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Oscillations in the ERG of the *Drosophila trpl*^{302} mutant are caused by an additional mutation in the *inebriated* gene.

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Introduction

Light-absorption in the *Drosophila* compound eye triggers a G protein- and PLCβ-mediated signalling cascade that finally leads to the opening of the cation channels TRP and TRPL. The influx of cations causes a depolarisation of the photoreceptor cells and, via histamine-dependant synaptic transmission, an activation of downstream neurons. Electroretinogram recordings (ERGs) reveal the depolarization of photoreceptor cells as a prominent voltage change while electrical responses of laminar neurons are recorded as on- and off-transients. Mutations in the *inebriated* (*ine*) gene, that encodes a putative neurotransmitter transporter, induce defects at the photoreceptor synapse and result in electrical oscillations superimposed on the depolarizing receptor potential (Gavin *et al.*, 2007). Gavin *et al.* have proposed a mechanism for the oscillations in the *ine* mutant. Histamine stored in the photoreceptor synapse is released into the synaptic cleft. Here, it activates postsynaptic Cl^- channels as well as presynaptic H_3 receptors. The activation of the H_3 receptor inhibits presynaptic Ca^{2+} channels and down-regulates histamine release. In the glia cells, histamine is converted to carcinine, which is transported into the photoreceptor cell by Inebriated. If Inebriated is missing, carcinine accumulates in the synaptic cleft and competes with histamine binding to the H_3 receptor. This leads to fluctuations of the current through the presynaptic Ca^{2+} channel. The resulting Ca^{2+} fluctuations finally cause oscillations in the response of downstream neurons. Similar oscillations were observed in the *trpl*^{302} mutant (Leung *et al.*, 2000), giving rise to speculations whether TRPL, in addition to its function in the phototransduction cascade, might have a function at the photoreceptor synapse. Interestingly, the *arrestin1*^{1} mutant also displays ERG oscillations. Here we show that oscillations observed in the ERG of *trpl*^{302} or *arr1*^{1} mutant flies are due to a secondary mutation in the *ine* gene.

Material and Methods

Drosophila stocks

Oregon R *w*^{1118} (here referred to as wild type), *y*; *ine*^{MI05077} [*y*$^{+}$], and the deletion strain *w*^{1118}; Df(2R)BSC131/CyO (46A1-46B4 covering trpl at 46B2) were obtained from the Bloomington *Drosophila* Stock Center. *trpl*^{302} *cn bw* (Niemeyer *et al.*, 1996), *arr1*^{1} *cn bw* (Dolph *et al.*, 1993) were obtained from C.S. Zuker. For the generation of a pure *trpl* mutant, *trpl*^{302} *cn bw ine* flies were crossed with *yw;* + mutant flies and female *trpl*^{302} *cn bw ine* / + flies were crossed with a *CyO*-balancer stock. Single male offspring flies carrying a recombined second chromosome and the *CyO* balancer were crossed with a *Sco*/*CyO* balancer stock. In the next generation, flies carrying the recombined second chromosome and *CyO* were crossed *inter se* and finally stocks carrying the recombined second chromosome homozygously were established. The mutant carrying the *trpl*^{302} mutation but lacking the *ine* second site mutation is now called *trpl*^{302NO} (No Oscillations) and still carries the *cn* and *bw* mutations (*trpl*^{302NO} *cn bw*). Flies were raised on standard cornmeal food at 25°C and crossings were carried out using standard *Drosophila* genetics.

Electroretinogram recordings (ERG)

For electroretinogram recordings, flies were immobilized in a pipette tip and mounted with a mixture of colophonium and bee's wax (1:3). Electroretinogram recordings were performed at room temperature after 3 minutes of dark adaptation prior to the first orange light-stimulus. Light-stimuli of 5 s duration were delivered by an orange light-emitting diode (580 nm, Roithner, Austria) and a blue light-emitting diode (470 nm, Roithner, Austria), collimated and combined by a 50% beam splitter (Linos, Germany) within the light path. The light intensity at the position of the fly eye was 2.15 mW/cm^2 for orange light and 1.3 mW/cm^2 for blue light. A DPA-2FS amplifier (NPI electronic, Germany) with a low pass filter (700 Hz) was used for signal amplification. Analog-to-digital conversion was accomplished with a PCI-6221 PC card (National Instruments, Germany). Data recording was achieved by the Whole Cell Analysis Program software WinWCP 4.7.6. (University of Strathclyde). The recording electrode glass capillary was filled with Davenport solution (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1.8 mM $NaHCO_3$, pH 7.2). In all experiments the following stimulus protocol (OBBOO) was used: 5 s orange light, 10 s dark, 5 s blue light, 10 s dark, 5 s blue light, 10 s dark, 5 s orange light, 10 s dark, 5 s orange light.

Western Blot analysis

Western blot analysis with anti-TRP and anti-TRPL antibodies was carried out as previously described (Cerny *et al.*, 2015).

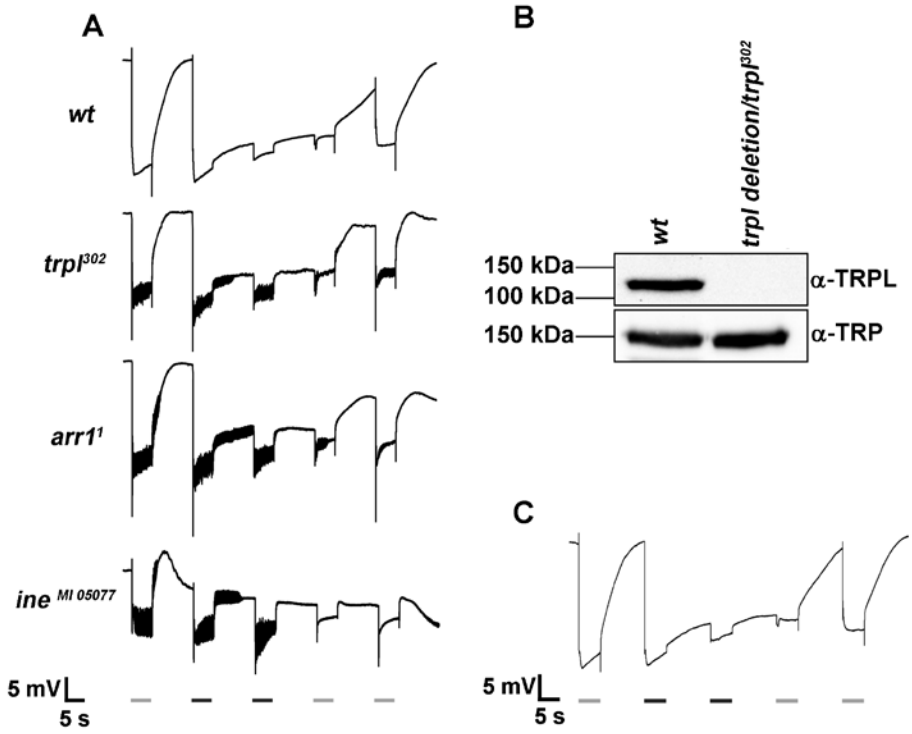

Figure 1. (A) ERGs of 3 day old flies with the indicated genotypes that were subjected to an OBBOO-protocol (see Material and Methods). The *ine*^{MI05077} mutant did not show a prolonged depolarization afterpotential (PDA) after blue light illumination due to its red eye color (*white*$^+$). Oscillations are superimposed on the depolarization and sometimes remain after termination of the light stimulus. (B) Western blot analysis of wild type (wt) and the *trpl deletion/trpl*^{302} mutant (Df(2R)BSC131/*trpl*^{302}). No TRPL protein was detected in the mutant while TRP that served as a loading control was present at wild type levels. (C) ERG of the *trpl deletion/trpl*^{302} mutant exposed to OBBOO protocol. No oscillations were observed.

Results

Figure 1A shows the oscillations in the ERG recordings of *trpl*^{302}, *arr1*^{1}, and *ine*^{MI05077} mutants. The oscillations superimposed on the depolarizing receptor potential have a frequency of 50-60 Hz and sometimes remain even after termination of the light pulse. In our stocks 92% of *trpl*^{302} flies, 57% of *arr1*^{1} flies, and 78% of *ine*^{MI05077} flies (*N* = 7-18) but none of the wild type flies showed oscillations.

To analyse whether the oscillations in the *trpl*^{302} mutant are really caused by a mutation in the *trpl* gene, we crossed the *trpl*^{302} mutant with a deletion mutant covering the *trpl* locus. These mutants were tested for presence of TRPL by Western blot analysis (Figure 1B) and analysed by ERG recordings (Figure 1C). Although the mutants lacked TRPL, none of seven flies tested showed oscillations. This finding revealed that the oscillations are not caused by the defective *trpl* gene.

Since *trpl*, *arr1*, and *ine* are all located on the second chromosome, we hypothesized that the oscillations in the *trpl*^{302} mutant as well as in the *arr1*^{1} mutant are caused by an additional mutation in the *ine* gene. To confirm this hypothesis we carried out complementation tests. For complementation analysis we first tested whether *ine*^{MI05077} is a recessive mutation with respect to ERG oscillations. This is the case, because heterozygous *ine* mutants showed no oscillations (Figure 2). However, ERG oscillations were readily observed in *ine*^{MI05077}/*trpl*^{302}, *ine*^{MI05077}/*arr1*^{1}, and *arr1*^{1}/*trpl*^{302} trans-heterozygous mutants (Figure 2).

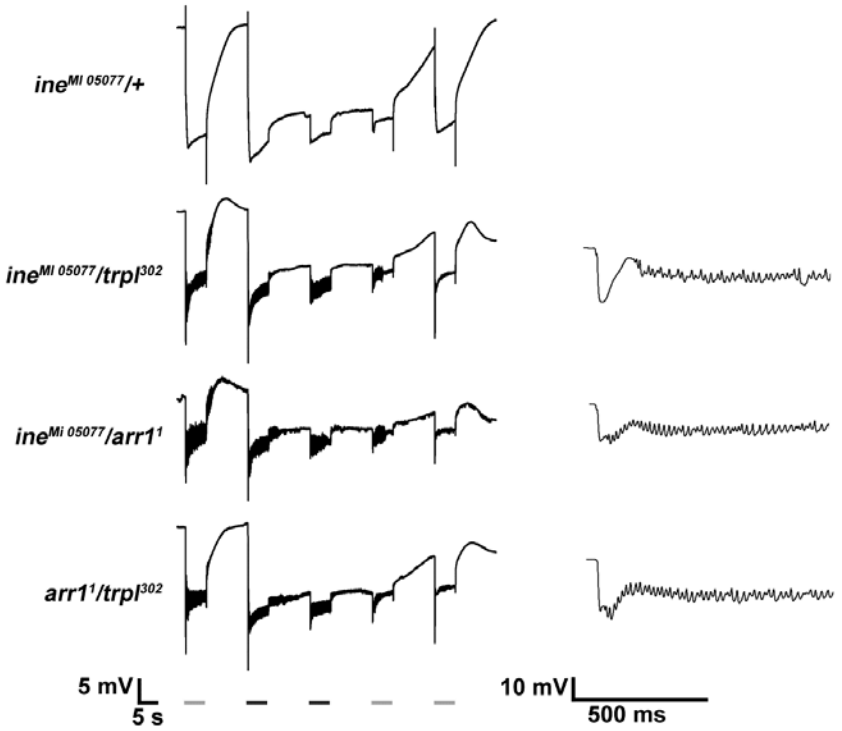

Figure 2. ERG recordings of 3 days old heterozygous *ine*^{MI05077} mutant and trans-heterozygous mutants, measured with an OBBOO protocol. None of the heterozygous *ine*^{MI05077} mutants (6 flies tested) but all of the tested trans-heterozygous mutants (9 flies *ine*^{MI05077}/*trpl*^{302}, 4 flies *ine*^{MI05077}/*arr1*^{1}, and 2 flies *arr1*^{1}/*trpl*^{302}) showed oscillations similar to the oscillations observed in the homozygous mutants. Recordings at the right show magnified views of the ERGs during the first orange light pulse.

As a conclusion, our results indicate that electric oscillations in the ERG recordings of *trpl*^{302} or *arr1*^{1} mutant flies are not caused by the mutations in the respective gene, but instead by an additional mutation in the *inebriated* gene. Therefore, ERG oscillations observed in *trpl*^{302} or *arr1*^{1} mutants cannot be taken as an

indication for a possible role of TRPL and arrestin in the phototransduction or synaptic transmission of photoreceptor cells. In order to generate a pure *trpl* mutant without an *ine* second site mutation, we performed a meiotic recombination between the *trpl* and *ine* loci which are 48 cM apart from each other. Among the 13 stocks carrying a recombined second chromosome, five stocks inherited the $trpl^{302}$ mutation (Figure 3A). Among these stocks, one stock was devoid of oscillations (Figure 3B,C) indicating a loss of the *ine* second site mutation. This mutant is now called $trpl^{302NO}$ (No Oscillations) and can be obtained from the authors.

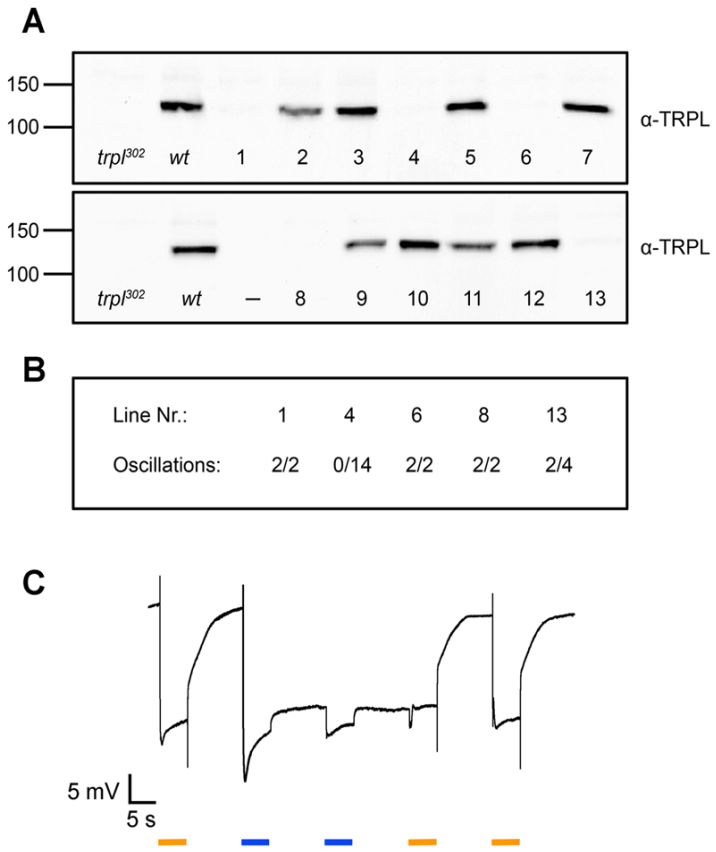

Figure 3. Generation of a pure $trpl^{302}$ mutant. (A) Stocks derived from recombination of the $trpl^{302}$ *cn bw ine* chromosome with a wild type chromosome (see Material and Methods) were analyzed for the $trpl^{302}$ mutation as indicated by the absence of a TRPL signal in Western-Blot analysis. (B) Stocks carrying the $trpl^{302}$ mutation were analyzed for the presence of oscillations in ERG recordings; fraction indicates the number of flies with oscillations/all flies tested. (C) ERG recording of a fly from recombination stock 4 using the OBBOO protocol. No oscillations could be detected.

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Cyp28AI gene variability in *Drosophila eremophila.*

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Abstract

We sequenced and analyzed gene *Cyp28A1* segment in cactophilic fly *Drosophila eremophila* samples obtained from the Mexican states of Guanajuato, Hidalgo, and San Luis Potosi. The results showed high