Received: 1 November 2022 | Revised: 1 January 2023 | Accepted: 10 January 2023

DOI: 10.1002/arch.22001

SHORT COMMUNICATION

Archives of INSECT BIOCHEMISTRY AND PHYSIOLOGY WILEY

Pan-neuronal knockdown of Ras GTPase-activating protein 1 alters *Drosophila* activity and sleep behavior

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Abstract

Ras signaling pathways are involved in numerous cellular functions and, for this reason, are highly regulated. In addition to alterations in the Ras proteins themselves, defects in Ras regulatory proteins, such as GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), may be relevant to disease development. *Drosophila* RasGAP1 is a protein with important physiological implications in flies due to its participation in the signaling of different pathways. In this work, the changes that occur in *Drosophila* behavior by reducing the pan-neuronal expression of RasGAP1 were investigated. Thus, RasGAP1 knockdown was found to cause a significant increase in total activity ($p \leq 0.001$) and activity at 30 min ($p \leq 0.001$). In contrast, total sleep duration ($p \leq 0.001$), sleep within 30 min ($p \leq 0.001$), and mean duration of sleep episodes ($p \leq 0.0001$) were all reduced. Furthermore, circulating levels of glucose ($p \leq 0.05$) and triacylglycerol ($p \leq 0.05$) were found to be elevated. No significant changes were found in feeding behavior, food source selection, trehalose, or glycogen levels. All these results show new functions of RasGAP1 in *Drosophila* physiology and may also serve to explain some functions of human orthologs (RasGAP2/3 [RASA2/3]).

KEYWORDS

circulating glucose, dopamine, feeding behavior, fly movement, RasGAP1

Research Highlights

- Pan-neuronal knockdown of RasGAP1 alters activity and sleep behavior of flies.
- Pan-neuronal knockdown of RasGAP1 does not modify the feeding behavior of flies.
- Pan-neuronal knockdown of RasGAP1 increases circulating glucose and triacylglyceride levels in flies.

1 | INTRODUCTION

Members of the Ras superfamily of small monomeric guanosine triphosphate (GTP)-binding proteins play roles in diverse biological processes, ranging from transmembrane signal transduction to various processes involving cytoskeletal rearrangements, vesicular trafficking, and nucleocytoplasmic transport, to mention only the most prominent (Mitin et al., 2005). These proteins switch between an active form bound to GTP and an inactive form bound to guanosine diphosphate (GDP), and whose cycles are regulated by guanine nucleotide exchange factors (GEFs), which promote the exchange of GDP for GTP, and by GTPase activating proteins (GAPs), which enhance its low intrinsic GTPase activity (Pamonsinlapatham et al., 2009). Most G proteins have a cognate GAP protein as well as a cognate GEF protein. Ras-specific GTPase-activating proteins (RasGAPs) act as negative regulators for different activated Ras proteins, and from a systematic point of view, six groups of homologous mammalian RasGAP sequences are commonly distinguished: p120-RasGAP (RasGAP1), neurofibromin (NF1), GAP1 subfamily members (GAP1M [RasGAP2], GAP1IP4BP [RasGAP3], CAPRI [RasGAP4], and RASAL1), SynGAP family members, Plexins and IQGAP family members (Scheffzek & Shivalingaiah, 2019).

Given the importance of Ras signaling pathways in numerous cellular functions, in addition to alterations in Ras proteins themselves, defects in Ras regulatory protein components may also be relevant to disease development (Bernards, 2003). In the case of RasGAPs, the most prominent example in this context is neurofibromatosis type 1, which is caused by deleterious mutations in the NF1 gene and is characterized by high Ras signaling and overactivation of phosphoinositide 3-kinase (PI3K)/AKT, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK), and mammalian target of rapamycin (mTOR) pathways that lead to the development of benign tumors (Amaravathi et al., 2021; Hennig et al., 2016). Similarly, genetic alterations in the RASA1 gene (encoding RasGAP1) have been reported to be responsible for capillary malformation-arteriovenous malformation (CM-AVM) syndrome, a genetic disorder characterized by defects in vascular development (Orme et al., 2013). Germline SynGAP1 loss-of-function mutations are causally associated with intellectual disability, severe epilepsy, autism spectrum disorder, and schizophrenia (Kilinc et al., 2018). Notably, the T/T genotype of the single-nucleotide polymorphism (SNP) rs16851483 in RASA2, the gene that encodes RasGAP2, was associated with increased body mass index (BMI) (Ahmad et al., 2015; Locke et al., 2015) and breast cancer risk (Zhu et al., 2017), although no clear mechanism to explain these associations has been provided so far.

It was previously reported that the long-term survival of adult neurons in *Drosophila* crucially depends on the activities of two GTPases, Ras and Rab5, regulated by the interaction of Vap-encoded RasGAP and Sprint (Rowshanravan et al., 2014). In this sense, deficiency of Vap-encoded RasGAP causes age-related brain degeneration due to autophagic cell death of neurons (Botella et al., 2003). Similarly, the *Drosophila* NF1 protein affects learning and short-term memory by regulating the cAMP pathway involving rutabaga (rut)-encoded adenylyl cyclase (Guo et al., 2000). PlexA RasGAP was also shown to be important in the control of *Drosophila* intersegmental motor nerve axon guidance during neural development (Yang et al., 2016).

Drosophila RasGAP1 was identified as a negative regulator of Sevenless pathway by downregulating Ras1 activity and consequently preventing the differentiation of precursor cone cells into R7 photoreceptor cells (Gaul et al., 1992). Ectopic expression of RasGAP1 in the wing imaginal disc reduced the size of the adult wing to nearly half normal size and suppressed the formation of ectopic wing veins due to the inactivation of Breathless and Heartless signaling (Feldmann et al., 1999; Woodcock & Hughes, 2004). Likewise, the participation of RasGAP1 has been described in other pathways such as Torso signaling during fly embryogenesis (Cleghon et al., 1998), in EGFR signaling where it altered the wing shape (Dworkin & Gibson, 2006), in MAPK/ERK signaling triggered by insulin (Zhang et al., 2011) and in poliovirus receptor (Pvr) signaling that controls cell size (Sims et al., 2009). Other studies have shown that this protein can stimulate the intrinsic GTPase activity of mammalian H-Ras (Bernards, 2003; Feldmann et al., 1999). Remarkably, a search in the FlyAtlas2 (Krause et al., 2022) showed that *Drosophila* RasGAP1 is mainly expressed in the brain/central nervous system of adult males (Supporting Information: Table 1) and thus may be involved in fly behavior. In this way, the purpose of the present work was to evaluate the effects of RasGAP1 pan-neuronal knockdown in *Drosophila* at the level of their activity, sleeping, and feeding behavior. Furthermore, it was analyzed whether these changes had an effect on carbohydrate metabolism, triacylglyceride levels, lifespan and/or starvation resistance of the flies.

2 | MATERIALS AND METHODS

2.1 | Fly stocks and maintenance

All fly strains were maintained with Jazz-Mix Drosophila food (AS153; Thermo-Fisher Scientific) supplemented with 1.5% yeast extract (20-254; Apex Bioresearch Products), 0.3% propionic acid (402907; Sigma-Aldrich) and 0.05% tegosept (20-258; Apex Bioresearch Product). The flies were maintained at 25°C and 60% humidity in a 12:12 h light:dark (L:D) cycle. The flies were obtained from the Bloomington Stock Center, and the strains corresponded to one that expresses an RNA interference (RNAi) directed against RasGAP1 (FBgn0004390) under UAS control: *y[1] v[1]; P{y[+t7.7] v[+t1.8]=TRiP.GL01258}attP2* (designated as RasGAP1^{RNAi}), as well as a strain that expresses the GAL4 protein under the control of the *elav* promoter: *P{w[+mW.hs]=GawB}elav[C155]* (designated as elav-GAL4). Unless otherwise indicated, all flies used were adult males 5–7 days after hatching. The flies with the GAL4 drivers and the UAS transgenic flies were crossed with each other and with w1118 flies for the controls, and their F1 progeny were reared at 29°C for the appropriate time to carry out the experiments.

2.2 | RNA purification, cDNA synthesis, and qRT-PCR

Total RNA was extracted from fly heads (Tito et al., 2016), midgut (Tauc, Tasdogan & Pandur, 2014), Malpighian tubules (Rossano & Romero, 2017), heart (Vogler & Oncorr, 2009), testis (Zamore & Ma, 2011) and fat body (Gupta & Lazzaro, 2022) using TRIzol reagent (15596026; Invitrogen). Twenty-five flies per group were used for each tissue. RNA was transformed into cDNA using the High Capacity RNA-to-cDNA Kit (4387406; Applied Biosystems) according to the supplier's instructions. Quantitative polymerase chain reaction (qPCR) was performed with iTaq Universal SYBR Green Supermix (1725121; Bio-Rad) and using a CFX connect real-time PCR detection system (Bio-Rad Laboratories). The protocol used was a 30-s denaturation at 95°C followed by 40 cycles of 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C. The primers for each gene are shown in Supporting Information: Table 2. Relative gene expression was determined using the $2^{-\Delta\Delta C_t}$ method (Lagunas-Rangel et al., 2021; Livak & Schmittgen, 2001; Rao et al., 2013) with the gene RpL32 as normalizer. Each experiment considered at least three samples and each sample was performed in triplicate.

2.3 | Activity and sleep analysis

The drosophila activity monitoring system (DAMS) from TriKinetics was used to analyze changes in locomotion and sleep behavior. Each individual fly was placed in a 5 mm diameter tube and then monitored with the DAMS. The tubes were sealed with common fly food on one side and a cotton plug on the other side. All tubes were placed at 25°C on monitors that determine the activity of the flies by beam crossing numbers. Flies were placed in the DAMS on a 12:12 LD cycle for 5 days, starting at 8 a.m. Each group had a minimum of 25 flies per trial and was repeated at least three times.

2.4 | Fly proboscis and activity detector (FlyPAD) feeding assays

The feeding experiments were performed in a similar manner as Itskov et al. (2014). For feeding behavior experiments, starved male flies 5–9 days old were individually transferred to the flyPAD behavioral arena and allowed to feed 2 µl of 150 mM sucrose (S9378; Sigma-Aldrich) in 1% of agarose (A9539; Sigma-Aldrich) for 1 h. Starvation of the flies used lasted 18 h. For food preference experiments, starved flies were transferred to the flyPAD behavior arena and allowed to freely choose between 100 mM L-fucose (F2252; Sigma-Aldrich) and 100 mM D-mannose (M6020; Sigma-Aldrich) solutions that were dissolved in 1% agarose. Flies were allowed to eat ad libitum for 1 h. Throughout the duration of the experiments, the flies were kept at a humidity of 55%–60% and 25–27°C. Control and knockdown groups were tested simultaneously under the same conditions. Each group sustained a minimum of 15 flies per feeding trial and was repeated at least three times.

2.5 | Carbohydrate assay

Body glycogen and trehalose concentrations were assessed using the Liquick Cor GLUCOSE kit (2-218; Cormay). At least six biological replicates were prepared from both the control and experimental groups. For each replicate, 10 male flies were decapitated and homogenized in 100 µl of 1× PBS buffer. Samples were deproteinized at 70°C for 5 min, then incubated on ice for 5 min and centrifuged at 12,900*g* at 4°C for 15 min. The supernatant was collected and used as a sample. Subsequently, 10 µl of sample was mixed with 10 µl of trehalase from porcine kidney (T8778; Sigma-Aldrich) and incubated at 37°C overnight. Separately, 10 µl of sample was mixed with 10 µl of *Aspergillus niger* amyloglucosidase (10115; Sigma-Aldrich) and incubated at 25°C overnight. In both cases, after incubation, 10 µl of sample was diluted in 90 µl of deionized water and mixed with 650 µl of glucose reaction mix (2-218; Cormay) in a 96-well plate. The absorbance at 500 nm was measured with a Multiskan GO microplate spectrophotometer (Thermo Scientific). The concentration was calculated based on a standard curve that was made with a series of dilutions of a 10 µg/µl glucose solution.

2.6 | Triacylglycerol determination

Male flies were collected and homogenized with 100 µl cold PBST buffer (1× PBS [20012027; Gibco] with 10% Tween 20 [P1379; Sigma-Aldrich]). They were incubated at 70°C for 10 min and then centrifuged at 14,000 rpm for 5 min. The supernatant was collected and used as a sample. To 10 µl of the sample, 100 µl of free glycerol reagent (F6428; Sigma-Aldrich) was added and incubated at 37°C for 15 min. Absorbance at 540 nm was measured in a Multiskan GO microplate spectrophotometer (Thermo Scientific) and glycerol concentration was calculated according to a standard curve generated with 1.0, 0.8, 0.6, 0.4, and 0.2 mg/ml triolein equivalent concentration (G7793; Sigma-Aldrich). Subsequently, 20 µl of triglyceride reagent (T2449; Sigma-Aldrich) were added to each

standard and sample and incubated at 37°C for 15 min. The absorbance at 540 nm was measured again and the triacylglyceride concentration was calculated using the formula: Triacylglyceride concentration = Total glycerol concentration − Free glycerol concentration. To normalize the results, the protein concentration in each sample was quantified with the Bradford assay (23246; Thermo Scientific). Triacylglycerol concentrations in the samples were calculated from five samples (five flies per sample) for each group.

2.7 | Lifespan assay

The lifespan assay was performed similarly to Linford et al. (2013). At least 30 male flies (5–7 days old) from each group were collected in vials with normal food and maintained at 29°C on a 12:12 L:D cycle. Each day the vials were carefully inspected looking for dead flies and a record was made.

2.8 | Starvation assay

The starvation resistance assay was performed similarly to Hergarden et al. (2012). Starvation resistance was measured by placing 30 male flies (5–7 days old) in individual tubes containing 1% agarose in DAMS. The DAMS assay was performed at 25°C in an incubator, in a L:D cycle of 12:12 h.

2.9 | Statistical analysis

Data were analyzed with the Shapiro–Wilk test or the Kolmogorov–Smirnov test depending on the number and type of samples. Data that followed a normal distribution were analyzed with Student's *t* test or one-way analysis of variance (ANOVA) with a Tukey post hoc as appropriate. Data that did not follow a normal distribution were analyzed with a Kruskal–Wallis test with Dunn's post hoc. Survival curves were evaluated using the Mantel-Cox log-rank test. GraphPad Prism 9 (GraphPad Software) was used to perform the analysis.

3 | RESULTS AND DISCUSSION

3.1 | Pan-neuronal knockdown of RasGAP1 alters the activity and sleep behavior of flies

Since *Drosophila* RasGAP1 is mainly expressed in the brain/central nervous system of adult males (Supporting Information: Table 1), it was decided to investigate the effects of reducing its expression in this region. For this purpose, flies expressing specifically in neurons (using the *elav* pan-neuronal promoter) an RNAi targeting the *Drosophila* RasGAP1 transcript and controlled by the GAL4/UAS system were used ($RasGAP1^{RNAi}$ × elav-GAL4 flies), as well as their controls with a background strain w1118 ($RasGAP1^{RNAi}$ × w1118 and w1118 x elav-GAL4 flies). First, it was confirmed that the expression of *Drosophila* RasGAP1 was significantly reduced in the brain of flies by qRT-PCR, obtaining an expression decrease of approximately 59.5% compared to controls (Figure 1a). Other fly tissues showed no significant changes in RasGAP1 expression, demonstrating that the reduction occurred specifically in neurons (Supporting Information: Figure 1). Subsequently, the 5-day activity and sleep behaviors of $RasGAP1^{RNAi}$ × elav-GAL4 flies and their controls were analyzed and compared. Thus, relative to both controls, RasGAP1 knockdown was found to cause a significant increase in several activity parameters, such as total activity counts (Figure 1b), activity counts/30 min (Figure 1c), mean activity/minutes while moving (Figure 1d)

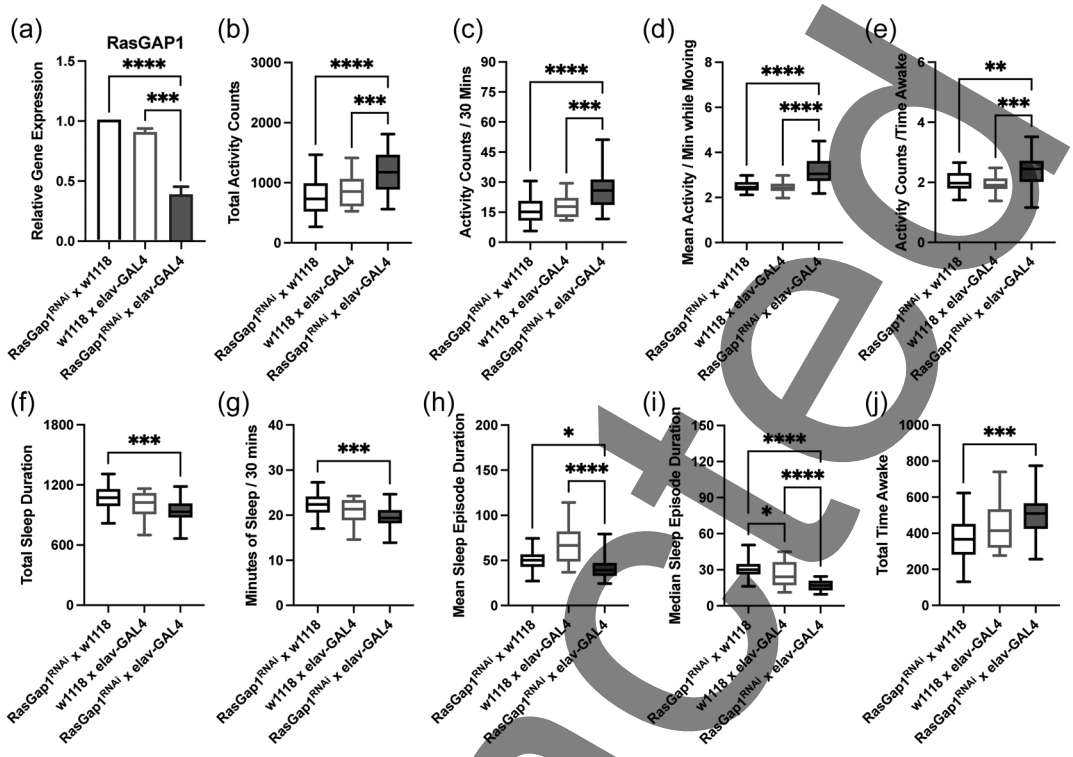

FIGURE 1 *Drosophila* RasGAP1 knockdown alters activity and sleep behavior. The graphs show the relative expression of RasGAP1 (a), total activity counts (b), activity counts/30 min (c), mean activity/minutes while moving (d), activity counts/time awake (e), total sleep duration (f), minutes of sleep/30 min (g), mean sleep episode duration (h), median sleep episode duration (i) and time awake (j) in flies with the RasGAP1 knockdown (gray boxes) and their controls (white boxes). For the statistical analysis of A, the Student's *t* test was used, in (b–g and i,j), one-way analysis of varience with Tukey's post hoc was used, and for (h) the Kruskal–Wallis test with Dunn's post hoc was used. Statistical significance is indicated as $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, and $^{****}p \leq 0.0001$.

and activity counts/time awake (Figure 1e). This might be related, at least partially, to overactivation of Ras with the consequent neuronal overexcitation (Ahmad et al., 2015; Locke et al., 2015; Machado Almeida et al., 2021). In contrast, decreases in some sleep parameters were found for RasGAP1^{RNAi} × elav-GAL4 flies compared to controls. For example, a trend toward decreased total sleep duration (Figure 1f) and minutes of sleep/30 min (Figure 1g) was found. Likewise, a significant reduction was found in mean sleep episode duration (Figure 1h) and median sleep episode duration (Figure 1i). A trend of increasing total awake time was also found (Figure 1j). These changes in sleep might be related to overactivation of RAS signaling in GABA-sensitive sleep-promoting neurons, and in a similar way to what happens with the loss of NF1 (Maurer et al., 2020). Another possible explanation that would account for both, increased activity and decreased sleep, is that RasGAP1 knockdown somehow affects neuronal dopamine signaling. Four G protein-coupled dopamine receptors have been identified in *Drosophila*: two D1-like receptors (D1Rs) (DopR1 and DopR2) that activate the cAMP pathway, a D2-like receptor (D2R) that inhibits the cAMP pathway and a noncanonical receptor (DopEcR) (Yamamoto & Seto, 2014). In this sense, one of the human orthologs of RasGAP1, RasGAP3 (RASA3), binds to Gαi/o inhibitory proteins activated by the dopamine D2S receptor and thereby inhibits thyrotropin-releasing hormone (TRH)-stimulated ERK1/2 activation (Nafisi et al., 2008). Notably, increased D2R receptor signaling in *Drosophila* neurons stimulates locomotor activity (Draper et al., 2007; Lee et al., 2013). Meanwhile, *Drosophila* knockdown of DopR1 and DopR2 in Kenyon cells and fungal body output neurons projecting the γ5 and β′2 compartments increased the duration and number of sleep

sessions (Driscoll et al., 2021). Similarly, DopR2 knockdown in sleep-promoting protocerebral bridging interneurons also increased sleep in flies (Tomita et al., 2021). With all this background, it can be suggested that RasGAP1 knockdown in *Drosophila* may alter dopaminergic signaling, activating both D1-like and D2Rs, and causing ERK1/2 to remain active for a longer time (Mikhail et al., 2017). As a result, the duration of sleep decreases and the duration of waking periods increases.

3.2 | Pan-neuronal knockdown of RasGAP1 does not modify the feeding behavior of flies

Since the dopamine reward system also influences foraging behavior (Baik, 2021), it was investigated whether RasGAP1 knockdown modifies feeding behavior in flies. In FlyPAD feeding assays, no significant differences in total number of sips (Figure 2a), sip duration (Figure 2b), activity bouts (Figure 2c), or feeding bouts (Figure 2d) were found between RasGAP1 knockdown flies and control flies. These results could be related to what has been observed in D2R knockdown mice, which are more vulnerable to obesity due to reduced physical activity rather than increased appetitive motivation (Beeler et al., 2016; Mourra et al., 2020). In this case, *Drosophila* D2R could remain active due to RasGAP1 knockdown and thus increase the physical activity of the flies, but without changing the amount of food ingested. Then, flies were given a choice between a nutritive and a nonnutritive sugar by placing equal molar amounts of palatable but nonnutritive L-fucose or nonpalatable but nutritious D-mannose in opposite positions on the FlyPAD arena and the number of sips taken for each sugar were measured. In this sense, no significant differences were found in the number of sips that *Drosophila* RasGAP1 knockdown gave to each sugar with respect to both controls, but there was a small tendency to reduce the number of sips that the knockdown flies gave to both sugars, mainly to D-mannose (Figure 2e,f). Activation of D1-like dopaminergic receptors in mice results in an increased preference for palatable food (Durst et al., 2019), whereas mouse models with inactivation of D2R receptors have not been shown to cause a change in food preference (Mourra et al., 2020). This may indicate that the effects of RasGAP1 knockdown in *Drosophila* are more closely linked to the activation of D2Rs than D1-like receptors.

3.3 | Pan-neuronal knockdown of RasGAP1 increases circulating glucose and triacylglyceride levels in flies

Since Ras overactivation could enhance insulin signaling, thus altering glucose, lipid, and protein metabolism (Banks et al., 2012), it was decided to analyze if these changes had any effect at the metabolic level. In this regard, the levels of some important carbohydrates and lipids of fly metabolism were assessed in RasGAP1 knockdown flies. A significant increase in circulating glucose was found in RasGAP1 knockdown flies compared to both controls (Figure 2g). It should be noted that this increase in circulating glucose could be associated with an increase in activity (Zametkin et al., 1990), although D2 receptor stimulation is also known to produce large increases in blood glucose in mammals (Hong et al., 2020; Saller & Kreamer, 1991) and could be related to our observations. Meanwhile, significant differences were found compared to either, but not both, controls in circulating (Figure 2h) or stored (Figure 2i) trehalose levels or glycogen content (Figure 2j). A significant increase in triacylglyceride content was also found in RasGAP1 knockdown flies with respect to both controls (Figure 2k). Interestingly, a previous study reported that deletion of *Drosophila* RasGAP1 in the brain and fat pad did not cause a change in body fat percentage distributions in adult flies (Baranski et al., 2018). Unlike this work, the Cg-GAL4 driver was used in this study, thus the differences between the two studies could be due to differences in promoter strength, persistence into adulthood, and different levels of silencing in neuronal subpopulations, among other factors. In addition, there

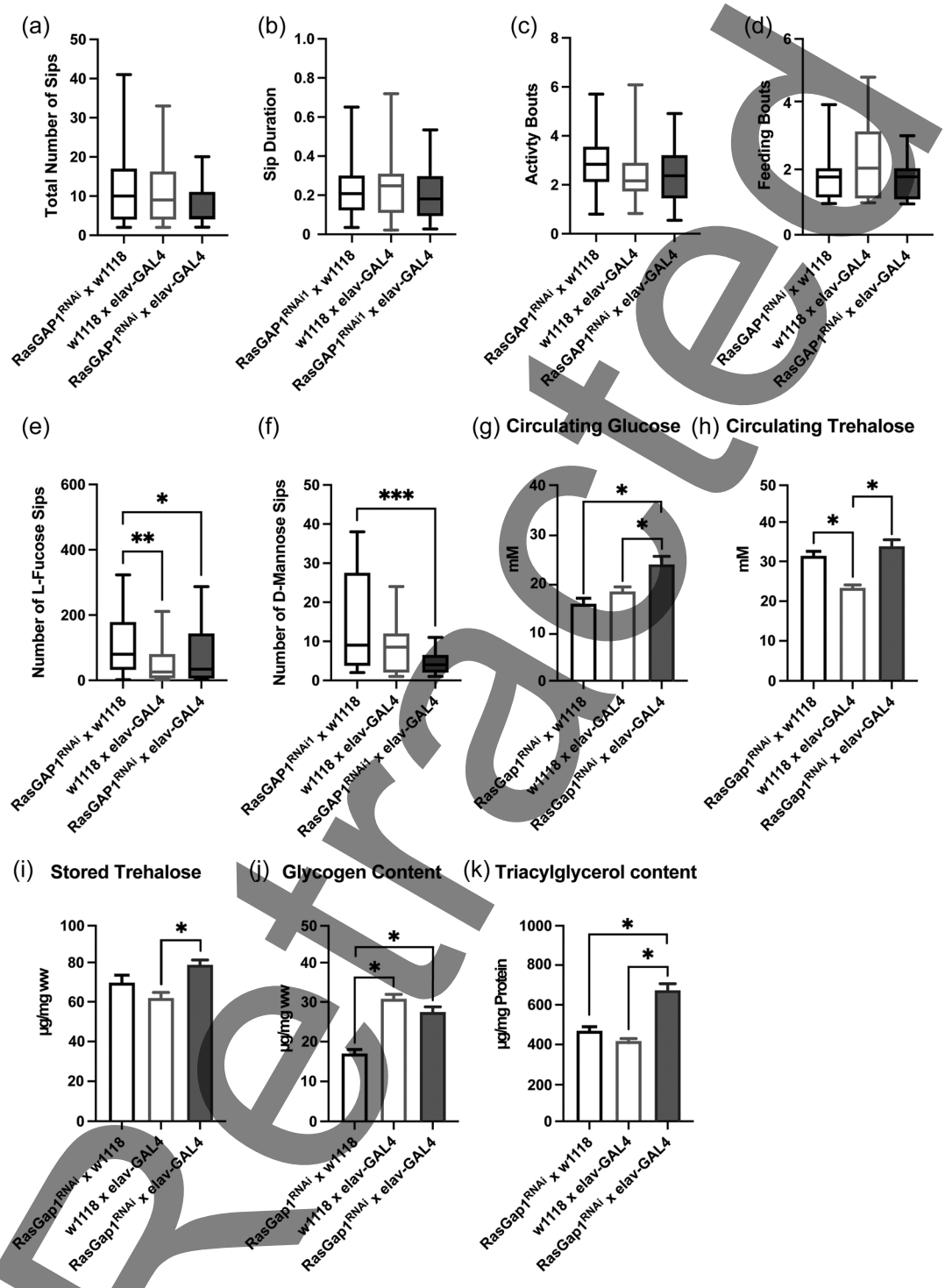

FIGURE 2 *Drosophila* RasGAP1 knockdown does not alter feeding behavior but causes circulating glucose and triacylglyceride levels to increase. The graphs show the total number of sips (a), sip duration (b), activity bouts (c), feeding bouts (d), number of L-fucose sips (e), number of D-mannose sips (f), circulating glucose (g), circulating trehalose (h), stored trehalose (i), glycogen content (j) and triacylglyceride content (k). For (a–f), the Kruskal–Wallis test with Dunns' post hoc was used, for (g–k), one-way analysis of variance with Tukey's post hoc was used. Statistical significance is indicated as $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, and $^{****}p \leq 0.0001$.

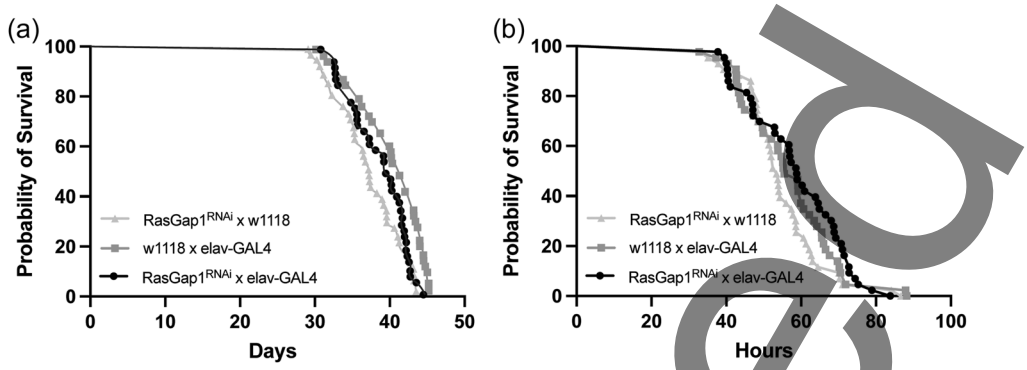

FIGURE 3 *Drosophila* RasGAP1 knockdown does not alter lifespan or starvation resistance. Lifespan (a) and starvation survival (b) curves in flies with the RasGAP1 knockdown (gray boxes and bars) and their controls (white boxes and bars). The Mantel-Cox log-rank test was used to analyze differences in survival between groups. No statistically significant differences were found.

are also other differences in the genetic background of flies. It has also been reported that, in humans, activation of the dopamine D2R receptor increases circulating levels of free fatty acids (Kok et al., 2006).

3.4 | Pan-neuronal knockdown of RasGAP1 does not alter lifespan or resistance to starvation in flies

In *Drosophila*, it has been shown that Ras inhibition is sufficient to extend lifespan and increase stress resistance (Slack et al., 2015). Therefore, there was interest in knowing if the neuronal knockdown of RasGAP1 also had an effect on this aspect. Lifespan assays showed no significant changes in the survival of RasGAP1 knockdown flies compared to their controls (Figure 3a). Regarding the possible involvement of dopaminergic signaling, in *Drosophila* it was identified that the D2R receptor mediates the increase in life expectancy when the progesterone antagonist mifepristone is administered (Lagunas-Rangel, 2022; Landis et al., 2015). However, one study reported that levels of this neurotransmitter in *Drosophila* do not influence survival in male flies (Vermeulen et al., 2006). Meanwhile, starvation resistance curves also showed no significant difference in the survival of RasGAP1 knockdown flies compared to both controls (Figure 3b). These observations could be because, as mentioned above, there is no increase in nutrient storage molecules, such as glycogen and trehalose, that allow RasGAP1-knockdown flies to more resist starvation.

4 | CONCLUSION

The experiments shown in this work demonstrate that RasGAP1 influences activity and sleep in *Drosophila*. Clearly, further research is needed to determine the molecular mechanisms involved, although in the first instance it is suspected that these effects are related to changes in dopamine signaling, in particular of the D2R receptor. In addition, increased circulating levels of glucose and triglycerides were observed due to RasGAP1 knockdown, which reinforces the idea of the involvement of dopaminergic signaling. No significant changes were found in feeding behavior, food source selection, trehalose, or glycogen levels. All these results show new functions of RasGAP1 in *Drosophila* physiology and may also serve to explain some functions of human orthologs (RasGAP2/3 [RASA2/3]) and their relationship with diseases such as obesity and neurological diseases.

AUTHOR CONTRIBUTIONS

Francisco Alejandro Lagunas-Rangel: conceptualization (lead), data curation (lead), formal analysis (lead), funding acquisition (lead), investigation (lead), methodology (lead), project administration (lead), resources (lead), supervision (lead), validation (lead), visualization (lead), writing – original draft (lead), writing – review and editing (lead).

CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Fly work do not require an ethical permit and the goal is to reduce animal work.

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How to cite this article: Lagunas-Rangel, F. A. (2023) Pan-neuronal knockdown of Ras GTPase-activating protein 1 alters *Drosophila* activity and sleep behavior. *Archives of Insect Biochemistry and Physiology*, e22001. https://doi.org/10.1002/arch.22001