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Electronic Supplementary Material

# Social interactions elicit rapid shifts in functional connectivity in the social decision-making network of zebrafish

Magda C. Teles, Olinda Almeida, João S. Lopes and Rui F. Oliveira

Correspondence to:
Rui F. Oliveira,
Instituto Gulbenkian de Ciência,
Rua da Quinta Grande 6,
2780-156 Oeiras, Portugal

Email: ruiol@ispa.pt

## Microdissection of the regions of interest in the SDM network

To identify and microdissect specific brain nuclei, slides were placed on a glass petri dish, filled with dry ice to maintain low temperatures, and viewed under a stereoscope (Zeiss; Stemi 2000). Tissue was collected with a modified 27G needle (inner diameter = 210μm) attached to a syringe. To prevent cross contamination between brain nuclei, one needle per nuclei was used and the needles were cleaned sequentially with distilled water and ethanol 70% between individuals. The nuclei in the vicinity of the ventricular area (i.e. Vv, Vs, and POA) were collected from both hemispheres at a single sampling point, due to their small size when compared to the diameter of the microdissection tool and due to their closeness to other nuclei. The remaining nuclei (i.e. Dm and Dl) were sampled from both hemispheres separately, and tissue from the two hemispheres were then pooled directly into lysis buffer (RNeasy Lipid Tissue Mini Kit, Qiagen) and stored at -80 until mRNA extraction.

**RNA extraction**

Tissue was homogenised in qiazol lysis reagent and incubated for 7 min at room temperature (RT). Chloroform (1:2) was added, and the sample incubated at RT for 5 min. Samples were subsequently centrifuged at 13000 *g* for 20 min at 4ºC, after which the upper aqueous phase was transferred to a new tube where 1 volume of 70% ethanol was added. This mixture was then transferred to an RNEasy column, remained 5 min at RT, and was centrifuged for 1 min at 9000 *g*. A sequence of buffers (provided by the RNeasy Lipid Tissue Mini Kit) was added to the Rneasy column: 700 µl of Buffer RW1, 500 µl of Buffer RPE and an additional 500 µl Buffer RPE. After each buffer, samples were centrifuged for 1 min at 9000 *g* and the flow-through was discarded. The RNeasy column was then placed in a new 2 ml tube and centrifuged for 3 min at 14000g. The column was transferred to a new 1.5 ml tube, RNA eluted with 25 µl of RNAse-free water, and centrifuged for 2 min at 9000 *g*. The elution step was repeated with the same 25 µl of RNAse-free water in order to increase RNA recovery efficiency. RNA concentration and purity of all samples was estimated by spectophotometric absorbance (260 nm and 280nm) in the Nanodrop (Thermo Scientific NanoDrop 2000), and the RNA integrity of a random group of samples was checked using Bionalyzer ( Agilent 2100 Bioanalyzer).

**Quantitative RT-PCR (qRT-PCR)**

Primer sequences for qRT-PCR were designed on Primer 3 (Premier Biosoft International, Palo Alto, CA, USA), tested for quality in the FastPCR 5.4., and the PCR products were sequenced to confirm the amplicon (Table S1). qRT-PCR reactions were performed in an Applied Biosystems 7900HT Fast thermocycler in 8 µl triplicate reactions with SYBR Green PCR Master Mix (Applied Biosystems, Life Technologies) and primers at 50 µM. Thermocycling conditions were 5 min at 95º C, followed by 40 cycles of: 95ºC for 30 s, specific annealing temperature for each primer for 30 s (Table S1), and 72 ºC

for 30 s. After PCR, a melting curve program from 55 to 95ºC with 0.5 º C changes was applied and the presence of a single reaction product in each well was confirmed. All reactions were performed in triplicate and technical replicates were run on the same plate. Before the analysis, the threshold value was adjusted manually for each plate at the inflection point of the amplification curve, and the same threshold was used in all assays of the same gene.

**Table S1**- Primer sequences and qRT-PCR parameters.

| Gene | Accession No. | Primer sequence (5' → 3') | Annealing temperature (Cº) | Amplicon length (pb) |
|---|---|---|---|---|
| *eef1a1l1* | NM_131263 | F-CAAGGAAGTCAGCGCATACA<br>R-TCTTCCATCCCTTGAACCAG | 60 | 134 |
| *c-fos* | NM_205569 | F-CCGATACACTGCAAGCTGAA<br>R- CGGCGAGGATGAACTCTAAC | 59 | 111 |
| *egr-1* | NM_131248 | F- GTGAGCCCAACCCCATCTAT<br>R- CCAGGCTGATCTCACTTTGC | 58 | 216 |

F - primer forward; R - primer reverse

**Table S2**- Final sample sizes (n) after outliers removal. Brain nuclei: medial zone of the dorsal telencephalic area (Dm); lateral zone of the dorsal telencephalic area (Dl); ventral nucleus of the ventral telencephalic area (Vv); supracommissural nucleus of the ventral telencephalic area (Vs); and preoptic area (POA).

| | | Social behaviour state | | | |
|---|---|---|---|---|---|
| Brain Nuclei | Gene | Isolation | Mirror–fighters | Winners | Losers |
| Dm | *cfos* | 12 | 11 | 12 | 13 |
| | *egr-1* | 11 | 11 | 12 | 11 |
| Dl | *cfos* | 12 | 10 | 12 | 13 |
| | *egr-1* | 12 | 10 | 12 | 13 |
| Vv | *cfos* | 12 | 11 | 12 | 12 |
| | *egr-1* | 12 | 11 | 12 | 11 |
| Vs | *cfos* | 7 | 7 | 10 | 12 |
| | *egr-1* | 9 | 8 | 10 | 12 |
| POA | *cfos* | 11 | 11 | 12 | 13 |
| | *egr-1* | 11 | 11 | 12 | 12 |

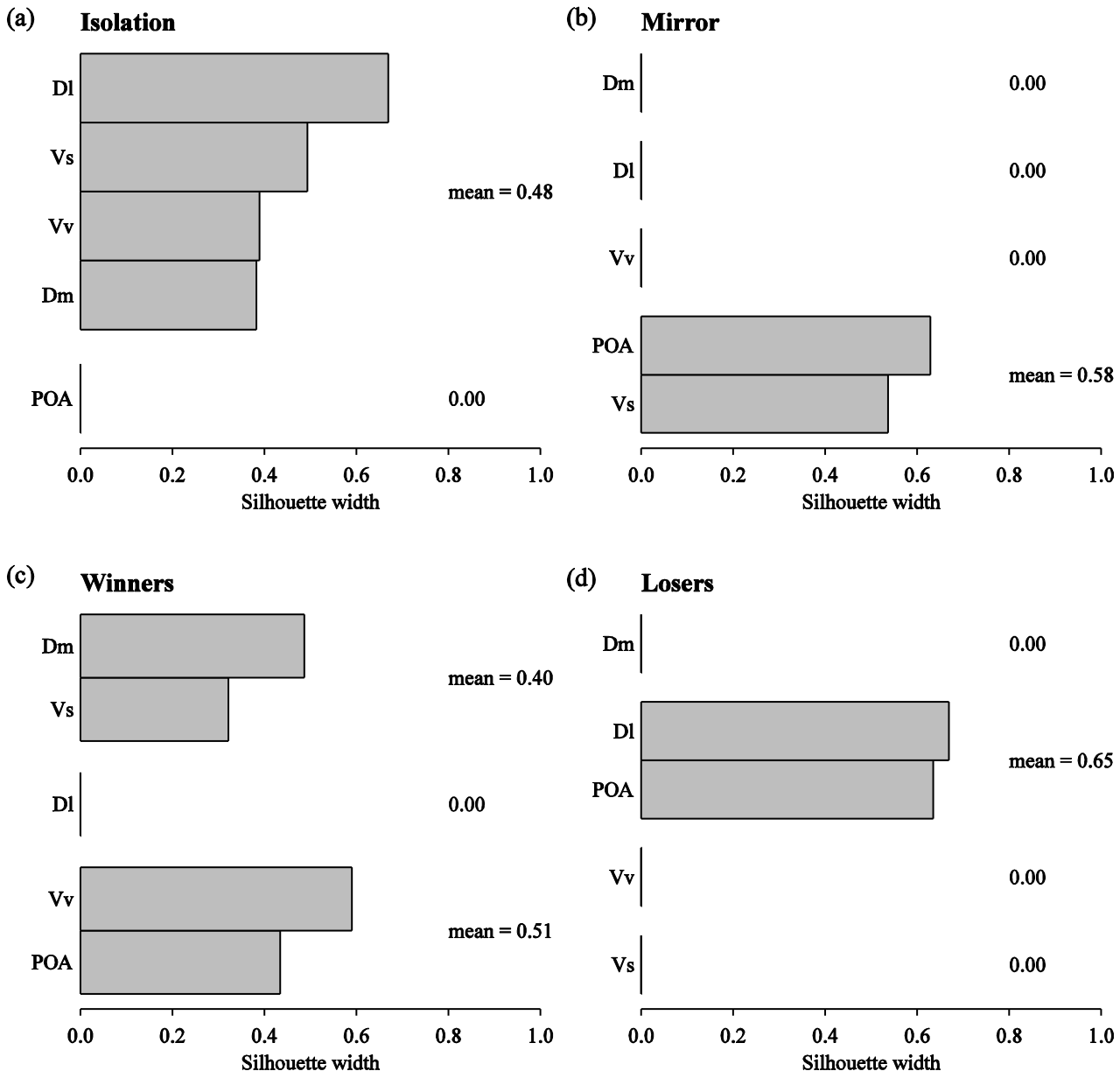


**Figure S1** – Clustering of different brain regions and their silhouette width according to correlation values of *c-fos* expression for various social contexts: (a) no interaction (Isolation); (b) unsolved mirror interaction (Mirror); winning conspecific interaction (Winners); and losing conspecific interaction (Losers). Considered brain regions were: medial zone of the dorsal telencephalic area (Dm); lateral zone of the dorsal telencephalic area (Dl); ventral nucleus of the ventral telencephalic area (Vv); supracommissural nucleus of the ventral telencephalic area (Vs); and preoptic area (POA). Average silhouette width per cluster is presented right to the silhouettes cluster (AS < 0.25 = no structure; 0.25 < AS < 0.5 = weak structure; 0.5 < AS < 0.7 = reasonable structure; AS > 0.7 = strong structure).

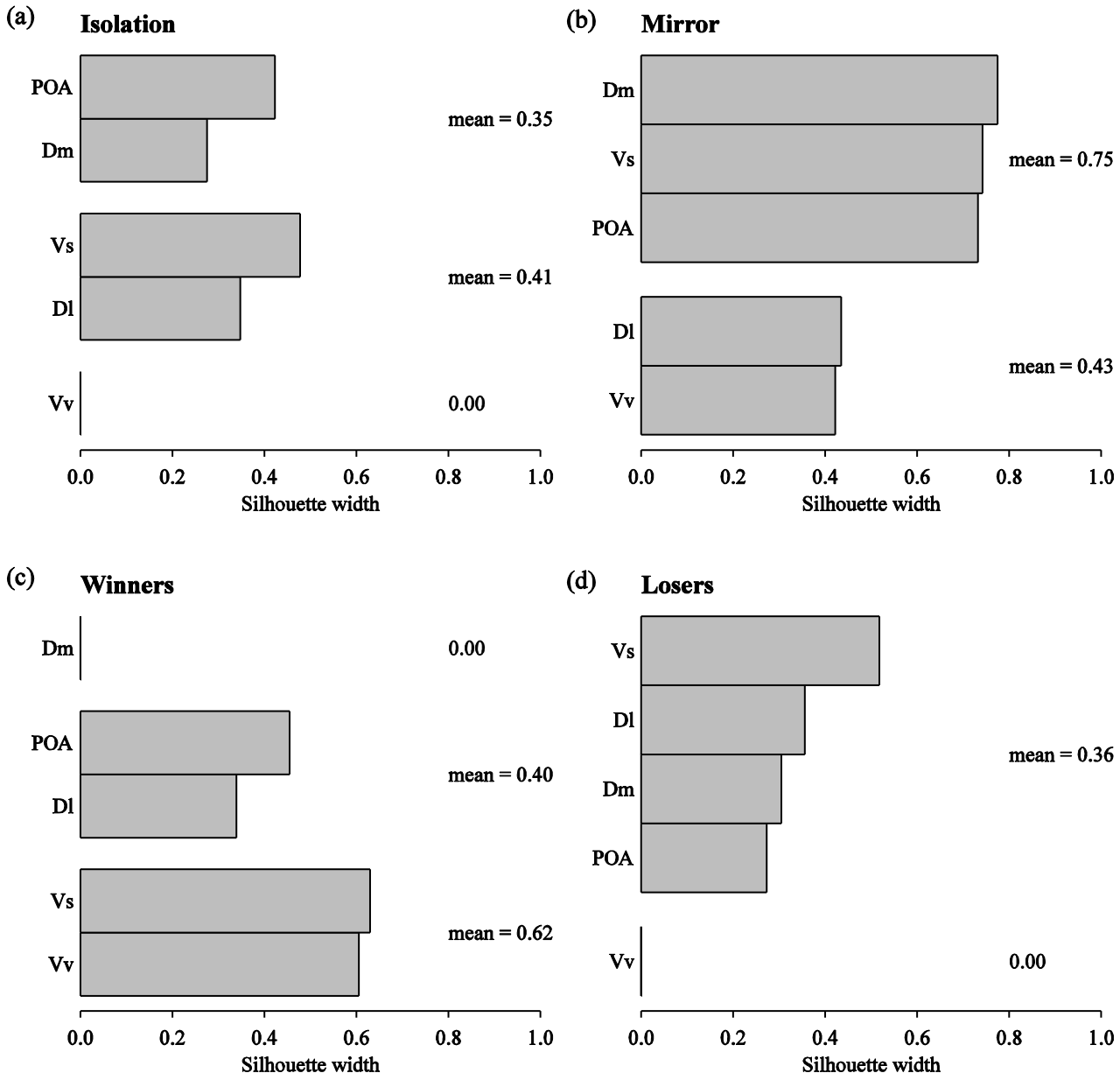


**Figure S2** – Clustering of different brain regions and their silhouette width according to *egr-1* expression for various social contexts: (a) no interaction (Isolation); (b) unsolved mirror interaction (Mirror); winning conspecific interaction (Winners); and losing conspecific interaction (Losers). Considered brain regions were: medial zone of the dorsal telencephalic area (Dm); lateral zone of the dorsal telencephalic area (Dl); ventral nucleus of the ventral telencephalic area (Vv); supracommissural nucleus of the ventral telencephalic area (Vs); and preoptic area (POA). Average silhouette width per cluster is presented right to the silhouettes cluster (AS < 0.25 = no structure; 0.25 < AS < 0.5 = weak structure; 0.5 < AS < 0.7 = reasonable structure; AS > 0.7 = strong structure).

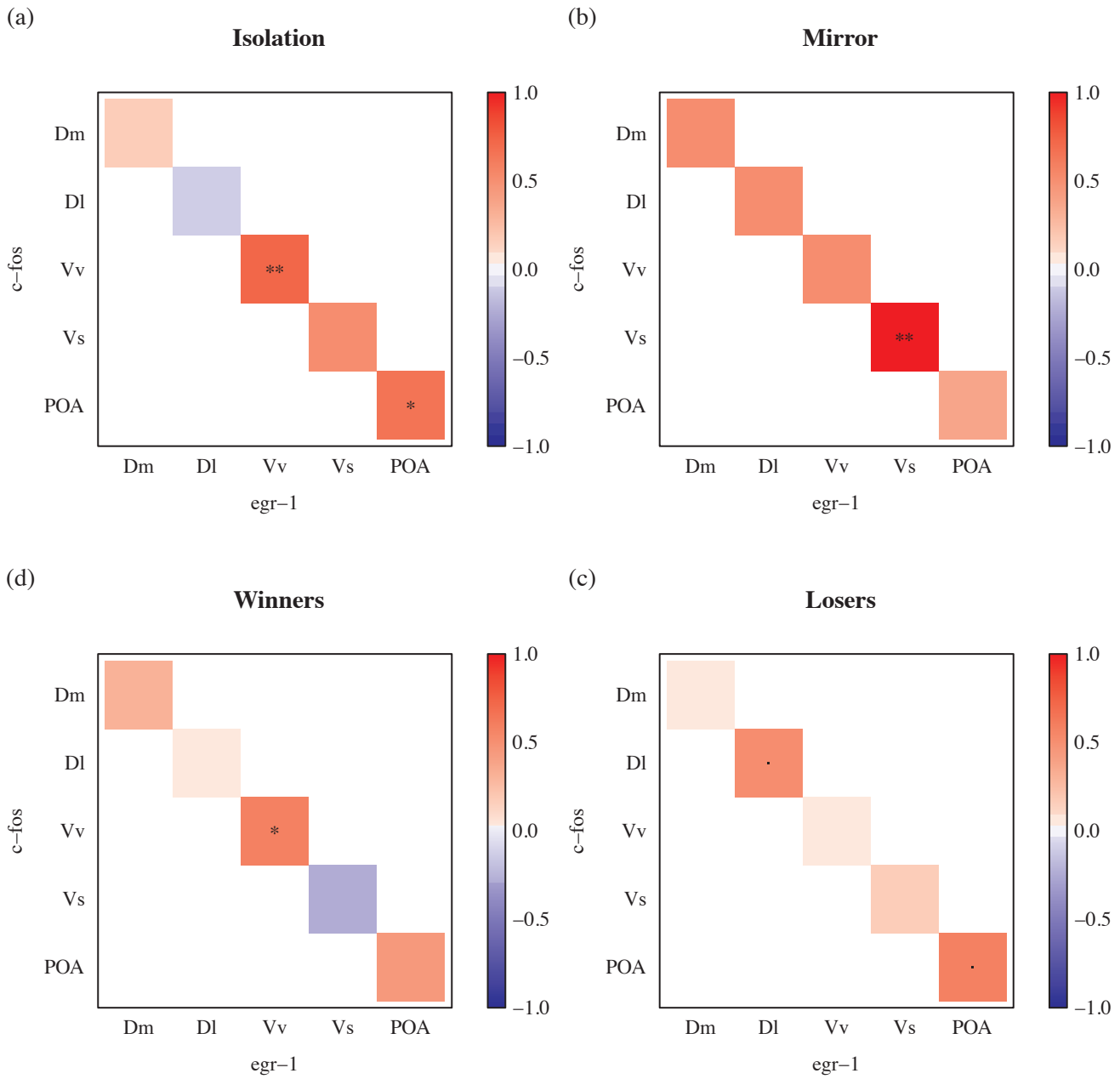


**Figure S3** – Pearson correlations between *c-fos* and *egr-1* expressions for various social contexts: (a) no interaction (Isolation); (b) unsolved mirror interaction (Mirror); winning conspecific interaction (Winners); and losing conspecific interaction (Losers). Considered brain regions were: medial zone of the dorsal telencephalic area (Dm); lateral zone of the dorsal telencephalic area (Dl); ventral nucleus of the ventral telencephalic area (Vv); supracommissural nucleus of the ventral telencephalic area (Vs); and preoptic area (POA). Colour scheme represents correlation values from -1 (blue) to 1 (red). Asterisks indicate significant correlations after p-value adjustment (dot (.), $p < 0.1$; *, $p < 0.05$; **, $p<0.01$; ***, $p< 0.001$).

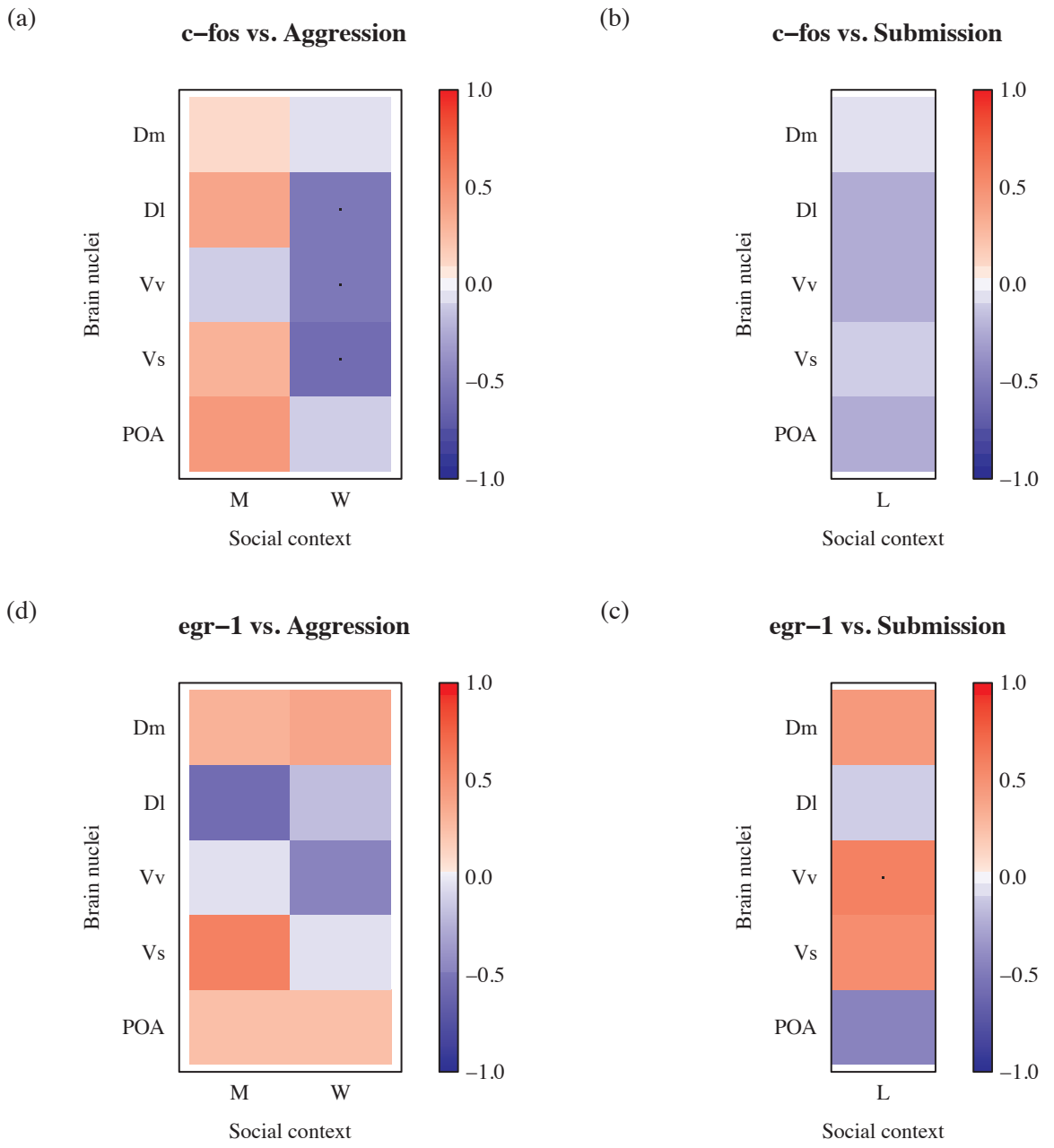


**Figure S4** – Pearson correlations between immediate early genes expression and behaviour towards conspecifics for various social contexts: (a) association between *c-fos* expression in various brain regions and aggressive behaviour in relevant social contexts; (b) association between *c-fos* expression in various brain regions and submissive behaviour in relevant social contexts; (c) association between *egr-1* expression in various brain regions and aggressive behaviour in relevant social contexts; and (d) association between *egr-1* expression in various brain regions and submissive behaviour in relevant social contexts. Considered brain regions were: medial zone of the dorsal telencephalic area (Dm); lateral zone of the dorsal telencephalic area (Dl); ventral nucleus of the ventral telencephalic area (Vv); supracommissural nucleus of the ventral telencephalic area (Vs); and preoptic area (POA). Considered social contexts were: winning conspecific interaction (W); losing conspecific interaction (L), unsolved mirror interaction (M); and no interaction (I). Colour scheme represents correlation values from -1 (blue) to 1 (red). Asterisks indicate significant correlations after p-value adjustment (dot (.), $p < 0.1$; *, $p < 0.05$; **, $p<0.01$; ***, $p< 0.001$).

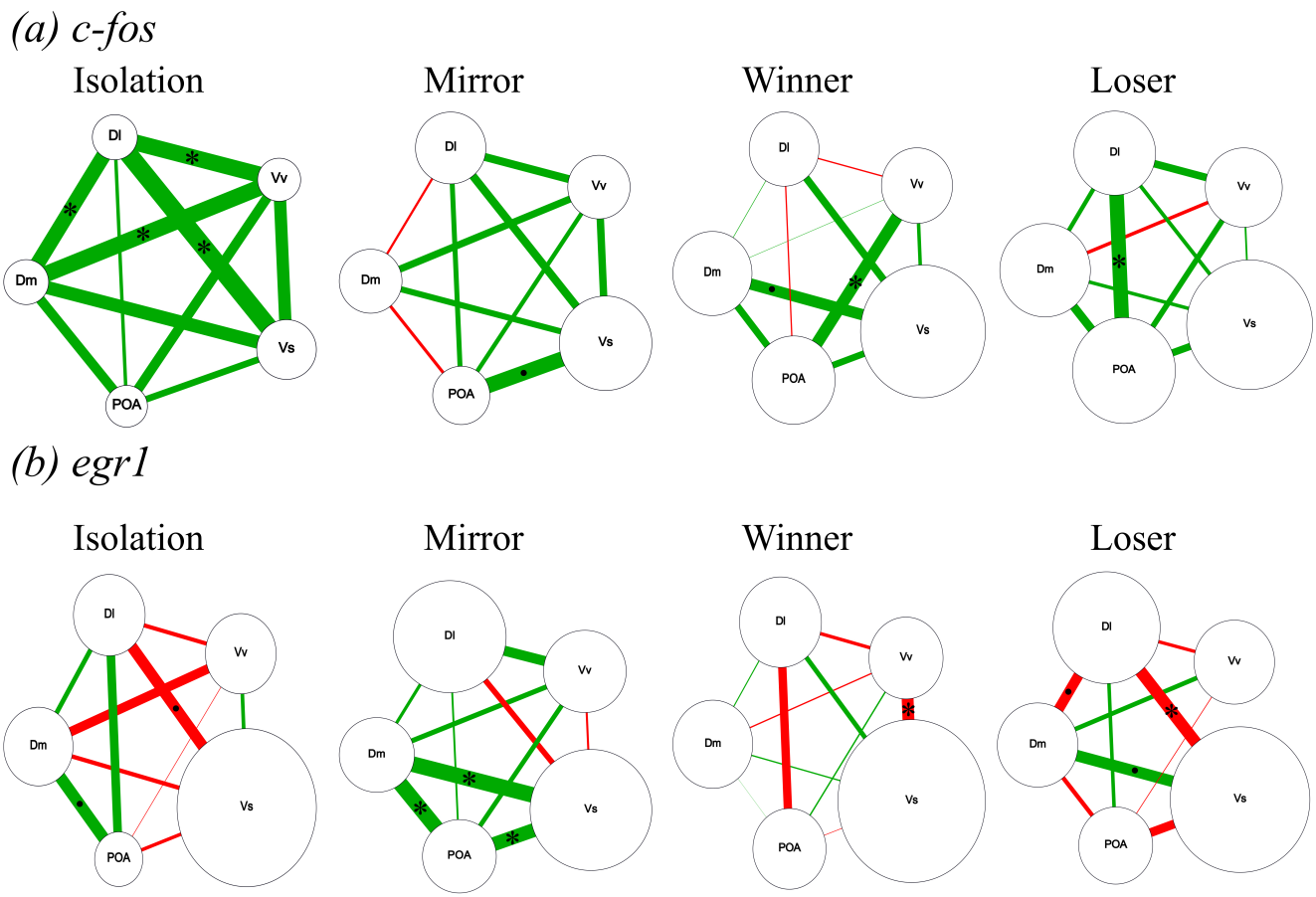


**Figure S5** – Representation of the state of the social decision-making network for each social behavioural state using: *(a) c-fos* expression and *(b)* egr-1 expression as reporters of neuronal activity. Circle diameter represents the activity level at each network node: medial zone of the dorsal telencephalic area (Dm); lateral zone of the dorsal telencephalic area (Dl); ventral nucleus of the ventral telencephalic area (Vv); supracommissural nucleus of the ventral telencephalic area (Vs); and preoptic area (POA). Lines linking pairs of nodes represent the functional connectivity between them as measured by Pearson correlation coefficients of IEG expression, such that: the thickness of the line is proportional to the R value and the colour scheme represents positive (green) or negative (red) correlations. Asterisks indicate significant correlations after p-value adjustment: dot (.) $p < 0.1$; * $p < 0.05$; ** $p<0.01$; *** $p< 0.001$.