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Abstract

Cockroach neurosecretory cells, dorsal unpaired median (DUM) neurons, express two distinct α-bungarotoxin-insensitive nicotinic acetylcholine receptor subtypes, nAChR1 and nAChR2 which are differently sensitive to the neonicotinoid insecticides and intracellular calcium pathways. The aim of this study is to determine whether sulfoxaflor acts as an agonist of nAChR1 and nAChR2 subtypes. We demonstrated that 1 mM sulfoxaflor induced high current amplitudes, compared to acetylcholine, suggesting that it was a full agonist of DUM neuron nAChR subtypes. Sulfoxaflor evoked currents were not inhibited by the nicotinic acetylcholine receptor antagonist d-tubocurarine (dTC) which reduced nAChR1. But, sulfoxaflor evoked currents were reduced in the presence of 5 µM mecamylamine which is known to reduce nAChR2 subtype. Interestingly, when 1 µM imidacloprid was added in the extracellular solution, sulfoxaflor-induced currents were significantly suppressed. Moreover, when extracellular calcium concentration was increased, bath application of 1 µM imidacloprid partially reduced sulfoxaflor activated currents when nAChR1 was inhibited with 20 µM dTC and completely suppressed sulfoxaflor currents when nAChR2 was inhibited with 5 µM mecamylamine. Our data demonstrated therefore that sulfoxaflor activates both nAChR1 and nAChR2 subtypes.

1. Introduction

Insect nicotinic acetylcholine receptors (nAChRs) play a central role in the synaptic transmission and they are the main targets of several insecticides including neonicotinoids (Ihara and Matsuda, 2018; Taillebois *et al*., 2018; Tomizawa and Casida, 2003, 2005) and sulfoximine derivatives (Cutler *et al*., 2013; Watson *et al*., 2011; Watson *et al*., 2017). They are ligand gated ion channels composed of five subunits which form homomeric or heteromeric receptors. The pharmacological properties of their native forms are currently studied using cockroach neurons expressing nAChR subtypes (Salgado, 2016; Salgado and Saar, 2004; Tan *et al*., 2007). It was found that neurons from the thoracic ganglia expressed two types of α-bungarotoxin-sensitive receptors: the desensitized subtype called nAChRD and nondesensitized (nAChRN) receptors. The nAChRN subtypes are inhibited by the nAChR antagonist methyllicaconitine (MLA) while the nAChRD subtypes are inhibited by neonicotinoid insecticides such as imidacloprid (IMI) (Salgado and Saar, 2004). Using cockroach terminal abdominal ganglia, it was found that the cockroach neurosecretory cells, called dorsal unpaired median (DUM) neurons, expressed two α-Bgt-insensitive nAChR subtypes. nAChR1 was blocked by d-tubocurarine (dTC) and was sensitive to IMI while nAChR2 was blocked by mecamylamine (MECA) and was insensitive to protein kinases C (PKC) 1 and 2, respectively (Courjaret *et al*., 2003; Courjaret and Lapied, 2001; Thany *et al*., 2008). In addition, increasing extracellular calcium concentration resulted to an increase of nicotine evoked current amplitudes through nAChR2. This increase occurred when nAChR1 was blocked with dTC (Thany *et al*., 2008). Moreover, pressure ejection of IMI on DUM neuron nAChRs induced only a monophasic current-voltage curve compared to that induced with acetamiprid (ACE) and clothianidin (CLT) which evoked biphasic curves (Bodereau-Dubois *et al*., 2012). Thus, it was suggested that ACE and CLT were able to activate both

nAChR1 and nAChR2 whereas IMI activated only nAChR2 subtype (Bodereau-Dubois *et al*., 2012).

Sulfoximine insecticides such as sulfoxaflor (SFX) form a new class of active compounds which exhibited structure-activity relationships different from neonicotinoid ones (Babcock *et al*., 2011). They have been included in the 4C subgroup (nAChR competitive modulators) by the IRAC committee while neonicotinoids are in the 4A subgroup (Sparks *et al*., 2013). They are used for the control of sap-feeding insects such as *Myzus persicae*, *Aphis gossypii*, *Bemissia tabaci* and *Nilaparvata lugens* for which a strong resistance against neonicotinoid insecticides was found (Sparks *et al*., 2013). Indeed, SFX is a potential new alternative over the current neonicotinoids such as IMI because of the lack of cross-resistance with neonicotinoids (Wang *et al*., 2017). Like neonicotinoids, sulfoximine potent insecticidal activity was associated to their efficacy at nAChR subtypes (Watson *et al*., 2011). Thus, it was found that SFX evoked very high amplitude currents through the activation of *Drosophila melanogaster* Dα2/β2 hybrid receptor expressed in *Xenopus laevis* oocytes. But, SFX displaced [^{3}H]IMI from *Myzus persicae* nAChR membrane preparations with weak affinity (Watson *et al*., 2011). These studies demonstrated that the mode of action of SFX on insect nAChRs needs to be further explored. Indeed, it was not clear if it can activate similar or distinct insect neuronal nAChRs with IMI (Cutler et al., 2013; Watson et al., 2011). In the present study, we evaluated the mode of action of SFX on cockroach DUM neurons. In particular, we were interested to evaluate its effect on the known α-Bgt-insensitive nAChR1 and nAChR2 subtypes.

2. Materials and methods

2.1. Insects

The experiments were carried out on DUM neuron cell bodies isolated from the dorsal midline of the terminal abdominal ganglion of the nerve cord from adult male cockroaches *Periplaneta americana*.

2.2. Cells isolation

DUM neurons were isolated following enzymatic treatment and mechanical dissociation as previously described (Lapied *et al*., 1990). Cells were incubated overnight at 29°C before electrophysiological experiments. Currents were recorded using the patch-clamp technique in the whole-cell recording configuration (Lapied *et al*., 1990). The Petri dish containing isolated cell bodies was placed onto the inverted microscope (CK2: Olympus), and continuously bathed with the standard extracellular solution (in mM: NaCl 200, KCl 3.1, $MgCl_2$ 4, $CaCl_2$ 5, sucrose 50, HEPES 10, pH 7.4 adjusted with NaOH) using a gravity perfusion system positioned at a distance of 150 µm from the cell body.

2.3. Patch clamp recordings

Signals were recorded using an Axopatch 200B amplifier (Axon instruments, Foster City, CA), connected to a computer with the pClamp software control (pClamp 10.0, Axon Instruments). The liquid junction potential between the bath and the internal pipette solution was compensated before the formation of a gigaOhm seal (>5 GΩ). Patch pipettes were pulled from borosilicate glass capillary tubes (GC 150T-10; Clark Electromedical Instruments,

Harvard Apparatus) and had resistance of 2 MΩ when filled with the standard pipette solution (osmolarity : 227.5) containing (in mM) : NaCl 10; $MgCl_2$ 1; $CaCl_2$ 0.5; HEPES 10; ATPMg 1; EGTA 10; K aspartate 160; KF 10; and adjusted to pH 7.4 with KOH. SFX was applied by pneumatic pressure ejection (15 psig, 300 ms, Miniframe, Medical System Corporation, USA) through a glass micropipette (resistance 2.0 MΩ when filled with agonist) positioned in solution at a distance of 150 µm from the isolated cell body. All currents were recorded under bath application of 0.5 µM α-Bgt to block α-Bgt-sensitive nAChRs (Courjaret and Lapied, 2001). Currents were filtered using the Clampfit software.

2.5. Compounds

All compounds were purchased from SIGMA Aldrich France, except for SFX which was prepared according to data previously published (Arndt *et al*., 2015).

2.6. Statistical analysis

For the statistical analysis, one way ANOVA and Bonferroni post-hoc test were employed, using the Prism program (GraphPAD Software, San Diego, CA). For each data, I/Imax were normalized according to the Imax recorded in the same experimental condition. In the results, 'n' represents the number of recorded cells. Normalized data were fitted by use of GraphPad Prism (GraphPad Software, UK) to the following equation : $y = I_{min} + (I_{max} + I_{min})/(1 + 10^{(\log(EC_{50} - X)H)}$ where y is the normalized response, I_{max} and I_{min} are the maximum and the minimum responses, H is the Hill coefficient, EC_{50} is the concentration giving half the maximum response and X is the logarithm of the compound concentration.

3. Results

3.1. Effect of sulfoxaflor on DUM neurons α-bungarotoxin-insensitive nicotinic acetylcholine receptors

Previous studies demonstrated that DUM neurons expressed two distinct α-Bgt-insensitive nAChRs, named nAChR1 and nAChR2 (Courjaret *et al*., 2003; Courjaret and Lapied, 2001; Thany *et al*., 2008). Thus, all experiments were performed under bath application of 0.5 µM α-Bgt to inhibit α-Bgt-sensitive nAChRs (Bodereau-Dubois et al., 2012; Courjaret et al., 2003; Courjaret and Lapied, 2001). Pressure application of 1 mM SFX (300 ms pulse duration) induced a mean inward current of -1.56 ± 0.21 nA at a holding membrane potential of -50 mV. The amplitude of the SFX-evoked currents was higher compared to 1 mM ACh, suggesting that SFX was a full activator of α-Bgt-insensitive nAChRs expressed on cockroach DUM neurons (Figure 1). Note that, no desensitized currents were found during successive applications of 1 mM SFX (Figure 1C). Using the equation describes above, we found that successive applications of SFX at different concentrations induced a concentration-dependent response with an EC_{50} value of 153.7 ± 10.2 µM and a Hill coefficient of 0.9 (Figure 2). As illustrated in figure 3, currents measured in the absence and presence of 20 µM dTC are not significantly different. Currents are respectively of -1.44 ± 0.08 nA and -1.43 ± 0.1 nA ($p > 0.05$, n = 12, figure 3A and B). However, significant reduced current amplitudes were found when 5 µM MECA was applied in the bath. As such, currents were reduced from -1.63 ± 0.08 nA under control condition to -0.87 ± 0.05 nA under bath application of 5 µM MECA ($p < 0.05$, n = 10, figure 3C and D). These results suggested that dTC was not able to inhibit SFX current amplitudes, leading to the assumption that SFX activated only nAChR2 subtype.

3.2. Imidacloprid reduces sulfoxaflor activated current through nAChR1 and nAChR2 subtypes

To test further the involvement of nAChR1 and nAChR2 subtypes on SFX evoked currents, 1 µM IMI was applied in the bath during pulse application of 1 mM SFX (300 ms pulse duration). Indeed, it was suggested that IMI acted preferentially on nAChR1 subtype (Courjaret and Lapied, 2001). Note that at 1 µM, IMI was not able to elicit any current, even when applied on DUM neurons (Data not shown). But, it strongly reduced currents evoked by SFX at different holding potentials (Figure 4). Indeed, the currents induced by 1 mM SFX at different holding potentials were strongly reduced in the presence of bath application of 1 µM IMI (Figure 4A). At -50 mV holding potential, currents were reduced from -1.61 ± 0.8 nA to -0.35 ± 0.03 nA ($p < 0.05$, $n = 8$, figure 4B) and SFX-induced currents were partially reversed after 20 min washout (Figure 4B). These results demonstrated that IMI was able to inhibit SFX evoked current amplitudes. Nevertheless, we noted a residual current when 1 µM IMI was applied in the bath. This current could suggest a partial blocking effect or the presence of nAChRs insensitive to IMI. Thus, we studied the involvement of nAChR1 and nAChR2 subtypes. As previously shown (see figure 3) when nAChR1 was blocked with 20 µM dTC, SFX currents were not reduced. But, co-application of both 1 µM IMI and 20 µM dTC reduced currents amplitudes to -0.436 ± 0.2 nA (Figure 5A). We suggested that it existed at least two nAChR2 subtypes, IMI-sensitive and –insensitive which were activated by SFX. Under similar conditions, when nAChR2 was blocked with 5 µM MECA, we found a significant decrease of SFX evoked current amplitudes and currents were completely blocked when we added 1 µM IMI in the bath (Figure 5B). Our data demonstrated that SFX was able to activate nAChR1 subtypes. We proposed that IMI could also activate SFX evoked currents through the activation of both nAChR1 and nAChR2 subtypes.

Effect of extracellular calcium concentration on SFX evoked currents

In a second series of experiments we used a cell preparation with 9 mM Ca^{2+}. We previously demonstrated that when nAChR1 was blocked with 20 µM dTC, increasing extracellular Ca^{2+} concentration strongly increased nicotine current amplitudes (Thany et al., 2008). We found that, when extracellular Ca^{2+} concentration was increased from 5 mM (normal saline solution) to 9 mM Ca^{2+}, SFX-induced currents were not increased: currents were -1.52 ± 0.16 nA and -1.43 ± 0.11 nA under control and 9 mM Ca^{2+} solution (data not shown). These data suggested that SFX evoked currents on DUM neuronal nAChRs were not sensitive to extracellular Ca^{2+} increase, compared to nicotine (Thany et al., 2008). Moreover, no significant effect on SFX evoked current amplitudes was found during bath application of 20 µM dTC used to inhibit nAChR1 (currents were -1.56 ± 0.09 nA and -1.41 ± 0.08 nA, respectively). However, SFX evoked current were reduced when 1 µM IMI was applied in the bath. Currents were reduced from -1.56 ± 0.09 to -0.47 ± 0.12 nA ($p < 0.05$, $n = 9$, figure 6A and B), and were not reversed after washout (data not shown). When nAChR2 was inhibited with 5 µM MECA, SFX evoked currents were strongly reduced (currents were -0.49 ± 0.04 nA, $p < 0.05$, $n = 8$, figure 6C and D). In addition, when 5 µM MECA was co-applied with 1 µM IMI currents were completely inhibited ($p < 0.05$, $n = 8$, figure 6C and D). Our data confirmed that SFX was able to activate both nAChR1 and nAChR2 subtypes.

4. Discussion

The effects of the sulfoximine insecticide, SFX, on cockroach DUM neurons were tested in the present study. DUM neurons were chosen because they expressed different α-Bgt-sensitive and -insensitive nAChR subtypes, more precisely, α-Bgt-insensitive nAChR1 and nAChR2 which were differently sensitive to IMI (Courjaret and Lapied, 2001). Our studies provide evidence that SFX is able to activate DUM neuronal nAChRs and is a full agonist of DUM neuronal nAChRs. We also found that SFX evoked currents were not blocked by bath application of dTC but they were reduced in the presence of MECA which was known to block nAChR2 (Courjaret *et al*., 2003; Courjaret and Lapied, 2001). The finding that IMI blocked SFX evoked current amplitudes suggested that SFX activated both nAChR1 and nAChR2 subtypes. This finding was confirmed when SFX was tested in bath solution containing higher Ca^{2+} concentration. Indeed, we previously demonstrated that nicotine evoked current amplitudes were affected by an increase of extracellular calcium concentration when nAChR1 was blocked by dTC (Thany *et al*., 2008). Moreover, our data suggested that SFX was able to activate DUM neuronal α-Bgt-insensitive nAChRs which are sensitive to IMI but not blocked by dTC. Thus, at least more than three α-Bgt-insensitive nAChR subtypes may be sensitive to SFX. The mode of action of SFX on nAChR1 and nAChR2 subtypes may reflect their subunit combinations. Indeed, Sun *et al*, demonstrated that cockroach DUM neuronal α-Bgt-insensitive nAChR1 was potentially composed of Paα3, Paα8 and Paβ1 subunits whereas, Paα1, Paα2 and Paβ1 may contribute to nAChR2 (Sun *et al*., 2017). Considering the number of subunit combinations to form functional receptors, we propose that both nAChR1 and nAChR2 subtypes could reflect the presence of several nAChR populations which are activated by SFX. Thus, nAChR1 could include only IMI-sensitive nAChR subtypes which represents low currents induced by SFX whereas nAChR2 could include nAChR2 subtypes-sensitive and -insensitive to IMI. We propose that the effect

of SFX on nAChR1 may be due to this complex subunit combination. This finding was also in accordance with data demonstrating that SFX elicited higher current amplitudes on hybrid drosophila Dα2β2 than Dα1β2 receptors, expressed on *Xenopus oocytes* (Watson *et al*., 2011; Zhu *et al*., 2011). Our data also point out that SFX may interact with IMI-sensitive receptors as found by Cutler et al. who demonstrated that IMI displaced the tritiated analogue of SFX ([^{3}H]-methyl-SFX) with a K_i value in the pM range leading to the assumption that SFX interacted specifically with the high-affinity IMI binding site present in a subpopulation of the total nAChR pool (Cutler *et al*., 2013) which is in accordance with previous studies demonstrating that resistance of field populations of *Nilaparvata lugens* and *Bemicia tabaci* to sulfoxaflor was significantly correlated with all neoncotinoids, including IMI (Liao et al.; Liao et al., 2017; Longhurst et al., 2013). Here, we advance the hypothesis that the correlation between SFX and neonicotinoids might be due to the same mode of action at insect neuronal nAChRs. In final, the present work add informations to our understanding on the mode of action of SFX on insect nAChRs.

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Legends to the figures

Figure 1

(A) Chemical structure of sulfoxaflor (SFX) prepared according to literature data (Arndt *et al*., 2015). (B) Currents represent the effects of 1 mM sulfoxaflor (SFX), acetylcholine (ACh) and imidacloprid (IMI) on DUM neuron nAChRs. Arrows indicate 300 ms pulse application (15 psig). (C) Successive applications of 1 mM SFX (300 ms pulse application) recorded at -50 mV holding potential. Each point represents mean ± SEM of n = 12 cells.

Figure 2

Effect of sulfoxaflor on DUM neuron nAChRs. (A) Representative responses of sulfoxaflor on DUM neurons. Arrows indicate pulse application of 300 ms sulfoxaflor. (B) Dose-response curve for sulfoxaflor. Data are mean ± SEM of n = 8 independent experiments.

Figure 3

The inhibitory effects of the nAChR antagonists, d-tubocurarine (dTC) and mecamylamine (MECA), on the responses evoked by 1 mM SFX. (A and B) Representative currents after 300 ms pulse application (arrows) of 1 mM SFX during control condition (Ctl) and bath application of 20 µM dTC. Histograms represent the mean SFX-induced currents recorded at -50 mV holding potential under 20 µM dTC. In each case, data are mean ± SEM of n = 8 cells. NS : non significant. (C and D) Examples of SFX-induced currents under control condition (Ctl) and bath application of 5 µM MECA. Arrows indicate 300 ms pulse application of 1 mM SFX. (D) Histograms summarizing the effect of 1 mM SFX under control condition (Ctl)

and bath application of 5 µM MECA. Each histogram represents means ± SEM of n = 10 cells. * $p < 0.05$

Figure 4

Effect of SFX on DUM neuron nAChRs. (A) Current-voltage relationships of SFX evoked current amplitudes plotted as a function of steady-state holding potentials 5 min after establishing the whole-cell configuration. Values are means ± SEM. (B) Currents induced by 1 mM SFX under control condition (Ctl) and during bath application of 1 µM Imidacloprid (IMI). The reduction by 1 µM IMI was partially reversible after washout.

Figure 5

Effect of SFX on α-bungarotoxin insensitive nAChR1 and nAChR2 under bath application of 1 µM IMI. Data were recorded using the same cells. Thus, we found that (A) 20 µM dTC has no effect on SFX-induced currents but currents are strongly reduced when we added 1 µM imidacloprid (IMI). (B) Histograms summarize data obtained in the same conditions. Data are means ± SEM. * $p < 0.05$. In each case, n = 10 cells. Using the same conditions, (C) bath application of 5 µM MECA strongly reduced SFX evoked currents which were completely inhibited by under bath application of 1 µM MECA. (D) Histograms summarize data obtained with MECA. Data are means ± SEM. * $p < 0.05$. In each case, n = 12 cells.

Figure 6

Effects of SFX on α-bungarotoxin-insensitive, nAChR1 and nAChR2, under bath application of 9 mM Ca^{2+}. (A) Currents induced by 1 mM SFX are not affected by 20 µM dTC but they are reduced by bath application of 1 µM IMI. (B) Histograms illustrating the effect of bath application of dTC and IMI. Histograms are means ± SEM of n = 8 cells. Data were obtained using the same cell. (C) Effect of 5 µM MECA on SFX evoked currents. Currents are reduced under bath application of 5 µM MECA and completely reduced when 1 µM IMI was added in the bath. (D) Histograms represent the means ± SEM of n = 8 cells. * $p < 0.05$. NS : no significant.

References

Arndt, K.E., Bland, D.C., Irvine, N.M., Powers, S.L., Martin, T.P., McConnell, J.R., Podhorez, D.E., Renga, J.M., Ross, R., Roth, G.A., Scherzer, B.D., Toyzan, T.W., 2015. Development of a Scalable Process for the Crop Protection Agent Isoclast. Organic Process Research & Development 19(3), 454-462.

Babcock, J.M., Gerwick, C.B., Huang, J.X., Loso, M.R., Nakamura, G., Nolting, S.P., Rogers, R.B., Sparks, T.C., Thomas, J., Watson, G.B., Zhu, Y., 2011. Biological characterization of sulfoxaflor, a novel insecticide. Pest Manag Sci 67(3), 328-334.

Bodereau-Dubois, B., List, O., Calas-List, D., Marques, O., Communal, P.Y., Thany, S.H., Lapied, B., 2012. Transmembrane potential polarization, calcium influx, and receptor conformational state modulate the sensitivity of the imidacloprid-insensitive neuronal insect nicotinic acetylcholine receptor to neonicotinoid insecticides. J Pharmacol Exp Ther 341(2), 326-339.

Courjaret, R., Grolleau, F., Lapied, B., 2003. Two distinct calcium-sensitive and -insensitive PKC up- and down-regulate an alpha-bungarotoxin-resistant nAChR1 in insect neurosecretory cells (DUM neurons). Eur J Neurosci 17(10), 2023-2034.

Courjaret, R., Lapied, B., 2001. Complex intracellular messenger pathways regulate one type of neuronal alpha-bungarotoxin-resistant nicotinic acetylcholine receptors expressed in insect neurosecretory cells (dorsal unpaired median neurons). Mol Pharmacol 60(1), 80-91.

Cutler, P., Slater, R., Edmunds, A.J., Maienfisch, P., Hall, R.G., Earley, F.G., Pitterna, T., Pal, S., Paul, V.L., Goodchild, J., Blacker, M., Hagmann, L., Crossthwaite, A.J., 2013. Investigating the mode of action of sulfoxaflor: a fourth-generation neonicotinoid. Pest Manag Sci 69(5), 607-619.

Ihara, M., Matsuda, K., 2018. Neonicotinoids: molecular mechanisms of action, insights into resistance and impact on pollinators. Curr Opin Insect Sci 30, 86-92.

Lapied, B., Le Corronc, H., Hue, B., 1990. Sensitive nicotinic and mixed nicotinic-muscarinic receptors in insect neurosecretory cells. Brain Res 533(1), 132-136.

Liao, X., Jin, R., Zhang, X., Ali, E., Mao, K., Xu, P., Li, J., Wan, H., Characterization of sulfoxaflor resistance in the brown planthopper, Nilaparvata lugens (Stål). 0(0).

Liao, X., Mao, K., Ali, E., Zhang, X., Wan, H., Li, J., 2017. Temporal variability and resistance correlation of sulfoxaflor susceptibility among Chinese populations of the brown planthopper Nilaparvata lugens (Stål). Crop Protection 102, 141-146.

Longhurst, C., Babcock, J.M., Denholm, I., Gorman, K., Thomas, J.D., Sparks, T.C., 2013. Cross-resistance relationships of the sulfoximine insecticide sulfoxaflor with neonicotinoids and other insecticides in the whiteflies Bemisia tabaci and Trialeurodes vaporariorum. Pest Manag Sci 69(7), 809-813.

Salgado, V.L., 2016. Antagonist pharmacology of desensitizing and non-desensitizing nicotinic acetylcholine receptors in cockroach neurons. Neurotoxicology 56, 188-195.

Salgado, V.L., Saar, R., 2004. Desensitizing and non-desensitizing subtypes of alpha-bungarotoxin-sensitive nicotinic acetylcholine receptors in cockroach neurons. J Insect Physiol 50(10), 867-879.

Sparks, T.C., Watson, G.B., Loso, M.R., Geng, C., Babcock, J.M., Thomas, J.D., 2013. Sulfoxaflor and the sulfoximine insecticides: chemistry, mode of action and basis for efficacy on resistant insects. Pestic Biochem Physiol 107(1), 1-7.

Sun, H., Liu, Y., Li, J., Cang, X., Bao, H., Liu, Z., 2017. The potential subunits involved in two subtypes of alpha-Bgt-resistant nAChRs in cockroach dorsal unpaired median (DUM) neurons. Insect Biochem Mol Biol 81, 32-40.

Taillebois, E., Cartereau, A., Jones, A.K., Thany, S.H., 2018. Neonicotinoid insecticides mode of action on insect nicotinic acetylcholine receptors using binding studies. Pestic Biochem Physiol (In press).

Tan, J., Galligan, J.J., Hollingworth, R.M., 2007. Agonist actions of neonicotinoids on nicotinic acetylcholine rececptors expressed by cockroach neurons. NeuroToxicology 28, 829-842.

Thany, S.H., Courjaret, R., Lapied, B., 2008. Effect of calcium on nicotine-induced current expressed by an atypical alpha-bungarotoxin-insensitive nAChR2. Neurosci Lett 438(3), 317-321.

Tomizawa, M., Casida, J.E., 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. Annu Rev Entomol 48, 339-364.

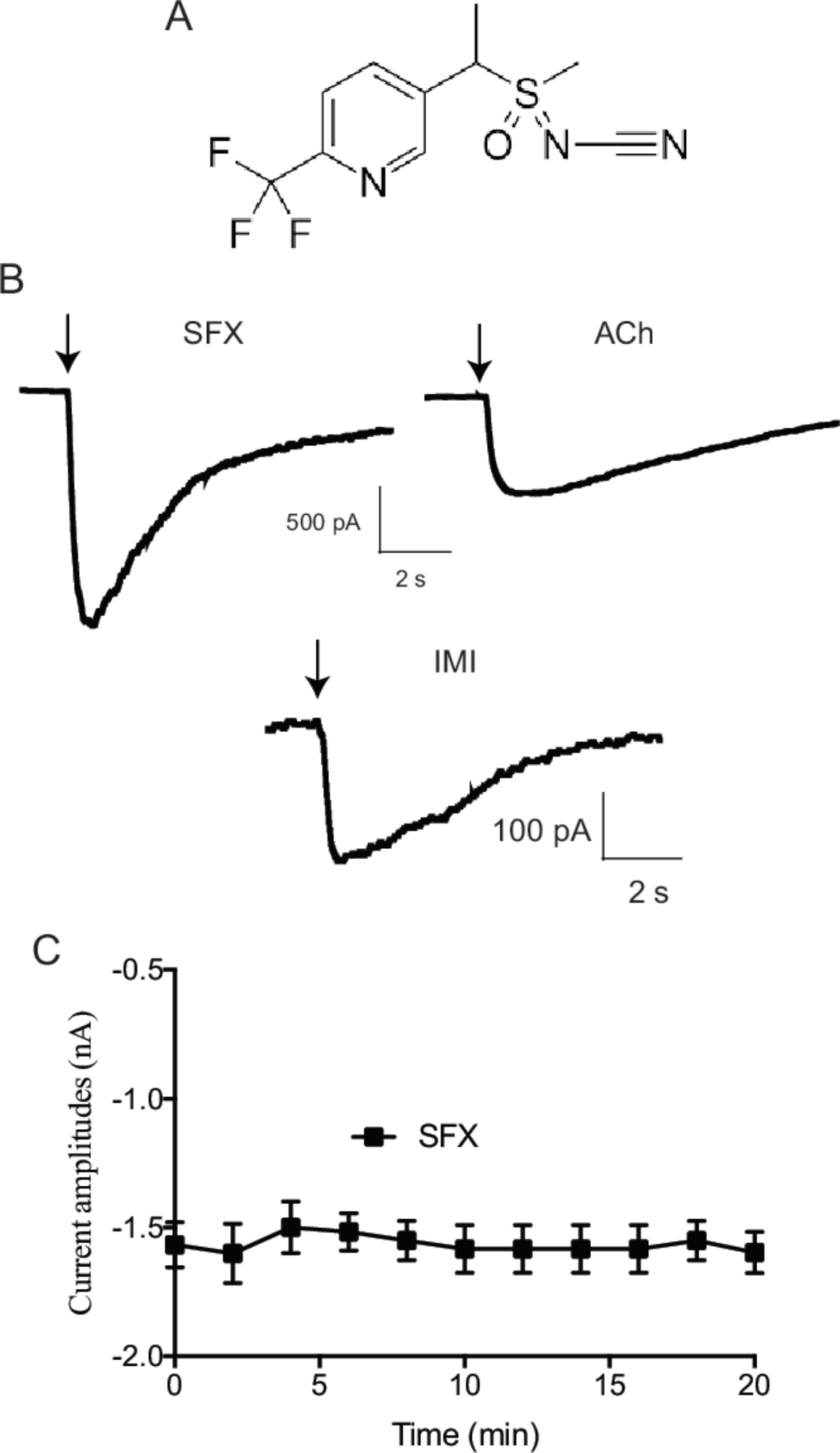
Tomizawa, M., Casida, J.E., 2005. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu Rev Pharmacol Toxicol 45, 247-268.

Wang, W., Wang, S., Han, G., Du, Y., Wang, J., 2017. Lack of cross-resistance between neonicotinoids and sulfoxaflor in field strains of Q-biotype of whitefly, Bemisia tabaci, from eastern China. Pestic Biochem Physiol 136, 46-51.

Watson, G.B., Loso, M.R., Babcock, J.M., Hasler, J.M., Letherer, T.J., Young, C.D., Zhu, Y., Casida, J.E., Sparks, T.C., 2011. Novel nicotinic action of the sulfoximine insecticide sulfoxaflor. Insect Biochem Mol Biol 41(7), 432-439.

Watson, G.B., Olson, M.B., Beavers, K.W., Loso, M.R., Sparks, T.C., 2017. Characterization of a nicotinic acetylcholine receptor binding site for sulfoxaflor, a new sulfoximine insecticide for the control of sap-feeding insect pests. Pestic Biochem Physiol 143, 90-94.

Zhu, Y., Loso, M.R., Watson, G.B., Sparks, T.C., Rogers, R.B., Huang, J.X., Gerwick, B.C., Babcock, J.M., Kelley, D., Hegde, V.B., Nugent, B.M., Renga, J.M., Denholm, I., Gorman, K., DeBoer, G.J., Hasler, J., Meade, T., Thomas, J.D., 2011. Discovery and characterization of sulfoxaflor, a novel insecticide targeting sap-feeding pests. J Agric Food Chem 59(7), 2950-2957.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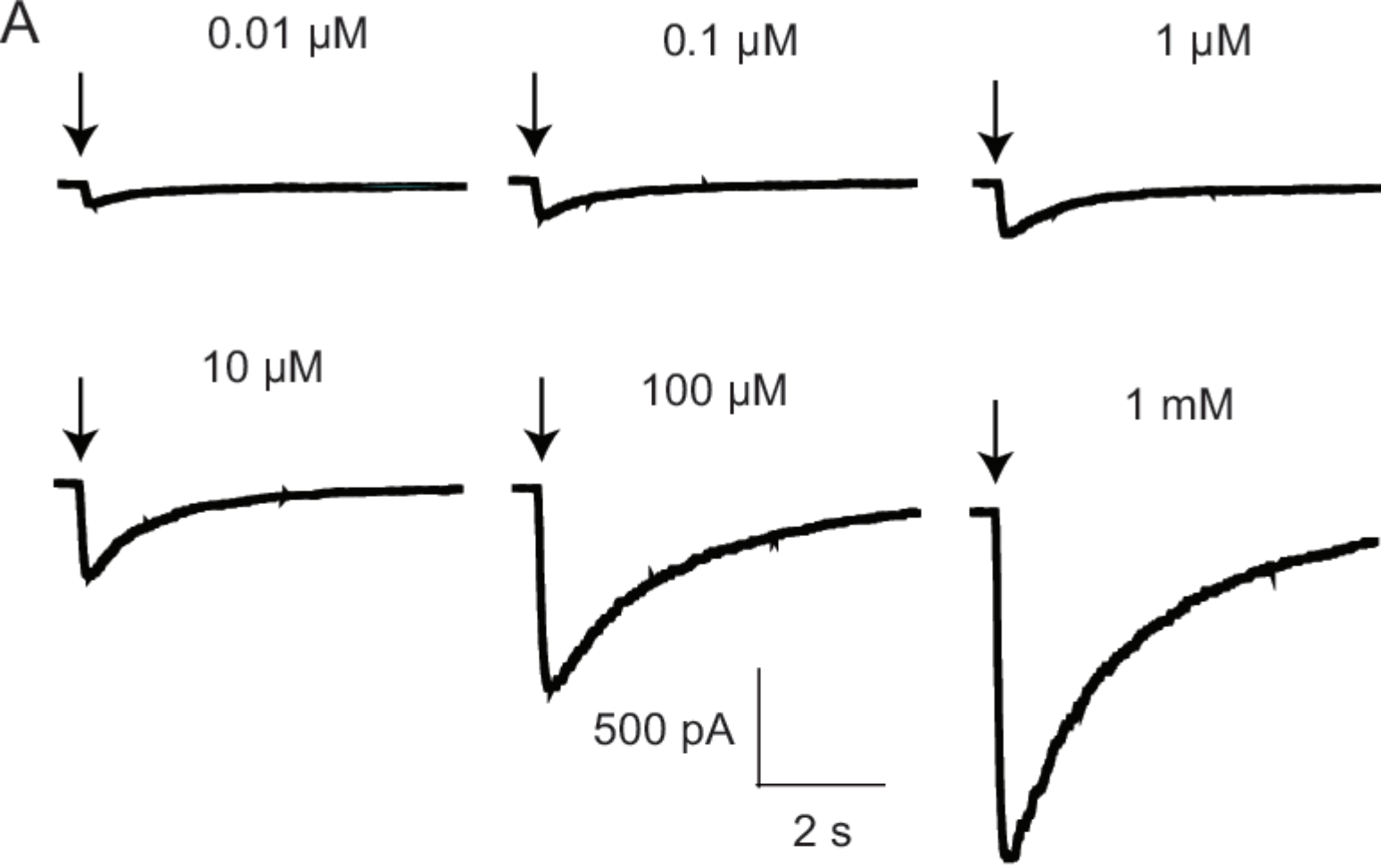
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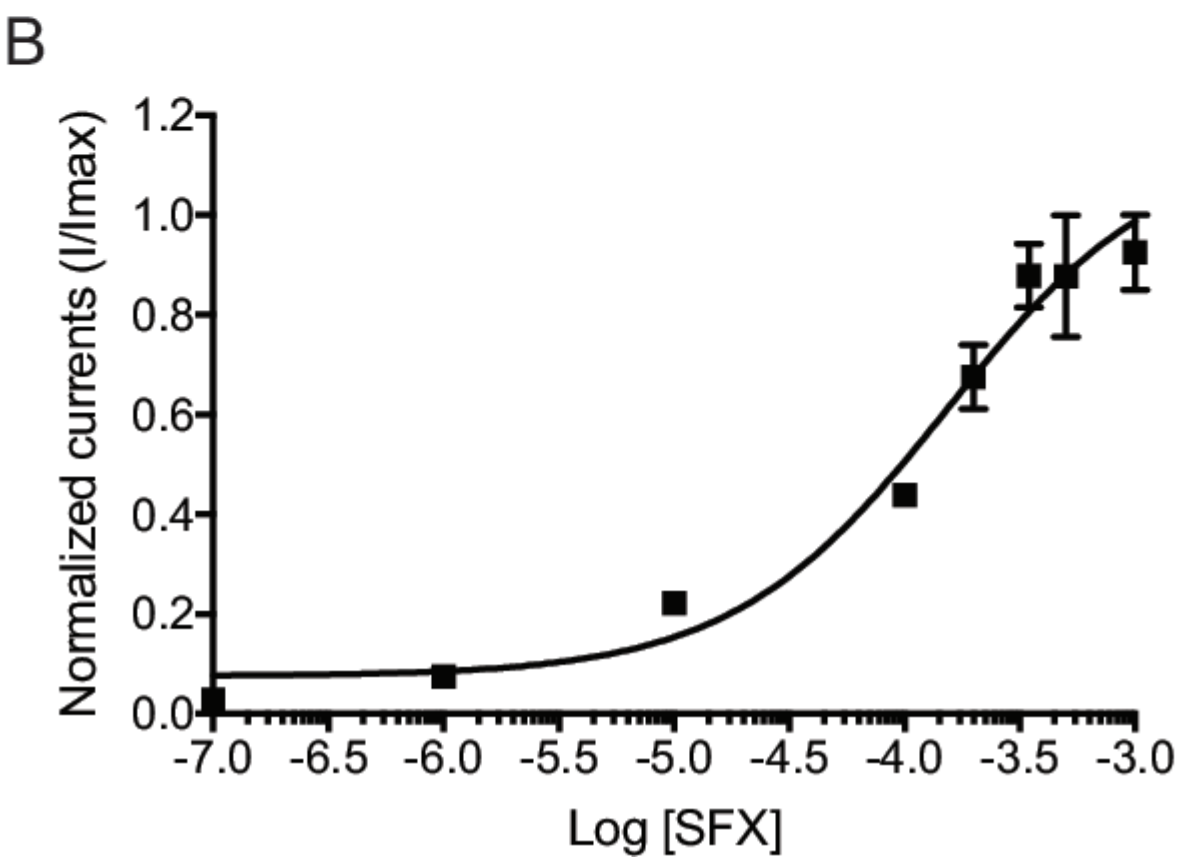
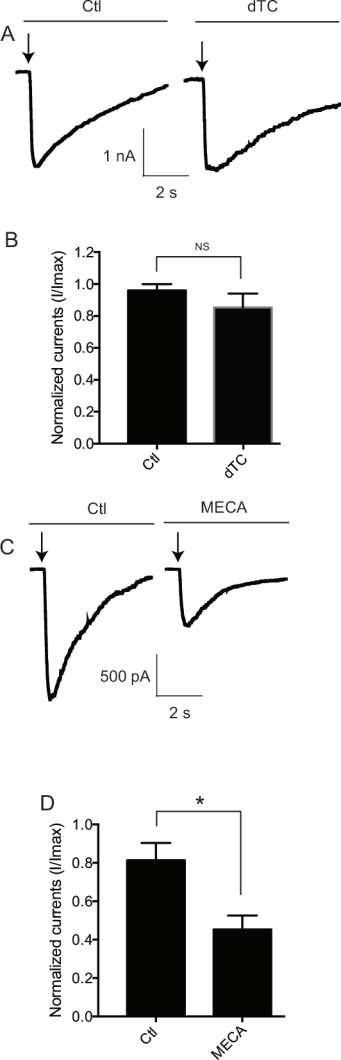
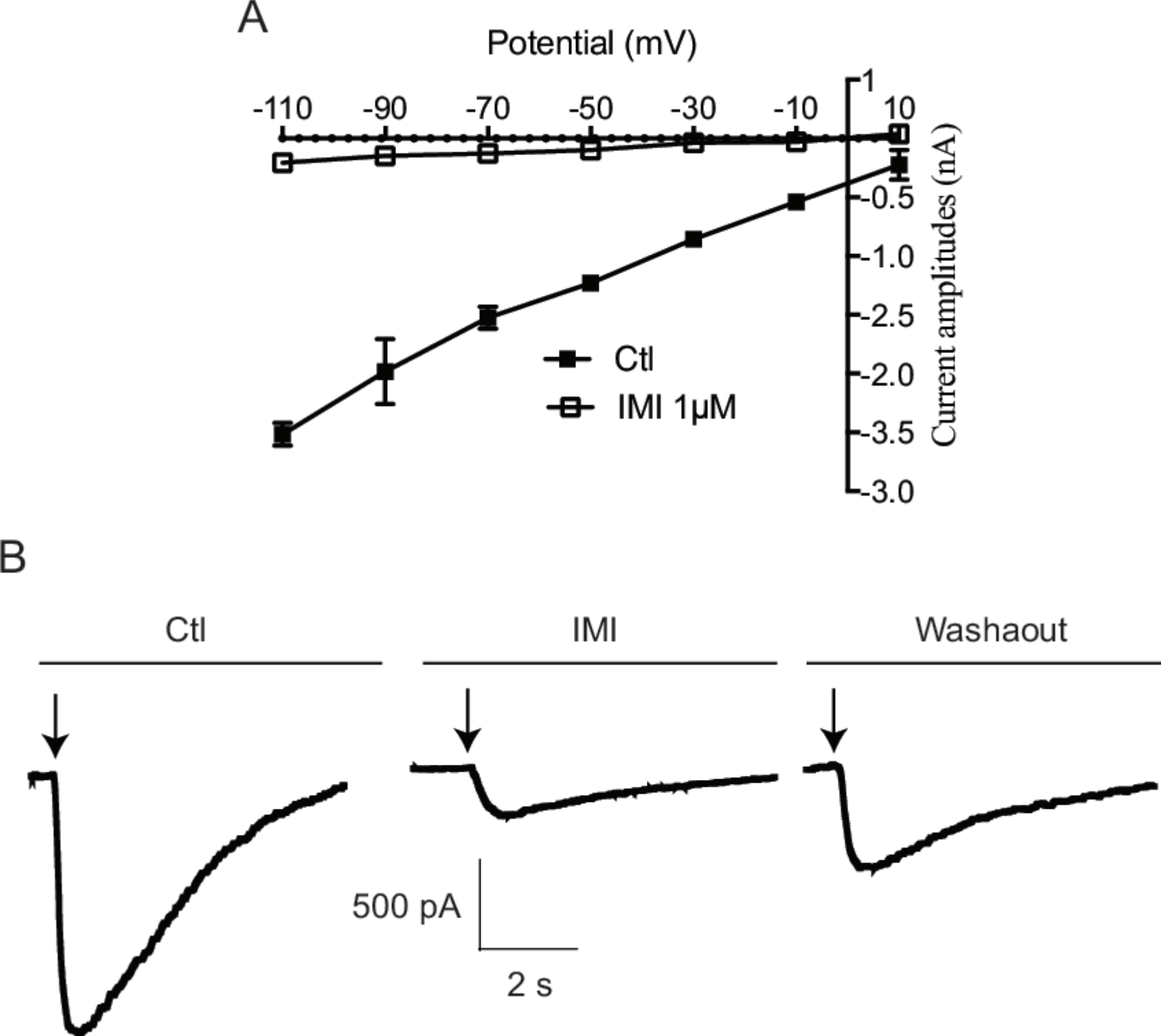
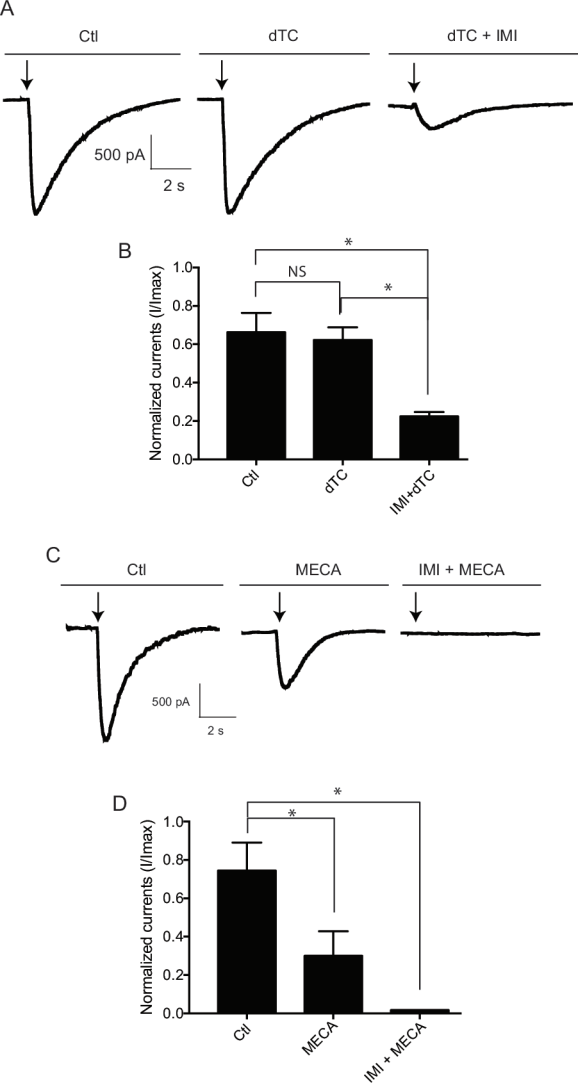
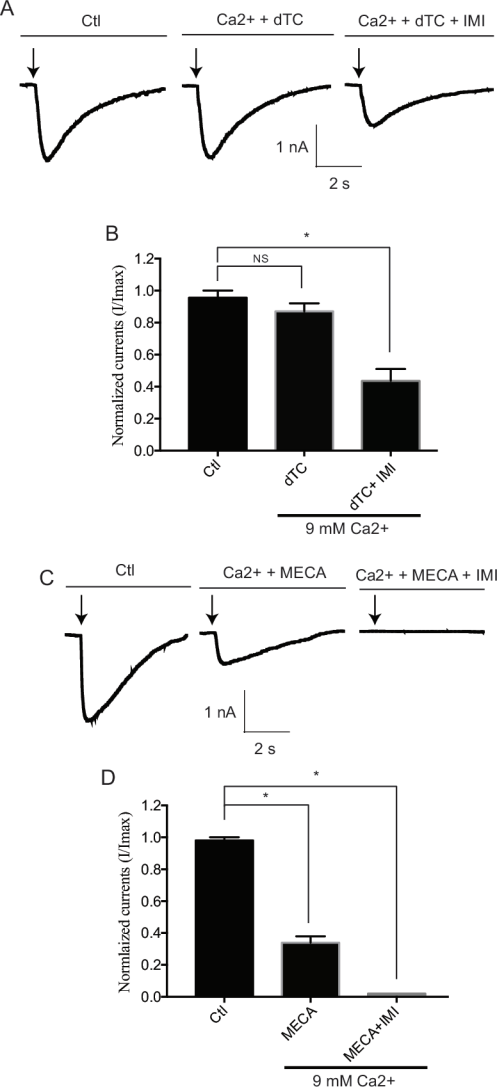
500 pA
2 s

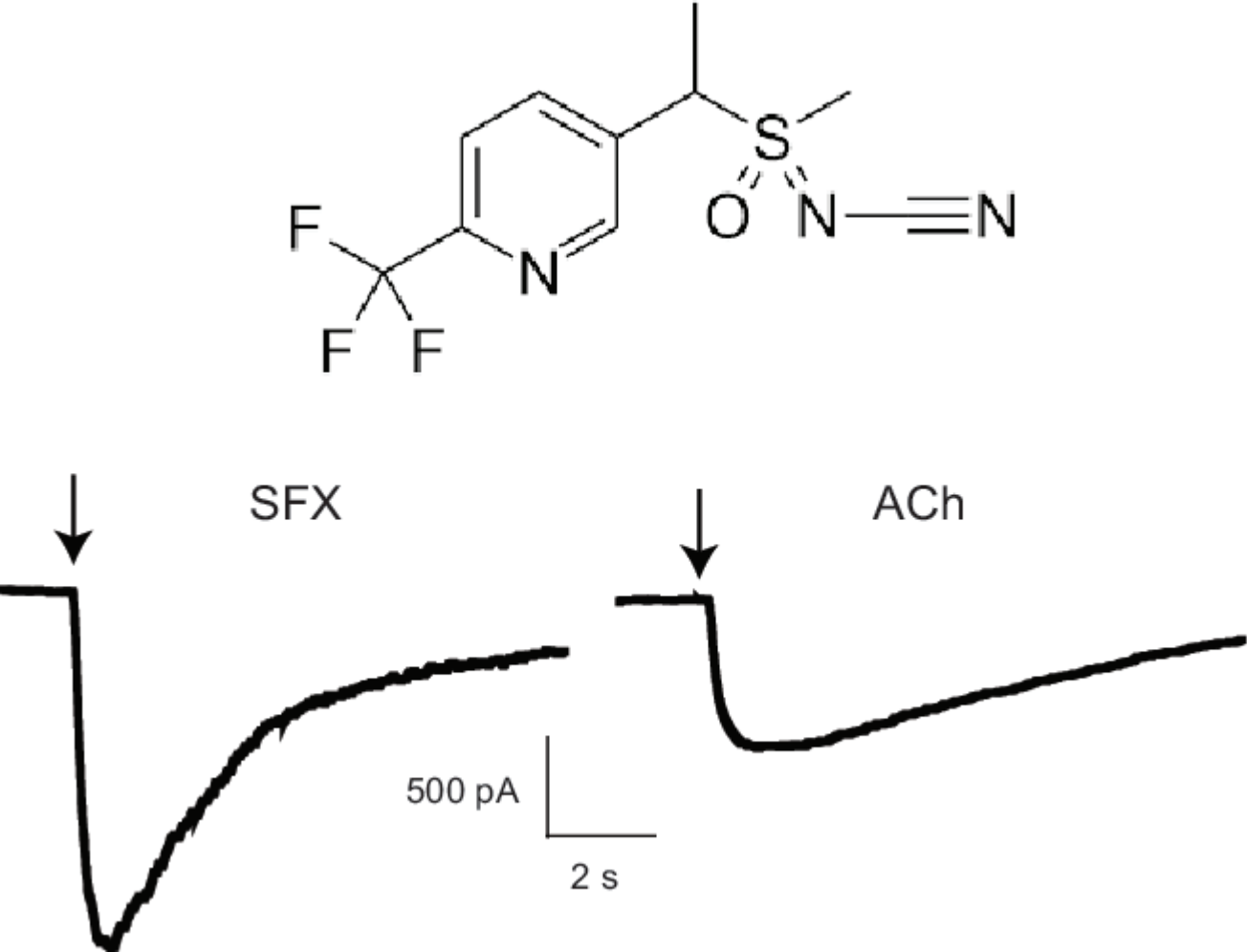

B
Normalized currents (I/Imax)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-7.0
-6.5
-6.0
-5.5
-5.0
-4.5
-4.0
-3.5
-3.0
Log [SFX]


A
Ctl
dTC
1 nA
2 s
B
NS
Normalized currents (I/Imax)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ctl
dTC
C
Ctl
MECA
500 pA
2 s
D
*
Normalized currents (I/Imax)
1.0
0.8
0.6
0.4
0.2
0.0
Ctl
MECA


A
Potential (mV)
-110
-90
-70
-50
-30
-10
10
1
-0.5
-1.0
-2.5
-2.0
-3.5
-3.0
Current amplitudes (nA)
Ctl
IMI 1μM
B
Ctl
IMI
Washaout
500 pA
2 s


A
Ctl
dTC
dTC + IMI
500 pA
2 s
B
Normalized currents (I/Imax)
1.0
0.8
0.6
0.4
0.2
0.0
NS
*
*
Ctl
dTC
IMI+dTC
C
Ctl
MECA
IMI + MECA
500 pA
2 s
D
Normalized currents (I/Imax)
1.0
0.8
0.6
0.4
0.2
0.0
*
*
Ctl
MECA
IMI + MECA


A
Ctl
Ca2+ + dTC
Ca2+ + dTC + IMI
1 nA
2 s
B
*
NS
Normalized currents (I/Imax)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ctl
dTC
dTC+ IMI
9 mM Ca2+
C
Ctl
Ca2+ + MECA
Ca2+ + MECA + IMI
1 nA
2 s
D
*
*
Normlaized currents (I/Imax)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Ctl
MECA
MECA+IMI
9 mM Ca2+


F
F
F
N
S
O
N
N
SFX
ACh
500 pA
2 s