**Table 1.** Squid behaviours shown to be altered by OA in Thomas *et al.* (2021) and used alongside transcriptomic data within the current study. ↑ = increase in the behaviour at elevated compared with current-day CO2 conditions.

|  |  |
| --- | --- |
| **Behaviour** | **CO2 Treatment Effect** |
| **Activity** |  |
| Active time (s) | ↑ |
| Total distance moved (cm) | ↑ |
| Average speed (cm/s) | ↑ |
| **Visually-mediated aggressive conspecific-directed behaviour** |  |
| Proportion of squid displaying one or more aggressive interactions | ↑ |
| Number of aggressive interactions per individual | ↑ |
| **Visually-mediated exploratory conspecific-directed behaviour** |  |
| Time spent in Zone A (3 cm closest to the mirror) | ↑ |
| Proportion of squid displaying one or more exploratory interactions | ↑ |
| Number of exploratory interactions per individual | ↑ |

**Table 2.** Results across all three analysis methods in the CNS and eyes related to neurotransmission. Genes identified as significantly differentially expressed (DE) between current-day and elevated CO2 conditions, GO Terms/functional categories significantly affected by elevated CO2 treatment as identified by gene set enrichment analysis (GSEA), and genes identified as correlated with both CO2 treatment and one or more OA-affected behaviours by weighted gene co-expression network analysis (WGCNA). DE: ↑ or ↓ = significant upregulation or downregulation of this gene at elevated compared to current-day CO2 conditions. GSEA: ↑ or ↓ = significant upregulation or downregulation of this GO Term/functional category, indicating small, coordinated upregulation or downregulation of the genes belonging to this functional category, at elevated compared to current-day CO2 conditions. WGCNA: CO2 ↑ or ↓ = positive or negative correlation between the expression of the gene and CO2 treatment, Behaviour ↑ or ↓ = positive or negative correlation between the expression of the gene and 1 or more OA-affected behaviours, ↑/↓ = different transcripts of the same gene were found to be positively and negatively correlated. \* = GO Term contains core enrichment genes for this type of neurotransmission.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **DE** | | | **GSEA** | | | **WGCNA** | | | | |
|  | **Gene** | **CNS** | **Eyes** | **GO Term / Functional Category** | **CNS** | **Eyes** | **Gene** | **CNS** | | **Eyes** | |
|  |  |  |  |  |  |  |  | **CO2** | **Behaviour** | **CO2** | **Behaviour** |
| **Neurotransmission** | |  |  |  |  |  |  |  |  |  |  |
| **Cholinergic** |  |  |  | 'acetylcholine-gated cation-selective channel activity', 'synapse\*', 'postsynaptic membrane\*', 'excitatory postsynaptic potential\*', 'transmembrane signaling receptor activity\*', 'extracellular ligand-gated ion channel activity\*' | ↓ |  | *chrna10* | ↓ | ↑ | ↑/↓ | ↓ |
|  |  |  |  | ‘ion transmembrane transport\*’ | ↓ | ↑ |  |  |  |  |  |
|  |  |  |  | ‘ion channel activity\*’ |  | ↑ |  |  |  |  |  |
| **GABAergic** | *syvn1-b* | ↑ |  | 'extracellular ligand-gated ion channel activity\*', 'G protein-coupled receptor signaling pathway\*' | ↓ |  | *phf24, rac1* | ↑ | ↑ |  |  |
|  | *slc18a2* |  | ↑ | 'ion transmembrane transport\*' | ↓ | ↑ | *aldh5a1* | ↓ | ↓ |  |  |
| **Glutamatergic** | *folh1* | ↑ |  | 'ionotropic glutamate receptor signalling pathway', 'ionotropic glutamate receptor activity', 'synapse\*', 'postsynaptic membrane\*', ''G protein-coupled receptor signaling pathway\*', 'G protein-coupled receptor activity\*' | ↓ |  |  |  |  |  |  |
|  | *celsr3* | ↓ |  | 'ion transmembrane transport\*' | ↓ | ↑ |  |  |  |  |  |
|  |  |  |  | 'ion channel activity\*' |  | ↑ |  |  |  |  |  |
| **Monoaminergic** | *maoa, slc18a2* |  | ↑ | 'G protein-coupled receptor signaling pathway\*', 'G protein-coupled receptor activity\*' | ↓ |  |  |  |  |  |  |
| **General Processes for Synaptic Neurotransmission** |  |  |  | 'potassium channel activity', 'potassium ion transmembrane transport', 'voltage-gated potassium channel activity', 'regulation of ion transmembrane transport', 'voltage-gated calcium channel complex', 'calcium ion transmembrane transport' | ↓ | ↓ | *futsch, dgkq* | ↓ | ↓ |  |  |
| **GPCR Trafficking** |  |  |  |  |  |  | *tmed2* | ↑ | ↑ |  |  |

**Table 3.** Results across all three analysis methods in the CNS and eyes related to the cell cycle, cell proliferation and differentiation, and neural wiring. Genes identified as significantly differentially expressed (DE) between current-day and elevated CO2 conditions, GO Terms / functional categories significantly affected by elevated CO2 treatment as identified by gene set enrichment analysis (GSEA), and genes identified as correlated with both CO2 treatment and one or more OA-affected behaviours by weighted gene co-expression network analysis (WGCNA). See Table 2 for a description of the symbols.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **DE** | | | **GSEA** | | | **WGCNA** | | | | |
|  | **Gene** | **CNS** | **Eyes** | **GO Term / Functional Category** | **CNS** | **Eyes** | **Gene** | **CNS** | | **Eyes** | |
|  |  |  |  |  |  |  |  | **CO2** | **Behaviour** | **CO2** | **Behaviour** |
| **Cell cycle** | | | |  |  |  |  |  |  |  |  |
| **Interphase: G1 phase** |  |  |  |  |  |  | *cdt1, snd1* | ↑ | ↑ |  |  |
| **Interphase: S phase** |  |  |  | 'DNA replication' | ↑ |  | *tubg1, nat10, psf2, cdt1, mcm5, dbf4, spt16, foxm1, bptf* | ↑ | ↑ |  |  |
| **Interphase: G2 phase** |  |  |  |  |  |  | *ranbp2, donson* | ↑ | ↑ |  |  |
| **Mitosis** |  |  |  | 'chromosome', 'chromatin organization' | ↑ |  | *ncaph, smc4, ttk, incenp, bub1, foxm1, cdt1* | ↑ | ↑ |  |  |
|  |  |  | 'spindle', 'spindle assembly' | ↑ |  | *bub3* | ↑ | ↑ |  |  |
|  |  |  | 'cell division' | ↑ |  | *zip, myh9/10, myl9, anln, prpf40a* | ↑ | ↑ |  |  |
| **Cell cycle regulation** |  |  |  | 'regulation of cell cycle', 'protein kinase binding', ‘protein kinase activity’ | ↑ |  | *foxm1, eif3b, ccnb3, melk, ttk, bub1, dbf4, anb32a, klf10, ecd* | ↑ | ↑ |  |  |
|  |  |  |  |  |  | *stk10, calm* | ↓ | ↓ |  |  |
| **Cell proliferation and differentiation** | | | |  |  |  |  |  |  |  |  |
| **Cell proliferation** |  |  |  | ‘protein serine/threonine kinase activity’ | ↑ |  | *ttk, eif3b, foxm1, melk, dbf4, srrt, tk1, tgfb1i1, pa2g4, ttc3, ptpr* | ↑ | ↑ |  |  |
|  |  |  | ‘protein serine kinase activity’, ‘protein threonine kinase activity’ | ↑ | ↓ | *gid-4* | ↑ |  | ↑ | ↓ |
|  |  |  |  |  |  | *bcar3* | ↓ | ↑ |  |  |
| **Cell differentiation** |  |  |  |  |  |  | *eif3b, rac1, ptpr, tbc1d1, tgfb1i1, itga4, pa2g4, slc4a11* | ↑ | ↑ |  |  |
|  |  |  |  |  |  | *btg1* | ↓ | ↓ |  |  |
| **Neuronal differentiation** |  |  |  |  |  |  | *ttc3, rac1* | ↑ | ↑ |  |  |
| **Neurogenesis** |  |  |  |  |  |  | *ncaph* | ↑ | ↑ |  |  |
|  |  |  |  |  |  | *cdk10* | ↑/↓ | ↓ | ↓ | ↑ |
| **Neural Wiring** | | |  |  |  |  |  |  |  |  |  |
| **Cell migration** |  |  |  | 'motor activity', 'actin binding' | ↑ |  | *arpc5, anln, myl9, slit3, mbtp, prpf40a* | ↑ | ↑ |  |  |
|  |  |  |  |  |  | *dag* | ↓ | ↓ |  |  |
|  |  |  |  |  |  |  | *zranb1* | ↓ | ↑ | ↓ | ↑ |
| **Cell adhesion** |  |  |  | 'cell adhesion', 'integrin complex' | ↑ |  | *itga4, itga9, slc4a11, pcdh1, vcl, rac1, ptpr, pa2g4* | ↑ | ↑ |  |  |
|  |  |  | 'homophilic cell adhesion via plasma membrane adhesion molecules' | ↓ | ↑ | *pcdh1* | ↓ | ↓ |  |  |
| **Neurite growth and synapse formation** |  |  |  |  |  |  | *adgrb3, rac1, apbb1, ptpr* | ↑ | ↑ |  |  |
|  |  |  |  |  |  | *futsch* | ↓ | ↓ |  |  |

**Table 4.** Results across all three analysis methods in the CNS and eyes related to transcription, RNA processing and protein processing. Genes identified as significantly differentially expressed (DE) between current-day and elevated CO2 conditions, GO Terms/functional categories significantly affected by elevated CO2 treatment as identified by gene set enrichment analysis (GSEA), and genes identified as correlated with both CO2 treatment and one or more OA-affected behaviours by weighted gene co-expression network analysis (WGCNA). See Table 2 for a description of the symbols.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **DE** | | | **GSEA** | | | **WGCNA** | | | | |
|  | **Gene** | **CNS** | **Eyes** | **GO Term / Functional Category** | **CNS** | **Eyes** | **Gene** | **CNS** | | **Eyes** | |
|  |  |  |  |  |  |  |  | **CO2** | **Behaviour** | **CO2** | **Behaviour** |
| **Transcription** | *chrac1, znf271* | ↑ |  | ‘DNA duplex unwinding', 'nuclear pore', 'regulation of transcription, DNA-templated', 'positive regulation of nucleic acid-templated transcription', 'transcription regulator complex', ‘transcription coactivator activity’ | ↑ |  | *polr1a, spt16, bptf, arpc5, nup160, nup155, nup205* | ↑ | ↑ |  |  |
|  | *nme6, gtf2e2* | ↓ |  |  |  |  |  |  |  |  |  |
| **RNA** |  |  |  |  |  |  |  |  |  |  |  |
| **RNA Processing** |  |  |  | ‘RNA processing', 'mRNA processing', 'rRNA processing', ‘mRNA transport', 'rRNA binding' | ↑ |  | *cstf1, exosc10, nat10, pa2g4* | ↑ | ↑ |  |  |
| **RNA Splicing** |  |  |  | ‘regulation of alternative mRNA splicing, via spliceosome', 'mRNA cis splicing, via spliceosome', 'U2snRNP', 'U4/U6 x U5 tri-snRNP complex' | ↑ |  | *melk, snrpa, ecd, prpf40a* | ↑ | ↑ |  |  |
|  |  |  |  |  |  |  | *snrnp200* | ↑/↓ | ↑/↓ |  |  |
| **Protein** |  |  |  |  |  |  |  |  |  |  |  |
| **Translation** |  |  |  | 'translation' | ↑ | ↑ | *eif3b, eif3d, eef1g* | ↑ | ↑ |  |  |
|  |  |  |  | 'translation initiation', 'translation initiation factor activity', 'eukaryotic 48S preinitiation complex', 'eukaryotic 43S preinitiation complex', 'formation of cytoplasmic translation initiation complex', 'cytoplasmic translation' | ↑ |  |  |  |  |  |  |
| **Ribosome** |  |  |  | ‘ribosome', 'structural constituent of ribosome' | ↑ | ↑ | *rpl23a, rpl4, rpl7l, rps27a, nop58* | ↑ | ↑ |  |  |
|  |  |  |  | 'cytosolic small ribosomal subunit', 'cytosolic large ribosomal subunit', 'small-subunit processome', 'ribosome biogenesis', 'ribosome binding' | ↑ |  |  |  |  |  |  |
| **Endoplasmic reticulum** |  |  |  | ‘endoplasmic reticulum’, ‘endoplasmic reticulum membrane’ | ↑ |  |  |  |  |  |  |
| **Protein trafficking** |  |  |  |  |  |  | *stt3a, tmed2, rpn2, rpn1* | ↑ | ↑ |  |  |
|  |  |  |  |  |  | *srp72* | ↓ |  | ↓ | ↑ |
| **Protein modification** |  |  |  | ‘peptide cross-linking’, ‘protein-glutamine gamma-glutamyltransferase activity’ | ↑ |  |  |  |  |  |  |
| **Protein folding** |  |  |  |  |  |  | *hspa5, pdia3, pdia4, pdia5, ssr1, ganab, ppib* | ↑ | ↑ |  |  |
| **Protein degradation: ubiquitination and proteasome** | *syvn1-b* | ↑ |  | ‘proteasome complex’ | ↑ | ↑ | *rpn1, ttc3, cblb* | ↑ | ↑ |  |  |
|  |  |  | ‘peptide metabolic process’ |  | ↑ | *vhl* | ↓ | ↑ | ↓ |  |
|  |  |  | ‘protein ubiquitination’, ‘protein deubiquitination’, ‘ubiquitin binding’, ‘ubiquitin-dependent protein catabolic process’, ‘thiol-dependent ubiquitin-specific protease activity’ | ↑ |  | *derl1* | ↓ | ↑ |  |  |
| **Protein degradation: lysosomal** | *ykt6* | ↑ | ↑ |  |  |  | *tmcc1/2* | ↓ | ↓ |  |  |

**Table 5.** Results across all three analysis methods in the CNS and eyes related to the immune response. Genes identified as significantly differentially expressed (DE) between current-day and elevated CO2 conditions, GO Terms/functional categories significantly affected by elevated CO2 treatment as identified by gene set enrichment analysis (GSEA), and genes identified as correlated with both CO2 treatment and one or more OA-affected behaviours by weighted gene co-expression network analysis (WGCNA). See Table 2 for a description of the symbols.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **DE** | | | **GSEA** | | | **WGCNA** | | | | |
|  | **Gene** | **CNS** | **Eyes** | **GO Term / Functional Category** | **CNS** | **Eyes** | **Gene** | **CNS** | | **Eyes** | |
|  |  |  |  |  |  |  |  | **CO2** | **Behaviour** | **CO2** | **Behaviour** |
| **Immune** |  |  |  |  |  |  |  |  |  |  |  |
| **Sensor** | *pglyrp2* |  | ↓ | 'scavenger receptor activity' |  | ↑ |  |  |  |  |  |
| **Immune signalling pathways** | *map4k5/3, syvn1-b* | ↑ |  |  |  |  | *abhd12* | ↑ | ↑ |  |  |
| *psenen* | ↓ |  |  |  |  |  |  |  |  |  |
| *cbs, maoa* |  | ↑ |  |  |  |  |  |  |  |  |
| **Effector: Controlling resources required by pathogens** | ***tf,*** *nme6* | ↑ |  |  |  |  | ***tf*** | ↑ | ↑ |  |  |
| **Effector: Autophagy** | *map1l3ca/b* | ↓ |  |  |  |  |  |  |  |  |  |
| **Effector: Phagocytosis** |  |  |  | 'cell adhesion', 'integrin complex', ‘motor activity’, ‘actin binding’, ‘microtubule cytoskeleton’ | ↑ |  | *itga4, itga9, rac1, ptpr* | ↑ | ↑ |  |  |
|  |  |  | ‘cytoskeleton’, ‘intermediate filament cytoskeleton organisation’ |  | ↓ |  |  |  |  |  |

**Table 6.** Results across all three analysis methods in the CNS and eyes related to cellular stress. Genes identified as significantly differentially expressed (DE) between current-day and elevated CO2 conditions, GO Terms/functional categories significantly affected by elevated CO2 treatment as identified by gene set enrichment analysis (GSEA), and genes identified as correlated with both CO2 treatment and one or more OA-affected behaviours by weighted gene co-expression network analysis (WGCNA). See Table 2 for a description of the symbols.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **DE** | | | **GSEA** | | | **WGCNA** | | | | |
|  | **Gene** | **CNS** | **Eyes** | **GO Term / Functional Category** | **CNS** | **Eyes** | **Gene** | **CNS** | | **Eyes** | |
|  |  |  |  |  |  |  |  | **CO2** | **Behaviour** | **CO2** | **Behaviour** |
| **Oxidative Stress** |  |  |  |  |  |  |  |  |  |  |  |
| **Antioxidant and ROS production** | ***tf*** | ↑ |  |  |  |  | ***tf****, slc4a11* | ↑ | ↑ |  |  |
|  | *cyb561d2* | ↓ |  | ‘glutathione metabolic process’ | ↓ |  |  |  |  |  |  |
|  | *cbs* |  | ↑ |  |  |  |  |  |  |  |  |
| **DNA damage and repair** | *chrac1* | ↑ |  | 'damaged DNA binding’, ‘DNA repair’ | ↑ |  | *spt16, foxm1, bptf, arpc5* | ↑ | ↑ |  |  |
|  |  |  |  |  |  |  | *nit1* | ↓ | ↓ |  |  |
| **Protein damage and ER stress** | *ykt6* | ↑ | ↑ | ‘endoplasmic reticulum’, ‘endoplasmic reticulum membrane’, 'protein ubiquitination', 'protein deubiquitination', 'ubiquitin binding', ubiquitin-dependent protein catabolic process', 'thiol-dependent ubiquitin-specific protease activity' | ↑ |  | *hspa5* | ↑ | ↑ |  |  |
|  |  |  |  | 'proteasome complex' | ↑ | ↑ |  |  |  |  |  |
|  |  |  |  | 'peptide metabolic process' |  | ↑ |  |  |  |  |  |
| **Cellular stress-induced apoptosis** | *syvn1-b* | ↑ |  | ‘regulation of apoptotic process’ | ↑ |  | *apbb5, tmem214-b* | ↑ | ↑ |  |  |