Journal of Translational Medicine

https://doi.org/10.1186/s12967-025-07609-6

Article in Press

Restoring brain wiring, mobility, sleep, and lifespan with a novel repurposed modulator of voltage-gated K^+ channels: an emerging perspective for channelopathies

Received: 16 October 2025

Accepted: 17 December 2025

Published online: 07 January 2026

Cite this article as: Setzu M.D., Casu M.A., Mocci I. *et al.* Restoring brain wiring, mobility, sleep, and lifespan with a novel repurposed modulator of voltage-gated K^+ channels: an emerging perspective for channelopathies. *J Transl Med* (2025). https://doi.org/10.1186/s12967-025-07609-6

Maria Dolores Setzu, Maria Antonietta Casu, Ignazia Mocci, Giuseppe Talani, Fatemah M. Haidar, Fabrizio Sanna, Cristina Manis, Pierluigi Caboni, Gilles Bru–Mercier, Francesca Biggio, Manigandan Krishnan, Patrizia Muroni, Bianca Baroli, Roberto Frau, Luca Concas, Monica Puligheddu, Sandeep B. Subramanya, Paola Imbrici, Maria Cristina D'Adamo & Mauro Pessia

Restoring brain wiring, mobility, sleep, and lifespan with a novel repurposed modulator of voltage-gated K^+ channels: an emerging perspective for channelopathies

Maria Dolores Setzu[a], Maria Antonietta Casu[b], Ignazia Mocci[b], Giuseppe Talani[c], Fatemah M. Haidar[d], Fabrizio Sanna[a], Cristina Manis[e], Pierluigi Caboni[e], Gilles Bru–Mercier[d], Francesca Biggio[e], Manigandan Krishnan[f], Patrizia Muroni[a], Bianca Baroli[e], Roberto Frau[a], Luca Concas[a], Monica Puligheddu[g], Sandeep B. Subramanya[d], Paola Imbrici[h], Maria Cristina D'Adamo[i] and Mauro Pessia[d,j,*]

[a] Department of Biomedical Sciences, University of Cagliari, Italy.
[b] CNR-Institute of Translational Pharmacology, UOS of Cagliari, Italy.
[c] CNR-Institute of Neuroscience, UOS of Cagliari, Italy.
[d] Department of Physiology, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain 15551, United Arab Emirates.
[e] Department of Life and Environmental Sciences, University of Cagliari, Italy.
[f] Department of Pediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain 15551, United Arab Emirates.
[g] Department of Medical Sciences and Public Health, Sleep Disorder Research Center, University of Cagliari, Cagliari, Italy.
[h] Department of Pharmacy–Drug Sciences, University of Bari ''Aldo Moro", Italy.
[i] Department of Medicine & Surgery, LUM University "Giuseppe DEGENNARO", 70010 Casamassima (BA), Italy.
[j] Department of Physiology & Biochemistry, Faculty of Medicine & Surgery, University of Malta, Msida, MSD2080, Malta.

[*]**Corresponding author**: Mauro Pessia**,** mauro.pessia@um.edu.mt

Abstract

Background

Voltage-gated K^+ channels (Kv) regulate various essential brain functions. Cognitive impairment, intellectual disability, and sleep disruption are prevalent in Kv1.1- and Kv1.2-related channelopathies, yet the mechanistic basis is unresolved and no symptom-specific repurposing candidates for pharmacotherapy have been identified. Furthermore, the role Kv channels play in establishing synaptic wiring—the intricate network of neuron-to-neuron connections that underpins communication within the brain that is fundamental to cognitive functions—remains poorly understood at ultrastructural level.

Method

We employed a multidisciplinary experimental approach integrating ultrastructural, biochemical, electrophysiological and behavioral analyses to investigate the impact of *Shaker* K^+ channel mutation on synaptic connections in the brain of *Drosophila melanogaster* flies *Sh* and to identify drugs with repurposing potential. In the protocerebrum, T-bars density and morphology was assessed using transmission electron microscopy while the concentration of the drug in the brain was measured by UHPLC–MS/MS. Survival and motor behavior were evaluated via lifespan and climbing assays, while sleep was quantified using the Drosophila Activity Monitoring System. The functional effects of the drug on ion channels were studied using two-electrode voltage-clamp in *Xenopus laevis* oocytes and whole-cell patch-clamp recordings in differentiated Neuro-2a cells. Finally, evoked post-synaptic potentials were recorded to assess neuromuscular transmission efficacy.

Results

Mutant *Sh* flies exhibit impaired neuromuscular transmission, climbing ability, and sleep duration, ultimately shortening lifespan. We observed that all these abnormalities in *Sh* flies were significantly alleviated by administering low dose of ibuprofen, a widely used non-steroidal anti-inflammatory drug. Through high-resolution ultrastructural analyses, we discovered decreased density of T-bars with morphologically abnormal presynaptic bouton active zones in the brains of *Sh* flies which were restored by nanomolar concentrations of ibuprofen. Mechanistically, the drug increased the amplitude of both *Shaker* currents in *Xenopus* oocytes and voltage-gated K^+ currents in Neuro-2a cells. Collectively, these results identify ibuprofen as a previously unrecognized modulator of Kv channels that restores neuronal network integrity compromised by *Shaker* K^+ channel dysfunction, thereby

normalizing neuromuscular signaling and enhancing motor performance, sleep, and longevity in *Sh* mutant flies. Notably, this study uncovers that genetically-induced dysfunction of Kv1 channels leads to disrupted brain wiring, highlighting the crucial role this K^+ channel type plays in establishing proper *neuron-to-neuron* connections, and offer a pioneering treatment strategy for symptoms-targeted Kv1.1 channelopathies.

Keywords

Shaker K^+ channels Kv1, T-bar, sleep disturbance, brain wiring, aging, longevity, ibuprofen.

Background

Genetic mutations in the *Shaker*-like K^+ channels Kv1.1 (*KCNA1*) [OMIM#160120] and Kv1.2 (*KCNA2)* [OMIM#176262, 616366] are associated with a spectrum of neurological disorders in humans, including episodic ataxia, myokymia, and epilepsy (1–4). While these disorders were initially considered primarily motor in nature, mounting evidence now highlights that both Kv1.1- and Kv1.2-related *channelopathies* frequently involve cognitive impairment and intellectual disability (5). In particular, individuals with mutations in either gene often

exhibit developmental delays and speech deficits—hallmarks of neurodevelopmental disorders. In line with this clinical findings, antisense-mediated suppression of *Shaker*-like Kv1.1 potassium channel expression disrupts associative memory in mice and rats (6). However, the mechanisms by which Kv1.1 and Kv1.2 dysfunction leads to cognitive impairment remain poorly understood. Indeed, although robust evidence linking these channels to altered network excitability in specific brain regions such as the cerebellum and hippocampus, a clear cellular explanation for the associated intellectual disabilities has yet to emerge.

The *Drosophila melanogaster* (*Dm*) strain *Sh* harbors a mutation in the *Shaker* K^+ channel gene, the orthologue of the human Kv1.1 channel, and replicates the key features of both Kv1.1- and Kv1.2-related *channelopathies* including learning and memory impairment (7). Furthermore, these flies exhibit a *mini-sleep* phenotype, and a markedly reduced lifespan (8,9).

Dm flies serves as an outstanding model for investigating synaptic connectivity in the brain (10–13). Combined with its rapid life cycle, *Dm* enables high-throughput studies that provide critical insights into the mechanisms underlying channelopathies and streamline the discovery of small molecules that preserve synaptic integrity and improve motor performance, sleep, and healthy aging.

Maintaining 7 to 8.5 hours of sleep without disturbances between the ages of 50 and 75 correlates with longer, disease-free life expectancy (14). While, sleep deprivation has been linked to poor quality of life, and early death across several species, including dogs, rats, cockroaches, and *Dm* flies (9,15–18). It is suggested that death from sleep deprivation is linked to impaired brain function, as cognitive performance noticeably declines following sleep loss (19–21). Indeed, several studies highlighted the importance of sleep in processes such as synaptic formation, plasticity, and homeostasis (22–26). Most individuals typically sleep 7–8 hours per night, although a minority appear to function well on just 3–4 hours of sleep. This trait tends to run in families, although the genetic basis remains unclear. Patients with episodic ataxia type 1 (EA1) carry *loss-of-function* (LoF) mutations in the Kv1.1 channel, and some have also been reported to exhibit short sleep duration (27). Consistent with these clinical findings, genetic deletion of *Kcna1* (Kv1.1) in mice leads to disrupted rest–activity rhythms (28) and significantly reduces the duration of both NREM and REM sleep

(29). Kv1.1 subunits can co-assemble with Kv1.2 to form heteromeric channels in multiple brain regions, including those that regulate the human sleep–wake cycle. Notably, mice lacking the *Kcna2* gene, encoding for the *Shaker*-related Kv1.2 channel, also have reduced non-rapid eye movement (NREM) sleep, increased waking episodes and died early (30). Hence, converging evidence from genetically determined phenotype in humans, rodent models, and *Drosophila* studies demonstrates that distinct voltage-gated K^+ channels—particularly Kv1.1, Kv1.2, and their homologs—are key regulators of sleep, and their dysfunction is linked to sleep disturbances.

In multiple species, drug-induced lifespan extension has been associated with the actions of ibuprofen (IBU) (31); however, the mechanisms driving this effect are not yet fully understood. In *Dm* flies, IBU has been shown to extend lifespan in females, while its effects in males remained inconsistent (31). Notably, this pro-longevity action occurs independently of cyclooxygenase inhibition, as flies lack the mammalian COX gene but instead express *pxt* (prostacyclin synthase-like or peroxidase). Given the sex-dependent variability in lifespan extension, the unclear mechanisms underlying IBU's effects—particularly on the *Dm* brain—along with its well-characterized pharmacological profile and strong potential for drug repurposing, we sought to investigate the impact of IBU on the longevity of wild-type (WT) male flies and the potential effects on *Sh* flies.

Here, we discovered that at nanomolar brain concentrations, IBU rescued synaptic transmission and motor deficits in *Sh* flies and extended both sleep duration and lifespan. We also unraveled that the brains of *Sh* flies possess a remarkable reduction in presynaptic T-bars compared to WT likely resulting from their genetic defect and associated *mini-sleep* trait. IBU Notably, IBU rescued the density of T-bars at presynaptic bouton active zones upregulating *Shaker* K^+ currents. This study reveals that genetically-induced dysfunction of K^+ channels disrupts brain wiring, underscoring their essential role in forming neuron-to-neuron connections and offering a groundbreaking approach for potential treatment of distinct human conditions such as EA1 and for longevity medicine.

Methods

Drosophila melanogaster stocks and culture

Drosophila melanogaster were maintained on standard cornmeal-agar-yeast medium in 50 mL plastic vials at 24–25 °C, 60% relative humidity, under a 12:12 h light/dark cycle. The strains used in this study were the *Shaker* mutant (Sh^{mns}) (https://flybase.org/reports/FBgn0003380.html#summaries_sub) and wild-type Canton-S (WT; obtained from the Bloomington Drosophila Stock Center; FlyBase: http://flybase.bio.indiana.edu) (31). Ibuprofen [(±)-Ibuprofen HCl; Toronto Research Chemicals, North York, Ontario, Canada] was first dissolved in distilled water or vehicle and then mixed into the fly food to obtain final concentrations of 0.5 µM or 1 µM. These concentrations were selected based on preliminary dose-finding experiments to determine effective, non-toxic levels for chronic treatment.

Survival Assay

To evaluate the effects of ibuprofen on lifespan and to establish appropriate dosing for behavioral assays, survival analysis was conducted using male *Drosophila melanogaster* (n= 100 per group). Wild-type (WT) males were collected within 24 hours from eclosion under CO_2 anesthesia; while, *Sh* mutants were identified based on their characteristic leg-shaking phenotype under light ether anesthesia. Flies were housed in standard vials (15–20 flies per vial) containing food supplemented with vehicle or ibuprofen at 0.5 µM or 1 µM, based on previously published protocols (6,32). Flies were maintained under standard laboratory conditions, and survival was monitored daily until all individuals had died. Mortality data were used to generate Kaplan–Meier survival curves. Statistical comparisons between groups were performed using the *Gehan–Breslow–Wilcoxon* test. All analyses were conducted using GraphPad Prism version 8, with significance set at $P<0.05$.

Sleep Assessment

Sleep was monitored in both wild-type (WT) and *Sh* mutant Drosophila melanogaster at 1, 7, and 14 days using the Drosophila Activity Monitoring System (DAMS; Trikinetics Inc.), as previously described (33). Flies were administered ibuprofen at concentrations of 0.5 µM or 1 µM for 1, 7, or 14 days via standard food medium. Locomotor activity was quantified by recording infrared beam crossings, and sleep was operationally defined as any period of

inactivity lasting ≥5 minutes. Following 24 hours of habituation to the monitoring environment, activity data were analyzed using pySolo software (34) in combination with custom-built analysis scripts.

Electron microscopy analysis and synaptic density assessment

Transmission electron microscopy (TEM) was conducted following the procedures described by (35). Wild-type (WT) and *Sh* mutant flies, either untreated or treated with ibuprofen (0.5 µM or 1 µM) for two weeks (n = 5 per group), were anesthetized on ice. Brains were rapidly dehydrated and fixed in a solution containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.15 M cacodylate buffer. After rinsing in the same buffer, brains were post-fixed in 1% osmium tetroxide in distilled water for 2 h and stained overnight with 0.5% uranyl acetate at 4°C. Tissues were dehydrated through a graded acetone series and embedded in EPON resin. To localize the protocerebrum, 1 µm semi-thin coronal sections of the whole brain were obtained using a Reichert Supernova ultramicrotome and stained with toluidine blue. Ultrathin sections (90 nm) were then collected and examined using a JEOL JEM 1400 Plus electron microscope equipped with a CCD camera, operating at 80 kV. T-bars were detected by eye, based on their typical morphology represented by electron-dense T-shaped structures located at presynaptic sites, in the protocerebrum, and labeled manually for quantification using NIH ImageJ software. T-bar numbers (synaptic density) were quantified within unit areas of 25 µm². The T-bar area was determined by surrounding the dense material and measuring the area of the region of interest created. For each brain, 40–50 non-overlapping fields were analyzed. In total, 3,275 T-bars were identified across 813 randomly sampled micrographs at a final magnification of 10,000X. Data were analyzed using GraphPad Prism v5.01.

Ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analysis

Chemicals. Analytical-grade acetonitrile and formic acid were obtained from Sigma-Aldrich (Milan, Italy). Ultrapure water was produced using a Milli-Q purification system (Millipore, Milan, Italy). (±)-Ibuprofen (HCl salt) was purchased from Toronto Research Chemicals (North York, Ontario, Canada).

Sample Preparation. A total of 400 *Sh* mutant *Drosophila* were divided into two groups (n = 200 each). One group was treated with ibuprofen (1 µM) for 7 days, while the control group received vehicle only. After treatment, flies were anesthetized, and heads were dissected for analysis. Samples were sonicated in 100 µL of ice-cold distilled water, followed by centrifugation at 15,000 × g for 15 minutes at 4°C. Supernatants were collected and stored at –20°C until analysis.

Quantification of Ibuprofen. Ibuprofen concentrations in fly heads were determined by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). Prior to analysis, 10 µL of each sample and its corresponding washing solution were diluted with 20 µL of a water:acetonitrile (5:95, v/v) mixture. Samples were vortexed and analyzed in negative ionization mode.

Calibration standards and quality controls. A stock solution of ibuprofen was prepared by dissolving 1 mg of standard in 1 mL of water:acetonitrile (5:95, v/v). Calibration standards were prepared at concentrations of 25, 50, 100, and 200 ng/mL and processed identically to biological samples.

UHPLC-MS/MS instrumentation and conditions. Analyses were performed using an Agilent 1290 Infinity II Bio LC system coupled via JetStream electrospray ionization (ESI) to an Agilent Ultivo LC/TQ mass spectrometer. A 15 µL aliquot from each sample was injected onto a Kinetex EVO C18 column (5 µm, 100 Å, 150 mm × 2.1 mm; Agilent Technologies, Palo Alto, CA), maintained at 50°C. The flow rate was set to 0.2 mL/min.

The mobile phase consisted of water with 0.01% formic acid and acetonitrile with 0.01% formic acid. The gradient elution profile was as follows: 95% (initial), decreased linearly to 60% A over 1.3 min, then to 40% in 1.1 min. A further reduction to 5% was reached in 0.5 min and held for 1.5 min, followed by re-equilibration to initial conditions over 3 min.

The mass spectrometer was operated in positive ion mode with the following source parameters: gas temperature, 325°C; gas flow (N_2), 7 L/min; nebulizer pressure, 20 psig; sheath gas temperature, 275°C; sheath gas flow, 12 L/min; capillary voltage, 4000 V; nozzle voltage, 1500 V; fragmentor voltage, 135 V; skimmer, 65 V; octopole RF, 7550 V. MS/MS acquisition was performed in multiple reaction monitoring (MRM) mode with two transitions for ibuprofen: m/z 205.1 → 161.2 and 205.1 → 205.1. Data acquisition and processing were carried out using Agilent MassHunter LC/MS software.

Electrophysiological recordings of Shaker K^+ currents in Xenopus laevis oocytes

Two-electrode voltage-clamp (TEVC) recordings were performed as previously described (27) Briefly, stage V–VI *Xenopus laevis* oocytes were obtained from the European Xenopus Resource Centre (University of Portsmouth, UK). Following enzymatic defolliculation with collagenase, each oocyte was injected with 50 nL of cRNA (5 ng/μL) encoding the *Shaker* K^+ channel using a Nanoject microinjector (Drummond Scientific, Broomall, PA, USA). Injected oocytes were incubated at 16 °C in ND96 solution containing (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 5 HEPES (pH 7.4), supplemented with 50 μg/mL gentamicin to prevent bacterial contamination. The recordings were performed 1–5 days post-injection at room temperature (21–23 °C). Oocytes were impaled with microelectrodes filled with 3 M KCl (resistance: 0.1–0.5 MΩ). Currents were recorded using a GeneClamp 500 amplifier (Axon Instruments) interfaced with a computer via an ITC-16 interface (Instrutech, Port Washington, NY, USA). Data acquisition and analysis were conducted using pCLAMP software (Molecular Devices). Oocytes were continuously perfused with a standard extracellular solution (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, and 5 HEPES (pH adjusted to 7.4). Membrane currents were evoked by applying voltage steps from a holding potential of –80 mV, with stimulation protocols detailed in the corresponding figure legends. Ibuprofen was applied by switching to a superfusion solution that differed only in its content of the drug. Complete exchange of the bath solution occurred in about 1 min.

Cell Culture and differentiation

Neuro-2a (N2a) cells (ATCC, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Serum Source International, Charlotte, NC, USA) and 1% Penicillin–Streptomycin (Gibco, Rockville, MD, USA). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. To preserve cellular integrity and prevent senescence, experiments were conducted using cells within 24 passages. Differentiation was performed according to a previously published method (36). When cultures reached 70–80% confluence, the growth medium was replaced with differentiation medium consisting of DMEM supplemented with 2% FBS and 20 μM all-trans retinoic acid (RA). Cells were maintained under differentiation conditions for up to 8 days, with media replaced every 2 days. Morphological and functional assessments were conducted at 4-, 6-, and 8-days post-induction. Neuronal differentiation

was confirmed by morphological analysis—based on the emergence of neurite-like processes resembling axons and dendrites—and by electrophysiological characterization as previously described (36).

Patch-clamp recordings of K^+ currents from Neuro-2a cells

Whole-cell patch-clamp recordings were performed to assess macroscopic delayed-rectifier K^+ currents in Neuro-2a cells at room temperature (21–23 °C), as previously described (36). Cells were continuously perfused with an external solution containing (in mM): 125 NaCl, 4 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 10 glucose, 0.3 tetrodotoxin (TTX), 0.2 $CdCl_2$, and 10 HEPES, adjusted to pH 7.4 with NaOH. The internal pipette solution contained (in mM): 140 KCl, 4 NaCl, 0.02 $CaCl_2$, 0.8 EGTA, 2 $MgCl_2$, 4 MgATP, and 10 HEPES, adjusted to pH 7.4 with KOH. Patch electrodes were pulled from borosilicate glass capillaries and had a resistance of 4–6 MΩ when filled with the internal solution. Data acquisition and voltage-clamp protocols were executed using an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA) and pCLAMP software suite (version 9.2 or higher). Signals were digitized at a sampling interval of 10 µs per point, filtered at 5 kHz, and corrected for series resistance using a 60–70% compensation protocol. Current-voltage (I–V) relationships and activation kinetics were analyzed offline using Clampfit (Axon Instruments) and OriginPro software (OriginLab, Northampton, MA). Voltage commands and analytical procedures are described in the relevant figure legends.

Electrophysiological recordings of evoked Post-Synaptic Potentials (ePSPs)

To assess the effects of chronic ibuprofen treatment on neuromuscular function, evoked post-synaptic potentials (ePSPs) were recorded from the dorsal longitudinal muscles (DLMs) of *Drosophila melanogaster* following electrical stimulation of the Cervical Giant Fiber System (GFS), as previously described (36,37,38). Consistent with our previous findings (36), ePSPs had an abnormal repolarization kinetic in *Sh* mutant flies, as shown by a significant increase in ePSPs decay times and amplitude. Flies from each experimental group (WT, *Sh*, WT+IBU, *Sh*+IBU) were anesthetized with CO_2 and mounted dorsal side up in wax-filled Petri dishes under a stereomicroscope (40× magnification). The GFS was activated via two tungsten stimulating electrodes inserted into the compound eyes and connected to a

stimulator (Master 8, A.M.P.I., Jerusalem, IL, USA) through a stimulus isolation unit (DS2A, Digitimer Ltd., Hertfordshire, UK). Stimulation intensity was gradually increased until a clear ePSP was evoked in the DLMs, with maximum voltage not exceeding 10 V. A third tungsten electrode was inserted into the abdomen to serve as ground. Recording electrodes were pulled from borosilicate glass capillaries using a horizontal puller (P97, Sutter Instruments, Novato, CA, USA), yielding tip resistances of 4–5 MΩ when filled with 3 M KCl. Electrodes were inserted into the 45a or 45b fibers of the DLMs (as illustrated in Fig.*B*). Signals were amplified using an Axopatch 2B amplifier (Axon Instruments, Foster City, CA, USA), low-pass filtered at 0.5 kHz, and digitized at 1 kHz. Recordings were acquired using pCLAMP 9.2 software and analyzed offline with Clampfit 10.7 (Molecular Devices). Each fly received single GFS stimuli delivered at 20-second intervals. For each animal, 3–5 traces were acquired, averaged, and analyzed. ePSP amplitude was measured from the pre-stimulation baseline to the peak. The decay phase was characterized using two parameters: (*i*) the decay time constant (τ), defined as the time required to reach 63.2% of peak amplitude, and (*ii*) the half-width decay time, calculated at 50% of peak amplitude. Additionally, the minimum stimulation intensity required to evoke a response was recorded. Recordings were performed on individual flies from each group: WT (n = 29), *Sh* (n = 24), WT+IBU (n = 17), and *Sh*+IBU (n = 40). The minimum stimulation intensity required to reliably evoke an ePSP was also determined and compared across experimental groups. Particular care was taken to ensure that the intensities of electrical stimulation used in each set of experiments were comparable, as dissimilar intensities may result in different properties of the evoked PSPs (*see* Fig.*F*). All flies were treated with vehicle or ibuprofen (1 µM) for 14 days. Data are presented as mean ± SEM and analyzed using one-way ANOVA followed by Tukey's post hoc test.

Climbing Assay (Negative Geotaxis Test)

The climbing performance was assessed using the *negative geotaxis* assay, as previously described (5,38). WT and *Sh* flies were treated with vehicle or ibuprofen (IBU; 0.5 µM or 1 µM) for 1, 7, or 14 days. For each experimental condition, groups of 12 flies were tested individually. Each fly was placed in a vertically oriented plastic tube (10 cm length × 1.5 cm diameter) and gently tapped to the bottom. The number of flies that reached a 6 cm mark

(*target level*) within 10 seconds was recorded and expressed as a percentage of the total. Each condition was tested in triplicate, and the average climbing performance was calculated from three independent experimental replicates.

Statistics

Data sets that passed the Shapiro–Wilk test for normality were analyzed with paired or unpaired Student's *t*–test. One–way ANOVAs followed by Bonferroni's post–hoc tests and Fisher's test on contingency tables were used in distinct cases (additional details concerning the statistical analysis of data are reported in methods, figure legends and main text). Where appropriate the significance level of probability (*p*) for the difference between mean values are given.

Results

Ibuprofen extends the lifespan of flies

The typical lifespan of male WT flies maintained in our laboratory setting is approximately 80 days post-eclosion (Fig.1*A*). To assess whether IBU affects the longevity of WT flies, they were cultured on fly food supplemented with either IBU or vehicle (H_2O) as a control and, their adult survival upon eclosion was monitored, continuously. The treatment of male WT flies with IBU (0.5 µM) significantly increased their lifespan (Fig.1*A*; *p<0.01*); while, the effect of IBU (1 µM) was less remarkable (Fig.1*A*), indicating a superior efficacy of the drug at a lower dosage. Compared to WT, the *Sh* strain of *Dm* flies exhibits a reduced life expectancy likely due to the deleterious missense mutation T248I in the *Shaker* K^+ channel gene that is carried by this strain (9,39). Consistent with previous findings, male *Sh* flies displayed reduced longevity compared to WT (~59 days; *p<0.0001;* Fig.1*A*,*B*), (9). To assess whether IBU also affects the longevity of *Sh* flies, they were reared on fly food supplemented with either IBU or vehicle as a control and, their adult survival upon eclosion was monitored, continuously. Notably, IBU significantly increased the median survival rate of *Sh* flies (Fig.1*B*). Moreover, their maximal survival time was prolonged from ~59 to ~75 days upon treatment with IBU (0.5µM) (Fig.2*B*; *p<0.0001*). Given the typical lifespan of *Dm* flies, the

impact of IBU could be considered remarkable. However, the longevity of *Sh* flies exposed to IBU (1 µM) was prolonged less compared to flies treated with IBU (0.5µM) (~70 days; Fig.2*B*; *p<0.001*), demonstrating again improved efficacy of the drug when administered at a lower dose.

Fig.1 Ibuprofen extends the lifespan of *Sh* flies. Survival rate of WT (*A*) and *Sh* flies (*B*) treated with IBU (0.5 and 1 µM) during their entire lifespan and, compared with flies treated with vehicle (CTRL). Cumulative survival curves data are expressed as mean±SEM of 60 flies per group (*the dashed lines indicate the median survival rate*). Data are mean±SEM and the statistical significance between groups was established using the *Gehan–Breslow–Wilcoxon* test (**p<0.01; ***p<0.001)

Fig.2 Ibuprofen improves the sleep phenotype of *Sh* flies. (*A-C*) Bar graphs showing the total sleep duration of WT and *Sh* flies assessed for 24 h. WT and *Sh* flies were treated with IBU or vehicle (CTRL) for 1 day (*A*), 7 days (*B*) and 14 days (*C*). (*D-F*) Bar graphs showing the sleep duration assessed for 12 h during day-time in WT and *Sh* flies treated with IBU or vehicle (CTRL) for 1 day (*D*), 7 days (*E*) and 14 days (*F*). (*G-I*) Bar graphs showing the sleep duration assessed for 12 h during night-time in WT and *Sh* flies treated with IBU or vehicle (CTRL) for 1 day (*G*), 7 days (*H*) and 14 days (*I*). Data are mean±SEM of activity counts collected from 20-40 flies for each group. Data are mean±SEM and the statistical significance was established using the Tukey's multiple comparisons test (*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001)

Ibuprofen improves the sleep phenotype in *Sh* flies

The reduced lifespan of *Sh* flies likely results from their well-established *mini-sleep* trait (9). We hypothesized that the enhancement of mutant flies' longevity caused by IBU treatment could be due at least in part to improvements in their sleep phenotype. Thus, the drug's effect on distinct sleep parameters was assessed in WT and *Sh* flies following 1 day (Fig.2*A*,*B*,*C*), 7 days (Fig.2*D*,*E*,*F*), and 14 days (Fig.2*G*,*H*,*I*) of dietary exposure to IBU (0.5–1 µM). Notably, IBU significantly increased the total-time, day-time and night-time of *Sh* flies sleep after both acute and chronic treatments (Fig.2*A*-*I*). While, after both acute and chronic treatment of WT flies, night-time sleep remained unaffected by the drug (Fig.2*G*-*I*). The greater efficacy of the drug in mutant flies compared to WT supports the notion that the sleep prolongation yielded by IBU in *Sh* flies contributes substantially to its mechanism of action.

Shaker K^+ channel mutation disrupts brain wiring in *Sh* flies that is restored by ibuprofen

Several studies highlighted the importance of sleep in processes such as synaptic formation, plasticity, and homeostasis (22–26). Based also on the notion that Kv channels control crucial brain functions including neurotransmitter release, we hypothesized that mutation-induced dysfunction in *Shaker* K^+ channels could impact not only the sleep but also the formation of synapses in *Sh* brains. Thus, images were acquired from *protocerebrum* samples of WT and *Sh* flies through *transmission electron microscopy* (TEM), and accurate morphometric analyses were performed. This assessment revealed a marked reduction in the number of presynaptic T-bars with most appearing reduced in size compared to WT flies (Fig.3*A,D,E*). We also found that a 14-day diet supplemented with IBU (1µM) restored both the density and size of T-bars at presynaptic active zones in the brains of *Sh* flies (Fig.1*A*-*C red arrows*; Fig.1*D,E*). This evidence implies that the brain of *Dm* flies is one of the target organs of IBU. Thus, to determine whether dietary incorporation of IBU leads to detectable drug levels in flies' brains, *ultra-high-performance liquid chromatography-tandem mass spectrometry* (UHPLC-MS/MS) was employed. This analysis demonstrated that the administration of IBU results in measurable concentrations of the drug in the brains of *Sh* flies (Fig.1*F*). From the UHPLC-MS/MS analysis of more than 400 *Dm* brain samples, the estimated drug concentration in a single *Dm* brain was approximately 4.7 nM, supporting its nanomolar-range activity and high potency.

Fig.3 Sh flies display a reduced number of brain T-bars with aberrant morphology that are restored by Ibuprofen treatment. Representative TEM image of T-Bars acquired from the *protocerebrum* of WT and *Sh* flies treated with vehicle (CTRL) (*A, top panels*) or IBU 0.5 µM (*B*, *central panels*) and IBU 1 µM (C, *lower panels*). Bar graphs illustrating the number of T-bars (*D*) and the total T-bar area (*E*) quantified in the protocerebrum of wild-type (WT) flies and *Sh* mutants, highlighting the effects of drug treatment on both parameters. A total number of non-overlapping 813 micrographs were randomly sampled and 3,275 T-bars were analyzed, blindly. Data are mean±SEM and the statistical significance was established using the Tukey's multiple comparisons test (***p<0.001; ****p<0.0001). (*F*) Representative UHPLC-MS/MS chromatograms obtained using the heads of flies fed with control diet (*top trace*), a diet containing IBU 1 µM (*middle trace*) and IBU analytical standard for comparison (*bottom trace*)

IBU enhances K^+ current amplitudes

Based on the impact of *Shaker* channels defects on lifespan, sleep and brain connectivity that are mitigated by IBU, we postulated that *Shaker* channels may serve as a potential drug target through which IBU increases the density and ameliorates the morphology of synaptic contacts in *Sh* flies. To test this hypothesis, we used *Xenopus* oocytes, a well-established model system known for its efficient translation of foreign genetic material (40). In particular, these oocytes have been widely utilized to express K^+ channels to study drug effects. Thus, the mRNA encoding for *Shaker* K^+ channels was injected into oocytes and electrophysiological recordings in *two–electrode voltage–clamp* (TEVC) mode were performed. It was found that K^+ current amplitudes were particularly enhanced at negative potentials by extracellular superfusion of IBU on *Xenopus* oocytes expressing *Shaker* channels (10 µM; Fig.4*A*-*G*); while unchanging endogenous oocyte currents (CTRL=18.0±2.0 nA; IBU 10 µM=15.7±2.3 nA at -35 mV; n=6; p=0.3; Supplementary Fig.1). On the other hand, at more depolarized potentials, the effect of IBU on the current amplitudes was less

remarkable (Fig.4*D*-*G*). Notably, IBU variably enhanced K^+ currents, with effects ranging from minimal to substantial across individual cells, suggesting that it functions as a facilitator—likely through metabolic pathways that modulates channel function rather than direct interaction with the channel protein.

Fig. 4 Ibuprofen increases the amplitude of Shaker K^+ currents. Representative K^+ current families recorded in TEVC mode from a *Xenopus* oocyte expressing wild-type *Shaker* channels under control conditions (*A*, *black trace*) and after the application of 10 µM IBU (*B*, *red trace*). Currents were elicited by steps from -80 mV to 60 mV (holding potential: -80 mV). (*C*) Sample traces of WT *Shaker* currents recorded under control condition (*black trace*) and after the application of 10 µM IBU (*red trace*). Currents were elicited by a depolarization step from -80 mV to -35 mV followed by a step to -40 (*C*) with a final repolarization step at -80 mV (holding potential: -80 mV). The traces show that the drug enhanced the amplitude of *Shaker* K^+ current particularly at threshold potential. (*D*,*F*) Fold of increase of *Shaker* peak (*D*) and steady-state (*F*) currents, as a function of step potentials, caused by the application of IBU 10 µM. The reported data represent the ratio of the current measured in the presence of the drug to the current measured under control conditions. The red symbols in panel *D* and panel *F* show the mean±SEM (both responsive unresponsive cells were included in this analysis; n=12 cells). (*E*, *G*) Box plots showing the percentage of increase of *Shaker* peak (*E*) and steady-state (*G*) currents, as a function of step potentials, caused by the application of IBU 10 µM (only responsive cells were included in this analysis; n=9)

To further validate these findings in a neuronal cell model, we examined the effects of IBU using *Neuro-2a* (N-2a) cells. This cell type exhibits neuronal stem cell properties (41) and can be differentiated into neurons able to discharge action potentials (Fig.5*A*,*B*)(36). Notably,

these neurons endogenously express orthologues of *Shaker* channels at high levels, as well as other voltage-gated K^+ channel types (41). Patch-clamp recordings showed that IBU increased significantly the amplitude of voltage-gated K^+ current between -40 mV and 10 mV (Fig.5*C*-*E*). Consistent with its effects on *Shaker* current amplitudes recorded in oocytes, IBU produced variable enhancement of K^+ currents—from negligible to pronounced depending on the cell (Fig.5*E*). These heterogeneous responses suggest that the drug acts as a facilitator, most likely by modulating channel-associated metabolic pathways rather than by directly engaging the channel protein. The steady-state current–voltage relationship, constructed before and after drug application, showed that IBU facilitated K^+ current activation by shifting the half-activation voltage ($V_{1/2}$) toward more hyperpolarized potentials (Fig.5*F*). Furthermore, the plot of time constants of ionic current activation and deactivation, as a function of membrane potentials, showed that IBU accelerated channel activation (Fig.5*G*), while slowed channel deactivation (Fig.5*H*). These results demonstrate that IBU significantly modulates several channel gating properties, providing compelling evidence that K^+ channels represent an additional pharmacological target of IBU.

Fig. 5 Ibuprofen increases the amplitude of voltage-gated K^+ currents in Neuro-2a cells . (*A*,*B*) Sample traces showing the recording of action potentials from neurons differentiated from N-2a cells and carried out in current–clamp mode. (*A*) Membrane potential changes were elicited by applying -0.7- 2.3 nA, 300 ms pulses (the resting membrane potential was held at -70 mV by injecting -0.7 nA). (*B*) Enlargement of the action potential highlighted in A as a red colored trace (the dashed line indicates 0 mV level). Traces were sampled at 50 kHz and filtered at 5 kHz. (*C,D*) Representative families of K^+ current recorded from N-2a cells in control conditions (*C*, *black trace*) and after the bath application of 30 µM IBU (*D*, *red trace*). Currents were recorded using the whole-cell patch-clamp technique in voltage-clamp mode and elicited by steps from -40 mV to 40 mV in 10 mV increments (holding potential: -80 mV) (*the protocol is shown as inset and reported below each current families*). (*E*) Fold of increase of K^+ currents calculated for individual cells, as a function of step potentials, caused by the bath application of IBU 30 µM. The reported data represents the ratio of the current

measured in the presence of the drug to the current measured under control conditions. The red symbols in panel *E* show the mean±SEM (n=4). Note that the drug enhanced the K^+ current amplitude significantly between -40mV and 10 mV. (*F*) Plot of normalized peak tail current amplitudes recorded at -40 mV as a function of depolarizing pre-pulse potentials (ΔV=10 mV) and constructed before (*open white circles*) and after (*closed red circles*) the superfusion of IBU 30 µM. Data points were fitted with a Boltzmann function (*solid curve*) from which the half-maximal activation voltage ($V_{1/2}$) and slope factor (*k*) were calculated. Note that IBU shifted I_{tail}/V relationships toward more hyperpolarized potentials. The best fit parameters are control: $V_{1/2}$ =1.2±0.1 mV and *k*=8.1; IBU: $V_{1/2}$ =-6.6±0.1*mV and *k* =7.6±0.1 (data are mean±SEM, n = 4; *P<0.05, paired student's t–test). (*G*) Plot of activation time constants as a function of membrane potential calculated from the best fit of fast activating current traces with a single-exponential function in the absence (*black circles*) and presence (*red squares*) of 30 µM IBU. Note that IBU accelerates the activation kinetics of the K^+ currents, significantly (data are mean±SEM, n = 4; *P<0.05, paired student's t–test). (*H*) Box plots of deactivation time constant as a function of depolarizing pre-pulse potentials and calculated from the best fit of tail currents with a single-exponential function in the absence (*black boxplots*) and presence (*red boxplots*) of 30 µM IBU. Tail currents were recorded at -40 mV following a pre-pulse depolarization to 10 mV (*empty boxplots*) or 20 mV (*patterned boxplots*). Note that IBU slowed-down the deactivation kinetics of K^+ currents, significantly (data are mean±SEM, n = 4; *P<0.05, paired student's t–test)

IBU restores the neuromuscular transmission in *Sh* flies

Sh files also exhibit altered neuromuscular transmission and impaired locomotor abilities (42–47) . Previous action potential recordings of *cervical giant axons* from *Sh* mutants and normal *Drosophilae* showed that the mutants exhibited abnormally long delays in K^+ channel–dependent repolarization likely resulting from the reduced delayed-rectifier K^+ channel function (44). Based on the evidence that IBU enhances the amplitude of *Shaker* current, we hypothesized that the drug could ameliorate both the neuromuscular transmission and locomotion of *Sh* flies. Thus, the neuromuscular junction of *Sh* mutant flies

was investigated to assess the mutation–induced changes in the function of *dorsal longitudinal muscle* (DLM) fibers. *Evoked Post–Synaptic Potentials* (ePSPs) were elicited by electrical stimulation of the *cervical giant fiber* (CGF) and recorded from the DLM fiber (Fig.6*A*,*B*; *see schematic*). The fundamental properties of ePSPs — including the decay time constant (τ) of the falling phase (Fig.6*C*), decay time at half-maximal amplitude (Fig.6*D*), and amplitude (Fig.6*E*) — were analyzed in both mutant and WT *Drosophila* strains. Particular care was taken to ensure that the intensities of electrical stimulation used in each set of experiments were comparable, as dissimilar intensities may result in different properties of the ePSPs (Fig.6*F*). Consistent with our previous findings (36), ePSPs had an abnormal repolarization kinetic in *Sh* mutant flies, as shown by a significant increase in ePSPs decay times and amplitude (Fig.6*A*,*C*,*D*). Notably, chronic treatment of *Sh* flies with IBU (1 μM for 14 days) completely normalized the kinetics and amplitudes of ePSPs, as the relevant parameters were indistinguishable from those observed in WT flies (Fig.6*A,C-E*). Conversely, IBU treatment had no effect on ePSP parameters in WT flies (Fig.6*A,C-E*).

Fig. 6 Ibuprofen restores the properties of evoked post-synaptic potentials at the neuromuscular junction of *Sh* flies and improves their climbing ability. (*A*) Representative traces showing typical ePSPs recorded from the DLM of a fly, evoked by CGF stimulation. The WT and *Sh* strains and treatments for all experimental groups are indicated above each sample trace (scale bar 10mV/10ms). The superimposed red curve shows the best fit of the decay phases with a single-exponential equation from which the time constants were calculated. (*B*) Illustration of the experimental setup for recording ePSPs in flies, highlighting the placement of the stimulation and recording electrodes along with a representative ePSP trace. (*C-E*) Bar graphs showing the ePSPs decay time constants (*C*), decay time constants calculated at half-maximal width of ePSPs (*D*) and ePSPs amplitudes (*E*) assessed in control condition and after treatment with vehicle (CTRL) or IBU (1μM) for 14 days. (*F*) Bar graph showing the minimal electrical stimulation values (mA) required to evoke a PSP (the data are the mean±SEM; the statistical significance was assessed using one-way ANOVA, followed by Tukey's post-hoc test; **p<0.01; ***p<0,001). (*G-I*) Bar graphs showing the climbing ability of flies as the percentage of climbers calculated in control conditions (CTRL) and after the administration of IBU (0.5 μM and 1 μM) for 1 day (*G*), 7 days (*H*), and 14 days (*I*). The data

are mean±SEM and represent the proportion of flies that crossed the 6 cm mark within 12 seconds. The statistical significance was assessed using Fisher's test on contingency tables (**p < 0.01; ****p < 0.0001).

IBU ameliorates the climbing ability in *Sh* flies

The evidence showing that IBU restores neuromuscular transmission in *Sh* flies prompted an investigation into whether the drug improves their *negative geotaxis* behavior, namely the flies' natural tendency to climb upwards against gravity. This behavior was evaluated using a climbing assay, which revealed that under control conditions a smaller proportion of *Sh* flies reached the target level compared to WT flies (Fig.6*G-I*). This result confirmed previous findings pointing out that *Sh* flies exhibit altered locomotor ability (44) Next, the animals were raised on fly food supplemented with either IBU or vehicle as a control. Notably, IBU-treated *Sh* flies exhibited a significant improvement in climbing ability following both acute and chronic treatments at concentrations of 0.5–1 μM, as a higher percentage of them crossed the target level (Fig.6*G-I*). Collectively, this evidence supports the notion that IBU, by increasing the availability of *Shaker* voltage-gated K^+ channels, ameliorates the neuromuscular transmission and locomotor performance of *Sh* flies to properties in line with those of WT animals.

Discussion

The central finding of this study is that IBU at low nanomolar concentrations in the brains of *Sh* flies, significantly ameliorated several genetically-induced defects, including impaired neuromuscular transmission, reduced motor performance, aberrant brain T-bar density and morphology, shortened sleep duration and early death. The unexpected findings that a mutation in *Shaker* K^+ channels leads to T-bar loss in the brains of *Dm* with IBU functioning as a novel K^+ channel facilitator are particularly significant. Indeed, IBU enhanced *Shaker* K^+ current amplitudes in oocytes and voltage-gated K^+ currents in neuronal cells at threshold potentials, uncovering a previously unrecognized mechanism of action for this widely used *over-the-counter* drug. By virtue of its multiple beneficial actions IBU prolonged Drosophila's

lifespan. Overall, the evidence presented here suggests that IBU is a promising candidate for repurposing and warrants evaluation in clinical trials for patients carrying mutations in the K^+ channel Kv1.1.

K^+ channels constitute one of the largest families of signaling molecules encoded by the human genome, playing a pivotal role in regulating essential physiological processes throughout the body. The essential nature of these channels is underscored by the pronounced phenotypic consequences observed in transgenic animal models and individuals carrying mutations in K^+ channel genes, which are frequently linked to severe human disorders (*channelopathies*). In particular, voltage-gated K^+ channels (Kv) are involved in the precise control of neuronal function, ensuring the proper generation and propagation of electrical signals in the brain. These channels open in response to membrane depolarization, allowing the outflux of K^+ ions, thereby repolarizing the membrane and modulating action potential duration. Their activity is critical for setting the resting membrane potential and governing repetitive firing patterns, which collectively tune-up synaptic transmission and network excitability (40). Disruptions in Kv channel function have been implicated in a range of neurological illnesses, including epilepsy, ataxia, and neurodevelopmental disorders, emphasizing their critical role in maintaining normal brain physiology. Understanding the functional diversity of Kv channels is essential for elucidating their contributions to neural circuitry and for developing targeted therapies for *channelopathies*. Although significant breakthroughs findings have unraveled the role of Kv channels in synaptic transmission, yet their contribution to the formation of synaptic wiring—the foundation of neural communication essential for cognitive and behavioral functions—remains unclear at cellular ultrastructural level. In patients, genetic mutations in *Shaker*-like K^+ channels Kv1.1 (*KCNA1*) and Kv1.2 (*KCNA2*) cause neurological disorders that extend beyond motor symptoms (48) to include cognitive impairment and intellectual disability (5). Yet, the cellular and ultrastructural mechanisms underlying these cognitive deficits remain unclear. Mutations in the *Shaker* (Kv1.1) K^+ channel in *Drosophila* lead to cognitive deficits, such as impairments in learning and memory (49). Here we show for the first time that a mutation in *Shaker* (Kv1.1) gene results in T-bar with reduced density and smaller size in the brain of flies. In *Dm'*s brain, T-bars (transmission bars) are specialized

presynaptic structures found at synaptic terminals where they play a crucial role in synaptic vesicle docking and neurotransmitter release ensuring the rapid and precise transmission of neural signals necessary for sensory processing and circuit function. The T-bars' abnormalities uncovered here provides an intriguing mechanistic rationale for the potential brain defect that may occur in the brains of patients with Kv1.1- or Kv1.2-related channelopathies, and contribute to their neurological and cognitive disabilities. The discovery that IBU, acting as a K^+ channel facilitator, can re-establish normal brain wiring and improve the motor performance in flies offers hope for effective therapeutic interventions in patients with movement disorders and cognitive deficits including EA1. *Niflumic acid*, a repurposable anti-inflammatory medication, was recently shown to activate Kv1.1 and Kv1.2 channels, thereby ameliorating EA1 phenotype (36). Plant-derived compounds traditionally used by Native Americans for ataxia also effectively modulate Kv1.1 channels (32). The findings presented here prompts investigations into the brain structure of mammalian models of these diseases to assess whether mutations in Kv1.1 and Kv1.2 channels lead to altered brain wiring, and to evaluate the efficacy of Kv1 channel activators, identified to date, in restoring normal connectivity to enhance their potential for translation into personalized medicine.

Patients and animal models with loss of Kv1.1 or Kv1.2 potassium channel function show disrupted sleep–wake regulation, including shorter sleep duration, reduced NREM/REM sleep, increased wakefulness, abnormal activity rhythms and early death. Sleep patterns change substantially during aging, with older adults experiencing lighter, more fragmented sleep, a decrease in deep sleep (*slow-wave*), increased night-time awakenings, and altered circadian rhythms including earlier sleep and wake times (50). In humans, insufficient sleep is linked to serious health issues, smaller brain volume (51) and reduced lifespan, though the underlying ultrastructural changes remain unclear. The reduction in T-bar density and aberrant T-bar size observed in the brains of *mini-sleep* flies—restored upon IBU administration—offers a compelling mechanistic explanation for both the neurodegenerative effects of sleep deprivation and the beneficial impact of IBU on sleep and lifespan. In *Dm*, two antagonistic K^+ conductance adjust the electrical output in sleep-inducing *fan-shaped body neurons* according to the sleep needs of flies. The two-pore domain K^+ channel (K2P) termed *Sandman* imposes silence during waking, whereas

enhanced *A-type* K^+ currents through *Shaker* channels support *tonic firing* during sleep. Notably, dopamine acts as a molecular awakening switch by down-regulating voltage-gated *A-type* currents mediated by *Shaker* and *Shab* channels, while up-regulating voltage-independent "*leak*" currents through the K2P *Sandman* (34). This notion raises the hypothesis that repurposable Kv1 channel activators (*e.g.* niflumic acid) (36) and the facilitator IBU restore sleep duration in *Dm* mutants by enhancing *A-type* currents mediated by *Shaker* channels.

IBU may improve neuronal connectivity, synaptic transmission, motor performance, sleep and longevity also via metabolic pathways or by modulating the activity of other channel types. Indeed, since the *Drosophila Shaker* locus generates multiple alternatively spliced transcripts encoding functionally distinct Kv channel isoforms, the point mutation harbored by *Sh* flies is unlikely to abolish all *Shaker*-mediated conductance. Residual expression of unaffected splice variants—and partial compensation by other Kv family members—likely preserves some channel function that can be modulated by IBU (52). The finding that a commonly used anti-inflammatory drug acts as a facilitator of *Shaker*-like K^+ channels merits consideration and prompts investigation into further IBU's mechanisms of action, which could pave the way for identifying more potent small-molecule modulators with signaling pathway selectivity.

Concerning the drug's adverse effects, the longest documented chronic IBU treatments in humans extend up to 5 years (53). The main side effects reported with long-term usage at moderate (1,200 mg daily) to high doses (2,400 mg daily) include increased risk for gastrointestinal issues (ulcers, bleeding), kidney damage, and at the highest doses, increased risk for cardiovascular events. However, in a nationwide phase II randomized controlled trial (*NCT03186638*), low-dose IBU (200 mg twice daily for 6 weeks) was well tolerated with minimal adverse events and no gastrointestinal bleeding in cancer patients experiencing chemotherapy-related cognitive impairment. Iterestingly, IBU treatment significantly improved processing speed and showed a trend toward enhanced visual memory, supporting its potential as a safe anti-inflammatory approach to alleviate cognitive dysfunction during or after chemotherapy (54).

Our study relies on the *Dm* model, which, although invaluable for genetic manipulation and large-scale drug screening, cannot fully recapitulate the structural complexity of the human brain or the intricate systemic interactions characteristic of higher organisms. Consequently, while findings in *Drosophila* provide critical mechanistic insights and a robust foundation for translational research, they may not directly reflect human physiology. This limitation should therefore be acknowledged when interpreting and generalizing the results to potential human applications. Therefore, although extrapolation to humans requires caution, the ability of IBU to extend lifespan across multiple species, combined with its beneficial effects exerted at low-dose on motor performance and sleep in our model, positions it as a compelling candidate for interventions aimed at promoting healthy aging. The *World Health Organization* estimated that by 2050, the world's population of individuals aged 60 years and older will exceed 2 billion. Interventions targeting aging to enhance the quality of the aging process, offer potentially larger economic gains than eradicating individual diseases (55). A central frontier of current research in this field is the development of anti-aging pharmacological agents that can delay or mitigate the physiological decline associated with aging and reduce age-related diseases to extend health span. Insufficient sleep is a common hallmark of aging, and numerous clinical as well as experimental studies have linked it to a wide range of serious health conditions (56,57).

Conclusions

This study highlights the therapeutic potential of IBU in improving key symptoms associated with Kv1.1- and Kv1.2-related channelopathies. Furthermore, our findings establish a mechanistic framework that bridges fundamental neurobiological insights with translational potential, paving the way for targeted interventions to mitigate the deleterious effects of pathological aging—including motor decline, cognitive deterioration, and sleep dysregulation—and ultimately advancing therapeutic strategies to restore neural and systemic homeostasis in aging populations. Given its excellent safety profile at low dose and cost-effectiveness, IBU appears to be a strong candidate for clinical trials to explore potential health benefits in individuals with genetic mutations that disrupt sleep and brain connectomics (*e.g.* EA1), and it may also serve as a valuable model for future drug discovery.

Acknowledgments

We are grateful to Dr. Ahmed Mustafa Ali Sultan and Dr. Balaji Venkataraman for their contributions to this work. We acknowledge the CeSAR (*Centro Servizi d'Ateneo per la Ricerca*) of the University of Cagliari, Italy, and Dr. Andrea Ardu for performing the electron microscopy analysis using the JEOL JEM-1400 Plus electron microscope.

Authors' contributions

M.C.D. and M.P. designed research; M.D.S, M.A.C., I.M., G.T., F.S., C.M., G.C., G.B.M., F.M.H, M.K., P.M., B.B., R.F., L.C., M.Pu., S.B.S., P.I., M.C.D. performed research and analyzed data; M.P. wrote the paper.

Funding

This study was supported by SENESCENCE2030.

Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Hasan SM, D'Adamo MC. Episodic Ataxia Type 1 [Internet]. GeneReviews®. 1993. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8845167

2. D'Adamo MC, Catacuzzeno L, Di Giovanni G, Franciolini F, Pessia M. K(+) channelepsy: progress in the neurobiology of potassium channels and epilepsy. Front Cell Neurosci [Internet]. 2013 Sep 13;7:134. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24062639

3. Imbrici P, Altamura C, Gualandi F, Mangiatordi GF, Neri M, De Maria G, et al. A novel KCNA1 mutation in a patient with paroxysmal ataxia, myokymia, painful contractures and metabolic dysfunctions. Mol Cell Neurosci [Internet]. 2017 Sep;83:6–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1044743116302755

4. D'adamo MC, Liantonio A, Rolland JF, Pessia M, Imbrici P. Kv1.1 channelopathies: Pathophysiological mechanisms and therapeutic approaches. Int J Mol Sci. 2020;21(8):1–21.

5. D'Adamo MC, Liantonio A, Conte E, Pessia M, Imbrici P. Ion Channels Involvement in Neurodevelopmental Disorders. Neuroscience [Internet]. 2020 Aug;440:337–59. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0306452220303298

6. Meiri N, Ghelardini C, Tesco G, Galeotti N, Dahl D, Tomsic D, et al. Reversible antisense inhibition of Shaker-like Kv1.1 potassium channel expression impairs associative memory in mouse and rat. Proc Natl Acad Sci [Internet]. 1997 Apr 29;94(9):4430–4. Available from: https://pnas.org/doi/full/10.1073/pnas.94.9.4430

7. Bushey D, Huber R, Tononi G, Cirelli C. Drosophila Hyperkinetic Mutants Have Reduced Sleep and Impaired Memory. J Neurosci [Internet]. 2007 May 16;27(20):5384–93. Available from: https://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.0108-07.2007

8. Schwarz TL, Tempel BL, Papazian DM, Jan YN, Jan LY. Multiple potassium-channel components are produced by alternative splicing at the shaker locus in Drosophila. Nature [Internet]. 1988 Apr;332(6166):740–740. Available from: https://www.nature.com/articles/332740a0

9. Cirelli C, Bushey D, Hill S, Huber R, Kreber R, Ganetzky B, et al. Reduced sleep in Drosophila Shaker mutants. Nature. 2005;434(7037):1087–92.

10. Buhmann J, Sheridan A, Malin-Mayor C, Schlegel P, Gerhard S, Kazimiers T, et al. Automatic detection of synaptic partners in a whole-brain Drosophila electron microscopy data set. Nat Methods [Internet]. 2021;18(7):771–4. Available from: http://dx.doi.org/10.1038/s41592-021-01183-7

11. Zheng Z, Lauritzen JS, Perlman E, Robinson CG, Nichols M, Milkie D, et al. A Complete Electron Microscopy Volume of the Brain of Adult Drosophila melanogaster. Cell [Internet]. 2018 Jul;174(3):730-743.e22. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867418307876

12. Schlegel P, Yin Y, Bates AS, Dorkenwald S, Eichler K, Brooks P, et al. Whole-brain annotation and multi-connectome cell typing of Drosophila. Nature [Internet]. 2024 Oct 3;634(8032):139–52. Available from: https://www.nature.com/articles/s41586-024-07686-5

13. Lin A, Yang R, Dorkenwald S, Matsliah A, Sterling AR, Schlegel P, et al. Network statistics of the whole-brain connectome of Drosophila. Nature [Internet]. 2024 Oct 3;634(8032):153–65. Available from: https://www.nature.com/articles/s41586-024-07968-y

14. Stenholm S, Head J, Kivimäki M, Magnusson Hanson LL, Pentti J, Rod NH, et al. Sleep Duration and Sleep Disturbances as Predictors of Healthy and Chronic Disease–Free Life Expectancy Between Ages 50 and 75: A Pooled Analysis of Three Cohorts. Journals Gerontol Ser A [Internet]. 2019 Jan 16;74(2):204–10. Available from: https://academic.oup.com/biomedgerontology/article/74/2/204/4837199

15. Bentivoglio M, Grassi-Zucconi G. The Pioneering Experimental Studies on Sleep Deprivation. Sleep [Internet]. 1997 Jul;20(7):570–6. Available from: https://academic.oup.com/sleep/article-lookup/doi/10.1093/sleep/20.7.570

16. Rechtschaffen A, Gilliland MA, Bergmann BM, Winter JB. Physiological Correlates of Prolonged Sleep Deprivation in Rats. Science (80-) [Internet]. 1983 Jul 8;221(4606):182–4. Available from: https://www.science.org/doi/10.1126/science.6857280

17. Shaw PJ, Tononi G, Greenspan RJ, Robinson DF. Stress response genes protect against lethal effects of sleep deprivation in Drosophila. Nature [Internet]. 2002 May;417(6886):287–91. Available from: https://www.nature.com/articles/417287a

18. Stephenson R, Chu KM, Lee J. Prolonged deprivation of sleep-like rest raises metabolic rate in the Pacific beetle cockroach, Diploptera punctata (Eschscholtz). J Exp Biol [Internet]. 2007 Jul 15;210(14):2540–7. Available from: https://journals.biologists.com/jeb/article/210/14/2540/16929/Prolonged-deprivation-of-sleep-like-rest-raises

19. Donlea JM. Roles for sleep in memory: insights from the fly. Curr Opin Neurobiol [Internet]. 2019 Feb;54:120–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0959438818301302

20. Killgore WDS. Effects of sleep deprivation on cognition. In: Progress in Brain Research [Internet]. 2010. p. 105–29. Available from:

https://linkinghub.elsevier.com/retrieve/pii/B9780444537027000075

21. Krause AJ, Simon E Ben, Mander BA, Greer SM, Saletin JM, Goldstein-Piekarski AN, et al. The sleep-deprived human brain. Nat Rev Neurosci [Internet]. 2017;18(7):404–18. Available from: http://dx.doi.org/10.1038/nrn.2017.55

22. Abel T, Havekes R, Saletin JM, Walker MP. Sleep, Plasticity and Memory from Molecules to Whole-Brain Networks. Curr Biol [Internet]. 2013 Sep;23(17):R774–88. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960982213008488

23. Frank MG. Sleep and developmental plasticity. In: Progress in Brain Research [Internet]. 2011. p. 221–32. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780444538390000144

24. Kayser MS, Yue Z, Sehgal A. A Critical Period of Sleep for Development of Courtship Circuitry and Behavior in Drosophila. Science (80-) [Internet]. 2014 Apr 18;344(6181):269–74. Available from: https://www.science.org/doi/10.1126/science.1250553

25. Li Q, Kellner DA, Hatch HAM, Yumita T, Sanchez S, Machold RP, et al. Conserved properties of Drosophila Insomniac link sleep regulation and synaptic function. Sehgal A, editor. PLOS Genet [Internet]. 2017 May 30;13(5):e1006815. Available from: https://dx.plos.org/10.1371/journal.pgen.1006815

26. Tononi G, Cirelli C. Sleep and the Price of Plasticity: From Synaptic and Cellular Homeostasis to Memory Consolidation and Integration. Neuron [Internet]. 2014 Jan;81(1):12–34. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0896627313011860

27. D'Adamo MC, Gallenmüller C, Servettini I, Hartl E, Tucker SJ, Arning L, et al. Novel phenotype associated with a mutation in the KCNA1(Kv1.1) gene. Front Physiol. 2015;6(JAN):1–10.

28. Fenoglio-Simeone KA, Wilke JC, Milligan HL, Allen CN, Rho JM, Maganti RK. Ketogenic diet treatment abolishes seizure periodicity and improves diurnal rhythmicity in epileptic Kcna1 -null mice. Epilepsia [Internet]. 2009 Sep 21;50(9):2027–34. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1528-1167.2009.02163.x

29. Dhaibar H, Gautier NM, Chernyshev OY, Dominic P, Glasscock E. Cardiorespiratory profiling reveals primary breathing dysfunction in Kcna1-null mice: Implications for sudden unexpected death in epilepsy. Neurobiol Dis [Internet]. 2019 Jul;127:502–11. Available from: https://linkinghub.elsevier.com/retrieve/pii/S096999611930097X

30. Douglas CL, Vyazovskiy V, Southard T, Chiu S-Y, Messing A, Tononi G, et al. Sleep in Kcna2 knockout mice. BMC Biol [Internet]. 2007 Dec 9;5(1):42. Available from: https://bmcbiol.biomedcentral.com/articles/10.1186/1741-7007-5-42

31. He C, Tsuchiyama SK, Nguyen QT, Plyusnina EN, Terrill SR, Sahibzada S, et al. Enhanced Longevity by Ibuprofen, Conserved in Multiple Species, Occurs in Yeast through Inhibition of Tryptophan Import. Kim SK, editor. PLoS Genet [Internet]. 2014 Dec 18;10(12):e1004860. Available from: https://dx.plos.org/10.1371/journal.pgen.1004860

32. Manville RW, Alfredo Freites J, Sidlow R, Tobias DJ, Abbott GW. Native American ataxia medicines rescue ataxia-linked mutant potassium channel activity via binding to the voltage sensing domain. Nat Commun [Internet]. 2023 Jun 6;14(1):3281. Available from: https://www.nature.com/articles/s41467-023-38834-6

33. Cowan TM, Siegel RW. Drosophila Mutations that Alter Ionic Conduction Disrupt Acquisition and Retention of a Conditioned Odor Avoidance Response. J Neurogenet [Internet]. 1986 Jan 11;3(4):187–201. Available from: https://www.tandfonline.com/doi/full/10.3109/01677068609106849

34. Pimentel D, Donlea JM, Talbot CB, Song SM, Thurston AJF, Miesenböck G. Operation of a homeostatic sleep switch. Nature [Internet]. 2016 Aug 18;536(7616):333–7. Available from: https://www.nature.com/articles/nature19055

35. Dorkenwald S, Matsliah A, Sterling AR, Schlegel P, Yu S, McKellar CE, et al. Neuronal wiring diagram of an adult brain. Nature [Internet]. 2024 Oct 3;634(8032):124–38. Available from: https://www.nature.com/articles/s41586-024-07558-y

36. Servettini I, Talani G, Megaro A, Setzu MD, Biggio F, Briffa M, et al. An activator of voltage-gated K + channels Kv1.1 as a therapeutic candidate for episodic ataxia type 1. Proc Natl Acad Sci [Internet]. 2023 Aug 24;120(31). Available from:

https://pnas.org/doi/10.1073/pnas.2207978120

37. Rogers JT, Liu C-C, Zhao N, Wang J, Putzke T, Yang L, et al. Subacute ibuprofen treatment rescues the synaptic and cognitive deficits in advanced-aged mice. Neurobiol Aging [Internet]. 2017 May;53:112–21. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0197458017300350

38. in 't Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, Stijnen T, et al. Nonsteroidal Antiinflammatory Drugs and the Risk of Alzheimer's Disease. N Engl J Med [Internet]. 2001 Nov 22;345(21):1515–21. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa010178

39. Mocci I, Casu MA, Sogos V, Liscia A, Angius R, Cadeddu F, et al. Effects of memantine on mania-like phenotypes exhibited by Drosophila Shaker mutants. CNS Neurosci Ther [Internet]. 2023 Jul 21;29(7):1750–61. Available from: https://onlinelibrary.wiley.com/doi/10.1111/cns.14145

40. Pessia M. Ion channels and electrical activity. In: Molecular Biology of the Neuron [Internet]. Oxford University Press; 2004. p. 103–38. Available from: https://academic.oup.com/book/8029/chapter/153407178

41. Leung Y, Huang C, Chao C, Lu D, Kuo C, Cheng T, et al. Voltage-gated K + channels play a role in cAMP-stimulated neuritogenesis in mouse neuroblastoma N2A cells. J Cell Physiol [Internet]. 2011 Apr 25;226(4):1090–8. Available from: https://onlinelibrary.wiley.com/doi/10.1002/jcp.22430

42. Lichtinghagen R, Stocker M, Wittka R, Boheim G, Stühmer W, Ferrus A, et al. Molecular basis of altered excitability in Shaker mutants of Drosophila melanogaster. EMBO J [Internet]. 1990 Dec;9(13):4399–407. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1702382

43. Rogina B, Helfand SL. Timing of Expression of a Gene in the Adult Drosophila Is Regulated by Mechanisms Independent of Temperature and Metabolic Rate. Genetics [Internet]. 1996 Aug 1;143(4):1643–51. Available from: https://academic.oup.com/genetics/article/143/4/1643/6016753

44. Ganetzky B, Wu C-F. INDIRECT SUPPRESSION INVOLVING BEHAVIORAL MUTANTS

WITH ALTERED NERVE EXCITABILITY IN DROSOPHILA MELANOGASTER. Genetics [Internet]. 1982 Apr 1;100(4):597–614. Available from: https://academic.oup.com/genetics/article/100/4/597/5995228

45. Gautam M, Tanouye MA. Alteration of potassium channel gating: Molecular analysis of the drosophila Sh5 mutation. Neuron [Internet]. 1990 Jul;5(1):67–73. Available from: https://linkinghub.elsevier.com/retrieve/pii/089662739090034D

46. Rogina B, Helfand SL. Spatial and temporal pattern of expression of the wingless and engrailed genes in the adult antenna is regulated by age-dependent mechanisms. Mech Dev [Internet]. 1997 Apr;63(1):89–97. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0925477397000336

47. Tanouye MA, Ferrus A, Fujita SC. Abnormal action potentials associated with the Shaker complex locus of Drosophila. Proc Natl Acad Sci [Internet]. 1981 Oct;78(10):6548–52. Available from: https://pnas.org/doi/full/10.1073/pnas.78.10.6548

48. Brunetti O, Imbrici P, Fm B, Ve P, Mc DA, Valentino M, et al. Dysfunction of voltage-gated K + Channels Kv1 . 1 in sciatic nerve causes spontaneous and stress-induced neuromuscular hyperexcitability. 2021;(256):1–40.

49. Cowan TM, Siegel RW. Drosophila mutations that alter ionic conduction disrupt acquisition and retention of a conditioned odor avoidance response. J Neurogenet [Internet]. 1986 Jul;3(4):187–201. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2427679

50. Li J, Vitiello M V., Gooneratne NS. Sleep in Normal Aging. Sleep Med Clin [Internet]. 2018 Mar;13(1):1–11. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1556407X17301029

51. Sun J, Zhao R, Yang X, Deng H, Zhu Y, Chen Y, et al. Alteration of Brain Gray Matter Density After 24 h of Sleep Deprivation in Healthy Adults. Front Neurosci [Internet]. 2020 Aug 11;14. Available from: https://www.frontiersin.org/article/10.3389/fnins.2020.00754/full

52. Kamb A, Tseng-Crank J., Tanouye MA, Multiple Products of the Drosophila Shaker

Gene May Contribute to Potassium Channel Diversity. Neuron 1988;1:421–30.

53. Salis Z, Sainsbury A. Association of long - term use of non - steroidal anti - inflammatory drugs with knee osteoarthritis : a prospective multi - cohort study over 4 - to - 5 years. Sci Rep [Internet]. 2024;1–12. Available from: https://doi.org/10.1038/s41598-024-56665-3

54. Janelsins M. C., Gada U., Culakova E, Tejani M. A., Ahles T., Bautista J., O'Rourke M. A., Berenberg J. L., Le-Lindqwister N., McCormack S. E., Rasheed H., Morrow G. R., Mustian K. M., Peppone L. J., Mohile S. G., Dunne, R. F. (2023). Nationwide phase II randomized controlled trial of low-dose ibuprofen vs placebo for cancer-related cognitive impairment (CRCI). Journal of Clinical Oncology, 41(15_suppl), 12116. https://doi.org/10.1200/JCO.2023.41.15_suppl.12116

55. Scott AJ, Ellison M, Sinclair DA. The economic value of targeting aging. Nat Aging [Internet]. 2021;1(7):616–23. Available from: http://dx.doi.org/10.1038/s43587-021-00080-0

56. Chattu VK, Manzar MD, Kumary S, Burman D, Spence DW, Pandi-Perumal SR. The Global Problem of Insufficient Sleep and Its Serious Public Health Implications. Healthcare [Internet]. 2018 Dec 20;7(1):1. Available from: https://www.mdpi.com/2227-9032/7/1/1

57. Medic G, Wille M, Hemels M. Short- and long-term health consequences of sleep disruption. Nat Sci Sleep [Internet]. 2017 May;Volume 9:151–61. Available from: https://www.dovepress.com/short--and-long-term-health-consequences-of-sleep-disruption-peer-reviewed-article-NSS