Fast-Scan Cyclic Voltammetry Analysis of Dynamic Serotonin Reponses to Acute Escitalopram

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ABSTRACT: The treatment of depression with selective serotonin reuptake inhibitors, SSRIs, is important to study on a neurochemical level because of the therapeutic variability experienced by many depressed patients. We employed the rapid temporal capabilities of fast scan cyclic voltammetry at carbon fiber microelectrodes to study the effects of a popular SSRI, escitalopram (ESCIT), marketed as Lexapro, on serotonin in mice. We report novel, dynamic serotonin behavior after acute ESCIT doses, characterized by a rapid increase in stimulated serotonin release and a gradual rise in serotonin clearance over 120 min. Dynamic changes after acute SSRI doses may be clinically relevant to the pathology of increased depression or suicidality after onset of antidepressant treatment. Due to the short-term variability of serotonin responses after acute ESCIT, we outline difficulties in creating dose response curves and we suggest effective means to visualize dynamic serotonin changes after SSRIs. Correlating chemical serotonin patterns to clinical findings will allow a finer understanding of SSRI mechanisms, ultimately providing a platform for reducing therapeutic variability.

KEYWORDS: 5-HT, FSCV, carbon fiber microelectrode, SSRI, mice
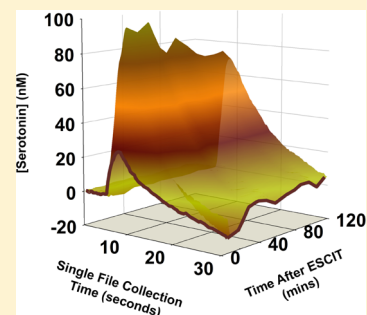
Depression is a highly prevalent neuropsychiatric disorder characterized by low mood or self-esteem. Fatigue, migraines, anxiety, and changes in weight are among the many additional symptoms of depression.[1] More than 9% of adult Americans are diagnosed with this disorder,[2] and in 2011 over $11 billion was spent on pharmacological treatments.[3] Selective serotonin-reuptake inhibitors (SSRIs) are the most frequently prescribed antidepressants and their usage appears to be rising.[4] SSRIs carry side effects,[5] and while their acute pharmacodynamic effects are rapid, several weeks of chronic administration may be required before clinical effectiveness can be achieved.[6] During the initial dosing period, highly vulnerable patients are encouraged to seek psychological therapy to counteract increased suicide risks.[7,8] Therefore, it is valuable to understand serotonin neurochemistry in experimental models in vivo after acute and chronic SSRI doses to elucidate the underlying mechanisms of SSRI action.

In this Letter, as a first step toward this goal, we seek to understand serotonin responses to escitalopram (ESCIT), one of the most popular SSRIs commercially known as Lexapro. While responses to ESCIT have previously been studied behaviorally[9] and neurochemically with microdialysis,[10] we are interested in characterizing the rapid serotonin responses afforded by fast-scan cyclic voltammetry (FSCV). FSCV at carbon fiber microelectrodes dynamically measures serotonin in the rat substantia nigra, pars reticulata (SNr) upon electrical stimulation of the dorsal raphe nucleus (DRN) or the medial forebrain bundle (MFB).[11,12] Here we find the same to be true in mice. Additionally, we find that an acute dose of ESCIT dramatically alters both stimulated serotonin release and reuptake. This effect is both dose and time dependent in the short term (0−120 min). Interestingly, we find that there is a complex relationship between serotonin amplitude and clearance time after drug administration (amplitude and clearance vary differently and independently with time for each dose). We discuss these important findings in the context of the short-term effects of SSRIs and describe difficulties in constructing dose response curves.

RESULTS AND DISCUSSION

SSRIs inhibit serotonin reuptake via the serotonin transporter (SERT). Researchers have traditionally studied the neurochemical mechanisms of acute SSRI uptake inhibition using microdialysis. Many studies show that acute SSRIs, independent of administration mode, augment basal serotonin levels.[13−16] In the brain region studied here, the SNr, citalopram (CIT) was reported to increase serotonin levels by 660%.[17] Because SSRIs prolong serotonin's lifetime in the extracellular space,[18] an increase in microdialysate serotonin supports a straightforward mechanism of uptake inhibition. However, because traditional microdialysis cannot distinguish dynamic events, a more rapid chemical analysis may increase our understanding of in vivo SSRI mechanisms.

FSCV can differentiate between changes in serotonin release and uptake. FSCV signals typically constitute an initial rapid amplitude increase due to electrical stimulation, which reaches a maximum at the end of the stimulation. At the cessation of stimulation, the signal is dominated by a decay curve, the $t_{1/2}$ of which is an index of serotonin clearance. As such, we will refer to the components of this signal as amplitude and $t_{1/2}$. We

explored the effect of an acute dose of ESCIT (10 mg kg^{-1}) on serotonin release and uptake in SNr in mice in vivo. Figure 1A
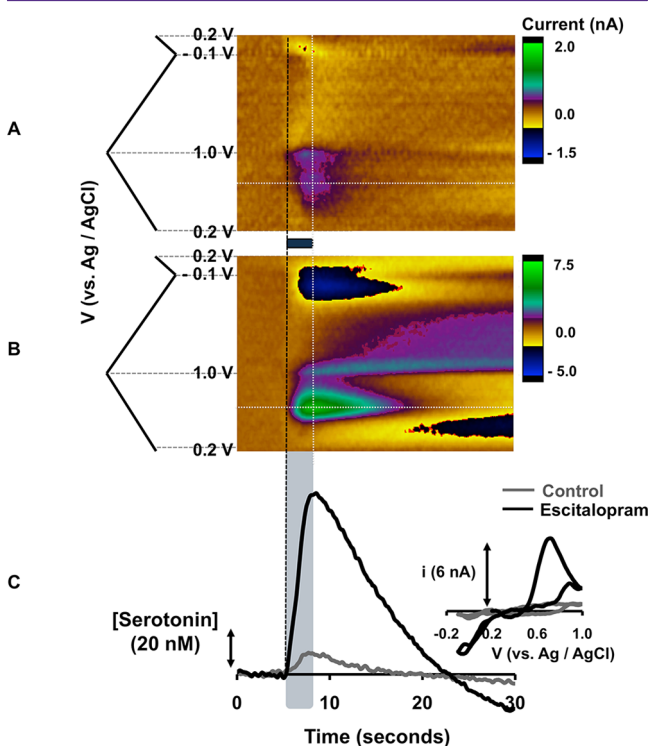

Figure 1. (A,B) Color plots with potential on the *y*-axis plotted against time on the *x*-axis and the current response represented in false color. These plots represent (A) the signal obtained in the SNr of an anesthetized mouse upon MFB stimulation (black bar under the color plot denotes the stimulation time and duration) (B) The same signal 1 h after ESCIT (10 mg kg^{-1}) administration. (C) [Serotonin] vs time extrapolated from the horizontal dashed lines in (A) and (B) with inset cyclic voltammograms taken from the vertical white dashed lines.

displays a typical color plot for serotonin release in the SNr evoked by electrical stimulation of the MFB (the stimulation is denoted by the horizontal blue bar between and the vertical gray box underlying the color plots). The amplitude (13.7 nM) and $t_{1/2}$ (3.0 s) agree well with values reported for rats in vivo.[19] Figure 1B shows the same response 60 min after ESCIT (10 mg kg^{-1}) administration. The $t_{1/2}$ of the signal increased to 5.8 s, and was accompanied by a 10-fold increase in the amplitude (132 nM) of the signal. Figure 1C is a superposition of [serotonin] versus time taken at the voltage indicated by the horizontal white dashed lines in Figure 1A and B. The inset in Figure 1C displays the cyclic voltammograms obtained at the vertical white dashed lines from both color plots. These cyclic voltammograms identify serotonin based on the positions of the oxidation and reduction peaks.[11]

We chose to study ESCIT because it is one of the most clinically useful SSRIs.[3,20] However, other SSRIs such as fluoxetine and sertraline exhibit similar modes of action (selective SERT inhibition), and we have observed similar increases in serotonin amplitude in vivo with these two agents (unpublished observations).

A comparable potentiation of serotonin amplitude has previously been observed in vivo in anesthetized rats with CIT.[11,19] Specifically, serotonin was found to increase by 380%[11] and 480%.[19] Current and previous findings in the intact brain of rats and mice are not in agreement with the data from

tissue slice preparations. For example, Bunin et al. reported on the effects of fluoxetine on serotonin in slice preparations in the rat SNr.[21,22] A modest increase in serotonin release (~10%) was attributed to delayed clearance (increased $t_{1/2}$). John and Jones reported similar findings in mouse SNr slices.[23] It is not likely that the affinity of SERT for serotonin is different between in vivo and slice preparations. Therefore, the increase in stimulated serotonin release in response to ESCIT administration, of up to 700% of pretreatment values, appears to be specific to intact brain studies. These findings suggest that synaptic processes in intact brains additionally modulate SSRI mechanisms.

Figure 1 displays the serotonin response 60 min after ESCIT administration. We initially chose this time based on the pharmacodynamics of ESCIT (maximum transporter occupancy at 60 min[33]). However, if measurements are taken at 5–10 min intervals from 0 to 120 min, a more complete profile emerges. Figure 2A displays color plots taken at different intervals after ESCIT administration (10 mg kg^{-1}) in a representative experiment. Figure 2B shows averaged [serotonin] versus time traces taken at the voltage indicated by the horizontal white dashed lines in each color plot in Figure 2A. The stimulation is denoted by the blue bar directly below the traces. Averaged serotonin release before drug administration (t = 0) was 28.0 ± 6.4 nM, and $t_{1/2}$ was 3.7 ± 0.4 s. Ten minutes after drug administration, there was a significant increase in serotonin amplitude (98.1 ± 19.7 nM, $p < 0.001$) and a significant increase in $t_{1/2}$ (5.7 ± 1.2 s, $p < 0.001$). Over the short-term, the $t_{1/2}$ further significantly increased from the 10 min value, reaching 7.1 ± 1.1 s ($p < 0.001$) at 120 min. A significant decrease in amplitude occurred from 10 to 120 min (to 71.5 ± 14.2 nM, $p = 0.011$). These changes in serotonin amplitude and $t_{1/2}$ over time are displayed in the histograms (inset Figure 2B).

The underlying cause of our primary finding (increased stimulated serotonin release after SSRIs in vivo) could be synaptic processes occurring in the intact brain that additionally modulate SSRI mechanisms. The data in Figure 2 show that these processes are rapid. Since evoked serotonin release is not expected to saturate the SERT,[34] then a rapid increase in amplitude supports independence between release and uptake. Additionally, despite the rapid pharmacodynamics of ESCIT,[33] the $t_{1/2}$ of serotonin clearance continues to increase up to 120 min after administration. If we assume that ESCIT does not change its structure or function, then it is possible that SSRIs cause dynamic physiological changes outside of their accepted mode of action.

The data we have presented in Figures 1 and 2 were after a single dose of ESCIT (10 mg kg^{-1}). To gain a better understanding of ESCIT using FSCV, it is useful to create dose response curves. There are two dynamic variables in our data that we could potentially utilize for a dose−response: serotonin amplitude and $t_{1/2}$. It is difficult to assess which of the two components of our signal is more physiologically relevant for a dose−response. Additionally, the signal is not stable with time. It would be desirable to create a standardized method for dose response curves (i.e., to take the amplitude at a particular time after ESCIT for all doses). For this to hold, it is critical that the amplitude/clearance patterns we observed for 10 mg kg^{-1} change in a similar manner for other doses. In Figure 3A−C we compare the effects of 3 doses of ESCIT (1, 10, and 100 mg kg^{-1}). We display this in 3-dimensions allowing amplitude (*y*-axis) and $t_{1/2}$ (*x*-axis) to be observed with time after ESCIT (*z*-
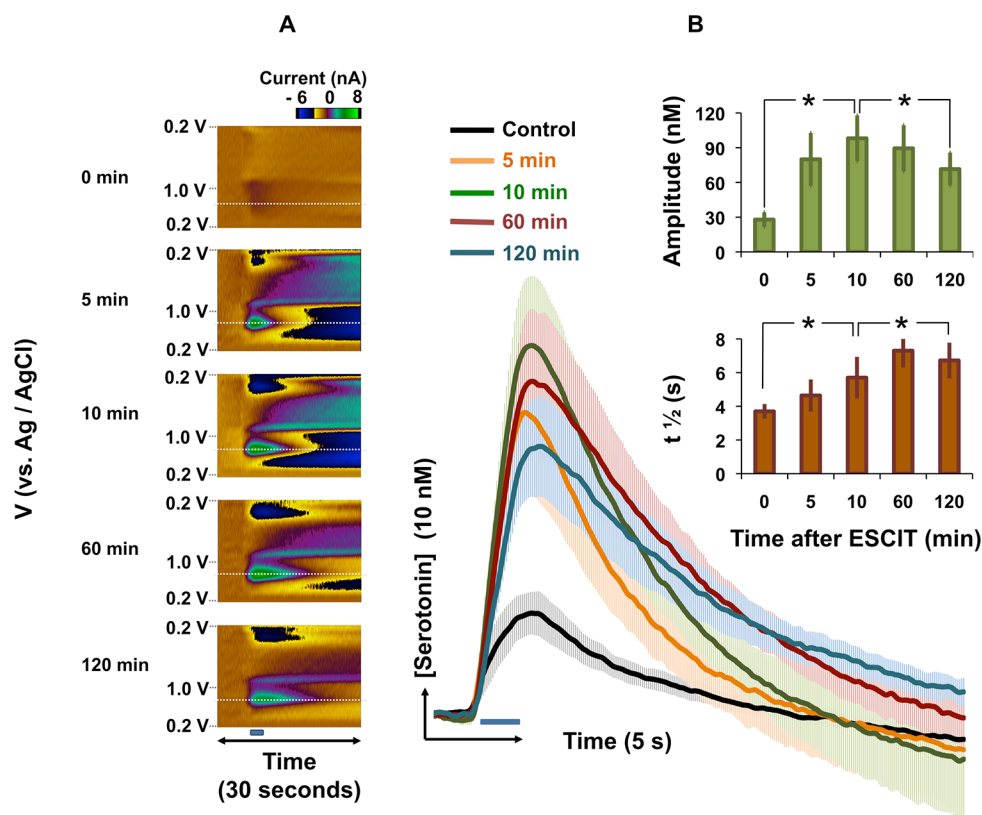
Figure 2. (A) Color plots with potential on the y-axis plotted against time on the x-axis and the current response represented in false color. These plots represent the time course of the serotonin signal after ESCIT administration in a representative animal. (B) [Serotonin] vs time, averaged for five animals ($\pm$SEM) taken from the maximum current value from the horizontal white dashed lines from each time course. The blue bar denotes stimulation onset and duration. Inset: Histograms showing maximum serotonin amplitude (green) and $t_{1/2}$ (orange) with time after ESCIT administration. Stars represent statistically different significance of comparisons between data sets ($p = 0.05$ is used as a two-tailed cutoff for statistical significance).

axis) (measurements were taken at 5−10 min intervals after ESCIT administration). The control (no-drug) response is highlighted in dark brown. The amplitude and clearance profiles varied across doses. The maximum amplitude and $t_{1/2}$ at each time point are plotted separately, as a percentage from baseline, for ease of interpretation (Figure 3D and E).

For $t_{1/2}$, there was a clear response order with progressive doses. The maximum $t_{1/2}$ was 202 ± 32.1% for 1 mg kg^{-1}, 211 ± 14.6% for 10 mg kg^{-1}, and 478 ± 205% for 100 mg. Over 120 min, all three doses displayed a continued $t_{1/2}$ increase, but the time to reach a significant increase again also followed the dose order. Significance was achieved after 20 min for 1 mg kg^{-1} (147 ± 8.60% ($p = 0.025$)), 7 min for 10 mg kg^{-1} (131 ± 16.0% ($p = 0.02$)), and 5 min for 100 mg kg^{-1} (277 ± 101% ($p = 0.026$)).

The dose response order for $t_{1/2}$ did not hold for amplitude. Here, the highest response was elicited by the middle dose, a phenomenon previously observed with DAT inhibition.[35] For 1 mg kg^{-1}, the maximum amplitude was 165 ± 19.7%, for 10 mg kg^{-1} this was 441 ± 145%, and for 100 mg kg^{-1} this was 257 ± 26.0%. These maxima were reached at 80, 30, and 7 min, respectively. The time taken to reach significantly increased amplitude was 20 min for 1 mg kg^{-1} and 5 min for both 10 and 100 mg kg^{-1} (all $p < 0.001$). For all three doses, there was a significant decrease at 120 min compared to the maximum amplitude ($p < 0.01$ for 1 mg kg^{-1}, $p = 0.01$ for 10 mg kg^{-1}, and $p = 0.007$ for 100 mg kg^{-1}).

We are unable to find a consistent relationship in the serotonin profile after ESCIT with different doses. While this is physiologically interesting, it creates difficulties in reporting standard dose responses. This challenge highlights a necessity for multivariate modeling of the data to mathematically describe the serotonin profile in three dimensions with each dose. In the absence of multivariate models, dose response data is best visualized in the 3-D formats shown in Figure 3.

Understanding the clinical variability of SSRIs is further confounded by our poor understanding of their in vivo mechanisms. We studied the effects of acute doses of ESCIT on serotonin with FSCV at carbon fiber microelectrodes in mice. We showed that dynamic neurochemical changes accompanied ESCIT; notably that, over the short-term, serotonin release was greatly and rapidly augmented while its clearance only gradually increased. Finding that dynamic changes accompany acute SSRI doses are clinically interesting, since there is little clinical evidence for therapeutic relief after an acute dose. Our results underline the depth of information that can be offered by FSCV, and with relevant mathematical modeling of SSRI responses we can start to compare neurochemical profiles to clinical profiles. Ultimately, key chemical characteristics of SSRIs may be identified and correlated to specific clinical effects.

■ METHODS

Animals and Surgery. Handling and surgery on male C57BL/6J mice weighing 20−25 g (Jackson Laboratory, Bar Harbor, ME) were

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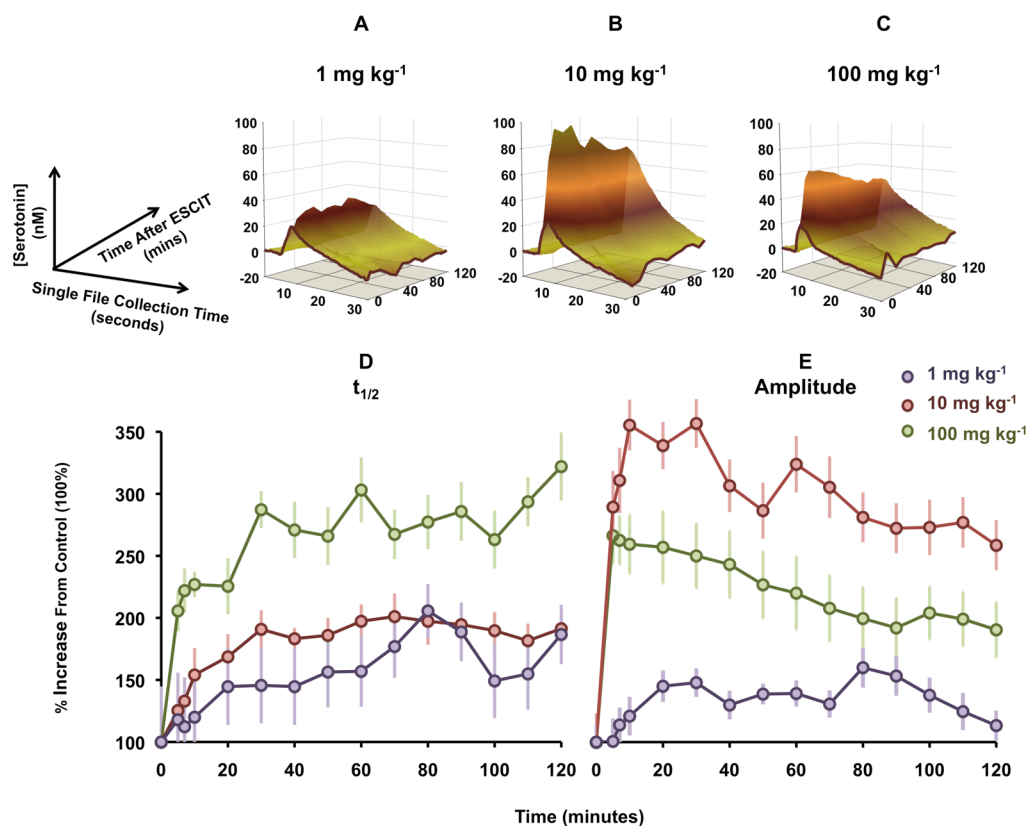dx.doi.org/10.1021/cn4000378 | ACS Chem. Neurosci. 2013, 4, 715−720

Figure 3. Dose responses to varying doses of ESCIT (A–C). The three-dimensional plots are [serotonin] vs time vs time after ESCIT administration. (D,E) Maximal values of amplitude and $t_{1/2}$, respectively, taken from (A)–(C) at each time course for each dose. Values are presented as a percent change from baseline.

in agreement with The Guide for the Care and Use of Laboratory Animals, approved by the Institutional Animal Care and Use Committees (IACUC) of Wayne State University. Food and water were offered ad libitum to the mice housed with a 12 h light/dark cycle. After urethane (25% dissolved in 0.9% NaCl solution, Hospira, Lake Forest, IL) was injected intraperitoneally, stereotaxic surgery (David Kopf Instruments, Tujunga, CA) was performed. A heating pad sustained mouse body temperature around 37 °C (Braintree Scientific, Braintree, MA). Stereotaxic coordinates were taken in reference to bregma.[36] A Nafion modified carbon fiber microelectrode was lowered into the SNr (AP: −1.58, ML: +1.40, DV: −4.2 to −4.8). The procedure for nafion electrodeposition is described in ref 11. This electrode was adjusted in the dorsal/ventral plane until a desired serotonin signal was observed. A stainless steel stimulating electrode (diameter: 0.2 mm, Plastics One, Roanoke, VA) was positioned into the MFB (AP: −3.28, ML: +1.10, DV: −5.0). Biphasic pulse trains applied through a linear constant current stimulus isolator (NL800A, Neurolog, Medical Systems Corp., Great Neck, NY) provoked serotonin efflux. The 60 Hz trains were 350 μA each phase, 2 ms in width, and 2 s in length. An Ag/AgCl reference electrode was implanted into the brain's opposite hemisphere. Chloride was electroplated onto silver wire using 0.1 M HCl (13 V vs tungsten). After the experiment, 13 V (vs AgAgCl) was applied to the electrode surface in order to lesion tissue directly surrounding the carbon fiber. This lesion aids in verifying electrode placement using histology.

FSCV Procedures. T-650 carbon fibers (diameter: 7 μm, Goodfellow, Oakdale, PA) were aspirated into glass capillaries (internal diameter: 0.4 mm, external diameter: 0.6 mm, AM Systems, Carlsborg, WA). A vertical pipet puller (Narishige Group, Tokyo, Japan) was used to taper the filled capillaries under gravity. The protruding carbon fibers were cut to approximately 150 μm, and Nafion was electrodeposited as described previously.[11] The serotonin or "5-HT waveform" was used:[37] the electrode was scanned at 1000 V s^{-1} from 0.1 to 1.0 V at 10 Hz and was held at 0.2 V. Electrodes with

this waveform have a precalibration sensitivity of 49.5 nA ± 10.2 μM^{-1} to serotonin,[11] and this value was used as a calibration factor. Post calibrations were not performed because the working electrode was utilized to create a lesion by applying 13 V to the electrode surface. Custom software, written using LabVIEW 2009 (National Instruments, Austin, TX), and hardware were used to collect data. A PCIe-6341 DAC/ADC Card (National Instruments) generated the waveform and collected the data. Potentials were measured against an Ag/AgCl reference electrode.

Data Analysis. Custom built software, written in LabVIEW 2009, was used for background subtraction, data analysis, and signaling processing including digital filtering (zero phase, Butterworth, fourth order, 5 kHz). The Butterworth filter is a maximally flat magnitude filter that applies an algorithm to remove noise with a cutoff of 5 kHz (based on serotonin waveform scan rate of 1000 V s^{-1}). Two-dimensional moving average smoothing was performed on nine points with zero-phase correction. Using all temporal data points for amplitude and $t_{1/2}$, one way repeated measures ANOVAs were conducted. Post hoc tests of the significance of linear combinations of ANOVA parameter estimates were used to determine the statistical significance of changes between different time points. This analysis was performed on Stata (StataCorp, version 12.1, College Station, TX) and $p = 0.05$ was used as a two-tailed cutoff for statistical significance. When baselines were subject to drift, a manual baseline correction was performed. Three-dimensional plots were created using SigmaPlot (Systat Software, San Jose, CA). The data in Figure 3 were analyzed using eDAQ Chart Software (Denistone East, Australia). Pooled data is presented as $n = 5 ±$ standard error of the mean (SEM).

Drugs. Escitalopram oxalate was obtained from Sigma-Aldrich (St. Louis, MO). It was dissolved in 0.9% NaCl Hospira, (Lake Forest, IL) and injected into the intraperitoneal space at a volume of 0.1 mL 20 g^{-1} body weight (based on the molecular weight of the escitalopram salt). ESCIT (10 mg kg^{-1}) was chosen as a high to intermediate dose based on previous studies in mice and rats.[10,38] The low dose, 1 mg

kg^{-1}, was chosen because doses lower than this do not typically have an effect on the FSCV signal. The high dose, 100 mg kg^{-1}, was chosen to have the same order of magnitude difference as the other two doses.

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Author Contributions
P.H. and K.M.W. designed the experiments. K.M.W. performed the experiments. P.H. and K.M.W. analyzed data and wrote the manuscript.

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