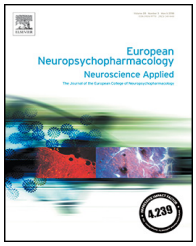
European Neuropsychopharmacology (2019) 29, 493–500

www.elsevier.com/locate/euroneuro



Cortical and striatal serotonin transporter binding in a genetic rat model of depression and in response to electroconvulsive stimuli

Anna Sophie Thue Hvilsom[a,b], Thea P. Lillethorup[b], Peter Iversen[b], Doris J. Doudet[b,c], Gregers Wegener[a,d], Anne M. Landau[a,b,*]

[a] *Translational Neuropsychiatry Unit, Aarhus University, Denmark*
[b] *Department of Nuclear Medicine and PET Center, Aarhus University, Nørrebrogade 44, Building 10G, 8000 Aarhus C, Denmark*
[c] *Department of Medicine/Neurology, University of British Columbia, Canada*
[d] *Centre for Pharmaceutical Excellence, School of Pharmacy (Pharmacology), North-West University, Potchefstroom, South Africa*

Received 11 October 2018; received in revised form 10 January 2019; accepted 14 February 2019

KEYWORDS
Flinders sensitive line rats;
Flinders resistant line rats;
Serotonin transporter;
Electroconvulsive therapy;
Autoradiography;
DASB

Abstract
Depression is a debilitating mental illness and two thirds of patients respond insufficiently to conventional antidepressants. Electroconvulsive therapy (ECT) remains the most effective treatment to alleviate drug-refractory depression, however the neurobiological mechanisms are mostly unknown. The serotonergic system plays an important role in depression and alterations in the serotonin transporter (SERT) are seen both in depression and response to antidepressant pharmacotherapies. The first aim of this study was to investigate SERT density in a genetic rat model of depression, Flinders Sensitive Line (FSL), compared to control Flinders Resistant Line (FRL) and Sprague-Dawley (SD) rats. The second aim was to investigate SERT density in response to electroconvulsive stimuli (ECS), an animal model of ECT. Female rats of each strain were treated with ECS or sham (ear-clip placement with no current) for 10 days before brains were removed, frozen and cut into 20 μm thick sections. SERT density was measured in striatal and cortical regions by quantitative *in vitro* autoradiography using the SERT-radioligand, [^{3}H]-DASB. Higher SERT density was observed in FSL rats compared to SD rats by 36–48% in motor

* Corresponding author at: Department of Nuclear Medicine and PET Center, Aarhus University, Nørrebrogade 44, Building 10G, 8000 Aarhus C, Denmark.
E-mail address: alandau@clin.au.dk (A.M. Landau).

https://doi.org/10.1016/j.euroneuro.2019.02.009

cortex and striatum under sham conditions. In response to ECS, SD rats displayed a significant effect of treatment, whereas no changes were observed in FRL and FSL rats. Increased SERT binding in FSL rats compared to SD supports a dysfunction of the serotonergic system in depression. The increased SERT density after ECS, seen in SD rats but not FSL rats, suggests a different mechanism of action between depressive-like rats and controls.

1. Introduction

Depression is a complex mental illness that affects more than 300 million people worldwide (Ferrari et al., 2013; GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). The lifetime prevalence of depression is 10–15% and women are affected more often than men (Lepine and Briley, 2011). Symptoms include low mood, decreased level of energy, low appetite, fatigue and anhedonia. Depressed patients have an increased risk of committing suicide compared to the general population; this risk is 21 times higher for men and 27 times higher for women (Ösby et al., 2001). Despite treatment, around 60% of depressed patients experience relapse and after each relapse episode this probability further increases (Solomon et al., 2000).

The biological mechanisms underlying depression are still poorly understood. The monoamine hypothesis of depression states that an imbalance in monoaminergic neurotransmission causes depression (Schildkraut, 1965), which is supported by the antidepressant efficacy of drugs that increase extracellular monoamine concentrations. However, it has now become clear that the pathology is more complex and may involve other mechanisms such as impaired neuroplasticity and hippocampal neurogenesis (Kempermann and Kronenberg, 2003), hyperactivity of the hypothalamic stress axis (Binder and Nemeroff, 2010) and neuroinflammation (Maes, 2008). Nevertheless, the importance of the monoaminergic systems in depression should not be underestimated.

Serotonin, a monoamine synthesized from tryptophan, is produced in several raphe nuclei in the brainstem and pons. Serotonergic projections cover a wide range of brain areas and spinal cord. The neurotransmitter is involved in the regulation of mood, sleep, appetite, memory and social behavior (reviewed in Sghendo and Mifsud, 2012). The "serotonin hypothesis" of depression is decades old and stems from the depressogenic effect of drugs such as reserpine, and the serendipitous observation of the antidepressant effects of tricyclic and monoamine oxidase inhibitors later found, in animal studies, to potentiate the synaptic effect of serotonin. This observation is supported by acute tryptophan depletion that leads to lowered mood in patients vulnerable to depression (Ruhe et al., 2007) but remains unsubstantiated due to the erratic relationship between serotonin levels and mood. Serotonin levels are modulated through the controlling action of the serotonin transporter (SERT) located in the membrane of the presynaptic serotonergic neurons and glia cells. Due to the antidepressant efficacy of drugs that block serotonin reuptake, i.e. selective serotonin reuptake inhibitors (SSRIs), many studies have examined the availability, density and gene expression of SERT in depression. *In vivo* imaging studies in depressed humans have produced inconsistent results (Cannon et al., 2007; Ichimiya et al., 2002; Joensuu et al., 2007; Meyer et al., 2004; Reimold et al., 2008; Reivich et al., 2004; Selvaraj et al., 2011). However, a meta-analysis including 18 studies and a total of 364 unmedicated depressed patients showed a significant reduction in SERT binding in midbrain, amygdala, thalamus, striatum and brainstem (Gryglewski et al., 2014).

The major shortcomings of SSRIs are that they typically take several weeks to take effect and only about one third of patients respond sufficiently to treatment (Tranter et al., 2002). Moreover, one third are entirely drug-resistant (Tranter et al., 2002). This creates an urgent need to gain a more detailed understanding of the biological basis of depression to enable the development of more effective, faster-acting antidepressants. Electroconvulsive therapy (ECT) remains the most effective treatment to alleviate depression and is significantly more effective than pharmacotherapy (UK ECT Review Group, 2003) despite its current almost exclusive use in treatment resistant patients. ECT involves the induction of generalized seizures by applying an electrical stimulus to the head. According to a multi-center randomized trial from the Consortium of Research in ECT, 50% of patients had an initial response after 3 ECT treatments (1 week) with a reduction of the Hamilton Rating Scale for Depression of at least 50%. After an average of 8 treatments this reduction was achieved in approximately 90% and full remission was attained in 75% of patients (Husain et al., 2004). Importantly, ECT also markedly reduces suicidal intent (Kellner et al., 2005).

Despite the robust antidepressant efficacy of ECT, the neurobiological mechanisms behind the therapeutic effect are still mostly unknown. Suggested mechanisms include normalization of the neuroendocrine system (Fosse and Read, 2013; Haskett, 2014), neurotrophic effects such as neurogenesis, synaptogenesis and gliogenesis (Bouckaert et al., 2014; McCall et al., 2014) and alterations in monoaminergic transmission such as the serotonergic system (Baldinger et al., 2014; Fosse and Read, 2013).

There are currently no published studies investigating the effect of ECT on SERT availability in human brain. Research investigating the corresponding animal model, electroconvulsive stimuli (ECS), has shown no difference in SERT binding after ECS in dorsal raphe and cortex (Burnet et al., 1999; Gleiter and Nutt, 1988) and upregulation in frontal cortex (Hayakawa et al., 1995; Shen et al., 2003) in healthy rats. In the current study, we use the well-validated Flinders Sensitive Line (FSL) genetic rat model of depression (Overstreet et al., 2005; Wegener et al., 2012) in order to study potential changes in SERT-radioligand [^{3}H]-DASB binding in response to depression and to ECS. FSL rats were inbred on an SD background for an increased sensitivity to anticholinesterase (Overstreet et al., 1979) and with behavioral symptoms of reduced activity, anhedonia, reduced appetite, disturbed rapid eye movement sleep and

neuroendocrine dysfunction (Wegener et al., 2012). In this study both outbred SD rats and the inbred Flinders Resistant Line (FRL) strain, bred for resistance to anticholinesterase (Overstreet et al., 1979), are used as controls since previous findings have shown that, despite often being used as "controls" to FSL, the FRL strain may also display abnormalities (Hasegawa et al., 2006; Lillethorup et al., 2015b; Nishi et al., 2009). The current study has two primary aims: (1) to investigate the involvement of SERT density in depression by evaluating differences at baseline, after daily handling, between female FSL, FRL and SD rats in striatum and cortex, and (2) to investigate the effects of ECS on SERT density in the same regions of female FSL, FRL and SD rats.

2. Experimental procedures

2.1. Animals

Adult female Flinders Sensitive Line (FSL) rats ($n = 18$) and Flinders Resistant Line (FRL) rats ($n = 16$) were bred at the Translational Neuropsychiatry Unit, Aarhus University Hospital and Sprague-Dawley (SD) rats ($n = 15$) were obtained from Taconic (Denmark). Animals were pair-housed and kept on a 12 h light/dark cycle with ad libitum access to food and water. Animals weighed between 200 and 250 g and were handled for 10 consecutive days before ECS treatment. All procedures were carried out under the approval of the Danish Committee on Ethics in Animal Experimentation (2007/561-1378).

2.2. Treatment

Rats were randomly assigned to receive either sham or ECS treatment, which was carried out on 10 consecutive days with ear clips being briefly attached in both cases as previously described (Lillethorup et al., 2015a, 2015b). In order to (1) investigate if the individual threshold needed to elicit a seizure was the same in each strain and (2) if not, determine each strain threshold, an initial pilot study was conducted to tailor the current necessary to induce adequate seizures for each animal strain: FSL rats required currents of 70 mA, FRL rats 55 mA and SD rats 50 mA. Currents were given for 0.5 s at a frequency of 100 Hz square wave pulses to induce full tonic-clonic seizures, whereas no current was applied to the sham animals. Rats were observed to ensure that the full tonic-clonic seizure lasted for a minimum of 10 s.

2.3. Tissue preparation

Rats were decapitated 48 h after the last treatment to avoid possible confounds by the acute effects of seizures on neurotransmitter release, blood flow and metabolic responses, in accordance with previous studies (Lillethorup et al., 2015a, 2015b; Strome et al., 2007). Brains were quickly taken out and flash frozen in isopentane at −40 °C. Samples were stored at −80 °C until being cut into 20 μm coronal sections using a cryostat at −20 °C (Microm HM 500 OM Cryostat). Sections were thaw-mounted on Polysine adhesion microscope slides (Thermo Scientific, Germany) and stored at −80 °C until the autoradiography experiment.

2.4. In vitro autoradiography

For each rat, sections at the anterior-posterior coordinates of 1 mm (Motor cortex (MCx), Caudate-Putamen dorsolateral (CP(dl)), ventrolateral (CP(vl)) and ventromedial (CP(vm)), Nucleus accumbens (ACC) and olfactory tubercle (OT)) and −3 mm from bregma (Sensory cortex (SCx) and Insular cortex (ICx)) were selected using a rat brain atlas (Paxinos and Watson, 1998). Striatal and cortical regions were selected based on a previous study of SERT binding in SD, FRL and FSL animals exposed to fluoxetine (Kovacevic et al., 2010), and our own studies of 5-HT_{2A} binding in a minipig ECS model (submitted). For each coordinate, three adjacent slides were used for autoradiography; two for total binding and one for non-specific binding. Each slide contained 3–4 brain sections. The procedure was carried out according to Zeng et al. (2006) with slight modifications. Buffer containing 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$ was used. Slides were thawed at room temperature (22 °C) for 15 min and pre-incubated for 20 min in buffer. Incubation was 60 min in buffer with 1 nM [^{3}H]-DASB (specific activity: 76 Ci/mmol, Vitrax) to assess total binding. Non-specific binding was measured in the presence of 10 μM Citalopram. After incubation, slides were washed for 3 min in cold (4 °C) buffer ending with a dip in cold (4 °C) distilled water and dried using a stream of cool air. After overnight storage in a desiccator, blank imaging storage phosphor screens (BAS-IP TR2025 Fuji Imaging Plate, VWR, Denmark) in standard film cassettes (BAS-Cassette 2025, Fujifilm) were exposed to slides and tritium standards (American Radiolabeled Chemicals, St. Louis) for 7 days. Images were read using a Fujifilm BAS-5000 Phosphorimager.

2.5. Analysis

The analysis was performed using Image Gauge 4.0 software. For total binding we used the average of 6–8 brain sections per region (2 slides per rat) and for non-specific binding, we used the average of 3–4 brain sections per region (1 slide per rat). Sections with tissue damage from either the cutting or the autoradiography experiment were excluded at the level of SCx and ICx (−3 mm from bregma) for one SD sham rat, one FRL sham rat, two FSL sham rats, and two FSL ECS rats. For one FSL sham animal, brain sections (1 mm from bregma) were found damaged only in the striatum (CP and ACC), while information from OT and MCx were obtained. Brain regions were drawn manually by a researcher blinded to the treatment groups. Briefly, photostimulated luminescence values/mm^2 were obtained for each rat in each region after background noise subtraction and area correction to provide total and non-specific binding values. Values were calibrated to known tritium standard concentrations (μCi/g, Amarican Radiolabeled Chemicals, St. Louis). Specific binding for each animal was obtained by subtracting the average non-specific binding (3–4 brain sections per region) from average total binding (6–8 brain sections per region).

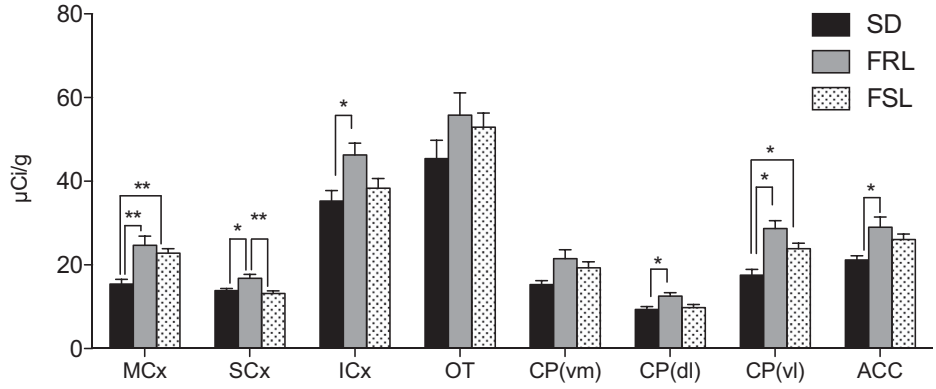


Fig. 1 A graphical presentation of the regional means ± SEM of [^{3}H]-DASB densities in rats at baseline, after sham handling. $^*p < 0.05$; $^{**}p < 0.01$, SD $n = 6$-7, FRL $n = 7$-8, FSL $n = 8$-10.

2.6. Statistics

To examine SERT binding in FSL rats compared to the two control strains a one-way multivariate analysis of variance (MANOVA) including all brain regions with strain as a factor was carried out. Subsequently, independent one-way ANOVAs were performed in each region followed by post hoc evaluation with Bonferroni corrections for multiple comparisons if the MANOVA was significant.

To examine the effects of ECS on SERT binding in each of the strains, we performed two-way ANOVAs with treatment and region as factors, and did post hoc evaluation with Bonferroni correction for multiple comparisons if the ANOVA was significant.

Normal distribution was evaluated with QQ-plots and Shapiro–Wilk Test and tests for equal variance were performed using Levene's test. Significance was obtained with a probability error of less than 5%. Data was analyzed using Stata software 13.1 (StataCorp) and Graphpad Prism (version 5.0).

3. Results

In the first part of the study comparing SERT binding in FSL ($n = 10$), FRL ($n = 8$) and SD ($n = 7$) sham-treated rats, one-way MANOVA with strain as a factor was significant ($p<0.05$). Subsequent one-way ANOVAs were significant in motor cortex, MCx ($p<0.01$), sensory cortex, SCx ($p<0.01$), insular cortex, ICx ($p<0.05$), dorsolateral caudate putamen, CP(dl) ($p<0.05$), ventrolateral caudate putamen, CP(vl) ($p<0.001$) and nucleus accumbens, ACC ($p<0.05$). Post hoc evaluations with Bonferroni corrections revealed significant differences between the strains (Fig. 1). FSL rats had significantly higher binding than control SD rats by 48% in the MCx and by 36% in the CP(vl). We also observed trends towards higher binding in FSL vs SD in the OT (16.5%), CP(vm) (26%) and ACC (23%), which did not reach statistical significance in our small groups. Surprisingly, the largest strain differences were observed when comparing the two control groups to one another (SD and FRL) with significantly higher binding in FRL animals in the three cortical regions (MCx (60%), SCx (21%) and ICx (31%)), in the CP(dl) (34%), CP(vl) (64%) and the ACC (23%). When comparing FSL rats to the second control group, FRL, we found significantly lower binding in only the SCx of FSL rats (21%).

In the second part of the study, the effects of ECS were investigated in each strain. Two way ANOVAs revealed a significant effect of region in all three strains, but an effect of treatment was only observed in the SD animals ($p<0.01$). However, none of the regions in the SD animals survived the Bonferroni correction for multiple comparisons. Although none of the individual regions reached statistical significance in these small samples, we would like to point out some regions in which large % increases were observed in SD animals. We found higher binding in the MCx (25%), the CP(vm) (19%) and the CP(dl) (25%). On the contrary, no remarkable increase or decrease binding was noted in FSL and FRL animals (Table 1).

4. Discussion

4.1. Strain differences in SERT density

The first part of the study aimed to investigate the SERT binding in the FSL rat model of depression. The FRL animals have routinely been used as "controls" for FSL data. However, since our previous study of the noradrenergic system in these same female animals showed striking differences between the two commonly used control strains for FSL animals (Lillethorup et al., 2015b), and studies by other groups have shown abnormalities in the FRL strain (Hasegawa et al., 2006; Nishi et al., 2009), both SD and FRL control groups were included in the current study. FSL rats exhibited significantly higher SERT densities in MCx and CP(vl) compared to SD rats. The same trend was seen in the OT, CP(vm) and ACC but did not reach significance. The upregulation in CP is consistent with some findings in positron emission tomography (PET) studies of depression in humans (Cannon et al., 2007; Meyer et al., 2004), but in contrast with others (Selvaraj et al., 2011). Increased SERT binding in ACC was previously found in FSL rats compared to SD rats (Kovacevic et al., 2010), which is consistent with the trend found in this study. Although Kovacevic et al. (2010) also used SD, FRL and FSL rats, their study showed no significant changes in motor cortex or striatum between FSL and SD rats, but found differences in sensory cortex (Kovacevic et al., 2010), not observed in the current study. These incon-

Table 1 Regional values of [^{3}H]-DASB densities and percent changes after ECS.

Regions	Mean ± SEM	(N)	Mean ± SEM	(N)	% Change
	SD sham		**SD ECS**		
MCx	15.43 ± 1.10	(7)	19.37 ± 1.51	(8)	25.5
SCx	13.84 ± 0.53	(6)	13.94 ± 0.65	(8)	0.2
ICx	35.29 ± 2.47	(6)	35.29 ± 1.72	(8)	0.0
OT	45.43 ± 4.35	(7)	52.20 ± 3.99	(8)	14.9
CP(vm)	15.32 ± 0.91	(7)	18.19 ± 1.13	(8)	18.8
CP(dl)	9.35 ± 0.71	(7)	11.72 ± 0.80	(8)	25.4
CP(vl)	17.55 ± 1.37	(7)	21.48 ± 2.02	(8)	22.4
ACC	21.20 ± 0.99	(7)	24.35 ± 1.99	(8)	14.8
	FRL sham		**FRL ECS**		
MCx	24.73 ± 2.17	(8)	24.37 ± 1.40	(8)	−1.4
SCx	16.81 ± 0.92	(7)	15.88 ± 0.55	(8)	−1.3
ICx	46.29 ± 2.84	(7)	39.33 ± 3.53	(8)	−9.9
OT	55.80 ± 5.31	(8)	59.49 ± 4.58	(8)	6.6
CP(vm)	21.47 ± 2.14	(8)	24.04 ± 1.95	(8)	11.9
CP(dl)	12.53 ± 0.81	(8)	12.28 ± 0.63	(8)	−2.0
CP(vl)	28.70 ± 1.89	(8)	27.34 ± 1.80	(8)	−4.8
ACC	29.03 ± 2.43	(8)	25.88 ± 2.25	(8)	−10.9
	FSL sham		**FSL ECS**		
MCx	22.80 ± 1.07	(10)	22.74 ± 0.88	(8)	−0.3
SCx	13.21 ± 0.62	(8)	14.69 ± 0.56	(6)	2.1
ICx	38.34 ± 2.34	(8)	39.83 ± 1.92	(6)	2.1
OT	52.91 ± 3.42	(10)	47.96 ± 4.06	(8)	−9.4
CP(vm)	19.29 ± 1.47	(9)	17.38 ± 1.04	(8)	−9.9
CP(dl)	9.80 ± 0.77	(9)	9.98 ± 0.64	(8)	1.9
CP(vl)	23.91 ± 1.27	(9)	22.45 ± 1.39	(8)	−6.1
ACC	26.05 ± 1.35	(9)	23.20 ± 1.94	(8)	−11.0

Data are represented as Mean [μCi/g] ± Standard Error of the Mean (N: number of measurements).

sistencies may be explained by a combination of differences in handling: sham electrode placement vs. saline injections, and thereby stress exposure, as well as the origin of the animals (commercial supplier for the SD vs. in house breeding for FSL and FRL). Sex may also have played a role: in humans, differences in depressive features are known to exist between males and females (reviewed in Altemus et al., 2014), and female rats were selected in the current study since depression is more prevalent and more severe in females than in males.

A possible explanation for the increase in SERT densities in the FSL rat model of depression found in this study as well as in a number of other pre-clinical and human studies (Cannon et al., 2007; Ichimiya et al., 2002; Reivich et al., 2004; Sato et al., 2010), may be due to an increase in SERT density in the raphe nucleus, leading to higher clearance of serotonin, which would decrease activation of 5-HT$_{1A}$ autoreceptors on the cell bodies. This would lead to increased raphe cell firing, higher serotonin levels in projection areas and a compensatory increase in SERT in those areas (Hahn et al., 2014). Hahn and colleagues have shown a positive association between the density of SERT in the raphe nucleus and projection areas (Hahn et al., 2014). Unfortunately, brainstem sections were not available in this study, and it was therefore not possible to investigate this correlation.

Both SD and FRL are commonly used as control strains for FSL rats and therefore would be expected to exhibit the same properties. In most regions investigated here, FRL rats had significantly higher SERT binding compared to SD rats, and were even higher than FSL rats. Other serotonergic differences have also been found between FRL and SD rats in other studies, namely in serotonin synthesis (Hasegawa et al., 2006) and 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor densities (Nishi et al., 2009). Therefore, although FRL rats have been used as a single control strain for FSL rats in many studies, the results from this and other studies underline the necessity of also using SD rats as controls in future studies until a consensus is reached in the scientific community. In this study, trends towards higher SERT binding, some of which are significant, are observed in both FSL and FRL rats compared to SD rats. Thus, it appears that the two inbred Flinders strains possess similar properties when compared to outbred SD rats. Additionally, it seems that SERT binding is higher in FRL compared to FSL rats, which could suggest an abnormal functioning of the serotonergic system in both strains, which is even more pronounced in FRL rats.

It is important to note that this experiment was performed in female rats while most published studies have previously used male rats. Thus, direct comparisons of the differences found between SD and FRL rats in different studies should be made with caution. The differences between the two sexes of these strains have not yet been thoroughly investigated. Additionally, the SD rats used in this study were bred at a different location than the FRL and FSL rats. Differences in early life experiences can therefore

not be excluded and could potentially have led to the differences observed between the two control strains. Finally, the rats used to compare baseline differences, were in fact sham-treated as part of the ECS study. Therefore, their handling over 10 consecutive days and the resulting mild stress may have exacerbated the differences observed between the three groups. Previous studies have demonstrated that stressful situations can lead to larger differences between FRL and FSL rats (Pucilowski et al., 1993; Wegener et al., 2010). Therefore, the differences in DASB density may have been a result of the combination of strain and mild stress.

4.2. Effects of ECS on SERT binding

The second part of the study aimed to investigate the effect of ECS on SERT binding in the FSL rat model of depression and in FRL and SD rats. We found a significant effect of treatment in the SD animals, with no effect in the FSL or FRL animals. Although none of the regions survived testing for multiple corrections in our small samples, increased SERT density of 15–25% was found in a number of regions in response to ECS. The majority of animal studies point towards enhanced neurotransmission after ECS treatment and an upregulation in SERT density is hypothesized to be a compensatory mechanism to maintain regulation of synaptic serotonin levels (Baldinger et al., 2014). This may explain the effect of ECS in SD rats in this study.

In contrast, the same effect was not observed after ECS treatment in the FRL or FSL rats, suggesting that the effect of ECS on SD rats is different from FRL and FSL rats. Both FRL and FSL rats display no changes in SERT binding after ECS, although a trend of 11% decrease in SERT binding was found in the ACC in both FRL and FSL groups. No previous studies have investigated SERT binding in animal models of depression after ECS. Since the pathophysiology of depression involves many different neuronal systems including the serotonergic system, the brains of depressed subjects may function differently than those of healthy subjects. Indeed, previous studies have shown a differential antidepressant-like effect of running in FSL vs. control animals (Bjornebekk et al., 2005, 2006). It is therefore possible that the effects and mechanisms of action of ECS also differ between normal and depressive-like rats. As discussed earlier, FRL rats seem to have different properties compared to SD rats and this could explain the different SERT binding responses of FRL and SD rats to ECS.

Studies in humans and non-human primates have found downregulation of cortical serotonergic receptors after ECT (Lanzenberger et al., 2013; Strome et al., 2005; Yatham et al., 2010), opposing most rodent ECS findings (Baldinger et al., 2014; Burnet et al., 1999; Green et al., 1983; Vetulani et al., 1981) and our own study in Göttingen minipig (Landau et al., 2019). Thus, there appears to be species differences in the serotonergic pathways between rodents and primates regarding the effects of ECT/ECS with depressed human and healthy non-human primates showing similar response to a course of ECT. In this study, increased SERT binding in SD rats and no change or decreased SERT binding in FRL and FSL rats were observed after ECS. This suggests that one mechanism of action could be present in the SD rats with “normal” brain function and a different mechanism in inbred FSL and FRL rats with altered neurocircuitry. Thus, the increased SERT in FSL baseline may reflect a maximized endogenous compensatory increase that precludes further increases in response to treatment. Yatham et al. (2010) has proposed a similar mechanism in depressed individuals to justify the poor response to antidepressants exhibited by some individuals (Yatham et al., 2010).

FSL rats were bred to express a particular biochemical phenotype and presented with a depressive behavioral phenotype (Overstreet et al., 2005). Phenotypic expression of depression may be a result of varied alterations in circuitry and biochemical interactions and it is well known that there are multiple presentations of depression in animal models of depression. It is also biologically heterogeneous in the human condition. FSL animals may only represent the biochemical make up of a small type of human depression, and various biochemical background underlying a general similar phenotype may also explain the variability in therapeutic response to treatments, such as ECS. A final point to consider is that the measured differential response to ECS in the different groups may not stem only from the ECS effects, but also represent the mild-stress induced by the sham treatment, which may have been more pronounced in the rats with a depressive phenotype or altered brain circuitry. Due to ECS-induced memory disturbances, the ECS groups may have forgotten the handling from the previous day, and experienced less stress than the sham groups. We cannot exclude the possibility that if the ECS effects on SERT binding had been considered against non-handled, non stressed baseline animals, significant differences may have been found.

4.3. Conclusion

In conclusion, this study supports an involvement of the serotonergic system in the pathophysiology of depression with indications of higher SERT density in the FSL rat model of depression compared to control SD rats. Furthermore, it appears that ECS has a different effect in depressed FSL rats compared to SD rats. Future studies in animal models of depression are required to further investigate the effects of ECS. Finally, our findings underline the necessity of having both FRL and SD rats as controls for the FSL rat model of depression in future studies.

Conflict of interest

All authors declare that they have no conflict of interest.

Contributors

GW, DD and AML designed the study. GW bred the FSL and FRL rats at the Translational Neuropsychiatry Unit, Aarhus University. AML treated the rats with ECS and PI conducted the tissue preparation. ASTH and TPL performed the autoradiography experiments and analysis, under the supervision of AML. ASTH and AML prepared the first draft of the manuscript. All authors commented on the manuscript and have approved the final version.

Role of funding source

Funding for this study was provided by the Danish Medical Research Council to DJD, by the Th. Maigaards Eftf. Fru Lily Benthine Lunds Fond to AML and by salary support to ASTH from Aarhus University. The funding sources had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

References

Altemus, M., Sarvaiya, N., Neill Epperson, C., 2014. Sex differences in anxiety and depression clinical perspectives. Front. Neuroendocrinol. 35, 320–330.

Baldinger, P., Lotan, A., Frey, R., Kasper, S., Lerer, B., Lanzenberger, R., 2014. Neurotransmitters and electroconvulsive therapy. J. ECT 30, 116–121.

Binder, E.B., Nemeroff, C.B., 2010. The CRF system, stress, depression and anxiety-insights from human genetic studies. Mol. Psychiatry 15, 574–588.

Bjornebekk, A., Mathe, A.A., Brene, S., 2005. The antidepressant effect of running is associated with increased hippocampal cell proliferation. Int. J. Neuropsychopharmacol. 8, 357–368.

Bjornebekk, A., Mathe, A.A., Brene, S., 2006. Running has differential effects on NPY, opiates, and cell proliferation in an animal model of depression and controls. Neuropsychopharmacology 31, 256–264.

Bouckaert, F., Sienaert, P., Obbels, J., Dols, A., Vandenbulcke, M., Stek, M., Bolwig, T., 2014. ECT: its brain enabling effects: a review of electroconvulsive therapy-induced structural brain plasticity. J. ECT 30, 143–151.

Burnet, P.W., Sharp, T., LeCorre, S.M., Harrison, P.J., 1999. Expression of 5-HT receptors and the 5-HT transporter in rat brain after electroconvulsive shock. Neurosci. Lett. 277, 79–82.

Cannon, D.M., Ichise, M., Rollis, D., Klaver, J.M., Gandhi, S.K., Charney, D.S., Manji, H.K., Drevets, W.C., 2007. Elevated serotonin transporter binding in major depressive disorder assessed using positron emission tomography and [11C]DASB; comparison with bipolar disorder. Biol. Psychiatry 62, 870–877.

Ferrari, A.J., Charlson, F.J., Norman, R.E., Patten, S.B., Freedman, G., Murray, C.J., Vos, T., Whiteford, H.A., 2013. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. PLoS Med. 10, e1001547.

Fosse, R., Read, J., 2013. Electroconvulsive treatment: hypotheses about mechanisms of action. Front. Psychiatry 4, 94.

GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388, 1545–1602.

Gleiter, C.H., Nutt, D.J., 1988. Repeated electroconvulsive shock does not change [3H]-paroxetine binding to the 5-HT uptake site in rat cortical membranes. Psychopharmacology (Berl) 95, 68–70.

Green, A.R., Johnson, P., Nimgaonkar, V.L., 1983. Increased 5-HT2 receptor number in brain as a probable explanation for the enhanced 5-hydroxytryptamine-mediated behaviour following repeated electroconvulsive shock administration to rats. Br. J. Pharmacol. 80, 173–177.

Gryglewski, G., Lanzenberger, R., Kranz, G.S., Cumming, P., 2014. Meta-analysis of molecular imaging of serotonin transporters in major depression. J. Cereb. Blood Flow Metab. 34, 1096–1103.

Hahn, A., Haeusler, D., Kraus, C., Hoflich, A.S., Kranz, G.S., Baldinger, P., Savli, M., Mitterhauser, M., Wadsak, W., Karanikas, G., Kasper, S., Lanzenberger, R., 2014. Attenuated serotonin transporter association between dorsal raphe and ventral striatum in major depression. Hum. Brain Mapp. 35, 3857–3866.

Hasegawa, S., Nishi, K., Watanabe, A., Overstreet, D.H., Diksic, M., 2006. Brain 5-HT synthesis in the flinders sensitive line rat model of depression: an autoradiographic study. Neurochem. Int. 48, 358–366.

Haskett, R.F., 2014. Electroconvulsive therapy's mechanism of action: neuroendocrine hypotheses. J. ECT 30, 107–110.

Hayakawa, H., Okamoto, Y., Shimizu, M., Nishida, A., Motohashi, N., Yamawaki, S., 1995. Single or repeated treatment with electroconvulsive shock increases number of serotonin uptake binding sites in the frontal cortex. Neuropsychobiology 31, 1–5.

Husain, M.M., Rush, A.J., Fink, M., Knapp, R., Petrides, G., Rummans, T., Biggs, M.M., O'Connor, K., Rasmussen, K., Litle, M., Zhao, W., Bernstein, H.J., Smith, G., Mueller, M., McClintock, S.M., Bailine, S.H., Kellner, C.H., 2004. Speed of response and remission in major depressive disorder with acute electroconvulsive therapy (ECT): a consortium for research in ECT (CORE) report. J. Clin. Psychiatry 65, 485–491.

Ichimiya, T., Suhara, T., Sudo, Y., Okubo, Y., Nakayama, K., Nankai, M., Inoue, M., Yasuno, F., Takano, A., Maeda, J., Shibuya, H., 2002. Serotonin transporter binding in patients with mood disorders: a PET study with [11C](+)McN5652. Biol. Psychiatry 51, 715–722.

Joensuu, M., Tolmunen, T., Saarinen, P.I., Tiihonen, J., Kuikka, J., Ahola, P., Vanninen, R., Lehtonen, J., 2007. Reduced midbrain serotonin transporter availability in drug-naive patients with depression measured by SERT-specific [(123)I] nor-beta-CIT SPECT imaging. Psychiatry Res. 154, 125–131.

Kellner, C.H., Fink, M., Knapp, R., Petrides, G., Husain, M., Rummans, T., Mueller, M., Bernstein, H., Rasmussen, K., O'Connor, K., Smith, G., Rush, A.J., Biggs, M., McClintock, S., Bailine, S., Malur, C., 2005. Relief of expressed suicidal intent by ECT: a consortium for research in ECT study. Am. J. Psychiatry 162, 977–982.

Kempermann, G., Kronenberg, G., 2003. Depressed new neurons?—Adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biol. Psychiatry 54, 499–503.

Kovacevic, T., Skelin, I., Diksic, M., 2010. Chronic fluoxetine treatment has a larger effect on the density of a serotonin transporter in the flinders sensitive line (FSL) rat model of depression than in normal rats. Synapse 64, 231–240.

Landau, A.M., Alstrup, A.K.O., Noer, O., Winterdahl, M., Audrain, H., Møller, A., Videbech, P., Wegener, G., Gjedde, A., Doudet, D.J., 2019. Electroconvulsive stimulation differentially affects [^{11}C]MDL100,907 binding to cortical and subcortical $5HT_{2A}$ receptors in porcine brain. J. Psychopharmacol. (in press).

Lanzenberger, R., Baldinger, P., Hahn, A., Ungersboeck, J., Mitterhauser, M., Winkler, D., Micskei, Z., Stein, P., Karanikas, G., Wadsak, W., Kasper, S., Frey, R., 2013. Global decrease of serotonin-1A receptor binding after electroconvulsive therapy in major depression measured by PET. Mol. Psychiatry 18, 93–100.

Lepine, J.P., Briley, M., 2011. The increasing burden of depression. Neuropsychiatr. Dis. Treat. 7, 3–7.

Lillethorup, T.P., Iversen, P., Fontain, J., Wegener, G., Doudet, D.J., Landau, A.M., 2015a. Electroconvulsive shocks decrease alpha2-adrenoceptor binding in the Flinders rat model of depression. Eur. Neuropsychopharmacol. 25, 404–412.

Lillethorup, T.P., Iversen, P., Wegener, G., Doudet, D.J., Landau, A.M., 2015b. Alpha2-adrenoceptor binding in Flinders-sensitive line compared with Flinders-resistant line and Sprague-Dawley rats. Acta Neuropsychiatr. 27, 345–352.

Maes, M., 2008. The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuro Endocrinol. Lett. 29, 287–291.

McCall, W.V., Andrade, C., Sienaert, P., 2014. Searching for the mechanism(s) of ECT's therapeutic effect. J. ECT 30, 87–89.

Meyer, J.H., Houle, S., Sagrati, S., Carella, A., Hussey, D.F., Ginovart, N., Goulding, V., Kennedy, J., Wilson, A.A., 2004. Brain serotonin transporter binding potential measured with carbon 11-labeled DASB positron emission tomography: effects of major depressive episodes and severity of dysfunctional attitudes. Arch. Gen. Psychiatry 61, 1271–1279.

Nishi, K., Kanemaru, K., Diksic, M., 2009. A genetic rat model of depression, Flinders sensitive line, has a lower density of 5-HT(1A) receptors, but a higher density of 5-HT(1B) receptors, compared to control rats. Neurochem. Int. 54, 299–307.

Ösby, U., Brandt, L., Correia, N., Ekbom, A., Sparén, P., 2001. Excess mortality in bipolar and unipolar disorder in Sweden. Arch. Gen. Psychiatry 58, 844–850.

Overstreet, D.H., Friedman, E., Mathe, A.A., Yadid, G., 2005. The Flinders Sensitive Line rat: a selectively bred putative animal model of depression. Neurosci. Biobehav. Rev. 29, 739–759.

Overstreet, D.H., Russell, R.W., Helps, S.C., Messenger, M., 1979. Selective breeding for sensitivity to the anticholinesterase DFP. Psychopharmacology 65, 15–20.

Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates, fourth ed. Academic Press, San Diego.

Pucilowski, O., Overstreet, D.H., Rezvani, A.H., Janowsky, D.S., 1993. Chronic mild stress-induced anhedonia: greater effect in a genetic rat model of depression. Physiol. Behav. 54, 1215–1220.

Reimold, M., Batra, A., Knobel, A., Smolka, M.N., Zimmer, A., Mann, K., Solbach, C., Reischl, G., Schwarzler, F., Grunder, G., Machulla, H.J., Bares, R., Heinz, A., 2008. Anxiety is associated with reduced central serotonin transporter availability in unmedicated patients with unipolar major depression: a [11C]DASB PET study. Mol. Psychiatry 13, 606–613 557.

Reivich, M., Amsterdam, J.D., Brunswick, D.J., Shiue, C.Y., 2004. PET brain imaging with [11C](+)McN5652 shows increased serotonin transporter availability in major depression. J. Affect. Disord. 82, 321–327.

Ruhe, H.G., Mason, N.S., Schene, A.H., 2007. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. Mol. Psychiatry 12, 331–359.

Sato, H., Skelin, I., Diksic, M., 2010. Chronic buspirone treatment decreases 5-HT1B receptor densities and the serotonin transporter but increases the density of 5-HT2A receptors in the bulbectomized rat model of depression: an autoradiographic study. Brain Res. 1345, 28–44.

Schildkraut, J.J., 1965. The catecholamine hypothesis of affective disorders: a review of supporting evidence. Am. J. Psychiatry 122, 509–522.

Selvaraj, S., Murthy, N.V., Bhagwagar, Z., Bose, S.K., Hinz, R., Grasby, P.M., Cowen, P.J., 2011. Diminished brain 5-HT transporter binding in major depression: a positron emission tomography study with [11C]DASB. Psychopharmacology (Berl) 213, 555–562.

Sghendo, L., Mifsud, J., 2012. Understanding the molecular pharmacology of the serotonergic system: using fluoxetine as a model. J. Pharm. Pharmacol. 64, 317–325.

Shen, H.-w., Numachi, Y., Yoshida, S., Fujiyama, K., Toda, S., Awata, S., Matsuoka, H., Sato, M., 2003. Electroconvulsive shock increases serotonin transporter in the rat frontal cortex. Neurosci. Lett. 341, 170–172.

Solomon, D.A., Keller, M.B., Leon, A.C., Mueller, T.I., Lavori, P.W., Shea, M.T., Coryell, W., Warshaw, M., Turvey, C., Maser, J.D., Endicott, J., 2000. Multiple recurrences of major depressive disorder. Am. J. Psychiatry 157, 229–233.

Strome, E.M., Clark, C.M., Zis, A.P., Doudet, D.J., 2005. Electroconvulsive shock decreases binding to 5-HT2 receptors in nonhuman primates: an in vivo positron emission tomography study with [18F]setoperone. Biol. Psychiatry 57, 1004–1010.

Strome, E.M., Zis, A.P., Doudet, D.J., 2007. Electroconvulsive shock enhances striatal dopamine D1 and D3 receptor binding and improves motor performance in 6-OHDA-lesioned rats. J. Psychiatry Neurosci. (JPN) 32, 193–202.

Tranter, R., O'Donovan, C., Chandarana, P., Kennedy, S., 2002. Prevalence and outcome of partial remission in depression. J. Psychiatry Neurosci. (JPN) 27, 241–247.

UK ECT Review Group, 2003. Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis. Lancet 361, 799–808.

Vetulani, J., Lebrecht, U., Pilc, A., 1981. Enhancement of responsiveness of the central serotonergic system and serotonin-2 receptor density in rat frontal cortex by electroconvulsive treatment. Eur. J. Pharmacol. 76, 81–85.

Wegener, G., Harvey, B.H., Bonefeld, B., Muller, H.K., Volke, V., Overstreet, D.H., Elfving, B., 2010. Increased stress-evoked nitric oxide signalling in the Flinders sensitive line (FSL) rat: a genetic animal model of depression. Int. J. Neuropsychopharmacol. 13, 461–473.

Wegener, G., Mathe, A.A., Neumann, I.D., 2012. Selectively bred rodents as models of depression and anxiety. Curr. Top. Behav. Neurosci. 12, 139–187.

Yatham, L.N., Liddle, P.F., Lam, R.W., Zis, A.P., Stoessl, A.J., Sossi, V., Adam, M.J., Ruth, T.J., 2010. Effect of electroconvulsive therapy on brain 5-HT(2) receptors in major depression. Br. J. Psychiatry 196, 474–479.

Zeng, Z., Chen, T.B., Miller, P.J., Dean, D., Tang, Y.S., Sur, C., Williams Jr., D.L., 2006. The serotonin transporter in rhesus monkey brain: comparison of DASB and citalopram binding sites. Nuclear Med. Biol. 33, 555–563.