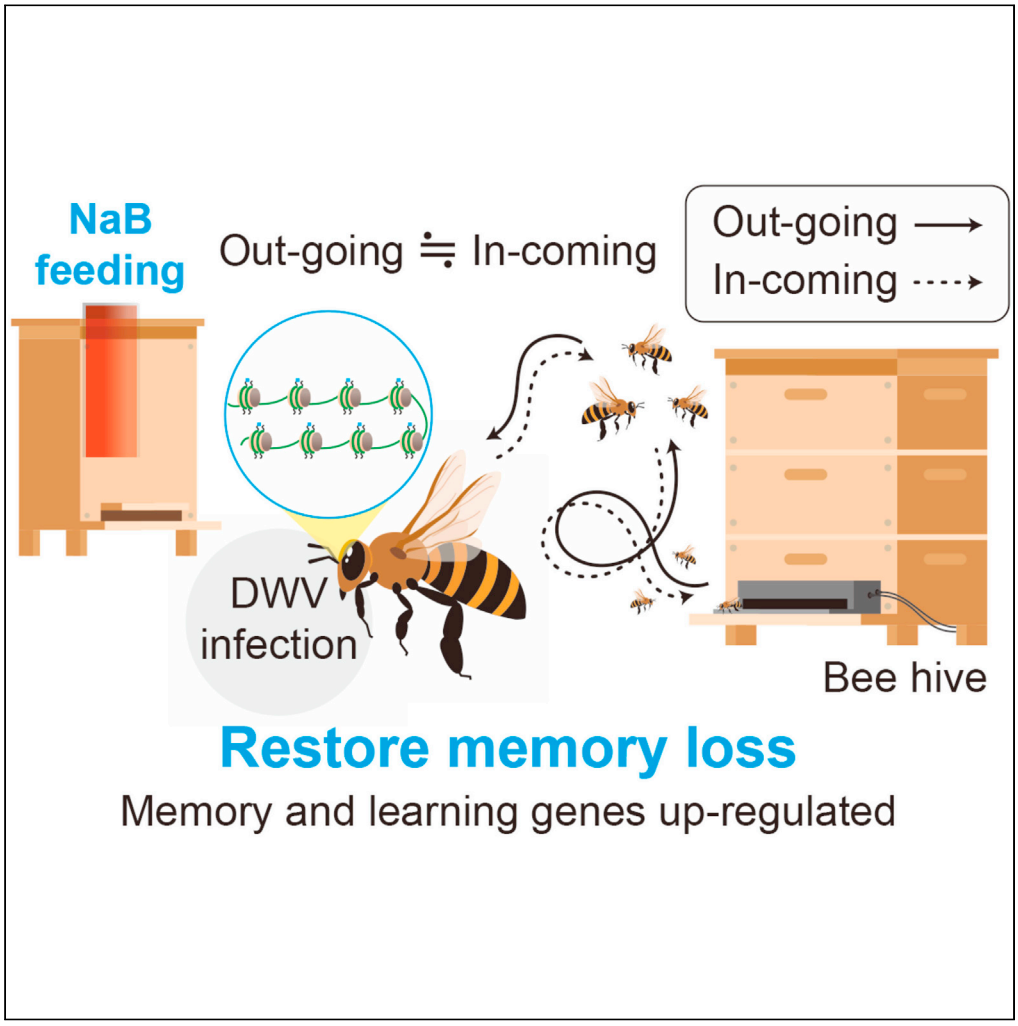
iScience

CellPress
OPEN ACCESS

Article

Real-time monitoring of deformed wing virus-infected bee foraging behavior following histone deacetylase inhibitor treatment



Cheng-Kang Tang, Yu-Hsien Lin, Joe-Air Jiang, ..., Yun-Ru Chen, Carol-P Wu, Yueh-Lung Wu

runwu@ntu.edu.tw

Highlights

Sodium butyrate (NaB) reversed learning ability of bees infected by deformed wing virus

RNA-Seq showed NaB restored the expression of genes involved in glycolysis and memory

NaB improved the homing ability of deformed wing virus-infected bees

Tang et al., iScience 24, 103056
October 22, 2021
https://doi.org/10.1016/j.isci.2021.103056

iScience

CellPress
OPEN ACCESS

Article

Real-time monitoring of deformed wing virus-infected bee foraging behavior following histone deacetylase inhibitor treatment

Cheng-Kang Tang,[1] Yu-Hsien Lin,[2] Joe-Air Jiang,[3,4] Yun-Heng Lu,[1] Chih-Hsuan Tsai,[1] Yu-Chun Lin,[1] Yun-Ru Chen,[1] Carol-P Wu,[1] and Yueh-Lung Wu[1,5,*]

SUMMARY

Impairment in the learning/memory behavior of bees is responsible for the massive disappearance of bee populations and its consequent agricultural economic losses. Such impairment might be because of o both pesticide exposure and pathogen infection, with a key contributor deformed wing virus (DWV). The present study found that sodium butyrate (NaB) significantly increased survival and reversed the learning/memory impairment of DWV-infected bees. A next-generation sequencing analysis showed that NaB affected the expression of genes involved in glycolytic processes and memory formation, which were suppressed by DWV infection. In addition, we performed a large-scale movement tracking experiment by using a wireless sensor network-based automatic real-time monitoring system and confirmed that NaB could improve the homing ability of DWV-infected bees. In short, we demonstrated the mechanism of how epigenetic regulation can resume the memory function of honeybees and suggest strategies for applying NaB to reduce the incidence of colony losses.

INTRODUCTION

Apis mellifera are globally distributed, economically important insects, having particular value as pollinators (Hladun et al., 2012; Potts et al., 2010; Pardo and Borges, 2020). Through ever-improving bee rearing technology, the number of bee farms has increased over the years (Zhou et al., 2017), as well as the variety of bee-derived products. According to the Food and Agriculture Organization of the United Nations, the number of artificial beehives reached 90 million in 2017 (Aizen and Harder, 2009). However, over the last few decades the numbers of both wild and managed bee pollinators have been declining, consequently decreasing their beneficial effects on ecosystems. Although the reasons for this decline are under debate, it is highly likely that a combination of multiple stressors is to blame, in particular deformed wing virus (DWV). This virus has been associated with winter mortality and colony losses, is globally distributed, and has been shown to affect 50–75% of honeybee hives (Ryabov et al., 2014). DWV is an RNA virus with a positive single-stranded RNA genome of 10,140 nt (GenBank accession NC_004830.2) and belongs to the *Picornaviridae* family (Lanzi et al., 2006). DWV infects bee species such as *Apis mellifera*, *Apis cerana*, and *Bombus terrestris* (Ai et al., 2012; Kajobe et al., 2010; Genersch et al., 2006), with all growth stages susceptible (di Prisco et al., 2016; Martin et al., 2012; de Miranda and Genersch, 2010). Previous studies have demonstrated that the Varroa mite can increase the frequency of DWVs from 10 to 100%, making it one of the biggest factors causing the worldwide decimation of bee colonies. *Varroa destructor* harbors virus levels higher than those seen in severely infected bees, and *V. destructor* beehive infestations may be related to severe DWV infections (Bowen-Walker et al., 1999). Thus, *V. destructor* may serve as both a concentrating vector and replicating incubator for the virus as it can magnify and increase the effects of the virus on bees and beehives. Even though most infected bees show no obvious symptoms before metamorphosis, seriously infected bees will have wrinkled and deformed wings after metamorphosis, resulting from the replication and accumulation of DWV in the brain and in the three/four segments of the abdomen. Infection leads to deprivation of nutrients which may cause further impairment of the nervous system and therefore impact on survival (Brettell et al., 2017). The DWV infection rate reaches a peak in winter, leading to the massive death of the worker bees (Genersch et al., 2010; Berthoud et al., 2010).

[1]Department of Entomology, National Taiwan University, 27, Lane 113, Roosevelt Road Sec. 4, Taipei 106, Taiwan

[2]Department of Plant Physiology, Swammerdam Institute for Life Sciences, University of Amsterdam, 1098 XH Amsterdam, The Netherlands

[3]Department of Biomechatronics Engineering, National Taiwan University, Taipei 106, Taiwan

[4]Department of Medical Research, China Medical University Hospital, China Medical University, Taichung 40447, Taiwan

[5]Lead contact

*Correspondence: runwu@ntu.edu.tw

https://doi.org/10.1016/j.isci.2021.103056

In addition to lethality, it has also been reported that replication of DWV in the mushroom body of the bee brain results in decreased neural signal transduction (Shah et al., 2009). The learning/memory ability of worker bees infected with DWV was found to be significantly impaired as compared to uninfected worker bees, based on learning behavioral studies (Traniello et al., 2020; de Miranda and Genersch, 2010). This may explain the massive disappearance of worker bees in hives positive for DWV infection, because impairment of the brain would prevent them from successfully navigating back to their hives. Whether if it is by direct death or reduced homing ability, the loss of worker bees in hives results in insufficient food (pollen and honey) for the developing larvae. The hives become poorly maintained, leading to the eventual collapse of the colony.

DWV infections were first reported in Japan, and have since been reported in Europe, North America, and Africa. At least three main strains, including DWV-A, DWV-B, and DWV-C, have been reported. DWV-A is classified into two subtypes: DWV and Kakugo virus (KV) (Lanzi et al., 2006; Fujiyuki et al., 2005). DWV-B, which can replicate in *V. destructor* and honeybees (Ongus et al., 2004), has a nucleotide identity, which is 84% similar to that of DWV-A. A third variant, DWV-C, reported by Mordecai et al. (2016), is an established variant rather than a recently emerged strain (Mordecai et al., 2016). Among them, DWV-B and DWV-A-VDV1 recombinant are known to be the most virulent variants (Moore et al., 2011; Ryabov et al., 2014).The transmission modes of DWV have been studied, and show that the virus can be vertically or orally transmitted through nursing bees to larvae and horizontally transmitted through predators feeding on infected bees (Amiri et al., 2018; Yanez et al., 2012; Chen et al., 2006). However, an efficient strategy to prevent and manage viral infection in bee rearing has yet to be developed.

Histone deacetylase inhibitors (HDACi) are a class of compounds which prevent deacetylation of histones and therefore increase gene expression (Marks et al., 2000; Fuchikami et al., 2016). These epigenetic regulators have been evaluated for their application in medical treatments, including anti-inflammatory response, anti-neural degeneration, anti-cancer, and anti-Alzheimer's disease (Shein and Shohami, 2011; Mckinsey, 2011; Consalvi et al., 2011; Wu et al., 2007). Feeding aged mice with sodium butyrate (NaB; Formula name: NaC4H8O2), a HDACi, can significantly improve their memory (Garcez et al., 2018; Blank et al., 2015). NaB is found in plants and also in human guts as a fermentation product. NaB could work on histone deacetylase (HDAC) class I, including HDAC1, HDAC2, HDAC3, and HDAC8. In our previous study (Hu et al., 2018), we found that including NaB in the diet of honeybees resulted in a significant increase in the expression of acetyl histone H3 and H4 and the genes involved in immune response, detoxification, and learning/memory. Furthermore, the addition of NaB restored learning ability in neonicotinoid-treated bees, proving that the functionality of NaB could enhance the ability of bees to resist outer stress (Hu et al., 2017, 2018).

As a valuable economic insect, the loss of honeybees, particularly through Colony Collapse Disorder (CCD), has attracted much attention; yet, much remains unknown about the causes, mechanisms, and potential treatment or prevention of CCD. Because of the previously found anti-stress effects of NaB on bees, the present study set out to evaluate the potential of NaB in preventing DWV-induced colony collapse. We determined the survival rate of DWV-infected bees after feeding them with NaB and investigated the learning/memory ability of bees by a proboscis extension response (PER) assay. Next-generation sequencing (NGS) was performed to comprehensively assess genes that underwent significant changes in expression as a result of DWV infection and dietary sodium butyrate. As NGS revealed that NaB increased the expression of genes involved in carbohydrate metabolism and learning/memory, we examined the detailed physiological changes in bee brains upon DWV infection and NaB addition. Finally, to validate the feasibility of applying NaB in hive maintenance, a real-time monitoring system, the "wireless sensor network (WSN)-based automatic monitoring system (Jiang et al., 2016)", was utilized to track the movement of DWV-infected bees with or without a NaB diet on a large scale that simulated real field conditions. This study uncovered gene regulations under DWV infection and proved that the epigenetic regulator NaB could be used to rescue the impaired physiological functions after virus infection. A detailed mechanism of the NaB effects is discussed and feasible in-field treatment strategies are provided.

RESULTS

Sodium butyrate (NaB) significantly reversed the low survival rate and learning ability of DWV-infected bees

In the present study, we first examined whether the intake of NaB can decrease the negative impacts of DWV infection in bees. It has been suggested that severe infection by DWV may lead to a high mortality

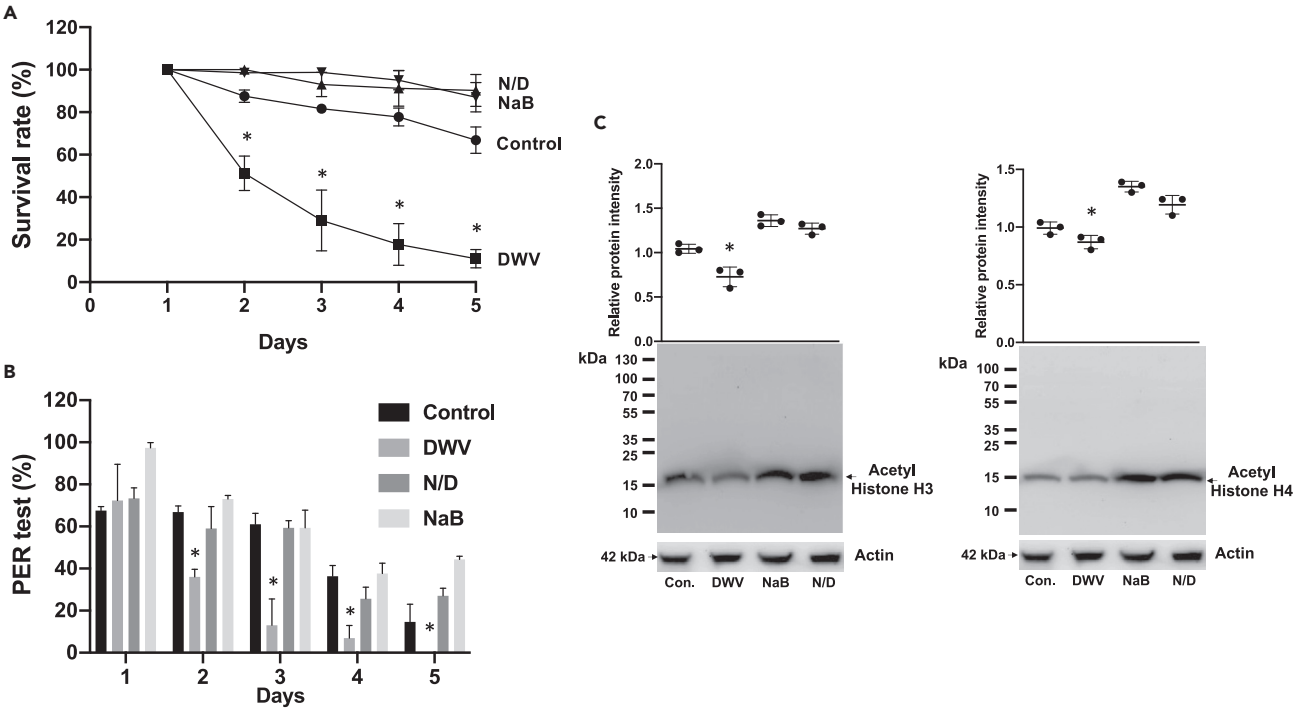


Figure 1. Sodium butyrate increases the survival rate of DWV-infected bees by reversing memory loss

(A) Survival rate of DWV-infected (DWV), sodium butyrate-fed (NaB), DWV-infected and sodium butyrate-fed (N/D) bees and bees without any treatment (Control). For the DWV group, bees were fed with a solution containing 10^6 virus copy number/bee. For the NaB group, bees were fed with 10 mM of sodium butyrate. For the N/D group, bees were fed with 10 mM sodium butyrate for 7 days, followed by infection with DWV. Three independent experiments were performed and 35 bees were included in each group for every experiment. The survival rate after DWV infection was recorded for 5 days. Statistical analysis was performed using Student's t test; "*" indicates $p < 0.05$ as compared to the Control group. The data are presented as mean ± SD.

(B) PER assay results from days 1–5 post-DWV infection with or without 10 mM sodium butyrate treatment. The memory formation was expressed as the percentage of PER in each group (n = 35 bees) to antennal stimulation. The data are presented as mean ± SD from 3 independent experiments and analyzed using a Mann–Whitney U test. An asterisk indicates a statistically significant difference between the treatment group and the Control group (*$p < 0.05$).

(C) Western blotting analysis of the expression of histone proteins in the brain. Bees were treated with DWV or sodium butyrate and brain lysates were prepared after treatment to analyze the expression of acetylated histone protein H3 and H4. Lysate prepared from bees without any treatment served as the control. The expression level of actin in each sample was also detected and used as a loading control. Signals of acetyl-histone H3 and H4 were first normalized to that of actin and the normalized acetyl-histone H3 and H4 signals of the control group were set to 1. Signals from treatment groups were adjusted accordingly and expressed as relative protein intensity. Statistical analysis was performed using Student's t test; an asterisk indicates a statistically significant difference between the treatment and control groups (*$p < 0.05$). The data are presented as mean ± SD.

rate in worker bees (Schroeder and Martin, 2012). Therefore, we determined the survival rate of DWV-infected bees with and without the addition of NaB to their diet. First, in order to confirm that DWV can infect bees through feeding, we measured the virus copy number in the hemolymph and brain of the bees after five days of feeding. The results showed that the virus successfully infected bees through this method. In addition, we also analyzed the effect of dietary NaB on virus replication; the results showed that NaB could inhibit DWV replication (Figure S1). When bees were infected with DWV, 50% of the bees died by the end of day 2 post-infection and only 10% survived by the end of day 5 post-infection (Figure 1A) ($p < 0.05$). When NaB was added to the diet prior to DWV infection, the survival rate of DWV-infected bees (N/D group) remained >90% after 5 days (Figure 1A). It is interesting to note that under these laboratory rearing conditions, around 30% of the control bees died over a period of 5 days (Control group); however, when NaB was included in the diet of the uninfected bees, less than 15% of the bees died. These results indicate that feeding bees with NaB could significantly increase their survival with or without DWV infection.

NaB has been shown to reverse memory impairment in bees caused by exposure to pesticides (Hu et al., 2017). The results of our proboscis extension reflex (PER) experiment showed that DWV infection significantly impaired the learning/memory ability of bees (Figure 1B). While bees were fed with NaB prior to DWV infection, the infected bees exhibited a similar level of learning/memory ability as those uninfected

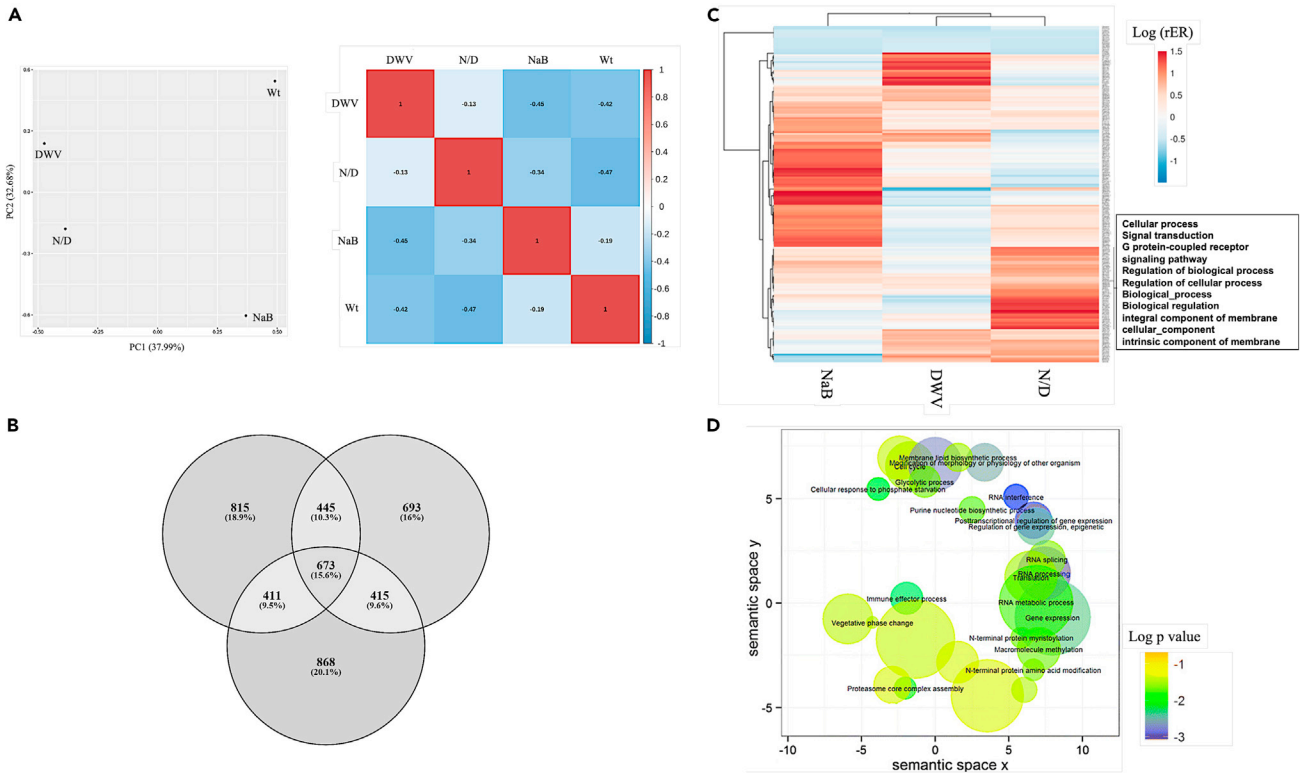


Figure 2. Analysis of gene expression profile in experimental groups by using next-generation sequencing (NGS)

(A) Genes were differentially expressed between the treatment groups. A scatterplot of the principal component analysis of the gene expression profile of the 3 experiment groups and 1 control group is shown on the left. The maximum variance is 37.99% for PC1 and 32.68% for PC2. A correlation heatmap was constructed and shown on the right. The number indicates the degree of correlation in the gene expression profile between 2 indicated groups and the strongest correlation was 1 by comparing between the same group. Red and blue color indicate a positive and negative correlation, respectively. Control: no treatment; DWV: DWV-infected; NaB: sodium butyrate-treated; N/B: DWV-infected with sodium butyrate treatment.

(B) Venn diagram showing the concordance of differentially expressed genes in treatment groups.

(C) Hierarchical clustering of gene expression in the brain of bees in treatment groups. Each column represents the gene expression profile from a treatment group and each row represents the logarithm-transformed expression values of a particular gene in 3 samples. Red and blue color indicate up- and down-regulation, respectively. The functions of genes in the clustering with the most diverse expression profiles in 3 groups are listed on the lower-right side of the heatmap.

(D) Semantic space scatterplot of gene ontology annotations associated with the most differentially-expressed genes in the heads harvested from DWV-infected bees with or without sodium butyrate treatment. Clusters of highly similar GO terms were formed using REVIGO with enrichment p value ≤ 0.05. Two-dimensional spatial organization of the enriched annotations was based on semantic similarity. The color of the bubble indicates the logarithm of the p value used for each annotation with yellow representing p values closer to 0.05 and purple colors representing p values much smaller than 0.05. The size of the bubble represents the number of genes included in each cluster.

($p < 0.05$). This suggests that DWV infection could cause learning and memory impairment in bees, and this could be reversed or prevented by NaB supplement. NaB is an HDACi that inhibits the deacetylation of the histone core, resulting in increased gene expression. The expression of acetylated histones, specifically H3 and H4, in the brains of NaB-fed bees was analyzed; it was found that NaB significantly increased acetylated H3 and H4 expression (acetyl-H3 and acetyl-H4) (Figure 1C) ($p < 0.05$). Based on these results, we postulate that NaB helped to improve learning and memory by up-regulating histone modification and subsequently the expression of genes involved in learning and memory.

NaB enhances the expression of genes involved in diverse biological processes in bees

To identify the possible gene regulation that rescues the negative effects of DWV infection on memory in NaB treatment, NGS was used to elucidate gene expression profiles in untreated bees (Control), DWV-infected bees (DWV), NaB-fed bees (NaB), and DWV-infected bees fed with NaB (N/D). After aligning and quantifying the NGS reads with the genes in the honeybee genome, an expression library for each treatment group was established. In the principal component analysis (PCA), each treatment group had a distinct gene expression profile and did not cluster with one another (Figure 2A). A total of 4,320 genes

showed differential expression in the treatment groups compared to the control group: 2,344 genes in the DWV group, 2,367 genes in the NaB group, and 2,226 genes in the N/D group (Figure 2B and Data S1). Among these genes, 673 were found in all 3 groups and the DWV and NaB groups had the most uniquely expressed genes (868 genes), which were not found in the other 2 groups.

Gene ontology of these differentially expressed genes was constructed with their relative respective expression in each group. There were 219 genes whose expressions were highly up-regulated in the N/D group but significantly suppressed in the DWV group. These genes were involved in signal transduction, the G protein-coupled receptor signaling pathway, regulation of biological and cellular processes, biological regulation, integral component of membrane, cellular component, and intrinsic component of membrane (Figure 2C). Of these 219 genes, 53 exhibited Log rER >0.05 between the N/D group and the DWV group (Table S1) and many were involved in memory behavior. A semantic scatterplot of these 53 genes yielded clusters with high levels of relatedness (Log p value < −2.5) including RNA processing, the glycolytic process, regulation of gene expression and epigenetic, as well as learning and memory (Figure 2D). The NGS results thus indicate that NaB might help repair the impairments caused by DWV infection through several different mechanisms, including directly inducing the expression of memory and learning-related genes or modulating the cellular processes.

NaB restores neurotransmitters in the bee brain via the glycolytic process

In NGS analysis, genes involved in the glycolytic process were among the genes whose expression levels were significantly different in DWV-infected bees with or without NaB treatment. Glycolysis is important for energy generation and involves a sequence of enzymatic reactions that convert a glucose molecule into two pyruvate molecules and generates adenosine triphosphate as a product. Previous research found that mushroom body glycolysis is required for olfactory memory in *Drosophila* (Wu et al., 2018).

Disruption of glycolysis in the brain may be one pathogenesis of memory impairment caused by DWV infection, and NaB might antagonize the disruption to restore memory functions. To verify this assumption, we analyzed the expression of glycolytic enzyme genes in the brain of honeybees after exposure to DWV, NaB, or a combination of both. In order to confirm that DWV can successfully infect bees via feeding, we measured the virus copy number in the brain of bees after five days post infection. After confirming virus infection, the same samples were used for viral gene expression assay (Figure S2). The expressions of glycolytic enzymes glucokinase (*gk*), phosphofructokinase (*pfk*), and pyruvate kinase (*pyk*) were determined by qPCR. The results showed that expression levels decreased significantly after bees were infected with DWV, whereas the decrease was not as significant in the N/D group, suggesting that NaB might help prevent the negative effect on gene expression induced by DWV (Figure 3A). At the same time, we also analyzed the ATP level, which yielded a similar result (Figure 3B). ATP provides energy for neurotransmission and is also involved in regulating neurotransmitters (Gold et al., 1986). The main neurotransmitter in bees is glutamate, which is normally synthesized via the Glu-Gln cycle. The concentration of glutamate and the expression level of glutaminase mRNA in the brain was measured; the result showed that glutamate concentration in the brain decreased significantly after bees were infected with DWV (Figure 3C) ($p < 0.05$) and that glutaminase mRNA expression decreased (Figure 3D). When bees were administered NaB prior to DWV infection (N/D group), the decrease in glutaminase gene expression was not as significant. This suggests that DWV infection negatively influences glutamate synthesis (via a decrease in glutaminase) and can be partially recovered by NaB as it mediates glutaminase gene expression (Figure 3D). N-methyl-D-aspartate receptor (NMDAR) is a major subunit of the glutamate receptor and is involved in the plasticity of dendrites and memory function. The expression levels of NMDA receptor subunits NR1 and NR2 were measured to assess whether their expression was affected by different levels of glutamate. Similarly, to the results for glutaminase, the expression levels of *nr1* and *nr2* decreased in DWV-infected bees compared to the control, and the pre-treatment of bees with NaB rescued this decrease (Figure 3E). In a previous study, it was shown that the activation of NMDAR was accompanied by calpain activation (Wu et al., 2005), and it was subsequently demonstrated that calpain is involved in learning and memory functions (Chakrabarti et al., 2015). In the present study, Calpain 1 expression was found to be significantly lower in the brains of bees from the DWV group than that of the N/D group, indicating that NaB could help restore its expression in DWV-infected bees (Figure 3F).

Overall, the above results suggest that NaBcould help rescue the negative effects on memory caused by DWV infection by resuming the normal glycolysis and the synthesis of factors involved in neural transmission in honeybees.

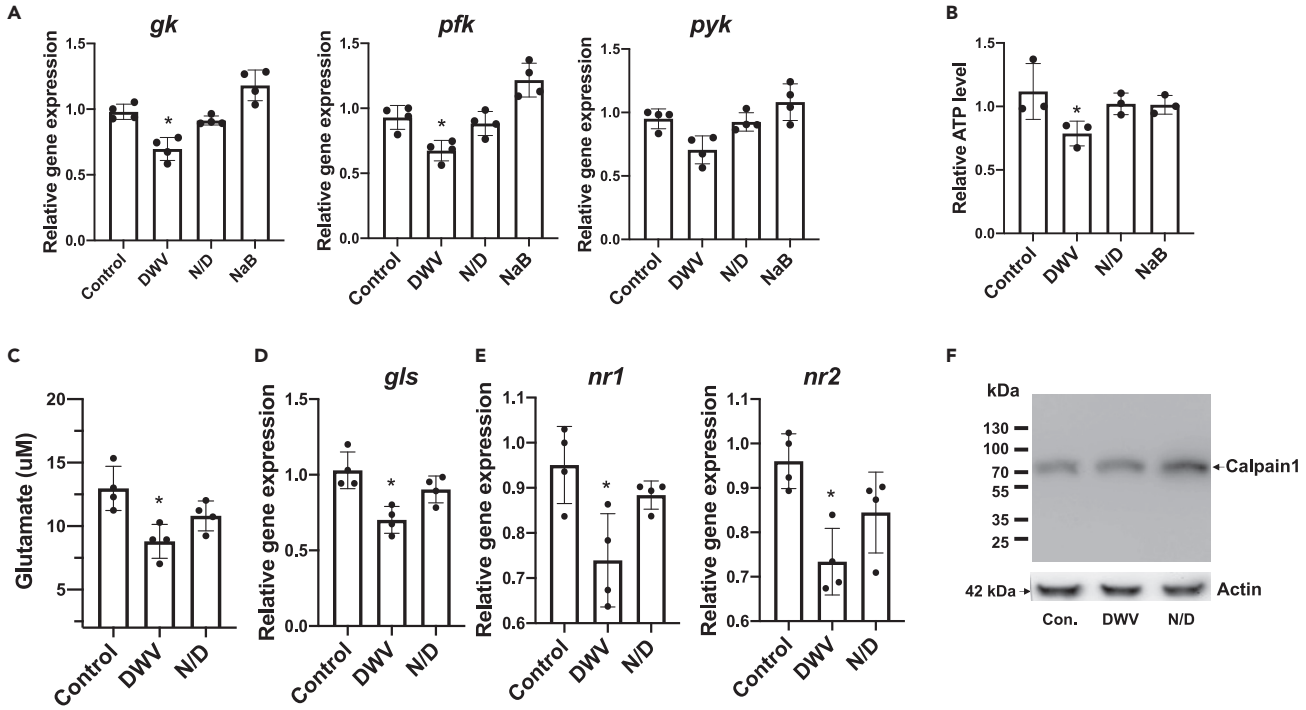


Figure 3. Sodium butyrate restores ATP and neurotransmitter glutamate in bee brains via glycolysis

(A) RT-qPCR analysis of glycolytic genes glucokinase (*gk*), phosphofructokinase (*pfk*), and pyruvate kinase (*pyk*) in honeybee brains 48 hr after DWV infection with or without sodium butyrate treatment. All results were normalized to the expression of the *18S* rRNA gene and non-infected control (ΔΔCt).

(B) ATP levels in honeybee brains were measured 48 hr after DWV infection with or without sodium butyrate treatment. All results were normalized to those of the control group.

(C) Glutamate levels in honeybee brains were measured 48 hr after DWV infection with or without sodium butyrate treatment.

(D) Expression of glutaminase (*gls*).

(E) Expression of NMDA receptor subunits *nr1* and *nr2*, respectively. For the NaB group, bees were fed with 10 mM of sodium butyrate. For the N/D group, bees were fed with 10 mM sodium butyrate for 7 days, followed by infection with DWV. Three independent experiments were performed and 35 bees were included in each group for every experiment.

(F) Western blotting analysis of the expression of Calpain-1 in the brain. Bees were treated with DWV or sodium butyrate and brain lysates were prepared after treatment to analyze the expression of Calpain-1. Lysate prepared from bees without any treatment served as the control. The expression level of actin in each sample was also detected and used as a loading control. All the bar graphs are presented as mean ± SD. p value were calculated using Student's t-test (*, $p<0.05$) as compared to the control group.

Short treatment of NaB reduces the negative physiological effect of DWV infection in bees and increases their field activity

The results so far indicated that NaB could help reverse the damage induced by DWV infection in bees, especially in learning/memory behavior. To validate this further, a field trial was devised. Hives were divided into 4 groups with different treatments as described above. As the queen of each hive came from the same source, thus each colony shared identical genetic traits, generating a comparable result. We also confirmed the successful DWV inoculation in DWV-infected hives by monitoring the viral loads with qPCR (Figure S3). Each hive was wired to a WSN-based automatic monitoring system, which recorded outgoing and incoming activity in addition to the ambient environmental parameters (Figure S4). Bees in the N/D groups were fed with NaB (10 mM/L) prepared in 40% sucrose water for 7 days; those in the infection groups (DWV and N/D) were infected with DWV after following the 7-day NaB diet (Figure 4A). The activity of these bees was monitored and recorded for 37 days post-DWV infection, during which bees were fed with sucrose water only. The activity of DWV-infected bees deviated significantly from other groups on the second day of recording, with an in/out ratio of 0.4971, indicating that at least 70% of the outgoing bees did not return to the hive, whereas the other 2 groups maintained an in/out ratio around 0.8250 (Figure 4B). This suggests that the bees from the DWV-infected hive may have lost their homing ability. A statistical analysis of the in/out ratio showed that DWV infection decreased the in/out ratio, and the DWV-infected colony supplied with NaB had a significantly higher in/out ratio (Figure 4C). This suggests

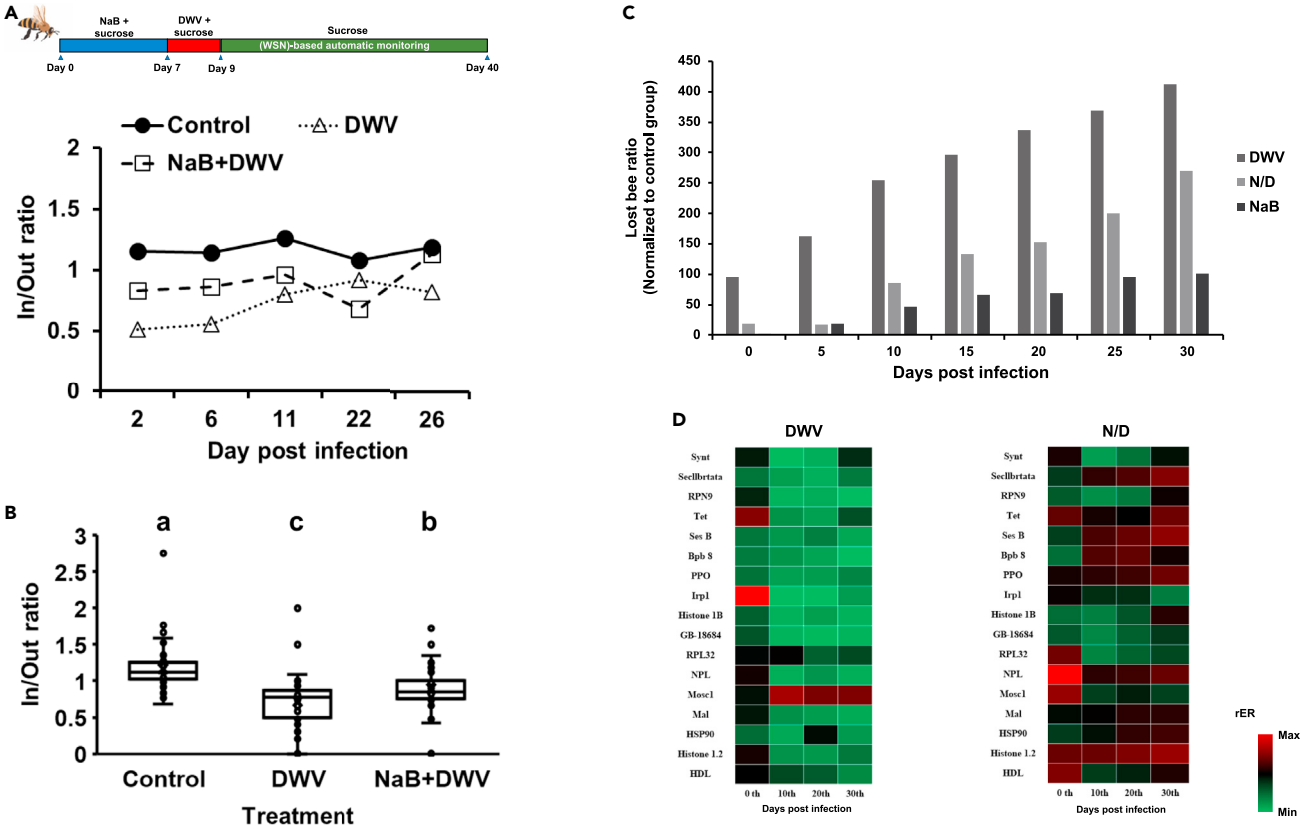


Figure 4. Short-term supply of sodium butyrate could restore the bee activity and the ratio of lost bees after DWV infection

(A) Real-time monitoring of the incoming and outgoing activity of DWV-infected bees with or without sodium butyrate treatment for 30 days. For DWV infection groups (DWV and N/D), bees were infected with the DWV solution containing 10^6 virus copy numbers in a time frame of 48 hr before their activities were monitored. Bees without any treatment were assigned to the Control group. Bee activity is expressed as an activity ratio, defined as the ratio of incoming to outgoing activities on the indicated day post-infection. The activity ratio on days 2, 6, 11, 22, and 26 post-infection was recorded. The highest activity ratio on an indicated day was set to 1 and the ratios from other groups were adjusted accordingly.

(B) The activity ratio on days 2, 6, 11, 22, and 26 post-infection was recorded. Significance was determined by one-way ANOVA with Post-hoc analysis (Tukey test); different letters for the treatment group indicate significant differences at $p < 0.05$. The data are presented as mean ± SD.

(C) Sodium butyrate treatment decreased the ratio of lost bees after DWV infection. The lost bee ratio was calculated by subtracting the number of incoming bees from the number of outgoing bees on an indicated day and then dividing that number by the number of lost bees in the Control group on the same day.

(D) Expression analysis of genes involved in learning and memory. DWV-infected bees with (N/D) or without (DWV) sodium butyrate treatment were collected on days 0, 10, 20, and 30 post-infection. The expression of 17 genes involved in learning and memory was analyzed by real-time qPCR; the expression level of *18S* rRNA was also measured and used as an internal control. These 17 genes included *Synt*, Syntaxin 1A; *Sec61Beta*, Sec61 b subunit FBgn0010638; *RPN9*, regulatory particle non-ATPase 9; *Tet*, tetracycline resistance protein; *SesB*, stress-sensitive B; *Rpb8*, subunit 8 of RNA polymerase II; *PPO*, prophenol oxidase; *Irp 1*, iron regulatory protein 1; *RPL32*, ribosomal protein L32; *NPL*, N-acetylneuraminate pyruvate lyase; *Mosc1*, mitochondrial mosc domain-containing protein 1; *Mal*, myelin and lymphocyte protein; *HSP90*, heat shock protein 90; and *HDC*, histidine decarboxylase. The expression levels of these 17 genes were normalized with that of *18S* rRNA. Normalized expressions of these genes are presented with red and green color denoting up- and down-regulation, respectively. Triplicate samples were prepared from each group and each sample contained brains from 5 bees in each group

that NaB treatment prior to DWV infection was sufficient to suppress the negative effect induced by DWV throughout the course of the infection. The DWV group lost the greatest number of bees during the course of the 30-day experiment (Figure 4C), which was 1.53-fold greater than that of the N/D group.

Worker bees from the 4 hives were collected on day 0, 10, 20, and 30 for gene expression analysis. Genes that had been previously reported to be involved in learning/memory in bees were investigated, including *synt*, *secllbrtata*, *rpn9*, *tet*, *sesB*, *bpb8*, *ppo*, *irp1*, *histone 1B*, *gb-18684*, *rpl32*, *npl*, *mosc1*, *mal*, *hsp90*, *histone1.2*, and *hdl*. The gene expressions were mostly suppressed in DWV-infected bees from day 0 to day 30, except for *irp* and *tet* on day 0 and *mosc1* from day 10 to day 30 (Figure 4D). For the N/D group bees, even though the expression of some genes was still suppressed (*irp1*, *gb-18684*, *histone 1B*, *rpn9*, *mosc1*, and *rpl32*), a greater number showed active expression, in particular

histone 1.2, *ppo*, *ses-b*, *tet*, and *secllbrtata*, which were up-regulated throughout the experiment (Figure 4D; Table S2).

Steady dietary supply of NaB significantly improves homing ability of DWV-infected worker bees by upregulating the expression of genes involved in learning and memory

In the first WSN monitoring experiment, it was found that a relatively short period of NaB treatment (7 days prior to DWV infection) was able to minimize the number of bees lost from a colony. To confirm whether a long-term supply of NaB had a negative effect on bee homing behavior, we performed another experiment by constantly supplying the NaB for more than 30 days and monitoring the number of bees entering and exiting the hive (Figure 5A). The in/out ratio of DWV group bees declined from day 2 and started to increase from day 17 (Figure 5B). The activity ratio of the N/D group bees, however, resembled that of the control and NaB only group bees (Figure 5B). Figure 5B shows the in/out ratio for Figure 5A at the time of drawing out for statistics [i.e., Figure 5B shows 7 time points (Figure 5A), and each treatment group has 7 data points]. The results highlighted in Figure 5C show that the continuous feeding of NaB has the ability to restore, and that there was no difference between the NaB treatment group and the control. This outcome is also reflected in lost bee ratio during the course of experiment, with the DWV group exhibiting the highest ratio (Figure 5C). There was no significant difference in the ratio of losing bees between the N/D and the NaB groups, which were both significantly lower than that of the DWV group. In addition, an analysis of the gene expression in experimental bees showed that those involved in learning/memory were highly up-regulated in NaB-fed bees despite being infected with DWV (Figure 5D; Table S3). These results demonstrate that a continuous supply of NaB helped bees counteract the negative effects of DWV infection on homing ability by up-regulating the expression of genes involved in learning/memory processes. In addition, we did not observe a significant impact of the NaB treatment on homing behavior.

DISCUSSION

In the present study, a comprehensive elucidation of DWV infection and the effect of sodium butyrate (NaB) treatment on bees is presented. We found that NaB could increase the survival rate of bees infected with DWV (Figure 1A). Furthermore, NaB was able to reverse the negative effects on learning/memory ability caused by DWV infection (Figure 1B). Genes that underwent a significant alteration in expression level by NaB and DWV were identified through NGS. The identified genes were found to be involved in the following physiological functions: epigenetics, signal transduction, regulation of biological process, regulation of cellular process, biological process, and biological regulation (Figures 2C and 2D). By functionally clustering genes which exhibited significantly different expression levels between DWV-infected bees with and without NaB treatment, it was found that many were involved in metabolic processes and the learning/memory pathway (Table S1). Previous studies have demonstrated that NaB could counteract the negative effects of imidacloprid (a nicotine-like pesticide) in bees, in addition to enhancing their immune responses and detoxification processes, and restore learning behavior (Hu et al., 2017, 2018). This suggests that NaB might have a use in preventing virus-induced CCD.

As an epigenetic regulator, the treatment of NaB might affect the expression of genes associated with diverse biological processes. A gene cluster identified by NGS analysis with significantly different expression levels between the DWV and N/D groups was the glycolytic process cluster. Detailed gene expression analysis showed that DWV infection down-regulated the expression of enzymes involved in the glycolysis pathway (Figure 3A) and disrupted the energy supply in the brain of bees (Figure 3B). The shortage in energy further affected neurotransmission. In honeybees, glutamate is one of the most abundant neurotransmitters. The majority of glutamate is produced through the conversion of glutamine to glutamate, which is mediated by glutaminase. Glutamine, one of the few amino acids which can cross blood-brain barriers, is primarily stored in muscles and is transported to the brain, where it is converted to glutamate. When the glutamate concentration reaches a certain level, it binds to glutamate receptors, e.g., NMDA receptors, which subsequently activate downstream calpain. This eventually forms long-term potentiation, which aids in memory formation and the consolidation of long-term memory (Chakrabarti et al., 2015). The present study showed that infection with DWV decreased the expression of glutaminase, calpain and the subunits of NMDA receptors, and resulted in impairments to both memory and homing behavior in bees (Figure 3). This correlates to studies in humans, where patients with age-related brain/neuronal diseases have been found to display significantly lower levels of both glutamate and NMDA receptors (Newcomer et al., 2000). We further demonstrated that NaB treatment is able to reduce the impairments caused by DWV infection by stimulating the expression of both glutamate and NMDA receptor

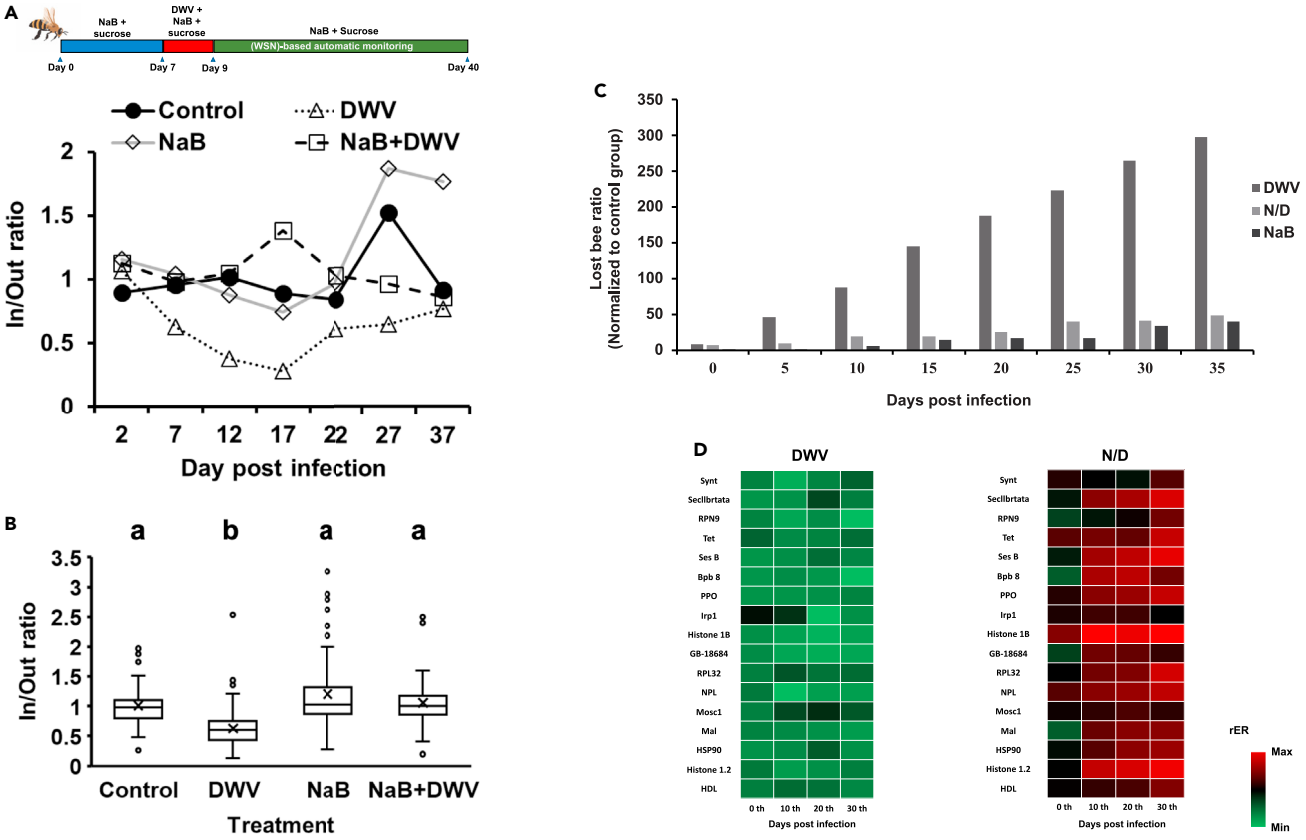


Figure 5. Routine supply of sodium butyrate in the diet significantly improves the activity ratio and minimizes the ratio of losing bees after DWV infection

(A) Real-time monitoring of the incoming and outgoing activity of DWV-infected bees with or without sodium butyrate treatment for 37 days. For the NaB group, bees were fed with sodium butyrate for the duration of the experiment. For the DWV and N/D groups, bees were infected with the DWV solution containing 10^6 virus copy numbers in a time frame of 48 hr before their activities were monitored. Bees without any treatment were assigned to the Control group. The activities of bees are expressed as activity ratios, i.e. the ratio of incoming to outgoing movement on the indicated day post-infection. The activity ratio on days 2, 7, 12, 17, 22, 27, and 37 post-infection was recorded. The highest activity ratio on an indicated day was set to 1 and the ratios from other groups were adjusted accordingly.

(B) The ratio of in and out of Figure 5A at the time of drawing out for statistics. Figure 5A records 7 time points, and each treatment group has five data points. Significance was determined by one-way ANOVA with Post-hoc analysis (Tukey test); different letters for the treatment group indicate significant differences at $p < 0.05$. The data are presented as mean ± SD.

(C) Sodium butyrate treatment significantly decreased the ratio of losing bees after DWV infection. The lost bee ratio was calculated by subtracting the number of incoming bees from the number of outgoing bees on an indicated day and then divided that number by the number of lost bees in the Control group on the same day.

(D) Expression analysis of genes involved in learning and memory. DWV-infected bees with (N/D) or without (DWV) sodium butyrate treatment were collected on days 0, 10, 20, and 30 post-infection. The expression of 17 genes involved in learning and memory was analyzed by real-time qPCR and the expression level of *18S* rRNA was also measured as an internal control. The expression levels of 17 genes were normalized with that of 18S rRNA. Normalized expressions of these genes are presented with red and green color denoting up- and down-regulation, respectively. Triplicate samples were prepared from each group and each sample contained heads from 5 bees in each group

subunits, which could therefore decrease colony loss (Figure 3). A similar phenomenon has been observed in mammals, where histone deacetylase inhibitors have been shown to enhance learning ability in mice (Malvaez et al., 2010).

The ability of NaB to reverse memory loss or impaired learning ability in bees infected with DWV was further demonstrated in field studies, which showed that pre-treating bees with NaB significantly restored the homing behavior of infected bees (Figure 4). Furthermore, DWV-infected bees provided with a continuous supply of NaB exhibited activity similar to uninfected bees (Figure 5). Gene expression profile analysis showed that genes involved in learning/memory ability exhibited up-regulated expression in DWV-infected bees on a NaB diet (Figure 5D). This suggests that NaB helps bees recover from the memory loss effects of DWV by up-regulating

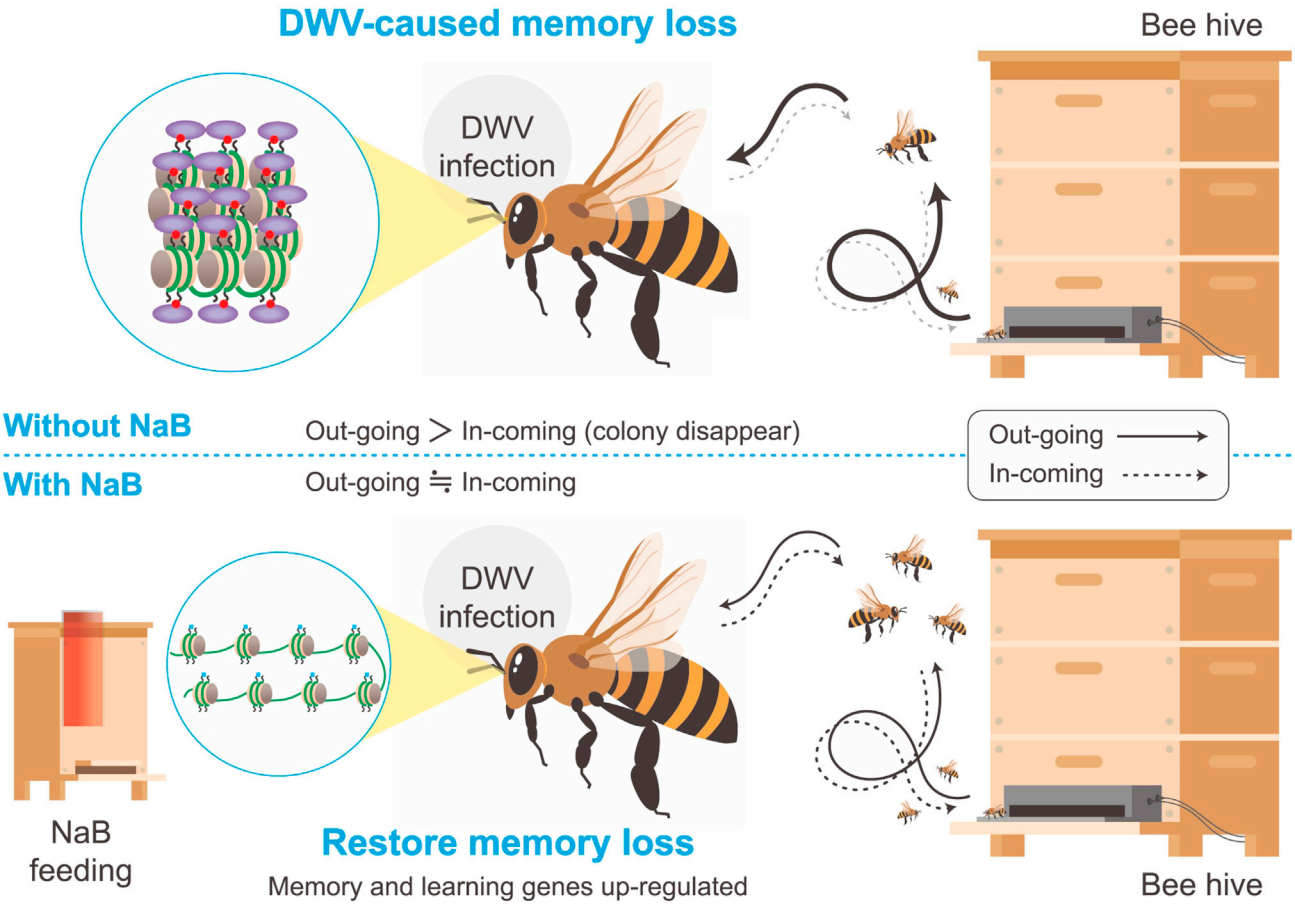


Figure 6. Schematic diagram of how epigenetics is involved in regulating the memory function of honeybees.
HDACi restored the memory impairment of DWV-infected bees. The WSN-based automatic monitoring system confirmed that sodium butyrate could improve the ability of DWV-infected bees to return to their hives.

learning/memory gene expression, either by direct regulation or by in-direct regulation through resumed neurotransmission activity. These results further demonstrate that NaB could counteract colony collapse by restoring the homing behavior upon DWV infection. Our monitoring system was able to continuously track the in-out frequency of bees. Although only one hive was used for each treatment, each hive contained more than ten thousand bees. In addition, two separate experiments were performed, with either shorter or continuous NaB treatment, and they were conducted during different dates with different hives. These two independent experiments showed a similar trend that NaB could decrease the replication of DWV in bees.

Many countries have now banned farmers from using nicotine-like pesticides (Nicholls et al., 2018; Dewar, 2017; Gross, 2013); however, despite this measure the incidence of reported colony collapse has not decreased significantly. Therefore, more attention has been directed toward pathogens as a cause, since the reduced use of pesticides has resulted in an increased number of pathogens present in the environment. *Varroa destructor*, an external parasitic mite that was found cohabiting in hives, is the main carrier of DWV (Rosenkranz et al., 2010; Le Conte et al., 2010; Yang and Cox-Foster, 2005). At present, removal of the infected hives from the farms is used to manage the DWV infection, with chemical-based pesticides to eliminate *V. destructor* as an alternative (Locke et al., 2017). However, it has been noted that *V. destructor*-targeted pesticide is toxic to bees. Although some products have been developed to boost bees' immunity against bacteria, fungi, and microbes by promoting digestive health, or fast expansion and restoration of the colony by providing sufficient nutrients (Tauber et al., 2019), no product or regimen has been developed to promote bees' ability to fight against viral pathogens thus far. The results of the present study demonstrate that routinely including NaB in the bee diet can prevent memory loss and colony decline by DWV infection without significant adverse effects (Figures 1B and 5).

Previous studies have found that histone deacetylase inhibitors increase long-term memory in mice (Davie, 2003; Vecsey et al., 2007; Dokmanovic et al., 2007). Experiments in the present study also present a similar conclusion, because bees receiving a NaB-containing diet exhibited a similar or better memory ability in the PER assays (Figure 1B) and real-time activity monitoring study, even in DWV-infected bees (Figures 4 and 5). The present study provides evidence that NaB could help bees fight against DWV infection and reverse the adverse effects of the infection by up-regulating learning/memory gene expression. Our investigation provides valuable information on HDACi gene regulation associated with memory mechanisms at the epigenetic level (Figure 6). Combining this with the results of our previous studies (Hu et al., 2017) (i.e., that NaB could enhance immunity and detoxification), a diet incorporating histone deacetylase inhibitors could be used to maintain the overall wellbeing of the bees and integrity of the colony.

Limitations of the study

In the present study, we showed the beneficial effects by short- and long-term treatment of NaB in DWV-infected bees. Although we conducted two experiments independently in different periods and hives, we could not ignore the fact that we only used one hive for each treatment because of the limited amount of our WSN monitor systems. Future work is required to expand our WSN systems for surveying higher number of colonies at the same time.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Purification of deformed wing virus (DWV)
  - Determination of virus copy number
  - Proboscis extension reflex assay
  - Western-blot analysis
  - Extraction of total RNA and cDNA synthesis
  - Analysis of gene expression by real-time qPCR
  - Sample preparation for next-generation sequencing
  - ATP analysis
  - Determination of glutamate concentration
  - Real-time recording of bee activity by a wireless sensing network (WSN)-based automatic monitoring system
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - NGS data analysis
  - Statistical analysis

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103056.

ACKNOWLEDGMENTS

We thank Mr. Alexander Barton for kindly revising the English language of the manuscript. This research was funded by the grant MOST107-2311-B-002-024-MY3 and MOST110-2313-B-002-043 to Y.L.W. from the Ministry of Science and Technology, Taiwan.

AUTHOR CONTRIBUTIONS

C.K.T. and Y.L.W. designed research; C.K.T., Y.H.L., Y.C.L., C.H.T. and Y.L.W. performed research; C.K.T., Y.H.L., J.A.J., Y.C.L., and Y.L.W. analyzed data; and C.K.T., Y.H.L., Y.R.C., C.P.W. and Y.L.W. wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: April 8, 2021
Revised: July 5, 2021
Accepted: August 25, 2021
Published: September 28, 2021

REFERENCES

Ai, H.X., Yan, X., and Han, R.C. (2012). Occurrence and prevalence of seven bee viruses in *Apis mellifera* and *Apis cerana* apiaries in China. J. Invertebr. Pathol. *109*, 160–164.

Aizen, M.A., and Harder, L.D. (2009). The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. Curr. Biol. *19*, 915–918.

Amiri, E., Kryger, P., Meixner, M.D., Strand, M.K., Tarpy, D.R., and Rueppell, O. (2018). Quantitative patterns of vertical transmission of deformed wing virus in honey bees. PLoS One *13*, e0195283.

Berthoud, H., Imdorf, A., Haueter, M., Radloff, S., and Neumann, P. (2010). Virus infections and winter losses of honey bee colonies (*Apis mellifera*). J. Apic. Res. *49*, 60–65.

Blank, M., Werenicz, A., Velho, L.A., Pinto, D.F., Fedi, A.C., Lopes, M.W., Peres, T.V., Leal, R.B., Dornelles, A.S., and Roesler, R. (2015). Enhancement of memory consolidation by the histone deacetylase inhibitor sodium butyrate in aged rats. Neurosci. Lett. *594*, 76–81.

Bowen-Walker, P.L., Martin, S.J., and Gunn, A. (1999). The transmission of deformed wing virus between honeybees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni* Oud. J. Invertebr. Pathol. *73*, 101–106.

Brettell, L.E., Mordecai, G.J., Schroeder, D.C., Jones, I.M., da Silva, J.R., Vicente-Rubiano, M., and Martin, S.J. (2017). A comparison of deformed wing virus in deformed and asymptomatic honey bees. Insects *8*, 28.

Chakrabarti, P., Rana, S., Bandopadhyay, S., Naik, D.G., Sarkar, S., and Basu, P. (2015). Field populations of native Indian honey bees from pesticide intensive agricultural landscape show signs of impaired olfaction. Sci. Rep. *5*, 12504.

Chen, Y.P., Evans, J., and Feldlaufer, M. (2006). Horizontal and vertical transmission of viruses in the honeybee, *Apis mellifera*. J. Invertebr. Pathol. *92*, 152–159.

Consalvi, S., Saccone, V., Giordani, L., Minetti, G., Mozzetta, C., and Puri, P.L. (2011). Histone deacetylase inhibitors in the treatment of muscular dystrophies: epigenetic drugs for genetic diseases. Mol. Med. *17*, 457–465.

Davie, J.R. (2003). Inhibition of histone deacetylase activity by butyrate. J. Nutr. *133*, 2485s–2493s.

de Miranda, J.R., and Genersch, E. (2010). Deformed wing virus. J. Invertebr. Pathol. *103*, S48–S61.

Dewar, A.M. (2017). The adverse impact of the neonicotinoid seed treatment ban on crop protection in oilseed rape in the United Kingdom. Pest. Manag. Sci. *73*, 1305–1309.

di Prisco, G., Annoscia, D., Margiotta, M., Ferrara, R., Varricchio, P., Zanni, V., Caprio, E., Nazzi, F., and Pennacchio, F. (2016). A mutualistic symbiosis between a parasitic mite and a pathogenic virus undermines honey bee immunity and health. Proc. Natl. Acad. Sci. U. S. A. *113*, 3203–3208.

Dokmanovic, M., Clarke, C., and Marks, P.A. (2007). Histone deacetylase inhibitors: overview and perspectives. Mol. Cancer Res. *5*, 981–989.

Fuchikami, M., Yamamoto, S., Morinobu, S., Okada, S., Yamawaki, Y., and Yamawaki, S. (2016). The potential use of histone deacetylase inhibitors in the treatment of depression. Prog. Neuropsychopharmacol. Biol. Psychiatry *64*, 320–324.

Fujiyuki, T., Takeuchi, H., Ono, M., Ohka, S., Sasaki, T., Nomoto, A., and Kubo, T. (2005). Kakugo virus from brains of aggressive worker honeybees. Adv. Virus Res. *65*, 1–27.

Garcez, M.L., de Carvalho, C.A., Mina, F., Bellettini-Santos, T., Schiavo, G.L., da Silva, S., Campos, A., Varela, R.B., Valvassori, S.S., Damiani, A.P., et al. (2018). Sodium butyrate improves memory and modulates the activity of histone deacetylases in aged rats after the administration of D-galactose. Exp. Gerontol. *113*, 209–217.

Genersch, E., von der Ohe, W., Kaatz, H., Schroeder, A., Otten, C., Buchler, R., Berg, S., Ritter, W., Muhlen, W., Gisder, S., et al. (2010). The German bee monitoring project: a long term study to understand periodically high winter losses of honey bee colonies. Apidologie *41*, 332–352.

Genersch, E., Yue, C., Fries, I., and de Miranda, J.R. (2006). Detection of deformed wing virus, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. J. Invertebr. Pathol. *91*, 61–63.

Gold, P.E., Vogt, J., and Hall, J.L. (1986). Glucose effects on memory: behavioral and pharmacological characteristics. Behav. Neural Biol. *46*, 145–155.

Gross, M. (2013). EU ban puts spotlight on complex effects of neonicotinoids. Curr. Biol. *23*, R462–R464.

Hladun, K.R., Smith, B.H., Mustard, J.A., Morton, R.R., and Trumble, J.T. (2012). Selenium toxicity to honey bee (*Apis mellifera* L.) pollinators: effects on behaviors and survival. PLoS One *7*, e34137.

Hu, Y.T., Tang, C.K., Wu, C.P., WU, P.C., YANG, E.C., TAI, C.C., and WU, Y.L. (2018). Histone deacetylase inhibitor treatment restores memory-related gene expression and learning ability in neonicotinoid-treated *Apis mellifera*. Insect Mol. Biol. *27*, 512–521.

Hu, Y.T., Wu, T.C., Yang, E.C., Wu, P.C., Lin, P.T., and Wu, Y.L. (2017). Regulation of genes related to immune signaling and detoxification in *Apis mellifera* by an inhibitor of histone deacetylation. Sci. Rep. *7*, 41255.

Jiang, J.A., Wang, C.H., Chen, C.H., Liao, M.S., Su, Y.L., Chen, W.S., Huang, C.P., Yang, E.C., and Chuang, C.L. (2016). A WSN-based automatic monitoring system for the foraging behavior of honey bees and environmental factors of beehives. Comput. Electron. Agric. *123*, 304–318.

Kajobe, R., Marris, G., Budge, G., Laurenson, L., Cordoni, G., Jones, B., Wilkins, S., Cuthbertson, A.G.S., and Brown, M.A. (2010). First molecular detection of a viral pathogen in Ugandan honey bees. J. Invertebr. Pathol. *104*, 153–156.

Lanzi, G., de Miranda, J.R., Boniotti, M.B., Cameron, C.E., Lavazza, A., Capucci, L., Camazine, S.M., and Rossi, C. (2006). Molecular and biological characterization of deformed wing virus of honeybees (*Apis mellifera* L.). J. Virol. *80*, 4998–5009.

Le Conte, Y., Ellis, M., and Ritter, W. (2010). Varroa mites and honey bee health: can Varroa explain part of the colony losses? Apidologie *41*, 353–363.

Lin, Y.H., Tai, C.C., Broz, V., Tang, C.K., Chen, P., Wu, C.P., Li, C.H., and Wu, Y.L. (2020). Adenosine receptor modulates permissiveness of baculovirus (budded virus) infection via regulation of energy metabolism in *Bombyx mori*. Front. Immunol. *11*, 763.

Locke, B., Semberg, E., Forsgren, E., and de Miranda, J.R. (2017). Persistence of subclinical deformed wing virus infections in honeybees following Varroa mite removal and a bee population turnover. PLoS One *12*, e0180910.

Lu, Y.H., Wu, C.P., Tang, C.K., Lin, Y.H., Maaroufi, H.O., CHUANG, Y.C., and WU, Y.L. (2020). Identification of immune regulatory genes in *Apis mellifera* through Caffeine treatment. Insects *11*, 516.

Malvaez, M., Sanchis-Segura, C., Vo, D., Lattal, K.M., and Wood, M.A. (2010). Modulation of chromatin modification facilitates extinction of cocaine-induced conditioned place preference. Biol. Psychiatry *67*, 36–43.

Marks, P.A., Richon, V.M., and Rifkind, R.A. (2000). Histone deacetylase inhibitors: inducers of

differentiation or apoptosis of transformed cells. J. Natl. Cancer Inst. *92*, 1210–1216.

Martin, S.J., Highfield, A.C., Brettell, L., Villalobos, E.M., Budge, G.E., Powell, M., Nikaido, S., and Schroeder, D.C. (2012). Global honey bee viral landscape altered by a parasitic mite. Science *336*, 1304–1306.

Mckinsey, T.A. (2011). Targeting inflammation in heart failure with histone deacetylase inhibitors. Mol. Med. *17*, 434–441.

Moore, J., Jironkin, A., Chandler, D., Burroughs, N., Evans, D.J., and Ryabov, E.V. (2011). Recombinants between deformed wing virus and *Varroa destructor* virus-1 may prevail in *Varroa destructor*-infested honeybee colonies. J. Gen. Virol. *92*, 156–161.

Mordecai, G.J., Brettell, L.E., Pachori, P., Villalobos, E.M., Martin, S.J., Jones, I.M., and Schroeder, D.C. (2016). Moku virus; a new Iflavirus found in wasps, honey bees and Varroa. Sci. Rep. *6*, 34983.

Newcomer, J.W., Farber, N.B., and Olney, J.W. (2000). NMDA receptor function, memory, and brain aging. Dialogues Clin. Neurosci. *2*, 219–232.

Nicholls, E., Botias, C., Rotheray, E.L., Whitehorn, P., David, A., Fowler, R., David, T., Feltham, H., Swain, J.L., Wells, P., et al. (2018). Monitoring neonicotinoid exposure for bees in rural and peri-urban areas of the UK during the transition from pre- to post-moratorium. Environ. Sci. Technol. *52*, 9391–9402.

Ongus, J.R., Peters, D., Bonmatin, J.M., Bengsch, E., Vlak, J.M., and van Oers, M.M. (2004). Complete sequence of a picorna-like virus of the genus Iflavirus replicating in the mite *Varroa destructor*. J. Gen. Virol. *85*, 3747–3755.

Pardo, A., and Borges, P.A.V. (2020). Worldwide importance of insect pollination in apple orchards: a review. Agric. Ecosyst. Environ. *293*, 17.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., and Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. *25*, 345–353.

Ray, S., and Ferneyhough, B. (1997a). The effects of age on olfactory learning and memory in the honey bee *Apis mellifera*. Neuroreport *8*, 789–793.

Ray, S., and Ferneyhough, B. (1997b). Seasonal variation of proboscis extension reflex conditioning in the honey bee (*Apis mellifera*). J. Apic. Res. *36*, 108–110.

Rosenkranz, P., Aumeier, P., and Ziegelmann, B. (2010). Biology and control of *Varroa destructor*. J. Invertebr. Pathol. *103*, S96–S119.

Ryabov, E.V., Wood, G.R., Fannon, J.M., Moore, J.D., Bull, J.C., Chandler, D., Mead, A., Burroughs, N., and Evans, D.J. (2014). A virulent strain of deformed wing virus (DWV) of honeybees (*Apis mellifera*) prevails after *Varroa destructor*-mediated, or in vitro, transmission. PLoS Pathog. *10*, e1004230.

Schroeder, D.C., and Martin, S.J. (2012). Deformed wing virus: the main suspect in unexplained honeybee deaths worldwide. Virulence *3*, 589–591.

Shah, K.S., Evans, E.C., and Pizzorno, M.C. (2009). Localization of deformed wing virus (DWV) in the brains of the honeybee, *Apis mellifera* Linnaeus. Virol. J. *6*, 182.

Shein, N.A., and Shohami, E. (2011). Histone deacetylase inhibitors as therapeutic agents for acute central nervous system injuries. Mol. Med. *17*, 448–456.

Tauber, J.P., Collins, W.R., Schwarz, R.S., Chen, Y.P., Grubbs, K., Huang, Q., Lopez, D., Peterson, R., and Evans, J.D. (2019). Natural product medicines for honey bees: perspective and protocols. Insects *10*, 356.

Traniello, I.M., Bukhari, S.A., Kevill, J., Ahmed, A.C., Hamilton, A.R., Naeger, N.L., Schroeder, D.C., and Robinson, G.E. (2020). Meta-analysis of honey bee neurogenomic response links deformed wing virus type A to precocious behavioral maturation. Sci. Rep. *10*, 3101.

Vecsey, C.G., Hawk, J.D., Lattal, K.M., Stein, J.M., Fabian, S.A., Attner, M.A., Cabrera, S.M., Mcdonough, C.B., Brindle, P.K., Abel, T., and Wood, M.A. (2007). Histone deacetylase inhibitors enhance memory and synaptic plasticity via CREB: CBP-dependent transcriptional activation. J. Neurosci. *27*, 6128–6140.

Wu, C.L., Chang, C.C., Wu, J.K., Chiang, M.H., Yang, C.H., and Chiang, H.C. (2018). Mushroom body glycolysis is required for olfactory memory in Drosophila. Neurobiol. Learn Mem. *150*, 13–19.

Wu, H.Y., Yuen, E.Y., Lu, Y.F., Matsushita, M., Matsui, H., Yan, Z., and Tomizawa, K. (2005). Regulation of N-methyl-D-aspartate receptors by calpain in cortical neurons. J. Biol. Chem. *280*, 21588–21593.

Wu, Y., Starzinski-Powitz, A., and Guo, S.W. (2007). Trichostatin A, a histone deacetylase inhibitor, attenuates invasiveness and reactivates E-cadherin expression in immortalized endometriotic cells. Reprod. Sci. *14*, 374–382.

Yanez, O., Jaffe, R., Jarosch, A., Fries, I., Moritz, R.F.A., Paxton, R.J., and de Miranda, J.R. (2012). Deformed wing virus and drone mating flights in the honey bee (*Apis mellifera*): implications for sexual transmission of a major honey bee virus. Apidologie *43*, 17–30.

Yang, X.L., and Cox-Foster, D.L. (2005). Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host immunosuppression and viral amplification. Proc. Natl. Acad. Sci. U. S. A. *102*, 7470–7475.

Zhou, Y.H., Badgett, M.J., Billard, L., Bowen, J.H., Orlando, R., and Willis, J.H. (2017). Properties of the cuticular proteins of *Anopheles gambiae* as revealed by serial extraction of adults. PLoS One *12*, e0175423.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
40% sucrose water	Taiwan Sugar Corporation	Q-103794905-00052-8
10 mM/L sodium butyrate	Tokyo Chemical Industry Co., Ltd	S0519
Phosphate-buffered saline (PBS)	Protech Technology Enterprise Co., Ltd.	J373-4L
pKShGP1	Welgene Biotech Co., Ltd.	CAT#212206
4 μL/mL limonene	Elevation Terpenes, LLC	CAT#92354
Sodium butyrate (10 mM) Formula name: NaC4H8O2	Tokyo Chemical Industry Co., Ltd	S0519
SDS-PAGE gels	Merck Millipore	MP42G10
5% skimmed milk	Fonterra Brands (Far East) Limited, Taiwan Branch (H.K.)	A-112430309-00000-0
0.05% Tween-20	Promega Corporation	H5152
Acetyl-H3	Merck Millipore	Cat# 06-599; RRID:AB_2115283
Acetyl-H4	Merck Millipore	Cat# 06-866; RRID:AB_310270
Calpain-1	ABcam	Cat# ab28258; RRID:AB_725819
Actin	Merck Millipore	Cat# MAB1501; RRID:AB_2223041
Trizol™ Reagent	Thermo Fisher Scientific Inc.	CAT#15596026
Chloroform	Sigma-Aldrich, Inc	C7559
Isopropanol	Echo Chemical Co., Ltd	CAT#423830010
Ethanol	Sigma-Aldrich, Inc	V001229
RNase-free water	QIAGEN	CAT#129112
Assay buffer	Cell Biolabs, Inc.	CAT#268002
Enzyme mix	BioVision, Inc.	K413-100-3
Substrate mix	BioVision, Inc.	K413-100-4
PicoProbe	BioVision, Inc.	K413-100-2
Critical commercial assays		
CloneJET PCR Cloning Kit	Thermo Fisher Scientific	K1231
Presto™ Mini Plasmid Kit	Geneaid	PDH300
Western blot analysis kit from Millipore	Merck Millipore	C72652
NEBNext® Ultra™ RNA Library Prep Kit for Illumina®	New England BioLabs	NEB #E7770
High Capacity cDNA Reverse Transcription Kit	Applied Biosystems	4368813
SensiFAST™ SYBR® Hi-ROX Kit	Bioline	BIO-92005
ATP determination kit	Molecular Probes, Inc.	P11496
Glutamate Determination Kit	BioVision, Inc.	K688-100
Oligonucleotides		
DWV qPCR forward primer: 5′-gTgTTgCAACTCgCTTCgTT-3′	Tri-I Biotech Inc. (See STAR Methods section)	N/A
DWV qPCR reverse primer: 5′ CgCgCTgTTTTTgACgTCTC 3′	Tri-I Biotech Inc. (See STAR Methods section)	N/A

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
gp1 qPCR forward primer: 5′-gATCgCTgAACgTTgTACgC-3′	Tri-I Biotech Inc. (See STAR Methods section)	N/A
gp1 qPCR reverse primer: 5′- TTgCCTgCACTggATTCgAT-3′	Tri-I Biotech Inc. (See STAR Methods section)	N/A
Software and algorithms		
Prism 9.2	GraphPad	https://www.graphpad.com/scientific-software/prism/
NanoDrop 2000 spectrophotometer	Thermo Fisher Scientific	CAT#ND-2000
DNA Copy Number and Dilution Calculator	Thermo Fisher Scientific	https://www.thermofisher.com/tw/zt/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/dna-copy-number-calculator.html
Enhanced chemiluminescence system	Immobilon Western, Millipore	N/A
ABI StepOnePlus Real-Time PCR	Thermo Fisher Scientific	CAT#4376600
Agilent 2100 Bioanalyzer	Agilent Technologies, Inc.	G2939BA
Illumina HiSeq 2000™	Illumina	N/A
Data analysis and expression library	Genomics, Taiwan	N/A
FlexCycler^2 PCR Thermal Cycler	VWR International, LLC.	16055039
ABI PlusOne real-time system	Applied Biosystems	N/A
Epoch Microplate Spectrophotometer	BioTek Instruments, Inc.	N/A
SpectraMax Gemini EM Microplate Reader	Molecular Devices, LLC.	N/A
Wireless sensing network (WSN)-based automatic monitoring system	Department of Electrical Engineering at National Taiwan University	N/A
Deposited data		
Apis mellifera RNA-seq control group	This paper	Accession: SRR15373629
Apis mellifera RNA-seq NaB/DWV group	This paper	Accession: SRR15373630
Apis mellifera RNA-seq NaB group	This paper	Accession: SRR15373631
Apis mellifera RNA-seq DWV group	This paper	Accession: SRR15373632

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Yueh-Lung Wu (runwu@ntu.edu.tw).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The NGS data have been deposited at SAR (NCBI) and are publicly available as of the date of publication. Accession numbers are listed in the key resources table. The transcriptional profile of the NGS data is also available in Data S1.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Western honey bees (*Apis mellifera*) were collected from a bee farm in Hsinchu County, Taiwan. Queen of each beehive was taken from the same source to reduce genetic differences between colony. Prior to the experiments, worker bees were placed in insect rearing cages [30 × 30 × 30 cm; BugDorm (Naturestore Co., Ltd)] and fed with 40% sucrose water containing 10 mM/L of sodium butyrate (Tokyo Chemical Industry Co., Ltd) for 7 days prior to DWV infection or as otherwise stated (N/D group) (Hu et al., 2017). For DWV infection, bees were placed in 1000-μL pipette tips with their necks taped at the openings of the tips such that their mandibles and proboscis were protruding from the tip. Each bee was then fed with 10^6 virus copy number of DWV prepared in 40% sucrose water over a period of 2 days (DWV group). Fifty foragers were kept in a cage for each treatment group (Control, DWV, NaB, and N/D) at the indicated time points. Three to five bees were collected and pooled together as one replicate for Western blot, qPCR, ATP and glutamate measurements, respectively. For survival and PER tests, each replicate contained 35 to 50 bees. For RNA-Seq, each RNA sample contained ten bees per treatment. For field experiment, one colony per treatment was used and each colony contained more than ten thousand bees.

METHOD DETAILS

Purification of deformed wing virus (DWV)

To locate a suitable source of DWV, we conducted a survey of *Varroa* mite-infested beehives at the farm. Hives with an infestation level of at least 50% (from inspected combs) were chosen and around 80–100 worker bees were directly collected. DWV was then purified from the infected worker bees, which was confirmed by sequence-specific PCR reaction (in-house) (PCR primers: DWV-F:5′-gTgTTg CAACTCgCTTCgTT-3′; DWV-R:5′ CgCgCTgTTTTTgACgTCTC 3′). Worker bees with obvious infection symptoms were collected. After removal of their appendages, they were homogenized in 1× phosphate-buffered saline (PBS). The supernatant was passed through a 400-mesh filter (approximately 0.037 mm), followed by centrifugation at 1,000× *g* for 30 min at room temperature. The supernatant was collected and used for subsequent DWV infection experiments (Lu et al., 2020). Each sample was then diluted to a titer of 1 × 10^7 virus copy number in 50 ml.

Determination of virus copy number

A plasmid was first constructed to establish the standard curve. A plasmid (pKShGP1) containing the *gp1* gene (NC_004830.2) of DWV (3,350 bp) was obtained using a CloneJET PCR Cloning Kit (Thermo Fisher Scientific). The product of DWV *gp1* is polyprotein located at bases 1145 to 9826 of DWV genome. Plasmid extraction was conducted using a Presto™ Mini Plasmid Kit (Geneaid) and the concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). DNA Copy Number and Dilution Calculator (Thermo Fisher Scientific) were used to determine the amount of DNA sample equivalent to 10^{10} plasmid copies. An RT-qPCR reaction was carried out using 10-fold serially-diluted plasmid as templates and *gp1*-specific primer pairs (gp1f: 5′-gATCgCTgAACgTTgTACgC-3′; gp1r: 5′- TTgCCTg CACTggATTCgAT-3′) the Ct values obtained were used to plot a standard curve. The virus copy number was then calculated using DNA Copy Number and Dilution Calculator (Lu et al., 2020). The DWV titer ranged from 8.85 × 10^4 to 5.05 × 10^8 virus copy number. We then diluted the virus to 1 × 10^7 virus copy number for the infection experiment.

Proboscis extension reflex assay

A proboscis extension reflex (PER) assay was used to assess the learning and memory behavior of experimental bees. When training the bees to associate, the honey bees were offered 50% sucrose solution (unconditioned stimulus) for 3 sec, followed by odor stimulation (4 μL/mL of limonene, conditioned stimulus) for 4 sec and then co-exposure to odor stimulation and sugar water for 5 sec. These steps accounted for 1 training trial. The training trial was repeated 20 times with a 5 min inter-trial interval (Fig. S5). The odor stimulation used for the subsequent experiment was 4 μL/mL of limonene. The PER response rate was calculated according to Ray and Ferneyhough (Ray and Ferneyhough, 1997a, 1997b), as shown: PER response rate = (no. of honey bees with response/no. of honey bees tested) × 100%. The PER responses of experimental bees without any treatment (Control), receiving sodium butyrate diet prior to DWV infection (N/D), receiving sodium butyrate diet only (NaB), and with DWV-infection only (DWV) were recorded daily for 5 days after DWV infection (Hu et al., 2018).

Western-blot analysis

The honey bees used for protein level analysis were divided into four groups: no treatment (con.), sodium butyrate only (NaB), DWV only (DWV) and NaB+DWV (N/D). Sodium butyrate (10 mM) in ddH_2O was used in the feeding assay for 7 days. Protein was extracted from the brains of the honey bees for Western blot analysis using a kit from Millipore (Hu et al., 2017). The samples were subjected to electrophoresis on SDS-PAGE gels with equal amounts of loaded samples. The proteins were transferred onto nitrocellulose membranes (Schleicher & Schuell) by electroblotting for 1 h. Membranes were then blocked with PBS containing 5% skimmed milk and 0.05% Tween-20 and incubated with primary anti-mouse antibodies against Acetyl-H3 (1:5000 dilution) (Millipore), Acetyl-H4 (1:5000 dilution) (Millipore), Calpain-1 (1:5000 dilution) (ABcam) and Actin (1:3000 dilution) (Millipore), followed by secondary rabbit anti-mouse antibody conjugated with horseradish peroxidase (Millipore). The proteins were detected with an enhanced chemiluminescence system (Immobilon Western, Millipore) (Hu et al., 2018). Each group included three biological replicates.

Extraction of total RNA and cDNA synthesis

Total RNA was extracted from bee brains or DWV virus solution using Trizol™ Reagent (Thermo Fisher Scientific) (Hu et al., 2018; Lu et al., 2020). One hundred milligrams of bee brains (five to ten whole brains) was placed in a microcentrifuge tube and ground with a pestle in 1,000 μL of Trizol™ reagent. They were then incubated for 5 min, followed by high-speed centrifugation at 12,000× *g* for 10 min at 4 °C. The supernatant was transferred to a clean tube and mixed with 200 μL of chloroform. The mixture was vortexed for 15 sec and then incubated for 3 min at room temperature, followed by centrifugation at 12,000× *g* for 15 min at 4 °C. The aqueous phase was collected, mixed with 500 μL of isopropanol and incubated for 10 min at room temperature, followed by centrifugation at 12,000× *g* for 10 min at 4 °C. The pellet was washed with 75% ethanol, followed by centrifugation at 7,500× *g* for 5 min at 4 °C and vacuum-dried for 3 min to remove residual ethanol. The pellet was resuspended in 30 μL of RNase-free water and stored at -80 °C until use.

Complementary DNA (cDNA) synthesis was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). One microgram of total RNA was used for cDNA synthesis following the manufacturer's protocol. The synthesis reaction was performed on a FlexCycler2 PCR Thermal Cycler (Thomas Scientific) with the following program: 25 °C for 10 min, 37 °C for 120 min, 85 °C for 5 min, then 4 °C on hold.

Analysis of gene expression by real-time qPCR

The expression levels of learning/memory-related genes in each group were quantitatively measured using a SensiFAST™ SYBRreg; Hi-ROX Kit (Bioline). Sequences of the primer pairs used are listed in Table S4. Real-time qPCR was performed on an ABI PlusOne real-time system (StepOnePlus™, Applied Biosystems) with the following program: initial denaturation at 95 °C for 20 sec, followed by 40 cycles of 95 °C for 3 sec and 59 °C for 30 sec. Finally, a melting curve analysis was undertaken following the manufacturer's protocol.

Sample preparation for next-generation sequencing

Total RNA was extracted from bees and an RNA library of each group was constructed using a NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (New England BioLabs) and validated quantitatively using ABI StepOnePlus Real-Time PCR and qualitatively using an Agilent 2100 Bioanalyzer. The samples were subjected to paired-end sequencing on Illumina HiSeq 2000™ and 10 million paired-end 150-bp reads were obtained.

ATP analysis

ATP levels in the brains of each treatment and control group were assessed using an ATP determination kit (Molecular Probes). The brains from 3-5 bees were placed in a microcentrifuge tube and homogenized using a pestle. The tube was centrifuged at 7,500× *g* at 4°C for 1 min. Ten microliters of supernatant was then transferred to a designated well of a 96-well plate, each containing 90 μL of assay buffer. Standard solutions of ATP were prepared in the same manner. The reaction was carried out at 28 °C, after which the relative light units (RLUs) of the sample and standard solution were simultaneously measured using a SpectraMax Gemini EM Microplate Reader (BioTek) at a maximum emission of 560 nm. A standard curve was plotted using the measured RLUs (Lin et al., 2020).

Determination of glutamate concentration

The glutamate concentration for each group was determined using a Glutamate Determination Kit (PicoProbe). The brains from 3-5 bees were dissected and placed in a microcentrifuge tube and homogenized using a pestle. The tube was centrifuged at 7,500× *g* at 4°C for 1 min. Ten microliters of supernatant was then transferred to a designated well of a black/opaque 96-well plate, each well containing 85 μL of assay buffer, 2 μL of enzyme mix, 2 μL of substrate mix, and 1 μL of PicoProbe. Glutamate standard solution samples were prepared similarly. The sample plate was incubated at 37°C for 1 h and then placed in a fluorescence microplate reader (SpectraMax Gemini EM Microplate Reader) with the excitation wavelength set at 535 nm. The intensity of the fluorescence emission at 587 nm was recorded as a relative fluorescence unit (RFU). The results obtained from standard samples were used to plot the standard curve to determine the glutamate concentration in the experimental samples.

Real-time recording of bee activity by a wireless sensing network (WSN)-based automatic monitoring system

The wireless sensing network (WSN)-based automatic monitoring system was developed by the Department of Electrical Engineering at National Taiwan University (Jiang et al., 2016). The system was installed at the entrance of beehives with 40 openings; both sides of each opening were equipped with a passing infrared beam. The passing of one bee through both infrared beams was considered a complete incoming or outgoing activity and thus recorded. The system was also equipped with a recording system for ambient temperature, pressure, sound and light exposure. Each beehive contained more than 10,000 bees, and to ensure similar genetic traits, all bees were from the same queen. The large-scale return-to-nesting test was divided into two treatment methods. As shown in Figure 4, the first involved including NaB in the diet for 7 days, followed by infection with DWV on day 7 and no NaB in the diet thereafter. As shown in Figure 5, for the second treatment NaB was included in the diet for 7 days, followed by infection with DWV on day 7, with the bees then fed continuously with NaB in their diet thereafter. Each hive was then fed with 50 mL of virus solution (10^7 virus copy number per 1 μL of DWV) prepared in 40% sucrose water (500 mL) over a period of 2 days (DWV group). After 2 days of virus exposure, the virus-containing solution was removed and supplanted with 40% sucrose water without the virus, which was provided daily. For the field experiments, we used qPCR to determine DWV replication profile in the hive (Figure S3). Bees in both methods were monitored for 26-37 days.

QUANTIFICATION AND STATISTICAL ANALYSIS

NGS data analysis

Data analysis and the establishment of an expression library for each group were outsourced (Genomics, Taiwan). Raw data were trimmed by Trimmomatic (v0.38) to obtain clean reads. HISTA2 was used to align reads to genes in the bee genome and FeatureCounts was used to quantify the reads. Reads in each sample were normalized by FDR/FPKM. DEGseq was used to identify the differentially expressed genes in each treatment group (Data S1).

Statistical analysis

Statistical analysis was performed using Mann–Whitney U test for PER test and one-way ANOVA followed by post hoc analysis (Tukey test) for WSN-based field experiment. Virus number data, gene expression and western blot were analyzed by using Student's t-test. A p-value < 0.05 was considered statistically significant. A heat map was plotted using the logarithm of the standardized *Ct* values (Log_{10}) using Prism 9.2 (Hu et al., 2018).