

Evolutionary conserved neural signature of early life stress affects animal social competence

Cecilia Nyman¹, Stefan Fischer², Nadia-Aubin-Horth³ & Barbara Taborsky¹

¹Div. of Behavioural Ecology, Institute of Ecology and Evolution, University of Bern, Switzerland

5 ²Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK

³Département de Biologie and Institut de Biologie Intégrative et des Systèmes, Université Laval, Quebec, Canada

Proceedings of the Royal Society B

10

Supplementary Information (SI)

Sample preparation and qPCR

15

Primers for qPCR

We measured the gene expression of *egr-1* and *gr1* in the hypothalamus and in the telencephalon of *N. pulcher*. We used the gene 18S as a control or 'housekeeping' gene. Primers for *gr1* were the same as used in [1], while 18S primers were the same as used in [2].

20 Primers for *egr-1* were designed from the genome of *N. brichardi* (<http://cichlid.umd.edu/cichlidlabs/kocherlab/bouillabase.html>) using the *A. burtoni* sequence as a search template (NCBI database ID number: AY493348.1, for-
CGGCGATATATCCTAAAATC; rev-TCCCATGCCTATAAACACT).

Sample preparation

25 RNA extraction was done from hypothalamus and telencephalon, for each brain part separately, using the Qiagen miRNeasy micro kit. We chose this kit, because the samples were very small and yielded low amounts of RNA. The protocol was modified to avoid sampling miRNAs. To make sure to get an end product free of DNA we carried out an
30 DNase treatment. In brief, we placed the brain tissue in a 1.5 ml Eppendorf tube with 200 µl of QIAzol lysis reagent (Qiagen), homogenized it and added further 500 µl of QIAzol lysis reagent. After that we placed the tube on the benchtop at room temperature (RT) for 5 min, after which we added 140 µl of chloroform, we shook the sample for 1 minute, and left it for 15 min (RT) and centrifuged it at 12 000 g at 4°C for 20 min. After centrifugation we moved

35 the upper aqueous phase to a new clean Eppendorf tube, added 140 μ l chloroform, shook the sample for 1 minute and centrifuged it at 12 000 g at 4°C for 20 min for a second phase separation step. After centrifugation we moved again the upper aqueous phase to a new clean Eppendorf tube, added 250 μ l of 70% Ethanol, mixed the sample thoroughly, and pipetted the whole sample onto a RNeasy MinElute spin column (SC, Qiagen). Then we centrifuged the
40 SC at 10 000 rpm for 30 s (RT) and discarded the flow-through. After this we carried out an DNase treatment by pipetting 350 μ l of buffer RWT (prepared with isopropanol, Qiagen) onto the spin column, centrifuged the column (10 000 rpm, 30 s, RT), discarded the flow-through, pipetted 80 μ l of DNase I incubation mix (10 μ l DNase stock solution + 70 μ l Buffer RDD, Qiagen) onto the SC and left the tube at RT for 15 min. After the DNase treatment we
45 pipetted 500 μ l of Buffer RWT onto the SC, centrifuged it (10 000 rpm, 30 s, RT), reapplied the flow-through onto the SC, centrifuged the SC (10 000 rpm, 30 s, RT) and discarded the flow-through. Then we pipetted 500 μ l buffer RPE (Qiagen) onto the SC, centrifuged the SC (10 000 rpm, 30 s, RT) and discarded the flow-through. To collect total RNA instead of miRNA we pipetted 500 μ l of 80% Ethanol onto the SC, left the tube 5 min at RT and then
50 centrifuged the SC (10 000 rpm, 30 s, RT) and discarded the flow-through. We repeated this step once. After the ethanol wash we placed the SC into a new 2 ml collection tube, centrifuged it (15 000 rpm, 5 min, RT) with the lid open, removed the SC and placed in into a final 1.5 ml collection tube, added 14 μ l of RNase free water (Qiagen) to the SC and centrifuged the SC (15 000 rpm, 1 min, RT) to elute the RNA. At the end we checked the
55 RNA concentration and sample composition with a Nanodrop microvolume spectrophotometer.

We carried out reverse transcription using the same amount of RNA from each sample (200 ng RNA from hypothalamus and 304 ng RNA from telencephalon) using a standard
60 Superscript protocol (Invitrogen). To confirm the expression of each gene and success of RT, we used a small amount of cDNA from random samples from both treatments in a PCR using both genes and visualised it using an electrophoretic gel. To determine amplification efficiency, the absence of primer dimers and the specificity of amplification for each primer pair, we run qPCR experiments and melting curves (50 to 90 Celsius) using standard curves
65 consisting of 5 x 10-fold dilutions (of pooled samples) in duplicates (Aubin-Horth *et al.*, 2012). We prepared the primers (Eurofins) and 5 μ l of sample cDNA on a 384-well plate (axigen) by using an epmotion liquid handler (Eppendorf) which is used for quantitative real-time PCR experiments following the scaled-down version of the Quantitect SYBRGreen PCR

kit manufacturer's protocol (Qiagen) using a 384-well plate qRT-PCR machine (Light Cycler,
70 Roche). We run each sample (from each individual) for hypothalamus and telencephalon in
triplicate for a given gene together with no primers and no template controls. To verify that
only a single amplified product was present and that no primer dimers were produced, we also
performed a melting curve on each replicate. We calculated the coefficient of variation (CV)
of the three replicates and use a CV cut-off of 5% for all genes. The CV value is used for
75 checking the repeatability and precision of the qPCR assay. Using the expression of a control
gene (18S) [3], we calculated the relative gene expression for each individual / brain region
combination.

80

85 References

1. Taborsky B, Tschirren L, Meunier C, Aubin-Horth N. 2013 Stable reprogramming of
brain transcription profiles by the early social environment in a cooperatively breeding
fish Stable reprogramming of brain transcription profiles by the early social
environment in a cooperatively breeding fish. *Proc. Biol. Sci.* **208**, 1–7.
- 90 2. O'Connor CM, Marsh-rollo SE, Ghio SC, Balshine S, Aubin-horth N. 2015 Is there
convergence in the molecular pathways underlying the repeated evolution of sociality
in African cichlids? *Horm. Behav.* **75**, 160–168. (doi:10.1016/j.yhbeh.2015.07.008)
3. Pfaffl MW. 2001 A new mathematical model for relative quantification in real-time
RT-PCR. *Nucleic Acids Res.* **29**, e45. (doi:10.1093/nar/29.9.e45)

95