Pesticide Biochemistry and Physiology 148 (2018) 190–198

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

# DSC1 channel-dependent developmental regulation of pyrethroid susceptibility in Drosophila melanogaster

Xueting Chen[a,b,1], Yuanyuan Wang[c,1], Wenjun Wu[a,b], Ke Dong[d], Zhaonong Hu[a,b,c,*]

[a] Provincial Key Laboratory for Botanical Pesticide R&D of Shaanxi Province, Yangling, Shaanxi 712100, PR China
[b] Institute of Pesticide Science, College of Plant Protection, Northwest A&F University, Yangling, Shaanxi 712100, PR China
[c] Key Laboratory of Crop Pest Integrated Management on the Loess Plateau, Ministry of Agriculture, Yangling, Shaanxi 712100, PR China
[d] Department of Entomology, Michigan State University, East Lansing, MI 48824, USA

ARTICLE INFO

*Keywords:*
Sodium channel
*DSC1* channel
pyrethroid insecticides
*Drosophila*

ABSTRACT

Pyrethroid insecticides modify the gating of voltage-gated sodium channels, thus disrupting the function of the nervous system. In *Drosophila melanogaster*, *para* encodes a functional sodium channel. *Drosophila Sodium Channel 1* (*DSC1*), although considered as a putative sodium channel gene for decades due to its high sequence similarity with sodium channels, encodes a voltage-gated cation channel with high permeability to $Ca^{2+}$. Previous study showed that knockout of the *DSC1* gene ($DSC1^{-/-}$) caused *Drosophila* adults to be more susceptible to pyrethroids and the adult giant fiber (GF) neural circuit were more susceptible to pyrethroids. Considering distinct expression of DSC1 transcripts in adults and larvae, we examined the role of DSC1 channels in regulating pyrethroid susceptibility in *Drosophila* larvae. We conducted insecticide bioassays and examined the susceptibility of the larval neuromuscular junction (NMJ) to pyrethroids using $w^{1118}$, an insecticide-susceptible line, $DSC1^{-/-}$, $para^{ts1}$ (a pyrethroid-resistant line carrying a mutation in *para*) and a double mutation line $para^{ts1}$; $DSC1^{-/-}$. We found that, like the adult GF system, the NMJ of $DSC1^{-/-}$ flies is more susceptible to pyrethroids than that of $w^{1118}$ with the pyrethroid susceptibility ranked as $DSC1^{-/-} > w^{1118} > para^{ts1}$; $DSC1^{-/-} > para^{ts1}$. However, $DSC1^{-/-}$ larvae were about two-fold more resistant to pyrethroids than $w^{1118}$ larvae, and the pyrethroid susceptibility of larvae ranked as $w^{1118} > DSC1^{-/-} > para^{ts1}$; $DSC1^{-/-} > para^{ts1}$. These results reveal common and distinct roles of DSC1 channels in regulating the action of pyrethroids in adults and larvae of *D. melanogaster*.

## 1. Introduction

*Drosophila Sodium Channel 1* (*DSC1*) was considered to be a putative sodium channel gene for decades largely because of the significant sequence similarity of the DSC1 protein with vertebrate sodium channels [1,2]. Like voltage-gated sodium channels, the DSC1 protein has four homologous domains, each containing six transmembrane segments. However, functional characterization of *DSC1* and a *DSC1* ortholog, *BSC1* from the German cockroach *Blattella germanica*, in *Xenopus* oocytes revealed that *DSC1/BSC1* encoded a novel and new voltage-gated cation channel, not a sodium channel [3,4]. DSC1/BSC1channels represent a novel family of voltage-gated cation channels with high permeability to $Ca^{2+}$, whereas another sodium channel gene, *para* ($DmNa_v$) in *D. melanogaster* [5], and *para*-orthologs from other insects encode functional sodium channels [6]. Phylogenetic analyses revealed that DSC1 and its orthologs formed a separate group distinct from the classical voltage-gated sodium and calcium channels [7].

To understand the role of DSC1 in neurophysiology and neurotoxicology, *DSC1* knockout flies were generated and characterized [8]. The most prominent defect of *DSC1* knockout was a jumpy phenotype when disturbed and this feature was intensified under heat shock or starvation. Functional characterization of a well-defined neural circuit, the giant fiber system (GFS) revealed specific defects in the circuitry of the GFS in *DSC1* knockout flies. These results collectively suggest that the DSC1 channel plays a critical role in maintaining the stability of the GFS, particularly under the conditions of heat shock and starvation [8].

*DSC1* knockout adults were more susceptible to pyrethroid insecticides which enhance sodium channel activation, but not to indoxacarb, which is a sodium channel blocker insecticide, or fipronil which induces neuronal hyper excitability by blocking GABA-gated $Cl^-$ channels [8]. The specific effect of *DSC1* knockout on pyrethroid susceptibility suggests that *DSC1* knockout flies are more susceptible to

* Corresponding author at: Provincial Key Laboratory for Botanical Pesticide R&D of Shaanxi Province, Yangling, Shaanxi 712100, PR China.
*E-mail address:* huzhaonong@nwsuaf.edu.cn (Z. Hu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.pestbp.2018.04.014
Received 18 January 2018; Received in revised form 10 April 2018; Accepted 25 April 2018
Available online 27 April 2018
0048-3575/ 

**Table 1**
Toxicity of eight pyrethroids against $w^{1118}$, $para^{ts1}$, $DSC1^{-/-}$, and $para^{ts1}$; $DSC1^{-/-}$ adult flies.

| Pesticide | Lines | n | $LC_{50}$(μg/mL) | r | *df* | $X^2$ | *P* | Resistance Ratio to $w^{1118}$ |
|---|---|---|---|---|---|---|---|---|
| Permethrin | $w^{1118}$ | 750 | 3.714(3.307 ~ 4.171) | 0.9906 | 3 | 5.21 | 0.16 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 1.655(1.470 ~ 1.862) | 0.9873 | 3 | 6.04 | 0.11 | -2.24 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 8.709(8.074 ~ 9.393) | 0.9904 | 3 | 3.32 | 0.34 | 2.34 |
| | $para^{ts1}$ | 750 | 19.834(18.199 ~ 21.615) | 0.9963 | 3 | 1.50 | 0.68 | 5.34 |
| Ethofenprox | $w^{1118}$ | 750 | 30.509(28.456 ~ 32.709) | 0.9925 | 3 | 2.95 | 0.40 | 1.00 |
| | $DSC1^{-/-}$ | 900 | 25.893(23.356 ~ 28.706) | 0.9911 | 4 | 1.60 | 0.76 | -1.18 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 40.435(37.466 ~ 43.639) | 0.9861 | 3 | 5.38 | 0.14 | 1.33 |
| | $para^{ts1}$ | 750 | 58.435(54.005 ~ 63.228) | 0.9930 | 3 | 2.20 | 0.53 | 1.92 |
| Allthrin | $w^{1118}$ | 750 | 2.718(2.612 ~2.828) | 0.9799 | 3 | 6.08 | 0.11 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 1.675(1.573 ~ 1.784) | 0.9787 | 3 | 7.14 | 0.07 | -1.61 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 2.898(2.794 ~ 3.005) | 0.9868 | 3 | 4.87 | 0.12 | 1.07 |
| | $para^{ts1}$ | 750 | 4.376(4.167~4.595) | 0.9908 | 3 | 2.91 | 0.40 | 1.61 |
| Tetramethrin | $w^{1118}$ | 750 | 12.666(11.465 ~ 13.993) | 0.9891 | 3 | 3.17 | 0.37 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 10.061(9.240 ~ 10.957) | 0.9909 | 3 | 3.29 | 0.35 | -1.27 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 16.578(15.530~17.696) | 0.9947 | 3 | 2.54 | 0.46 | 1.31 |
| | $para^{ts1}$ | 750 | 26.118(23.956 ~ 28.475) | 0.9939 | 3 | 1.66 | 0.64 | 2.06 |
| Deltamethrin | $w^{1118}$ | 750 | 0.243(0.211 ~ 0.279) | 0.9953 | 3 | 1.96 | 0.58 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.115(0.099 ~ 0.134) | 0.9910 | 3 | 3.12 | 0.37 | -2.11 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.271(0.237 ~ 0.309) | 0.9908 | 3 | 3.79 | 0.28 | 1.12 |
| | $para^{ts1}$ | 750 | 0.544(0.468 ~ 0.632) | 0.9931 | 3 | 2.24 | 0.53 | 2.24 |
| Cypermethrin | $w^{1118}$ | 600 | 1.357(1.166 ~ 1.579) | 0.9820 | 3 | 6.78 | 0.07 | 1.00 |
| | $DSC1^{-/-}$ | 600 | 0.616(0.524~0.723) | 0.9813 | 3 | 7.31 | 0.06 | -2.22 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 1.986(1.699 ~ 2.320) | 0.9919 | 3 | 3.33 | 0.35 | 1.46 |
| | $para^{ts1}$ | 750 | 4.243(3.619 ~ 4.975) | 0.9897 | 3 | 2.91 | 0.29 | 3.13 |
| Lambda - Cyhalothrin | $w^{1118}$ | 750 | 0.338(0.292 ~ 0.392) | 0.9842 | 3 | 6.65 | 0.08 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.135(0.118 ~ 0.156) | 0.9974 | 3 | 1.01 | 0.80 | -2.5 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.396(0.343 ~ 0.458) | 0.9969 | 3 | 1.24 | 0.74 | 1.17 |
| | $para^{ts1}$ | 750 | 0.802(0.705 ~ 0.912) | 0.9942 | 3 | 2.99 | 0.40 | 2.37 |
| Fenvalerate | $w^{1118}$ | 750 | 0.869(0.749 ~ 1.008) | 0.9941 | 3 | 2.11 | 0.55 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.515(0.445 ~ 0.596) | 0.9944 | 3 | 2.08 | 0.56 | -1.69 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 1.098(0.950 ~ 1.271) | 0.9944 | 3 | 2,12 | 0.55 | 1.26 |
| | $para^{ts1}$ | 750 | 2.272(1.984 ~ 2.601) | 0.9888 | 3 | 4.53 | 0.21 | 2.61 |

**Table 2**
Time (min) to knockdown ($KT_{50}$) for 50% of adult flies exposed to 8 insecticide.

| Pesticides | Concentration (μg/mL) | $KT_{50}$ (min) | | | |
|---|---|---|---|---|---|
| | | $w^{1118}$ | $DSC1^{-/-}$ | $para^{ts1}$; $DSC1^{-/-}$ | $para^{ts1}$ |
| Permethrin | 100 | 18.09(17.28 ~ 18.93) | 16.58(15.82 ~ 17.38) | 18.99(17.81 ~ 20.26) | 21.75(20.63 ~ 22.93) |
| Ethofenprox | 320 | 11.98(10.89 ~ 13.17) | 9.72(8.94 ~ 10.57) | 12.87(11.75 ~ 14.10) | 15.26(14.34~16.23) |
| Allthrin | 32 | 24.75(23.72 ~ 25.84) | 21.34(20.30 ~ 22.42) | 26.88(25.79 ~ 28.02) | 32.57(31.04 ~ 34.17) |
| Tetramethrin | 100 | 19.20(18.06 ~ 20.40) | 14.77(13.79 ~ 15.82) | 20.56(19.45 ~ 21.73) | 25.98(24.67 ~ 27.35) |
| Deltamethrin | 10 | 15.75(14.74 ~ 16.82) | 12.86(11.89 ~ 13.92) | 20.46(19.19 ~ 21.80) | 30.22(28.24 ~ 32.34) |
| Cypermethrin | 40 | 13.91(12.74 ~ 15.20) | 11.41(10.58 ~ 12.31) | 18.15(16.78 ~ 19.63) | 24.34(22.95 ~ 25.81) |
| Lambda - cyhalothrin | 10 | 11.78(11.04 ~ 12.58) | 10.36(9.61 ~ 11.17) | 16.57(15.63 ~ 17.57) | 23.37(22.31 ~ 24.49) |
| Fenvalerate | 40 | 18.58(17.17 ~ 20.11) | 15.29(14.24 ~ 16.42) | 22.23(21.11 ~ 23.41) | 37.03(33.49 ~ 40.96) |

neuronal stimulation by sodium channel activators. Enhanced hypersensitivity of the GFS to pyrethroids in *DSC1* knockout flies further supports the notion that the DSC1 channel normally suppresses the response of the nervous system to pyerthroid exposure [8].

The *DSC1* transcript was widely expressed in the central nervous system (CNS) and peripheral nervous system (PNS) at the adult stage [9]. However, very few *DSC1*-expressing cells were found in either the PNS or CNS during larval stage [10]. To investigate the contribution of the DSC1 channel in the action of pyrethroids in *Drosophila* larvae, we conducted insecticide bioassays using $w^{1118}$, the "wild-type" control; $para^{ts1}$, a pyrethroid-resistant line carrying a mutation in the sodium channel; $DSC1^{-/-}$, and the double mutation $para^{ts1}$; $DSC1^{-/-}$ to evaluate their susceptibility to eight pyrethroid insecticides. Furthermore, we performed intracellular microelectrode recording to measure the susceptibility of *Drosophila* larval neuromuscular junction potential (NMJ) to three pyrethroids. Our results revealed the common and distinct roles of DSC1 channels in the action of pyrethroids in *D. melanogaster*. The *Drosophila* larvae NMJ represents an excellent system for further elucidating the functional interaction between DSC1 and sodium channels.

## 2. Materials and methods

### 2.1. Fly lines

Four *D. melanogaster* lines used in this study were $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$; $DSC1^{-/-}$ and $para^{ts1}$, which were obtained from the Michigan State University and maintained in the Northwest A&F University. $w^{1118}$ is a control line, $DSC1^{-/-}$ is a *DSC1* knockout line, $para^{ts1}$ is a pyrethroid-resistant line carrying a mutation in the sodium channel [11], and $para^{ts1}$; $DSC1^{-/-}$ is a double mutation line [12].

### 2.2. Insecticides

Eight technical grade compounds used in this study were supplied by Sunger (Shaanxi Sunger Road Bio-science Co., Ltd, China), including a pseudo-pyrethroid etofenprox, three type I pyrethroids, allethrin, permethrin, and tetramethrin as well as four type II pyrethroids,

**Table 3**
Toxicity of eight pyrethroids against $w^{1118}$, $para^{ts1}$, $DSC1^{-/-}$ and $para^{ts1}$; $DSC1^{-/-}$ larvae.

| Pesticide | Lines | n | $LD_{50}$ (μg/larva) | r | *df* | $X^2$ | *P* | Resistance Ratio to $w^{1118}$ |
|---|---|---|---|---|---|---|---|---|
| Permethrin | $w^{1118}$ | 750 | 0.073(0.064 ~ 0.084) | 0.9895 | 3 | 1.90 | 0.59 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.146(0.127 ~ 0.168) | 0.9974 | 3 | 0.49 | 0.92 | 2.00 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 900 | 0.480(0.405 ~ 0.569) | 0.9978 | 4 | 0.64 | 0.95 | 6.58 |
| | $para^{ts1}$ | 900 | 0.770(0.614 ~ 0.965) | 0.9710 | 4 | 5.15 | 0.23 | 10.55 |
| Etofenprox | $w^{1118}$ | 750 | 0.083(0.073 ~ 0.094) | 0.9982 | 3 | 0.92 | 0.82 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.153(0.133 ~ 0.177) | 0.9977 | 3 | 0.81 | 0.85 | 1.84 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.404(0.344 ~ 0.475) | 0.9985 | 3 | 0.42 | 0.94 | 4.86 |
| | $para^{ts1}$ | 750 | 0.747(0.637 ~ 0.875) | 0.9996 | 3 | 0.12 | 0.93 | 9.00 |
| Allethrin | $w^{1118}$ | 750 | 0.026(0.023 ~ 0.031) | 0.9977 | 3 | 0.78 | 0.86 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.046(0.040 ~ 0.053) | 0.9988 | 3 | 0.46 | 0.93 | 1.77 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.086(0.076 ~ 0.097) | 0.9997 | 3 | 0.18 | 0.92 | 3.31 |
| | $para^{ts1}$ | 750 | 0.139(0.121 ~ 0.161) | 0.9987 | 3 | 0.50 | 0.92 | 5.34 |
| Tetramethrin | $w^{1118}$ | 750 | 0.020(0.017 ~ 0.023) | 0.9971 | 3 | 0.90 | 0.83 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.036(0.032 ~ 0.042) | 0.9992 | 3 | 0.32 | 0.96 | 1.80 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.064(0.055 ~ 0.075) | 0.9993 | 3 | 0.22 | 0.97 | 3.20 |
| | $para^{ts1}$ | 750 | 0.091(0.079 ~ 0.105) | 0.9956 | 3 | 1.65 | 0.65 | 4.55 |
| Deltamethrin | $w^{1118}$ | 750 | 0.006(0.005 ~ 0.007) | 0.9980 | 3 | 0.91 | 0.82 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.011(0.010 ~ 0.013) | 0.9959 | 3 | 1.53 | 0.68 | 1.83 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.021(0.018 ~ 0.024) | 0.9935 | 3 | 2.48 | 0.48 | 3.50 |
| | $para^{ts1}$ | 750 | 0.032(0.027 ~ 0.038) | 0.9949 | 3 | 1.47 | 0.69 | 5.33 |
| Cypermethrin | $w^{1118}$ | 900 | 0.015(0.014 ~ 0.017) | 0.9960 | 4 | 1.25 | 0.87 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.027(0.022 ~ 0.032) | 0.9947 | 3 | 0.39 | 0.67 | 1.80 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.045(0.038 ~ 0.053) | 0.9968 | 3 | 1.21 | 0.75 | 3.00 |
| | $para^{ts1}$ | 900 | 0.087(0.073 ~ 0.103) | 0.9981 | 4 | 0.72 | 0.95 | 5.80 |
| Lambda - cyhalothrin | $w^{1118}$ | 750 | 0.013(0.013 ~ 0.014) | 0.9968 | 3 | 1.51 | 0.68 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.022(0.019 ~ 0.025) | 0.9948 | 3 | 1.83 | 0.61 | 1.69 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.041(0.036 ~ 0.047) | 0.9972 | 3 | 1.05 | 0.79 | 3.15 |
| | $para^{ts1}$ | 750 | 0.071(0.059 ~ 0.085) | 0.9978 | 3 | 0.50 | 0.92 | 5.46 |
| Fenvalerate | $w^{1118}$ | 750 | 0.040(0.035 ~ 0.046) | 0.9955 | 3 | 1.59 | 0.66 | 1.00 |
| | $DSC1^{-/-}$ | 750 | 0.076(0.066 ~ 0.087) | 0.9990 | 3 | 0.39 | 0.94 | 1.90 |
| | $para^{ts1}$; $DSC1^{-/-}$ | 750 | 0.172(0.147 ~ 0.199) | 0.9930 | 3 | 2.15 | 0.54 | 4.30 |
| | $para^{ts1}$ | 900 | 0.375(0.308 ~ 0.456) | 0.9977 | 4 | 0.45 | 0.98 | 9.38 |

deltamethrin, cypermethrin, lambda-cyhalothrin and fenvalerate. The purity of the compounds ranged from 92% to 98%.

### 2.3. Insecticide bioassays

A contact bioassay was used to evaluate the toxicity of eight pyrethroid insecticides against adults, which was partly modified from the method previously described in Rinkevich and Scott [13]. One to three-day-old adults were used. Insecticides were dissolved in acetone solution. Scintillation vials of 38.6 $cm^2$ were coated with 0.5 mL of test insecticide solution. The vials were rolled in a fume hood and left there for 1 h to let acetone evaporate. Ten adults were placed in each treated vial and plugged with a degreasing cotton ball and then placed into an incubator (25 °C ± 0.1). Each degreasing cotton ball was wetted with 10% sugar water. Test compounds were set 5 or 6 concentrations. Each bioassay was repeated at least four times with three replicates for each concentration. Acetone was used as solvent control. Mortality, which was defined as no movement upon touched with a needle, was assessed 24 h after insecticide application.

The same contact bioassay described above was also used to measure the time to knockdown by pyrethroids [12]. Scintillation vials were coated with one concentration of permethrin, ethofenprox, allethrin, tetramethrin, deltamethrin, cypermethrin, lambda-cyhalothrin or fenvalerate at 100 μg/mL (approximately 28× the $LC_{50}$ for $w^{1118}$), 320 μg/mL(approximately 11× the $LC_{50}$ for $w^{1118}$), 32 μg/mL(approximately 12× the $LC_{50}$ for $w^{1118}$), 100 μg/mL(approximately 8× the $LC_{50}$ for $w^{1118}$), 10 μg/mL(approximately 42× the $LC_{50}$ for $w^{1118}$), 40 μg/mL(approximately 31× the $LC_{50}$ for $w^{1118}$), 10 μg/mL(approximately 30× the $LC_{50}$ for $w^{1118}$), or 40 μg/mL(approximately 47× the $LC_{50}$ for $w^{1118}$), respectively. The number of flies that were knocked down was recorded at 5 min intervals for a total of 40 min. Each bioassay was repeated at least four times with three replicates.

A topical bioassay was used to evaluate the toxicity of the eight pyrethroid insecticides against larvae [14]. At room temperature, the heated 2% agar (2.5 mL) was poured into a 3.5 cm diameter petri dish to solidify. Ten third instar larvae were placed in the dish and 0.21 μL insecticide solution diluted with acetone was delivered onto the dorsal side of the thorax of individual larva. The treated groups set 5 or 6 dosages. The control group was treated with an equal amount of acetone. Each bioassay was repeated five times with three replicates for each dose. The number of flies that knocked down after 10 min was recorded. When the needle burned with the alcohol lamp stabbed the end of the larva, if the larva immediately curled, which indicated that the larva was not knocked down, otherwise knocked down.

Probit analyses were performed by SPSS software (version 12.0, SPSS Inc., Chicago, IL, USA, 2003). The median lethal concentration ($LC_{50}$) values were considered as significantly different when the 95% confidence intervals did not overlap.

### 2.4. Electrophysiology

Third-instar larvae were first fixed on a 4-cm diameter petri dish with pins and dissected by making a longitudinal mid-dorsal incision. Then internal organs were carefully removed to expose the body wall muscles and the nervous system as described [15,16]. The dissected larva was put in HL3 (hemolymph-like 3) physiological solution which was similar to that of *Drosophila* hemolymph [15]. Microelectrodes (20–30 MΩ) were pulled from borosilicate glasses with a micropipette puller (Sutter Instrument, CA, USA) and filled with 3 M KCl.

Glass microelectrodes impaled the ventral longitudinal muscle cell, without stimulating the motor nerve. Some slight potential changes were recorded in the screen, which were called miniature excitatory junctional potentials (mEJPs). The mEJPs were amplified by an Axonclamp 900A amplifier (Axon Instruments, CA, USA) and displayed through a Digidata 1440A interface, and then stored on a personal computer [17].

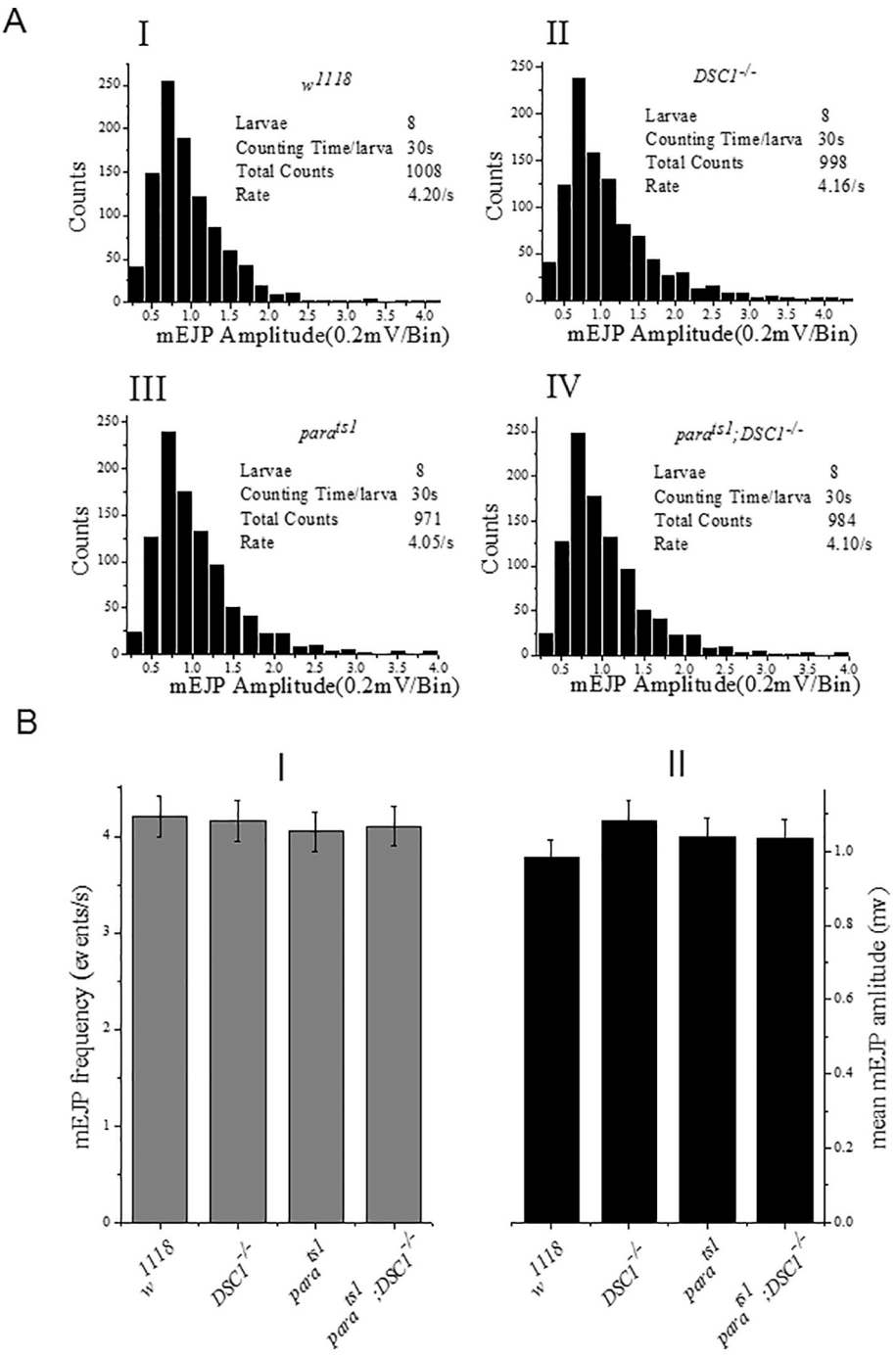


**Fig. 1.** Frequency and amplitude of mEJP of $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$, and $para^{ts1}$; $DSC1^{-/-}$ before insecticide treatment. A: Histogram of four lines of larval mEJP frequency and amplitude. Each line recorded total 240 s (n = 8 NMJ); B: Column of mean mEJP frequency and amplitude of four lines of larval NMJ. No significantly differences were found in the frequency and average amplitude of mEJP among the four lines.

To measure the effect of pyrethroids on the neuromuscular junction potential of the four lines of *D. melanogaster*, the glass microelectrode was inserted into the ventral longitudinal muscle cell. After the potential was stable, 1 mL of physiological solution was extracted. Subsequently, 1 mL of the physiological solution including tested pyrethroids was added, which made the final concentration to 100 nM. The solvent dimethyl sulfoxide (DMSO) could not exceed one thousandth of the solution. The changes of the resting potential for 10 min after adding the tested pyrethroids were recorded. When sudden rise and repetitive firing of the rest potential occurred, the experiments were stopped. Each concentration treatment of three pyrethroids for each strain was done using 35 larvae (n = 35).

Data analyses were performed by SPSS software (version 12.0, SPSS Inc., Chicago, IL, USA, 2003) and Microcal Origin 8.6 (Origin Lab Corp, Northampton, MA, USA, 2013). Statistical significance was determined by student's *t*-test. $P \leq 0.05$ was for the significant difference.

## 3. Results

### 3.1. Sensitivities of $w^{1118}$, $para^{ts1}$, $DSC1^{-/-}$, and $para^{ts1}$; $DSC1^{-/-}$ to eight pyrethroids

To compare the effects of *DSC1* knockout on the potency of pyrethroids on larvae and adults, we conducted bioassays to examine the

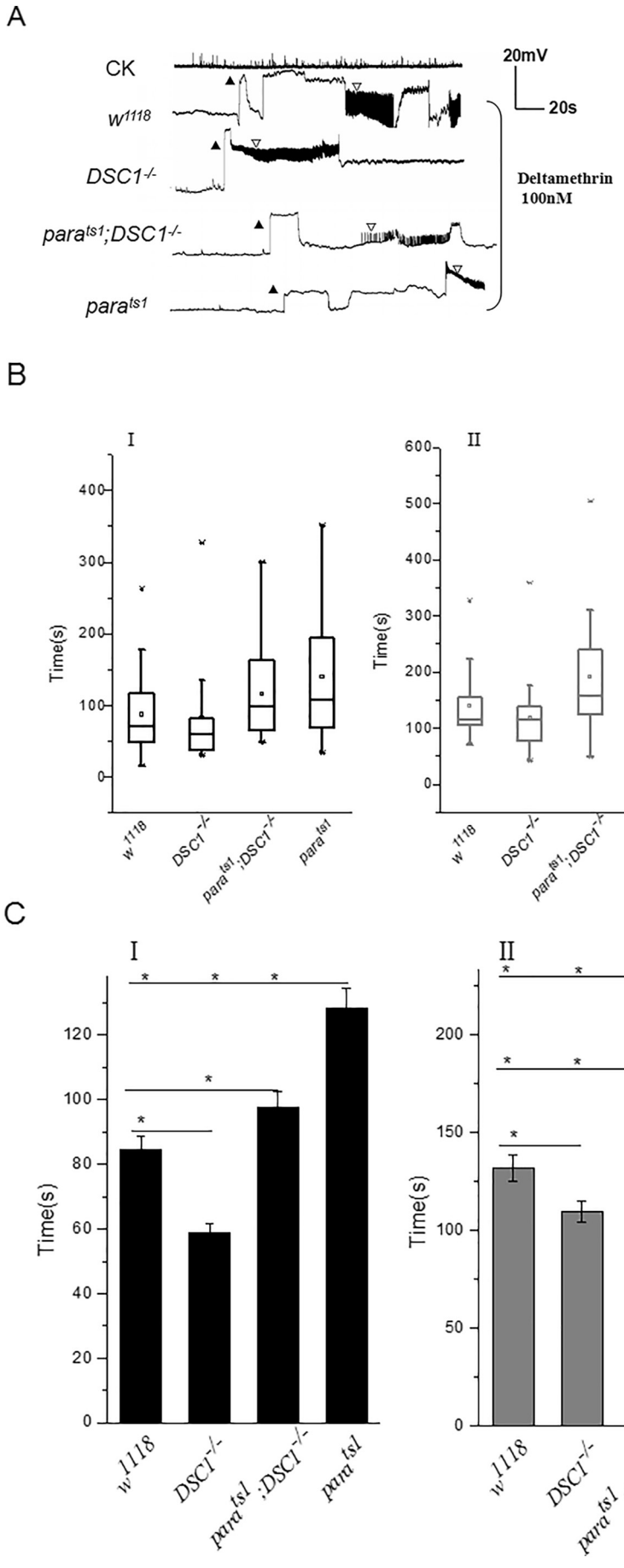


**Fig. 2.** Effects of deltamethrin on larval NMJ potentials of $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$, and $para^{ts1}$; $DSC1^{-/-}$. A: Changes of larval NMJ potentials after treatment with 100 nM deltamethrin: ▲ means resting potential that suddenly rise, ▽ means repetitive firing of mEJP; B: I, box plot of the onset time of rest potential sudden rise; II, box plot of the onset time of repetitive firing of mEJP; C: I, column of mean onset time of rest potential; II, column of mean onset time of repetitive firing of mEJP. n = 35 NMJ. Statistical significance was determined by student's *t*-test. * indicates significant difference in $P < 0.05$.

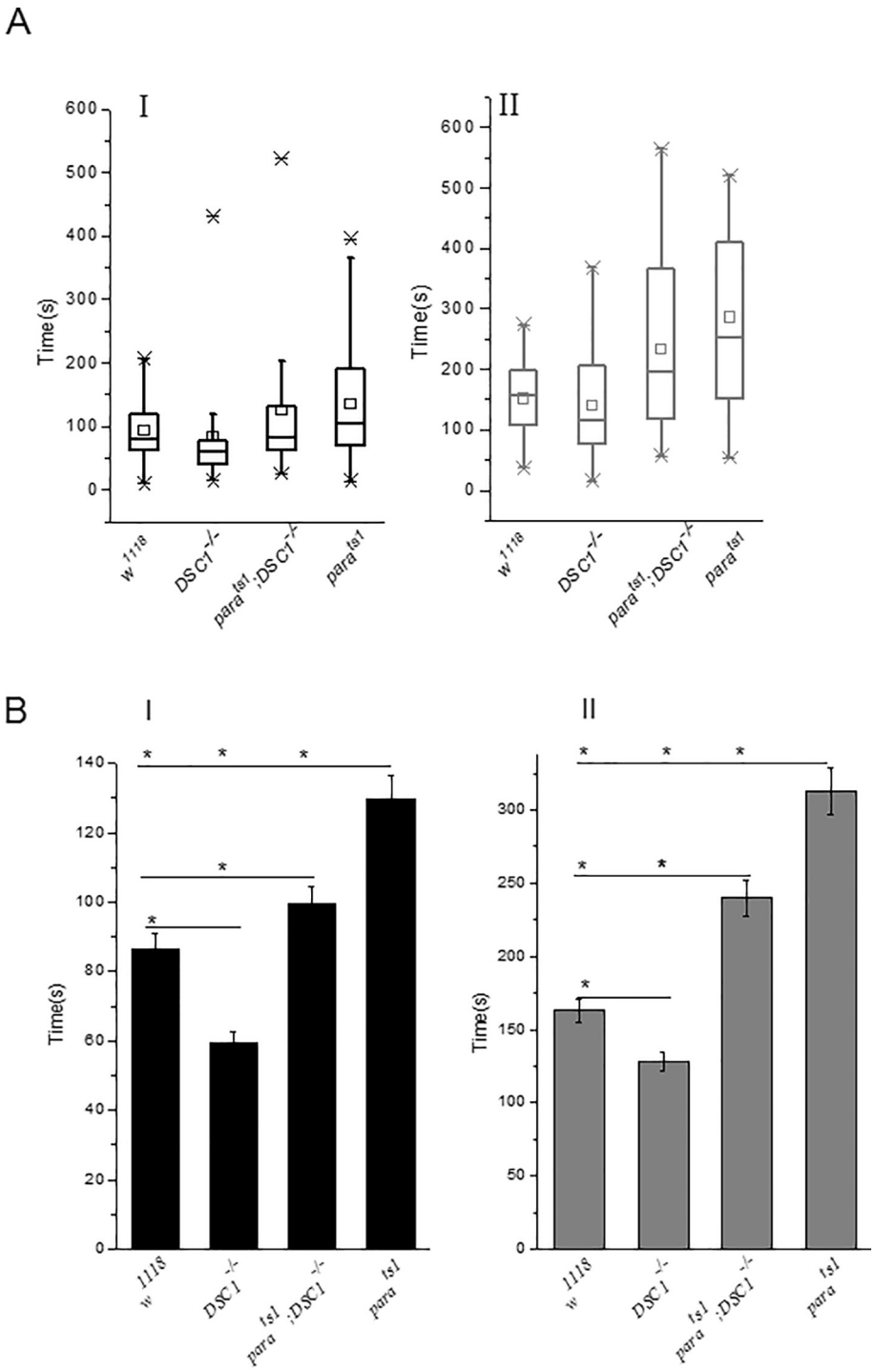


**Fig. 3.** Effects of cypermethrin on larval NMJ potentials of $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$, and $para^{ts1}$; $DSC1^{-/-}$. A: I, box plot of the onset time of rest potential sudden rise; II, box plot of the onset time of repetitive firing of mEJP; B: I, column of the mean onset time of rest potential; II, column of the mean onset time of repetitive firing of mEJP. n = 35 NMJ. Statistical significance was determined by student's *t*-test. * indicates significant difference in $P < 0.05$

sensitivities of adults and larvae of $w^{1118}$, $para^{ts1}$, $DSC1^{-/-}$, and $para^{ts1}$; $DSC1^{-/-}$ to eight pyrethroids (permethrin, etofenprox, allethrin, tetramethrin, deltamethrin, cypermethrin, lambda-cyhalothrin, and fenvalerate). As shown in Table 1, adult flies of $DSC1^{-/-}$ were 2.24-, 1.18-, 1.61-, 1.27-, 2.11-, 2.22-, 2.5-, and 1.69-fold more susceptible to permethrin, etofenprox, allethrin, tetramethrin, deltamethrin, cypermethrin, lambda-cyhalothrin, and fenvalerate than adults of $w^{111}$, respectively (Table 1). Compared with that of $w^{1118}$, adult flies of $para^{ts1}$ were 5.34-, 1.92-, 1.61-, 2.06-, 2.24-, 3.13-, 2.37-, and 2.61-fold more resistant to permethrin, etofenprox, allethrin, tetramethrin, deltamethrin, cypermethrin, lambda-cyhalothrin and fenvalerate, respectively (Table 1). Compared with $para^{ts1}$ flies, $para^{ts1}$; $DSC1^{-/-}$ flies were less resistant to these pyrethroids. The susceptibility order of the four lines to the pyrethroids was $DSC1^{-/-} > w^{111} \geq para^{ts1}$; $DSC1^{-/-} > para^{ts1}$. In addition, significant differences were found in knockdown in terms of $KT_{50}$ between the four lines to the eight pyrethroids (Table 2). The susceptibility order was ranked as $DSC1^{-/-} > w^{1118} \geq para^{ts1}$; $DSC1^{-/-} > para^{ts1}$, which was consistent with the results from the mortality assessment.

In contrast, compared with that of $w^{1118}$, $DSC1^{-/-}$ larvae were 2-,

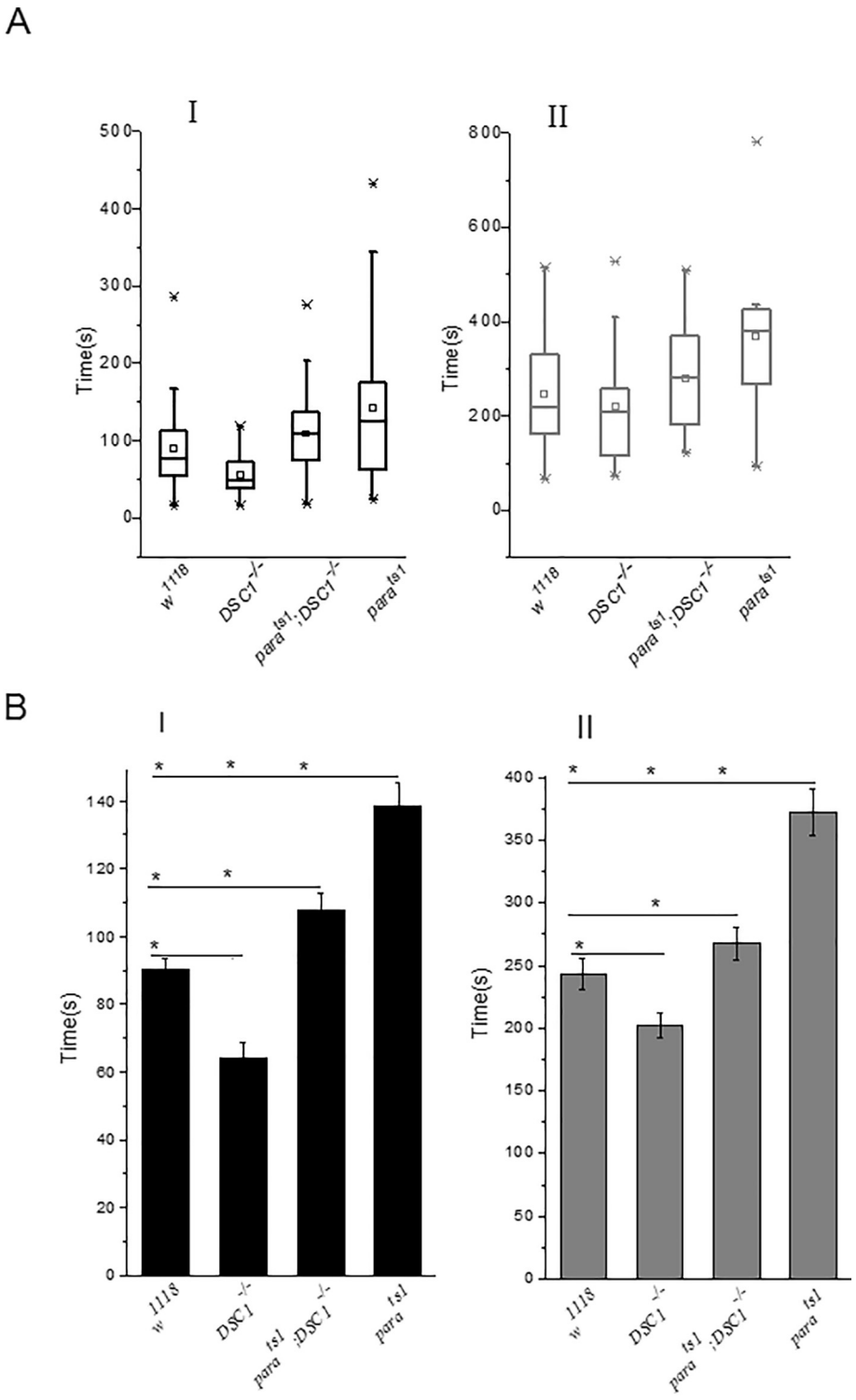


**Fig. 4.** Effects of permethrin on larval NMJ potentials of $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$, and $para^{ts1}$; $DSC1^{-/-}$. A: I, box plot of the onset time of rest potential sudden rise; II, box plot of the onset time of repetitive firing of mEJP; B: I, column of the mean onset time of rest potential; II, column of mean onset time of repetitive firing of mEJP. n = 35 NMJ. Statistical significance was determined by student's *t*-test. * indicates significant difference in $P < 0.05$.

1.84-, 1.77-, 1.8-, 1.83-, 1.8-, 1.69-, and 1.9-fold more resistant, and $para^{ts1}$ larvae were 10.55-, 9-, 5.34-, 4.55-, 5.33-, 5.8-, 5,46-, and 9.38-fold more resistant to permethrin, etofenprox, allethrin, tetramethrin, deltamethrin, cypermethrin, lambda-cyhalothrin and fenvalerate, respectively. However, $para^{ts1}$; $DSC1^{-/-}$ double-mutant larvae were 1.71-fold, 1.85-, 1.62-, 1.42-, 1.52-, 1.89-, 1.73-, and 2.2-fold more susceptible to the eight pyrethroids than those of $para^{ts1}$ larvae. Therefore, the susceptibility order of the four *Drosophila* larval strains to the pyrethroids was $W^{1118} > DSC1^{-/-} > para^{ts1}$; $DSC1^{-/-} > para^{ts1}$ (Table 3).

**Table 4**
Time of the onset of membrane potential changes after the treatment of 100 nM of deltamethrin, cypermethrin and permethrin.

| | | $w^{1118}$ | $DSC1^{-/-}$ | $para^{ts1}$ | $para^{ts1}$; $DSC1^{-/-}$ |
|---|---|---|---|---|---|
| Deltamethrin | Fluctuation | 84.52 ± 4.27 | 58.62 ± 2.93* | 128.12 ± 6.41* | 97.65 ± 4.88 |
| | Repetitive firing | 131.96 ± 6.60 | 109.57 ± 5.48* | 217 ± 10.85* | 172.71 ± 8.64* |
| Cypermethrin | Fluctuation | 86.73 ± 4.34 | 59.73 ± 3.00* | 129.81 ± 6.49* | 99.37 ± 4.97 |
| | Repetitive firing | 162.82 ± 8.14 | 128.24 ± 6.41* | 312.96 ± 15.66* | 239.77 ± 11.99* |
| Permethrin | Fluctuation | 90.23 ± 4.51 | 64.25 ± 3.21* | 138.29 ± 6.91* | 107.58 ± 5.38* |
| | Repetitive firing | 243.26 ± 12.16 | 202.50 ± 10.12* | 371.93 ± 18.60* | 267.65 ± 13.38* |

Note: The data is presented by mean ± SEM. Statistical significance was determined by student's *t*-test.* indicates significantly different compared with $w^{1118}$, $P < 0.05$.

### 3.2. Effects of pyrethroids on the Drosophila larval NMJ potential

#### 3.2.1. mEJP amplitude and frequency

Miniature excitatory junctional potentials (mEJPs) were observed and recorded with glass microelectrode impaled longitudinal abdominal muscle cells without stimulating motor nerve. The frequencies of mEJPs from $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$; $DSC1^{-/-}$, and $para^{ts1}$ larvae were 4.20 ± 0.21/s, 4.16 ± 0.21/s, 4.05 ± 0.20/s and 4.10 ± 0.20/s, respectively. The amplitudes of mEJPs were between 0.4 and 1.2 mV (Fig. 1A). No significantly differences were found in the frequency and average amplitude of mEJP among the four lines (Fig. 1B).

#### 3.2.2. Deltamethrin affects Drosophila larval NMJ potential

Pyrethroid insecticides, including deltamethrin, depolarize motor nerves resulting in increased neurotransmitter release, fluctuations of membrane potential and ultimate blocking of neuromuscular transmission due to continued neurotransmitter release in the house fly neuromuscular junction (NMJ) [18]. Similarly, after the application of 100 nM of deltamethrin, we observed that a sudden rise or fluctuations of resting potential caused muscle contraction and twitch of larvae and followed by potential decline and then repetitive firing of mEJPs from the NMJ of the $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$; $DSC1^{-/-}$ and $para^{ts1}$ larvae, but the onset of the events was different among the lines (Fig. 2). On average, the sudden change in resting potential appeared 26 s earlier in the $DSC1^{-/-}$ NMJ and 44 s later in the $para^{ts1}$ NMJ compared with $w^{1118}$. However, no distinct difference was observed between the $w^{1118}$ and $para^{ts1}$; $DSC1^{-/-}$ lines (Fig. 2A, Fig. 2BI, and Fig. 2CI). With the extension of insecticide exposure, repetitive firing of mEJP gradually appeared from $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$; $DSC1^{-/-}$, and $para^{ts1}$ preparations. The onset time of $w^{1118}$, $DSC1^{-/-}$, $para^{ts1}$:$DSC1^{-/-}$, and $para^{ts1}$ were 131.96 ± 6.60 s, 109.57 ± 5.48 s, 172.71 ± 8.64 s and 217 ± 10.85 s, respectively (Fig. 2B II and Fig. 2CII). The shorter onset time of resting potential rise or mEJP repetitive firing means more susceptible of the NMJ to deltamethrin. Therefore, based on onset time of mEJP repetitive firing, the susceptibility of the larval NMJ to deltamethrin can be ranked as: $DSC1^{-/-}$ > $w^{1118}$ > $para^{ts1}$:$DSC1^{-/-}$ > $para^{ts1}$. The tendency of the onset time of mEJP repetitive firing of four lines of larval NMJ was not coincident with the results of larval bioassays. Notably, the onset time of the membrane potential changes of $para^{ts1}$:$DSC1^{-/-}$ and $para^{ts1}$ compared with those of other lines was more dispersed, especially $para^{ts1}$. Within the 10 min recording, some $para^{ts1}$ larvae did not exhibit sudden changes in resting potential but showed directly mEJP repetitive firing (9%) likely due to individual variations. Some of them only showed sudden changes in resting potential but not repetitive firing of mEJP (17%), or some exhibited neither of these two phenomena (6%).

#### 3.2.3. Cypermethrin and permethrin affect Drosophila larval NMJ potential similar to that of deltamethrin

As shown in Fig. 3 and 4, the order of onset time of the sudden change in resting potential and mEJP repetitive firing of four lines of larval NMJ was similar to that of deltamethrin after treatment with 100 nM of cypermethrin or permethrin. Accordingly, the susceptibility of the larval NMJ to cypermethrin and permethrin was ranked as $DSC1^{-/-}$ > $w^{1118}$ > $para^{ts1}$; $DSC1^{-/-}$ > $para^{ts1}$ (Table 4). The results further revealed that the NMJ of $DSC1^{-/-}$ flies was more susceptible to cypermethrin and permethrin than that of $w^{1118}$.

## 4. Discussion

Previous studies showed that $DSC1^{-/-}$ adults and the GFS of $DSC1^{-/-}$ adults were more susceptible to pyrethroids compared to wild-type flies, indicating that *DSC1* knockout enhances the susceptibility of the nervous system to pyrethroids [8,12]. In this study, we investigated the effects of *DSC1* knockout on the susceptibility of *Drosophila* larvae to pyrethroids and the susceptibility of the larval NMJ to pyrethroids. We found that similar to the adults, the larva NMJ in $DSC1^{-/-}$ larvae was more susceptible to pyrethroids than those in wild-type larvae. However, $DSC1^{-/-}$ larvae were more resistant to pyrethroids than wild-type larvae. These results suggest the DSC1 channel-dependent developmental regulation of pyrethroid susceptibility in *D. melanogaster*.

The intrinsic activities of the larval NMJ, such as frequency and amplitude of the mEJP, seem to be not affected by *DSC1* knockout. However, when challenged by pyrethroids, impairments in the NMJ function including membrane depolarization and repetitive firing were more profound in $DSC1^{-/-}$ larvae than in wild-type larvae. These results are consistent with what were reported from the adult GFS in an earlier study [8]. Like in adults, DSC1 channels in the larval NMJ seem to play a similar role in stabilizing the membrane potential counteracting the repetitive firing and membrane depolarization induced by pyrethroids. However, even though the NMJ is more susceptible to pyrethorids, $DSC1^{-/-}$ larvae are more resistant to pyrethroids than wild-type larvae. A *in situ* hybridization study revealed overlapping expression of *para* and *DSC1* transcripts in the central nervous system (CNS) in adults [10]. However, no expression of *DSC1* was detected in the CNS in third instar larvae, where the expression of *para* in the larval CNS was confirmed [10]. These results suggest that roles of DSC1 channels in regulating neuronal excitability in larvae and adults are different.

Whether the DSC1 channel is a target of pyrethroids remains elusive. Knockout of *DSC1* made *Drosophila* larvae more resistant to pyrethroids, suggesting that DSC1 channels in larvae may be targeted for the action of pyrethroids. Both sodium channel and *DSC1* genes undergo extensive alternative splicing and RNA editing and development-specific and tissues-specific regulations of these two post-transcriptional mechanisms have been documented [6,19]. It is possible that distinct expression of unique DSC1 and sodium channels variants in the larval and adult nervous system contributes to the different sensitivities of $DSC1^{-/-}$ adults and larvae to pyrethroids.

Compared with $w^{1118}$ flies, $para^{ts1}$ flies (both larvae and adults) are more resistant to pyrethroids due to a mutation in the sodium channel [12] (and this study). However, larvae carrying the double mutation $para^{ts1}$; $DSC1^{-/-}$ were more susceptible to pyrethroids than $para^{ts1}$ larvae. Somehow, knockout of *DSC1* in the wild-type and $para^{ts1}$ background had seemingly opposite effects on the action of pyrethroids

in larvae. These results suggest potential functional interactions of DSC1 channels and sodium channels in regulating the neuronal excitability and action of pyrethroids.

## 5. Conclusions

Although knockout of *DSC1* increased the susceptibility of *Drosophila* adults to pyrethroids, knockout of *DSC1* reduced the susceptibility of larvae to pyrethroids. These results suggest that the roles of DSC1 channels in regulating the action of pyrethroids in larvae and adults are different. Further investigation is needed to explore the role of DSC1 channels in regulating neuronal excitability in both larvae and adults and how DSC1 channels modulate the action of pyrethroids on sodium channels.

## Acknowledgements

The authors thank Dr. Yuzhe Du for critical review of this manuscript. The research was supported by grants from the National Natural Science Foundation of China (31672055) and from the National Institutes of Health (GM080255 to K.D. and C.-F. Wu).

## References

[1] L. Salkoff, A. Butler, A. Wei, N. Scavarda, K. Giffen, C. Ifune, G. Mandel, Genomic organization and deduced amino acid sequence of a putative sodium channel gene in *Drosophila*, Science 237 (1987) 744–749.
[2] M. Ramaswami, M.A. Tanouye, Two sodium-channel genes in Drosophila: implications for channel diversity, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 2079–2082.
[3] W. Zhou, I. Chung, Z. Liu, A.L. Goldin, K. Dong, A voltage-gated calcium-selective channel encoded by a sodium channel-like gene, Neuron 42 (2004) 101–112.
[4] T. Zhang, Z. Liu, M. Song, Y. Du, K. Dong, Molecular characterization and functional expression of the DSC1 channel, Insect. Biochem. Physiol. 41 (2011) 451–458.
[5] K. Loughney, R. Kreber, B. Ganetzky, Molecular analysis of the *para* locus, a sodium channel gene in *Drosophila*, Cell 58 (1989) 1143–1154.
[6] K. Dong, Y. Du, F.D. Rinkevich, Y. Nomura, P. Xu, L. Wang, K.S. Silver, B.S. Zhorov, Molecular biology of insect sodium channels and pyrethroid resistance, Insect. Biochem. Physiol. 50 (2014) 1–17.
[7] Y.J. Cui, L.L. Yu, H.J. Xu, K. Dong, C.X. Zhang, Molecular characterization of DSC1 orthologs in invertebrate species, Insect. Biochem. Physiol. 42 (2012) 353–359.
[8] T. Zhang, Z. Wang, L. Wang, N. Luo, L. Jiang, Z. Liu, K. Dong, Role of the DSC1 channel in regulating neuronal excitability in *Drosophila melanogaster*: extending nervous system stability under stress, PLoS Genet. 9 (2013) e1003327.
[9] C. Castella, M. Amichot, J.B. Bergé, D. Pauron, DSC1 channels are expressed in both the central and the peripheral nervous system of adult *Drosophila melanogaster*, Invert. Neurosci. 4 (2001) 85–94.
[10] C.S. Hong, B. Ganetzky, Spatial and temporal expression patterns of two sodium channel genes in *Drosophila*, J. Neurosci. 14 (1994) 5160–5169.
[11] B. Pittendrigh, R. Reenan, R.H. French-Constant, B. Ganetzky, Point mutations in the Drosophila sodium channel gene para associated with resistance to DDT and pyrethroid insecticides, Mol. Gen. Genet. 256 (1997) 602–610.
[12] F.D. Rinkevich, Y. Du, J. Tolinski, A. Ueda, C.F. Wu, B.S. Zhorov, K. Dong, Distinct roles of the $DmNa_v$ and DSC1 channels in the action of DDT and pyrethroids, Neurotoxicology 47 (2015) 99–106.
[13] F.D. Rinkevich, J.G. Scott, Reduction of dADAR activity affects the sensitivity of *Drosophila melanogaster* to spinosad and imidacloprid, Pestic. Biochem. Physiol. 104 (2012) 163–169.
[14] J.R. Bloomquist, T.A. Miller, Sodium channel neurotoxins as probes of the knockdown resistance mechanism, Neurotoxicology 7 (1986) 217–224.
[15] B.A. Stewart, H.L. Atwood, J.J. Renger, J. Wang, C.F. Wu, Improved stability of *Drosophila* larval neuromuscular preparations in haemolymph-like physiological solutions, J. Comp. Physiol. 175 (1994) 179–191.
[16] B.A. Stewart, C.M. Schuster, C.S. Goodman, H.L. Atwood, Homeostasis of synaptic transmission in *Drosophila* with genetically altered nerve terminal morphology, J. Neurosci. 16 (1996) 3877–3886.
[17] Z. Hu, Y. Du, X. Xiao, K. Dong, W. Wu, Insight into the Mode of Action of Haedoxan A from *Phryma leptostachya*, Toxins 8 (2016) 53.
[18] V.L. Salgado, S.N. Irving, T.A. Miller, Depolarization of motor nerve terminals by pyrethroids in susceptible and kdr-resistant house flies, Pestic. Biochem. Physiol. 20 (1983) 100–114.
[19] K. Dong, Y. Du, F.D. Rinkevich, L. Wang, P. Xu, The *Drosophila* Sodium Channel 1 (DSC1): the founding member of a new family of voltage-gated cation channels, Pestic. Biochem. Physiol. 120 (2015) 36–39.