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Abstract

Compounds targeting serotonin (5-HT) are widely used as antidepressants. However, the role of 5-HT in mediating the effects of electroconvulsive seizure (ECS) therapy remains undefined. Using *Tph2*$^{-/-}$ mice depleted of brain 5-HT, we studied the effects of ECS on behavior and neurobiology. ECS significantly prolonged the start latency in the Elevated O-Maze test, an effect that was abolished in *Tph2*$^{-/-}$ mice. Furthermore, in the absence of 5-HT, the ECS-induced increase in adult neurogenesis and in brain-derived neurotrophic factor (BDNF) signaling in the hippocampus was significantly reduced. Our results indicate that brain 5-HT critically contributes to the neurobiological responses to ECS.

Keywords

Neurogenesis, BDNF, TPH2, depression, antidepressants

Conflict of interest

The authors have no conflicts of interest.

Introduction

Accumulating evidence from rodent models indicates that adaptive responses such as increased neurogenesis in the adult hippocampus and increased BDNF signaling contribute to the action of most antidepressants and of electroconvulsive therapy (ECT) [1-3]. Experiments using selective serotonin reuptake inhibitors (SSRIs) clearly demonstrate that the serotonin (5-HT) system may be harnessed to increase neurogenesis and BDNF concentrations [2, 4, 5]. At the same time, the precise role of 5-HT in mediating the biological effects of ECS has remained undefined. Here, we addressed this question by examining the response to ECS of tryptophan hydroxylase 2 deficient (*Tph2*$^{-/-}$) mice selectively depleted of brain 5-HT [6]. Physiology and behavior of *Tph2*$^{-/-}$ mice have been characterized in detail earlier [7, 8]. Briefly, these mice exhibit transient growth retardation during the first six postnatal weeks. In adulthood, *Tph2*$^{-/-}$ mice reveal aggressive behavior, lack of maternal care, and increased despair-like responses [7, 8]. This combination of symptoms closely represents the clinical features of depressed patients with reduced central 5-HT function (i.e., low 5-hydroxyindoleacetic acid [5-HIAA] levels in cerebrospinal fluid) [9, 10].

We have previously shown that *Tph2*$^{-/-}$ mice lack exercise-induced cell proliferation in the adult hippocampus [11], and that deficiency of brain 5-HT impacts BDNF protein levels [12]. In this study, *Tph2*$^{-/-}$ mice and wild type [13] controls were subjected to a series of 5 daily ECS treatments to test the hypothesis that 5-HT signaling is involved in the biological responses to ECS. Besides behavioral measures, we examined survival of newly generated cells in the dentate gyrus, and quantified BDNF levels in micro-dissected hippocampus.

Methods

Animals and treatment. *Tph2*$^{-/-}$ mice [6] were bred onto the C57BL/6N background for more than 10 generations. Three-month old female mice were randomly assigned into groups (ECS vs. control, CTR). ECS followed an established protocol with pentobarbital pretreatment and unilateral electrode placement [14]. Seizure threshold was defined as minimum current required to elicit an ictal motor response of at least 20 sec. Subsequently on day 6, all animals received three intraperitoneal injections of BrdU (5-bromo-2′-deoxyuridine, 50 mg/kg; Sigma-Aldrich) 6 h apart followed by behavioral tests on day 8 – *Elevated O-Maze test* [15, 16], day 9 – *Open field test* [17, 18], and day 10 – *Forced swim test* [19, 20] (Fig. 1a). Animals were sacrificed on day 28 to determine BDNF protein levels and survival of newly generated cells in the hippocampus. All procedures were approved by the respective official committees and carried out in accordance with the Animal Welfare Act and the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Tissue preparation and procedures. Mice were deeply anesthetized and perfused transcardially with 0.9% sodium chloride. Brains were removed and hemispheres separated. The right one was placed into 4% paraformaldehyde for immunohistochemistry while the dissected hippocampus of the left hemisphere was snap-frozen. Endogenous levels of BDNF were measured in the thawed homogenates using a commercial enzyme-linked immunosorbent assay kit (Promega); the manufacturer's instructions were adapted to a highly sensitive fluorometric technique [21]. BrdU immunohistochemistry followed the peroxidase method in accordance with an established protocol [11]. Briefly, one in-six series of sequential 40 μm coronal sections were stained, and immunoreactive cells were counted throughout the rostro-caudal extent of the dentate gyrus. The total number of BrdU-positive cells was estimated by multiplying cell counts by six.

Statistical analysis. Two-way ANOVA analysis was followed by Tukey's *post hoc* test (GraphPad PRISM 6.01 software). All values are expressed as means ± SEM. *P* values of 0.05 were considered statistically significant.

Results

Tph2$^{-/-}$ mice revealed with 30 ± 0 mA on day 1 a significantly higher seizure threshold compared to WT animals (23.8 ± 1.4 mA, $p < 0.001$), which was reversed on day 5 (35 ± 0 mA vs. 37 ± 0.4 mA, $p = 0.0001$; Fig. 1b). Notably, *Tph2*$^{-/-}$ mice already reached the seizure response of 35 ± 0 mA on day 3 of the ECS course.

ECS does not affect anxiety levels but attenuates the highly active phenotype of Tph2$^{-/-}$ mice

All animals were subjected to behavior tests on day 8 to 10 to compare locomotor activity, stress, and antidepressant response to ECS. In the Elevated O-Maze test, a robust treatment and genotype effect was observed in WT as start latency to exit into open arms was increased three times ($F(1,24)=7.175$, $p_{\text{interaction}} = 0.0131$; $F(1,24)=13.21$, $p_{\text{treatment}} = 0.0233$; $F(1,24)=5.870$, $p_{\text{genotype}} = 0.0013$; Fig. 1c). In *Tph2*$^{-/-}$ mice, start latency was not affected (Fig. 1c), and mice spent more time in the open arms of the O-Maze independently of treatment ($F(1,28)=9.004$, $p = 0.0056$; Fig. 1d). When assessing exploratory behavior in the Open field test, a genotype effect was observed ($F(1,28)=12.99$, $p = 0.0012$; Fig. 1e). *Tph2*$^{-/-}$ mice spent significantly more time in the center of the arena, an effect diminished by treatment (Fig. 1e). This is consistent with *Tph2*$^{-/-}$ mice staying in greater distances to walls ($F(1,28)=24.60$, $p < 0.0001$; Fig. 1f). The activity level of *Tph2*$^{-/-}$ mice upon ECS was decreased as shown by total velocity ($F(1,28)=13.73$, $p_{\text{genotype}} = 0.0009$; Fig. 1g). No effect of ECS was observed for WT in the Forced swim test. In *Tph2*$^{-/-}$ mice, latency to immobility was significantly shorter regardless of treatment ($F(1,27)=75.04$, $p < 0.0001$; Fig. 1h). *Tph2*$^{-/-}$ mice revealed an increased immobility time that was abolished upon ECS ($F(1,26)=5.037$, $p_{\text{treatment}} = 0.0335$; $F(1,27)=16.59$, $p_{\text{genotype}} = 0.0004$; Fig. 1i). Vice versa, ECS therapy seems to attenuate the highly active phenotype of *Tph2*$^{-/-}$ mice as shown by shorter distances traveled ($F(1,28)=13.20$, $p = 0.0011$; Fig. 1k).

Reduced neurobiological responses to ECS in Tph2$^{-/-}$ mice

On day 28, we assessed 1) the survival of BrdU labeled cells, and 2) BDNF protein levels in the hippocampus. In response to ECS, the number of newly generated cells was significantly increased 21 days after BrdU injections ($F(1,26)=55.26$, $p_{\text{treatment}} < 0.0001$; Fig. 1m), which led to the characteristic boost in WT (CTR 190 ± 32 cells vs. ECS 695 ± 73 cells, $p = 0.0001$; Fig. 1m), and a significantly attenuated increase in *Tph2*$^{-/-}$ mice (CTR 179 ± 48 cells vs. ECS 496 ± 50 cells, $p < 0.01$; Fig. 1m). Likewise, a treatment as well as genotype effect for BDNF protein levels was observed in the hippocampus in response to ECS ($F(1,26)=6.120$, $p_{\text{treatment}} = 0.0202$; $F(1,26)=10.83$, $p_{\text{genotype}} = 0.0029$; Fig. 1n) which led to an increase by 66% in WT mice. In *Tph2*$^{-/-}$ mice, BDNF protein levels increased by 43% upon treatment but this was markedly reduced by half compared with WT (Tukey's *post hoc* test $p = 0.0219$; Fig. 1n). Notably, our data reveal a positive correlation between the number of newly generated cells in the dentate gyrus and BDNF protein levels in WT ($R^2 = 0.4213$, $p = 0.0065$; Fig. 1o) that was absent in *Tph2*$^{-/-}$ mice (Fig. 1o).

Discussion

In this study, we show 5-HT signaling as prerequisite in mediating key neurobiological effects of ECS. Our main finding is a diminished treatment-induced increase in cell survival and BDNF concentrations in the hippocampus of *Tph2*$^{-/-}$ mice. Interestingly, both biological systems correlate with each other in WT animals but were uncoupled in the absence of 5-HT. Serotonin has long been recognized for its role in the regulation of mood. Manipulations increasing 5-HT levels lead to clinical improvement from depression that is associated with a delayed increase in adult neurogenesis [2, 22]. Furthermore, 5-HT neurotransmission is under the influence of BDNF [23], which is believed to contribute to the effects of antidepressants and ECT [5]. We have shown previously that the 5-HT system interacts with BDNF to maintain homeostasis in the dentate gyrus [12]. However, whether the strong increase in cell survival after ECS is directly mediated by increased BDNF or whether both systems require 5-HT signaling independently remains undefined.

The current finding of reduced BDNF levels in sham-treated *Tph2*$^{-/-}$ mice as compared with WT is in apparent contradiction to our earlier report [12]. We speculate that *Tph2*$^{-/-}$ mice are highly sensitive to stress as a result of sham ECS [14], while all animals in the earlier study were virtually unhandled.

Antidepressant efficacy of ECS sessions can be examined by behavioral tests in rodents [24, 25], e.g. Elevated plus maze is used to analyze changes in anxiety-related behavior and has recently been translated to humans as "unconditioned approach-avoidance conflict" test [26]. Besides alleviating depressed mood, ECS therapy is used to improve despair-like behavior and to ameliorate symptoms of anxiety. In animal models, decreased thigmotaxis or increased open arm entries might refer to less anxious behavior. However, changes in behavior upon ECS may not be detectable in 'non-depressed' wild type groups *per se*, and are time-depended as shown in rats [24, 25]. Our short-term analysis scheme of up to only 3 days after a 5-days ECS series may not have been enough to detect profound alterations, and the behavior was largely unaffected in WT mice. In the more susceptible *Tph2*$^{-/-}$ model, previous observations in behavior at baseline were confirmed: *Tph2*$^{-/-}$ mice are highly active and impulsive [8] accompanied by a reduced anxious phenotype that results in decreased thigmotaxis. Therefore, it might be difficult to further detect treatment effects on anxiety levels. However, in response to ECS, explorative behavior of *Tph2*$^{-/-}$ mice was reduced and the highly active phenotype was attenuated.

To summarize, our results indicate that brain 5-HT critically contributes to the physiological outcome of ECS, by possibly coupling adult neurogenesis with BDNF signaling. These findings add to our understanding of 5-HT action and intertwined biological systems.

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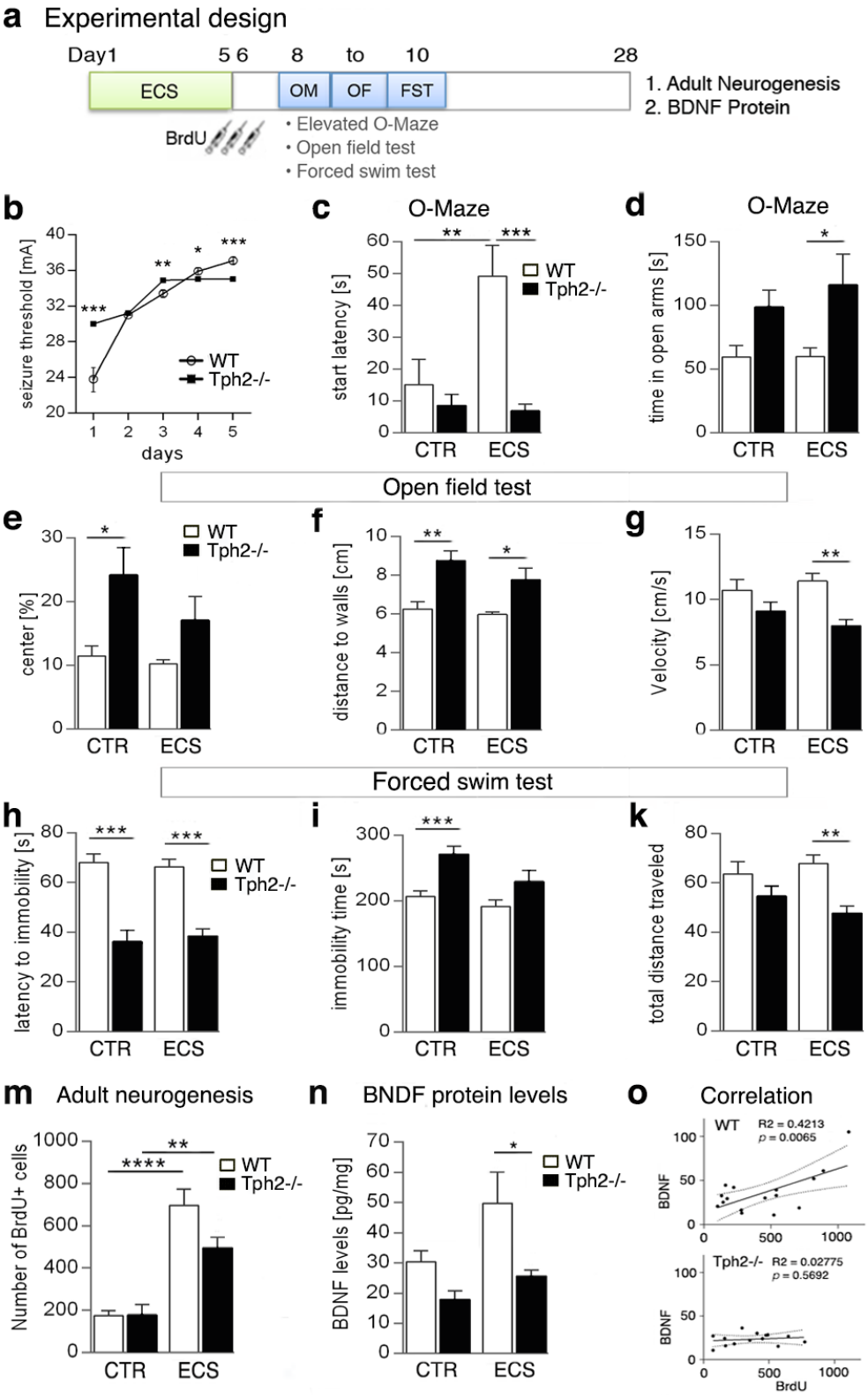
Fig. 1 Physiology and behavior responses to ECS. **a** Experimental design. **b** Mean seizure thresholds for 5 days of *Tph2*$^{-/-}$ and WT mice. **c** Start latency in the Elevated O-Maze test was increased in WT animals upon ECS. **d** *Tph2*$^{-/-}$ mice spent more time in open arms independently of treatment. **e-g** Open field test: *Tph2*$^{-/-}$ mice spent more time in the center (e, percentage) and off the walls (f) which was diminished in response to ECS, as was the activity index (mean velocity, g). **h-k** Forced swim test: latency to immobility time was significantly shorter in *Tph2*$^{-/-}$ mice and unaffected by ECS (h). However, the longer immobility time of *Tph2*$^{-/-}$ mice was abolished after treatment (i), and mice traveled shorter distances (k). **m** In response to ECS, the survival of newly generated cells was robustly increased 21 days following BrdU in WT mice, with an attenuated increase in *Tph2*$^{-/-}$ animals. **n** At 28 days, BDNF protein levels were increased in the hippocampus of WT mice, with a markedly attenuated response in *Tph2*$^{-/-}$ animals. **o** The positive correlation of BDNF levels with adult neurogenesis in WT mice was absent in *Tph2*$^{-/-}$ mice. CTR, control sham w/o seizure; Two-way ANOVA followed by Tukey's *post hoc* test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, data are presented as mean ± SEM.

Figure 1


a Experimental design
Day1
5 6
8
to
10
28
ECS
OM
OF
FST
1. Adult Neurogenesis
2. BDNF Protein
BrdU
• Elevated O-Maze
• Open field test
• Forced swim test
b
seizure threshold [mA]
days
WT
Tph2-/-
c O-Maze
start latency [s]
CTR
ECS
d O-Maze
time in open arms [s]
Open field test
e
center [%]
f
distance to walls [cm]
g
Velocity [cm/s]
Forced swim test
h
latency to immobility [s]
i
immobility time [s]
k
total distance traveled
m Adult neurogenesis
Number of BrdU+ cells
n BNDF protein levels
BDNF levels [pg/mg]
o Correlation
WT R2 = 0.4213
p = 0.0065
Tph2-/- R2 = 0.02775
p = 0.5692
BDNF
BrdU