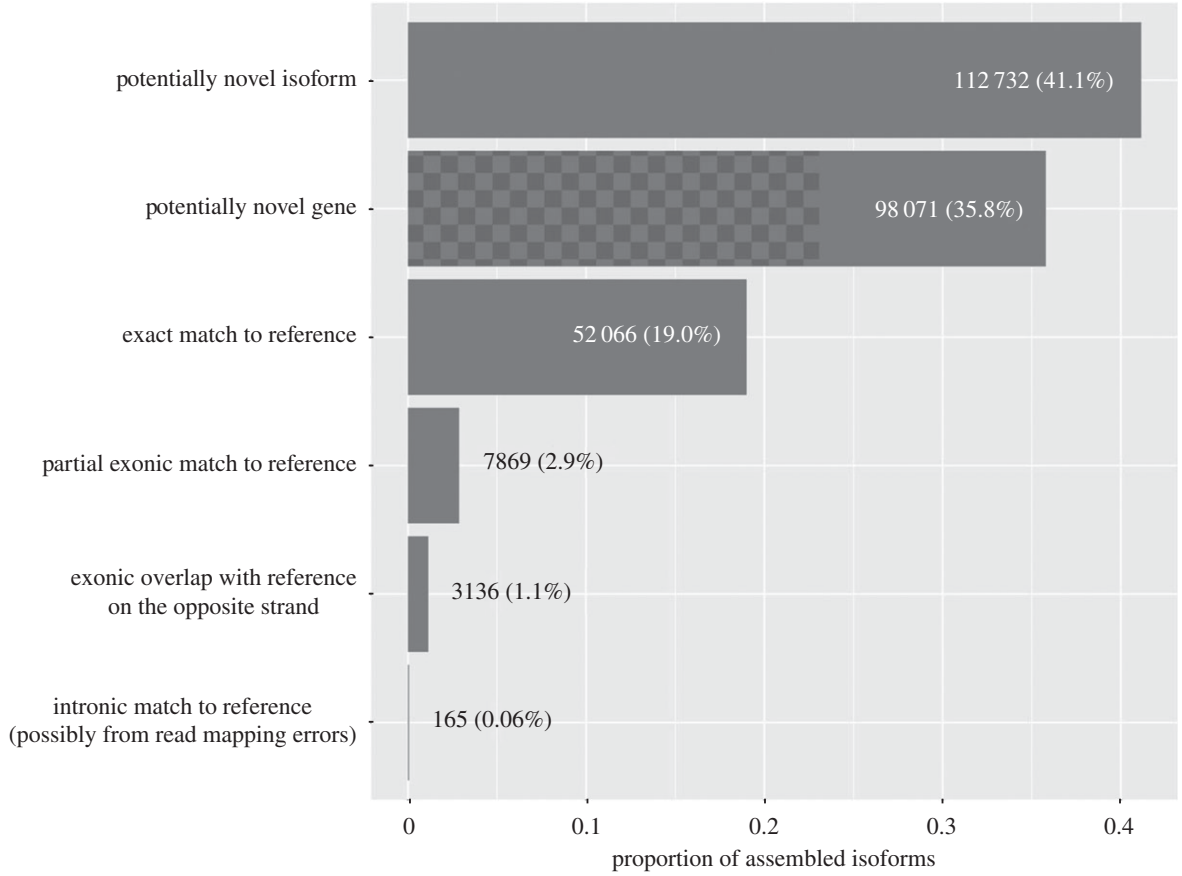

Figure 1. Distribution of contigs generated by the reference-guided assembly. The checkered area represents contigs matching to known long non-coding RNAs.

gene [11]:

$$y \sim \text{retina} + \text{treatment} + \text{retina} : \text{treatment}$$

where the response y, or expression, is a function of the predictors 'retina' (left versus right), 'treatment' (pulsed versus control), and their interaction (retina : treatment). We contrasted both the 'treatment' effect and the interaction effect. In all comparisons, only genes with an alignment count greater than or equal to 10 were included. We retained genes with a false discovery rate (FDR) less than 0.05 as differentially expressed.

3. Results

We obtained approximately 1.2 billion paired-end sequencing reads which are summarized in table 1. The merged, reference-guided assembly generated 126 080 genes comprised from 274 039 isoforms, including 42 700 (91.7%) of the annotated reference genes. The distribution of novel isoforms is illustrated in figure 1. Of the isoforms representing novel genes, 62 932 (64.2%) were assigned to 20 884 unique lncRNAs previously identified in rainbow trout (mean length and *e*-value were 377 bp and 1.6×10^{-8}, respectively; electronic supplementary material, figure S1).

Our first approach, using the model in Cufflinks [10], found little variation in gene expression in response to the magnetic pulse (figure 2). When comparing control retinas (LC versus RC), no differences in expression were observed ($n = 73\ 992$ genes). When assessing the effect of the magnetic pulse on retinas from the left (LP versus LC; $n = 73\,964$ genes) and right (RP versus RC; $n = 74\,998$ genes) sides separately, $\log_2$FC estimates were moderately correlated ($r = 0.42$, $n = 64\,803$, $p < 2.2 \times 10^{-16}$; electronic supplementary material, figure S2); and again, no differential expression was observed. Additionally, there were no differences in isoform expression, promoter usage or splicing in all comparisons. The second approach, using a generalized linear model, produced results similar to the first, with the exception of a single gene, *gamma* (γ)*-crystallin M3-like* (*crygm3*; GenBank accession XP_021435344.1; $\log_2\text{FC} = 20.8$; FDR = 0.025; electronic supplementary material, figure S3), that was affected by the treatment. We found no genes with a significant interaction effect.

4. Discussion

As a first step towards determining if magnetite-based magnetoreceptors in trout are associated with the retina, we investigated gene expression in the retina following a brief magnetic pulse known to alter magnetic orientation behaviour in animals [1]. Our retina transcriptome, the first in rainbow trout, identified many known genes, in addition to some potential retina-specific lncRNAs. The latter were included in examinations of differential expression because lncRNAs are known to be important regulators of genes [12].

Contrary to expectations, the pulse affected expression of only a single gene (see below) in the retina. Additionally, no evidence of gene expression lateralization was detected despite reports that retinal-associated magnetoreceptors may be lateralized in birds [13]. The single gene affected by the pulse was identified when modelling the effect of the pulse while controlling for retina lateralization. The near absence of an effect of the magnetic pulse on retina gene expression differs from the large number of genes (181) that were differentially expressed in brain tissue after exposure

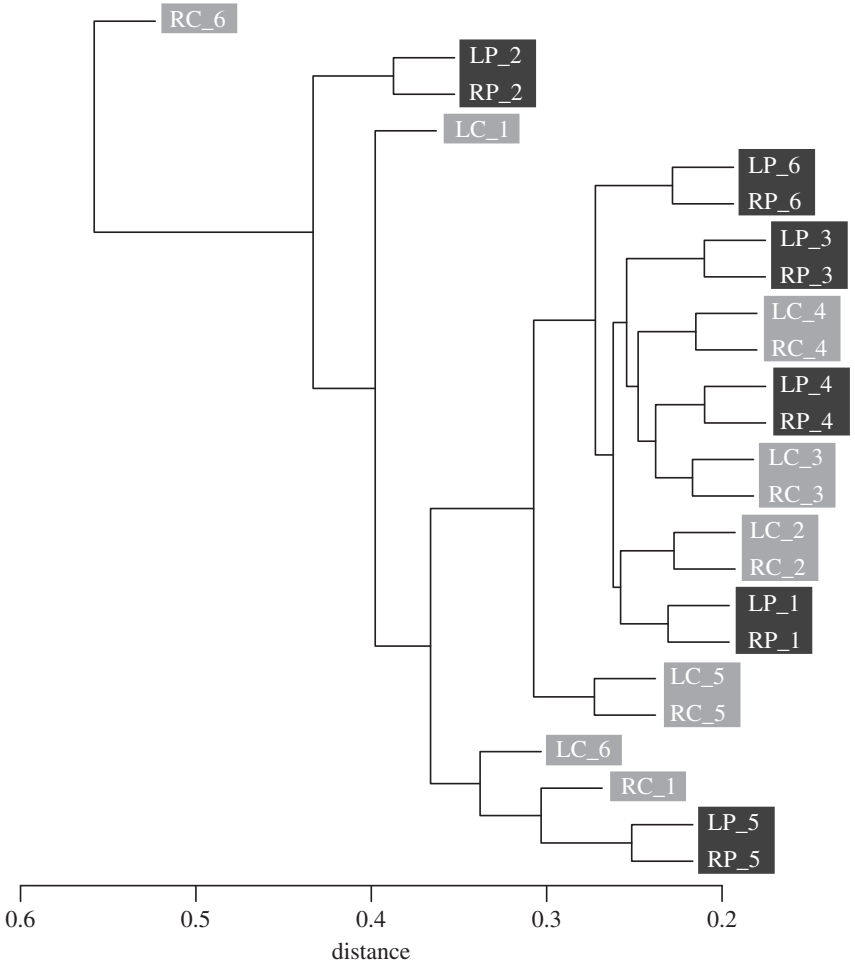

Figure 2. Hierarchical clustering diagram. Clustering based upon Euclidean distances of gene expression in the six pairs (left and right) of control (grey boxes) and pulsed (black boxes) rainbow trout retinas. LP, left pulsed; LC, left control; RP, right pulsed; RC, right control.

to the same pulse [6]. Taken together, the results suggest either that magnetite-based magnetoreceptors in trout are not located in the retina, or else that retinal magnetoreceptors, if they do exist, use a different mechanism (e.g. a chemical mechanism based on the protein cryptochrome [14]) that is thought to be largely unaffected by magnetic pulses). A dual-mechanism magnetoreception system, in which retinal cryptochromes function in a magnetic compass and magnetite-based receptors function in a magnetic map, has been proposed in birds [1] and cannot be ruled out in fish. Nevertheless, dissimilarities between the light-dependent compasses of birds and the light-independent compasses of teleosts suggest the possibility that the two are based on different underlying mechanisms [1,15].

The single gene affected by the pulse was γ-crystallin M3-like (*crygm3*). Interestingly, a previous study found that a different copy of *crygm3* was significantly reduced in the brain of rainbow trout in response to a magnetic pulse [6]. Although one possible interpretation is that *crygm3* is involved in magnetoreception (e.g. through magnetite-based magnetoreceptors in the crystallin-rich cornea [16]), an alternative possibility is that *crygm3* might respond to stress or damage induced by the pulse, perhaps in a tissue-specific manner. Consistent with the latter, γ-crystallins, such as *crygm3*, belong to a complex family of proteins that are expressed in the retina where they might protect neurons from environmental damage or metabolic stress [17,18].

In summary, the same magnetic pulse that elicited changes in expression of genes in brain tissue of trout, including a number of genes associated with visual processes, had little to no effect on expression, promoter usage or splicing in the retinas. A single gene, *crygm3*, was affected, but whether this gene is associated with magnetoreception or instead with a generalized, non-specific effect of the pulse cannot be determined at present.

The lack of differential gene expression elicited by the pulse in retinal tissue, combined with the stronger response elicited in brain tissue, provides support for two provisional conclusions. First, magnetite-based magnetoreceptors in trout are probably not located in the retina. Second, if photoreceptive tissue is involved in trout magnetoreception, then either a magnetite-independent mechanism is used (such as a mechanism based upon radical electron pairs associated with the blue-light sensitive cryptochrome protein), or structures closely associated with the brain (e.g. the pineal gland), are better candidates than the retina. Future studies will be needed to confirm or refute these inferences.

Ethics. Experimental methods were approved by Duke University's IACUC (protocol A175-15-06).

Data accessibility. Sequence data were deposited into GenBank under accession PRJNA391176 and computer code is available at https://github.com/rfitak/RETINA_RNA-SEQ. All other data are archived in Dryad (http://dx.doi.org/10.5061/dryad.24732) [19]. Accession numbers in table 1 for sequences are deposited in the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra).

Authors' contributions. R.R.F. conceived and designed the study, performed experiments, analysed the data and drafted the manuscript; L.E.S. performed dissections; B.R.W. helped design the study and performed experiments; D.A.E. and K.J.L. helped to conceive and

design the study; S.J. conceived, designed and coordinated the study. All authors helped draft the manuscript, interpreted results, approved the final manuscript for publication and agree to be held accountable for the content therein.

Competing interests. We have no competing interests.

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