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Virus specific impacts on honey bee flight performance are mediated by the octopamine pathway

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eLife Assessment

This **important** study investigates how distinct honey bee viruses differentially alter flight performance through interactions with octopamine signaling pathways. The combination of behavioral flight assays, pharmacological perturbation, and transcriptomic analyses provides **solid** evidence that virus-specific effects on flight are associated with octopamine signaling. However, some of the stronger mechanistic conclusions regarding direct regulation of octopamine signaling remain **incomplete** without more specific validation of receptor-level effects and direct quantification of octopamine levels or signaling activity.

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Abstract

High annual honey bee colony losses are associated with environmental and biological stressors, including virus infections. In insects, the octopamine pathway orchestrates the “fight-or-flight” response, regulating energy mobilization, temperature, and flight. We determined that sacbrood virus (SBV) infections induce expression of an octopamine receptor and enhance honey flight performance, whereas deformed wing virus (DWV) infections reduce flight performance, but how viruses interface with this pathway remained unknown. To elucidate the relationships between the octopamine response, virus infection, and flight, honey bees were infected with SBV or DWV and exposed to octopamine (OA), epinastine (EP)–an OA receptor antagonist, or both OA and EP; flight and gene expression were assessed. Pharmacologic manipulation revealed that octopamine supplementation rescued flight deficits in DWV-infected bees, but diminished performance in SBV-infected bees, while blocking octopamine receptors altered these effects. Transcriptome analyses indicated that SBV infections, and DWV infection with OA treatment, activated honey bee metabolic pathways, and that SBV infected bees had greater expression of genes involved in OA synthesis, unless treated with OA. These results provide a mechanistic explanation for virus-specific impacts on honey bee flight, which may have consequences on foraging efficiency, colony health and virus transmission.

Introduction

Honey bees (*Apis mellifera*) are eusocial, generalist pollinators that live in densely populated colonies of ~30,000 individuals (1, 2). The survival of each colony depends on the coordinated activities of thousands of individual bees that provision nutritional resources, rear brood, and regulate internal hive temperature (3–5). Colony losses in the U.S. averaged 40% from 2008-2025 (6–10), these losses are associated with several abiotic and biotic stressors, including parasites and pathogens (11–14). Honey bee pathogens include bacteria, microsporidia, and viruses (15, 16). Viruses may be transmitted vertically, horizontally, or by vectors, including the *Varroa destructor* mite (17–21). *Varroa* mites feed on honey bees and infestations may kill colonies (22). In addition,

Varroa mites vector viruses, including deformed wing virus (DWV), which replicates in the mite, and others that are mechanically transmitted such as sacbrood virus (SBV) (23). Most characterized bee infecting viruses have positive single-stranded RNA (+ssRNA) genomes including members of *Iflaviridae* (e.g., DWV and SBV). Virus infections in honey bees may remain seemingly asymptomatic, cause morphological symptoms, paralysis, and/or result in death (15, 24–28). Honey bees that lack morphological symptoms may still harbor high virus levels that impact lifespan and flight performance (29–32).

Flight is an energetically taxing behavior that is essential for honey bee foraging, defecation, and mating (33–38), and flight muscles are essential for temperature regulation (i.e., fanning to cool and 'shivering' for heat) (17, 39–42). In our previous work, we used flight as a proxy for overall bee health and demonstrated that honey bees harboring high levels of DWV without obvious symptoms had impaired flight performance (29). Unexpectedly, we determined that the flight performance of SBV infected honey bees was enhanced and linked to the metabolism-stimulating, stress-induced octopamine pathway (29). Abiotic and biotic stressors may induce enhanced activity of stress pathways including the octopamine response. Invertebrates synthesize octopamine (OA), a 'fight or flight' neurohormone analogous to norepinephrine, which facilitates energy production for taxing activities including flight (43–47). The octopamine response may also be triggered by other stressors, including pathogen infections (29, 48, 49).

Whether octopamine signaling is immunosuppressive or immunoenhancing is dependent on multiple factors and its role in honey bee antiviral defense has not been thoroughly investigated (50–52). Honey bee behaviors may be influenced by multiple monoamine neurohormones including OA, serotonin, dopamine, and tyramine (44, 53–58). Octopamine promotes movement, neuromuscular signaling, and metabolism. To synthesize OA, tyrosine is first converted to tyramine by Tyrosine decarboxylase (TDC); tyramine is the precursor neurohormone to OA and is generally associated with reduced flight and movement (45, 59, 60). Tyramine may serve as an antagonistic modulator of behavior to OA or Tyramine β-hydroxylase (TβH) may convert it to OA (Fig 1A). One of the most abundant OA receptors in honey bees is a G-coupled protein receptor, Oβ-2R (61). When OA binds to Oβ-2R, it activates adenylyl cyclase (AC), which increases intracellular concentration of cyclic adenylyl monophosphate (cAMP). Elevated cAMP activates cAMP-dependent Protein kinase A (PKA) which phosphorylates metabolic enzymes and transcription factors that generally enhance metabolic activity (Fig 1B) (40, 46, 61, 62).

We hypothesized that SBV infections, which correlate with higher expression of an OA receptor (Oβ-2R) (29), activate the OA response which results in enhanced flight performance. Therefore, we predicted that OA treatment would enhance honey bee flight performance regardless of virus infection. Epinastine (EP) is a highly specific OA receptor antagonist that does not appreciably interfere with other neurohormone receptor binding (63, 64). Therefore, we hypothesized that EP treatment would reduce honey bee flight performance and negate SBV-associated enhanced flight performance.

Results

To test these hypotheses, we exposed virus infected honey bees to OA, EP, or both OA and EP and assessed flight performance and gene expression (e.g., *tdc*, *tβh*, and *oβ-2R*) in three independent experiments (i.e., exp- 1, 2, and 3) (Fig 1 and Data S1). Specifically, age-matched adult bees (n = 336) were experimentally infected with DWV (n = 117), SBV (n = 75), or mock infected (buffer injection, n = 144) and treated with OA (10 mM), EP (4 mM), or both OA and EP via injection or feeding, or not treated; all bees were fed 50% sucrose syrup *ad libitum*.

Treatments of OA and EP were given in 2 μL injections or fed at the same concentration in sucrose syrup; doses were in-line with previous studies (29, 40, 45, 63). Exposure to OA and/or EP via injection was temporally limited, whereas *ad libitum* feeding provided continuous exposure (Fig 1).

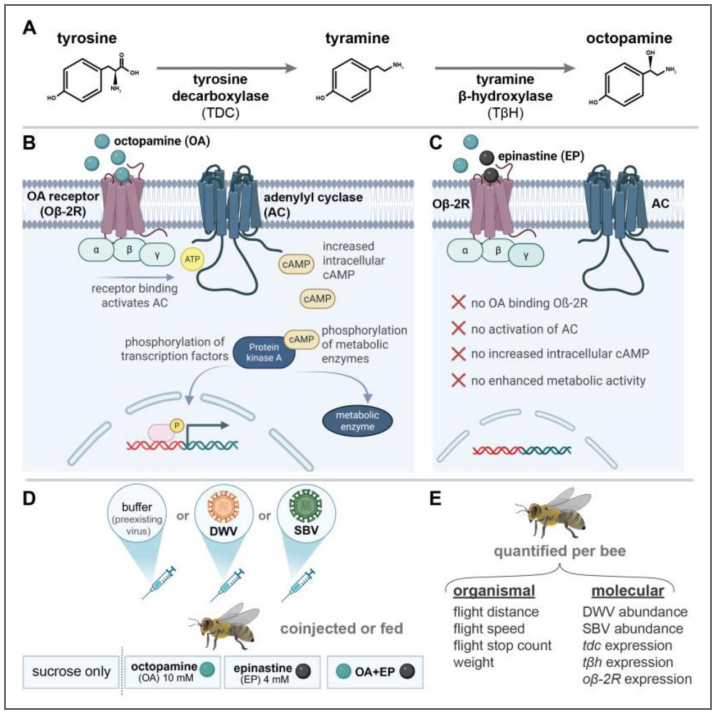


Figure 1. Octopamine signaling pathway (i.e., synthesis, activation, inhibition) and experimental design

(A) Octopamine (OA) is synthesized by conversion of tyrosine to tyramine by tyrosine decarboxylase (TDC) and tyramine conversion to OA by tyramine β-hydroxylase (TβH). (B) OA β-2 receptor (Oβ-2R) is one of the most abundant and specific OA-detecting receptors. Upon activation of Oβ-2R, a G-coupled protein receptor, adenylyl cyclase (AC) is activated and increases intracellular cyclic adenylyl monophosphate (cAMP) levels. High cAMP levels induce protein kinase A activity which phosphorylates metabolic enzymes and transcription factors that enhance metabolic activity. (C) An OA receptor antagonist, epinastine (EP), inhibits OA binding (D) To evaluate relationships between virus infection and the OA response, and prevents activation of the Oβ-2R pathway honey bees were inoculated with either deformed wing virus (DWV) or sacbrood virus (SBV) via injection or mock infected. Bees were exposed to either OA, EP, or both OA and EP via co-injection or feeding, all bees were fed sucrose. (E) Impacts of experimental conditions were quantified in individual bees 72 hours post infection. Panels B-D reproduced from Kaku, 2025.

Quantification of experimentally introduced and naturally occurring honey bee virus infections

Honey bees were tested for preexisting infections using pathogen-specific polymerase chain reactions (PCR) and detected pathogens were quantified using quantitative PCR, as described previously (29) (Data S1-S3). We determined that honey bees in exp-1 had preexisting DWV infections and bees in exp-2 and exp-3 had preexisting infections of DWV and SBV (S1 Fig).

Most bees analyzed herein had either experimentally introduced or preexisting DWV infections (n = 325/336) and 40% had experimentally introduced or preexisting SBV infections (n = 134/336). DWV infections ranged between 1×10^3 and 7.1×10^{10} copies per 2 µg of RNA ($\sim7\times10^3$ – 8×10^{11} DWV copies per bee). SBV infections were between 1.2×10^3 and 1.2×10^{10} copies per 2 µg of RNA ($\sim8.3\times10^4$ – 8.3×10^{11} SBV copies per bee). Due to high DWV and SBV prevalence there were no virus-free honey bees. Mock infected bees harbored low virus levels (i.e., an average of 3×10^5 DWV copies and 2×10^3 SBV copies per 2 µg RNA) and inoculation resulted in higher virus loads (i.e., an average of 2×10^8 DWV copies and 2×10^8 SBV copies per 2 µg RNA), $p = 2\times10^{-16}$ and 5×10^{-11}, respectively). To compare the impact of varying virus levels and treatments, we evaluated several flight metrics (i.e., flight distance, duration, speed, and stop count; Data S1) using linear mixed effect models (LMMs) (complete information for each model and model selections in methods and Data S4).

OA and EP altered flight performance of virus infected bees

The previously described DWV-associated flight impairment relative to mock infected bees (29), was recapitulated in this study (i.e., DWV infected bees flew shorter distances ($p = 0.005$)). Mock infected bees from this sample cohort, which harbored low virus levels, flew an estimated 45 meters, whereas bees harboring high DWV levels (i.e., 10^8 DWV RNA copies / 2 µg RNA) flew only ~9 meters. As expected, DWV-associated flight impairment was alleviated by OA feeding or injection (i.e., average flight distance was similar to mock infected bees fed sucrose, $p = 0.61$ and 0.08, respectively) (Fig 2A). Honey bees that received EP flew shorter distances ($p = 0.002$), but the effect of feeding EP was not assessable since bees with experimentally introduced DWV infections that were fed EP died, and there were only 14 bees with low DWV infection levels (Fig 2B, Data S4). Co-injection of OA and EP negatively impacted the distance DWV infected honey bees flew ($p = 0.020$) (Fig 2C). Counter to our hypothesis, OA-treatment did not enhance flight performance of SBV infected bees ($p = 0.69$ and 0.60) (Fig 2D, Data S4). SBV infected bees that were treated with EP or co-treated with both EP and OA via injection flew shorter distances than untreated SBV infected bees ($p = 0.022$ and 0.004, respectively), whereas feeding had no impact (Fig 2E-F).

The amount of time a honey bee was actively flying was also assessed as an indicator of energetic capacity (S2 Fig). Honey bees harboring high DWV levels (i.e., $>10^8$ DWV copies / 2 µg RNA) flew only 14 minutes ($p = 0.022$), relative to mock infected bees with low virus infection levels, which flew for 26 minutes. Honey bees with high SBV levels flew for similar durations to mock infected bees (i.e., 28 minutes, $p = 0.58$), which is consistent with previous results (29). In line with our hypotheses, mock infected bees with low virus infection levels flew longer when they were fed OA (i.e., 49 minutes) ($p = 0.0001$). DWV-associated flight impairment was mitigated in honey bees that were supplemented with OA, which flew for similar durations to mock infected bees (i.e., 28 minutes, $p = 0.76$). However, counter to our hypothesis, when SBV infected bees were injected or fed OA, flight duration was significantly reduced (i.e., 18 and 12 minutes, respectively; $p = 0.04$ and 0.0009).

To quantify the impact of virus and OA and/or EP treatment on honey bee flight speed, we compared average and peak speeds. Average flight speed (i.e., total distance / total flight duration) was similar across all conditions except in bees fed EP, which flew at slower average speeds ($p = 3\times10^{-4}$) (Data S4). We also compared peak speed (i.e., maximum speed reached during flight sustained >10 seconds) (S2 Fig). Similar to previously reported results (29), DWV infected bees had slower peak speeds than mock infected bees ($p = 0.016$). SBV infected bees flew similar speeds to

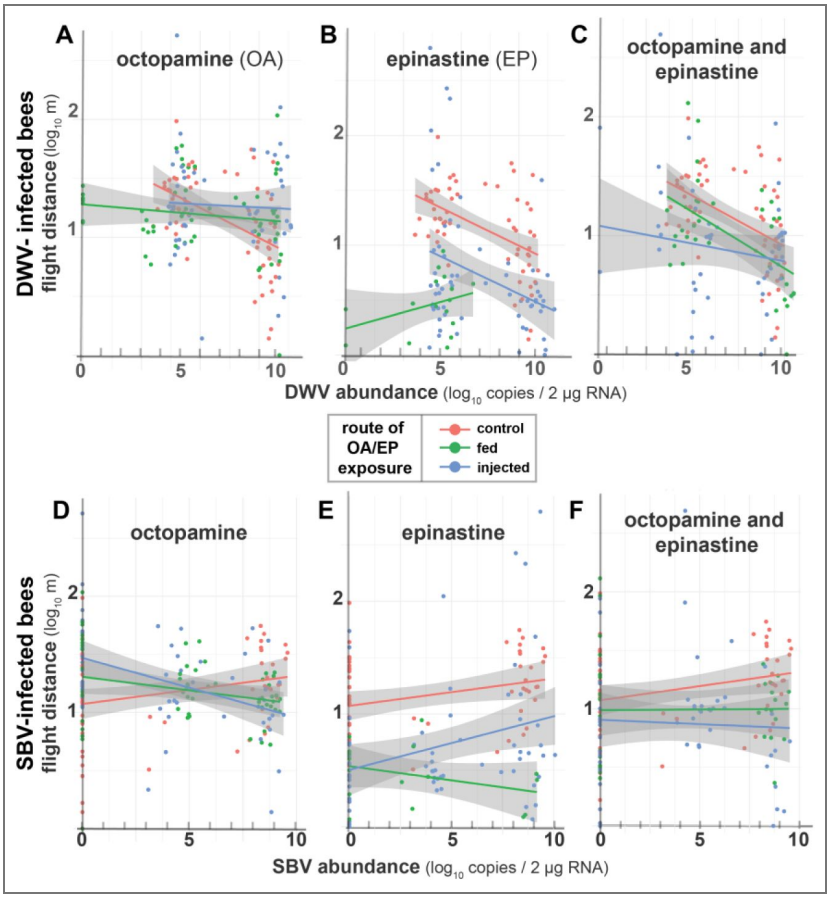


Figure 2. The flight performance of honey bees infected with DWV and/or SBV is differentially impacted by OA and EP treatment

To evaluate the involvement of the OA pathway on virus infection and/or flight performance, virus- or mock- infected honey bees were exposed to OA, EP, or both OA and EP via co-injection (blue) or feeding (green). Honey bees were experimentally infected with or had preexisting infections of DWV (A-C) or SBV (D-F) in three experiments. Flight distance (log_{10} m) was evaluated using linear mixed effect models to quantify impacts of DWV and/or SBV infection in the context of OA and/or EP treatment. Each data point represents an individual bee (n = 336); colors indicate treatment (i.e., bees fed only sucrose in pink, bees fed OA and/or EP in green, and bees injected with OA and/or EP in blue). (A) In bees fed only sucrose, DWV infections negatively impacted flight distance, whereas DWV infected bees treated with OA flew further distances. (B) DWV infected bees fed or injected with EP flew shorter distances than DWV infected bees fed only sucrose. (C) DWV infected bees fed or injected with OA and EP flew similar distances to DWV infected bees fed only sucrose. (D) In the control group, SBV infected bees flew greater distances than uninfected bees. SBV infected bees fed OA flew shorter distances than SBV infected bees fed only sucrose. (E) Honey bees with SBV infections fed or injected with EP flew shorter distances than SBV infected bees fed only sucrose. (F) Bees with SBV infections fed or injected with OA and EP resulted in similar flight distances regardless of SBV infection level.

mock infected bees (p = 0.07), but peak speeds were reduced when SBV infected bees were fed or injected with OA (p = 0.004, p = 0.0006) (Data S4). Mock infected bees flew peak speeds of ~3.3 km/h, honey bees with high levels of SBV (i.e., 10^8 SBV copies / 2 µg RNA) flew ~3.8 km/h, whereas highly SBV infected bees injected or fed OA flew an estimated 2.0 or 2.5 km/h, respectively (p = 0.001 and 0.022).

In addition to flight metrics, post-flight thorax temperatures were measured within a subset of individual bees (n = 130) since flight induces heat and the octopamine response is involved in honey bee temperature regulation (40, 61) (Data S4). The ambient temperature for all flight assays was 22°C, and post-flight thorax temperatures in honey bees maintained in control conditions averaged 28°C. DWV infected bees had similar post-flight temperatures, and highly SBV infected bees were 2°C cooler than control (p = 0.04). Honey bees with DWV infections that were fed OA had thorax temperatures that were 2.5°C higher than bees not fed OA. Whereas the thorax temperatures of bees with DWV infections were 5.6°C lower in bees co-fed OA and EP (p = 0.027 and 1×10^{-6}). Likewise, honey bees with high SBV levels that were injected with OA also had reduced thorax temperatures post-flight (i.e., ~ 3°C lower) (p = 0.03; Data S4).

Virus specific outcomes of experimentally manipulated octopamine pathway explained by differential expression of key genes

Induction of the OA pathway was previously implicated in the enhanced flight performance of SBV infected honey bees, which had elevated expression of *oβ-2R* (29). When OA binds to Oβ-2R, it activates AC, increases cAMP concentration, thereby activating cAMP-dependent PKA, which phosphorylates enzymes and transcription factors that enhance metabolic activity (Fig 1B) (40, 46, 61, 62). To determine if DWV, SBV, or different experimental treatments were associated with differences in OA synthesis, we quantified the expression of *tdc* and *tβh* relative to mock infected honey bees fed only sucrose (Data S1, Fig 3). We evaluated the relationship between *tdc* expression and multiple factors including DWV abundance, SBV abundance, experimental treatments (i.e., OA and/or EP, fed/injection) using LMMs which explained 91% of the data variation (Data S4). SBV infection was associated with increased *tdc* expression (p = 0.021), and SBV infected bees fed OA or co-fed OA and EP also expressed higher *tdc* levels (p = 0.030 and 2×10^{-10}, respectively). Expression of *tdc* was lower in SBV infected bees co-injected with EP, and SBV and DWV-coinfected bees that were fed OA or both OA and EP (p-values = 0.033, 0.022, 0.006, 0.004, respectively) (Fig 3A). Expression of *tdc* was not associated with DWV infection, or DWV infection with OA and/or EP treatment (p-values all >0.2, Data S4). We also examined the effects of virus infection in the context of experimental OA and EP treatments on *tβh* expression (i.e., in bees with detectable *tβh* levels n=306/336) (Fig 3B). An LMM that included DWV abundance, SBV abundance, treatment, and *tdc* expression as explanatory variables explained 85% of the variation in *tβh* expression (Data S4). Expression of *tdc* and *tβh* were positively correlated (with a *tdc*:*tβh* ratio of 1:1.6, p = 0.004) (S4 Fig). Expression of *tβh* was higher in SBV and DWV infected bees (p =0.022 and 1×10^{-9}, respectively), suggesting more tyramine is converted to OA during DWV or SBV infection (Fig 3B). Results from these models indicate that only SBV infections were associated with greater expression of both *tdc* and *tβh* relative to mock infected bees with low preexisting virus levels. Whereas SBV infected honey bees that were co-injected with OA expressed similar levels of *tdc* or *tβh* as mock infected bees (p = 0.421 and 0.437, respectively). This result suggests that the elevated expression of genes involved in OA synthesis in SBV infected honey bees is reduced by OA supplementation and partially explains the impaired flight performance of these bees relative to untreated SBV infected honey bees (Fig 4).

For OA to stimulate metabolic activity and energy production, OA must activate receptors including Oβ-2R (61). To identify variables that contribute to the range of *oβ-2R* expression, we compared LMMs including *tβh* expression, *tdc* expression, SBV abundance, and OA/EP treatment, which explained 95% of the data variation (S4 Fig). We considered including DWV abundance as a fixed effect in this model, but it did not appreciably contribute to *oβ-2R* expression (Data S4). These results corroborate our previous findings that SBV, but not DWV, was associated with

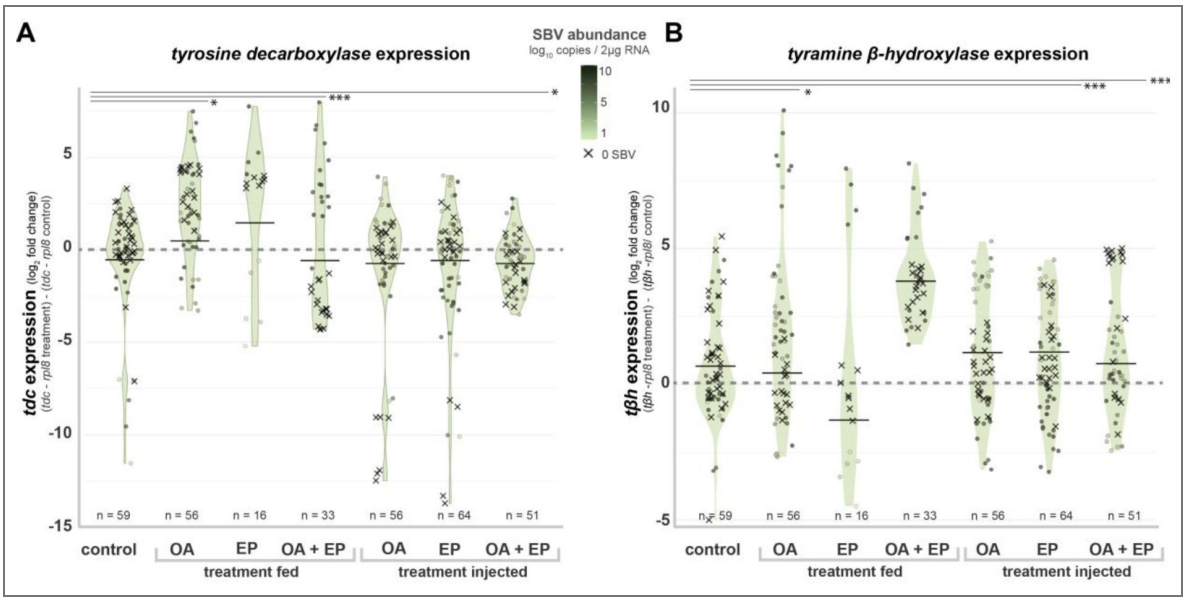
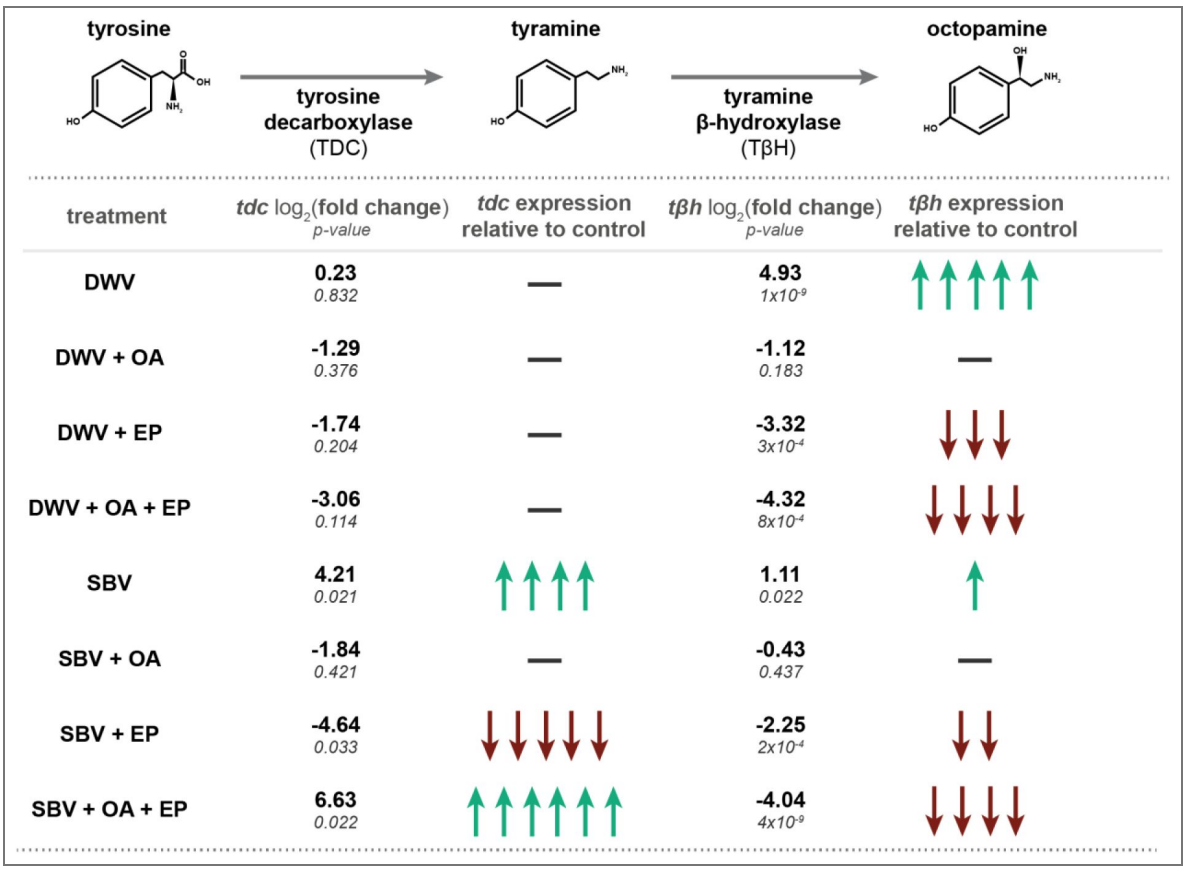


Figure 3. Expression of *tyrosine decarboxylase* (*tdc*) and *tyramine β-hydroxylase* (*tβh*) across treatments and SBV infection

The relationships between honey bee *tyrosine decarboxylase* (*tdc*) and *tyramine β-hydroxylase* (*tβh*) expression in the context of virus infection and treatments were evaluated using a linear mixed effect model that included SBV abundance and OA/EP treatments as fixed effects with an interaction. Individual honey bee data plotted by treatment group with either (A) *tdc* expression or (B) *tβh* expression. Gene expression was calculated as $\log_2$ fold change using the ΔΔCt method relative to housekeeping *rpl8* and compared to mock infected bees. SBV abundance was calculated as $\log_{10}$ copies / 2 µg RNA. The background violin plots represent the 95% confidence interval, and the horizontal line in each violin represents the median. SBV abundance is represented by a green color scale and SBV-negative samples with an "X". When SBV abundance did not impact *tdc* or *tβh* expression the dark green points are evenly distributed above and below the control '0' fold change line, whereas unequal distribution indicates an SBV-specific effect; * p<0.05, **p<0.01, and ***p<0.001. (A) Positive association between SBV abundance and *tdc* expression (p = 0.021). SBV infected bees fed OA or both OA and EP had greater *tdc* expression levels than uninfected bees fed only sucrose (p = 0.03 and 2×10^{-8}). (B) Expression of *tβh* was greater in SBV infected bees than uninfected bees (p =0.022). SBV infected bees fed OA had greater *tβh* expression levels than uninfected bees fed sucrose ($p = 2\times10^{-5}$). SBV infected bees injected with EP or co-injected with OA and EP had lower *tβh* expression ($p = 2\times10^{-5}$ and 4×10^{-9}).



treatment	*tdc* $\log_2$(fold change) *p-value*	*tdc* expression relative to control	*tβh* $\log_2$(fold change) *p-value*	*tβh* expression relative to control
DWV	0.23 *0.832*	—	4.93 *1x10⁻⁹*	↑↑↑↑↑
DWV + OA	-1.29 *0.376*	—	-1.12 *0.183*	—
DWV + EP	-1.74 *0.204*	—	-3.32 *3x10⁻⁴*	↓↓↓
DWV + OA + EP	-3.06 *0.114*	—	-4.32 *8x10⁻⁴*	↓↓↓↓
SBV	4.21 *0.021*	↑↑↑↑	1.11 *0.022*	↑
SBV + OA	-1.84 *0.421*	—	-0.43 *0.437*	—
SBV + EP	-4.64 *0.033*	↓↓↓↓↓	-2.25 *2x10⁻⁴*	↓↓
SBV + OA + EP	6.63 *0.022*	↑↑↑↑↑↑	-4.04 *4x10⁻⁹*	↓↓↓↓

Figure 4. Relative *tdc* and *tβh* expression in honey bees with high DWV or SBV levels

(A) Tyrosine is converted via tyrosine decarboxylase (TDC) to tyramine. Tyramine is a neurohormone that acts as a behavioral antagonist to octopamine (OA), resulting in reduced movement in invertebrates. Tyramine may act as a signaling neurohormone, or tyramine β-hydroxylase (TβH) may convert it to OA. (B) A linear mixed model was used to assess the relative expression of *tdc* and *tβh* in honey bees harboring high levels of either deformed wing virus (DWV) or sacbrood virus (SBV) (i.e., 108 virus copies / 2μg RNA) in the context of OA and/or epinastine (EP) injection. Estimates of relative *tdc* and *tβh* expression were generated based on data from 336 honey bees. The single bold dash indicates *tdc* or *tβh* expression was similar in virus infected bees and mock infected bees and arrows indicate estimates that were higher or lower than controls.

increased *oβ-2R* expression (29). The strongest predictors of *oβ-2R* expression levels were *tdc* and *tβh* expression ($p = 4.4\times10^{-5}$ and 3.9×10^{-5}) and SBV was positively associated with *tdc* and *tβh* expression ($p = 0.0006$) (Data S4).

Transcriptome level impacts of virus infection, flight, and OA treatment

We hypothesized that flight-impacting virus infections would result in differential expression of genes involved in stress, metabolic, and immune pathways. To test this, the transcriptomes of SBV, DWV, DWV and OA, or mock infected honey bees were compared. Honey bees were obtained from a previous experiment and included individuals that did or did not fly (29). RNA was prepared for high throughput sequencing on an Illumina NovaSeq X Plus 25B (n = 36 total individual bee samples, 3-5 per treatment group; Data S6). An average of 25 million reads was obtained for each sequencing library and mapped to the *A. mellifera* assembly HAv3.1 (65, 66). To evaluate similarities and differences between the transcriptional responses of honey bees with flight-impacting virus infections, we compared DWV, SBV, and DWV and OA injected bees relative to mock infected bees immediately post-flight since these bees had little to no detectable preexisting infections, whereas the no-flight mock infected bees had preexisting DWV infections (Data S5-S6).

Hundreds of differentially expressed genes (DEGs) were identified in SBV, DWV, and OA treated DWV infected bees (Fig 5). DWV infected bees with and without OA treatment shared 95 DEGs, which was more than they shared with SBV infected bees, indicating that there are virus specific transcriptional responses. There were 24 genes shared among SBV, DWV, and DWV and OA injected bees including elevated expression of those involved in the Ras-Raf-MEK-ERK pathway (including *mesr3* and *rap2l*) which is associated with metabolism, muscle development, and the cAMP/PKA pathway (67). Genes with lower expression were involved in muscle function (i.e., *mlc2*, *mlc1*, *cher*, *sar*), energy production (i.e., *cnn*, *ant*, *pka-c3*), and an inhibitor of proteolytic activity (i.e., *cyp4g11*).

There were many genes unique to SBV and DWV infections (Fig 5). Honey bees with SBV infections exhibited lower expression of several genes associated with the citric acid cycle (*acsl6*, *cg5065*, *acyl-coA delta*(11), *mcad*, *nd-13a*, *nd-13b*, *nc73ef*, and *mdh2*) and general muscle function and tissue repair genes (*tmp-1*, *tmod*, *plastin-2*, *and fgf17*). SBV infected bees had elevated expression of *rgs11*–which increases G-protein α-subunit active state to inactive to expedite the G-coupled protein response (68), *titin*–which contracts stretched/striated muscle tissues (69), and several immune genes such as *argonaute-2*, *relish*, and *domeless* (Data S6-S7). DWV infected bees exhibited greater expression of several genes associated with immune function including *protein mahjong*, *spastin*, and a small heat shock protein, *protein-lethal*(2) *essential for life-like* (Data S6). The DEGs unique to DWV infected bees post-flight that exhibited lower expression included genes involved with muscle function and structure (*obscurin*, a titin homolog, *ryr*, *actin*, *mhc*, *stacl*, *tpm1*, and *zasp66*) and ATP generation (*citrate synthase*, *adenylate kinase 1*, *ADP/ATP translocase*).

Since OA supplementation mitigated DWV-associated flight impairment, we identified differences associated with OA by comparing DEGs to untreated DWV infected honey bees post-flight. As hypothesized, DWV infected bees that were co-injected with OA treatment had greater expression of OA pathway genes (*oβ-2R*, *adenylyl cyclase (ac)*, and CREB binding proteins) and genes involved in glucose metabolism (*gale*, *gdh*, *adpgk*, *putative glucosylceramidase 4*, *gba1lp*, *g6pd*, *sugarless*, *pdp*, *ND-24*, *NAD kinase*, *tret1*, *1,5-anhydro-D-fructose reductase, and igfals*) (Data S8-S9). These bees also expressed higher levels of genes induced by high cyclic guanine monophosphate (cGMP) levels (i.e., *burgundy*, *cGMP-dependent protein kinase-like*, and *cGMP-dependent protein kinase 1*) and genes in the RAS/MAPK-pathway (*rho1*, *rac1*, and *rab5*). Untreated, DWV infected bees expressed higher levels of several immune genes (*abaecin*, *domeless*, *echinoid*, and *defensin-1*) and heat shock protein encoding genes (*hsp83*, *hsp90*, *hspβ-1*) relative to DWV infected bees treated with OA (Data S7-S9).

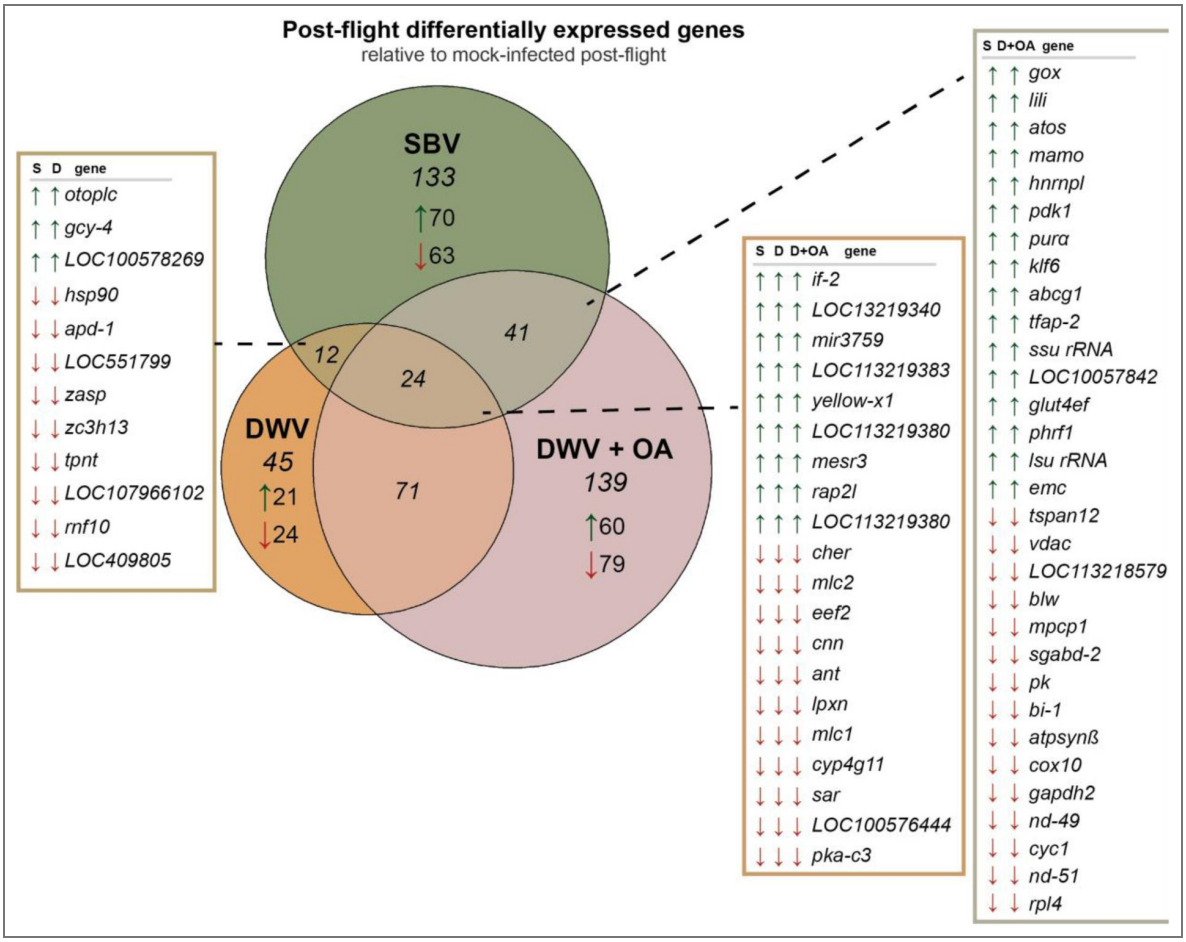


Figure 5. Transcriptome level comparison of honey bees that were SBV infected, DWV infected, or DWV infected and OA-treated

Gene expression was evaluated in honey bees that were mock infected, SBV infected, DWV infected, or DWV infected bees that were co-injected with OA (DWV+OA) after flight. Differentially expressed genes (DEGs) for each treatment group were identified relative to mock infected bees and compared using a Venn diagram. Full list of DEGs, fold change, and Benjamini-Hochberg corrected significance values are included in SI Appendix Table S6.

After flight, both SBV infected and OA treated DWV infected bees expressed lower levels of several mitochondrial associated genes (*vdac, blw, atpsynβ*, *cox10*, *klf6,* and *mcmp1*) and genes involved in energy generation (*pyruvate kinase*, *gapdh2,* and 2 *ND-49,* and *ND-51*) than mock infected bees (Fig 5) This result suggested that flight was more taxing for virus infected bees. In addition, transcriptome level results indicated that OA treatment during DWV inoculation resulted in enhanced energy production, since these bees expressed higher levels of genes involved in energy production post-flight relative to DWV infected bees that did not fly (Data S10).

Since OA treatment resulted in recovered flight performance in DWV infected bees, we compared the transcriptomes of honey bees with similar DWV-levels that were either OA-treated or untreated. The 50 genes with the greatest difference in mean expression were identified in bees that did or did not fly (i.e., 100 total possible genes) (Data S10). There were 30 shared genes associated with OA-treatment regardless of flight status. This list included *defensin-1*, *apidermin-2*, *melittin*, *odorant binding protein 3*, *chymotrypsin-1*, two α-glucosidases, *peritrophin*, 2 chitinase proteins, *fibrillin-2*, *muscle protein 20*, and 7 uncharacterized genes. The OA-associated list also included *vegetative cell wallprotein-like (gpl-1),* which exhibited higher expression in bees that flew further distances (mock, SBV, DWV and OA), than in DWV infected bees. Previous studies determined that *gpl-1* expression was greater in *Nosema ceranae* infected honey bees (70), and that octopamine signaling was also impacted (48, 49). While the bees in our study were not *Nosema* infected (S1 Fig), we used qPCR to confirm that bees expressed higher *gpl-1* levels when injected with OA or EP (LMM, $p = 2\times10^{-7}$ and 4×10^{-8}, respectively); data from additional experiments (i.e., exp-1, n = 61 and exp-2, n = 69; Data S1). We also examined expression of a subset of OA-associated genes including OA receptors (i.e., *oα-1R*, *oβ-1R*, *oβ-2R*, *oβ-3R*), enzymes involved in OA synthesis (*tdc* and *tβh*), and genes associated with Oβ-2R activation (*adenylyl cyclase* (*ac*), *protein kinase A* (*pka*), and CREB-associated protein encoding genes) (Data S9 and S5 Fig). Expression of *tdc*, *tβh*, *oβ-1R*, *crebA*, and *creb-binding isoform X1* was generally higher in SBV infected honey bees, and greater *tdc* and *tβh* expression was confirmed via qPCR (exp 1-3, n = 336) (Fig 3, Data S1 and S4). Honey bees that were simultaneously DWV infected and treated with OA expressed higher levels of *oβ-2R*, *oα-1R*, and *creb binding isoform X2* than untreated DWV infected bees (S5 Fig); greater *oβ-2R* expression was also confirmed by qPCR (LMM, n = 336; $p = 9\times10^{-4}$; Data S1). Expression of *oα-1R* and *tyrR* was assessed via qPCR in only one experiment since expression was generally low regardless of treatment (exp-3, n = 69; Data S1). SBV infected bees had greater expression of *oα-1R* ($p = 0.017$), and SBV infected bees co-injected with EP had significantly lower expression of both *oα-1R* and *tyrR* ($p = 0.0006$ and 0.04, respectively).

Discussion

The interactions between virus infections, host immune and stress responses, and organismal health are complex. Insects rely on OA as a neurohormone and neuromodulator to regulate physiological processes and behaviors (43, 45, 71–73), and our previous studies indicated that viruses differentially impacted honey bee octopamine signaling and flight performance. Therefore, we used honey bees as a model system to further investigate these interactions at organismal and transcriptional levels.

As previously described and shown herein, inapparent (or asymptomatic) DWV infections negatively impact honey bee flight performance (i.e., flight distance, duration, and peak flight speed). Evaluation of transcriptional responses to DWV infection suggest that DWV-associated flight impairment may be partially explained by lower expression of genes involved in energy production, which were elevated in OA treated honey bees (Fig 5, S5 Fig, Data S7-S8). These results are supported by previous studies that showed DWV infected honey bees expressed lower levels of G-coupled protein receptor encoding genes, as well as other genes involved in metabolism (74–76). Previously, we demonstrated that the flight performance of SBV infected honey bees was enhanced and that these bees expressed more *oβ-2R* (29). Herein, we showed that SBV-associated flight enhancement was reduced when OA signaling was inhibited by EP, an OA receptor antagonist (Fig 2 and 4). We expected that SBV infected bees would have compounding, enhanced flight performance when treated with OA, but unexpectedly, OA supplementation

negatively impacted flight performance (Fig 2). To further investigate this physiological response, we examined the expression key genes in the octopamine pathway and determined that SBV infected honey bees expressed greater levels of *tdc*, *tβh*, and an OA receptor, *oβ-2R*. Whereas, expression of *tdc* and *tβh* was lower in SBV infected bees that were treated with OA (Data S1 and S4, Fig 3-4). Reduced expression of these genes would likely limit OA synthesis, OA signaling, reduce metabolic activity, and result in less available energy for flight (43). Therefore, the reduced flight performance of SBV infected bees treated with OA may be explained by a feedback loop that regulates intracellular OA concentrations.

Depending on OA concentration and specific pathogen challenge, OA may enhance or inhibit immune function in many invertebrates, though mechanistic studies have been primarily restricted to two model organisms (51, 52, 77–79). In *Drosophila*, OA treatment impacts intracellular OA concentration, likely in a feedback mechanism that regulates neurohormonal signaling (56, 80, 81). In *Caenorhabditis elegans*, OA pathways are hypothesized to be constitutively active, but downregulated upon pathogen infection to enable an effective immune response while limiting immune activity in the absence of pathogen challenge (77).

Transcriptomic analysis of honey bees infected with a model virus (Sindbis) that were co-injected with double-stranded RNA, a virus-associated molecular pattern, had greater expression of an OA receptor (*oβ-2R*) (82). Results presented herein indicate that OA treatment of DWV infected honey bees alters expression of several genes associated with immune function including *apidermin*, *defensin*, and *melittin* (83–86). Additionally, DWV infected bees with OA treatment had reduced expression of an odorant binding protein that was previously associated with SBV infection, indicating that it may be involved in honey bee antiviral defense (87). Together, these results highlight virus-specific transcriptional responses in honey bees that may impact virus dynamics and flight performance during commonly occurring coinfections (29, 88).

Multiple OA receptors have been identified in honey bee flight muscles and neural tissues including OαR1, Oβ-2R, Oβ-1R, and Oβ-3/4R (40). OA receptors have also been detected on invertebrate immune cells (51). In addition to their involvement in flight, OA receptors are also involved in honey bee hygienic, communicative dance, and thermoregulatory behaviors (40, 53, 57, 61, 89). Typically around 12 days post-emergence honey bees change from nurse bees, which care for developing brood, to pre-forager bees and undergo physiological changes required for flight (90, 91). As honey bees age and become foragers, OA levels in the brain increase (44, 55, 92). Honey bees infected with the microsporidian parasite, *Nosema*, have greater OA levels than uninfected bees (48, 49) and are prone to precocious foraging (93), though *Nosema* levels did not appreciably impact honey bee flight capability (30). These findings are in-line with SBV-induced flight enhancement that correlated with higher *oβ-2R* expression levels (29). Previous laboratory-based studies demonstrated that precocious foraging may be induced by OA supplementation (44).

Honey bees are exposed to environmental OA sources including citrus blossom nectar, which may contain tyramine and OA at concentrations up to 256 μM (94–97). In addition, acaracides that are OA receptor agonists including amitraz and chlordimeform are commonly used in agriculture (98). Amitraz is commonly used to mitigate *Varroa* mite infestations in honey bee colonies (99). *Varroa* mites with amitraz-resistant Oβ-2R mutations have been reported (100, 101). Amitraz has generally been considered safe for honey bees since honey bee OA receptors are divergent from *Varroa* mite OA receptors (i.e., 48% identical at the amino acid level) (99, 102). However, honey bees exposed to amitraz had higher mortality when co-stressed via infection with a model virus (Flock House virus) (103). These results in conjunction with our findings, linking virus infection with the OA stress response pathway, may indicate previously unrecognized impacts at the organismal level. *Varroa* mites vector many honey bee viruses and infested colonies often have coinfections and greater average virus levels (104).

Therefore, application of miticide to reduce *Varroa* and control vectored viruses, or the accumulation miticide metabolites in wax or other hive matrices, may inadvertently harm co-stressed, virus infected honey bees (103, 105, 106). Collectively, the data presented herein demonstrate that the OA pathway is critical for mediating virus-specific effects on honey bee flight performance, which may in turn influence viral transmission and colony health.

Materials & Methods

Honey bees

Honey bees (*Apis mellifera,* primarily *carnica*), were maintained with standard apicultural practices at Montana State University's Horticulture Farm and bees were collected 24 hours prior to eclosion for each experiment. Each experiment was performed with honey bees from different colonies to obtain robust results from genetically diverse bees (Data S1). Bees were cohoused in modified deli containers (n = 10 per house), maintained at 32°C in an incubator and fed 50% sucrose syrup and water *ad libitum* (107). All bees were fed only sucrose syrup, unless octopamine (OA) and/or epinastine (EP) oral treatment was otherwise specified. OA was administered at a concentration of 10 mM and EP at 4 mM in fresh sucrose syrup daily, and honey bees were given unrestricted access to the supplemented sucrose syrup for the duration of the experiment.

Virus stock preparation

Deformed wing virus (DWV) and sacbrood virus (SBV) inoculums were propagated in white-eyed pupae as previously described (29). In brief, pupae were injected with 2μL of Tris-HCl containing either 4.0×10^6 DWV RNA copies or 3.5 x 10^4 SBV RNA, between the 2^{nd}-3^{rd} abdominal tergites using a Harbo syringe and a disposable borosilicate needle made from modified capillary tubes. Pupae were maintained in individual wells in a 24-well plate with a piece of Whatman filter paper at the bottom of each well to prevent pupal tissue damage. In each plate, four wells were filled with sterile water for humidity, at 32°C for 7 days. Pupae were then homogenized in a 2 mL microfuge tube with a sterile, metal, 3 mm bead in 1 mL 1X PBS (pH 7.4) using the TissueLyser II (Qiagen) at 30 Hz for 2 min. Homogenates were centrifuged at 14,000 x g for 15 min at 4°C and supernatant was transferred to a fresh tube. The virus-containing lysates were filtered through a 0.45 μm filter, then a 0.22 μm filter to remove large particles and non-viral microbial contaminants, respectively. RNA was isolated from the virus-containing filtrate (20 μL) using TRIzol (Thermo) according to manufacturer's instructions. RNA was quantified using a NanoDrop 2000c Spectrophotometer, cDNA was synthesized with reverse transcriptase, and virus RNA copies (including both genomes and transcripts) were quantified from cDNA using qPCR. The inoculum was tested for copurifying/contaminating viruses via PCR (Data S2, S2 Fig). RNA copy concentration in the purified virus stock was quantified relative to a standard curve using qPCR (Data S3). DWV genome consensus sequences were assembled from short-read sequencing data obtained from sequencing RNA isolated from the inoculum (DWV-lab 2024; GenBank PV821422) and SBV sequencing data (sacbrood virus isolate MT 2023; GenBank PV788228.1). Virus inoculum aliquots were stored at −80°C until each experiment.

Experimentally introduced honey bee virus infections

Honey bees were infected with DWV using previously described methods (107). In brief, age-matched bees were cold anesthetized at 4°C for 10 min and infected via intra-thoracic injection using a Harbo syringe with 3.5 x 10^4 DWV RNA copies, 3.5 x 10^4 SBV RNA copies, or coinfected with 3.5 x 10^4 DWV RNA copies and 9.1×10^3 SBV RNA copies. If octopamine (OA) and/or epinastine (EP) treatment was administered via injection, the injection inoculum also contained 10 mM OA and/or 4 mM EP so that each bee only experienced one injection to reduce injection-associated stress. Each inoculum dose was suspended in 2 μL10 mM Tris HCl buffer, pH 7.5. Buffer injected/ mock infected bees were injected with 2 μL buffer. The DWV doses utilized in this study were similar to previous studies and resulted in virus levels commonly found in naturally infected bees (8, 30, 108–111). As previously described (29), bees had #000 steel washers affixed to their thoraces using rubber cement immediately after injections; washers had an outside diameter of 0.19 cm and weighed 4.3 mg, less than half of an average pollen load (112).

Flight mill

The flight mills used in this study were based on flight mills used in previous honey bee studies (29, 39, 113, 114) and included a magnet to tether bees from the affixed steel washer on each thorax, which enabled their removal from flight mills with minimal damage. Flight assays were performed as previously described (29). In brief, bees 72 hpi (i.e., 4-days post eclosion) were immobilized via incubation at 4°C for ~12 minutes and tethered to flight mills; counterweights were adjusted for each bee. Temperature and humidity were maintained the same for each experiment (23°C and 31±4% relative humidity). Flight was instigated by tapping the flight arm downward and was re-initiated up to three times before bees were considered 'exhausted' and the data collected was representative of 'total flight' capability (Data S1). Bees were stored at - 80°C immediately after flight. Flight distance, duration, and speed calculations were based on redlight detector counts which were recorded on an attached Raspberry Pi as previously described (29). Active flight duration was calculated as the sum time moving >5.5 cm (one encoder count) (Data S1). Flight stop count was defined as any stop that exceeded 1.5 seconds. Honey bees may stop to seemingly engage in grooming behaviors, stops were counted if the bee re-initiated flight (29). DWV infected, EP injected, SBV infected bees fed OA or EP or injected with OA, or SBV infected bees fed both OA and EP had fewer stops than mock infected bees (Data S4).

Honey bee RNA isolation

RNA was isolated from the abdomen of each individual honey bee since abdomens were representative of the entire bee (29). Each abdomen was individually homogenized in sterile water (300 µL) with one sterile steel bead (4.5 mm) using a Qiagen TissueLyser for 2 min at 30 Hz. Lysates were centrifuged at 4°C for 5 minutes at 7,500xg. RNA was isolated from the supernatants using equal volumes of TRIzol reagent according to manufacturer's instructions. The concentration and quality of RNA samples were assessed on a ThermoFisher Nanodrop 2000 spectrophotometer. RNA was stored at −80°C.

Reverse transcription / cDNA synthesis

Reverse transcription reactions were performed by incubating 2 µg RNA, 200 U M-MLV reverse-transcriptase and 500 ng random hexamer primers in 25 µL reactions for 1h at 37°C, according to the manufacturer's instructions. cDNA was diluted with sterile water (1:2) and 2 µL were used for polymerase chain reactions (PCR).

Polymerase Chain Reaction

Since bees were obtained from colonies that are subject to natural infections (107, 108), PCR was performed to test for preexisting virus infections. Specifically, 2 µL cDNA template was combined with 10 pmol of each forward and reverse primers (Data S2) and amplified with ChoiceTaq polymerase according to the manufacturer's instructions using the following conditions: 95°C for 5min; 95°C for 30s, 57°C for 30s, 72°C for 30s, 35 cycles, followed by final elongation at 72°C for 4min. PCR products were assessed by gel electrophoresis (1.5% agarose with SYBR safe) and visualized using a Syngene U:Genius 3 imaging system (S1 Fig). PCR products were previously verified by sequencing (108). Analysis of pooled cDNA samples from mock infected honey bees from each of these experiments revealed that many bees had preexisting DWV and SBV infections, therefore qPCR was used to assess their abundance in all individual samples (Data S1). The qPCR primers used in this study to target DWV, bind to both DWV-A (GenBank AY292384) and DWV-B (GenBank MN565036), in addition to numerous other DWV sequences on NCBI including the consensus sequences of the DWV inoculum as part of this study (Datal S3).

Quantitative PCR

Quantitative PCR (qPCR) was used to assess virus abundance and relative abundance of honey bee transcripts. All qPCR reactions were performed in triplicate with 2 µL of 1:2 diluted cDNA template. Each reaction contained 1× ChoiceTaq Mastermix, 0.4 µM each forward and reverse

primer, 1× SYBR Green (Life Technologies), and 3 mM $MgCl_2$ for a total volume of 20 µL per reaction. A CFX Connect Real Time instrument (BioRad) was used for the following: pre-incubation 95°C for 1 minute followed by 40 cycles of 95°C for 10s, 58°C for 20s, and 72°C for 15s, with a final melt curve analysis at 65°C for 5s to 95°C. To quantify virus copies in each sample, plasmid standards (i.e., virus-specific PCR amplicons cloned into the pGEM-T (Promega) vector and sequence verified) for each virus were used as templates, ranging from 10^3-10^9 copies per reaction for each standard curve as previously described (Data S3) (29). The honey bee gene *rpl8* was used as a housekeeping gene for each sample for comparison and for relative fold change calculations (Data S1-S2). Sterile water containing no DNA template was used for negative controls. qPCR specificity was verified by melt point analysis, gel electrophoresis, and sequencing (108). The starting quantity (SQ) of cDNA template was calculated for each honey bee sample as previously described (29). For each honey bee sample, the starting quantity (SQ) of cDNA template (representing 80 ng total RNA) was calculated based on the standard curve and subtracting the average SQ of the no template control reaction (<600 copies). Samples with virus RNA copies below the limit of detection for qPCR (<1000 copies / 2 µg RNA) were listed as 0 (Data S1). Virus abundance was reported as virus RNA copies, including genomes and transcripts, per 2 µg total RNA (i.e., per RT reaction) and ranged up to 7×10^{10} virus RNA copies / 2µg RNA. Whole bee data was estimated based on the previous estimate of 138 µg RNA per whole bee (29). Comparison of virus abundance levels using estimated copy numbers enabled inclusion of values of 0. Using the ΔΔCt method to calculate virus levels in bees with no detectable virus would inaccurately result in 'missing' data and consequently skew model interpretations. Relative host gene expression was calculated using the average ΔΔCt method where ΔCt was determined by subtracting *rpl8* Ct from the gene of interest Ct. Average ΔCt was calculated by the mean ΔCt of the buffer-injected bees in each experiment, which harbored the lowest virus levels. The ΔΔCt was calculated by subtracting the average control ΔCt and fold change was determined by the equation $2^{-\Delta\Delta Ct}$ (Data S3).

RNA sequencing and analysis

Honey bee samples with low/no preexisting infections (n = 3-5) were selected per treatment from previously published data (29) (n=36 total) (Data S5). RNA from each sample was DNase treated using Qiagen RNeasy columns. RNA samples were sent to the Roy J. Carver Biotechnology Center at University of Illinois; quality was assessed with an Agilent 3500 fragment analyzer. Libraries were prepared via Watchmaker polyA RNA-Seq prep kit according to manufacturer instructions. Libraries were sequenced on an Illumina NovaSeq X Plus 25B (paired 150 nt reads), approximately 3.2 billion total paired end reads were obtained.

Sequencing quality of reads representing individual samples using FastQC, and filtering of the comprehensive dataset was done using MultiQC versions 0.12.1 (115). Illumina adaptors were trimmed and low quality reads were omitted when Q<30 with BBduk from BBTools version 39.26 (116). Reads were normalized with shrinkage estimations for dispersion using DESeq2 l (correction with q-value<0.05) (117). Sequencing libraries were aligned to the *Apis mellifera* genome, Amel_HAv3.1 (i.e, GCA_003254395.2_Amel_HAv3.1_genomic.fna, downloaded from NCBI 2019–02-07) (65, 66). Sequencing data from individual samples were uploaded to the NCBI short read archive (SRA) (BioProject ID: PRJNA1414634). Genes were considered differentially expressed if $\log_2$ fold change of transcript abundance had adjusted p-values <0.05. Venn diagrams were produced using the eulerr package in R (118).

For virus quantification, trimmed paired end read files were aligned to a nonredundant version of the Holobee database (i.e., *https://doi.org/10.15482/USDA.ADC/1255217*) (119) which considered 32 potential virus alignments, using HISAT2 version 2.2.1 (120). Default parameters were used during alignment to allow multimapping of reads. Samples with unexpected infections were omitted from analyses (n = 6/36; Data S5). Alignment files were converted to compressed alignment files and sorted using samtools version 1.21 and unmapped reads were discarded (121). The samtools built-in idxstats command was used to quantify virus abundance in sorted alignment files and generate read counts. Read counts of each trimmed FastQ file were quantified using the

Seqkit stats command (122). Computational efforts were performed on the Tempest High Performance Computing System, operated and supported by University Information Technology Research Cyberinfrastructure (*RRID:SCR_026229*) at Montana State University.

Statistics

Statistical analyses were performed in R 4.3.3. Log-transformations and non-parametric tests were performed as necessary. Specifically, all relative gene expression data was $\log_2$ transformed and all virus abundance data were $\log_{10}(1+)$ transformed, which enabled inclusion of 0s. All mixed models were assessed using the lmer4 package (123). Assumptions for linear models were verified by diagnostic plots and histograms using base R functions and the performance package (124, 125). All models included experiment ID as a random effect and where relevant, virus injections and flight mill IDs were also included as random effects (Data S4). Models were compared using corrected Aikike information criterion (AICc) and Bayesian information criterion (BIC). Models were assessed via maximum likelihood (ML) when random effects varied and via restricted maximum likelihood (REML) when fixed effects varied. We reported only results from the best model for each analysis, but other considered models were included in supplementary material (Data S4). For each linear mixed model with reported estimates, *p*-values were estimated from *t*-statistics via Satterthwaite's method (126).

Figures

All figures were generated through GraphPad Prism software for windows using version 10.6.0, in RStudio using version R 4.3.3, in BioRender, and Adobe Illustrator.

Data availability

All data are available in the main text or the supplementary materials. Sequencing data from individual samples were uploaded to the NCBI short read archive (SRA) (BioProject ID: PRJNA1414634).

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Author Contributions

N.G.K. and M.L.F. designed research; performed research; analyzed data; and wrote the paper. M.L.F. acquired funding, provided resources, supervised the research, and administered the project.

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Additional files

Supplementary file 1. Supplementary Figures.

Supplementary file 2. Supplementary Data Tables.

References

1. **Farrar C** (1937) The influence of colony populations on honey production. *J Agric Res* **54**:945-54
2. **Chabert S,** Requier F, Chadoeuf J, Guilbaud L, Morison N, Vaissière BE (2021) Rapid measurement of the adult worker population size in honey bees. *Ecological Indicators* **122**:107313 https://doi.org/10.1016/j.ecolind.2020.107313
3. **Simpson J** (1961) Nest Climate Regulation in Honey Bee Colonies. *Science* **133**:1327-33 https://doi.org/10.1126/science.133.3461.1327 | PubMed
4. **Jones JC,** Oldroyd BP (2006) Nest Thermoregulation in Social Insects. In: Simpson SJ (Ed). *Advances in Insect Physiology* **33** Academic Press. pp. 153-91 https://doi.org/10.1016/s0065-2806(06)33003-2
5. **Kleinhenz M,** Bujok B, Fuchs S, Jr Tautz (2003) Hot bees in empty broodnest cells: heating from within. *Journal of Experimental Biology* **206**:4217-31 https://doi.org/10.1242/jeb.00680 | PubMed
6. **Bruckner S,** Wilson M, Aurell D, Rennich K, et al. (2023) A national survey of managed honey bee colony losses in the USA: results from the Bee Informed Partnership for 2017–18, 2018–19, and 2019–20. *Journal of Apicultural Research* **62**:429-43 https://doi.org/10.1080/00218839.2022.2158586
7. **Anonymous** Severe and Sudden Losses of Managed Honey Bees Across the Nation [press release]. Project Apis m.. Accessed February 6th, 2025
8. **Traynor KS,** Rennich K, Forsgren E, Rose R, Pettis J, Kunkel G, et al. (2016) Multiyear survey targeting disease incidence in US honey bees. *Apidologie* **47**:325-47 https://doi.org/10.1007/s13592-016-0431-0
9. **Seitz N,** Traynor KS, Steinhauer N, Rennich K, Wilson ME, Ellis JD, et al. (2015) A national survey of managed honey bee 2014–2015 annual colony losses in the USA. *Journal of Apicultural Research* **54**:292-304 https://doi.org/10.1080/00218839.2016.1153294
10. **van Engelsdorp D,** Hayes J, Underwood RM, Pettis JS (2010) A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. *Journal of Apicultural Research* **49**:7-14 https://doi.org/10.3896/ibra.1.49.1.03
11. **Genersch E,** von der Ohe W, Kaatz H, Schroeder A, Otten C, Büchler R, et al. (2010) The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. *Apidologie* **41**:332-52 https://doi.org/10.1051/apido/2010014
12. **Genersch E,** Aubert M (2010) Emerging and re-emerging viruses of the honey bee (*Apis mellifera L.*). *Vet Res* **41**:54 https://doi.org/10.1051/vetres/2010027 | PubMed
13. **Goulson D,** Nicholls E, Botias C, Rotheray EL (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **347**:1255957 https://doi.org/10.1126/science.1255957 | PubMed

14. **Nazzi F,** Brown SP, Annoscia D, Del Piccolo F, Di Prisco G, Varricchio P, et al. (2012) Synergistic Parasite-Pathogen Interactions Mediated by Host Immunity Can Drive the Collapse of Honeybee Colonies. *Plos Pathog* **8**:e1002735 https://doi.org/10.1371/journal.ppat.1002735 | PubMed
15. **Grozinger CM,** Flenniken ML (2019) Bee Viruses: Ecology, Pathogenicity, and Impacts. *Annu Rev Entomol* **64**:205-26 https://doi.org/10.1146/annurev-ento-011118-111942 | PubMed
16. **de Guzman LI,** Williams GR, Khongphinitbunjong K, Ecology Chantawannakul P., History Life (2017) and Management of Tropilaelaps Mites. *Journal of Economic Entomology* **110**:319-32 https://doi.org/10.1093/jee/tow304 | PubMed
17. **de Miranda JR,** Bailey L, Ball BV, Blanchard P, Budge GE, Chejanovsky N, et al. (2015) Standard methods for virus research in *Apis mellifera*. *Journal of Apicultural Research* **52**:1-56 https://doi.org/10.3896/ibra.1.52.4.22
18. **Chen Y,** Evans J, Feldlaufer M (2006) Horizontal and vertical transmission of viruses in the honey bee, Apis mellifera. *J Invertebr Pathol* **92**:152-9 https://doi.org/10.1016/j.jip.2006.03.010 | PubMed
19. **Amiri E,** Seddon G, Zuluaga Smith W, Strand M, Tarpy D, Rueppell O (2019) Israeli Acute Paralysis Virus: Honey Bee Queen–Worker Interaction and Potential Virus Transmission Pathways. *Insects* **10**:9 https://doi.org/10.3390/insects10010009 | PubMed
20. **Amiri E,** Meixner MD, Kryger P (2016) Deformed wing virus can be transmitted during natural mating in honey bees and infect the queens. *Scientific Reports* **6**:33065 https://doi.org/10.1038/srep33065 | PubMed
21. **Fievet J,** Tentcheva D, Gauthier L, de Miranda J, Cousserans F, Colin ME, et al. (2006) Localization of deformed wing virus infection in queen and drone Apis mellifera L. *Virol J* **3** https://doi.org/10.1186/1743-422x-3-16 | PubMed
22. **Nazzi F,** Le Conte Y (2016) Ecology of Varroa destructor, the Major Ectoparasite of the Western Honey Bee, Apis mellifera. *Annu Rev Entomol* **61**:417-32 https://doi.org/10.1146/annurev-ento-010715-023731 | PubMed
23. **Damayo JE,** McKee RC, Buchmann G, Norton AM, Ashe A, Remnant EJ (2023) Virus replication in the honey bee parasite, Varroa destructor. *J Virol* **97**:e0114923 https://doi.org/10.1128/jvi.01149-23 | PubMed
24. **Tentcheva D,** Gauthier L, Zappulla N, Dainat B, Cousserans F, Colin ME, et al. (2004) Prevalence and seasonal variations of six bee viruses in Apis mellifera L. and Varroa destructor mite populations in France. *Appl Environ Microbiol* **70**:7185-91 https://doi.org/10.1128/aem.70.12.7185-7191.2004 | PubMed
25. **Gauthier L,** Tentcheva D, Tournaire M, Dainat B, Cousserans F, Colin ME, et al. (2007) Viral load estimation in asymptomatic honey bee colonies using the quantitative RT-PCR technique. *Apidologie* **38**:426-35 https://doi.org/10.1051/apido:2007026
26. **Chen YP,** Pettis JS, Corona M, Chen WP, Li CJ, Spivak M, et al. (2014) Israeli Acute Paralysis Virus: Epidemiology, Pathogenesis and Implications for Honey Bee Health. *Plos Pathog* **10**:e1004261 https://doi.org/10.1371/journal.ppat.1004261 | PubMed
27. **McMenamin AJ,** Flenniken ML (2018) Recently identified bee viruses and their impact on bee pollinators. *Current Opinion in Insect Science* **26**:120-9 https://doi.org/10.1016/j.cois.2018.02.009 | PubMed
28. **Chen YP,** Siede R (2007) *Honey Bee Viruses. Advances in Virus Research* **70** Elsevier. pp. 33-80
29. **Kaku NG,** Jankauski MA, Doyle BF, Collins CJ, Flenniken ML (2025) Inapparent virus infections differentially affect honey bee flight. *Science Advances* **11**:eadw8382 https://doi.org/10.1126/sciadv.adw8382 | PubMed
30. **Wells T,** Wolf S, Nicholls E, Groll H, Lim KS, Clark SJ, et al. (2016) Flight performance of actively foraging honey bees is reduced by a common pathogen. *Env Microbiol Rep* **8**:728-37 https://doi.org/10.1111/1758-2229.12434 | PubMed

31. **Osabutey AF,** Hamdo S, Erez T, Bonda E, Otmy A, Soroker V (2025) Natural viral infection, individual immunity, and flight performance in honey bee Apis mellifera drones. *Apidologie* **56**:35 https://doi.org/10.1007/s13592-025-01157-0

32. **Benaets K,** Van Geystelen A, Cardoen D, De Smet L, de Graaf DC, Schoofs L, et al. (1848) Covert deformed wing virus infections have long-term deleterious effects on honeybee foraging and survival. *Proc Biol Sci* 284 https://doi.org/10.1098/rspb.2016.2149 | PubMed

33. **Heidinger IM,** Meixner MD, Berg S, Büchler R (2014) Observation of the Mating Behavior of Honey Bee (Apis mellifera L.) Queens Using Radio-Frequency Identification (RFID): Factors Influencing the Duration and Frequency of Nuptial Flights. *Insects* **5**:513-27 https://doi.org/10.3390/insects5030513 | PubMed

34. **Floris I,** Pusceddu M, Niolu P, Satta A (2021) Where Is the Honey Bee Queen Flying? The Original Case of a Foraging Queen. *Insects* **12**:1035 https://doi.org/10.3390/insects12111035 | PubMed

35. **Gmeinbauer R,** Crailsheim K (1993) Glucose utilization during flight of honeybee (Apis mellifera) workers, drones and queens. *J Insect Physiol* **39**:959-67 https://doi.org/10.1016/0022-1910(93)90005-c

36. **Klein S,** Pasquaretta C, He XJ, Perry C, Søvik E, Devaud J-M, et al. (2019) Honey bees increase their foraging performance and frequency of pollen trips through experience. *Scientific Reports* **9**:6778 https://doi.org/10.1038/s41598-019-42677-x | PubMed

37. **Drummond F** (2022) Honey bee Cleansing Flights . .. Just Cleaning?. *Journal of the Kansas Entomological Society* **94**:158-62 https://doi.org/10.2317/0022-8567-94.2.158

38. **Graham L. A,** Langstroth J. (1992) *The Hive and the Honey Bee* Hamilton, Illinois, USA: Dadant.

39. **Brodschneider R,** Omar E, Crailsheim K (2022) Flight performance of pollen starved honey bees and incomplete compensation through ingestion after early life pollen deprivation. *Frontiers in Physiology* **13**:1004150 https://doi.org/10.3389/fphys.2022.1004150 | PubMed

40. **Kaya-Zeeb S,** Engelmayer L, Straßburger M, Bayer J, Bähre H, Seifert R, et al. (2022) Octopamine drives honeybee thermogenesis. *eLife* **11** https://doi.org/10.7554/elife.74334 | PubMed

41. **Stabentheiner A,** Kovac H, Hetz SK, Käfer H, Stabentheiner G (2012) Assessing honeybee and wasp thermoregulation and energetics—New insights by combination of flow-through respirometry with infrared thermography. *Thermochimica Acta* 77-86 https://doi.org/10.1016/j.tca.2012.02.006 | PubMed

42. **Stabentheiner A,** Kovac H, Brodschneider R (2010) Honeybee colony thermoregulation--regulatory mechanisms and contribution of individuals in dependence on age, location and thermal stress. *PLoS ONE* **5**:e8967 https://doi.org/10.1371/journal.pone.0008967 | PubMed

43. **Li Y,** Hoffmann J, Li Y, Stephano F, Bruchhaus I, Fink C, et al. (2016) Octopamine controls starvation resistance, life span and metabolic traits in Drosophila. *Sci Rep* **6**:35359 https://doi.org/10.1038/srep35359 | PubMed

44. **Schulz DJ,** Robinson GE (1999) Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *Journal of Comparative Physiology A* **184**:481-8 https://doi.org/10.1007/s003590050348 | PubMed

45. **Fussnecker BL,** Smith BH, Mustard JA (2006) Octopamine and tyramine influence the behavioral profile of locomotor activity in the honey bee (Apis mellifera). *J Insect Physiol* **52**:1083-92 https://doi.org/10.1016/j.jinsphys.2006.07.008 | PubMed

46. **Blenau W,** Wilms JA, Balfanz S, Baumann A (2020) AmOctα2R: Functional Characterization of a Honeybee Octopamine Receptor Inhibiting Adenylyl Cyclase Activity. *Int J Mol Sci* **21** https://doi.org/10.3390/ijms21249334 | PubMed

47. **Adamo SA** (2008) Norepinephrine and octopamine: linking stress and immune function across phyla. *Invertebrate Survival Journal* **5**

48. **Gage SL,** Kramer C, Calle S, Carroll M, Heien M, DeGrandi-Hoffman G (2018) Nosema ceranae parasitism impacts olfactory learning and memory and neurochemistry in honey bees (Apis mellifera). *Journal of Experimental Biology* **221** https://doi.org/10.1242/jeb.161489 | PubMed

49. **Mayack C,** Natsopoulou ME, McMahon DP (2015) Nosema ceranae alters a highly conserved hormonal stress pathway in honeybees. *Insect Mol Biol* **24**:662-70 https://doi.org/10.1111/imb.12190 | PubMed

50. **Adamo SA,** Lovett MME (2011) Some like it hot: the effects of climate change on reproduction, immune function and disease resistance in the cricket Gryllus texensis. *Journal of Experimental Biology* **214**:1997-2004 https://doi.org/10.1242/jeb.056531 | PubMed

51. **Huang J,** Wu S-F, Li X-H, Adamo SA, Ye G-Y (2012) The characterization of a concentration-sensitive α-adrenergic-like octopamine receptor found on insect immune cells and its possible role in mediating stress hormone effects on immune function. Brain. *Behavior, and Immunity* **26**:942-50 https://doi.org/10.1016/j.bbi.2012.04.007 | PubMed

52. **Liu K-F,** Kuo H-W, Chang C-C, Cheng W (2019) The intracellular signaling pathway of octopamine upregulating immune resistance functions in Penaeus monodon. *Fish & Shellfish Immunology* **92**:188-95 https://doi.org/10.1016/j.fsi.2019.06.007 | PubMed

53. **Barron AB,** Maleszka R, Vander Meer RK, Robinson GE (2007) Octopamine modulates honey bee dance behavior. *Proceedings of the National Academy of Sciences* **104**:1703-7 https://doi.org/10.1073/pnas.0610506104 | PubMed

54. **White MA,** Chen DS, Wolfner MF (2021) She's got nerve: roles of octopamine in insect female reproduction. *J Neurogenet* **35**:132-53 https://doi.org/10.1080/01677063.2020.1868457 | PubMed

55. **Schulz DJ,** Pankiw T, Fondrk MK, Robinson GE, Page RE (2004) Comparisons of Juvenile Hormone Hemolymph and Octopamine Brain Titers in Honey Bees (Hymenoptera: Apidae) Selected for High and Low Pollen Hoarding. *Annals of the Entomological Society of America* **97**:1313-9 https://doi.org/10.1603/0013-8746(2004)097[1313:cojhha]2.0.co;2

56. **Patil YP,** Joshi RS (2025) From Signals to Sustenance: The Role of Biogenic Amines in Insect Feeding Behavior. *Journal of Insect Behavior* **38**:15 https://doi.org/10.1007/s10905-025-09879-w

57. **Linn M,** Glaser SM, Peng T, Grüter C (2020) Octopamine and dopamine mediate waggle dance following and information use in honeybees. *Proc Biol Sci* **287**:20201950 https://doi.org/10.1098/rspb.2020.1950 | PubMed

58. **Cook CN,** Brent CS, Breed MD (2017) Octopamine and tyramine modulate the thermoregulatory fanning response in honey bees (Apis mellifera). *Journal of Experimental Biology* **220**:1925-30 https://doi.org/10.1242/jeb.149203 | PubMed

59. **Brembs B,** Christiansen F, Pflüger HJ, Duch C (2007) Flight Initiation and Maintenance Deficits in Flies with Genetically Altered Biogenic Amine Levels. *The Journal of Neuroscience* **27**:11122-31 https://doi.org/10.1523/jneurosci.2704-07.2007 | PubMed

60. **Saraswati S,** Fox LE, Soll DR, Wu C-F (2004) Tyramine and octopamine have opposite effects on the locomotion of Drosophila larvae. *Journal of Neurobiology* **58**:425-41 https://doi.org/10.1002/neu.10298 | PubMed

61. **Kaya-Zeeb S,** Delac S, Wolf L, Marante AL, Scherf-Clavel O, Thamm M (2022) Robustness of the honeybee neuro-muscular octopaminergic system in the face of cold stress. *Front Physiol* **13**:1002740 https://doi.org/10.3389/fphys.2022.1002740 | PubMed

62. **Sassone-Corsi P** (2012) The Cyclic AMP Pathway. *Cold Spring Harbor Perspectives in Biology* **4** https://doi.org/10.1101/cshperspect.a011148 | PubMed

63. **Roeder T,** Degen J, Gewecke M (1998) Epinastine, a highly specific antagonist of insect neuronal octopamine receptors. *European Journal of Pharmacology* **349**:171-7 https://doi.org/10.1016/s0014-2999(98)00192-7 | PubMed

64. **Degen J,** Gewecke M, Roeder T (2000) Octopamine receptors in the honey bee and locust nervous system: pharmacological similarities between homologous receptors of distantly related species. *British Journal of Pharmacology* **130**:587-94 https://doi.org/10.1038/sj.bjp.0703338 | PubMed

65. **Weinstock GM,** Robinson GE, Gibbs RA, Weinstock GM, Weinstock GM, Robinson GE, et al. (2006) Insights into social insects from the genome of the honeybee Apis mellifera. *Nature* **443**:931-49 https://doi.org/10.1038/nature05260 | PubMed

66. **Elsik CG,** Worley KC, Bennett AK, Beye M, Camara F, Childers CP, et al. (2014) Finding the missing honey bee genes: lessons learned from a genome upgrade. *Bmc Genomics* **15** https://doi.org/10.1186/1471-2164-15-86 | PubMed

67. **Dumaz N,** Marais R (2005) Integrating signals between camp and the ras/raf/mek/erk signalling pathways: Based on the anniversary prize of the gesellschaft für biochemie und molekularbiologie lecture delivered on 5 July 2003 at the special febs meeting in brussels. *The FEBS journal* **272**:3491-504 https://doi.org/10.1111/j.1742-4658.2005.04763.x | PubMed

68. **Snow BE,** Krumins AM, Brothers GM, Lee S-F, Wall MA, Chung S, et al. (1998) A G protein subunit-like domain shared between RGS11 and other RGS proteins specifies binding to G subunits. *Proceedings of the National Academy of Sciences* **95**:13307-12 https://doi.org/10.1073/pnas.95.22.13307 | PubMed

69. **Wang K,** McClure J, Tu A (1979) Titin: major myofibrillar components of striated muscle. *Proc Natl Acad Sci U S A* **76**:3698-702 https://doi.org/10.1073/pnas.76.8.3698 | PubMed

70. **Chang Z-T,** Ko C-Y, Yen M-R, Chen Y-W, Nai Y-S (2020) Screening of Differentially Expressed Microsporidia Genes from Nosema ceranae Infected Honey Bees by Suppression Subtractive Hybridization. *Insects* **11**:199 https://doi.org/10.3390/insects11030199 | PubMed

71. **Adamo SA,** Linn CE, Hoy RR (1995) The Role of Neurohormonal Octopamine During 'Fight or Flight' Behaviour in the Field Cricket Gryllus Bimaculatus. *Journal of Experimental Biology* **198**:1691-700 https://doi.org/10.1242/jeb.198.8.1691 | PubMed

72. **Corby-Harris V,** Deeter ME, Snyder L, Meador C, Welchert AC, Hoffman A, et al. (2020) Octopamine mobilizes lipids from honey bee (Apis mellifera) hypopharyngeal glands. *Journal of Experimental Biology* **223** https://doi.org/10.1242/jeb.216135 | PubMed

73. **Bisen RS,** Liessem S, Held M, Ache JM (2026) The role of insulin and octopamine in regulating energy homeostasis and locomotion in insects. *Current Opinion in Insect Science* **73**:101457 https://doi.org/10.1016/j.cois.2025.101457 | PubMed

74. **Tang C-K,** Lin Y-H, Jiang J-A, Lu Y-H, Tsai C-H, Lin Y-C, et al. (2021) Real-time monitoring of deformed wing virus-infected bee foraging behavior following histone deacetylase inhibitor treatment. *iScience* **24**:103056 https://doi.org/10.1016/j.isci.2021.103056 | PubMed

75. **Pizzorno MC,** Field K, Kobokovich AL, Martin PL, Gupta RA, Mammone R, et al. (2021) Transcriptomic Responses of the Honey Bee Brain to Infection with Deformed Wing Virus. *Viruses* **13** https://doi.org/10.3390/v13020287 | PubMed

76. **Chen P,** Lu Y-H, Lin Y-H, Wu C-P, Tang C-K, Wei S-C, et al. (2021) Deformed wing virus infection affects the neurological function of Apis mellifera by altering extracellular adenosine signaling. *Insect Biochem Mol Biol* **139**:103674 https://doi.org/10.1016/j.ibmb.2021.103674 | PubMed

77. **Sellegounder D,** Yuan C-H, Wibisono P, Liu Y, Sun J (2018) Octopaminergic Signaling Mediates Neural Regulation of Innate Immunity in Caenorhabditis elegans. *mBio* **9**:10.1128/mbio.01645-18 https://doi.org/10.1128/mbio.01645-18 | PubMed

78. **Kong H,** Dong C, Tian Z, Mao N, Wang C, Cheng Y, et al. (2018) Altered immunity in crowded Mythimna separata is mediated by octopamine and dopamine. *Scientific Reports* **8**:3215 https://doi.org/10.1038/s41598-018-20711-8 | PubMed

79. **Kuo H-W,** Cheng W (2018) Octopamine enhances the immune responses of freshwater giant prawn, Macrobrachium rosenbergii, via octopamine receptors. *Developmental & Comparative Immunology* **81**:19-32 https://doi.org/10.1016/j.dci.2017.11.002 | PubMed

80. **El-Kholy S,** Stephano F, Li Y, Bhandari A, Fink C, Roeder T (2015) Expression analysis of octopamine and tyramine receptors in Drosophila. *Cell and Tissue Research* **361**:669-84 https://doi.org/10.1007/s00441-015-2137-4 | PubMed

81. **Damrau C,** Colomb J, Brembs B (2021) Sensitivity to expression levels underlies differential dominance of a putative null allele of the Drosophila tβh gene in behavioral phenotypes. *PLOS Biology* **19**:e3001228 https://doi.org/10.1371/journal.pbio.3001228 | PubMed

82. **Brutscher LM,** Daughenbaugh KF, Flenniken ML (2017) Virus and dsRNA-triggered transcriptional responses reveal key components of honey bee antiviral defense. *Scientific reports* **7**:6448 https://doi.org/10.1038/s41598-017-06623-z | PubMed

83. **McMenamin AJ,** Daughenbaugh KF, Parekh F, Pizzorno MC, Flenniken ML (2018) Honey Bee and Bumble Bee Antiviral Defense. *Viruses-Basel* **10** https://doi.org/10.3390/v10080395 | PubMed

84. **Seyam H,** Metwally AAA, El-Deeb AH, El-Mohandes S, Badr MS, Abd-El-Samie EM (2022) Effect of honeybee venom and Egyptian propolis on the honeybee (Apis mellifera L.) health in vivo. *Egyptian Journal of Biological Pest Control* **32**:78 https://doi.org/10.1186/s41938-022-00580-0

85. **Mahmoud SH,** Kandel M, El-Seedi H, Al Naggar Y (2024) Honey bee venom promotes the immune system and reduces Vairimorpha (Nosema) ceranae infection in honey bees (Apis mellifera L.. *Apidologie* **55**:8 https://doi.org/10.1007/s13592-023-01048-2

86. **Hejníková M,** Tomčala A, Černý J, Kodrík D (2024) Melittin—The principal toxin of honeybee venom—Is also produced in the honeybee fat body. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **281** https://doi.org/10.1016/j.cbpc.2024.109928 | PubMed

87. **Al Naggar Y,** Shafiey H, Paxton RJ (2023) Transcriptomic Responses Underlying the High Virulence of Black Queen Cell Virus and Sacbrood Virus following a Change in Their Mode of Transmission in Honey Bees (Apis mellifera). *Viruses* **15**:1284 https://doi.org/10.3390/v15061284 | PubMed

88. **Durand T,** Bonjour-Dalmon A, Dubois E (2023) Viral Co-Infections and Antiviral Immunity in Honey Bees. *Viruses* **15**:1217 https://doi.org/10.3390/v15051217 | PubMed

89. **Spivak M,** Masterman R, Ross R, Mesce KA (2003) Hygienic behavior in the honey bee (Apis mellifera L.) and the modulatory role of octopamine. *Journal of Neurobiology* **55**:341-54 https://doi.org/10.1002/neu.10219 | PubMed

90. **Scofield SL,** Amdam GV (2024) Fat body lipogenic capacity in honey bee workers is affected by age, social role and dietary protein. *Journal of Experimental Biology* **227** https://doi.org/10.1242/jeb.247777 | PubMed

91. **Johnson BR** (2008) Within-nest temporal polyethism in the honey bee. *Behavioral Ecology and Sociobiology* **62**:777-84 https://doi.org/10.1007/s00265-007-0503-2

92. **Kaatz H,** Eichmüller S, Kreissl S (1994) Stimulatory effect of octopamine on juvenile hormone biosynthesis in honey bees (Apis mellifera): Physiological and immunocytochemical evidence. *J Insect Physiol* **40**:865-72 https://doi.org/10.1016/0022-1910(94)90020-5

93. **Goblirsch M,** Huang ZY, Spivak M (2013) Physiological and Behavioral Changes in Honey Bees (Apis mellifera) Induced by Nosema ceranae Infection. *PLoS ONE* **8**:e58165 https://doi.org/10.1371/journal.pone.0058165 | PubMed

94. **Muth F,** Philbin CS, Jeffrey CS, Leonard AS (2022) Discovery of octopamine and tyramine in nectar and their effects on bumblebee behavior. *iScience* **25** https://doi.org/10.1016/j.isci.2022.104765 | PubMed

95. **Barberis M,** Calabrese D, Galloni M, Nepi M (2023) Secondary Metabolites in Nectar-Mediated Plant-Pollinator Relationships. *Plants* **12**:550 https://doi.org/10.3390/plants12030550 | PubMed

96. **Barberis M,** Bogo G, Bortolotti L, Flaminio S, Giordano E, Nepi M, et al. (2023) Floral nectar and insect flower handling time change over the flowering season: Results from an exploratory study. *Acta Oecologica* **120**:103937 https://doi.org/10.1016/j.actao.2023.103937

97. **Tang F,** Tao L, Luo X, Ding L, Guo M, Nie L, et al. (2006) Determination of octopamine, synephrine and tyramine in Citrus herbs by ionic liquid improved 'green' chromatography. *Journal of Chromatography A* **1125**:182-8 https://doi.org/10.1016/j.chroma.2006.05.049 | PubMed

98. **Ahmed MAI,** Vogel CFA (2020) Hazardous effects of octopamine receptor agonists on altering metabolism-related genes and behavior of Drosophila melanogaster. *Chemosphere* **253**:126629 https://doi.org/10.1016/j.chemosphere.2020.126629 | PubMed

99. **Guo L,** Fan X-y, Qiao X, Montell C, Huang J (2021) An octopamine receptor confers selective toxicity of amitraz on honeybees and Varroa mites. *eLife* **10**:e68268 https://doi.org/10.7554/eLife.68268 | PubMed

100. **Rinkevich FD,** Moreno-Martí S, Hernández-Rodríguez CS, González-Cabrera J (2023) Confirmation of the Y215H mutation in the β2-octopamine receptor in Varroa destructor is associated with contemporary cases of amitraz resistance in the United States. *Pest Manag Sci* **79**:2840-5 https://doi.org/10.1002/ps.7461 | PubMed

101. **Hernández-Rodríguez CS,** Moreno-Martí S, Emilova-Kirilova K, González-Cabrera J (2025) A new mutation in the octopamine receptor associated with amitraz resistance in Varroa destructor. *Pest Manag Sci* **81**:308-15 https://doi.org/10.1002/ps.8434 | PubMed

102. **NCBI** (2024) Apis mellifera Octopamine beta 2-R. NCBI Gene. ID 412896 https://www.ncbi.nlm.nih.gov/gene/412896

103. **O'Neal ST,** Brewster CC, Bloomquist JR, Anderson TD (2017) Amitraz and its metabolite modulate honey bee cardiac function and tolerance to viral infection. *J Invertebr Pathol* **149**:119-26 https://doi.org/10.1016/j.jip.2017.08.005 | PubMed

104. **Doublet V,** Oddie MAY, Mondet F, Forsgren E, Dahle B, Furuseth-Hansen E, et al. (2024) Shift in virus composition in honeybees (Apis mellifera) following worldwide invasion by the parasitic mite and virus vector Varroa destructor. *Royal Society Open Science* **11**:231529 https://doi.org/10.1098/rsos.231529 | PubMed

105. **Bischoff K,** Baert N, McArt S (2023) Pesticide contamination of beeswax from managed honey bee colonies in New York State. *J Vet Diagn Invest* **35**:617-24 https://doi.org/10.1177/10406387231199098 | PubMed

106. **Mullin CA,** Frazier M, Frazier JL, Ashcraft S, Simonds R, vanEngelsdorp D, et al. (2010) High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. *PLoS ONE* **5**:e9754 https://doi.org/10.1371/journal.pone.0009754 | PubMed

107. **Brutscher LM,** Daughenbaugh KF, Flenniken ML (2017) Virus and dsRNA-triggered transcriptional responses reveal key components of honey bee antiviral defense. *Sci Rep* **7**:6448 https://doi.org/10.1038/s41598-017-06623-z | PubMed

108. **Faurot-Daniels C,** Glenny W, Daughenbaugh KF, McMenamin AJ, Burkle LA, Flenniken ML (2020) Longitudinal monitoring of honey bee colonies reveals dynamic nature of virus abundance and indicates a negative impact of Lake Sinai virus 2 on colony health. *PLoS One* **15**:e0237544 https://doi.org/10.1371/journal.pone.0237544 | PubMed

109. **Glenny W,** Cavigli I, Daughenbaugh KF, Radford R, Kegley SE, Flenniken ML (2017) Honey bee (*Apis mellifera*) colony health and pathogen composition in migratory beekeeping operations involved in California almond pollination. *PLoS One* **12**:e0182814 https://doi.org/10.1371/journal.pone.0182814 | PubMed

110. **Runckel C,** Flenniken ML, Engel JC, Ruby JG, Ganem D, Andino R, et al. (2011) Temporal analysis of the honey bee microbiome reveals four novel viruses and seasonal prevalence of known viruses, Nosema, and Crithidia. *PLoS One* **6**:e20656 https://doi.org/10.1371/journal.pone.0020656 | PubMed

111. **D'Alvise P,** Seeburger V, Gihring K, Kieboom M, Hasselmann M (2019) Seasonal dynamics and co-occurrence patterns of honey bee pathogens revealed by high-throughput RT-qPCR analysis. *Ecol Evol* **9**:10241-52 https://doi.org/10.1002/ece3.5544 | PubMed

112. **Winston ML** (1991) *The biology of the honey bee* Cambridge, MA. USA: Harvard University Press.

113. **Tosi S,** Burgio G, Nieh JC (2017) A common neonicotinoid pesticide, thiamethoxam, impairs honey bee flight ability. *Sci Rep* **7**:1201 https://doi.org/10.1038/s41598-017-01361-8 | PubMed

114. **Scheiner R,** Abramson CI, Brodschneider R, Crailsheim K, Farina WM, Fuchs S, et al. (2013) Standard methods for behavioural studies of Apis mellifera. *Journal of Apicultural Research* **52**:1-58 https://doi.org/10.3896/ibra.1.52.4.04

115. **Andrews S** (2015) FastQC.

116. **Bushnell B** (2014) BBMap: A Fast, Accurate, Splice-Aware Aligner. https://bbmap.org

117. **Love MI,** Huber W, Anders S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**:550 https://doi.org/10.1186/s13059-014-0550-8 | PubMed

118. **Larsson J,** Gustafsson P (2018) A Case Study in Fitting Area-Proportional Euler Diagrams with Ellipses using eulerr. CRAN: Contributed Packages. https://doi.org/10.32614/cran.package.eulerr

119. **Evans JD,** Schwarz R, Childers A (2016) HoloBee Database. figshare. version: 2016.1 https://doi.org/10.15482/USDA.ADC/1255217

120. **Kim D,** Paggi JM, Park C, Bennett C, Salzberg SL (2019) Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nature Biotechnology* **37**:907-15 https://doi.org/10.1038/s41587-019-0201-4 | PubMed

121. **Li H,** Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**:2078-9 https://doi.org/10.1093/bioinformatics/btp352 | PubMed

122. **Shen W,** Le S, Li Y, Hu F (2016) SeqKit: A Cross-Platform and Ultrafast Toolkit for FASTA/Q File Manipulation. *PLoS ONE* **11**:e0163962 https://doi.org/10.1371/journal.pone.0163962 | PubMed

123. **Bates D,** Machler M, Bolker BM, Walker SC (2015) Fitting Linear Mixed-Effects Models Using lme4. *J Stat Softw* **67**:1-48 https://doi.org/10.18637/jss.v067.i01

124. **Lüdecke D,** Ben Shachar M, Patil I, Waggoner P (2021) Makowski D. performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *The Journal of Open Source Software* **6**:3139 https://doi.org/10.21105/joss.03139

125. **Schielzeth H,** Dingemanse NJ, Nakagawa S, Westneat DF, Allegue H, Teplitsky C, et al. (2020) Robustness of linear mixed-effects models to violations of distributional assumptions. *Methods in Ecology and Evolution* **11**:1141-52 https://doi.org/10.1111/2041-210x.13434

126. **Kuznetsova A,** Brockhoff P, Christensen R (2015) LmerTest: Tests in linear mixed effects models. CRAN. https://doi.org/10.32614/CRAN.package.lmerTest

Peer reviews

Reviewer #1 (Public review):

Summary:

Kaku and Flenniken investigate the mechanistic pathways through which specific viral infections alter the flight capabilities of honey bees. Building on their previous discovery that DWV impairs flight while SBV unexpectedly enhances it, the authors hypothesized that these behavioral shifts are driven by interactions with the insect's octopamine (OA) signaling pathway, which is responsible for the "fight-or-flight" neurohormonal stress response and energy mobilization. To test this, the authors experimentally infected adult honey bees with DWV or SBV and pharmacologically manipulated the OA pathway using either octopamine supplementation or epinastine (EP), an OA-receptor antagonist. They then evaluated the bees' flight performance (distance, duration, and speed) on custom flight mills and profiled their gene expression using qPCR and RNA sequencing.

Strengths:

A major strength of this study is the high prevalence of preexisting background DWV and SBV infections in the honey bee cohorts, which meant there were no completely "virus-free" control groups. However, the authors successfully mitigated this limitation by rigorously quantifying viral RNA copies for every individual bee via qPCR and utilizing these viral abundances as continuous variables in powerful linear mixed-effect models.

Weaknesses:

The primary weakness lies in the methodology used for targeted pharmacological manipulations, as well as the lack of OA quantification across different treatments. Thus, their claims are not sufficiently supported by the current data.

(1) The authors utilize Epinastine to block octopamine signaling, describing it as a highly specific OA receptor antagonist. However, pharmacological inhibitors often lack absolute specificity. Epinastine might bind to other octopamine receptor subtypes present in honey bee neural and flight muscle tissues, or it could potentially cross-react with tyramine and dopamine receptors. Without further genetic validation (e.g., RNA interference targeting specific receptors), it is difficult to definitively conclude that the altered flight performance is solely due to the blockade of the specific Oβ−2R pathway.

(2) As a natural neurotransmitter, insects have evolved highly efficient "cleanup" mechanisms. OA is rapidly cleared from the synaptic cleft via reuptake transporters and quickly inactivated by enzymes such as N-acetyltransferase (NAT) or Monoamine Oxidase (MAO). Consequently, an injection of OA produces only a transient "pulse" of activity. It is often a poor "tool" for inducing prolonged physiological effects compared to synthetic formamidines like Amitraz.

(3) The study relies heavily on transcriptomics and quantitative PCR to measure the mRNA expression of key synthesizing enzymes, namely tyrosine decarboxylase (tdc) and tyramine β-hydroxylase (tβh), to infer the activation or suppression of the octopamine pathway. However, changes in enzyme synthesis at the RNA level are often insufficient to accurately reflect the true physiological levels of biogenic amines. To robustly prove the authors' hypothesis of a "feedback loop that regulates intracellular OA concentrations", direct quantification of actual octopamine and tyramine titers in the bees (e.g., using high-performance liquid chromatography or mass spectrometry) is necessary.

https://doi.org/10.7554/eLife.111072.1.sa2

Reviewer #2 (Public review):

Summary:

This highly original and well-designed study provides insight into how honeybee picorna-like viruses, Deformed wing virus (DWV) and Sacbrood virus (SBV), affect flight performance, and reveals the role of the octopamine (OA) pathway in virus-honeybee interactions. The authors used a flight mill to quantify the flight performance of bees with different levels of DWV and SBV. Bees were treated with OA and/or epinastine (EP) - an OA receptor antagonist; the study also quantified virus loads and expression of two key genes involved in OA biosynthesis.

The results showed that reduced flight performance associated with high DWV levels could be alleviated by OA administration. In contrast, increased levels of SBV had the opposite effect, leading to enhanced flight performance. This suggests distinct physiological responses to DWV and SBV infections. Administration of EP had led to a reduction of flight performance in SBV-infected bees, indicating the involvement of the OA pathway.

The authors also quantified levels of mRNAs of enzymes involved in OA synthesis, tyrosine decarboxylase (TDC) and tyramine beta-hydroxylase (TbH), and concluded that DWV induced expression of TbH, while SBV upregulated expression of TDC. Furthermore, the study identified upregulated and downregulated genes in response to SBV, DWV and DWV in combination with OA.

Strengths:

The study reported opposing effects of infections of related viruses, SBV and DWV, on honeybee flight performance, and identified the central role of the octopamine (OA) signaling pathway in the effect of viruses on honeybee flights.

These findings were achieved by using a combination of approaches, including experimental measurement of flight distance, virus infections, and introduction of OA and EP. Experimental work with honeybees is technically challenging and requires specialized expertise, which makes the results produced in this study more valuable.

DWV and SBV are among the most important honeybee pathogens affecting honeybee health and threatening the pollination service. Therefore, an understanding of the mechanisms underlying DWV and SBV pathogenesis has the potential to develop novel approaches to mitigate the negative impact of these viruses.

Weaknesses:

No weaknesses were identified by this reviewer.

https://doi.org/10.7554/eLife.111072.1.sa1

Author response:

> ***Reviewer #1 (Public review):***
>
> *Summary:*
>
> *Kaku and Flenniken investigate the mechanistic pathways through which specific viral infections alter the flight capabilities of honey bees. Building on their previous discovery that DWV impairs flight while SBV unexpectedly enhances it, the authors hypothesized that these behavioral shifts are driven by interactions with the insect's octopamine (OA) signaling pathway, which is responsible for the "fight-or-flight" neurohormonal stress response and energy mobilization. To test this, the authors experimentally infected adult honey bees with DWV or SBV and pharmacologically manipulated the OA pathway using either octopamine supplementation or epinastine (EP), an OA-receptor antagonist. They then evaluated the bees' flight performance (distance, duration, and speed) on custom flight mills and profiled their gene expression using qPCR and RNA sequencing.*
>
> *Strengths:*
>
> *A major strength of this study is the high prevalence of preexisting background DWV and SBV infections in the honey bee cohorts, which meant there were no completely "virus-free" control groups. However, the authors successfully mitigated this limitation by rigorously quantifying viral RNA copies for every individual bee via qPCR and utilizing these viral abundances as continuous variables in powerful linear mixed-effect models.*
>
> *Weaknesses:*
>
> *The primary weakness lies in the methodology used for targeted pharmacological manipulations, as well as the lack of OA quantification across different treatments. Thus, their claims are not sufficiently supported by the current data.*

We thank Reviewer #1 for these comments.

> *(1) The authors utilize Epinastine to block octopamine signaling, describing it as a highly specific OA receptor antagonist. However, pharmacological inhibitors often lack absolute specificity. Epinastine might bind to other octopamine receptor subtypes present in honey bee neural and flight muscle tissues, or it could potentially cross-react with tyramine and dopamine receptors. Without further genetic validation (e.g., RNA interference targeting specific receptors), it is difficult to definitively conclude that the altered flight performance is solely due to the blockade of the specific Oβ−2R pathway.*

We thank the reviewer for this thoughtful comment and agree that pharmacological approaches have inherent limitations with respect to receptor specificity. However, among the available octopamine receptor antagonists, epinastine is considered one of the most selective compounds for insect octopamine receptors. Roeder et al. (1998) reported that epinastine exhibits affinities for octopamine receptors that are at least four orders of magnitude greater than those for other insect biogenic amine receptors, including dopamine, tyramine, histamine, and serotonin receptors.

Honeybees encode four β-adrenergic-like receptors AmOARβ1- AmOARβ4) and one αadrenergic-like receptor (AmOARα1). Our transcriptomic analyses indicated that expression of AmOARβ2 was substantially higher than that of other octopamine receptor genes. Specifically, AmOARβ4 transcripts were not detected in our RNA-seq datasets, while AmOARβ1 and AmOARβ3 were expressed at very low levels in most samples (Supplementary Table S9; Figure S5). Although AmOARα1 transcripts were detected in some samples, expression levels were consistently lower than those of AmOARβ2. These observations support the interpretation that the physiological effects observed following epinastine treatment are primarily mediated through disruption of AmOARβ2 signaling. We agree that

receptor-specific genetic approaches would provide valuable complementary evidence. RNAi-mediated knockdown of AmOARβ2 is an attractive future direction; however, RNAi efficacy in honey bees is variable and influenced by factors including transcript turnover rates. In addition, dsRNA treatments can induce sequence independent antiviral effects that could confound interpretation in studies involving viral infection (Flenniken and Andino, 2013). We have revised the manuscript to more explicitly acknowledge these limitations and to clarify the basis for our interpretation of the epinastine experiments.

> *(2) As a natural neurotransmitter, insects have evolved highly efficient "cleanup" mechanisms. OA is rapidly cleared from the synaptic cleft via reuptake transporters and quickly inactivated by enzymes such as N-acetyltransferase (NAT) or Monoamine Oxidase (MAO). Consequently, an injection of OA produces only a transient "pulse" of activity. It is often a poor "tool" for inducing prolonged physiological effects compared to synthetic formamidines like Amitraz.*

We thank the reviewer for this important point regarding the pharmacokinetics of octopamine. We agree that octopamine is rapidly metabolized and cleared under physiological conditions and that exogenous administration is unlikely to precisely mimic endogenous signaling dynamics. Our goal was not to induce a prolonged pharmacological activation of octopamine signaling comparable to that produced by synthetic agonists such as amitraz, but rather to determine whether increasing octopaminergic signaling could mitigate the flight impairments associated with DWV infection. Octopamine was administered either by injection or through feeding (Lines 86-89), both of which resulted in significant improvements in flight performance in DWV-infected bees (Figure 2). The observation that two independent delivery methods produced similar outcomes supports the conclusion that enhanced octopaminergic signaling can partially rescue the DWV-associated flight phenotype. We have revised the manuscript to clarify this distinction and to acknowledge that exogenous octopamine administration likely produces transient elevations in signaling rather than sustained receptor activation.

> *(3) The study relies heavily on transcriptomics and quantitative PCR to measure the mRNA expression of key synthesizing enzymes, namely tyrosine decarboxylase (tdc) and tyramine βhydroxylase (tβh), to infer the activation or suppression of the octopamine pathway. However, changes in enzyme synthesis at the RNA level are often insufficient to accurately reflect the true physiological levels of biogenic amines. To robustly prove the authors' hypothesis of a "feedback loop that regulates intracellular OA concentrations", direct quantification of actual octopamine and tyramine titers in the bees (e.g., using high-performance liquid chromatography or mass spectrometry) is necessary.*

We thank the reviewer for this comment and agree that octopamine and tyramine quantification would strengthen the mechanistic interpretation of our findings. Previous studies have successfully quantified OA in honey bees using HPLC-based approaches, including KayaZee et al. (2022, eLife), who measured OA in honey bee muscle tissue (both naturally occurring levels and levels post-treatment with 10 mM OA), and Cook et al. (2017, J. Exp. Bio) who quantified OA in pooled honey bee brain samples.

Prior to submission, we inquired with our institutional mass spectrometry facility regarding the feasibility of measuring OA in individual honey bee samples. The expected concentrations of OA in our samples was below their limit of detection, so we did not pursue these analyses at that time.

We are exploring the possibility of analyzing a subset of samples at external facilities that may have the sensitivity required to quantify OA and tyramine in honey bee tissues. However, our initial discussions indicate that such analyses would require substantial resources, with estimated costs of approximately $5,000–10,000 for 12–15 samples. While we acknowledge that direct measurements of OA and tyramine would provide valuable

complementary evidence, the current study relies on multiple independent lines of evidence including gene expression analyses, OA supplementation experiments, and behavioral measurements that collectively support a role for octopaminergic signaling in mediating the observed effects.

> ***Reviewer #2 (Public review):***
>
> *Summary:*
>
> *This highly original and well-designed study provides insight into how honeybee picorna-like viruses, Deformed wing virus (DWV) and Sacbrood virus (SBV), affect flight performance, and reveals the role of the octopamine (OA) pathway in virus-honeybee interactions. The authors used a flight mill to quantify the flight performance of bees with different levels of DWV and SBV. Bees were treated with OA and/or epinastine (EP) - an OA receptor antagonist; the study also quantified virus loads and expression of two key genes involved in OA biosynthesis.*
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>
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> *The study reported opposing effects of infections of related viruses, SBV and DWV, on honeybee flight performance, and identified the central role of the octopamine (OA) signaling pathway in the effect of viruses on honeybee flights.*
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> *These findings were achieved by using a combination of approaches, including experimental measurement of flight distance, virus infections, and introduction of OA and EP. Experimental work with honeybees is technically challenging and requires specialized expertise, which makes the results produced in this study more valuable.*
>
> *DWV and SBV are among the most important honeybee pathogens affecting honeybee health and threatening the pollination service. Therefore, an understanding of the mechanisms underlying DWV and SBV pathogenesis has the potential to develop novel approaches to mitigate the negative impact of these viruses.*
>
> *Weaknesses:*
>
> *No weaknesses were identified by this reviewer.*

We thank Reviewer #2 for these comments

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