Altered neuronal activity and differential sensitivity to acute antidepressants of locus coeruleus and dorsal raphe nucleus in Wistar Kyoto rats: a comparative study with Sprague Dawley and Wistar rats.

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Short Title: A comprehensive analysis of Wistar Kyoto Rats

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Abstract

The Wistar Kyoto rat (WKY) has been proposed as an animal model of depression. The noradrenergic nucleus, locus coeruleus (LC) and the serotonergic nucleus, dorsal raphe (DRN) have been widely implicated in the ethiopathology of this disease. Thus, the goal of the present study was to investigate *in vivo* the electrophysiological properties of LC and DRN neurons from WKY rats, using single-unit extracellular techniques. Wistar (Wis) and Sprague Dawley (SD) rats were used as control strains**.** In the LC from WKY rats the basal firing rate was higher than that obtained in the Wis and SD strain, and burst firing activity also was greater compared to that in Wis strain but not in SD. The sensitivity of LC neurons to the inhibitory effect of the α_2-adrenoceptor agonist, clonidine and the antidepressant reboxetine was lower in WKY rats compared to Wis, but not SD. Regarding DRN neurons, in WKY rats burst activity was lower than that obtained in Wis and SD rats, although no differences were observed in other firing parameters. Interestingly, while the sensitivity of DRN neurons to the inhibitory effect of the 5-HT_{1A} receptor agonist, 8-OH-DPAT was lower in the WKY strain, the antidepressant fluoxetine had a greater inhibitory potency in this rat strain compared to that recorded in the Wis group. Overall, these results point out important electrophysiological differences regarding noradrenergic and serotonergic systems between Wis and WKY rats, supporting the utility of the WKY rat as an important tool in the research of cellular basis of depression

Keywords

Locus coeruleus, dorsal raphe, Wistar Kyoto, SERT

1. Introduction

Major depression is a mood disorder that represents a severe and long lasting clinical problem with a big impact on society, both in social and economic aspects. The prevalence rate today is between 15 and 20% (Kessler et al., 2005) and progressively increases instead of decreasing. Despite the great improvement that has been done in terms of tolerability of antidepressant drugs, there is still an elevated number of patients that does not respond to the treatment or that responds but exhibit some residual depressive symptoms (Zajecka et al., 2013). Current first line pharmacological agents for the treatment of depression share monoaminergic neurotransmission as a single common target. Thus nowadays, the most commonly used antidepressants are the selective serotonin reuptake inhibitors (SSRI) followed by the noradrenaline reuptake inhibitors (NRI) that modulate serotonergic and/or noradrenergic neurotransmission. Although it is well accepted that the serotonin and noradrenaline function are altered from the first antidepressant administration, the therapeutic effect is delayed several weeks. The reason of this fenomenon and the total or partial resistance to the treatment showed by a high percentage of patients are still unknown matters in the pharmacological therapy.

The brainstem monoaminergic nuclei, the noradrenergic *locus coeruleus* (LC) and serotonergic *dorsal raphe nucleus* (DRN) are characterized by their extensive efferent projections throughout the neuroaxis to all brain areas related to depression. Both nuclei are under the control of somatodendritic 5-HT_{1A} or α_2-adrenoceptors (Cedarbaum and Aghajanian, 1976; Williams et al., 1985). Activation of these receptors, directly by agonists or indirectly by increasing neurotransmitter levels at the synapses cleft using SSRI and NRI, inhibits neuronal activity of the DRN and LC (Szabo and Blier 2001a, 2001b; Miguelez et al.,

2009, 2011). Thus, changes in the spontaneous neuronal activity and/or the sensitivity of 5-HT_{1A} and α_2-adrenoceptors may be of relevance not only in the etiology of depression but also in the response to antidepressant drugs.

Animal models are pivotal in the effort to understand the neurobiology of major depressive disorder and to develop new treatments (Berton et al., 2012). In this regard, Wistar Kyoto (WKY) rat strain has been proposed as an animal model of depression since several studies have shown that WKY rats exhibit inherent depressive-like behavior in different behavioral tests (Lahmame and Armario 1996; Paré and Tejani-Butt, 1996; Lahmame et al., 1997; López-Rubalcava and Lucki 2000; Tejani-Butt et al 2003; Will et al., 2003). In addition, exaggerated endocrine response to stressors has been described in this rat strain (Rittenhouse et al., 2002; De la Garza et al., 2004), as well as reduced efficacy of specific antidepressant drugs alleviating symptoms of depression (Lahmame and Armario 1996; Lahmame et al., 1997; López-Rubalcava and Lucki 2000; Tejani-Butt et al 2003; Will et al., 2003). WKY rats show reduced basal levels of NE and 5-HT in LC and DRN, as well as significant modifications on the distribution and density of dopamine transporter sites in the mesolimbic areas, that may lead to altered dopaminergic transmission (De la Garza et al., 2004; Heal et al., 2008; Scholl et al., 2010; Yamada et al., 2013;). Indeed, in several brain areas of this rat strain dopamine release is greater than that observed in spontaneous hypertensive rats, an inbred genetic strain derived from the WKY (see Heal et al., 2008). Moreover, several differences have been found in the LC and DRN of WKY rats in comparison to other experimental strains. Thus, gene expression for enzymes involved in noradrenaline turnover, amino-acid receptors, and certain G-protein-couple receptors, such as k-opioid receptor, are increased in the LC of WKY rats (Pearson et al., 2006). Interestingly, k-opioid receptor antagonists produce antidepressant-like effects selectively in this rat strain

(Carr et al., 2010). In the DRN of WKY rats decreased intrinsic excitability in 5-HT neurons has also been reported as compared to SD rats (Lemos et al., 2011).

The main goal of this study was to characterize *in vivo* basal electrophysiological properties of LC and DRN neurons in WKY rats and their response to acute administration of serotonergic and noradrenergic agonists and antidepressants. For that purpose, we used as control strains the Wistar (Wis) rat, which is the original strain from which WKY rats were derived, and the Sprague Dawley (SD) rat, a type of rat widely used in brain research studies.

2. Experimental procedures

2.1. Animals

Male SD, Wis and WKY rats weighting 250–300 g at the beginning of experiments, were used for the electrophysiological recordings (SD: n=55; Wis: n=29 and WKY: n=50) and immunohistochemical experiments (SD: n=7; Wis: n=6 and WKY: n=9). Every effort was made to minimize the animals' suffering and to use the minimum number of animals possible. Experimental protocols were reviewed and approved by the Local Committee for Animal Experimentation at the University of the Basque Country (CEBA/17-P07-02/2009/ UGEDO URRUELA), and performed in compliance with the European Community Council Directive on 'The Protection of Animals Used for Experimental and Other Scientific Purposes' 86/609/EEC) and with the Spanish Law (RD 1201/2005) for the care and use of laboratory animals.

2.2. Drugs

The drugs used in this study were chloral hydrate (Sigma-Aldrich, USA), clonidine hydrochloride (Sigma-Aldrich, USA), reboxetine mesylate (Sigma-Aldrich, USA), RX-

821002 hydrochloride (Sigma-Aldrich, USA), 8-OH-DPAT (Sigma-Aldrich, USA) fluoxetine hydrochloride (Tocris Bioscience, UK), citalopram hydrobromide (Tocris Biosciencie, UK) and WAY-100635 (Sigma-Aldrich, USA). Chloral hydrate, clonidine hydrochloride, reboxetine mesylate, RX-821002 hydrochloride, 8-OH-DPAT and citalopram hydrobromide were prepared in 0.9% saline, and fluoxetine in distilled water.

2.3. Electrophysiological procedures

Single-unit extracellular recordings of LC and DRN neurons were performed as previously described (Miguelez et al. 2009, 2011). Animals were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame.

For LC recordings, the head was oriented at 15º to the horizontal plane (nose down). For DRN recordings, ligature and subsequent cutting of the sagittal sinus was needed. The recording electrode was lowered into the LC (relative to lambda: AP -3.7 mm, ML +1.1 mm, DV -5.5 to -6.5 mm) or DRN (relative to lambda: AP +1.0 mm, ML 0 mm, DV -4.5 to -6.0 mm). LC neurons were identified by standard criteria which included: spontaneous activity displaying a regular rhythm and a firing rate between 0.5–5 Hz; characteristic spikes with a long-lasting, positive-negative waveform, and a biphasic excitation–inhibition response to pressure applied to the contralateral hindpaw (paw pinch). In LC neurons burst firing onset and end was defined as the concurrence of two spikes with the first interspike interval ≤80 ms and a termination interval ≥160 ms, as previously described in publications from our group (Miguelez et al., 2009). DRN serotonergic neurons were identified by established criteria, which include wide-duration action potentials between 1–2 ms, positive-negative spikes, a regular rhythm and a slow firing rate (0.5–3 Hz). Burst-firing DRN serotonergic neurons were

identified as described previously (Hajos et al. 1995; Miguelez et al. 2011), by criteria including fired spike doublets or triplets with an intraburst time-interval <20 ms.

Firing patterns were analyzed offline, using the computer software Spike2 (Cambridge Electronic Design, UK) and the following parameters were calculated: firing rate, coefficient of variation (percentage ratio of standard deviation to the mean interval value of an interspike time-interval histogram), percentage of spikes in burst, percentage of cells exhibiting burst firing, and response to intravenous drug administration. Only one cell was studied in each animal when any drug was administered. All recording sites were located within the LC and DRN.

2.4. Immunohistochemistry procedures

2.4.1. Fixation and tissue processing

Animals of different strain groups (SD, Wis and WKY n=5-9) were anaesthetized with an intraperitoneal injection of sodium pentobarbital (200 mg/kg). Rats were perfused through the aortic arch with 3.75% acrolein (50 ml, TAAB, UK) in a solution of 2% paraformaldehyde (Sigma, UK) and 0.1 M phosphate buffer (PB) pH 7.4, followed by 2% paraformaldehyde (125 ml). Brain blocks were post-fixed in 2% paraformaldehyde for 24 hours and kept in 0.1 M PB, pH 7.4. Coronal sections of the brain were cut into 40 μm thickness using a vibrating microtome (VT1000 S, Leica, Milton Keynes, UK). Free floating brain sections in 0.1 M PB, pH 7.4 were collected and stored in cryoprotectant solution containing 25% sucrose and 3.5% glycerol in 0.05 M PB at pH 7.4. Coronal vibratome sections at levels 1.70mm/ -0.30mm (prefrontal cortex, PFC) from bregma, -1.58 mm/-2.46 mm (hippocampus, HPP) and

-7.04mm/-9.30mm (raphe nuclei, RN) posterior to bregma were selected for immunohistochemistry according to the rat brain atlas of Paxinos and Watson, (1997).

2.4.2. Antibodies

A polyclonal rabbit antibody raised against a synthetic peptide sequence corresponding to amino acids 602–622 of rat 5HT transporter (Immunostar, Hudson, WI, USA) was used for determination of serotonin transporter (SERT)-positive axons and terminals in the HPP, RN and PFC. The specificity of the antibodies has been reported previously using immunohistochemistry (Mamounas et al., 2000) and Western blots (Albright et al., 2007). To determine the specificity of the antibodies adsorption controls were done using SERT and 5-HT peptides, respectively, which resulted in total absence of target labelling. Furthermore, omission of primary and/or secondary antibodies also showed no immunoreactivity (Noristani et al., 2010; Supplementary Figure 1).

2.4.3. Immunohistochemistry

To minimize methodological variability, sections through the dorsal and ventral hippocampus (DHPP and VHPP, respectively), PFC and RN containing both hemispheres of all animals were processed at the same time using precisely the same experimental conditions. For this procedure, the vibratome sections were first incubated for 30 min in 30% methanol in 0.1 M PB and 30% hydrogen peroxide (H_2O_2; Sigma, UK). Sections were then rinsed with 0.1 M PB for 5 min and placed in 1% sodium borohydride (Aldrich, UK) for 30 min. The sections were then washed with PB profusely before rinsing in 0.1 M Trizma base saline (TS) for 10 min. Brain sections were then incubated in 0.5% bovine serum albumin (Sigma, UK) in 0.1 m TS and 0.25% Triton X-100 (Sigma, UK) for 30 min. Sections were incubated for 48 h at room temperature in primary antibody (rabbit anti-SERT, 1: 2500, Immunostar, Hudson, WI,

USA). The sections were rinsed in 0.1 m TS for 30 min and incubated in 1: 400 dilutions of biotinylated donkey anti-rabbit IgG (Jackson Immunoresearch, Stratech Scientific, Soham, UK) for 2 h at room temperature. Sections were rinsed with 0.1 M TS for 30 min followed by incubation for 30 min in avidin-biotin peroxidase complex (Vector Laboratories, Peterborough, UK). The peroxidase reaction product was visualized by incubation in a solution containing 0.022% 3,3'diaminobenzidine (DAB; Aldrich, Gillingham, UK) and 0.003% $H2O_2$ for 6 min, as described previously (Rodriguez et al., 2008). The reaction was stopped by rinsing the sections in 0.1 M TS for 6 min followed by 0.1 M PB for 15 min. Brain sections were permanently mounted onto gelatinized slides and allowed to dry overnight. Sections were then dehydrated in ascending concentration of ethanol (50, 70, 80, 90, 95 and 100%) and, finally, xylene. Coverslips were applied using Entellan (Merck KGaA, Germany) and slides were left to dry overnight.

2.5. Optical Density (OD) measurement

Using computer-assisted imaging analysis (Image J 1.32j, NIH, USA), we analyzed the expression and density of SERT labeling on SD, Wis and WKY strains by measuring their optical density (OD) as described previously (Cordero et al., 2005). Briefly, to exclude any experimental errors and/or bias, all images were taken at constant light intensity. Optical filters were used to ensure the specificity of the signal recorded by the camera. The staining was observed throughout the thickness of the section (40 μm) using light microscopy (Nikon Eclipse 80i). No differences were observed in SERT immunoreactivity throughout the thickness of the section between strains control animals; hence the changes in OD were used as measure of increased SERT expression. The OD was calculated from a relative scale of intensity ranging from 0 to 250, with a measurement of 250 corresponding to the area with very low SERT and 0 corresponding to the densest area of labeling. The calibration density

was kept constant for measuring all sections to avoid experimental variances. Non-specific OD in sections was measured from the *corpus callosum*.

SERT density of each area was measured independently and a single measurement was obtained from every sub-region in each hemisphere. To analyze the change in SERT density against constant control, 250 was divided by control region (*corpus callosum*) and the obtained factor was multiplied by the region of interest in every given section. Inverse OD was obtained by subtracting from the obtained background level (250). Measurement of mean density were taken and averaged, after background subtraction, from each area in both the left and the right hemisphere of each slice. The results are shown as inverse SERT density (IOD/pixel).

2.6. Statistical analysis of data

Changes in firing rate are expressed as percentages of the baseline firing rate (mean firing rate during 3 min prior to drug injection). The response to drug administration was evaluated according to dose–response curves nonlinearly fitted to a logistic three-parameter equation (Parker and Waud, 1971) using GraphPad Prism Software (v.5.01; GraphPad Software Inc., USA) as described previously (Miguelez et al., 2009). The following equation was used:

$$E = E_{max} [A]^n / (ED^n_{50} + [A]^n),$$

Where [A] is the dose of the drug, E is the effect on the firing rate induced by A, E_{max} is the maximal effect, ED_{50} is the effective dose for eliciting 50% of E_{max} and n is the slope factor of the dose-response curve. Statistical comparison between dose-response curves parameters was done using the extra sum-of-squares F test (GraphPad Prism 5.01, San Diego, USA). Differences in the percentage of neurons presenting burst firing were statistically evaluated by

two-tailed χ^2 analysis of contingency tables. Parameters derived from burst pattern were analyzed by the non-parametric Kruskal-Wallis test followed by Dunn´s *post-hoc* test. Spontaneous firing rate, coefficient of variation and immunochemical data were compared through strains by one-way analysis of variance (ANOVA) followed by Newman-Keuls *post-hoc* test. The level of significance was considered as $p<0.05$.

3. Results

3.1. Electrophysiology

3.1.1. Electrophysiological properties of LC neurons in anaesthetized SD, Wis and WKY rats.

All the recorded neurons fitted the standard criteria previously described (see methods). A total of 175 LC noradrenergic neurons were recorded: 69 neurons from the SD group (n=22 animals), 40 neurons from the Wis group (n=12 animals) and 57 neurons from the WKY group (n=20 animals). The basal firing rate statistically varied among the groups ($p<0.001$, $F_{(2,163)}=10.91$, one way ANOVA) (Table 1). Thus, neurons recorded from WKY rats showed a significant higher basal firing rate than those recorded from Wis and SD rats ($p<0.001$ *vs* Wis; $p<0.01$ *vs* SD, Newman Keuls multiple comparison test). No differences were found in the coefficient of variation among the three groups. The percentage of LC noradrenergic neurons discharging in burst changed among rat strains (Table 1) since, in the Wis group a smaller proportion of cells showed burst activity compare to that recorded in the WKY group ($\chi^2=20.90$, df=2 $p<0.0001$). Other burst patter parameters such as the number of burst per cell, the percentage of spikes in burst, the mean spikes per burst and the mean burst interspike did not differ statistically among groups (Table 1).

3.1.2. The inhibitory effect of clonidine and reboxetine on the firing rate of LC noradrenergic neurons from SD, Wis and WKY rats.

To evaluate potential changes on the sensitivity of LC α_2-adrenoceptors, we compared the inhibitory effect of cumulative increasing doses of the α_2-adrenoceptor agonist, clonidine (0.625-10 µg/kg, i.v.), onto basal firing rate of LC neurons from SD, Wis and WKY rats. In all cases, clonidine administration caused a dose-dependent inhibition of the basal firing rate (Fig.1*a* and *b*). In WKY rats the dose-response curve for clonidine was shifted to the right, so that the ED_{50} mean value was significantly higher than that obtained in the Wis group (ED_{50}: 3.36±0.36 µg/kg, n= 6; ED_{50}: 2.70±0.36 µg/kg, n=6, for WKY and Wis respectively, $p<0.001$, Nonlinear fit analysis, extra sum-of-squares *F* test) (Fig.1*c*). On the other hand, the clonidine dose-response curves obtained in WKY and SD rats were not statistically different (ED_{50}: 4.03±0.29 µg/kg, n= 6). Following administration of α_2-adrenoceptor antagonist RX 821002 (0.2mg/kg, i.v.) firing activity was completely recovered in all groups (SD: 110.0±23%, n=4; Wis: 116.9±13%, n=5, and WKY: 134.6±16%, n=5). No significant differences were observed among groups.

Then, we assessed if sensitivity of LC neurons to the effect of the NRI, reboxetine was altered in the WKY group compared to SD and Wis. As we have previously reported (Miguelez et al. 2011), reboxetine administration (0.025-0.8 mg/kg, i.v.) produced a progressive and complete inhibition of LC firing rate in all groups tested. However, in WKY rats reboxetine was less potent inhibiting LC firing rate as compared to Wis strain. Consequently, ED_{50} value for WKY rats was significantly higher compared to that obtained in the Wis group (ED_{50}: 0.12±0.01 mg/kg, n=6; ED_{50}: 0.08±0.01 mg/kg, n=7, for WKY and Wis rats respectively, $p<0.001$, Nonlinear fit analysis, extra sum-of-squares *F* test) (Fig.1*d*). No

differences were observed in the reboxetine effect between WKY and SD groups (ED_{50}: 0.12 ±0.01 mg/kg, n=7, for SD strain).

3.1.3. Electrophysiological properties of DRN neurons in anaesthetized SD, Wis and WKY rats.

A total of 139 neurons in the DRN were recorded: 45 neurons from the SD group (n=26 animals), 43 neurons from the Wis group (n=11 animals) and 53 neurons from the WKY group (n=21 animals). The basal firing rate and coefficient of variation did not differ among groups (Table 2). However, the percentage of DRN serotonergic neurons discharging in burst varied among groups since, a smaller percentage of DRN neurons in the WKY strain showed burst firing pattern as compared to that recorded in the Wis and SD strain (χ^2=10.59, df=2 $p<0.01$) (Table 2). The burst pattern was similar in all groups.

3.1.4. The inhibitory effect of 8-OH-DPAT, fluoxetine and citalopram on the firing rate of DRN neurons from SD, Wis and WKY rats.

To evaluate central serotonergic transmission of the three rat strains, we compared the effect of cumulative doses of the 5-HT_{1A}-receptor agonist, 8-OH-DPAT (0.05-10µg/kg, i.v.) onto basal firing rate of DRN from SD, Wis and WKY rats. In all cases 8-OH-DPAT administration reduced neuronal firing rate dose-dependently (Fig. 2*a* and *b*). However, in WKY rats the dose-response curve for 8-OH-DPAT was shifted to the right compared to that obtained in Wis rats, so that 8-OH-DPAT was less potent inhibiting DRN neurons activity in the WKY strain (ED_{50}: 2.27±0.26 µg/kg, n=6; ED_{50}: 1.33±0.17 µg/kg, n=6, for WKY and Wis rats respectively, $p<0.001$, Nonlinear fit analysis, extra sum-of-squares *F* test). The dose-response curves in the WKY and SD groups were also different (ED_{50}: 1.13±0.15 µg/kg, n=7, $p<0.001$, Nonlinear fit analysis, extra sum-of-squares *F* test) (Fig. 2*c*). Following administration of 5-HT_{1A}-receptor antagonist, WAY 100635 (100µg/kg, i.v.) firing rate was

completely recovered in all groups (SD: 140.7±14%, n=7; Wis: 80.2±30%, n=6; WKY: 94.9±20%, n=7). No significant differences were observed among groups.

Then, we assessed if sensitivity of DRN neurons to the effect of SSRI, fluoxetine was altered in the WKY compared to the SD and Wis group. Fluoxetine administration (0.12-14 mg/kg, i.v.) progressive and completely inhibited DRN firing rate in all groups tested. Fluoxetine administration was more potent inhibiting DRN serotonergic neurons in the WKY group, so that the ED_{50} value for WKY rats was significantly lower than that obtained in the Wis group (ED_{50}: 0.89±0.13 mg/kg, n=5; ED_{50}: 2.81±0.22 mg/kg, n=5, for WKY and Wis rats respectively, $p<0.0001$, Nonlinear fit analysis, extra sum-of-squares *F* test). No differences were observed in the fluoxetine effect between WKY and SD group (ED_{50}: 0.98±0.13 mg/kg, n=7, for SD rats) (Fig. 2*d*).

In two additional groups of Wis and WKY rats, we examined the effect of other SSRI, citalopram (0.02-0.62 mg/kg, i.v). In this case, we observed a slight, although significant increase in the inhibitory potency of citalopram in WKY rats compared to that in Wis rats (ED_{50}: 0.12±0.01 mg/kg, n=6; ED_{50}: 0.16±0.01 mg/kg, n=7, for WKY and Wis rats respectively, $p<0.01$, Nonlinear fit analysis, extra sum-of-squares *F* test) (Fig. 2*e*).

3.2. SERT density in the HPC, RN and PFC from SD, Wis and WKY rats.

To further investigate whether the increased potency of SSRIs inhibiting serotonergic transmission in WKY strain was related to an alteration of SERT immunoreactivity (SERT-IR) expression, SERT-IR IOD was analyzed in the RN and projecting areas, such as HPC and PFC from the above mentioned rat strains. No significant differences were reached in any of the analyzed areas (Fig. 3). The overall SERT-IR density was similar in all groups both in the DHPC (WKY: 37.65 ± 4.45, SD: 35.37 ± 2.85 and Wis: 43.41 ± 2.69) and the VHPC (WKY:

36.42 ± 3.37; SD: 34.77 ± 4.49; 4.56 and Wis: 34.21 ± 4.11). Similar pattern of expression was also obtained in the DRN (WKY: 69.26 ± 9.17, SD: 83.00 ± 11.90 and Wis: 81.96 ± 4.91) and the VRN (WKY: 83.87 ± 7.23, SD: 92.88± 9.17 and Wis: 85.58 ± 5.27). In the PFC no differences were shown among the three strains either in the CG1 (WKY: 14.03 ± 2.89, SD: 16.47 ± 1.66 and Wis: 13.44 ± 1.17) or in the CG2 (WKY: 16.70 ± 2.61, SD: 18.73 ± 2.41 and Wis: 17.57 ± 1.62).

4. Discussion

In WKY rats, many studies have mainly investigated the effect of stress onto monoaminergic systems (Pare and Tejani-Butt, 1996; Zafar et al., 1997; López-Rubalcava and Lucki, 2000; Sands et al., 2000; De la Garza et al., 2004). To the best of our knowledge, so far no electrophysiological characterization of LC and DRN neurons in WKY rats has been performed in basal conditions *in vivo*. Thus, the purpose of this investigation was to study whether noradrenergic and serotonergic neurotransmission were altered in the WKY rat. Our results show that basal activity of LC and DRN neurons as well as sensitivity of α_2-adrenoceptors and serotonergic 5-HT_{1A} autoreceptors, were modified in WKY rats as compared to the strain from which it was outbred, the Wis rat. One key finding in this *in vivo* study is that serotonergic and noradrenergic antidepressants induce differential electrophysiological effects in WKY rats.

Regarding noradrenergic transmission, the present study shows that LC neurons from WKY rats display increased basal activity compared to that recorded from control groups, since augmented firing rate and a greater proportion of neurons displaying burst activity were recorded in the WKY strain. The hyperactivity of LC neurons described in this study could be

related to the lower concentration of noradrenaline reported in the LC of WKY rats (Scholl et al., 2010), which exerts an inhibitory action onto neuronal activity by activation of α_2-adrenoreceptors. Our results also show that the α_2-adrenoceptor agonist, clonidine was less potent inhibiting LC neurons from WKY rats as compared to the effect induced by this drug in the Wis rats, supporting the contention that noradrenergic signaling is altered in the LC of WKY rats. Activation of noradrenergic transmission is involved in behavioral and neuroendocrine response to stress. In this sense, noradrenergic reactivity induced by acute stress is attenuated in WKY rats since expression of tyrosine hydroxylase mRNA in the LC and release of noradrenaline in projecting areas are significantly reduced comparing with those obtained in other rat strains (Sands et al., 2000; Pardon et al., 2002). Lower concentrations of noradrenaline have been reported in specific terminal sites of WKY rats, such as the DRN and VHPC, although not in the PFC (De La Garza and Mahoney, 2004; Scholl et al., 2010). Noradrenaline uptake and expression level of noradrenergic transporters are reduced in the cortex of WKY rats compared to Wis rats (Jeannotte et al., 2009). In line with this, our results show that acute administration of the antidepressant reboxetine, which increases noradrenaline levels by blocking noradrenaline transporters, was less potent inhibiting LC basal activity in the WKY rats compared to Wis. Despite altered noradrenergic transmission, acute and chronic administrations of noradrenergic antidepressants are able to improve behavioral responses in WKY rats (Lahmame and Armario, 1996; López-Rubalcava and Lucki, 2000; Tejani-Butt et al., 2003; Will et al., 2003; Jeannotte et al., 2009).

Dysfunctional serotonergic transmission has been associated with depressive-like states. In fact, altered DRN neuron activity and reduced 5-HT_{1A} sensitivity have been observed in several well established and putative animal models of depression (Gobbi et al., 2001; Gartside et al., 2003; Froger et al., 2004; Lanfumey et al., 1999; Bambico et al, 2009). Here, a

smaller population of DRN neurons from WKY rats showed burst firing activity compared to that recorded from the Wis and SD strain, although DRN firing rate was not significantly different among strains. Interestingly, burst activity is related with a greater 5-HT release in terminal areas (Gartside et al., 2000). This reduced burst activity supports the idea that DRN neurons from WKY rats have altered electric properties. According to this, DRN neurons from WKY rats have a more hyperpolarized resting membrane potential than those from SD rats (Lemos et al., 2011). This serotonergic alteration could be a consequence of lower contents of noradrenaline in DRN tissue (Scholl et al., 2010), which controls neuronal firing rate through α_1-adrenoreceptor activation (Baraban and Aghajanian, 1980; Vandermaelen and Aghajanian, 1983). In addition, our results show that *in vivo* sensitivity of 5-HT_{1A} autoreceptors from DRN neurons of WKY rats was reduced, as the ED_{50} value from the 8-OH-DPAT dose-response curve was increased 1.71-fold and 2.0-fold compared with that obtained from the Wis and SD strain, respectively. Conversely, the efficacy of the 8-OH-DPAT to reach maximal inhibition of DRN firing rate remained unaltered among groups. In line with this, a maximal concentration of the 5-HT_{1A} receptor agonist, 5-CT, induces similar hyperpolarizing response in the membrane potential of DRN neurons from WKY and SD *in vitro* (Lemos et al., 2011). The present work agrees with functional desensitization of 5-HT_{1A} autoreceptors from DRN previously described on several animal models that exhibit hallmark characteristics of anxiety/depression (Lanfumey et al., 1999; Froger et al., 2004; Gobbi et al., 2001; Bambico et al., 2009). It has been recently shown that the hypothermic and electrophysiological responses induced by the 5-HT_{1A} agonist 8-OH-DPAT are significantly reduced in a mouse model of anxiety/depression based on addition of corticosterone to drinking water (Rainer et al., 2012). Dysregulation of glucocorticoid system has also been

extensively described in the WKY rats (Rittenhouse et al., 2002; De la Garza et al., 2004; Lemos et al., 2011)

Data from the present study suggest that the acute electrophysiological response to SSRIs could uncover differential adaptations of the serotonergic system in the WKY strain. Indeed, fluoxetine had a greater inhibitory potency in this rat strain. No statistical differences were observed between fluoxetine dose-response curve obtained in WKY and SD group, and the maximal inhibitory effect of the drug was reached in all tested rat strains. Citalopram, another SSRI, was slightly but significantly more potent inhibiting basal firing rate of DRN neurons in WKY compared to Wis rats, reaching maximal inhibition in both groups. Thus, this study reveals that DRN neurons from WKY rats were more sensitive to the inhibitory effect of SSRIs, even though 5-HT_{1A} autoreceptors from that nucleus were desensitized. On one hand, this discrepancy could be explained by a greater expression or activity of SERT in the DRN of WKY rats. However, present inmunohistochemical data showed no differences regarding optical densities of SERT in the DRN and projecting areas of WKY and Wis rats. In line with this, an earlier study shows no differences in ^{3}H-CNIMI binding to SERT site under basal and stress condition in the DRN of WKY and SD rats (Pare et al., 1996). Although increased serotonin turnover has been described in the DRN of WKY rats (Scholl et al., 2010), a recent study using dual-probe microdialysis has reported lower extracellular basal levels of serotonin in the DRN of WKY rats but a similar relative increase of serotonin level in response to escitalopram, another SSRI, in WKY and Wis rats (Yamada et al., 2013). On the other hand, it should be taken into account that fluoxetine has been proposed as an atypical SSRI since, unlike citalopram, also increases extracellular levels of noradrenaline (Bymaster et al., 2002; Koch et al., 2002; Kobayashi et al., 2008). Accordingly, noradrenaline-deficient mice lack behavioral responses to fluoxetine (Cryan et al., 2004). In

the same line, we have previously shown that the inhibitory effect induced by fluoxetine on LC activity is completely reversed by antagonizing α_2-adrenoceptors (Miguelez et al., 2009), while the citalopram-induced effect is only partially reversed (Grandoso et al., 2005). Therefore, the direct impact of fluoxetine on altered noradrenergic transmission of WKY rats could underlie the increased sensitivity of DRN neurons to the inhibitory effect of this SSRI in this rat strain That is, fluoxetine could induce a greater additional inhibition of DRN neurons in WKY rats by decreasing α_1-adrenoceptor-mediated activation driven by noradrenergic inputs from the LC (Baraban and Aghajanian, 1980; Clement et al., 1992). In fact, DRN from WKY rat exhibits lower noradrenaline content as compared to control strains (Scholl et al., 2010). Further electrophysiological studies on DRN slices would be of great interest to clarify the neuroanatomical origin as well as the pharmacological profile of this adaptation, which is selectively revealed in the WKY rat. In order to detect electrophysiological adaptations on central neurotransmission in the WKY rat, it is important to use both strains as control, Wis and SD rats.

The present work extends the knowledge of the neurophysiology of WKY rats pointing out important differences regarding *in vivo* basal activity of DRN and LC nucleus, and electrophysiological responses induced by the antidepressant reboxetine and fluoxetine. Overall, these data support the utility of the WKY strain in the research of the neurobiological basis of depression as well as cellular effects of diverse antidepressant drugs in this pathological condition.

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Figure legends

Figure 1. The inhibitory effect of clonidine and reboxetine on the basal firing rate of locus coeruleus noradrenergic neurons in SD, Wis and WKY rats. (*a*) Representative firing rate histograms illustrate the inhibitory effect of clonidine (0.625-10 µg/kg, i.v.) on LC basal activity in Wis and *(b)* WKY rats. Note that following administration of the noradrenergic antagonist RX 821002 (0.2 mg/kg, i.v.) completely reversed the clonidine-induced effect. *(c)* Dose-effect curves for clonidine and *(d)* reboxetine (0.025-0.8 mg/kg i.v.) on LC basal firing rate in SD, Wis and WKY strains. Each point of the curve represents the mean ± S.E.M of *n* experiments (n=6-7 animals per strain).

Figure 2. The inhibitory effect of 8-OH-DPAT, fluoxetine and citalopram on the basal firing rate of dorsal raphe serotonergic neurons in SD, Wis and WKY rats. (*a*) Representative firing rate histograms illustrate the inhibitory effect of 8-OH-DPAT (0.05-10µg/kg, i.v.) on DRN basal activiy in Wis and *(b)* WKY rats. Note that following administration of the serotonergic antagonist WAY 100635 (100µg/kg, i.v.) reversed the 8-OH-DPAT-induced effect. *(c)* Dose-effect curves for 8-OH-DPAT, *(d)* fluoxetine and *(e)* citalopram on the DRN basal firing rate in SD, Wis and WKY rats. Each point of the curve represents the mean ± S.E.M of *n* experiments (n=5-7 animals in each group).

Figure 3. Brigh field micrographs showing serotonergic fibre expression of SERT in the hippocampus, raphe nuclei and prefrontal cortex from SD, Wis and WKY rats. *(a)* Light microscopy images of SERT-IR expression in the different analyzed areas. *(b)* Bar graphs showing SERT fibre presence and density content as determined by its inverted optical density (IOD) from SD, Wis and WKY rats Bars represent mean ± S.E.M.of *n* experiments (n=5-7 animals). RN: raphe nuclei; DRN: dorsal raphe nucleus; VRN: ventral raphe nucleus;

HPC: hippocampus; DHPC: dorsal hippocampus; VHPC: ventral hippocampus; PFC: prefrontal cortex.

Supplementary Figure 1. Brightfield micrographs showing the control conditions used to determine the specificity of the SERT antibody. No labelling was observed in the dorsal hippocampus following omission of primary (A), secondary (B) as well as combined omission of primary and secondary antibodies (C). In addition, the adsorption control using the SERT specific peptide also showed no labelling (D). **Key: DG:** dentate gyrus, **CA:** cornu ammonis.

Table 1. *In vivo* electrophysiological characteristics of locus coeruleus neurons recorded under basal conditions in Sprague Dawley, Wistar and Wistar Kyoto rats.

	Rat strain		
	SD (n=47)	Wis n=(13)	WKY (n=37)
Basal firing rate (Hz)	2.10±0.12*	1.78±0.11**	2.69±0.14
Coefficient of variation (%)	40.5±1.28	39.4±1.45	41.2±0.94
Neurons with burst firing (%)	58	33***	67
Number of burst	15.74±3.54	4±1.00	11.65±2.75
Spikes in burst (%)	6.12±1.15	2.07±0.41	3.72±0.69
Mean spikes per burst	2.08±0.04	2.23±0.12	2.11±0.06
mean burst interspike (ms)	63.88±1.89	60.62±6.54	68.05±1.45

All values represent the mean ± S.E.M. of *n* experiments.

*p<0.01, **p<0.001, ***p<0.0001 *vs* WKY (For firing rate analysis, *One-way ANOVA following Newman-Keuls test;* for neurons with burst firing χ^2 test, otherwise *Kruskal-Wallis test followed Dunn's test*).

Each cell was recorded for 3 min.

SD: Sprague Dawley; Wis: Wistar; WKY: Wistar Kyoto

Table 2. *In vivo* electrophysiological characteristics of dorsal raphe neurons recorded under basal conditions in Sprague Dawley, Wistar and Wistar Kyoto rats.

	Rat strain		
	SD (n=4)	Wis (n=4)	WKY (n=1)
Basal firing rate (Hz)	1.32±0.08	1.49±0.08	1.47±0.06
Coefficient of variation (%)	31.9±1.73	34.2±2.34	31.2±2.14
Neurons with burst firing (%)	18*	17*	4
Number of burst	2.25±1.25	8.75±2.69	4.00
Spikes in burst (%)	2.99±1.75	11.03±3.45	2.82
Mean spikes per burst	2.50±0.28	2.50±0.28	3.00
mean burst interspike (ms)	10.45±2.35	10.20±1.82	19.97

All values represent the mean ± S.E.M. of *n* experiments.

*$p<0.01$ vs WKY (χ^2 test).

Each cell was recorded for 3 min.

SD: Sprague Dawley; Wis: Wistar; WKY: Wistar Kyoto

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Individual contribution: CM, LU and MT designed the study. CB-C and CM performed electrophysiological experiments. CB-C, RG-L and JJR the performed inmunohistochemistry assay. CB-C carried out the data analysis. LU and MT drafted the manuscript. All the authors critically reviewed and approved the final manuscript.

Statement of Interest: None

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FIGURE 1

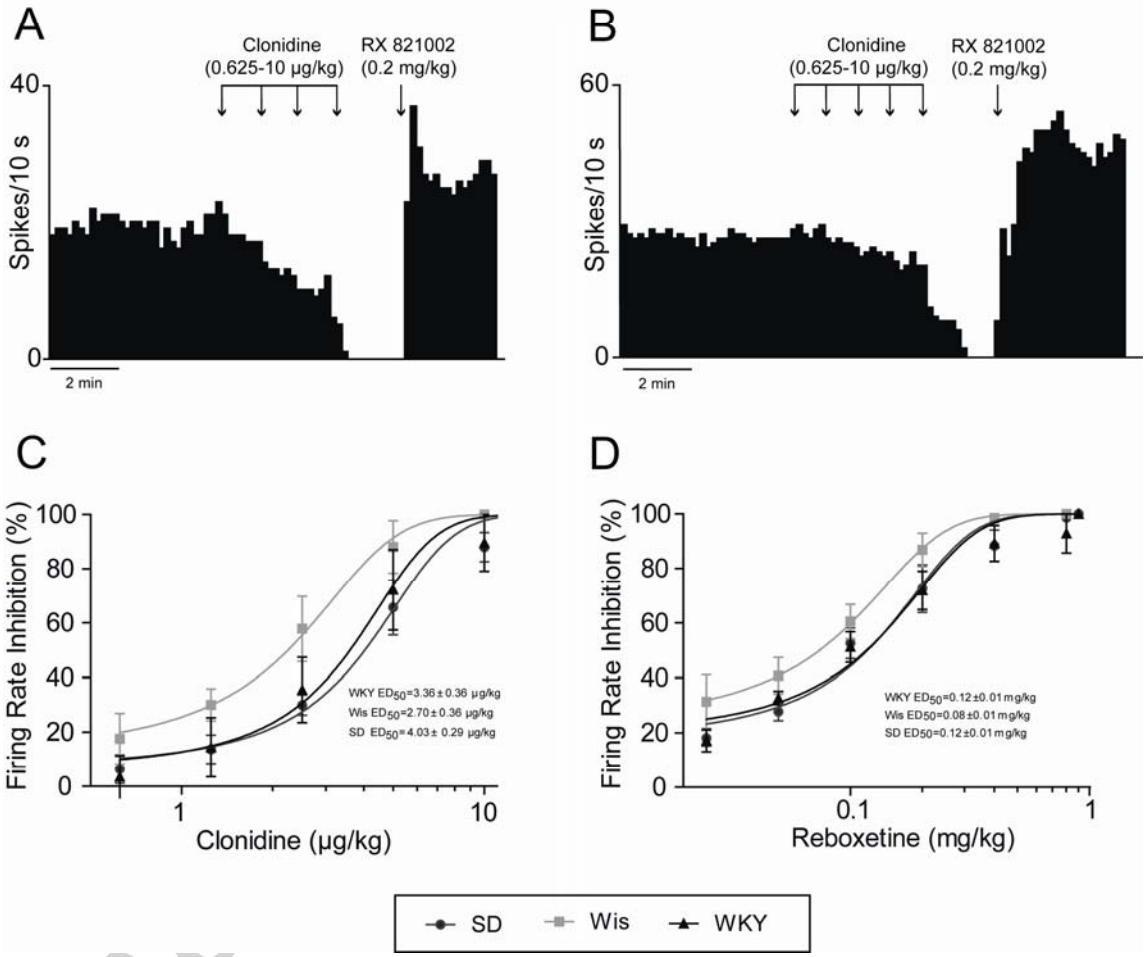

FIGURE 2

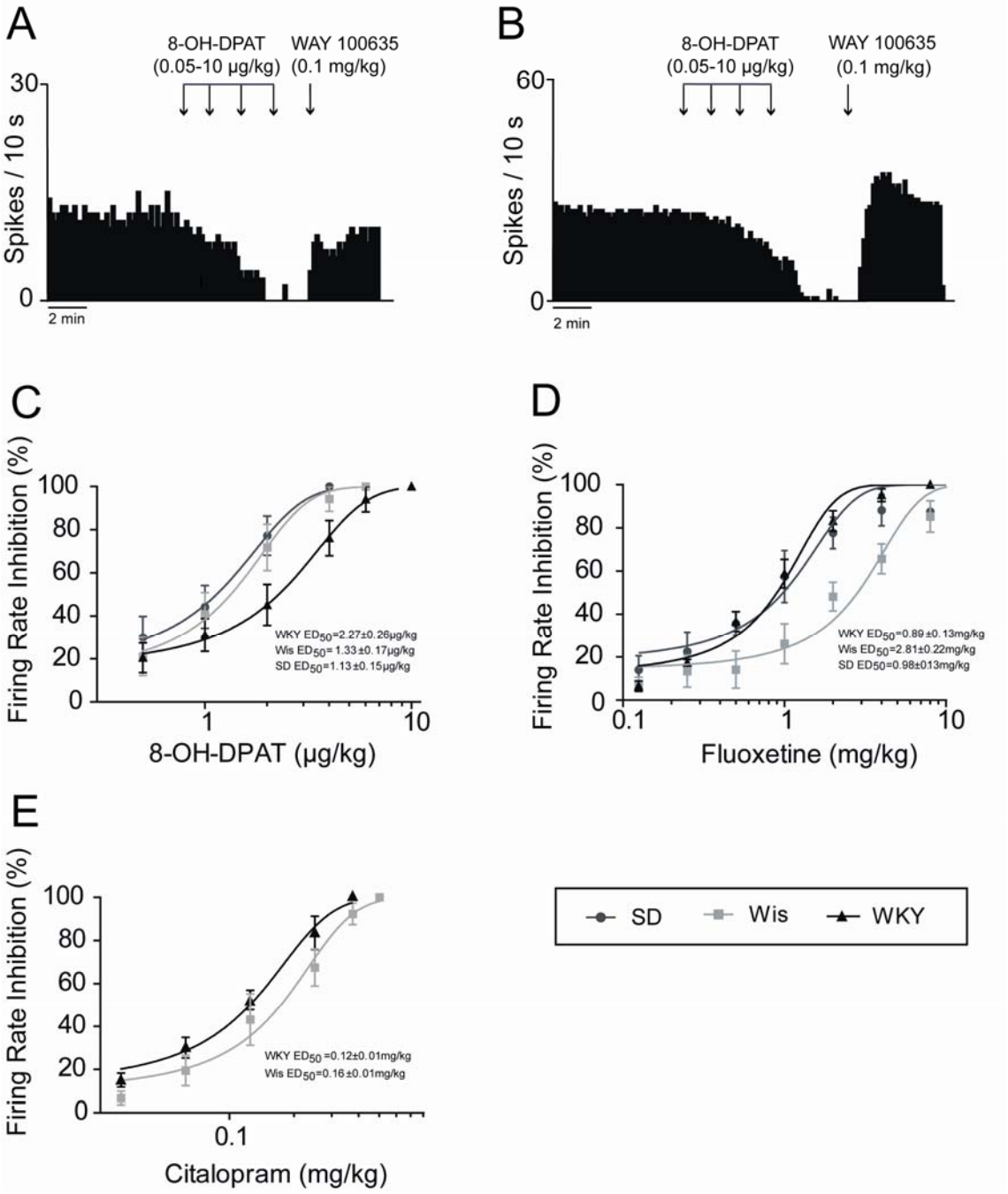

FIGURE 3