



**HAL**  
open science

## **Pallidin function in *Drosophila* surface glia regulates sleep and is dependent on amino acid availability**

Hui Li, Sami Aboudhiaf, Sandrine Parrot, Céline Scote-Blachon, Claire Benetollo, Jian-Sheng Lin, Laurent Seugnet

► **To cite this version:**

Hui Li, Sami Aboudhiaf, Sandrine Parrot, Céline Scote-Blachon, Claire Benetollo, et al.. Pallidin function in *Drosophila* surface glia regulates sleep and is dependent on amino acid availability. *Cell Reports*, 2023, 42 (9), pp.113025. 10.1016/j.celrep.2023.113025 . hal-04783458

**HAL Id: hal-04783458**

**<https://hal.science/hal-04783458v1>**

Submitted on 14 Nov 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

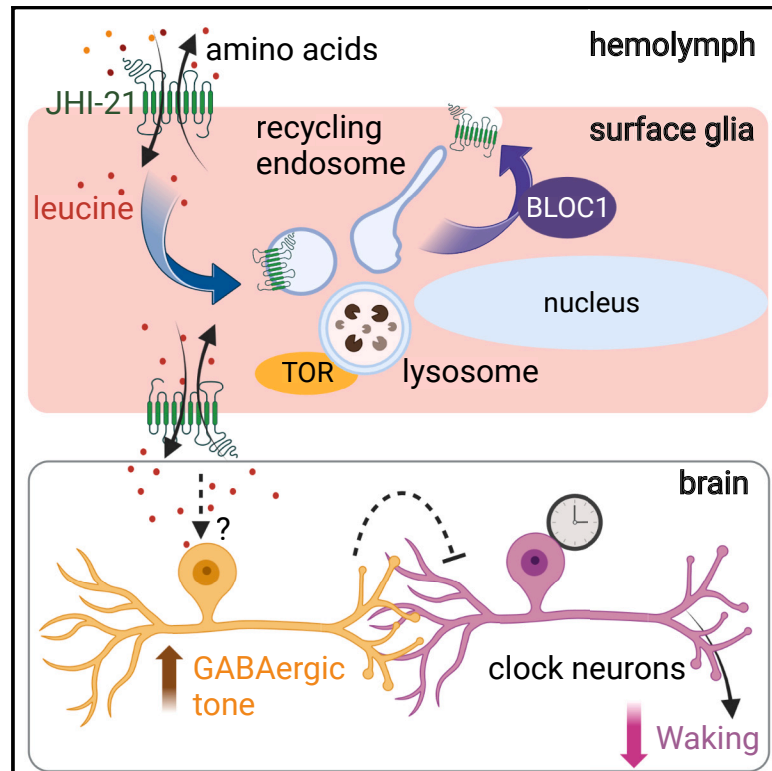
L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

# Pallidin function in *Drosophila* surface glia regulates sleep and is dependent on amino acid availability

## Graphical abstract



## Authors

Hui Li, Sami Aboudhief, Sandrine Parrot, Céline Scote-Blachon, Claire Benetollo, Jian-Sheng Lin, Laurent Seugnet

## Correspondence

laurent.seugnet@inserm.fr

## In brief

The *Pallidin* gene is a central component of an endosomal protein complex. Li et al. provide evidence that *Pallidin* is required in *Drosophila* surface glia, the equivalent of the blood-brain barrier, to initiate sleep at the beginning of the night through regulating leucine transport and GABAergic tone on circadian clock neurons.

## Highlights

- Knockdown of *Pallidin* and LAT1-like transporters in surface glia delays night sleep
- Inhibiting PDF-expressing clock neurons rescues *Pallidin* knockdown sleep phenotype
- Supplementing food with leucine rescues *Pallidin* knockdown sleep phenotype
- *Pallidin* regulates the subcellular trafficking of the LAT1-like transporter JHI-21



## Article

# Pallidin function in *Drosophila* surface glia regulates sleep and is dependent on amino acid availability

Hui Li,<sup>1</sup> Sami Aboudhraf,<sup>1</sup> Sandrine Parrot,<sup>2</sup> Céline Scote-Blachon,<sup>3</sup> Claire Benetollo,<sup>3</sup> Jian-Sheng Lin,<sup>1</sup> and Laurent Seugnet<sup>1,4,\*</sup>

<sup>1</sup>Centre de Recherche en Neurosciences de Lyon, Team WAKING, Université Claude Bernard Lyon 1, INSERM U1028, CNRS UMR 5292, 69675 Bron, France

<sup>2</sup>Centre de Recherche en Neurosciences de Lyon, NeuroDialyTics Facility, Université Claude Bernard Lyon 1, INSERM U1028, CNRS UMR 5292, 69675 Bron, France

<sup>3</sup>Centre de Recherche en Neurosciences de Lyon, GenCyTi Facility, Université Claude Bernard Lyon 1, INSERM U1028, CNRS UMR 5292, 69675 Bron, France

<sup>4</sup>Lead contact

\*Correspondence: [laurent.seugnet@inserm.fr](mailto:laurent.seugnet@inserm.fr)

<https://doi.org/10.1016/j.celrep.2023.113025>

## SUMMARY

The Pallidin protein is a central subunit of a multimeric complex called biogenesis of lysosome-related organelles complex 1 (BLOC1) that regulates specific endosomal functions and has been linked to schizophrenia. We show here that downregulation of *Pallidin* and other members of BLOC1 in the surface glia, the *Drosophila* equivalent of the blood-brain barrier, reduces and delays nighttime sleep in a circadian-clock-dependent manner. In agreement with BLOC1 involvement in amino acid transport, downregulation of the large neutral amino acid transporter 1 (LAT1)-like transporters *Jhl-21* and *mnd*, as well as of TOR (target of rapamycin) amino acid signaling, phenocopy *Pallidin* knockdown. Furthermore, supplementing food with leucine normalizes the sleep/wake phenotypes of *Pallidin* downregulation, and we identify a role for *Pallidin* in the subcellular trafficking of *Jhl-21*. Finally, we provide evidence that *Pallidin* in surface glia is required for GABAergic neuronal activity. These data identify a BLOC1 function linking essential amino acid availability and GABAergic sleep/wake regulation.

## INTRODUCTION

Biogenesis of lysosome-related organelles complex 1 (BLOC1) is an octameric complex linked to endosomal compartments and the cytoskeleton.<sup>1–3</sup> The genes coding for the 8 subunits in mice (*Pallidin*, *dysbindin*, *BLOS1*, *BLOS2*, *BLOS3*, *cappuccino*, *muted*, and *snapin*) are broadly expressed within the brain and in peripheral tissues. The complex regulates the trafficking of various receptors and transporters and appears to play a prominent role in the biogenesis of recycling endosomes.<sup>4–7</sup> Mice bearing severe or complete loss-of-function mutations in BLOC1 genes are viable and fertile.<sup>1,8</sup> They display common phenotypes, originating from defects in highly specialized lysosome-related organelles, such as reduced pigmentation due to impaired retinal and epidermal melanosomes or extended bleeding times resulting from the lack of dense granules in platelets. In humans, mutations in BLOC1 genes and other functionally related genes are found in the Hermansky-Pudlak syndrome.<sup>9</sup> In addition, genetic studies have identified variants of the *dysbindin* gene and other BLOC1 genes as risk factors for developing schizophrenia. Although the latter results are debated,<sup>10</sup> several postmortem studies have reported reduced levels of *dysbindin* mRNA and protein in the brain of

schizophrenics.<sup>1,3,11–13</sup> Furthermore, genome-wide association studies also reported a link between *dysbindin* genetic variants and cognitive abilities.<sup>14,15</sup> These findings have led to investigations aiming at deciphering the role of BLOC1 in neuronal function<sup>1</sup> using not only mouse models defective for individual BLOC1 gene function but also the *Drosophila* model, in which the complex is well conserved.<sup>16–21</sup> These studies confirmed the involvement of BLOC1 in behavior and memory. They identified potential cellular and molecular mechanisms such as abnormal glutamatergic, GABAergic, and dopaminergic transmission.<sup>1,16–18,20,22–24</sup> However, the vast majority of these studies have been carried out in animals with spontaneous or artificial mutations. This approach makes the interpretation of the resulting phenotypes challenging, given the broad expression of BLOC1 in many cell types. Furthermore, the complex is expressed during development, when it can be involved in neurodevelopmental diseases such as autism spectrum disorders.<sup>1,10,25,26</sup>

Apart from one recent report,<sup>23</sup> the involvement of BLOC1 in sleep/wake regulation has not been investigated. Interestingly, we have recently found that *Pallidin* is upregulated in a somnolent mouse model with defective histaminergic transmission.<sup>27</sup> Thus, further investigation is required given the critical



implication of sleep in brain function, and in particular in schizophrenia, in which sleep disruption could be in part a consequence of the pathology,<sup>28–30</sup> or an aggravating factor as seen in human<sup>31</sup> and rodent models.<sup>32</sup> Here, we investigated the potential role of BLOC1 in sleep/wake regulation in *Drosophila*. Neurotransmission systems, ion channels, and glial functions in *Drosophila* and mammals are globally conserved.<sup>33,34</sup> There are differences regarding sleep/wake in insects compared with mammals, such as the absence of slow wave and paradoxical sleep. Nevertheless, the evidence for conserved regulatory principles and functions is compelling.<sup>35</sup> The flexibility and short generation time of the *Drosophila* model is an asset in molecular genetic studies, providing hypotheses that can be tested in rodents and ultimately used for therapy. We used a conditional knockdown strategy to target Pallidin, a major component of BLOC1. The pool of Pallidin in the cell is associated almost entirely with the complex, and this protein plays a central role through its interactions with dysbindin, BLOS1, and cappuccino,<sup>2,19</sup> with its loss leading to their degradation.<sup>1,25</sup> We focused our study on glia, as our previous results suggest a function for Pallidin in non-neuronal cells.<sup>27</sup> Glial cells are increasingly proven to play very significant roles in the control of sleep/wake and circadian rhythms both in *Drosophila* and in rodent models.<sup>36,37</sup> Glia-dependent neurotransmitter reuptake, neurotransmitter metabolism, and glial calcium transients critically influence neuronal networks that control sleep timing and sleep homeostasis.<sup>38–40</sup> Neuroglia signaling pathways have been shown to modulate sleep-deprivation-induced learning impairments.<sup>41,42</sup> Recent reports have demonstrated the prominent role of glial cells at the interface between the brain and the circulating fluids during the sleep/wake cycle.<sup>40,43,44</sup>

## RESULTS

### Pallidin is required in adult *Drosophila* surface glia for normal sleep/wake regulation in a circadian-clock-dependent manner

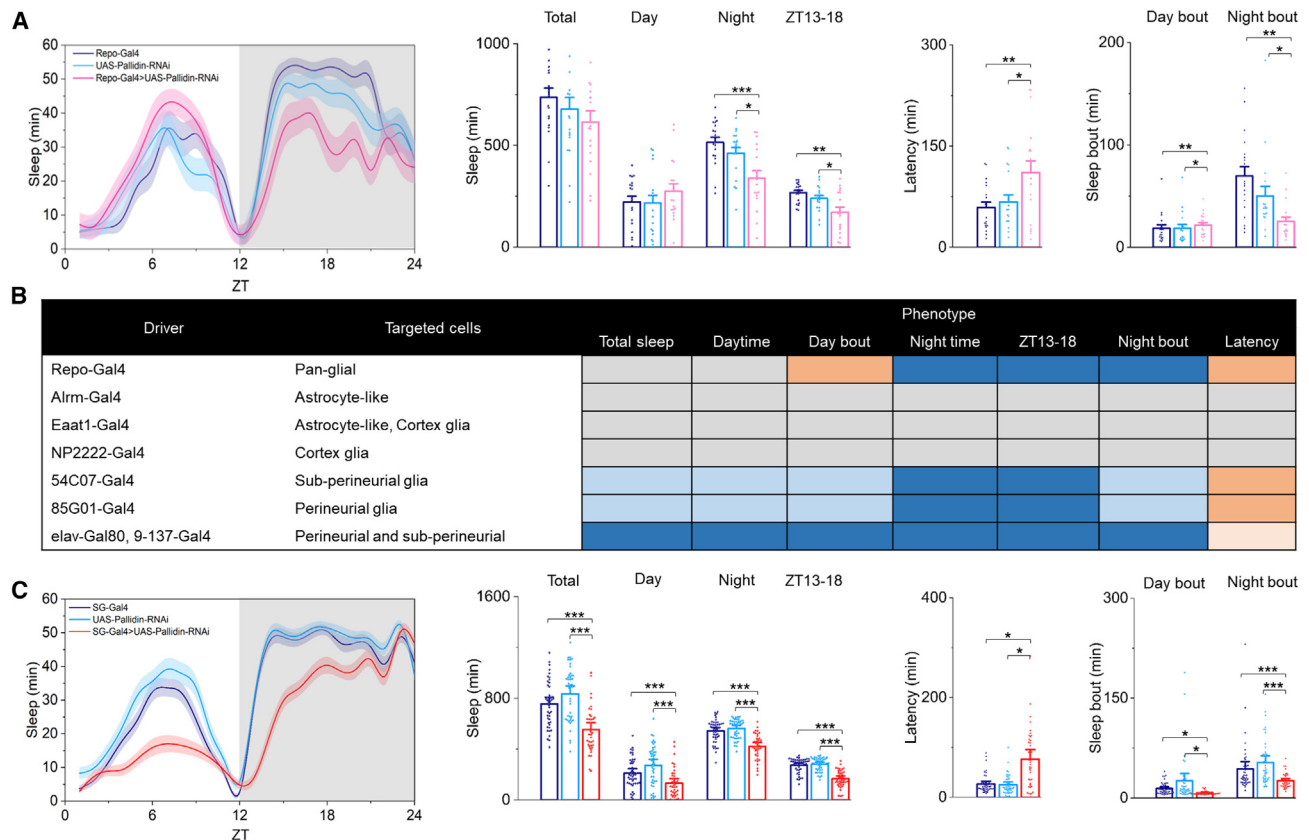
We used the *Drosophila* model and the Gal4-UAS system to downregulate Pallidin with UAS-Pallidin-RNAi transgenes in different cell types<sup>45</sup> to investigate its functional implication in sleep/wake regulation. Pallidin knockdown in all glial cells resulted in a significant reduction of night sleep, especially in the first half of the dark period, the primary sleep period in *Drosophila* (Figure 1A).<sup>46,47</sup> Specific Gal4 drivers were used to determine which glial cell type was responsible for the phenotype observed with pan-glial knockdown. As shown in Figures 1B and 1C, the knockdown of Pallidin expression in perineurial or in subperineurial cells was sufficient to replicate the delayed and reduced night sleep phenotype obtained upon pan-glial knockdown, using two independent UAS-RNAi constructs (Figure S1). These cells constitute the invertebrate equivalent of the blood-brain barrier (BBB) and are referred to here as surface glia (SG).<sup>48</sup> A reduction in sleep amounts in the first half of the night and a longer sleep latency at lights off were consistently observed, suggesting a difficulty initiating sleep at a time where flies display the highest amount of consolidated sleep.<sup>49</sup> Thus, less time is allowed for sleep each night despite similar sleep bout numbers, explaining why average sleep bout dura-

tion could be reduced. However, this latter phenotype was not consistent across drivers and RNAi constructs (Figures S2 and S3) and for one of the UAS-Pallidin-RNAi constructs, sleep reduction and fragmentation during daytime was also observed (Figure S2A). Given that the daytime sleep reduction was not consistently observed across genotypes and may occur from genetic background differences, we focused the rest of our analysis on the nighttime sleep phenotype induced by Pallidin downregulation: longer latency, which results in lower sleep amounts in the first half of the night. Expression of a UAS-RNAi against *sugar baby*, a gene sharing sequences with Pallidin and co-targeted by the UAS-RNAi-Pallidin<sup>GD13391</sup> construct, did not affect sleep (Figure S1). The 9-137-Gal4 driver line, which is expressed in both perineurial and subperineurial glia,<sup>50</sup> displayed a severe sleep loss phenotype during the nighttime as well as during the daytime. Although reported as SG specific,<sup>43,50</sup> 9-137-Gal4 is also expressed at low levels in neurons of the pars intercerebralis (Figure S4), which include insulin-like-peptide 2 (*ilp2*) producing neurons, reported to play a role in sleep/wake regulation.<sup>51–54</sup> We combined 9-137-Gal4 with the *elav-Gal80* transgene to inhibit Gal4 activity in all neurons and restrict it to SG. This compound driver (SG-Gal4) showed no detectable expression in pars intercerebralis neurons and was still able to induce sleep loss with UAS-Pallidin-RNAi (Figures S4 and 1C). In addition, driving UAS-Pallidin-RNAi specifically in the *ilp2* producing neurons with an *Ilp2-Gal4* driver did not affect sleep/wake (Figure S5).

To determine whether Pallidin is required for sleep regulation in the adult stage, we used the TARGET system to control the timing of UAS-Pallidin-RNAi expression<sup>55</sup> (Figure 2). At 18°C, the UAS-Pallidin-RNAi expression is inhibited by the thermosensitive Gal80<sup>ts</sup> inhibitor of Gal4 and no sleep reduction was observed during the night (Figure 2C). This latter lack of effect was not due to differences in sleep/wake regulation at 18°C, as nighttime sleep reduction was readily observed at 18°C in absence of Gal80<sup>ts</sup> expression (Figure 2A). When the adult flies were transferred to 25°C, however, we observed reduced sleep amounts, especially during the first half of the night (Figure 2D). This phenotype was milder than in the absence of the *tub-Gal80<sup>ts</sup>* transgene. Increasing the temperature to 30°C or 32°C did not result in a more severe phenotype, which could be attributed to the fact that all flies show nocturnal behavior at that temperature, independently of circadian clock regulation.<sup>56</sup> These results suggest that Pallidin is required in the adult stage to regulate sleep/wake during the first half of the night.

To determine whether nighttime sleep quality was affected by the knockdown of Pallidin in SG, we assessed sleep depth using video recording<sup>57,58</sup> (see STAR Methods). Nighttime sleep depth in SG>Pallidin-RNAi flies was similar to controls, suggesting that sleep loss is not originating from the inability of the flies to reach deep sleep stages (Figure S6). The knockdown flies also showed normal localization close to the food during sleep (Figure S6).

In constant darkness SG-Pallidin knockdown flies displayed a typical sleep/wake rhythm with reduced sleep during the pre-sumptive light phase and a free-running period of 25.1 ± 0.6 h, comparable with 25 ± 0.4 and 24.3 ± 0.4 h observed for the UAS and Gal4 controls, respectively (N = 30–32, 2 replicates). As in light/dark conditions, SG-Pallidin knockdown flies



**Figure 1. Pallidin knockdown in surface glia results in delayed, reduced, and fragmented night sleep**

(A) Baseline daily sleep of flies expressing a UAS-Pallidin-RNAi driven in all glial cells (repo-Gal4 driver) in red compared with the genetic control groups in blue. (B) Comparison of sleep parameters upon expression of UAS-Pallidin-RNAi under the control of several Gal4 drivers. 54C07-Gal4, 85G01-Gal4, and 9-137-Gal4 elav-Gal80 were crossed to both UAS-Pallidin-RNAi<sup>GD13391</sup> and UAS-Pallidin-RNAi<sup>HMS05728</sup> (Figures S1–S3). Dark blue color indicates significant increase and salmon color significant decrease in the amount/duration of the parameters in the knockdown condition compared with both the Gal4 and UAS controls ( $p < 0.05$ , Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions). Lighter color indicates that the changes were observed with the UAS-Pallidin-RNAi<sup>HMS05728</sup> or UAS-Pallidin-RNAi<sup>GD13391</sup> only.

(C) Baseline daily sleep of flies expressing UAS-Pallidin-RNAi, driven in subperineurial and perineurial cells (9-137-Gal4 combined with elav-Gal80 [SG]). As in (A), night sleep is reduced, especially during the second half of the night (zeitgeber time [ZT] 13–18; middle left bar graph), as a consequence of prolonged night sleep latency (far right bar graph). Night sleep is also significantly fragmented (middle right bar graph).  $N = 40$ – $45$  for each condition.  $*p < 0.05$ ,  $**p < 0.005$ , and  $***p < 0.0005$ , Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions.

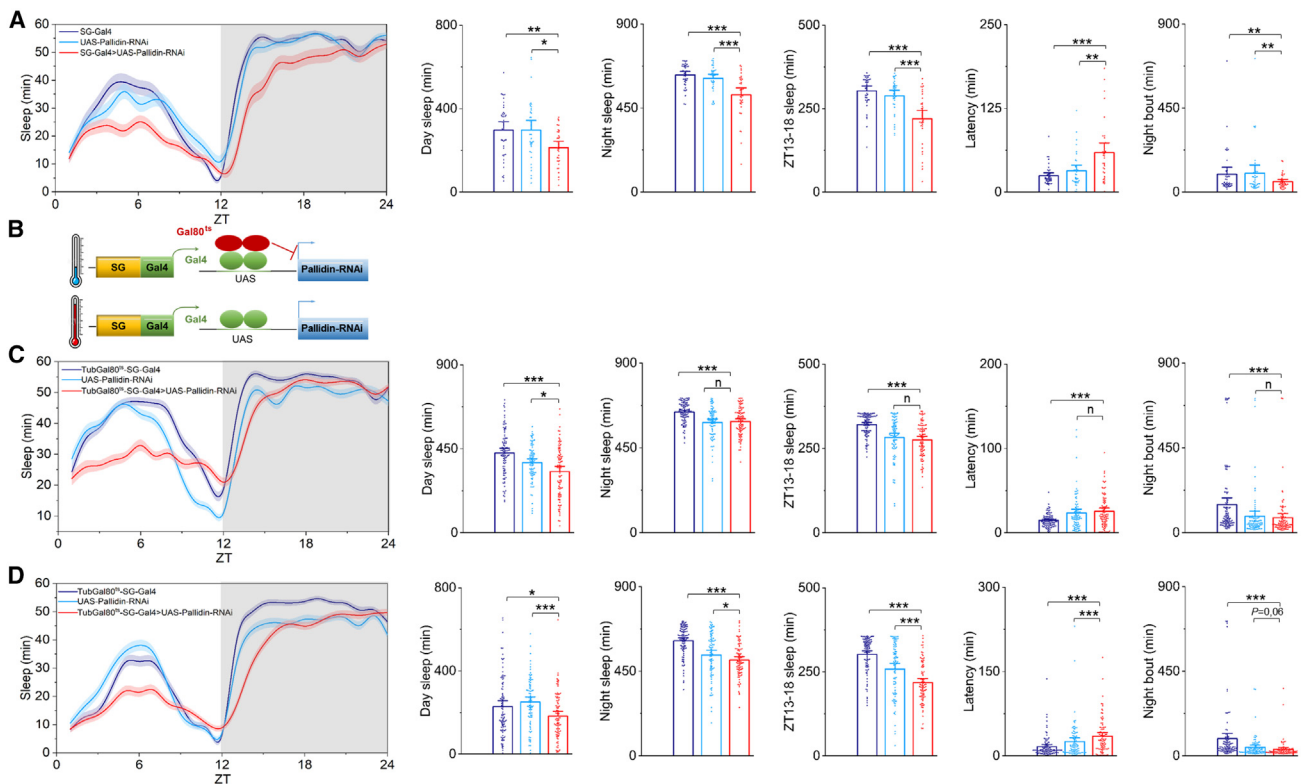
See Table S1 for genotypes and Figure S1–S4 for qPCR data, further control experiments, and surface glia-specific Gal4 drivers. Mean and SEM are shown.

displayed lower sleep amounts compared with the controls during the early part of the presumptive night (Figures 3A and 3B). These results indicate that the circadian clock is functional and that early-night sleep loss in knockdown flies is independent from light exposure. To assess the implication of the circadian clock, the period<sup>0</sup> (per<sup>0</sup>) mutation, known to completely abolish the cycling of the molecular oscillator,<sup>59</sup> was introduced into the genetic background. The downregulation of Pallidin in SG was no longer disrupting sleep in presence of the per<sup>0</sup> mutation, suggesting that the processes affected by Pallidin are dependent on the circadian clock (Figures 3C and 3D). Among the 150 neurons expressing the molecular clock, the pigment dispersing factor (PDF) expressing ventrolateral neurons promote wakefulness at specific times of the day. At the beginning of the night phase, GABAergic input to the large ventral lateral clock neurons (ILNv) is playing a critical role in promoting

sleep.<sup>60–63</sup> To evaluate whether insufficient inhibitory input to LNv could be involved in the inability of Pallidin knockdown flies to initiate sleep, we used the LexA/LexAOP system to express the potassium rectifier channel Kir2.1 and inhibit depolarization in all LNv clock neurons. As shown in Figures 3E and 3F, this manipulation was able to reduce latency in Pallidin knockdown flies to a level similar to the controls, suggesting that Pallidin in SG modulates LNv inhibitory input. However, nighttime sleep amounts remained lower than controls in this experiment, indicating that potentially other mechanisms are also involved regarding that aspect of the phenotype.

### Pallidin is required for sustained GABAergic neuronal activity

To assess the implication of GABAergic transmission in the SG-Pallidin knockdown phenotype, we first fed the flies with



**Figure 2. *Pallidin* downregulation in surface glia during adulthood is sufficient to affect sleep/wake regulation**

(A) *Pallidin* knockdown flies (red) display delayed, reduced, and fragmented sleep at 18°C, compared with controls (blue), similar to results obtained at 25°C. \* $p < 0.05$ , \*\* $p < 0.005$ , and \*\*\* $p < 0.0005$ , Kruskal-Wallis with post hoc comparisons between control and knockdown conditions;  $N = 38$ –42.

(B) *Pallidin* knockdown was restricted to adulthood using the *Tub-Gal80<sup>TS</sup>* construct and allowing the flies to develop at 18°C.

(C) Nighttime sleep in *Pallidin* knockdown flies is similar to the control groups at 18°C.

(D) At 25°C, the night sleep of *Pallidin* knockdown flies was reduced and delayed. Average night sleep bout duration was marginally reduced compared with controls. \* $p < 0.05$ , \*\* $p < 0.005$ , and \*\*\* $p < 0.0005$ , Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions;  $N = 84$ –93.

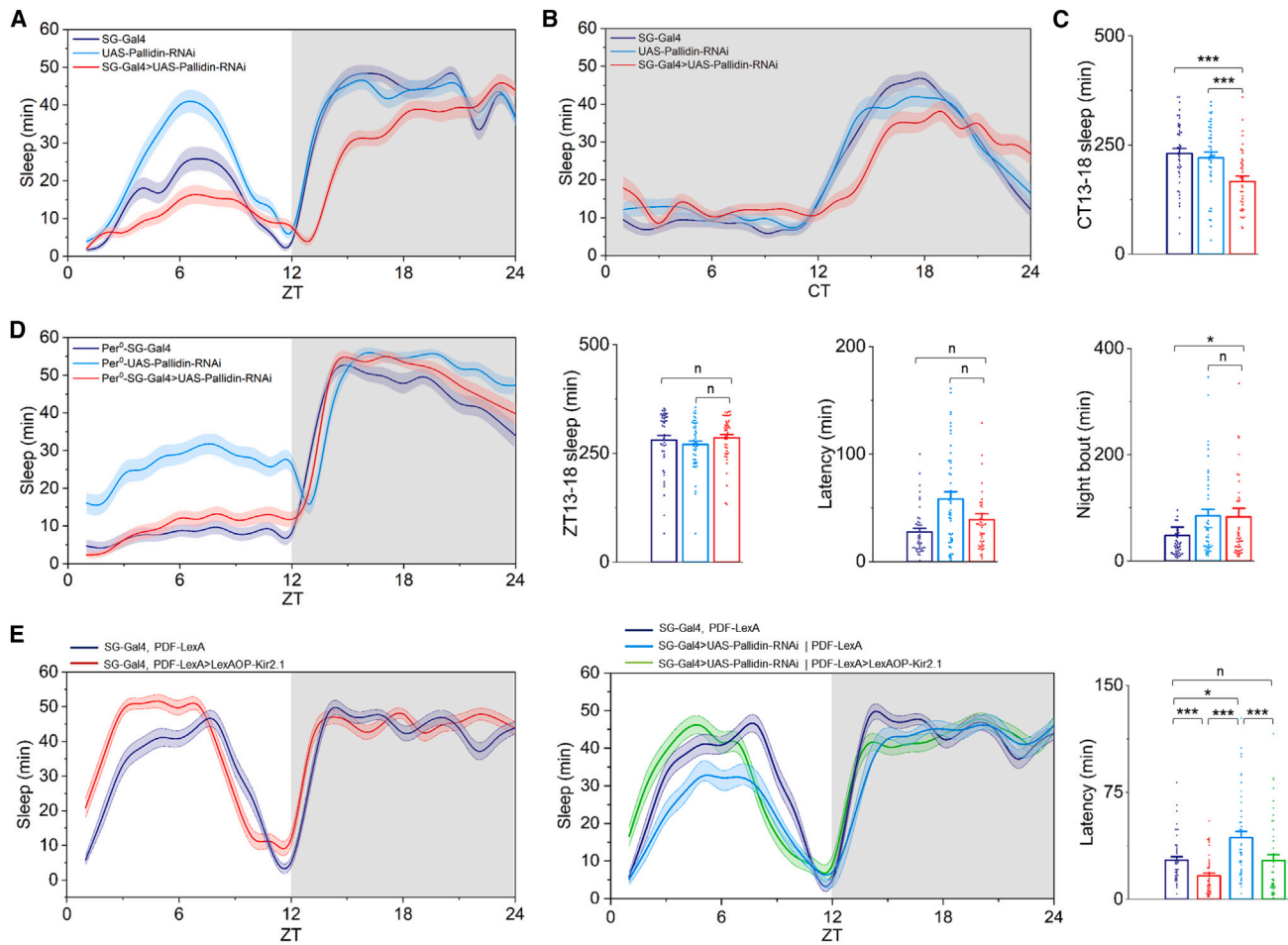
See Table S1 for genotypes. Mean and SEM are shown.

the GABA-A agonist 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol (THIP). This treatment potently increased sleep, as previously reported,<sup>64,65</sup> and *Pallidin* knockdown flies were no longer significantly different from the controls, suggesting that the phenotype originates from impaired release of GABA (Figure 4A). However, this strong potentiation of GABAergic transmission could potentially increase sleep independently of the origin of the deficit. To further evaluate the implication of GABAergic transmission in the *Pallidin* knockdown sleep phenotype, we expressed the heat-dependent cation channel dTrpA1 with the LexA/LexAOP system in GABAergic neurons, while downregulating *Pallidin* in SG (Figure 4B). We do observe a significant, although milder, increase in latency in the SG-*Pallidin* knockdown condition, similarly to the previous experiments. We observed that raising the temperature to 30°C induced lethality in vGAT-lexA>LexAOP-TrpA1 flies, we therefore used a lower temperature to activate the TrpA1 channel. After transfer from 18°C to 25°C the vGAT-lexA>LexAOP-TrpA1 flies displayed reduced latency and larger nighttime sleep amounts, as expected following and increase in GABAergic tone (Figures 4C, 4D, and S7). *Pallidin* downregulation in SG completely

prevented this effect, suggesting that it is required for the sustained activity of GABAergic neurons.

GABA is synthesized by the decarboxylation of glutamate, which is also an excitatory neurotransmitter. Using individual brain high-performance liquid chromatography (HPLC), evaluation of the global content of GABA, glutamate, and aspartate, an amino acid metabolically linked to glutamate, showed a trend for a reduction of the GABA/glutamate ratio and an increase in the glutamate/aspartate ratio (Figure 4E). Taken together, these pharmacological, behavioral, and neurochemical data suggest that *Pallidin* affects GABAergic neurons on a global level in the brain. However, other neuronal subtypes could also be affected.

In *Drosophila*, most neuronal cell bodies are surrounded by cortex glia. However, a previous anatomical description reported direct subperineurial glia contacts with a subset of neuronal cell bodies.<sup>66</sup> To evaluate whether GABAergic neurons, or a subset of them could be in direct contact with the SG, we used GFP reconstitution across synaptic partners.<sup>67,68</sup> As shown in Figure 4F, we observe extensive cellular contacts between the cell bodies of numerous GABAergic neurons and SG.



**Figure 3. The circadian clock is required to observe the sleep/wake phenotype of *Pallidin* downregulation in surface glia**  
 (A and B) Baseline daily sleep of *Pallidin* knockdown flies in light dark condition (LD) (A) and following transfer to constant darkness (DD) (B). *Pallidin* knockdown flies displayed reduced sleep during the presumptive night in constant darkness.  
 (C and D) Combining the period<sup>0</sup> (*per*<sup>0</sup>) mutant with *Pallidin* downregulation prevents nighttime sleep loss (C), fragmentation, or longer latency (D).  
 (E) Hyperpolarization of PDF-expressing clock neurons rescues the night sleep latency of *Pallidin* knockdown flies. \**p* < 0.05 and \*\*\**p* < 0.0005, Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions.  
 N = 30–43 (A–C) and N = 46–48 (E). See Table S1 for genotypes. Mean and SEM are shown.

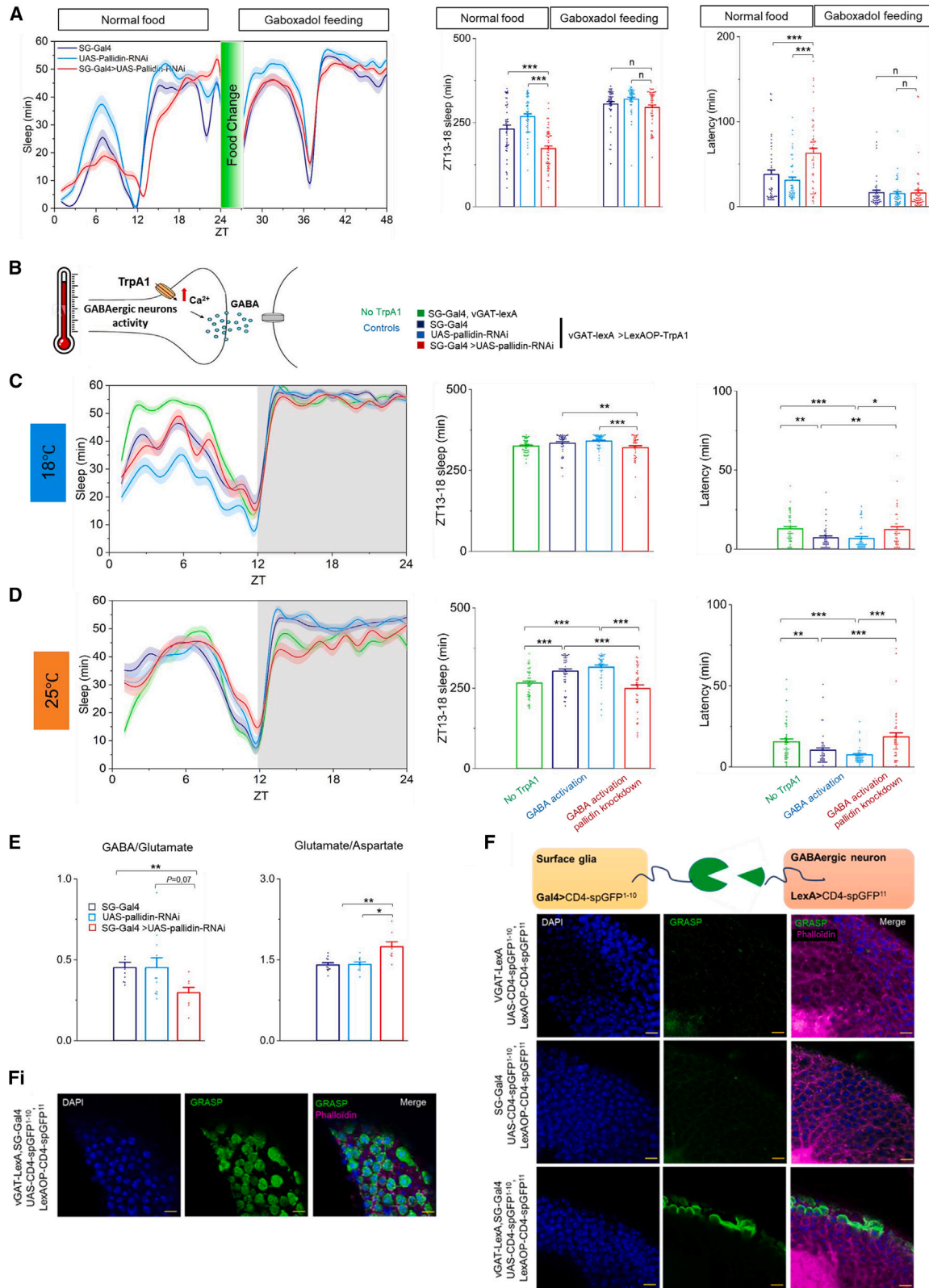
### Other members of the BLOC1 complex genes are required in SG for sleep/wake regulation

To determine whether the sleep-regulatory function of *Pallidin* in SG is shared by other BLOC1 genes, we used available UAS-RNAi lines to inhibit their expression in SG. As shown in Figure 5, downregulation of several BLOC1 members presented a phenotype similar to that seen with *Pallidin*—*dysbindin*, *Blos1*, and *Blos2* (Figures 5A–5C)—while the downregulation of *snapin* did not affect significantly sleep/wake (Figure 5D).

### LAT1-like transporters and TOR signaling knockdown in SG affect early-night sleep

The function of *Pallidin* in the mouse brain has been linked to the transport and/or the availability of large neutral amino acids,<sup>69,70</sup> which are controlled to a large extent by the heterodimeric large neutral amino acid transporter 1 (LAT1) transporter localized in

BBB cells.<sup>71–75</sup> We therefore conducted a set of experiments in *Drosophila* to test the hypothesis that the sleep phenotype observed upon *Pallidin* downregulation could be linked to amino acid availability in the brain. We first found that downregulation of the *Drosophila* homologs of LAT1 light-chain (*slc7a5*): *Jhl-21* (*Juvenile Inducible 21*), *mnd* (*Minidisc*), as well as *CD98Hc*, the *Drosophila* homolog of *slc3a2*, coding for the heavy-chain subunit, replicates the *SG>UAS-Pallidin-RNAi* sleep phenotype (Figures 6A, 6B, and S8). Second, knockdown of the target of rapamycin (TOR) cellular amino acid sensing pathway using *UAS-TOR-RNAi* or a *UAS-raptor-RNAi* resulted in the same effect (Figures 6C and 6D). These results suggest that LAT1-like transporters, TOR, and *Pallidin* could participate in a process regulating both amino acids intake and sleep. In support of this idea, the intake of leucine through LAT1 is required to activate TOR signaling in some cellular contexts<sup>76</sup> (Figure 6E).



(legend on next page)



### Leucine supplementation normalizes the *Pallidin* and LAT1-like transporters knockdown phenotypes

To test the hypothesis that *Pallidin* knockdown flies suffer from a deficit in amino acids, notably essential amino acids provided by feeding, we attempted to rescue the phenotype with a nutritional approach. We find that supplementing food with leucine rescues the *SG>UAS-Pallidin-RNAi*, the *SG>UAS-Jhl-21-RNAi* and *SG>UAS-Blos2-RNAi* sleep phenotypes (Figures 6F, 6G, and S9), while having little or no detectable effect in the genetic controls. The effect of amino acid supplementation was prominent on night sleep, reducing latency, increasing sleep amounts and sleep bout duration. Importantly, food intake was unaffected by the knockdown (Figure S10E). Supplementing food with other essential amino acid such as valine or tryptophan rescued the *Jhl-21* knockdown but, interestingly, not *Pallidin* knockdown (Figure S10). As mated female flies have recently been shown to eat more amino acids at night, a behavior absent in males and in non-mated females,<sup>77</sup> we evaluated the *SG>UAS-Pallidin-RNAi* night sleep phenotype in mated females and in males. The sleep phenotype was unaffected by the mating status of females and was observed in males, with the exception of longer latency for the latter (Figure S11).

### *Pallidin* downregulation results in an abnormal trafficking of the LAT1-like *Jhl-21* transporter

As *Pallidin* and the BLOC1 complex can regulate transmembrane protein trafficking, in particular transporters,<sup>78,79</sup> we evaluated the subcellular localization of *Jhl-21* in SG using a validated antibody.<sup>80,81</sup> To unambiguously identify perineurial and subperineurial glia cells, we labeled their nuclei by the expression of a nuclear- $\beta$ -galactosidase (Figure 7A) or by the expression of a *UAS-GFP-mcherry-ATG8* transgene to label both the nuclei and lysosomal organelles, including autophagosomes<sup>82</sup> (Figure 7B). *Jhl-21* was detected in punctae in the perinuclear region of perineurial and subperineurial cells, as well as in neurons, and colocalized partially with GFP-mcherry-ATG8 suggesting that at least a subset of the transporter is internalized in lysosomes. We observed that the knockdown of *Pallidin* in SG resulted in an accumulation of *Jhl-21* immunofluorescence in punctae within the nuclei of

both perineurial and subperineurial glia, suggesting abnormal trafficking of the transporter (Figures 7A–7C). In contrast, no significant changes were observed in neighboring cells, mostly neurons (Figure 7C). *Pallidin* knockdown did not result in major changes in autophagy, as detected with the *UAS-GFP-mcherry-ATG8* construct<sup>82</sup> (Figure S12).

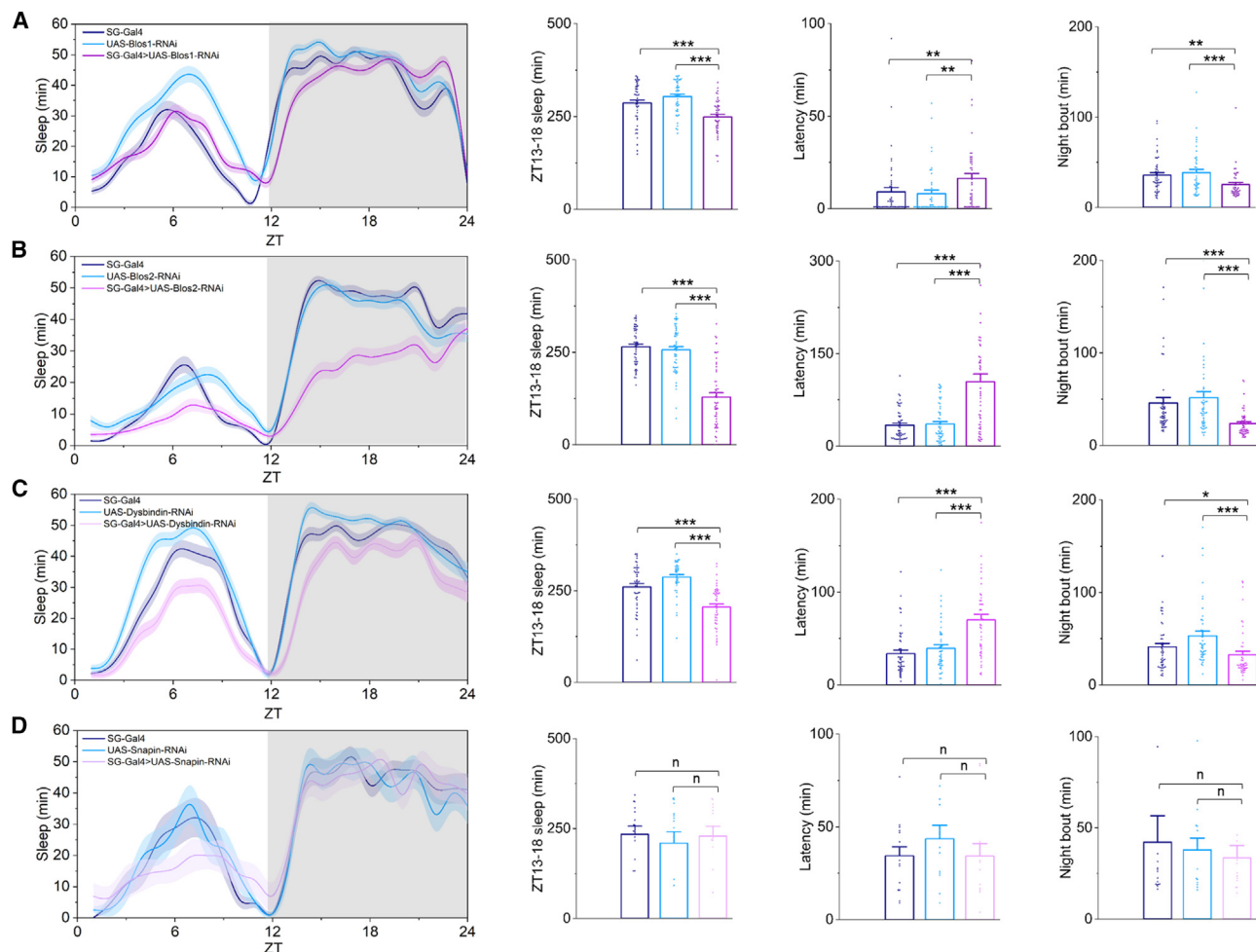
### *Pallidin* in SG does not modulate dopamine synthesis

Although the data outlined so far suggest a role for *Pallidin* in the regulation of GABAergic transmission, previous reports suggested that this gene could modulate monoaminergic transmission. In particular, it has been shown previously that mice with a defective *Pallidin* gene were less sensitive to intraperitoneal injections of tryptophan and of levodopa (L-DOPA).<sup>70</sup> A similar hypothesis has been proposed for *dysbindin* in *Drosophila*, although in the latter case, a deficit in dopamine recycling has been proposed.<sup>1,18,21,83</sup> To test whether dopamine synthesis could explain the sleep/wake phenotype of *SG>UAS-Pallidin-RNAi* flies we conducted similar experiments in *Drosophila*. Feeding flies with L-DOPA leads to nocturnal insomnia within 24 h and a large increase in dopamine synthesis.<sup>81</sup> The sensitivity to L-DOPA as evaluated by sleep loss was not significantly different between *SG>UAS-Pallidin-RNAi* flies and controls, while food intake remained equal in all the groups (*SG-Gal4*,  $0.79 \pm 0.4 \mu\text{L}$ ; *UAS-RNAi*,  $0.71 \pm 0.4 \mu\text{L}$ ; *SG-Gal4 >UAS-RNAi*,  $0.68 \pm 0.3 \mu\text{L}$  over 24 h; N = 6; Figure S13A). Furthermore, the global levels of dopamine measured on HPLC were increased to a similar extent in *Pallidin* knockdown flies compared with the control groups (Figure S13B). We observed no difference in dopamine or 5-hydroxytryptamine (5-HT) levels in *SG>UAS-Pallidin-RNAi* in the vehicle control condition. To further test the involvement of SG and *Pallidin* in dopaminergic transmission, we combined the knockdown of *Pallidin* with the *fumin* mutation of the *dopamine transporter* (*DAT*) gene. This mutation impairs the reuptake of dopamine and leads to a hyperdopaminergic state and severe insomnia.<sup>84,85</sup> Given the lack of dopamine reuptake, it can be assumed that dopaminergic transmission in this mutant relies heavily on *de novo* dopamine synthesis.<sup>86</sup> It could then be assumed that *Pallidin* knockdown would attenuate the *fumin* phenotype by preventing the transport of amino acids.

### Figure 4. Effect of *Pallidin* knockdown in surface glia on GABAergic transmission

- (A) *Pallidin* knockdown flies (in red) and genetic controls (in blue) recorded on normal food, then transferred to food supplemented with 0.1 mg/mL Gaboxadol (THIP) for 2 days. The amount of sleep increased in all groups. Hourly sleep, latency, and sleep at ZT 13–18 in the knockdown flies become similar to the control groups. \*\*\*  $p < 0.0005$ , Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions; N = 45–53.
- (B) Schematic of GABAergic neurons activation by targeted expression of the TrpA1 heat-inducible channel.
- (C) Expression of TrpA1 in GABAergic neurons using the vGAT-lexA transgene combined with LexAOP-TrpA1 has no effect on sleep at 18°C.
- (D) Expression of TrpA1 in GABAergic neurons results in higher sleep amounts and longer sleep bout duration at 25°C compared with non-TrpA1-expressing controls (green). *Pallidin* knockdown flies (red) do not display higher night sleep amounts and shorter sleep latency compared with non-TrpA1-expressing flies (green), in contrast to the genetic background controls (dark and light blue). Similar results were obtained with an independent *UAS-Pallidin-RNAi* transgene (Figure S6). \* $p < 0.05$ , \*\* $p < 0.005$ , and \*\*\* $p < 0.0005$ ; Kruskal-Wallis test with post hoc comparisons; N = 37–43.
- (E) HPLC analysis of GABA, glutamate (Glu), and aspartate (Asp) contents in individual brains. The ratio of GABA to Glu concentrations is slightly but not significantly decreased in *Pallidin*-downregulation flies (red) compared with the genetic control (blue). The ratio of Glu to Asp was significantly increased in *Pallidin*-downregulation. \* $p < 0.05$  and \*\* $p < 0.005$ , Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions; N = 8–12.
- (F) Schematic of the GRASP system (see STAR Methods) and confocal images (z stack, maximal-intensity projection of 5 images, 1  $\mu\text{m}$  apart) of cross sections in the dorsal brain for each genotype tested. GFP fluorescence is observed on the top half of the GABAergic neurons when both surface glia and GABAergic neurons express the GRASP constructs. (Fi) Tangential view of the GRASP signal between GABAergic neurons and surface glia (z stack, maximal-intensity projection of 2 images, 1  $\mu\text{m}$  apart). Scale bar: 5  $\mu\text{m}$ .

See Table S1 for genotypes, and Figure S5 for further controls. Mean and SEM are shown.



**Figure 5. Downregulation of BLOC1 complex genes in surface glia**

(A–D) Baseline daily sleep of flies expressing UAS-Blos1-RNAi, UAS-Blos2-RNAi, UAS-Dysbindin-RNAi, and UAS-Snapin-RNAi in surface glial cells (in magenta) compared with the respective genetic control groups (in blue). In former three cases, nighttime sleep is reduced, delayed (longer latency), and fragmented (shorter average bout duration). \* $p < 0.05$ , \*\* $p < 0.005$ , and \*\*\* $p < 0.0005$ , Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions;  $N = 42$ – $52$ , except  $N = 13$ – $15$  for snapin (one replicate). See Table S1 for genotypes. Mean and SEM are shown.

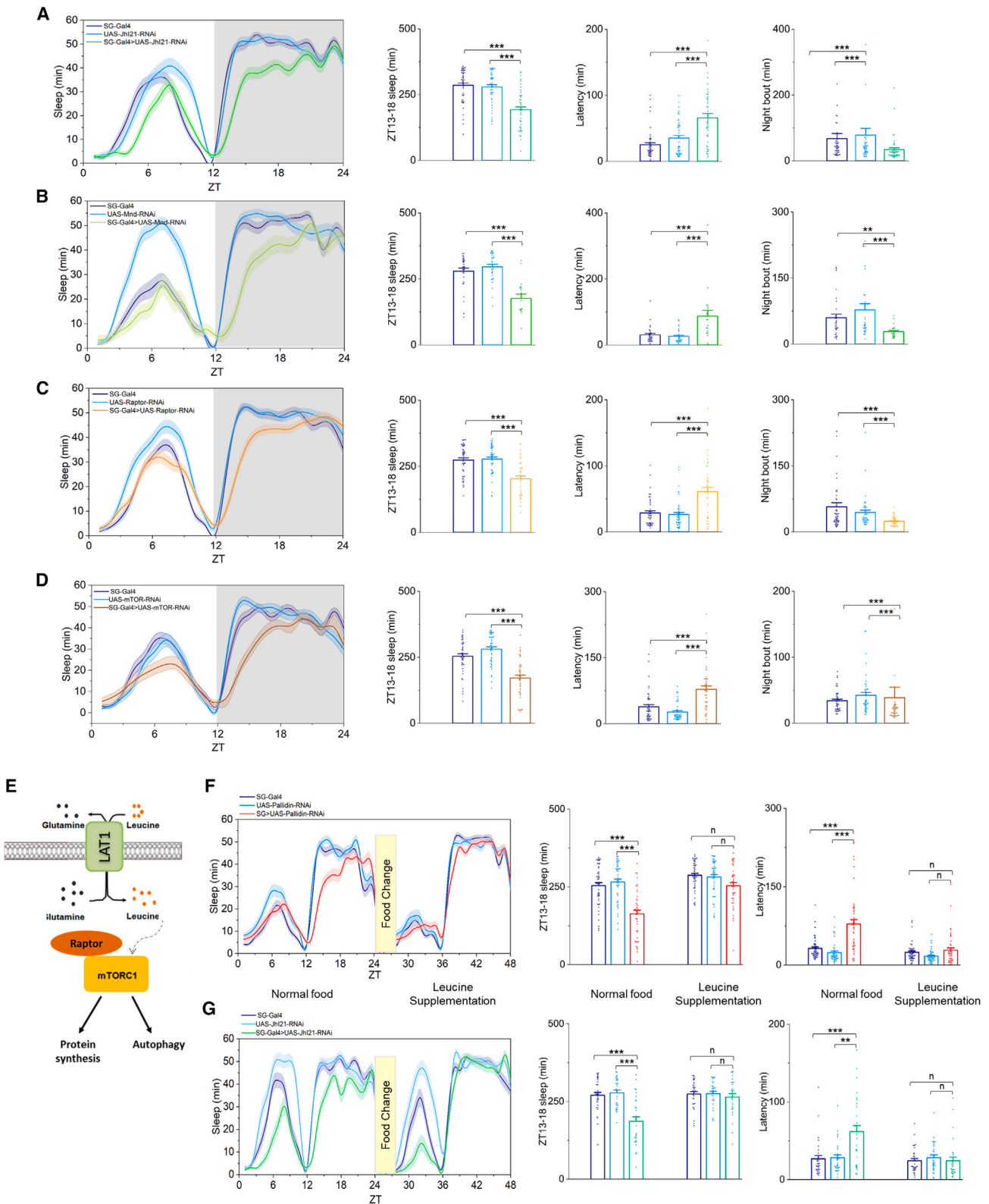
*Pallidin* downregulation in SG failed to attenuate the severity of *fumin*-driven insomnia, suggesting that it does not affect dopaminergic synaptic transmission directly (Figure S13C).

## DISCUSSION

We provide evidence in *Drosophila* that *Pallidin* and other BLOC1 components regulate the initiation of sleep in the early part of the night, at the level of SG cells. In *Drosophila*, SG cells express tight junctions and molecular components in common with the mammalian BBB. As in the BBB this organization prevents the entry of macromolecules and tightly regulates the exchanges of solutes between the circulatory system (the hemolymph in *Drosophila*) and the brain, thus maintaining the particular interstitial fluid composition necessary for neuronal activity.<sup>48</sup> This function relies on intense transporter activity. The data support a mechanism whereby BLOC1 regulates LAT1-like transporters

subcellular trafficking in both perineurial and subperineurial cells, leading to adequate essential amino acid supply and GABAergic neuronal activity promoting sleep, in a circadian-clock-dependent manner (Figure 7D). In this study, we observed that downregulating *Pallidin* using a pan-neuronal driver also resulted in reduced night sleep, suggesting that SG is not the only cell type in the brain where *Pallidin* is required to regulate sleep (Figure S14).

Blocking the cycling of the molecular clock by placing flies in a *per<sup>0</sup>* mutant background completely abolishes the *Pallidin* knockdown sleep/wake phenotype, suggesting that the circadian clock is implicated. Accordingly, we find that hyperpolarization of PDF-expressing clock neurons is sufficient to normalize the main *Pallidin* knockdown phenotype: increased sleep latency at night. This raises the possibility that *Pallidin* affects clock-dependent sleep/wake regulatory networks and in particular, those under the control of the large PDF-expressing



(legend on next page)

neurons (ILNv). In ILNv neurons, GABAergic input is modulated in a circadian manner, resulting in higher inhibition in the early night, promoting sleep, while a disruption of this input results in delayed and fragmented sleep.<sup>61–63,87</sup> The GABAergic neurons responsible for ILNv inhibition are so far unidentified. The delayed and reduced night sleep observed upon *Pallidin* knockdown in SG is also similar to the phenotype previously reported with pan-GABAergic neuron inhibition.<sup>50</sup> Conversely, feeding flies the GABA agonist THIP<sup>65</sup> or increasing GABAergic neuronal firing by expressing *TrpA1* (Figure 4) can potently induce sleep. We find here that the latter manipulation cannot promote sleep when *Pallidin* is downregulated in SG, suggesting that the gene is required to allow sustained GABAergic neuronal activity. Alternatively, *Pallidin* function could be regulated directly by the clock present in perineurial cells, which modulates the efflux properties of subperineurial cells in a circadian manner.<sup>88</sup>

Downregulation of several members of BLOC1 in SG phenocopies the knockdown of *Pallidin*, strongly suggesting that the whole complex is involved in this *Pallidin*-dependent sleep/wake regulation. This is consistent with the observation that the pool of *Pallidin* and *dysbindin* present in the cell is almost entirely associated with the complex<sup>1,25</sup> and that the lack of one subunit leads to a destabilization of the others.<sup>16,89</sup> The lack of sleep alteration with *snarin* manipulation may result from lower efficiency of the genetic tools used for downregulation. Alternatively, it may also reflect different gene dosage sensitivity and functionality for the different members of the complex, as previously reported,<sup>16,20,90</sup> or the existence of multiple subunits within the complex<sup>2</sup> or different complexes containing a subset of the subunits.<sup>10</sup> In *Drosophila* larval neuromuscular junction, *Snarin* has, for example, been shown to regulate synaptic homeostasis,<sup>17</sup> while *Pallidin* does not seem to affect baseline neurotransmission and is required during sustained neuronal activity to replenish the pool of releasable synaptic vesicles.<sup>16</sup> BLOC1 has been shown to be required for the biogenesis of recycling endosomes through its interactions with sorting endosomes and the cytoskeleton.<sup>4</sup> These BLOC1 functions and the regulation of sleep by BLOC1 members reported in this study are in agreement with a recent report showing bidirectional interactions between sleep and endocytosis in SG.<sup>43</sup> Intriguingly, the endocytosis in SG was reported to be the most intense in the early part of the night,<sup>43</sup> when sleep is the deepest and the phenotype of *Pallidin* downregulation is the most pronounced. In addition, the activity of the recycling endosome associated small GTPase *rab11* appears to play a prominent role in this context.<sup>43</sup> Consistent with these findings, extensive

protein-protein interaction analyses identified *rab11* as the best interacting partner for BLOC1 in both *Drosophila* and humans.<sup>5</sup> Thus, BLOC1 may facilitate the high endocytic activity of SG during the early part of the night, notably the biogenesis of recycling endosomes, while having a less prominent role at other times during the day. In line with our results, *Pallidin* mutant mice display reduced sleep amount during the light phase of the day, the primary sleep period in rodents, and shorter average sleep-bout duration.<sup>23</sup> However, BBB-specific and conditional knockout models in mice would be necessary to determine whether the model outlined here applies to mammals. In humans, the incidence of sleep/wake disorders in the rare Hermansky-Pudlak syndrome, bearing deficits in BLOC1 or other functionally related complexes, is unknown but deserves scrutiny.<sup>91</sup> In contrast, sleep disruption is common among patients suffering from schizophrenia, another pathology associated with BLOC1 deficits. Interestingly, one of the prominent sleep abnormalities in schizophrenia patients is a reduction in sleep spindle density,<sup>30</sup> which strongly relies on GABAergic activity,<sup>92,93</sup> and the latter is also affected in this pathology.<sup>10</sup> Accordingly, previous studies in *dysbindin* mutant mice have shown that BLOC1 can disrupt GABAergic activity.<sup>22</sup> Thus, a potential role for amino acid transport at the BBB in those contexts would deserve investigation given our results.

Early studies of *Pallidin* function pointed to a role in amino acid import into the brain transport, as suggested by lower sensitivity to intraperitoneal injections of the LAT1 substrates L-DOPA and tryptophan.<sup>70</sup> The LAT1 transporter plays a major role in the import of these amino acids and in the import of large neutral essential amino acids such as leucine, isoleucine, and histidine.<sup>73,94</sup> A conditional knockout of LAT1 in brain endothelial cells confirmed the crucial role of LAT1 in the regulation essential amino acid abundance within the brain, its impact on GABAergic transmission, and its potential implication in autism spectrum disorders.<sup>95</sup> Intriguingly, the relative abundance of amino acids in the brain of *Pallidin* mutant mice resembles those found in mice with a BBB specific knockout LAT1.<sup>69</sup> Here, we provide evidence that *Pallidin* function in SG is linked to essential amino acid supply and to LAT1-like transporter activity. First, downregulation of the LAT1-like transporters and of TOR signaling phenocopy knock down, to a large extent or completely, the BLOC1 genes. Second, supplementing the food with leucine can normalize the *Jhl-21*, *Pallidin*, and *Blos2* phenotypes. Interestingly, the phenotype of the *Jhl-21* transporter knockdown could be rescued by valine and tryptophan supplementation, while *Pallidin* knockdown could be rescued only by leucine. This suggests

#### Figure 6. Downregulation of LAT1-like homologs and TOR, Raptor phenocopy *Pallidin* downregulation in surface glia

Food supplemented with leucine normalizes the sleep phenotypes of *Pallidin* and *Jhl-21* downregulation in surface glia.

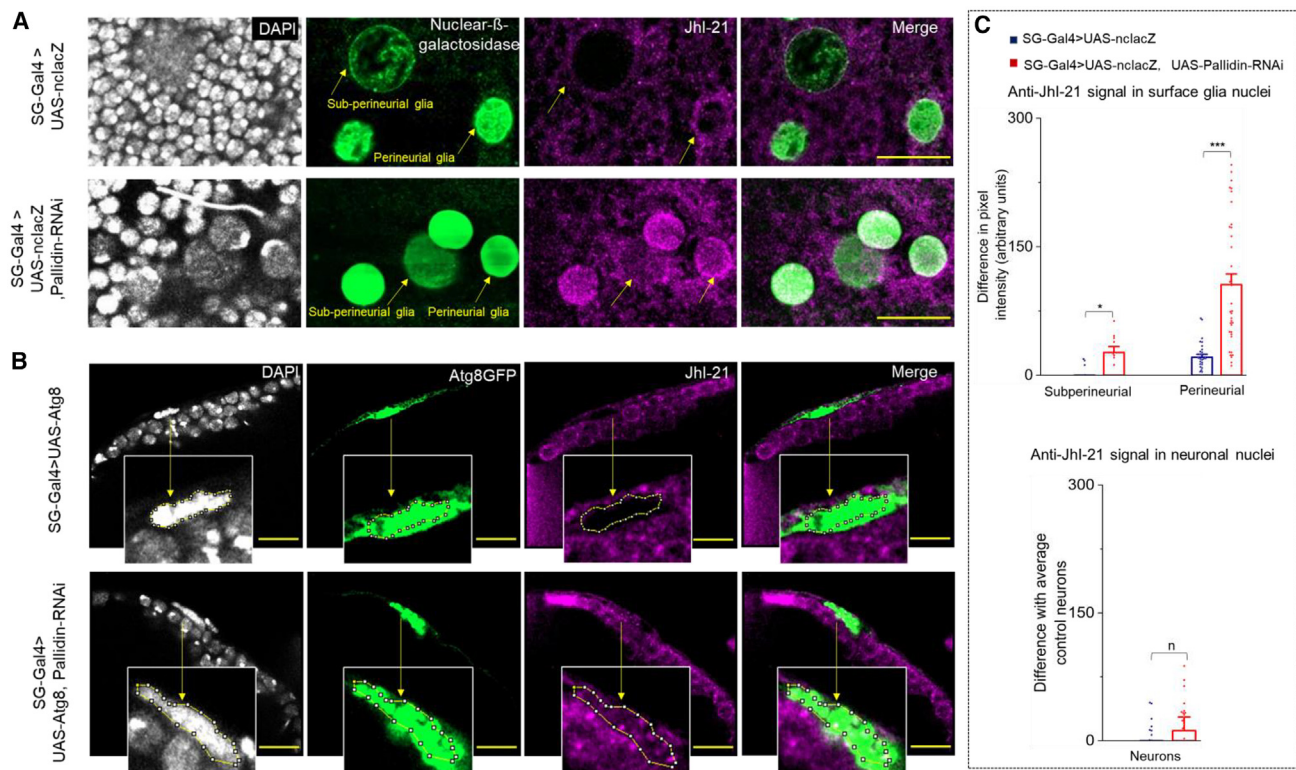
(A–D) Baseline daily sleep of flies expressing UAS-*Jhl-21*-RNAi, UAS-*Mnd*-RNAi (A and B), UAS-*Raptor*-RNAi, and UAS-*TOR*-RNAi (C and D) in surface glial cells (in green, orange, or brown) compared with the respective genetic control groups (in blue). In all cases, latency was increased and night sleep reduced compared with controls. Average night bout was also reduced except for the *TOR*-RNAi-expressing flies. \**p* < 0.05, \*\**p* < 0.005, \*\*\**p* < 0.0005, Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions; N = 44–46.

(E) Schematic of potential interactions between LAT1-like, TOR, and amino acids based on literature.

(F) *Pallidin* knockdown flies (in red) and genetic controls (in blue) recorded on normal food, then transferred to normal food or normal food supplemented with 50 mM leucine for 1 day. Hourly sleep, latency, and sleep at ZT 13–18 in the knockdown flies become similar to the control groups.

(G) The same protocol was applied to *Jhl-21* knockdown flies and similarly normalized nighttime sleep. \**p* < 0.05, \*\**p* < 0.005, \*\*\**p* < 0.0005, Kruskal-Wallis test with post hoc comparisons between control and knockdown conditions; N = 29–45.

See Table S1 for genotypes and Figures S6–S9 for complementary experiments. Mean and SEM are shown.



**Figure 7. The knockdown of *Pallidin* modifies *Jhl-21* subcellular localization in surface glia**

(A) Tangential sections of the surface of brains expressing either the *UAS-nuclear-LacZ* (*UAS-ncLacZ*) alone or together with *UAS-Pallidin-RNAi* under the control of *9-137-gal4*. Perineurial and subperineurial cells are identified by virtue of the size and location of the anti- $\beta$ -galactosidase labeled nuclei (green). The anti-*Jhl-21* antibody reveals a substantial signal within the nuclei of both perineurial and subperineurial glia of *UAS-Pallidin-RNAi*-expressing brains, not observed in the control condition.

(B) Cross-sectional images showing a single subperineurial cell nucleus in brains expressing either the *UAS-GFP-mcherry-ATG8* construct alone or together with *UAS-Pallidin-RNAi* under the control of *9-137-Gal4*. *GFP-mcherry-ATG8* expression is revealed by an anti-GFP antibody and is present both in the cytoplasm and in the nucleus of surface glial cells. The anti-*Jhl-21* antibody signal is absent from the nucleus in the control condition but detected as punctae double labeled with *ATG8-mcherry-GFP* within the *UAS-Pallidin-RNAi*-expressing flies. In (A) and (B), scale bar: 10  $\mu$ m; N = 4–8. Single confocal sections are shown.

(C, top) Quantification of the anti-*Jhl-21* signal in the nuclei of subperineurial (N = 8–10) and perineurial (N = 28–36) cells in 5 independent brains of each condition presented in (B).

(C, bottom) Quantification of the anti-*Jhl-21* signal in the nuclei of neurons (N = 18–24) in 5 independent brains of each condition presented in B shows no significant difference between *Pallidin* knockdown and control conditions. \* $p < 0.05$  and \*\*\* $p < 0.0005$ , Mann-Whitney test between control and knockdown conditions.

See Table S1 for genotypes and Figure S10 for lysosome detection. Mean and SEM are shown.

that *Pallidin* does not solely modulate LAT1-like transporter function and has complex effect on multiple transport systems, as previously suggested.<sup>69,70</sup> *Jhl-21* and *minidisc* are the *Drosophila* closest homologs of LAT1 light-chain.<sup>96</sup> *Jhl-21* is expressed broadly in the *Drosophila* brain, with most of the signal in perinuclear punctae that colocalizes partially with the lysosomal marker ATG8 (Figure 7). The subcellular trafficking of *Jhl-21* in SG is abnormal following *Pallidin* downregulation, with a substantial fraction of the transporter localized in the nucleus (Figure 7). This abnormal trafficking is likely to reduce the functionality of the transporter, explaining the similar sleep/wake phenotypes of *Jhl-21* and *Pallidin* downregulation in SG and their normalization by essential amino acid supplementation. A nuclear localization has already been reported in glioma cell lines for the solute carrier (SLC) *Eaat1* and *Eaat2* transporters' and

for the K<sup>+</sup> inwardly rectifying channel.<sup>97,98</sup> In both cases, this unusual subcellular localization was associated with an overall reduced functionality of the protein in the cell. Aside transporters, several full-length transmembrane receptors have been repeatedly reported to be localized in the nucleus where some of them may act as transcription factors.<sup>99,100</sup> The mechanisms underlying this phenomenon are mostly unknown. In this study, we were not able to detect obvious changes in autophagy at our level of analysis, suggesting that *Pallidin* downregulation does not induce major changes in lysosomal activity and rather affects more specifically particular cargoes, as previously suggested.<sup>4,79,101</sup> Interestingly, in addition to their cytoplasmic localization, a nuclear localization has previously been reported for two BLOC1 subunits: *BLOS2*<sup>102</sup> and, in particular, *dysbindin*.<sup>1,11,103</sup> ATG8 is also found both in lysosomes and in the

nucleus where it can regulate gene expression in association with transcription factors.<sup>82,104</sup> Such lysosomal-nuclear connections open the possibility of trapping a diversity of unexpected proteins in the nucleus in normal as well as in abnormal conditions.

In *Drosophila*, protein intake, threonine intake, and D-serine levels play a role in sleep/wake regulation.<sup>52,105–107</sup> Although the changes in amino acid intake presumably change global free amino acid levels within the brain, the effect on sleep/wake regulation seems to originate from specific sleep/wake regulatory networks or neurotransmission systems. In humans, amino-acid-supplemented diets have been designed to improve sleep and wakefulness, on the basis of the principle that this will increase the synthesis of monoamines.<sup>71,108–110</sup> Our results point to a model independent from these previously identified mechanisms: higher recycling endosome activity in the early part of the night, mediated by BLOC1, would lead to high LAT1-like activity and essential amino acid import in the brain. The TOR signaling appears also to be required and may facilitate LAT1-like transporter function. Our assessment suggests that this *Pallidin*-dependent sleep regulation does not involve a modulation of monoamine levels, in contrast with previous reports.<sup>18,70</sup> However, in these reports the conclusions were reached using complete knock out and not by downregulation in specific cell types, making comparison with the present results difficult. For example, *Drosophila* dysbindin has been suggested to regulate global brain dopamine level by modulating its recycling through glial cells.<sup>18</sup> In contrast, our data suggest that essential amino acid import to the brain facilitated by *Pallidin* would enhance GABAergic transmission required for sleep. We show here that a large number of GABAergic neurons in the adult fly brain are in direct cellular contacts with SG. Although the significance and the function of these contacts remain to be determined, these observations fit the idea that a significant subset of these neurons have particular metabolic needs.

How could amino acid transport regulate GABAergic transmission? Amino acids are at the interplay among many processes, being involved in protein synthesis, energy metabolism, neurotransmitter synthesis, and degradation. Amino acid transporters may further intertwine these processes: as a prime example, LAT1 is an antiporter that can associate the import of leucine to the export of glutamine, two amino acids that are crucial in the glutamate/GABA/glutamine cycle in the brain.<sup>76,111</sup> Pharmacological experiments<sup>112</sup> have indeed suggested that LAT1 in the BBB regulates GABA homeostasis in the interstitial fluid. BCAA and leucine in particular are thought to be critical providers of nitrogen for glutamate synthesis through transamination,<sup>111</sup> affecting glutamate/GABA/glutamine cycling. Enzymes for BCAA metabolism are conserved in *Drosophila*, suggesting similar metabolic regulation in this model organism. An interesting example is *Drosophila* mutants for GABA transaminase (GABAT),<sup>39</sup> which sleep 2–3 h longer than their genetic controls because of impaired GABA degradation. For survival, these mutants require that food include glutamate or BCAAs (leucine and valine), whose transamination can provide glutamate in the cells. These data indicate that GABA metabolism and BCAAs as a source of glutamate play a substantial role in sleep/wake regulation and brain energy metabolism. Interestingly, we find

here that the GABA/glutamate ratio tends to be decreased in *Pallidin* knockdown flies, while the glutamate/aspartate ratio is increased compared with controls. These results suggest that BLOC1 function in SG modulates neurotransmitter and brain amino acid metabolism on a global level and may control the inhibitory/excitatory balance.

The data presented here emphasize the implication of circulating amino acids, in particular BCAA, in sleep/wake regulation, corroborating several recent metabolomics studies in mammalian models and in humans,<sup>113,114</sup> in which branched-chain amino acid (BCAA) levels have been repeatedly shown to be modulated by the circadian clock and/or the sleep homeostat. For example, a recent study identified in insomniac patients prominent changes in the levels of several circulating BCAA, including increased levels of leucine during the night.<sup>115</sup> Food supplemented with BCAA can correct sleep disorders in a mouse model with chronic sleep disruption.<sup>116</sup> It is worth noting that genes involved in amino acid transport are among those commonly disrupted in autism and schizophrenia.<sup>117</sup> In conclusion, this study provides potential mechanisms at the blood-brain interface that may be relevant to both sleep disruption and psychosis and emphasizes the possibility of diet-based therapies.

#### Limitations of the study

One limitation of this study has been our inability to monitor *Pallidin* protein expression using immunofluorescence. A previously published antibody<sup>16</sup> produced a non-specific signal in the adult brain, and we unsuccessfully attempted to generate our own antibody. This precluded us from determining the subcellular localization of the protein and assessing the local efficiency of the knockdown constructs. The lack of appropriate drivers prevented us to manipulate more precisely the neuronal targets affected by *Pallidin* function: the GABAergic neurons presynaptic to ILNv neurons are unknown, and therefore there is no identified driver making it possible to monitor or control their activity, and in addition, there are no available LexA construct specifically expressed in ILNv neurons. For future investigations, it will be important to develop an efficient conditional system, independent of temperature, to limit the knockdown of *Pallidin* to the adult stage. Finally, further work is required to elucidate the involvement of the TOR signaling pathway in this context and the *Pallidin*-Jhl-21-dependent interactions between SG and GABAergic neurons.

#### STAR★METHODS

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

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
  - Fly stocks and husbandry

● **METHOD DETAILS**

- Sleep recording, circadian rhythms evaluation
- Immunofluorescence
- GRASP experiment
- Leucine supplementation
- L-DOPA feeding
- Feeding and negative geotaxis assays
- Analysis of tissue amino acid content
- Analysis of dopamine and serotonin content
- Quantitative real-time PCR

● **QUANTIFICATION AND STATISTICAL ANALYSIS**

**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at <https://doi.org/10.1016/j.celrep.2023.113025>.

**ACKNOWLEDGMENTS**

We thank the Flybase, the Bloomington Drosophila Stock Center, the Vienna Drosophila RNAi Center, S. Birman, M. Bourouis, B. Mollereau, and I. Alliaga for fly stocks, Lorie Meunier and Paul Sauvageot for the HPLC protocol implementation for amino acid content analysis, and Serge Birman for critical reading of the manuscript. This work was funded by Inserm (<https://www.inserm.fr>), CNRS (<https://www.cnrs.fr/fr/page-daccueil>), Université Claude Bernard Lyon 1 (<https://www.univ-lyon1.fr>), région Auvergne-Rhône-Alpes, and SFRMS ([www.sfrms-sommeil.org](http://www.sfrms-sommeil.org)). H.L. received a scholarship from the China Scholarship Council (CSC, <https://www.chinesescholarshipcouncil.com>), and S.A. received a scholarship from Université de Tunis El Manar (<http://www.utm.mu.tn>).

**AUTHOR CONTRIBUTIONS**

H.L. performed the experiments, analyzed and interpreted data, provided visualization of the data, and edited the manuscript. S.A. performed the experiments, analyzed and interpreted data, and provided visualization of the data. S.P. designed and performed the HPLC experiments, developed the HPLC protocol for single brains, and edited the manuscript. C.S.-B. performed the qPCR experiments. C.B. developed and produced transgenes for the project. J.-S.L. contributed to funding and initial data. L.S. designed and performed the experiments, analyzed and interpreted data, and wrote the first draft of the manuscript.

**DECLARATION OF INTERESTS**

The authors declare no competing interests.

Received: December 28, 2022

Revised: June 16, 2023

Accepted: August 9, 2023

Published: September 7, 2023

**REFERENCES**

1. Ghiani, C.A., and Dell'Angelica, E.C. (2011). Dysbindin-containing complexes and their proposed functions in brain: from zero to (too) many in a decade. *ASN NEURO* 3, e00058. <https://doi.org/10.1042/AN20110010>.
2. Lee, H.H., Nemecek, D., Schindler, C., Smith, W.J., Ghirlando, R., Steven, A.C., Bonifacino, J.S., and Hurley, J.H. (2012). Assembly and Architecture of Biogenesis of Lysosome-related Organelles Complex-1 (BLOC-1). *J. Biol. Chem.* 287, 5882–5890. <https://doi.org/10.1074/jbc.M111.325746>.
3. Ryder, P.V., and Faundez, V. (2009). Schizophrenia: The 'BLOC' May Be in the Endosomes. *Sci. Signal.* 2, pe66. <https://doi.org/10.1126/scisignal.293pe66>.
4. Delevoeye, C., Heiligenstein, X., Ripoll, L., Gilles-Marsens, F., Dennis, M.K., Linares, R.A., Derman, L., Gokhale, A., Morel, E., Faundez, V., et al. (2016). BLOC-1 brings together the actin and microtubule cytoskeletons to generate recycling endosomes. *Curr. Biol.* 26, 1–13. <https://doi.org/10.1016/j.cub.2015.11.020>.
5. Rodriguez-Fernandez, I.A., and Dell'Angelica, E.C. (2009). A data-mining approach to rank candidate protein-binding partners—The case of biogenesis of lysosome-related organelles complex-1 (BLOC-1). *J. Inherit. Metab. Dis.* 32, 190–203. <https://doi.org/10.1007/s10545-008-1014-7>.
6. Dell'Angelica, E.C. (2016). Melanosomes made from recycling (endosomes): A tubule-stabilizing function revealed for Biogenesis of Lysosome-related Organelles Complex-1. *Pigment Cell Melanoma Res.* 29, 258–259. <https://doi.org/10.1111/pcmr.12456>.
7. Dennis, M.K., Delevoeye, C., Acosta-Ruiz, A., Hurbain, I., Romao, M., Hesketh, G.G., Goff, P.S., Sviderskaya, E.V., Bennett, D.C., Luzio, J.P., et al. (2016). BLOC-1 and BLOC-3 regulate VAMP7 cycling to and from melanosomes via distinct tubular transport carriers. *J. Cell Biol.* 214, 293–308. <https://doi.org/10.1083/jcb.201605090>.
8. Li, W., Rusiniak, M.E., Chintala, S., Gautam, R., Novak, E.K., and Swank, R.T. (2004). Murine Hermansky-Pudlak syndrome genes: regulators of lysosome-related organelles. *Bioessays* 26, 616–628. <https://doi.org/10.1002/bies.20042>.
9. Li, W., Hao, C.-J., Hao, Z.-H., Ma, J., Wang, Q.-C., Yuan, Y.-F., Gong, J.-J., Chen, Y.-Y., Yu, J.-Y., and Wei, A.-H. (2022). New insights into the pathogenesis of Hermansky-Pudlak syndrome. *Pigment Cell Melanoma Res. n/a.* 35, 290–302. <https://doi.org/10.1111/pcmr.13030>.
10. Hartwig, C., Monis, W.J., Chen, X., Dickman, D.K., Pazour, G.J., and Faundez, V. (2018). Neurodevelopmental Disease Mechanisms, Primary Cilia, and Endosomes Converge on the BLOC-1 and BORC Complexes. *Dev. Neurobiol.* 78, 311–330. <https://doi.org/10.1002/dneu.22542>.
11. Talbot, K., Louneva, N., Cohen, J.W., Kazi, H., Blake, D.J., and Arnold, S.E. (2011). Synaptic Dysbindin-1 Reductions in Schizophrenia Occur in an Isoform-Specific Manner Indicating Their Subsynaptic Location. *PLoS One* 6, e16886. <https://doi.org/10.1371/journal.pone.0016886>.
12. Talbot, K., Eidem, W.L., Tinsley, C.L., Benson, M.A., Thompson, E.W., Smith, R.J., Hahn, C.-G., Siegel, S.J., Trojanowski, J.Q., Gur, R.E., et al. (2004). Dysbindin-1 is reduced in intrinsic, glutamatergic terminals of the hippocampal formation in schizophrenia. *J. Clin. Invest.* 113, 1353–1363. <https://doi.org/10.1172/JCI200420425>.
13. Tang, J., LeGros, R.P., Louneva, N., Yeh, L., Cohen, J.W., Hahn, C.-G., Blake, D.J., Arnold, S.E., and Talbot, K. (2009). Dysbindin-1 in dorsolateral prefrontal cortex of schizophrenia cases is reduced in an isoform-specific manner unrelated to dysbindin-1 mRNA expression. *Hum. Mol. Genet.* 18, 3851–3863. <https://doi.org/10.1093/hmg/ddp329>.
14. Luciano, M., Miyajima, F., Lind, P.A., Bates, T.C., Horan, M., Harris, S.E., Wright, M.J., Ollier, W.E., Hayward, C., Pendleton, N., et al. (2009). Variation in the dysbindin gene and normal cognitive function in three independent population samples. *Gene Brain Behav.* 8, 218–227. <https://doi.org/10.1111/j.1601-183X.2008.00462.x>.
15. Zhang, J.-P., Burdick, K.E., Lencz, T., and Malhotra, A.K. (2010). Meta-analysis of Genetic Variation in DTNBP1 and General Cognitive Ability. *Biol. Psychiatr.* 68, 1126–1133. <https://doi.org/10.1016/j.biopsych.2010.09.016>.
16. Chen, X., Ma, W., Zhang, S., Paluch, J., Guo, W., and Dickman, D.K. (2017). The BLOC-1 Subunit Pallidin Facilitates Activity-Dependent Synaptic Vesicle Recycling. *eNeuro* 4, ENEURO.0335.16.2017. <https://doi.org/10.1523/ENEURO.0335-16.2017>.
17. Dickman, D.K., Tong, A., and Davis, G.W. (2012). Snapin is critical for presynaptic homeostatic plasticity. *J. Neurosci.* 32, 8716–8724. <https://doi.org/10.1523/JNEUROSCI.5465-11.2012>.

18. Shao, L., Shuai, Y., Wang, J., Feng, S., Lu, B., Li, Z., Zhao, Y., Wang, L., and Zhong, Y. (2011). Schizophrenia susceptibility gene dysbindin regulates glutamatergic and dopaminergic functions via distinctive mechanisms in *Drosophila*. *Proc. Natl. Acad. Sci. USA* *108*, 18831–18836. <https://doi.org/10.1073/pnas.1114569108>.
19. Cheli, V.T., Daniels, R.W., Godoy, R., Hoyle, D.J., Kandachar, V., Starcevic, M., Martinez-Agosto, J.A., Poole, S., DiAntonio, A., Lloyd, V.K., et al. (2010). Genetic modifiers of abnormal organelle biogenesis in a *Drosophila* model of BLOC-1 deficiency. *Hum. Mol. Genet.* *19*, 861–878. <https://doi.org/10.1093/hmg/ddp555>.
20. Mullin, A.P., Sadanandappa, M.K., Ma, W., Dickman, D.K., VijayRaghavan, K., Ramaswami, M., Sanyal, S., and Faundez, V. (2015). Gene Dosage in the Dysbindin Schizophrenia Susceptibility Network Differentially Affect Synaptic Function and Plasticity. *J. Neurosci.* *35*, 325–338. <https://doi.org/10.1523/JNEUROSCI.3542-14.2015>.
21. Hidalgo, S., Castro, C., Zárate, R.V., Valderrama, B.P., Hodge, J.J.L., and Campusano, J.M. (2020). The behavioral and neurochemical characterization of a *Drosophila* dysbindin mutant supports the contribution of serotonin to schizophrenia negative symptoms. *Neurochem. Int.* *138*, 104753. <https://doi.org/10.1016/j.neuint.2020.104753>.
22. Ji, Y., Yang, F., Papaleo, F., Wang, H.-X., Gao, W.-J., Weinberger, D.R., and Lu, B. (2009). Role of dysbindin in dopamine receptor trafficking and cortical GABA function. *Proc. Natl. Acad. Sci. USA* *106*, 19593–19598. <https://doi.org/10.1073/pnas.0904289106>.
23. Lee, F.Y., Wang, H.-B., Hitchcock, O.N., Loh, D.H., Whittaker, D.S., Kim, Y.-S., Aiken, A., Kokikian, C., Dell'Angelica, E.C., Colwell, C.S., and Ghiani, C.A. (2018). Sleep/Wake Disruption in a Mouse Model of BLOC-1 Deficiency. *Front. Neurosci.* *12*, 759. <https://doi.org/10.3389/fnins.2018.00759>.
24. Dickman, D.K., and Davis, G.W. (2009). The schizophrenia susceptibility gene dysbindin controls synaptic homeostasis. *Science* *326*, 1127–1130. <https://doi.org/10.1126/science.1179685>.
25. Ghiani, C.A., Starcevic, M., Rodriguez-Fernandez, I.A., Nazarian, R., Cheli, V.T., Chan, L.N., Malvar, J.S., de Vellis, J., Sabatti, C., and Dell'Angelica, E. (2010). The dysbindin-containing complex (BLOC-1) in brain: developmental regulation, interaction with SNARE proteins, and role in neurite outgrowth. *Mol. Psychiatr.* *15*, 115–215. <https://doi.org/10.1038/mp.2009.58>.
26. Larimore, J., Ryder, P.V., Kim, K.-Y., Ambrose, L.A., Chapleau, C., Calfa, G., Gross, C., Bassell, G.J., Pozzo-Miller, L., Smith, Y., et al. (2013). MeCP2 regulates the synaptic expression of a Dysbindin-BLOC-1 network component in mouse brain and human induced pluripotent stem cell-derived neurons. *PLoS One* *8*, e65069. <https://doi.org/10.1371/journal.pone.0065069>.
27. Seugnet, L., Anaclet, C., Perier, M., Ghersi-Egea, J.-F., and Lin, J.-S. (2022). A marked enhancement of a BLOC-1 gene, Pallidin, associated with somnolent mouse models deficient in histamine transmission. *CNS Neurosci. Ther.* *29*, 483–486. <https://doi.org/10.1111/cns>.
28. Ferrarelli, F. (2020). Sleep disturbances in Schizophrenia and Psychosis. *Schizophr. Res.* *221*, 1–3. <https://doi.org/10.1016/j.schres.2020.05.022>.
29. Waite, F., Sheaves, B., Isham, L., Reeve, S., and Freeman, D. (2020). Sleep and schizophrenia: From epiphenomenon to treatable causal target. *Schizophr. Res.* *221*, 44–56. <https://doi.org/10.1016/j.schres.2019.11.014>.
30. Lai, M., Hegde, R., Kelly, S., Bannai, D., Lizano, P., Stickgold, R., Manooch, D.S., and Keshavan, M. (2022). Investigating sleep spindle density and schizophrenia: A meta-analysis. *Psychiatr. Res.* *307*, 114265. <https://doi.org/10.1016/j.psychres.2021.114265>.
31. Ettinger, U., and Kumari, V. (2015). Effects of sleep deprivation on inhibitory biomarkers of schizophrenia: implications for drug development. *Lancet Psychiatr.* *2*, 1028–1035. [https://doi.org/10.1016/S2215-0366\(15\)00313-2](https://doi.org/10.1016/S2215-0366(15)00313-2).
32. Petrovsky, N., Ettinger, U., Hill, A., Frenzel, L., Meyhöfer, I., Wagner, M., Backhaus, J., and Kumari, V. (2014). Sleep deprivation disrupts prepulse inhibition and induces psychosis-like symptoms in healthy humans. *J. Neurosci.* *34*, 9134–9140. <https://doi.org/10.1523/JNEUROSCI.0904-14.2014>.
33. Yoshihara, M., Ensminger, A.W., and Littleton, J.T. (2001). Neurobiology and the *Drosophila* genome. *Funct. Integr. Genomics* *1*, 235–240. <https://doi.org/10.1007/s101420000029>.
34. Nichols, C.D. (2006). *Drosophila melanogaster* neurobiology, neuropharmacology, and how the fly can inform central nervous system drug discovery. *Pharmacol. Ther.* *112*, 677–700. <https://doi.org/10.1016/j.pharmthera.2006.05.012>.
35. Chakravarti, L., Moscato, E.H., and Kayser, M.S. (2017). Chapter Eight - Unraveling the Neurobiology of Sleep and Sleep Disorders Using *Drosophila*. In *Current Topics in Developmental Biology Fly Models of Human Diseases*, L. Pick, ed. (Academic Press), pp. 253–285. <https://doi.org/10.1016/bs.ctdb.2016.07.010>.
36. Ingiosi, A.M., and Frank, M.G. (2023). Goodnight, astrocyte: waking up to astroglial mechanisms in sleep. *FEBS J.* *290*, 2553–2564. <https://doi.org/10.1111/febs.16424>.
37. Artiushin, G., and Sehgal, A. (2020). The Glial Perspective on Sleep and Circadian Rhythms. *Annu. Rev. Neurosci.* *43*, 119–140. <https://doi.org/10.1146/annurev-neuro-091819-094557>.
38. Farca Luna, A.J., Perier, M., and Seugnet, L. (2017). Amyloid Precursor Protein in *Drosophila* Glia Regulates Sleep and Genes Involved in Glutamate Recycling. *J. Neurosci.* *37*, 4289–4300. <https://doi.org/10.1523/JNEUROSCI.2826-16.2017>.
39. Chen, W.-F., Maguire, S., Sowcik, M., Luo, W., Koh, K., and Sehgal, A. (2015). A neuron-glia interaction involving GABA transaminase contributes to sleep loss in sleepless mutants. *Mol. Psychiatr.* *20*, 240–251. <https://doi.org/10.1038/mp.2014.11>.
40. Coll-Tané, M., Gong, N.N., Belfer, S.J., van Renssen, L.V., Kurtz-Nelson, E.C., Szuperak, M., Eidhof, I., van Reijmersdal, B., Terwindt, I., Durkin, J., et al. (2021). The CHD8/CHD7/Kismet family links blood-brain barrier glia and serotonin to ASD-associated sleep defects. *Sci. Adv.* *7*, eabe2626. <https://doi.org/10.1126/sciadv.abe2626>.
41. Seugnet, L., Suzuki, Y., Merlin, G., Gottschalk, L., Duntley, S.P., and Shaw, P.J. (2011). Notch signaling modulates sleep homeostasis and learning after sleep deprivation in *Drosophila*. *Curr. Biol.* *21*, 835–840. <https://doi.org/10.1016/j.cub.2011.04.001>.
42. Halassa, M.M., Florian, C., Fellin, T., Munoz, J.R., Lee, S.-Y., Abel, T., Haydon, P.G., and Frank, M.G. (2009). Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron* *61*, 213–219. <https://doi.org/10.1016/j.neuron.2008.11.024>.
43. Artiushin, G., Zhang, S.L., Tricoire, H., and Sehgal, A. (2018). Endocytosis at the *Drosophila* blood-brain barrier as a function for sleep. *Elife* *7*, e43326. <https://doi.org/10.7554/eLife.43326>.
44. Hablitz, L.M., Plá, V., Giannetto, M., Vinitzky, H.S., Stæger, F.F., Metcalfe, T., Nguyen, R., Benrais, A., and Nedergaard, M. (2020). Circadian control of brain glymphatic and lymphatic fluid flow. *Nat. Commun.* *11*, 4411. <https://doi.org/10.1038/s41467-020-18115-2>.
45. Duffy, J.B. (2002). GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. *Genesis* *34*, 1–15. <https://doi.org/10.1002/gene.10150>.
46. Shaw, P.J., Cirelli, C., Greenspan, R.J., and Tononi, G. (2000). Correlates of sleep and waking in *Drosophila melanogaster*. *Science* *287*, 1834–1837. <https://doi.org/10.1126/science.287.5459.1834>.
47. Hendricks, J.C., Finn, S.M., Panckeri, K.A., Chavkin, J., Williams, J.A., Sehgal, A., and Pack, A.I. (2000). Rest in *Drosophila* is a sleep-like state. *Neuron* *25*, 129–138. [https://doi.org/10.1016/s0896-6273\(00\)80877-6](https://doi.org/10.1016/s0896-6273(00)80877-6).
48. DeSalvo, M.K., Mayer, N., Mayer, F., and Bainton, R.J. (2011). Physiologic and anatomic characterization of the brain surface glia barrier of *Drosophila*. *Glia* *59*, 1322–1340. <https://doi.org/10.1002/glia.21147>.
49. Andretic, R., and Shaw, P.J. (2005). Essentials of Sleep Recordings in *Drosophila*: Moving Beyond Sleep Time. In *Methods in Enzymology*



- Circadian Rhythms, M.W. Young, ed. (Academic Press), pp. 759–772. [https://doi.org/10.1016/S0076-6879\(05\)93040-1](https://doi.org/10.1016/S0076-6879(05)93040-1).
50. DeSalvo, M.K., Hindle, S.J., Rusan, Z.M., Orng, S., Eddison, M., Halliwill, K., and Bainton, R.J. (2014). The *Drosophila* surface glia transcriptome: evolutionary conserved blood-brain barrier processes. *Front. Neurosci.* **8**, 346. <https://doi.org/10.3389/fnins.2014.00346>.
  51. Crocker, A., Shahidullah, M., Levitan, I.B., and Sehgal, A. (2010). Identification of a neural circuit that underlies the effects of octopamine on sleep:wake behavior. *Neuron* **65**, 670–681. <https://doi.org/10.1016/j.neuron.2010.01.032>.
  52. Brown, E.B., Shah, K.D., Faville, R., Kottler, B., and Keene, A.C. (2020). *Drosophila* insulin-like peptide 2 mediates dietary regulation of sleep intensity. *PLoS Genet.* **16**, e1008270. <https://doi.org/10.1371/journal.pgen.1008270>.
  53. Cong, X., Wang, H., Liu, Z., He, C., An, C., and Zhao, Z. (2015). Regulation of Sleep by Insulin-like Peptide System in *Drosophila melanogaster*. *Sleep* **38**, 1075–1083. <https://doi.org/10.5665/sleep.4816>.
  54. Metaxakis, A., Tain, L.S., Grönke, S., Hendrich, O., Hinze, Y., Birras, U., and Partridge, L. (2014). Lowered Insulin Signalling Ameliorates Age-Related Sleep Fragmentation in *Drosophila*. *PLoS Biol.* **12**, e1001824. <https://doi.org/10.1371/journal.pbio.1001824>.
  55. McGuire, S.E., Mao, Z., and Davis, R.L. (2004). Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in *Drosophila*. *Sci. STKE* **2004**, pl6. <https://doi.org/10.1126/stke.2202004pl6>.
  56. Parisky, K.M., Agostó Rivera, J.L., Donelson, N.C., Kotecha, S., and Griffith, L.C. (2016). Reorganization of Sleep by Temperature in *Drosophila* Requires Light, the Homeostat, and the Circadian Clock. *Curr. Biol.* **26**, 882–892. <https://doi.org/10.1016/j.cub.2016.02.011>.
  57. Faville, R., Kottler, B., Goodhill, G.J., Shaw, P.J., and van Swinderen, B. (2015). How deeply does your mutant sleep? Probing arousal to better understand sleep defects in *Drosophila*. *Sci. Rep.* **5**, 8454. <https://doi.org/10.1038/srep08454>.
  58. van Alphen, B., Yap, M.H.W., Kirszenblat, L., Kottler, B., and van Swinderen, B. (2013). A Dynamic Deep Sleep Stage in *Drosophila*. *J. Neurosci.* **33**, 6917–6927. <https://doi.org/10.1523/JNEUROSCI.0061-13.2013>.
  59. Yu, Q., Jacquier, A.C., Citri, Y., Hamblen, M., Hall, J.C., and Rosbash, M. (1987). Molecular mapping of point mutations in the period gene that stop or speed up biological clocks in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **84**, 784–788. <https://doi.org/10.1073/pnas.84.3.784>.
  60. Agosto, J., Choi, J.C., Parisky, K.M., Stilwell, G., Rosbash, M., and Griffith, L.C. (2008). Modulation of GABAA receptor desensitization uncouples sleep onset and maintenance in *Drosophila*. *Nat. Neurosci.* **11**, 354–359. <https://doi.org/10.1038/nn2046>.
  61. Liu, S., Lamaze, A., Liu, Q., Tabuchi, M., Yang, Y., Fowler, M., Bharadwaj, R., Zhang, J., Bedont, J., Blackshaw, S., et al. (2014). WIDE AWAKE Mediates the Circadian Timing of Sleep Onset. *Neuron* **82**, 151–166. <https://doi.org/10.1016/j.neuron.2014.01.040>.
  62. Chung, B.Y., Kilman, V.L., Keath, J.R., Pitman, J.L., and Allada, R. (2009). The GABAA Receptor RDL Acts in Peptidergic PDF Neurons to Promote Sleep in *Drosophila*. *Curr. Biol.* **19**, 386–390. <https://doi.org/10.1016/j.cub.2009.01.040>.
  63. Li, Q., Li, Y., Wang, X., Qi, J., Jin, X., Tong, H., Zhou, Z., Zhang, Z.C., and Han, J. (2017). Fbxl4 Serves as a Clock Output Molecule that Regulates Sleep through Promotion of Rhythmic Degradation of the GABAA Receptor. *Curr. Biol.* **27**, 3616–3625.e5. <https://doi.org/10.1016/j.cub.2017.10.052>.
  64. Yap, M.H.W., Grabowska, M.J., Rohrscheib, C., Jeans, R., Troup, M., Paulk, A.C., van Alphen, B., Shaw, P.J., and van Swinderen, B. (2017). Oscillatory brain activity in spontaneous and induced sleep stages in flies. *Nat. Commun.* **8**, 1815. <https://doi.org/10.1038/s41467-017-02024-y>.
  65. Dissel, S., Angadi, V., Kirszenblat, L., Suzuki, Y., Donlea, J., Klose, M., Koch, Z., English, D., Winsky-Sommerer, R., van Swinderen, B., and Shaw, P.J. (2015). Sleep restores behavioral plasticity to *Drosophila* mutants. *Curr. Biol.* **25**, 1270–1281. <https://doi.org/10.1016/j.cub.2015.03.027>.
  66. Kremer, M.C., Jung, C., Batelli, S., Rubin, G.M., and Gaul, U. (2017). The glia of the adult *Drosophila* nervous system. *Glia* **65**, 606–638. <https://doi.org/10.1002/glia.23115>.
  67. Feinberg, E.H., VanHoven, M.K., Bendesky, A., Wang, G., Fetter, R.D., Shen, K., and Bargmann, C.I. (2008). GFP Reconstitution Across Synaptic Partners (GRASP) Defines Cell Contacts and Synapses in Living Nervous Systems. *Neuron* **57**, 353–363. <https://doi.org/10.1016/j.neuron.2007.11.030>.
  68. Otto, N., Marejka, Z., Schoofs, A., Kranenburg, H., Bittern, J., Yildirim, K., Berh, D., Bethke, M., Thomas, S., Rode, S., et al. (2018). The sulfite oxidase Shopper controls neuronal activity by regulating glutamate homeostasis in *Drosophila* ensheathing glia. *Nat. Commun.* **9**, 3514. <https://doi.org/10.1038/s41467-018-05645-z>.
  69. van Liempd, S.M., Cabrera, D., Lee, F.Y., González, E., Dell'Angelica, E.C., Ghiani, C.A., and Falcon-Perez, J.M. (2017). BLOC-1 deficiency causes alterations in amino acid profile and in phospholipid and adenosine metabolism in the postnatal mouse hippocampus. *Sci. Rep.* **7**, 5231. <https://doi.org/10.1038/s41598-017-05465-z>.
  70. Cotzias, G.C., Tang, L.C., Miller, S.T., Sladic-Simic, D., and Hurley, L.S. (1972). A mutation influencing the transportation of manganese, L-dopa, and L-tryptophan. *Science* **176**, 410–412. <https://doi.org/10.1126/science.176.4033.410>.
  71. Wurtman, R.J., and Fernstrom, J.D. (1976). Control of brain neurotransmitter synthesis by precursor availability and nutritional state. *Biochem. Pharmacol.* **25**, 1691–1696. [https://doi.org/10.1016/0006-2952\(76\)90400-7](https://doi.org/10.1016/0006-2952(76)90400-7).
  72. Kageyama, T., Nakamura, M., Matsuo, A., Yamasaki, Y., Takakura, Y., Hashida, M., Kanai, Y., Naito, M., Tsuruo, T., Minato, N., and Shimohama, S. (2000). The 4F2hc/LAT1 complex transports L-DOPA across the blood-brain barrier. *Brain Res.* **879**, 115–121. [https://doi.org/10.1016/S0006-8993\(00\)02758-X](https://doi.org/10.1016/S0006-8993(00)02758-X).
  73. Duelli, R., Enerson, B.E., Gerhart, D.Z., and Drewes, L.R. (2000). Expression of large amino acid transporter LAT1 in rat brain endothelium. *J. Cerebr. Blood Flow Metabol.* **20**, 1557–1562. <https://doi.org/10.1097/00004647-200011000-00005>.
  74. Verrey, F. (2003). System L: heteromeric exchangers of large, neutral amino acids involved in directional transport. *Pflügers Archiv* **445**, 529–533. <https://doi.org/10.1007/s00424-002-0973-z>.
  75. Fernstrom, J.D., and Fernstrom, M.H. (2007). Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J. Nutr.* **137**, 1539S–1547S. <https://doi.org/10.1093/jn/137.6.1539S>.
  76. Nicklin, P., Bergman, P., Zhang, B., Triantafellow, E., Wang, H., Nyfeler, B., Yang, H., Hild, M., Kung, C., Wilson, C., et al. (2009). Bidirectional transport of amino acids regulates mTOR and autophagy. *Cell* **136**, 521–534. <https://doi.org/10.1016/j.cell.2008.11.044>.
  77. Uchizono, S., Tabuki, Y., Kawaguchi, N., Tanimura, T., and Itoh, T.Q. (2017). Mated *Drosophila melanogaster* females consume more amino acids during the dark phase. *PLoS One* **12**, e0172886. <https://doi.org/10.1371/journal.pone.0172886>.
  78. Gokhale, A., Vrillas-Mortimer, A., Larimore, J., Comstra, H.S., Zlatic, S.A., Werner, E., Manvich, D.F., Iuvone, P.M., Weinschenker, D., and Faundez, V. (2015). Neuronal copper homeostasis susceptibility by genetic defects in dysbindin, a schizophrenia susceptibility factor. *Hum. Mol. Genet.* **24**, 5512–5523. <https://doi.org/10.1093/hmg/ddv282>.
  79. Setty, S.R.G., Tenza, D., Sviderskaya, E.V., Bennett, D.C., Raposo, G., and Marks, M.S. (2008). Cell-specific ATP7A transport sustains copper-dependent tyrosinase activity in melanosomes. *Nature* **454**, 1142–1146. <https://doi.org/10.1038/nature07163>.

80. Ziegler, A.B., Manière, G., and Grosjean, Y. (2018). Jhl-21 plays a role in *Drosophila* insulin-like peptide release from larval IPCs via leucine transport. *Sci. Rep.* **8**, 1908. <https://doi.org/10.1038/s41598-018-20394-1>.
81. Aboudhief, S., Alves, G., Parrot, S., Amri, M., Simonnet, M.M., Grosjean, Y., Manière, G., and Seugnet, L. (2018). LAT1-like transporters regulate dopaminergic transmission and sleep in *Drosophila*. *Sleep* **41**. <https://doi.org/10.1093/sleep/zsy137>.
82. Jacomin, A.-C., Petridi, S., Di Monaco, M., Bhujabal, Z., Jain, A., Mulakkal, N.C., Palara, A., Powell, E.L., Chung, B., Zampronio, C., et al. (2020). Regulation of Expression of Autophagy Genes by Atg8a-Interacting Partners Sequoia, YL-1, and Sir2 in *Drosophila*. *Cell Rep.* **31**, 107695. <https://doi.org/10.1016/j.celrep.2020.107695>.
83. Murotani, T., Ishizuka, T., Hattori, S., Hashimoto, R., Matsuzaki, S., and Yamatodani, A. (2007). High dopamine turnover in the brains of Sandy mice. *Neurosci. Lett.* **421**, 47–51. <https://doi.org/10.1016/j.neulet.2007.05.019>.
84. Kume, K., Kume, S., Park, S.K., Hirsh, J., and Jackson, F.R. (2005). Dopamine is a regulator of arousal in the fruit fly. *J. Neurosci.* **25**, 7377–7384. <https://doi.org/10.1523/JNEUROSCI.2048-05.2005>.
85. Ueno, T., and Kume, K. (2014). Functional characterization of dopamine transporter in vivo using *Drosophila melanogaster* behavioral assays. *Front. Behav. Neurosci.* **8**, 303. <https://doi.org/10.3389/fnbeh.2014.00303>.
86. Sotnikova, T.D., Caron, M.G., and Gainetdinov, R.R. (2006). DDD mice, a novel acute mouse model of Parkinson's disease. *Neurology* **67**, S12–S17. [https://doi.org/10.1212/wnl.67.7\\_suppl\\_2.s12](https://doi.org/10.1212/wnl.67.7_suppl_2.s12).
87. Parisky, K.M., Agosto, J., Pulver, S.R., Shang, Y., Kuklin, E., Hodge, J.J.L., Kang, K., Liu, X., Garrity, P.A., Rosbash, M., and Griffith, L.C. (2008). PDF cells are a GABA-responsive wake-promoting component of the *Drosophila* sleep circuit. *Neuron* **60**, 672–682. <https://doi.org/10.1016/j.neuron.2008.10.042>.
88. Zhang, S.L., Yue, Z., Arnold, D.M., Artiushin, G., and Sehgal, A. (2018). A Circadian Clock in the Blood-Brain Barrier Regulates Xenobiotic Efflux. *Cell* **173**, 130–139.e10. <https://doi.org/10.1016/j.cell.2018.02.017>.
89. Li, W., Zhang, Q., Oiso, N., Novak, E.K., Gautam, R., O'Brien, E.P., Tinsley, C.L., Blake, D.J., Spritz, R.A., Copeland, N.G., et al. (2003). Hermansky-Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). *Nat. Genet.* **35**, 84–89. <https://doi.org/10.1038/ng1229>.
90. Larimore, J., Zlatic, S.A., Gokhale, A., Tornieri, K., Singleton, K.S., Mullin, A.P., Tang, J., Talbot, K., and Faundez, V. (2014). Mutations in the BLOC-1 Subunits Dysbindin and Muted Generate Divergent and Dosage-dependent Phenotypes. *J. Biol. Chem.* **289**, 14291–14300. <https://doi.org/10.1074/jbc.M114.553750>.
91. Wei, M.L. (2006). Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. *Pigm. Cell Res.* **19**, 19–42. <https://doi.org/10.1111/j.1600-0749.2005.00289.x>.
92. Ulrich, D., Lalanne, T., Gassmann, M., and Bettler, B. (2018). GABAB receptor subtypes differentially regulate thalamic spindle oscillations. *Neuropharmacology* **136**, 106–116. <https://doi.org/10.1016/j.neuropharm.2017.10.033>.
93. Zhang, J., Yetton, B., Whitehurst, L.N., Naji, M., and Mednick, S.C. (2020). The effect of zolpidem on memory consolidation over a night of sleep. *Sleep* **43**, zsa084. <https://doi.org/10.1093/sleep/zsaa084>.
94. Zaragoza, R. (2020). Transport of Amino Acids Across the Blood-Brain Barrier. *Front. Physiol.* **11**, 973. <https://doi.org/10.3389/fphys.2020.00973>.
95. Tărlungeanu, D.C., Deliu, E., Dotter, C.P., Kara, M., Janiesch, P.C., Scalise, M., Galluccio, M., Tesulov, M., Morelli, E., Sonmez, F.M., et al. (2016). Impaired Amino Acid Transport at the Blood Brain Barrier Is a Cause of Autism Spectrum Disorder. *Cell* **167**, 1481–1494.e18. <https://doi.org/10.1016/j.cell.2016.11.013>.
96. Reynolds, B., Roversi, P., Laynes, R., Kazi, S., Boyd, C.A.R., and Gorbodhan, D.C.I. (2009). *Drosophila* expresses a CD98 transporter with an evolutionarily conserved structure and amino acid-transport properties. *Biochem. J.* **420**, 363–372. <https://doi.org/10.1042/BJ20082198>.
97. OLSEN, M.L., and SONTHEIMER, H. (2004). Mislocalization of Kir Channels in Malignant Glia. *Glia* **46**, 63–73. <https://doi.org/10.1002/glia.10346>.
98. Varini, K., Benzaria, A., Taïeb, N., Di Scala, C., Azmi, A., Graoudi, S., and Maresca, M. (2012). Mislocalization of the excitatory amino-acid transporters (EAATs) in human astrocytoma and non-astrocytoma cancer cells: effect of the cell confluence. *J. Biomed. Sci.* **19**, 10. <https://doi.org/10.1186/1423-0127-19-10>.
99. Jans, D.A., and Hassan, G. (1998). Nuclear targeting by growth factors, cytokines, and their receptors: a role in signaling? *Bioessays* **20**, 400–411. [https://doi.org/10.1002/\(SICI\)1521-1878\(199805\)20:5<400::AID-BIES7>3.0.CO;2-R](https://doi.org/10.1002/(SICI)1521-1878(199805)20:5<400::AID-BIES7>3.0.CO;2-R).
100. Lin, S.Y., Makino, K., Xia, W., Matin, A., Wen, Y., Kwong, K.Y., Bourguignon, L., and Hung, M.C. (2001). Nuclear localization of EGF receptor and its potential new role as a transcription factor. *Nat. Cell Biol.* **3**, 802–808. <https://doi.org/10.1038/ncb0901-802>.
101. Setty, S.R.G., Tenza, D., Truschel, S.T., Chou, E., Sviderskaya, E.V., Theos, A.C., Lamoreux, M.L., Di Pietro, S.M., Starcevic, M., Bennett, D.C., et al. (2007). BLOC-1 Is Required for Cargo-specific Sorting from Vacuolar Early Endosomes toward Lysosome-related Organelles. *Mol. Biol. Cell* **18**, 768–780. <https://doi.org/10.1091/mbc.E06-12-1066>.
102. Felten, A., Leister, P., Burgdorf, S., Uhlmann, L., and Scheidtman, K.H. (2007). Characterization of rat BLOS2/Ceap, a putative yeast She3 homolog, as interaction partner of apoptosis antagonizing transcription factor/Che-1. *Biol. Chem.* **388**, 569–582. <https://doi.org/10.1515/BC.2007.073>.
103. Fei, E., Ma, X., Zhu, C., Xue, T., Yan, J., Xu, Y., Zhou, J., and Wang, G. (2010). Nucleocytoplasmic Shuttling of Dysbindin-1, a Schizophrenia-related Protein, Regulates Synapsin I Expression. *J. Biol. Chem.* **285**, 38630–38640. <https://doi.org/10.1074/jbc.M110.107912>.
104. Jacomin, A.-C., Petridi, S., Di Monaco, M., and Nezis, I.P. (2020). A nuclear role for Atg8-family proteins. *Autophagy* **16**, 1721–1723. <https://doi.org/10.1080/15548627.2020.1794356>.
105. Dai, X., Zhou, E., Yang, W., Zhang, X., Zhang, W., and Rao, Y. (2019). D-Serine made by serine racemase in *Drosophila* intestine plays a physiological role in sleep. *Nat. Commun.* **10**, 1986. <https://doi.org/10.1038/s41467-019-09544-9>.
106. Ki, Y., and Lim, C. (2019). 17jul2019). Sleep-promoting effects of threonine link amino acid metabolism in *Drosophila* neuron to GABAergic control of sleep drive. *Elife* **8**, e40593. <https://doi.org/10.7554/eLife.40593>.
107. Murphy, K.R., Deshpande, S.A., Yurgel, M.E., Quinn, J.P., Weissbach, J.L., Keene, A.C., Dawson-Scully, K., Huber, R., Tomchik, S.M., and Ja, W.W. (2016). Postprandial sleep mechanics in *Drosophila*. *Elife* **5**, e19334. <https://doi.org/10.7554/eLife.19334>.
108. Zhao, M., Tuo, H., Wang, S., and Zhao, L. (2020). The Effects of Dietary Nutrition on Sleep and Sleep Disorders. *Mediators Inflamm.* **2020**, 3142874. <https://doi.org/10.1155/2020/3142874>.
109. Silber, B.Y., and Schmitt, J.A.J. (2010). Effects of tryptophan loading on human cognition, mood, and sleep. *Neurosci. Biobehav. Rev.* **34**, 387–407. <https://doi.org/10.1016/j.neubiorev.2009.08.005>.
110. Halson, S.L. (2014). Sleep in Elite Athletes and Nutritional Interventions to Enhance Sleep. *Sports Med.* **44**, 13–23. <https://doi.org/10.1007/s40279-014-0147-0>.
111. Yudkoff, M. (2017). Interactions in the Metabolism of Glutamate and the Branched-Chain Amino Acids and Ketoacids in the CNS. *Neurochem. Res.* **42**, 10–18. <https://doi.org/10.1007/s11064-016-2057-z>.
112. Dolgodilina, E., Imobersteg, S., Laczko, E., Welt, T., Verrey, F., and Makrides, V. (2016). Brain interstitial fluid glutamine homeostasis is controlled by blood-brain barrier SLC7A5/LAT1 amino acid transporter. *J. Cerebr.*

- Blood Flow Metabol. 36, 1929–1941. <https://doi.org/10.1177/0271678X15609331>.
113. Bourdon, A.K., Spano, G.M., Marshall, W., Bellesi, M., Tononi, G., Serra, P.A., Baghdoyan, H.A., Lydic, R., Campagna, S.R., and Cirelli, C. (2018). Metabolomic analysis of mouse prefrontal cortex reveals upregulated analytes during wakefulness compared to sleep. *Sci. Rep.* 8, 11225. <https://doi.org/10.1038/s41598-018-29511-6>.
114. Malik, D.M., Paschos, G.K., Sehgal, A., and Weljie, A.M. (2020). Circadian and Sleep Metabolomics Across Species. *J. Mol. Biol.* 432, 3578–3610. <https://doi.org/10.1016/j.jmb.2020.04.027>.
115. Gehrman, P., Sengupta, A., Harders, E., Ubeydullah, E., Pack, A.I., and Weljie, A. (2018). Altered diurnal states in insomnia reflect peripheral hyperarousal and metabolic desynchrony: a preliminary study. *Sleep* 41, zsy043. <https://doi.org/10.1093/sleep/zsy043>.
116. Lim, M.M., Elkind, J., Xiong, G., Galante, R., Zhu, J., Zhang, L., Lian, J., Rodin, J., Kuzma, N.N., Pack, A.I., and Cohen, A.S. (2013). Dietary Therapy Mitigates Persistent Wake Deficits Caused by Mild Traumatic Brain Injury. *Sci. Transl. Med.* 5, 215ra173. <https://doi.org/10.1126/scitranslmed.3007092>.
117. Guan, J., Cai, J.J., Ji, G., and Sham, P.C. (2019). Commonality in dysregulated expression of gene sets in cortical brains of individuals with autism, schizophrenia, and bipolar disorder. *Transl. Psychiatry* 9, 152. <https://doi.org/10.1038/s41398-019-0488-4>.
118. Shaw, P.J., Tononi, G., Greenspan, R.J., and Robinson, D.F. (2002). Stress response genes protect against lethal effects of sleep deprivation in *Drosophila*. *Nature* 417, 287–291. <https://doi.org/10.1038/417287a>.
119. Schmid, B., Helfrich-Förster, C., and Yoshii, T. (2011). A new ImageJ plug-in “ActogramJ” for chronobiological analyses. *J. Biol. Rhythm.* 26, 464–467. <https://doi.org/10.1177/0748730411414264>.
120. Sun, J., Xu, A.Q., Giraud, J., Poppinga, H., Riemensperger, T., Fiala, A., and Birman, S. (2018). Neural Control of Startle-Induced Locomotion by the Mushroom Bodies and Associated Neurons in *Drosophila*. *Front. Syst. Neurosci.* 12, 6. <https://doi.org/10.3389/fnsys.2018.00006>.
121. Mertens, P., Ghaemmaghami, C., Bert, L., Perret-Liaudet, A., Guenot, M., Naous, H., Laganier, L., Later, R., Sindou, M., and Renaud, B. (2001). Microdialysis study of amino acid neurotransmitters in the spinal dorsal horn of patients undergoing microsurgical dorsal root entry zone lesioning. Technical note. *J. Neurosurg.* 94, 165–173. <https://doi.org/10.3171/spi.2001.94.1.0165>.

## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
Rabbit anti-JHI-21	Y. Grosjean, University of Burgundy, France	N/A
Mouse anti-GFP	University of Iowa Developmental Studies Hybridoma Bank	RRID:AB_2617423
Mouse anti-β-galactosidase	Promega	RRID:AB_2313752
Anti-rabbit IgG-Alexa Fluor 633	Thermo Fisher Scientific	RRID:AB_2535731
Anti-mouse IgG-Alexa Fluor 488	Thermo Fisher Scientific	RRID:AB_2534069
<b>Chemicals, peptides, and recombinant proteins</b>		
Normal Goat Serum	SIGMA	Cat# G9023
Triton X100	SIGMA	Cat# T8787
Rhodamine Phalloidin	ABCAM	Cat# ab235138
DAPI	SIGMA	Cat# MBD0015
Vectashield medium	Vector Laboratory	Cat# H-1200
THIP	SIGMA	Cat# T101
Leucine	SIGMA	Cat# L8000
Valine	SIGMA	Cat# V0500
Tryptophan	SIGMA	Cat# T0254
L-DOPA	SIGMA	Cat# D9628
Methanol for analysis	Prolabo	Cat# 20847295
Acetonitrile for analysis	Merck	Lichrosolv
Tetrahydrofuran for analysis	Sigma	T5267
Ringer's solution	Cold Spring Harbor Protocols	<a href="https://cshprotocols.cshlp.org/content/2007/4/pdb.rec10919.full">https://cshprotocols.cshlp.org/content/2007/4/pdb.rec10919.full</a>
<b>Experimental models: Organisms/strains</b>		
Cs (Canton S)	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0064349
UAS-Pallidin-RNAi <sup>GD13391</sup>	Vienna <i>Drosophila</i> Resource Center	Flybase:FBst0454949 FBst0454948
UAS-Pallidin-RNAi <sup>HMS05728</sup>	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0067884
UAS-dicer2	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0024646
UAS-Sugb-RNAi <sup>KK111268</sup>	Vienna <i>Drosophila</i> Resource Center	Flybase:FBst0478221
da-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBal0042573
Tub-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0005138
repo-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0007415
Eaat1-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0008849
Alrm-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0067031
NP2222-Gal4	Kyoto Stock Center	Flybase:FBst0315565
54C07-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0050472
85G01-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0040436
9-137-Gal4	Dr. Ulrike Heberlein	(DeSalvo et al. 2014) <sup>50</sup>
UAS-lacZ	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0008529
llp2-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0037516
elav-Gal80	Dr. Véronique Morel	(Dai et al. 2019) <sup>107</sup>
Tub-Gal80 <sup>ts</sup>	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0007016
per <sup>0</sup>	Dr. François Rouyer	Flybase:FBst0080928

(Continued on next page)

<b>Continued</b>		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
LexAOP-Kir2.1	Dr. François Rouyer	N/A
PDF-LexA	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0084429
vGAT-LexA	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0084441
LexAOP-TrpA1	Dr. Pierre-Yves Plaçais	Flybase: FBal0335427
UAS-CD4-spGFP1-10, LexAOP-CD4-spGFP11	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0058755
UAS-Blos1-RNAi <sup>30077R-3</sup>	National Institute of Genetics - FLY (NIG-FLY)	NIG-Fly:30077R-3
UAS-Blos2-RNAi <sup>HMJ21554</sup>	Vienna <i>Drosophila</i> Resource Center	Flybase:FBst0054835
UAS-dysbindin-RNAi <sup>6856R-1</sup>	National Institute of Genetics - FLY (NIG-FLY)	(Shao et al. 2011) <sup>18</sup>
UAS-snapin- RNAi <sup>JF02692</sup>	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0027541
UAS-Jhl21-RNAi <sup>KK112996</sup>	Vienna <i>Drosophila</i> Resource Center	Flybase:FBst0480319
UAS-Minidisc-RNAi <sup>KK102686</sup>	Vienna <i>Drosophila</i> Resource Center	Flybase:FBst0481798
UAS-Raptor-RNAi <sup>HMS02306</sup>	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0041912
UAS-TOR-RNAi <sup>HMS00904</sup>	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0033951
UAS-CD98hc-RNAi <sup>46622GD</sup>	Vienna <i>Drosophila</i> Resource Center	Flybase:FBst0464680
UAS-ncLacZ	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0003956
UAS-mcherry-GFP-LC3	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0037749
DAT <sup>fumin</sup>	Dr. Serge Birman	Flybase: FBal0197506 (Kume et al. 2005) <sup>85</sup>
elav-Gal4	Bloomington <i>Drosophila</i> Stock Center	Flybase:FBst0000458
<b>Oligonucleotides</b>		
RP49 forward primer: 5'-ACGACAATCTCCTTGGCCTTCT-3'	(Farca et al. 2017) <sup>38</sup>	N/A
RP49 reverse primer: 5'-ATCGTTACGGATC GAACAA-3'	(Farca et al. 2017) <sup>38</sup>	N/A
pallidin forward primer: 5'-GATTCCACCGCCCAATCTTC-3'	This Paper	N/A
pallidin reverse primer: 5'-GACTGGCCATGACTTCGTTG-3'	This Paper	N/A
<b>Software and algorithms</b>		
GraphPad Prism	GraphPad Software	<a href="https://www.graphpad.com/scientific-software/prism/">https://www.graphpad.com/scientific-software/prism/</a>
ImageJ	National Institutes of Health, USA	<a href="https://imagej.net/software/fiji/downloads">https://imagej.net/software/fiji/downloads</a>
Statel	Adscience	<a href="http://www.adscience.fr/logiciels/contenu-de-statel">http://www.adscience.fr/logiciels/contenu-de-statel</a>
Origin Software	OriginLab	<a href="https://www.originlab.com/">https://www.originlab.com/</a>
LabSolutions software	Shimadzu	V 5.32 SP1
<b>Other</b>		
Zeiss LSM 800 confocal microscope	Zeiss	N/A
<i>Drosophila</i> activity monitor	Trikinetics	DAM2 system
<i>Drosophila</i> ARousal Tracking System	(Faville et al. 2015) <sup>57</sup>	N/A
HPLC system including a Prominence degasser, a high-pressure LC-30 AD pump, a low-pressure LC-20 pump, a 20- $\mu$ L mixer, an SIL-30AC autosampler	Shimadzu	<a href="https://www.shimadzu.com/">https://www.shimadzu.com/</a>
ZETALIF laser induced fluorescence detector	Adelis	<a href="https://www.adelis-tech.com/">https://www.adelis-tech.com/</a>
DECADE II detector	Antec Leyden	<a href="https://antecscientific.com/">https://antecscientific.com/</a>

## RESOURCE AVAILABILITY

### Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Laurent Seugnet ([laurent.seugnet@inserm.fr](mailto:laurent.seugnet@inserm.fr))

### Materials availability

This study did not generate new unique reagents.

### Data and code availability

- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- Data: All datasets presented in the figures have been deposited at Mendeley Data: DOI: <https://doi.org/10.17632/65g72527mv.2> (<https://data.mendeley.com/datasets/65g72527mv/2>) and are publicly available as of the date of publication.
- Code: This paper does not report original code.

## EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

### Fly stocks and husbandry

Flies were raised on autoclaved food containing inactivated yeast (50 g/L), cornmeal (33 g/L), agar (10 g/L), molasses (30 mL/L), and sucrose (18 g/L) and maintained at 25°C, 60% humidity in a 12hr: 12hr Light: Dark (LD) cycle, except otherwise mentioned. The following stocks obtained were used in this study: from the Bloomington Drosophila Stock Center: *Canton S*, *UAS-dicer2*, *UAS-Pallidin-RNAi<sup>HMS05728</sup>*, *repo-Gal4*; *GMR54C07-Gal4*, *GMR85G01-Gal4*, *Tub-Gal80<sup>ts</sup>*, *LexAOP-Kir2.1*; *PDF-LexA*; *UAS-snapin-RNAi<sup>JF02692</sup>*, *UAS-Jhl-21-RNAi<sup>HMS02271</sup>*, *UAS-Raptor-RNAi<sup>HMS02306</sup>*, *UAS-TOR-RNAi<sup>HMS00904</sup>*, *UAS-nuclear-lacZ*, *vGAT-Gal4*; from the Vienna Drosophila Resource Center: *UAS-Pallidin-RNAi<sup>GD13391</sup>*, *UAS-Blos2-RNAi<sup>HMJ21554</sup>*, *UAS-Jhl-21-RNAi<sup>KK112996</sup>*, *UAS-Mnd-RNAi<sup>KK102686</sup>*, *UAS-Mnd-RNAi<sup>GD453</sup>*, *UAS-RNAi-CD98Hc<sup>GD46622</sup>*, *UAS-RNAi-CD98Hc<sup>HMC04939</sup>*; from the National Institute of Genetics, Mishima: *UAS-Blos1-RNAi<sup>CG30077R-3</sup>*, *UAS-dysbindin-RNAi<sup>CG6856R-1</sup>*; from A. Borst, Max Planck Institute of neurobiology: *VGAT-lexA*.

From Y. Aso, Janelia farm, HHMI: *TH-lexA*; from S. Birman, Center National de La recherche Scientifique: *LexAOP-TrpA1*, *DAT<sup>lumin</sup>*; from I. Alliaga London Institute of Medical Sciences: *Ilp2-Gal4*; from B. Mollereau, Ecole Normale Supérieure de Lyon: *UAS-GFP*, *UAS-mCherry-GFP-LC3*; from C Klämbt, Munster University: *UAS-CD4-spGFP1-10*, *LexAOP-CD4-spGFP11/TM6*. All lines were outcrossed 3 times to a Canton S reference strain or to other lines previously outcrossed to Canton S before being used for experimentation. For standard Gal4-UAS experiments, the Gal4 and UAS parental lines outcrossed to Canton S served as genetic background controls. In most experiments, the UAS-dicer2 transgene was added to the genetic background to increase the efficiency of the knockdown. At least one wild type copy of the *white* gene was present in each genotype, unless otherwise indicated. For the experiments involving the TARGET system<sup>54</sup> or *LexAOP-TrpA1* expression, flies were raised at 18°C to prevent Gal4 and TrpA1 activity, respectively. Evaluation of *Pallidin* transcripts by QPCR shows that *UAS-Pallidin-RNAi<sup>HMS05728</sup>* combined with *da-Gal4* significantly downregulates the *Pallidin* transcripts in the whole body (Supplemental data, Figure S1). Except otherwise mentioned, virgin female flies 3–10 days old were used for experiments.

## METHOD DETAILS

### Sleep recording, circadian rhythms evaluation

Freshly eclosed female flies were collected daily under CO<sub>2</sub> over 2–3 days. 2–5 days old flies were then transferred individually to 5 × 65 mm long glass tubes containing regular food medium. Sleep was recorded in a 12h:12h Light: Dark (LD) cycle at 25°C, 60% humidity using the Trikinetics DAMS system ([www.trikinetics.com](http://www.trikinetics.com)), unless otherwise mentioned. Sleep parameters were evaluated across 3–6 days of baseline as described previously using an excel macro and 5 min immobility as criteria.<sup>46,49</sup> We used the Drosophila Arousal Tracking (DART) system<sup>56</sup> for the evaluation of sleep depth. The stimulus used to evaluate sleep depth was provided by two vibrating motors stuck underneath the platform hosting the flies, for 1 s every hour, during two days. The percentage of locomotor responses were binned according to the time of immobility previous to the stimulation.<sup>58,57</sup> Sleep experiments were repeated at least 3 times unless otherwise mentioned. Distribution and homogeneity as well as statistical group comparisons were evaluated using the Microsoft Excel plugin software Statel. Mean ± SEM is plotted and the p value shown is the highest obtained among post hoc comparisons. Black box areas indicate the scotophase in the 24h hourly sleep graphs. Sleep deprivation was carried out using a Sleep Nullifying Apparatus system (SNAP) as previously described,<sup>46,118</sup> for 24hr starting at ZT (zeitgeber time) 0. For the evaluation of circadian rhythms, flies were recorded in constant darkness for 6 days, and the free-running period was calculated using locomotor data and the  $\chi^2$  method in ActogramJ.<sup>119</sup>

### Immunofluorescence

Flies were anesthetized on ice and brains dissected quickly in cold phosphate-buffered saline (PBS) solution before being fixed for 25min in a 4% paraformaldehyde PBS solution at room temperature. Flies were dissected at ZT0-2. A 5% normal goat serum (Sigma) in PBS with 0,3% Triton X- was used for blocking during at least 45min, before overnight incubation at 4°C in 0,3% Triton X- PBS with the primary antibody: rabbit anti-Jhl-21 (1/250; kindly provided by Y. Grosjean, University of Burgundy, France<sup>16,26</sup>), combined with mouse anti-GFP (8H11, from University of Iowa Developmental Studies Hybridoma Bank, 1/200), or mouse anti-β-galactosidase (Z378A from Promega, 1/1000). Secondary antibodies (anti-rabbit IgG-Alexa Fluor 633 and anti-mouse IgG-Alexa Fluor 488) were purchased from Thermo Fisher Scientific and used at 1/1000. Brains were mounted in Vectashield medium containing DAPI (Vector Laboratories). Fluorescence was observed using a confocal microscope (Zeiss LSM 800) with a 40× objective and ImageJ (FIJI) was used for image processing. DAPI fluorescence and β-galactosidase immunofluorescence were used to delineate the nuclei in neurons and surface glia, respectively. For the quantification of anti-Jhl-21 immunofluorescence in neuronal nuclei, the average pixel intensity in control brains was used to normalize the signal. For the quantification of anti-Jhl-21 immunofluorescence in perineurial and subperineurial cells, the average pixel intensity of 5 neuronal nuclei was subtracted to the signal detected in surface glial cells in the same optical section. The average pixel intensity in control subperineurial cells was the lowest observed in any cells and arbitrarily set to zero.

### GRASP experiment

The *vGAT-LexA* transgene was used to drive expression of *LexAOP-CD4-sp-GFP<sup>11</sup>* in all GABAergic neurons, while *SG-Gal4* was used to drive *UAS-CD4-sp-GFP<sup>1-10</sup>* in surface glia. Flies were dissected in PBS, fixed for 5min in a 4% paraformaldehyde PBS solution at room temperature, incubated for 10 min in 0,3% Triton X- PBS containing 1 μg/mL DAPI and 1:100 Phalloidin-rhodamine (*Abcam* ab235138), rinsed and mounted in PBS solution. Fluorescence was observed using a confocal microscope (Zeiss LSM 800) with a 40× objective and ImageJ (FIJI) was used for image processing.

### Leucine supplementation

Flies in Trikinetics tubes were recorded for 2 days and transferred at ZT0 from standard food to food containing either Leucine, valine or Tryptophan at 50mM or to tubes containing fresh standard food (control condition).

### L-DOPA feeding

For L-DOPA feeding experiments, flies in Trikinetics tubes were transferred at ZT0 from standard food to vehicle (1% agar 1% sucrose) for 24h (day 1). At ZT0 the following day, flies of each genotype were divided into two groups, one placed on fresh vehicle for 24h (“vehicle” condition) and the other one on vehicle plus L-DOPA dissolved at 3 mg/ml (day 2). The % of nighttime sleep change: (day 2 sleep – day 1 sleep)/day 1 sleep is displayed as a boxplot.

### Feeding and negative geotaxis assays

Food intake was evaluated using the CAFE assay<sup>27</sup>: in vials containing 5 female flies, food was provided by a 5 μL calibrated capillary filled with liquid food containing 5% sucrose, 5% yeast extract, 1% blue food coloring dye and either Leucine, valine or Tryptophan at 50mM or L-DOPA (3 mg/ml). Experimental readings and capillaries replacement were carried out every 12 h. An identical CAFE chamber without flies was used to determine evaporative losses (typically <10% of ingested volumes), which were subtracted from experimental readings. Startle Induced Negative Geotaxis assay (SING) assays were performed as previously described.<sup>120</sup> For each assay, 10 female flies were placed in a vertical column (25 cm long, 1.5 cm diameter) and left for about 30 min for habituation. The columns were then gently tapped down (startle) to through all the flies at the bottom. Flies having reached at least once the 22cm line after 1 min were counted. Each fly group was assayed 2 times at 15 min intervals. The SING performance index is defined as  $\frac{1}{2}[1 + (n_{top} - n_{bot})/n_{tot}]$ , where  $n_{tot}$  is the total number of flies in the column,  $n_{top}$  the number of flies at the top and  $n_{bot}$  the number of flies at the bottom after 1 min.

### Analysis of tissue amino acid content

Single brain samples from female flies were quickly dissected in 1 μL of Ringer’s solution and frozen at –80°C until tissue content analysis. The extraction was made similarly as for dopamine and serotonin content analysis, but with 3 μL of perchloric acid/EDTA/sodium bisulfite solution. Aspartate, glutamate, and GABA levels were quantified using HPLC with laser induced fluorescence detector (HPLC-LIFD) from adapted chromatographic conditions<sup>121</sup> in a low-pressure binary gradient mode. The HPLC system (Shimadzu, Japan) consisted of a Prominence degasser, a high-pressure LC-30 AD pump (as pump A), a low-pressure LC-20 pump (as pump B), a 20-μL mixer and an SIL-30AC autosampler. The mixing of the mobile phases from both pumps and fluorescence detection using a ZETALIF laser induced fluorescence detector (Picometrics, France) were performed at 23°C–24°C before injection and after separation, respectively. The detection was carried out at the outlet of the column in a fused-silica capillary serving as detection cell (i.d. 75 μm, OD 375 μm, stripped out of its polyimide layer at 50 cm (effective length), total length 60 cm, toward waste). Excitation was performed using a 410-nm diode laser (Melles Griot, USA) and the emission wavelength was set at 490 nm. Separations were performed using a 100 × 2.1 mm Kinetex C18 core-shell 2.6 μm column equipped with a KrudKatcher Ultra HPLC in-line filter. The column oven was set to 45°C. 1 μL of each extract sample was diluted 100-fold in a Ringer’s solution. The derivatization reagents were

added offline in the 100  $\mu\text{L}$  of the diluted extracts, by adding 20  $\mu\text{L}$  of borate/NaCN solution (mixing solution [250:50, v/v] of 500 mmol/L borate buffer pH 8.7, and 87 mmol/L NaCN in water), 10  $\mu\text{L}$  of ultrapure water, and, in the end, 10  $\mu\text{L}$  of NDA solution (2.925 mmol/L in acetonitrile/water, 50:50, v/v) for a 15-min reaction at 23°C–24°C.<sup>30</sup> 10  $\mu\text{L}$  of the derivatized samples were placed in the autosampler and kept at 4°C before injection. The injection volume was 2  $\mu\text{L}$ . Separations were performed at a flow rate of 0.3 mL/min ( $\sim$ 260 bars). The gradient elution conditions were as follows: buffer: 50 mmol/L phosphate buffer pH 6.8; mobile phase A: buffer/tetrahydrofuran [97:3 v/v]; mobile phase B: buffer/methanol/acetonitrile [35:10:55, v/v:v]; gradient: 22% B increased to 27% B from 0.01 to 5.08 min, increased to 33% B from 5.08 min to 6.41 min, increased to 41% B from 6.41 to 14.52 min, increased to 100% B from 14.52 to 15.85 min, kept at 100% until 21.19 min, then decreased to 22% B from 21.19 min to 22.52 min; kept at 22% B until the end of the run. The acquisition time was 27.5 min. Chromatograms were acquired at a rate of 10 Hz (Lab Solutions software). The retention times were  $\sim$ 1.8 min,  $\sim$ 2.5 min, and  $\sim$ 11.2 min for Asp, Glu, and GABA, respectively. Concentrations of the analytes in the extracts were determined by comparison of chromatographic peak areas with calibration curves derived from a mixture of the 3 synthetic standards (two points:  $10^{-6}$ ,  $5 \times 10^{-6}$  mol/L).

### Analysis of dopamine and serotonin content

5 brains from female flies were quickly dissected from cold-anesthetized flies and frozen in 5  $\mu\text{L}$  of ringer solution at  $-80^\circ\text{C}$  until being processed for tissue content analysis. More precisely, 15  $\mu\text{L}$  of ice-cold 0.1 mol/L perchloric acid containing 1.34 mmol/L EDTA and 0.05% w/v sodium bisulfite were added to each sample and samples were then sonicated in an ultra-sonic bath for 12 min. The homogenates were centrifuged at  $15,000 \times g$  for 20 min at  $+4^\circ\text{C}$ , and the supernatants analyzed for monoamine content on the same day or the following day when a dilution of sample was required due to some very high dopamine concentrations (saturated peaks). The HPLC system consisted of a Prominence degasser, an LC-30 AD pump, and an SIL-30AC autosampler from Shimadzu. Detection was carried out at  $43^\circ\text{C}$  using a Decade II electrochemical detector fitted with a 0.7 mm glass carbon working electrode, an Ag/AgCl reference electrode, and a 25- $\mu\text{m}$  spacer (cell volume 80 nL, Antec). Separations were performed in the detector oven at  $43^\circ\text{C}$  using a capillary 150  $\times$  0.5 mm Zorbax-SB C18 5  $\mu\text{m}$  column. The mobile phase, which was pumped at a flow rate of 10  $\mu\text{L}/\text{min}$ , consisted of 100 mmol/L sodium phosphate containing 0.1 mmol/L EDTA, 2 mmol/L octane sulfonate, and 0.01% triethylamine, with a pH adjusted to 5.5 with sodium hydroxide, filtered through a 0.22  $\mu\text{m}$  membrane before use and mixed with 17% methanol (v/v). Analytes were detected at an oxidation potential of 700 mV versus the reference electrode. Chromatograms were acquired at a rate of 10 Hz using Lab Solutions software. The acquisition time was 40 min per sample. The injection volume was 1  $\mu\text{L}$ . Concentrations of dopamine and serotonin in the extracts were determined by comparison of chromatographic peak areas with calibration curves derived from a mixture of synthetic standards containing dopamine and serotonin (two points:  $2.5 \times 10^{-7}$  and  $5 \times 10^{-7}$  mol/L). The average concentration in the group on vehicle was used to normalize the results which are expressed as percentage of vehicle controls.

### Quantitative real-time PCR

Total RNA was extracted from 10 homogenized bodies using the RNeasy Minikit with DNase treatment (Qiagen) and reverse transcription was performed using SuperScript IV Reverse Transcriptase (Invitrogen). qPCR amplifications were performed with the Rotor-Gene SYBR Green PCR Kit (Qiagen), on a Rotor-Gene Qcycler (Qiagen). Absolute quantification of cDNA copy number was obtained by relating the Ct value to a standard curve made with serial dilutions of a reference DNA. The copy numbers obtained for the *Ribosomal Protein 49 (RP49)* gene were used as controls. The values shown correspond to the copy number of *Pallidin* divided by the copy number for RP49 amplified transcripts. Primers were designed with Primer-Blast software (National Center for Biotechnology Information/NCBI, Bethesda, USA) and purchased from Eurogentec.

### QUANTIFICATION AND STATISTICAL ANALYSIS

Distribution and homogeneity as well as statistical group comparisons were tested using Microsoft Excel plugin software Statel. The statistical test used, sample sizes and p values can be found in the figure legends. Except otherwise mentioned averages with Standard Error of the Mean (SEM) are plotted. The Origin software was used for data visualization.