Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Restoration of STIM-dependent systemic and cellular Ca 2+ responses in Drosophila dopaminergic neurons by herbal formulations of Bacopa monnieri

Ashwini Thakare
University of Trans-Disciplinary Health Sciences and Technology
Nandashree Kasturacharya
National Centre for Biological Sciences
Syed Kavish Nizami
National Centre for Biological Sciences
Ashwini Godbole
University of Trans-Disciplinary Health Sciences and Technology
Gaiti Hasan
gaiti@ncbs.res.in

National Centre for Biological Sciences

Article

Keywords: Growth, neuronal excitability, GCaMP6m, flight, Parkin, muscarinic acetylcholine receptor

Posted Date: November 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11264097/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Title: Restoration of STIM-dependent systemic and cellular Ca^{2+} responses in *Drosophila* dopaminergic neurons by herbal formulations of *Bacopa monnieri*

Authors: Ashwini Thakare[1,4], Nandashree Kasturacharya[1,2,4,6], Syed Kavish Nizami[1,2], Ashwini Godbole[1,5], Gaiti Hasan [2,3,5]

1. The University of Trans-Disciplinary Health Sciences and Technology (TDU), Bengaluru, India
2. National Centre for Biological Sciences, TIFR, Bellary Road, Bengaluru, India
3. Centre for High Impact Neuroscience and Translational Applications (CHINTA), TCG-Crest, Kolkata 700091, India.
4. Equal contribution
5. Corresponding authors
6. Current address: Department of Neuroscience, Brown University, Providence, RI 02912, USA

Author for correspondence: Gaiti Hasan email: gaiti@ncbs.res.in

Abstract:

The fruit fly *Drosophila melanogaster* is a useful model for investigating mechanisms of neurodegeneration due to its well characterised genetics and brain morphology. Validated disease models can then be tested for novel therapeutic drugs. *Drosophila* mutants in genes underlying familial Parkinson's disease (PD) have contributed to the cellular and molecular understanding of PD. For example, Parkin/PINK1 pathway mutants placed the focus of dopaminergic neurodegeneration on mitochondrial function and recent studies have identified maintenance of endoplasmic reticular (ER) Ca^{2+} regulation as an important contributor to mitochondrial health. The ER-Ca^{2+} sensor and Store-operated Ca^{2+} entry regulator protein STIM plays an integral role in ER-Ca^{2+} homeostasis. *Drosophila* mutants in STIM exhibit deficits in larval viability and growth. The focus of these deficits is a small set of dopaminergic neurons in the *Drosophila* larval brain. Here we show that feeding STIM deficient larvae with the fat-based extract of *Bacopa monnieri* (Brahmi), rescues larval viability, growth and excitability of dopaminergic neurons. Importantly, flight motor deficits and cellular Ca^{2+} responses of dopaminergic neurons in adult *Drosophila* mutant heterozygotes for STIM and Parkin, also improved upon feeding the Brahmi extract. Our study provides a mechanistic basis for the use of Brahmi in treating patients with Parkinson's disease.

Key words: Growth, neuronal excitability, GCaMP6m, flight, Parkin, muscarinic acetylcholine receptor.

Introduction:

Bacopa monnieri, commonly referred to as Brahmi, is a well-known nootropic herb described in Indian traditional medicine, especially Ayurveda [1]. Different formulations of Brahmi continue to be used in clinical practice, for treating functional and degenerative disorders of the nervous system, including Parkinson's disease [2], where a subset of dopaminergic neurons undergo neurodegeneration [3]. Mechanisms underlying clinical effects of Brahmi extracts have been studied in neurodegenerative disease models of rats [4,5], *Drosophila* [6] and *C. elegans* [7]. In line with clinical observations, such experimental studies have also shown memory-enhancing, stress-reducing, motor function-augmenting, anti-oxidant, neurotrophic and disease-modifying ability of the extract [8,9]. The few mechanistic studies performed suggest that the Brahmi extract can ameliorate oxidative stress [5,10] and a well-established consequence of oxidative stress is loss of dopaminergic neuron function in PD patients and *Drosophila* models of PD [11–13]. To better understand the systemic and cellular basis of Brahmi action we have explored nootropic effects of two traditional, clinically preferred Brahmi formulations namely Brahmi swarasa (juice) [14] and Brahmi ghrita (milk-fat infused with Brahmi juice) [15] on dopaminergic neurons of the model organism *Drosophila melanogaster*.

Our previous work has shown that *Drosophila* mutants in genes for intracellular Ca^{2+} signaling attenuate excitability of a subset of dopaminergic neurons in larvae and adults [16,17]. The larval phenotype, studied in a genetic knockout for the endoplasmic reticulum (ER) calcium sensor protein STIM ($STIM^{KO}$), effects feeding and growth resulting in mid-larval lethality [18,19]. In adult *Drosophila*, expression of dominant negative transgenes effecting either ER-Ca^{2+} release (IP_3R^{DN}; [17]) or ER-Store operated Ca^{2+} entry ($Orai^{DN}$; [16]), in the dopaminergic neuron subset exhibit attenuated of flight durations. Moreover, recent studies have demonstrated genetic synergism between *Drosophila* mutants for the IP_3R and the PD causing gene *parkin* [20] and a role for the *parkin/PINK1* pathway in intracellular Ca^{2+} release and mitochondrial Ca^{2+} entry through regulation of the IP_3R [12]. These interactions manifest in the PPL1 subset of dopaminergic neurons of adult *Drosophila* and in the case of genetic synergism are identified by attenuation of flight bout durations. Given

the neuronal basis of these phenotypes, we sought to determine if Brahmi formulations and active components restored function to dopaminergic neurons that are the focus of larval deficits in *Drosophila* mutants for the endoplasmic reticular Ca^{2+} sensor and Store-operated Ca^{2+} entry channel regulator, STIM [18]. To directly assess Brahmi effects on PD-associated motor deficits, adults with a single copy of a *parkin* null mutation were tested for flight in the presence of one copy of either a knock out of STIM ($STIM^{KO}$) or a mutant of IP_3R ($itpr^{ug3}$).

Results:

Viability and growth improve in $STIM^{KO}$ larvae upon feeding *Bacopa monnieri* extracts (Brahmi)

Brahmi ghrita (BG) is a traditional Ayurvedic formulation made from milk-fat infused with *Bacopa monnieri* juice (see methods). To determine the growth and viability of $STIM^{KO}$ larvae fed with BG (1%), control *Canton S* (*CS*) and mutant $STIM^{KO}$ larvae were collected as first instars aged 30-34 h after egg laying (AEL). Their growth and viability were assessed at 90-96 h AEL (Fig. 1). As expected, a majority of *CS* larvae reached the third instar stage, with 20.6±0.3 and 21.3±0.3 larvae surviving out of 25 in common *Drosophila* growth media (CM) and BG-supplemented media, respectively (Fig. 1-a). In $STIM^{KO}$ larvae, there was a marked difference between those fed on CM and those fed with BG. Out of 25 larvae, 13.75±0.47 survived on CM, while 21.25±1.4 survived on BG-supplemented media (Fig. 1-b). Although $STIM^{KO}$ larvae in both conditions remained at the second instar stage, those fed with BG appeared healthier, with an overall improvement in larval length (Fig. 1c-d). The concentration of 1% BG used for this experiment was determined as an effective dose by measuring the growth and viability of $STIM^{KO}$ larvae fed on common media (CM) and CM containing either 1%, 2.5% or 5% BG. We observed improved growth of $STIM^{KO}$ larvae across all concentrations of BG (Supplementary Fig. S1), with the 1% BG concentration significantly enhancing both larval growth and survival (Supplementary Fig. S1).

To test the consistency of rescue by Brahmi, we repeated the experiment using a different batch of Brahmi ghrita (Fig. 1 e-h). In *CS*, the results were consistent with Batch 1 (Fig. 1a and Fig. 1e). However, in $STIM^{KO}$, although significant rescue of growth was observed, the rescue of viability was not as pronounced as with Batch 1 (Fig. 1b and 1f-h). The variability in rescue levels between batches of BG suggests

that the concentration of active compounds in *Bacopa monnieri* plants, previously identified as Bacosides [21] may differ between extracts.

To address this variability, we prepared a third batch of Brahmi juice extract from *Bacopa monnieri* plants referred to as Brahmi swarasa (BS) and a corresponding Brahmi ghrita (BG) made from same batch of the juice. Amount of tThe major phyto active compound, Bacoside A (BA), in the Brahmi swarasa extract was detected and quantified using HPLC (Fig. 2a-b; [22,23]). The BA compound was quantified based on a standard curve (Supplementary Fig. S2). The chromatogram indicated that 0.79 mg/ml of Bacoside A was present in Brahmi swarasa. We then repeated the growth and viability experiments for $STIM^{KO}$ larvae fed with the quantified third batch of Brahmi ghrita (BG), Brahmi swarasa (BS), 100 µg of purified bacosides (92.68% BA), and their respective solvent and base controls.

In *CS*, viability was consistent across the three formulations of BA, BS and BG, with no significant changes (Fig. 2c). However, larvae fed on ghee-based media showed a significant increase in body size, suggesting that a lipid-rich diet enhances over all body growth [24] (Fig. 2d-e). Interestingly, BG-fed *CS* larvae did not show this size increase, despite the presence of ghee (Fig. 2d-e). $STIM^{KO}$ larvae fed with BG, BS, and ghee showed significant rescue of viability. Survival on ghee was 16.3±0.6 larvae, on BS it was 16.6±0.3 larvae and on BG it was 21.6±0.6 larvae as compared with 13±0 on CM calculated from three batches of 25 larvae each. In contrast, larvae fed on BA and the solvent control DMSO, had lower survival rates of 15±0.57 larvae and 14.6±0.6 larvae respectively (Fig. 2f).

In terms of larval growth, significant differences were observed among $STIM^{KO}$ larvae fed on BA (2.46±0.05 mm), BS (2.46±0.057 mm) and BG (2.54±0.052 mm) as compared to CM (2.22±0.055 mm) (Fig. 2g-h). $STIM^{KO}$ larvae fed with DMSO (2.23±0.041 mm) and ghee (2.34±0.048 mm) were not significantly different in length from $STIM^{KO}$ larvae fed on CM. Next we counted the number of larvae on each diet based on larval length in ranges of 1-2 mm, 2-3 mm, and 3-4 mm (Fig. 2i). $STIM^{KO}$ larvae fed on BG had the highest proportion of larvae in the 3-4 mm range (Fig. 2j). These findings confirm the medicinal properties of Brahmi, and suggest that lipid-based Brahmi extracts prove more effective than either the aqueous extract of Brahmi (BS) or purified bacosides (BA). We speculate that the presence of lipids in BG may facilitate the transport of active compounds across the plasma membrane, enhancing their cellular effects.

Brahmi supplemented diet maintain excitability of dopaminergic neurons in *STIM^{KO}*

Following the significant rescue of growth in *STIM^{KO}* larvae with three independent batches of BG supplements, we investigated neuronal excitability of central dopaminergic neurons of the DL1/DL2 cluster. These neurons are marked by *THD'GAL4,* a transgene that expresses GAL4 under the control of a part of the Tyrosine Hydroxylase promoter [25]. In *STIM^{KO}* larvae at 76–80 h AEL the DL1/DL2 dopaminergic neurons lose excitability and the ability to release dopamine, which contributes significantly to larval lethality [18]. To evaluate the effect of Brahmi supplementation on DL1/DL2 neurons, we measured KCl-evoked calcium transients in *THD'*-positive neurons using the genetically encoded Ca^{2+} indicator GCaMP6m [26][1] in larvae aged 82–86 h AEL (Figure 3), when *STIM^{KO}* larvae are viable.

In all brains from the 7 control larvae (*THD'>GCaMP6m*) tested, neurons marked by *THD' GAL4* responded to the addition of KCl with a sharp increase in fluorescence, indicating Ca^{2+} entry through voltage-gated Ca^{2+} channels upon neuronal depolarisation (Fig. 3a-d). In contrast a depolarisation response was observed only in 2 out of 7 brains of *STIM^{KO}* larvae raised on CM (Fig. 3a and b). The majority of *STIM^{KO}* brains remained unresponsive to KCl (Fig. 3a-c, Fig. 3e). Moreover, the response to KCl in *STIM^{KO}* brains was muted as compared with controls (compare Fig. 3e with 3d). These data are in agreement with previously published data that also measured excitability of THD' marked neurons from *STIM^{KO}* larval brains [18]. When *STIM^{KO}* larvae were raised on Brahmi supplements the response to KCl was significantly enhanced (Fig. 3a-3c, 3f and 3g). Brains from all 7 *STIM^{KO}* larvae fed on BS responded to KCl, whereas 5 out of 7 brains responded from larvae fed on BG (Fig. 3b-c). The response from *STIM^{KO}* larvae fed on Brahmi supplements was comparable to the response from control larvae (compare Fig. 3f-g with 3d). These data demonstrate that Brahmi supplements restore neuronal health to dopaminergic neurons in *STIM^{KO}* larvae.

Brahmi rescues development and flight defects in the heteroallelic combination of *STIM^{KO}/park^{13}*

Our findings demonstrate that Brahmi treatment partially rescues dopaminergic neuron dependent *STIM^{KO}* larval lethality and maintains dopaminergic neuron

excitability, highlighting its potential neuroprotective effects in dopaminergic neurons. However, Brahmi treatment did not extend to adult survival of *STIM*^{KO} animals, limiting our capacity to evaluate its neuroprotective role in dopaminergic neurons of adult flies. Dopaminergic neuron dysfunction is a key feature of neurodegenerative diseases such as Parkinson's disease (PD). Moreover, dysregulated ER-Ca^{2+} homeostasis, observed in dopaminergic neurons of *STIM*^{KO} larvae, is associated with progression of PD [27]. During our experiments, we found that in the heteroallelic combination of one copy of *STIM*^{KO} with one copy of a *parkin* gene null mutant, *park*^{13} (*STIM*^{KO} /+; *park*^{13}/+ or SP) the number of adult eclosing flies was reduced as compared to the wild type (CS) controls and improved upon feeding Brahmi starting at 86 h AEL (Fig. 4a). This observation also suggested synergism between *Drosophila* STIM and Parkin.

Here we collected adult viable *STIM*^{KO}/+; *park*^{13}/+ (SP) flies and their genetic controls to examine flight bout durations, a motor function directly impacted by PPL1 dopaminergic neuronal function [17]. Adult SP flies (aged 5-6 days) were tested for flight in a tethered flight assay [28] (Fig. 4b). As compared with wild type flies (*CS*) and the appropriate genetic controls, SP flies exhibit a significantly reduced mean flight performance (Fig. 4b). Notably, overexpressing wild-type *STIM*$^{+}$ in THD'-positive dopaminergic neurons restored normal flight ability, underscoring the critical requirement for STIM function in these neurons in maintaining flight (Fig. 4b).

To study the effect of Brahmi on adult dopaminergic neuron function in *Drosophila* and in the context of PD progression, we analysed the flight behaviour of SP flies fed Brahmi ghrita either from the larval stage or as adults. Strikingly, Brahmi feeding (1% BG) during larval+adult stages rescued flight behaviour in SP flies when compared to SP flies reared on common media (Fig. 4c). We also tested SP flies fed on Brahmi (1% BG) for 5-6 days after eclosion (Fig. 4d), where a mild rescue was observed, that did not reach significance. In contrast, control flies (both *CS* and genetic controls) showed no significant differences in flight performance between fed and non-fed groups (Fig. 4c-d, Supplementary Fig. S3a). Collectively, these results support a role for Brahmi's neuroprotective effects on adult dopaminergic neuronal function and agree with previous findings demonstrating that loss of either *STIM* or *Parkin* by RNAi in adult *Drosophila* dopaminergic neurons leads to motor deficits including loss of flight [19,20,29,30].

Flies exhibit reduced flight times with ageing (Supplementary Fig. S3b). To further test Brahmi's efficacy in age-related decline of flight, we fed Brahmi to aged wild type (*CS*) flies and a heteroallelic combination of *parkin* and *itpr* mutants (*park^{13}/itpr^{ug3}* or *PU*) known to exhibit age-dependent flight defects [20]. Brahmi feeding in *CS* and *PU* flies began 20 days post eclosion and was continued for 5-7 days, till the day of testing for flight (Fig. 4e). Both Brahmi-fed *CS* and PU flies displayed a significant improvement in flight time compared to their non-fed counterparts (Fig. 4e). Collectively, these findings indicate Brahmi's ability in enhancing motor function across both developmental and late-onset aged conditions, further emphasizing its potential neuroprotective role.

Dynamics of intracellular calcium responses in dopaminergic neurons of *STIM^{KO}/park^{13}* mutants improve upon Brahmi treatment:

To understand the cellular basis of flight defect rescue by Brahmi in *STIM^{KO}/park^{13}* animals we measured neuromodulatory responses of adult dopaminergic neurons to an agonist (carbachol) for the muscarinic acetylcholine receptor (mAchR) using ex-vivo brain preparations. Our previous work has identified sustained mAchR-stimulated dopamine release from the PPL1 cluster of dopaminergic neurons as essential for maintenance of long flight durations [17,31] (Fig. 5a). mAchR stimulation of PPL1 neurons leads to IP_3R mediated Ca^{2+} release[17] and ER-Store operated Ca^{2+} entry through the STIM/Orai pathway [16]. Ca^{2+} responses from PPL1 neurons were visualised by expression of GCaMP6m, a genetically encoded Ca^{2+} sensor [26]. A robust Ca^{2+} response was observed in control PPL1 neurons (*THD'>GCaMP6m*) upon stimulation with carbachol added at 120 seconds (CCh; Fig. 5b and 5c - grey trace). The Ca^{2+} response, which is a composite of ER-Ca^{2+} release through the IP_3R followed by SOCE through STIM/Orai, peaked rapidly (168s $\pm$11s) after addition of carbachol and then showed a gradual decrease (Fig. 5b and 5c; grey trace). Ca^{2+} responses of control flies fed with 1% BG appeared similar to control flies on CM (Fig. 5b-5d, compare grey and blue traces and columns), with a peak time of 167s$\pm$10s. In *STIM^{KO}/+*, *park^{13}/+* animals (*STIM^{KO}*; *THD'>GCaMP6m; park^{13}*) the kinetics of carbachol-stimulated Ca^{2+} response was altered. The Ca^{2+} peak was delayed significantly to 369s$\pm$9s and the slope of reaching the peak was significantly slower (Fig. 5b-5e; red trace, column and box). On feeding of 1% BG to *STIM^{KO}/park^{13}* flies, two out of the three altered parameters were

restored. Peak time returned to 233s±21s and the slope of the peak response was closer to control (Fig. 5b - 5e, green trace, column and box).

Discussion:

In this study, we identified systemic amelioration of viability and motor deficits in *Drosophila* mutants for the ER-Ca^{2+} sensor STIM, by feeding Brahmi extracts. The focus of these deficits have been identified earlier as dopaminergic neurons in the larval [18] and adult brains [16,17,31]. Importantly, we demonstrate that functional rescue is accompanied by improved cellular calcium responses of dopaminergic neurons. Feeding Brahmi formulations improved neuronal excitability in larval dopaminergic neurons (Fig. 3), whereas in adult dopaminergic neurons the Ca^{2+} response to an acetylcholine-like agonist (carbachol) was partially rescued (Fig. 5). Previous studies have demonstrated that loss of ER-Ca^{2+} and SOCE affects the long-term expression of membrane-localised ion channels in *Drosophila* larval brains and adult *Drosophila* dopaminergic neurons, leading to chronic loss of excitability [16,32,33], whereas the Ca^{2+} response to the muscarinic acetylcholine agonist carbachol is an acute response, that is a composite of ER-Ca^{2+} release through the IP_3R and SOCE through the STIM/Orai channel at the plasma membrane [16–18]. Thus, stimulation of the muscarinic acetylcholine receptor (mAchR) followed by ER-Ca^{2+} release and Store-operated Ca^{2+} entry play an integral role in the chronic and acute functioning of both larval and adult dopaminergic neuron subsets investigated here. In these contexts it is interesting that a component of *Bacopa monnieri* extract, Ebelin lactone, binds mAchR1 at relatively high affinity [34], suggesting a possible cellular mechanism by which Brahmi extract improves cellular calcium responses in dopaminergic neurons, thus rescuing both chronic and acute deficits. Moreover, poor rescue by purified Bacosides as compared with whole plant extracts of *Bacopa monnieri* (BS and BG; Fig. 2), supports the idea that multiple phytocomponents and their secondary products, such as Ebelin lactone may act synergistically with Bacosides to enhance the overall biological effect of Brahmi.

In Ayurveda, different Brahmi formulations are recommended differentially according to the individual's health condition and other factors like resource availability. Brahmi swarasa (BS) is considered a potent formulation [14], while Bramhi ghrita (BG) is preferred for mitigation of degenerative changes and age-related decline [15]. In our studies we find the best systemic and cellular rescues with the BG

formulation, agreeing with Ayurvedic practice where ghrita (or ghee) is considered as one of the best vehicles for herbal components. Moreover, the ghrita itself appears to have beneficial effects in both CS and *STIM*^{KO} animals (Fig. 2) and these are further enhanced by the addition of the *Bacopa monnieri* extract (or Brahmi). Possibly, Brahmi extract has better stability and cellular availability in the presence of a fat base like ghee.

Multiple case studies have documented the beneficial effects of Brahmi extract for learning, memory and cognition [35]. Here we have focussed on testing how feeding clinically relevant Brahmi formulations might ameliorate dopaminergic neuron function in order to understand the less well catalogued benefits of Brahmi formulations for PD [2,7,36]. Due to the high energy demand of dopaminergic neurons, ATP production through the electron transport chain leads to oxidative stress and this is one of the key drivers of neurodegeneration in PD. ER-mitochondrial Ca^{2+} homoeostasis plays a key role in maintaining healthy mitochondria in dopaminergic neurons through balancing the production of reactive oxygen species and hydrogen peroxide [20,27]. Our findings that Brahmi feeding can restore cellular and systemic deficits linked to dysregulated Ca^{2+} signaling, taken together with studies demonstrating a close connection between ER-Ca^{2+} release and oxidative stress, support careful testing of Brahmi in age-related decline of motor function and in patients with PD, where it might help reduce dopaminergic neuron degeneration.

Materials and Methods:

Fly rearing:

Drosophila strains were reared on standard cornflour agar media consisting of 80 g corn flour, 20 g glucose, 40 g sugar, 15 g yeast extract, 4 mL propionic acid, 5 mL *p*-hydroxybenzoic acid methyl ester in ethanol, and 5 ml ortho butyric acid in 1l at 25°C, unless otherwise specified, under a 12:12 hr light:dark cycle. In all studies, the *Canton S* (*CS*) strain was used as a wild-type control and CRISPR-*Cas9* generated deficiency for *STIM,* referred to as *STIM*^{KO} served as a null mutant for the *Drosophila STIM* gene[19], *itpr*^{ug3} was the mutant tested for the *itpr* (IP_3R) gene[37] and *park*^{13}, a null mutant was tested for the *Parkin* gene [38]

Larval Rearing:

Age synchronized larvae of the indicated ages were collected and transferred to agar-less media containing yeast (4 g/100 mL), sucrose (8 g/100 mL), corn flour (16 g/100 mL), propionic acid (1 mL/100 mL), and benzoic acid (0.05 g dissolved in 1 mL

absolute alcohol). Larval, lethality and developmental profiles of specified genotypes were assessed under different feeding conditions as previously described [18,37]. Timed, synchronized egg-laying was performed for 6 hours, and larvae were then collected at either 30-34 h or 66-72 h after egg laying (AEL) in batches of 25 for larval studies and 10 for adult studies. Heterozygous larvae were identified and excluded using dominant markers (*FM7iGFP, TM6Tb,* and *CyOGFP*). Each batch of larvae was placed in a separate vial, with a minimum of three vials containing agar-less media tested per genotype at each feeding condition. At 94-98 hours AEL, larvae were screened for number of survivors and their developmental stage, based on the morphology of anterior spiracles and mouth hooks[37]. For adult studies, eclosed flies were collected, counted, and used for further analysis. For a single experiment all experimental genotypes and corresponding genetic controls were tested simultaneously.

Preparations of different Brahmi formulations:

*1. Plant Authentication, Collection, Source and Permissions***:** The formal identification and authentication of *Bacopa monnieri* (L.) Wettst. (Brahmi) samples used in this study were conducted by Dr. S. Noorunnisa Begum, Associate Professor and Curator of the FRLH Herbarium and Raw Drug Repository of Medicinal Plants Used in Indian Systems of Medicine, Foundation for Revitalisation of Local Health Traditions (FRLHT), Bengaluru, India. A voucher specimen of the identified plant material was deposited in the FRLH Herbarium and was assigned an accession number (FRLH Acc.No.7087) for future reference.

The recommendations of the IUCN Policy Statement on Research Involving Species at Risk of Extinction and relevant provisions of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) were also considered. *Bacopa monnieri* (L.) Wettst. is currently listed as Least Concern (LC) on the IUCN Red List of Threatened Species (IUCN Red List Taxon ID: 164168). That means, *Bacopa monnieri* is not currently classified by the IUCN as threatened or at risk of extinction. The present study did not involve international trade or commercial exploitation of *Bacopa monnieri* material. All relevant considerations concerning the responsible collection and use of plant material were taken into account before the plant material of *Bacopa monnieri* (L.) Wettst. used in this study was collected from the vicinity of Mavallipura Lake, Bengaluru, Karnataka, India. for non-commercial academic research purposes. No specific biological-resource collection permit or

licence was obtained prior to collection of the plant material. The quantity collected was limited to less than a kilogram of fresh weight, that was sufficient for the study.

2. Preparation of Brahmi Swarasa

The preparation of Brahmi Swarasa (fresh juice) extract was from the paste of whole plants. Fresh *Bacopa monnieri* plants were washed with distilled water to remove contaminants. A required amount of whole cleaned plants were chopped and ground to fine paste using an end-runner stone grinder. The paste was then placed in a clean cotton cloth, tied, and manually compressed to extract the juice. The juice was filtered to remove plant fibres and stored in a sterile tubes at -80°C till further use.

3. Preparation of Brahmi Ghrita:

Brahmi Ghrita was prepared by infusing Brahmi fresh juice in cow ghee (clarified butter) according to general procedure of preparation of herbal ghee described in a traditional text for Ayurvedic formulations[39]. Briefly, 400 ml of fresh juice and 25 gm of fresh paste from whole Brahmi plants were mixed in 100 ml of cow ghee. The mixture was boiled in a thick bottom vessel on continuous low-medium heat until the water content evaporated and clear fat remain in the vessel. The characteristic indicators of successful processing, including the formation of a wick-like shape and the absence of crackling sounds upon heating. The preparation was filtered through a clean cotton cloth to remove any residual plant material. The resulting Brahmi Ghrita was then stored in dry air-tight containers at room temperature for further use in this study.

Quantification of Bacoside A by High-Performance Liquid Chromatography (HPLC)

Bacoside A, (mixture of Bacoside A3, Bacopaside II , jujubogenin isomer of Bacopasaponin C and Bacopasaponin C) was quantified in *Bacopa monnieri* swarasa by High-Performance Liquid Chromatography (HPLC) following reported methods [22], with minor modifications. HPLC analysis was performed on a Thermo Scientific Dionex UltiMate 3000 HPLC/UHPLC equipped with an UV–Vis Variable Wavelength Detector. Separation was achieved using a C18 column. The mobile phase consisted of 0.2% phosphoric acid and acetonitrile (65:35, v/v), with the aqueous phase adjusted to pH 3.0 using 5M NaOH. The flow rate was maintained at 1.0 mL/min, with an injection volume of 20 µL and a total run time of 25 min. Bacoside A was detected at a wavelength of 205nm and the chromatographic response recorded in milli-absorbance units (mAU) plotted against retention time (minutes). The Bacoside A reference

standard was obtained from Natural Remedies (92.68% purity) and dissolved in methanol to obtain a 1.0 mg/mL stock solution. Serial dilutions were prepared in methanol to obtain standard concentrations ranging from 0.1 to 1.0 mg/mL. The standards were analysed under the same HPLC conditions, and a calibration curve was generated by plotting peak area against the corresponding Bacoside A concentration. Linear regression was used to obtain the calibration equation.

For analysis of Brahmi swarasa, the sample was diluted 1:1 (v/v) with methanol, mixed well, and filtered through a 0.45 µm syringe filter. The filtered sample was then injected at a volume of 20 µL. Bacoside A was identified by comparing the retention time of the sample peak with that of the reference standard. Quantification was performed using the calibration curve, and the calculated concentration was corrected for the dilution factor.

Preparation of Brahmi media of different formulations for larval study:

1.For Brahmi ghrita treatment:

Agar-less media, as described above, was prepared, and while the media was still warm, melted Brahmi ghrita was added and mixed thoroughly. Approximately 3 mL of this media was poured into each vial, maintaining Brahmi ghrita concentrations of either 1%, 2.5%, or 5% (e.g., 100 µL of Brahmi ghrita per 10 mL of agar-less media for a 1% concentration). After the media solidified and cooled, larvae of the desired age were transferred to the appropriate food vials and reared at 25°C in an incubator with a 12:12-hour light-dark cycle. For control media, agar-less media was prepared without Brahmi ghrita following the same procedure as described above.

2.For ghee media treatment:

Agar-less media was prepared as described above. After cooling, thawed ghee was added to the media, mixed thoroughly, and approximately 3 mL was poured into each vial. The solidified, cooled media was then used to assess the effect of ghee on larval lethality.

3.For Brahmi swarasa treatment:

Agar-less media was prepared as above, and after cooling, thawed Brahmi swarasa was added and mixed thoroughly. Approximately 3 mL was poured into each vial, and the solidified, cooled media was used to assess the effect of Brahmi swarasa on larval lethality. For 1% BS, 100 µl of BS is added to 10 ml of agar less media.

4.For Bacoside (BA) treatment:

A stock solution of BA (100 mg) was prepared by dissolving in 1ml of 100% DMSO. This DMSO solution containing BA was added to agar-less media to achieve a concentration of 100 µg of BA per ml of media. For the Bacoside control media, the same volume of DMSO (without BA) was added to agar-less media and used for the study.

4.6 Brahmi ghrita media for adult studies:

For adult treatments, standard corn flour agar media as described above was prepared. While media was still hot and in liquid form, melted Brahmi ghrita was added and mixed thoroughly. Approximately 3 ml of this media was poured into vials and allowed to solidify. Flies were starved overnight (12-14 hours) and then transferred to Brahmi ghrita media for 6 hours, followed by normal media for 4-6 hours. This cycle of starvation, Brahmi treatment followed by normal media was repeated for 5-6 days before the flies were tested for flight. During starvation, flies were kept in vials containing sterile water-soaked tissue paper. No more than 10 flies were maintained per vial during treatment. SP flies received this treatment from the day of eclosion for 5-6 days, while for PU flies, treatment began on the 20th day post-eclosion and continued until the flight assay was conducted.

For ghee treatment, the same procedure was followed as for Brahmi ghrita, except melted ghee was added instead of Brahmi ghrita.

Larval imaging and measurement:

Staged larvae from specified genotypes were collected at defined developmental time points, anesthetized on ice for 1 hour, and mounted in ice-cold HL3 buffer. Immediately following mounting, larvae were imaged using an Olympus MVX10 stereo microscope. Larval length, measured from the mouth hook to the posterior spiracle, was quantified using FIJI software.

Ex-vivo imaging of larval brains:

GCaMP6m signals were obtained from appropriately aged larval brains taken from the specified genotypes and dissected in haemolymph like saline (HL3) (70mM NaCl, 5mM KCl, 20mM MgCl2, 10mM NaHCO3, 5mM trehalose, 115mM sucrose, 5 mM HEPES, 1.5mM Ca^{2+}, pH 7.2). Dissected brains were transferred to a 35mm punched dish with a cover slip adhered to the bottom. Brains were embedded in ~5µl of 0.8% low melt agarose (Invitrogen, Cat#16520-100) and bathed in 86µl of HL3. Images were acquired as a time series on an XY plane at an interval of 2sec using a

20X-oil objective on an Olympus FV3000 inverted confocal microscope (Olympus Corp., Japan). For KCl stimulation, at the 30th frame, 7μl of HL3 was added and at the 60th frame 7μl of 1M KCl was added. The final concentration of KCl in the solution surrounding the brain was 70mM [18].

A minimum of 7 independent brain preparations Control (*THD'>GCaMP6m*) aged 60-66 h and *STIM^{KO}; THD'>GCaMP6m* aged 80-86 h AEL were used for all live imaging experiments. The exact number of cells imaged for each genotypes are indicated in the figures and figure legends. Raw fluorescence data were extracted from the marked ROIs using a time series analyser plugin in Fiji[2]. ΔF/f was calculated using the following formula for each time point (t): $\Delta F/f = (F_t/F_0)/F_0$, where F_0 is the average basal fluorescence obtained from the first 30 frames.

Flight assays

Flight assays were performed as described by Manjila and Hasan [28]. Briefly, on the day of testing, flies were anesthetized on ice for approximately 8-10 minutes, and each fly was prepared by tethering a thin metal wire between the head and thorax using nail polish. After recovery, a gentle mouth-blown air puff was administered, and flight duration was recorded with a stopwatch, with a maximum of 15 minutes per fly. Female flies of the genotype *STIM^{KO}*/+; *park^{13}*/+ and the respective controls were aged to 5 – 7 days prior to testing for flight bout durations. Either sex was tested for flies of the genotype *park^{13}/itpr^{ug3}* and respective controls after aging them to 25 -27 days. Flies were tested in batches of 5-8. All mutant genotypes were crossed to the wild-type strain *Canton S*, with control genotypes consisting of GAL4 and UAS strains also crossed to *Canton S*. Flight times were plotted as boxplots using Origin software (Origin Lab, Northampton, MA); each box represents the 25th to 75th percentiles, solid squares indicate the mean, the horizontal line in each box represents the median, and individual data points are shown as open circles.

Immunohistochemistry

Hand dissected and fixed adult fly brains were incubated overnight at 4^{o}C in anti-GFP antibody diluted 1/8000 (Abcam, ab13970; RRID: AB_300798). After washing, the brains were incubated in an Alexa-Fluor 488 labelled secondary antibody (1/400; Thermo Fisher Scientific, A 11039; RRID: AB_2534096) for 2 hours at room temperature. Washed brains were mounted in Vectashield anti-fade mounting medium (Vector Laboratories, SKU: H-1000-10). Confocal sections of 1μm were obtained using

an Olympus FV3000 with a X20, 0.85 NA objective and visualised using Image J 1.54p [40].

Ex-vivo imaging of adult brains

Adult brains of the appropriate genotypes were dissected in adult haemolymph-like saline (AHL; 2 mM $CaCl_2$, 5 mM KCl_2, 5 mM HEPES, 8.2 mM $MgCl_2$, 108 mM NaCl, 4 mM $NaHCO_3$, 1 mM NaH_2PO_4, 10 mM sucrose, 5 mM trehalose at pH 7.5), and placed on a poly-lysine coated coverslip adhered to a punched 35mm petridish. The dissected brains were bathed in a small volume (~5ml) of AHL. Time-series of images were obtained on an Olympus FV3000 inverted microscope using an *xy* plane and a X20, 0.85 NA oil objective at intervals of 1.5s. Ca^{2+} dynamics were monitored by measuring changes in the fluorescence intensity of GCaMP6m under 488 nm excitation. Carbachol (Cch) was added at 120 s to a final concentration of 50 µM and in a total volume of 100 µl. Raw fluorescence data were extracted by marking regions of interest (ROIs) using the time series analyzer plugin of Image J1.54p [3]. ΔF/f was computed for each time point (t) using the formula $\Delta F/F_0=(F_t-F_0)/F_0$, where F_0 represents the average basal fluorescence of the initial 40 frames (0-60s).

Cellular Ca^{2+} changes were analysed for peak times from the fluorescence intensity traces (ΔF/F0) and quantified for Ca^{2+} influx rates via a linear regression using the linear model lm() function in R and R scripts utilizing the packages readxl, dplyr, and ggplot2. Statistical significance of activation slopes and peak times were assessed using the non-parametric Kruskal-Wallis test for overall variance, followed by pairwise Mann-Whitney U tests.

Statistical analysis

For normally distributed data, significance of $p<0.05$ was calculated using a student's *t* test. Non parametric distributions were checked by the non-parametric Kruskall-Wallis test, followed by obtaining *p* values with Mann-Whitney *U* tests. All calculated *p* values are available in supplementary table 1. Numbers of individuals (n) and groups (N) tested are mentioned in individual figure legends**.**

Data and Resource availability:

All relevant data and details of resources can be found within the manuscript and supplementary information. Additional data supporting the findings of this study are available from the corresponding authors on reasonable request.

References:

1. Dash, V. & Kashyap, V. Dash, V. & Kashyap, V. *Materia Medica of Ayurveda.* Concept Publishing Company (1980).
2. Santos, A. F. D. *et al. Bacopa monnieri* in Patients with Parkinson's Disease: A Pilot Study. *J Med. Food* 26, 114–119 (2023).
3. Pissadaki, E. K. & Bolam, J. P. The energy cost of action potential propagation in dopamine neurons: clues to susceptibility in Parkinson's disease. *Front. Comput. Neurosci.* 7, (2013).
4. Saraf, M. K., Prabhakar, S. & Anand, A. Neuroprotective effect of *Bacopa monniera* on ischemia induced brain injury. *Pharmacol. Biochem. Behav.* 97, 192–197 (2010).
5. Ghosh, S. *et al.* Assessment of the mechanistic role of an Indian traditionally used ayurvedic herb *Bacopa monnieri (L.)*Wettst. for ameliorating oxidative stress in neuronal cells. *J. Ethnopharmacol.* 328, 117899 (2024).
6. Jansen, R. L. M., Brogan, B., Whitworth, A. J. & Okello, E. J. Effects of Five Ayurvedic Herbs on Locomotor Behaviour in a *Drosophilla Melanogaster* Parkinson's Disease Model. *Phytother. Res.* 28, 1789–1795 (2014).
7. Jadiya, P. *et al.* Anti-Parkinsonian effects of *Bacopa monnieri*: Insights from transgenic and pharmacological *Caenorhabditis elegans* models of Parkinson's disease. *Biochem. Biophys. Res. Commun.* 413, 605–610 (2011).
8. Tiemtad, P. *et al.* Comparative effects of *Bacopa monnieri* and *Ginkgo biloba* on cognitive functions: A systematic review and network meta-analysis. *Phytomedicine* 153, 157915 (2026).
9. Srivastava, P. *et al.* Investigating *Bacopa monnieri L.* Therapeutic Potential for the Treatment of Neurological Diseases. *Curr. Pharm. Des.* 30, 1016–1030 (2024).
10. Valotto Neto, L. J. *et al.* Investigating the Neuroprotective and Cognitive-Enhancing Effects of *Bacopa monnieri*: A Systematic Review Focused on Inflammation, Oxidative Stress, Mitochondrial Dysfunction, and Apoptosis. *Antioxidants* 13, 393 (2024).
11. Pereira, S. L., Grossmann, D., Delcambre, S., Hermann, A. & Grünewald, A. Novel insights into Parkin-mediated mitochondrial dysfunction and neuroinflammation in Parkinson's disease. *Curr. Opin. Neurobiol.* 80, 102720 (2023).
12. Ham, S. J. *et al.* PINK1 and Parkin regulate IP3R-mediated ER calcium release. *Nat. Commun.* 14, 5202 (2023).
13. Whitworth, A. J. *et al.* Increased glutathione *S* -transferase activity rescues dopaminergic neuron loss in a *Drosophila* model of Parkinson's disease. *Proc. Natl. Acad. Sci. USA* 102, 8024–8029 (2005).
14. Aggarwal, S. & Patil, V.B. A Scientific Review on Selected Herbal *Medhya Rasayanas* from *Sushruta Samhita*. *J. Ayurveda Integr. Med. Sci.* 10, 106–116 (2025).
15. Singh, A. D. *et al.* Neurometabolite changes in cingulate cortex regions in healthy Indian population and effect of *Bacopa monnieri* intervention: a H1 MRS study. *Neurochem. Int.* 197, 106163 (2026).
16. Mitra, R., Richhariya, S. & Hasan, G. Orai-mediated calcium entry determines activity of central dopaminergic neurons by regulation of gene expression. *eLife* 12, RP88808 (2024).
17. Sharma, A. & Hasan, G. Modulation of flight and feeding behaviours requires presynaptic IP3Rs in dopaminergic neurons. *eLife* 9, e62297 (2020).

18. Kasturacharya, N., Dhall, J. K. & Hasan, G. A STIM dependent dopamine-neuropeptide axis maintains the larval drive to feed and grow in *Drosophila*. *PLoS Genet*. 19, e1010435 (2023).
19. Pathak, T., Trivedi, D. & Hasan, G. CRISPR-Cas-Induced Mutants Identify a Requirement for dSTIM in Larval Dopaminergic Cells of *Drosophila melanogaster*. *G3 [Bethesda]* 7, 923–933 (2017).
20. Dileep, M. *et al.* Synergism of IP3R and Parkin mutants identifies mitochondrial stress as an early feature of Parkinson's disease. *Dis. Model. Mech.* 19, dmm052146 (2026).
21. Deepak, M., Sangli, G. K., Arun, P. C. & Amit, A. Quantitative determination of the major saponin mixture bacoside A in *Bacopa monnieri* by HPLC. *Phytochem. Anal.* 16, 24–29 (2005).
22. Taratima, W., Trunjaruen, A., Kaenpho, K., Maneerattanarungroj, P. & Kunpratum, N. Physiological and metabolic responses of *Bacopa monnieri* to salt-induced stress: implications for secondary metabolite elicitation. *BMC Plant Biol*. 25, 991 (2025).
23. Murthy, P. B. S. *et al.* Estimation of Twelve Bacopa Saponins in *Bacopa monnieri* Extracts and Formulations by High-Performance Liquid Chromatography. *Chem. Pharm. Bull.* 54, 907–911 (2006).
24. Texada, M. J. *et al.* Insulin signaling couples growth and early maturation to cholesterol intake in *Drosophila*. *Curr. Biol.* 32, 1548-1562.e6 (2022).
25. Liu, Q., Liu, S., Kodama, L., Driscoll, M. R. & Wu, M. N. Two Dopaminergic Neurons Signal to the Dorsal Fan-Shaped Body to Promote Wakefulness in *Drosophila*. *Curr. Biol.* 22, 2114–2123 (2012).
26. Chen, T.-W. *et al.* Ultrasensitive fluorescent proteins for imaging neuronal activity. *Nature* 499, 295–300 (2013).
27. Zaichick, S. V., McGrath, K. M. & Caraveo, G. The role of Ca2+ signaling in Parkinson's disease. *Dis. Model. Mech.* 10, 519–535 (2017).
28. Manjila, S. & Hasan, G. Flight and Climbing Assay for Assessing Motor Functions in *Drosophila*. *Bio. Protoc.* 8, (2018).
29. Richhariya, S., Jayakumar, S., Abruzzi, K., Rosbash, M. & Hasan, G. A pupal transcriptomic screen identifies Ral as a target of store-operated calcium entry in *Drosophila* neurons. *Sci. Rep.* 7, 42586 (2017).
30. Park, J. *et al.* Mitochondrial dysfunction in *Drosophila* PINK1 mutants is complemented by parkin. *Nature* 441, 1157–1161 (2006).
31. Pathak, T., Agrawal, T., Richhariya, S., Sadaf, S. & Hasan, G. Store-Operated Calcium Entry through Orai Is Required for Transcriptional Maturation of the Flight Circuit in *Drosophila*. *J. Neurosci.* 35, 13784–13799 (2015).
32. Mitra, R., Richhariya, S., Jayakumar, S., Notani, D. & Hasan, G. IP3-mediated Ca2+ signals regulate larval to pupal transition under nutrient stress through the H3K36 methyltransferase Set2. *Development* 148, dev199018 (2021).
33. Jayakumar, S., Richhariya, S., Deb, B. K. & Hasan, G. A Multicomponent Neuronal Response Encodes the Larval Decision to Pupariate upon Amino Acid Starvation. *J. Neurosci.* 38, 10202–10219 (2018).
34. Ramasamy, S. *et al.* In Silico and In Vitro Analysis of Bacoside A Aglycones and Its Derivatives as the Constituents Responsible for the Cognitive Effects of *Bacopa monnieri*. *PLoS ONE* 10, e0126565 (2015).
35. Kongkeaw, C., Dilokthornsakul, P., Thanarangsarit, P., Limpeanchob, N. & Norman Scholfield, C. Meta-analysis of randomized controlled trials on cognitive effects of *Bacopa monnieri* extract. *J. Ethnopharmacol.* 151, 528–535 (2014).

36. Singh, B. *et al.* Neuroprotective and Neurorescue Mode of Action of *Bacopa monnieri (L.)* Wettst in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-Induced Parkinson's Disease: An In Silico and In Vivo Study. *Front. Pharmacol.* 12, 616413 (2021).
37. Joshi, R., Venkatesh, K., Srinivas, R., Nair, S. & Hasan, G. Genetic Dissection of *itpr* Gene Function Reveals a Vital Requirement in Aminergic Cells of *Drosophila* Larvae. *Genetics* 166, 225–236 (2004).
38. Greene, J. C. *et al.* Mitochondrial pathology and apoptotic muscle degeneration in *Drosophila parkin* mutants. *Proc. Natl. Acad. Sci. USA* 100, 4078–4083 (2003).
39. Srivastava, S. Srivastava, S. Sharangadhara Samhita, *Madhyama Khanda.* (Chaukhamba Orientalia, 2009).
40. Schindelin, J. *et al.* Fiji: an open-source platform for biological-image analysis. *Nat. Methods* 9, 676–682 (2012).
41. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675 (2012).

Acknowledgments:

The authors thank FlyBase for access to various databases; the BDSC and the fly facility at National Centre for Biological Sciences (NCBS) for fly lines; the Central Imaging and Flow Cytometry Facility at NCBS and NCBS laboratory support for timely help. Special thanks go to Mr. Pushpendra Jat for his support in standardizing the HPLC method, and to Dr. Subramanya Kumar of Trans-Disciplinary University (TDU), Bengaluru, for helping prepare Brahmi ghrita.

Funding:

This work was supported by a Science, Education and Research Board fellowship (SB/DF/001/2019) from the Department of Biotechnology, Ministry of Science and Technology, India to GH and a fellowship from the Council of Scientific and Industrial Research (CSIR), Govt. of India to SKN. AT was supported by a grant from Pratiksha Trust and Rural India Supporting Trust (RIST).

Author Contributions:

Conceptualization: NK, AG, AT and GH; Supervision: NK, AG and GH; Data curation: AT, NK, and SKN; Formal analysis: NK, AT and SKN; Funding acquisition: GH and AG; Methodology: GH, AG, NK, AT and SKN. Project administration: NK, GH and AG. Resources: GH and AG; Writing: NK, AT, AG and GH.

Competing interests: The authors declare no competing or financial interests.

Figure 1:

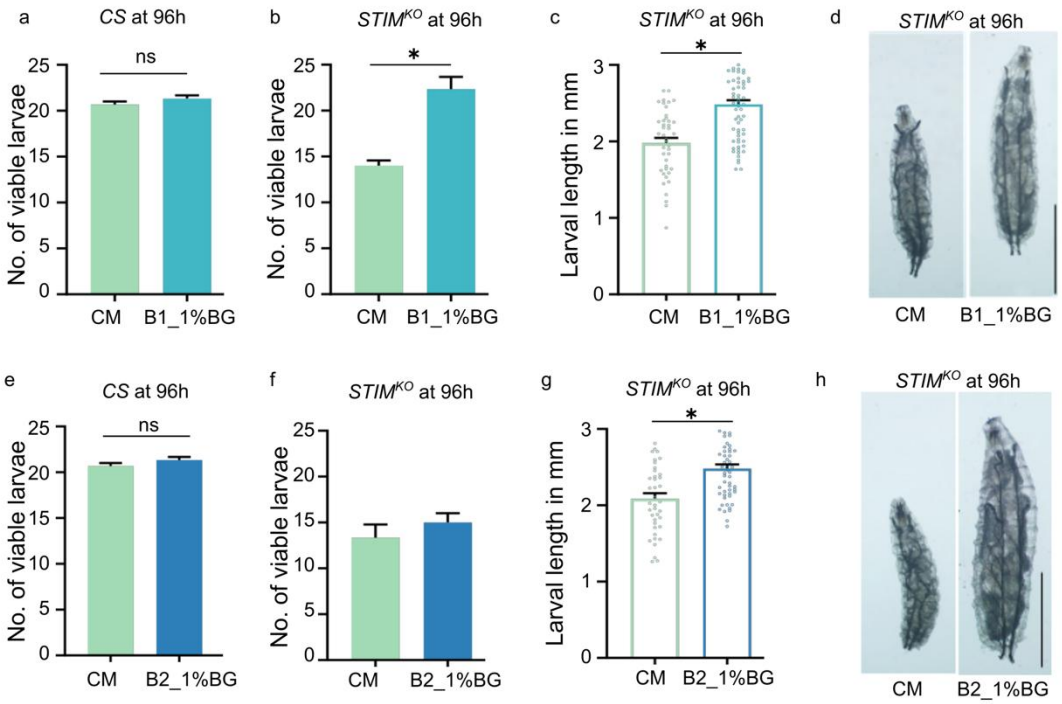


Figure 2:

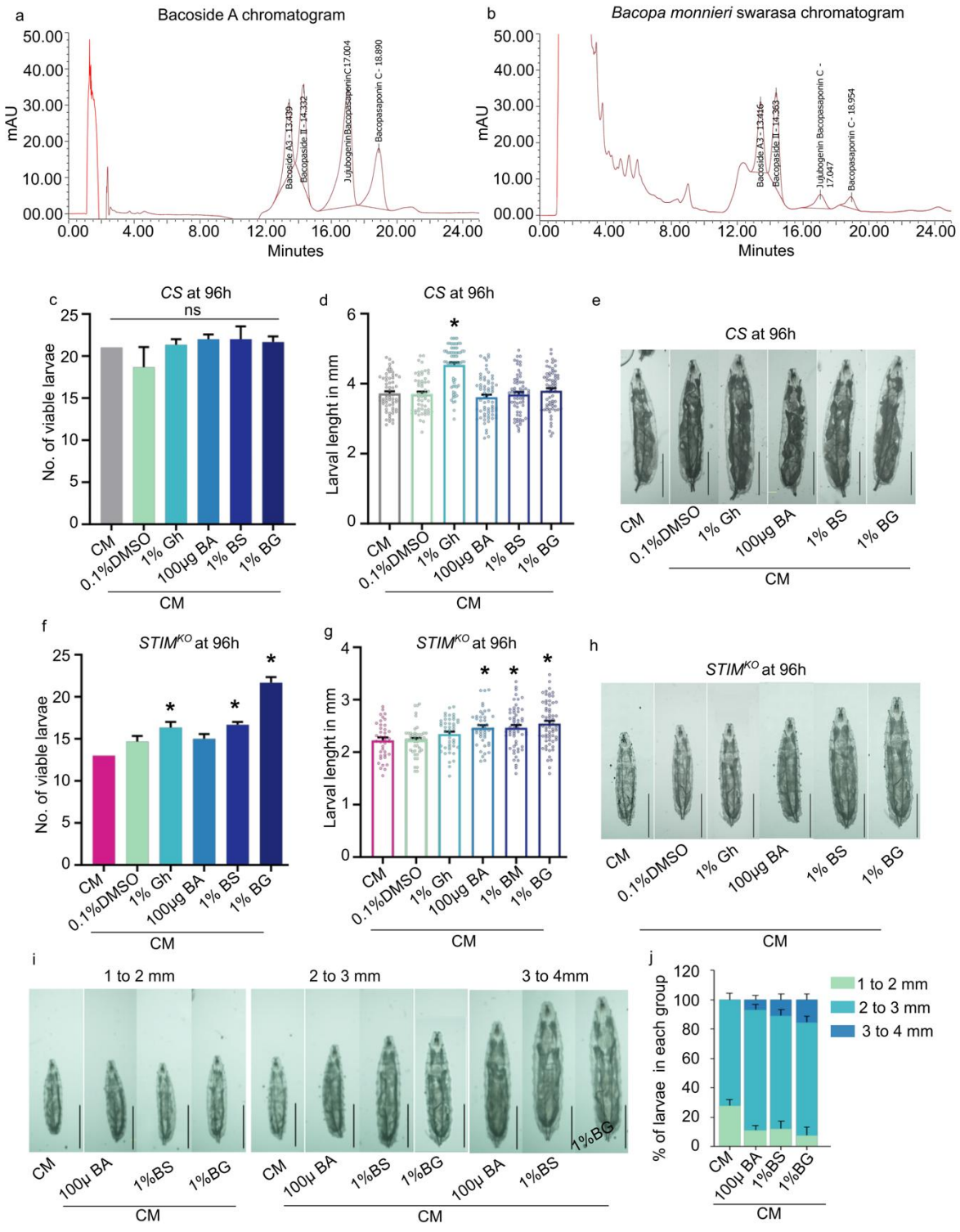

a
Bacoside A chromatogram
mAU
Minutes
Bacoside A3 - 13.439
Bacopaside II - 14.332
Jujubogenin Bacopasaponin C 17.004
Bacopasaponin C - 18.890
b
Bacopa monnieri swarasa chromatogram
Bacoside A3 - 13.416
Bacopaside II - 14.363
Jujubogenin Bacopasaponin C - 17.047
Bacopasaponin C - 18.954
c
CS at 96h
ns
No. of viable larvae
d
CS at 96h
Larval lenght in mm
e
CS at 96h
f
STIMKO at 96h
No. of viable larvae
g
STIMKO at 96h
Larval lenght in mm
h
STIMKO at 96h
i
1 to 2 mm
2 to 3 mm
3 to 4mm
1%BG
j
% of larvae in each group
1 to 2 mm
2 to 3 mm
3 to 4 mm
CM
0.1%DMSO
1% Gh
100µg BA
1% BS
1% BG
1% BM
100µ BA
1%BS
1%BG

Figure 3:

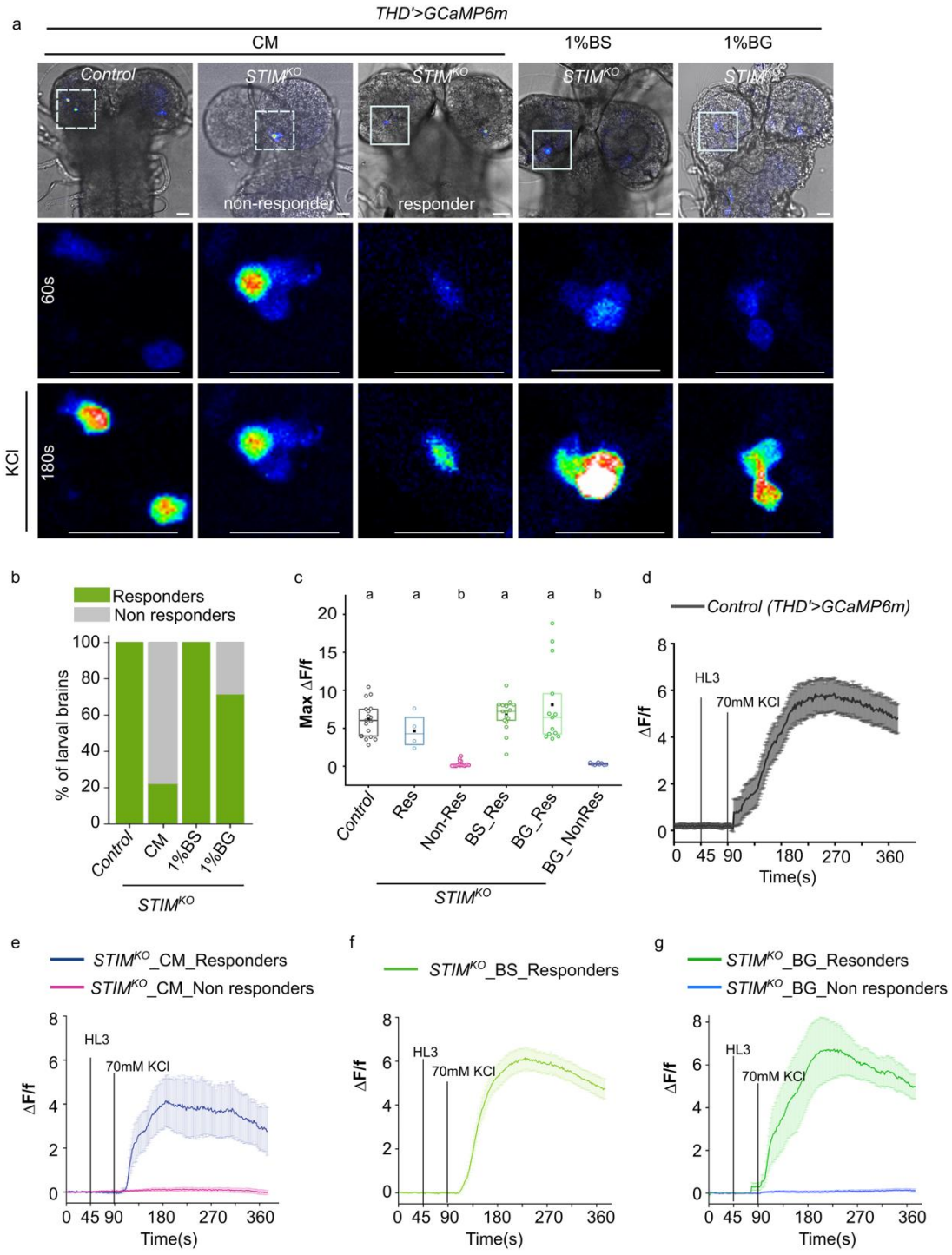

a
THD'>GCaMP6m
CM
1%BS
1%BG
Control
STIM^KO
non-responder
responder
60s
180s
KCl
b
Responders
Non responders
% of larval brains
Control
CM
1%BS
1%BG
STIM^KO
c
Max ΔF/f
Control
Res
Non-Res
BS_Res
BG_Res
BG_NonRes
STIM^KO
d
Control (THD'>GCaMP6m)
ΔF/f
HL3
70mM KCl
Time(s)
e
STIM^KO_CM_Responders
STIM^KO_CM_Non responders
f
STIM^KO_BS_Responders
g
STIM^KO_BG_Resonders
STIM^KO_BG_Non responders

Figure 4:

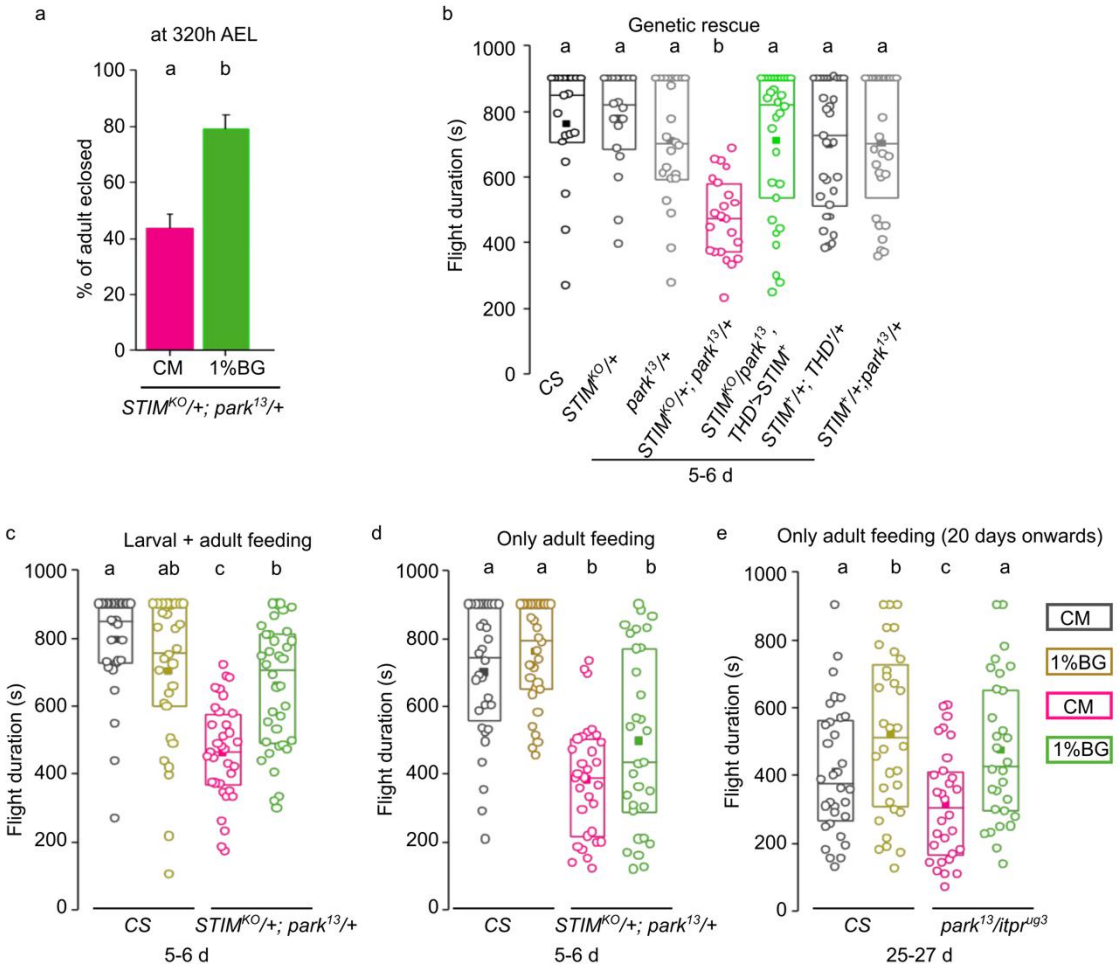

a
at 320h AEL
% of adult eclosed
CM
1%BG
STIM^KO/+; park^13/+
b
Genetic rescue
Flight duration (s)
CS
STIM^KO/+
park^13/+
STIM^KO/+; park^13/+
STIM^KO/park^13; THD'>STIM+
STIM+/+; THD'/+
STIM+/+;park^13/+
5-6 d
c
Larval + adult feeding
d
Only adult feeding
e
Only adult feeding (20 days onwards)
25-27 d
park^13/itpr^ug3

Figure 5:

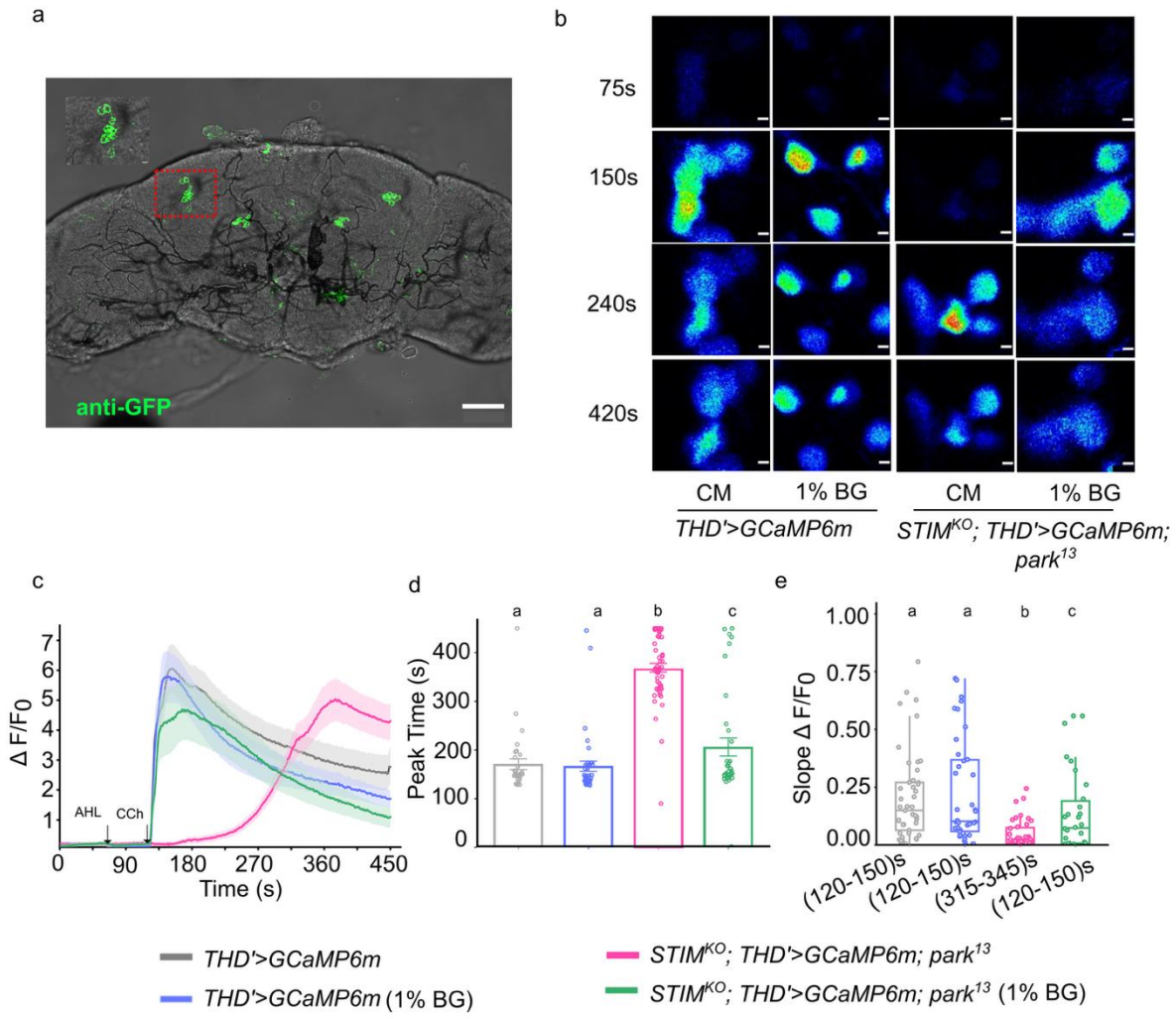


Figure Legends:

Figure 1: Lethality and growth of *STIM*^{KO} larvae are rescued partially by feeding Brahmi ghrita, a lipid-based extract from *Bacopa monnieri* plants.

a and e: Bar graphs showing the number (mean ± SEM) of viable *Canton S* (*CS*) larvae at 96 h after egg laying. Larvae were reared in common media (CM) and compared to those reared in media containing 1% BG from Batch 1 (B1, Fig 1A) and Batch 2 (B2, Fig 1E). N = 3 independent experiments, each with n=25 larvae.

b and f: Bar graphs showing the number (mean ± SEM) of viable *STIM*^{KO} larvae at 96 h after egg laying reared in common media (CM) compared to those reared in media containing 1% BG from Batch 1 (B1, Fig 1B) and Batch 2 (B2, Fig 1F). N = 3 independent experiments, each with n=25 larvae. **P<0.05 (unpaired t-test).

c and g. Quantification of larval length of *STIM*^{KO} larvae that survived the staging experiment at 96 h from N=3 experiments shown in Fig 1B and Fig 1F. The box plots display the 25th and 75th percentiles, with the median represented by a line, the mean by a square, and individual larval sizes shown as circles*P<0.05 (unpaired t-test).

d and h. Representative images of *STIM*^{KO} larvae at 96 h after egg laying, reared in common media (CM) and in media containing 1% BG. Scale bar = 1 mm

Figure 2: Comparison of the viability of *CS* and *STIM*^{KO} larvae with pure bacosides and different formulations of Brahmi

a. An HPLC chromatogram with peaks of individual components of commercial Bacoside A (92.68%).

b. An HPLC chromatogram from *Bacopa monnieri* extract. Four peaks near the retention time of 13 to 19 minutes correspond to the peaks seen in the commercial Bacosides A compound in (a) as marked.

c. Number (mean ± SEM) of viable *Canton S* (*CS*) larvae at 96 h after egg laying (AEL). Larvae were reared in common media (CM) or in CM supplemented with either one of the following: 0.1% DMSO, ii) Ghee (1% Gh), iii) Bacoside 92.68% (100 µg BA), iv) Brahmi swarasa (1% BS), and v) Brahmi ghrita (BG) from Batch 3. N = 3 and n= 25 larvae were reared in each food media plus its supplement.

d. Quantification of larval length for *CS* larvae at 96 h (mean ± SEM). As in Fig 2c larvae were reared in the indicated media and supplements. N = 3 and n = 25, *P<0.05 (unpaired t-test).

e. Representative images of *CS* larvae at 96 h AEL in the indicated media and supplements as in Fig 2c.

f. Number (mean ± SEM) of viable *STIM*^{KO} larvae at 96 h AEL. Larvae were reared in common media (CM) or in CM supplemented with either one of the mentioned supplements as in Fig 2c. N = 3 and n= 25 larvae were reared in each food media plus its supplement. *P<0.05 (unpaired t-test).

g. Quantification of larval length for *STIM*^{KO} larvae at 96 h (mean ± SEM). As in Fig 2f larvae were reared in the indicated media and supplements. N = 3 and n = 25, *P<0.05 (unpaired t-test).

h. Representative images of *CS* larvae at 96 h AEL in the indicated media and supplements as in Fig 2f.

i. Representative images of *STIM^{KO}* larvae sorted by length into groups by the indicated length obtained after being reared in the indicated media plus supplements.
j. A stack bar graph showing the percentage of *STIM^{KO}* larvae (mean ± SEM) that were 1-2 mm, 2-3 mm, and 3-4 mm in length after being reared in the indicated media plus supplements.

Figure 3: *STIM^{KO}* larvae fed with Brahmi formulations maintain excitability of DL1/DL2 central dopaminergic neurons
Representative images of brain lobes from larvae of the indicated ages and genotypes, fed with the indicated formulations. The first column contains images with the region of interest boxed, followed by columns with images of either DL1 or DL2 clusters of dopaminergic cells expressing the Ca^{2+} indicator GCaMP6m. Fluorescence of Ca^{2+} transients is shown before (60 s) and after (180 s) the addition of a depolarizing agent (70 mM KCl). Ca^{2+} transients were assessed by measuring changes in GCaMP6m fluorescence intensity in *THD'>GCaMP6m* (control at 60-66 h AEL) and *STIM^{KO}; THD'>GCaMP6m* (82-86h AEL) reared in common media, compared to *STIM^{KO}; THD'>GCaMP6m* larvae reared in media supplemented with either 1% BS or 1% BG. Scale bar = 20μm
a. Stacked bar chart representing the number of larval brains that either responded or did not respond to KCl stimulation across the specified genotypes reared in different media conditions.
b. Changes in GCaMP6m fluorescence (mean ± SEM of $\Delta F/F$) from responding and non-responding DL1/DL2 neurons marked by *THD'GAL4* at the indicated time points before and after addition of KCl (70mm). The genotypes shown are *THD'>GCaMP6m* (control) and *STIM^{KO}; THD'>GCaMP6m* (reared in common media) and compared with *STIM^{KO}; THD'>GCaMP6m* larvae reared in media supplemented with either 1%BS or 1%BG. For each genotype and condition the number of brains = 7, number of cells $\geq$ 15.
c. Maximal GCaMP6m fluorescence intensities (Δ_{max}) in DL1/DL2 cells from the respective genotypes under different media conditions. Box plots display the 25th and 75th percentiles, the median (line), and the mean (square), with individual cell's ΔF maximum values represented by small circles

Figure 4: Viability and flight of *STIM^{KO}/park^{13}* flies is rescued by food supplemented with BG

a. Bar graph showing the percentage of *STIM^{KO}/park^{13}* adult flies that eclosed upon transfer of 64 larvae when grown in media with and without BG. No more than 12 larvae were fed in each vial. n=8-12, N=6, $p>0.05$ Unpaired *t* test

b. Box plots depict air-puff stimulated flight durations of flies from the indicated genotypes The box plots display the 25th and 75th percentiles, the median (line), and the mean (square), with flight bouts of individual flies represented by circles. The "+" symbol denotes a wild-type allele in the parental control genotypes. n ≥ 25.

c. Box plot depict air-puff stimulated flight duration of flies from the indicated genotypes, comparing conditions with or without 1% BG. The supplementation started from the late second instar larval stage through to 5 days post-adult eclosion. The box plots display the 25th and 75th percentiles, the median (line), and the mean (square), with individual flight bouts represented by round circles. N ≥ 25.

d. Box plot depict air-puff stimulated flight duration of flies from the indicated genotypes, comparing conditions with or without 1% BG supplementation administered only during the adult stage, starting 6 hours post-eclosion. The box plots display the 25th and 75th percentiles, the median (line), and the mean (square), with individual flight bouts represented by round circles. N ≥ 25.

e. Box plot depict air-puff stimulated flight duration of flies from the indicated genotypes, comparing conditions with or without 1% BG. The supplementation provided from the 20th day to 27th days post-adult eclosion. The box plots display the 25th and 75th percentiles, the median (line), and the mean (square), with individual flight bouts represented by round circles. N ≥ 25.

Non-parametric, Mann–Whitney *U* test was used to compare the experimental genotypes with both heterozygous parental controls and effect under different feeding conditions. Individual comparisons of genotypes or feeding condition with their *p*-values are provided in Table S1. Lower case letters above each genotype, when different, represent significant differences from flight times of other genotypes ($P<0.05$).

Figure 5. Ca^{2+} responses of central flight promoting dopaminergic neurons in *STIM^{KO}/park^{13}* adults are partially restored by feeding of Brahmi ghrita.

a. Image of an adult *Drosophila* brain from flies of a control genotype (*THD'>GCamP6m*) immunostained for GFP. The PPL1 cluster of one brain hemi-lobe is marked by a red box and shown at a higher magnification on the top left. Scale bar indicates 50μm.

b. Images from ex-vivo preparations of adult brains from the indicated genotypes after addition of Adult Haemolymph-like saline (AHL, 75s) and after addition of carbachol (Cch at 120s; images shown from 150s onwards). A shift toward warmer colours indicates elevated cytosolic [Ca^{2+}]. Columns marked as CM indicate brains obtained from flies fed on common medium. Columns marked as 1% BG indicated brains obtained from flies fed with CM containing 1% Brahmi ghrita. Scale bars indicate 3μm.

c. Median GCaMP6m responses from PPL1 neurons of the indicated genotypes and feeding conditions plotted as a function of time. A shaded region around the solid line represents 95% confidence interval of 35 or more cells imaged from 7 or more brains.

d. Bar graph with average peak times of PPL1 cells from the indicated genotypes and feeding conditions. Individual cellular peak times are shown as circles. Peak times are summarized as means with standard error of the mean (SEM) error bars.

e. Feeding Brahmi improves the rate of Ca^{2+} entry in dopaminergic neurons from $STIM^{KO}$/$park^{13}$ adults (compare red with green boxes), as indicated by the faster Ca^{2+} peak response in D and the greater slope of the Ca^{2+} response curve. The time intervals on the X axis indicate the period taken for calculating the slope. Activation slope distributions are represented via boxplots combined with individual cell-level jittered strip points.

Mann-Whitney U test was used to compare the experimental genotype with control genotypes and effect under different feeding conditions; Different letters represent significant difference from other genotypes or feeding condition.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.docx