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Supplemental information

Glial glucose fuels the neuronal pentose phosphate pathway for long-term memory

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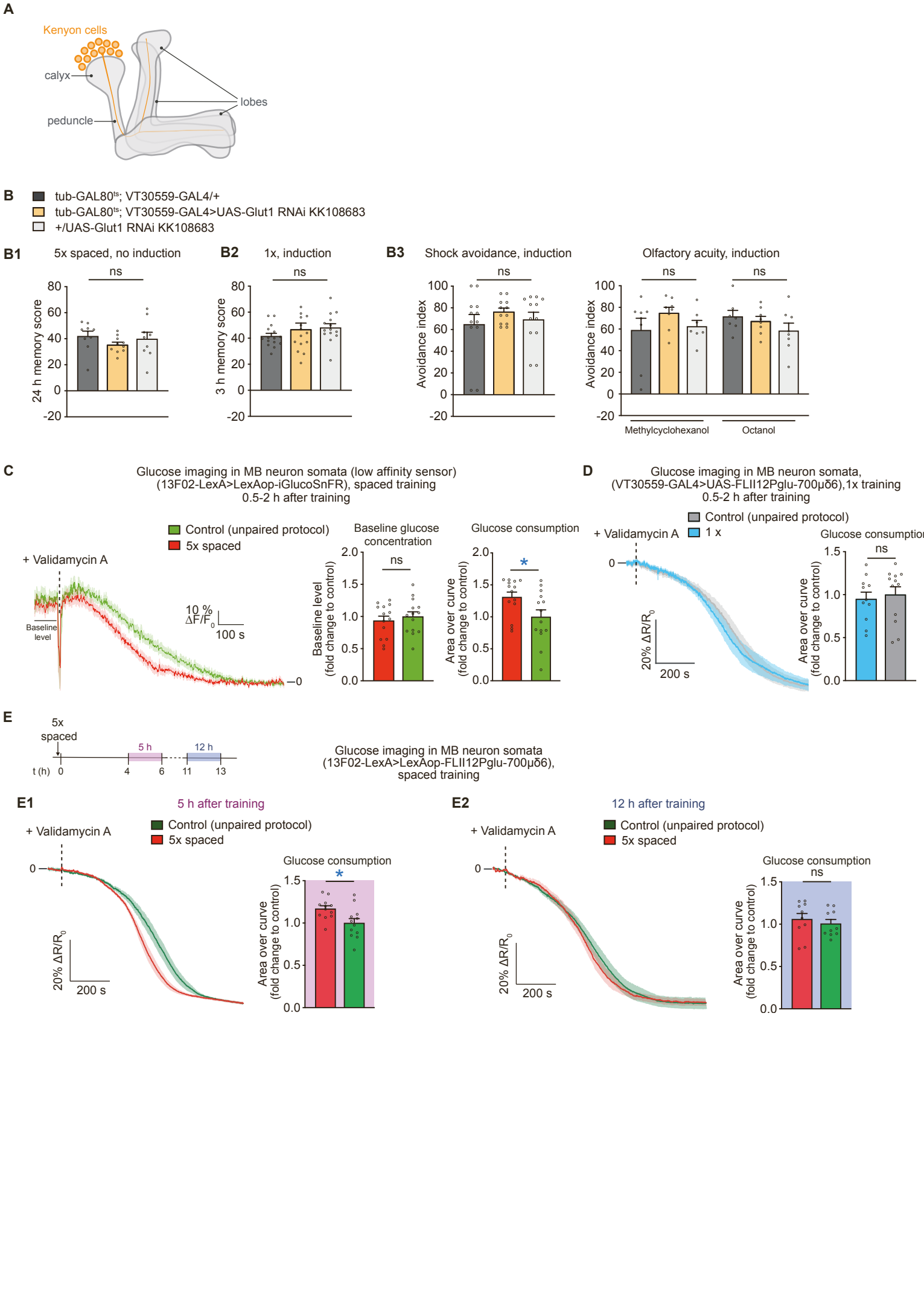
A
Kenyon cells
calyx
peduncle
lobes
B
tub-GAL80ts; VT30559-GAL4/+
tub-GAL80ts; VT30559-GAL4>UAS-Glut1 RNAi KK108683
+/UAS-Glut1 RNAi KK108683
B1
5x spaced, no induction
24 h memory score
B2
1x, induction
3 h memory score
B3
Shock avoidance, induction
Olfactory acuity, induction
Avoidance index
ns
Methylcyclohexanol
Octanol
C
Glucose imaging in MB neuron somata (low affinity sensor)
(13F02-LexA>LexAop-iGlucoSnFR), spaced training
0.5-2 h after training
Control (unpaired protocol)
5x spaced
+ Validamycin A
Baseline level
10 % ΔF/F0
100 s
Baseline glucose concentration
Baseline level (fold change to control)
Glucose consumption
Area over curve (fold change to control)
D
Glucose imaging in MB neuron somata,
(VT30559-GAL4>UAS-FLII12Pglu-700μδ6),1x training
0.5-2 h after training
1 x
20% ΔR/R0
200 s
E
5x spaced
5 h
12 h
t (h) 0 4 6 11 13
Glucose imaging in MB neuron somata
(13F02-LexA>LexAop-FLII12Pglu-700μδ6),
spaced training
E1
5 h after training
E2
12 h after training

Figure S1. Related to Figure 1.

A. Illustration of mushroom body organization. Kenyon cells (KC) are the intrinsic mushroom body (MB) neurons. MB neuron dendritic processes are bundled into the calyx, where they receive olfactory input from projection neurons. MB neurons then extend parallel axonal fibres bundled in the peduncle. At the distal end of the peduncle, axons show distinct patterns of bifurcation or branching within medial and vertical lobes. MB lobes receive dopaminergic innervation conveying reinforcement signalling (here, through aversive shock stimulation) during olfactory conditioning. B. Control experiments corresponding to Glut1 knock-down in MB neurons. B1. Without induction of the RNAi against Glut1 in MB neurons, LTM in the flies of interest was normal (n = 9-10, $F_{2,27}$ = 0.84, P = 0.45). B2. Glut1 knock-down in MB neurons did not alter 3-h memory after 1 x training (n = 14-15, $F_{2,43}$= 1.00, P = 0.38). B3. Shock reactivity (n = 12, $F_{2,35}$ = 0.75, P = 0.48) and olfactory acuity (methylcyclohexanol: n = 7–8, $F_{2,22}$ = 1.15, P = 0.34, octanol: n = 7-8, $F_{2,22}$ = 1.3, P = 0.29) were normal in the flies of interest. C. Variations in glucose concentration in the somata of MB neurons expressing the low-affinity iGlucoSnFR glucose sensor were monitored following the application of validamycin A (4 mM), at the time point marked by a dashed line. The injection induced a transient disturbance in the focus, which in turn induced a drop in the recorded signal, as this single-fluorophore sensor is not ratiometric. Data were normalized to the last 150 s of the recording, when a stable floor value was reached corresponding to the unbound state of the sensor. The baseline glucose concentration was then quantified before validamycin A application during the first 90 s of the recording (solid line). No difference in glucose concentration in the somata of MB neurons was observed between flies subjected to 5 x spaced training and flies conditioned with a non-associative spaced unpaired training protocol (n = 14, t_{26} = 0.63, P = 0.53). The glucose concentration in the somata of MB neurons decreased faster following validamycin application in flies after 5 x spaced training as compared to flies conditioned with a non-associative spaced unpaired training protocol (n = 14, t_{26} = 2.30, P = 0.030), similar to what we observed with the high-affinity FRET sensor. D. No difference in glucose consumption was observed between flies subjected to 1 x training or to a non-associative control protocol (n = 11–12, t_{21} = 0.41, P = 0.69), as measured with the glucose FRET sensor. E. Variations in glucose concentration in the somata of MB neurons were monitored following the application of validamycin A (4 mM), at the time point marked by a dashed line. The scheme indicates the time window during which imaging data were collected. E1. Glucose concentration in the somata of MB neurons decreased faster in flies after 5 x spaced training as compared to flies conditioned with a non-associative spaced unpaired training protocol 4 to 6 h after olfactory conditioning (n = 12, t_{22} = 2.65 P = 0.015). E2. Between 11 and 13 h after training, no difference in glucose decrease was observed following validamycin application between flies subjected to 5 x spaced training and a non-associative unpaired protocol (n = 10, t_{18} = 0.65, P = 0.52). Data in C and D were collected between 0.5 and 2 h following the end of the indicated conditioning protocol.

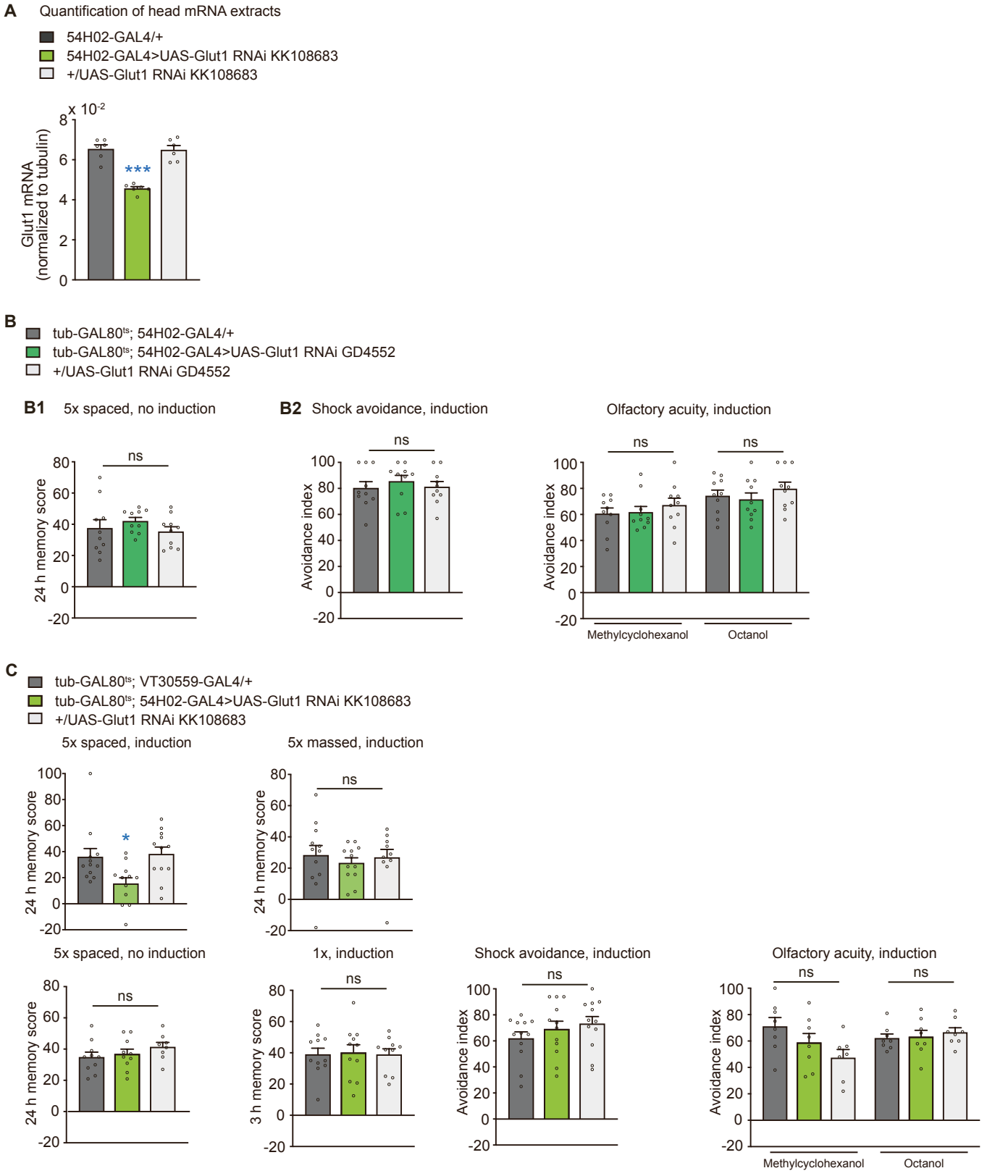
A
Quantification of head mRNA extracts
54H02-GAL4/+
54H02-GAL4>UAS-Glut1 RNAi KK108683
+/UAS-Glut1 RNAi KK108683
x 10-2
Glut1 mRNA (normalized to tubulin)
***
B
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-Glut1 RNAi GD4552
+/UAS-Glut1 RNAi GD4552
B1
5x spaced, no induction
24 h memory score
ns
B2
Shock avoidance, induction
Avoidance index
Olfactory acuity, induction
Methylcyclohexanol
Octanol
C
tub-GAL80ts; VT30559-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-Glut1 RNAi KK108683
+/UAS-Glut1 RNAi KK108683
5x spaced, induction
5x massed, induction
5x spaced, no induction
1x, induction
3 h memory score
Shock avoidance, induction
Olfactory acuity, induction
D
Perineurial glia
Subperineurial glia
MB neurons
cortex glia
astrocyte-like glia
ensheathing glia
E
Glut1 KD in adult astrocyte-like glia
tub-GAL80ts; Alrm-GAL4/+
tub-GAL80ts; Alrm-GAL4>UAS-Glut1 RNAi KK108683
+/UAS-Glut1 RNAi KK108683
5x spaced, induction
F
Glut1 KD in adult ensheathing glia
tub-GAL80ts; 56F03-GAL4/+
tub-GAL80ts; 56F03-GAL4>UAS-Glut1 RNAi KK108683
+/UAS-Glut1 RNAi KK108683
5x spaced, induction

Figure S2. Related to Figure 2.

A. Glut1 knock-down in cortex glia resulted in a significant decrease in overall Glut1 mRNA concentration in the fly brain as measured by RT-qPCR (N = 3 independent extracts, n = 6 replicates, $F_{2,17}$ = 36.0, P < 0.0001). B. Control experiments corresponding to Glut1 knock-down (GD4552) in cortex glia. B1. Without RNAi induction, the flies of interest displayed normal LTM (n = 10, $F_{2,29}$ = 0.78, P = 0.47). B2. Shock reactivity (n = 10, $F_{2,29}$ = 0.36, P = 0.70) and olfactory acuity (methylcyclohexanol: n = 10, $F_{2,29}$ = 0.54, P = 0.59, octanol: n = 10, $F_{2,29}$ = 0.72, P = 0.49) were not altered by Glut1 knock-down in cortex glia. C. The whole series of experiments was performed with a second RNAi against Glut1. Knock-down of Glut1 (RNAi KK108683) in adult cortex glia disrupted LTM as compared to the genotypic controls after 5 x spaced training (n = 12, $F_{2,35}$ = 5.11, P = 0.012), but did not affect 24-h memory after 5 x massed training (n = 10/12, $F_{2,33}$ = 0.27, P = 0.77). Without induction of the RNAi, LTM in the flies of interest was normal (n = 8-10, $F_{2,27}$ = 1.05, P = 0.37). 3-h memory after 1 x training was not affected upon Glut1 knock-down (n = 10/11, $F_{2,31}$ = 0.023, P = 0.98). Shock reactivity (n = 12, $F_{2,35}$ = 1.04, P = 0.36) and olfactory acuity (methylcyclohexanol: n = 7/8, $F_{2,22}$ = 3.03, P = 0.07, octanol: n = 8, $F_{2,23}$ = 0.33, P = 0.72) were normal after Glut1 knock-down in cortex glia. D. Subtypes and localization of Drosophila glia. Perineurial (yellow) and subperineurial (blue) glia form a chemical and physical barrier for the brain. In the cortical region (dark grey), cortex glia (green) encase cell bodies of neurons (orange). In the neuropile (light grey), astrocyte-like glia (purple) extend processes close to the synapses. Ensheathing glia (pink) processes delimit brain anatomical structures. E. LTM was normal when Glut1 RNAi (KK108683) was expressed in adult astrocyte-like glia using the Alrm-GAL4 driver (n = 15, $F_{2,44}$ = 2.76, P = 0.07). F. LTM was normal when Glut1 RNAi (KK108683) was expressed in adult ensheathing glia using the 56F03-GAL4 driver (n =11/12, $F_{2,34}$ = 0.75, P = 0.48).

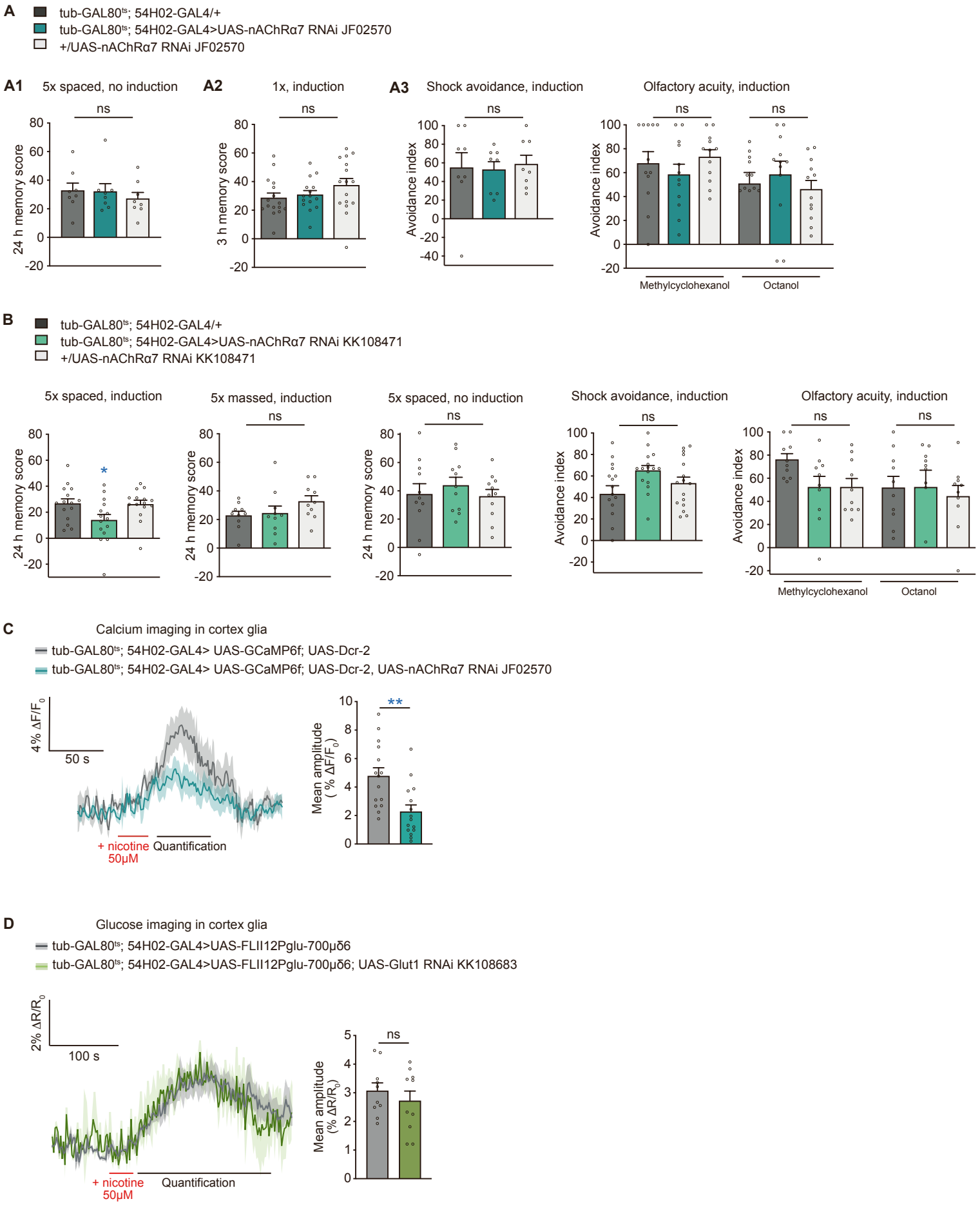
A
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-nAChRα7 RNAi JF02570
+/UAS-nAChRα7 RNAi JF02570
A1
5x spaced, no induction
24 h memory score
ns
A2
1x, induction
3 h memory score
ns
A3
Shock avoidance, induction
Avoidance index
ns
Olfactory acuity, induction
Avoidance index
ns
ns
Methylcyclohexanol
Octanol
B
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-nAChRα7 RNAi KK108471
+/UAS-nAChRα7 RNAi KK108471
5x spaced, induction
24 h memory score
*
5x massed, induction
24 h memory score
ns
5x spaced, no induction
24 h memory score
ns
Shock avoidance, induction
Avoidance index
ns
Olfactory acuity, induction
Avoidance index
ns
ns
Methylcyclohexanol
Octanol
C
Calcium imaging in cortex glia
tub-GAL80ts; 54H02-GAL4> UAS-GCaMP6f; UAS-Dcr-2
tub-GAL80ts; 54H02-GAL4> UAS-GCaMP6f; UAS-Dcr-2, UAS-nAChRα7 RNAi JF02570
4% ΔF/F0
50 s
+ nicotine 50μM
Quantification
Mean amplitude (% ΔF/F0)
**
D
Glucose imaging in cortex glia
tub-GAL80ts; 54H02-GAL4>UAS-FLII12Pglu-700μδ6
tub-GAL80ts; 54H02-GAL4>UAS-FLII12Pglu-700μδ6; UAS-Glut1 RNAi KK108683
2% ΔR/R0
100 s
+ nicotine 50μM
Quantification
Mean amplitude (% ΔR/R0)
ns

Figure S3. Related to Figure 3.

A. Control experiments corresponding to nAChRα7 knock-down in cortex glia. A1. Without thermal induction of nAChRα7 JF02570 RNAi expression, no LTM defect was displayed by the flies of interest as compared to the genotypic controls (n = 8, $F_{2,23}$ = 0.38, P = 0.69). A2. 3-h memory after 1 x training was not affected upon nAChRα7 knock-down (n = 16, $F_{2,46}$ = 1.52, P = 0.23). A3. Shock reactivity (n = 8, $F_{2,23}$ = 0.064, P = 0.94) and olfactory acuity (methylcyclohexanol: n = 12, $F_{2,35}$ = 0.82, P = 0.45, octanol: n = 12, $F_{2,35}$ = 0.44, P = 0.65) were not altered by nAChRα7 knock-down. B. The whole series of experiments was performed with a second RNAi against nAChRα7. RNAi induction caused impaired LTM (n = 15, $F_{2,44}$ = 3.76, P = 0.031) while leaving 24-h memory unaffected after 5 x massed training (n = 10, $F_{2,29}$ = 1.72, P = 0.20). Without RNAi expression, LTM in the flies of interest was normal (n = 11, $F_{2,32}$ = 0.46, P = 0.64). Shock reactivity (n = 16, $F_{2,47}$ = 3.09, P = 0.055) and olfactory acuity (methylcyclohexanol: n = 10, $F_{2,29}$ = 3.36, P = 0.05, octanol: n = 10, $F_{2,29}$ = 0.15, P = 0.86) were normal after nAChRα7 knock-down. C. Calcium dynamics in cortex glia were monitored by *In vivo* imaging following nicotine application onto the brain. Dicer-2 (Dcr-2) expression was used to increase the efficiency of RNA interference knock-down of nAChRα7. Following nicotine perfusion, the induced calcium increase was reduced when nAChRα7 was knocked-down in cortex glia (n = 15, t_{28} = 3.32, P = 0.0025). D. The FRET glucose sensor expression was targeted to cortex glia in 54H02-GAL4>UAS-FLII12Pglu-700μδ6 flies. After 30 s of 50 μM nicotine stimulation (indicated by a red line), the glucose concentration was sustainably increased in cortex glia. Decreasing the expression levels of Glut1 in adult cortex glia had no effect on the nicotine-induced glucose elevation as measured during the 200-s quantification window (black line), starting 10 s after the end of nicotine perfusion (n = 10, t_{18} = 0.80, P = 0.44).

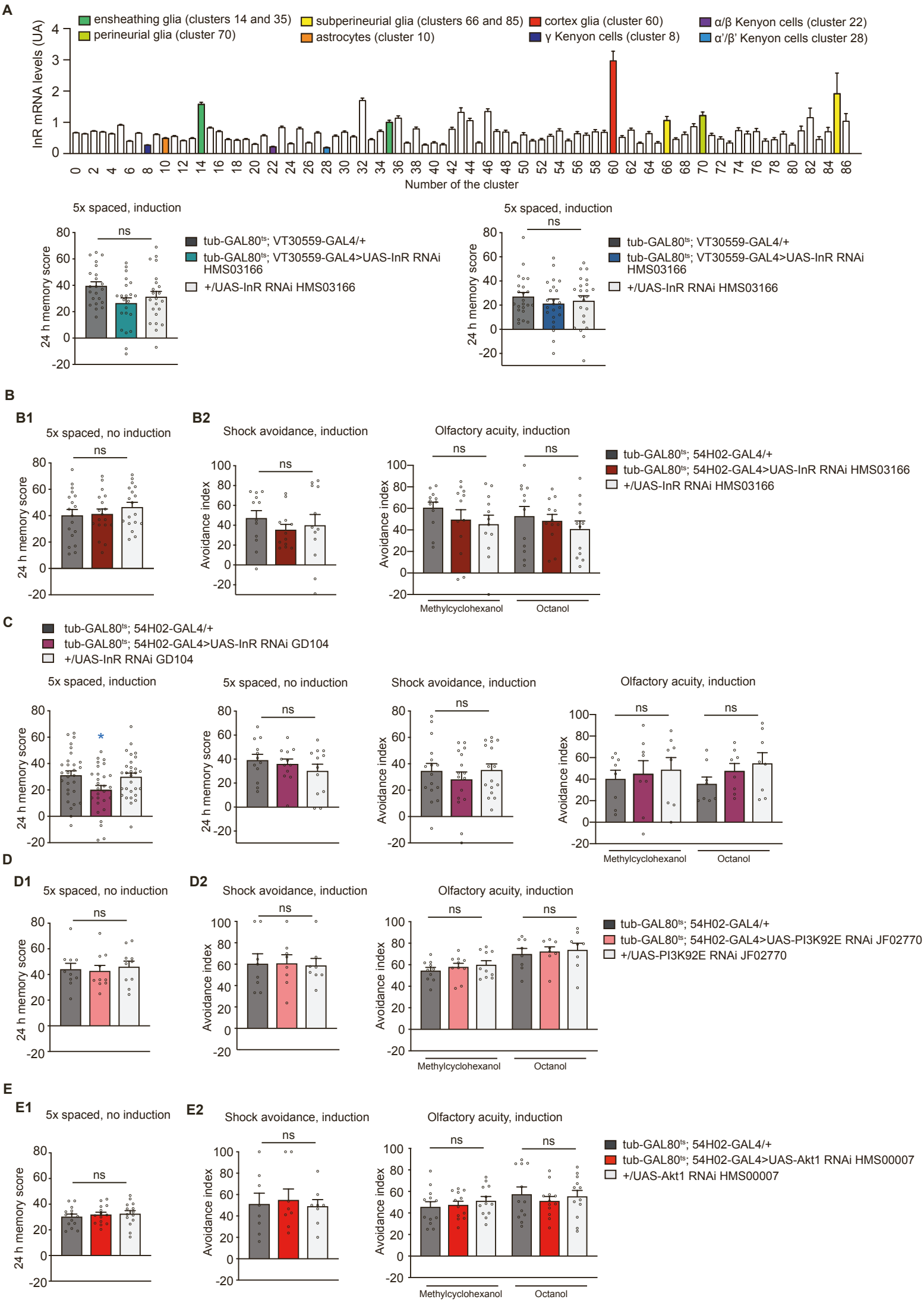
A
ensheathing glia (clusters 14 and 35)
perineurial glia (clusters 70)
subperineurial glia (clusters 66 and 85)
astrocytes (cluster 10)
cortex glia (cluster 60)
γ Kenyon cells (cluster 8)
α/β Kenyon cells (cluster 22)
α'/β' Kenyon cells cluster 28)
InR mRNA levels (UA)
Number of the cluster
5x spaced, induction
24 h memory score
ns
tub-GAL80ts; VT30559-GAL4/+
tub-GAL80ts; VT30559-GAL4>UAS-InR RNAi HMS03166
+/UAS-InR RNAi HMS03166
B
B1
5x spaced, no induction
B2
Shock avoidance, induction
Olfactory acuity, induction
Avoidance index
Methylcyclohexanol
Octanol
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-InR RNAi HMS03166
+/UAS-InR RNAi HMS03166
C
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-InR RNAi GD104
+/UAS-InR RNAi GD104
D
D1
D2
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-PI3K92E RNAi JF02770
+/UAS-PI3K92E RNAi JF02770
E
E1
E2
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-Akt1 RNAi HMS00007
+/UAS-Akt1 RNAi HMS00007

Figure S4. Related to Figure 4.

A. Relative expression of InR in various cell clusters according to published single-cell transcriptomics data (Davie et al., 2018). The number of the cluster in the original study is indicated under each bar, and relevant clusters are displayed in colour and notated. Levels of InR mRNA are higher in glial cells, particularly in cortex glia (cluster 60) as compared to MB neurons (clusters 8, 22, 28). A low level of InR expression in MB neurons is consistent with our behavioural data showing no involvement of the MB neuron InR in LTM. InR knock-down in MB neurons induced at the adult stage in all MB neurons (using the VT30559-GAL4 line) or in α/β MB neurons (using the c739-GAL4 line) did not disrupt LTM (all MB neurons: n = 22, $F_{2,65} = 3.05$, P = 0.054; α/β MB neurons: n = 23, $F_{2,68} = 0.56$, P = 0.58). This result is in apparent contradiction to a previous report concluding that insulin signalling is required in MB neurons for LTM formation (Chambers et al., 2015). The conclusion from this study was exclusively drawn from experiments using the constitutive knock-down of InR. Given that insulin signalling in neuroblasts is crucial during development (Chell and Brand, 2010) and that mutants of the insulin receptor substrate *chico* show cell-autonomous developmental defects in MB size and number of neurons (Naganos et al., 2012), it is possible that phenotypes observed with constitutively decreased InR expression were due to subtle developmental defects in MB physiology. Chambers and colleagues also reported that a knock-down of InR at the adult stage impairs LTM, although this result was obtained using a ubiquitous, heat shock inducible driver that includes neuronal and glial cells. Therefore, considering only the experiments performed with inducible tools that are devoid of any possible developmental issues, the published results can be explained by an acute role for InR signalling in cortex glia during LTM formation. B. Control experiments corresponding to InR knock-down in cortex glia. B1. In cortex glia, in the absence of thermal induction of the RNAi against InR, LTM in the flies of interest was normal (n = 18, $F_{2,53} = 0.69$, P = 0.51). B2. Shock reactivity (n = 12, $F_{2,35} = 0.50$, P = 0.61) and olfactory acuity (methylcyclohexanol: n = 12, $F_{2,35} = 1.00$, P = 0.38, octanol: n = 12, $F_{2,35} = 0.62$, P = 0.54) were normal after InR knock-down. C. The series of experiments concerning InR knockdown in cortex glia was performed with another RNAi. InR knock-down in cortex glia at the adult stage disrupted LTM (n = 28-30, $F_{2,85} = 3.41$, P = 0.038). Without RNAi induction, LTM in the flies of interest was normal (n = 12, $F_{2,35} = 0.83$, P = 0.45). Shock reactivity (n = 16, $F_{2,47} = 0.52$, P = 0.60) and olfactory acuity (methylcyclohexanol: n = 8, $F_{2,23} = 0.67$, P = 0.49, octanol: n = 8, $F_{2,23} = 1.45$, P = 0.27) were normal after InR knock-down. D. Control experiments corresponding to PI3K92E knock-down in cortex glia. D1. In cortex glia, in the absence of thermal induction of the RNAi against PI3K92E, LTM in the flies of interest was normal (n = 10, $F_{2,29} = 0.13$, P = 0.87). D2. Shock reactivity (n = 8, $F_{2,23} = 0.02$, P = 0.98) and olfactory acuity (methylcyclohexanol: n = 10, $F_{2,29} = 0.61$, P = 0.55, octanol: n = 8, $F_{2,23} = 0.13$, P = 0.88) were normal after PI3K92E knock-down. E. Control experiments corresponding to Akt1 knock-down in cortex glia. E1. In cortex glia, in the absence of thermal induction of the RNAi against Akt1, LTM in the flies of interest was normal (n = 14, $F_{2,41} = 0.30$, P = 0.75). E2. Shock reactivity (n = 8, $F_{2,23} = 0.10$, P = 0.90) and olfactory acuity (methylcyclohexanol: n = 12, $F_{2,35} = 0.46$, P = 0.64, octanol: n = 12, $F_{2,35} = 0.31$, P = 0.73) were normal after Akt1 knock-down.

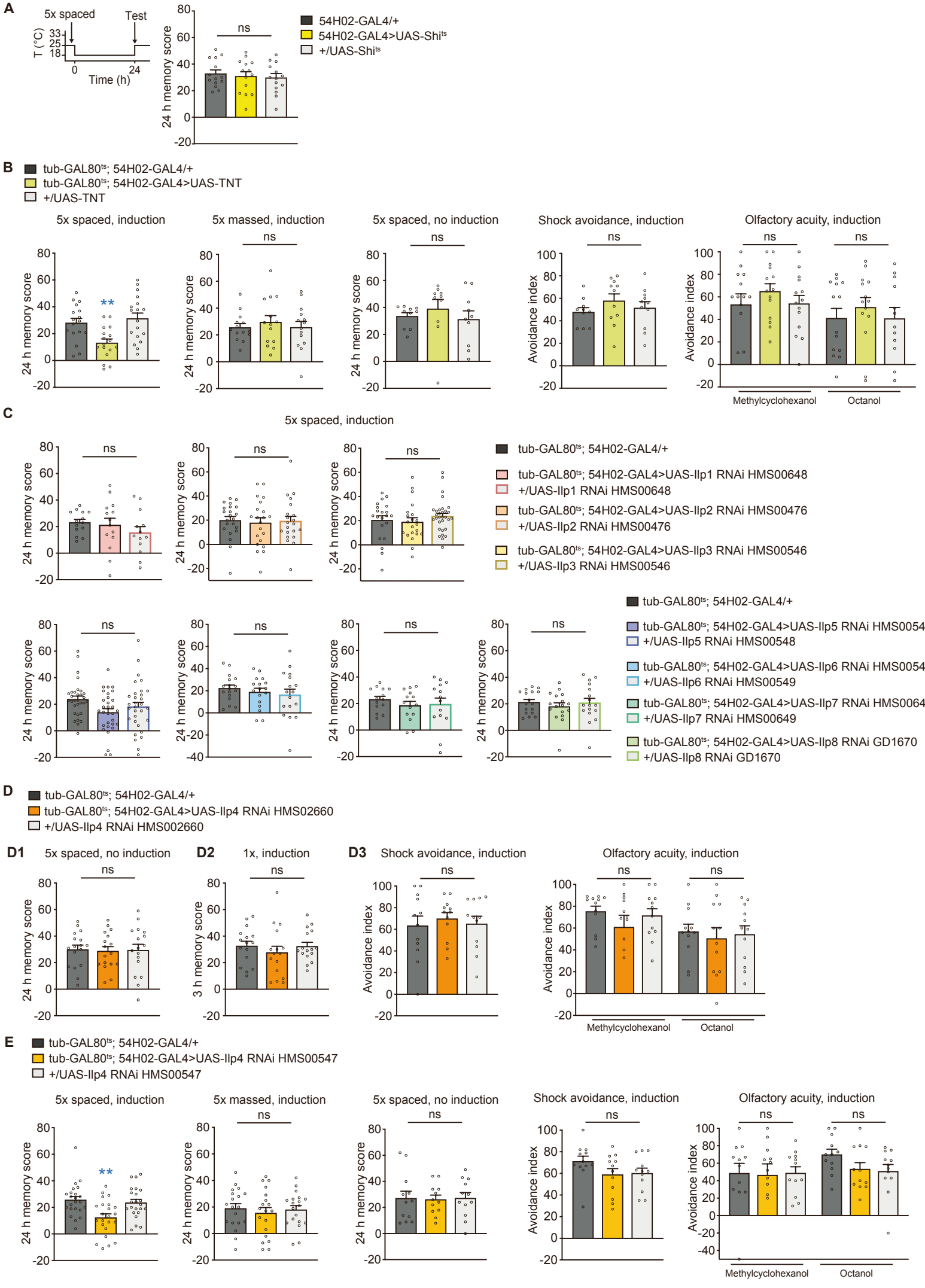
A
5x spaced
Test
T (°C)
33
25
18
0
24
Time (h)
24 h memory score
ns
54H02-GAL4/+
54H02-GAL4>UAS-Shits
+/UAS-Shits
B
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-TNT
+/UAS-TNT
5x spaced, induction
5x massed, induction
5x spaced, no induction
Shock avoidance, induction
Olfactory acuity, induction
Avoidance index
Methylcyclohexanol
Octanol
C
5x spaced, induction
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-Ilp1 RNAi HMS00648
+/UAS-Ilp1 RNAi HMS00648
tub-GAL80ts; 54H02-GAL4>UAS-Ilp2 RNAi HMS00476
+/UAS-Ilp2 RNAi HMS00476
tub-GAL80ts; 54H02-GAL4>UAS-Ilp3 RNAi HMS00546
+/UAS-Ilp3 RNAi HMS00546
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-Ilp5 RNAi HMS00548
+/UAS-Ilp5 RNAi HMS00548
tub-GAL80ts; 54H02-GAL4>UAS-Ilp6 RNAi HMS00549
+/UAS-Ilp6 RNAi HMS00549
tub-GAL80ts; 54H02-GAL4>UAS-Ilp7 RNAi HMS00649
+/UAS-Ilp7 RNAi HMS00649
tub-GAL80ts; 54H02-GAL4>UAS-Ilp8 RNAi GD1670
+/UAS-Ilp8 RNAi GD1670
D
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-Ilp4 RNAi HMS02660
+/UAS-Ilp4 RNAi HMS002660
D1
5x spaced, no induction
D2
1x, induction
3 h memory score
D3
Shock avoidance, induction
Olfactory acuity, induction
Avoidance index
Methylcyclohexanol
Octanol
E
tub-GAL80ts; 54H02-GAL4/+
tub-GAL80ts; 54H02-GAL4>UAS-Ilp4 RNAi HMS00547
+/UAS-Ilp4 RNAi HMS00547
5x spaced, induction
5x massed, induction
5x spaced, no induction
Shock avoidance, induction
Olfactory acuity, induction
Avoidance index
Methylcyclohexanol
Octanol

Figure S5. Related to Figure 5.

A. Control experiment corresponding to Shi^{ts} expression in cortex glia. The scheme next to the memory graph indicates the time-course of temperature treatments. LTM after spaced training was normal in 54H02-GAL4>UAS-shi^{ts} flies that were not subjected to a restrictive temperature treatment (n = 14, $F_{2,41} = 0.25$, P = 0.78). B. Blocking vesicular release in adult cortex glia through expression of tetanus toxin (TNT) yielded a significant LTM defect (n = 17/18, $F_{2,51} = 7.79$, P = 0.001), but did not affect memory tested 24 h after massed training (n = 14, $F_{2,39} = 0.30$, P = 0.74). Without induction, LTM performance was normal (n = 10, $F_{2,29} = 0.52$, P = 0.60). Shock reactivity (n = 10-12, $F_{2,31} = 0.88$, P = 0.43) and olfactory acuity (methylcyclohexanol: n = 14, $F_{2,41} = 0.68$, P = 0.51, octanol n = 12-14, $F_{2,39} = 0.42$, P = 0.66) were normal after TNT expression. C. Screen for the role of Ilps in LTM. With the exception of Ilp4 (see Figure 3), knocking down any insulin-like protein in the adult cortex glia did not affect LTM (Ilp1: n = 13/14, $F_{2,40} = 0.93$, P = 0.40; Ilp2: n=21/22, $F_{2,63} = 0.084$, P = 0.92; Ilp3: n=21, $F_{2,62} = 0.70$, P = 0.50; Ilp5: n = 31, $F_{2,92} = 2.96$, P = 0.057; Ilp6: n = 17, $F_{2,50} = 0.57$, P = 0.57; Ilp7: n = 16, $F_{2,47} = 0.49$, P = 0.61; Ilp8: n = 17, $F_{2,50} = 0.38$, P = 0.68). D. Control experiments corresponding to Ilp4 knock-down in cortex glia. D1. Without thermal induction of Ilp4 RNAi expression, LTM in the flies of interest was normal (n = 17, $F_{2,50} = 0.027$, P = 0.97). D2. Ilp4 knock-down in the adult cortex glia did not affect memory performance tested at 3 h after 1 x conditioning (n = 16, $F_{2,47} = 0.55$, P = 0.58). D3. Shock reactivity (n = 12, $F_{2,35} = 0.21$, P = 0.81) and olfactory acuity (methylcyclohexanol: n = 12, $F_{2,35} = 0.91$, P = 0.41, octanol: n = 12, $F_{2,35} = 0.15$, P = 0.86) after Ilp4 knock-down were normal. E. The whole series of experiments was performed with a second RNAi against Ilp4. Ilp4 knock-down in cortex glia exclusively at the adult stage disrupted LTM tested 24 h after 5 x spaced training (n = 23, $F_{2,68} = 7.59$, P = 0.0011), but did not affect memory tested 24 h after 5 x massed training (n = 19, $F_{2,56} = 0.25$, P = 0.78). Without Ilp4 RNAi induction, LTM in the flies of interest was normal (n = 12, $F_{2,35} = 0.016$, P = 0.98). Shock reactivity (n = 12, $F_{2,35} = 1.76$, P = 0.19) and olfactory acuity (methylcyclohexanol: n = 12, $F_{2,35} = 0.016$, P = 0.98, octanol: n = 12, $F_{2,35} = 2.13$, P = 0.13) were normal after Ilp4 knock-down.

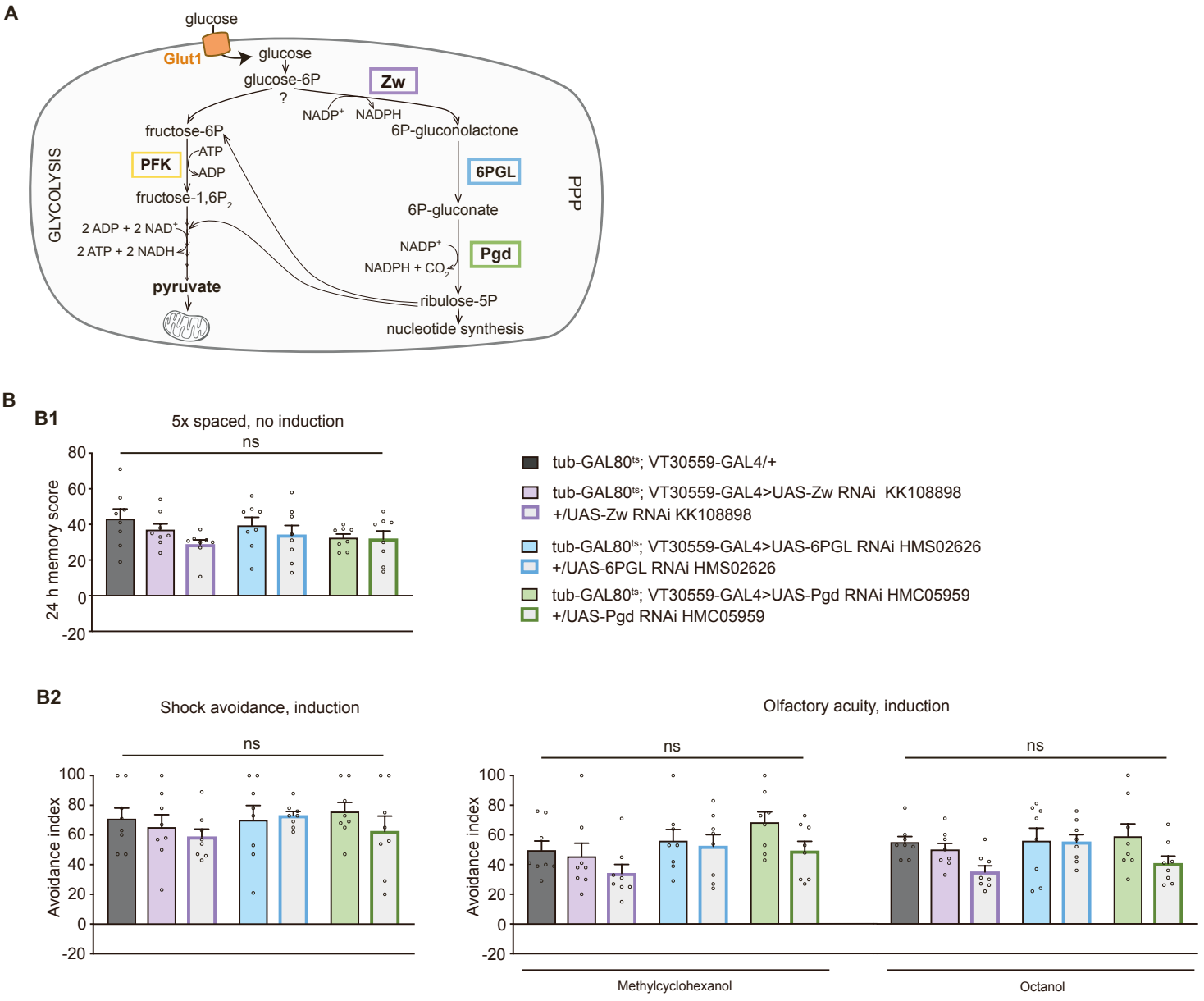
A
glucose
Glut1
glucose
glucose-6P
Zw
?
NADP+
NADPH
6P-gluconolactone
fructose-6P
ATP
PFK
ADP
6PGL
GLYCOLYSIS
PPP
fructose-1,6P2
6P-gluconate
2 ADP + 2 NAD+
2 ATP + 2 NADH
NADP+
NADPH + CO2
Pgd
pyruvate
ribulose-5P
nucleotide synthesis
B
B1
5x spaced, no induction
ns
24 h memory score
80
60
40
20
0
-20
tub-GAL80ts; VT30559-GAL4/+
tub-GAL80ts; VT30559-GAL4>UAS-Zw RNAi KK108898
+/UAS-Zw RNAi KK108898
tub-GAL80ts; VT30559-GAL4>UAS-6PGL RNAi HMS02626
+/UAS-6PGL RNAi HMS02626
tub-GAL80ts; VT30559-GAL4>UAS-Pgd RNAi HMC05959
+/UAS-Pgd RNAi HMC05959
B2
Shock avoidance, induction
ns
Avoidance index
100
80
60
40
20
0
-20
Olfactory acuity, induction
ns
ns
Avoidance index
Methylcyclohexanol
Octanol

Figure S6. Related to Figure 6.

A. Scheme of the two pathways of cellular glucose catabolism. Glucose is phosphorylated into glucose-6-phosphate (Glucose-6P). Glucose-6P can then be routed into glycolysis, where it is converted into fructose-6-phosphate (Fructose-6P), and in turn phosphorylated into fructose-1,6-bisphosphate (Fructose-1,6P_2) by phosphofructokinase (PFK). Glycolysis produces ATP and pyruvate, which can enter the mitochondrial TCA cycle to fuel electron transport chain and produce ATP. Alternatively, glucose-6P can be directed through the pentose phosphate pathway (PPP) and metabolized into 6-phospho-gluconolactone (6P-gluconolactone) by the glucose-6-phosphate dehydrogenase Zw, with the concomitant reduction of $NADP^+$ to NADPH. 6P-gluconolactone is then metabolized into 6-phospho-gluconate (6P-gluconate) by 6-phosphogluconolactonase (6PGL). 6P-gluconate is catalysed to ribulose-5-phosphate (ribulose-5P) *via* phosphogluconate dehydrogenase (Pgd), accompanied by the reduction of $NADP^+$ to NADPH and release of CO_2. Ribulose-5P can be used for generating glycolytic intermediates or for the biosynthesis of nucleotides and thus nucleic acids. These two functions of PPP, anti-oxidant protection and biosynthesis, are not exclusive. B. Control experiments corresponding to the knock-down of the PPP enzymes Zw, 6PGL and Pgd in MB neurons. B1. Without thermal induction of RNAi expression against Zw (RNAi KK108898), 6PGL (RNAi HMS02626) or Pgd (RNAi HMC05959), no LTM defect was displayed by the flies of interest as compared to the genotypic controls ($n = 8$, $F_{6,55} = 1.34$, $P = 0.26$). B2. Shock reactivity ($n = 8$, $F_{6,55} = 0.63$, $P = 0.70$) and olfactory acuity (methylcyclohexanol: $n = 8$, $F_{6,55} = 2.05$, $P = 0.08$, octanol: $n = 8$, $F_{6,55} = 2.22$, $P = 0.06$) were not altered by Zw, 6PGL or Pgd knock-down.