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Reduction of *dADAR* activity affects the sensitivity of *Drosophila melanogaster* to spinosad and imidacloprid

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ABSTRACT

The insecticides spinosad and imidacloprid cause toxicity by acting at the nicotinic acetylcholine receptor (nAChR), but at distinct sites on the receptor. Mutations or deletions of specific nAChR subunits cause resistance to these compounds. Transcripts of nAChR subunits are subject to A-to-I RNA editing by adenine deaminase acting on RNA (ADAR), which may result in altered receptor physiology. However, reports on the influence of A-to-I RNA editing on insecticide sensitivity have been very limited. We used the Gal4–UAS system in *Drosophila melanogaster* to reduce the expression of *dAdar* in specific tissues and evaluated the toxicity of spinosad and imidacloprid. Ubiquitous reduction of *dAdar* increased spinosad sensitivity, while *dAdar* reduction in cholinergic neurons and muscle reduced spinosad sensitivity. Imidacloprid sensitivity was reduced by decreased *dAdar* expression in cholinergic neurons, muscle and glia. While reduction in editing of nAChR subunits may explain some of these changes in insecticide toxicity, it is likely that editing of genes other than nAChRs can affect toxicity of these insecticides.

1. Introduction

Spinosyns such as spinosad, and neonicotinoids such as imidacloprid, are two widely used classes of insecticides [1,2]. Spinosad and imidacloprid both exert their toxic effects through interactions with the nicotinic acetylcholine receptor (nAChR), although their mechanisms of action on nAChRs are distinct [2–4].

Nicotinic acetylcholine receptors (nAChRs) are members of the cys-loop ligand-gated ion channel superfamily that includes receptors for serotonin, glycine and GABA [5]. Ligand binding occurs at the extracellular N-terminal domain [6]. Ion currents through the receptor are mediated by the second of four transmembrane domains [7]. The intracellular linker between the third and fourth transmembrane domains is responsible for receptor desensitization and intracellular trafficking [8]. In insects, nAChRs are found on post synaptic sites of neurons in the central nervous system and native nAChRs of insects are homo or heteropentamers of α and β subunits, although the exact composition is largely unresolved [9–11]. Insects have between 10 and 16 nAChR genes [9,12–14]. Evolutionarily conserved patterns of post-transcriptional modifications such as alternative splicing and A-to-I RNA editing create a diverse array of transcripts [15–18].

Insecticide resistance to spinosad or imidacloprid can arise though gene deletions, altered post-transcriptional modifications, or single nucleotide polymorphisms in nAChR subunits. Null mutations in *Dα6* of *Drosophila melanogaster* are 370–1100-fold resistant to spinosad [19,20]. The highly spinosad resistant Pearl-Sel strain of the diamondback moth, *Plutella xylostella*, produces no full length transcripts of *Pxylα6*, and this trait is genetically linked with resistance [21]. Neonicotinoid resistance due to changes in nAChR has been investigated in the brown planthopper, *Nilaparvata lugens*, and a single Y151S mutation in *Nlα1* of the R-T35 strain was associated with imidacloprid resistance [22]. Receptors containing the $Nl\alpha1^{Y151S}$ mutation were less sensitive to imidacloprid [22,23]. An A-to-I RNA editing site that introduces an N133D substitution in *Nlβ1* was edited at a significantly higher rate in an imidacloprid resistant strain of *N. lugens* compared to a susceptible strain and receptors with this edited site were less sensitive to imidacloprid [24]. Imidacloprid resistance in *Myzus persicae* is due to a mutation (R81T) in *Mpβ1* [25]. These results indicate that the *α6*, and the *α1* and *β1* nAChR subunits are targets of spinosad and imidacloprid, respectively. These subunits undergo A-to-I RNA editing [18,24,26], but the contribution of A-to-I RNA to *in vivo* sensitivity to spinosad and imidacloprid has not been evaluated.

A-to-I RNA editing is catalyzed by the enzyme adenine deaminase acting on RNA (ADAR), which converts adenosine to inosine in mRNA. Inosine is recognized as guanosine by the translational and splicing machinery of the cell. This genetic recoding may cause amino acid substitutions, splice site variations, or modify the levels of transcripts [27].

A-to-I RNA editing can affect behavior, neuroanatomy, and function of neuronal receptors and channels of insects. In *D. melanogaster*, *dAdar* null mutants have a disorganized nervous system morphology and deficits in motor control that grow progressively

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worse with age [28]. Flies lacking *dAdar* did not fly or exhibit diurnal activity patterns and displayed temperature sensitive paralysis [29]. Additionally, *dAdar* deficient male flies take longer to initiate courtship behaviors and have an altered courtship song waveform [30]. Editing causes an R122G substitution in the extracellular ligand binding domain of the *Rdl* subunit of GABA-gated chloride receptors of *D. melanogaster*. GABA sensitivity and maximum current responses were significantly reduced in receptors with the R122G substitution [31]. In the cockroach *Blattella germanica*, the voltage-dependence of activation is hyperpolarized by 7 mV in sodium channels that are edited to introduce an R184 K substitution [32].

The goal of our research was to evaluate the effect of A-to-I RNA editing on the sensitivity of nAChRs to both spinosad and imidacloprid, *in vivo*. We used the Gal4–UAS system to drive expression of dsRNA for *dAdar* in different tissues of *D. melanogaster* to evaluate the role of A-to-I RNA editing on spinosad and imidacloprid toxicity. Spinosad sensitivity varied due to the tissue specific reduction of *dAdar*. Imidacloprid sensitivity decreased when *dAdar* expression was reduced in cholinergic neurons, glia and muscle. These results indicate that while A-to-I RNA editing may affect traditional insecticide targets (i.e. nAChRs), previously unknown factors that are subject to A-to-I RNA editing may also be important for insecticide interactions.

2. Materials and methods

2.1. Drosophila strains

Seven strains of *D. melanogaster* were used in this study (Table 1). Reciprocal crosses, of a strain expressing Gal4 under specific promoters with a strain (*dAdar*) possessing a construct for the expression of dsRNA under the control of UAS, were performed to activate the expression of dsRNA for *dAdar*. In all crosses, the parental female is indicated by the strain firstappearing in the cross description (i.e. actin♀ × *dAdar*♂). Flies were reared on cornmeal-agar based media supplemented with dextrose and held at 25 °C, ±30% RH, and 12:12 light–dark photoperiod. Flies were transferred to new vials weekly.

2.2. RNA isolation and reverse transcription

Total RNA was isolated from 10 flies (5♀ and 5♂) from each strain and cross using TRIzol (Invitrogen, Carlsbad CA) according to the manufacturer's directions. The RNA was dissolved in 50 μl of DEPC-treated water. Reverse transcription was performed with 5 μg of total RNA using Go-Script (Promega, Madison WI) according to the manufacturer's directions.

Table 1
Strains of *D. melanogaster* strains used.

Strain	Bloomington Stock #	Details
actin	25374	Gal4 expressed ubiquitously
elav[a]		Gal4 expressed in the nervous system
elav3	8760	Gal4 expressed in the nervous system
cha[a]		Gal4 expressed in cholinergic neurons
dj667	8171	Gal4 expressed in adult muscle
repo	7415	Gal4 expressed in glia
dAdar	28311	dsRNA for *dAdar* under UAS control

[a] Obtained from Hanna Kim, Department of Neurobiology and Behavior, Cornell University.

2.3. PCR and estimation of RNA editing

An 854 bp fragment corresponding to nt 93–947 of the open reading frame of *Dα6* was amplified with the primers Dα6ORF-F3 and Dα6ORF-R3 (Table 2). A positive control that amplified a 496 bp portion of the open reading frame of *actin5C* from nt 465–961 was performed using the primers DmelActinF and DmelActinR (Table 2). Reactions were performed in 100 μl Go-Taq reactions (Promega, Madison WI) under the following thermocycler conditions: 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 10 min. PCR products were purified with the Wizard PCR Purification kit (Promega, Madison WI) and sent for sequencing with the Dα6InternalR2 primer at Cornell's Biotechnology Resource Center. Electropherograms were visualized in Chromas (Technelysium, Brisbane AUS). The heights of the peaks at each editing site were determined using the measurement tool in Photoshop (Adobe Systems, San Jose CA). The extent of A-to-I RNA editing was determined by calculating the proportion of the total height of the both the *C* and *T* peaks at each editing site (representing edited and unedited transcripts, respectively) that was composed of the height of the *C* peak as previously described [33]. The editing rates of more than three independent batches of cDNA from flies from each cross were compared separately to the male and female parental strains by a paired *t*-test in Minitab (Minitab, State College PA).

2.4. Insecticides

Spinosad (98% purity) and imidacloprid (99.5%) were obtained from Chem Service (West Chester, PA). Stock solutions of both compounds were dissolved in acetone. All other chemicals were obtained from Fisher (St. Louis, MO).

2.5. Bioassays

One to three day old, mixed sex adults were used in both bioassays. The stock solutions of spinosad and imidacloprid were serially diluted in acetone and 10% sugar water (w/v), respectively, to find concentrations of each compound that provided >0% and <100% mortality. A contact bioassay was used to evaluate spinosad toxicity. Scintillation vials with an internal surface area of 38.6 cm^2 (Wheaton Scientific, Millville, NJ) were filled with 0.5 ml of spinosad solution. Plain acetone was used as a control. Vials were placed on their side in a fume hood and rolled to evenly coat the inner surface of the vials. Treated vials were allowed to dry for 1 h. Flies were placed in a vial covered with white nylon tulle and plugged with a cotton ball. The cotton balls were wetted daily with 10% sugar water.

A feeding bioassay was used to evaluate imidacloprid toxicity. Flies were placed in a scintillation vial that was covered with white nylon tulle and plugged with a cotton ball (cotton balls weighed 1.05 g ± 0.05 g). Imidacloprid solutions were applied to a cotton ball. Plain 10% sugar water was used as a control. The cotton for all treatments was wetted daily with 10% sugar water. Mortality was assessed after 72 h for both spinosad and imidacloprid. Bioas-

Table 2
Sequences of primers used.

Name	Sequence
DmelActinF	ACTCCGGCGATGGTGTCTCC
DmelActinR	GGGCGGTGATCTCCTTCTGC
Dα6InternalR2	TGGGCAGCAGGCGTAGACT
Dα6ORF-F3	GCGCCTGCTGAACCATCTGC
Dα6ORF-R3	ACCACCGACGAGGGCGACCAT

Table 3
Percentage of A-to-I RNA editing at 6 editing sites of *Dα6* in parental *D. melanogaster* strains used in this study.

	Editing site						
Strain	398	400	415	415	468	560	Average[a]
actin	14 ± 2	13 ± 2	67 ± 4	77 ± 3	81 ± 1	63 ± 3	53 ± 2
elav	10 ± 0	8 ± 2	59 ± 2	72 ± 1	75 ± 3	57 ± 1	47 ± 1
elav3	19 ± 0	20 ± 1	82 ± 2	91 ± 1	94 ± 1	66 ± 0	62 ± 1
cha	10 ± 0	9 ± 1	57 ± 2	72 ± 1	75 ± 1	57 ± 3	47 ± 1
dj667	16 ± 0	14 ± 2	67 ± 1	77 ± 2	81 ± 2	61 ± 1	53 ± 1
repo	10 ± 5	10 ± 1	66 ± 1	81 ± 4	79 ± 2	66 ± 3	52 ± 1
dAdar	13 ± 2	12 ± 1	68 ± 5	79 ± 3	80 ± 1	65 ± 4	53 ± 3

Values are the average (±standard deviation) percent of transcripts edited at the site indicated.
[a] Average for all six editing sites in the strain.

say data were pooled across replicates and analyzed by standard Probit analysis [34], as adapted to personal computer use [35]using Abbott's correctionfor control mortality [36].

The insensitivity ratio (IR) and corresponding 95% confidence interval (CI) were calculated by standardizing the LC_{50} values of each cross relative to the parental driver and *dAdar* values [37]. The IR values were considered significantly different when the 95% CI did not include 1. A-to-I RNA-editing in a specific tissue was considered to significantly affect the sensitivity to spinosad or imidacloprid when the IRs were significantly different from 1.0 for both parental comparisons of both reciprocal crosses.

3. Results

3.1. Suppression of A-to-I RNA editing

The percentage of A-to-I RNA editing (at six sites in *Dα6*) in all parental strains of *D. melanogaster* is shown in Table 3. The percentage of editing at each site was very similar in all parental strains, except for elav3 which had a higher percentage of editing. All crosses resulted in reduced RNA editing of at least one *Dα6* editing site relative to at least one parental strain (Table 4). There was variation in the relative suppression of *dAdar*, as measured by editing of *Dα6*, in each of the crosses. Greatest suppression of editing was seen using the actin driver, while little or no suppression was seen using the cha driver. The dj667 and repo drivers gave intermediate levels of editing suppression in muscle and glia, respectively (Table 4). The reduction in editing frequency was not uniform across editing sites (Table 4). Editing was completely-eliminated at some sites (i.e. site 398 of *dAdar* × actin), but only reduced at other sites (i.e. site 468 of *dAdar* × actin).

3.2. Spinosad bioassays

The LC_{50} for spinosad in the parental strains ranged from 4.7 ng/cm^2 in elav3 to 35.9 ng/cm^2 in *dAdar* (Table 5). Ubiquitous reduction in RNA editing (via the actin driver) increased sensitivity to spinosad by 1.7–5.9-fold. Reduction of RNA editing in cholinergic neurons (cha) and muscles (dj667) decreased spinosad sensitivity by1.2–2.6-fold and 1.6–6.0-fold, respectively (Fig. 1). There was no significant change in spinosad sensitivity due to reduced editing in the nervous system (elav and elav3) or glia (repo, Fig. 1).

3.3. Imidacloprid bioassays

The LC_{50} for imidacloprid in the parental strains ranged from 4.2 μg/cotton ball in repo to 30.3 μg/cotton ball in actin (Table 5). Reduction in A-to-I RNA editing in cholinergic neurons (cha), muscles (dj667) and glia (repo) was associated with a significant decrease in imidacloprid sensitivity (Fig. 2). The most dramatic reduction in imidacloprid sensitivity (2.1–14.7-fold) was seen when *dAdar* expression was reduced in glia. There was no significant change in imidacloprid sensitivity associated with reduced editing in all tissues (actin) or in the entire nervous system (elav and elav3, Fig. 2).

Table 4
Proportion of RNA editing in progeny of crosses relative to their parental strains.

	Editing Site						
Cross	**398**	**400**	**415**	**416**	**468**	**560**	**Average**
actin x	0.02 (0.03)	0.00 (0.00)	0.32 (0.13)	0.45 (0.11)	0.58 (0.12)	0.23 (0.13)	0.37 (0.11)
dAdar	0.02 (0.03)	0.00 (0.00)	0.32 (0.13)	0.43 (0.11)	0.59 (0.12)	0.22 (0.12)	0.37 (0.11)
dAdar x	0.00 (0.00)	0.04 (0.05)	0.35 (0.11)	0.44 (0.14)	0.59 (0.09)	0.21 (0.04)	0.38 (0.08)
actin	0.00 (0.00)	0.05 (0.05)	0.35 (0.11)	0.42 (0.14)	0.60 (0.09)	0.20 (0.03)	0.37 (0.08)
elav x	0.08 (0.12)	0.12 (0.08)	0.49 (0.09)	0.64 (0.08)	0.82 (0.09)	0.35 (0.10)	0.56 (0.08)
dAdar	0.06 (0.09)	0.09 (0.01)	0.43 (0.03)	0.58 (0.01)	0.77 (0.02)	0.31 (0.05)	0.50 (0.02)
dAdar x	0.53 (0.09)	0.62* (0.21)	0.74 (0.11)	0.83 (0.07)	0.93* (0.05)	0.68 (0.07)	0.79 (0.08)
elav	0.38 (0.07)	0.43 (0.14)	0.64 (0.09)	0.75 (0.07)	0.87 (0.05)	0.60 (0.06)	0.70 (0.07)
elav3 x	0.21 (0.05)	0.45 (0.16)	0.61 (0.05)	0.73 (0.02)	0.78 (0.08)	0.51 (0.09)	0.63 (0.04)
dAdar	0.30 (0.07)	0.76* (0.27)	0.73 (0.06)	0.84 (0.02)	0.91* (0.09)	0.52 (0.09)	0.74 (0.04)
dAdar x	0.22 (0.09)	0.23 (0.02)	0.49 (0.07)	0.66 (0.03)	0.80 (0.02)	0.36 (0.11)	0.56 (0.05)
elav3	0.32 (0.12)	0.38 (0.04)	0.59 (0.09)	0.75 (0.04)	0.94 (0.02)	0.37 (0.11)	0.65 (0.06)
cha x	1.02* (0.26)	1.10* (0.18)	1.09 (0.04)	1.04* (0.03)	1.05 (0.03)	1.04* (0.07)	1.04* (0.05)
dAdar	0.75* (0.19)	0.78* (0.12)	0.92* (0.04)	0.94* (0.03)	0.97* (0.03)	0.91* (0.06)	0.91 (0.04)
dAdar x	0.94* (0.21)	1.13* (0.13)	1.10 (0.02)	1.04* (0.02)	1.04 (0.01)	1.04* (0.03)	1.05* (0.03)
cha	0.69* (0.15)	0.80 (0.09)	0.93* (0.02)	0.94* (0.02)	0.96 (0.01)	0.91* (0.03)	0.92* (0.02)
dj667 x	0.59 (0.04)	0.61 (0.04)	0.86 (0.05)	0.94 (0.03)	0.97* (0.02)	0.83 (0.07)	0.88 (0.04)
dAdar	0.69 (0.05)	0.71 (0.05)	0.85 (0.04)	0.91 (0.03)	0.98* (0.02)	0.79 (0.06)	0.87 (0.04)
dAdar x	0.53 (0.05)	0.54 (0.08)	0.84 (0.04)	0.93 (0.03)	0.95 (0.01)	0.81 (0.06)	0.86 (0.03)
dj667	0.62 (0.06)	0.62 (0.09)	0.84 (0.04)	0.90 (0.03)	0.95 (0.01)	0.77 (0.05)	0.85 (0.03)
repo x	0.90* (0.06)	0.99* (0.05)	0.94* (0.04)	0.91 (0.01)	0.98* (0.00)	0.91 (0.03)	0.94 (0.02)
dAdar	0.70 (0.04)	0.79 (0.04)	0.92* (0.03)	0.93 (0.01)	0.96 (0.00)	0.93* (0.03)	0.92 (0.02)
dAdar x	0.95* (0.03)	1.00* (0.06)	0.95* (0.03)	0.92 (0.01)	0.99* (0.02)	0.89 (0.02)	0.94 (0.01)
repo	0.74* (0.02)	0.80 (0.05)	0.93* (0.03)	0.94 (0.01)	0.97* (0.02)	0.91* (0.02)	0.93* (0.01)

Shaded values represent RNA editing rates relative to the parental driver strain and unshaded values represent RNA editing rates relative to *dAdar* parental. Numbers in parentheses indicate the standard deviation. Values with an * are not different from the parental strain.

4. Discussion

4.1. Tissue-specific reductions in A-to-I RNA editing

The rate of A-to-I RNA editing suppression by *dADAR* silencing varied based on which editing site was evaluated and on the tissue(s) in which editing was reduced (i.e. driver used). Editing site 468 was the most resistant to reduction in editing rate among all crosses, which is in agreement with previous work [29]. The difference in editing reduction between the six sites was not correlated to the frequency of editing of the sites in the parental strains and may be due to differences in editing efficiency due to differences in the structure of dsRNA needed for *dADAR* activity [30]. Heterologous expression of these receptors with the appropriate combinations of different amino acids associated with the edited sites will provide more specific information on the role of A-to-I RNA editing on insecticide sensitivity. This future course of study is promising as it has been demonstrated that editing of *Nlβ1* reduces sensitivity of the receptor to imidacloprid, but not to acetylcholine [24]. We expected that the reduction in editing of *Dα6* (due to silencing of *dADAR*) that we measured (from a pool of whole animal RNA) would be influenced by the expression of *Dα6* (i.e. tissue specific silencing of *dADAR* would affect a different proportion of *Dα6* editing depending of the expression of *Dα6* in that tissue). This is generally what we observed, with the greatest reduction in *Dα6* editing found for global silencing of *dADAR*. Given that *Dα6* expression is primarily in the nervous system [38], the minimal changes seen for editing of *Dα6* with the cha driver (cholinergic neurons) was a bit surprising, but in this case cholinergic neurons are defined by the neurotransmitter they release, not by the receptors they possess.

4.2. Changes in spinosad sensitivity

Dα6 and Pxylα6 are target sites of spinosad [19–21]. Therefore, it is feasible that reduced editing of *Dα6* in cholinergic neurons (cha driven suppression of *dADAR*) is responsible for the decrease in spinosad sensitivity observed (Fig. 1). The reduction in *dADAR* expression in cholinergic neurons did not dramatically reduce the level of RNA editing (Table 4), but the change in spinosad sensitivity was also modest (1.2–3.0-fold).

Reduced expression of *dADAR* in muscle (via dj667 driver) caused the most significant decrease in spinosad sensitivity (Fig. 1). Although reduction in editing of *Dα6* was modest (85–88% relative to the parental strains) using the muscle driver, this is likely due to the relatively low level of expression of *Dα6* in muscle [38] (i.e. there was silencing of editing for only a fraction of the total *Dα6* transcripts). The reduction in editing seen for *Dα6* seen using the dj667 driver suggests that expression of *Dα6* may be slightly higher in muscle than previously thought [38]. Alternatively, the decrease in spinosad sensitivity associated with silencing of *dADAR* in muscle may be due to reduced editing of other nAChRs or GABA receptors (thought to represent the primary and secondary target sites of spinosad, respectively [2,4]) found in muscle [39–42].

Ubiquitous reduction of *dAdar* expression (actin driver) increased sensitivity to spinosad. In contrast, reduced *dAdar* expression with other drivers either had no effect (elav, elav3 and repo) or decreased sensitivity (cha or dj667) to spinosad (Fig. 1), suggesting that the increased spinosad sensitivity seen with ubiquitous reduction of *dAdar* expression was due to reduction in editing of genes expressed outside of the nervous system or muscle. It does not appear that the global silencing of *dADAR* resulted in a general weakening of the flies, as the increased sensitivity seen with the actin driver was specific to spinosad. There was no effect of reduced global editing on imidacloprid sensitivity. The exact factors responsible for the increased spinosad sensitivity seen with the global silencing of *dADAR* expression will require further study.

4.3. Changes in imidacloprid sensitivity

Imidacloprid sensitivity was significantly reduced when expression of *dAdar* was reduced in cholinergic neurons, muscle, and glia; which is consistent with the expression of AChRs in neurons and

Table 5
Toxicity of spinosad and imidacloprid to various strains and crosses of *D. melanogaster*.

Strain	Spinosad			Imidacloprid		
	n[a]	LC_{50}[b]	Slope[c]	*n*[a]	LC_{50}[d]	Slope[c]
actin	1100	25.0 (22.4–28.0)	2.4 (0.2)	505	30.3 (23.5–39.6)	1.8 (0.2)
elav	672	15.1 (12.0–18.5)	1.3 (0.1)	565	6.5 (5.5–7.8)	2.8 (0.4)
elav3	357	4.7 (2.7–6.5)	1.3 (0.2)	289	10.6 (6.4–15.0)	1.6 (0.3)
cha	313	29.3 (24.0–34.2)	2.9 (0.4)	461	18.5 (15.6–22.2)	2.5 (0.3)
dj667	915	11.8 (8.7–28.6)	1.8 (0.5)	512	20.4 (17.6–24.0)	2.6 (0.2)
repo	731	10.8 (9.45–12.2)	2.3 (0.2)	716	4.2 (3.5–5.0)	1.7 (0.1)
dAdar	1349	35.9 (30.6–42.4)	1.3 (0.1)	753	23.2 (20.3–26.7)	2.1 (0.2)
actin♀ × *dAdar*♂	340	6.1 (4.1–8.1) ‡*	1.8 (0.2)	770	15.0 (12.6–17.3) ‡*	1.9 (0.2)
dAdar♀ × actin♂	393	14.9 (9.8–20.6) ‡*	1.5 (0.2)	647	28.2 (24.5–32.0)	2.3 (0.2)
elav♀ × *dAdar*♂	355	19.3 (14.6–23.7)*	1.7 (0.2)	717	17.7 (15.3–20.6)‡	2.1 (0.2)
dAdar♀ × elav♂	378	48.7 (43.1–54.5) ‡*	2.7 (0.2)	965	18.9 (16.3–21.9)‡	2.1 (0.1)
elav3♀ × *dAdar*♂	614	12.7 (11.3–14.4)	2.6 (0.2)	881	27.1 (24.2–29.9)‡	3.9 (0.3)
dAdar♀ × elav3♂	707	13.2 (11.8–14.9)	2.4 (0.2)	1060	23.2 (21.3–25.3)‡	3.3 (0.2)
cha♀ × *dAdar* ♂	405	43.4 (35.1–52.8)‡	1.8 (0.2)	345	42.1 (38.0–46.3) ‡*	4.1 (0.5)
dAdar♀ × cha♂	484	75.5 (61.9–97.5) ‡*	1.6 (0.2)	371	35.5 (30.5–41.1) ‡*	2.4 (0.3)
dj667♀ × *dAdar*♂	480	70.6 (60.9–82.1) ‡*	2.3 (0.4)	548	38.2 (30.1–52.0) ‡*	1.7 (0.2)
dAdar♀ × dj667♂	389	58.8 (48.7–68.3) ‡*	2.4 (0.4)	446	47.1 (36.7–66.3) ‡*	1.7 (0.3)
repo♀ × *dAdar*♂	348	42.5 (35.0–48.7)‡	3.3 (0.5)	223	49.6 (39.2–64.7) ‡*	2.5 (0.4)
dAdar♀ × repo♂	447	31.0 (25.9–36.0)‡	2.4 (0.2)	251	61.8 (47.8–82.1) ‡*	2.2 (0.3)

For comparison purposes the LC_{50} (95% confidence intervals) values for spinosad and imidacloprid against Canton-S are 14.0 (12.3–16.1) and 31.0 (26.0–36.0), respectively.
[a] Number of flies per treatment.
[b] Units in ng/cm^2, numbers in parenthesis are the 95% CI of the LC_{50}.
[c] Number in parentheses are standard error of the slope.
[d] Units in μg/cotton ball, numbers in parenthesis are the 95% CI of the LC_{50}.
‡ LC_{50} significantly different than parental driver.
* LC_{50} significantly different than *dAdar*.

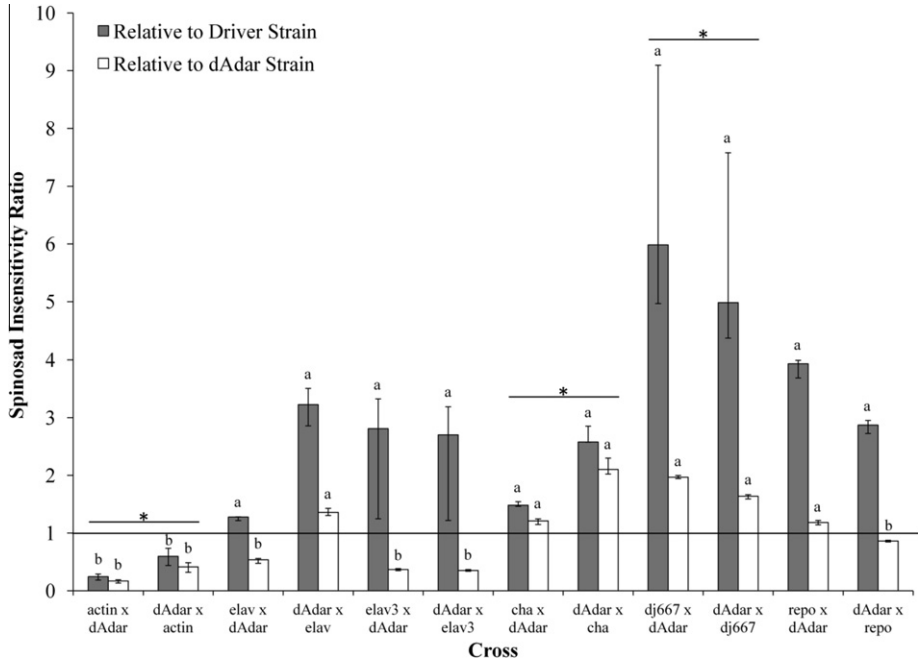

Fig. 1. Changes in spinosad sensitivity due to reduction of A-to-I RNA editing activity in specific tissues of *D. melanogaster*. Columns represent the insensitivity ratios and bars represent the 95% of the insensitivity ratio. Insensitivity ratios significantly greater than or less than 1 are indicated by "a" or "b", respectively. If both reciprocal crosses showed the same result (i.e. significantly greater or less than one) relative to both parental strains, they were deemed to have a significant driver specific effect indicated by an asterisk.

muscle [39–42]. Imidacloprid insensitivity has been demonstrated with a Y151S mutation in *Nlα1* of the brown planthopper [23] and a R81T mutation in *Mpβ1* in *Myzus persicae* [25]. Heterologous expression of receptors with an N133D editing substitution in *Nlβ1* results in reduced imidacloprid potency [24]. Lesions in *Dα1* and *Dβ2* cause reduced sensitivity to several neonicotinoids in larval feeding bioassays [43]. While *Dβ1* and *Dβ2* are edited, only *Dβ1* is edited in the ligand binding domain in loop D [9]. Amino acids in and around loop D are important for ligand interactions [44–46]. Therefore, reduced editing of *Dβ1* may be responsible for the reduction in imidacloprid sensitivity seen when *dADAR* silencing is driven by the cha driver. Clarification of this will require further study.

The observation of decreased imidacloprid sensitivity following suppression of dADAR in glia was unexpected. Glia serve a myriad of functions for neurons such as supplying nutrients, guiding axonal development, and synaptic modulation [47]. Olfactory receptor axons release ACh that cause ACh-inducible Ca^{2+} currents which are blocked by nAChR antagonists in glia in pupae of the tobacco hornworm, *Manduca sexta*. These currents, that are blocked by nAChR antagonists, are necessary and sufficient to cause migration of glial cells. The presence of ACh-inducible Ca^{2+} currents indicates nAChRs are expressed in glia [48]. The fact that such a large change in imidacloprid sensitivity is seen when *dAdar* expression is reduced in glia, indicates glial functions that are modified by A-to-I RNA editing are important features of imidacloprid toxicity.

4.4. Tissue specific silencing and insecticide sensitivity

Our results indicate that A-to-I RNA editing is an important process that influences the sensitivity to spinosad and imidacloprid *in vivo*. However, the tissue(s) in which *dADAR* expression was reduced did not alter the sensitivity to spinosad and imidacloprid equally. For example, ubiquitous silencing of *dADAR* editing increases sensitivity to spinosad, but has no effect on imidacloprid sensitivity. The greatest change in imidacloprid sensitivity was observed with reduced editing in glia, although this did not affect spinosad sensitivity. These results are consistent with spinosad and imidacloprid having different target sites.

Reduction in *Dα6* editing seen using different drivers to silence *dADAR* expression was generally consistent with the pattern of both *dAdar* and *Dα6* expression [9,49], although there was at least one exception. Reduction of *dAdar* expression in glia resulted in suppression of *Dα6* editing. However, *dADAR* does not co-localize with the glial nuclear marker repo [30]. Therefore, it is difficult to connect the change in editing frequency (and the change in imidacloprid sensitivity) with the reduction of *dAdar* expression driven by repo when immunohistochemistry shows that *dADAR* is not expressed in glia. A second possible exception was that editing of *Dα6* was reduced when muscle specific drivers were used to silence *dAdar*. While AChRs are known to exist in insect muscle [40,50], the levels of *Dα6* expression are thought to be very low [38]. It may be that even with a low level of *Dα6* expression in muscle, the abundance of muscle as a tissue using whole insects allows for the detection of silencing of *Dα6* in our experiments.

Our strategy to assess the role of editing on insecticide sensitivity focused on a tissue specific role of editing. The contribution of RNA editing of *Dα6* and *Dβ1* on sensitivity to spinosad and imidacloprid, respectively, is feasible based on previous work [19,24]. Future studies on the influence of editing on a particular nAChR subunit could be addressed using drivers that are specific for those genes. For example, a strain of transgenic fly that expresses Gal4 under the control of the *Dα6* promoter could be crossed to the *dAdar* strain to reduce A-to-I RNA editing only in those cells expressing *Dα6*. This would clarify the contribution of RNA editing of individual subunits on insecticide sensitivity.

We have demonstrated that A-to-I RNA editing influences the sensitivity to two classes of insecticides. Some of these changes in toxicity were unexpected as the results indicate a broader range of potential target sites for these insecticides, and other processes may have a greater influence on insecticide sensitivity than previously thought. It will be necessary to determine which specific

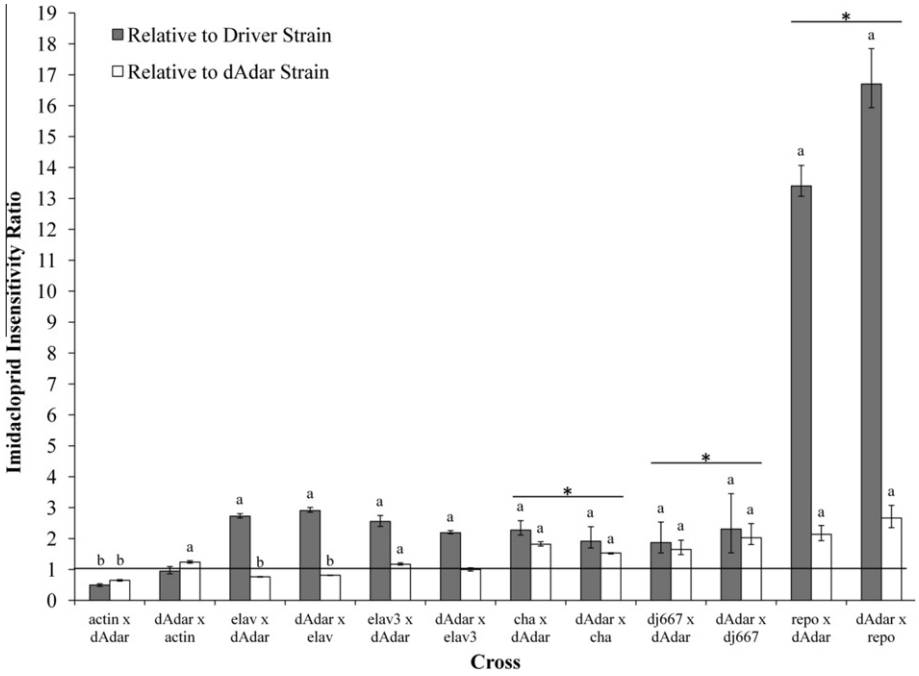

Fig. 2. Changes in imidacloprid sensitivity due to reduction of A-to-I RNA editing activity in certain tissues of *D. melanogaster*. Significant insensitivity ratios are indicated as described in Fig. 1.

transcripts that undergo A-to-I RNA editing are important for influencing insecticide sensitivity. These results provide a strong foundation to assess the role of A-to-I RNA editing of nAChR subunits on insecticide sensitivity, especially in the case of spinosad and *Dα6*.

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