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Search for factors contributing to resistance to the electroconvulsive seizure treatment model using adrenocorticotrophic hormone-treated mice

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ABSTRACT

Approximately one third of patients with depression remain treatment resistant with existing antidepressants, suggesting that the currently-available antidepressants cannot induce appropriate responses in the brains of all patients. Long-term exposure to adrenocorticotrophic hormone (ACTH) has been proposed as a model that mimics at least some aspects of clinical treatment-resistant depression in rodents. The purpose of this study was to explore potential causes of antidepressant treatment resistance using the chronic ACTH-treated mouse model. We subjected ACTH-treated mice to a rodent model of electroconvulsive therapy, i.e., electroconvulsive seizure (ECS), which induces various molecular and cellular changes, including in gene expression and adult neurogenesis in the hippocampus. First, behavioral effect of repeated ECS in the forced swim test (FST) was examined. In our experimental setting, ACTH-treated mice showed resistance to the antidepressant-like effect of ECS in the FST. We then examined which cellular and molecular changes induced by ECS were attenuated by ACTH administration. Chronic ACTH treatment suppressed the increase of gene expression such as of *Bdnf*, *Npy*, and *Drd1* induced by ECS in the hippocampus. In contrast, there was no difference in ECS-induced promotion of the early neurogenetic process in the hippocampus between ACTH-treated and control mice. Our results suggest the possibility that impaired neuromodulation and monoamine signaling in the hippocampus are among the factors contributing to antidepressant treatment resistance.

1. Introduction

Preclinical and clinical studies have shown that antidepressant treatments act by inducing a wide range of neurochemical and morphological changes in the brain including in the hippocampus (Manji et al., 2001; Duman and Aghajanian, 2012; Willner et al., 2013; Boku et al., 2018). However, approximately one third of patients with depression remain treatment resistant, suggesting that the currently-available antidepressants cannot induce appropriate responses in the brains of all patients (Mathew, 2008; Akil et al., 2018). Long-term exposure to adrenocorticotrophic hormone (ACTH) has been proposed as a model that mimics at least some aspects of clinical treatment-resistant depression in rodents (Kitamura et al., 2002). This model shows resistance to the antidepressant-like effects of tricyclic antidepressants in the forced swim test (FST). Recent studies have also revealed that an acute response to antidepressants including fluoxetine and duloxetine in the FST was not observed in ACTH-treated mice (Srikumar et al., 2017). In contrast, chronic treatment with the dopamine reuptake inhibitor bupropion or central administration of neuropeptide Y (NPY) led to decreased immobility in the FST in ACTH-treated animals (Kitamura et al., 2010; Antunes et al., 2015). However, it remains unknown which cellular signal of typical antidepressant treatments in the brain was suppressed and why the dopaminergic and NPY pathways exhibited antidepressant-like action in the ACTH-treated model. Identifying the cause of treatment resistance induced by ACTH administration would enable the identification of new therapeutic targets.

Our previous studies have revealed that electroconvulsive seizure (ECS), a rodent model of electroconvulsive therapy (ECT), induces various molecular and cellular changes, including in gene expression and adult neurogenesis in the hippocampus (Segi-Nishida et al., 2008, 2011; Imoto et al., 2017; Ueno et al., 2019). In a past study, Li et al. (2006) demonstrated that repeated ECS decreased the immobility time in the FST in ACTH-treated rats. However, when we examined the behavioral effect of ECS using the ACTH-administration mouse model in a preliminary study, we found that the mice showed resistance to ECS treatment. Therefore, in order to explore the cause of ECS treatment resistance in this model, we examined whether and which cellular and molecular changes induced by ECS would be attenuated in ACTH-treated mice.

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2. Materials and methods

2.1. Experimental animals

Five-week-old male ddY mice were purchased from Japan SLC (Hamamatsu, Japan). Mice were housed in groups of four per cage (32 cm length × 11 cm width × 13.5 cm height). All mice were housed under standard conditions (24 °C ± 1 °C, 55% ± 5% humidity) with a 12-h light/dark cycle and ad libitum access to water and food (MR standard, Sankyo Labo Service, Tokyo, Japan). All mice were habituated for longer than 1 week before any experimental procedures were performed. Body weight gain in each mouse was measured during the experiments to monitor the animal's physical condition. Animal use and procedures were in accordance with the National Institute of Health guidelines and approved by the Animal Care and Use Committee of Tokyo University of Science (approval number K18009).

2.2. Drug administration

Mice (6–7 weeks old) were arbitrarily assigned to each experimental group. Mice were subcutaneously administered ACTH-(1-24)-zinc (cortrosyn-Z: Daiich-Sankyo, Tokyo, Japan) once daily (09:00 to 13:00) at 0.45 mg/kg for 18 days. The dose of ACTH was based on previous studies (Sasaki-Hamada et al., 2015, 2017). Control mice received vehicle (saline) in the manner used for the ACTH administration. To label dividing cells, BrdU (150 mg/kg, i.p., Sigma-Aldrich, St. Louis, MI) was administered 2 h before sacrifice.

2.3. Electroconvulsive stimulation

Bilateral ECS (current, 30–40 mA; shock duration, 1 s; frequency, 100 pulses/s; pulse width, 0.5 ms) was administered via moistened, spring-loaded ear-clip electrodes (Bioresearch Center, Nagoya, Japan) with a pulse generator (ECT Unit; Ugo Basile, Gemonio, Italy), to mice that were anesthetized with isoflurane (1.5 to 2%, Pfizer, Tokyo, Japan) in order to minimize their suffering and avoid sudden, unexpected death associated with seizures (Imoto et al., 2017). ECS was administered daily for 11 days (Fig. 1A). The shock administered produced a tonic seizure phase, characterized by the extension of all four limbs, which lasted for longer than 5 s. After 3 min, the animal returned to a normal physiological condition. The sham-treated animals were handled in the same manner as the ECS-treated animals, but without the administration of shock.

2.4. Forced swim test

We used the FST, which is a modified version of the traditional mouse FST (Porsolt et al., 1977), to increase sensitivity for detecting the effect of chronic antidepressant treatment (Jiao et al., 2011). The FST was carried out in a cylindrical container (13 cm diameter, 25.5 cm height) filled with water to a height of 20 cm. The water was replaced between trials. One day before the ECS treatment (on day 7), each mouse was placed in the cylinder for a 10-min pre-swim period. After 10 ECS repetitions (on day 17), the test session was performed and lasted 8 min. Immobility times were measured for 4 min after 2 min of habituation and for 5 min after 3 min of habituation using a digital video camera. The duration of immobility was quantified autonomously by Smart 3.0 (Panlab, Barcelona, Spain).

2.5. Tissue dissection and assay for corticosterone levels

Mice were anesthetized using chloral hydrate (150 mg/kg, i.p., Nacalai Tesque, Japan). Blood samples were intracardially collected; subsequently, the mice were transcardially perfused with cold saline 24 h after the last ECS (from 9:00 to 12:30). The hippocampus from half the brain was dissected and used for RNA extraction. The other half of the brain was fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 72 h at 4 °C, cryoprotected in 20% sucrose for 72 h, and stored at −80 °C until further use in the immunohistochemical analysis. The adrenal glands were dissected and weighed. Blood was centrifuged at 12,000*g* for 15 min at 4 °C for serum isolation. The serum corticosterone levels were assayed using the Corticosterone ELISA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions.

2.6. RNA extraction and real time PCR

Total RNA was extracted using Reliaprep RNA Cell Miniprep System (Promega, Madison, WI) and subjected to the reverse transcription reaction with ReverTra Ace (Toyobo, Osaka, Japan), followed by real time PCR with the StepOne system (Applied Biosystems, Foster City, CA) using the Thunderbird SYBR qPCR mix (Toyobo). Crossing point values were acquired using the second derivative maximum method. The expression level of each gene was quantified using external standardized dilutions. Relative expression levels of target genes between samples were normalized to that of 18S rRNA. The specificity of each primer set was confirmed by examining the product size by gel electrophoresis. Primer sequences for each gene are shown in Table 1.

2.7. Immunohistochemistry

Serial sections (30-μm thickness) from half the brain were then cut through the entire hippocampus with a cryostat (Leica 1510, Leica Microsystems, Tokyo, Japan) and stored in a non-freezing solution at −20 °C until stained.

For BrdU immunostaining, every sixth hippocampal section was incubated in 50% formamide in 2× saline sodium citrate buffer for 2 h at 60 °C, followed by incubation in 2 M hydrogen chloride at 37 °C for 30 min, and neutralized with 0.1 M boric acid (pH 8.5) at room temperature for 10 min. For doublecortin (DCX) staining, these steps were skipped. The sections were blocked in 10% equine serum in phosphate-buffered saline (PBS) containing 0.3% Triton X-100 at room temperature for 60 min, followed by overnight incubation with monoclonal rat anti-BrdU (1:4000; Abcam, Cambridge, MA; ab6326) or polyclonal rabbit anti-DCX (1:5000; Cell Signaling, Danvers, MA; 4604) at 4 °C. After washing with PBS containing 0.3% Triton X-100, sections were incubated with donkey anti-rat IgG conjugated with AlexaFluor 555 (1:300, Abcam, ab150154) or biotinylated goat anti-rabbit IgG (1:1000; Vector Laboratories Inc., Burlingame, CA, BA1000) for 60 min. For immunofluorescence staining, the sections were mounted on slides after washing with Mowiol mounting media (Sigma-Aldrich). For biotin-labeled staining, the sections were incubated with ABC Vectastain Kit (Vector), and antigen detection was performed with 0.06% 3,3′-diaminobenzidine staining. After washing, the sections were mounted on slides with Entellan New (Merck Millipore, Burlington, MA).

2.8. Quantitation of BrdU-labeled cells and DCX-positive cells

For BrdU-labeled cell quantification, a modified unbiased stereological procedure was used as previously described (Imoto et al., 2015). Sections were coded to ensure that the analysis was performed by a blind observer, and BrdU(+) cells were counted in the dentate gyrus (DG) using a fluorescent microscope (Axiovert 200; Zeiss, Göttingen, Germany). Every sixth hippocampal section (30 μm) was counted, and the sum was multiplied by 6 to provide an estimate of the total number of BrdU(+) cells in the entire region. DCX-positive (+) cell quantification was performed as previously described (Ueno et al., 2019). Briefly, two sections of the DG were photographed using a light microscope (Nikon Eclipse E200; Nikon, Tokyo, Japan). Sections were coded to ensure that the analysis was performed by a blind observer. The number of DCX(+) cells is shown as the number of cells per 100 μm of length along the subgranular zone in the DG.

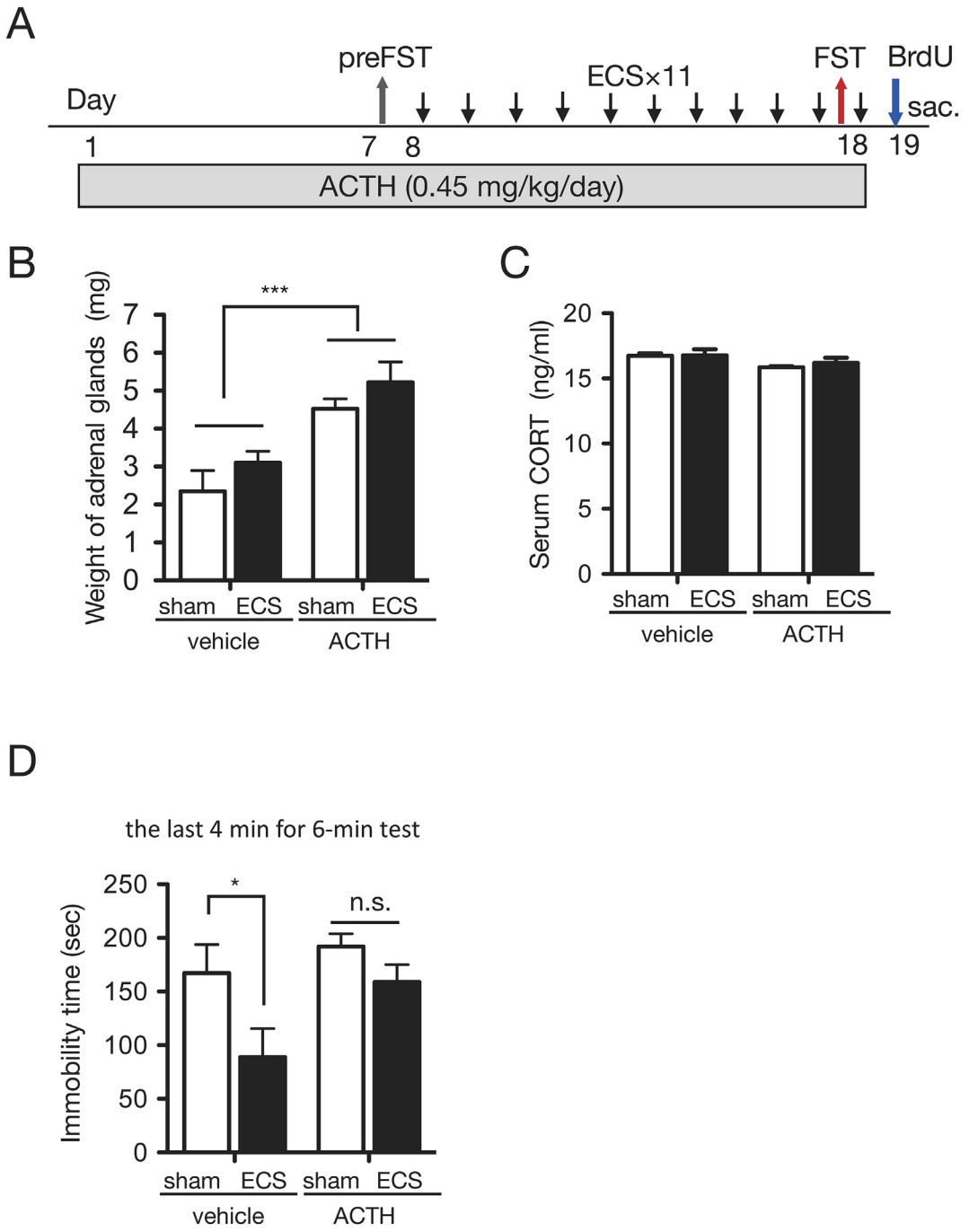


Fig. 1. The effect of ECS on the duration of immobility in the FST for ACTH-treated mice.
(A) Experimental scheme. ACTH was administered once a day for 18 days. ECS was administered once a day for the last 11 days during the ACTH treatment (from day 8 to day 18). On the day following the last administration of ECS, mice received BrdU and were sacrificed 2 h after the BrdU injection (on day 19). Two swim sessions were conducted in a 10-min FST (pre-FST) on day 7 and an 8-min FST on day 17. (B) The effect of ACTH administration on the weight of the adrenal glands (n = 4, Main effect of ACTH: $F_{(1, 12)} = 24.80$, ***P < 0.001 by two-way ANOVA). (C) The effect of ACTH administration on the serum corticosterone (CORT, n = 8) level. (D) The effect of ECS on the duration of immobility in the FST (n = 8, $t = 2.595$, *P < 0.05 and n. s., not significant for Bonferroni's post-hoc test following two-way ANOVA). The time of immobility was measured for 4 min after 2 min of habituation. Data are expressed as the mean ± SEM. ECS: electroconvulsive seizure, FST: forced swim test, ACTH: adrenocorticotrophic hormone, ANOVA: analysis of variance, SEM: standard error of the mean.

2.9. Statistical analyses

All data are presented as mean ± SEM. Statistical analysis was performed by means of unpaired *t*-test, the Mann–Whitney *U* test, or two-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test. Statistical significance was set at P < 0.05. Detailed statistical data are shown in Supplemental Table 1 (Table S1). All analyses were performed using PRISM 5 software (GraphPad, San Diego, CA).

3. Results

3.1. Chronic ACTH administration attenuated the behavioral effect of ECS in ddY mice

To examine the effect of ECS in ACTH-treated mice, we administered ACTH to ddY mice at a dose of 0.45 mg/kg (approximately 13–18 μg/mouse) once daily for 18 days (from day 1 to day 18; Fig. 1A).

Table 1
List of primers used for qPCR analysis.

Gene	Forward (5′ to 3′)	Reverse(5′ to 3′)
Bdnf	TCATACTTCGGTTGCATGAAGG	AGACCTCTCGAACCTGCCC
Npy	ATGCTAGGTAACAAGCGAATGG	TGTCGCAGAGCGGAGTAGTAT
Htr4	TCTGGATGTCCTACTTACCACAG	GCAGCAGATGGCGTAATACCT
Drd1a	ACAGCAGCCCCTCCGATAG	GTTAGACCTGGGCAGATGAAG
Gabrd	ATTGGGGACTACGTGGGCT	CCACATTCACAGGAGCACC
Tdo2	ATGAGTGGGTGCCCGTTTG	GGCTCTGTTTACACCAGTTTGAG
18S	GAGGCCCTGTAATTGGAATGAG	GCAGCAACTTTAATATACGCTATTGG

At this ACTH dosage, there was a significant increase in adrenal gland weight (Fig. 1B; Two-way ANOVA, ACTH effect: $F_{(1,\ 12)} = 24.8$, $P < 0.001$), suggesting that chronic ACTH treatment structurally affected the adrenal gland. Meanwhile, there was no difference in the baseline of serum corticosterone levels between saline and ACTH treatment on day 19 (Fig. 1C). We examined the behavioral effect of ECS in ACTH-treated mice using the FST. ECS was administered daily from day 8 to day 18. In vehicle-treated mice, ECS led to significant decrease in immobility (Fig. 1D; $t = 2.595$, $P < 0.05$ and Fig. S1; $t = 3.194$, $P < 0.01$) in the FST on day 17. In contrast, a behavioral effect of ECS on immobility was not observed in the ACTH-treated mice (Fig. 1D; $t = 1.104$, $P > 0.05$ and Fig. S1; $t = 0.670$, $P > 0.05$), suggesting that our ACTH-treated animal model showed resistance to the antidepressant-like behavioral effect of repeated ECS.

3.2. Effect of ECS on gene expression change in the hippocampus in ACTH-treated mice

To explore the cause of ECS treatment resistance in the ACTH-treated mouse model, we focused on the effect of ECS on gene expression change in the hippocampus. As we previously revealed that ECS induced enhancement of dopaminergic modulation at the hippocampal mossy fiber synapse likely via increased expression of the dopamine D1 receptor (*Drd1*) (Kobayashi et al., 2017), we examined the expression of *Drd1* in the hippocampus. As previously shown, repeated ECS caused a significant increase in the *Drd1* expression level in vehicle-treated mice ($t = 3.508$, $P < 0.01$), while the enhancement of *Drd1* expression by ECS was attenuated in ACTH-treated mice (Fig. 2A; $t = 1.596$, $P > 0.05$). We also examined the expression of the 5-HT type 4 receptor (*Htr4*), which plays an important role in the antidepressant effect in the hippocampus (Kobayashi et al., 2010; Mendez-David et al., 2014; Imoto et al., 2015). In vehicle-treated mice, there was a trend toward increased expression of *Htr4* by ECS ($t = 2.145$, $P = 0.054$), which was attenuated in ACTH-treated mice (Fig. 2B; $t = 1.003$, $P = 0.333$). We next examined the expression of *Bdnf* and *Npy*, the levels of which were increased by ECS in the hippocampus (Mikkelsen et al., 1994; Nibuya et al., 1995). The increased effect of ECS on the levels of *Bdnf* ($t = 2.538$, $P < 0.01$) and *Npy* ($U = 8$, $P < 0.05$ for Mann–Whitney *U* test) expression in vehicle-treated mice was also weakened in the ACTH-treated mice (Fig. 2C and D; $t = 0.238$, $P > 0.05$ for *Bdnf*, $U = 21$, $P > 0.05$ for Mann–Whitney U test for *Npy*). We further examined the expression levels of genes that were suppressed by ECS (Imoto et al., 2017). Expression analysis showed that ECS significantly decreased the expression of the $GABA_A$ receptor delta subunit (*Gabrd*) and tryptophan 2,3-dioxygenase (*Tdo2*) in the hippocampus of both vehicle- and ACTH-treated mice (Fig. 2E and F, Table S1).

3.3. Effect of ECS on the early neurogenic process in the hippocampus in ACTH-treated mice

Most types of antidepressant treatment including ECS increase neurogenesis in the adult hippocampus. Our previous studies showed that ECS facilitates several steps of neurogenesis including progenitor proliferation, survival, and dendritic complexity (Segi-Nishida et al., 2008; Ueno et al., 2019). We accordingly examined whether the neurogenic effect of ECS is altered in ACTH-treated mice. BrdU was administered 2 h before sacrifice on day 19 to label proliferating cells (Fig. 1A). Repeated ECS significantly increased the number of BrdU-positive cells in the subgranular zone of the DG compared with sham treatment in vehicle-treated mice ($t = 5.694$, $P < 0.001$), and this proliferative effect of ECS was similarly observed in ACTH-treated mice (Fig. 3A and B; $t = 5.257$, $P < 0.001$). We also assessed the number of immature neurons by immunostaining for DCX, a marker of the early process of neurogenesis (Fig. 4A). The number of DCX-positive cells in the DG was significantly enhanced by ECS in both vehicle- ($t = 3.99$, $P < 0.001$) and ACTH- ($t = 3.098$, $P < 0.01$) treated mice, suggesting that the early phase of the neurogenic effects of ECS was retained in ACTH-treated mice (Fig. 4B).

4. Discussion

In our experimental setting, ACTH-treated mice showed resistance to the antidepressant-like effect of ECS in the FST. We examined which cellular and molecular changes induced by ECS were attenuated by ACTH administration. We found that chronic ACTH administration suppressed the increase of gene expression such as of *Bdnf*, *Npy*, and *Drd1* induced by ECS in the hippocampus. Our results suggest the possibility that impaired neuromodulation and monoamine signaling in the hippocampus are among the factors that contribute to antidepressant treatment resistance.

4.1. Behavioral effect of ECS in the ACTH-treated mouse model

Abnormalities in the hypothalamus-pituitary-adrenal (HPA) axis have been repeatedly noted in patients with depression (Vreeburg et al., 2009; Zorn et al., 2017; Juruena et al., 2018). Past studies have reported that chronic ACTH administration to rats or mice counteracted the decrease in immobility induced by tricyclic antidepressants in the FST (Kitamura et al., 2002; Srikumar et al., 2017). Disturbed activity of the HPA axis by chronic exposure to ACTH has been implicated in treatment resistance (Kitamura et al., 2002) and the development of depressive phenotypes (Kim et al., 2016; Petrovic et al., 2018) in this model. Consistently, we observed increase in adrenal gland weight by chronic ACTH treatment (Fig. 1B), suggesting that the activity of the HPA axis was chronically changed. However, no basal increase in blood corticosterone concentration was observed (Fig. 1C), implying that responsivity to stress or ACTH stimulation may be altered in this model.

In this study, we showed that the effect of ECS on decreased immobility in the FST was blocked in ACTH-treated mice. In contrast, a previous study showed that ACTH-treated rats were sensitive to ECS in the FST (Li et al., 2006). How ACTH impacts the effect of ECS on decreased immobility may vary by species and our model is considered to be more resistant to antidepressant treatment. We do not exclude the possibility that antidepressant-like effects may be observed in the ACTH-treated mouse model by increasing ECS repetition or ECS intensity. However, we used ACTH-treated mice in this study as a suitable model to explore the cause of treatment resistance to ECS.

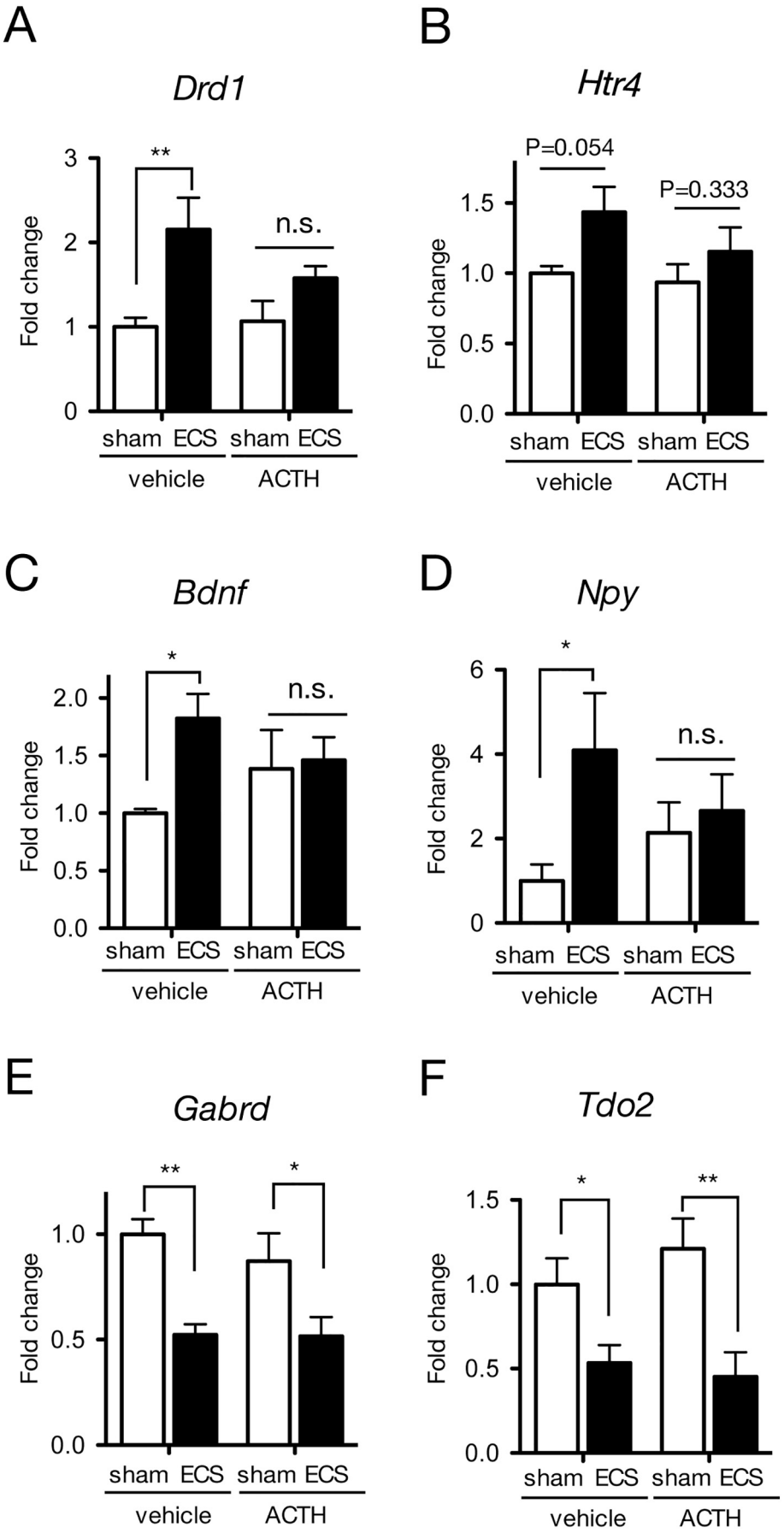
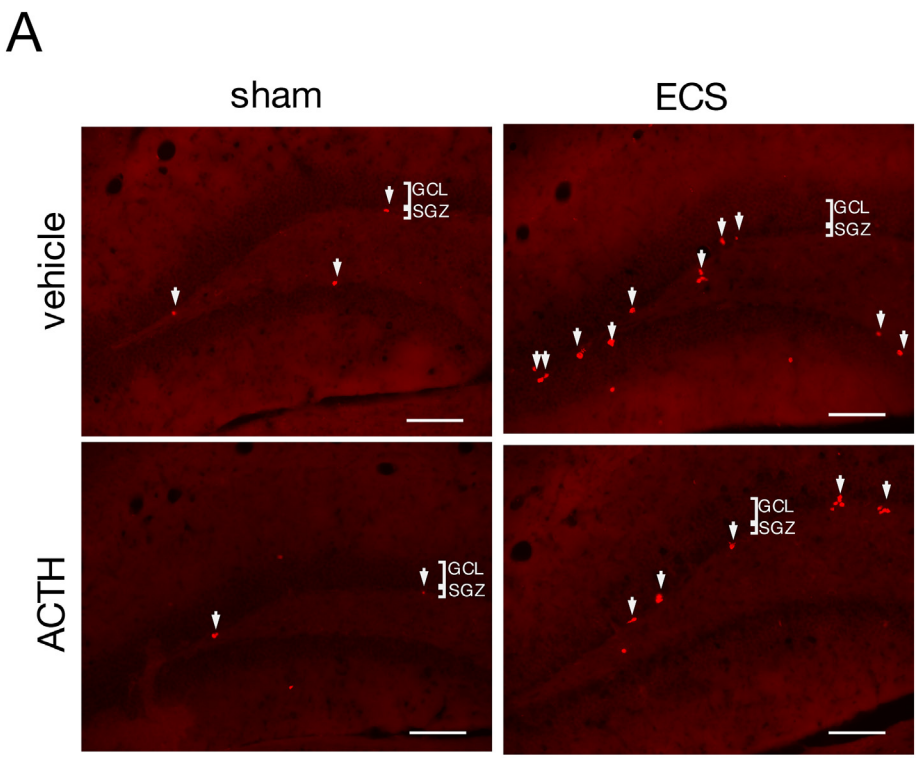


Fig. 2. The effect of ECS on gene expression change in the hippocampus for ACTH-treated mice.
The relative expression levels of *Drd1*, *Htr4*, *Bdnf*, *Npy*, *Gabrd*, and *Tdo2* at 24 h after repeated ECS. Relative expression levels of target genes between samples were normalized to that of 18S rRNA. (A) The expression of *Drd1* ($t = 3.508$, $P < 0.01$ in vehicle-treated mice and $t = 1.596$, $P > 0.05$ in ACTH-treated mice by unpaired *t*-test). (B) The expression of *Htr4* ($t = 2.145$, $P = 0.054$ in vehicle-treated mice and $t = 1.003$, $P = 0.333$ in ACTH-treated mice by unpaired *t*-test). (C) The expression of *Bdnf* ($t = 2.538$, $P < 0.05$ in vehicle-treated mice and $t = 0.238$, $P > 0.05$ in ACTH-treated mice by unpaired *t*-test). (D) The expression of *Npy* ($U = 8$, $P < 0.05$ in vehicle-treated mice and $U = 21$, $P > 0.05$ in ACTH-treated mice by the Mann–Whitney *U* test). (E) The expression of *Gabrd* ($t = 3.691$, $P < 0.01$ in vehicle-treated mice and $t = 2.751$, $P < 0.05$ in ACTH-treated mice by unpaired *t*-test). (F) The expression of *Tdo2* ($t = 2.423$, $P < 0.05$ in vehicle-treated mice and $t = 3.609$, $P < 0.01$ in ACTH-treated mice by unpaired *t*-test). *$P < 0.05$, **$P < 0.01$, and n. s., not significant. Data are expressed as the mean ± SEM (n = 7–8). ECS: electroconvulsive seizure, ACTH: adrenocorticotrophic hormone, SEM: standard error of the mean.



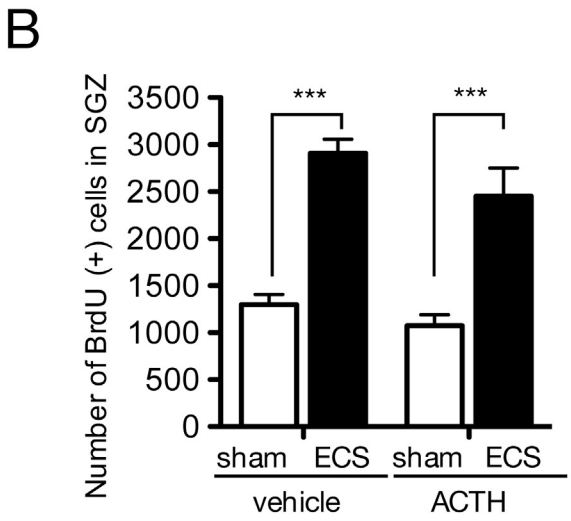


Fig. 3. The effect of ECS on cell proliferation in the hippocampus for ACTH-treated mice.
BrdU was administered on the day following the last ECS at 150 mg/kg and the mice were sacrificed 2 h after the injection. (A) Representative coronal images of BrdU immunostaining in the DG. The arrows represent BrdU(+) cells. Scale bars: 100 μm. (B) Quantification of BrdU-positive cells in the SGZ of the DG in vehicle and ACTH-treated mice ($t = 5.694$, $P < 0.001$ in vehicle-treated mice and $t = 5.257$, $P < 0.001$ in ACTH-treated mice by unpaired *t*-test). Data are expressed as the mean ± SEM (n = 4). ***P < 0.001. ECS: electroconvulsive seizure, ACTH: adrenocorticotrophic hormone, DG: dentate gyrus, GCL: granular cellular layer, SGZ: subgranular zone, SEM: standard error of the mean.

Several clinical studies have reported on the clinical effect of ECT and its regulation of the HPA axis. Yuuki et al. (2005) reported that remission in patients with depression that underwent ECT was accompanied by resolution of HPA dysregulation. Vukadin et al. (2011) showed that high levels of salivary cortisol after dexamethasone administration were associated with greater ECT efficacy. These results suggest the possibility that ECT-induced normalization of the HPA axis may be one of the mechanisms of ECT action. ECS-independent HPA axis abnormalities by continuous ACTH administration may have induced treatment resistance to ECS in our model.

4.2. *Effect of chronic ACTH administration on hippocampal gene expression change induced by ECS*

We found that the increased expression of *Bdnf* by ECS was attenuated in the ACTH-treated hippocampus (Fig. 2C). Conversely, past studies have shown that ECS significantly increased the expression level of *Bdnf* in ACTH-treated rats, in which, ECS had antidepressant-like effects (Li et al., 2006; Kuwatsuka et al., 2013). The increased expression of *Bdnf* in the hippocampus and the antidepressant-like effect by ECS were correlated in both studies, suggesting that BDNF elevation in the hippocampus by ECS may be important for the induction of the antidepressant-like effects in the FST.

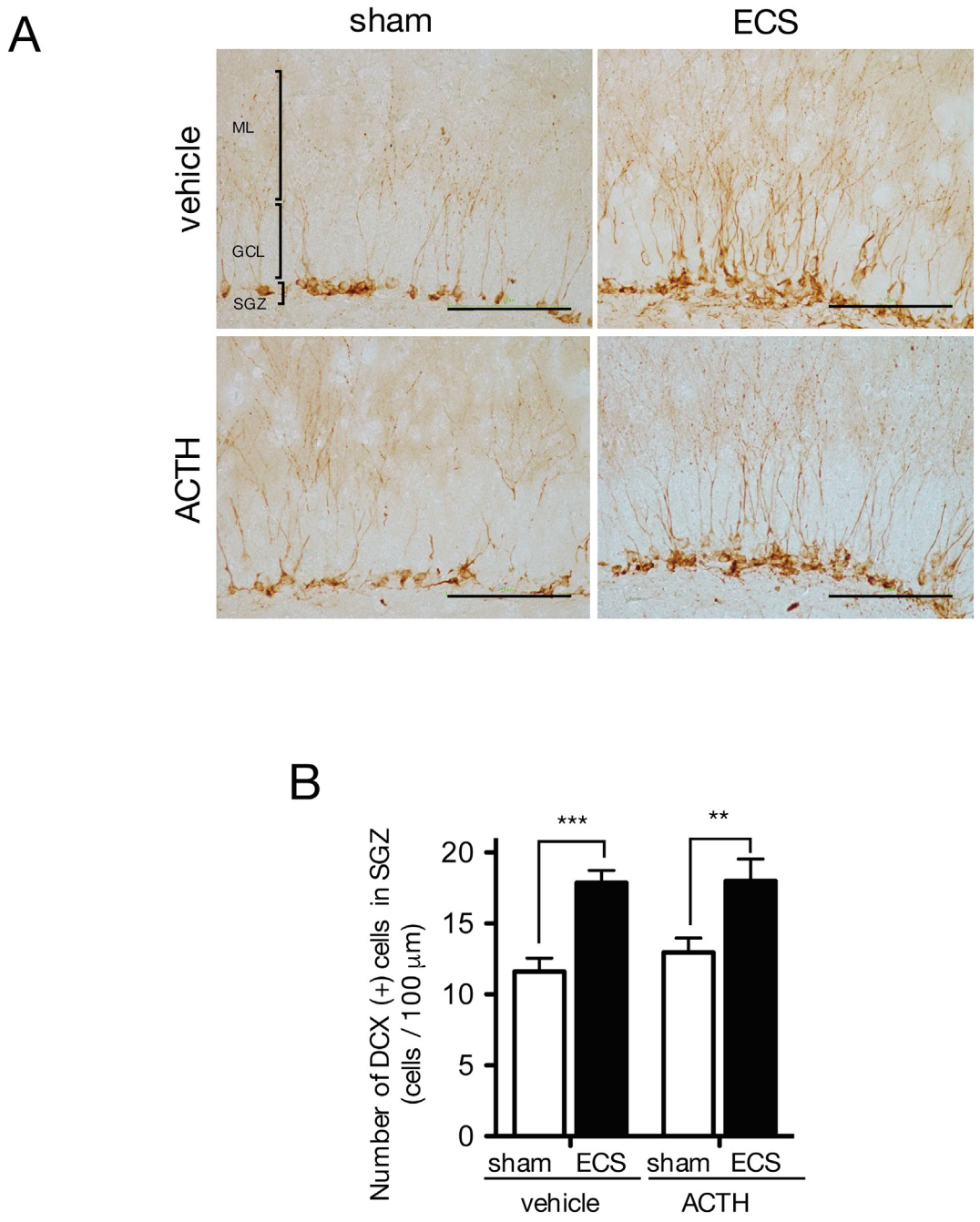


Fig. 4. The effect of ECS on the early phase of neurogenesis in the hippocampus for ACTH-treated mice.
(A) Representative coronal images of anti-DCX immunostaining in the DG. Scale bars: 100 μm. (B) Quantification of DCX-positive cells in the SGZ of the DG in vehicle and ACTH-treated mice. The number of DCX-positive cells in the DG is shown as the number of cells per 100 μm of length of the SGZ ($t = 3.99$, $P < 0.001$ in vehicle-treated mice and $t = 3.098$, $P < 0.01$ in ACTH-treated mice by unpaired t-test). Data are expressed as the mean ± SEM (n = 7–8). **P < 0.01, ***P < 0.001. ECS: electroconvulsive seizure, ACTH: adrenocorticotrophic hormone, DCX: anti-doublecortin, DG: dentate gyrus, ML: molecular layer; GCL: granular cellular layer, SGZ: subgranular zone, SEM: standard error of the mean.

We also showed that increased expression of *Drd1* and *Npy* by ECS was attenuated in this model (Fig. 2A and D). A previous study using ACTH-treated rats showed that the noradrenaline dopamine reuptake inhibitor bupropion led to a decrease in immobility in the FST (Kitamura et al., 2010). ACTH-treated mice also showed decreased immobility with a combination of bupropion and fluoxetine, neither of which alone showed a significant effect (Srikumar et al., 2017). These studies suggested that enhancement of the dopaminergic system is important for induction of the antidepressant-like effect in the ACTH-administration model. We previously demonstrated that ECS increases the expression of *Drd1* in the hippocampus and enhances dopaminergic modulation at the hippocampal mossy fiber synapse (Kobayashi et al., 2017). A recent study also showed that the dopamine D1 receptor in the hippocampus is important for the antidepressant effects of fluoxetine (Shuto et al., 2018), supporting the possibility that hippocampal D1 receptor signaling may be involved in the antidepressant-like effect of ECS. Preclinical studies have also shown that central NPY administration induced an antidepressant-like effect in the FST in ACTH-treated mice (Antunes et al., 2015). In agreement with the present results, ECS and other types of antidepressants increase the expression of *Npy* in the DG of the hippocampus (Mikkelsen et al., 1994; Husum et al., 2000). In the chronic ACTH-treatment models, the suppression of elevated

expression of *Drd1* and *Npy* by ECS may also be one of the factors contributing to treatment resistance.

It is unknown how ACTH administration affects the regulation of gene expression by ECS in the hippocampus. Previous studies have shown that several types of stress and glucocorticoid receptor activation suppressed BDNF expression in the hippocampus (Gronli et al., 2006; Fuchikami et al., 2009; Wosiski-Kuhn et al., 2014). It has been also suggested that the glucocorticoid receptor dimer adheres to the activation protein-1 (AP-1) transcription factor complex, thereby reducing expression of AP-1 target genes, such as *Bdnf* and *Npy* (Andersson et al., 1994; Diefenbacher et al., 2008; Wosiski-Kuhn et al., 2014). In contrast, chronic ECS highly induced a long-lasting AP-1 complex in the hippocampus (Hope et al., 1994). These studies suggest the possibility that hyper-modulation of the HPA axis suppresses the ECS-induced increase of *Bdnf* and *Npy* by glucocorticoid receptor-dependent repression via AP-1 in the hippocampus.

The promoter of the mouse D1 receptor gene has an AP-2 binding region, and it has been suggested that AP-2 suppresses the transcription of *Drd1* in the neuronal cell line (Lee et al., 1999; Takeuchi et al., 1999). As it has been reported that the overexpression of the corticotropin-releasing factor in the brain suppressed D1 receptor expression in the hippocampus (Kasahara et al., 2011), it is necessary to examine the transcriptional regulatory mechanism of AP-2 in the hippocampus using stress and antidepressant treatment.

4.3. Effects of repeated ECS on hippocampal neurogenesis in ACTH-treated mice

Most classes of antidepressants increase neurogenesis in the adult hippocampus, which is a multi-step process that spans proliferation, neuronal differentiation, survival, and functional maturation (Kheirbek et al., 2012). Adult-born neurons mediate some of the behavioral effects of antidepressants, including decreased time in novelty-suppressed feeding, and protect against stress-induced behavioral impairments (Wang et al., 2008; David et al., 2009). We showed that ECS-induced cell proliferation and the differentiation to neural progenitors in the hippocampus were promoted in ACTH-treated mice, as in control mice (Figs. 3 and 4). These results suggest that chronic ACTH administration does not impair the overall ECS responsiveness in the hippocampus. Consistent with this, the decreased expression of *Gabrd* and *Tdo2* by ECS in ACTH-treated mice was similar to that in vehicle-treated mice (Fig. 2E and F). As decreased immobility in the FST by ECS was not seen in the ACTH-treated mice in the present study, it is expected that the promotion of the early neurogenic process does not contribute to this type of antidepressant-like effect. Although there has been no report on the effect of ECS on neurogenesis-dependent antidepressant-like behaviors, it will be necessary to examine the effect of ECS on decreased time in the novelty-suppressed feeding test in the ACTH-treated model. It will also be necessary to evaluate the effect of ECS on the later neurogenic processes such as survival and neuronal maturation in ACTH-treated mice.

5. Conclusion

In this study, we explored the cause of treatment resistance to ECS using the ACTH-treated mouse model. Our results showed that the increase of gene expression such as of *Bdnf*, *Npy*, and *Drd1* by ECS was attenuated in the hippocampus, suggesting the possibility that suppression of ECS-induced expression of these factors may contribute to treatment resistance. As the ACTH-treated mice in this study did not show increase in immobility in the FST, this model should not be considered a depression model. However, it has the potential to explore the factors of antidepressant treatment resistance and identify novel targets for treatment-resistant depression.

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Declaration of competing interest

The authors declare that they have no conflicts of interest relevant to the content of the article.

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