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Electroconvulsive seizures lead to lipolytic-induced gene expression changes in mediobasal hypothalamus and decreased white adipose tissue mass

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Abstract

Aims: Electroconvulsive seizure (ECS) therapy is highly effective in the treatment of several psychiatric disorders, including depression. Past studies have shown that the rodent model of ECS reveals the activation of multiple brain regions including the hypothalamus, suggesting that this method of brain stimulation broadly regulates central neuronal function, which results in peripheral function. The ventromedial nucleus of the hypothalamus (VMH) plays an important role in feeding and energy homeostasis. Our previous study showed that ECS increases the expression of anorexigenic factors in the VMH and has an anorexigenic effect in a mouse model. Since the VMH is also suggested to play a critical role in the peripheral lipid metabolism of white adipose tissue (WAT), we hypothesized that ECS alters lipid metabolism via activation of the VMH.

Methods and Results: Here, we demonstrate that repeated ECS suppresses the fat mass of epididymal WAT and significantly increases the expression levels of lipolytic and brown adipose tissue markers such as Adrb3, Hsl/Lipe, and Ppargc1a. In the VMH, ECS increased the expression of multiple genes, notably Bdnf, Adcyap1, and Crhr2, which are not only anorexigenic factors but are also modulators of lipid metabolism. Furthermore, gold-thioglucose-induced hypothalamic lesions affecting the VMH abolished the effect of ECS on the WAT, indicating that hypothalamus activation is required for the phenotypic changes seen in the epididymal WAT.

Conclusion: Our data demonstrates a new effect of ECS on the lipid metabolism of WAT via induction of hypothalamic activity involving the VMH.

KEYWORDS

BDNF, electroconvulsive seizure, lipid metabolism, ventromedial hypothalamus, white adipose tissue

1 | INTRODUCTION

Electroconvulsive seizure (ECS) therapy has been known to be an effective treatment for drug-resistant depression.[1,2] This therapy also has beneficial effects on psychiatric symptoms in Alzheimer's disease,[3] motor symptoms in Parkinson's disease,[4] and chronic pain in neuropathy,[5] indicating that ECS broadly affects central and peripheral body function.

We have previously demonstrated using a rodent model that ECS activates neurons in the ventromedial nucleus of the hypothalamus (VMH), which plays an important role in feeding and energy homeostasis.[6] We showed that both single and repeated stimulation with ECS significantly induces the expression of immediate-early genes and anorexigenic factors, such as *Bdnf*, *Adcyap1*, and *Crhr2* in the VMH. Among these genes, the expression of *Bdnf* is also increased in the hippocampus by ECS; however, the anorexigenic effect of BDNF is limited in the VMH.[7] In fact, repeated ECS elicited an anorexigenic effect under both regular and high-fat diet conditions in C57BL/6 mice and the activation of the hypothalamus, including the VMH, was required for this ECS-induced anorexigenic effect.

During these experiments, we noticed that repeated ECS resulted in suppressed body weight gain prior to the anorexigenic effect, suggesting that ECS may lead to weight gain suppression through other mechanisms in addition to decreased food intake. The VMH is also suggested to play a critical role in peripheral lipid metabolism of white adipose tissue (WAT).[8,9] WAT is now known to be a complex organ with many functions other than energy storage, and is highly adaptive to external stimuli.[10] In particular, brown adipocyte-like cells found in the WAT of rodents and humans are greatly enhanced by a variety of stimuli.[11,12] Recent studies have demonstrated that living in an enriched environment with complex physical and social stimulation activates the hypothalamus, including the VMH, and upregulates markers of brown adipocyte-like cells in several types of WAT.[13,14] Furthermore, hypothalamic overexpression of BDNF alters the biology of WAT to induce "browning" and increase energy dissipation, resulting in a leaner phenotype. Another study showed that type 2 corticotropin-releasing factor receptor, a gene product of *Crhr2* present in the VMH, regulates lipid metabolism of WAT.[15] These studies suggest the importance of VMH signaling not only in regulating feeding behavior but also in modulating WAT functions.

Since we have previously demonstrated that repeated ECS induces *Bdnf* and *Crhr2* in the VMH, we hypothesized that ECS alters lipid metabolism and/or induces white-to-brown phenotypic changes via the activation of the VMH. In this study, we examined the effect of ECS on fat mass gain and phenotypic changes in WAT using ddY mice, a strain of mice in which marked increases in fat mass and body weight can occur in a short period of time. Furthermore, we explored the role of the hypothalamus, including the VMH, with regard to the effects of ECS on WAT.

2 | METHODS

2.1 | Experimental animals

Five- to eleven-week-old male Slc:ddY mice were purchased from Japan SLC (RRID:MGI_5558113). Mice were housed in groups of four per cage (32 cm length × 15 cm width × 13.5 cm height). All mice were housed under standard conditions (24°C ± 2°C, 55% ± 5% humidity) with a 12-hour light/dark cycle and ad libitum access to water and food (3.5 kcal/g, MR standard, Sankyo Labo Service). Body weight was measured once every 2 days during experiments.

When measuring food intake, the mice were housed individually (20 cm length × 12.5 cm width × 10.5 cm height). Food intake was measured once every 2 days during ECS or sham administration (on days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19), and the daily food consumption average was calculated. All mice were habituated for longer than 1 week before experimental procedures were performed. 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 K19010).

2.2 | Electroconvulsive stimulation

Bilateral ECS (current, 30-40 mA; shock duration, 1 second; 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), to mice that were anesthetized with isoflurane (2.0%, Pfizer) in order to minimize their suffering and avoid sudden, unexpected death associated with seizures.[16] ECS was administered once every 2 days. The shock administered produced a tonic seizure phase, characterized by the extension of all four limbs, which lasted for longer than 5 seconds. After 3 minutes, the animal returned to a normal physiological state. The sham-treated animals were handled in the same manner as the ECS-treated animals, but without the administration of shock.

2.3 | Locomotor activity

A spontaneous activity test was performed 23 hours after the last ECS or sham treatment with a transparent cage without beddings (20 cm length × 12.5 cm width) over a period of 30 minutes. The total distance of movements was tracked using SMART video tracking software (Panlab, RRID:SCR_002852) for the last 20 minutes of the test.

2.4 | Hypothalamus and white adipose tissue dissection

Mice were deeply anesthetized with chloral hydrate (400 mg/kg, i.p., Nacalai Tesque) and sacrificed. Mouse whole brain was quickly removed 24 hours after the last ECS or sham treatment, and a coronal slice was made between bregma −1.22 mm and −2.06 mm.[17] The mediobasal hypothalamus, including the VMH, was then excised under a microscope. Epididymal white adipose tissue (eWAT) was collected and weighed. Both samples were immediately frozen on dry ice and stored at −20°C until further processing.

2.5 | RNA extraction and real-time PCR

Total RNA from the hypothalamus was extracted using the Reliaprep RNA Cell Miniprep System (Promega). Total RNA from eWAT was extracted using RNAiso Plus (Takara Bio). The purity of all RNA samples was determined by the ratio of absorption readings at A_{260}/A_{280}, with all analyzed samples having a value above 1.8. The total RNA was subjected to reverse transcription reaction with ReverTra Ace (Toyobo), followed by real-time PCR with the StepOne system (Applied Biosystems) 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.

2.6 | Hypothalamic lesions by gold thioglucose treatment

Six-week-old mice were injected intraperitoneally with a single dose of 0.3 mg/g gold thioglucose (GTG; Wako, Japan) in saline, 2 weeks before starting either ECS or sham treatment. To confirm lesions of the VMH, serial coronal sections (30-μm thickness) were cut with a cryostat (Leica 1510, Leica Microsystems), and anterior, middle and posterior parts of the VMH sections were evaluated by Nissl staining. Area of the VMH was measured using Image J (NIH, Bethesda, USA, RRID:SCR_003070).

2.7 | Statistical analyses

All data are presented as mean ± SEM. Statistical analysis was performed by means of unpaired Student's *t* test or Welch's *t* test. Statistical significance was set at $P < .05$. All analyses were performed using PRISM 5 software (GraphPad, RRID:SCR_002798).

3 | RESULTS

3.1 | Repeated ECS suppressed body weight gain and decreased the weight of epididymal white adipose tissue (eWAT) in ddY mice

We have previously reported that repeated ECS suppresses body weight gain and food intake in C57BL/6N strain mice.[6] We first tried to confirm that repeated ECS suppresses body weight gain in ddY mice, which display rapid gain of body weight and fat mass.[18] In the first set of experiments, 14 repetitions of ECS were administered once every 2 days (Figure 1A). Consistent with the previous report, body weight gain was significantly suppressed during the later phase of ECS in ddY mice (Figure 1B). To examine the effect of ECS on food intake, mice were isolated in a single cage for the second set of experiments. In contrast to the previous report, the daily average of food intake was found to be no different between the sham and the repeated ECS-treated ddY mice (Figure 1D), while the weight of the epididymal white adipose tissue (eWAT) and body weight gain were significantly reduced in ECS-treated mice (Figure 1C,E), suggesting that ECS may modulate the energy metabolism of WAT. Since it has been known that repeated ECS can change several types of behaviors, including antidepressant-like effects,[19,20] we assessed the effect of ECS on locomotor activity. Repeated ECS-treated mice tended to experience increased locomotor activity in a new cage without beddings ($t_{(6)} = 2.329$, $P = .058$).

3.2 | Repeated ECS activated the hypothalamus, including the VMH, in ddY mice

We have previously reported that ECS increases the expression of anorexigenic genes in the VMH of C57BL/6N mice.[6] Therefore, we investigated whether neuronal activation was present in the hypothalamus and the VMH of ddY mice whose eWAT weight was reduced by ECS. The mediobasal hypothalamus including the VMH

TABLE 1 List of primers used for qPCR analysis

Gene	Forward	Reverse
18S rRNA	5′-GAGGCCCTGTAATTGGAATGAG-3′	5′-GCAGCAACTTTAATATACGCTATTGG-3′
Lipe(Hsl)	5′-CCAGCCTGAGGGCTTACTG-3′	5′-CTCCATTGACTGTGACATCTCG-3′
Mgll	5′-ACCATGCTGTGATGCTCTCTG-3′	5′-CAAACGCCTCGGGGATAACC-3′
Atgl	5′-GGATGGCGGCATTTCAGACA-3′	5′-CAAAGGGTTGGGTTGGTTCAG-3′
Ucp1	5′-AGCCATCTGCATGGGATCAAA-3′	5′-GGGTCGTCCCTTTCCAAAGTG-3′
Ucp2	5′-ATGGTTGGTTTCAAGGCCAVA-3′	5′-CGGTATCCAGAGGGAAAGTGAT-3′
Ppargc1a	5′-TATGGAGTGACATAGAGTGTGCT-3′	5′-CCACTTCAATCCACCCAGAAAG-3′
Adrb3	5′-AGAAACGGCTCTCTGGCTTTG-3′	5′-TGGTTATGGTCTGTAGTCTCGG-3′
Bdnf	5′-GACAAGGCAACTTGGCCTAC-3′	5′-ACTGTCACACGCTCAGC-3′
Crhr2	5′-GCCCTAGTAGAGAGACCGTG-3′	5′-TGTGAGTAGTTGACCCTTGAGG-3′
Adcyap1	5′-ACCATGTGTAGCGGAGCAAG-3′	5′-CTGGTCGTAAGCCTCGTCT-3′
Srxn1	5′-CCCAGGGTGGCGACTACTA-3′	5′-GTGGACCTCACGAGCTTGG-3′

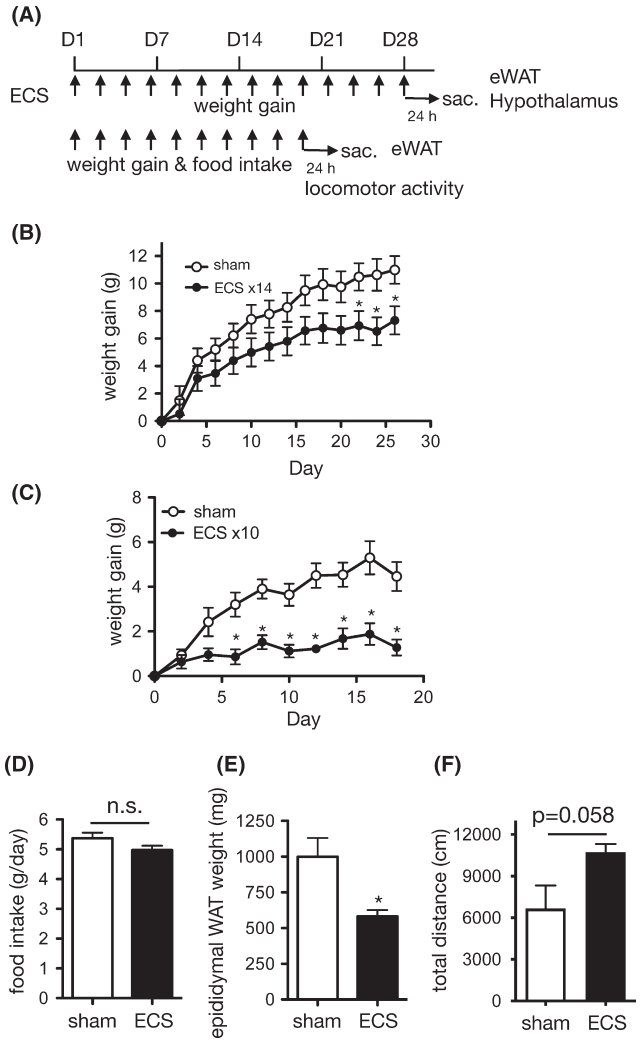

FIGURE 1 Effect of ECS on weight gain and epididymal WAT weight. A, Experimental scheme. In the first set of experiments, mice were group-housed and 14 repetitions of ECS were administered and body weight was measured 3-4 times in a week. In the second set of experiments, mice were singly housed and 10 repetitions of ECS were administered and food consumption and body weight were measured 3-4 times in a week. Mice were sacrificed 24 h after the last ECS and the hypothalamus and/or epididymal WAT (eWAT) were isolated. B, The effect of repeated ECS on body weight gain (n = 10 each, the first set of experiment, $t_{(18)} = 2.432$ on day 22, $P = .026$, $t_{(18)} = 2.66$ on day 24, $P = .016$, $t_{(18)} = 2.56$ on day 26, $P = .02$). C, In the second set of experiments, mice were singly housed and 10 repetitions of ECS were carried out; body weight was measured 3-4 times in a week (n = 5 each). D, The effect of repeated ECS on food intake. The daily average of food intake during ECS stimulation is shown (n = 5 each, the second set of experiment, $t_{(8)} = 1.692$, $P = .129$). E, The effect of repeated ECS on the weight of eWAT (n = 10 each, $t_{(18)} = 3.014$ $P = .0075$). F, The effect of repeated ECS on the locomotor activity (n = 4 each, the second set of experiment, $t_{(6)} = 2.329$, $P = .058$). Data are expressed as the mean ± SEM. ECS, electroconvulsive seizure; sac, sacrificed; SEM, standard error of the mean, $^*P < .05$ and n. s., not significant

was isolated 24 hours after the last ECS and gene expression was assessed by qPCR analysis. Repeated ECS significantly increased the expression levels of *Bdnf*, *Adcyap1*, and *Crhr2* (Figure 2A-C). These genes have been suggested to regulate not only feeding but also lipid metabolism of WAT.[13,15,21] We also found that the expression level of *Srxn1*, known as an activity-dependent antioxidant factor,[22] was significantly increased by repeated ECS (Figure 2D).

3.3 | Repeated ECS increased gene expression of lipolytic enzymes and brown adipose tissue markers in eWAT

It has been reported that several environmental stimuli, such as the cold and environmental enrichment, induces upregulation of genes involved in lipolysis, beta-adrenergic signaling, and white-to-brown fat-like phenotypic changes in WAT via activation of the hypothalamus.[14,23] To explore the effect of ECS on the phenotypic changes in the eWAT, we examined the expression levels of lipolytic and brown adipose tissue (BAT) markers. We found that repeated ECS significantly increased the expression of beta3 adrenergic receptor (*Adrb3)*, hormone-sensitive lipase (*Hsl/Lipe*), and PCG-1a (*Ppargc1a*), known as a BAT marker (Figure 3A). There was a trend toward increase in the expression of *Ucp1*, a BAT specific thermogenic factor. These results demonstrate that repeated ECS promotes lipolytic-related gene expression in the eWAT (Figure 3B).

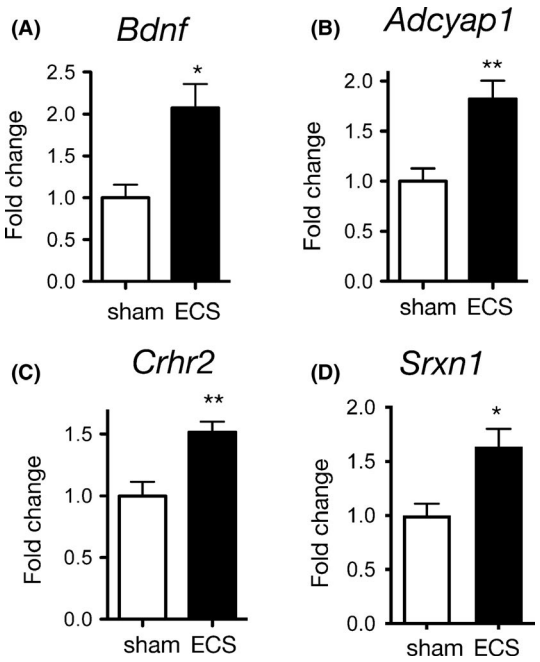

FIGURE 2 Effect of ECS on gene expression in the mediobasal hypothalamus. The relative expression levels of *Bdnf*, *Adcyap1*, *Crhr2*, *and Srxn1* at 24 h after repeated ECS in the mediobasal hypothalamus including the VMH. A, The expression of *Bdnf* ($t_{(8)} = 3.302$, $P = .011$). B, The expression of *Adcyap1* ($t_{(8)} = 3.696$, $P = .006$). C, The expression of *Crhr2* ($t_{(8)} = 3.595$, $P = .007$). D, The expression of *Srxn1* ($t_{(8)} = 3.253$, $P = .012$). $^*P < .05$, $^{**}P < .01$. Data are expressed as the mean ± SEM (n = 5 each). ECS, electroconvulsive seizure; SEM, standard error of the mean

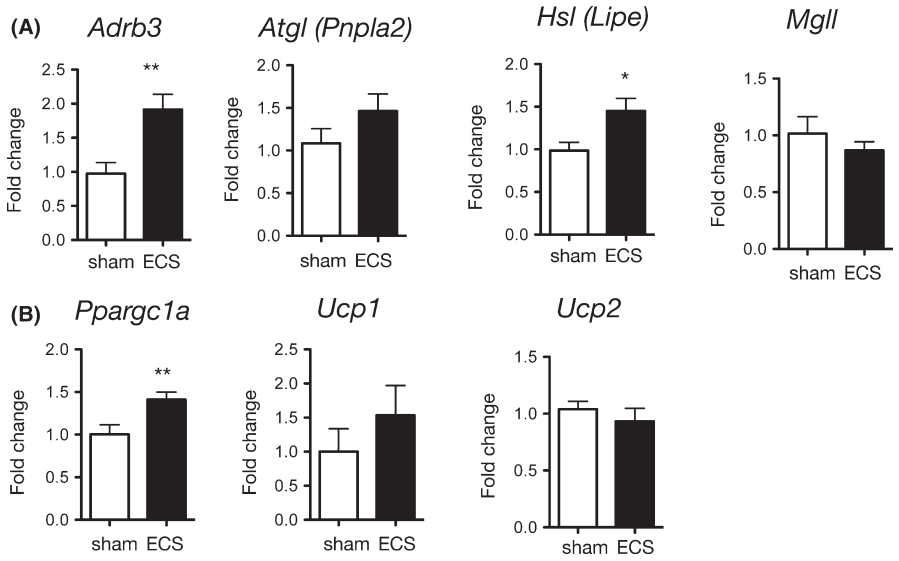

FIGURE 3 Effect of ECS on gene expression in eWAT. The relative expression levels of lipolytic and brown fat marker genes in eWAT. A, The expression of lipolytic genes (Adrb3, adipocyte triglyceride lipase *Atgl/Pnpla2*, hormone-sensitive lipase [*Hsl/Lipe*], monoglyceride lipase [*Mgll*]) were examined (*Adrb3*, $t_{(18)} = 3.422$, $P = .003$; *Atgl/Pnpla2*, $t_{(18)} = 1.419$, $P = .173$; *Hsl/Lipe*, $t_{(18)} = 2.627$, $P = .017$; *Mgll*, $t_{(18)} = 1.419$, $P = .173$). B, The expression of brown fat marker genes (Ppargc1a, Ucp1, Ucp2) were examined (*Ppargc1a*, $t_{(18)} = 2.893$, $P = .0097$, *Ucp1*, $t_{(18)} = 0.97$, $P = .345$; *Ucp2*, $t_{(18)} = 0.789$, $P = .44$) . $^{*}P < .05$, $^{**}P < .01$. Data are expressed as the mean ± SEM (n = 10 each). ECS: electroconvulsive seizure, SEM: standard error of the mean

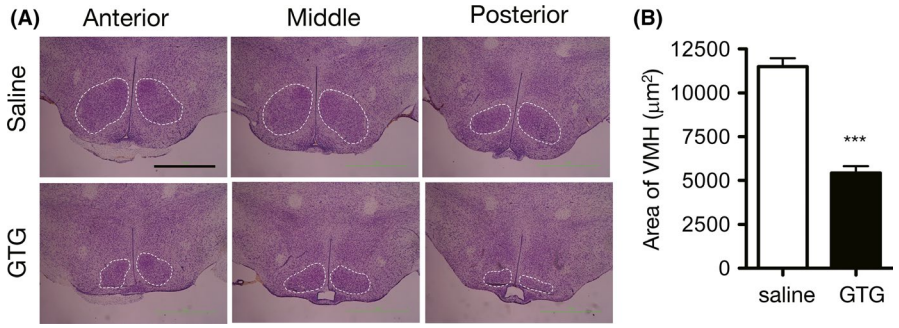

FIGURE 4 Effect of gold thioglucose (GTG) on the lesions of hypothalamus including the VMH. A, Representative coronal images of the anterior, middle, and posterior part of the VMH (white dashed area) 3 wk after GTG administration. Scale bar: 100 μm. B, Quantification of the area of the VMH. The anterior, middle, and posterior parts of the VMH area were measured in saline- (n = 6) and GTG-treated (n = 9) mice ($t_{(13)} = 9.703$, $^{***}P < .0001$)

3.4 | Activation of the hypothalamus, including the VMH, is required for the reduced eWAT weight and phenotypic change induced by repeated ECS

We then assessed whether repeated ECS-induced activation of the hypothalamus, including the VMH, is required for the reduced weight and gene expression changes of eWAT. Treatment with GTG, which is selectively toxic to glucose-sensitive neurons, results in chemical lesions of the hypothalamus involving the VMH and the arcuate nucleus, with subsequent development of obesity.[24–26] Mice were housed isolated in a single cage and GTG was intraperitoneally administered, and 2 weeks later, significant lesions of the hypothalamus including the VMH was observed in consecutive coronal sections of the brain (Figure 4A,B). ECS administration was started 2 weeks after GTG treatment. We observed that GTG administration increased body weight and eWAT mass (see Figures 1 and 5). However, ECS could not suppress body weight gain, food intake, and eWAT mass in GTG-treated mice (Figure 5B-D). We also found that the expression of lipolytic-related factors was not different between the sham and ECS groups among GTG-treated mice (Figure 5E). These results suggest that the activation of the hypothalamus, including the VMH, is required for the decreased fat mass and gene expression changes in eWAT induced by repeated ECS.

4 | DISCUSSION

Our previous study has demonstrated that ECS activates anorexigenic signals in the mouse VMH.[6] In the present study, we

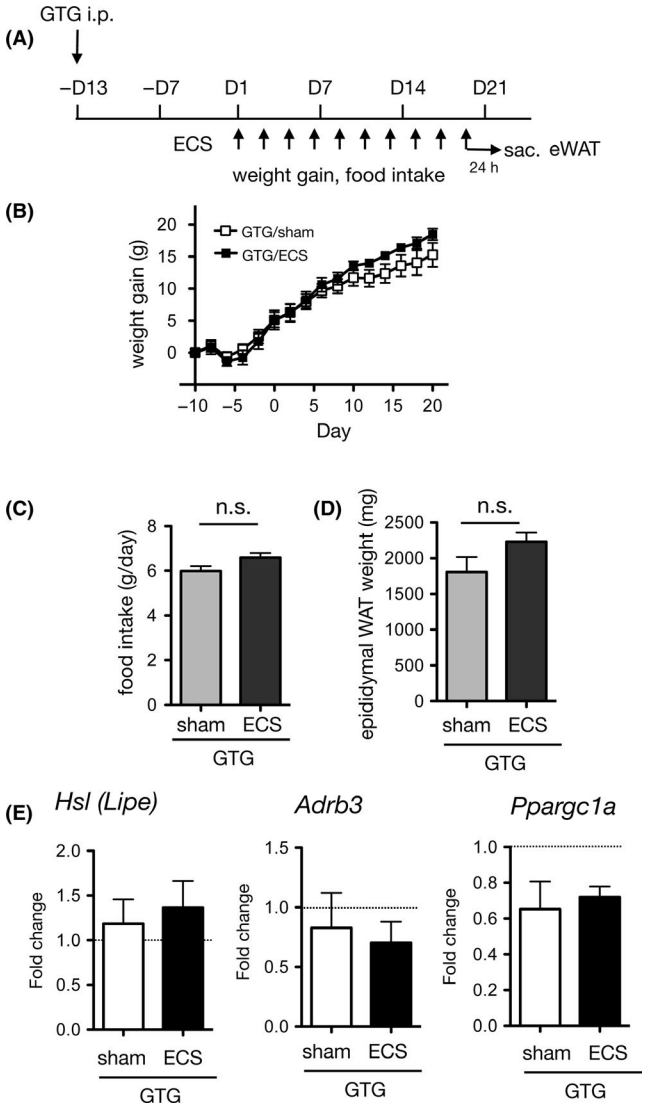

FIGURE 5 Effect of GTG-induced hypothalamic lesions on the ECS-induced suppression of weight gain and epididymal WAT weight. A, Experimental scheme. GTG was administered 13 d before ECS stimulation. Food consumption and body weight were measured 3-4 times in a week. Mice were sacrificed 24 h after the last ECS and eWAT was isolated. B, The effect of the hypothalamic lesions on ECS-induced suppression of body weight gain (n = 5 each). C, The effect of the hypothalamic lesions on food intake (n = 5 each), $t_{(8)} = 2.07$, $P = .072$). D, The effect of the hypothalamic lesions on ECS-induced suppression of eWAT weight (n = 5 each), $t_{(8)} = 1.72$, $P = .123$). E, The effect of the hypothalamic lesions on ECS-induced gene expression change of eWAT (n = 5 each), *Hsl (Lipe)*, $t_{(8)} = 0.444$, $P = .668$; *Adrb3*, $t_{(8)} = 0.37$, $P = .72$; *Ppargc1a*, $t_{(8)} = 0.41$, $P = .695$). Relative expression levels of target genes were compared to that of non-GTG/sham-treated mice as shown in Figure 3. Data are expressed as the mean ± SEM. ECS, electroconvulsive seizure; n.s., not significant; sac, sacrificed; SEM, standard error of the mean

demonstrated that repeated ECS suppresses the fat mass of eWAT, and the expression levels of lipolytic-related genes and BAT markers in eWAT are increased by ECS. We also showed that ECS increased the gene expression of not only anorexigenic factors but also lipid metabolism modulators in the VMH. We also found that the effects of repeated ECS on the body weight gain and phenotypic changes of eWAT were eliminated by GTG hypothalamic lesions including the VMH. To the best our knowledge, this is the first study to show that ECS exerts effects on adipose tissue and suggest the requirement of the hypothalamus, including the VMH, for the lipolytic effect of ECS.

4.1 | Repeated ECS stimulation leads to decreased WAT mass and increased lipolytic-related gene expression

In this study, we found that repeated ECS increases the gene expression of lipolytic enzymes and BAT markers in eWAT. Lipolysis is a catabolic process that leads to the breakdown of triacyl glycerol to glycerol and fatty acids. Hormone-sensitive lipase (HSL/Lipe) is one of the main enzymes involved in WAT lipolysis. This enzyme is activated by cAMP-dependent protein kinase A phosphorylation such as during β-adrenergic signaling.[27] Therefore, the increased gene expression of β3 receptor (*Adrb3*) and *Hsl* by ECS may promote lipolytic signals in the eWAT.

We also demonstrated that ECS increased the gene expression of PGC-1α (*Ppargc1a*) in the eWAT. PGC-1α is a transcriptional coactivator that plays an important role in the expression of thermogenic factors including uncoupling protein 1 (UCP1) and mitochondrial biogenesis factors in the BAT and muscle tissues.[28] In WAT, exercise, environmental enrichment, and adrenergic stimulation increases PGC-1α expression,[14,29,30] which has been suggested as one of the mechanisms of white-to-brown fat-like phenotypic changes.[11] The reduction of eWAT mass by ECS may be involved in the accelerated degradation of triacyl glycerol and heat production due to oxidative degradation of fatty acids. However, in this study, we observed a trend toward increased expression of *Ucp1* by ECS, but this change was not statistically significant. Histological analysis of brown fat-like components in the eWAT is necessary to identify functional changes induced by ECS stimulation.

4.2 | Involvement of hypothalamic activity, including the VMH, in the changes to eWAT by repeated ECS

In the hypothalamus, neurons in the VMH have been suggested to play crucial roles in both feeding behavior and peripheral lipid metabolism. We observed that repeated ECS increases the expression levels of *Bdnf*, *Adcyap1*, and *Crhr2* in the hypothalamus including the VMH. These gene products in the hypothalamus have been suggested to regulate not only feeding but also lipid metabolism in WAT. It has been reported that hypothalamic overexpression of BDNF activates white-to-brown fat-like phenotypic switch in WAT and induces a lean phenotype.[14] In addition,

enriched environment-induced "browning" phenotypes are blocked by the inhibition of hypothalamic BDNF signaling. PACAP (*Adcyap1*) is one of the VMH-enriched genes.[31] PACAP neurons in the VMH are targets of central leptin signaling, which mediates anti-adiposity effects.[32,33] Central PACAP activates sympathetic nerve activity directed toward the adipose tissue.[34] In addition, the expression of *Ucp1* and *Adrb3* in the eWAT of PACAP$^{-/-}$ mice was decreased in a cold environment,[21] suggesting that PACAP is involved in the cold-induced "browning" phenotypes. CRFR2 (*Crhr2*) is a high-affinity receptor of urocortin 3. It has been reported that reduced CRFR2 expression in the VMH increases body weight and WAT mass.[15] Furthermore, the expression of *Hsl* is significantly reduced in the WAT when CRFR2 is knocked down in the VMH, implying that CRFR2 signaling in the VMH activates lipolytic signaling in the WAT. These studies support the idea that the increased gene expression of *Bdnf*, *Adcyap1*, and *Crhr2* in the VMH by ECS promotes lipolytic signaling and white-to-brown fat-like phenotypic changes in WAT. The sympathetic nervous system is expected to play a role in the mechanism by which VMH activation changes the phenotype of WAT.[35] Since the locomotor activity in ECS-treated mice tended to increase, the effects of ECS on energy expenditure, including locomotor activity, metabolic rate, and body temperature, may also be involved in these processes. Recent studies indicate that distinct hypothalamic neuronal populations differentially regulate white-to-brown fat-like change in WAT, BAT thermogenesis, and hepatic lipogenesis. It has been recently reported that the activation of a subset of VMH neurons preferentially promotes "browning" phenotypes in WAT via a selective activation of the sympathetic nervous system, but has no effect on BAT.[23] In our preliminary study, no significant change in BAT weight due to repeated ECS was observed (data not shown). However, it is necessary to examine the effects of ECS on thermogenesis in BAT and hepatic metabolism in future studies.

We showed that GTG-induced hypothalamic lesions, including the VMH, eliminated the phenotypic changes of eWAT by repeated ECS, pointing to the requirement of the VMH for the effects of ECS. However, we could not rule out the possibility that ECS regulates weight gain and lipolytic function via functions other than the VMH since the hyperphagic effect of the VMH lesion might be too strong to mask the specific effects of ECS. VMH-specific gene knockdown, such as of *Bdnf*, *Adcyap1*, and *Crhr2*, may be useful in elucidating the molecular mechanisms of ECS in the VMH.

We also found that the expression level of *Srxn1* in the VMH was significantly increased by repeated ECS. Sulfiredoxin1 (*Srxn1*) is known as an endogenous antioxidant protein.[22] A high fat diet induces changes in the hypothalamus such as an increase in markers of oxidative stress and endoplasmic reticulum stress.[36] Recent studies suggested that a reduction of hypothalamic cellular stress activates "browning" phenotypes of WAT and ameliorates obesity.[37,38] Increased expression levels of *Srxn1* in the VMH may affect phenotypic changes in WAT by lowering oxidative stress levels.

5 | CONCLUSION

In this study, we demonstrate a new ECS-induced effect on adipose tissue via hypothalamic activity including the VMH. The VMH is suggested to be involved in the manifestation of stress and anxiety-related behavior.[39,40] Since ECS therapy is known to an effective treatment for multiple types of psychiatric disorders,[1–3,41,42] it is important to investigate the role of ECS in controlling emotional state via the VMH. Our results point to the potential for ECS or other modalities of brain stimulation to reduce body weight or activate lipid metabolism through VMH activation.[43–45]

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

MT and ESN designed the study, conducted the experiments, analyzed the data, and drafted the manuscript. MT, YK, and MO conducted the experiments and analyzed the data. All authors approved the final manuscript.

ANIMAL STUDIES

Animal use and procedures were approved by the Animal Care and Use Committee of Tokyo University of Science (approval number K19010).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are included in Supporting Information.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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