Upregulation of the dorsal raphe nucleus-prefrontal cortex serotonin system by chronic treatment with escitalopram in hyposerotonergic Wistar-Kyoto rats

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Abstract

Wistar-Kyoto (WKY) rats are sensitive to chronic stressors and exhibit depression-like behavior. Dorsal raphe nucleus (DRN) serotonin (5-HT) neurons projecting to the prefrontal cortex (PFC) comprise the important neurocircuitry underlying the pathophysiology of depression. To evaluate the DRN-PFC 5-HT system in WKY rats, we examined the effects of escitalopram (ESCIT) on the extracellular 5-HT level in comparison with Wistar rats using dual-probe microdialysis. The basal levels of 5-HT in the DRN, but not in the PFC, in WKY rats was reduced as low as 30% of Wistar rats. Responses of 5-HT in the DRN and PFC to ESCIT administered systemically and locally were attenuated in WKY rats. Feedback inhibition of DRN 5-HT release induced by ESCIT into the PFC was also attenuated in WKY rats. Chronic ESCIT induced upregulation of the DRN-PFC 5-HT system in WKY rats, with increases in basal 5-HT in the DRN, responsiveness to ESCIT in the DRN and PFC, and feedback inhibition, whereas downregulation of these effects was induced in Wistar rats. Thus, the WKY rat is an animal model of depression with low activity of the DRN-PFC 5HT system. The finding that chronic ESCIT upregulates the 5-HT system in hyposerotonergic WKY rats may contribute to improved understanding of mechanisms of action of antidepressants, especially in depression with 5-HT deficiency.

Keyword: microdialysis; depression; animal model; SSRIs

1. Introduction

Dysfunction of serotonin (5-HT) neurotransmission is a common hallmark in major depression and the 5-HT system is a therapeutic target of antidepressants (Hirschfeld, 2000). Dorsal raphe nucleus (DRN) 5-HT neurons projecting to the prefrontal cortex (PFC) comprise the important neurocircuitry underlying the pathophysiology of depression (Azmitia, 1999). Selective 5-HT reuptake inhibitors (SSRIs), which are widely used as antidepressants, rapidly increase extracellular 5-HT levels in the DRN and PFC, but show a delay in therapeutic onset for a few weeks. Chronic administration of SSRIs reduces the sensitivity of 5-HT autoreceptors and enhances 5-HT neurotransmission (Artigas et al., 1996; Blier, 2001; Gardier et al., 1996; Rausch et al., 2006). Such neural adaptation of the DRN-PFC 5-HT system may mediate the clinical effects of antidepressants.

Wistar-Kyoto (WKY) rats have been proposed as a genetic animal model of depression (Overstreet, 2012; Solberg et al., 2004). A number of loci mapped for behavioral despair in WKY rats overlap with regions associated by linkage or genome scan analyses with major depression and bipolar disorder in humans (Overstreet, 2012; Solberg et al., 2004). WKY rats display depression-like behavior in a wide range of behavioral paradigms (Malkesman and Weller, 2009; Paré, 1989; Tejani-Butt et al., 2003), including reduced exploration in the open-field test (Paré, 1989) and decreased struggling and increased immobility in the forced swim test (Lahmame et al., 1997) (Rittenhouse et al., 2002), in comparison with Sprague-Dawley (SD) and Wistar rats.

Altered behavioral responses to SSRIs (Lahmame and Armario, 1996; Tejani-Butt et al., 2003) and differential regulation of 5-HT transporter sites in the cortex in response to chronic stressors (Paré and Tejani-Butt, 1996) suggest dysfunction of the 5-HT system in WKY rats. Electrophysiological studies have revealed decreased excitability of DRN 5-HT neurons in these rats (Lemos et al., 2011) and low tissue contents of 5-HT in the DRN, but not in the medial PFC, are found in WKY rats compared with SD rats (Scholl et al., 2010). The decreased expression of tryptophan hydroxylase 2 (TPH2) mRNA in DRN 5-HT neurons of WKY rats (Lemos et al., 2011) supports the idea that synthesis and release of 5-HT in the DRN are low in these rats. Consistent with this, polymorphisms in the TPH2 gene that result in reduction of 5-HT synthesis are associated with depression in various human populations (Jacobsen et al., 2012). Among TPH2 variants, the TPH2 $^{Arg}439^{His}$ knock-in mouse exhibits depression-like behavior related to 5-HT deficiency (Jacobsen et al., 2012).

We hypothesized that the genetic background of WKY rats is associated with dysfunction of the

DRN-PFC 5-HT system, resulting in expression of depression-like behavior. To characterize the DRN-PFC 5-HT system and its responses to acute and chronic SSRIs in WKY rats, the extracellular 5-HT levels in the DRN and PFC of WKY rats were measured using dual-probe microdialysis under naïve conditions and after chronic treatment with the most selective SSRI, escitalopram (ESCIT) (Kennedy et al., 2009). Our results show low activity of the DRN-PFC 5-HT system and upregulation of the 5-HT system with chronic ESCIT in WKY rats, opposite to the downregulation response in Wistar rats.

2. Materials and methods

2.1. Animals

Male Wistar (280–340 g, Kyudo, Tosu, Japan) and WKY (280–320 g, SLC, Japan) rats were housed at 23 ± 2 °C under a 12-h light–dark cycle with free access to food and water. All rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the U.S. National Institutes of Health. Specific protocols were approved by the Committee for Animal Experimentation, Kurume University School of Medicine. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

Escitalopram oxalate (generously provided by H. Lundbeck A/S, Copenhagen, Denmark) was dissolved in saline (0.2 ml) and Ringer's solution for systemic injection and local application, respectively.

2.3. Experimental set-up

Rats in each strain were divided into three experimental groups; naïve (untreated), chronic vehicle-treated and chronic ESCIT-treated groups. In chronic vehicle- and ESCIT-treated groups, rats were treated with saline (0.2 mL) and ESCIT (5 mg/kg s.c.) once daily for 14 days, respectively. Experiments were conducted 48 h after the last injection

In the microdialysis experiments, Wistar and WKY rats in three experimental groups received a systemic injection of ESCIT (5 mg/kg in 0.2 mL saline s.c.) or local infusion of ESCIT (0.1 or 1.0 μM in Ringer's solution) into the DRN and PFC by retrograde microdialysis, in which ESCIT was added to the perfusion fluid of Ringer's solution at these concentrations. The tissue concentration provided by proper in vivo calibration procedures (Stahle, 1991), was shown approximately 10–20% (Bundgarrd, 2007).

2.4. Surgery and brain dialysis

Microdialysis was performed with an I-shaped cannula. Microdialysis probes were implanted in the unilateral PFC (exposed length 5.0 mm) and the ipsilateral DRN (exposed length 1.5 mm) for dual-probe microdialysis under pentobarbital (50 mg/kg i.p.) and xylazine (8 mg/kg i.p.) anesthesia and local application of 10% lidocaine. The coordinates of the implantation were A/P 3.2 mm, L/M 2.4 mm, V/D −5.0 mm from the bregma and dura for the PFC and A/P −7.8 mm, L/M 4.0 mm, V/D −7.4 mm at an angle of 34° in the sagittal plane for the DRN. After surgery, the rats were housed individually in plastic cages (30 × 30 × 40 cm).

Microdialysis experiments were conducted 24 h after implantation of the probe, as previously described (Kawahara et al., 2007). An on-line approach for real-time quantification of 5-HT was used, in which the probes were perfused with Ringer's solution at a flow rate of 2.0 μl/min. The 15-min sample fractions collected through dialysis probes were directly injected to high-performance liquid chromatography using a reverse-phase column (100 × 2.1 mm; BetaBasic-18, Thermo, Waltham, MA, USA) with electrochemical detection. An EICOM EP-300 pump (Kyoto, Japan) was used in conjunction with an electrochemical detector (ESA; potential first cell, +230 mV; potential second cell, +50 mV). The mobile phase was a mixture of 4.1 g/l sodium acetate, 100 mg/l Na_2EDTA, 30 mg/l octanesulfonic acid, 30 μl/l triethylamine, and 7% v/v methanol, pH 4.65. The flow rate was 1.0 ml/min. The detection limit of assay was about 0.3 fmol per sample (on-column). The composition of the Ringer's solution (in mM) was: NaCl 140.0, KCl 3.0, $CaCl_2$ 1.2, $MgCl_2$ 1.0 for the DRN and NaCl 147.0, KCl 4.0, $CaCl_2$ 3.4 for the PFC. Ringer's solution with high Ca^{2+} was used to recover dialysate containing detectable concentrations of 5-HT from the PFC. At the end of the experiments, the rats were given an overdose of chloral hydrate and brains were fixed with 4% paraformaldehyde via intracardiac infusion. Sagittal sections (50 μm) were cut and dialysis probe placement was localized using the atlas of Paxinos and Watson (2007) (Supplementary Fig. 1). Rats, in which dialysis probes were misplaced, were not included in data analysis.

2.5. Forced swim test (FST)

The method of Porsolt et al. (1977) with modification by Malkesman et al. (2006) was used to assess the immobility of rats as a measure of their helplessness or depressive-like behavior. After 26–27 h of isolation, rats were placed individually in a round Pyrex cylinder pool measuring 28.0 cm in diameter and 45.5 cm in height for 5 min. The cylinder was filled with 30 cm of water (34 ± 1 °C) to ensure that animals could not touch the bottom of the container with their hind paws or tails, as described in previous studies using putative genetic rat models of depression, WKY and FSL (Malkesman et al., 2006, 2008). It should be noted that the water temperature is different from standard protocols (Slattery and Cryan, 2012). Fresh water was used for each FST in every animal. Immobility was defined as no additional activity other than that required to keep the head above water. Most behavioral tests assessing the antidepressant effects of drugs use a model in which rats undergo a 15 min pretest 18–24 h before the actual test (Porsolt et al., 1977; Overstreet et al., 2005). However, WKY rats and other genetically selected models of depression (e.g. Flinders sensitive line (FSL) and Fawn-Hooded rats) have the advantage of exhibiting depressive-like characteristics without the need for a pretest (Overstreet et al., 2005; Tizabi et al., 1999, Tizabi et al., 2000 and Tizabi et al., 2009).

2.6. Measurement of locomotor activity

The number of horizontal and vertical (or rearing) movements was determined as activity counts using an infrared sensor (NS-AS01; Neuroscience, Japan) for 24 h.

2.7. Sucrose preference test

The sucrose preference test is a measure of the hedonic state of an animal or the ability to experience pleasure (De la Garza, 2005; Kalueff et al., 2006; Jones et al., 2008). Impairment in this test is a fundamental feature of clinical depression (American Psychiatric Association, 2000). The animals were tested for 4 days, with a free choice between two bottles, one with 1% sucrose in tap water and the other with tap water alone. To eliminate potential side preferences, the positions of the bottles were switched after 2 days. Consumption of water, sucrose solution and total liquid intake (ml) was assessed daily for 4 days. The preference for sucrose was calculated as a percentage of the consumed sucrose solution out of the total volume of consumed liquid.

2.8. Novelty-suppressed feeding test (NSFT)

The NSFT was performed as previously described (Bodnoff et al., 1988; Santarelli and Saxe, 2003; Zhang et al., 2010) with modifications. Briefly, the test was conducted in an open field box measured 55 × 45 × 40 cm^3. All food was removed from the home cage 24 h before the test. A single pellet of food was placed on a white round paper (diameter = 6.25 cm) in the center of the open field box. During the test, the rat was put at the corner of the test box for 5 min to measure the latency to bite the food pellets. The rat was then put back in its cage with food. The amount of food that the rat ate during the next 5-min period was measured.

2.9. Statistical analysis

All values are expressed as a percentage of the average of three baseline samples. The average concentration of three stable baseline samples was set at 100%. Repeated measures one-way analysis of variance (ANOVA) and a Dunnett multiple comparison test for post-hoc determination were performed using the SAS mixed procedure (SAS Institute, Cary, N.C., USA). One-way ANOVA and a Scheffe multiple comparison test for post-hoc determination were used for comparison of naïve experimental groups. Repeated measures two-way ANOVA and a Bonferroni multiple comparison test for post-hoc determination were used for comparison between experimental groups (GraphPad Prism, GraphPad Software, San Diego, CA, USA). The level of significance was set at $p < 0.05$. Details of statistical data are listed in Supplementary Table 1.

3. Results

3.1. Depression- and anxiety-like behaviors with or without repeated ESCIT treatment

3.1.1. FST in naïve rats

Immobility in the forced swim test was analyzed to examine depression-like behavior. WKY rats showed a markedly longer duration of immobility in comparison with Wistar rats (Fig. 1A). Locomotor activities assessed throughout the 12-h light/dark cycle were similar in Wistar and

WKY rats for both light and dark cycles (Supplementary Fig. 2), suggesting that the longer duration of immobility in WKY rats is not due to a difference in basal motor activity between the two strains.

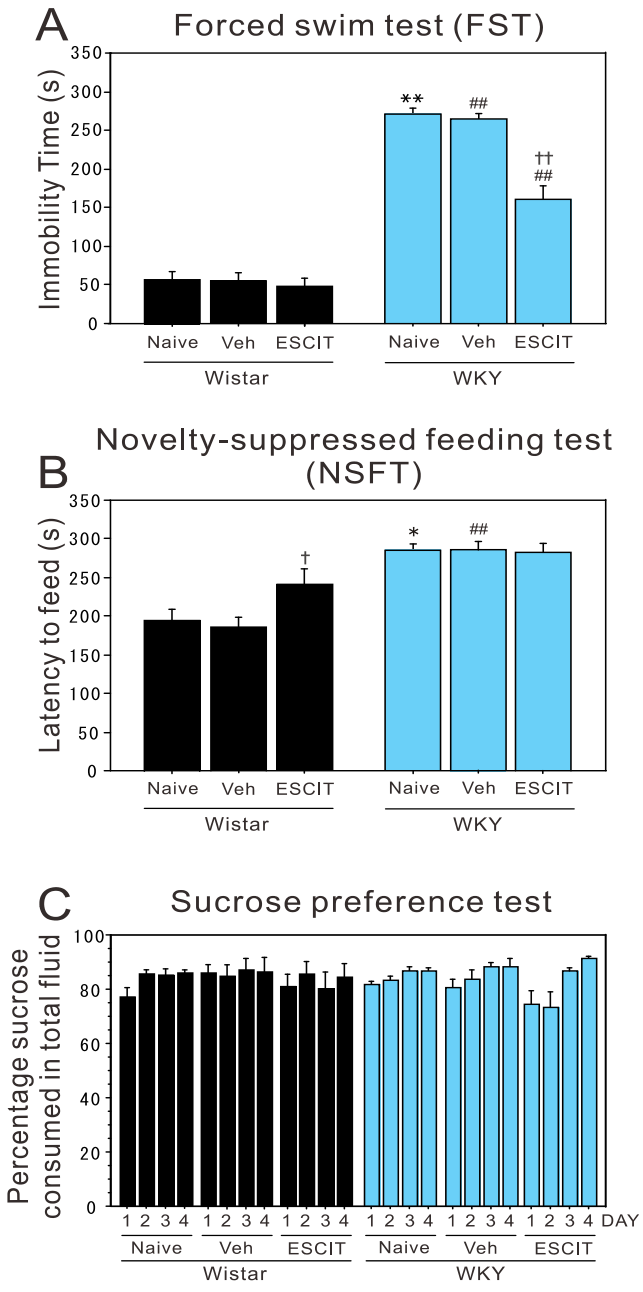

Fig. 1.

Depression- and anxiety-related behaviors in Wistar and WKY rats. Immobility time in the forced swim test (A), latency to feed in the novelty-suppressed feeding test (B), and sucrose solution consumption (% of total liquid

consumption) in the sucrose preference test (C) in naïve rats and rats treated with vehicle (Veh; saline 0.2 ml s.c.) or escitalopram (ESCIT; 5 mg/kg s.c.) for 14 days. Data are expressed as mean ± S.E.M. (n = 10 rats in each group). *p < 0.05, **p < 0.01 vs. naïve Wistar; ##p < 0.01 vs. corresponding Wistar; †p < 0.05 vs. vehicle-treated Wistar; ††p < 0.01 vs. vehicle-treated WKY.

3.1.2. FST in ESCIT or vehicle-treated rats

To examine the effects of repeated ESCIT treatment, rats received vehicle (saline; 0.2 ml s.c.) or ESCIT (5 mg/kg/day s.c.) for 14 days. Repeated treatment with vehicle did not affect immobility in each strain. Repeated treatment with ESCIT significantly reduced the duration of immobility in WKY rats, but had no effect in Wistar rats.

3.1.3. NFST in naïve rats

Latency to feed in a novel environment in the NSFT gives an indication of anxiety levels and was significantly longer in WKY rats compared with Wistar rats (Fig. 1B).

3.1.4. NSFT in ESCIT or vehicle-treated rats

Vehicle-treated Wistar and WKY rats showed a similar latency to naïve rats in each strain. Repeated treatment with ESCIT increased the latency to feed in Wistar rats, but not in WKY rats.

3.1.5. Sucrose preference test in naïve rats

The sucrose preference test for anhedonia assesses loss of appetitive motivation, which is a core symptom of depression. The sucrose preference (% of total liquid consumption) determined over 4 consecutive days was similar in Wistar and WKY rats (Fig. 1C). However, WKY rats had significantly lower food and water intakes (Supplementary Fig. 3A–D) and sucrose consumption (data not shown) compared to Wistar rats. In microdialysis experiments, Wistar rats at 9 weeks of age and WKY rats at 11 weeks of age were used, because the brain coordinates of the DRN and PFC were anatomically similar across the strains when body weights were matched (Wistar 309.44 ± 4.67 g; WKY 303.71 ± 2.72 g) (Supplementary Fig. 1) and 5-HT system in Wistar rats was found to be similar during aging from 9 to 11 weeks (Supplementary Fig. 3E, F). However,

the possible impact of different ages, such as difference of neuronal development or 5-HT system, cannot be completely ruled out.

3.1.6. Sucrose preference test in ESCIT or vehicle-treated rats

Vehicle-treated Wistar and WKY rats showed similar sucrose preference to naïve rats in each strain. Repeated treatment with ESCIT had no effect in either strain.

3.2. Extracellular 5-HT levels with or without repeated ESCIT treatment

The basal levels of 5-HT in the DRN and PFC were determined by microdialysis in Wistar and WKY rats (Fig. 2).

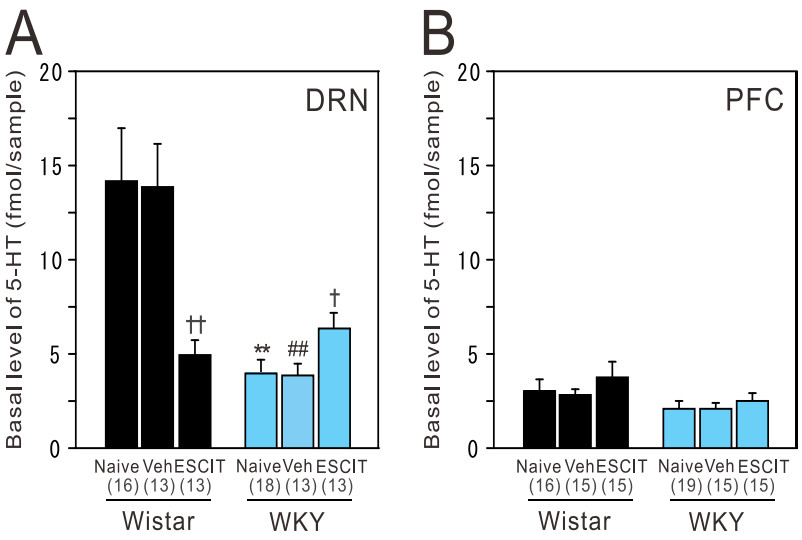

Fig. 2.

Extracellular 5-HT contents in dialysates from the DRN (A) and PFC (B) in Wistar and WKY rats. Basal values of extracellular 5-HT were determined by microdialysis in naïve rats and rats treated with vehicle (Veh) or ESCIT for 14 days. Data are expressed as mean ± S.E.M. (number of rats shown in parentheses). $^{**}p < 0.01$ vs. naïve Wistar; $^{\#\#}p < 0.001$ vs. vehicle-treated Wistar; $^{\dagger\dagger}p < 0.01$ vs. vehicle-treated Wistar; $^{\dagger}p < 0.05$ vs. vehicle-treated WKY

3.2.1. Naïve rats

Basal 5-HT was significantly lower in WKY rats than in Wistar rats in the DRN (3.93 ± 0.77 vs. 14.16 ± 2.80 fmol/sample) and slightly (but not significantly) lower in the PFC (2.12 ± 0.36 vs. 3.02 ± 0.57 fmol/sample).

3.2.2. ESCIT or vehicle-treated rats

Vehicle treatment did not affect 5-HT in the DRN or PFC in either strain. In the DRN, repeated treatment with ESCIT decreased the 5-HT level in Wistar rats, but increased this level in WKY rats. In the PFC of vehicle and ESCIT-treated rats, 5-HT in WKY rats was slightly lower than that in Wistar rats (strain effect, $p < 0.05$) and ESCIT treatment had a significant effect (treatment effect, $p < 0.05$).

3.3. Effects of systemic administration of ESCIT on 5-HT levels

3.3.1. Naïve rats

Systemic administration of ESCIT (5 mg/kg s.c.) induced increases in 5-HT levels in the DRN (Fig. 3A) and PFC (Fig. 3B) in Wistar and WKY rats, but the increases in the DRN and PFC were both smaller in WKY rats.

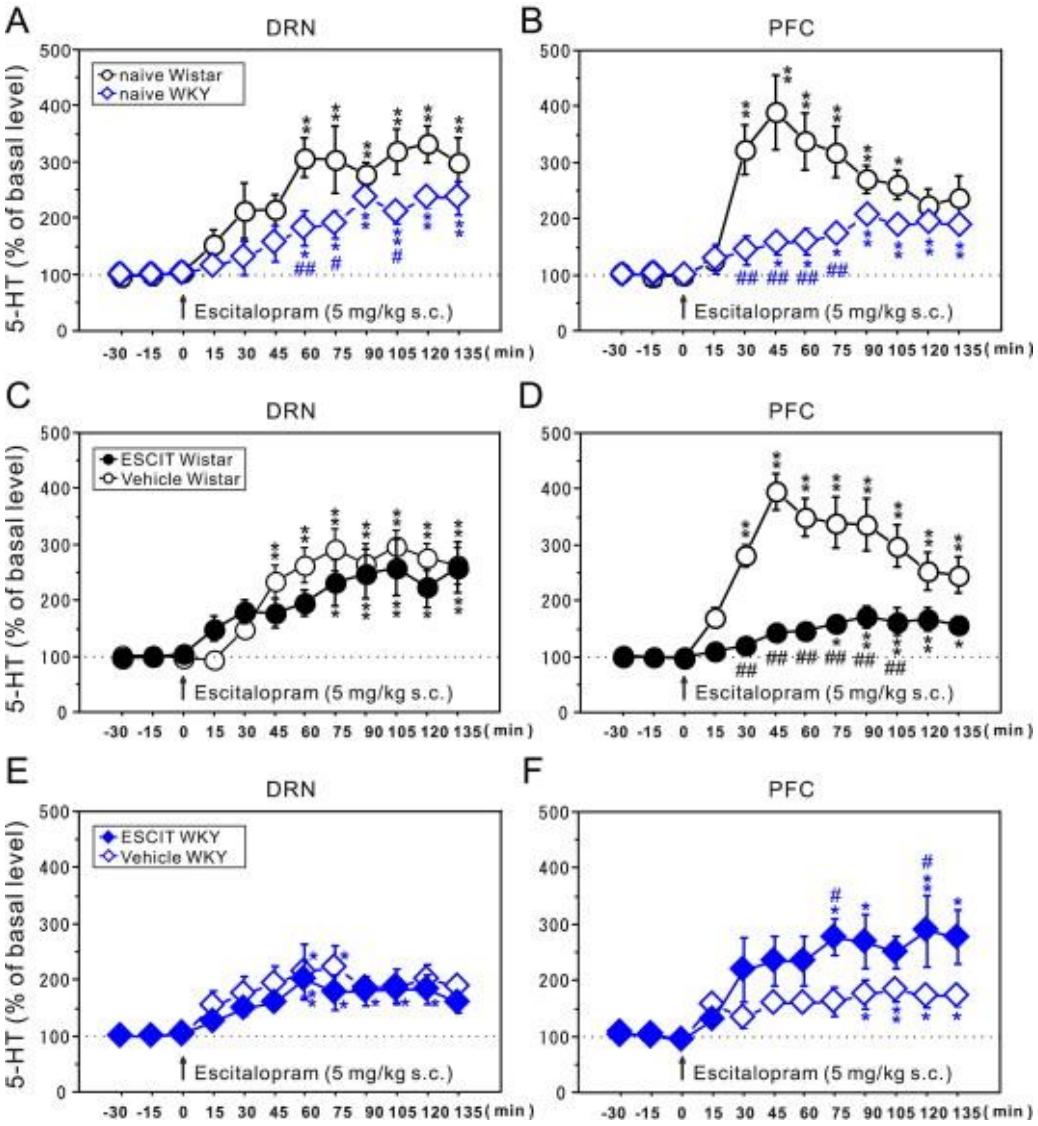

Fig. 3.

Effects of systemic ESCIT administration (5 mg/kg s.c.) on 5-HT in dialysates from the DRN and PFC in Wistar and WKY rats. (A, B) 5-HT contents in the DRN (A) and PFC (B) in naïve Wistar (black open circles) and WKY (blue open squares) rats. (C, D) 5-HT contents in the DRN (C) and PFC (D) in Wistar rats treated with vehicle (open circles) or ESCIT (closed circles). (E, F) 5-HT contents in the DRN (E) and PFC (F) in WKY rats treated with vehicle (blue open squares) or ESCIT (blue closed squares). All values are calculated as a percentage of basal values within the same group. Data are expressed as mean ± S.E.M. ($n = 5$ rats in each group). *$p < 0.05$, **$p < 0.01$ vs. basal values; #$p < 0.05$, ##$p < 0.001$ vs. naïve Wistar (A, B), vehicle-treated Wistar (C, D), or vehicle-treated WKY (E, F). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3.2. ESCIT or vehicle-treated rats

In the DRN, repeated treatment with ESCIT did not affect the relative increases in 5-HT (% of basal value in each experimental group) in response to systemic administration of ESCIT (5 mg/kg s.c.) in Wistar (Fig. 3C) or WKY (Fig. 3E) rats. Since the basal levels of 5-HT in the DRN in naïve (and vehicle-treated) Wistar (100%) and WKY (30% of naïve Wistar) rats were changed by repeated ESCIT treatment to 35% and 45% of those in naïve Wistar rats, respectively, the effects of systemic ESCIT administration were re-evaluated by normalizing to the 5-HT levels to naïve Wistar rats (100% control) (Supplementary Fig. 5A). This re-evaluation showed that the responses of 5-HT in the DRN to systemic ESCIT administration in WKY rats are similar to those in Wistar rats after repeated treatment with ESCIT.

In the PFC, repeated ESCIT treatment largely attenuated the increase in the 5-HT level induced by systemic ESCIT administration in Wistar rats (Fig. 3D). In contrast, in WKY rats, ESCIT-induced increase in 5-HT was enhanced (Fig. 3F). Since the basal levels of 5-HT in the PFC were relatively constant with or without repeated ESCIT treatment in both strains, the responses of 5-HT in the PFC to systemic ESCIT become comparable in Wistar and WKY rats after repeated ESCIT (Supplementary Fig. 5B).

3.4. Effects of local infusion of ESCIT into the DRN on 5-HT levels

3.4.1. Naïve rats

Local infusion of ESCIT at concentrations of 0.1 μM (Fig. 4A) and 1 μM (Supplementary Fig. 4A) into the DRN caused similar relative increases in 5-HT in the DRN in Wistar and WKY rats. In Wistar rats, local infusion of ESCIT in the DRN simultaneously decreased 5-HT in the PFC (Fig. 4B, Supplementary Fig. 4B). The decreases in 5-HT in the PFC were similar in Wistar and WKY rats. These results indicate that 5-HT release in the PFC in Wistar rats is regulated by serotonergic autoinhibition of DRN neurons (Sharp et al., 2007; Aso et al., 2009) and that this mechanism is functional in WKY rats.

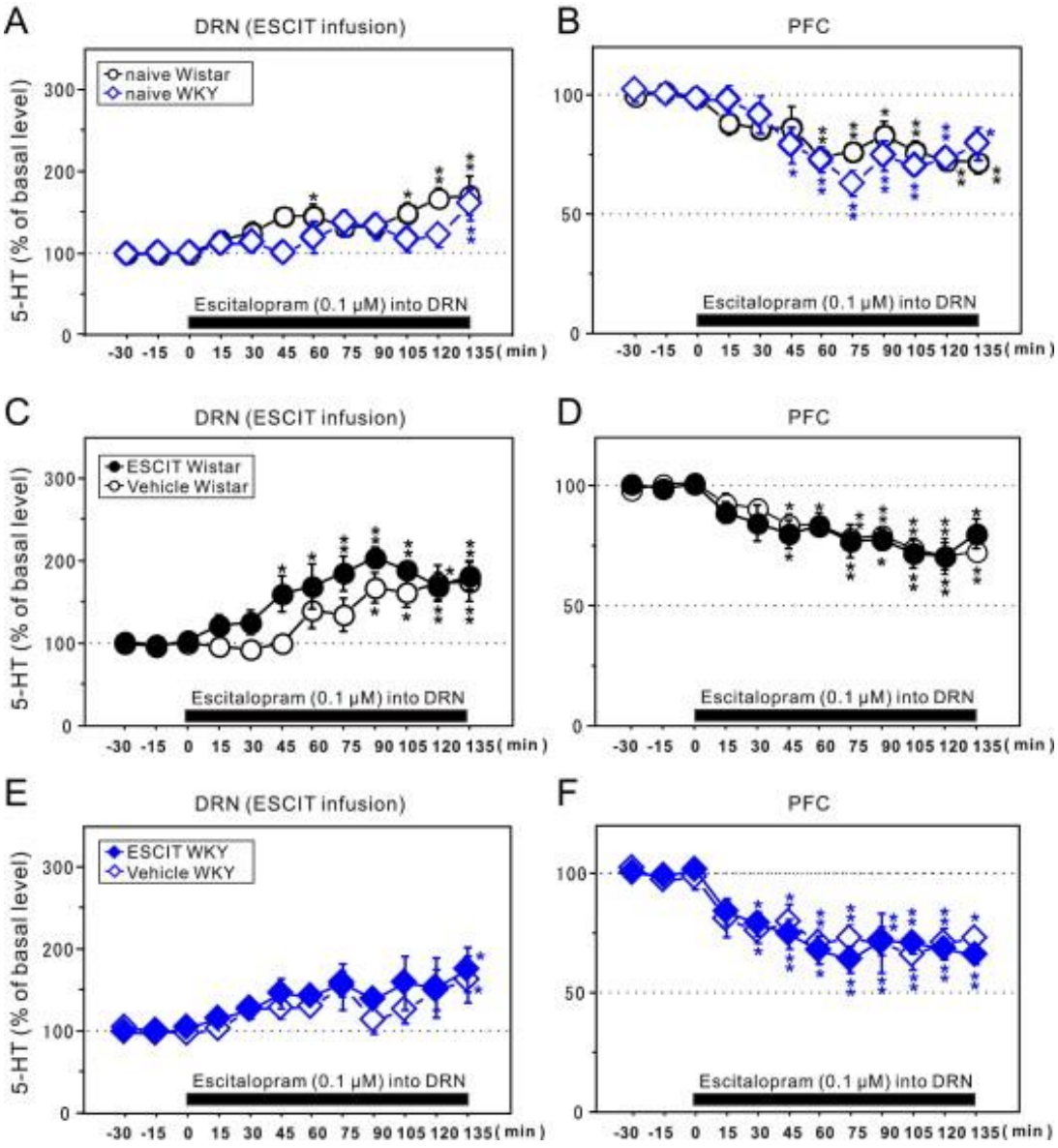

Fig. 4.

Effects of local infusion of ESCIT (0.1 μM) into the DRN on 5-HT in dialysates from the DRN and PFC in Wistar and WKY rats. (A, B) 5-HT contents in the DRN (A) and PFC (B) in naïve Wistar (black open circles) and WKY (blue open squares) rats. (C, D) 5-HT contents in the DRN (C) and PFC (D) in Wistar rats treated with vehicle (open circles) or ESCIT (closed circles). (E, F) 5-HT contents in the DRN (E) and PFC (F) in WKY rats treated with vehicle (blue open squares) or ESCIT (blue closed squares). All values are calculated as a percentage of basal values within the same group. Data are expressed as mean ± S.E.M ($n = 4$ rats in each naïve group (A, B), $n = 5$ in each treated group (C–F)). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. basal values. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4.2. ESCIT or vehicle-treated rats

In the DRN, repeated treatment with ESCIT did not affect the relative increases in 5-HT induced by local infusion of ESCIT into the DRN in Wistar (Fig. 4C) or WKY (Fig. 4E) rats. When the increases were normalized to the basal 5-HT level in naïve (or vehicle-treated) Wistar rats (100%), the increases in absolute 5-HT levels in response to local infusion of ESCIT in WKY rats were smaller than those in Wistar rats due to the low basal levels (30% of naïve Wistar rats), but became comparable to Wistar rats after repeated ESCIT treatment due to the similar basal levels (Supplementary Fig. 5A).

In the PFC, the decreases in 5-HT caused by local infusion of ESCIT into the DRN were not affected by repeated ESCIT treatment in Wistar or WKY rats (Fig. 4D, F). Thus, repeated ESCIT treatment did not modulate serotonergic autoinhibition of 5-HT release in the PFC.

3.5. Effects of local infusion of ESCIT into the PFC on 5-HT levels

3.5.1. Naïve rats

Local infusion of ESCIT at concentrations of 0.1 μM (Fig. 5A) and 1 μM (Fig. 5B) into the PFC induced larger increases in 5-HT in the PFC in Wistar rats than in WKY rats. In Wistar rats, local infusion of ESCIT at 0.1 and 1 μM into the PFC simultaneously decreased the 5-HT level in the DRN (Fig. 5C, D). In WKY rats, ESCIT infusion at the high concentration (1 μM), but not at the low concentration (0.1 μM), decreased 5-HT in the DRN (Fig. 5K, L). These results suggest that the activity of DRN 5-HT neurons and 5-HT release are regulated by feedback inhibition mediated through activation of postsynaptic 5-HT receptors in the PFC (Sharp et al., 2007; Celada et al., 2001) and that feedback inhibition of DRN 5-HT neurons is attenuated in WKY rats.

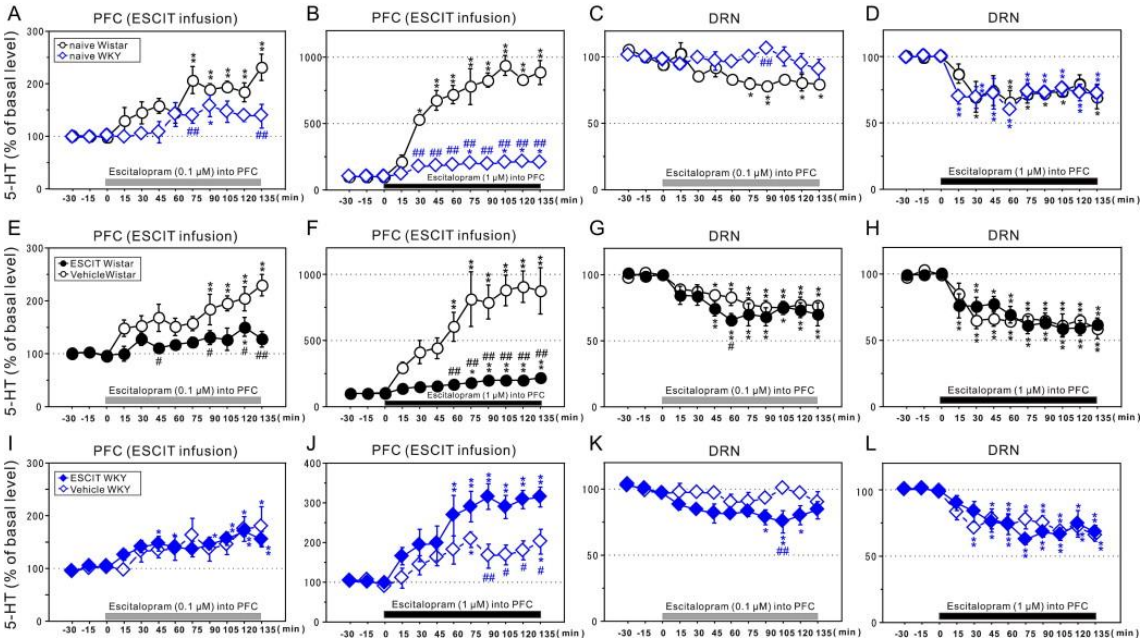

Fig. 5.

Effects of local infusion of ESCIT (0.1 and 1.0 μM indicated as gray bars and black bars, respectively) into the PFC on 5-HT in dialysates from the PFC and DRN in Wistar and WKY rats. (A–D) 5-HT contents in PFC (A, B) and DRN (C, D) in naïve Wistar (black open circles) and WKY (blue open squares) rats. (E–H) 5-HT contents in the PFC (E, F) and DRN (G, H) in Wistar rats treated with vehicle (open circles) or ESCIT (closed circles). (I–L) 5-HT contents in the PFC (I, J) and DRN (K, L) in WKY rats treated with vehicle (blue open squares) or ESCIT (blue closed squares). All values are calculated as a percentage of basal values within the same group. Data are expressed as mean ± S.E.M (n = 4 rats in each group for (A-D, H, J, L), n = 5 in each group for (G, I, K), n = 6 in each group for (E, F)). *p < 0.05, **p < 0.01 vs. basal values; #p < 0.05, ##p < 0.001 vs. naïve Wistar (A–C), vehicle-treated Wistar (E–G), or vehicle-treated WKY (J,K). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5.2. ESCIT or vehicle-treated rats

Repeated treatment with ESCIT strongly suppressed the relative increases in 5-HT induced by local infusion of ESCIT (0.1 and 1 μM) into the PFC in Wistar rats (Fig. 5E, F), but enhanced the relative increases induced by local infusion of ESCIT at 1 μM, but not at 0.1 μM, into the PFC in WKY rats (Fig. 5I, J).

Repeated treatment with ESCIT did not affect the decreases in 5-HT in the DRN induced by local infusion of ESCIT into the PFC in Wistar rats (Fig. 5G and H), but enhanced the decrease in

5-HT induced by ESCIT infusion at the low concentration (0.1 μM) in WKY rats (Fig. 5K). These results suggest that repeated ESCIT treatment enhances feedback inhibition of DRN 5-HT neurons in WKY rats.

4. Discussion

In this study, extracellular 5-HT levels were analyzed simultaneously in two brain regions, the DRN and PFC, in WKY and Wistar rats. In the DRN, WKY rats showed a low basal level of 5-HT and a small absolute increase in 5-HT in response to ESCIT in the DRN. In the PFC, despite the similar basal levels of 5-HT, WKY rats showed small absolute and relative increases in 5-HT in response to ESCIT in the PFC. Feedback inhibition of DRN 5-HT neurons by ESCIT in the PFC (through postsynaptic 5-HT receptors in the PFC) was also attenuated in WKY rats. After chronic treatment with ESCIT, the low basal level of 5-HT in the DRN, the small increases of 5-HT in response to ESCIT in the DRN and PFC, and feedback inhibition of DRN 5-HT neurons were upregulated in WKY rats (Fig. 6, Supplementary Fig. 5). The effect of chronic ESCIT in WKY rats was opposite to that in Wistar rats, in which chronic ESCIT induced downregulation of the 5-HT system. The differential adaptation of the DRN-PFC 5-HT system to chronic ESCIT observed in WKY rats may play a role in the therapeutic effect of antidepressants on depression-like behavior.

4.1. 5-HT system in the DRN and its response to ESCIT in WKY rats

WKY rats had a low basal level of extracellular 5-HT in the DRN, in agreement with previous reports showing a low tissue content of 5-HT in the DRN of WKY rats compared with SD rats (Nguyen et al., 2009; Scholl et al., 2010) and low expression of mRNA for TPH2, a rate-limiting enzyme for 5-HT synthesis, in DRN 5-HT neurons (Lemos et al., 2011). The excitability of DRN 5-HT neurons has also been reported to be decreased in WKY rats (Lemos et al., 2011). These findings suggest that TPH2-mediated 5-HT synthesis and release of 5-HT from somata, dendrites and axon collaterals of DRN 5-HT neurons are decreased in WKY rats. Interestingly, low basal levels of 5-HT in the DRN in adulthood have also been found in two other rat models of depression, in which rats receive chronic mild stress or a tricyclic antidepressant (clomipramine) in the neonatal period (Yang et al., 2008).

In comparison with Wistar rats, absolute increases in 5-HT induced by ESCIT in the DRN were attenuated in WKY rats due to the low basal level of 5-HT (Supplementary Fig. 5A). However,

the relative increase in 5-HT over the basal level in response to ESCIT in WKY rats was similar to that in Wistar rats, suggesting that the function of 5-HT transporters, such as the sensitivity of the high-affinity 5-HT transporter to ESCIT, may be maintained in WKY rats. Taken together, these results indicate that low activity of DRN 5-HT neurons contributes to the pathophysiology of depression (Hirschfeld, 2000).

4.2. 5-HT system in the PFC and its response to ESCIT in WKY rats

In contrast to the DRN, WKY and Wistar rats had similar basal levels of 5-HT in the PFC. Our findings are consistent with previous measurements of tissue 5-HT contents in the PFC (De La Garza and Mahoney, 2004; Scholl et al., 2010). However, the increase of 5-HT induced by ESCIT in the PFC was extremely low in WKY rats (Supplementary Fig. 5B). The mechanisms underlying the low response of 5-HT in the PFC to ESCIT are unknown. It is possible that 5-HT turnover at axon terminals is reduced since activity of DRN 5HT neurons is low. Alternatively, there may be functional alterations of 5-HT transporters in the PFC, and it is of note that decreased densities of 5-HT transporters in the cortex and hippocampus have been reported in WKY rats (Paré and Tejani-Butt, 1996). The contributions of 5-HT_{1A} autoreceptors (Ceglia et al., 2004; Kosofsky and Molliver, 1987) and 5-HT_{2C} receptors, which are involved in regulation of extracellular 5-HT in the PFC under SSRI-treated conditions (Cremers et al., 2004; Sotty et al., 2009), need to be evaluated.

4.3. Feedback inhibition of DRN 5-HT neurons by postsynaptic 5-HT receptors in the PFC

Intracortical infusion of ESCIT resulted in reduction of extracellular 5-HT in the DRN. The findings fit to the model of feedback inhibition of DRN 5-HT neurons by postsynaptic 5-HT receptors in the PFC (Sotty et al., 2009). Activation of these receptors on non-5-HT neurons in the PFC modulates neural inputs to DRN 5-HT neurons [e.g. stimulation of GABAergic interneurons (via $5\text{-HT}_{2A/2C}$ and 5-HT_{3} receptors) connected to pyramidal neurons (Liu et al., 2007; Puig et al., 2004), inhibition of pyramidal neurons (via 5-HT_{1A} receptors) (Yuen et al., 2008), or stimulation of pyramidal neurons (via 5-HT_{2A} receptors) connected to GABAergic interneurons in the DRN (Sharp et al., 2007)] (Fig. 6), resulting in inhibition of DRN 5-HT neurons and 5-HT release (Puig and Gulledge, 2011; Romero et al., 1996). In the present study, feedback inhibition of 5-HT release in the DRN was found to be attenuated in WKY rats (Fig. 6). This attenuation

may be explained by the small increases of 5-HT in the PFC in response to ESCIT or by alteration of postsynaptic 5-HT receptor pathways. Postsynaptic 5-HT receptor-mediated feedback inhibition of DRN 5-HT neurons may be a mechanism to control excessive activation of DRN 5-HT neurons associated with uncontrollable stress (Amat et al., 2005; Puig and Gulledge, 2011; Romero et al., 1996), which is consistently reported as defective in WKY rats (Braw et al., 2008; Jiao et al., 2011).

Feedback inhibition of DRN 5-HT neurons was enhanced by chronic ESCIT in WKY rats (Fig. 6). Since basal 5-HT in the DRN of WKY rats increased after chronic ESCIT, feedback inhibition of DRN 5-HT neurons may not have a tonic effect, but may function only when 5-HT in the PFC is increased excessively under certain conditions, such as psychological stress and stimulation of emotional processes (Miyata et al., 2007; Robbins and Roberts, 2007; Roy et al., 2006). This adaptation might be involved in the improvement of stress-controllability in WKY rats (Amat et al., 2005).

4.4. Upregulation of 5-HT system by chronic ESCIT in WKY rats and its contribution to antidepressant effects

In WKY rats, the activity of the DRN-PFC 5-HT system was found to be low and chronic ESCIT upregulated this system. In contrast, chronic ESCIT downregulated this system in control Wistar rats. Thus, an upregulatory effect of chronic ESCIT on the DRN-PFC 5-HT system was observed only in the depressed WKY animal model and may have been associated with improvement of depressed behavior, such as that observed in the forced swim test. Similar changes of 5-HT in the DRN have been found after treatment in other animal models of depression. For example, basal 5-HT in the DRN is low in rats with depression after chronic mild stress, and lateral habenula lesions increase 5-HT and improve depressive behavior (Yang et al., 2008). These findings indicate that upregulation of the 5-HT level in the DRN may underlie the therapeutic effects of antidepressants in a depressive state.

In the depressive state with 5-HT deficiency, chronic treatment with antidepressants including SSRIs is generally thought to increase 5-HT levels and induce desensitization of inhibitory 5-HT_{1A} autoreceptors (Blier, 2001). This proposed model of antidepressant action fits with the chronic ESCIT-induced upregulation in the DRN-PFC 5-HT system in WKY rats. These rats are a genetic animal model of depression with a feature of low activity of the DRN-PFC 5-HT system. It is conceivable that responses to chronic ESCIT are determined by the tone of DRN 5-HT neurons:

upregulation when hypofunctional and downregulation when normofunctional. The 5-HT deficiency in human depression is supported with findings of polymorphisms in the TPH2 gene that result in reduction of 5-HT synthesis (Jacobsen et al., 2012). Among TPH2 variants, the TPH2 $^{Arg}439^{His}$ knock-in mouse exhibits depression-like behavior related to 5-HT deficiency (Jacobsen et al., 2012). Identification of adaptive mechanisms to chronic antidepressants in the hyposerotonergic/depressive state in WKY rats will be extremely important for development of new therapeutic strategies for depression with 5-HT deficiency.

Emerging preclinical studies of the therapeutic action of antidepressants are using 'non-depressed' animals and/or 'depressed' animals, but most do not differentiate between these types of animals in interpreting the action of antidepressants. However, the results in the current study in WKY and Wistar rats with different serotonergic states show opposite responses to chronic ESCIT and suggest the importance of use of the optimal animal model. In this regard, the WKY rat is a useful model of depression with low activity of the DRN-PFC 5-HT system.

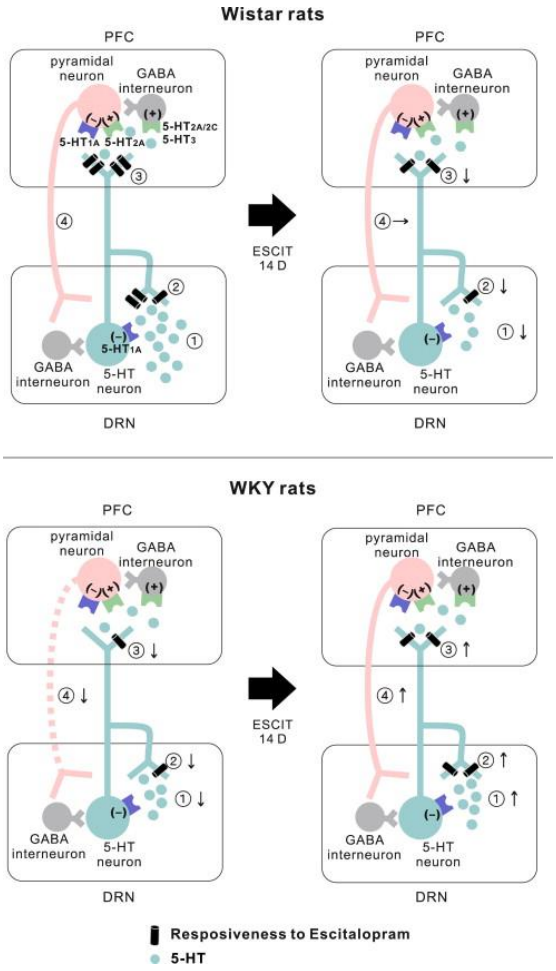

Fig. 6.

Schematic representation of the DRN-PFC 5-HT system in Wistar (upper left panel) and WKY (lower left panel) rats and their adaptation to chronic treatment with ESCIT (right panels). Basal 5-HT release (indicated by blue circles) in the DRN (1) and the responses of 5-HT to ESCIT in the DRN (2) and PFC (3) in WKY rats (indicated by black rods) are lower than those in Wistar rats. In the PFC, basal 5-HT release and inhibition of 5-HT release in the PFC by ESCIT in the DRN [e.g. through inhibition of DRN 5-HT neurons via 5-HT_{1A} autoreceptors (Barnes and Sharp, 1999)] are equivalent in the two strains. Feedback inhibition of DRN 5-HT neurons by ESCIT in the PFC [e.g. through stimulation of GABAergic interneurons (via $5\text{-HT}_{2A/2C}$ and 5-HT_{3} receptors) connected to pyramidal neurons (Liu et al., 2007; Puig et al., 2004), inhibition of pyramidal neurons (via 5-HT_{1A} receptors) (Yuen et al., 2008), or stimulation of pyramidal neurons (via 5-HT_{2A} receptors) connected to GABAergic interneurons in the DRN (Sharp et al., 2007)] (4) is attenuated in WKY rats (pink dotted line). Chronic ESCIT upregulates basal 5-HT release in the DRN (1), responses of 5-HT to ESCIT in the DRN (2) and PFC (3), and feedback inhibition of DRN 5-HT neurons (4) in WKY rats (Liu et al., 2007). In Wistar rats, the effects of chronic ESCIT are opposite, inducing downregulation of basal 5-HT release in the DRN (1) and responses of 5-HT to ESCIT (2, 3).

5. Conclusion

The WKY rat is a genetic animal model of depression with dysfunction of the DRN-PFC 5-HT system, which resembles a type of human depression with 5-HT deficiency (Jacobsen et al., 2012). In this study in WKY rats, we showed that chronic treatment with an antidepressant upregulated the DRN-PFC 5-HT system, which had low activity before treatment, and that this upregulation was dependent on the hyposerotonergic state. These results indicate that elucidation of the mechanisms through which antidepressants regulate the 5-HT system in WKY rats is critical for understanding the action of antidepressants.

Disclosure

The authors declare no conflict of interest.

Acknowledgments

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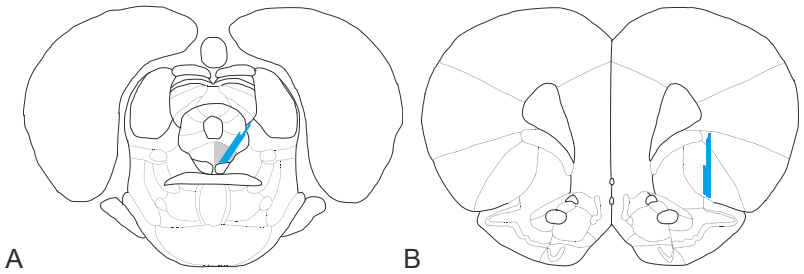
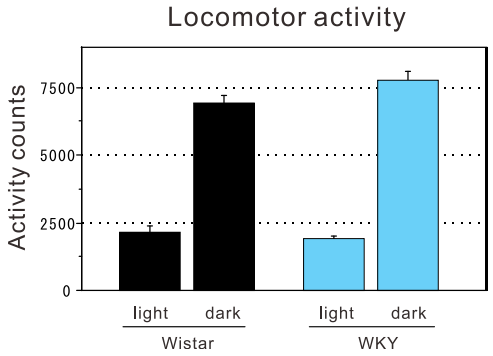
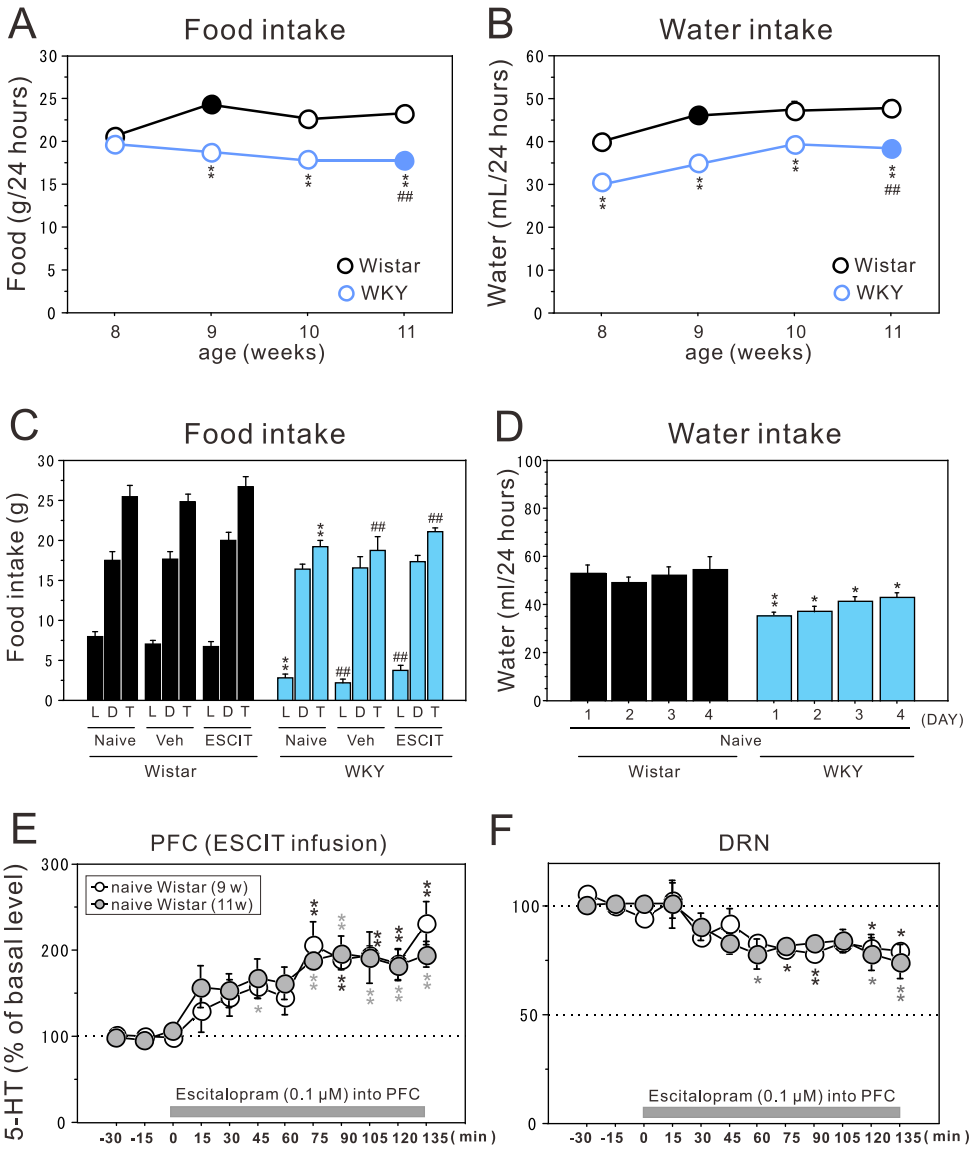


Supplementary Fig. 1. Representative coronal diagrams of microdialysis probe placements for DRN (A) and PFC (B). Blue lines indicate dialysis probe tracks from Wistar and WKY rats, if more than one probe was located in the same place only one representative line is depicted. Since there was no regional difference of the DRN and PFC between Wistar and WKY, dialysis probe tracks from both strains were indicated together in the same coronal section of Wistar. Distance relative to bregma (mm) is -7.8 (A) and 3.2 (B). Figures are adapted from Paxinos and Watson (2007). In all the experiments, the microdialysis probe covered the DRN and PFC.

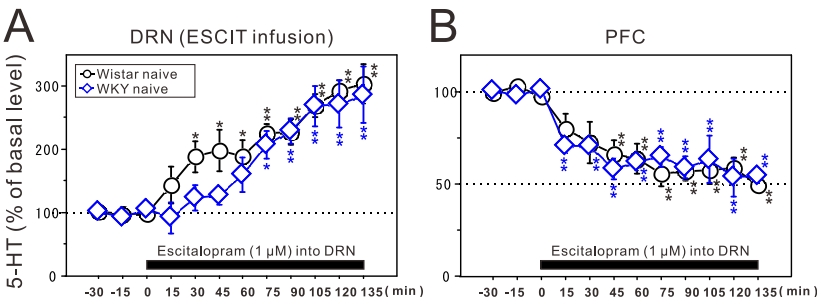


Supplementary Fig. 2. Locomotor activities during a dark and light cycle (12h/12h) in naïve Wistar and WKY rats. Data are expressed as mean ± S.E.M (n=8 rats in each strain).

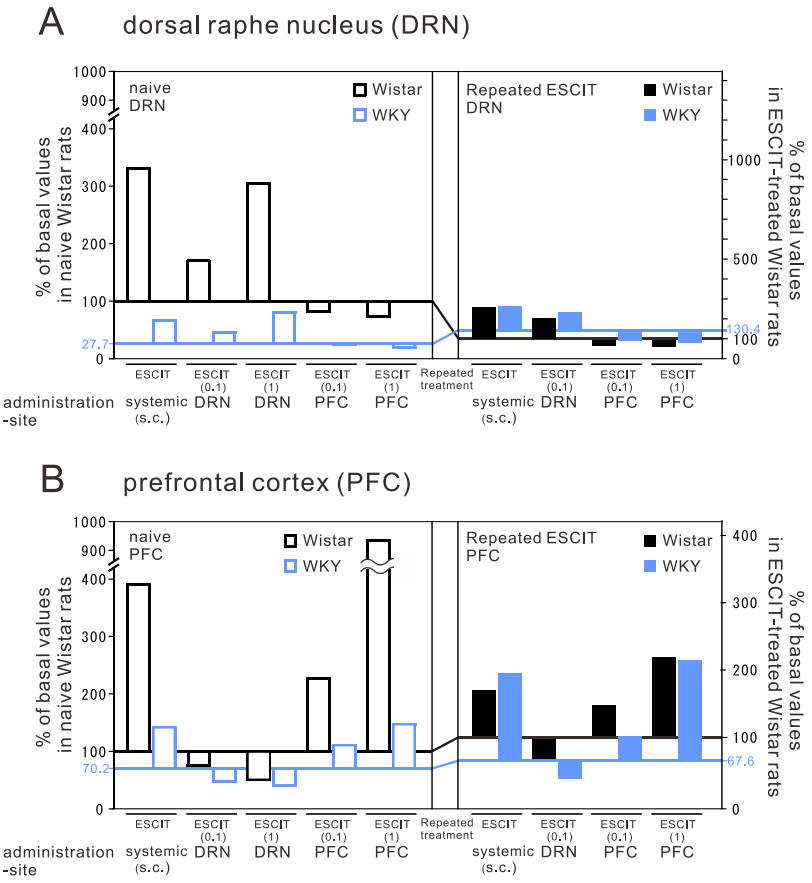


Supplementary Fig. 3. (A, B) Food (A) and water (B) intakes for 24 h in naïve Wistar and WKY rats from 8 to 11 weeks of age. Data are expressed as means ± S.E.M. (n=10 rats for each strain). ***p*< 0.01 vs. Wistar group at corresponding ages; ## *p*<0.001 vs. Wistar group at 9 weeks of age, as indicated with closed circles. (C) Food intake was recorded for the light (L) and dark (D) cycle (12h/12h) and total (T) in Wistar rats at 9 weeks of age (black bars) and WKY rats at 11 weeks of age (blue bars). Rats were untreated (naïve) or treated with vehicle (Veh) or ESCIT for 14 days. Data are expressed as mean ± S.E.M. (n=10 rats in each group). ***p*< 0.01 vs. naïve Wistar rats at corresponding phases; ## *p*< 0.001 vs. Wistar rats treated with vehicle or ESCIT at corresponding phases. (D) Water intake was recorded for 4 consecutive days in naïve Wistar rats at 9 weeks of age and naïve WKY rats at 11 weeks of age. Data are expressed as means ± S.E.M. (n=10 rats in each group). **p*< 0.05, ***p*< 0.01 vs. Wistar rats on corresponding days. (E, F) Comparison of serotonergic system in naïve Wistar rats at 9 and 11 weeks of age. Basal levels of 5-HT at 9 and 11 weeks of age were similar in the DRN (16.32 ± 5.43 and 17.46 ±

3.22 fmol/sample) and the PFC (2.45 ± 0.35 and 2.84 ± 1.20 fmol/sample). Effects of local infusion of ESCIT (0.1 μM) into the PFC on the 5-HT in dialysates from the PFC (E) and DRN (F) were similar in Wistar rats at 9 and 11 weeks of age. Data are expressed as means ± SEM (n=4 rats in each group). *p< 0.05, **p< 0.01 vs. basal value.



Supplementary Fig. 4. Effects of local infusion of ESCIT (1.0 μM) into the DRN on 5-HT in dialysates from the DRN (A) and PFC (B) in naïve Wistar (black open circles) and WKY (blue open squares) rats. All values are calculated as a percentage of basal values within the same group. Data are expressed as mean ± SEM (n=4 rats in each naïve group). *p< 0.05, **p< 0.01 vs. basal values.



Supplementary Fig. 5. Overall comparison of the maximum effects of ESCIT administered systemically or locally into the DRN and PFC on 5-HT levels in the DRN (A) and PFC (B) of Wistar and WKY rats. The relative increases in 5-HT over basal in each condition were recalculated by setting the basal level of 5-HT in naïve Wistar rats as 100%. Left panels: 5-HT levels in naïve Wistar and WKY rats, which are essentially identical to 5-HT levels in vehicle-treated Wistar and WKY rats. Right panels: 5-HT levels in ESCIT-treated Wistar and WKY rats. Left Y-axis shows the percentage of basal values in naïve Wistar rats, and right Y-axis shows the percentage of basal values in ESCIT-treated Wistar rats.

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Set of data	Type of ANOVA	F	p
Figure 1			
A			
strain-treatment interaction	Two-way	$F_{(1,36)}=18.68$	0.0001
strain effect	Two-way	$F_{(1,36)}=176.8$	<0.0001
treatment effect	Two-way	$F_{(1,36)}=20.97$	<0.0001
naïve: Wistar, WKY	t-test		<0.0001
B			
strain-treatment interaction	Two-way	$F_{(1,36)}=2.527$	0.1207
strain effect	Two-way	$F_{(1,36)}=22.98$	<0.0001
treatment effect	Two-way	$F_{(1,36)}=1.527$	0.2246
naïve: Wistar, WKY	t-test		0.0008
C			
Wistar naïve vs. WKY naïve			
strain-treatment interaction	Two-way	$F_{(3,72)}=1.093$	0.3579
strain effect	Two-way	$F_{(1,72)}=0.03828$	0.8454
day effect	Two-way	$F_{(3,72)}=4.583$	0.0054
Wistar vehicle vs. Wistar ESCIT			
strain-treatment interaction	Two-way	$F_{(3,72)}=0.3130$	0.8159
strain effect	Two-way	$F_{(1,72)}=0.09840$	0.7547
day effect	Two-way	$F_{(3,72)}=0.1413$	0.9349
WKY vehicle vs. WKY ESCIT			
strain-treatment interaction	Two-way	$F_{(3,72)}=0.5457$	0.6526
strain effect	Two-way	$F_{(1,72)}=1.057$	0.3075
day effect	Two-way	$F_{(3,72)}=4.363$	0.007
Wistar naïve vs. Wistar vehicle			
strain-treatment interaction	Two-way	$F_{(3,72)}=0.5655$	0.6395
strain effect	Two-way	$F_{(1,72)}=1.337$	0.2514
day effect	Two-way	$F_{(3,72)}=0.5614$	0.6422
WKY naïve vs. WKY vehicle			
strain-treatment interaction	Two-way	$F_{(3,72)}=0.1749$	0.913
strain effect	Two-way	$F_{(1,72)}=0.4547$	0.5023
day effect	Two-way	$F_{(3,72)}=4.077$	0.0099
Figure 2			
A			
strain-treatment interaction	Two-way	$F_{(1,48)}=18.35$	<0.0001
strain effect	Two-way	$F_{(1,48)}=113.75$	0.0005
treatment effect	Two-way	$F_{(1,48)}=12.685$	0.1079
Naïve: Wistar, WKY	t-test		0.0008
B			
strain-treatment interaction	Two-way	$F_{(1,56)}=0.1124$	0.7387
strain effect	Two-way	$F_{(1,56)}=5.565$	0.0218
treatment effect	Two-way	$F_{(1,56)}=4.984$	0.0296
Naïve: Wistar, WKY	t-test		0.1779

Figure 3. Escitalopram s.c.

A			
Wistar naïve	One-way	$F_{(12,52)}=9.311$	<0.0001
WKY naïve	One-way	$F_{(12,52)}=8.065$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=2.150$	0.0341
strain effect	Two-way	$F_{(1,48)}=45.34$	<0.0001
time effect	Two-way	$F_{(11,48)}=11.97$	<0.0001
B			
Wistar naïve	One-way	$F_{(12,52)}=9.738$	<0.0001
WKY naïve	One-way	$F_{(12,52)}=8.128$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=6.614$	<0.0001
strain effect	Two-way	$F_{(1,48)}=69.42$	<0.0001
time effect	Two-way	$F_{(11,48)}=10.31$	<0.0001
C			
Wistar ESCIT	One-way	$F_{(12,52)}=4.419$	<0.0001
Wistar Vehicle	One-way	$F_{(12,52)}=14.573$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=0.3077$	0.9809
strain effect	Two-way	$F_{(1,48)}=0.1911$	0.664
time effect	Two-way	$F_{(11,48)}=37.21$	<0.0001
D			
Wistar ESCIT	One-way	$F_{(12,52)}=5.134$	<0.0001
Wistar vehicle	One-way	$F_{(12,52)}=14.015$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=5.912$	<0.0001
strain effect	Two-way	$F_{(1,48)}=129.7$	<0.0001
time effect	Two-way	$F_{(11,48)}=19.91$	<0.0001
E			
WKY ESCIT	One-way	$F_{(12,52)}=4.173$	0.0001
WKY vehicle	One-way	$F_{(12,52)}=3.266$	0.001
strain-time interaction	Two-way	$F_{(11,48)}=0.1764$	0.9982
strain effect	Two-way	$F_{(1,48)}=2.522$	0.1188
time effect	Two-way	$F_{(11,48)}=7.221$	<0.0001
F			
WKY ESCIT	One-way	$F_{(12,52)}=4.510$	<0.0001
WKY vehicle	One-way	$F_{(12,52)}=4.122$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=1.839$	0.073
strain effect	Two-way	$F_{(1,48)}=30.08$	<0.0001
time effect	Two-way	$F_{(11,48)}=6.459$	<0.0001

Figure 4. Escitalopram (0.1) into DRN

A			
Wistar naïve	One-way	$F_{(12,39)}=5.525$	<0.0001
WKY naïve	One-way	$F_{(12,39)}=2.351$	0.215
strain-time interaction	Two-way	$F_{(11,36)}=0.9450$	0.5108
strain effect	Two-way	$F_{(1,36)}=9.649$	0.0037
time effect	Two-way	$F_{(11,36)}=5.806$	<0.0001
B			

Wistar naïve	One-way	$F_{(12,39)}=7.055$	<0.0001
WKY naïve	One-way	$F_{(12,39)}=7.533$	<0.0001
strain-time interaction	Two-way	$F_{(11,36)}=1.006$	0.4607
strain effect	Two-way	$F_{(1,36)}=0.3811$	0.5409
time effect	Two-way	$F_{(11,36)}=11.21$	<0.0001
C			
Wistar ESCIT	One-way	$F_{(12,52)}=7.211$	<0.0001
Wistar vehicle	One-way	$F_{(12,52)}=5.706$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=0.6713$	0.758
strain effect	Two-way	$F_{(1,48)}=8.356$	0.0058
time effect	Two-way	$F_{(11,48)}=15.95$	<0.0001
D			
Wistar ESCIT	One-way	$F_{(12,52)}=5.503$	<0.0001
Wistar vehicle	One-way	$F_{(12,52)}=9.504$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=0.2930$	0.9842
strain effect	Two-way	$F_{(1,48)}=0.3533$	0.5551
time effect	Two-way	$F_{(11,48)}=12.34$	<0.0001
E			
WKY ESCIT	One-way	$F_{(12,52)}=2.316$	0.0186
WKY vehicle	One-way	$F_{(12,52)}=2.029$	0.0353
strain-time interaction	Two-way	$F_{(11,48)}=0.1450$	0.9993
strain effect	Two-way	$F_{(1,48)}=0.2962$	0.5888
time effect	Two-way	$F_{(11,48)}=5.154$	<0.0001
F			
WKY ESCIT	One-way	$F_{(12,52)}=7.588$	<0.0001
WKY vehicle	One-way	$F_{(12,52)}=5.578$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=0.2462$	0.9923
strain effect	Two-way	$F_{(1,48)}=0.3474$	0.5584
time effect	Two-way	$F_{(11,48)}=16.24$	<0.0001

Figure 5. Escitalopram (0.1) into PFC

A			
Wistar naïve	One-way	$F_{(12,39)}=8.055$	<0.0001
WKY naïve	One-way	$F_{(12,39)}=2.677$	0.0097
strain-time interaction	Two-way	$F_{(11,36)}=2.813$	0.0094
strain effect	Two-way	$F_{(1,36)}=44.16$	<0.0001
time effect	Two-way	$F_{(11,36)}=5.856$	<0.0001
B			
Wistar naïve	One-way	$F_{(12,39)}=32.524$	<0.0001
WKY naïve	One-way	$F_{(12,39)}=4.241$	0.0001
strain-time interaction	Two-way	$F_{(11,36)}=16.68$	<0.0001
strain effect	Two-way	$F_{(1,36)}=370.9$	<0.0001
time effect	Two-way	$F_{(11,36)}=30.32$	<0.0001
C			
Wistar naïve	One-way	$F_{(12,39)}=4.806$	<0.0001
WKY naïve	One-way	$F_{(12,39)}=0.797$	0.6513
strain-time interaction	Two-way	$F_{(11,36)}=2.552$	0.0168

strain effect	Two-way	$F_{(1,36)}=25.33$	<0.0001
time effect	Two-way	$F_{(11,36)}=2.796$	0.0098
D			
Wistar naïve	One-way	$F_{(12,39)}=4.269$	0.0002
WKY naïve	One-way	$F_{(12,39)}=6.852$	<0.0001
strain-time interaction	Two-way	$F_{(11,36)}=0.5140$	0.8811
strain effect	Two-way	$F_{(1,36)}=0.7597$	0.3892
time effect	Two-way	$F_{(11,36)}=6.328$	<0.0001
E			
Wistar ESCIT	One-way	$F_{(12,65)}=2.011$	0.0371
Wistar vehicle	One-way	$F_{(12,65)}=6.641$	<0.0001
strain-time interaction	Two-way	$F_{(11,60)}=2.336$	0.0181
strain effect	Two-way	$F_{(1,60}=50.33$	<0.0001
time effect	Two-way	$F_{(11,60)}=9.941$	<0.0001
F			
Wistar ESCIT	One-way	$F_{(12,65)}=4.875$	<0.0001
Wistar vehicle	One-way	$F_{(12,65)}=10.767$	<0.0001
strain-time interaction	Two-way	$F_{(11,60)}=6.757$	<0.0001
strain effect	Two-way	$F_{(1,60)}=134.3$	<0.0001
time effect	Two-way	$F_{(11,60)}=14.86$	<0.0001
G			
Wistar ESCIT	One-way	$F_{(12,52)}=6.556$	<0.0001
Wistar vehicle	One-way	$F_{(12,52)}=7.760$	<0.0001
strain-time interaction	Two-way	$F_{(11,48)}=0.8337$	0.6079
strain effect	Two-way	$F_{(1,48)}=10.38$	0.0023
time effect	Two-way	$F_{(11,48)}=9.807$	<0.0001
H			
Wistar ESCIT	One-way	$F_{(12,39)}=11.753$	<0.0001
Wistar vehicle	One-way	$F_{(12,39)}=11.092$	<0.0001
strain-time interaction	Two-way	$F_{(11,36)}=0.6984$	0.7314
strain effect	Two-way	$F_{(1,36)}=0.1928$	0.6632
time effect	Two-way	$F_{(11,36)}=17.32$	<0.0001
I			
WKY ESCIT	One-way	$F_{(12,52)}=6.225$	<0.0001
WKY vehicle	One-way	$F_{(12,52)}=2.435$	0.0175
strain-time interaction	Two-way	$F_{(11,48)}=0.5755$	0.839
strain effect	Two-way	$F_{(1,48)}=0.04792$	0.8276
time effect	Two-way	$F_{(11,48)}=4.354$	0.0002
J			
WKY ESCIT	One-way	$F_{(12,39)}=10.118$	<0.0001
WKY vehicle	One-way	$F_{(12,39)}=3.432$	0.001
strain-time interaction	Two-way	$F_{(11,36)}=2.208$	0.0364
strain effect	Two-way	$F_{(1,36)}=41.30$	<0.0001
time effect	Two-way	$F_{(11,36)}=9.402$	<0.0001
K			

WKY ESCIT	One-way	$F_{(12,52)}=3.722$	0.0004
WKY vehicle	One-way	$F_{(12,52)}=0.703$	0.7352
strain-time interaction	Two-way	$F_{(11,48)}=1.811$	0.0783
strain effect	Two-way	$F_{(1,48)}=37.23$	<0.0001
time effect	Two-way	$F_{(11,48)}=2.777$	0.0071
L			
WKY ESCIT	One-way	$F_{(12,39)}=7.490$	<0.0001
WKY vehicle	One-way	$F_{(12,39)}=8.292$	<0.0001
strain-time interaction	Two-way	$F_{(11,36)}=0.9025$	0.5474
strain effect	Two-way	$F_{(1,36)}=0.01320$	0.9092
time effect	Two-way	$F_{(11,36)}=8.072$	<0.0001
Supplementary figure 2.			
Locomotor activity			
strain-time interaction	Two-way	$F_{(1,36)}=4.280$	0.0479
strain effect	Two-way	$F_{(1,36)}=1.014$	0.3226
light/dark cycle effect	Two-way	$F_{(1,36)}=504.4$	<0.0001
Supplementary figure 3.			
Food intake (**A**)			
strain-treatment interaction	Two-way	$F_{(1,72)}=9.288$	<0.0001
strain effect	Two-way	$F_{(1,72)}=138.2$	<0.0001
age effect	Two-way	$F_{(1,72)}=3.151$	0.0301
food intake: 9 weeks Wistar, 11 weeks WKY	t-test		<0.0001
Water intake (**B**)			
strain-time interaction	Two-way	$F_{(1,72)}=0.8528$	0.4697
strain effect	Two-way	$F_{(1,72)}=102.6$	<0.0001
age effect	Two-way	$F_{(1,72)}=15.45$	<0.0001
water intake: 9 weeks Wistar, 11 weeks WKY	t-test		0.0001
Light Food intake (**C**)			
strain-time interaction	Two-way	$F_{(1,36)}=7.331$	0.0103
strain effect	Two-way	$F_{(1,36)}=86.97$	<0.0001
time effect treatment	Two-way	$F_{(1,36)}=1.217$	0.2773
Naïve: Wistar, WKY	t-test		<0.0001
Dark Food intake (**C**)			
strain-time interaction	Two-way	$F_{(1,36)}=0.1227$	0.7281
strain effect	Two-way	$F_{(1,36)}=3.429$	0.0723
time effect treatment	Two-way	$F_{(1,36)}=0.9043$	0.348
naïve: Wistar, WKY	t-test		0.3867
Total Food intake (**C**)			
strain-time interaction	Two-way	$F_{(1,36)}=6.255$	0.0171
strain effect	Two-way	$F_{(1,36)}=49.9$	<0.0001
time effect treatment	Two-way	$F_{(1,36)}=7.641$	0.0089
naïve: Wistar, WKY	t-test		0.0004
Water intake (**D**)			
DAY 1	t-test		0.0001
DAY 2	t-test		0.0006
DAY 3	t-test		0.0066

DAY 4	t-test		0.0461
PFC (**E**)			
naive Wistar (9 w)	One-way	$F_{(12,39)}=8.055$	<0.0001
naive Wistar (11 w)	One-way	$F_{(12,39)}=5.667$	<0.0001
age (w)-time interaction	Two-way	$F_{(11,36)}=0.4114$	0.9415
age (w) effect	Two-way	$F_{(1,36)}=0.00109$	0.9737
time effect	Two-way	$F_{(11,36)}=12.29$	< 0.0001
DRN (**F**)			
naive Wistar (9 w)	One-way	$F_{(12,39)}=4.806$	<0.0001
naive Wistar (11 w)	One-way	$F_{(12,39)}=3.758$	0.0008
age (w)-time interaction	Two-way	$F_{(11,36)}=0.3389$	0.9706
age (w) effect	Two-way	$F_{(1,36)}=0.4557$	0.5039
time effect	Two-way	$F_{(11,36)}=9.670$	< 0.0001
Supplemetary figure 4. Escitalopram (1) into DRN			
A			
Wistar naïve	One-way	$F_{(12,39)}=13.243$	<0.0001
WKY naïve	One-way	$F_{(12,39)}=6.852$	<0.0001
strain-time interaction	Two-way	$F_{(11,36)}=0.8773$	0.5696
strain effect	Two-way	$F_{(1,36)}=7.767$	0.0084
time effect	Two-way	$F_{(11,36)}=17.12$	<0.0001
B			
Wistar naïve	One-way	$F_{(12,39)}=10.156$	<0.0001
WKY naïve	One-way	$F_{(12,39)}=4.241$	0.0001
strain-time interaction	Two-way	$F_{(11,36)}=0.5763$	0.8351
strain effect	Two-way	$F_{(1,36)}=0.03180$	0.8595
time effect	Two-way	$F_{(11,36)}=14.23$	<0.0001

Supplementary Table 1. Results of statistical analysis using ANOVA and t-tests for data shown in Figs. 1–5 and Supplementary Figs. 2–4.