Electroconvulsive shocks decrease α_2 -adrenoceptor binding in the Flinders rat model of depression

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Abstract

Despite years of drug development, electroconvulsive therapy (ECT) remains the most effective treatment for severe depression. The exact therapeutic mechanism of action of ECT is still unresolved and therefore we tested the hypothesis that the beneficial effect of ECT could in part be the result of increased noradrenergic neurotransmission leading to a decrease in α_2 -adrenoceptor binding.

We have previously shown that both the Flinders sensitive line (FSL) and Flinders resistant line (FRL) rats had altered α_2 -adrenoceptor binding compared to control Sprague-Dawley (SD) rats. In this study, we treated female FSL, FRL and SD rats with electroconvulsive shock (ECS), an animal model of ECT, or sham stimulation for 10 days before brains were removed and cut into 20 μ m thick sections. Densities of α_2 -adrenoceptors were measured by quantitative autoradiography in the hippocampus, thalamic nucleus, hypothalamus, amygdala, frontal cortex, insular cortex, and perirhinal cortex using the α_2 -adrenoceptor antagonist, [³H]RX 821002.

ECS decreased the binding of α_2 -adrenoceptors in cortical regions in the FSL and cortical and amygdaloid regions in the control FRL rats compared to their respective sham treated group. The normal SD controls showed no significant response to ECS treatment. Our data suggest that the therapeutic effect of ECS may be mediated through a decrease of α_2 -adrenoceptors,

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probably due to a sustained increase in noradrenaline release. These data confirm the importance of the noradrenergic system and the α_2 -adrenoceptor in depression and in the mechanism of antidepressant treatments.

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1. Introduction

Depression does not only affect an individual's mood but also their sleep, appetite and interest in daily activities resulting in insomnia, weight loss, overall disability and loss of quality of life in affected individuals. Moreover, symptoms of anxiety often coexist with depression. The individual and societal costs associated with depression are pervasive, and include higher suicide rates, complications in consequence of chronic disease, higher health care cost, and loss of productive work time (American Psychiatric Association, 2013).

In cases where pharmacological antidepressants provide insufficient relief of symptoms, electroconvulsive therapy (ECT), a non-pharmacological treatment, shows quick onset of clinical improvement (Sackeim et al., 1995). ECT was introduced to psychiatric practice 80 years ago and remains the most effective antidepressant therapy.

The exact mechanism of action of ECT remains to be elucidated, although prior studies in human and animals support the involvement of monoaminergic systems in the effects of ECT, including noradrenaline (Landau et al., 2011; Stanford and Nutt, 1982; Yatham et al., 2010). We investigated the effect of electroconvulsive shock (ECS), the corresponding animal procedure of ECT, on the α_2 -adrenoceptors in cortical and limbic brain regions involved in mood disorders in a rat model of depression. The relationship between down-regulation of the α_2 -adrenoceptors and antidepressant efficacy has been postulated for several decades (Cohen et al., 1982) but the lack of reliable direct *in vivo* markers has impeded studies in depressed patients.

The vast majority of α_2 -adrenoceptors are located postsynaptic to noradrenaline terminals (Arnstén et al., 2007; U'Prichard and Snyder, 1979) but they can also be located presynaptically as autoreceptors on terminals or cell bodies of noradrenergic neurons where they modulate the release of noradrenaline through a negative feedback mechanism (Langer, 1974). Post-mortem studies on brains of suicide victims show increased α_2 -adrenoceptor binding in depressed patients, particularly in the frontal cortex (De Paermentier et al., 1997; Meana and Garcia-Sevilla, 1987), hippocampus (Gonzalez et al., 1994) and the pontine regions including locus ceruleus (Ordway et al., 2003).

The up-regulation of α_2 -adrenoceptors as a result of decreased noradrenaline release in depressed patients is reversed by antidepressants with either reuptake inhibition and/or antagonism properties (Andrade and Rao, 2010). ECS also potentiates the release of noradrenaline; rats chronically treated with ECS have significantly increased release of noradrenaline from the frontal cortex as compared to chronic sham controls, which probably mediates a compensatory down-regulation of the presynaptic α_2 -adrenoceptors (Thomas et al., 1992).

Most studies of the effects of ECS have been performed in normal animals and it is unclear if the neurochemical abnormalities underlying depression may also affect response to

antidepressants. To better understand the neurochemical make-up of depression, many attempts have been made to develop animal models of the disease. Such a model is represented by the Flinders sensitive line (FSL) rat, a genetic model developed from the selective breeding of Sprague-Dawley (SD) rats for sensitivity to an anticholinesterase agent (Overstreet and Wegener, 2013). In parallel to FSL, the selective breeding led to development of a 'resistant' line, the Flinders resistant line (FRL) rat (Overstreet et al., 1979; Russell et al., 1982).

The FSL rat model displays features similar to some human features of depression including cholinergic supersensitivity, abnormal immune function, compromised psychomotor function, impaired sleep pattern, reduced appetite and anxiety (Overstreet et al., 2005). The FSL strain has phenotype sensitivity for antidepressant drugs, and both increased immobility in the forced swim test and suppression of neuronal plasticity can be reversed by some antidepressant treatments reflecting the predictive validity of the depressed FSL rats (Chen et al., 2010; Overstreet and Wegener, 2013).

We previously used *in vitro* autoradiography to investigate the baseline α_2 -adrenoceptor levels in female FSL, FRL and SD rats where we found increased α_2 -adrenoceptor binding in cortical regions in the FSL strain compared to SD rats (Lillethorup et al., submitted for publication), which resembles the pathology found in depressed humans. In this study, we aimed to determine the effects of ECS on α_2 -adrenoceptor densities in FSL, FRL and SD rats, in order to further investigate the mechanism of ECT and potential differences between the strains.

2. Experimental procedure

2.1. Animals

Adult female Flinders sensitive line (FSL) rats ($n=18$, 10/ECS, 8/sham) and Flinders resistant line (FRL) rats ($n=12$, 6/ECS, 6/Sham) from breeding colonies maintained in the animal quarters of Centre for Psychiatric Research, Aarhus University Hospital were used along with Sprague-Dawley (SD) rats ($n=10$, 5/ECS 5/sham) from Taconic farms located in Denmark. All rats were kept on a normal 12 h light/12 h dark cycle, had free access to food and water and were housed two per cage. The animals were weighed prior to the first and after the 10th day of sham or ECS treatment (average weight: 220–250 g; age: 12–14 weeks old at the start of the study). This study was approved by the Danish Committee on Ethics in Animal Experimentation (2007/561-1378).

2.2. ECS treatment

ECS or sham treatments were administered every morning for 10 consecutive days. ECS was given via ear clip electrodes using 55–70 mA for 0.5 s at a frequency of 100 Hz square wave pulses (UgoBasile, Comerio, Italy). The aim of the treatment was to achieve a full

tonic-clonic seizure, which was reached between currents of 50 and 70 mA. During ECS treatments, all rats were monitored for seizures ensuring that clonic movements of the face and forelimbs (indicating generalized seizures) lasted at least for 10 s. The same procedure was used for sham treatment with ear clip electrodes attached but without any current passing or seizure generation.

2.3. Tissue preparation

Forty-eight hours after the last ECS or sham session, the rats were decapitated using a guillotine. The brains were rapidly removed, the hemispheres separated and frozen in isopentane cooled to -40°C with dry ice. One hemisphere was sliced into 20 μm thick coronal sections using a cryostat (Microm HM 500 OM Cryostat) at -20°C and thaw-mounted on polysine adhesion microscope slides (ThermoScientific, Germany). The slides were stored in a -80°C freezer until the binding studies were performed.

2.4. Autoradiography

According to the stereotaxic coordinates of the rat brain atlas of Paxinos and Watson (1998), slices at the approximate levels of the frontal cortex (FC, anterior-posterior: 12.24–11.76 mm), the insular cortex (IC, anterior-posterior: 12.24–11.76 mm), the perirhinal cortex (PRh, anterior-posterior: 6–5.76 mm), the basolateral amygdaloid nucleus (BLA, anterior-posterior: 6.00–5.76 mm), the medial amygdaloid nuclei (MeA, anterior-posterior: 6.00–5.76 mm), the ventromedial hypothalamic nucleus (VMH, anterior-posterior: 6.00–5.76 mm), the laterodorsal thalamic nucleus (LDT, anterior-posterior: 6.00–5.76 mm) and the dorsal hippocampus (HIP, anterior-posterior: 6.00–5.76 mm) were selected.

Autoradiography was carried out with slight modifications of the method described by Booze et al. (2006): frozen slides were thawed at room temperature (22°C) for 5 min before they were preincubated in 25 mM GLYGLY (Sigma, Germany) buffer (pH 7.6) for 5 min. Subsequently, the slides were incubated for 90 min at 22°C with 2 nM [^3H]RX 821002 (Specific activity: 40–70 Ci/mmol, PerkinElmer, Denmark) mixed with buffer. The non-specific binding was determined by 10 μM unlabeled phentolamine (Sigma, Germany). After incubation, slides were transferred into 4°C buffer and washed for 2×2 min to terminate the incubation. Slides were then quickly rinsed in 4°C distilled water to remove buffer salts, blow-dried with cool air and stored overnight for further drying. On day 2, slides were exposed to blank tritium storage phosphor screens (BAS-IP TR2025 Fuji Imaging Plate, VWR, Denmark) for 6 days at room temperature in standard film cassettes (BAS-Cassette 2025, Fuji-film) along with tritium standard microscale with concentrations ranging from 0.1 to 15.9 $\mu\text{Ci/g}$ (American Radiolabeled Chemicals, St. Louis). The phosphor screens were quickly removed from the cassettes and scanned using a Fujifilm BAS-5000 Phosphorimager on day 7.

2.5. Autoradiography analyses

The imaging plates were analyzed with the software ImageGauge 4.0 provided with the imager. Photostimulated luminescence values/ mm^2 obtained after background subtraction were calibrated using the Tritium microscale. Regions of interest encompassing layers II and III of the FC, the IC, the PRh, the BLA, the MeA, the VMH, the LDT and a dorsal hippocampal region that includes the combined CA1–CA3 fields (HIP) were manually drawn as seen in Figure 1 to obtain the regional binding values. For each region, two adjacent slides were used to measure total binding (incubated with tracer) and a third was used to measure nonspecific binding (incubated with both tracer and blocker). On the slides determining the non-specific binding, circular regions were drawn on each brain slice to provide an average for each

individual rat since the distribution of the non-specific binding was homogeneous. To determine the specific binding densities in each rat and in each region, the average non-specific binding values were subtracted from the average regional total binding values. Some slides were excluded due to tissue damage.

2.6. Statistical analyses

All data are shown as the specific binding mean $\pm$ SEM obtained from the indicated number of measurements (N: beside each graph). Initially, the data was confirmed to be normally distributed using the Normal Q-Q Plot and a Shapiro-Wilk Test for each strain and treatment and to have equal variance across treatment groups using the Bartlett's test (R Core Team, 2014). Two-way independent ANOVA was performed for each brain region with strain (FSL/FRL/SD) and treatment (Sham/ECS) as main factors followed by a Bonferroni correction. Differences were considered significant if the probability of error was less than 5%.

3. Results

Figure 1 displays representative autoradiograms of the regional distribution of α_2 -adrenoceptor binding attributed to photostimulated luminescence signals of [^3H]RX 821002 at different coronal sections with labeled regions of interest. The quantitative results are expressed as mean $\pm$ SEM and presented in Table 1 and Figure 2.

In the HIP, there was a statistically significant effect of strain ($F_{2,28}=3.80$, $p<0.05$), but no effect of treatment or interaction between the two factors. No significant differences were observed in any of the 3 strains. When investigating the LDT, a significant effect of strain ($F_{2,28}=7.01$, $p<0.01$) and a significant interaction between strain and treatment ($F_{2,28}=4.66$, $p<0.05$) was observed. Two-way ANOVA revealed no significant differences between any of the 3 strains. There was a significant effect of strain ($F_{2,25}=8.07$, $p<0.01$) but no significant interaction or effect of treatment in the VMH. No significant differences were observed in any of the 3 strains.

In the MeA, there was a statistically significant effect of treatment ($F_{1,25}=13.56$, $p<0.01$) but no effect of strain or interaction between the two factors in the medial amygdaloid nucleus. The two-way ANOVA analysis showed that ECS resulted in a 21% decrease in the binding to α_2 -adrenoceptors in the FRL animals ($p<0.01$) but no differences were found in the FSL or SD rats in the MeA. Similar to the MeA, a significant effect of treatment was found ($F_{1,28}=20.85$, $p<0.0001$) in the BLA, but no effect of strain or any interaction between treatment and strain were observed. Two-way ANOVA revealed only a significant decrease of 32% in the FRL strain following ECS ($p<0.01$).

Statistically significant effects of strain ($F_{2,26}=5.27$, $p<0.05$) and treatment ($F_{1,26}=68.20$, $p<0.0001$), as well as an interaction between the two factors ($F_{2,26}=8.16$, $p<0.01$) were obtained in the PRh. The two-way ANOVA analysis revealed a significant 31% decrease in the FSL ($p<0.0001$) and a 34% decrease in the FRL strain ($p<0.0001$) in the α_2 -adrenoceptor binding following ECS treatment. There were statistically significant effects of strain ($F_{2,33}=25.36$, $p<0.0001$) and treatment ($F_{1,33}=9.48$, $p<0.01$) but no interaction between the two factors in the FC. The two-way ANOVA analysis found that ECS treatment significantly decreased α_2 -adrenoceptor binding by 9% in the FSL animals ($p<0.05$). In the IC, statistically

Figure. 1 A set of representative autoradiograms illustrating the regional distribution of α_2 -adrenoceptor binding in sham or ECS treated FSL, FRL and SD groups obtained at different coronal sections corresponding to the anterior-posterior: 12.24-11.76 mm (two upper rows) and 6.00-5.76 mm (two lower rows) (Paxinos and Watson, 1998). The range of standards used and a non-specific binding autoradiogram displaying four brain slices are shown in the bottom of the image. Abbreviations: FC (frontal cortex), IC (insular cortex), HIPP (hippocampus), LDT (laterodorsal thalamic nucleus), VMH (ventromedial hypothalamic nucleus), MeA (medial amygdaloid nucleus), BLA (basolateral amygdaloid nucleus) and PRh (perirhinal cortex).

Table 1 Regional values of [3 H]RX 821002 densities in all six groups of animals.

ROIs	FSL sham Mean $\pm$ SEM (N)	FSL ECS Mean $\pm$ SEM (N)	FSL sham vs. FSL ECS <i>p</i>
HIPP	7.28 $\pm$ 1.29 (8)	9.90 $\pm$ 0.53 (7)	NS* (<i>p</i> =0.09)
LDT	17.64 $\pm$ 1.24 (8)	21.23 $\pm$ 0.93 (7)	NS* (<i>p</i> =0.04)
VMH	18.93 $\pm$ 1.24 (8)	15.68 $\pm$ 1.38 (6)	NS
MeA	28.78 $\pm$ 1.26 (8)	25.30 $\pm$ 1.38 (6)	NS* (<i>p</i> =0.09)
BLA	25.04 $\pm$ 1.41 (8)	21.37 $\pm$ 1.36 (7)	NS* (<i>p</i> =0.09)
PRh	25.96 $\pm$ 0.51 (6)	17.79 $\pm$ 1.06 (7)	< 0.0001
FC	12.66 $\pm$ 0.44 (8)	11.59 $\pm$ 0.18 (10)	< 0.05
IC	17.35 $\pm$ 0.61 (7)	15.88 $\pm$ 0.24 (10)	NS* (<i>p</i> =0.03)
FRL sham vs. FRL ECS			
	FRL sham Mean $\pm$ SEM (N)	FRL ECS Mean $\pm$ SEM (N)	<i>p</i>
HIPP	8.20 $\pm$ 0.61 (6)	9.41 $\pm$ 0.86 (4)	NS
LDT	22.69 $\pm$ 0.91 (6)	19.26 $\pm$ 1.33 (4)	NS* (<i>p</i> =0.06)
VMH	22.12 $\pm$ 1.43 (4)	21.68 $\pm$ 1.12 (4)	NS
MeA	33.81 $\pm$ 1.51 (4)	26.65 $\pm$ 0.91 (4)	< 0.01
BLA	28.09 $\pm$ 0.96 (6)	19.19 $\pm$ 0.88 (4)	< 0.01
PRh	29.18 $\pm$ 0.91 (6)	19.32 $\pm$ 1.80 (4)	< 0.0001
FC	12.15 $\pm$ 0.22 (6)	11.83 $\pm$ 0.51 (6)	NS
IC	17.20 $\pm$ 0.24 (6)	16.32 $\pm$ 0.69 (6)	NS
SD sham vs. SD ECS			
	SD sham Mean $\pm$ SEM (N)	SD ECS Mean $\pm$ SEM (N)	<i>p</i>
HIPP	6.49 $\pm$ 0.80 (4)	6.20 $\pm$ 0.85 (5)	NS
LDT	16.33 $\pm$ 0.74 (4)	17.42 $\pm$ 1.20 (5)	NS
VMH	17.45 $\pm$ 1.64 (4)	16.20 $\pm$ 0.99 (5)	NS
MeA	28.54 $\pm$ 0.80 (4)	26.63 $\pm$ 1.47 (5)	NS
BLA	23.41 $\pm$ 2.97 (4)	18.38 $\pm$ 1.32 (5)	NS
PRh	22.24 $\pm$ 0.84 (4)	20.06 $\pm$ 0.51 (5)	NS
FC	10.49 $\pm$ 0.26 (5)	9.136 $\pm$ 0.38 (4)	NS
IC	15.28 $\pm$ 0.49 (5)	14.00 $\pm$ 0.52 (5)	NS

Data are represented as mean [μ Ci/g] $\pm$ standard error of the mean (N: number of measurements).

Keys: ROIs, regions of interest; NS, non-significant; FSL, Flinders sensitive line; FRL, Flinders resistant line; SD, Sprague-Dawley; HIPP, hippocampus; LDT, laterodorsal thalamic nucleus; VMH, ventromedial hypothalamic nucleus; MeA, medial amygdaloid nucleus; BLA, basolateral amygdaloid nucleus; PRh, perirhinal cortex; FC, frontal cortex; and IC, insular cortex.

NS*: *p*-values are obtained from single *t*-tests comparing Sham and ECS in selected groups and regions. They indicate a trend toward significance or significance for a particular comparison.

significant effects of strain ($F_{2,33}=12.10$, $p<0.001$) and treatment ($F_{1,33}=9.47$, $p<0.01$) were found, but no significant interaction between the two factors. No significant differences were found under ECS conditions.

4. Discussion

In a separate study we reported that in the sham 'baseline' condition, the binding of [3 H]RX 821002 to α_2 -adrenoceptors was increased in the 'depressed' FSL group when compared to the control SD group (10% across regions) (Lillethorup et al., submitted for publication).

Here we studied the effects of ECS on α_2 -adrenoceptor binding in all three strains in female animals. Overall, examination of Table 1 suggests that ECS treatment preferentially decreased α_2 -adrenoceptor binding towards control SD levels in the cortical regions in the FSL rats and in the perirhinal cortex

and amygdaloid regions in the FRL rats, a finding that may reflect a strain differentiation. However, due to the variability of the data and the small number of subjects in some groups, caution should be exerted in interpretation and conclusions regarding strain differences.

The α_2 -adrenoceptor binding in control female SD animals showed no significant response to ECS treatment. The lack of effect in SD animals is somewhat surprising as many studies have reported large effects of ECS in normal rats with various neurotransmitters. Specifically, Pilc and Vetulani (1982) and Stanford and Nutt (1982) have reported downregulation of α_2 -adrenoceptors in various brain regions, including cortical regions, following 10 days of ECS in young Wistar and SD rats, respectively. These findings were however obtained using clonidine, an agonist tracer of the α_2 -adrenoceptors as opposed to an antagonist such as RX 821002 used in our study. Compared to antagonist tracers, agonist ligands may be more sensitive to functional receptor changes due to their preference for the

Figure. 2 Graphical presentations of the regional [^3H]RX 821002 density means $\pm$ SEM in FSL, FRL and SD in sham and ECS conditions. The significant differences indicated are the results of the two-way ANOVA analysis. Abbreviations: HIPP (hippocampus), LDT (laterodorsal thalamic nucleus), VMH (ventromedial hypothalamic nucleus), MeA (medial amygdaloid nucleus), BLA (basolateral amygdaloid nucleus), PRh (perirhinal cortex), FC (frontal cortex) and IC (insular cortex). * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$.

high affinity binding sites of the receptor, sites reported to disappear after chronic ECS (Pilc and Vetulani, 1982). Another interesting observation from our own ECS studies may suggest another possible explanation: our initial dopamine D1 receptor study (Strome et al., 2007) was like many others, including Pilc and Vetulani (1982) and Stanford and Nutt (1982), performed in male rats. A recent pilot study, which was a replication of our ECS D1 study in female SD rats, also revealed no significant change in D1 receptor binding following 10 days of ECS. The

animals in the current study are also female. Indeed, the role of sex in disease and response to therapy is becoming an important factor in evaluating therapeutic outcomes.

Relatively little is known of the relationship between female hormones and noradrenaline in the brain. Estradiol attenuates presynaptic inhibition of noradrenaline release in female compared to ovariectomized SD rats through desensitization of the α_2 -adrenoceptor (Ansonoff and Etgen, 2001). In the current study, we made a point to use animals in each of

the 3 groups that were in the same part of their cycle at the start of the study, in an effort to decrease possible variability in α_2 -adrenoceptor binding and response to the chronic 10 days of ECS. However, we cannot exclude that female rats may have a more variable response to ECS and its noradrenergic modulation than male rats. Our data are in line with and support new research guidelines preconizing a careful consideration of the sex of the animals used for research and recommending the use of both sexes when possible (Clayton and Collins, 2014).

As in animal research, few studies have investigated human gender differences in response to ECT treatment (Bloch et al., 2005; Coryell and Zimmerman, 1984; Herrington et al., 1974), despite the fact that human females experience higher depression rates than males. Together with our data, these human studies suggest that future investigations should carefully consider the inclusion of both male and females in clinical and basic research studies.

While the reasons for the large effect of ECS on FRL rats and the lack of effect of ECS in control SD rats remain unclear, the decreases in binding post-ECS found in most cortical regions and amygdaloid regions in the FSL rats is in agreement with clinical data.

Presynaptic and postsynaptic α_2 -adrenoceptors have important roles in regulation of both noradrenaline synthesis and release from the noradrenergic terminals, respectively (Knaus et al., 2007; Langer, 1974). Moreover, they mediate agonistic effects of sedation, analgesia and enhancing working memory (Gilsbach et al., 2011; Wang et al., 2007) as well as, like other monoamines, maintaining inhibitory control over higher cortical functions (Arnsten et al., 2007). Their role in regulating the noradrenergic system in the pathophysiology of depression has thus captured attention and there is growing evidence for increased α_2 -adrenoceptor density in untreated suicide victims. Indeed, suicide victims treated with antidepressants have however significantly lower levels of α_2 -adrenoceptors in cortex, hippocampus, caudate and amygdala compared to antidepressant-free suicides (De Paermentier et al., 1997). Chronic antidepressant treatment has also repeatedly been shown to induce down-regulation of α_2 -adrenoceptors in rat brain (Barturen and Garcia-Sevilla, 1992; Cottingham et al., 2011; Smith et al., 1981). However, it must be noted that post-mortem changes in binding do not always correlate with the *in vivo* functional response of receptors and increased sensitivity of amygdaloid neurons to monoaminergic transmitters has been found following antidepressant treatment (Wang and Aghajanian, 1980).

A decreased density in α_2 -adrenoceptors was found by Subhash et al. (2003) in cortical areas, but not in hippocampal areas, in the rat brain after chronic treatment with tricyclic antidepressants suggesting region-specific down-regulation and Thomas et al. (1992) found a release of noradrenaline 24 h after the last chronic ECS treatment session in rat frontal cortex, but not in hippocampus, compared to sham treated controls. Furthermore, when Thomas et al. (1992) challenged these rats with the α_2 -adrenoceptor antagonist idazoxan, increased release of noradrenaline was found in the sham treated rats in both brain regions, but in the chronically ECS treated rats, the release was only observed in the hippocampus. These data suggest that a sustained release of noradrenaline over the course of ECS in the frontal cortex may have induced a compensatory down-regulation of the presynaptic

α_2 -adrenoceptors in the frontal cortex but not in hippocampus, explaining why idazoxan did not have any effect on the α_2 -adrenoceptors in frontal cortex of the ECS treated rats (Thomas et al., 1992). Our data are consistent with the observations of Subhash et al. (2003) and Thomas et al. (1992), finding no significant differences in the hippocampal region of the ECS treated FSL and FRL rats compared to the sham treated rats, but demonstrating down-regulation in cortical regions in the FSL rats and in cortical and amygdaloid regions in the FRL rats. Moreover, the lack of alteration in receptor binding in the hippocampal region is in line with the unresponsiveness of hippocampal pyramidal neurons to nor-epinephrine found following ECS (de Montigny, 1984).

In fact, the reason why we see a small trend toward increase in α_2 -adrenoceptor density in the hippocampal region in the FSL and FRL ECS groups may be related to the increased hippocampal volume that was observed in FSL and FRL rats after ECS treatment when compared with FSL and FRL sham groups (Kaae et al., 2012).

The region with the most pronounced decrease under ECS conditions compared to sham conditions, in both the FSL (–31%) and FRL (–34%) rats, was the perirhinal cortex. The perirhinal cortex is mainly involved in visual perception and recognition memory (Meunier et al., 1993; Murray et al., 2007) and it is tightly connected to amygdala and hippocampus, structures primarily involved in emotional (Klüver and Bucy, 1997) and memory processes (Scoville and Milner, 2000), respectively. This suggests that the perirhinal cortex may play a key role both in the phenotypic expression of depressive symptoms as well as in the antidepressant response after ECS. Both stress and depression are associated with decreased neuroplasticity in hippocampus (Videbech and Ravnkilde, 2004), increased aberrant neuroplasticity in the amygdala and decreased neuroplasticity in the perirhinal cortex (Andrade and Rao, 2010). As a more potent stimulator of neuroplasticity than antidepressants, ECT has been shown to normalize these aberrant changes (Chen et al., 2009; Hellsten et al., 2002). Moreover, the increased response and sensitivity of the cortical noradrenergic system and trend toward decrease in the amygdaloid regions in the FSL animals following ECS (Table 1) may be related to the anxious traits it exhibits, since heightened anxiety is associated with impaired cortical top-down control mechanisms (Bishop et al., 2004) and increased amygdala activation (Wang et al., 2011).

The results of this study are based on binding in the tissue in only one hemisphere of the brain as the other hemisphere was dedicated to a different study. The separation of the two hemispheres led to some damage in the brain stem areas, especially medially, which precluded appropriate sectioning in the locus coeruleus and verification of our hypothesis that ECS-induced down-regulation of its inhibitory α_2 autoreceptors and consequent increased noradrenaline release in terminal fields led to down-regulation of the post synaptic α_2 -adrenoceptors in projection area(s). Furthermore, the effects observed in this study are based on small groups of animals, which might have masked significance in some regions and groups.

In conclusion, our data suggest that ECS exerts a down-regulating effect on α_2 -adrenoceptors in both FSL and FRL animals and that these effects may be dependent on the individual strains and baseline density of receptors. It will be necessary to replicate these findings in a larger cohort composed of both male and female animals of all strains.

Nevertheless, our data demonstrate a normalizing effect of ECS on the cortical noradrenergic systems in female Flinders rats.

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Contributors

GW, DD and AML designed the study. GW bred the FSL and FRL rats at the Centre for Psychiatric Research, Aarhus University Hospital. AML and JF treated the rats with ECS and PI conducted the tissue preparation. TPL performed the autoradiography experiments and analysis, under the supervision of AML, and prepared the first draft of the manuscript and the figures. All authors commented on the manuscript and have approved the final version.

Conflict of interest

All authors declare that they have no conflict of interest.

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