Fluoxetine Blocks \( \text{Na},1.5 \) Channels Via a Mechanism Similar to That of Class 1 Antiarrhythmics

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Fluoxetine blocks Na,1.5 channels

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Abbreviations: CNS, central nervous system; DAT, dopamine transporter; HP, holding potential; IC50, dose producing 50% maximum current inhibition; OCD, obsessive compulsive disorders; MAT, monoamine transporter; NET, norepinephrine transporter; SCD, sudden cardiac death; SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor
Abstract

The voltage-gated Na,1.5 channel is essential for the propagation of action potentials in the heart. Malfunctions of this channel are known to cause hereditary diseases. It is a prime target for class 1 antiarrhythmic drugs and a number of antidepressants. The purpose of the present study was to investigate the Na,1.5 blocking properties of fluoxetine, a selective serotonin re-uptake inhibitor. Na,1.5 channels were expressed in HEK-293 cells, and Na⁺ currents were recorded using the patch-clamp technique. Dose-response curves of racemic fluoxetine (IC₅₀ = 39 μM) and its optical isomers had similar IC₅₀ (40 and 47 μM for the (+) and (–) isomer, respectively). Norfluoxetine, a fluoxetine metabolite, had a higher affinity than fluoxetine, with an IC₅₀ of 29 μM. Fluoxetine inhibited currents in a frequency-dependent manner, shifted steady-state inactivation to more hyperpolarized potentials, and slowed the recovery of Na,1.5 from inactivation. Mutating a phenylalanine (F1760) and a tyrosine (Y1767) in DIVS6, significantly reduced the affinity of fluoxetine and its frequency-dependent inhibition. We used a non-inactivating Na,1.5 mutant to show that fluoxetine displays open-channel block behavior. The molecular model of fluoxetine in Na,1.5 was in agreement with mutational experiments, in which F1760 and Y1767 were found to be the key residues in binding fluoxetine. We concluded that fluoxetine blocks Na,1.5 by binding to the class 1 antiarrhythmic site. The blocking of cardiac Na⁺ channels should be taken into consideration when prescribing fluoxetine alone, or in association with other drugs that may be cardiotoxic or for patients with conduction disorders.
Introduction

Fluoxetine (Prozac) is a selective serotonin re-uptake inhibitor (SSRI) (Wong et al., 1995) that is widely prescribed for the treatment of Central Nervous System (CNS)-linked cognitive, emotional, and behavioral disorders. Since its discovery in 1974 (Wong et al., 1974), the beneficial psychotropic effects of fluoxetine have led to its being used to treat disorders other than depression, including obsessive compulsive disorders (OCD) and bulimia nervosa (Wong et al., 1995). The multiple side effects of fluoxetine (Sghendo and Mifsud, 2012) have raised questions about its supposed selective 5-HT-mediated effect. While fluoxetine inhibits serotonin transporter (SERT) in the low nanomolar range (Torres et al., 2003), its therapeutic effect appears only at much higher plasma and brain concentrations (Muscettola et al., 1978; Bolo et al., 2000). At low micromolar concentrations, fluoxetine also targets other proteins and inhibits several types of ion channels and receptors, including the nicotinic acetylcholine receptor (Hennings et al., 1999; Eisensamer et al., 2003), voltage-gated Ca^{2+} channels (Deák et al., 2000; Pacher et al., 2000), volume-regulated anion channels (Maertens et al., 2002), neuronal Na^{+} channels (Lenkey et al., 2006), and hERG, a cardiac K^{+} channel (Thomas et al., 2002). The inhibition of the hERG K^{+} channel by fluoxetine occurs via two different mechanisms: (i) direct channel blockade and (ii) disruption of channel protein trafficking (Rajamani et al., 2006). This may explain some of the cardiovascular side effects observed during chronic fluoxetine treatments, including bradycardia and QT prolongation (Pacher and Kecskemeti, 2008; Timour et al., 2012). Dysfunctions of Na_{1.5}, which are responsible for the rapid upstroke of the action potential caused by the rapid entry of Na^{+} ions into cardiomyocytes, also lead to arrhythmia complications. The
prolongation of QT intervals may be due to the improper inactivation of the Na\textsubscript{v}1.5 as in Romano-Ward syndrome (LQT3), while the reduction of Na\textsuperscript{+} currents through Na\textsubscript{v}1.5 may lead to arrhythmias such as Brugada syndrome (Herbert and Chahine, 2006). The major cause of the higher mortality rate in psychiatric patients versus the general population is sudden cardiac death (SCD), which mainly results from arrhythmias that occur during treatments with psychotropic drugs. It has been reported that fluoxetine decreases the maximum rate of rise of the depolarization phase (V\textsubscript{max}) of ventricular cell preparations (Pacher \textit{et al.}, 2000; Magyar \textit{et al.}, 2003), but little is known about the direct effect of fluoxetine on the biophysical properties of Na\textsubscript{v}1.5.

In the present study, we investigated the electrophysical properties of fluoxetine (racemic and enantiomers) and its metabolite norfluoxetine, as well as other psychotropic drugs, on Na\textsubscript{v}1.5 stably expressed in HEK-293 cells. We showed that racemic fluoxetine, its metabolite norfluoxetine, and its enantiomers act as potential antagonists of human Na\textsubscript{v}1.5 unlike the other classes of antidepressants tested.

We also studied the effect of the F1760C and Y1767C mutations of the class I antiarrhythmic binding site on the use-dependent blockade of cardiac Na\textsuperscript{+} channels by fluoxetine and showed that fluoxetine behaves like a class I antiarrhythmic drug.
Materials and Methods

Cell cultures

Human embryonic kidney (HEK-293) cells stably expressing human Na\textsubscript{v}1.5 were used as previously described (Huang et al., 2011). In brief, the cells were grown under standard tissue culture conditions (5% CO\textsubscript{2}, 37°C) in high-glucose DMEM supplemented with 10% FBS, 2 mM L-glutamine, 100 U/ml of penicillin, and 10 mg/ml of streptomycin (Gibco-BRL Life Technologies, Burlington, ON). For experiments with the F1760C, Y1767C, and L409C/A410W mutants, the HEK-293 cells were transiently transfected with the pcDNA3.1 vector containing mutant Na\textsubscript{v}1.5 cDNA (5 μg) or with the empty vector pIRES/CD8 (5 μg) in 10 cm petri dishes using the calcium phosphate method as previously described (Huang et al., 2011). Transfected cells were briefly preincubated with CD8 antibody-coated beads (Dynabeads M450 CD8-a, (Life Technologies Inc., Burlington, ON, Canada) before recording currents. HEK-293 cells expressing the pIRES/CD8 vector were decorated with CD8 beads, which were used to identify cells for recording Na\textsuperscript{+} currents.

Whole-cell patch-clamp recordings

Macroscopic Na\textsuperscript{+} currents from HEK-293 cells were recorded using the whole-cell patch-clamp technique. Patch-clamp recordings were obtained using low-resistance, fire-polished electrodes (<1 MΩ) made from 8161 Corning borosilicate glass coated with Sylgard (Dow-Corning, Midland, MI) to minimize electrode capacitance. Currents were recorded using an Axopatch 200 amplifier with the pClamp software (Molecular Devices Sunnyvale, CA). The series resistance was 70-80% compensated. Whole-cell currents
were filtered at 5 kHz, digitized at 10 kHz, and stored on a microcomputer equipped with an analog-to-digital converter (Digidata 1300, Molecular Devices). The cells were allowed to stabilize for 5 min after the whole-cell configuration was established before recording the currents. The experiments were performed at room temperature (22°C). The pipettes were filled with an intracellular solution composed of 35 mM NaCl, 105 mM CsF, 10 mM EGTA, and 10 mM Cs-HEPES. The pH was adjusted to 7.4 with CsOH. The external solution was composed of 150 mM NaCl, 2 mM KCl, 1.5 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES. The pH was adjusted to 7.4 with NaOH.

The drugs were applied using a constantly running ValveLink8.2® gravity-driven perfusion system (Automate Scientific, Berkeley, CA) equipped with a glass syringe with a 250 µM tip. Different concentrations of the same drug were applied on the same cell. We used silicone-free tubing since we had observed changes in fluoxetine concentrations when silicon tubing was used, most likely because fluoxetine adheres to silicone, which can change the applied concentrations considerably.

The peak current amplitudes at different drug concentrations were subtracted from the value obtained with the control solution and were normalized to the control value in order to obtain the dose-response curves and IC₅₀ values. Each point on the dose-response curves represents the mean of inhibition calculated from all recorded cells at a specific drug concentration. The values were fit to a Hill equation of the following form:

\[ \frac{(I_{\text{control}} - I_{\text{fluoxetine}})}{I_{\text{control}}} = \alpha \frac{t^b}{(c^b + x^b)}, \]

where \( I \) is the peak current, \( \alpha \) is the maximum inhibition, \( b \) is the Hill coefficient, \( c \) is the IC₅₀, and \( x \) is the concentration of agonist. To obtain activation curves, Na⁺ conductance (\( G_{\text{Na}} \)) was calculated from the peak current (\( I_{\text{Na}} \)) using the following equation: \( G_{\text{Na}} = \)
\[ I_{Na}(V - E_{Na}), \text{ where } V \text{ is the test potential and } E_{Na} \text{ is the reversal potential. Normalized } G_{Na} \text{ values were plotted against the test potentials. To obtain the inactivation curves, the peak current was normalized to the maximal value and was plotted against the conditioning pulse potential. Steady-state activation and inactivation curves were fit to a Boltzmann equation of the following form:} \]

\[ \frac{G}{G_{max}} \text{ (or } I/I_{max}) = \frac{1}{1 + \exp \left( V_{1/2} - \frac{V}{k_v} \right)}, \]

where \( G \) is the conductance, \( I \) is the current, \( V_{1/2} \) is the voltage at which the channels are half-maximally activated or inactivated, and \( k_v \) is the slope factor. To determine the recovery from inactivation, the test pulse peak current (\( I_{test} \)) was normalized to the corresponding prepulse current (\( I_{cont} \)). \( I_{test}/I_{cont} \) was plotted against the pulse interval and was fitted to a double or triple exponential function of the following form:

\[ \frac{I}{I_{max}} = A_1(1 - \exp (-t/\tau_1)) + A_2(1 - \exp (-t/\tau_2)) \]

or

\[ \frac{I}{I_{max}} = A_1(1 - \exp (-t/\tau_1)) + A_2(1 - \exp (-t/\tau_2)) + A_3(1 - \exp (-t/\tau_3)), \]

where \( \tau_1, \tau_2 \text{ and } \tau_3 \) are the time constants, \( t \) is the time and \( A_1, A_2 \text{ and } A_3 \) are the amplitudes of the time constants.

The results were analyzed using a combination of pCLAMP software v10.2 (Molecular Devices), Microsoft Excel, and SigmaPlot v11.0 (SSPSS, Chicago, IL).

**Statistical analysis**

Results are expressed as means ± SEM. Statistical significance was calculated using Student’s unpaired \( t \) test, and the level of statistical significance was set at \( P < 0.05 \). The
statistical significances for the IC₅₀ were calculated using R software and the *drc* package (The R Foundation for Statistical Computing, Vienna, Austria).

**Drugs**

Racemic fluoxetine, S (+) fluoxetine, S (−) fluoxetine, norfluoxetine, and (+) fenfluramine were from Sigma-Aldrich (St. Louis, MO). Nisoxetine and methylphenidate were from Tocris Bioscience (Bristol, UK). Stock solutions (5 mM) were prepared in water and were diluted in the external solution prior to use.

**Homology modeling of fluoxetine binding site in the Naᵥ1.5**

The human cardiac Naᵥ1.5 was modeled in the closed and open states based on the closed NaᵥAb (3RVY.pdb) and open NaᵥMs (3ZJZ.pdb) X-ray structures (Payandeh *et al.*, 2011; Bagnéris *et al.*, 2013). To describe the symmetric positions of residues in four homologous domains in the channel, we used a universal residue-labeling scheme (Zhorov and Tikhonov, 2004). A residue is labeled by its domain number (1–4), segment (i, inner helix S6; o, outer helix S5; p, P-loop), and the relative number from the N end of a transmembrane helix or from the DEKA-locus positions 1p50, 2p50, etc. For example, F⁴i₁₅(1760) designates phenylalanine in the domain IV inner helix, 15 positions downstream from the start of the segment. In some cases, the sequence-based residue number is included in the label in parentheses.

The alignment of bacterial NaᵥAb and NaᵥMs with eukaryotic sodium channels was taken as previously proposed (Payandeh *et al.*, 2011; McCusker *et al.*, 2012; Tikhonov and Zhorov, 2012). An insertion downstream from the DEKA locus was proposed (Tikhonov and Zhorov, 2012), however, in our models, this insertion was not introduced.
as the ligand was docked in the pore and residues above the DEKA locus would not affect ligand binding. The models contained the pore region (S5, P, and S6) of the human Na\textsubscript{v}1.5. The closed model also contained the L4-5 linker (the linker between domain 4 and 5) because it is available in the X-ray structure. The extracellular linkers between P-loops and transmembrane helices were truncated to match the length of the X-ray structure templates, which does not affect ligand docking in the inner pore as they are distant. Ionizable residues were modeled as neutral, but the ionizable residues of DEKA locus were modeled as charged. S-fluoxetine was modeled as protonated, since its ammonium group has a pK\textsubscript{a} of ~10.

All calculations were performed using the ZMM program (www.zmmsoft.com, ZMM Software Inc., Flamborough, Ontario, Canada). The nonbonded energy was calculated using the AMBER force field (Weiner \textit{et al.}, 1984, 1986) with a cutoff distance of 8 Å. Atomic charges at fluoxetine were calculated with the MOPAC software using the semi-empirical method AM1 (Dewar \textit{et al.}, 1985). The hydration energy was calculated by using the implicit-solvent method (Lazaridis and Karplus, 1999). Electrostatic energy was calculated using the environment- and distance-dependent dielectric function without desolvation energy (Garden and Zhorov, 2010). The DEKA locus was loaded with an explicit water molecule, which was initially constrained to the Asp and Lys side chains, subsequently once constraints were removed, the water did not move away from the DEKA locus. The Monte Carlo minimization (MCM) method (Li and Scheraga, 1987) was used to optimize the models. All torsional angles of the protein and ligand were allowed to vary during energy calculations, while bond angles were rigid in the protein and flexible in the ligand. To prevent large deviations of the channel
models from the X-ray structure templates during energy minimizations, the \( \alpha \)-carbons of the model were constrained to the template using a flat-bottom energy function that allows atoms to deviate penalty-free up to 1 Å, but imposes a penalty of 10 kcal mol\(^{-1}\) Å\(^{-1}\) for larger deviations. All molecular images were created using MVM. No specific energy terms were used for cation-\( \pi \) interactions, which were accounted for with partial negative charges at the aromatic carbons (Bruhova et al., 2008).

The homology models were first MC-minimized without ligand until the 3000 consecutive energy minimizations did not improve the apparent global minimum found. The optimal binding modes of S-fluoxetine were searched by a two-stage random-docking approach. In the first stage, 60 000 different binding modes of the ligand were randomly generated within a cube with 14 Å edges. This sampling volume covered the entire inner pore including the domain interfaces. Each binding mode was MC-minimized for only 5 steps to remove steric overlaps with the protein. Energetically favorable conformations within 200 kcal/mol from the apparent global minimum were accumulated and then clustered based on ligand generalized coordinates. In the second stage, the 500 energetically best conformations found in the first stage were further MC-minimized for 1000 MC-minimization steps. The energetically most favorable ligand-receptor complexes within 4 kcal/mol were collected and analyzed.
Results

*Fluoxetine and its optical isomers block the Na\(_v\)1.5 channel*

We studied the effect of fluoxetine on Na\(_v\)1.5 stably expressed in HEK-293 cells. Figure 1A shows an example of whole-cell current traces before (control) and after superfusion of 25 and 100 μM racemic fluoxetine. Fluoxetine inhibited Na\(^+\) currents, with a maximum blockade occurring at 100 μM. The inhibition was partially reversible. The superfusion of increasing concentrations of fluoxetine (1, 10, 25, 50, 100, and 200 μM) showed that the blockade by fluoxetine was dose dependent. The dose-response curves (Fig. 1B) showed that the sensitivities of the optical isomers were similar, with an IC\(_{50}\) of 39.4 μM for racemic fluoxetine, and 40.0 μM and 46.7 μM for the (+) and (–) isomers, respectively. When the cells were maintained at a holding potential of -90 mV instead of -140 mV, where a proportion of the channels are inactivated, the affinity of racemic fluoxetine for Na\(_v\)1.5 significantly increased with an IC\(_{50}\) of 4.7 μM. Surprisingly, norfluoxetine, a fluoxetine metabolite, displayed a significantly higher affinity than fluoxetine, with an IC\(_{50}\) of 29.5 μM at holding potential of -140 mV. Surprisingly, norfluoxetine, a fluoxetine metabolite, displayed a significantly higher affinity than fluoxetine, with an IC\(_{50}\) of 29.5 μM at holding potential of -140 mV.

The effects of three other monoamine transporters (MAT) -targeting drugs were also tested using HEK-293 cells stably expressing Na\(_v\)1.5. The norepinephrin reuptake inhibitor, nisoxetine, the dopamine reuptake inhibitor, methylphenidate, and fenfluramine, which like fluoxetine, targets SERT, were all less effective in blocking the channels than fluoxetine, with IC\(_{50}\) of 104.5, 618.7 and 203.5 μM, respectively at a holding potential of -140 mV (Fig. 1C). The inhibition potency of these three compounds
was also increased at a holding potential of -90 mV, with IC$_{50}$ of 20.2, 239.5 and 65.5 μM for nisoxetine, methylphenidate and fenfluramine, respectively (Fig. 1C).

**Effect of fluoxetine on the steady-state gating properties of Na$_v$1.5 channels**

The availability of Na$^+$ channels following depolarization depends on a number of parameters, including the membrane potential. Fewer channels become available as the membrane potential progressively becomes more depolarized. This is due to the buildup of channels in the inactivated non-conducting state. We studied this phenomenon using a double-pulse protocol: a 500 ms conditioning pulse to voltages ranging from -140 mV to 0 mV, and a test pulse to -30 mV. The current measured following the test pulse is an indicator of the fraction of available channels. The normalized currents following the test pulse were plotted against the conditioning voltage (Fig. 2A). Fluoxetine (30 μM) significantly shifted the $V_{1/2}$ of inactivation of Na$_v$1.5 by 6.7 mV toward more hyperpolarized voltages and resulted in a less steep slope factor (Table 1 and Fig. 2A).

We also investigated the effect of fluoxetine on the steady-state activation of Na$_v$1.5. The activation curves were derived from I/V curves (see Materials and Methods). The activation curves of Na$_v$1.5 in the absence and presence of 30 μM fluoxetine were plotted against voltage (Fig. 2A). Fluoxetine did not significantly shift the midpoint of steady-state activation but had a little effect on the slope factor by reducing its steepness.

**Fluoxetine slows the recovery from inactivation of Na$_v$1.5 channels**

A prominent characteristic of many class 1 antiarrhythmics is their ability to slow the recovery from inactivation of drug-modified Na$^+$ channels. We used a two-pulse protocol
to investigate the effect of fluoxetine on the recovery from inactivation. We used a 40 ms, –30 mV conditioning pulse and a 20 ms, –30 mV test pulse, with an interval ranging from 0.1 to 4000 ms to induce recovery from inactivation. The amplitudes of the Na+ currents measured following the test pulse were then normalized to the control currents and were plotted against the duration of the recovery interval. Channels that recovered from inactivation displayed a progressive increase in currents following the increase in the recovery interval (Fig. 2B). The recovery from inactivation of Na\textsubscript{v}1.5 after fluoxetine treatment was strongly slowed with the appearance of a third time constant. In comparison, the control curve had a $\tau_1$ and $\tau_2$ of 1.50 and 9.13 ms, respectively, while the fluoxetine had a $\tau_1$, $\tau_2$ and $\tau_3$ of 1.63, 14.90 and 1598.23 ms, respectively (Table 1).

**Fluoxetine blocks Na\textsubscript{v}1.5 channels in a use-dependent manner**

During depolarization, Na\textsuperscript{+} channels cycle from the resting to the activated and inactivated states. However, when they are subjected to a train of depolarizing pulses, the number of channels available to open is reduced because they are gradually trapped in the inactivated state. This phenomenon is referred to as use-dependence or “frequency-dependent” current reduction. In the presence of a drug, further decreases in currents are likely due to the accumulation of drug-modified channels. For example, lidocaine, a class 1 antiarrhythmic drug, is known to cause the use-dependent inhibition of Na\textsuperscript{+} channels. We tested the effect of rapid pulsing on Na\textsubscript{v}1.5 by applying a series of 50 short 10 ms depolarizing –30 mV pulses. We first characterized the effect of fluoxetine on Na\textsubscript{v}1.5/WT, and then on the Na\textsubscript{v}1.5/F1760C and Na\textsubscript{v}1.5/Y1767C mutant channels. We previously reported that these highly conserved residues of the D4S6 directly contribute
to local anesthetic binding to cardiac channels (O’Leary and Chahine, 2002). As shown in Figure 3A, in the absence of fluoxetine, there was no significant change in the availability of Na\textsubscript{v},1.5/WT channels when they were pulsed up to 10 Hz. However, in the presence of 30 μM fluoxetine, the availability of Na\textsubscript{v},1.5/WT channels was dramatically reduced by 44% (P50/P1) when they were pulsed at 2 Hz (Fig. 3B-C) in comparison with the control without drug. When 5 and 10 Hz pulses were used, 30 μM fluoxetine reduced the currents of the Na\textsubscript{v},1.5/WT by 58% and 67%, respectively compared to the control without drug. To further investigate the role of class 1 antiarrhythmic binding in the current block caused by fluoxetine, we inserted the F1760C or Y1767C mutation into Na\textsubscript{v},1.5. As shown in Figure 3B-C, 30 μM fluoxetine reduced the current by 8%, 15%, and 20% when Na\textsubscript{v},1.5/F1760C were pulsed at 2, 5, and 10 Hz, respectively, in comparison with the control without drug. The Y1760C mutation almost completely prevented the use-dependent inhibition of fluoxetine, with a maximal current inhibition of 5% when pulsed at 10 Hz. These results indicated that fluoxetine blocks Na\textsubscript{v},1.5/WT currents in a use-dependent manner, and that the F1760C and Y1767C mutations dramatically reduce the use-dependent inhibition.

**Fluoxetine had a lower affinity for Na\textsubscript{v},1.5/F1760C mutant channels**

We studied the effect of the F1760C and Y1767C mutations on the concentration-dependent block of Na\textsubscript{v},1.5 currents by fluoxetine. Figure 4A shows examples of current traces recorded from Na\textsubscript{v},1.5/WT and the mutant channels before and after a treatment with 50 μM fluoxetine. As shown in Figure 4B, while the IC\textsubscript{50} value of fluoxetine for
Nav1.5/Y1767C (50.1 μM) was slightly higher to that of Nav1.5/WT (39.4 μM), the IC50 value for Nav1.5/F1760C (82.8 μM) was more than twice that of the WT channel.

**Fluoxetine act as an open-channel blocker**

To investigate the role of inactivation in the blockade of Na,1.5 by fluoxetine in greater detail, we used Na,1.5/L409C/A410W mutant stably expressed in HEK-293 cells. These channels exhibit a significant reduction in fast inactivation in HEK-293 (Wang et al., 2013). A large persistent current was detected in the absence of fluoxetine (Fig. 5A). We applied different concentrations of fluoxetine and determined the IC50 at the peak current and at the end of the test pulse (90-100 ms). The block at the end of the pulse represents the affinity of the fluoxetine for open channels. As shown in Figure 5B, the IC50 (3.5 μM) at the end of the pulse was slightly lower than the IC50 at the peak current (9.6 μM), suggesting that fluoxetine is an open-channel blocker.

**Molecular modeling of fluoxetine in the Na,1.5**

To discover the molecular details of the fluoxetine binding site, we have homology modeled the pore domain of the cardiac Na,1.5 in the closed and open states based on the X-ray structures of bacterial Na channels, Na,Ab (Arcobacter butzleri sodium channel) (Payandeh et al., 2011) and Na,Ms (Magnetococcus sp sodium channel) (Bagnéris et al., 2013), respectively (see PDB file in Data Supplement). A random sampling approach was used to search for the energetically most favorable binding modes of fluoxetine in the Na,1.5. 60 000 random orientations of fluoxetine were seeded inside the channel within a volume to cover the entire pore cavity and inner helix interfaces (Fig. 6A, B). After two
rounds of Monte-Carlo energy minimizations, the energetically best fluoxetine complexes bound inside the inner pore. Fluoxetine adopts two distinct binding modes, a horizontal and a vertical binding mode (Fig. 6C-F). These two binding modes were energetically favorable in both the closed and the open channel pore.

Fluoxetine resembles a three-pointed star with a chiral center in the middle linking three arms comprising an ammonium group, a benzene ring, and a trifluoromethyl benzene ring. In both of the two binding modes of fluoxetine, its ammonium group localizes to the channel’s central axis under the DEKA locus, near the focus of the P-helices (Figure 1C-F). Just one position upstream of the DEKA locus, a ring of QGFS residues in position p49 (see description of relative number scheme in Methods) favorably interact with fluoxetine since their side chains face downward into the pore. Particularly, Q^{1p49(372)} and S^{4p49(1712)} form favorable electrostatic contacts with fluoxetine’s nitrogen, each contributing 4-9% to the ligand-receptor energy. The ammonium group of fluoxetine was also attracted by the two negatively charged residues of the DEKA locus, which outweighed the repulsion from the Lys of the DEKA locus. Further, the backbone carbonyl groups of residues two to three positions upstream of the DEKA locus (positions p47 and p48) also stabilize fluoxetine.

The horizontal and vertical binding modes are distinguished by the two benzene arms of fluoxetine. In the vertical binding mode (Fig. 6C, E), one benzene arm is parallel and the other arm is perpendicular to the pore axis. In this mode, one benzene ring π-stacks with Y^{4i22(1767)} and the other interacts with F^{4i15(1760)}. Y^{4i22} and F^{4i15} were found to be the two most significant residues in binding fluoxetine; each contributes 16-33% to the ligand-receptor energy. In the horizontal binding mode (Fig. 6D, F), the two benzene
arms point away ~45-90° from the pore axis. The ligand leans against the P-loop and protrudes between the III-IV domain inner helices. Here, one of the benzene rings π-stacks with F³₁₁₅, while the other arm extends towards F³₃₉⁴⁹(1236) and IIIS6. In the horizontal binding mode, F³₁₁₅ has the strongest interaction with fluoxetine contributing 23-33% to the ligand-receptor energy, while other residues contributed <10%.

The closed NaᵥAb-based and open NaᵥMs-based models exhibit similar channel geometry, except for the intracellular half of the inner helices of the open state that bend to widen the pore. In both the closed and open channel models, residues in position i15 (which includes F³₁₁₅) and position i22 (which includes Y³₁₂₂) are pore-facing. Thus, a vertical and a horizontal binding mode of fluoxetine were found in both the closed and open pore. However, in terms of energy, the horizontal binding mode was favored in the closed state, because the fluoxetine experienced ligand strain in the narrower closed pore. On the other hand, the fluoxetine preferred to adopt the vertical binding mode in the open state as it formed better ligand-receptor contacts with F³₁₁₅ and Y³₁₂₂. In summary, the molecular model of fluoxetine in Naᵥ1.5 was in agreement with mutational experiments, suggesting that F1760 and Y1767 are the two key residues for its binding.

**Discussion**

In the present study, we characterized the effects of fluoxetine, a widely used antidepressant drug, on Naᵥ1.5, the cardiac voltage-gated Na⁺ channel.

Our results showed that racemic fluoxetine and its optical isomers are equally effective blockers of Naᵥ1.5 when current were recorded at a holding potential of -140 mV. Similar results have been reported for cardiac voltage-gated Ca²⁺ channels in canine
ventricular cardiomyocytes, where both fluoxetine enantiomers have similar IC$_{50}$ (Magyar et al., 2003). We also conducted dose-response curves experiments for racemic fluoxetine in HEK-293 at a holding potential of -90 mV, which is near the resting potential of cardiomyocytes. These experiments showed that the IC$_{50}$ of fluoxetine is eight time lower at a holding potential of -90 mV compared to -140 mV, going from 39.4 µM to 4.7 µM. In a manner that is hard to explain, these data are in contradiction with those published by Rajamani et al. who reported that fluoxetine does not inhibit Na$^+$ currents in HEK-293 cells expressing Na$_{v}$1.5 (Rajamani et al., 2006). However, our IC$_{50}$ of 4.7 µM is very similar with that published by Harmer et al., who reported an IC$_{50}$ of 4.9 µM using IonWorks$^{\text{TM}}$ assays from hNa$_{v}$1.5-expressing CHO cells maintained at a holding potential of -90 mV (Harmer et al., 2011). These results suggest that the holding potential of the cell is very important to the affinity of fluoxetine for the channel, as it has been also shown in rat hippocampi neurons (Lenkey et al., 2006), suggesting that the fluoxetine binds with higher affinity to inactivated than to resting channels.

In the nervous system, fluoxetine primary targets SERT, which, together with dopamine transporter (DAT) and norepinephrine transporter (NET), make up the three major MAT classes. In order to investigate the effect of other MAT-targeting drugs, we investigated the effect of nisoxetine (NET-targeting drug) (Tejani-Butt, 1992), methylphenidate (DAT-targeting drug) (Han and Gu, 2006), and fenfluramine (SERT-targeting drug) (Cosgrove et al., 2010) on Na$_{v}$1.5 currents. Our results showed that the affinity of these drugs for Na$_{v}$1.5 is dependent of the holding potential. The IC$_{50}$ of nisoxetine, methylphenidate and fenfluramine are respectively 5, 2.5 and 3 times lower at a holding potential of -90 mV compared to -140 mV. Like the fluoxetine, the decrease of
IC$_{50}$ at more depolarized potential suggests a higher affinity of these three compounds to inactivated than to resting channels. Furthermore, these compounds also exhibited a use-dependent inhibition, especially nisoxetine with a significant current reduction of 26%, 36% and 38% when pulsing at 2, 5 and 10 Hz, respectively (data not shown). However, these three compounds are still less potent than fluoxetine to inhibit Na$_v$1.5.

The present study was designed to investigate the biophysical mechanism of the Na$_v$1.5 block by fluoxetine as well as the possible pro-arrhythmic properties of this drug. A major finding of our work was that fluoxetine shifts the steady-state inactivation curve by 6.7 mV toward more hyperpolarized values, indicating that it binds to the inactivated state of Na$_v$1.5, as is the case with neuronal Na$^+$ channels (Lenkey et al., 2006). In addition to a tonic block, fluoxetine decreased Na$_v$1.5 currents in a use-dependent manner when pulsing at 2, 5 and 10 Hz. The affinity of fluoxetine for Na$_v$1.5 thus appears to be modulated by the state of the channel, which rapidly switches between the open and inactivated configurations, leading to the progressive accumulation of inactivated Na$_v$1.5. Use-dependence occurs because drug-modified channels slowly recover only at hyperpolarized voltages. Class 1 antiarrhythmic drugs and local anesthetics have a similar effect (Chahine et al., 1992). We thus determined whether fluoxetine could inhibit Na$^+$ currents by mutating residues in the class 1 antiarrhythmic drug binding site. Amino acids situated near the cytoplasmic ends of the membrane-spanning S6 $\alpha$-helixes of all four homologous domains (D1S6-D4S6) form the cytoplasmic entrance of the pore and contribute to the binding sites of both the native inactivation gate and class 1 antiarrhythmic drugs. We previously reported that two highly conserved residues of the D4S6 segment (F1760, Y1767) contribute directly to the local anesthetic binding site of
cardiac Na\(^+\) channels (O’Leary and Chahine, 2002). We showed that both mutations (F1760C and Y1767C) markedly reduced the frequency-dependent effect, with the Y1767C mutation having the greatest effect. However, in tonic block, the F1760C increased the IC\(_{50}\) of fluoxetine more significantly than the Y1767C. These results showed that these residues of D4S6 are an integral part of the binding site of fluoxetine, as is the case with many class 1 antiarrhythmic drugs. Our data also suggest that F1760 appears to be more involved in binding fluoxetine when the channel is in the resting state, while Y1767 appears to be key for fluoxetine binding when the channel is in the open/inactivated state.

Molecular modeling fluoxetine in Na\(_v\),1.5 was in agreement with mutational experiments, in which F\(^{4i15(1760)}\) and Y\(^{4i22(1767)}\) were found to be the key residues in binding fluoxetine. However, the models predicted that the ligand is able to assume two energetically favorable binding modes. The vertical binding mode was favored in the open state model, while the horizontal mode in the closed state model. This could suggest that open channel block involves both F\(^{4i15(1760)}\) and Y\(^{4i22(1767)}\) as visualized in the vertical binding mode. With the same assumption, the horizontal binding mode could represent resting channel block with F\(^{4i15(1760)}\) as the essential residue. Fluoxetine share similarities to local anesthetics. Both are drugs sensitive to mutations at F\(^{4i15(1760)}\) and Y\(^{4i22(1767)}\). Structurally, fluoxetine resembles most classical local anesthetics in approximate size and by possessing an ammonium group and a benzene ring. Fluoxetine adopts similar binding modes in the closed channel homology model as QX-314, cocaine, and tetracaine (Bruhova \textit{et al.}, 2008; Tikhonov and Zhorov, 2012). Since fluoxetine can protrude between the III-IV inner helix interface while in the horizontal binding mode, it could
suggest that fluoxetine may enter or exit through the III-IV domain interface pathway from the extracellular side of the membrane as it has been demonstrated with local anesthetics (Qu et al., 1995; Sunami et al., 2001). Experiments with fluoxetine with a quaternarized ammonium could reveal whether the ligand can block from the extracellular side.

The blockade of the Na\(_{\,1.5}\) by fluoxetine should be taken into consideration when prescribing this drug. Blocking the cardiac Na\(^+\) channel may cause an intracardiac conduction delay, which may in turn cause a prolongation of the QRS complex on the electrocardiogram (Delk et al., 2007). Given the association between QRS prolongation and mortality, and the potential for drug-induced arrhythmia, caution is required when prescribing fluoxetine (Thanacoody and Thomas, 2005; Delk et al., 2007), especially given that inhibiting the Na\(_{\,1.5}\) by as little as 10% may cause a prolongation of the QRS complex in humans (Cordes et al., 2009). However, a question remains as to how to transpose the significance of the IC\(_{50}\) value of fluoxetine to a pathophysiological setting. The IC\(_{50}/fC_{\text{max}}\) ratio, where fC\(_{\text{max}}\) represents the unbound (free) plasma concentration in a clinical setting, of a drug that evokes a QRS or a change in QT has been proposed as a tool for determining whether a drug can be safely prescribed (Redfern et al., 2003; Harmer et al., 2011). An IC\(_{50}/fC_{\text{max}}\) ratio above 30 to 100 has been shown to ensure a suitable degree of safety in terms of drug-induced QRS complex prolongation. The fC\(_{\text{max}}\) for fluoxetine is 93 nM (Harmer et al., 2011). Thereby, when we mimic the membrane potential of cardiomyocytes in patch-clamp by imposing a holding potential of -90 mV to HEK-293 cells, the IC\(_{50}/fC_{\text{max}}\) ratio is 50. This is within the 30 to 100 margin and it should act as a safety flag for a possible cardiotoxicity.
Furthermore, in the case of fluoxetine, fC\textsubscript{max} may not be a good indicator of actual plasma concentrations of total Na\textsubscript{v}1.5 blockers in vivo since norfluoxetine, an active metabolite of fluoxetine, has a higher affinity for Na\textsubscript{v}1.5 than fluoxetine itself. Given that norfluoxetine has a half-life of more than a week compared to 70 h for a single dose of fluoxetine (Schepens, 1996), there is a possibility of a long-lasting additive effect on cardiac Na\textsuperscript{+} channels. In fact, in the calculation of the IC\textsubscript{50}/fC\textsubscript{max} ratio, we should take into consideration the unbound (free) plasma concentration of norfluoxetine. Despite the lack of information about the fC\textsubscript{max} after a single dose, it is known that the plasma concentration of total (unbound and bound) fluoxetine and norfluoxetine at steady state are very similar after chronic treatment (91 to 302 ng/ml and 72 to 258 ng/ml, respectively) (U.S. Food and Drug Administration). These suggest that the IC\textsubscript{50}/fC\textsubscript{max} ratio following fluoxetine treatment is probably underestimated.

In conclusion, caution should be taken when prescribing fluoxetine at the same time as other Na\textsuperscript{+} channel inhibitors such as class 1 anti-arrhythmic drugs, especially class 1A and 1C drugs. In addition, fluoxetine should be prescribed with extreme care for patients suffering from ventricular conduction disorders or liver disease. Indeed, as the liver is the primary site of fluoxetine metabolism, its impairment, such as cirrhosis, affects the elimination half-life of fluoxetine and norfluoxetine (Schenker \textit{et al.}, 1988).
Authorship Contributions:

Conducted experiments: Poulin, Chahine, Beaulieu

Participated in research design: Poulin, Chahine, Beaulieu, Timour, Theriault

Wrote and contributed to the writing of the manuscript: Poulin, Chahine, Beaulieu, Timour, Frassati
References


Footnotes

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Figure legends:

Figure 1

Tonic block of Na\textsubscript{v}1.5/WT currents. (A) Superimposed $I_{Na}$ recordings obtained before and after perfusion with two concentrations of racemic fluoxetine from a holding potential of -140 mV. The dashed line represents zero current. (B) Dose-response curves of the inhibitory effect of norfluoxetine and different optical isomers of fluoxetine on Na\textsubscript{v}1.5/WT currents. HEK-293 cells stably expressing Na\textsubscript{v}1.5/WT were perfused with different concentrations of norfluoxetine, racemic fluoxetine, S(+) fluoxetine or R(−) fluoxetine. There was no significant difference between the IC\textsubscript{50} of racemic fluoxetine (IC\textsubscript{50} = 39.4 ± 2.0 μM, n = 3-7) and its two optical isomers (IC\textsubscript{50} = 40.0 ± 2.6 μM, n = 6-14 and 46.7 ± 3.1 μM, n = 3-10). However, norfluoxetine had a significant lower IC\textsubscript{50} (29.5 ± 1.0 μM, n = 8-15). The IC\textsubscript{50} of fluoxetine was significantly reduced to 4.7 ± 0.5 μM (n = 7-10) when recorded at a holding potential of -90 mV (B, open triangle). (C) Dose-response curves of the inhibitory effect of nisoxetine (n = 3-11), methylphenidate (n = 4-9), and fenfluramine (n = 4-6) on Na\textsubscript{v}1.5/WT currents recorded at a holding potential of -140 or -90 mV. The IC\textsubscript{50} of the three drugs at a holding potential of -90 mV were significantly lower than those recorded at -140 mV. Insets in B and C show the IC\textsubscript{50} for each compound. The values were fitted to a Hill equation. Currents were elicited from a holding potential of -140 mV or -90 mV, and a-30 mV test pulse lasting 50 ms was delivered every 5 s. ***$P < 0.001$
Figure 2

Gating properties of Na\textsubscript{v}1.5/WT treated with fluoxetine. (A) Voltage-dependence of steady-state activation and inactivation of Na\textsubscript{v}1.5. Cells were perfused with Ringer’s solution as a control (activation, n = 14; inactivation, n = 19) or with 30 µM racemic fluoxetine (activation, n = 18; inactivation, n = 17). Activation curves were elicited with 50 ms depolarizing steps from –100 to 80 mV in 10 mV increments. Cells were held at a holding potential of –140 mV. Fluoxetine caused no significant shift in the activation curve. Steady-state inactivation was determined using 4 ms test pulses to –30 mV after a 500 ms prepulse to potentials ranging from –140 mV to 0 mV (see the inset under the inactivation curves for the protocol). The application of 30 µM fluoxetine induced a significant –6.7 mV shift of the inactivation curve (***, P < 0.001) (Table 1). The activation and inactivation curves were fitted to a single Boltzman function (see Materials and Methods). (B) Recovery from inactivation of Na\textsubscript{v}1.5 in the absence (n = 10) or presence (n = 9) of 30 µM fluoxetine. The cells were depolarized to –30 mV for 40 ms from a holding potential of –140 mV to inactivate all the Na\textsuperscript{+} channels. Test pulses were then applied to –30 mV for 20 ms to measure current amplitudes, with an interval ranging from 0.1 to 4000 ms. The resulting curves were fitted to a double (control) or a triple (+ fluoxetine) exponential equation, which yielded two or three time constants (τ\textsubscript{1}, τ\textsubscript{2}, τ\textsubscript{3}). The application of 30 µM fluoxetine strongly slowed the recovery from inactivation with the appearance of a third time constant (Table 1).
Figure 3

Frequency-dependent inhibition. (A) Representative whole-cell traces recorded from Na\textsubscript{v}1.5/WT (+/- 30 µM fluoxetine), Na\textsubscript{v}1.5/F1760C (+ 30 µM fluoxetine), and Na\textsubscript{v}1.5/Y1767C (+ 30 µM fluoxetine) when pulsing at 10 Hz. The dashed line represents zero current. (B) Use-dependent blocks of Na\textsubscript{v}1.5/WT, Na\textsubscript{v}1.5/F1760C, and Na\textsubscript{v}1.5/Y1767C currents in the presence of 30 µM fluoxetine. A 50 pulse train was applied at −30 mV for 10 ms from a holding potential of −140 mV when pulsing at 2 Hz, 5 Hz, and 10 Hz. Peak currents were measured, normalized to the peak amplitude at P\textsubscript{1}, and plotted against the corresponding pulses. (C) Relative currents amplitudes (P\textsubscript{50}/P\textsubscript{1}) of the 50th sweep recorded from Na\textsubscript{v}1.5/WT, Na\textsubscript{v}1.5/F1760C and Na\textsubscript{v}1.5/Y1767C. After the fluoxetine treatment, Na\textsubscript{v}1.5/WT (n = 8) currents were significantly reduced by 44%, 58%, and 67% compared to the control when pulsing at 2, 5, and 10 Hz, respectively (\(**\) *P* < 0.001). Fluoxetine significantly reduced Na\textsubscript{v}1.5/F1760C (n = 11) currents by 15% and 20% when pulsing at 5 and 10 Hz (\(###\) *P* < 0.001), respectively, and Na\textsubscript{v}1.5/Y1767C (n = 6) currents by 5% when pulsing at 10 Hz (\(\varphi\varphi\) *P* < 0.01) compared with control. There was no significant use-dependent inhibition of Na\textsubscript{v}1.5/WT (n = 7), Na\textsubscript{v}1.5/F1760C (n = 5), or Na\textsubscript{v}1.5/Y1767C (n = 6) currents before the fluoxetine treatment. The controls curves of Na\textsubscript{v}1.5/WT, Na\textsubscript{v}1.5/F1760C and Na\textsubscript{v}1.5/Y1767C without fluoxetine treatment were removed from the graphic B and C for clarity.

Figure 4

Tonic block of Na\textsubscript{v}1.5/F1760C and Na\textsubscript{v}1.5/Y1767C by fluoxetine. (A) Representative whole-cell traces recorded from Na\textsubscript{v}1.5/WT (left), Na\textsubscript{v}1.5/F1760C (middle), and
Nav1.5/Y1767C (right) channels before and after the application of 50 μM fluoxetine. The dashed line represents zero current. (B) Dose-response curves of the inhibitory effect of racemic fluoxetine on Nav1.5/WT, Nav1.5/F1760C, and Nav1.5/Y1767C. The IC\textsubscript{50} values of Nav1.5/F1760C (83 μM) and Nav1.5/Y1767C (50 μM) were significantly higher than Nav1.5/WT value (39 μM), **P < 0.01 and ***P < 0.001. The different concentrations of drugs were applied using a perfusion system. Currents were elicited from a holding potential of –140 mV with a 50 ms test pulse at –30 mV delivered every 5 s. Insets in B shows the IC\textsubscript{50} for each compound. Normalized current (I\textsubscript{Na}) values were fitted to a Hill equation.

**Figure 5**
Open-channel block of Nav1.5 by fluoxetine. (A) Superimposed I\textsubscript{Na} recordings obtained following the application of different concentrations of fluoxetine on Nav1.5/L409C/A410W expressing cells. The dashed line represents zero current. (B) Dose-response curves of the inhibitory effect of fluoxetine on Nav1.5/L409C/A410W at the peak current (blue circle) and 90-100 ms after the beginning of the pulse (green square). The IC\textsubscript{50} value at the end of the pulse (3.5 μM) was significantly lower than the IC\textsubscript{50} value at the peak current (9.6 μM) (**P < 0.001). Currents were elicited from a holding potential of –140 mV with a 50 ms test pulse at 0 mV delivered every 5 s. Normalized current (I\textsubscript{Na}) values were fitted to a Hill equation. Dotted grey boxes represent the peak current (left boxe) and the 90-100 ms (right boxe) areas used to construct the dose-response curves.
Figure 6

Searching for the binding site of fluoxetine in the closed and open Na\(_v\)1.5. The P-loops and S6 helices of domains I, II, III, and IV are colored blue, orange, green, and violet, respectively. The outer helices and the L45 linker are shown as gray strands. The side chains of residues in the DEKA locus, Q\(^{1p49(372)}\), S\(^{4p49(1712)}\), F\(^{4i15(1760)}\) and Y\(^{4i22(1767)}\) are shown as sticks with yellow carbons. The water molecule at the DEKA locus is rod-shaped. (A and B) The side and extracellular views of the randomly generated starting points of fluoxetine in the closed Na\(_v\)1.5. Fluoxetine is presented in wire-frame with gray carbons. For clarity, only 6000 of the 60,000 starting points are shown. (C-F) The side views of the lowest energy vertical (C and E) and horizontal (D and F) binding modes of fluoxetine in the closed (C and D) and open (E and F) channel. Fluoxetine is shown in thick sticks with gray carbons. The side chain of F\(^{3p49(1236)}\) is shown in D and F. For clarity the outer helices are not shown in C–F (see PDB file in Data Supplement).
## Table 1. Biophysical properties of Nav1.5 channels

<table>
<thead>
<tr>
<th></th>
<th>Na&lt;sub&gt;1.5&lt;/sub&gt;/WT Control</th>
<th>Na&lt;sub&gt;1.5&lt;/sub&gt;/WT Fluoxetine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± sem</td>
<td>n</td>
</tr>
<tr>
<td><strong>Steady-state activation</strong></td>
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<td></td>
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<tr>
<td>$V_{1/2}$, mV</td>
<td>- 43.49 ± 1.46</td>
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</tr>
<tr>
<td>$k_V$</td>
<td>- 6.13 ± 0.32</td>
<td>14</td>
</tr>
<tr>
<td><strong>Steady-state inactivation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{1/2}$, mV</td>
<td>-87.34 ± 0.94</td>
<td>19</td>
</tr>
<tr>
<td>$k_V$</td>
<td>6.37 ± 0.19</td>
<td>19</td>
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<tr>
<td><strong>Recovery from inactivation</strong></td>
<td></td>
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<tr>
<td>$\tau_1$</td>
<td>1.50 ± 0.1</td>
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<tr>
<td>$A_1$</td>
<td>76.3 ± 2.7</td>
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</tr>
<tr>
<td>$\tau_2$</td>
<td>9.13 ± 1.0</td>
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<tr>
<td>$A_2$</td>
<td>23.7 ± 0.8</td>
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</tr>
<tr>
<td>$\tau_3$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$A_3$</td>
<td>–</td>
<td>–</td>
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</tbody>
</table>

$V_{1/2}$, midpoint for activation or inactivation; $k_V$, slope factor for activation or inactivation; $\tau$, time constant; $A$, fraction of the $\tau$ components (%); $n$, number of cells. Values are means ± sem, **$P < 0.01$, ***$P < 0.001$
Figure 1

A

Na\textsubscript{v}1.5/WT

Control
25 \mu M racemic Fluoxetine
100 \mu M racemic Fluoxetine
Wash-out

2000 pA
1 ms

B

Fluoxetine (\mu M)

Block of sodium current (%)

0
20
40
60
80
100

1
10
100
1000
10000

Drug concentration (\mu M)

Block of sodium current (%)

0
20
40
60
80
100

Figure 1

Control
25 \mu M racemic Fluoxetine
100 \mu M racemic Fluoxetine
Wash-out

IC\textsubscript{50} (\mu M)

S (+) Fluoxetine 40.0 ± 2.6
R (-) Fluoxetine 46.7 ± 3.1
racemic Fluoxetine 39.4 ± 2.0
Norfluoxetine 29.5 ± 1.0
racemic Fluoxetine (-90 mV) 4.7 ± 0.5

∗∗∗
∗∗
∗∗

IC\textsubscript{50} (\mu M)

Nisoxetine 104.5 ± 5.5
Fenfluramine 203.5 ± 5.5
Methylphenidate 618.7 ± 99.7
Nisoxetine (-90 mV) 20.2 ± 1.5
Fenfluramine (-90 mV) 65.5 ± 5.2
Methylphenidate (-90 mV) 239.5 ± 17.5

∗∗∗
∗∗
∗∗
∗∗∗
Figure 2

A

B
Figure 3

A) Graph showing normalized currents for different mutants of Na,1.5/WT, Na,1.5/F1760C, and Na,1.5/Y1767C with and without Fluoxetine.

B) Graph showing normalized currents over 50 pulses at different frequencies (2 Hz, 5 Hz, 10 Hz) for Na,1.5/WT, Na,1.5/F1760C, and Na,1.5/Y1767C with and without Fluoxetine.

C) Graph showing normalized currents as a function of frequency (Hz) for Na,1.5/WT + Fluoxetine, Na,1.5/F1760C + Fluoxetine, Na,1.5/Y1767C + Fluoxetine.
Figure 4

A

Na\textsubscript{v}1.5/WT

Na\textsubscript{v}1.5/F1760C

Na\textsubscript{v}1.5/Y1767C

Control
50 µM Fluoxetine

B

Block of sodium current (%)

0 20 40 60 80 100

Fluoxetine (µM)

1 10 100 1000 10000

IC\textsubscript{50} (µM)

Na\textsubscript{v}1.5/F1760C 82.8 ± 3.2

Na\textsubscript{v}1.5/Y1767C 50.1 ± 1.8

Na\textsubscript{v}1.5/WT 39.4 ± 2.0

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Figure 5

A

Na_{1.5}/L409C-A410W

B

Block of sodium channel (%)

Fluoxetine (µM)

Control
1 µM
5 µM
10 µM
25 µM
50 µM
75 µM
100 µM

0.1 1 10 100 1000 µM

1000 pA

10 ms

IC_{50}(µM)

Block at 90-100 ms

3.4 ± 0.2

Block at peak current

9.6 ± 0.5

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