Figures and figure supplements

Seminal fluid compromises visual perception in honeybee queens reducing their survival during additional mating flights

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Figure 1. Semen and seminal fluid inseminations cause expression changes in honeybee queen brains, as revealed by Non-metric Multidimensional Scaling (NMDS) of Bray-Curtis dissimilarities between brain samples based on the 1327 differentially expressed genes identified with DESeq2 analyses. Samples from truly inseminated queens (semen and pure seminal fluid without sperm) are separated from controls (Hayes saline and mechanical mock insemination) after removal of batch effect caused by two distinct RNA-sequencing experiments being combined. A stress value well below 0.2 of the NMDS analysis indicates that after reduction to two dimensions the plot captures the relevant variation fairly well.

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**Figure 1—figure supplement 1.** Volcano plots presenting significance (-log10 (P value)) versus log2 (fold change) for all pair-wise comparisons between treatment groups in RNA-sequencing Experiments 1 and 2. Genes with unchanged expression are plotted as black dots, differentially expressed genes with FDR < 0.05 as red dots, genes with log2 (fold change)>1 as orange dots, and genes with both FDR < 0.05 and log2 (fold change)>1 as green dots.

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Figure 1—figure supplement 2. Venn diagram showing overlap between differentially expressed genes identified in each of the pair-wise comparisons of insemination treatments against controls across the two experiments. DOI: https://doi.org/10.7554/eLife.45009.005
Figure 1—figure supplement 3. Networks representing interactions between enriched GO terms (p<0.05) in all pair-wise comparisons between insemination treatment groups in Experiments 1 and 2. Circle colours represent enrichment P values (red more significant, white less significant, but still significant).
below 0.05) and circle sizes are proportional to the frequency of GO terms in the underlying Gene Ontology Annotation (GOA) database (Barrell et al., 2009). Highly similar GO terms are linked by grey-line edges with line-width indicating degree of semantic similarity (the distance between terms is based on the likeness of their meaning). Nodes are placed based on an edge-weighted spring-embedded layout algorithm, which keeps nodes with higher semantic similarity closer together.

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**Figure 2.** Seminal fluid and semen induce expression changes in honeybee queen brains for genes mapping to the phototransduction pathway of *Drosophila* in both Experiment 1 (A) and Experiment 2 (B). The plotted KEGG model represents the microvillus of a photoreceptor cell with the major proteins in the pathway shown by rectangles. Photoreceptors use the pigment rhodopsin (bottom grey panel) to absorb light, after which phospholipase protein C (PLC), upon activation via rhodopsin and the G-protein Gq, hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to generate diacylglycerol (DAG), inositol 1,4,5-trisphosphate (InsP3) and a proton, resulting in the activation of two classes of Ca$^{2+}$-permeable cation channels, TRP and TRPL (Hardie, 2012) (top right). Several components of the cascade, including TRP, PLC, and protein kinase C (PKC), are assembled into multimolecular signaling complexes by the scaffolding protein INAD (Hardie, 2012), which has been suggested to be linked to the central F-actin filament via the ninaC class III myosin (Hicks et al., 1996) (bottom centre). The electrical impulses generated by light absorption reach the brain...
Figure 2 continued

through the visual fibres of photoreceptor cells, which extend into the optic lobes (Ehmer and Gronenberg, 2002; Wernet et al., 2015). Results of Experiment 1 and Experiment 2 are shown in separate panels with quadrants within each protein-rectangle showing differences in the expression of the underlying coding genes of the first treatment group in each pair-wise comparison, relative to the second treatment group as shown above each of the figure panels (red for up-regulation, green for down-regulation, grey if no significant difference between treatments was detected but the gene was expressed in our datasets, white if the gene was not expressed).

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Figure 3. Heatmaps of gene expression for genes mapping to the phototransduction pathway in Experiment 1 (A) or Experiment 2 (B). Colours in each cell represent counts normalized by variance stabilizing transformation (Huber et al., 2003), which were then centred over the mean across samples. Only genes having expression differences between treatment groups above noise levels based on separate analyses performed with the essGene function (R Bioconductor package GAGE) in Experiment 1 or two are shown (legends to the right). Column and row dendrograms represent sample and gene clustering, respectively, based on Euclidean distances, reflecting that samples represent different treatments (central legend at the bottom) and that gene expression reacted differently to treatments. Small plots towards the top left of panels represent colour legends for expression values (x axis), and the distribution of gene counts (y axis) for these values across all genes and samples is depicted by blue lines. Bottom legends: MI = mock insemination, SF = seminal fluid insemination, INS = semen insemination, and HS = Hayes saline insemination.

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Figure 3—figure supplement 1. Differences in expression between pair-wise combinations of treatment groups of genes mapping to the KEGG neuroactive ligand-receptor interaction pathway for Experiments 1 and 2. Separately colour-marked quadrants within each gene box show differences between gene expression in different treatment conditions.
in expression for the first treatment group relative to the second in each pair-wise comparison as explained in the legends above the figure panels (red for up-regulation, green for down-regulation, grey if no significant difference between treatments was detected but the gene was expressed in our datasets, white if the gene was not expressed).

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Figure 3—figure supplement 2. Heatmaps of gene expression for genes mapping to the KEGG neuroactive ligand-receptor interaction pathway in Experiments 1 (A) or 2 (B). Colours in each cell represent counts normalized by variance stabilizing transformation (Huber et al., 2003), which were then centred over the mean across samples. Only genes having expression differences between treatment groups above noise levels based on separate analyses performed with the essGene function (R, R Core Development Team, 2015; Bioconductor package GAGE, Luo et al., 2009) in Experiment one and/or two are shown (legends to the right). Column and row dendrograms represent sample and gene clustering, respectively, based on Euclidean distances, reflecting that samples represent different treatments (central legend at the bottom). Small plots towards the top left of panels represent colour legends for expression values (x axis), and the distribution of gene counts (y axis) for these values across all genes and samples is depicted by blue lines. Bottom legends: MI = mock insemination, SF = seminal fluid insemination, INS = semen insemination, and HS = Hayes saline insemination.

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Figure 3—figure supplement 3. Receptor genes for, respectively, glutamate (first plot), serotonin (second and third plots), and N-Methyl-D-aspartic acid (last plot), showing consistent expression differences between

Figure 3—figure supplement 3 continued on next page
Figure 3—figure supplement 3 continued

insemination treatments in individual RNA-sequencing samples of honeybee brains in Experiment 1 (left panels) and Experiment 2 (right panels).

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Figure 4. Response amplitude of electroretinograms (ERGs) measured from compound eyes (A) and ocelli (B) and mean contrast sensitivity (the lowest contrast to produce a detectable response) of ERGs from compound eyes (C) of honeybee queens stimulated with flickering lights of different frequencies (Hz). All measurements were taken one and two days after artificial inseminations with either semen, seminal fluid or Hayes saline control solution (see colour legend). Panel C includes all measurements taken at all stimulus intensities. Total sample sizes (n) are shown in the top centre of each panel and differ because some queens were exclusively measured at day 1 (N = 10; seminal fluid: N = 5, semen: N = 3, Hayes saline: N = 2) or at day 2 (N = 9; seminal fluid: N = 4, semen: N = 3, Hayes saline: N = 2), whereas the remaining queens were consecutively assessed on both days (N = 18; seminal fluid: N = 5, semen: N = 6, Hayes saline: N = 7). Two queens were excluded from the ocelli dataset because of large technical noise. See Materials and Methods for additional details.

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Figure 4—figure supplement 1. Experimental set-up for electroretinography to measure honeybee queen eyesight responses to flickering light or short (1 ms) light impulses.
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Figure 4—figure supplement 2. An example of response amplitude (mV) at different contrasts to calculate a contrast threshold measured at the highest intensity and temporal frequency of 4 Hz. The amplitudes to the flickering light stimuli typically decreased as stimulus contrast reduced. The horizontal black line represents the noise threshold of 0.04 mV, determined from the control measurement with blocking the light stimuli. Red data points indicate responses that were significantly different from the surrounding noise, whereas black data points indicate responses that were not significantly different. The blue line is the fitted regression line of the response amplitude. The contrast threshold was determined as the intercept of the fitted response amplitude line and the noise threshold (red circle) for each temporal frequency. Contrast sensitivity was defined as the inverse of the contrast threshold.

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Figure 4—figure supplement 3. Response duration (A), latency (B) and amplitude (C) of electroretinograms (ERGs) measured from compound eyes of queens treated with semen, seminal fluid or Hayes saline after exposure to 1 ms light impulses.

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Figure 4—figure supplement 4. Examples of impulse responses to 1 ms flashes of light in (A) median ocellus and (B) compound eye of honeybee queens. Peak amplitude (green point) was determined by the minimum amplitude of response. The latency of impulse response (red point) was defined as the time taken for the response to attain −0.5 mV amplitude. The duration of impulse response (blue line) was measured as the half-width of response size. The impulse response of compound eye has an inset (black point) considered as an input from high order neurons, such as a lamina neuron.

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Figure 5. Seminal fluid and semen induce alterations of mating flight behaviour in honeybee queens. (A) Stacked bars showing the percentage of queens in each artificial insemination treatment that were lost and how many of them produced brood after returning from their last mating flight, with absolute sample sizes printed on top of the bars. (B) Effects of insemination and control treatments on the time from artificial insemination to making their first and last flight. Bars show mean ±SE.
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