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Electroconvulsive seizures protect against methamphetamine-induced inhibition of neurogenesis in the rat hippocampus

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ABSTRACT

Following methamphetamine consumption and during abstinence many behavioral consequences emerge (i.e., cognitive deficits, ongoing episodes of psychosis, depression, severe cravings, brain neurotoxicity), which are likely linked to propensity to relapse. In this line of thought, we recently showed that binge methamphetamine administration enhanced negative affect and voluntary drug consumption in rats, while it induced persistent neurotoxic effects (i.e., impaired hippocampal neurogenesis), effects that emerged long after drug removal. To date, no pharmacological strategies have been proven to be effective for the treatment of methamphetamine toxicity. A few studies have evaluated the impact of combining methamphetamine pretreatment with electroconvulsive seizures (ECS) post-treatment, an alternative non-pharmacological option used in psychiatry for resistant depression that offers a safe and really potent therapeutic response. Against this background, the present study aimed at testing whether repeated ECS treatment could ameliorate some of the long-term neurotoxicity effects induced by adolescent methamphetamine exposure in rats and emerging after drug removal. At the behavioral level, the main results showed that methamphetamine administration did not alter negative affect immediate during adolescence or later on in adulthood. Interestingly, repeated ECS improved the negative impact of methamphetamine administration on reducing hippocampal neurogenesis, demonstrating that ECS can attenuate certain degree of methamphetamine-induced neurotoxicity in rats, and suggesting ECS as a good therapeutical candidate that deserves further studies.

1. Introduction

Methamphetamine is not only the synthetic stimulant most consumed at world level (UNODC, 2018, 2019), but its use showed an upward trend in the last National Survey on Drug Use and Health (SAMHSA, 2020). Methamphetamine is typically self-administered in a binge manner (i.e., frequent administrations in short periods of time; UNODC, 2018) and early age drug initiation represents a vulnerability factor for how it will impact brain function and behavior (Spear, 2007). In fact, an early methamphetamine initiation during adolescence and/or young adulthood is crucial for its long-term neurotoxic outcome (Brunell and Spear, 2006; Teixeira-Gomes et al., 2015). During methamphetamine dependence and/or abstinence many behavioral consequences emerge, such as cognitive deficits, ongoing episodes of psychosis, depression, severe cravings, brain neurotoxicity, etc. (e.g., Rogers et al., 2008; Karila et al., 2010). This negative impact on brain and behavior is likely linked to propensity to relapse (reviewed in Moszczynska and Callan, 2017). In this line of thought, we recently showed that binge methamphetamine administration enhanced negative affect and voluntary drug consumption in rats following prolonged forced abstinence (García-Cabrerizo and García-Fuster, 2019a). The neurotoxic effects of methamphetamine in experimental animals are particularly damaging in certain brain regions, such as the observed reduced hippocampal volume paired with cell death (Thompson et al., 2004; Hori et al., 2010; Thanos et al., 2016), and/or the described impaired hippocampal neurogenesis (reviewed in Eisch and Harburg, 2006; Mandyam and Koob, 2012). In this context, recent data from our group demonstrated that adolescent methamphetamine binge administration in rats induced hippocampal cell damage (i.e., neurotoxic effects) immediately after drug exposure during adolescence (i.e., inhibited cell proliferation; García-Cabrerizo and García-Fuster, 2016), and in adulthood long after drug removal (i.e., decreased cell survival – over 40 days old cells;

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García-Cabrerizo et al., 2018).

Up to date, no pharmacological strategies have been proven to be effective for the treatment of methamphetamine dependence (reviewed in Karila et al., 2010 and Siefried et al., 2020, showing no real progress in the past 10 years). Nevertheless, some novel therapeutical approaches are at an early stage of proving certain benefits in treating methamphetamine use and deserve further investigation in larger scale studies (e.g., agonist therapies; see further details in Siefried et al., 2020). Interestingly, a few studies have evaluated the impact of combining methamphetamine pre-treatment with electroconvulsive seizures (ECS) post-treatment, an alternative non-pharmacological option used in psychiatry for resistant depression that offers a safe and really potent therapeutic response (UK ECT Review Group, 2003), that could be easily and reliably modeled in rodents (see for example prior data demonstrating an antidepressant-like potential of ECS in male rats; Li et al., 2007; Kyeremanteng et al., 2014; García-Cabrerizo et al., 2020). One study evaluated several behavioral phenotyping assays in mice (Chao et al., 2012), while others were performed in clinical situations; interestingly, they all suggested that ECS could improve negative behavioral symptoms associated with methamphetamine consumption (Grelotti et al., 2010; Roshanaei-Moghaddam and Pauly, 2014; Ziaaddini et al., 2015; Ahmadi et al., 2015). Against this background, and since ECS is capable of inducing potent antidepressant-like effects along with vast increases in hippocampal neurogenesis (e.g., García-Cabrerizo et al., 2020), the present study aimed at testing whether repeated ECS treatment could improve some of the neurotoxic consequences induced by methamphetamine administration in rats.

2. Material and methods

2.1. Animals

This study used 40 Sprague-Dawley male rats bred in the animal facility (University of the Balearic Islands, Spain) and housed under specific environmental settings (22 °C, 70 % humidity, 12 h light/dark cycle, lights on at 8:00 AM) in standard cages with *ad libitum* food (standard diet) and tap water. All experimental procedures followed the ARRIVE Guidelines (Percie du Sert et al., 2020), and complied with the European Communities Council Directive 86/609/EEC (Directive 2010/63/EU and Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research, National Research Council 2003), as well as the Spanish Guidelines (Royal Decree 53/2013). All procedures were performed during the light period (between 8:30 and 13:00 h).

2.2. Experimental procedures

All rats received 5-bromo-2'-deoxyuridine (BrdU, 2 × 50 mg/kg, one pulse every 12 h, i.p.; Calbiochem, USA) for 3 days (PND 48-50; see Fig. 1a) as described before (García-Fuster et al., 2010; García-Cabrerizo et al., 2018). Rats were then randomly allocated in two groups (n = 20 each) that were treated with (+)-methamphetamine hydrochloride (M, 5 mg/kg, i.p.; Sigma-Aldrich, MO, USA) or saline (C, 0.9 % NaCl,1 mL/kg, i.p.) following a binge paradigm (3x pulses per day separated 3 h) for 4 days (PND 54-57; Fig. 1a). The age of administration (PND 54-57) and the dose of methamphetamine (pulses of 5 mg/kg) were selected based on prior studies from our group showing immediate (García-Cabrerizo and García-Fuster, 2016) and enduring (García-Cabrerizo et al., 2018; García-Cabrerizo and García-Fuster, 2019a) changes in brain neurochemistry and/or neurotoxicity (i.e., diminished hippocampal neurogenesis), as well as by other studies with similar drug schedules (reviewed in Teixeira-Gomes et al., 2015; Moszczynska and Callan, 2017). Weights were monitored every day throughout the course of treatment as detailed in Fig. 1. To corroborate the expected effects of binge methamphetamine on increasing core body temperature (García-Cabrerizo and García-Fuster, 2016; García-Cabrerizo et al., 2018), rectal temperature was measured every treatment day 30-min after the last daily pulse of methamphetamine (PND 54-57; Fig. 1a).

Potential changes in affective-like behavior were assessed by two consecutive tests (i.e., forced-swim, novelty-suppressed feeding) as described earlier (García-Cabrerizo and García-Fuster, 2019a,b; García-Cabrerizo et al., 2020). To evaluate the possible immediate negative effects of drug treatment on behavioral despair rats were exposed to the forced-swim test – a 15 min pre-test on PND 58 followed by a 5 min test on PND 59 (Fig. 1a). Following standard protocols (Slattery and Cryan, 2017), rats were placed in individual tanks (41 cm high x 32 cm diameter) filled with water (25 ± 1 °C, 25 cm depth) for the duration of the test (for further details see García-Cabrerizo et al., 2015; Bis-Humbert et al., 2020). Test sessions on PND 59 were videotaped and blindly coded for posterior behavioral analysis (i.e., immobility time as measured with Behavioral Tracker software, CA, USA).

Two-days later, on PND 61, rats were exposed to the novelty-suppressed feeding test that measures changes in affective-like responses in an anxiogenic-like environment following 48 h of food deprivation (Bodnoff et al., 1988). This is a 5 min test performed in a wall-enclosed square arena (60 × 60 cm × 40 cm of height), in which three food pellets are placed in the center of the arena (see Bis-Humbert et al., 2021 for the same test protocol). As mentioned, and since motivation for food is needed, rats were food deprived for 48 h prior to

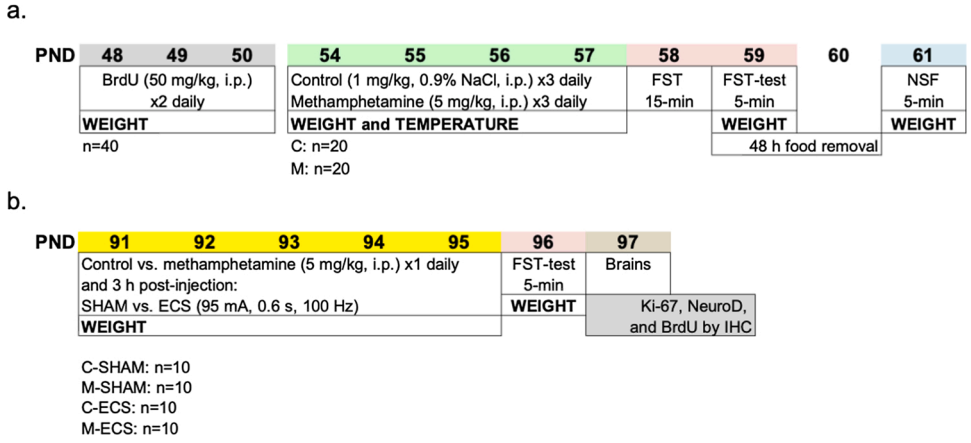

Fig. 1. Experimental timeline. a Male Sprague-Dawley rats were exposed to the procedures described at the indicated postnatal days (PND). Adolescent rats were treated with BrdU (2 pulses of 50 mg/kg, i.p., 3 days, PND 48-50) and then with a binge paradigm (3 pulses per day, i.p., every 3 h, for 4 days, PND 54-57) of methamphetamine (5 mg/kg, n = 20, M) or saline (0.9 % NaCl, 1 mL/kg, n = 20, C). Rats were then exposed to the forced-swim test (FST) on PND 58 (15 min pre-test) and on PND 59 (5 min test), and then food deprived for 48 h prior to exposure to the novelty-suppressed feeding (NSF) test on PND 61. **b** In adulthood, and following prolonged abstinence, rats were re-exposed to methamphetamine (5 mg/kg) or saline for 5 days (1 injection per day, PND 91-95) followed by a daily post-treatment (3 h later) with ECS (95 mA, 0.6 s, 100 Hz; C-ECS, n = 10 and M-ECS, n = 10) or SHAM (electrodes with no current applied; C-SHAM, n = 10 and M-SHAM, n = 10). Finally, rats were tested in the FST on PND 96, killed 24 h later on PND 97, and their brains removed and dissected for further neurogenesis analysis (Ki-67, NeuroD, BrdU) by immunohistochemistry.

testing (see Bodnoff et al., 1988) and their weight was monitored before and after food removal (PND 58 and 61; see details in Fig. 1a). Test sessions were videotaped to later blindly score latency to center (sec) and feeding time (sec) (Bis-Humbert et al., 2021).

Subsequently, rats were left undisturbed for 34 days (i.e., drug forced removal) since the last methamphetamine administration on PND 57 (see Fig. 1b). This was chosen based on earlier experiments from our group showing that waiting at least 4 weeks after drug exposure led to enduring hippocampal cell damage (García-Cabrerizo et al., 2018), increased negative affect and increased voluntary methamphetamine consumption (García-Cabrerizo and García-Fuster, 2019a), in line with the notion that the delayed neurobiological effects emerging during persistent drug removal could increase relapse susceptibility. In this context, our goal was to ascertain whether repeated ECS could improve some of the negative consequences emerging during methamphetamine abstinence and/or following drug re-exposure as reported in some of our prior work (see for example García-Cabrerizo et al., 2018; García-Cabrerizo and García-Fuster, 2019a). To do so, on PND 91 rats were challenged with a daily dose of methamphetamine (5 mg/kg, i.p.) or saline (C, 0.9 % NaCl,1 mL/kg, i.p.) for 5 consecutive days; according to what they received in late adolescence. Every day (PND 91-95; 3 h post-injection, see Fig. 1b), rats were treated with a daily single ECS session (i.e., inducing the characteristic tonic and clonic convulsions; M-SHAM, n = 10) which was administered via earclip electrodes using 95 mA for 0.6 s at a frequency of 100 Hz square wave pulses (pulse width 0.6 ms) through a pulse generator (ECT Unit 7801; Ugo Basile, Italy) as previously described (García-Fuster and García-Sevilla, 2016; García--Cabrerizo et al., 2020). Control rats were connected to earclip electrodes with no electrical current (C-SHAM, n = 10). Although other alternative dosing parameters for the seizure dosing could potentially be more effective than the ones used here, the exact same repeated ECS parameters proved, in our hands, potent antidepressant-like responses in rodents, as well as increases in some early markers of hippocampal neurogenesis (e.g., García-Cabrerizo et al., 2020), effects that were not observed following a single ECS exposure (García-Cabrerizo et al., 2017). Rats were then scored in the FST on PND 96 (5 min test as detailed above) to evaluate the possible emergence of negative affect and/or the potential antidepressant-like effects induced by ECS (i.e., highest antidepressant-like efficacy observed 1-day post-ECS treatment; see García-Cabrerizo et al., 2020).

2.3. Hippocampal neurogenesis

Rats were sacrificed by rapid decapitation on PND 97 (Fig. 1b) and the left half-brain was quickly frozen in −30 °C isopentane and stored at −80 °C to later evaluate hippocampal neurogenesis by immunohistochemistry at different stages of regulation (i.e., Ki-67 for recent cell proliferation, NeuroD for early neuronal survival, and BrdU for long-term survival of labelled cells; 47–49 days of age). Tissue was cryostat-cut (30 μm sections) and slide-mounted throughout the whole extent of the hippocampus (−1.72 to −6.80 mm from Bregma) as described in detail before (García-Fuster et al., 2010; García-Cabrerizo and García-Fuster, 2016; García-Cabrerizo et al., 2018, 2020). For each marker, experiments were performed in 3 slides containing every 8th section from the anterior, middle and posterior part of the hippocampus respectively (i.e., 8 tissue-sections per slide, and for a total of 24 sections per animal). Tissue was post-fixed in 4% paraformaldehyde and following several steps (e.g., antigen retrieval, blocking in peroxidase solution and BSA) it was incubated overnight with the corresponding primary polyclonal antibody: rabbit anti-Ki-67 (1:40000) and rabbit anti-BrdU (1:40000) (both kindly provided by Drs. Huda Akil and Stanley J. Watson, University of Michigan, MI, USA), and goat anti-NeuroD (1:25000; Santa Cruz Biotechnology, CA, USA). Afterwards, sections were sequentially incubated with the appropriate secondary antibody (biotinylated anti-rabbit or anti-goat, 1:1000, Vector Laboratories, CA, USA), an Avidin/Biotin complex (Vectastain Elite ABC kit; Vector Laboratories), and the chromogen 3,3′-diaminobenzidine (DAB) (with nickel chloride for NeuroD) for signal detection. Tissue for Ki-67 and BrdU labeling was counterstained with cresyl violet. The final steps included tissue dehydration in graded alcohols, immersion in xylene and cover-slipping with Permount®. Positive cells for each animal were blinded quantified with a Leica DMR light microscope (63x objective lens) following a modified unbiased stereological procedure (Malberg and Duman, 2003) that counts every 8th section throughout the entire extent of the hippocampus and multiplies the resulting sum by the sampling factor 8 to provide an estimate of the total number of positive cells per marker and rat (see our prior publications following the same procedure in the past 10 years; e.g., García-Fuster et al., 2010; García-Cabrerizo et al., 2020).

2.4. Data analysis and data availability statement

All data analysis was done with GraphPad Prism, Version 9 (GraphPad Software, Inc., CA, USA) and followed the guidelines for displaying data and statistical methods in experimental pharmacology (Michel et al., 2020). Results are reported as mean values ± standard error of the mean (SEM), and individual symbols for each rat are shown within bar graphs. One-, two- or three-way ANOVA, with repeated measures (RM) and followed by Sidak's multiple comparisons test (when appropriate), or Student's *t*-tests were used for statistical comparisons. The level of significance was set at $p \leq 0.05$. The data that supports the findings of this study are available from the corresponding author upon reasonable request.

3. Results

3.1. Body weight and core temperature

While BrdU pre-treatment (PND 48-50) did not induce changes in body weight (lack of treatment x day interaction: $F_{2,38} = 0.32$, $p = 0.728$; two-way ANOVA Fig. 2a), binge methamphetamine treatment (PND 54-57) induced changes in body weight during adolescence as observed by a significant treatment x day interaction ($F_{3,57} = 15.22$, $p < 0.001$; two-way ANOVA). In particular, adolescent rats treated with methamphetamine showed reduced normal weight gain from the second day of treatment (PND 55: −9 g, ***$p < 0.001$; PND 56: −5 g, ***$p < 0.001$ and PND 57: −4 g, ***$p < 0.001$ vs. control rats; Fig. 2a). Then, all rats were food deprived for 48 h (to exhibit motivation for food in the novelty-suppressed feeding test), which caused an overall drop in body weight (about -30 to −32 g less when comparing PND 61 vs. PND 59; effect of Day: $F_{1,38} = 412.2$, $p < 0.001$; two-way ANOVA; significance not represented, see Fig. 2a). Following prolonged abstinence, rats were weighted on PND 91 prior to any drug treatment in adulthood and the results showed an increase in body weight in rats with a prior history of adolescent methamphetamine exposure (+23 g, $t = 2.27$, $df = 19$, *$p <$ 0.05 vs. C-treated rats in adolescence). Finally, rats re-exposed to methamphetamine in adulthood with or without a daily post-treatment with ECS were weighted from PND 92 to PND 95; although a three-way RM ANOVA did not show a significant treatment x age x post-treatment interaction ($F_{3,108} = 0.60$, $p = 0.618$, Fig. 2a), there was a significant effect of treatment ($F_{1,36} = 32.06$, $p < 0.001$), manifested as an overall lower body weight in rats treated with methamphetamine (SHAM and ECS groups) that showed significance on PND 92 for the SHAM group (−9 g, *$p < 0.05$ vs. control rats; Fig. 2a).

Core body temperature was measured for each rat before (basal; C, 37.6 °C vs. M, 37.8 °C, see left panel on Fig. 2b) and daily during drug treatment (PND 54-57) in adolescence. The present results showed a significant effect of treatment ($F_{1,38} = 13.93$, $p < 0.001$; two-way RM ANOVA), with methamphetamine-treated rats showing increased rectal temperature across days (PND 54: +0.69 °C, **$p < 0.01$; PND 55: +0.61 °C **$p < 0.01$ and PND 56: +0.65 °C, **$p < 0.01$; PND 57: +0.40 °C, n.s. vs. control rats; Fig. 2b).

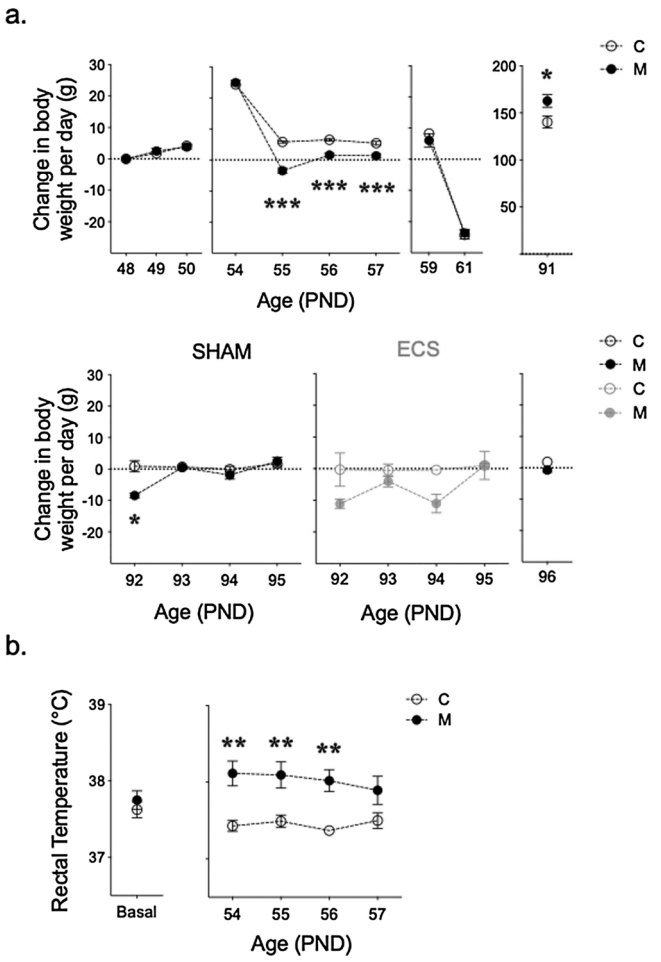

Fig. 2. Body weight and core temperature. a Changes in body weight across days (g). Groups of treatment: control (0.9 % NaCl, 1 mL/kg, i.p., n = 20, C) vs. methamphetamine (5 mg/kg, i.p., n = 20, M) with a binge paradigm (3 pulses per day, every 3 h, for 4 days, PND 54-57). Groups were then split in four: C-SHAM, n = 10 and C-ECS, n = 10; and M-SHAM, n = 10 and M-ECS, n = 10; daily dose of methamphetamine (5 mg/kg) or saline from PND 91-95, and 3 h later post-treated with ECS (daily pulse of 95 mA, 0.6 s, 100 Hz) or SHAM. Data represent mean ± SEM of change in body weight (g). Student's *t*-test or two- or three-way RM ANOVAs followed by Sidak's multiple comparisons tests: $*p < 0.05$ and $***p < 0.001$ vs. C. **b** Basal rectal temperature and daily temperature changes (°C). Groups of treatment: control (0.9 % NaCl, 1 mL/kg, i.p., n = 20, C) vs. adolescent methamphetamine (5 mg/kg, i.p., n = 20, M) with a binge paradigm (3 pulses per day, every 3 h, for 4 days, PND 54-57). Data represent mean ± SEM of core body temperature (°C). $**p < 0.01$ vs. C (two-way RM ANOVA followed by Sidak's).

3.2. Affective-like behavior

When evaluating changes in affective-like behavior, the results showed no immediate significant effects of binge methamphetamine administration in the forced-swim test on PND 59 (immobility: $t = 0.93$, $df = 38$, $p = 0.359$; Fig. 3a) or in the novelty-suppressed feeding test on PND 61 (latency to eat: $t = 0.69$, $df = 38$, $p = 0.496$; feeding time: $t = 0.26$, $df = 38$, $p = 0.797$; Fig. 3b). Note that a significant number of rats reached the time limit when measuring latency to eat (n = 4 for controls and n = 5 for methamphetamine-treated rats; Fig. 3b). Moreover, following prolonged methamphetamine abstinence, drug re-exposure during adulthood did not induce changes in affective-like behavior in the forced-swim test (no effect of treatment on PND 96: $F_{1,34} = 0.90$, $p = 0.350$), but showed a significant treatment (M vs. C) x post-treatment (ECS vs. SHAM) interaction ($F_{1,34} = 5.61$, $p < 0.05$), probably driven by the observed significant effect of post-treatment ($F_{1,34} = 6.67$, $p = 0.01$, Fig. 3c). In fact, *post-hoc* comparisons revealed the expected decrease in immobility caused by ECS (i.e., antidepressant-like effect) in control rats (with no methamphetamine exposure; −66 sec, $**p < 0.01$ vs. C-SHAM, Fig. 3c). ECS did not improve immobility in rats treated with methamphetamine (−3 sec, n.s. vs. M-SHAM, Fig. 3c).

3.3. Hippocampal neurogenesis

When evaluating recent cell proliferation, the results showed a lack of interaction between treatment x post-treatment ($F_{1,35} = 0.10$, $p = 0.752$), and no effect of treatment ($F_{1,35} = 0.46$, $p = 0.504$), but a significant effect of post-treatment ($F_{1,35} = 166.7$, $p < 0.001$). ECS increased Ki-67+ cells independently of prior drug history (C-ECS: +638 Ki-67+ cells, $***p < 0.001$; M-ECS: +621 Ki-67+ cells, $***p < 0.001$; vs. SHAM- respective groups; Fig. 4a). As for the survival of young neurons, although no interaction treatment x post-treatment was observed ($F_{1,34} = 0.74$, $p = 0.397$), there was a significant effect of treatment ($F_{1,34} = 4.92$, $p < 0.05$) and of post-treatment ($F_{1,34} = 22.01$ $p < 0.001$). *Post-hoc* comparisons revealed that while methamphetamine decreased NeuroD+ cells (M-SHAM: -1995 NeuroD+ cells, $\#p < 0.05$ vs. C-SHAM, Fig. 4b), ECS increased them (C-ECS: +2486 NeuroD+ cells, $*p < 0.05$; M-ECS: +3599 NeuroD+ cells, $***p < 0.001$; vs. SHAM-respective groups; Fig. 4b). Interestingly, when evaluating BrdU results (i.e., 47–49 days old surviving cells), a significant treatment x post-treatment interaction was observed ($F_{1,35} = 5.41$, $p < 0.05$). *Post-hoc* analysis revealed that methamphetamine decreased the number of BrdU+ cells (M-SHAM: -1289 BrdU+ cells, $\#p < 0.05$ vs. C-SHAM, Fig. 4c) and, while ECS did not alter BrdU+ cells in control rats (C-ECS vs. C-SHAM), it was capable of normalizing the decrease induced by methamphetamine (M-ECS: +1212 BrdU+ cells, $*p < 0.05$ vs. M-SHAM, Fig. 4c).

4. Discussion

The present study evaluated whether repeated ECS treatment could improve some of the neurotoxicity induced by methamphetamine exposure in rats. The main results showed that although methamphetamine (binge administration during adolescence followed by drug re-exposure in adulthood after prolonged drug removal) did not alter negative affect, it decreased the rate of hippocampal neurogenesis. Interestingly, repeated ECS improved the negative impact of methamphetamine administration on hippocampal neurogenesis. These results suggest that repeated ECS could serve as a potential therapeutical treatment to ameliorate certain neurotoxic correlates dysregulated following methamphetamine administration.

The effects of methamphetamine on body weight and core temperature were evaluated throughout treatment as a control of the drug administration procedures. Rats treated with methamphetamine during adolescence displayed reduced normal weight gain after the second day of treatment. These results replicated prior general (e.g., Steinkellner et al., 2011) as well as specific findings from our group (see García-Cabrerizo and García-Fuster, 2016; García-Cabrerizo et al., 2018) describing the anorexigenic effects of the same binge paradigm of methamphetamine administration in rats, and at the same age-window of exposure during late adolescence. In line with these changes in body weight, methamphetamine during adolescence induced the expected increase in core temperature across days, similarly to what was earlier reported (García-Cabrerizo et al., 2018). As described before, the emergence of tolerance to the hyperthermic effects of methamphetamine appeared across time during repeated treatment (e.g., Myles et al., 2008; García-Cabrerizo et al., 2018). Thus, adolescent methamphetamine binge administration induced the expected anorexigenic- and hyperthermic-like effects in rats (García-Cabrerizo and García-Fuster, 2016; García-Cabrerizo et al., 2018). Long after forced drug removal, rats with a prior history of adolescent methamphetamine exposure showed an increase in body weight gain when compared to controls. Although food intake was not monitored in the present experiment,

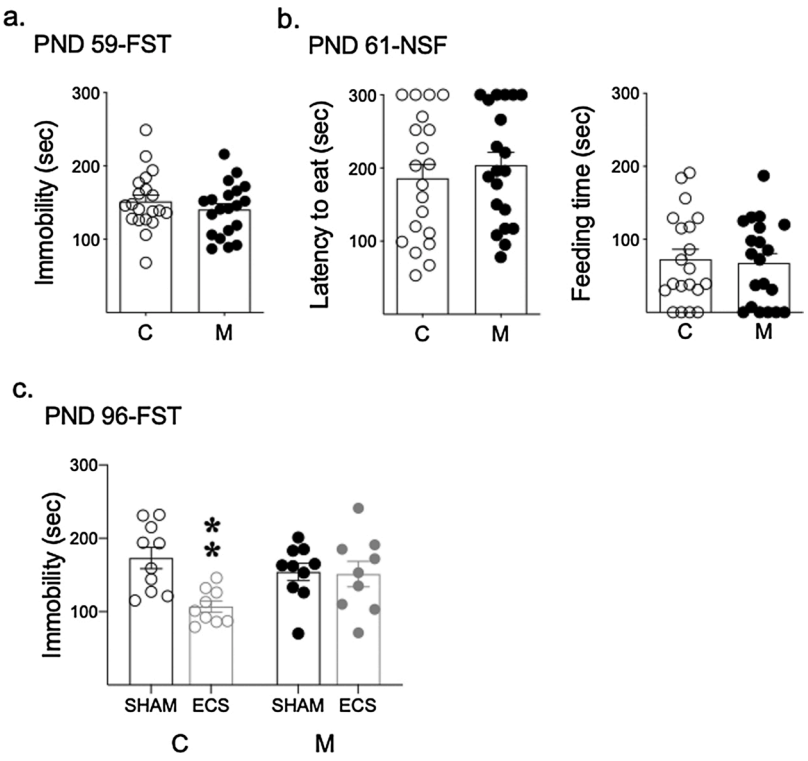

Fig. 3. Affective-like behavior. a Forced-swim test (FST, PND 59) and **b** Novelty-suppressed feeding test (NSF, PND 61). Groups of treatment: control (0.9 % NaCl, 1 mL/kg, i.p., n = 20, C) vs. adolescent methamphetamine (5 mg/kg, i.p., n = 20, M) with a binge paradigm (3 pulses per day, every 3 h, for 4 days, PND 54-57). Data represent mean ± SEM of the time (sec) spent immobile in the FST or the latency to eat (sec) and the feeding time (sec) in the NSF. **c** FST (PND 96). Groups of treatment: C-SHAM, n = 10 and C-ECS, n = 10; and M-SHAM, n = 10 and M-ECS, n = 10. Data represent mean ± SEM of the time (sec) spent immobile. Two-way ANOVA followed by Sidak's multiple comparisons test: $^{**}p < 0.01$ vs. C-SHAM.

prior results showed increased food intake after methamphetamine exposure and during drug abstinence (e.g., Torney and Lasagna, 1960; Kraeuchi et al., 1985), in line with the present results. Finally, methamphetamine re-exposure in adulthood decreased the overall body weight of rats as compared to controls, an effect that normalized over time (as observed on PND 96), and as earlier reported by our group (García-Cabrerizo and García-Fuster, 2019a).

When assessing changes in affective-like behavior, adolescent methamphetamine exposure did not alter the behavioral outcome during adolescence, as observed in the forced-swim and novelty-suppressed feeding tests. These lack of immediate effects during adolescence extend previous reports on adolescent insensitivity (i.e., better adaptation) to certain methamphetamine effects (e.g., see Teixeira-Gomes et al., 2015; García-Cabrerizo and García-Fuster, 2016 and references within). Besides, long after methamphetamine adolescent exposure and following drug re-exposure in adulthood, no changes were observed in the forced-swim test either, in line with our prior study (García-Cabrerizo and García-Fuster, 2019a). Moreover, and as expected, repeated ECS induced a potent antidepressant-like effect (García-Cabrerizo et al., 2020) in control-treated rats, but it was not capable of inducing such beneficial effects in rats with a history of methamphetamine (i.e., dysregulation that impeded such powerful effect). In this line of thought a prior study reported changes in the incidence and duration of ECS after repeated treatment with methamphetamine in mice (Nakamura et al., 1993), suggesting that maybe this interaction impeded the normal antidepressant-like therapeutic response of ECS following methamphetamine pre-treatment (i.e., changes in the primary features of the seizures as observed in our rats).

To our knowledge no prior studies have evaluated the potential of ECS on reverting certain neurotoxicity actions induced by methamphetamine, such as the inhibition of hippocampal neurogenesis. In this context, methamphetamine alone did not alter cell proliferation (Ki-67 labeling), but induced hippocampal neurotoxicity by decreasing both early neuronal survival (NeuroD+ cells) and long-term cell survival (i.e., 47–49 days old surviving BrdU+ cells; persistent effects after adolescent drug exposure), in line with our prior study (García-Cabrerizo et al., 2018), and demonstrating enduring neurotoxic effects induced by methamphetamine. These results help us to better understand how methamphetamine administration affect the distinct stages of neurogenesis, since each cell marker labeled different time points of regulation: Ki-67 for dividing cells (25 h; Kee et al., 2002); NeuroD for immature young neurons (early cell survival, 4–7 days old; von Bohlen Und Halbach, 2007); and BrdU for mature 47–49 days old cells (long-term survival; Dayer et al., 2003). Thus, jointly with our prior results (García-Cabrerizo and García-Fuster, 2016; García-Cabrerizo et al., 2018), the present data extend the research done also by others (e.g., Eisch and Harburg, 2006; Mandyam et al., 2008; Loxton and Canales, 2017), to propose that the immediate effects of methamphetamine on impairing cell proliferation (García-Cabrerizo and García-Fuster, 2016) led to a delayed decreased number of neural progenitors (early survival, NeuroD+ cells) and long-term surviving cells (BrdU+ cells), emphasizing the persistent negative effects of methamphetamine on neurogenesis regulation. Interestingly, repeated ECS, which has been described as a potent modulator of neurogenesis by others and our own (see García-Cabrerizo et al., 2020 and references within), increased cell proliferation and early neuronal survival (early stages of the neurogenic process) in control rats, but did not alter the fate of cells born several weeks prior to its exposure (BrdU+ cells). Interestingly, when ECS was administered in rats treated with methamphetamine, it reverted the decrease in young neuronal survival (NeuroD) and long-term cell survival (BrdU), demonstrating, for the first time, that ECS could serve as a neuroprotective therapeutic tool for reverting methamphetamine-induced neurotoxicity. As proven by these results, both novel NeuroD+ cells (being born in adulthood during methamphetamine re-exposure), as well as old BrdU+ cells (born in adolescence prior to any drug treatments), were negatively affected by methamphetamine re-exposure in adulthood. A daily post-treatment with ECS was capable of reverting these decays, demonstrating that ECS could improve some of the negative impact emerging following adolescent methamphetamine exposure and after drug re-exposure. Since hippocampal neurotoxicity could account for some of the addictive-like behaviors emerging after drug removal and relapse (e.g., increased

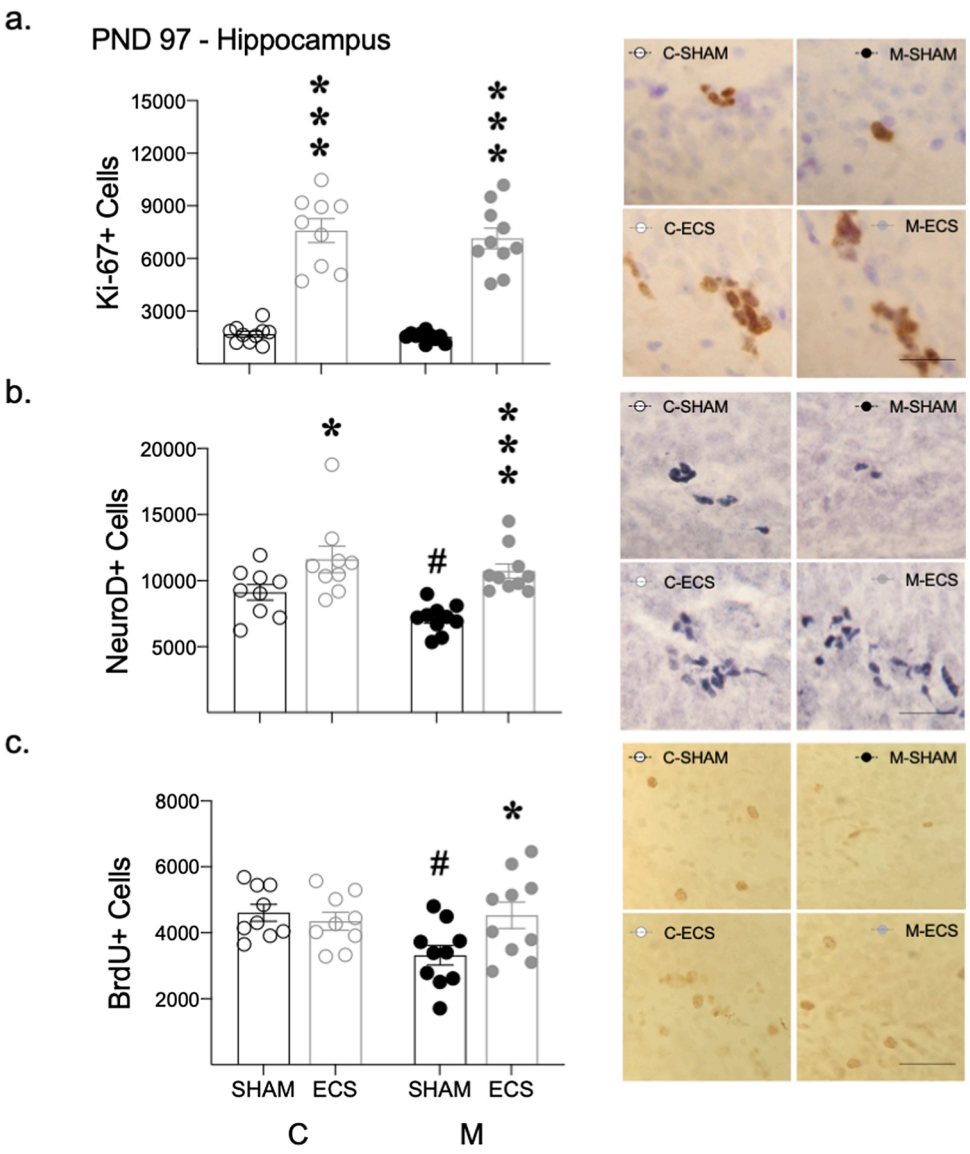

Fig. 4. Hippocampal neurogenesis. a Quantitative analysis of Ki-67+, **b** NeuroD+ and **c** BrdU+ cells in the left dentate gyrus of the hippocampus on PND 97. Groups of treatment: C-SHAM, n = 10 and C-ECS, n = 10; and M-SHAM, n = 10 and M-ECS, n = 10. Data represents mean ± SEM of the number of + cells quantified in every 8th section throughout the entire extent of the hippocampus and multiplied by the sampling factor 8 providing an estimate of the total number of + cells per marker (individual values are shown for each rat: symbols). Two-way ANOVAs followed by Sidak's multiple comparisons tests: $*p < 0.05$ and $***p < 0.001$ when comparing the effects of post-treatment (ECS vs. SHAM groups), and $\#p < 0.05$ when comparing the effects of treatment (M-SHAM vs. C-SHAM). Right panels: representative images showing individual Ki-67+ (brown labeling in the blue granular layer), NeuroD+ (dark blue labeling in the blue granular layer), and BrdU+ (brown labeling in the light brown granular layer) cells taken with a light microscope (63x objective lens; scale bar = 30 μm).

voluntary methamphetamine consumption; García-Cabrerizo and García-Fuster, 2019a), the improved rates of cell survival (young neurons, old cells) could help ease the negative impact of methamphetamine.

To sum up, these results report that ECS attenuated methamphetamine-induced neurotoxicity by protecting against hippocampal neurogenesis reduction, and thus, together with the prior literature, support a possible role for ECS in the treatment of methamphetamine-induced abnormalities. Therefore, this therapeutical approach is presented as a suitable option for improving methamphetamine-induced neurotoxicity likely leading to addiction and/or associated comorbidities.

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CRediT authorship contribution statement

Rubén García-Cabrerizo and **M. Julia García-Fuster** were responsible for the study concept and design. **Rubén García-Cabrerizo** conducted the experiments and analyzed the behavioral and molecular data, with the participation of **Cristian Bis-Humbert**. **M. Julia García-Fuster** revised the data, plotted the figures and drafted the manuscript. **Rubén García-Cabrerizo, Cristian Bis-Humbert, M. Julia García-Fuster** contributed and approved the final version for publication.

Conflict of interest

The authors declare that they have no conflict of interest.

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