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Supplemental information

Adenosine signaling in glia modulates metabolic state-dependent behavior in *Drosophila*

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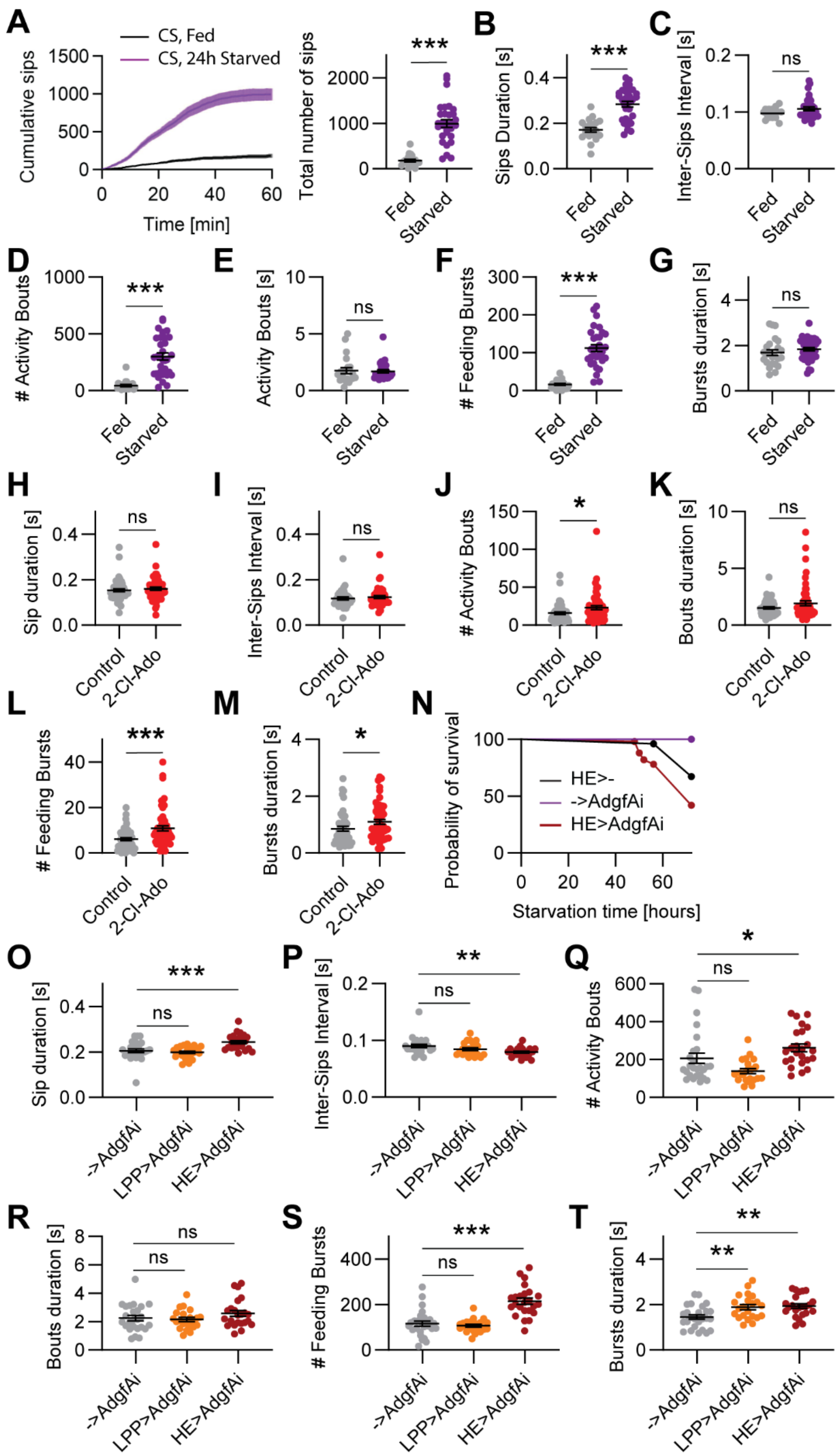
A
CS, Fed
CS, 24h Starved
Cumulative sips
Time [min]
Total number of sips
***
Fed
Starved
B
Sips Duration [s]
***
C
Inter-Sips Interval [s]
ns
D
Activity Bouts
***
E
Activity Bouts [s]
ns
F
Feeding Bursts
***
G
Bursts duration [s]
ns
H
Sip duration [s]
ns
Control
2-Cl-Ado
I
Inter-Sips Interval [s]
ns
J
Activity Bouts
*
K
Bouts duration [s]
ns
L
Feeding Bursts
***
M
Bursts duration [s]
*
N
Probability of survival
HE>-
->AdgfAi
HE>AdgfAi
Starvation time [hours]
O
Sip duration [s]
***
ns
->AdgfAi
LPP>AdgfAi
HE>AdgfAi
P
Inter-Sips Interval [s]
**
ns
Q
Activity Bouts
*
ns
R
Bouts duration [s]
ns
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S
Feeding Bursts
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ns
T
Bursts duration [s]
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**

Figure S1: Extracellular adenosine modifies flies' feeding pattern similarly to starvation (related to Figure 1)

(A) Cumulative number of sips (mean ± SEM) and scatter plot of the total number of sips on 10% sucrose drops in fed and 24-h-starved CS flies in one hour (n = 29/45; Mann-Whitney test $p < 0.0001$). (B) Averaged sip duration (Mann-Whitney test $p < 0.0001$). (C) Averaged time interval between sips (Mann-Whitney test $p = 0.09$). (D) Total number of activity bouts (Mann-Whitney test $p < 0.0001$). (E) Averaged duration of activity bouts (Mann-Whitney test $p = 0.31$). (F) Total number of feeding bursts (Mann-Whitney test $p < 0.0001$). (G) Averaged duration of feeding bursts (Mann-Whitney test $p = 0.13$).

(H) Averaged sip duration in fed WT flies kept for 5 days on standard fly food supplemented with the AdoR agonist 2-chloroadenosine (2-Cl-Ado; 1.65 mM; n = 57/57; Mann-Whitney test $p = 0.29$). (I) Averaged time interval between sips (Mann-Whitney test $p = 0.43$). (J) Total number of activity bouts (Mann-Whitney test $p = 0.0179$). (K) Averaged duration of activity bouts (Mann-Whitney test $p = 0.37$). (L) Total number of feeding bursts (Mann-Whitney test $p = 0.0006$). (M) Averaged duration of feeding bursts (Mann-Whitney test $p = 0.0182$).

(N) Survival curves over 72 h of starvation for control flies (HE>- and ->AdgfAi) or upon knockdown of AdgfA in hemocytes (HE>AdgfAi; n = 50/50/50; Log-rank test $p < 0.0001$).

(O) Averaged sip duration in 24-h-starved control flies (->AdgfAi) or upon knockdown of AdgfA in the fat body (LPP>AdgfAi) or hemocytes (HE>AdgfAi; n = 25/22/24; Kruskal-Wallis test $p = 0.0002$). (P) Averaged time interval between sips (Kruskal-Wallis test $p = 0.0056$). (Q) Total number of activity bouts (Kruskal-Wallis test $p < 0.0001$). (R) Averaged duration of activity bouts (one-way ANOVA $p = 0.25$). (S) Total number of feeding bursts (Kruskal-Wallis test $p < 0.0001$). (T) Averaged duration of feeding bursts (one-way ANOVA $p = 0.0017$).

Scatter plots display individual data points, as well as the mean ± SEM for each group. Pair-wise comparisons are indicated as follow: ns, non-significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.

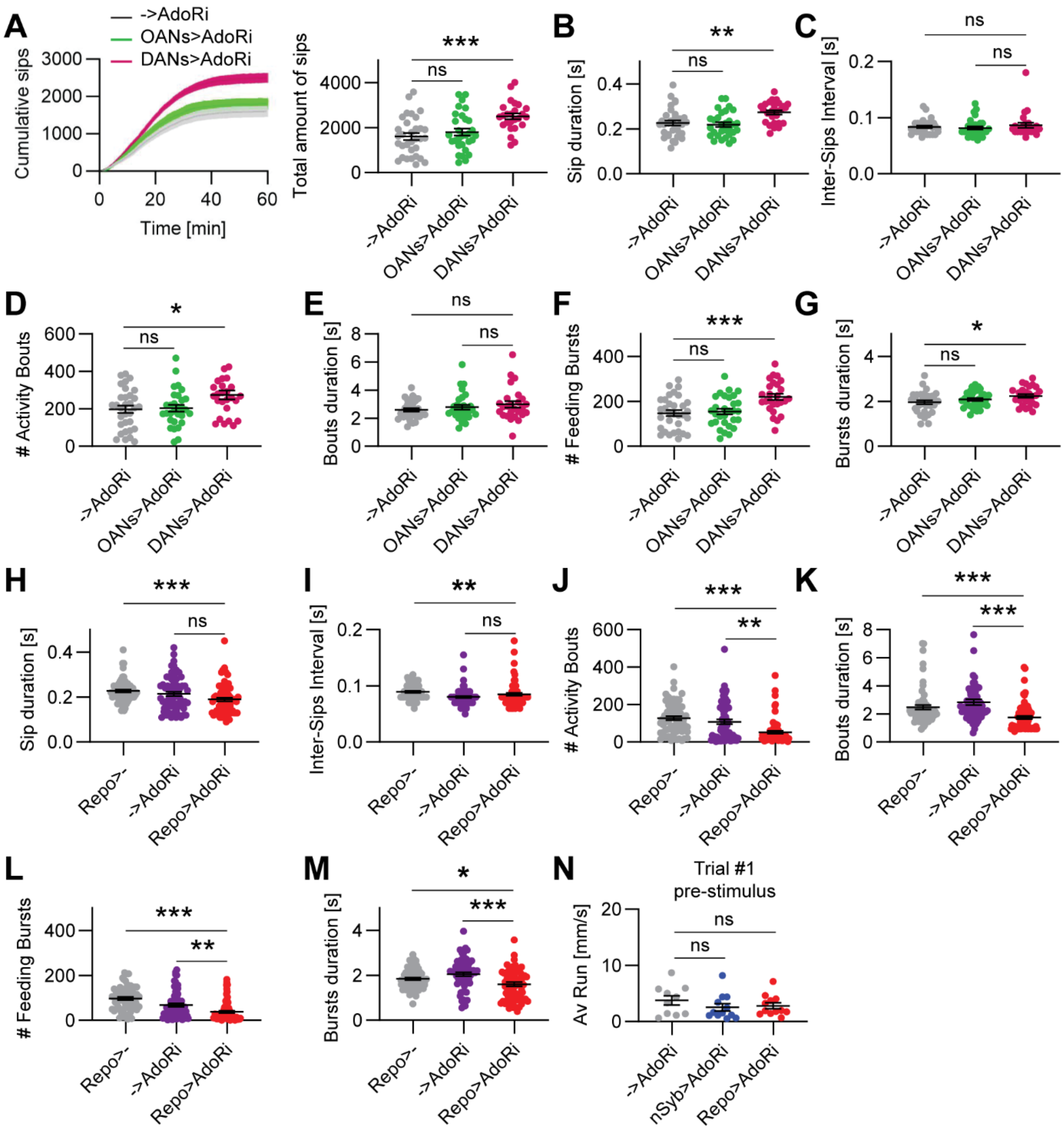


Figure S2: Adenosine signaling in dopaminergic neurons inhibits feeding behavior (related to Figure 2)

(A) Cumulative number of sips (mean ± SEM) and scatter plot of the total number of sips on 10% sucrose drops in 24-h-starved control flies (->AdoRi) or upon knockdown of AdoR in octopaminergic (OANs>AdoRi) and dopaminergic neurons (DANs>AdoRi; n = 32/30/25; one-way ANOVA $p = 0.0002$). (B) Averaged sip duration (one-way ANOVA $p = 0.0002$). (C) Averaged time interval between sips (Kruskal-Wallis test $p = 0.39$). (D) Total number of activity bouts (Kruskal-Wallis test $p = 0.0272$). (E) Averaged duration of activity bouts (Kruskal-Wallis test $p = 0.43$). (F) Total number of feeding bursts (one-way ANOVA $p = 0.0004$). (G) Averaged duration of feeding bursts (one-way ANOVA $p = 0.0437$).

(H) Averaged sip duration in 24-h-starved control flies (Repo>- and ->AdoRi) or upon knockdown AdoR in glia (Repo>AdoRi; n = 20/20/32; Kruskal-Wallis test p=0.0003). (I) Averaged time interval between sips (Kruskal-Wallis test $p = 0.0001$). (J) Total number of activity bouts (Kruskal-Wallis test $p < 0.0001$). (K) Averaged duration of activity bouts (Kruskal-Wallis test $p < 0.0001$). (L) Total number of feeding bursts (Kruskal-Wallis test $p < 0.0001$). (M) Averaged duration of feeding bursts (Kruskal-Wallis test $p < 0.0001$).

(N) Average forward running speed during the pre-stimulus phase of the 1st trial in 24-h-starved control (->AdoRi) flies or upon knockdown of AdoR in neurons (nSyb>AdoRi) and glia (Repo>AdoRi), respectively (n = 10/12/12; Kruskal-Wallis test $p = 0.40$).

Scatter plots display individual data points, as well as the mean ± SEM for each group. Pair-wise comparisons are indicated as follow: ns, non-significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.

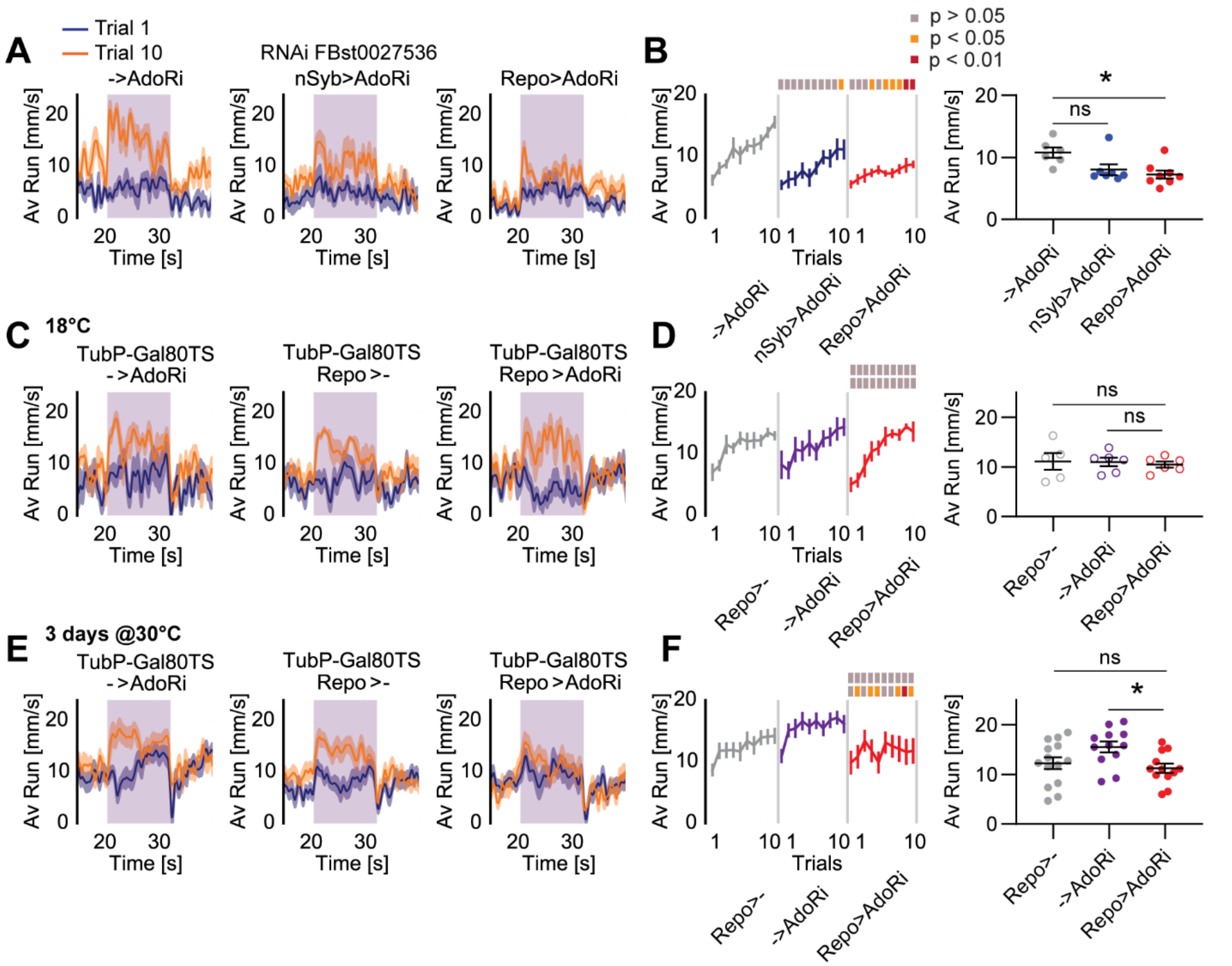
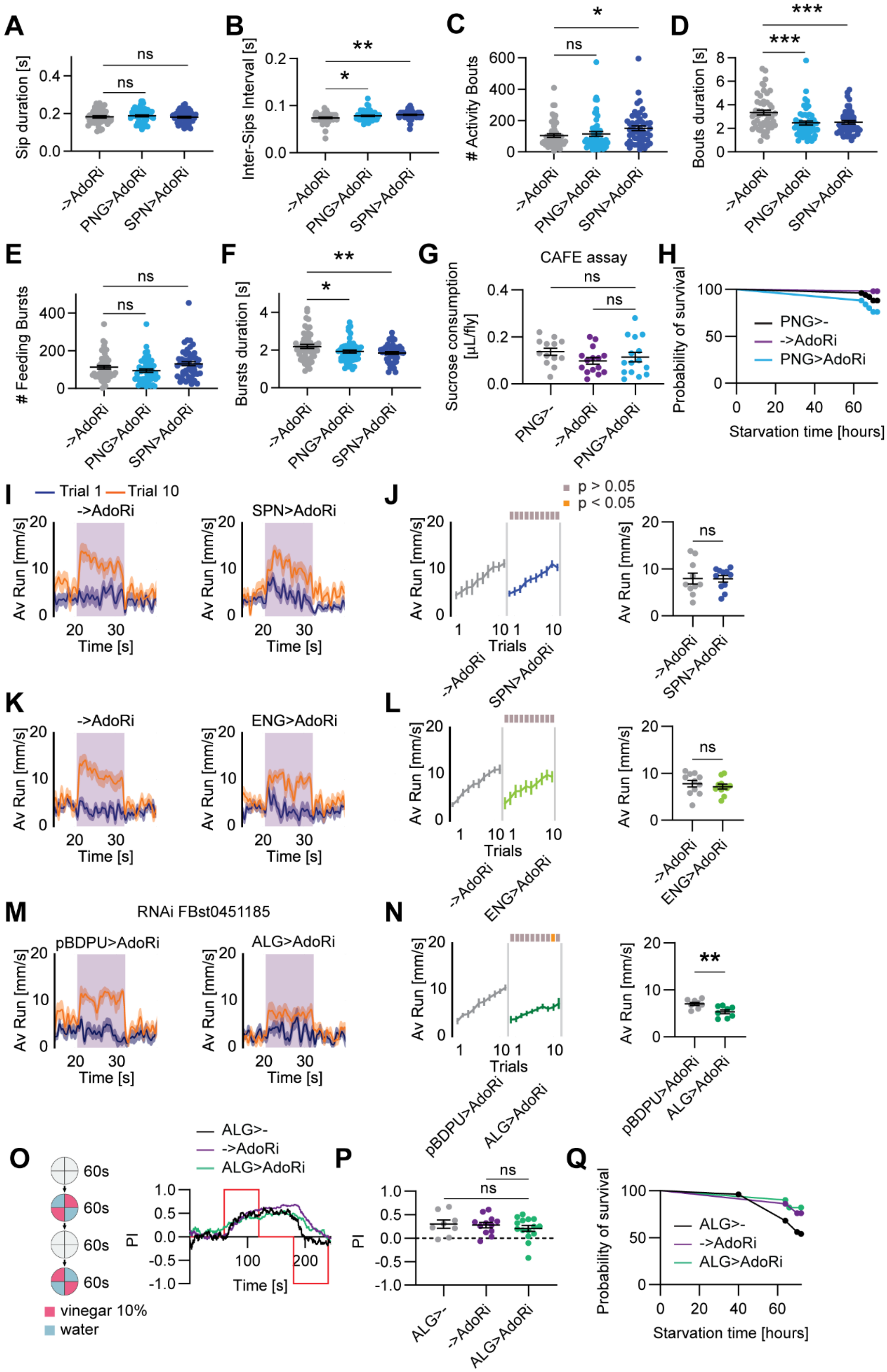


Figure S3: Adenosine signaling in glia is necessary for food-odor tracking behavior in adult flies (related to Figure 2)

(A) Averaged forward running speed (± SEM) in 24-h-starved control flies (->AdoRi) or upon knockdown of AdoR in neurons (nSyb>AdoRi) and glia (Repo>AdoRi) using an alternative RNAi line (FBst0027536), respectively. (B) Running speed during the stimulus phase over 10 successive trials (mean ± SEM; n = 6/7/8; 2-way RM ANOVA p(groups) = 0.0147; p(trials) < 0.0001; p(interaction) = 0.0830). The scatter plot displays the averaged running speed over the 10 trials for individual flies (Kruskal-Wallis test $p = 0.0087$). (C-F) Restriction of AdoR knockdown in adult flies using the temperature-sensitive TARGET system. (C) Averaged forward running speed (± SEM) in 24-h-starved control flies (TubP-Gal80^{TS}, Repo>- and TubP-Gal80^{TS}, ->AdoRi) or upon knockdown of AdoR in glia (TubP-Gal80^{TS}, Repo>AdoRi) in flies raised and kept at 18°C to prevent RNAi expression. (D) Running speed during the stimulus phase over 10 successive trials (mean ± SEM; n = 6/5/6; 2-way RM ANOVA p(groups) = 0.90; p(trials) < 0.0001; p(interaction) = 0.13). The scatter plot displays the averaged running speed over the 10 trials for individual flies (Kruskal-Wallis test $p = 0.85$). (E) Averaged forward running speed (± SEM) in 24-h-starved control flies (TubP-Gal80^{TS}, Repo>- and TubP-Gal80^{TS}, ->AdoRi) or upon knockdown of AdoR in glia (TubP-Gal80^{TS}, Repo>AdoRi) in flies raised at 18°C to prevent RNAi expression. Flies were kept for 2 days at 30°C before experiment to enable RNAi expression. (F) Running speed during the stimulus phase over 10 successive trials (mean ± SEM; n = 14/12/12; 2-way RM ANOVA p(groups) = 0.0275; p(trials) < 0.0001;

p(interaction) = 0.54). The scatter plot displays the averaged running speed over the 10 trials for individual flies (one-way ANOVA $p = 0.0275$).

Sidak's post hoc trial-to-trial comparisons are depicted on the top of the graphs as color-coded boxes (grey $p > 0.05$, orange $p < 0.05$ and red $p < 0.01$). Scatter plots display individual data points, as well as the mean ± SEM for each group. Pair-wise comparisons are indicated as follow: ns, non-significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.



A
Sip duration [s]
ns
ns
->AdoRi
PNG>AdoRi
SPN>AdoRi
B
Inter-Sips Interval [s]
**
*
C
Activity Bouts
*
ns
D
Bouts duration [s]
***
***
E
Feeding Bursts
ns
ns
F
Bursts duration [s]
**
*
G
CAFE assay
Sucrose consumption [µL/fly]
ns
ns
PNG>-
H
Probability of survival
PNG>-
->AdoRi
PNG>AdoRi
Starvation time [hours]
I
Trial 1
Trial 10
->AdoRi
SPN>AdoRi
Av Run [mm/s]
Time [s]
J
p > 0.05
p < 0.05
Trials
ns
K
ENG>AdoRi
L
ns
M
RNAi FBst0451185
pBDPU>AdoRi
ALG>AdoRi
N
**
O
60s
vinegar 10%
water
ALG>-
->AdoRi
ALG>AdoRi
PI
Time [s]
P
ns
ns
Q
Probability of survival
Starvation time [hours]

Figure S4: Adenosine signaling in subperineurial and ensheating glia is not necessary for food-odor tracking behavior (related to Figure 3)

(A) Averaged sip duration in 24-h-starved control flies (->AdoRi) or flies deficient for AdoR in PNG (PNG>AdoRi) and SPN (SPN>AdoRi), respectively ($n = 49/51/48$; one-way ANOVA $p = 0.60$). (B) Averaged time interval between sips (Kruskal-Wallis test $p = 0.0013$). (C) Total number of activity bouts (Kruskal-Wallis test $p = 0.0117$). (D) Averaged duration of activity bouts (Kruskal-Wallis test $p = 0.0005$). (E) Total number of feeding bursts (Kruskal-Wallis test $p = 0.0270$). (F) Averaged duration of feeding bursts (Kruskal-Wallis test $p = 0.0481$).

(G) Total sucrose consumption in control flies (PNG>- and ->AdoRi) or upon knockdown of AdoR in PNG (PNG>AdoRi) after 16h in the CAFE ($n = 13/15/15$; Kruskal-Wallis test $p = 0.23$).

(H) Survival curves over 72 h of starvation for control flies (PNG>- and ->AdoRi) or upon knockdown of AdoR in PNG (PNG>AdoRi; $n = 50/50/50$; Log-rank test $p = 0.0036$).

(I) Averaged forward running speed (± SEM) in 24-h-starved control flies (->AdoRi) or upon knockdown of AdoR in SPN (SPN>AdoRi) (J) Running speed during the stimulus phase over 10 successive trials (mean ± SEM; $n = 10/11$; 2-way RM ANOVA p(groups) = 0.98; p(trials) < 0.0001; p(interaction) = 0.85). The scatter plot displays the averaged running speed over the 10 trials for individual flies (Unpaired t-test $p = 0.98$).

(K) Averaged forward running speed (± SEM) in 24-h-starved control flies (->AdoRi) or upon knockdown of AdoR in ENG (ENG>AdoRi). (L) Running speed during the stimulus phase over 10 successive trials (mean ± SEM; $n = 10/11$; 2-way RM ANOVA p(groups) = 0.47; p(trials) < 0.0001; p(interaction) = 0.77). The scatter plot displays the averaged running speed over the 10 trials for individual flies, as well as their mean ± SEM (Unpaired t test $p = 0.47$).

(M) Averaged forward running speed (± SEM) in 24-h-starved control flies (pBDPU>AdoRi) or upon knockdown of AdoR in ALG (ALG>AdoRi) using an alternative RNAi line (FBst041185). (N) Running speed during the stimulus phase over 10 successive trials (mean ± SEM; $n = 8/8$; 2-way RM ANOVA p(groups) = 0.0070; p(trials) < 0.0001; p(interaction) = 0.0393). The scatter plot displays the averaged running speed over the 10 trials for individual flies (Unpaired t test $p = 0.0070$).

(O) Schematic representation of the experimental protocol used for 4-arm maze behavioral assay and average time course of preference indices (PI) during vinegar (10%) presentation for 24-h-starved control (ALG>- and ->AdoRi) flies or upon knockdown of AdoR in ALG (ALG>AdoRi). The red rectangles represent the stimulus presentations. (P) Averaged PIs during vinegar presentation ($n = 8/12/15$; Kruskal-Wallis test $p = 0.80$).

(Q) Survival curves over 72 h of starvation for control flies (ALG>- and ->AdoRi) or upon knockdown of AdoR in ALG (ALG>AdoRi; $n = 50/50/50$; Log-rank test $p = 0.0041$).

For food-odor tracking behavior experiments, Sidak's post hoc trial-to-trial comparisons are depicted on the top of the graphs as color-coded boxes (grey $p > 0.05$, orange $p < 0.05$ and red $p < 0.01$). Scatter plots display individual data points, as well as the mean ± SEM for each group. Post hoc pair-wise comparisons are indicated as follow: ns, non-significant ($p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.

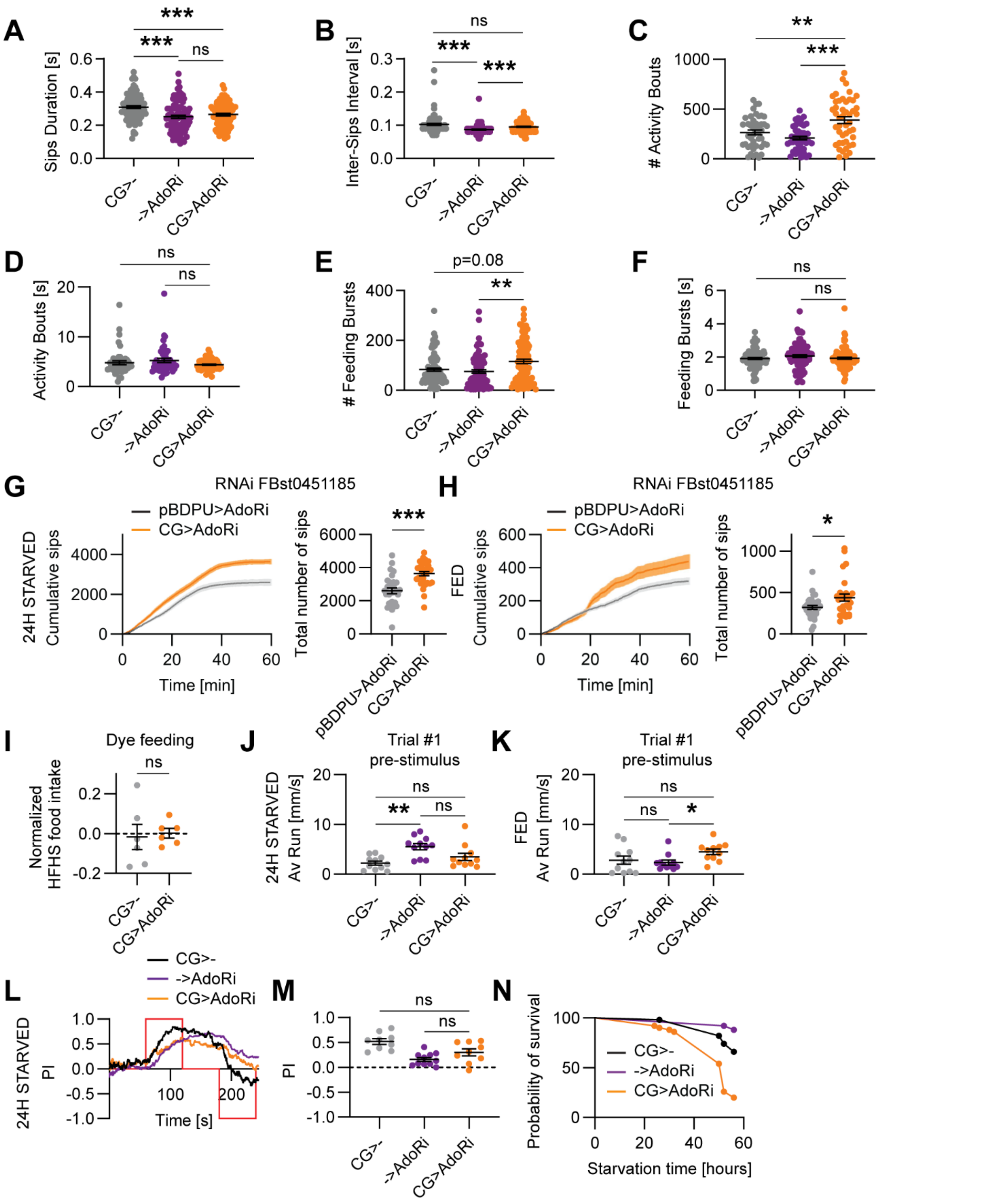


Figure S5: Adenosine signaling in cortex glia inhibits feeding and food-odor tracking behavior (Related to Figure 4)

(A) Averaged sip duration in 24-h-starved control flies (CG>- and ->AdoRi) or flies deficient for AdoR in CG (CG>AdoRi; n = 45/44/44; one-way ANOVA $p < 0.0001$). (B) Averaged time interval between sips

(Kruskal-Wallis test $p < 0.0001$). (C) Total number of activity bouts (Welch's ANOVA $p < 0.0001$). (D) Averaged duration of activity bouts (Kruskal-Wallis test $p = 0.53$). (E) Total number of feeding bursts (Kruskal-Wallis test $p = 0.0024$). (F) Averaged duration of feeding bursts (Kruskal-Wallis test $p = 0.18$).

(G) Cumulative number of sips (mean ± SEM) and scatter plot of the total number sips on 10% sucrose drops in 24-h-starved control flies (pBDPU>AdoRi) and AdoR-deficient flies in CG (CG>AdoRi) using an alternative RNAi line (FBst041185; $n = 29/30$; Unpaired t test $p < 0.0001$).

(H) Cumulative number of sips (mean ± SEM) and scatter plot of the total number sips on 10% sucrose drops in fed control flies (pBDPU>AdoRi) or AdoR-deficient flies in CG (CG>AdoRi) using an alternative RNAi line (FBst041185; $n = 32/28$; Mann Whitney test $p = 0.0406$).

(I) Normalized HFHS food intake measured by colorimetry in control flies (CG>-) and AdoR-deficient flies in CG (CG>AdoRi; $n = 6/6$; Mann Whitney test $p = 0.48$).

(J) Average forward running speed during the pre-stimulus phase of the 1st trial in 24-h-starved control (CG>- and ->AdoRi) flies or upon knockdown of AdoR in CG (CG>AdoRi; $n = 11/11/11$; Kruskal-Wallis test $p = 0.0046$).

(K) Average forward running speed during the pre-stimulus phase of the 1st trial in 24-h-starved control (CG>- and ->AdoRi) flies or upon knockdown of AdoR in CG (CG>AdoRi; $n = 11/11/11$; Kruskal-Wallis $p = 0.0323$).

(L) Average time course of preference indices (PI) during vinegar (10%) presentation for 24-h-starved control (CG>- and ->AdoRi) flies or upon knockdown of AdoR in CG (CG>AdoRi). The red rectangles represent the stimulus presentations. (M) Averaged PIs during vinegar presentation ($n = 9/11/9$; Kruskal-Wallis test $p = 0.0013$).

(N) Survival curves over 56 h of starvation for control flies (ALG>- and ->AdoRi) or upon knockdown of AdoR in ALG (ALG>AdoRi; $n = 50/50/50$; Log-rank test $p < 0.0001$).

Scatter plots display individual data points, as well as the mean ± SEM for each group. Post hoc pair-wise comparisons are indicated as follow: ns, non-significant ($p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.

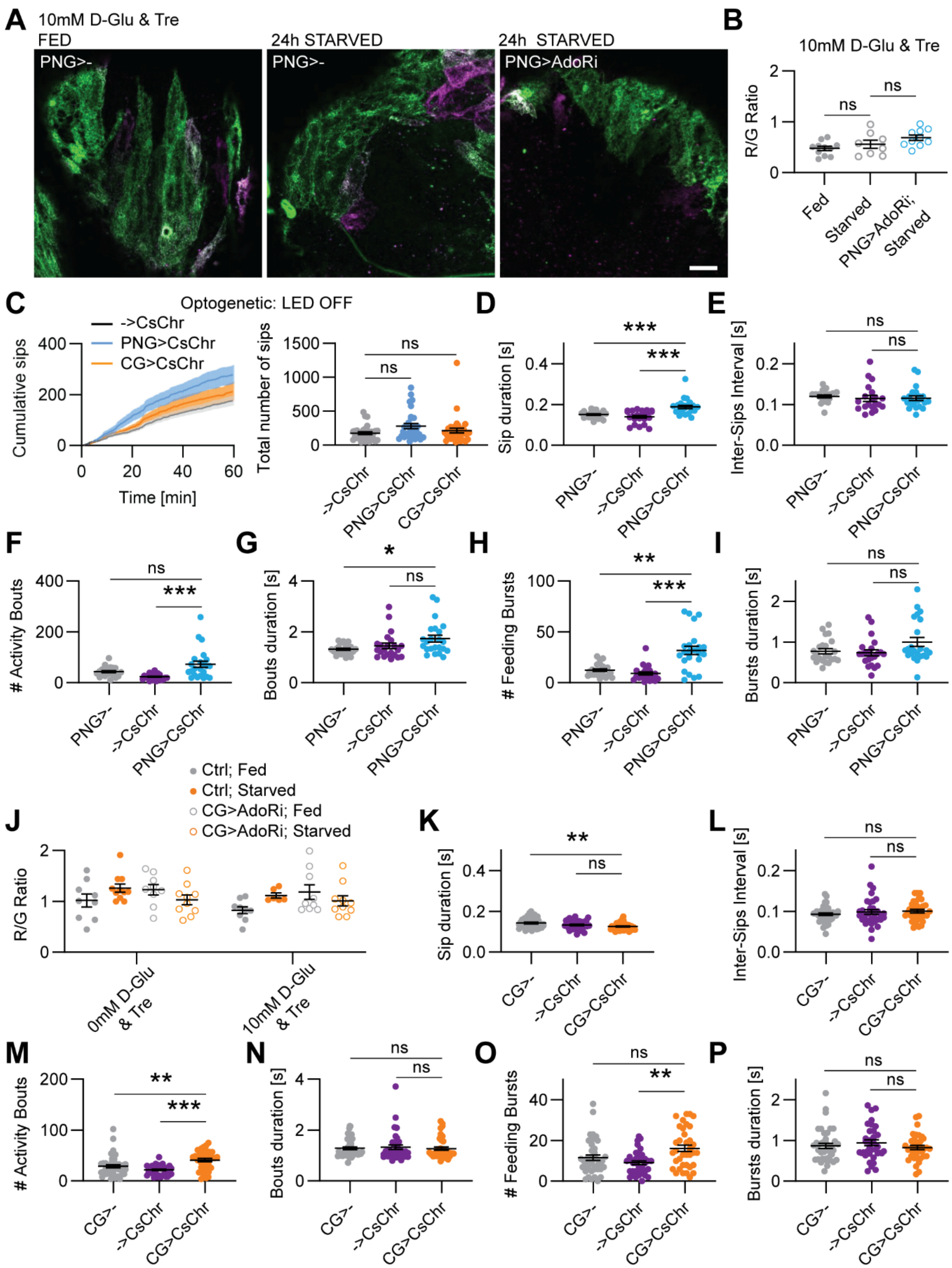


Figure S6: Starvation and adenosine signaling modulate intra-cellular calcium levels in perineurial and cortex glia (related to Figure 5)

(A) Example images of CaMPARI2-L398T-expressing PNG cells in dorso-caudal brain explants from fed and 24-h-starved control flies (PNG>-) and upon knockdown of AdoR (PNG>AdoRi), in standard AHL (10 mM D-Glu and 10 mM Tre), after photoconversion (scale bar = 20 µm). (B) CaMPARI2 photoconversion ratios in fed and 24-h-starved control flies (n = 11/8), as well as 24-h-starved PNG>AdoRi flies (n = 10; 2-way ANOVA p(AHL) = 0.0076; p(groups) = 0.06; p(interaction) = 0.06).

(C) Cumulative number of sips (mean ± SEM) and scatter plot of the total number sips on 10% sucrose drops in fed control flies (->CsChr) and flies expressing CsChrimson in PNG (PNG>CsChr) and CG (CG>CsChr), respectively, without light stimulation (n = 32/32/32; Kruskal-Wallis test p = 0.10).

(D) Averaged sip duration in fed flies upon closed-loop optogenetic activation of PNG (n = 23/22/23; Kruskal-Wallis test $p < 0.0001$). (E) Averaged time interval between sips (Kruskal-Wallis test p = 0.17). (F) Total number of activity bouts (Kruskal-Wallis test $p < 0.0001$). (G) Averaged duration of activity bouts (Kruskal-Wallis test p = 0.0283). (H) Total number of feeding bursts (Kruskal-Wallis test $p < 0.0001$). (I) Averaged duration of feeding bursts (Kruskal-Wallis test p = 0.10).

(J). CaMPARI2 photoconversion ratios in fed and 24-h-starved control and CG>AdoRi flies imaged in standard (n_{fed}=9/11; $n_{starved}$=9/10) or sugar-free AHL (n_{fed}=9/8; $n_{starved}$=9/10; 3-way ANOVA p(state) = 0.43; p(genotype) = 0.59; p(AHL) = 0.18; p(state x genotype) = 0.0022; p(state x AHL) = 0.35; p(genotype x AHL) = 0.79; p(state x genotype x AHL) = 0.95). Data from standard and sugar-free AHL are pooled for display in Figure 5F.

(K) Averaged sip duration in fed flies upon closed-loop optogenetic activation of CG (n = 44/41/37; one-way ANOVA p = 0.0037). (L) Averaged time interval between sips (Kruskal-Wallis test p = 0.48). (M) Total number of activity bouts (Kruskal-Wallis test $p < 0.0001$). (N) Averaged duration of activity bouts (Kruskal-Wallis test p = 0.85). (O) Total number of feeding bursts (Kruskal-Wallis test p = 0.0059). (P) Averaged duration of feeding bursts (Kruskal-Wallis test p = 0.62).

Scatter plots display individual data points, as well as the mean ± SEM for each group. Post hoc pair-wise comparisons are indicated as follow: ns, non-significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.

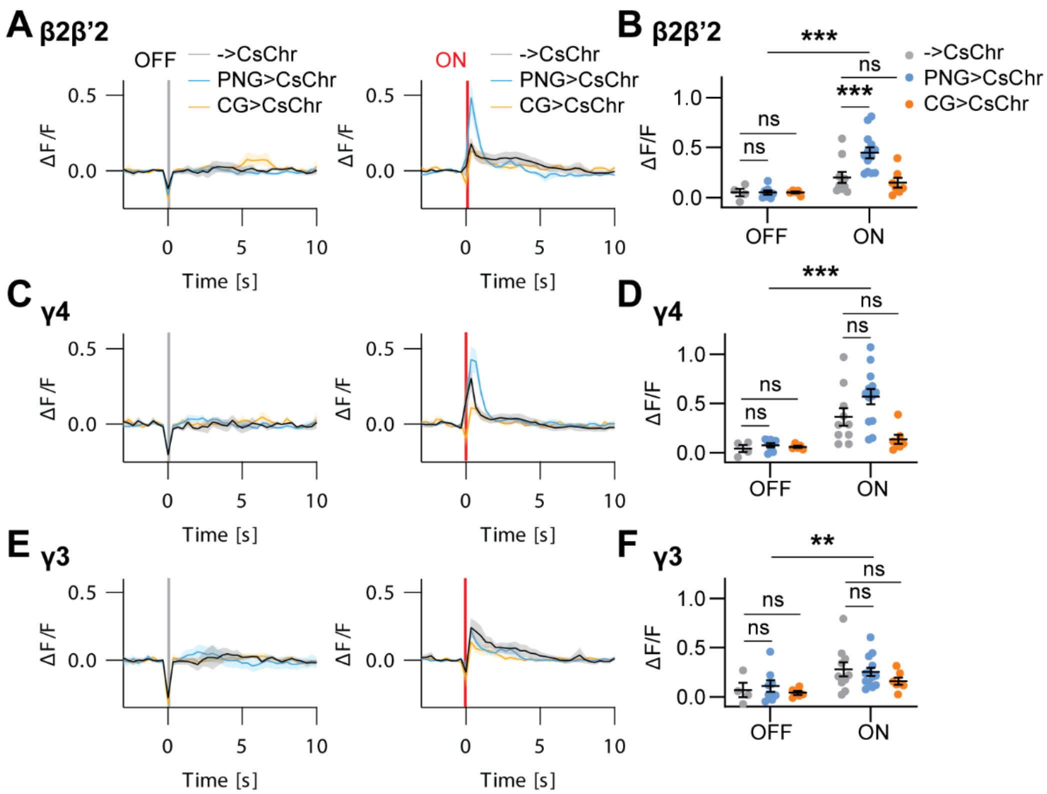


Figure S7: Optogenetic PNG stimulation evokes calcium transients in specific PAM dopaminergic neurons (related to Figure 5)

(A) Average (± SEM) ΔF/F calcium responses in PAM-DANs innervating the β2β'2 compartments of the MB to 100 ms optogenetic stimulation (*grey* line: LED OFF; *red* line: LED ON) of PNG (PNG>CsChr) and CG (CG>CsChr), respectively. (B) Calcium peak response following the stimulus (LED OFF: n = 4/8/5; LED ON n = 10/13/7; 2-way ANOVA p(genotype) = 0.0128; p(LED) < 0.0001; p(interaction) = 0.0122).

(C) Average (± SEM) ΔF/F calcium responses in PAM-DANs innervating the γ4 compartment of the MB to 100 ms optogenetic stimulation (*grey* line: LED OFF; *red* line: LED ON) of PNG (PNG>CsChr) and CG (CG>CsChr), respectively. (D) Calcium peak response following the stimulus (LED OFF: n = 4/8/5; LED ON n = 10/13/7; 2-way ANOVA p(genotype) = 0.0200; p(LED) < 0.0001; p(interaction) = 0.0364).

(E) Average (± SEM) ΔF/F calcium responses in PAM-DANs innervating the γ3 compartment of the MB to 100 ms optogenetic stimulation (*grey* line: LED OFF; *red* line: LED ON) of PNG (PNG>CsChr) and CG (CG>CsChr), respectively. (F) Calcium peak response following the stimulus (LED OFF: n = 4/8/5; LED ON n = 10/13/7; 2-way ANOVA p(genotype) = 0.37; p(LED) = 0.0037; p(interaction) = 0.77).

Scatter plots display individual data points, as well as the mean ± SEM for each group. Post hoc pair-wise comparisons are indicated as follow: ns, non-significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.

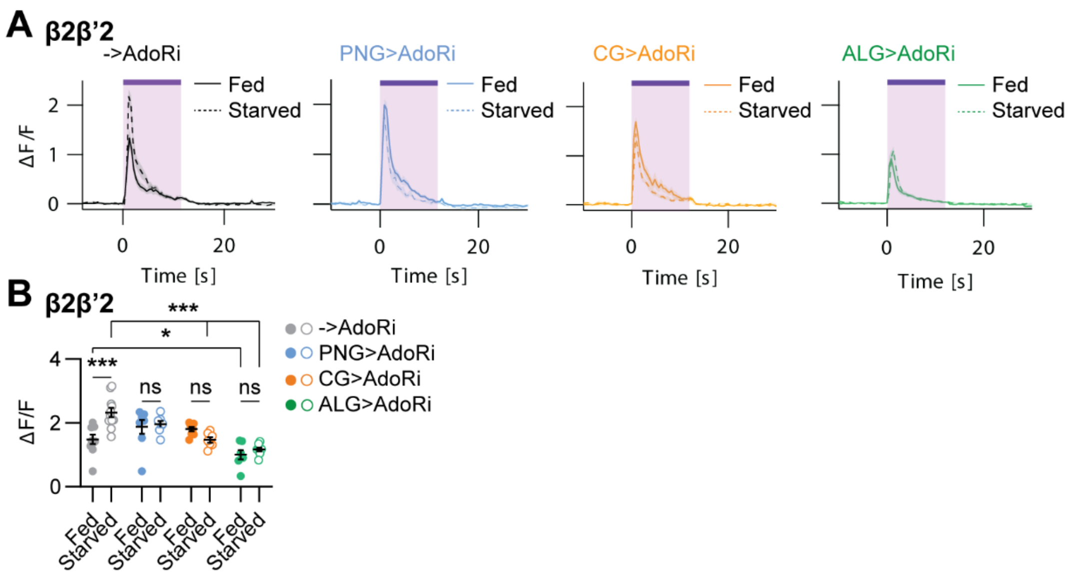


Figure S8: Adenosine signaling in perineurial, cortex and astrocyte-like glia differentially modulates PAM-β2β'2 dopaminergic neurons response to vinegar in fed and starved flies (related to Figure 7)
(A) Averaged ΔF/F calcium responses to vinegar-odor stimulation (*purple*) in the β2β'2 compartments in fed and 24-h-starved control flies (->AdoRi) or in flies expressing *AdoR*-RNAi in PNG (*blue*; PNG>AdoRi), CG (*orange*; CG>AdoRi) and ALG (*green*; ALG>AdoRi), respectively. (B) Maximal peak responses to vinegar-odor in the different groups ($n_{\text{->AdoRi}}$=10/11; $n_{\text{PNG>AdoRi}}$=8/7; $n_{\text{CG>AdoRi}}$=8/7; $n_{\text{ALG>AdoRi}}$ = 7/8; 2-way ANOVA p(genotype) < 0.0001, p(state) = 0.06, p(interaction) = 0.0004).
Scatter plots display individual data points, as well as the mean ± SEM for each group. Post hoc pair-wise comparisons are indicated as follow: ns, non-significant (p >0.05); *, p < 0.05; **, p < 0.01; *** p < 0.001.

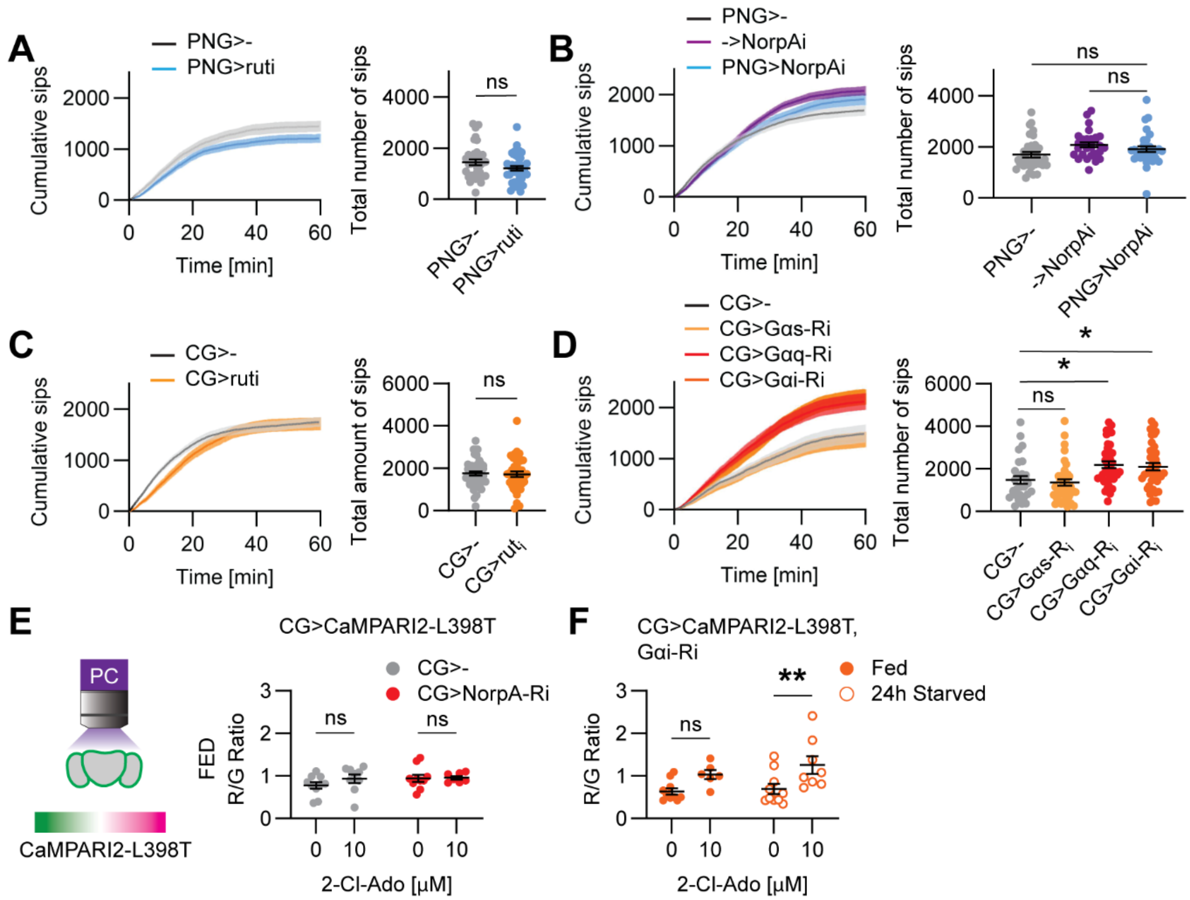


Figure S9: Adenosine signals hunger state through distinct pathways in astrocyte-like and in cortex glia (related to Figure 6)

(A) Cumulative number of sips (mean ± SEM) and scatter plot of the total number sips on 10% sucrose drops in 24-h-starved control flies (PNG>-) or upon knockdown of *rutabaga* in PNG (PNG>ruti; $n = 36/40$; Unpaired t test $p = 0.09$).

(B) Cumulative number of sips (mean ± SEM) and scatter plot of the total number sips on 10% sucrose drops in 24-h-starved control flies (PNG>- and ->NorpAi) or upon knockdown of NorpAi in PNG (PNG>NorpAi; $n = 35/31/32$; Kruskal-Wallis test $p = 0.0043$).

(C) Cumulative number of sips (mean ± SEM) and scatter plot of the total number sips on 10% sucrose drops in 24-h-starved control flies (CG>-) or upon knockdown of *rutabaga* in CG (CG>ruti; $n = 45/39$; Unpaired t test $p = 0.82$).

(D) Cumulative number of sips (mean ± SEM) and scatter plot of the total number sips on 10% sucrose drops in 24-h-starved control flies (CG>-) or upon knockdown of the Gα-proteins -s, -q and -i in CG (CG>Gαs-Ri, CG>Gαq-Ri and CG>Gαi-Ri; $n = 30/40/38/40$; Kruskal-Wallis test $p < 0.0001$).

(E) CaMPARI2 photoconversion ratios measured in explant brains from fed control flies (CG>-) or upon knockdown of *NorpA* in CG (CG>NorpAi). Before photoconversion, explant brains were kept for 10 minutes in saline solution containing or not 10 µM of 2-Cl-Ado (CG>- $n = 10/9$; Mann-Whitney test p(2-Cl-Ado) = 0.13; CG>NorpAi $n = 10/7$; Welch's t test p(2-Cl-Ado) = 0.91; CG>- vs. CG>NorpAi Unpaired t test $p = 0.22$).

(F) CaMPARI2 photoconversion ratios measured in explant brains from fed or 24-h-starved flies upon knockdown of Gαi in CG (CG> Gαi-Ri). Before photoconversion, explant brains were kept for 10 minutes in saline solution containing or not 10 μM of 2-Cl-Ado (n_{Fed} = 10/6; $n_{Starved}$ = 10/8; 2-way ANOVA p(feeding state) = 0.31, p(2-Cl-Ado) = 0.0015, p(interaction) = 0.55).

Scatter plots display individual data points, as well as the mean ± SEM for each group. Post hoc pair-wise comparisons are indicated as follow: ns, non-significant ($p > 0.05$); *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$.

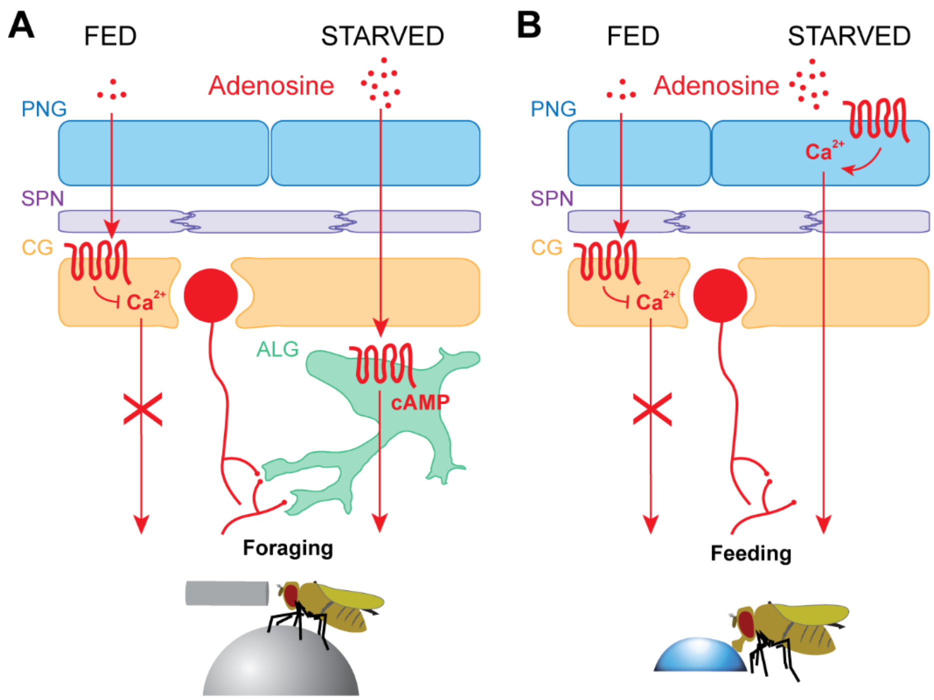


Figure S10: Summary model for the modulation of metabolic state-dependent behavior by adenosine signaling in glia

(A) Adenosine signaling prevents calcium increase in CG, refraining fed flies from tracking food-odor. In starved flies, increased extracellular adenosine concentration promotes cAMP-related signaling in ALG that in turn promotes food-odor tracking behavior (B) Adenosine signaling prevents calcium increase in CG, refraining fed flies from feeding. In starved flies, adenosine induces cytoplasmic calcium increase in PNG and promotes feeding behavior.