Dysfunctional Inhibitory Mechanisms in Locus Coeruleus Neurons of the Wistar Kyoto Rat

C Bruzos-Cidón, PhD; N Llamosas, PhD; L Ugedo, PhD; and M Torrecilla, PhD

Department of Pharmacology, Faculty of Medicine and Dentistry, University of the Basque Country UPV/EHU, 48940 Leioa, Spain (Drs Bruzos-Cidón, Ugedo, and Torrecilla, and Llamosas).

Correspondence: Maria Torrecilla, PhD, Department of Pharmacology, Faculty of Medicine and Dentistry, University of the Basque Country, 48940 Leioa, Spain (maria.torrecilla@ehu.es).

Abstract

Background: The noradrenergic nucleus locus coeruleus (LC) has functional relevance in several psychopathologies such as stress, anxiety, and depression. In addition to glutamatergic and GABAergic synaptic inputs, the activation of somatodendritic \( \alpha_2 \)-adrenoceptors is the main responsible for LC activity regulation. The Wistar Kyoto (WKY) rat exhibits depressive- and anxiety-like behaviors and hyperresponse to stressors. Thus, the goal of the present study was to investigate in vitro the sensitivity of \( \alpha_2 \)-adrenoceptors, as well as the glutamatergic and GABAergic synaptic activity on LC neurons of the WKY strain.

Methods: For that purpose patch-clamp whole-cell recordings were done in LC slices.

Results: The \( \alpha_2 \)-adrenoceptors of LC neurons from WKY rats were less sensitive to the effect induced by the agonist UK 14 304 as compared to that recorded in the Wistar (Wis) control strain. In addition, the GABAergic input to LC neurons of WKY rats was significantly modified compared to that in Wis rats, since the amplitude of spontaneous GABAergic postsynaptic currents was reduced and the half-width increased. On the contrary, no significant alterations were detected regarding glutamatergic input to LC neurons between rat strains.

Conclusions: These results point out that in WKY rats the inhibitory control exerted by \( \alpha_2 \)-adrenoceptors and GABAergic input onto LC neurons is dysregulated. Overall, this study supports in this animal model the hypothesis that claims an imbalance between the glutamatergic-GABAergic systems as a key factor in the pathophysiology of depression.

Keywords: depression, GABA, glutamate, patch-clamp

Introduction

Animal models, despite inherent limitations, are critical to achieve substantial progress in understanding the neurobiological bases of psychiatric disorders (Nestler and Hyman, 2010; Overstreet, 2012; Armario and Nadal, 2013). Among these models, the Wistar Kyoto (WKY) rat is frequently used to study the pathophysiology of depression. A substantial amount of work has shown that WKY rats present innate depressive-like behavior in different tests, such as the forced swimming test and the learned helplessness test (Pare, 1989; Pare and Redei, 1993; Lahmame and Armario, 1996; Pare and Tejani-Butt, 1996; Lahmame et al., 1997; Lopez-Rubalcava and Lucki, 2000; Rittenhouse et al., 2002; Tejani-Butt et al., 2003; Will et al., 2003; Malkesman, Braw, et al., 2006; Carr et al., 2010; Yamada et al., 2013; Kyeremanteng et al., 2014; Nam et al., 2014). They also exhibit disturbances in the sleep-wake cycle, including increased rapid-eye-movement sleep and sleep fragmentation (Dugovic et al., 2000), which are prevalent symptoms in depressive patients (Bunney and Bunney, 2013). In addition, hormonal abnormalities of the hypothalamic-pituitary-adrenal axis, often disrupted in depressive patients, have been extensively reported in this rat strain as compared to the Wistar (Wis) control strain (Pare and Redei, 1993; Redei et al., 2001; Solberg et al.,...
which WKY rats were derived (Okamoto and Aoki, 1963).

in the WKY rat. As a control group we used the Wis strain, from
and GABAergic synaptic activity of this noradrenergic nucleus
(De La Garza and Mahoney, 2004; Malkesman, Maayan, et al.,
1976; Williams et al., 1985; Aghajanian and Wang, 1986; Torrecilla
et al., 2008, 2013). Additionally, major glutamatergic input from
anterior to the genu of the facial nucleus (Figure 1A).

Male Wis and WKY rats weighing 175–250 g were provided by
Harlan Interfauna Ibérica. This weight range ensures the use of
age-matched animals, according to the animal supplier. Every
effort was made to minimize the suffering of the animals 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. All the experiments were 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

Drugs
(2S)-3-[(1S)-1-{3,4-Dichlorophenyl}ethyl]amino-2-hydroxypropyl](phenylmethyl) phosphinic acid hydrochloride (CGP 55845
hydrochloride), D-(-)-2-Amino-5-phosphonopentanoic acid
(D-APS), 6,7-Dinitroquinoxalin-2,3-dione disodium salt (DNQX
disodium salt), 4-Hydroxyquinoline-2-carboxylic acid (kynurenic
acid), 55,10R)-(+)-5-Methyl-10,11-dihydro5H-dibenzo[a,d]
cyclohepten-5,10-imine maleate [(+)-MK-801 maleate], picrotoxin,
and 17-Hydroxyyohimbane-16-carboxylic acid methyl
ester hydrochloride (yohimbine hydrochloride) were obtained
from Tocris Bioscience. Chlroly hydrate and 5-Bromo-6-(2-
imidazolin-2-ylamino) quinoxaline (UK 14 304) were obtained
from Sigma-Aldrich and (S)-1-Aminopropane-1,3-dicarboxylic
acid (glutamate) from Research Biochemicals International.

Drug stocks, except chloral hydrate, were prepared in distilled
water and diluted in artificial cerebrospinal fluid (ACSF) right before
use. Chloral hydrate was prepared in 0.9% saline and applied by
one intraperitoneal injection to achieve animal anaesthesia.

Path-Clamp Recordings of Locus Coeruleus
Neurons from Rat Brain Slices
Slice preparation and whole-cell patch-clamp recordings were
performed as described by Torrecilla et al. (2008).

Brain Slice Preparation
Animals were anaesthetized with chloral hydrate (400 mg/kg,
i.p) and decapitated. The brain was immediately extracted and
placed in cooled ACSF containing (in mM): 125 NaCl, 2.5 KCl, 1.2
MgCl2, 26 NaHCO3, 1.25 NaH2PO4, 2.4 CaCl2, and 11 D-glucose satu-
rated with 95% O2 and 5% CO2 (pH 7.3–7.4). In order to reduce
neuronal damage, (+)-MK-801 maleate (10 μM) was added to
ACSF. To record excitatory synaptic activity, kynurenic acid
(100 μM) was substituted for (+)-MK-801 maleate. Horizontal
brainstem sections (220 μm) containing the LC were cut using
a vibrtoome (VT1200S; Leica Microsystems). LC slices were
incubated in warmed (35°C) ACSF for at least 30 min before recording.

Neuronal Identification and Recording
The slice mounted on a recording chamber was maintained at
35–37 °C and perfused with ACSF at a flow rate of 1.5–2 ml/
min, through bath perfusion by gravity. LC neurons were visual-
ized, using an upright microscope with infrared optics (Eclipse
E600FN, Nikon), as a dense and compact group of cells located
on the lateral border of the central gray and the fourth ventricle,
just anterior to the genu of the facial nucleus (Figure 1A).

Electrophysiological parameters, UK 14 304, and glutamate-
induced whole-cell currents, as well as spontaneous glutamatergic
and GABAergic postsynaptic currents (eEPSC and sIPSC, respec-
tively) and evoked glutamatergic and GABAergic postsynaptic
currents (eEPSC and sIPSC, respectively) were recorded in voltage-
clamp mode. For that purpose, glass pipettes (2–4 MΩ) prepared
from borosilicate glass capillaries (World Precision Instruments)
with a micropipette puller (PC-10, Narishige CO., LTD) were filled
with an internal solution containing (in mM): 130 K-Gluconate, 5
NaCl, 1 MgCl2, 1 ethylene glycol-bis[2-aminoethyl] ether]-N,N,N',N'-
tetraacetic acid (EGTA), 10 4-[2-hydroxyethyl]piperazine-1-ethane-
sulfonic acid (HEPES), 2 adenosine 5'-triphosphate magnesium
salt (Mg-ATP), 0.5 guanosine 5'-triphosphate sodium salt hydrate
(Na-GTP), and 10 phosphocreatine disodium salt hydrate (pH: 7.4,
280 mOsm). Currents were recorded with the membrane potential
held at -50 mV. LC neurons were identified by the presence of
a resting inwardly-rectifying potassium (IRK) conductance by
stepping the membrane potential from -40 to -120 mV in -10

Experimental Procedures

Animals
Male Wis and WKY rats weighing 175–250 g were provided by
Harlan Interfauna Ibérica. This weight range ensures the use of
age-matched animals, according to the animal supplier. Every
effort was made to minimize the suffering of the animals 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. All the experiments were 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.
mV increments (100 ms/step; Figure 1B; Williams et al., 1988). The term inward rectification refers to an increase in slope of the current-voltage (I/V) relationship near the reversal potential (Nernst prediction for potassium, -105 mV for our recording conditions). Immediately after neuron identification membrane capacitance (Cm), series resistance and input resistance (Rm) were measured using Clampex software. In some experiments, resting membrane potential (Vm) was also recorded (I = 0). Two concentrations of UK (0.15 µM and 1.5 µM) were administered to calculate UK ratio. For that purpose, the peak current induced by 0.15 µM UK was divided by the peak current induced by 1.5 µM UK. In addition, activation of GIRK currents by UK were detected using the above described I/V protocol before and during drug application. Activation of G-protein coupled receptors opens a GIRK conductance, evidenced by an increase in the resting I/V (Williams et al., 1988). The amplitude of UK-induced currents in LC neurons was also normalized to the capacitance of the neuron, resulting in a current density. Series resistance was monitored throughout the experiment and the cell was discarded if it exceeded 15 MΩ.

To evaluate sEPSCs and eEPSCs, voltage-clamp recordings were performed with 100 µM picrotoxin in the ACSF to block GABA<sub>A</sub> receptors. The glass pipettes were filled with an internal solution containing (in mM): 70 CsSO<sub>4</sub>, 20 CsCl, 20 NaCl, 1.5 MgCl<sub>2</sub>, 5 HEPES, 1 EGTA, 2 Mg-ATP, and 0.5 Na-GTP (pH: 7.4, 280 mOsm). To trigger both α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors-mediated responses, eEPSCs were recorded at a holding potential of +40 mV. By passing constant current pulses (15–50 µA, at every 20 s, using the Iso-Flex stimulus isolator (A.M.P.I)) through a glass pipette situated into the LC and filled with external solution, 30 eEPSCs were evoked. After events stabilization, NMDA receptors antagonist D-AP5 (100 µM) was applied in order to isolate AMPA receptors-mediated eEPSCs. Glutamatergic total response and AMPA receptors-mediated eEPSCs were calculated from the average of at least seven consecutive events. NMDA receptors-mediated eEPSCs were calculated by subtraction of AMPA receptors-mediated response to total eEPSCs. Peak currents of isolated AMPA and NMDA receptors-mediated eEPSC were used to calculate the AMPA/NMDA ratio.

To record sIPSCs and eIPSCs, we added 1 µM CGP55845, 50 µM D-AP5 and 20 µM DNQX to the ACSF to block GABA<sub>B</sub>, NMDA and AMPA/kainate receptors, respectively. The internal solution contained (in mM): 115 KCl, 20 NaCl, 1.5 MgCl<sub>2</sub>, 5 HEPES, 10 1,2-bis(2-aminophenoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA), 2 Mg-ATP, and 0.5 Na-GTP (pH: 7.4, 280 mOsm). eIPSCs were recorded at a holding potential of -50 mV and one (1–10 mA) or five pulse trains (1–10 mA) were triggered at 10 Hz using a bipolar tungsten electrode situated posterior to the LC. GABA<sub>B</sub> receptors-mediated eIPSCs were calculated by averaging at least seven consecutive events. Changes in the probability of GABA release were investigated by measuring the IPSCn/IPSC 1 ratio. To do that the amplitude of a consecutive IPSC (IPSCn) was divided by the amplitude of the first IPSC (IPSC1).

**Data Acquisition and Analysis**

Recordings were detected with an Axopatch-200B (Axon Instruments), filtered at 5 kHz and digitized with a Digidata 1322A (Axon Instruments). Data were sampled at 10 kHz and analyzed with Clampex 10.2 software (Axon Instruments).

Recordings were filtered post hoc at 1 kHz and visually inspected to select manually synaptic events. Template detection was used to select spontaneous synaptic events. Template was generated from an average of multiple spontaneous events, the selection was fitted to the 3.5 threshold of the template, and each peak was visually inspected. The duration of synaptic events was determined by measuring the width at 50% of the peak amplitude (half-width). In all cases, spontaneous activity from each neuron was measured for 1 minute. All data were extracted using Clampfit 10.3 software.

Graph Prism (v.5.01; GraphPad Software, Inc.) was used for statistical evaluations. Non-repeated two-way ANOVA was used to compare the outward current induced by two different concentrations of UK in both rat strains, Wis and WKY. The amplitude of spontaneous synaptic currents was analyzed by the Kolmogorov-Smirnov test (Clampfit 10.3 software). The rest of the electrophysiological parameters evaluated in the present work were statistically compared through strains using Student’s t-test. The level of significance was considered as p < 0.05.

---

**Figure 1.** Localization and electrophysiological identification of noradrenergic locus coeruleus (LC) neurons in vitro. (A) Representative picture of LC localization in the horizontal plane (Paxinos and Watson, 1997). (B) Representative traces of inwardly-rectifying potassium currents in a LC neuron triggered by stepping the membrane potential in -10 mV increments for 100 ms.
Results

Electrophysiological Findings

Intrinsic Electrophysiological Properties of the Locus Coeruleus Neurons from Wistar Kyoto Rats

To study whether LC neurons from WKY and Wis rats had substantial differences in their intrinsic electrical properties, besides visual localization, we first identify LC neurons by the presence of a resting IRK conductance (see in Experimental Procedures, the Neuronal Identification section and Figure 1B). A total of 138 LC neurons were recorded: 65 neurons from the Wis strain (n = 12 animals) and 73 neurons from the WKY strain (n = 12 animals). The basal properties of these LC cells, such as Cm, Rm, and Vm, did not statistically differ between strains (Table 1).

In Vitro Noradrenergic Transmission in Locus Coeruleus Neurons of Wistar Kyoto Rats

To evaluate the noradrenergic transmission on LC neurons from Wis and WKY strains, we recorded the whole-currents induced by two different concentrations of the α1-adrenoceptor agonist UK (0.15 µM and 1.5 µM). In LC neurons of both Wis and WKY rats, 0.15 µM and 1.5 µM UK application caused a concentration-dependent outward current at a holding potential of -50 mV (p < 0.001, two-way ANOVA; Figure 2A and B). The amplitude of the peak current induced by each concentration was not statistically different between strains (0.15 µM UK, Wis: 124.9 ± 7.79 pA, n = 7 and WKY: 110.1 ± 9.02 pA, n = 5; 1.5 µM UK, Wis: 152.1 ± 7.61 pA, n = 7 and WKY: 154.9 ± 14.52 pA, n = 5; Figure 2B, upper panel). Accordingly, the current densities induced by UK perfusion were not different between Wis and WKY rats (0.15 µM UK, Wis: 0.65 ± 0.06 pA/pF, n = 7 and WKY: 0.61 ± 0.07 pA/pF, n = 5; 1.5 µM UK, Wis: 0.82 ± 0.06 pA/pF, n = 7 and WKY: 0.94 ± 0.08 pA/pF, n = 5; Figure 2B, lower panel). However, when we analyzed the UK ratio of the peak current induced by both concentrations of UK, data showed that LC neurons from WKY rats were significantly less sensitive than those from the Wis strain (WKY: 0.64 ± 0.03, n = 5; Wis: 0.79 ± 0.04, n = 7; p = 0.028, unpaired two-tailed t-test; Figure 2C).

The UK-induced outward current was completely reversed by α1-adrenoceptor antagonist yohimbine (10 µM; Figure 2A).

To further characterize the agonist-induced outward current in the LC neurons, I/V relationships were obtained before and during UK perfusion, by hyperpolarizing the membrane potential (see Experimental Procedures)). While bath perfusion of 0.15 µM UK increased the I/V relationship recorded in LC neurons of Wis rats to a greater extent than that in WKY rats (Figure 2D), perfusion of 1.5 µM UK induced a similar increment in both strains (Figure 2D). The reversal potential of the I/V obtained during UK perfusion was similar to that predicted by Nernst for a potassium-sensitive IRK conductance (see Experimental Procedures). Both Wis and WKY rats showed similar AMPA/NMDA ratios regarding the amplitude and the half-width of eEPSCs in LC neurons (amplitude for Wis: 2.24 ± 0.55, n = 7 and WKY: 1.71 ± 0.27, n = 10; half-width for Wis: 0.44 ± 0.05, n = 7 and WKY: 0.32 ± 0.03, n = 10; Figure 5A and B).

Study of the GABAergic Input to Locus Coeruleus Neurons of Wistar Kyoto Rats

To evaluate fast inhibitory synaptic activity in LC neurons, GABA transmission was pharmacologically isolated by adding the following AMPA, NMDA, and GABA_A receptor blockers to the external bath solution (Figure 6A): DNQX diiodosalt (20 µM), D-AP5 (50 µM), and CGP 55845 hydrochloride (1 µM). The frequency of sIPSCs in LC neurons was not statistically different between strains (Wis: 2.76 ± 0.38 Hz, n = 22; WKY: 2.59 ± 0.24 Hz, n = 27; Figure 6B). However, in the WKY rats the mean amplitude of sIPSCs was significantly reduced as compared to that recorded in Wis rats (Wis: 45.27 ± 0.44 pA, n = 22; WKY: 34.33 ± 0.33 pA, n = 27; p < 0.01, Kolmogorov-Smirnov test; Figure 6B). The amplitude of the peak current induced by each concentration was not statistically different between strains (0.15 µM UK, Wis: 124.9 ± 7.79 pA, n = 6; 1.5 µM UK: 152.1 ± 7.61 pA, n = 6; Figure 2B, upper panel). Accordingly, the current densities induced by UK perfusion were not different between Wis and WKY rats (0.15 µM UK, Wis: 0.82 ± 0.06 pA/pF, n = 7 and WKY: 0.94 ± 0.08 pA/pF, n = 5; Figure 2B, lower panel). However, when we analyzed the UK ratio of the peak current induced by both concentrations of UK, data showed that LC neurons from WKY rats were significantly less sensitive than those from the Wis strain (WKY: 0.64 ± 0.03, n = 5; Wis: 0.79 ± 0.04, n = 7; p = 0.028, unpaired two-tailed t-test; Figure 2C).

The UK-induced outward current was completely reversed by α1-adrenoceptor antagonist yohimbine (10 µM; Figure 2A).

To further characterize the agonist-induced outward current in the LC neurons, I/V relationships were obtained before and during UK perfusion, by hyperpolarizing the membrane potential (see Experimental Procedures). While bath perfusion of 0.15 µM UK increased the I/V relationship recorded in LC neurons of Wis rats to a greater extent than that in WKY rats (Figure 2D), perfusion of 1.5 µM UK induced a similar increment in both strains (Figure 2D). The reversal potential of the I/V obtained during UK perfusion was similar to that predicted by Nernst for a potassium-sensitive IRK conductance (see Experimental Procedures). Both Wis and WKY rats showed similar AMPA/NMDA ratios regarding the amplitude and the half-width of eEPSCs in LC neurons (amplitude for Wis: 2.24 ± 0.55, n = 7 and WKY: 1.71 ± 0.27, n = 10; half-width for Wis: 0.44 ± 0.05, n = 7 and WKY: 0.32 ± 0.03, n = 10; Figure 5A and B).

Study of the GABAergic Input to Locus Coeruleus Neurons of Wistar Kyoto Rats

To evaluate fast inhibitory synaptic activity in LC neurons, GABA transmission was pharmacologically isolated by adding the following AMPA, NMDA, and GABA_A receptor blockers to the external bath solution (Figure 6A): DNQX diiodosalt (20 µM), D-AP5 (50 µM), and CGP 55845 hydrochloride (1 µM). The frequency of sIPSCs in LC neurons was not statistically different between strains (Wis: 2.76 ± 0.38 Hz, n = 22; WKY: 2.59 ± 0.24 Hz, n = 27; Figure 6B). However, in the WKY rats the mean amplitude of sIPSCs was significantly reduced as compared to that recorded in Wis rats (Wis: 45.27 ± 0.44 pA, n = 22; WKY: 34.33 ± 0.33 pA, n = 27; p < 0.01, Kolmogorov-Smirnov test; Figure 6B). The amplitude of the peak current induced by each concentration was not statistically different between strains (0.15 µM UK, Wis: 124.9 ± 7.79 pA, n = 6; 1.5 µM UK: 152.1 ± 7.61 pA, n = 6; Figure 2B, upper panel). Accordingly, the current densities induced by UK perfusion were not different between Wis and WKY rats (0.15 µM UK, Wis: 0.82 ± 0.06 pA/pF, n = 7 and WKY: 0.94 ± 0.08 pA/pF, n = 5; Figure 2B, lower panel). However, when we analyzed the UK ratio of the peak current induced by both concentrations of UK, data showed that LC neurons from WKY rats were significantly less sensitive than those from the Wis strain (WKY: 0.64 ± 0.03, n = 5; Wis: 0.79 ± 0.04, n = 7; p = 0.028, unpaired two-tailed t-test; Figure 2C).

The UK-induced outward current was completely reversed by α1-adrenoceptor antagonist yohimbine (10 µM; Figure 2A).

To further characterize the agonist-induced outward current in the LC neurons, I/V relationships were obtained before and during UK perfusion, by hyperpolarizing the membrane potential (see Experimental Procedures). While bath perfusion of 0.15 µM UK increased the I/V relationship recorded in LC neurons of Wis rats to a greater extent than that in WKY rats (Figure 2D), perfusion of 1.5 µM UK induced a similar increment in both strains (Figure 2D). The reversal potential of the I/V obtained during UK perfusion was similar to that predicted by Nernst for a potassium-sensitive IRK conductance (see Experimental Procedures). Current densities of the basal and UK-induced I/V were also provided, and were all similar in both strains (Figure 2E).

Table 1. In vitro intrinsic electrophysiological properties of locus coeruleus neurons recorded in Wistar and Wistar Kyoto rats.

|            | Cm (pF) | Rm (MΩ) | Vm (mV) |
|------------|---------|---------|---------|
| Wistar     | 142.9 ± 6.6 | 260.8 ± 13.7 | -56.38 ± 2.81 |
| Wistar Kyoto | 153.0 ± 7.5 | 237.8 ± 12.2 | -52.14 ± 1.72 |

All values represent the mean ± standard error of the mean of experiments. To calculate Cm and Rm, Wis n = 65 and WKY n = 74. For Vm Wis n = 13 and WKY n = 25. No statistical significances were found between strains using unpaired two-tailed t-tests.

Cm, membrane capacitance; Rm, membrane resistance; Vm, resting membrane potential.
Figure 2. Whole-cell currents induced by UK 14,304 in locus coeruleus (LC) neurons of Wistar (Wis) and Wistar Kyoto (WKY) rats. (A) Representative recordings of the outward current induced by UK (0.15 µM and 1.5 µM) in LC neurons of Wis and WKY rats. Administration of the α₂-antagonist yohimbine (10 µM) completely reversed the UK-induced current. (B) Summary bar graphs displaying the mean amplitudes of the UK-induced outward currents and mean of current densities in a concentration-dependent manner (*p < 0.01 and **p < 0.001, unpaired two-tailed t-test). (C) Summary data of UK ratio (*p = 0.028, unpaired two-tailed t-test). (D) Whole-cell current-voltage relationship in LC neurons of Wis (grey symbols) and WKY (black symbols) rats at rest (circles) and during (squares) UK perfusion (n = 6–7). (E) Current-voltage relationship in LC neurons of Wis (grey symbols) and WKY (black symbols) rats for the data concerning basal (circles) and induced current densities (pA/pF) during (squares) UK perfusion (n = 6–7).
differences were observed in the amplitude of consecutive eIPSC or in the IPSCn/IPSC1 ratio between WKY and Wis rats. It is important to note that while the frequency of inhibitory synaptic events was statistically greater in the LC of Wis rats than the frequency of the excitatory events (sIPSCs: $2.76 \pm 0.38$ Hz, $n = 22$; sEPSCs: $1.86 \pm 0.31$ Hz, $n = 25$; $p < 0.05$, unpaired $t$-test; see Figures 4B and 6B), in WKY rats both frequencies were similar. Interestingly, a previous patch-clamp study showed that sEPSCs frequencies were lower than sIPSCs frequencies in the LC of Sprague-Dawley rats (Sugiyama et al., 2012), in line with what we observed in the Wis strain. This suggests that synaptic activity might be dysregulated in LC neurons of WKY rats.
Discussion

A recent in vivo study from our group reveals significant alterations in LC activity of the WKY rat (Bruzos-Cidon et al., 2014) in a proposed animal model of anxiety/depression (Lahmame et al., 1997; Lopez-Rubalcava and Lucki, 2000; Pare, 2000; Pardon et al., 2002; Tejani-Butt et al., 2003; De La Garza and Mahoney, 2004; Ma and Morilak, 2004; Tizabi et al., 2012). To further investigate the origin of those adaptations, the main goal of the present study was to determine whether WKY rats display a differential modulation of LC neuronal activity compared to that of Wis rats. For that purpose, intrinsic sensitivity of $\alpha_2$-adrenoceptors and extrinsic synaptic inputs to LC neurons were evaluated in Wis and WKY rats in vitro. Our results indicate that in LC neurons of WKY rats, $\alpha_2$-adrenoceptors are desensitized and, while spontaneous GABAergic synaptic activity is reduced, the glutamatergic activity is similar in both rat strains. The latter data may reflect an imbalance between the glutamatergic and GABAergic transmissions, which has been previously described in depressive patients (Krystal et al., 2002; Luscher et al., 2011) and WKY rats.

Regarding noradrenergic transmission in WKY rats, we have previously shown that in vivo LC neurons of this rat strain are less sensitive to the inhibitory effect of the $\alpha_2$-adrenoceptor agonist clonidine to the basal firing rate (Bruzos-Cidon et al., 2014). The present in vitro study indicates that WKY rats display lower sensitivity to $\alpha_2$-adrenoceptor agonist UK compared to the Wis strain, since the UK ratios of the peak current induced by two submaximal concentrations of the drug are significantly smaller in WKY rats compared to in Wis rats. These results confirm that noradrenergic transmission in LC neurons from WKY rats is intrinsically altered, since $\alpha_2$-adrenoceptors are desensitized in vitro. This could be due to a number of reasons, including both different functionality/density of $\alpha_2$-adrenoceptor and/or GIRK channels in this rat strain. Although so far expression of GIRK channels has not been investigated in the WKY rat, an early study using in situ hybridization showed that expression of $\alpha_2$-adrenoceptor mRNA in the LC of WKY rats is not different to that obtained in Wis rats (Sands et al., 2000). In line with this, specific binding to $\alpha_2$-adrenoceptors in the LC of WKY rats is similar to that in the control Sprague-Dawley strain (Tejani-Butt et al., 1994).

All in all, dysregulation of this signaling pathway in the LC of WKY rats could be due to altered expression levels or function of other downstream components such as Gi/o proteins and/or the GIRK channels. Evidence indicates that noradrenergic transmission is altered in WKY rats. Thus, deficient noradrenergic reactivity to acute stress (Pardon et al., 2002), low levels of NA in the LC and several of its terminal sites (De La Garza and Mahoney, 2004; Scholl et al., 2010), as well as reduced cortical NA reuptake (Jeannotte et al., 2009) have been described in this rat strain. Accordingly, it has been recently reported that acute administration of the noradrenergic antidepressant reboxetine is less potent at inhibiting in vivo basal activity of LC neurons in the WKY rats than that recorded in the Wis strain (Bruzos-Cidon et al., 2014). Overall, a dysregulation of the noradrenergic transmission, including the inhibitory control that $\alpha_2$-adrenoceptors exert onto neuronal activity of LC neurons, could be related to the in vivo LC hyperactivity observed in the WKY strain. Interestingly, chronic exposure to cold stress, which resembles alterations observed on central noradrenergic transmission of patients with mood and anxiety disorders, alters electrophysiological properties of LC neurons not only in vivo but also in vitro (Mana and Grace, 1997; Jedema and Grace, 2003).
Preclinical and clinical studies support a relevant role of the glutamatergic transmission in the pathology of psychiatric illnesses such as depression (Krystal et al., 2002; Pittenger et al., 2007). Our results show that postsynaptic sensitivity of LC neurons to glutamate is not different between the WKY and Wis strains. Regarding glutamatergic synaptic input to LC, no significant alterations have been detected in WKY rats. In the present study we separately evaluated AMPA and NMDA receptors-mediated eEPSC and we did not observe differences between the two strains. Although lower or similar NMDA receptor binding has been observed in the hippocampus, as well as in other brain areas of WKY compared to Wis rats (Lei et al., 2009), the ratio of AMPA/NMDA receptor densities is similar in both strains and is selectively increased in the WKY strain after chronic treatment with ketamine (Tizabi et al., 2012). Further experiments would be of interest to determine the electrophysiological response of noradrenergic neurons to antidepressant drugs that affect glutamatergic transmission in WKY rats. Despite lacking data about NMDA and AMPA receptor densities in LC of WKY rats, our data indicate that the synaptic activity of both NMDA and AMPA receptors is similar in the WKY and Wis strains.

The current study indicates that the frequency of sIPSC in LC neurons is similar in both strains, which suggests that release probability of GABA is not significantly altered in LC neurons of WKY strain. On the contrary, sIPSCs amplitude, which is also determined by postsynaptic factors such as receptor sensitivity, is reduced in LC neurons of WKY rats as compared with those of Wis rats. In addition, the half-width of sIPSCs is significantly greater in LC neurons of WKY rats, indicating that in this rat strain GABAergic spontaneous events are slower than in the Wis strain. Interestingly, several studies identify a GABAergic deficit as a possible cause of mood disorders. One hand, reduced plasma and brain concentrations of GABA have been reported in depressed patients (Gerner and Hare, 1981; Petty and Sherman, 1984; Honig et al., 1988; Hasler et al., 2007). On the other, animal studies demonstrate that GABAergic transmission is dysregulated in several animal models of depression (Dennis et al., 1994; Zink et al., 2009; Veeraiah et al., 2014; Venzala et al., 2013) and that chronic administration of antidepressants markedly changes GABAergic transmission (Wabno and Hess, 2013). As previously described in Sprague-Dawley rats (Sugiyama et al., 2012), the frequency of sEPSCs and sIPSCs are uneven in LC neurons of Wis rats. Conversely, LC neurons of WKY rats showed similar frequencies of both spontaneous synaptic events, suggesting synaptic dysregulation. Since the LC spontaneous firing rate is regulated by GABAergic input (Szabo and Blier, 2001; Belujon et al., 2009), the deficient inhibitory GABAergic transmission in this brain area may be underlying the in vivo hyperactivity of LC neurons in WKY rats (Bruzos-Cidon et al., 2014). Corticotropin-releasing factor fails to increase GABAergic activity in dorsal raphe neurons of WKY rats, as is normally seen in Sprague-Dawley rats (Lemos et al., 2011). Altogether, these results support the GABAergic deficit hypothesis as a cause of depressive disorder in the WKY strain. Importantly, reduced GABAergic transmission in LC of WKY rats could account for the disruption of the balance between glutamatergic and GABAergic transmission.
systems that has been reported as an important factor in the pathophysiology of mood disorders, including depression (Krystal et al., 2002; Luscher et al., 2011).

Our data indicate that LC neurons of WKY are differentially regulated as compared to those of Wis rats, since the noradrenergic and GABAergic systems are altered. Thus, both intrinsic and extrinsic inhibitory mechanisms are diminished in LC neurons of WKY rats. In summary, this study supports the utility of the WKY rat as a useful animal model to characterize the neurobiological bases of pathologies related to dysfunctions of multiple neurotransmitter systems, such as anxiety and depression.

Acknowledgements

This work was supported by grants from the Basque Government (S-PE11UN055); the University of the Basque Country, UPV/EHU (UFI 11/32); and the Spanish Government (FIS PI12/00613). Dr Bruzos-Cidón had a postdoctoral fellowship from the University of the Basque Country, UPV/EHU, and Dr Llamosas had a predoctoral fellowship from the University of the Basque Country, UPV/EHU.

These institutions had no further role in the present study. The authors were entirely responsible for the scientific content of the manuscript and the decision to submit it for publication. We thank The Advanced Research Facilities (SGIker) of the University of the Basque Country, UPV/EHU for animal care.

Statement of Interest

None

References

Aghajanian GK, Wang YY (1986) Pertussis toxin blocks the outward currents evoked by opiate and alpha 2-agonists in locus coeruleus neurons. Brain Res 371:390–394.

Armario A, Nadal R (2013) Individual differences and the characterization of animal models of psychopathology: A strong challenge and a good opportunity. Front Pharmacol 4:137.

Aston-Jones G, Shipley MT, Chouvet G, Ennis M, van Bockstaele E, Pieribone V, Shiekhattar R, Akaoka H, Drolet G, Astier B (1991) Afferent regulation of locus coeruleus neurons: Anatomy, physiology and pharmacology. Prog Brain Res 88:47–75.

Aston-Jones G, Zhu Y, Card JP (2004) Numerous GABAergic afferents to locus ceruleus in the pericerulear dendritic zone: Possible interneuronal pool. J Neurosci 24:2313–2321.

Belujon P, Baufreton J, Grandoso L, Boue-Grabo B, Batten TF, Ugedo L, Garret M, Taupignon Ai (2009) Inhibitory transmission in locus coeruleus neurons expressing GABA A receptor epsilon subunit has a number of unique properties. J Neurophysiol 102:2312–2325.

Bruzos-Cidón C, Miguelez C, Rodriguez JJ, Gutierrez-Lanza R, Ugedo L, Torrecilla M (2014) 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. Eur Neuropsychopharmacol 24:1122–1123.

Bunney BG, Bunney WE (2013) Individual differences and the characterization of animal models of psychopathology: A strong challenge and a good opportunity. Front Pharmacol 4:137.
Cedarbaum JM, Aghajanian GK (1976) Noradrenergic neurons of the locus coeruleus: Inhibition by epinephrine and activation by the alpha-antagonist piperoxane. Brain Res 112:413–419.

Corteen NL, Cole TM, Sarna A, Sieghart W, Swinny JD (2011) Localization of GABA-A receptor alpha subunits on neurochemically distinct cell types in the rat locus coeruleus. Eur J Neurosci 34:250–262.

De La Garza R, 2nd, Mahoney J, 3rd (2004) A distinct neurochemical profile in WKY rats at baseline and in response to acute stress: Implications for animal models of anxiety and depression. Brain Res 1021:209–218.

Dennis T, Beauchemin V, Lavoie N (1994) Antidepressant-induced modulation of GABAA receptors and beta-adrenoceptors but not GABAB receptors in the frontal cortex of olfactory bulbectomised rats. Eur J Pharmacol 262:143–148.

Dugovic C, Solberg LC, Redei E, Van Reeth O, Turek FW (2000) Sleep in the wistar-kyoto rat, a putative genetic animal model for depression. Neuroreport 11:527–531.

Geiger RH, Hare TA (1981) CSF GABA in normal subjects and patients with depression, schizophrenia, mania, and anorexia nervosa. Am J Psychiatr 138:1098–1101.

Hasler G, van der Veen JW, Tunonis T, Meyers N, Shen J, Drevets WC (2007) Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. Arch Gen Psychiatr 64:193–200.

Honig A, Bartlett JR, Bouras N, Bridges PK (1988) Amino acid levels in depression: A preliminary investigation. J Psychiatr Res 22:159–164.

Iijima K, Ohtomo K (1987) Immunocytochemical study using a GABA antiserum for the demonstration of inhibitory neurons in the rat locus ceruleus. Am J Anat 181:43–52.

Itti K, Sugimoto N (2010) The brainstem noradrenergic systems in stress, anxiety and depression. J Neuroendocrinol 22:355–361.

Jeannotte AM, McCarthy JG, Redei EE, Sidhu A (2009) Desipramine modulation of alpha-, gamma-synuclein, and the noradrenaline transporter in an animal model of depression. Neuropsychopharmacology 34:987–998.

Jedema HP, Grace AA (2003) Chronic exposure to cold stress alters electrophysiological properties of locus coeruleus neurons recorded in vitro. Neuropsychopharmacology 28:63–72.

Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, Epperson CN, Goddard A, Mason GF (2002) Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. Mol Psychiat 7(Suppl 1):71–80.

Kyeremanteng C, MacKay JC, James JS, Kent P, Cayer C, Anisman H, Merali Z (2014) Effects of electroconvulsive seizures on depression-related behavior, memory and neurochemical changes in wistar and wistar-kyoto rats. Prog Neuropsychopharmacol Biol Psychiatr 54C:170–178.

Lahmame A, Armario A (1996) Differential responsiveness of inbred strains of rats to antidepressants in the forced swimming test: Are wistar kyoto rats an animal model of subsensitivity to antidepressants? Psychopharmacology (Berl) 123:191–198.

Lahmame A, del Arco C, Pazos A, Yritia M, Armario A (1997) Are wistar-kyoto rats a genetic animal model of depression resistant to antidepressants? Eur J Pharmacal 337:115–123.

Lei Y, Yaroslavsky I, Tejani-Butt SM (2009) Strain differences in the distribution of N-methyl-d-aspartate and gamma (gamma)-aminobutyric acid-A receptors in rat brain. Life Sci 85:794–799.

Lemos JC, Zhang G, Walsh T, Kirby LG, Akanwa A, Brooks-Kayal A, Beck SG (2011) Stress-hyperresponsive WKY rats demonstrate depressed dorsal raphe neuronal excitability and dysregulated CRF-mediated responses. Neuropsychopharmacolology 36:721–734.

Lopez-Rubalcava C, Lucki I (2000) Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. Neuropsychopharmacology 22:191–199.

Luscher B, Shen Q, Sahir N (2011) The GABAergic deficit hypothesis of major depressive disorder. Mol Psychiatr 16:383–406.

Ma S, Morilik DA (2004) Induction of FOS expression by acute immobilization stress is reduced in locus coeruleus and medial amygdala of wistar-kyoto rats compared to sprague-dawley rats. Neuroscience 124:963–972.

Malkesman O, Braw Y, Maayan R, Weizman A, Overstreet DH, Shabat-Simon M, Kesner Y, Touati-Werner D, Yadid G, Weller A (2006) Two different putative genetic animal models of childhood depression. Biol Psychiatry 59:17–23.

Malkesman O, Maayan R, Weizman A, Weller A (2006) Aggressive behavior and HPA axis hormones after social isolation in adult rats of two different genetic animal models for depression. Behav Brain Res 175:408–414.

Mano MJ, Grace AA (1997) Chronic cold stress alters the basal and evoked electrophysiological activity of rat locus coeruleus neurons. Neuroscience 81:1055–1064.

Morilik DA, Barrera G, Echevarria DJ, Garcia AS, Hernandez A, Ma S, Petre CO (2005) Role of brain norpinephrine in the behavioral response to stress. Prog Neuropsychopharmacol Biol Psychiatr 29:1214–1224.

Nam H, Clinton SM, Jackson NL, Kerman IA (2014) Learned helplessness and social avoidance in the wistar-kyoto rat. Front Behav Neurosci 8:109.

Nestler EJ, Hyman SE (2010) Animal models of neuropsychiatric disorders. Nat Neurosci 13:1161–1169.

Noriega NC, Garyfallou VT, Kohama SG, Urbanski HF (2007) Glutamate receptor subunit expression in the rhesus macaque locus coeruleus. Brain Res 1173:53–65.

Okamoto K, Aoki K (1963) Development of a strain of spontaneously hypertensive rats. Jpn Circ J 27:282–293.

Olpe HR, Steinmann MW, Brugger F, Pozza MF (1989) Excitatory amino acid receptors in rat locus coeruleus: an extracellular in vitro study. Naunyn Schmiedebergs Arch Pharmacol 339:312–314.

Overstreet DH (2012) Modeling depression in animal models. Methods Mol Biol 829:125–144.

Pardon MC, Gould GG, Garcia A, Phillips L, Cook MC, Miller SA, Mason PA, Morilik DA (2002) Stress reactivity of the brain noradrenergic system in three rat strains differing in their neuroendocrine and behavioral responses to stress: Implications for susceptibility to stress-related neuropsychiatric disorders. Neuroscience 115:229–242.

Pare WP (1989) Stress ulcer susceptibility and depression in wistar kyoto (WKY) rats. Physiol Behav 46:993–998.

Pare WP (2000) Investigatory behavior of a novel conspecific by wistar kyoto, wistar and sprague-dawley rats. Brain Res Bull 53:759–765.

Pare WP, Redei E (1993) Depressive behavior and stress ulcer in wistar kyoto rats. J Physiol Paris 87:229–238.

Pare WP, Tejani-Butt SM (1996) Effect of stress on the behavior and 5-HT system in sprague-dawley and wistar kyoto rat strains. Integr Physiol Behav Sci 31:112–121.

Paxinos G, Watson C (1997) The Rat Brain in Stereotaxic Coordinates, Compact. San Diego, CA: Academic Press.
Petty F, Sherman AD (1984) Plasma GABA levels in psychiatric illness. J Affect Disord 6:131–138.

Pittenger C, Sanacora G, Krystal JH (2007) The NMDA receptor as a therapeutic target in major depressive disorder. CNS Neurol Drug Targets 6:101–115.

Redei EE, Solberg LC, Kluczynski JM, Pare WP (2001) Paradoxic hormonal and behavioral responses to hypothyroid and hyperthyroid states in the wistar-kyoto rat. Neuropsychopharmacology 24:632–639.

Rittenhouse PA, Lopez-Rubalcava C, Stanwood GD, Lucki I (2002) Amplified behavioral and endocrine responses to forced swim stress in the wistar-kyoto rat. Psychoneuroendocrinology 27:303–318.

Sanacora G, Treccani G, Popoli M (2012) Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders. Neuropsychopharmacology 62:63–77.

Sands SA, Strong R, Corbitt J, Morilak DA (2000) Effects of acute restraint stress on tyrosine hydroxylase mRNA expression in Locus coeruleus of Wistar and Wistar-Kyoto rats. Mol Brain Res 75:1–7.

Sara SJ, Bouret S (2012) Orienting and reorienting: The locus coeruleus mediates cognition through arousal. Neuron 76:130–141.

Scholl JL, Renner KJ, Forster GL, Tejani-Butt S (2010) Central monoamine levels differ between rat strains used in studies of depressive behavior. Brain Res 1355:41–51.

Solberg LC, Olson SL, Turek FW, Redei E (2001) Altered hormone levels and circadian rhythm of activity in the WKY rat, a putative animal model of depression. Am J Physiol Regul Integr Comp Physiol 281:R786–794.

Sugiyama D, Hur SW, Pickering AE, Kase D, Kim SJ, Kawamata M, Imoto K, Furue H (2012) In vivo patch-clamp recording from locus coeruleus neurones in the rat brainstem. J Physiol 590:2225–2231.

Szabo ST, Blier P (2001) Serotonin (1A) receptor ligands act on norepinephrine neuron firing through excitatory amino acid and GABA(A) receptors: A microiontophoretic study in the rat locus coeruleus. Synapse 42:203–212.

Tejani-Butt S, Pare WP, Yang J (1994) Effect of repeated novel stressor on depressive behavior and brain norepinephrine receptor system in Sprague-Dawley and Wistar Kyoto (WKY) rats. Brain Res 649:27–35.

Tejani-Butt S, Kluczynski J, Pare WP (2003) Strain-dependent modification of behavior following antidepressant treatment. Prog Neuropsychopharmacol Biol Psychiatry 27:7–14.

Tizabi Y, Bhatti BH, Manaye KF, Das JR, Akinfresoye L (2012) Anti-depressant-like effects of low ketamine dose is associated with increased hippocampal AMPA/NMDA receptor density ratio in female wistar-kyoto rats. Neuroscience 213:72–80.

Torrecilla M, Quillinan N, Williams JT; Wickman K (2008) Pre- and postsynaptic regulation of locus coeruleus neurons after chronic morphine treatment: A study of GIRK-knockout mice. Eur J Neurosci 28:618–624.

Torrecilla M, Fernandez-Aedo I, Arrue A, Zumarraga M, Ugedo L (2013) Role of GIRK channels on the noradrenergic transmission in vivo: An electrophysiological and neurochemical study on GIRK2 mutant mice. Int J Neuropsychophysiol 16:1093–1104.

Veeraiah P, Noronha JM, Maitra S, Bagga P, Khandelwal N, Chakravarty S, Kumar A, Patel AB (2014) Dysfunctional glutamatergic and gamma-aminobutyric acidergic activities in prefrontal cortex of mice in social defeat model of depression. Biol Psychiatry 76(3):231–8.

Venzala E, Garcia-Garcia AL, Elizalde N, Tordera RM (2013) Social vs. environmental stress models of depression from a behavioural and neurochemical approach. Eur Neuropsychopharmacol 23:697–708.

Wabno J, Hess G (2013) Repeated administration of imipramine modifies GABAergic transmission in rat frontal cortex. J Neural Transm 120:711–719.

Will CC, Aird F, Redei EE (2003) Selectively bred wistar-kyoto rats: An animal model of depression and hyper-responsiveness to antidepressants. Mol Psychiatry 8:925–932.

Williams JT, Henderson G, North RA (1985) Characterization of alpha 2-adrenoceptors which increase potassium conductance in rat locus coeruleus neurones. Neuroscience 14:95–101.

Williams JT, North RA, Tokimasa T (1988) Inward rectification of resting and opiate-activated potassium currents in rat locus coeruleus neurones. J Neurosci 8:4299–4306.

Williams JT, Bobker DH, Harris GC (1991) Synaptic potentials in locus coeruleus neurones in brain slices. Prog Brain Res 88:167–172.

Yamada M, Kawahara Y, Kaneko F, Kishikawa Y, Sotogaku N, Poppinga WJ, Folgering JH, Dremencov E, Kawahara H, Nishi A (2013) Upregulation of the dorsal raphe nucleus-prefrontal cortex serotonin system by chronic treatment with escitalopram in hyposerotonergic wistar-kyoto rats. Neuropharmacology 72:169–178.

Zink M, Vollmayr B, Gebicke-Haerter PJ, Henn FA (2009) Reduced expression of GABA transporter GAT3 in helpless rats, an animal model of depression. Neurochem Res 34:1584–1593.