# figuremaking

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*25 Sep 2015*

**A**

### Putative mutants: no phenotype

**B**

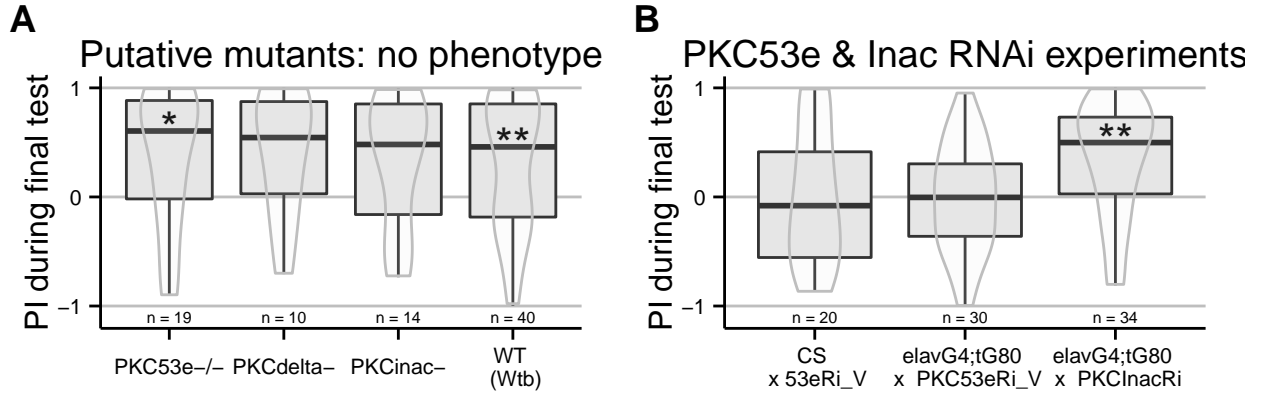### PKC53e & Inac RNAi experiments



Figure 1: Our attempt at determining the PKC gene involved has failed. Performance indexes (PI) during a test period following an 8 min. training session is reported. LEFT: Flies putatively mutants for PKC genes (PKC-53e, PKC-delta and PCK-InaC) performed well in the self-learning assay. Right: Flies possessing RNAi constructs designed against PKC53e and PKC InaC were crossed to elav-Gal4;tub-Gal80ts or to CS females. RNAi was induced for two days before the experiment via a 32 degrees heat shock. While the construct against PKC InaC had no effect, the construct for PKC53e prevented self-learning formation even in absence of Gal4 driven expression, such that no firm conclusion can be driven. Full genotype of the flies tested is indicated below. CS x 53eRi_V : ;;UAS_PKC53eRNAi_27696/+ . elavG4;tG80 x PKC53eRi_V : elavGal4/+;tubGal80ts/+;UAS_PKC53eRNAI_27696/+ . elavG4;tG80 x PKCInacRi : elavGal4/+;tubGal80ts/+;UAS_PKCInacRNAI_2895/+ . Stars indicate significant difference of the score against 0, using a non-parametrical wilcox test.
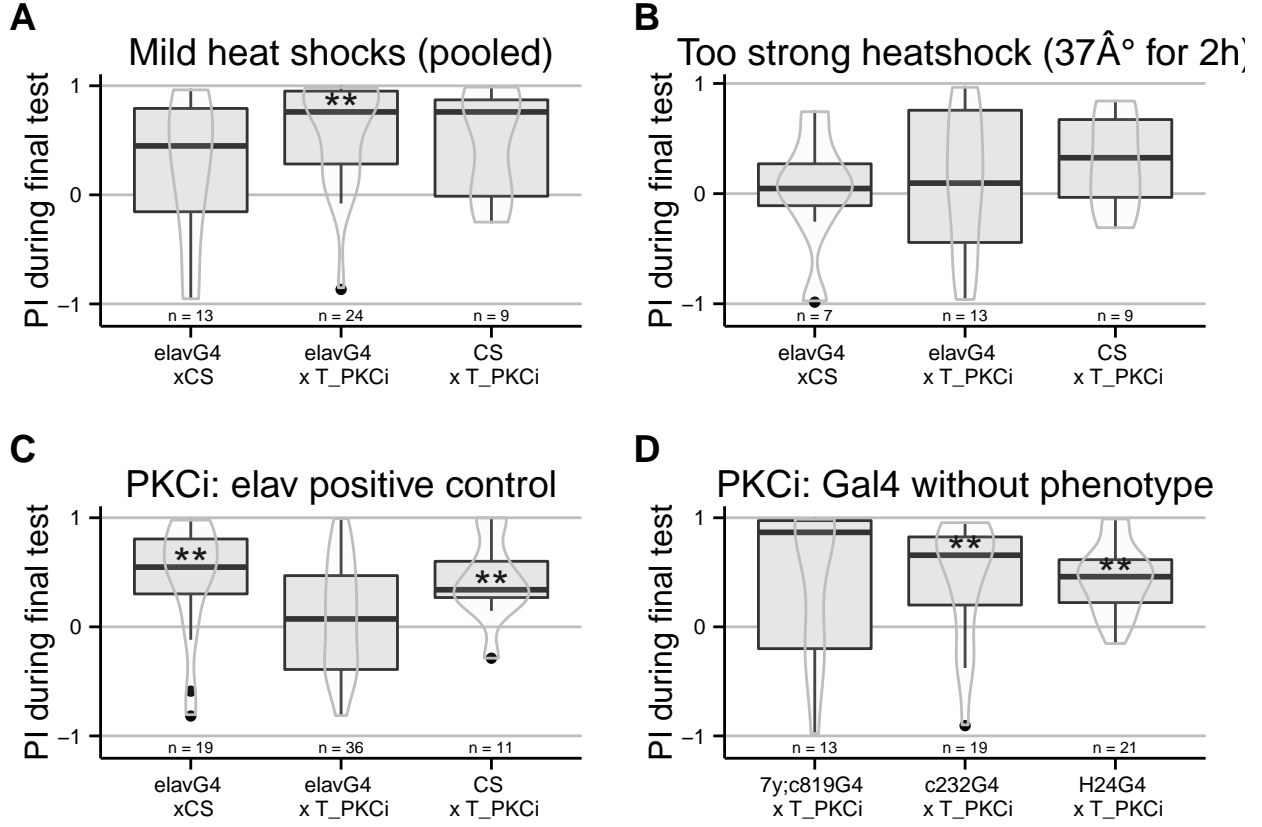
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Figure 2: PKC inhibition (achieved by using an effective heat shock protocol) in neurons, but not central brain regions, prevents self-learning formation. Driving Gal4 in all neurons using the elav-Gal4 driver while inactivating its ubiquitously expressed inhibitor Gal80 with a heat shock protocol can drive the expression of the PKC inhibitor. While test flies are still performing well after a mild heat shock (A, data pooled from different protocols: 33° for 15h, 36° for 2h, and 37°for 1h), astrong heat shock prevent learning in control flies (B, 37° for 2h). After a 4 hours heat shock at 35°C, test but not control flies were unable to form self-learning (C). Using this latter protocol, we restricted the expression of Gal4 in central brain regions using different drivers targetting central brain regions (D), which were all ineffective in preventing self-learning. Full genotype of the flies tested is indicated below. elavG4 xCS : elav-Gal4/+ . elavG4 x T_PKCi : elav-Gal4/+;tubGal80ts/+ ; UAS-PKCi/+ . CS x T_PKCi : tubGal80ts/+ ; UAS-PKCi/+. 7y;c819G4 x T_PKCi : tubGal80ts/+ ; UAS-PKCi/+ ___ H24-Gal4 . c232G4 x T_PKCi : tubGal80ts/+ ; UAS-PKCi/7y_Gal4,c819-Gal4 . H24G4 x T_PKCi : tubGal80ts/+ ; UAS-PKCi/c232-Gal4 . Stars indicate significant difference of the score against 0, using a non-parametrical wilcox test.
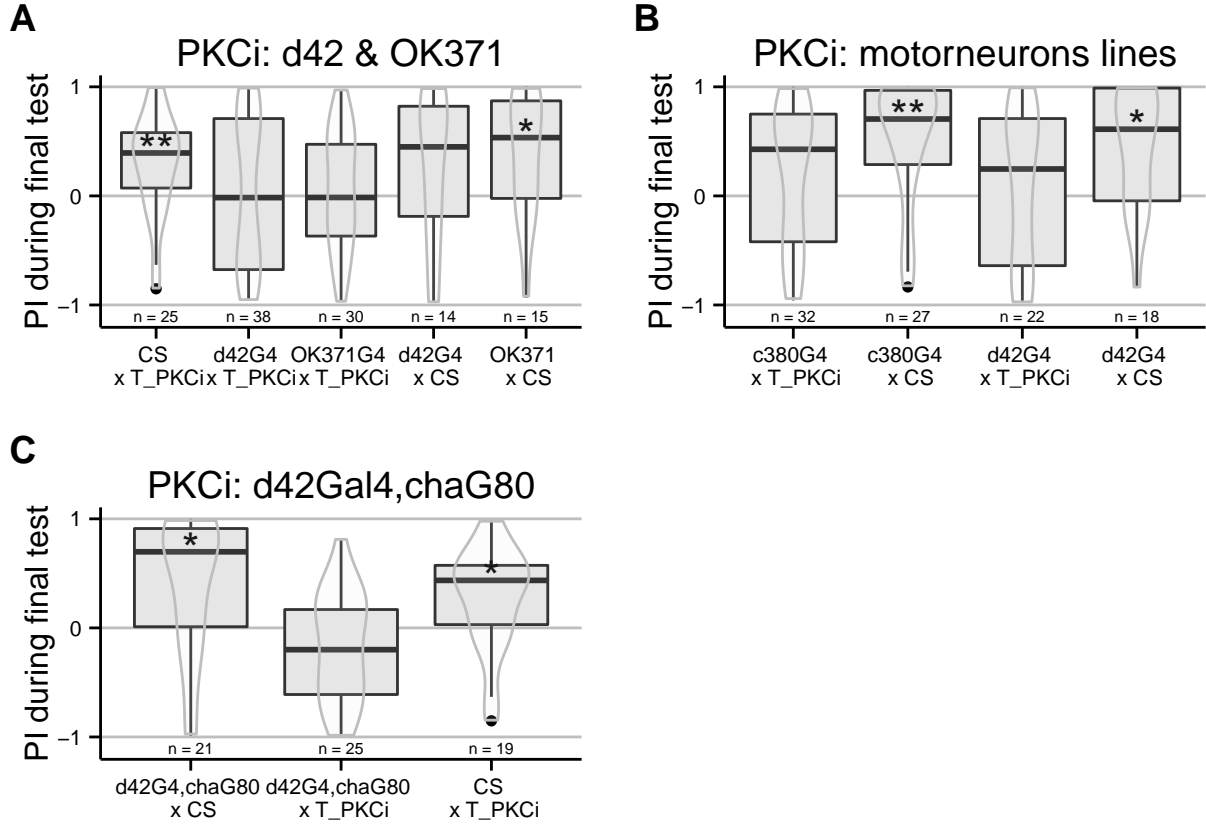
Figure 3: Flies with PKC inhibition using Gal4 line showing expression in motoneurons, were impaired in self-learning. A. Using OK371-Gal4 (expression in most glutamatergic neurons) or d42-Gal4 to drive PKC inhibition was effective in preventing self-learning formation, while the control flies seem to learn, although the score of the d42Gal4 x CS control was not statistically significantly positive. B. While the previous result with the D42Gal4 driver was reproduced, c380-Gal4, a second driver showing expression in motoneurons, lead to similar effects. C. The use of the d42Gal4,chaGal80 double construct as a driver was effective in preventing self-learning, while the controls did perform well Heat shock protocol was a 4 hours heat shock at 35°C. Full genotype of the flies tested is indicated below. CS x T_PKCi : tubGal80ts/+ ; UAS-PKCi/+ . d42G4 x T_PKCi : tubGal80ts/+ ; UAS-PKCi/d42-Gal4 . OK371G4 x T_PKCi : tubGal80ts/+ ; UAS-PKCi/+;OK371/+ . d42G4 x CS : d42Gal4/+ . OK371 x CS : OK371/+ .c380G4 x T_PKCi : c380-Gal4/+ . c380G4 x CS : c380Gal4/+; tubGal80ts/+ ; UAS-PKCi/+ . d42G4,chaG80 x CS : d42-Gal4, cha-Gal80/+ . d42G4,chaG80 x T_PKCi : tubGal80ts/+ ; UAS-PKCi/d42-Gal4, cha-Gal80 . CS x T_PKCi : tubGal80ts/+ ; UAS-PKCi/+ . Stars indicate significant difference of the score against 0, using a non-parametrical wilcox test.