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Analysis of a spatial orientation memory in *Drosophila*

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Supplementary Information

Figure S1 Persistence of orientation in mutant lines with structural central complex defects.

Figure S2 No thoracic expression in GAL4-lines c232, c481 and c105.

Figure S3 Comparison of the locomotor and orientation behaviour of wild-type Canton S (CS) and *ign*$^{58/1}$ mutant flies in Buridan`s paradigm.

Table S1 Statistical tests used and their results for all figures in the main article.

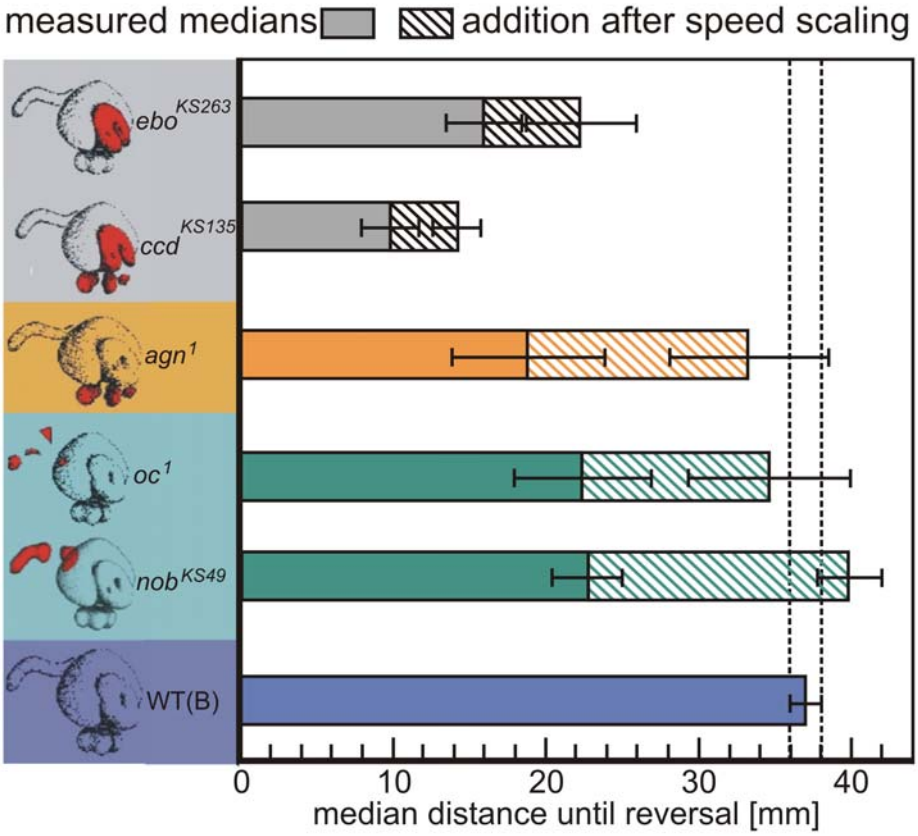

Figure S1 Persistence of orientation in mutant lines with structural central complex defects. Classical central complex mutant lines[10] were tested within the persistence-of-orientation paradigm[6] (no distracter). Summary sketches of their anatomical defects are given on the left side (*ebo, ellipsoid-body open*; *ccd, central complex deranged*; *agn, agnostic*; *oc, ocelliless*; *nob, no bridge*; WT(B), wild-type Berlin) Persistence of orientation is altered, whenever the ellipsoid body is anatomically impaired (ebo^{KS263}, ccd^{KS135}). Mutant lines affected in the structure of the noduli (agn^{1}) or the protocerebral bridge (oc^{1}, nob^{KS49}) showed no alteration. Hatched portions of bars: Central complex defective flies all display lower walking speeds[10] than wild-type flies. Consequently, they cannot cover the same distance in a given time. Data have been normalized accordingly, taking the independently determined mean walking speeds from Buridan`s paradigm[8,10] into account (N=10 flies, n=100 trials per genotype; error bars represent SEM values).

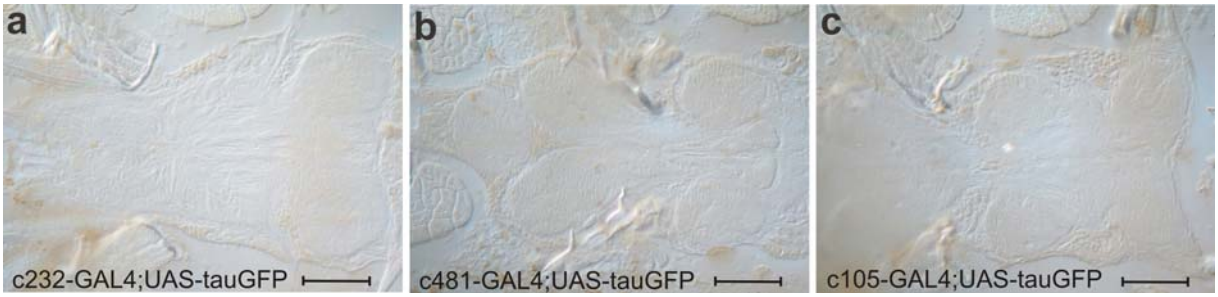

Figure S2 No thoracic expression in GAL4-lines c232, c481 and c105. Horizontal paraffin sections of adult fly thoraces. Expression was assessed after standard paraffin histology using a monoclonal anti-bovine TAU antibody (1:200; Sigma) as described[30] (scale bar = 50µm). **a**, **b**, **c**, Lines c232, c481 and c105, respectively, show no neuronal staining within their thoracic ganglia or in their leg neuromeres.

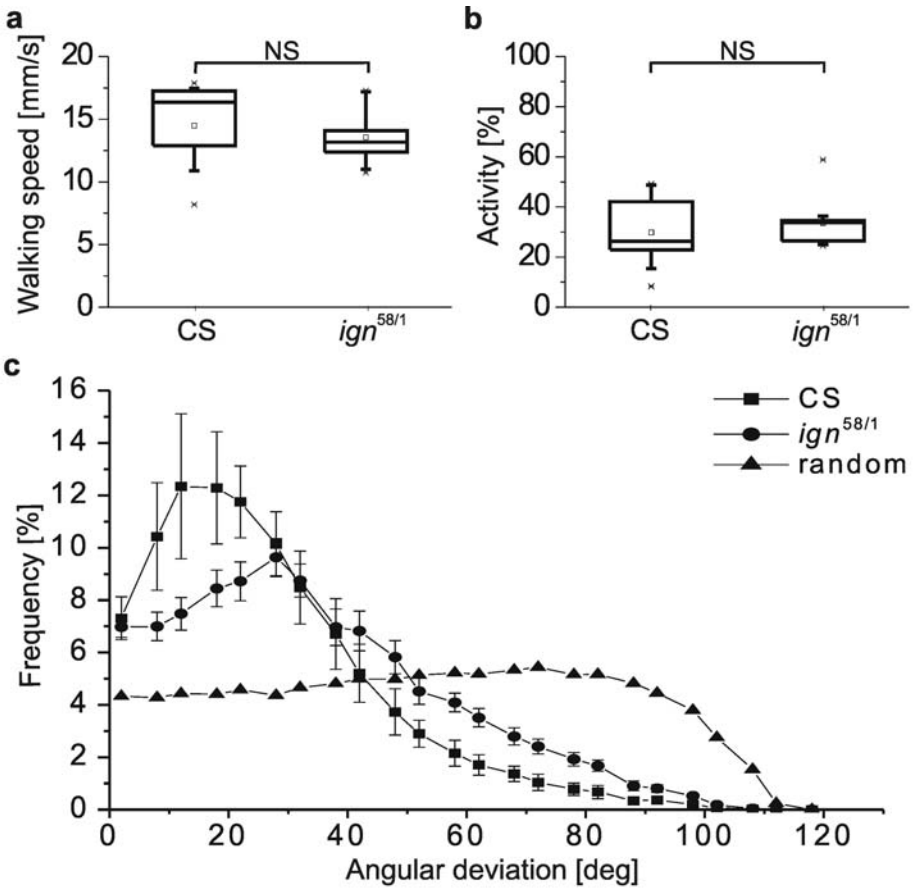

Figure S3 Comparison of the locomotor and orientation behaviour of wild-type Canton-S (CS) and *ign*$^{58/1}$ (ref. 5) mutant flies in Buridan`s paradigm[8,10]**.** Neither **a,** walking speed (p=0.44; N=24, 24) nor **b,** walking activity (p=0.44; N=24, 24) were significantly different between wild-type and mutant flies, suggesting an intact locomotor behaviour of *ign*$^{58/1}$ mutant flies. **c,** The histogram shows the absolute angular deviations from the direct course toward the opposing stripes. The variances of *ign*$^{58/1}$ mutant flies were not different from wild-type CS flies [F(24;24)=1.84, p=0.15], revealing a normal orientation ability of the *ignorant* mutant. The curve indicating random choice behaviour was generated using a Monte-Carlo calculation. **a, b,** Box-Whisker plots represent the median (bold line), the mean (square), 25% and 75% quartiles (box), 10% and 90% quantiles (whiskers) and extreme values (stars).

Fig. 1e	**1s** vs **2s** vs **3s** vs **4s**	Kruskal-Wallis Test	H=4.47	N=10,10,20,15	p=0.22
Fig. 1g	**CS** vs ***ebo*^{KS263}**	Mann-Whitney U-Test	U=2.5	N=11,13	$p=6.4\ 10^{-5}$
Fig. 1g	**HU-control** vs **HU**	Mann-Whitney U-Test	U=21	N=10,10	p=0.25

Fig. 2 a-c	**+/Y;UAS-TNT/+;*Tub*GAL80^{ts}/+ (18°C)** vs **(33°C)**	Sign-Test	Z=1.58	N=10,10	p=0.11
Fig. 2a	**+/Y;+/+;c232-GAL4/+ (18°C)** vs **(33°C)** but **(33°C)** above **58%-chance level**	Sign-Test One-Sample Sign Test	Z=2.33 t=6.19	N=10,10 N=10	p=0.02 $p=2\ 10^{-4}$
	+/Y;UAS-TNT/+;c232-GAL4/*Tub*-GAL80^{ts} (18°C) vs **(33°C)**	Sign-Test	Z=2.85	N=10,10	p=0.004
	+/Y;UAS-TNT/+;c232-GAL4/*Tub*-GAL80^{ts} (33°C) vs **58%-chance level**	One-Sample Sign-Test	t= -2.99	N=10	p=0.18
Fig. 2b	**c481-GAL4/Y (18°C)** vs **(33°C)**	Sign-Test	Z=1.77	N=10,10	p=0.08
	c481-GAL4/Y;UAS-TNT/+;*Tub*GAL80^{ts}/+ (18°C) vs **(33°C)**	Sign-Test	Z=2.48	N=10,10	p=0.01
	c481-GAL4/Y; UAS-TNT/+;*Tub*GAL80^{ts}/+ (33°C) vs **58%-chance level**	One-Sample Sign-Test	t= -0.84	N=10	p=0.43
Fig. 2c	**c105-GAL4/Y (18°C)** vs **(33°C)**	Sign-Test	Z=0.35	N=10,10	p=0.72
	c105-GAL4/Y;UAS-TNT/+;*Tub*GAL80^{ts}/+ (18°C) vs **(33°C)**	Sign-Test	Z=2.00	N=10,10	p=0.05
	c105-GAL4/Y;UAS-TNT/+;*Tub*GAL80^{ts}/+ (33°C) vs **58%-chance level**	One-Sample Sign-Test	t= -1.59	N=10	p=0.15

Fig. 3a	**CS** vs ***ign*$^{58/1}$/Y**	Mann-Whitney U-Test	U=16.5	N=11,10	p=0.012
	CS vs ***dnc*^{1}**	Mann-Whitney U-Test	U=37	N=11,10	p=0.34
Fig. 3b	**multiple comparison**	Kruskal-Wallis Test	H=6.51	N=10,14,13,10	p=0.09
	***ign*$^{58/1}$/Y** vs **+/Y ;;c232-GAL4/UAS-*ign*RNAi**	Mann-Whitney U-Test	U=31.5	N=10,10	p=0.27
	CS vs **+/Y ;;c232-GAL4/UAS-*ign*RNAi**	Mann Whitney U-Test	U=30.5	N=10,10	p=0.09

Fig. 4a	**multiple comparison**	Kruskal-Wallis Test	H=8.26	N=10 in all cases	p=0.04
	+/Y; +/+; *Appl*-GAL4/+ vs ***ign*$^{58/1}$/Y; UAS-*ign*/+; *Appl*-GAL4/+**	Mann-Whitney U-Test	U=14.5	N=10,10	p=0.58
	+/Y; +/+; *elav*-GAL4/+ vs ***ign*$^{58/1}$/Y; UAS-*ign*/+; *elav*-GAL4/+**	Mann-Whitney U-Test	U=27	N=10,10	p=0.63
	+/Y; +/+; c232-GAL4/+ vs ***ign*$^{58/1}$/Y; UAS-*ign*/+; c232-GAL4/+**	Mann-Whitney U-Test	U=19.5	N=10,10	p=0.83
Fig. 4b	***ign*$^{58/1}$/Y;; c232-GAL4/+ (18°C)** vs **(33°C)**	Sign-Test	Z=0.18	N=10,10	p=0.86
	+/Y;; *Tub*-GAL80^{ts} UAS-*ign* /+ (18°C) vs **(33°C)**	Sign-Test	Z=1.79	N=10,10	p=0.02
	***ign*$^{58/1}$/Y;; c232-GAL4/*Tub*-GAL80^{ts} UAS-*ign* (18°C)** vs **(33°C)**	Sign-Test	Z=3.33	N=13,13	p=0.001
	***ign*$^{58/1}$/Y ;; c232-GAL4/+ (18°C)** vs ***ign*$^{58/1}$/Y;; c232-GAL4/*Tub*-GAL80^{ts} UAS-*ign* (18°C)**	Mann-Whitney U-Test	U=48.5	N=10,13	p=0.36
	+/Y;; *Tub*-GAL80^{ts} UAS-*ign*/+ (33°C) vs ***ign*$^{58/1}$/Y;; c232-GAL4/*Tub*-GAL80^{ts} UAS-*ign* (33°C)**	Mann-Whitney U-Test	U=46.5	N=10,13	p=0.85

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