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Functional Recovery with Electro-Acupuncture Stimulation in an *Mecp2*-Knockout Rat Model of Rett Syndrome

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ABSTRACT

Rett syndrome is a progressive neurodevelopmental disorder that lacks effective treatments. Although deep-brain stimulation can alleviate some symptoms in Rett model mice, this interventional manipulation requires deliberate surgical operations. Here, we report that electro-acupuncture stimulation (EAS) can ameliorate symptoms of an *Mecp2*-knockout rat model of Rett syndrome from the remote acupoints Baihui (GV 20), Yongquan (KI 1), and Shenmen (HT 7). We find that EAS not only prolongs the survival time of Rett rats, but also improves their behavior ability, including locomotion, motor coordination, and social interaction. Neural activation was observed in the substantia nigra of the midbrain, corpus striatum, and cerebral cortex of wild-type and Rett model rats, as reflected by the increased expression of the c-Fos protein. Hence, EAS provides a potential promising therapeutic tool for treating neurodevelopmental diseases.

1. Introduction

Rett syndrome, as a subtype of autism spectrum disorders, is a progressive neurodevelopmental disorder that occurs mainly in female children with an incidence of 1 in 10 000–15 000. Most children with Rett syndrome have a shorter lifespan, along with an array of symptoms such as language retrogression, growth retardation, autonomic dysfunctions, ataxia, severe social skills impairment, and stereotypic hand movements [1]. At present, treatment of Rett syndrome is a great challenge due to the lack of effective treatments to improve the cognition and behavior of patients. Taking brain-derived neurotrophic factor (BDNF) or insulin-like growth factor 1 (IGF-1) can alleviate some symptoms of Rett syndrome; however, it remains a problem for these compounds to cross the blood–brain barrier efficiently [2–4]. It has been reported that physical intervenes, such as forniceal deep-brain stimulation (DBS) [5] and deep-brain magnetic stimulation (DMS) [6], can alleviate the symptoms of Rett model mice. DMS has been shown to promote hippocampal neurogenesis and synaptic plasticity and enhance neuronal activity in the hippocampus; moreover, DMS has been shown to alleviate anxiety-associated behaviors and strikingly prolong lifespan in a Rett mouse model [6]. Forniceal DBS has been shown to rescue the contextual fear memory and spatial memory, restoring the *in vivo* hippocampal neurogenesis of Rett model mice. However, DBS is an interventional manipulation that requires surgical operations and precise localization. In addition, DBS led to limited improvements in the levels of social behavior, locomotion, motor coordination, and growth in Rett model mice [5].

Acupuncture is a traditional Chinese therapeutic intervention that works on diseases through programmed distal stimulus on specific acupoints. Acupuncture has been widely accepted around

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the world for pain relief. Furthermore, acupuncture has been demonstrated to work well in treating other diseases, including motor disorders, stress urinary incontinence, and immuno-inflammatory conditions [7–10]. Electro-acupuncture stimulation (EAS) is a technique that strengthens stimulation using an electro-acupuncture device to output a pulse current, acting on the meridian and acupoints in traditional Chinese medicine. EAS has exhibited excellent analgesic and anti-inflammatory effects through the receptor- or neurotransmitter-mediated immune response [11,12]. EAS has been shown to improve memory and the behavioral ability of motor control in degenerative diseases (e.g., Alzheimer's disease and Parkinson's disease) by stimulating the expression of neurotrophic factors and neurotransmitters [13–15]. Recently, EAS was also reported to improve the social interaction behavior of autistic children and rats by upregulating the level of oxytocin (OXT) and arginine-vasopressin (AVP) associated with mammalian social behaviors [16,17]. However, whether or not EAS can be used for the treatment of other neurodevelopmental diseases remains less explored.

In this work, we treated Rett model rats with EAS and investigated social hormones, the expression of function proteins, neuronal activity in different regions of the brain, and individual behavior, which allowed us to determine the relationship between the EAS-activated brain regions and stress-related behavior changes, together with the effects of EAS on Rett model rats.

2. Experimental process

Male *Mecp2*$^{-/y}$ and wild-type (WT) Sprague Dawley rats aged 4–5 weeks were prepared according to previous study Ref. [18]. Typical symptoms of Rett syndrome in male rats appear relatively early, such as short survival time, decreased motor ability, and decreased social ability. Therefore, in this study, the male *Mecp2*$^{-/y}$ rats were used as the Rett model, and their littermate male WT rats were used as the control. The rats were lightly immobilized in the rat holder (Fig. S1(a) in Appendix A) and their feet were straightened and fixed with medical tape. EAS was performed with a distant-dense wave stimulation for 20 min with a pulse width of 0.2 ms and a frequency of 2 Hz:10 Hz (distant wave:dense wave, Fig. S1(b) in Appendix A) using an electrostimulator (Hwato SDZ-V nerve and muscle stimulator, Suzhou Medical Appliance Factory, China). The acupuncture needles (13 mm in length, 0.25 mm in diameter) were inserted to a depth of 3 mm at the points of HT 7, KI 1, and GV 20 according to Refs. [19,20]. Bilateral HT 7 and KI 1 were stimulated by electro-acupuncture, while GV 20 was performed with needle retention. The *Mecp2*$^{-/y}$ rats and WT rats were treated by electro-acupuncture six times per week for four continuous weeks. The WT and *Mecp2*$^{-/y}$ rats in the control group were also lightly immobilized in the rat holder without EAS.

Open-field, accelerating rotarod [19,21], and three-chamber interaction [22] behavior tests were performed one week before fear conditioning [5,23], according to previous procedure. Before each test, the rats were transported to the behavioral room to habituate for at least 30 min. The details of the experiment are presented in the Supplementary Information in Appendix A. After the detection of behavioral data, the rat brains were dissected to prepare 40 μm brain slices using a freezing microtome (Leica CM1950, Leica Biosystems, Germany) [24]. The brain sections were incubated with 0.25% Triton X-100 in 5% bovine serum albumen (BSA) for 1 h at room temperature [5]. Next, the sections were co-incubated with anti-c-Fos polyclonal (ab190289, rabbit, 1:5000; Abcam, UK) or doublecortin (DCX; 4604S, rabbit polyclonal, 1:400; Cell Signaling Technology, USA) and 5-bromo-2′-deoxyuridine (BrdU; mouse monoclonal, 5292S, 1:140; Cell Signaling Technology) for 24 h at 4 °C. The sections were subsequently co-incubated with secondary antibodies (ab150077 and ab150116, Alexa Fluor 488 and 594, 1:500; Abcam) for 2 h, and the cell nucleus was counterstained with 4′,6-diamidino-2-phenylindole (DAPI; 1:1000, Beyotime Biotechnology, China) for 15 min. Images were obtained with a confocal microscope (Leica Microsystems) and analyzed by Image J software.

3. Results and discussion

We first studied whether EAS could improve the lifespan of Rett rats, because a short lifespan is characteristic of most Rett children [1]. A deficiency of methyl CpG binding protein 2 (MeCP2) in the central nervous system neurons can result in a Rett-like phenotype in rodents [1,25]. Therefore, 4–5-week-old rats with a mutation in the *Mecp2* gene (*Mecp2*$^{-/y}$) were used to perform treatment with EAS (*Mecp2*$^{-/y}$ + EAS) at the acupoints Shenmen (HT 7), Yongquan (KI 1), and Baihui (GV 20) 20 min daily for four weeks (Figs. 1(a) and (b) and Fig. S1(b)). The acupoint locations were chosen according to previous methods [19,20]. In addition, 4–5-week-old littermate male WT and *Mecp2*$^{-/y}$ rats were used as the control and received the same treatment except for EAS. Surprisingly, the lifespan of the EAS-treated *Mecp2*$^{-/y}$ rats (median lifetime: 102 days) was extended by nearly 44% compared with the *Mecp2*$^{-/y}$ rats (median lifetime: 71 days) (Fig. 1(c)). That is, EAS greatly improved the median lifetime of *Mecp2*$^{-/y}$ rats to 102 days; the longest survival time of EAS-treated *Mecp2*$^{-/y}$ rats was 240 days. Growth retardation is a characteristic feature of *Mecp2*$^{-/y}$ pathology, which leads to lower body and brain weight. We found that acupoint EAS also increased the body and brain weight of *Mecp2*$^{-/y}$ rats (Figs. 1(d) and (e)). At 30 days of age, there was no difference between WT rats ((106.25 ± 17.65) g) and *Mecp2*$^{-/y}$ rats ((105.33 ± 19.50) g) in terms of body weight. At 60 days of age, the body weight of the *Mecp2*$^{-/y}$ rats ((205.7 ± 78.6) g) increased more slowly than that of the WT rats ((309.6 ± 39.6) g), and the body weight of the EAS-treated *Mecp2*$^{-/y}$ rats ((276.3 ± 78.6) g) was obviously higher than that of the *Mecp2*$^{-/y}$ rats. At 120 days of age, the body weight of the *Mecp2*$^{-/y}$ rats ((225.0 ± 42.5) g) was less than half that of the WT rats ((480.3 ± 46.7) g), and there was a significant difference between the WT and *Mecp2*$^{-/y}$ groups ($p < 0.01$). However, the body weight of the EAS-treated *Mecp2*$^{-/y}$ rats increased nearly 60% compared with that of the *Mecp2*$^{-/y}$ rats, suggesting that EAS improves the general growth of *Mecp2*$^{-/y}$ rats.

Next, we examined the effects of EAS on locomotor activity by means of an open-field test and those on motor coordination ability by means of a rotarod test in *Mecp2*$^{-/y}$ rats. *Mecp2*$^{-/y}$ and WT rats were subjected to a 10 min open-field test, and their movement traces were recorded by infrared camera. Rats with anxiety-like behavior spend less time exploring the center of the chamber and travel shorter distances [26]. As shown in Figs. 2(a)–(d), the *Mecp2*$^{-/y}$ rats traveled a shorter distance in the test ((1363.0 ± 447.6) cm) than the WT rats ((5482.0 ± 214.1) cm; Figs. 2(a) and (b)). Simultaneously, the *Mecp2*$^{-/y}$ rats spent a shorter time ((5.78 ± 2.27) s) in the center and exhibited a smaller center/total ratio (Figs. 2(c) and (d)) than the WT rats ((29.32 ± 5.99) s), suggesting decreased motor ability and anxiety-like behavior in the *Mecp2*$^{-/y}$ rats. The EAS-treated *Mecp2*$^{-/y}$ rats traveled a longer distance ((2989.0 ± 668.1) cm) in the open-field test and spent more time ((17.15 ± 13.04) s) in the center than the *Mecp2*$^{-/y}$ rats. Thus, EAS improved the locomotor activity of the *Mecp2*$^{-/y}$ rats, as shown by its effect of increasing the total distance traveled, and especially increasing the distance and time spent in the central region.

We further tested the effect of EAS on the coordination of motor ability. *Mecp2*$^{-/y}$ and WT rats were placed on the rotating cylinder of an accelerating rotarod apparatus (5–40 r·min^{-1}), and the latency to fall was recorded according to the procedure in Ref.

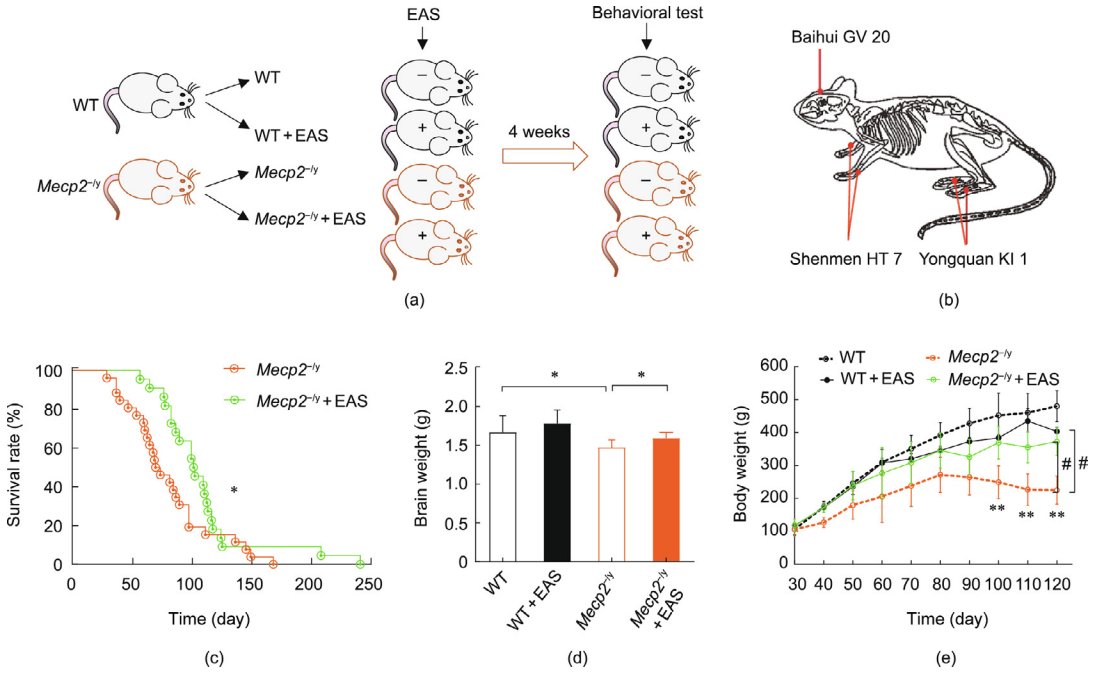

Fig. 1. EAS increased the lifespan and body weight of *Mecp2*$^{-/y}$ rats. (a) Experimental design and procedure; (b) selection and location of acupoints; (c) lifespan of *Mecp2*$^{-/y}$ rats (n = 26; median survival time: 71 days) and that of *Mecp2*$^{-/y}$ rats receiving EAS (n = 22; median survival time: 102 days) (long-rank test, $^{*}p < 0.05$, $^{**}p < 0.01$); (d) brain weight (WT, n = 10; WT + EAS, n = 10; *Mecp2*$^{-/y}$, n = 9; *Mecp2*$^{-/y}$ + EAS, n = 10), one-way analysis of variance (ANOVA; Bonferroni's *post hoc*) test ($^{*}p < 0.05$, $^{**}p < 0.01$); (e) body weights (mean ± standard error of the mean (SEM); $^{*}p < 0.05$, $^{**}p < 0.01$ compared with the WT group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the *Mecp2*$^{-/y}$ group).

[27]. As shown in Fig. 2(e), we found that the latency to fall from the rod for *Mecp2*$^{-/y}$ rats ((48.80 ± 11.89) s) was reduced to about a third of that in WT rats ((149.7 ± 15.93) s). The latency of the EAS-treated *Mecp2*$^{-/y}$ rats ((108.8 ± 19.54) s) was more than twice that of the *Mecp2*$^{-/y}$ rats. Moreover, the latency of the EAS-treated WT rats ((238.7 ± 20.59) s) significantly increased compared with that of the WT rats. Therefore, EAS also improved the motor coordination of the *Mecp2*$^{-/y}$ rats.

A three-chamber interaction test was performed to determine whether EAS would improve the social behavior of the *Mecp2*$^{-/y}$ rats [22,28]. In the habituation stage, WT or *Mecp2*$^{-/y}$ rats were placed in the middle chamber of the three-chamber apparatus, and two barred cages were equipped in the corners of the left and right chambers (Fig. 3(a)). The mice were allowed to explore freely for 10 min. Next, a strange rat was placed in the right barred cage, while the left barred cage remained empty. The time spent in each chamber and the sniffing frequency was recorded. As shown in Figs. 3(b)–(d), the WT rats spent more time in the chamber containing an unfamiliar rat ((366.6 ± 36.5) s) than in the empty side ((47.4 ± 13.1) s). The *Mecp2*$^{-/y}$ rats spent more time in the middle chamber ((384.2 ± 90.1) s) or the empty barred cage ((158.2 ± 52.6) s), whereas the time they spent in the chamber with the unfamiliar rat ((160.9 ± 48.8) s) was much shorter than that of the WT rats. The sniffing frequency of the *Mecp2*$^{-/y}$ rats (3.8 ± 2.4) with the unfamiliar rat was much less than that of the WT rats (29.6 ± 4.5, $p < 0.05$). EAS increased the interaction time of *Mecp2*$^{-/y}$ rats ((358.3 ± 78.0) s, $p = 0.07$) and their interaction frequency (10.0 ± 3.1, $p = 0.08$) with the unfamiliar rat. These results show that EAS can improve the social ability of *Mecp2*$^{-/y}$ rats. However, EAS did not promote the preference for social novelty of the *Mecp2*$^{-/y}$ rats (Figs. S2(b) and (c) in Appendix A).

Some neurotransmitter systems in the central nervous system play a central role in modulating mammalian social behavior. For example, AVP and OXT are closely related to the social behavior and cognition of patients with autistic spectrum disorders [24,29,30]. In a previous study, lower levels of AVP and OXT were exhibited in autistic children [29,30]. Other studies have shown that high plasma levels of OXT can cause anxiety and social phobia [31]. In the *Mecp2*$^{-/y}$ rats, we found a higher level of OXT in the *Mecp2*$^{-/y}$ rats ((36.68 ± 4.15) pg·mL^{-1}) than in the WT rats ((23.51 ± 3.82) pg·mL^{-1}; $p < 0.05$). The serum OXT level of the EAS-treated *Mecp2*$^{-/y}$ rats ((23.25 ± 3.56) pg·mL^{-1}; $p < 0.05$) was clearly lower than that of the *Mecp2*$^{-/y}$ rats (Fig. 3(e)). There were no obvious differences in the AVP levels of the WT rats and *Mecp2*$^{-/y}$ rats, but EAS significantly decreased the AVP level of the WT rats instead of that of the *Mecp2*$^{-/y}$ rats (Fig. 3(f)).

To investigate whether EAS would improve memory, we subjected the rats to a fear-conditioning paradigm to test their fear memory. EAS slightly improved contextual fear memory (3 h: WT, 21.41% ± 6.04%; WT + EAS, 24.65% ± 5.94%; *Mecp2*$^{-/y}$, 9.96% ± 4.92%; *Mecp2*$^{-/y}$ + EAS, 16.71% ± 8.11%) and cued fear memory (3 h: WT, 28.03% ± 7.45%; WT + EAS, 33.08% ± 7.24%; *Mecp2*$^{-/y}$, 60.77% ± 7.85%; *Mecp2*$^{-/y}$ + EAS, 69.39% ± 11.17%) in the WT and *Mecp2*$^{-/y}$ rats, but there was no significant statistical difference between the treated and untreated groups (Figs. 4(a)–(c)). We further investigated the neurogenesis by counting the positive cells for BrdU (to mark newborn cells) and DCX (to mark immature neurons). There was no obvious change in the number of BrdU^{+} and DCX^{+} cells between the WT and WT + EAS or the *Mecp2*$^{-/y}$ and *Mecp2*$^{-/y}$ + EAS groups (Figs. 4(d)–(f) and Fig. S3 in Appendix A), suggesting that EAS did not stimulate the hippocampal neurogenesis of the *Mecp2*$^{-/y}$ rats.

In order to understand how EAS improved social behavior and motor function, we detected the expression of c-Fos protein to monitor the brain activation. The c-Fos protein is a sensitive marker of neuronal activation, since its expression generally responds to various stimuli, including physical, chemical, and nonpathogenic environmental conditions [32–35]. We assessed the brain activation by quantifying the c-Fos^{+} cells of the immunofluorescence images (Figs. 5(a) and (b) and Figs. S4(a)–(d) in Appendix A).

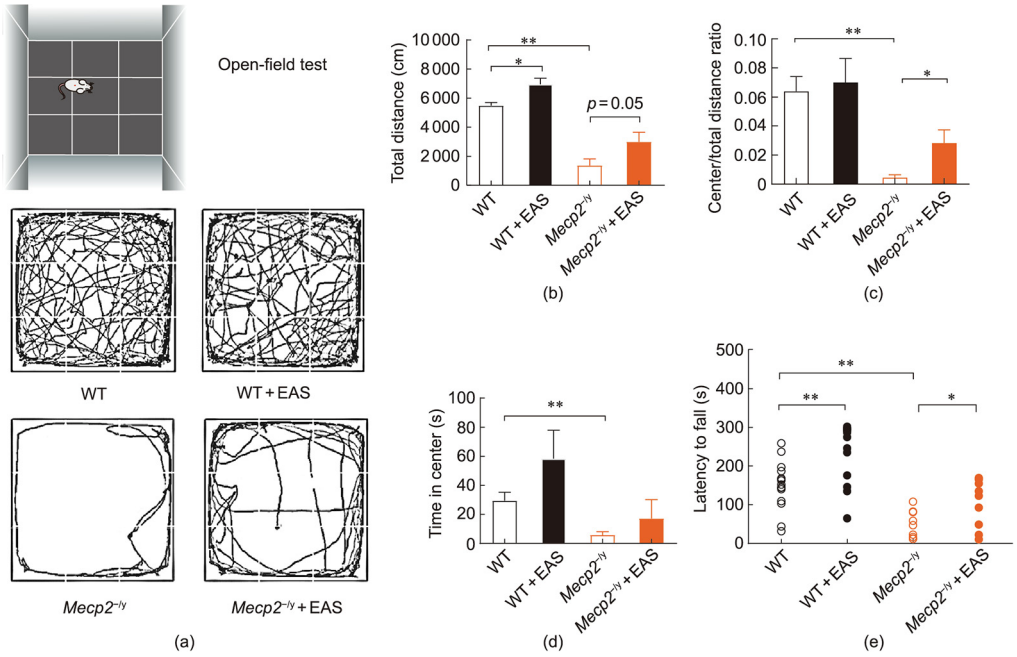

Fig. 2. EAS improved the locomotion and motor coordination of *Mecp2*$^{-/y}$ rats. (a) Representative traces of activity in the open-field test; (b) total distance traveled in the open-field chamber; (c) center-to-total distance ratio in the open-field chamber; (d) time spent in the center of the open-field chamber; (e) latency to fall in the rotarod test. All data are presented as mean ± SEM. WT, $n = 10$; WT + EAS, $n = 10$; *Mecp2*$^{-/y}$, $n = 9$; *Mecp2*$^{-/y}$ + EAS, $n = 10$; one-way ANOVA (Bonferroni's *post hoc*) test, $^{*}p < 0.05$, $^{**}p < 0.01$.

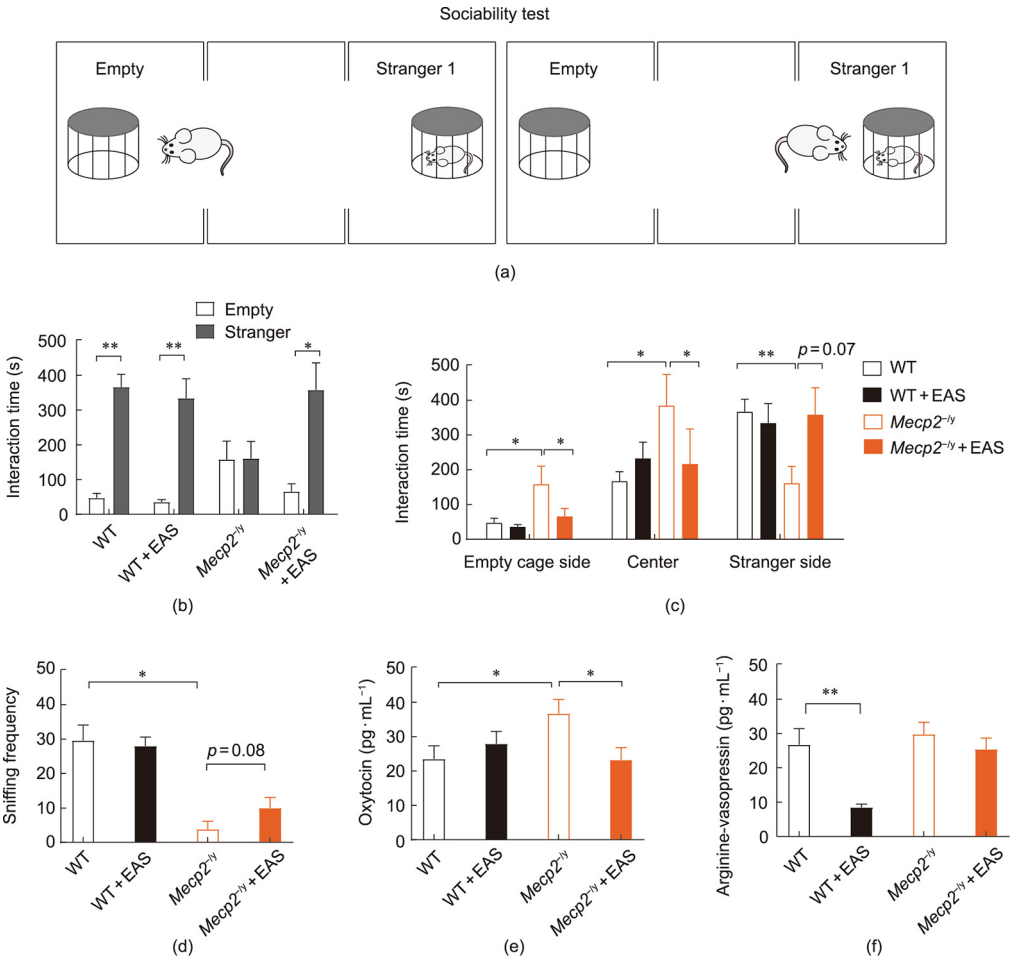

Fig. 3. EAS improved the social interaction behavior of *Mecp2*$^{-/y}$ rats. (a) Schematic diagram of the three-chamber apparatus test; (b) interaction time with the empty side and the strange rat side ($^{*}p < 0.05$, $^{**}p < 0.01$; two-tailed *t*-test); (c) interaction time in a different chamber of the three-chamber test; (d) sniffing frequency with the strange rat (Kruskal–Wallis test, $^{*}p < 0.05$, $^{**}p < 0.01$); (e) oxytocin level in serum; (f) arginine-vasopressin level in serum. (c, e, f) One-way ANOVA (Bonferroni's *post hoc*) test ($^{*}p < 0.05$, $^{**}p < 0.01$). All data are presented as mean ± SEM. WT, $n = 10$; WT + EAS, $n = 10$; *Mecp2*$^{-/y}$, $n = 9$; *Mecp2*$^{-/y}$ + EAS, $n = 10$.

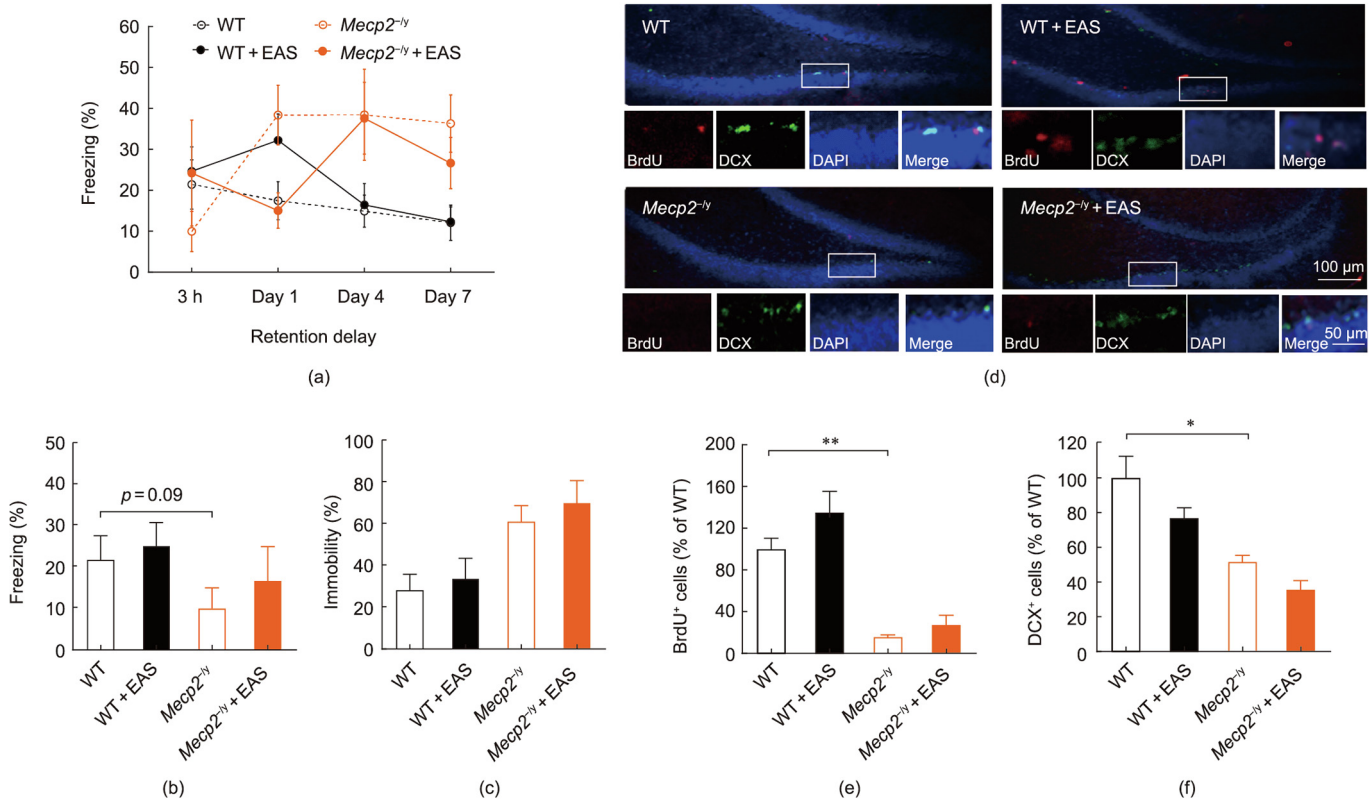

Fig. 4. EAS did not improve hippocampal neurogenesis or alter cued fear in WT and *Mecp2*$^{-/y}$ rats. (a) Effect of EAS on fear memory in our groups at different times; (b) contextual fear memory at 3 h; (c) cued fear memory at 3 h; (d) immunofluorescence images of BrdU and DCX cells at low magnification (top; scale bar, 100 μm) and high magnification (bottom; scale bar, 50 μm), showing BrdU$^+$ cells (red), DCX$^+$ cells (green), and the merging (yellow) of each group; (e) numbers of BrdU$^+$ cells; (f) numbers of DCX$^+$ cells. All data are presented as mean ± SEM. (a–c) WT, $n = 10$; WT + EAS, $n = 10$; *Mecp2*$^{-/y}$, $n = 9$; *Mecp2*$^{-/y}$ + EAS, $n = 10$. (d–f) $n = 6$. One-way ANOVA (Bonferroni's *post hoc*) test, $^*p < 0.05$, $^{**}p < 0.01$.

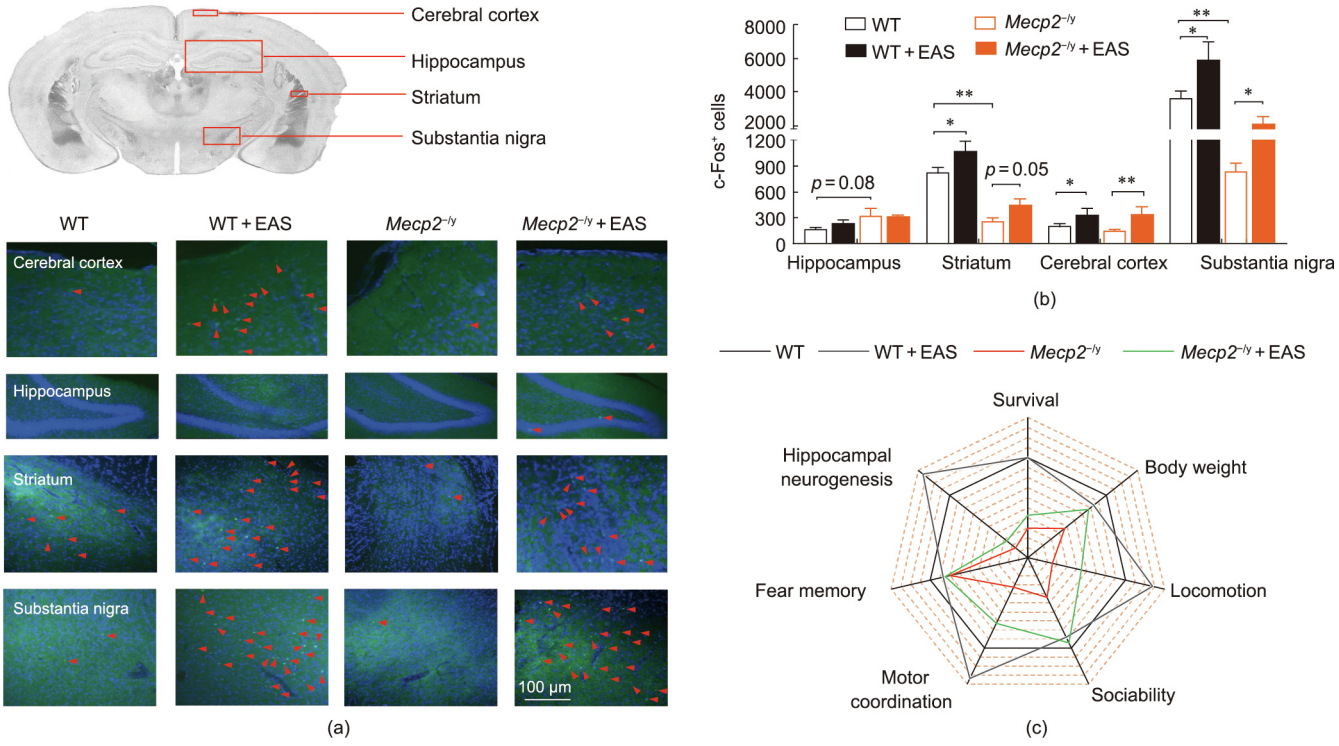

Fig. 5. EAS-induced neuronal activity in the cerebral cortex, hippocampus, corpus striatum, and substantia nigra. (a) Immunofluorescence image of c-Fos in the cerebral cortex, hippocampus, corpus striatum, and substantia nigra; (b) c-Fos$^+$ cell counts in the hippocampus, corpus striatum, cerebral cortex, and substantia nigra; (c) behavioral ability plot. All data are presented as mean ± SEM. $n = 6$, one-way ANOVA (Bonferroni's *post hoc*) test, $^*p < 0.05$, $^{**}p < 0.01$.

Comparing the WT and *Mecp2*$^{-/y}$ rats (WT, 209.7 ± 27.1; *Mecp2*$^{-/y}$, 154.7 ± 18.2), the numbers of c-Fos^{+} cells of EAS-treated WT and EAS-treated *Mecp2*$^{-/y}$ rats were significantly increased in the cerebral cortex (WT + EAS, 336.7 ± 78.9; *Mecp2*$^{-/y}$ + EAS, 336.3 ± 96.2). In the corpus striatum region, there was a significant difference in the number of c-Fos^{+} cells between EAS-treated (WT + EAS, 1070.0 ± 113.1; *Mecp2*$^{-/y}$ + EAS, 444.80 ± 73.18) and EAS-untreated (WT, 825.7 ± 51.2; *Mecp2*$^{-/y}$, 273.20 ± 34.09) WT or *Mecp2*$^{-/y}$ rats. Similar results were also found in the substantia nigra of the midbrain (WT, 3551.0 ± 408.7; WT + EAS, 5818 ± 1044; *Mecp2*$^{-/y}$, 839.5 ± 93.1; *Mecp2*$^{-/y}$ + EAS, 2021.0 ± 443.8). These results suggested that EAS can promote brain activation in the cerebral cortex, corpus striatum, and substantia nigra of the midbrain. In the hippocampus region, however, EAS did not lead to an increase in the number of c-Fos^{+} cells (WT, 177.0 ± 17.6; WT + EAS, 244.7 ± 28.3; *Mecp2*$^{-/y}$, 329.00 ± 78.85; *Mecp2*$^{-/y}$ + EAS, 323.3 ± 10.9), implying that the hippocampus did not respond to EAS as a main functional area of memory and no significant memory improvement occurred.

Rett syndrome is a progressive neurodevelopmental disorder in children with an array of symptoms including language retrogression, growth retardation, autonomic dysfunctions, ataxia, severe social skills impairment, and stereotypical hand movements [1]. Mice and rats with mutation in the *Mecp2* gene exhibit the characteristic symptoms of Rett syndrome [21,36–38]. In this study, we investigated the effect of EAS acupuncture on *Mecp2*$^{-/y}$ rats. We found that EAS at specific acupoints (HT 7, KI 1, and GV 20) significantly improved the locomotion, motor coordination, and social behavior of *Mecp2*$^{-/y}$ rats (Fig. 5(c)). In particular, the median survival time of *Mecp2*$^{-/y}$ rats was extended by 44%. Lack of MeCP2 in rats and mice leads to a significantly shorter lifespan [3,18], which was also observed in our experiments. The median survival time of *Mecp2*$^{-/y}$ rats was only 71 days. However, after treatment with EAS, the median survival time of EAS rats was greatly improved to 102 days, and the longest survival time was 240 days, indicating that EAS did indeed exert positive effects on a rat model of Rett syndrome. EAS provides a safe and effective method for the treatment of Rett syndrome, without the need to consider surgery or the blood–brain barrier.

EAS is a type of electrical stimulation that is performed by inserting a needle into an acupoint that is far from the central nervous system (i.e., the brain and spinal cord). Stimulus at some acupoints can be effectively transmitted over a long distance within the body and targets specific regions of the brain. Unlike DBS, EAS is a very safe way to treat brain disease, because there is no direct injury to the brain. Furthermore, the remote operation of EAS greatly reduces false probability from an inaccurate operation, making the popularization of EAS possible. Most importantly, the significant improvements it brings to both behavioral ability and lifespan make EAS an efficient means of Rett syndrome therapy. We found that EAS improved motor function and social behavior by activating the related brain regions. c-Fos can be used as a sensitive marker of neuronal activation, since its expression generally responds to various stimuli including physical, chemical, and nonpathogenic environmental conditions [32–35]. EAS increased the activation of the brain, including the cerebral cortex, corpus striatum, and substantia nigra of the midbrain, of the WT and *Mecp2*$^{-/y}$ rats—regions that are related to motor and social behaviors.

However, it is a great challenge to use a single therapy strategy to alleviate all the symptom of Rett syndrome, and we found that the effect of EAS on fear memory is not obvious. Poor activation of the hippocampus region and a low increase in the c-Fos expression level in the hippocampus region were responsible for EAS's poor improvement to intelligence. It is well known that the hippocampus is the main functional area of the memory, while the corpus striatum and substantia nigra are the main functional areas of motion [24,35]. This is a good explanation for why EAS improved social and motor skills instead of memory. In a previous study, forniceal DBS in Rett mice, which was performed by implanting DBS electrodes in the fimbria–fornix, was shown to rescue contextual fear memory and spatial memory; however, it did not improve other behavior abilities, such as locomotion, motor coordination, social behavior, and body weight in Rett mice [5]. According to the characteristics of DBS and EAS, it is possible that DBS could be combined with EAS to improve the social, motor, and memory abilities of rats with Rett syndrome. In addition, more acupoints are worth trying for significant improvement of Rett pathology in further studies.

4. Conclusions and perspectives

In summary, EAS at specific acupoints (HT 7, KI 1, and GV 20) significantly improved the locomotion, motor coordination, and social behavior of *Mecp2*$^{-/y}$ rats. EAS increased the activation of the brain, including the cerebral cortex, corpus striatum, and substantia nigra of the midbrain of the WT and *Mecp2*$^{-/y}$ rats—regions that are related to motor and social behavior. EAS provides a safe and effective method for the treatment of *Mecp2*$^{-/y}$ that is free of surgery. We also envision that the combination of EAS with other therapies (e.g., DBS and DMS) may further improve the social, motor, and memory abilities of rats with Rett syndrome.

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Authors' contributions

Zilong Qiu, Jun Hu, and Chunhai Fan conceptualized and directed the research; Yanhong Sun, Lihua Wang, Zilong Qiu, and Chunhai Fan designed the study. Yi Xu, Yanhong Sun, Zhifang Chen, and Yuefang Zhang performed the experiments. Chenglie Lin, Zhilei Ge, Fangfei Zhao, Xinyi Liu, and Meiling Yan participated in the experiments. Meiling Yan, Fangfei Zhao, Jimin Gao, Ying Zhu, Hongyi Li, and Yanhong Sun performed the data analysis for the study. Yanhong Sun, Lihua Wang, Zilong Qiu, and Chunhai Fan wrote the paper.

Compliance with ethics guidelines

Yanhong Sun, Zhifang Chen, Yi Xu, Yuefang Zhang, Zhilei Ge, Chenglie Lin, Yi Zhou, Fangfei Zhao, Meiling Yan, Xinyi Liu, Ying Zhu, Jimin Gao, Hongyi Li, Lihua Wang, Jun Hu, Zilong Qiu, and Chunhai Fan declare that they have no conflict of interest or financial conflicts to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.eng.2020.06.032.

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