

## RESEARCH PAPER

# Blockade of the high-affinity noradrenaline transporter (NET) by the selective 5-HT reuptake inhibitor escitalopram: an *in vivo* microdialysis study in mice

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### Keywords

antidepressant; behaviour; escitalopram; intracerebral microdialysis; frontal cortex; 5-HT selective reuptake inhibitor; 5-HT transporter

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## BACKGROUND AND PURPOSE

Escitalopram, the *S*(+)-enantiomer of citalopram is the most selective 5-HT reuptake inhibitor approved. Although all 5-HT selective reuptake inhibitors (SSRIs) increase extracellular levels of 5-HT ( $[5\text{-HT}]_{\text{ext}}$ ), some also enhance, to a lesser extent, extracellular levels of noradrenaline ( $[\text{NA}]_{\text{ext}}$ ). However, the mechanisms by which SSRIs activate noradrenergic transmission in the brain remain to be determined.

## EXPERIMENTAL APPROACH

This study examined the effects of escitalopram, on both  $[5\text{-HT}]_{\text{ext}}$  and  $[\text{NA}]_{\text{ext}}$  in the frontal cortex (FCx) of freely moving wild-type (WT) and mutant mice lacking the 5-HT transporter ( $\text{SERT}^{-/-}$ ) by using intracerebral microdialysis. We explored the possibilities that escitalopram enhances  $[\text{NA}]_{\text{ext}}$ , either by a direct mechanism involving the inhibition of the low- or high-affinity noradrenaline transporters, or by an indirect mechanism promoted by  $[5\text{-HT}]_{\text{ext}}$  elevation. The forced swim test (FST) was used to investigate whether enhancing cortical  $[5\text{-HT}]_{\text{ext}}$  and/or  $[\text{NA}]_{\text{ext}}$  affected the antidepressant-like activity of escitalopram.

## KEY RESULTS

In WT mice, a single systemic administration of escitalopram produced a significant increase in cortical  $[5\text{-HT}]_{\text{ext}}$  and  $[\text{NA}]_{\text{ext}}$ . As expected, escitalopram failed to increase cortical  $[5\text{-HT}]_{\text{ext}}$  in  $\text{SERT}^{-/-}$  mice, whereas its neurochemical effects on  $[\text{NA}]_{\text{ext}}$  persisted in these mutants. In WT mice subjected to the FST, escitalopram increased swimming parameters without affecting climbing behaviour. Finally, escitalopram, at relevant concentrations, failed to inhibit cortical noradrenaline and 5-HT uptake mediated by low-affinity monoamine transporters.

## CONCLUSIONS AND IMPLICATIONS

These experiments suggest that escitalopram enhances, although moderately, cortical  $[\text{NA}]_{\text{ext}}$  *in vivo* by a direct mechanism involving the inhibition of the high-affinity noradrenaline transporter (NET).

## Abbreviations

aCSF, artificial CSF; D22, 1,1'-diethyl-2,2'-cyanine iodide; DR, dorsal raphe; FCx, frontal cortex; LC, locus coeruleus; MDD, major depressive disorder; NET, noradrenaline transporter; SERT, 5-HT transporter; SSRIs, selective 5-HT reuptake inhibitors

## Introduction

Selective 5-HT reuptake inhibitors (SSRIs) have proved to be effective in the treatment of depression. This class of antidepressant drugs exerts their therapeutic effects by inhibiting the reuptake of 5-HT, thereby prolonging its duration of action at the postsynaptic level (Frazer, 2001). Although many patients benefit from SSRIs, approximately 50% of depressed individuals do not respond adequately to these agents (Berton and Nestler, 2006). The SSRI escitalopram is the active S(+)-enantiomer of the racemic molecule citalopram. (Sanchez *et al.*, 2003a; Jacquot *et al.*, 2007). *In vitro* studies on embryonic kidney cells heterologously expressing the human monoaminergic transporters have demonstrated that the affinity of escitalopram for the 5-HT transporter (SERT) is much greater than for the noradrenaline transporter (NET) or the dopamine transporter (DAT) ( $K_i$  values: 1.1; 7841 and 27 410 nM respectively) (Owens *et al.*, 2001; transporter and receptor nomenclature follow Alexander *et al.*, 2011). These data were confirmed in functional studies from rat brain synaptosomes showing that escitalopram blocked the NET and DAT with marginal potency (Sanchez *et al.*, 2003a). Consistent with its potent inhibitory action on the SERT, *in vivo* studies have reported that an acute administration of escitalopram suppressed the firing rate of dorsal raphe (DR) 5-HT neurons in rats with an ED<sub>50</sub> of 60  $\mu\text{g}\cdot\text{kg}^{-1}$  (El Mansari *et al.*, 2005). Escitalopram was also shown to enhance extracellular 5-HT levels in the rat frontal cortex (FCx) (Mork *et al.*, 2003) and produce antidepressant/anxiolytic-like effects in various animal models (Sanchez *et al.*, 2003a,b). Interestingly, these electrophysiological, neurochemical and behavioural responses are partially inhibited by R(-)-citalopram (Mork *et al.*, 2003; Sanchez *et al.*, 2003a,b; El Mansari *et al.*, 2005). After sustained administration, escitalopram produces a faster desensitization of somatodendritic 5-HT<sub>1A</sub> autoreceptors in the DR than citalopram (El Mansari *et al.*, 2005), an effect that probably accounts for the robust increase in cortical extracellular 5-HT levels ([5-HT]<sub>ext</sub>) observed after only 2 weeks of treatment (Ceglia *et al.*, 2004). In humans, escitalopram demonstrates a rapid onset of antidepressant action, and recent data suggest that it may be more effective than other SSRIs and at least as effective as dual 5-HT/noradrenaline reuptake inhibitors in the treatment of major depression (Kennedy *et al.*, 2009; Kornstein *et al.*, 2009; Garnock-Jones and McCormack, 2010). Interestingly, SSRIs, such as paroxetine, fluoxetine and citalopram can also inhibit uptake of [<sup>3</sup>H]noradrenaline in rat cortical synaptosomes *in vitro* (Hughes and Stanford, 1996) and consequently enhance extracellular noradrenaline levels ([NA]<sub>ext</sub>) in the FCx and hippocampus after acute administration in rodents (Jordan *et al.*, 1994; Shachar *et al.*, 1997; Millan *et al.*, 2001; Beyer *et al.*, 2002; Bymaster *et al.*, 2002; Koch *et al.*, 2002; David *et al.*, 2003; Kobayashi *et al.*, 2008). Although this property seems to be a common feature of SSRIs *in vivo* in rodents, it is still unknown whether SSRIs and more particularly escitalopram enhance the level of [NA]<sub>ext</sub> by a direct mechanism involving the inhibition of the high-affinity noradrenaline transporter (NET), or by an indirect mechanism in response to increases in [5-HT]<sub>ext</sub>.

Anatomical and functional studies have demonstrated that 5-HT and noradrenaline have reciprocal interactions at both somatodendritic and nerve terminal levels. The locus coeruleus (LC), the major noradrenergic brainstem nucleus, sends projections into the DR, while the DR projects into the LC, creating ample opportunity for cross-modulation (Pudovkina *et al.*, 2002; Guiard *et al.*, 2008a). The physiological importance of such connections is demonstrated, for example, by the observation that SSRIs modulate the activity of noradrenergic neurons. Escitalopram, but also the other SSRIs, can decrease the spontaneous neuronal activity of LC noradrenergic neurons through the local activation of postsynaptic 5-HT<sub>2A/C</sub> receptors (Szabo and Blier, 2001a,b; Dremencov *et al.*, 2007; Miguez *et al.*, 2009). Since it is difficult to reconcile these electrophysiological data with the fact that SSRIs increase [NA]<sub>ext</sub> at nerve terminals, the present study was aimed to evaluate the effects of an acute administration of escitalopram on cortical extracellular levels of both 5-HT and noradrenaline by using intracerebral microdialysis in awake, freely-moving wild-type (WT) and also in knockout mice lacking the 5-HT transporter (SERT<sup>-/-</sup>).

In addition to the high-affinity NET and SERT, other categories of transporters have recently been implicated in 5-HT and noradrenaline clearance in the brain. Organic cation transporters (OCTs; Breidert *et al.*, 1998; Amphoux *et al.*, 2006; Koepsell *et al.*, 2007) and the plasma membrane monoamine transporter (PMAT; Engel *et al.*, 2004; Engel and Wang, 2005) have been shown *in vitro* to transport these monoamines. OCT2, OCT3 and PMAT, in particular, are expressed in various brain areas including the cortex (Engel *et al.*, 2004; Vialou *et al.*, 2004; 2008; Dahlin *et al.*, 2007), and a direct role in 5-HT and noradrenaline clearance *in vivo* in the mouse brain was recently demonstrated for OCT2 (Bacq *et al.*, 2011). Interestingly, some of these transporters were shown to be moderately inhibited by antidepressants *in vitro* (Kekuda *et al.*, 1998; Wu *et al.*, 2000; Haenisch and Bönisch, 2010), raising the possibility that they could be secondary targets of these drugs. Indeed, it was recently suggested that a functionally up-regulated alternative transporter for 5-HT may prevent extracellular 5-HT from attaining levels sufficiently high enough to trigger the adaptive neurochemical events necessary for the antidepressant activity of SSRIs (Baganz *et al.*, 2008). In support of this hypothesis, systemic administration of the OCT blocker decynium 22 (D22) was found to decrease 5-HT clearance and exhibit antidepressant-like activity in 5-HT transporter knockout (SERT<sup>-/-</sup>) mice (Baganz *et al.*, 2008).

The main results presented in this present study show that the acute systemic administration, or local injection of escitalopram in the FCx, significantly increased both the extracellular levels of 5-HT and noradrenaline in the FCx of WT mice. Unexpectedly, the ability of escitalopram to increase cortical noradrenaline levels remained effective in SERT<sup>-/-</sup> mice, but not in SERT<sup>+/+</sup> mice administered with desipramine. In addition, escitalopram, at physiological concentrations, failed to inhibit cortical noradrenaline and 5-HT *ex vivo* uptake by the low-affinity monoamine transporters. These data suggest that this SSRI, previously considered highly selective for the SERT, may also non-selectively block the NET, when administered at high doses.

## Methods

### Animals

All animal care and experimental procedures were conducted in conformity with the institutional guidelines in compliance with national policy (Council directive #87–848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions #005037 to AM Gardier). Male WT and SERT<sup>-/-</sup> mice, 4–6 months old, weighing 25–35 g, were used in this study. Mutant mice originally produced by homologous recombination (Bengel *et al.*, 1998) were housed in our animal care facility in groups of three to six and kept under standard conditions (room temperature of 22–23°C, 12:12 light–dark cycle, free access to food and water). Mice were tested between 9:00 a.m. and 5:00 p.m. during the light phase.

### Drugs and administration

Escitalopram oxalate (H. Lundbeck, Paramus, NJ, USA) was administered by the i.p. route at a dose of 4, 8 or 16 mg·kg<sup>-1</sup>. In reverse microdialysis experiments, escitalopram (0.5 μM) or desipramine (50 or 100 μM) was dissolved in artificial CSF (aCSF; for composition see below) and then perfused locally into the FCx. The concentration of escitalopram was chosen on the basis of previous microdialysis experiments showing that the perfusion of 1 μM of the racemic compound (i.e. citalopram) into the FCx produced a significant increase in local extracellular 5-HT levels in mice (Guilloux *et al.*, 2006). The concentration of desipramine was selected on the basis of its previously reported enhancing effect on extracellular noradrenaline levels in mice (Fisher *et al.*, 2007).

### Microdialysis procedure

Mice anaesthetized with chloral hydrate (400 mg·kg<sup>-1</sup>, i.p.) were implanted with probes (CMA7 model, Carnegie Medicin, Stockholm, Sweden) located in the FCx (stereotaxic coordinates in mm from bregma) (Hof *et al.*, 2000): A = +1.6, L = +1.3, V = -1.6; A, anterior; L, lateral; and V, ventral (Guilloux *et al.*, 2006). Animals were allowed to recover from the surgery overnight. The next day, ~20 h after surgery, the probes were continuously perfused with aCSF (composition in mM: NaCl 147, KCl 3.5, CaCl<sub>2</sub> 1.26, MgCl<sub>2</sub> 1.2, NaH<sub>2</sub>PO<sub>4</sub> 1.0, pH 7.4 ± 0.2) at a flow rate of 1.0 μL·min<sup>-1</sup> in the cortex using a CMA/100 pump (Carnegie Medicin). The animals were awake and freely moving in their cage during the perfusion procedures. The noradrenaline and 5-HT microdialysis samples were collected from independent animals. One hour after the start of aCSF perfusion stabilization period, four fractions were collected (one every 20 min) to measure the basal monoamine values (mean ± SEM corresponding to B<sub>0</sub> at t<sub>0</sub>) calculated for each mouse before systemic administration or local perfusion of vehicle, escitalopram or desipramine. Subsequent dialysate samples were then collected for a 0–120 or 0–240 min post-treatment period and analysed for 5-HT and noradrenaline by a HPLC system (XL-ODS, 4.6 × 75 mm, particle size 3 μm; Beckman, Fullerton, CA, USA) coupled to an amperometric detector (1049 A, Hewlett-Packard, Les Ulis, France). The mobile phase for 5-HT and noradrenaline contained 107 or 100 mM NaH<sub>2</sub>PO<sub>4</sub>, 140 or 151 μM disodium EDTA; 0.77 or 3 mM I-octanesulphonic acid, respectively, and

20% (v/v) methanol (pH adjusted between 4.1 and 4.3 with phosphoric acid). Its flow rate through the HPLC column was set at 0.7 mL·min<sup>-1</sup> using a 118 pump (Beckman). The limit of sensitivity for 5-HT or noradrenaline was ~0.5 fmol per sample (signal-to-noise ratio = 2). At the end of the experiments, localization of microdialysis probes was verified histologically (Bert *et al.*, 2004; Guilloux *et al.*, 2006).

### Forced swimming test (FST) procedure

FST was performed in groups of mice, separate from those used in the microdialysis experiments. The FST procedure was modified to enhance the sensitivity for detecting the putative antidepressant-like activity of drugs (Porsolt *et al.*, 1977; Holick *et al.*, 2008). Mice were briefly placed into clear plastic buckets (20 cm in diameter and 23 cm deep), filled two-thirds with water at 23–25°C. Automated scoring in the FST was done using automated software X'PERT FST developed by Bioseb (Vitrolles, France) to assess antidepressant-like activity in mice (Rainer *et al.*, 2011). Each bucket was instrumented with a sensor recording the vibrations due to movements of the mice, and a video was recorded from above. The system synchronizes mouse position, data calculated from the video recording and the vibration data. This information allows the system to compute characteristic values (based on speed of the animal, as well as different frequencies and power of the vibrations (via a fast Fourier transform calculation), describing the animal behaviour every second. Dependent variables were mobility, swimming and climbing duration. This test was performed 30 min after drug administration. The mobility duration is an index of antidepressant-like activity. Swimming behaviour relies on the 5-hydroxytryptaminergic system and climbing behaviour on the noradrenergic system in mice (Dulawa *et al.*, 2004; Holick *et al.*, 2008).

### Monoamine uptake

Male WT mice (Janvier, St. Berthevin, France) were killed by decapitation, and the FCx was dissected, minced on ice and resuspended in 10 volumes (w/v) of ice-cold sucrose (0.32 M). The cells were dissociated by filtering through nylon mesh of decreasing pore size (180–60 μm; Small Parts, Miramar, FL) and resuspended in ice-cold sucrose. The cellular suspension was preincubated 10 min at 37°C in 3 vol of Krebs Ringer HEPES (KRH) LiCl buffer (25 mM HEPES-KOH, pH 7.4, 125 mM LiCl, 4.8 mM KCl, 5.6 mM D(+)-glucose, 1.2 mM CaCl<sub>2</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub> and 1.2 mM MgSO<sub>4</sub>). To determine specifically the potential role played by low-affinity monoamine transporters in the effects of escitalopram on 5-HT and noradrenaline, we examined the uptake of radiolabelled 5-HT and noradrenaline in cellular suspensions treated with inhibitors of the high-affinity monoamine transporters such as 10 μM of venlafaxine (Tocris Bioscience, Bristol, UK), 5 μM of desipramine, 100 μM of GBR12935 or 10 μM of pargyline (Sigma-Aldrich, St. Louis, MO). Reserpine (10 μM was used to block the vesicular transporter VMAT2 (Sigma-Aldrich). These cellular suspensions were incubated for 15 min at 37°C in the same buffer supplemented with 10 μM [<sup>3</sup>H]noradrenaline, [<sup>3</sup>H]5-HT (Perkin Elmer, Boston, MA). The effect of escitalopram on endogenous low-affinity transporter activity was evaluated in the presence of increasing concentrations of this antidepressant (0.1–10 mM) during preincubation and

uptake. The reaction was terminated by rapid filtration through Unifilter-96 GF/C filters (Perkin Elmer). The cells were washed four times with 3 vol of uptake buffer, and the radioactivity retained on the filters was assessed by liquid scintillation counting. The protein concentration in tissue extracts was measured by the method of Bradford standardized with BSA. Low-affinity transporter-mediated uptake was quantified by inhibition with the specific inhibitor decynium 22 (D22: 1,1'-diethyl-2,2'-cyanine iodide) (500 µM; Sigma-Aldrich). Data are expressed as mean ± SEM of low-affinity uptake from one experiment performed in triplicate. D22-sensitive uptake rates were defined by subtracting uptake in the presence of D22 from the uptake observed in its absence (D22 uptake fraction ± escitalopram was subtracted from the control fraction ± escitalopram) and were expressed as mean ± SEM of D22-sensitive uptake from three to four independent experiments.

### Data analysis

Statistical analyses were performed using the computer software StatView 5.0. (Abacus Concepts, Inc., Berkley, CA). Statistical comparisons between basal [5-HT]<sub>ext</sub> and [NA]<sub>ext</sub> in WT and SERT<sup>-/-</sup> mice were made using the nonparametric Newman-Keuls test. All others values for microdialysis studies were calculated as percentage change at each time point relative to the average of four baseline values. Significant differences were determined on AUC values using Student's *t*-test or a one- or two-way ANOVA followed by Fisher's protected last significance difference (PLSD) *post hoc* test when appropriate. For behavioural studies, values were calculated on swimming or climbing duration, and significant differences were determined on this parameter using a one-way ANOVA followed by Fisher's PLSD *post hoc* test when appropriate. With respect to the uptake experiments, one-way ANOVA followed by Fisher's PLSD *post hoc* was applied. The level of statistical significance was set at *P* < 0.05.

## Results

### Basal extracellular levels of 5-HT ([5-HT]<sub>ext</sub>) and of noradrenaline ([NA]<sub>ext</sub>) in the frontal cortex of WT and SERT<sup>-/-</sup> mice

The effects of systemic administration of escitalopram on [5-HT]<sub>ext</sub> and [NA]<sub>ext</sub> in the FCx were evaluated by conventional microdialysis. Table 1 shows the mean ± SEM of basal cortical [5-HT]<sub>ext</sub> and [NA]<sub>ext</sub> levels [in fmol·(20 µL)<sup>-1</sup>] in WT

**Table 1**

Basal [5-HT]<sub>ext</sub> and [NA]<sub>ext</sub> values in the FCx of WT and SERT<sup>-/-</sup> mice

Basal/ mice	WT	SERT <sup>-/-</sup>
[5-HT] <sub>ext</sub>	2.16 ± 0.19 (n = 32)	13.29 ± 3.02*** (n = 16)
[NA] <sub>ext</sub>	6.77 ± 0.57 (n = 35)	3.08 ± 0.78*** (n = 16)

\*\*\*Significantly different from WT mice.

and SERT<sup>-/-</sup> mice. 5-HT levels were sixfold higher in SERT<sup>-/-</sup> than in WT mice (*P* < 0.001), whilst noradrenaline levels were twofold lower (*P* < 0.001).

### Effects of systemic administration of escitalopram on cortical [5-HT]<sub>ext</sub> and swimming time in the FST in WT mice

A dose-response experiment was undertaken to determine the effects of systemic administration of escitalopram on [5-HT]<sub>ext</sub> and [NA]<sub>ext</sub> in the FCx (Figures 1 and 2). One-way ANOVA on 5-HT outflow in the FCx measured from AUC values calculated during the 120 min post-treatment period revealed significant treatment-related effects (*F*[3,31] = 12.19; *P* < 0.001). Escitalopram increased cortical [5-HT]<sub>ext</sub> at the doses of 8 and 16 mg·kg<sup>-1</sup>, i.p., compared with vehicle-treated mice (*P* < 0.001 and *P* < 0.001, respectively) (time course, Figure 1A). The maximal increase in AUC values was of 378 ± 80% of baseline for the dose of 16 mg·kg<sup>-1</sup> (Figure 1B).

FST was carried out in an independent group of mice during the peak of the escitalopram effect (30 min after the drug; see grey area in Figure 1A). The mobility and swimming times were measured during the last 4 min of the 6 min test in mice. One-way ANOVA revealed a significant effect of escitalopram on mobility (*F*[3,35] = 6.26; *P* = 0.01) and swimming times (*F*[3,35] = 6.26; *P* = 0.01; Figure 1C), suggesting an antidepressant-like activity in this test. Escitalopram induced a statistically significant increase in the mobility duration at the doses of 8 and 16 mg·kg<sup>-1</sup>, i.p. (52.9 ± 11.2 s and 49.4 ± 10.5 s, respectively), when compared with vehicle-treated group (24.0 ± 4.0 s).

### Effects of systemic administration of escitalopram on cortical [NA]<sub>ext</sub> levels and climbing time in the FST in WT mice

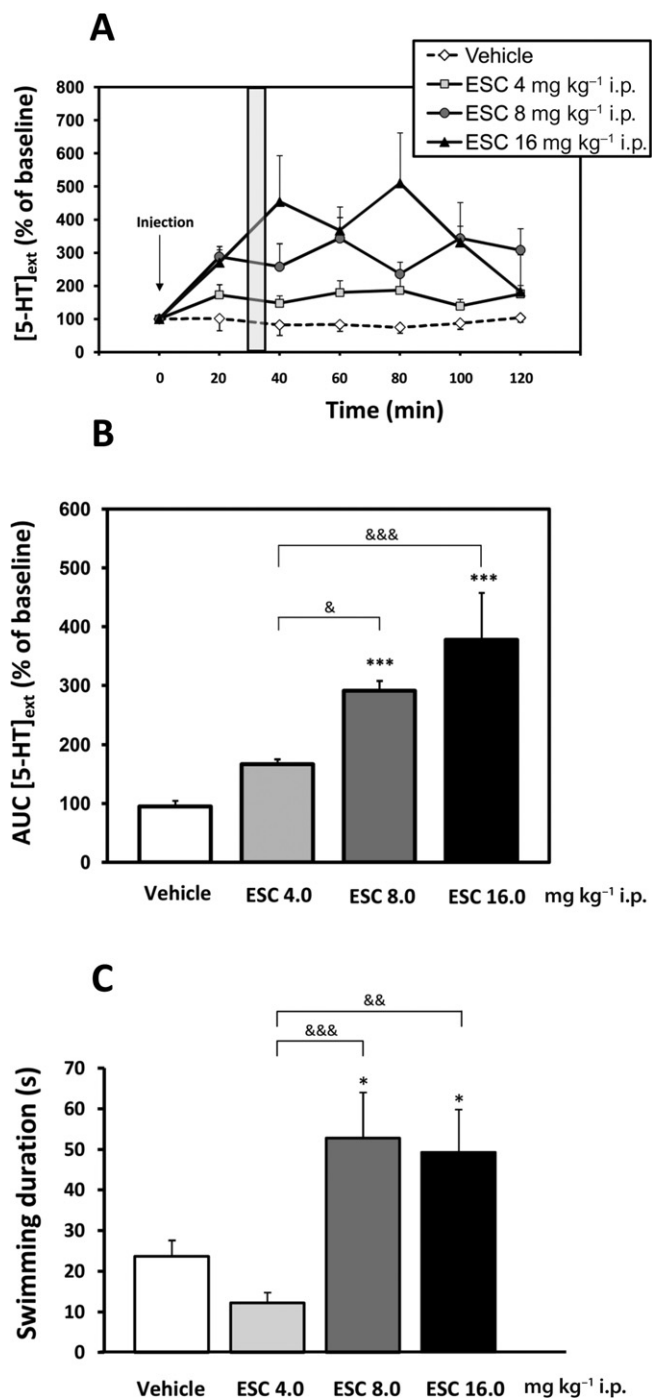
One-way ANOVA on noradrenaline outflow in the FCx revealed significant treatment-related effects (*F*[2,34] = 11.87; *P* < 0.001). Escitalopram increased cortical [NA]<sub>ext</sub> at the doses of 4 and 8 mg·kg<sup>-1</sup>, i.p., compared with vehicle-treated mice (*P* < 0.01 and *P* < 0.001, respectively) (time course, Figure 2A). The maximal increase in AUC values was 148 ± 14% of baseline at an escitalopram dose of 8 mg·kg<sup>-1</sup>, i.p. (Figure 2B).

In contrast, one-way ANOVA revealed no effect of escitalopram on climbing behaviour in the FST (*F*[2,27] = 3.1; *P* = 0.06) (Figure 2C).

### Effects of systemic administration of escitalopram on cortical [5-HT]<sub>ext</sub> and [NA]<sub>ext</sub> in SERT<sup>-/-</sup> mice and WT littermates

To test the hypothesis that the enhancement of noradrenergic transmission induced by escitalopram resulted from the blockade of the NET at nerve terminals, the neurochemical effects of escitalopram were studied by using intracerebral microdialysis in mice lacking the SERT (SERT<sup>-/-</sup> mice).

Figure 3 compares the effects of an acute administration of escitalopram (8 mg·kg<sup>-1</sup>, i.p.) on cortical [5-HT]<sub>ext</sub> and [NA]<sub>ext</sub> in WT and SERT<sup>-/-</sup> mice. A two-way ANOVA (treatment × genotype) on 5-HT outflow, measured from AUC calculated during a 120 min post-treatment period, revealed not only a significant effect of treatment (*F*[1,29] = 76.26; *P* = 0.001) and



genotype factors ( $F[1,29] = 55.19$ ;  $P = 0.001$ ) but also a significant treatment  $\times$  genotype interaction ( $F[1,29] = 61.97$ ;  $P = 0.001$ ). As expected, when the main target of the SSRIs, the SERT, was genetically inactivated, escitalopram failed to increase cortical [5-HT]<sub>ext</sub> in SERT<sup>-/-</sup> mice (Figure 3A,C).

Similarly, a two-way ANOVA (treatment  $\times$  genotype) on noradrenaline outflow indicated a significant effect of treatment ( $F[1,40] = 30.25$ ;  $P = 0.001$ ), but not of genotype ( $F[1,40] = 0.13$ ;  $P > 0.05$ ) (Figure 3B,C). Escitalopram increased [NA]<sub>ext</sub> in both WT and SERT<sup>-/-</sup> mice ( $P < 0.001$  and  $P < 0.001$  respectively).

**Figure 1**

Effects of acute escitalopram administration on extracellular 5-HT levels in the FCx and on swimming behaviour in the FST in WT mice. Mice received either vehicle or escitalopram (ESC; 4, 8, 16 mg·kg<sup>-1</sup>, i.p.). (A) Time course. Data are mean  $\pm$  SEM. Values of [5-HT]<sub>ext</sub> in the FCx are expressed as percentages of baseline ( $B_0$ ) following exposure to vehicle or escitalopram ( $n = 7$ –8 mice per group). Baseline 5-HT in vehicle, escitalopram 4, 8 and 16 mg·kg<sup>-1</sup>, i.p., were  $2.65 \pm 0.21$ ,  $2.21 \pm 0.18$ ,  $2.11 \pm 0.09$  and  $1.6 \pm 0.29$  fmol·(20  $\mu$ L)<sup>-1</sup> respectively. The grey area indicates the duration time of the FST (i.e. 6 min). Microdialysis and behavioural experiments were carried out with the same administration protocol, and the swimming parameter in the FST was measured, in a separate group of mice, at the maximum effect of escitalopram 8 mg·kg<sup>-1</sup>. (B) AUC values (mean  $\pm$  SEM) were calculated for the amount of 5-HT outflow collected during 0–120 min post treatment for escitalopram. (C) Antidepressant-like effect of the escitalopram on the swimming time in the FST in WT mice ( $n = 9$ –10 mice per group). \* $P < 0.05$ ; \*\*\* $P < 0.001$ : significantly different from vehicle-injected mice. & $P < 0.05$ ; && $P < 0.01$ ; &&& $P < 0.001$  compared with the escitalopram (8; 16 mg·kg<sup>-1</sup>, i.p.) group and escitalopram 4 mg·kg<sup>-1</sup>, i.p. (one-way ANOVA, Fisher's PLSD *post hoc* test).

As escitalopram was administered systemically in these experiments, it was not possible to determine the brain region where it exerted its neurochemical effect. To test the hypothesis that escitalopram acted preferentially at noradrenergic nerve terminals, without involving an action in the LC, its effects were studied after local perfusion of escitalopram in the FCx via reverse dialysis.

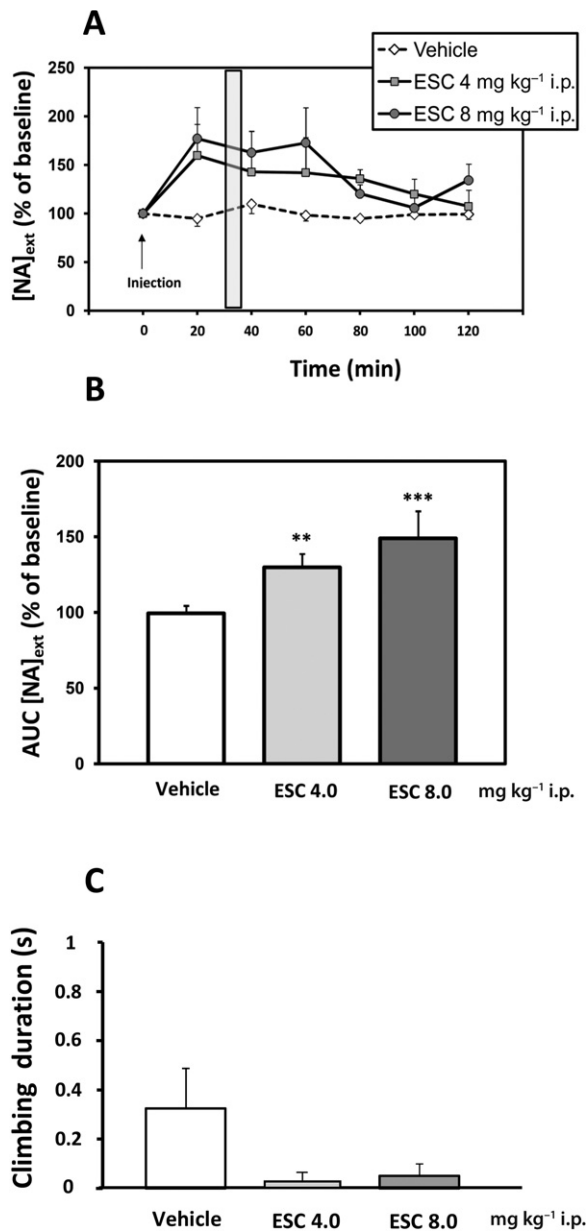
### Effects of continuous local perfusion of escitalopram on cortical [5-HT]<sub>ext</sub> and noradrenaline levels in SERT<sup>-/-</sup> mice and WT littermates

Figure 4 compares the effects of local perfusion of escitalopram (0.5  $\mu$ M) in both WT and SERT<sup>-/-</sup> mice. Under basal conditions (i.e. before treatment), 5-HT outflow in FCx of WT mice was stable. Local perfusion of escitalopram significantly increased cortical 5-HT levels in WT mice (AUC values = 569% of baseline;  $P < 0.001$ ) but had no significant effect on 5-HT outflow in SERT<sup>-/-</sup> mice ( $P > 0.05$ ; Figure 4A).

With respect to noradrenaline, local perfusion of escitalopram (0.5  $\mu$ M) significantly increased cortical noradrenaline levels in both WT and SERT<sup>-/-</sup> mice ( $P < 0.001$  and  $P < 0.001$  respectively). These increases reached a maximum of approximately 163% of baseline in both genotypes (Figure 4B). No differences were detected in [NA]<sub>ext</sub> between WT and SERT<sup>-/-</sup> mice after local perfusion of escitalopram ( $P > 0.05$ ).

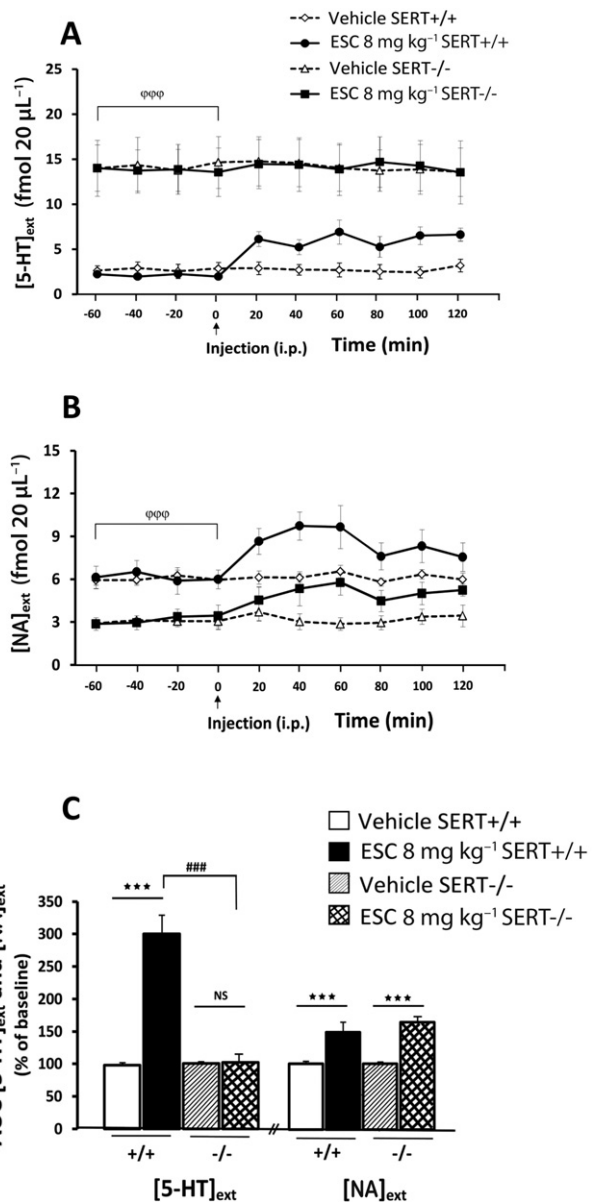
### Effects of pharmacological inactivation of the noradrenaline transporter on escitalopram-induced changes in cortical [NA]<sub>ext</sub> in WT mice

Figure 5 shows the effects of an acute administration of escitalopram (8 mg·kg<sup>-1</sup>, i.p.) on cortical [NA]<sub>ext</sub> in the presence of locally applied desipramine (50  $\mu$ M) in the perfusion medium in WT mice. Student's *t*-test on AUC<sub>0–120</sub> values for noradrenaline outflow in the FCx revealed no differences in the effects



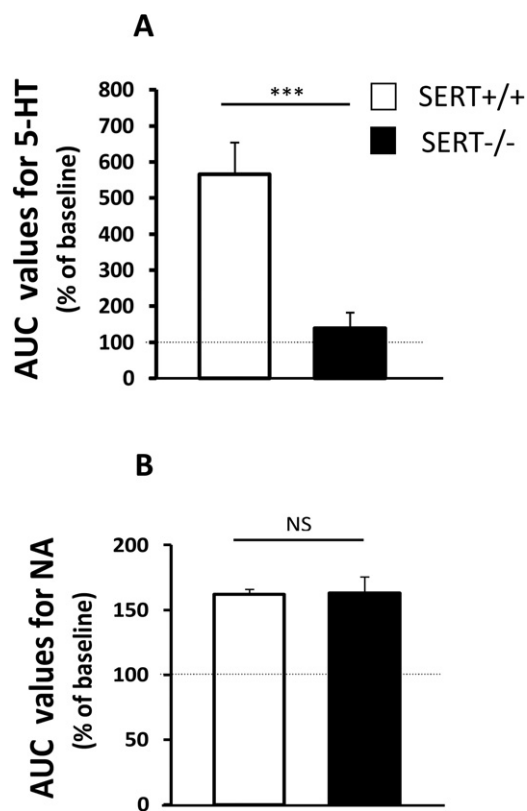
**Figure 2**

Effects of acute escitalopram administration on  $[NA]_{ext}$  in the FCx and on the climbing time in WT mice in the FST. Mice received either the vehicle or escitalopram (4 and 8 mg·kg<sup>-1</sup>, i.p.). (A) Time course. Data are mean ± SEM values of  $[NA]_{ext}$  in the FCx expressed as percentages of baseline ( $B_0$ ) following exposure to vehicle or escitalopram (ESC;  $n = 7-8$  mice per group). Baseline  $[NA]_{ext}$  in vehicle, escitalopram 4 and 8 mg·kg<sup>-1</sup>, i.p., were  $6.23 \pm 0.29$ ,  $8.01 \pm 0.91$  and  $6.01 \pm 0.76$  fmol·(20  $\mu$ L)<sup>-1</sup> respectively. The grey area indicates the duration time of the FST (i.e. 6 min). Microdialysis and behavioural experiments were carried out with the same protocol, and the climbing parameter in the FST was measured, in a separate group of mice, at the maximum effect of escitalopram 8 mg·kg<sup>-1</sup>. (B) AUC values (means ± SEM) were calculated for the amount of noradrenaline outflow collected during 0–120 min post treatment for escitalopram. (C) Antidepressant-like effect of escitalopram on climbing time in the FST in WT mice ( $n = 9-10$  mice per group). \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ : significantly different from vehicle-injected mice (one-way ANOVA, Fisher's PLSD *post hoc* test).



**Figure 3**

Effect of systemic administration of escitalopram on  $[5-HT]_{ext}$  and  $[NA]_{ext}$  in the FCx in WT and SERT<sup>-/-</sup> mice. (A, B) Time course. Data are mean ± SEM values of  $[5-HT]_{ext}$  and  $[NA]_{ext}$  expressed as percentages of baseline ( $B_0$ ) following exposure to vehicle or escitalopram (ESC;  $n = 5-7$  mice per group). Arrow indicates the time at which the injection of either vehicle in WT or SERT<sup>-/-</sup> mice, or escitalopram 8 mg·kg<sup>-1</sup>, i.p., in WT or SERT<sup>-/-</sup> mice was performed. Baseline  $[5-HT]_{ext}$  values were  $2.65 \pm 0.21$  and  $2.11 \pm 0.09$  fmol·(20  $\mu$ L)<sup>-1</sup> in WT mice administered vehicle and escitalopram 8 mg·kg<sup>-1</sup>, i.p., respectively, and  $13.01 \pm 3.28$  and  $13.69 \pm 2.04$  fmol·(20  $\mu$ L)<sup>-1</sup> in SERT<sup>-/-</sup> mice. Baseline  $[NA]_{ext}$  values were  $6.35 \pm 0.81$  and  $6.62 \pm 1.13$  fmol·(20  $\mu$ L)<sup>-1</sup> in WT mice administered vehicle and escitalopram 8 mg·kg<sup>-1</sup>, i.p., respectively, and  $2.93 \pm 0.87$  and  $3.15 \pm 0.97$  fmol·(20  $\mu$ L)<sup>-1</sup> in SERT<sup>-/-</sup> mice. (C) AUC values (means ± SEM) were calculated for the amount of 5-HT and noradrenaline outflow collected during 0–120 min post treatment for ESC. \*\*\* $P < 0.001$ : significantly different between controls and escitalopram-treated mice. &&& $P < 0.001$ : significantly different from SERT<sup>-/-</sup> mice.



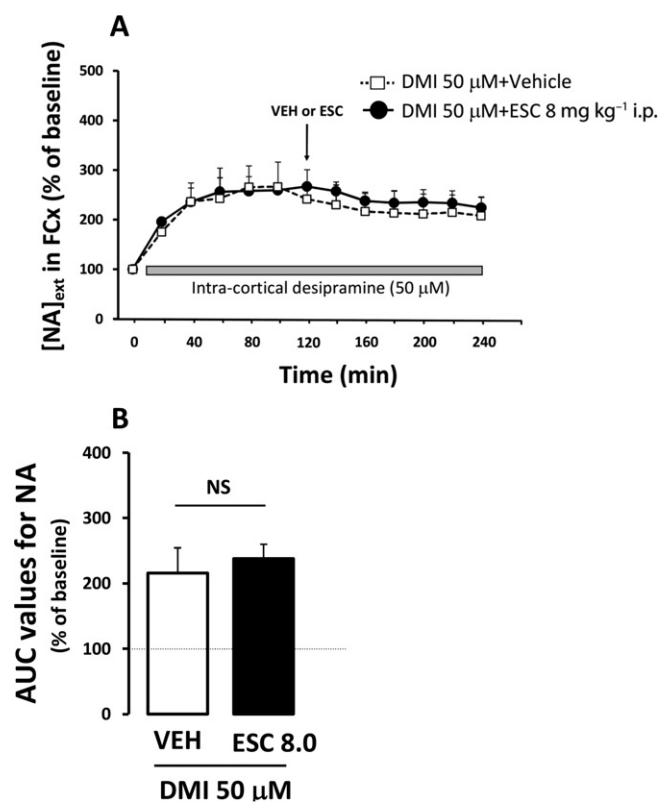
**Figure 4**

Effect of local perfusion of escitalopram on  $[5\text{-HT}]_{\text{ext}}$  and  $[\text{NA}]_{\text{ext}}$  in the FCx in WT and  $\text{SERT}^{-/-}$  mice. (A) AUC values (mean  $\pm$  SEM) were calculated for the amount of 5-HT outflow collected during the cortical 120 min perfusion of escitalopram (ESC;  $0.5\ \mu\text{M}$ ;  $n = 4\text{--}6$  mice per group).  $***P < 0.001$ : significantly different from  $\text{SERT}^{+/+}$  mice in the same drug condition. (B) AUC values (mean  $\pm$  SEM) were calculated for the amount of noradrenaline outflow collected during the cortical 120 min perfusion of escitalopram  $0.5\ \mu\text{M}$  ( $n = 4\text{--}6$  mice per group). NS, not statistically significant.

of desipramine between groups ( $P = 0.9$ ). Similarly, analysis on  $\text{AUC}_{120\text{--}240}$  values indicated no significant effect of escitalopram compared with vehicle-treated mice ( $P = 0.16$ ), demonstrating that the blockade of the NET by desipramine prevented escitalopram-induced increase in cortical  $[\text{NA}]_{\text{ext}}$  in WT mice.

#### Effects of escitalopram on noradrenaline and 5-HT uptake mediated by low-affinity monoamine transporters in ex vivo cell extracts from WT mice

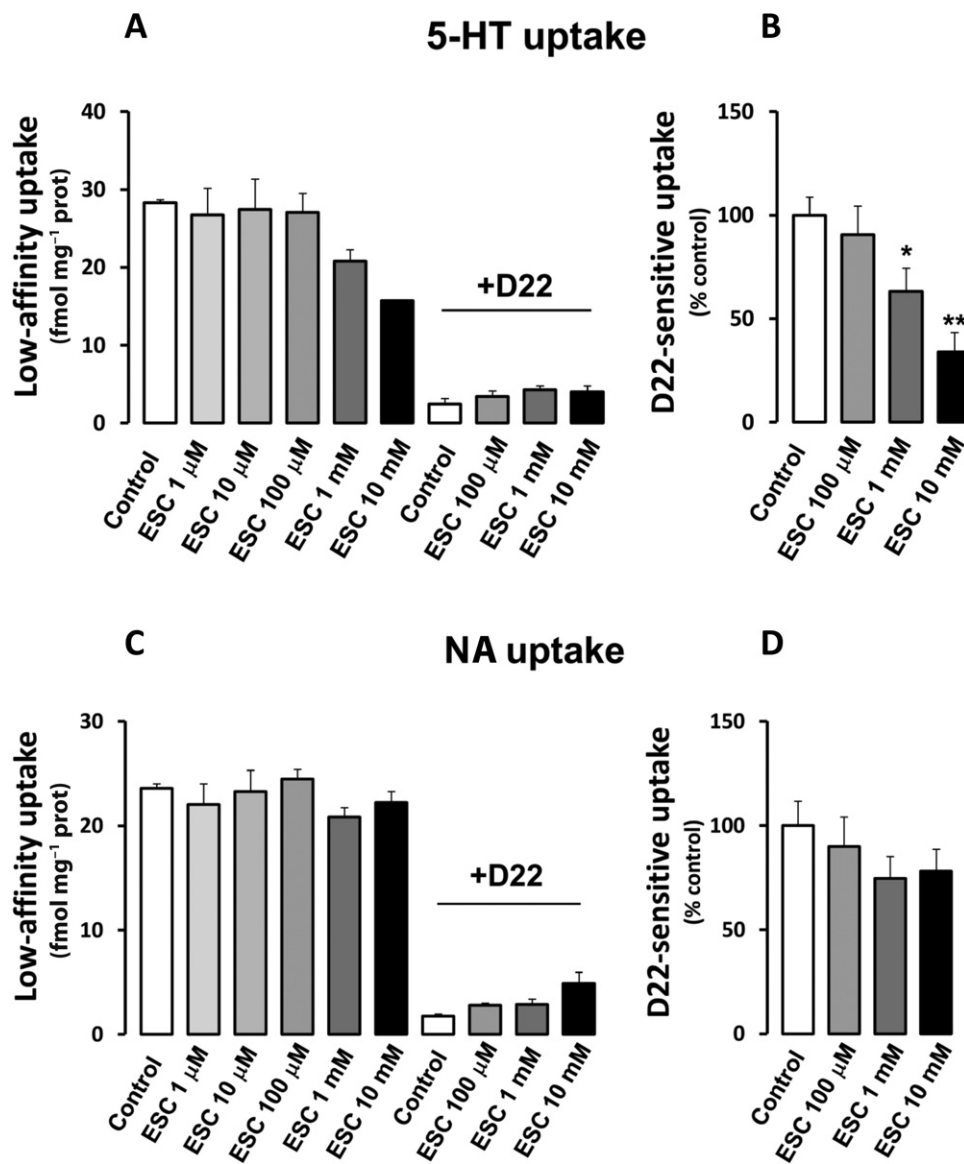
The atypical transporters OCT2, OCT3 and PMAT have recently been proposed to participate in low-affinity monoamine clearance in the brain, in complement to the high-affinity transporters. To investigate whether escitalopram could inhibit noradrenaline or 5-HT transport mediated by these low-affinity transporters, the effects of this SSRI on  $[^3\text{H}]$ noradrenaline and  $[^3\text{H}]$ 5-HT uptake were evaluated in cell



**Figure 5**

Effects of pharmacological inactivation of the NET on escitalopram-induced changes in cortical  $[\text{NA}]_{\text{ext}}$  in WT mice. (A) Time course. Data are means  $\pm$  SEM values of  $[\text{NA}]_{\text{ext}}$  expressed as percentages of baseline ( $B_0$ ) following exposure to vehicle or escitalopram ( $n = 5\text{--}7$  mice per group). Arrow indicates the time at which the injection of vehicle or escitalopram (ESC;  $8\ \text{mg}\cdot\text{kg}^{-1}$ , i.p.), was performed. Grey bar represent the local perfusion of desipramine (DMI,  $50\ \mu\text{M}$ ) into the FCx. (B) AUC values (mean  $\pm$  SEM) were calculated for the amount of noradrenaline outflow collected during 120–240 min post injection.

suspensions from WT mouse cortex, in the presence of inhibitors of the high-affinity monoamine transporters, venlafaxine, desipramine and GBR12935, and of an inhibitor of vesicular transporter VMAT2, reserpine. The specific contribution of low-affinity transporters was determined in the presence and absence of an inhibitor of low-affinity transporters, decynium 22 (D22). At the concentration used in the present study ( $500\ \mu\text{M}$ ), D22 is a potent inhibitor of OCTs (Hayer-Zillgen *et al.*, 2002) and PMAT (Engel and Wang, 2005), with affinities within the submicromolar range. In these *ex vivo* experiments, D22 alone decreased the uptake of 5-HT (Figure 6A) in agreement with previous *in vivo* microdialysis studies (Feng *et al.*, 2005; 2010), but also that of noradrenaline (Figure 6C). One-way ANOVA revealed a significant effect of escitalopram upon D22-sensitive 5-HT uptake ( $F[3,10] = 7.04$ ;  $P < 0.01$ ; Figure 6A,B), but no significant effect upon D22-sensitive noradrenaline uptake ( $F[3,12] = 1.08$ ,  $P > 0.05$ ), with an inhibition of noradrenaline uptake at 1 and 10 mM escitalopram (Figure 6C,D).



**Figure 6**

Effects of escitalopram on *ex vivo* noradrenaline and 5-HT uptake by low-affinity transporter in the frontal cortex in WT mice. (A–C) Representative [<sup>3</sup>H]noradrenaline and 5-HT uptake in cortex extracts in the presence of increasing concentrations of escitalopram (ESC; 1 μM to 10 mM) with or without the inhibitor D22 is expressed as mean fmol ± SEM, normalized by protein concentration ( $n = 3–5$  experiments). (B–D) D22-sensitive [<sup>3</sup>H]noradrenaline and 5-HT uptake in the presence of increasing concentrations of escitalopram (100 μM to 10 mM) is expressed as percentage of D22-sensitive uptake in absence of citalopram (Control;  $n = 3–5$ ). D22-sensitive uptake rates were defined by subtracting uptake in the presence of D22 from the uptake observed in its absence. \* $P < 0.05$ ; \*\* $P < 0.01$ : significantly different from control (one-way ANOVA followed by Fisher's *post hoc* test).

## Discussion

Antidepressants, such as paroxetine, fluoxetine, citalopram and escitalopram, used for the treatment of major depressive disorders, exert their therapeutic effects mainly by inhibiting 5-HT reuptake into presynaptic nerve terminals, thereby enhancing 5-HT neurotransmission which might be diminished in depressed patients (Invernizzi *et al.*, 1995; Sanchez *et al.*, 2003a). Although SSRIs share a common target (i.e. the SERT), the affinity, selectivity and potency to block monoaminergic transporters vary substantially. *In vitro*, the six SSRIs

approved in Europe are all potent 5-HT reuptake inhibitors (Richelson and Pfenning, 1984; Sanchez and Hyttel, 1999). In agreement with this inhibitory action, intracerebral *in vivo* microdialysis studies in mice revealed that an acute systemic administration of an SSRI significantly increases cortical extracellular 5-HT levels ( $[5\text{-HT}]_{\text{ext}}$ ) (David *et al.*, 2003; Guiard *et al.*, 2004; Guilloux *et al.*, 2006; Richardson-Jones *et al.*, 2010) in a strain-dependent manner (Calcagno *et al.*, 2007). The present study extends these neurochemical observations to escitalopram by showing that in WT mice, this SSRI induced a dose-dependent increase in cortical  $[5\text{-HT}]_{\text{ext}}$ . In



Table 2

*In vivo* selectivity of antidepressant drugs in mice measured by intracerebral microdialysis

Ratio of AUC values in WT mice	Escitalopram (8 mg·kg <sup>-1</sup> , i.p.) Present study	Citalopram (8 mg·kg <sup>-1</sup> , i.p.)	Paroxetine (8 mg·kg <sup>-1</sup> , i.p.) David <i>et al.</i> , 2003	Venlafaxine (8 mg·kg <sup>-1</sup> , i.p.)
5-HT/NE	2.03	1.68	0.91	2.91

addition, consistent with a previous study (Zomkowski *et al.*, 2010), similar doses of escitalopram produced a robust antidepressant-like effect as shown by a significant increase in swimming duration (Figure 1C). This parameter is believed to reflect the activation of the brain 5-hydroxytryptaminergic system in rodents (Detke *et al.*, 1995; Renner and Lucki, 1998; Cryan and Lucki, 2000) since pretreatment with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine, prevented SSRI-induced increase in swimming behaviour (Page *et al.*, 1999).

Considerable variations in the 5-HT/noradrenaline selectivity ratios of SSRIs have been reported *in vitro*. Based on their inhibition constants, citalopram and escitalopram emerge as the most selective compounds amongst the SSRIs, whilst fluoxetine is the least selective (Sanchez and Hyttel, 1999; Owens *et al.*, 2001). Despite this apparent selectivity for SERT, the present data indicate that the systemic or local injection of escitalopram also significantly increases [NA]<sub>ext</sub> in the FCx of WT mice. These results are consistent with previous *in vivo* microdialysis experiments, which revealed that the acute administration of fluoxetine, paroxetine or citalopram enhanced noradrenaline levels in the FCx and/or hippocampus of rodents (Jordan *et al.*, 1994; Hughes and Stanford, 1996; 1998; Shachar *et al.*, 1997; Millan *et al.*, 2001; Beyer *et al.*, 2002; Bymaster *et al.*, 2002; Koch *et al.*, 2002; David *et al.*, 2003; Kobayashi *et al.*, 2008). In the present study, despite the neurochemical data, escitalopram failed to increase climbing behaviour (Figure 2C), a parameter reflecting the activation of noradrenergic neurons arising from cells located in the lateral tegmentum as shown in rats (Cryan *et al.*, 2002) or mice (Dulawa *et al.*, 2004). This observation suggests that the 50% increase in cortical [NA]<sub>ext</sub> induced by escitalopram was not sufficient to affect this behavioural response. When comparing the relative ability of SSRIs in our microdialysis studies, escitalopram (8 mg·kg<sup>-1</sup>, i.p.) was more potent in increasing cortical [5-HT]<sub>ext</sub> than citalopram and paroxetine, but surprisingly less potent than the SNRI venlafaxine (8 mg·kg<sup>-1</sup>, i.p.) (Table 2). The fact that the most selective 5-HT reuptake inhibitor escitalopram also demonstrated a neurochemical noradrenergic-enhancing property was particularly intriguing. Our results suggest that noradrenaline may play an important role in mediating the acute neurochemical actions of this SSRI. This inference is in agreement with previous findings showing that the neurochemical effects of SSRIs are attenuated in noradrenaline-deficient mice (Cryan *et al.*, 2004). Several possibilities may explain these neurochemical effects on cortical [NA]<sub>ext</sub> in WT mice, such possibilities include direct or indirect actions at the SERT, the NET or an interaction with other transporters.

### Potential heterologous reuptake of noradrenaline through the SERT or the DAT

The cloning and sequencing of monoamine transporters revealed that this family shows a high degree of structural homology (Gether *et al.*, 2006). This observation may explain, at least in part, the fact that SSRIs, despite their supposed selectivity, can enhance not only 5-hydroxytryptaminergic but also noradrenergic neurotransmission. Vizi *et al.* (2004) showed previously that the noradrenaline uptake was substantially reduced, but not completely abolished, in the hippocampus and FCx of NET<sup>-/-</sup> mice. Interestingly, the neuronal component of this residual uptake was markedly decreased in the presence of citalopram, suggesting that noradrenaline can be taken up by 5-hydroxytryptaminergic varicosities through the SERT. This heterologous uptake could explain the increase in noradrenaline outflow induced by escitalopram in the present study. However, the fact that basal cortical levels of [NA]<sub>ext</sub> are markedly attenuated in SERT<sup>-/-</sup> mice suggests that the SERT does not contribute significantly to noradrenaline uptake in this region.

In addition, since the NET and DAT cooperate, to a certain degree, in the clearance of catecholamines (i.e. dopamine and noradrenaline) in the prefrontal cortex (Carboni *et al.*, 1990; Morón *et al.*, 2002; Guiard *et al.*, 2008b; Borgkvist *et al.*, 2011), the possibility that escitalopram blocked the DAT, and thereby enhancing [NA]<sub>ext</sub>, cannot be excluded. However, the lack of effect of escitalopram on cortical [DA]<sub>ext</sub> (see Figure S1) strongly argues against this hypothesis.

### Potential action of escitalopram at low-affinity monoamine transporters

Beside the high-affinity transporters, other transporters such as OCTs and PMAT have been proposed to participate in monoamine clearance in the brain, a role recently confirmed *in vivo* for OCT2 (Bacq *et al.*, 2011). The affinities of various monoamines for these transporters, as defined *in vitro* and tested in the millimolar range, have been shown to vary depending on transporter type and species. Specifically, rat OCT2 show affinities of 0.8–3.6 and 2–4.4 mM and rat OCT3 affinities of 0.5–1 and 0.4–1.9 mM for 5-HT and NE respectively (Grundemann *et al.*, 1998a,b; Wu *et al.*, 2000; Amphoux *et al.*, 2006), whilst human PMAT show affinities of approximately 0.1 and 2.6 mM for 5-HT and NE respectively (Engel *et al.*, 2004; Engel and Wang, 2005). Both OCTs and PMAT show a high transport capacity (Wu *et al.*, 2000; Engel *et al.*, 2004), which may compensate for their low substrate affinity and hence, improve transport efficacy. In addition, OCT3 from rodent species was shown to be inhibited by

antidepressants such as desipramine and imipramine (Kekuda *et al.*, 1998; Wu *et al.*, 2000). Human PMAT has been shown to be inhibited by a large array of antidepressants (Haenisch and Bönisch, 2010), albeit with affinities in the 5–200  $\mu\text{M}$  range. These findings raise the possibility that escitalopram could interact with some of these low-affinity transporters *in vivo*, thereby contributing to the increase in  $[\text{NA}]_{\text{ext}}$ .

To explore whether the escitalopram-mediated increase in cortical  $[\text{NA}]_{\text{ext}}$  could result from an action at low-affinity monoamine transporters, we evaluated the action of this SSRI on noradrenaline and 5-HT uptake in brain cortex cell suspensions in the presence of the high-affinity monoamine transporter inhibitors venlafaxine, desipramine and GBR12935 and of the vesicular transporter VMAT2 inhibitor reserpine. Specific uptake by low-affinity transporters was evaluated using a selective inhibitor of OCTs and PMAT, D22 (Hayer-Zillgen *et al.*, 2002; Engel and Wang, 2005). Under these conditions, most of the 5-HT and noradrenaline uptake could be inhibited by D22, indicating the presence in the cortex of the low-affinity transporters, insensitive to the high-affinity transporter blockers, as previously published (Bacq *et al.*, 2011). In these cortical extracts, escitalopram, even at high concentrations, had no significant action upon D22-sensitive noradrenaline uptake, whilst concentrations as high as 1 mM were required to inhibit D22-sensitive 5-HT uptake. This differential effect of high concentrations of escitalopram suggests that several transporters with distinct specificities contributed to D22-sensitive uptake in the cortex. PMAT, has been shown to transport 5-HT with a higher efficacy than noradrenaline and may be a more significant transport mechanism than OCT3 in certain heterologous systems (Duan and Wang, 2010). Hence PMAT could contribute notably to 5-HT clearance in certain brain areas and it might represent the component sensitive to 1–10 mM escitalopram. In any case, these inhibitory concentrations are far higher than the therapeutically active concentrations of escitalopram found in the brain of rodents after acute administration. Recent acute dosing studies in mice have shown that a 30 min escitalopram pretreatment (0.3 mg·kg<sup>-1</sup>, s.c.), produces a SERT occupancy of 77% and mean plasma and brain levels of approximately 15.9 and 10.6 ng·mL<sup>-1</sup> (38 and 26 nM) respectively. At the dose of 5 mg·kg<sup>-1</sup> of escitalopram, SERT occupancy reaches 99%, whereas plasma and brain levels were of 247 and 401 ng·mL<sup>-1</sup> (600 and 970 nM), respectively (unpubl. obs.). Importantly, in rodents, steady-state plasma levels have been evaluated in the range of 6–21 ng·mL<sup>-1</sup> for escitalopram (Kreilgaard *et al.*, 2008). Similarly, for citalopram, the concentrations found in rodent brains after chronic treatment are 1000-fold above the concentrations of citalopram required to inhibit uptake in this *ex vivo* model (Uhr and Grauer, 2003; Cervo *et al.*, 2005; Bacq *et al.*, 2011). Taken together, these data suggest that at therapeutically relevant concentrations, this SSRI does not interact directly with the low-affinity monoamine transporters present in the brain, OCT2, OCT3 or PMAT.

### Potential effect of escitalopram on the noradrenergic system through 5-HT or blockade of the NET

The present study explored the possibility that the effects of escitalopram on noradrenaline outflow could be attributable

to an excitatory effect of 5-HT on noradrenergic neurons. Close anatomical and functional interactions exist between the serotonergic and noradrenergic systems in the brain (Mongeau *et al.*, 1997; Nutt, 2002). In particular, it is well established that the increase in 5-HT transmission induced by SSRIs inhibits noradrenergic neuronal activity (Dremencov *et al.*, 2007), an effect believed to be mediated through activation of 5-HT<sub>2A</sub> and/or 5-HT<sub>2C</sub> receptors expressed on GABA neurons (Szabo and Blier, 2001b; Bymaster *et al.*, 2002; Miguez *et al.*, 2011). Accordingly, in the present study, basal cortical  $[\text{5-HT}]_{\text{ext}}$  was increased in knock-out SERT (SERT<sup>-/-</sup>) mice, whilst cortical  $[\text{NA}]_{\text{ext}}$  was significantly attenuated compared with WT littermates. Thus, a putative excitatory effect of 5-HT on noradrenaline neurons would most likely involve a local action at nerve terminals. Consistent with this hypothesis, initial microdialysis studies reported that the local perfusion of the SSRIs fluoxetine or citalopram can enhance cortical  $[\text{NA}]_{\text{ext}}$  (Hughes and Stanford, 1996; 1998). Interestingly, increases in the synaptic availability of 5-HT in the hippocampus have also been shown to activate the noradrenergic transmission through 5-HT<sub>1A</sub> (Hajos-Korcsok *et al.*, 1999) and/or 5-HT<sub>3</sub> receptors (Mongeau *et al.*, 1994). To clarify this point, we hypothesized that, if the escitalopram-induced increase in cortical  $[\text{NA}]_{\text{ext}}$  was due to its local action at 5-HT terminals, it should be attenuated in mice lacking the SERT, which display no detectable 5-HT reuptake in the FCx (Perez *et al.*, 2006). As expected, escitalopram did not increase cortical  $[\text{5-HT}]_{\text{ext}}$  in SERT<sup>-/-</sup> mice. However, the ability of the systemic administration or local perfusion of escitalopram to increase cortical  $[\text{NA}]_{\text{ext}}$  remained unchanged in SERT<sup>-/-</sup> as compared with WT mice (164% vs. 148% and 162% vs. 163%, respectively), indicating that elevation of 5-HT was not required to stimulate cortical noradrenaline release. These data strongly suggest that escitalopram can increase cortical  $[\text{NA}]_{\text{ext}}$  by a SERT-independent mechanism, involving a potentially direct inhibition of NET.

To explore this possibility further, we anticipated that, if the escitalopram-induced increase in cortical  $[\text{NA}]_{\text{ext}}$  resulted from the blockade of NET, it would be attenuated if this transporter had been previously inactivated. We thus evaluated the effect of escitalopram on cortical  $[\text{NA}]_{\text{ext}}$  in the presence of locally applied desipramine (50  $\mu\text{M}$ ) which inhibits only noradrenaline reuptake in the FCx (Figure S2A,B). Significantly, with respect to the occupancy of the NET, we can legitimately assume that 50  $\mu\text{M}$  desipramine optimally inactivated this transporter, since higher concentrations (100  $\mu\text{M}$ ) failed to enhance further  $[\text{NA}]_{\text{ext}}$  (Figure S2B). As previously demonstrated in anaesthetized mice (Fisher *et al.*, 2007), our results show that desipramine alone increased cortical  $[\text{NA}]_{\text{ext}}$  in awake WT mice. However, escitalopram when administered i.p. 2 h after the beginning of the intra-cortical desipramine perfusion, failed to enhance further cortical  $[\text{NA}]_{\text{ext}}$ . These findings strengthen the possibility that escitalopram can inhibit the NET. Importantly, apart from NET, desipramine can bind to various receptors including 5-HT<sub>1A/2A</sub> and  $\alpha_{1/2}$ -adrenoceptors, which are known to modulate both the 5-hydroxytryptaminergic and noradrenergic systems (Guiard *et al.*, 2008a). Indeed, a significant inhibition of specific radioligand binding by desipramine was shown *in vitro* on rat brain synaptosomes (Thomas *et al.*, 1987). On the other hand, paroxetine and citalopram have little affinity for

these receptor subtypes (Sanchez and Hyttel, 1999). It is thus conceivable that a direct interaction of desipramine with these receptors could account for its preventive effects. In addition, desipramine was also shown to block PMAT (Hae-nisch and Bönisch, 2010), which may consequently favour cortical [NA]<sub>ext</sub> accumulation. This effect could induce an eventual 'ceiling effect', which could hamper the detection of a further enhancement of [NA]<sub>ext</sub> by escitalopram. Finally, if desipramine enhanced [5-HT]<sub>ext</sub> by blocking either high- and/or low-affinity transporters, high levels of 5-HT could exert a negative feedback on noradrenergic systems, thereby masking an elevation of [NA]<sub>ext</sub> in response to escitalopram. Nevertheless, despite these conflicting hypotheses, the combination of our *in vivo* results with the uptake data strongly suggests that escitalopram acts primarily on the NET.

In conclusion, the present study suggests that *in vivo*, escitalopram is not as selective for transporters as it has been reported *in vitro* and is likely to interact with the NET locally in the FCx. So far, the explanation for this drastic difference between *in vivo* and *in vitro* observations remains unclear. Pharmacokinetic parameters may provide an interesting explanation since desmethylcitalopram, the major metabolite of escitalopram, displays a twofold higher affinity for the NET than citalopram (Tatsumi *et al.*, 1997). In addition, increased action of escitalopram at NET *in vivo* could be due to environment-based post-translational modifications of the transporter modulating its affinity, such as phosphorylation or interaction with regulatory proteins (Blakely and De Felice, 2007). Nevertheless, the increased noradrenaline efflux in the FCx appears to play a minor role in the antidepressant-like activity of escitalopram administered acutely, since climbing activity in the FST did not change in WT mice. On the other hand, it is possible that escitalopram increases dialysate noradrenaline levels also in other brain regions more directly involved in behavioural activity (the hippocampus, amygdala), than the brain region studied here. These effects of escitalopram on cortical [NA]<sub>ext</sub> are probably dependent on the doses given to mice. It is likely that the exposures produced by the doses used in the present study exceed those that will be achieved in clinical use. It will thus be interesting in future investigations to complete the present study by evaluating the antidepressant-like activity of escitalopram in rodents and explore the neurochemical and behavioural consequences of a repeated administration.

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## Conflict of interest

None.

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## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Effects of acute escitalopram administration on extracellular dopamine ( $[DA]_{ext}$ ) levels in the FCx in WT mice. Mice received either vehicle or escitalopram ( $8 \text{ mg}\cdot\text{kg}^{-1}$ , i.p.). (A) Time course. Data are mean  $\pm$  SEM. Values of  $[DA]_{ext}$  in the FCx are expressed as percentages of baseline ( $B_0$ ) following exposure to vehicle or escitalopram ( $n = 7\text{--}8$  mice per group). Baseline  $[DA]_{ext}$  in vehicle and escitalopram  $8 \text{ mg}\cdot\text{kg}^{-1}$ , i.p., were  $0.46 \pm 0.11$  and  $0.68 \pm 0.21 \text{ fmol}\cdot(20 \mu\text{L})^{-1}$  respectively. (B) AUC values (mean  $\pm$  SEM) were calculated for the amount of dopamine outflow collected during 0–120 min post treatment for escitalopram.

**Figure S2** Effects of intra-cortical perfusion of desipramine on extracellular 5-HT and noradrenaline levels in WT mice. Mice received an intra-cortical perfusion of desipramine (50 and  $100 \mu\text{M}$ ). (A, B) Time course. Data are mean  $\pm$  SEM. Values of  $[5\text{-HT}]_{ext}$  (A) or of  $[NA]_{ext}$  (B) in the FCx are expressed as percentages of baseline ( $B_0$ ) perfusion of desipramine ( $n = 4\text{--}5$  mice per group). The grey area indicates the duration of desipramine perfusion by reverse dialysis.