Experimental Neurology 320 (2019) 113005

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Research Paper

Comorbidities of early-onset temporal epilepsy: Cognitive, social, emotional, and morphologic dimensions

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ARTICLE INFO

Keywords:
Anxiety
Cognition
Depression
Epilepsy
Exploratory behavior
Hippocampus
Pilocarpine
Rats
Spatial learning
Status epilepticus

ABSTRACT

Epilepsy, the most common neurologic disorder in childhood, is associated with a subset of psychiatric dysfunctions, including cognitive deficits, and alterations in emotionality (e.g., anxiety and depression) and social functioning. In the present study, we evaluated an integrative set of behavioral responses, including cognitive/socio-cognitive and emotional dimensions, using a number of behavioral paradigms in the LiCl/pilocarpine model of status epilepticus (SE) in rats. The aims of the study were to examine whether SE affects: 1) non-associative learning (habituation of exploratory behavior); 2) investigatory response to an indifferent stimulus object; 3) sociability/social novelty preference; 4) social recognition or discrimination; and 4) short- and long-term memory in the Morris water maze (MWM). Finally, we investigated the morphology of key brain structures involved in the examined behavioral dysfunctions. SE did not affect habituation to an open-field arena in juvenile (P25), adolescent (P32), or adult (P80) rats. SE rats spent less time in the central part of the arena. SE adolescent rats (P32) displayed a higher number of rearings with a shorter duration. SE rats displayed a markedly attenuated investigatory response to an indifferent stimulus object. SE rats in all age groups demonstrated pronounced deficits in sociability and the preference for social novelty. In addition, SE rats spent a reduced amount of time investigating a juvenile rat upon first exposure. After 30 min re-exposure together with an additional, novel juvenile, the SE rats spent equal time investigating both juveniles. In the MWM task, acquisition was unimpaired but there was a deficit in delayed memory retention after 10 days. SE did not affect cognitive flexibility expressed by reversal learning. Together, these findings suggest that early-life SE leads to alterations in emotional/anxiety-related behavior and affects sociability/preference for social novelty and social discrimination. Early-life SE did not alter acquisition of spatial learning, but it impaired delayed retention. Using Fluoro Jade B staining performed 24 h after SE revealed apparent neurodegeneration in the dorsal hippocampus, mediodorsal thalamic nucleus and medial amygdala, brain areas that are critically involved in network underlying emotional behavior and cognitive functions.

1. Introduction

Epilepsy is the most common neurologic disorder in childhood and is often associated with neurobehavioral abnormalities, including cognitive and emotional dysfunction (e.g., anxiety and depression), and social interaction and communication deficits (Berg et al., 2008; Caplan, 2017; Holmes, 2015; Holmes, 2016; Holmes et al., 2015; Jones et al., 2016; Mohanraj and Brodie, 2013). In early childhood and adolescence, the peak periods for epileptic seizure onset, the prevalence of intellectual disabilities is highest and is associated with structural brain lesions (Amiet et al., 2008; Nishimura et al., 2011). Data from epidemiologic studies suggest a bidirectional relationship between several psychiatric disorders and epilepsy, highlighting common pathogenic mechanisms in both conditions (for review, see Ekinci et al., 2009; Verrotti et al., 2014). Moreover, a number of studies support a bidirectional relationship between early-life seizures (ELS) and an early neurodevelopmental syndrome, autism spectrum disorder (ASD), with an increased prevalence of ASD in children with epilepsy and of epilepsy in children with ASD (Amiet et al., 2008; Berg and Plioplys, 2012; Bernard and Benke, 2015; Brooks-Kayal, 2010; Holmes, 2015). The newly-proposed definition of epilepsy recognizes psychiatric comorbidities in epilepsy as a part of the seizure disorder that should be treated together with the seizures (for review, see Kanner, 2016). Available data suggest a higher incidence of psychiatric comorbidity in

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https://doi.org/10.1016/j.expneurol.2019.113005
Received 31 October 2018; Received in revised form 16 April 2019; Accepted 2 July 2019
Available online 03 July 2019
0014-4886/

children with temporal lobe epilepsy (TLE) than in adults, but the relationship of psychopathology to TLE has been less examined in children and adolescents (for review, see Ekinci et al., 2009). Children with TLE show impaired development of emotion recognition, perceptual deficits, abnormal social-behavioral traits, and disrupted cognition with or without intellectual deficiency. In addition, high levels of anxiety, depression, and seizure frequency are risk factors for impaired cognitive function (Laurent et al., 2014; Martinos et al., 2018).

In recent years, the extensive and complex comorbidities of ELS received significant attention as an important area of research in epilepsy (for review, see Brooks-Kayal et al., 2013; Danzer, 2012; Holmes et al., 2015; Stafstrom and Benke, 2015). Experimental studies conducted in commonly used rat models of epilepsy acquired because of ELS demonstrate an increased incidence of behavioral abnormalities. These include cognitive deficits, anxiety- and depression-like behavior, and profound deficits in social domains that mimic, to a certain extent, symptoms of neuropsychiatric comorbidities often present in humans (for review, see Bernard and Benke, 2015; Brooks-Kayal et al., 2013; Casanova et al., 2014). The majority of studies demonstrating cognitive impairment mainly examined spatial cognition (for review, see Barry et al., 2016; Casanova et al., 2014; Holmes, 2005; Holmes, 2015; Karnam et al., 2009; Kubová et al., 2004; Lugo et al., 2014; Rutten et al., 2002). ELS can also lead to alterations in social behavior, however, that in some models resemble the autistic features observed in humans (Castelhano et al., 2013; Hernan et al., 2014; Holmes et al., 2015; Lippman-Bell et al., 2013; Talos et al., 2012). The social tests were mainly interpreted in terms of pure socialization, but more sensitive tests of preference for a novel versus a familiar conspecific may also reflect abnormalities in socio-cognition (Sandi and Haller, 2015; Yang et al., 2011). Animal models of epilepsy, such as the pilocarpine model of acquired TLE are useful for studying the relationship between epilepsy and behavioral dysfunction. The temporal lobe and amygdala, in particular, play crucial roles in processing the appropriate cognitive and behavioral responses to emotionally-relevant stimuli (Twining et al., 2017). Dysregulation of the amygdala-hippocampal complex and entorhinal cortex function caused by neuronal hyperexcitability may lead to psychiatric comorbidities (Adolphs, 2010). Our data from immature rats with LiCl-pilocarpine–induced SE (Li-Pilo SE) suggest mild retardation of psychomotor development and persistent learning deficits in the hippocampal-dependent Morris water maze (MWM) task. In addition, continuous video-electroencephalographic monitoring indicates that the incidence of spontaneous non-convulsive seizures tends to progress over time (Kubová and Mareš, 2013; Kubová et al., 2004).

In the present study, we evaluated an integrative set of behavioral responses, including cognitive/socio-cognitive and emotional dimensions, using a number of behavioral paradigms in the Li-Pilo model of SE induced in immature rats at various developmental stages. The aims of the study were to examine whether ELS affect later 1) non-associative learning (habituation of exploratory behavior in an open-field arena); 2) investigatory response to an indifferent stimulus object; 3) sociability/social novelty preference; 4) social recognition or discrimination; 4) short- and long-term memory in the MWM. The final aim was to investigate the key brain structures involved in the examined behavioral dysfunctions.

The animals were tested repeatedly across multiple developmental time-points. Selected behavioral paradigms covered tasks of ethologic relevance to animals that differ in terms of the type of behavior they mediate. These behaviors involve neural circuits of interacting structures such as the hippocampus, perirhinal cortex, medial prefrontal cortex, medial dorsal thalamus, striatum, and amygdala. This neural circuitry plays a critical role in acquisition, discrimination learning, storage and retrieval of various memory processes, programming of social behavior, and mediation of behaviors with motivation/incentive and affective properties (Brown and Banks, 2015; Gasbarri et al., 2014; Hitti and Siegelbaum, 2014; Ouhaz et al., 2017; Ouhaz et al., 2015; Parnaudeau et al., 2018; van den Bos, 2015).

2. Materials and methods

Male outbred Wistar albino rats (Institute of Physiology of the Czech Academy of Sciences, Prague, $n = 59$ for behavioral study, and $n = 11$ animals for neuropathological examination) were used in the experiments. Animals were maintained under controlled temperature (22 ± 1 °C) and humidity (50 to 60%) with a 12/12 h light/dark cycle (lights on at 6:00 AM). Food and water were provided ad libitum (with the exception of the testing period). On day 5, (birth was defined as day 0), the pups were randomly fostered and each litter was adjusted to 10 males. At postnatal day (PD) 11, the animals were marked for identification and mixed by treatment. To exclude a litter effect, animals in any group were selected from different litters. One or two animals from the same litter were used. They were weaned at PD28. After weaning, the animals were housed in groups of 3–4 per cage. The number of animals is indicated for each experiment. Experiments were approved by The Central Committee for Animal Care of the Czech Academy of Sciences (approval # 131/2013). Animal care and experimental procedures were also conducted in accordance with the guidelines of the European Community Council directives 86/609/EEC. 0.2010/63/EC).

2.1. Induction of status epilepticus (SE)

SE was induced as described in detail previously (Kubová and Mareš, 2013). LiCl was administered intraperitoneally (3 mmol/ml/kg; # L-0505, Millipore Sigma, St. Louis, MO) to PD11 immature rats. After 24 h, pilocarpine (40 mg/ml/kg; # P-6503, Millipore Sigma) was administered to the LiCl-pretreated rats. The appearance of clonic motor seizures was considered to be the beginning of SE. To decrease mortality, paraldehyde (0.07 ml/kg, #76260, Fluka Chemie AG, Buchs, Switzerland) was injected intraperitoneally 1.5 h after the onset of SE (for details, see Kubová et al., 2005). Control animals were treated with equal doses of LiCl and paraldehyde, but the pilocarpine solution was replaced with saline.

Severity of motor SE was assessed according to the following scoring system: 0 = normal behavior; 1 = stereotypic behavior (face washing, scratching), isolated myoclonic jerks; 2 = head bobbing, pivoting, swimming movements; 3 = clonic seizures with preserved righting reflex; 4 = repeated episodes of wild running; and 5 = generalized tonic-clonic seizures with loss of the righting reflex. Animals were assigned a score for the most severe behavior observed. Latency to the onset of motor seizures was recorded. Only rats that exhibited behavioral manifestations of seizures progressing to forelimb clonus (i.e., score 3) for at least 1 h and without periods of wild running and generalized tonic-clonic seizures (score 4–5) were used.

During the entire period of separation from their mothers, P12 animals were maintained at + 34 °C with a Physiological-Biological Temperature Controller (TMP-5b; Supertech; Hungary) to compensate for the immature thermoregulation (Conklin and Heggeness, 1971). Approximately 3 h after pilocarpine injection, the pups were injected subcutaneously with 0.9% NaCl (up to 3% of body weight) to restore the volume loss, and then returned to their mothers (duration of separation from mothers was the same between groups).

2.2. Behavioral tests (experiment 1)

It is difficult to extrapolate age in rats to age in humans since it is highly dependent on what measure is being evaluated. Therefore to roughly estimate neurodevelopmental time in rats to age in human we relied on comparative studies from literature (Andersen, 2003; Clancy et al., 2007; McCutcheon and Marinelli, 2009; Pressler and Auvin, 2013; Workman et al., 2013). The testing cover the age of immaturity (P18), juvenility (P25), adolescence (P32), sexual maturity (P60), young adulthood (P80) an adulthood (P200). Compared to humans,

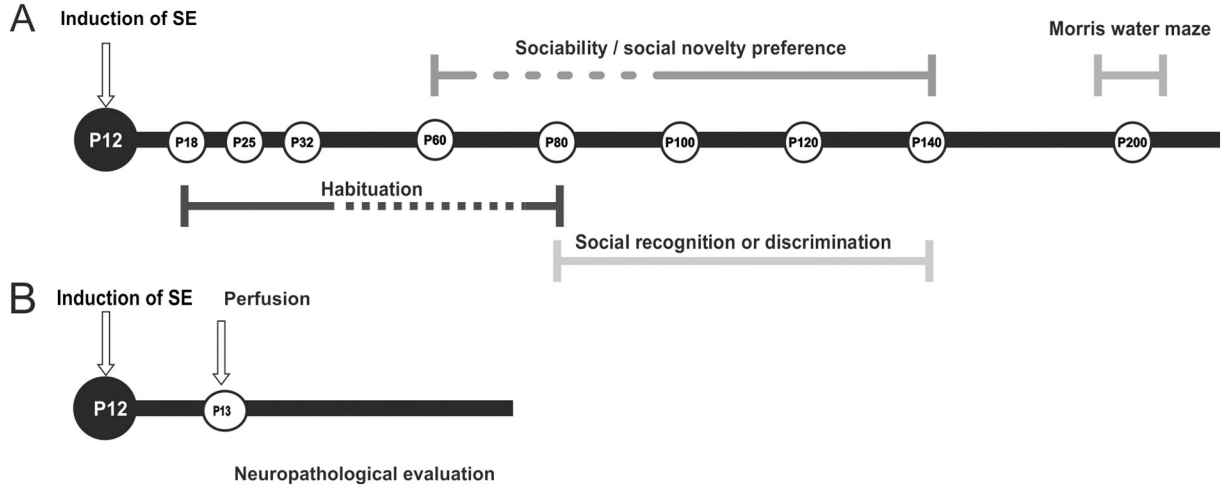


Fig. 1. Design/time diagram of experimental procedure. Fig. A: Time schedule of behavioral testing. Habituation tests started at P18 (age of immaturity) and covered the age of juvenility (P25), adolescence (P32) and young adulthood (P80). The sociability/social novelty preference started at the age of sexual maturity (P60), social recognition or discrimination tests started at the age of young adulthood (P80). The reference memory was tested in adulthood (P200). Further details are given in methods. Fig. B: Preparation of brains for neuropathological evaluation. Separate group of animals (was perfused 24 h after SE and brains were used for assessment of acute neurodegeneration. Further details in Methods.

selected ages cover the age of early-childhood, childhood, peri-adolescence, adolescence and adulthood. The ages cannot be considered very accurate, but merely as approximating certain aspect of development. A time diagram of experimental procedure is depicted in Fig. 1.

Behavioral manifestations related to seizures were not observed before or during the behavioral testing.

For the habituation tests, 22 rats were used. Social behavior tests were performed in two independent groups to avoid possible interaction effects; therefore, for the sociability/social novelty preference and social recognition or discrimination, a total of 17 and 20 rats, respectively, were used. The groups of animals tested for spatial learning and memory (MWM) comprised the animals tested in the social behavior tests. Due to a video tracking failure, only 30 rats were used in the MWM analysis. Animals used as social stimuli (adult, $n = 10$; juvenile, $n = 40$) were used in a different study. All behavioral tests were performed between 9:00 AM and 3:00 PM in a special room at a constant temperature (22 ± 2 °C) and dim light conditions (94–96 lx). Before testing, animals were adapted to the testing room for 30 min. Behavior was video-recorded and then analyzed using Observer and EthoVision software (Noldus Information Technology, The Netherlands).

2.3. Between-session habituation tests

Habituation is a decrease of the innate response to a repeated stimulus or environment, providing a simple but valid model of non-associative learning processes. The animals (controls, n = 10; SE, $n = 12$) were tested at P18, P25, and P32, and thus the age groups used spanned across three stages of ontogeny: preweaned, juveniles, and adolescents, respectively. The test was performed in an open-field arena that comprised a square black plastic box (48 × 48 cm, walls 30 cm). Rats were tested for 4 consecutive 5-min sessions with a 60-min interval between sessions. To assess *between-session habituation* (Mikulecká et al., 2014; Thiel et al., 1999), exploratory activity in the 1st session was compared with that in the 4th session. The decrease in activity with repeated sessions (habituation) served as an index of learning. Total distance moved (cm), center time (total time spent in the central section (30 × 30 cm section of the open field), and total number and total duration of rearing were evaluated.

At P80, the animals (control, $n = 10$; SE, $n = 11$,- 1 animal died) were tested in a different habituation test (OFF-OFF-ON) in the same open field using a similar procedure described elsewhere (Dai et al., 1995; Mikulecká et al., 1999). The animals were individually placed in the arena for two habituation sessions for 10 min with a 24-h delay between them (Test day 1 and Test day 2, OFF-OFF). Before the start of the 3rd session (Test day3, ON) an object measuring 4 × 4 × 2 cm was placed in the center of the arena. For each animal tested, a new clean object was used and the arena was carefully cleaned with a 70% pure ethanol solution before testing another rat. The distance moved (cm), and the total number and total duration of investigations of the stimulus object were evaluated. The OFF-OFF-ON test allows for testing the specific hypothesis of habituation (decreased response from one session to a subsequent session) and dishabituation (increased response after an object-elicited investigation).

2.4. Social behavior tests

To detect the possible progression of impairment with epilepsy duration in socially mediated behavior, two tests were selected: sociability/social novelty preference and social recognition or discrimination. Both tests rely on the intrinsic motivation of rodents to investigate novel conspecifics when introduced into their familiar territory. These paradigms represent good ethologically relevant models for the study of social olfactory learning and memory.

2.5. Sociability/social novelty preference

The sociability/social novelty preference was evaluated using a slightly modified procedure based on previous tasks The same groups of animals (controls, $n = 7$; SE, n = 10) were tested repeatedly at four different ages: P60, P100, P120, and P140. As a social stimulus, adult rats (n = 10) were used. The experiment was performed in an open-field arena (80 × 80 × 40 cm) where two empty identical round wire-mesh cages (20 cm high, 15.5 cm diameter, bars spaced 1.5 cm) were placed in line, directly across from each other on the right and left sides, allowing sufficient space for the rat to move around each cage. For the adaptation phase (habituation), the experimental rat was placed in the center of the arena and allowed to explore for 10 min. To test sociability, one of the cages was removed and a rat designated Stranger 1, aged-matched with the experimental rat but without prior contact, was enclosed in a wire cage and placed on the left or the right side of the arena and then the experimental rat was allowed to explore for 10 min. After the sociability phase, to test social novelty/preference, another novel rat, Stranger 2, was enclosed in the second wire cage and placed in the testing arena. The tested rat was allowed to explore both

strangers for 10 min. The tested rat had a choice between the already investigated rat and the novel unfamiliar rat. Cage location was counterbalanced between animals. The cages, which were identical, and the testing arena were cleaned with a 70% pure ethanol solution and completely dried between each test.

The total duration of contacts between the experimental rat and empty cage vs. cage housing Stranger 1 (sociability phase), or between the experimental rat and the cage housing Stranger 1 vs. cage housing Stranger 2 (social novelty/preference phase) was evaluated.

2.6. Social recognition or discrimination

A procedure similar to the one described previously (Engelmann et al., 2011; Lukas et al., 2013; Macbeth et al., 2009) was used. The same groups of animals (controls, $n = 11$; SE, $n = 9$) were tested repeatedly at four different ages: P80, P100, P120, and P140. Three hours before the start of the experiment, the animals were housed individually. The juveniles (P20–22, for each age group, $n = 10$) were used as social stimuli to prevent potential aggressive and/or sexual behavior. They were marked to allow the observer to distinguish between them and isolated in small cages for 30 min. During the first session, a juvenile (stimulus J1) was introduced into the home cage of an adult for 5 min. The juvenile was then removed and kept individually in a cage for an interval of 30 min. In the second session, two juveniles - the same one (J1, that was exposed previously, i.e. during the first exposure) and a novel one (stimulus J2) were simultaneously exposed to the adult for 5 min. The time spent investigating the juvenile (J1) during the first exposure, and the time spent investigating the previously exposed juvenile (J1) and the novel juvenile (J2) in the second exposure were evaluated.

2.7. Morris water maze

Animals tested for place learning and long-term spatial memory comprised the animals used in the social behavior tests (controls, $n = 13$; SE, $n = 17$). Testing in the MWM started at P200 (D'Hooge and De Deyn, 2001; Spreng et al., 2002; Washington et al., 2012). The MWM comprised a black circular pool (180 × 50 cm) filled with clear water (20 °C). A circular transparent Plexiglas platform (10 cm in diameter) was submerged 1.5 cm below the surface of the water, in the center of an arbitrarily defined quadrant of the pool (North-West) and remained in the same position throughout the whole testing. Before the start of acquisition, the animals were tested with a visible platform positioned in the same location as for the acquisition session and cued by a tennis ball that had a black lower hemisphere. The rats' performance on the visual platform task indicated that they had sufficient vision to perform the task. None of the tested rats were excluded due to poor performance. In the acquisition test, each rat received one session (8 trials) per day for five consecutive days. A trial was initiated by placing the animal into one of the four pseudo-random starting positions (N, W, S, or E). In case the rat failed to locate the platform within 60 s, the experimenter guided the rat to the platform where it was allowed to rest for 30 s. After each session, the rat was dried with a towel and kept in a warmed cage. To test the rat's knowledge of the hidden platform location, a spatial probe trial was run immediately after completion of the 5th session. The platform was removed from the maze and the rat was allowed to swim freely for 90 s. The time spent in the target quadrant where the platform was formerly located during the acquisition was measured (PT1) to test immediate retention. Ten days after the acquisition, a second test (PT2) was performed to test for delayed retention. This trial was followed by an eight-trial reversal learning session with the platform located in the opposite quadrant to evaluate the behavioral flexibility of the animals. This last session was terminated by a 3rd probe trial (PT3) to test for the immediate retention of the re-learned position. In the probe trials, all rats started from the same position opposite to the quadrant where the escape platform was positioned during acquisition. The following parameters were analyzed: escape latency (time from start to goal), distance traveled (distance from start to target quadrant), and swimming speed to control whether or not groups had the same motor and motivational competencies. For the probe trials, time spent in the target quadrant (platform position) was evaluated.

2.8. Histologic processing of tissue (experiment 2)

Separate groups of control ($n = 3$) and SE ($n = 8$) animals were used to assess the extent and distribution of neuronal damage. The rats were deeply anesthetized by intraperitoneal injection of urethane (2 g/kg; #U-2500, Millipore Sigma) 24 h after SE and transcardially perfused with saline followed by 4% paraformaldehyde (0.01 MPBS, PH 7.4). The brains were post-fixed in phosphate-buffered 4% paraformaldehyde for 3 h and then cryoprotected in an ascending series of sucrose concentrations in 0.01 M sodium phosphate buffer (pH 7.4) at + 4 °C. The brains were frozen in dry ice, and sectioned in the coronal plane (50 μm, 1-in-5 series) with a Cryocut Leica CM1900 and one series of sections was used for cresyl violet staining. To assess neuronal damage, Fluoro-Jade B (FJB) histochemistry was performed as described previously (Schmued et al., 1997). To detect labeled cells, one series of sections through the entire rostrocaudal axis of the brain was stained and examined by an experienced observer using an Olympus AX53 microscope and the structures containing FJB-positive cells were determined. Brain areas containing >10 FJB-labeled cells per area per section were considered positive. Based on this screening, three brain areas exhibiting consistent damage in majority of SE animals - the CA1 field of the dorsal hippocampus (CA1), the mediodorsal nucleus of the thalamus (MD) and the medial amygdalar nuclei (M) were selected for quantification. The extent of damage was expressed as a number of FJB-positive cells/mm^2.

Three sequential sections containing the CA1, MD and M taken from comparable AP levels at 250 μm intervals were used to manually count FJB-positive cells. Two images of CA1 field at 20× magnification (in total 1.4 mm^2) one image of MD at 10× magnification (1.67 mm^2) and one image of M at 20× magnification were taken from each of three sections from each animal. Mean number of FJB-positive cells per 1mm^2 was calculated in the evaluated structures. Structures with number of FJB-positive cells lower than 10 per brain area on one section were taken as not damaged. In text, data are presented as median with 25–75%. In graphs, data is presented in box plots (median with 25–75%) with whiskers indicating minimal and maximal values and symbols indicated values for each subject.

2.9. Statistical analysis

The data from habituation tests and MWM were analyzed by a two-way repeated measures ANOVA with one between-group factor (control, SE) and one within-subject factor (repeated session). Two-way repeated measure ANOVA was used to analyze the age differences in both sessions. The number and the time spent in object investigation were analyzed by *t*-test. The data set from the sociability/social novelty preference were analyzed by a two-way ANOVA to compare the duration of investigation (empty cage vs. Stranger 1) and the preference for Stranger 1 or Stranger 2 in the second phase of the test. Two-way repeated measure ANOVA was used to compare the investigation time for repeated exposure to Stranger 1. The data from the social investigation or discrimination test were analyzed by a two-way repeated measure ANOVA with one between-group factor (control and SE) and one within-group factor (repeated session). Two-way ANOVA with between-group factor (control and SE) and condition (J1vs J2) was used to compare the investigation time in the second session.

For all experiments, the post hoc Student-Newman-Keuls method was used to explore main significant effects or interactions resulting from ANOVA. The significance was set at $P < .05$ (Sigma Stat®, SPSS

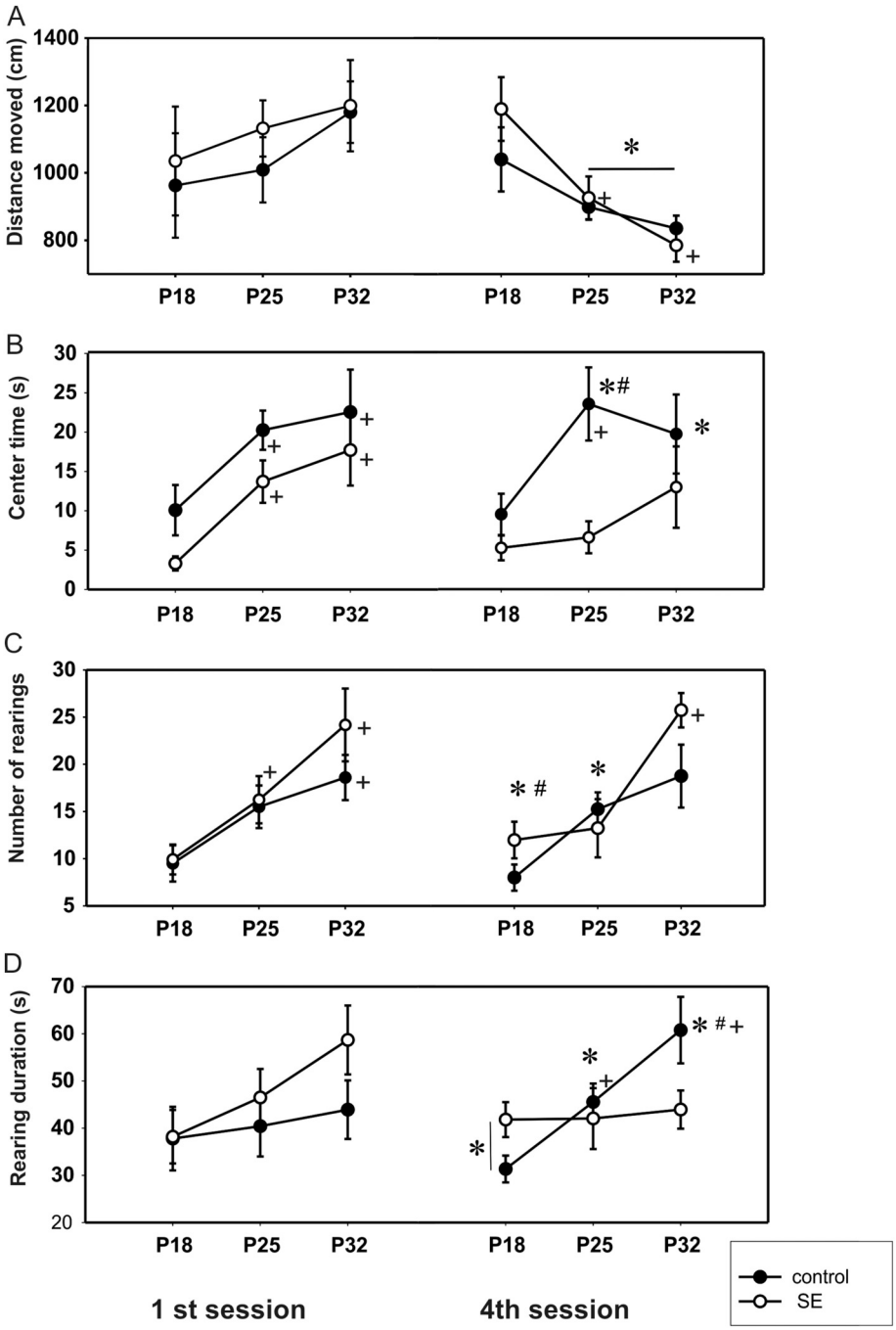


Fig. 2. Effect of early-life SE on habituation in preweaned, juvenile, and adolescent rats. The animals were tested at P18, P25, and P32 (controls, $n = 10$; SE, $n = 12$). A, distance moved; B, time spent in center part; C, number of rearings, and D, rearing duration. Abscissae: Between-session habituation (1st session vs. 4th session). Ordinate: mean and SEM (±) values for distance moved (cm), total center time (s), total number of rearings and total duration of rearings. * Significant difference compared to 1st session, # significant difference compared with controls, + significant difference compared with P18 and/or P25.

Inc., Chicago, IL). The significant differences of multiple comparisons are depicted in the figures.

3. Results (experiment 1)

3.1. Effects of early-life SE on between-session habituation

Early SE had no effect on the development of between-session habituation. The distance moved was decreased between the 1st and the 4th session in both controls and SE groups at P25 and P32 (Fig. 2A, $F_{(1,20)} = 24.98$, $p < .001$; $F_{(1,20)} = 34.63$, $p < .001$, respectively). No habituation with repeated exposure to the open-field arena was observed at P18 (Fig. 2A, $F_{(1,20)} = 0.71$, $p = .40$), suggesting a lack of habituation in both control and SE animals at this age. No age differences were found in the distance moved in the 1st session (Fig. 2A, $F_{(2,40)} = 1.36$, $p = .26$). In the 4th session SE animals at P25 and P32 walked significantly shorter distance compared with P18 (Fig. 2A, $F_{(2,40)} = 5.01$, $p = .01$).

SE increased *anxiety-like behavior* assessed based on the amount of time spent in the center part of the open-field arena. Whereas control animals spent nearly the same amount of time in the central part, SE animals spent significantly less time in the central part during the 4th

session compared with the 1st session at P25 and P32 (Fig. 2B, $F_{(1,20)} = 4.91$, $p = .03$; $F_{(1,20)} = 9.96$, $p = .005$, respectively). In addition, at P25, SE animals spent significantly less time in the center compared with controls in the 4th session ($p = .004$, see Fig. 2B). In the 1st session both controls and SE animals spend significantly more time in the central part of arena at P25 and at P32 compared to P18. In the 4th session, only control animals at P25 spent more time in the central part compared with P18 (Fig. 2B, $F_{(2,40)} = 9.48$, $p < .001$; F (2.20) = 5.12, $p = .03$).

In both control and SE animals, the number of rearings tended to increase with age, reflecting normal development of exploratory activity. In both control and SE animals, the number of rearings tended to increase with age, reflecting normal development of exploratory activity. SE resulted in an increased number of rearings in the 4th session and the number of rearings was significantly higher at P18 and P32 in SE animals compared with controls. In accordance with the increase in the number of rearings, total time spent rearing also tended to increase with maturation in both groups. This trend was particularly noticeable in controls in the 4th session. Compared with the 1st session, time spent rearing was significantly shorter at P18 in the 4th session in both groups (Fig. 2D, $F_{(1,20)} = 13.78$, $p = .001$) and also in SE animals at P25 and P32 (Fig. 2D, $F_{(1,20)} = 4.40$, $p = .04$; $F_{(1,20)}$, $p = .07$, respectively). Despite the higher rearing frequency, SE animals at P32 spent less time rearing than controls in the 4th session and the mean duration of individual rearing was 59% shorter in SE animals than in controls.

Repeated measure ANOVA yielded a significant age effect for the number of rearings in both sessions (Fig. 2C, $F_{(2,40)} = 17.27$, $p < .001$; $F_{(2,40)} = 12.18$, $p < .001$, respectively). For the total time spent in rearing ANOVA did not show overall age differences in the 1st session ($F_{(2,40)} = 2.90$, $p = .06$). In the 4th session, there were a significant effect of age ($F_{(2,40)} = 5.76$, $p = .006$) and treatment x age interaction ($F_{(2,40)} = 4.29$, $p = .02$). Multiple comparisons revealed an increased time spent in rearing in control but not in SE animals.

These findings suggest that early-life SE does not affect the development of habituation with repeated exposure to the arena, but increases anxiety-like behavior expressed by decreased time spent in the central part of arena. The increased number of rearings with shorter duration observed in P32 animals suggests that early-life SE affects non-specific excitability (emotionality) in adolescence (Fig. 2C, D).

3.2. Effects of early-life SE on habituation (OFF-OFF-ON)

Comparison of OFF sessions with the ON session revealed significant increases in the distance moved in both the control and SE rats. This finding suggests that both groups of animals were able to perceive the change in the arena in the 3rd session (Fig. 3B, effect of session $F_{(1,38)} = 23.10$, $p < .001$). In addition, analysis of the number and duration of object investigations revealed that SE animals approached the object less frequently and spent significantly less time investigating the object (Fig. 3C number and duration $t = 2.48$, df = 19, $p = .03$; t = 2.48, df = 19, $p = .02$, respectively). Decreased exploration of the object placed into arena known to be safe could reflect increased anxiety in SE animals.

3.3. Effects of SE on social-mediated behaviors in adulthood

3.3.1. Sociability/social novelty preference

In the sociability phase at P60, both control and SE rats preferred to explore the unfamiliar rat (Stranger 1) over a non-social stimulus (empty cage), but SE animals spent less time investigating Stranger 1 compared with controls (treatment effect $F_{(1,30)} = 97.15$, $p < .001$; stimulus effect $F_{(1,30)} = 640.19$, $p < .001$, and treatment x stimulus effect $F_{(1,30)} = 82.51$, $p < .001$). In the social novelty/preference test, animals could choose between Stranger 1 and Stranger 2, and animals in both groups demonstrated a preference for social novelty, i.e., for Stranger 2 (treatment effect $F_{(1,30)} = 11.18$, $p = .002$; stimulus effect $F_{(1,30)} = 42.31$, $p < .001$; and stimulus x treatment effect $F_{(1,30)} = 12.73$, $p = .001$). However, in social novelty/preference SE animals spent less time investigating a new social stimulus, Stranger 2. When the animals were tested repeatedly at the P100, P120, and P140 the social mediated behavior remain unchanged. Sociability phase: P100 (treatment effect $F_{(1,30)} = 14.95$, $p < .001$, stimulus effect $F_{(1,30)} = 40.54$, $p < .001$, and treatment x stimulus effect $F_{(1,30)} = 10.74$, $p = .003$); P120, treatment effect $F_{(1,30)} = 7.60$, $p = .01$; stimulus effect $F_{(1,30)} = 37.32$, $p < .001$ and treatment x stimulus effect $F_{(1,30)} = 6.11$, $p < .01$; P140, treatment effect $F_{(1,30)} = 8.82$, $p = .006$, stimulus effect $F_{(1,30)} = 27.08$, $p < .001$ and treatment x stimulus effect $F_{(1,30)} = 7.42$, $p = .01$.

Social novelty/preference: P100 (treatment effect $F_{(1,30)} = 15.40$, $p = .001$; stimulus effect $F_{(1,30)} = 61.08$, $p < .001$; and treatment x stimulus effect $F_{(1,30)} = 21.13$, $p = .001$. P120 (treatment effect $F_{(1,30)} = 7.78$, $p = .009$; stimulus effect $_{(1,30)} = 45.97$, $p < .001$ and treatment x stimulus effect $F(_{1,30}) = 7.78$, $p = .009$. P140 (treatment effect $F_{(1,30)} = 15.54$, $p < .001$; stimulus effect $F_{(1,30)} = 35.14$, $p < .001$ and treatment x stimulus effect $F_{(1,30)} = 11.66$, $p < .002$ (Fig. 4B). Finally, repeated encounter with Stranger 1 in the social novelty/preference phase, revealed decrease in social investigation in both control and SE animals. At P60, treatment effect $F_{(1,15)} = 55.67$, $p < .001$; session effect $F_{(1,15)} = 441.85$, $p < .001$ and treatment x session effect $F_{(1,15)} = 77.77$, $p < .001$. At P100, treatment effect F $_{(1,15)} = 10.78$, $p < .005$; session effect $F_{(1,15)} = 39.32$, $p < .001$ and treatment x session effect F $_{(1,15)} = 14.82$, $p = .002$. At P120, treatment effect F $_{(1,15)} = 6.33$, $p = .02$, session effect $F_{(1,15)} = 38.29$, $p < .001$ and treatment x session effect $F_{(1,15)} = 7.49$, $p = .01$. At P140, treatment effect $F_{(1,15)} = 7.58$, $p = .01$, session effect $F_{(1,15)} = 30.20$, $p < .001$ and treatment x session effect $F_{(1,15)} = 8.73$, $p = .01$.

Taken together, the sociability and social novelty /preference expressed as the drive to approach and explore conspecifics were markedly lower in SE animals and persisted with time after SE.

3.3.2. Social recognition or discrimination

Early SE negatively affected the ability of animals to recognize and discriminate conspecifics in adulthood. Except at P120, in the 1st session, SE animals spent significantly less time investigating the juvenile rat (J1) compared with controls. Repeated exposure to the same juvenile (J1) during the 2nd session resulted in a reduction in time spent investigating the previously encountered juvenile in both groups (session effect: P80, $F_{(1,18)} = 17.09$, $p < .001$; P100, $F_{(1,18)} = 73.55$, $p < .001$; P120, $F_{(1,18)} = 106.72$, $p < .001$; and P140, $F_{(1,18)} = 83.27$, $p < .001$) and (treatment effect: P80, $F_{(1,18)} = 27.80$, $p < .001$; P100, $F_{(1.18)} = 14.19$, $p = .001$; and P140, $F_{(1,18)} = 10.69$, $p = .004$). Except at P80, SE animals investigated both encountered juveniles equally, whereas controls spent more time investigating the novel juvenile (J2) at all time-points during testing (treatment effect: P80, $F_{(1,36)} = 6.12$, $p < .018$; P100, $F_{(1,36)} = 4.43$, $p = .042$; P120, $F_{(1,36)} = 42.73$, $p < .001$; P140, $F_{(1,36)} = 19.26$, $p = .001$), and (discrimination effect: P80, $F_{(1,36)} = 0.20$, $p = .66$; P100, $F_{(1,36)} = 4.47$, $p = .041$; P120, $F_{(1,36)} = 3.53$, $p = .006$; and P140, $F_{(1,36)} = 19.08$, $p < .001$). These findings suggest decreased social investigation by SE animals compared with controls. Further, control animals were able to recognize and discriminate between the previously encountered (J1) and the novel juvenile (J2). On the contrary, SE animals were not able to discriminate between the previously encountered juvenile (J1) and the novel juvenile (J2). The results are depicted in Fig. 5B. Therefore, SE animals were not able to form a short-term olfactory memory for the previously encountered juvenile.

3.3.3. Morris water maze (MWM)

The time required to locate the platform and the swimming distance to reach the submerged platform decreased across the five successive sessions at a similar rate in both groups (control, $F_{(4,112)} = 38.72$, $p < .001$ and SE, $F_{(4,112)} = 32.43$, $p < .001$). Although the SE animals

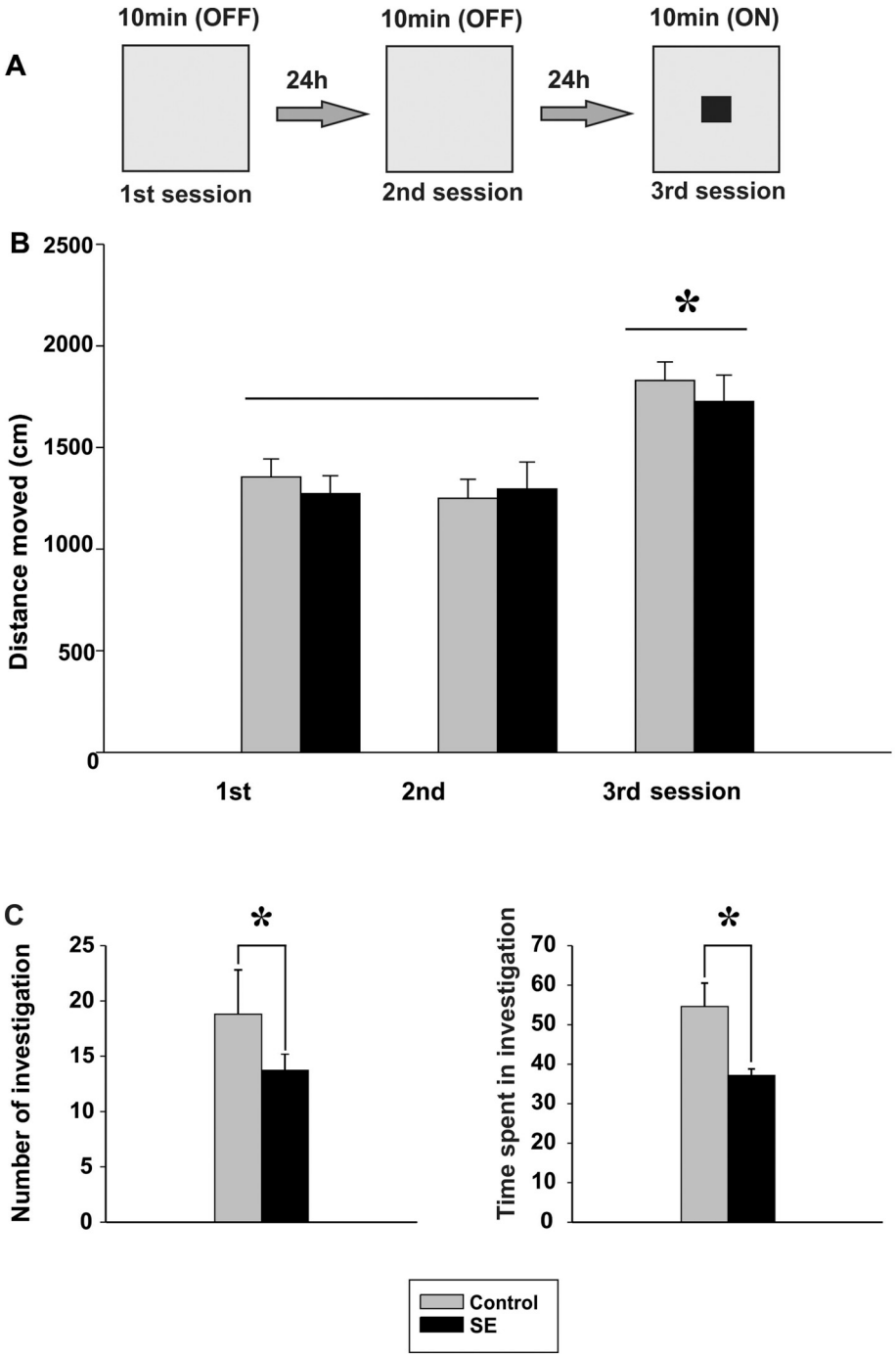


Fig. 3. Effect of early-life SE on habituation in adult rats (controls, n = 10; SE, n = 11). A, Scheme of the experiment; B, distance moved during the 1st, 2nd, and 3rd sessions. The 1st and the 2nd sessions with an empty arena, and the 3rd session with a solid object placed in the center of arena. C, Object response. Mean and SEM (±) values of total number (left graph), and the total duration (right graph), of the object investigation. * Significant difference between sessions, or significant difference compared with controls.

performed worse, as indicated by an increase in the time required to find the platform and longer distance swum, the differences between groups were not statistically significant in either the time ($F_{(1,112)}$ = 3.34, p = .08) or swimming distance to reach the platform ($F_{(1,112)}$ = 2.06, p = .16). The post hoc test revealed that only in the 4th session the SE animals tend to have longer latencies and to swim a longer distance to escape onto the platform (Fig. 6B). Also, across sessions, the swimming speed decreased to a similar extent in both the control and SE groups ($F_{(4,112)}$ = 6.29, p < .001). Swimming speed was not significantly different between groups ($F_{(1,112)}$ = 0.99, NS.). The acquisition of an eight-trial reversal learning session with the platform located in the opposite quadrant also revealed no significant differences in any of the evaluated parameters, suggesting that cognitive flexibility was not affected in either the controls or the SE animals.

The immediate-retention probe trial (PT1) performed shortly after the last learning trial showed intact retention of recent training in both the control and SE groups. With repeated probe trials (PT2), the time spent in the training quadrant decreased in both the control and SE groups (Fig. 6C effect of session $F_{(1,28)}$ = 5.46, p = .02). The post hoc analysis, however, showed a significant decrease only in SE animals, indicating impairment in long-term memory retention after a 10-day interruption. Comparison between PT2 and PT3 after the reversal

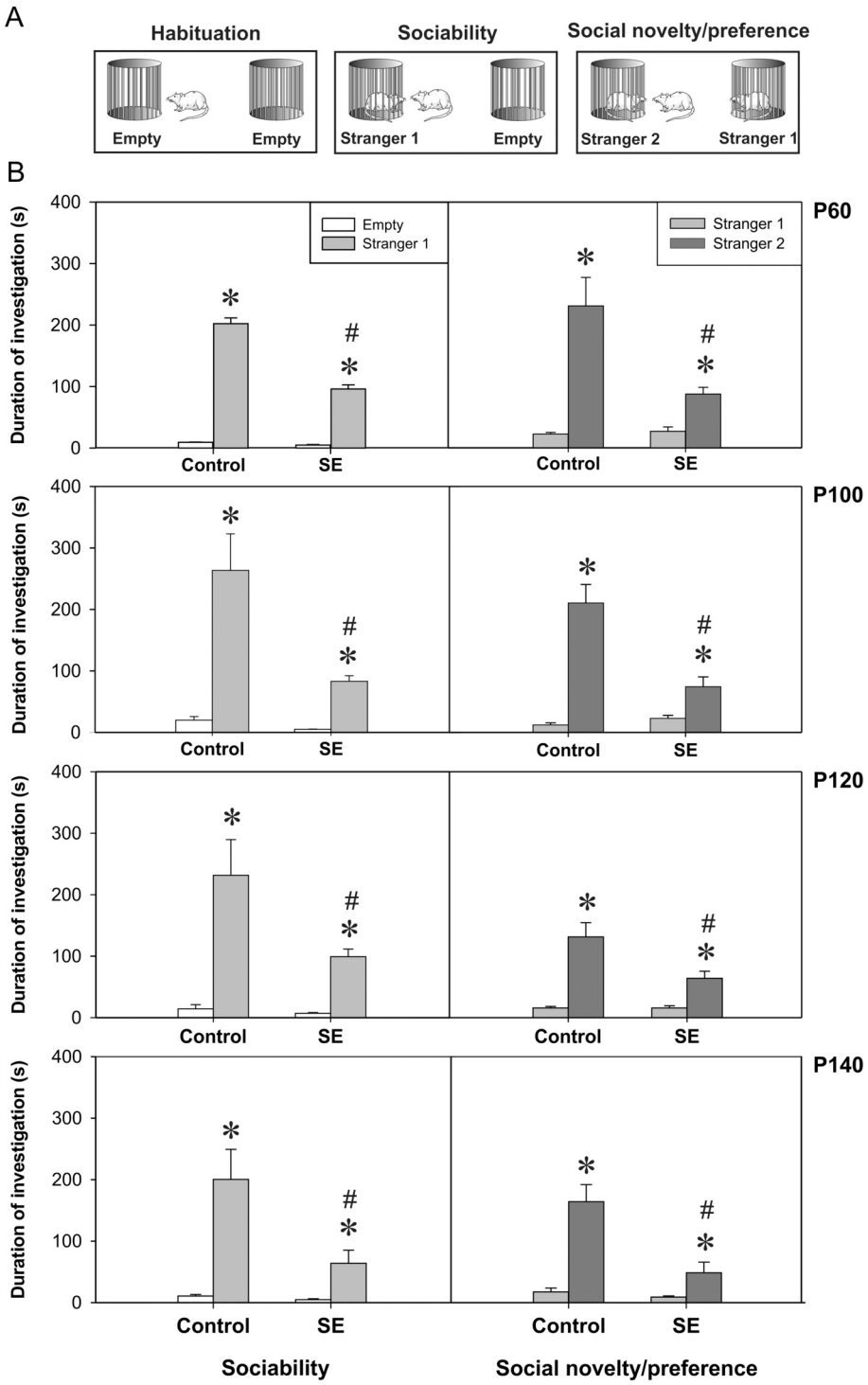


Fig. 4. A, Scheme of the sociability/social novelty preference: Habituation phase consisting of exposure to two empty cages; Sociability phase consisting of presentation to an unfamiliar rat (S1) and, social novelty/preference phase consisting of exposure to one familiar rat (S1) and a novel rat (S2). B, Effect of early-life SE on sociability and social novelty/preference. Animals, (controls, $n = 7$; SE, n = 10) were tested at P60, P100, P120, and P140 (from top to bottom). Abscissae: The sociability phase and social/novelty preference phase for both control and SE groups. Ordinates: mean and SEM (±) values for total duration of investigation in seconds. * Significant difference compared with non-social stimulus (empty cage) for both controls and SE rats; # significant difference between controls and SE rats in investigation time of a stranger rat (S1) in the sociability phase. * Significant difference between a novel stranger (S2) and stranger (S1) for both controls and SE animals in the social novelty/preference phase. #Significant difference between control and SE rats in investigation time of a novel stranger (S2) in the social novelty/preference phase.

training, i.e., with the platform relocated to the opposite quadrant, showed a similar increase in the time spent to relocate the platform in both the control and SE groups, indicating intact short-term retention of the reversal learning in both groups (effect of session $F_{(1,28)} = 7.93$, $p = .009$).

These results indicate that acquisition of the MWM task was unimpaired but there was a deficit in the memory retention with a 10-day delay.

3.3.4. *Damage to cortical and subcortical structures after SE (experiment 2)*

Systematic neuropathological examination of brains collected 24 h after SE revealed apparent neuronal degeneration in the hippocampus, medial amygdala, and the mediodorsal thalamus (i.e these brain areas contained >10 labeled cells per area per section) (Fig. 7) in 75–87% of examined animals. Considerable variability however appeared in severity and distribution of neuronal damage among individual animals. In the hippocampus, FJB-positive cells appeared in 7 of 8 animals in the pyramidal layer of the CA1 subfield (median 27.5 FJB-positive cells/mm^2 with 25 and 75% - (18.6, 79.6)). Only scattered degenerated neurons were detected in the pyramidal layer of the CA3 subfield and in the inner part of the granular cell layer of the dentate gyrus. These results are in line with our early observation (Druga et al., 2010). As published before (Kubová et al., 2002) neuronal damage was consistently observed in the mediodorsal nucleus of the thalamus. Fluoro-

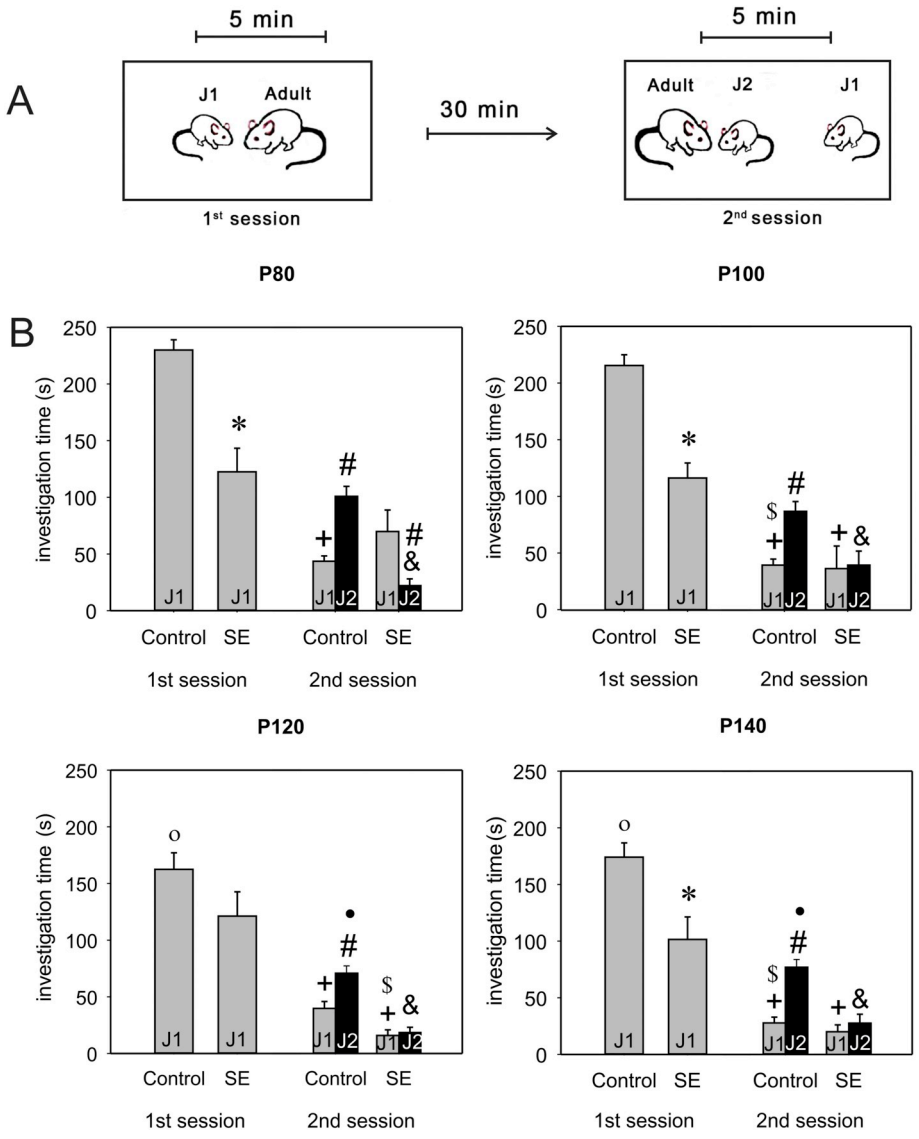


Fig. 5. Scheme of the social recognition or discrimination procedure. A, 1st session, exposure of adult rat to a juvenile (J1), 2nd session, exposure of adult rat to the same juvenile (J1) and a novel juvenile (J2). B, Effect of early-life SE on social recognition or discrimination. Animals (controls, n = 11; SE, $n = 9$) were tested at P80, P100, P120, and P140. Abscissae: the 1st (recognition) and 2nd (discrimination) session for both control and SE groups. Ordinate: mean and SEM (±) of total investigation time in seconds. *Significant difference compared with control group in the 1st session; + significant difference compared with the 1st session; # significant difference compared with J1 in the 2nd session; significant difference compared to control group in the 2nd session; ○ significant difference compared with P80 and P100.

Jade B-positive cells were found in 7 of 8 animals throughout the entire rostrocaudal extent of MD, most of which were located in the periphery of the central segment of the nucleus (median with 25 and 75% - 41.9 FJB-positive cells/mm^2 (37.0, 47.7)). In the amygdala, neurodegeneration was detected in 6 of 8 animals and most of degenerating neurons were found in medial amygdalar nuclei (median with 25 and 75% - 61.4 FJB-positive cells/mm^2 (11.8, 282.8). In all animals, scattered FJB-positive neurons appeared in the lateral dorsal and lateral posterior nucleus of the thalamus, in the second and third layer of the piriform cortex, in the dorsal endopiriform nucleus, and in the claustrum. No damage was detected in the prefrontal cortex. There were no FJB-positive cells in any of control animals.

4. Discussion

The results of the present study support the growing body of literature showing that early-life SE result in long-term behavioral alterations. Here we provide evidence that early-life SE decreases the responsiveness of animals to indifferent or social cues, process them, and elicit the appropriate behavioral response. Especially, the decreased social investigation and social discrimination by animals exposed to SE indicate disruption of the social motivation/incentive state essential for normal displays of social behavior and subsequently socio-cognition. As for tasks with aversive motivation, such as the MWM, early-life SE did not impair acquisition but leads to delayed memory retention. In parallel study, we demonstrate acute neuronal damage in several brain areas involved cognitive and emotional brain domains that can significantly contribute to development of functional impairment.

Early SE did not cause disruption of a between-session habituation in the open field test, which is considered to be an index of non-associative memory (Grissom and Bhatnagar, 2009; Leussis and Bolivar, 2006; Schmid et al., 2014) in juvenile (P25) and adolescent (P32) animals. According to cognitive map theory, exploration of a novel environment over time helps an animal to construct a cognitive map of that environment in the hippocampus and as this map is established, exploration is reduced (O'Keefe and Nadel, 1978). Both controls and SE animals exhibited a decrease in distance moved over time. As demonstrated before between-session habituation appears at the end of the 4th postnatal week, reflecting maturation of the inhibitory circuits in the forebrain (Campbell and Stehouwer, 1980). The ability to habituate is highly dependent on hippocampal integrity (Roberts et al., 1962). Results of neuropathological examination show that SE induces neurodegeneration in the hippocampus only in a subpopulationof neurones. Hence limited effect of SE on habituation seen in P18 rats reflect less severe initial damage to the hippocampus. It also raises the possibility

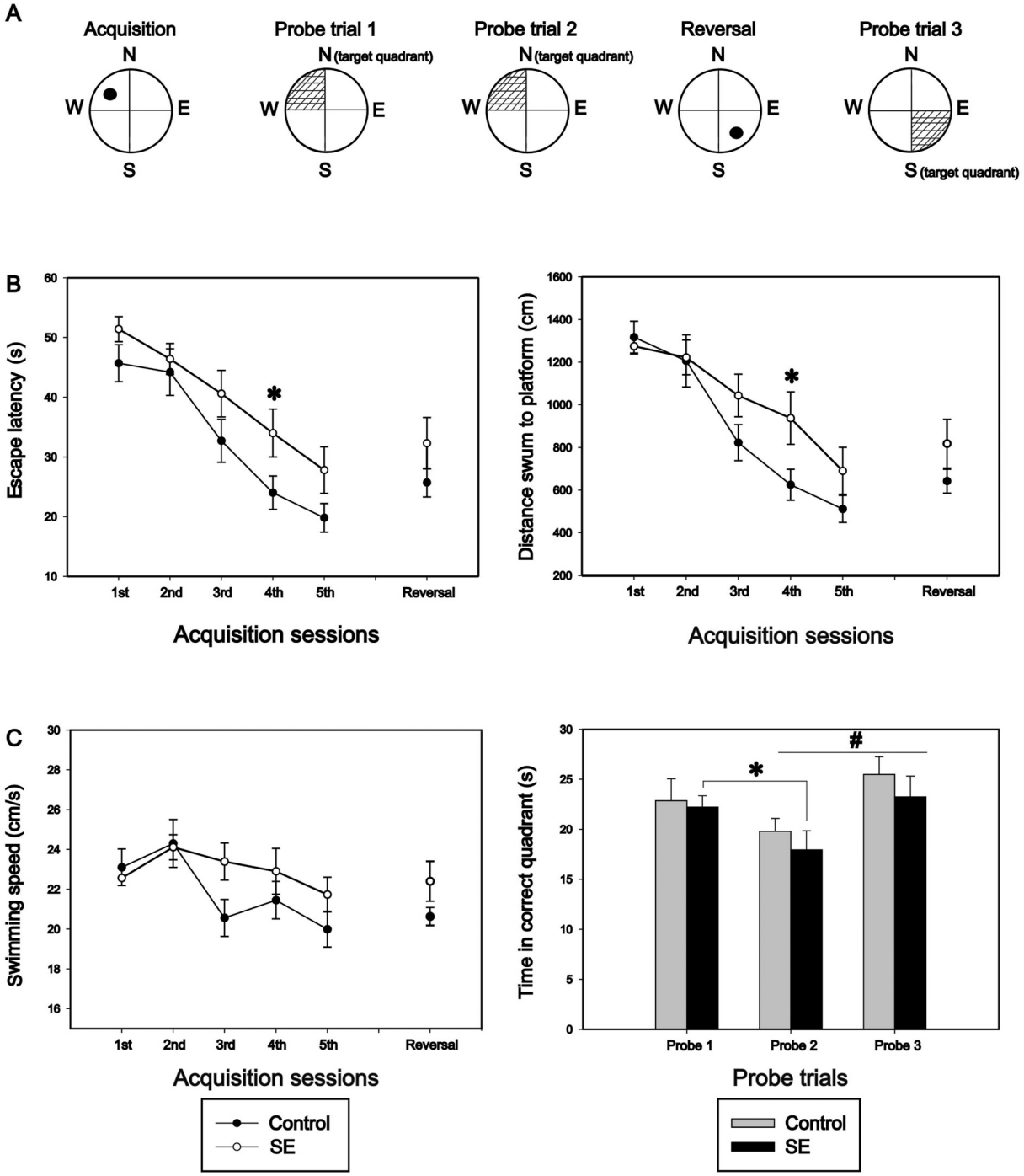
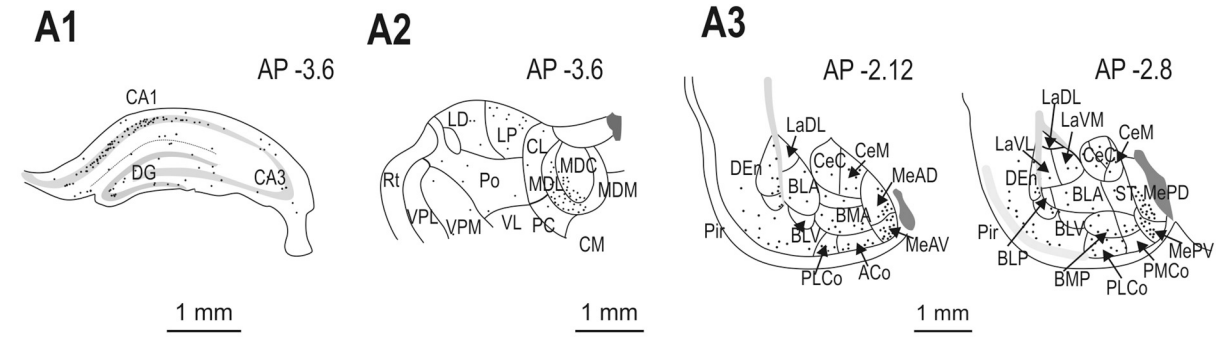
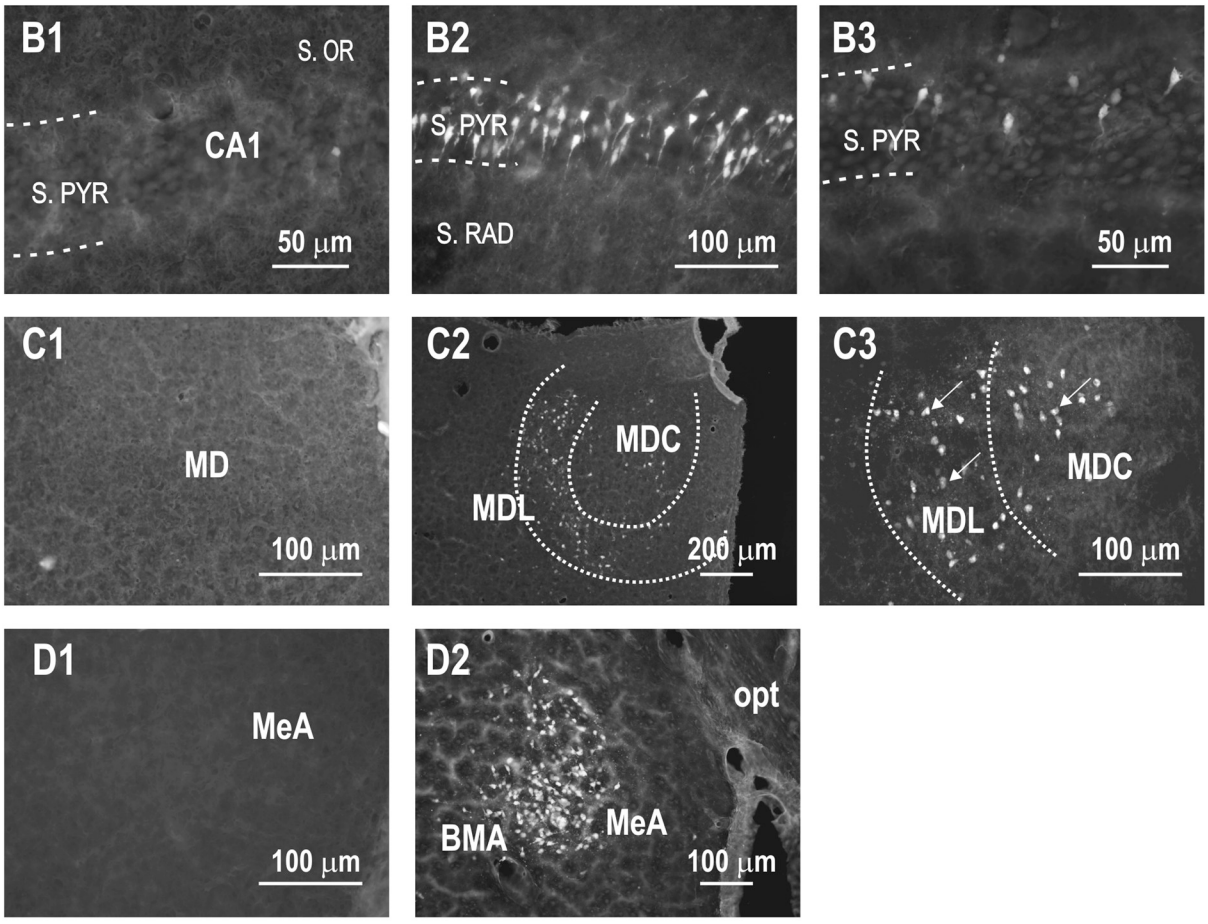


Fig. 6. Spatial learning and memory in the Morris Water Maze (MWM). A, Diagram of the experimental design. Acquisition sessions: the rats (controls, $n = 13$; SE, $n = 17$) were trained to find a hidden platform located in the northwest quadrant of the water maze in five daily sessions (each session consists of 8 trials). Probe trial one (PT1) was performed after the final acquisition session with the platform removed to test for immediate retention. Probe trial two (PT2) was performed 10 days after the PT1 to test for delayed retention. After PT2, reversal learning, consisting of one session (8 trials), was performed followed by PT3 to test spatial memory of the novel location. B, Upper graphs: mean and SEM (±) values for escape latencies and distance swum (cm) to platform; C, Lower graphs: mean and SEM (±) values for swimming speed (cm/s) and mean and SEM (±) values for quadrant time (s) in the probe trials. *Significant difference compared to controls in the 4th session and significant difference compared to PT1, # significant difference compared to PT2.

that ongoing maturation of the hippocampal formation and its functions can compensate for moderate injury.

SE animals were more anxious compared to controls. Both P25 and P32 with early SE spent a shorter time in the central part of open field arena. This behavioral response may be due to different rates of habituation to novelty/a different exploratory drive and anxiety-like behavior (Salomons et al., 2012). Moreover, adolescent (P32) animals displayed a greater number of rearing episodes with shorter duration than controls. Rearing behavior is one of several ethologic measures modulated by various factors such as anxiety/emotionality (for review, see Lever et al., 2006). Between juvenility and/or adolescence the brain is in transition differing markedly both anatomically and neurochemically from that of newborns, weanlings, or adults. During this period, substantial remodeling occurs in brain areas that are involved in





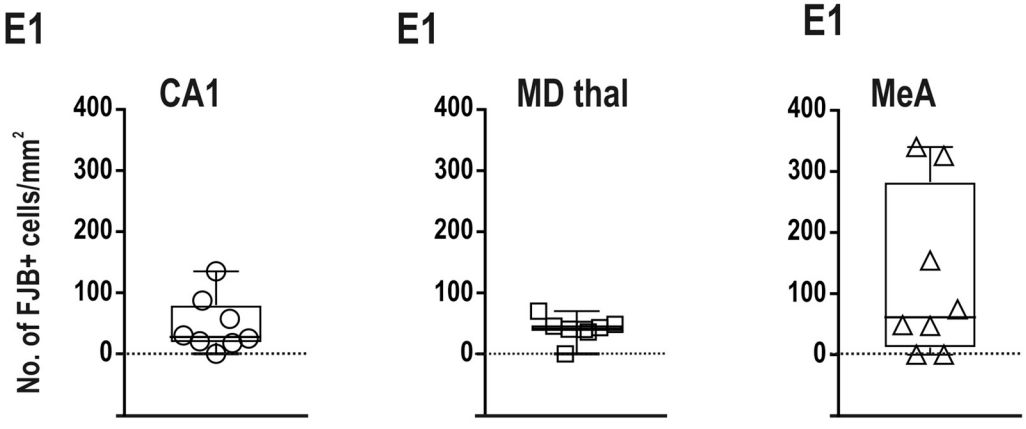


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Fig. 7. The distribution and severity of SE-induced damage 24 h after SE. Computer-generated plots demonstrating the distribution of degenerating cells in the hippocampus (A1), thalamus (A2), and amygdala (A3). Degenerating cells were labeled with Fluoro-Jade B and plotted from sections with a computer-aided digitizing system (Minnesota Datametrics, St. Paul, MN). The anatomic boundaries were drawn from adjacent cresyl violet-stained sections using a stereomicroscope equipped with a drawing tube. The corresponding rostrocaudal level relative to bregma is shown in the upper right corner of each plot (according to Paxinos and Watson, 1986). Each black dot represents one labeled cell. Microphotographs B2 and B3 illustrate the distribution of Fluoro-Jade B-positive cells (white arrows) in the CA1 subfield of the hippocampus in the animal exhibiting the most extensive damage (B2), from the animal with mild damage (B3) and from the control animal (B1) - (s. or – stratum oriens, s. pyr - stratum pyramidale, s. rad – stratum radiatum). Typical distribution of Fluoro-Jade B-positive cells in the mediodorsal thalamic nucleus (MD) is shown in microphotographs C2 (lower magnification) and C3 (higher magnification). Panel C1 shows MD in control animal. Panel D2 illustrates distribution of Fluoro-Jade B-positive cells in the medial amygdaloid nuclei nucleus of the amygdala (MeA) in the animal with SE (D2) and in control (D1). In controls, Fluoro-Jade B-positive cells were not detected (microphotographs on the left B1, C1 and D1). High power photomicrographs were taken with a Olympus BX53 microscope.

Quantification of neuronal damage in the CA1 subfield of the hippocampus (E1), the mediodorsal nucleus of the thalamus (E2) and the medial amygdaloid nuclei (E3). Numbers of neurons in individual animals are plotted over box plots (median with 25–75%) with whiskers indicating minimal and maximal values. Values are expressed as a mean of 3 sequential 50 μm thick sections and symbols represent number of FJB-positive cells per mm^2 in individual animals. Circles – CA1; squares – MD; triangels - MeA.

Abbreviations: CA1 = CA1 subfield of the hippocampus, CA3 = CA3 subfield of the hippocampus, DG = dentate gyrus; MDM = mediodorsal thalamic ncl., medial part, MDC = mediodorsal thalamic ncl., central part, MDL = mediodorsal thalamic ncl., lateral part, CL = centrolateral thalamic ncl., PC = paracentral thalamic ncl., CM = central medial thalamic ncl., VL = ventrolateral thalamic ncl., VPM = ventral posteromedial thalamic ncl., LD = laterodorsal thalamic ncl., LD = laterodorsal thalamic ncl., Rt = reticular thalamic ncl; LaDL = lateral amygdalar ncl., dorsolateral; Den = dorsal endopiriform ncl.; Pir = piriform cx.; BLA = basolateral amygdaloid ncl, anterior part; BLP = basolateral amygdaloid ncl, posterior part; BLM = basolateral amygdaloid ncl, medial part; BLV = basolateral amygdaloid ncl, ventral part; CeC = central amygdaloid ncl, capsular; CeM = amygdaloid ncl, medial division; BMA = basomedial amygdaloid ncl, anterior part; PLCo = posterolateral cortical amygdaloid ncl.; PMCo = posteromedial cortical amygdaloid ncl.; ACo = anterior cortical amygdaloid ncl.; MeAD = medial amygdaloid ncl., anterodorsal part; MeAV = medial amygdaloid ncl., anteroventral part; MePV = medial amygdaloid ncl., posteroventral part; MePD = medial amygdaloid ncl., posterodorsal part; LaDL = lateral amygdaloid ncl., dorsolateral part; LaVM = lateral amygdaloid ncl., ventromedial part; AHi = amygdalohippocampal area. Scale bars: panels A1, B1, C1 = 1 mm; panels A2, B2, B3, C2 = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

emotional and learning processing (Tsoory and Richter-Levin, 2006). In terms of neural, hormonal, and behavioral responsiveness, adolescent rats differ from their counterparts of other ages. Behavioral changes that characterize the period of adolescence include emotional instability and impulsivity. The differences in rearing behavior observed between control and SE rats can be due to differences in emotional dimensions such as anxiety, curiosity, arousal and locomotor activity (Semple et al., 2013; Spear, 2000). In light of this, our results indicate that adolescent-age animals experiencing seizures are more vulnerable to emotional disturbances, but this possibility has to be further confirmed.

Similarly to younger animals, adults with early SE did not show any signs of altered habituation in new context, expressed by an increase in distance moved when a new stimulus was present in the environment. In accordance with previous studies (Groticke et al., 2007) the SE animals failed to investigate a novel object in the object exploration task. Decreased exploration of a new object placed in known, safe area has been claimed to reflect rather trait than state anxiety (Belzung and Griebel, 2001; van Gaalen and Stecker, 2002), but because individuals with high trait-anxiety often show an increased tendency to display high state anxiety as well, it is not easy to separate these phenomena one from the other.

Early SE did not affect learning ability in a spatial reference memory task. Animals learned the task at the same rate, but in accordance with our previous study, SE animals never reached the same level as controls (Kubová and Mareš, 2013). During acquisition, the shape of the learning curve did not differ from that of the control group; nevertheless escape latency and swimming distance to the platform were longer in SE animals. The SE rats swam faster in the 3rd, 4th, and 5th sessions, suggesting that the animals were not equivalent across sessions in their swimming ability and/or motivation to escape from water. Reversal learning in the MWM reveals whether or not animals can extinguish their initial learning of the platform's position and acquire a direct path to the new goal position. In SE rats, standard performance measures such as transfer latency, swimming distance, and swimming speed revealed that SE rats did not exhibit perseveration for the previous location during reversal learning. Taken together, cognitive flexibility of adult animals with early SE was not impaired because they learned the new location of the platform as rapidly as controls. Also, the results of the probe trials revealed that immediate retention of reference memory (PT1) and reversal learning (PT3) were not affected in the SE animals, indicating no differences in the consolidation of immediate retention of spatial information. Interestingly, the animals experiencing early-life SE had a less accurate spatial response after a 10-day delay indicating an altered delayed retention. Our study confirms the finding of (Barry et al., 2016) showing that early-life seizure may not necessarily lead to MWM spatial deficits in adulthood.

As reported before, the extent and location of hippocampal damage associates with the severity and pattern of spatial memory deficits (Broadbent et al., 2004; Kubová and Mareš, 2013; Lee and Kesner, 2003; Maia et al., 2014). Neuropathological examination of parallel group of animals with SE revealed acute neurodegeneration in the septal hippocampus, primarily in the CA1 subfield, with considerable variability among animals in the damage extent. Extensive damage occurred only in subpopulation of animals and damage to other hippocampal subfields, the dentate gyrus and CA3, was only negligible. Long-term, early SE resulted in hippocampal atrophy that was proven with MRI (Nairismägi et al., 2006) morphometry and stereology (Kubová and Mareš, 2013; Suchomelová et al., 2015) in adults with SE at P10–12. Interestingly, we found that the best performance in MWM expressed as cumulative latency was significantly shorter in animals with early SE than in controls and that there was negative correlation between cumulative latency and the hippocampal volume.

SE animals displayed marked deficits in sociability and in a preference for social novelty. These findings are consistent with the results of previous studies showing that early-life seizures in rats lead to social behavior impairments (Castelhano et al., 2013; Castelhano et al., 2015; Hernan et al., 2014; Holmes et al., 2015). Moreover, this study revealed that animals experiencing seizures displayed social deficits over a long period of their life, suggesting persistent impairment in normal sociability. When confronted with a juvenile, investigative behavior declined over time in both control and SE animals. Upon re-exposure to the same juvenile and a new one, however, the investigative behavior of SE animals was directed toward both juveniles. In terms of social recognition ability, these findings indicate that SE animals were not able to discriminate between the two juveniles. The ability to distinguish familiar from novel conspecifics through individual odor cues is relevant to both socialization and social memory (Sandi and Haller, 2015; Yang et al., 2011). This behavioral phenomenon is interpreted to mean that adult animals form a transient short-term working memory for olfactory

characteristics of particular juveniles (for review, see Meira et al., 2018; van der Kooij and Sandi, 2012). In addition to disruption of learning and memory the diminished discrimination by SE rats may also involve disruption of motivation-emotionality. Impairments in emotional-motivational behavior, decreased investigation, and low preference for social novelty are reported in a pilocarpine model of early-life seizures, (Castelhano et al., 2013) and also in a model of fluorothyl-induced ELS (Holmes et al., 2015) resembling symptoms of ASD. Nevertheless, the present study did not address how to separate them. Alternatively, the social memory deficit seen in social discrimination test could reflect impairment of sociability as well as social memory encoding and/or maintenance.

Consistently with our previous studies (Kubová et al., 2002; Kubová et al., 2001) neuropathological examination revealed consistent neuronal damage in the mediodorsal nucleus of the thalamus (MD), particularly in its central and lateral segments. The mediodorsal nucleus acts as a critical link between the basal forebrain and the prefrontal cortex (Krettek and Price, 1977). Behavioral studies have indicated that the MD is involved in learning, memory, decision making, anxiety and social behavior. Particularly, tasks assigned to the central and lateral segments of the mediodorsal nucleus include the olfactory related functions, memory, and eye-movements (McCrea and Baker, 1985). Recent studies demonstrated that early postnatal damage to the MD disrupt development of connections between MD, the prefrontal cortex (PFC) and basolateral amygdala (BLA) and impairs various types of visual-recognition-related memory and social behavior later in life. Animals with early damage to the MD display more anxiety-like behavior and disrupted recognition memory in the novel object recognition paradigm (Ouhaz et al., 2017; Ouhaz et al., 2015; Parnaudeau et al., 2018). In addition to neurodegeneration observed in the MD, degenerating neurons were found also in other brain areas involved in emotional and social behavior. The high number of FJB positive cells occurred in the medial amygdala (MeA) that plays a key role in a variety of mammalian social behaviors, anxiety and innate emotional behavior (Adolphs, 2010; Twining et al., 2017). Taken together, we hypothesize that neuronal damage to “brain hubs” in networks essential for emotional and social behavior and memory formation and consequent disruption of normal network development and maturation is responsible for behavioral alterations detected in animals with early SE.

Early remodeling of brain circuitry seem to be a most intriguing explanation for our findings, but it must be acknowledged that this is not the only possible mechanism. Other explanation involve interrupted hippocampal neurogenesis (Dunleavy et al., 2014), reduction in the dendritic spine density and decrease of dendritic arbors (Jiang et al., 1998) or changes in neurotransmission (Lauren et al., 2013) reported in various models of early seizures or SE. In addition, early stress associated with SE can participate in functional alterations (for review see (Avishai-Eliner et al., 2002; Holmes et al., 2005). These widespread changes could contribute significantly to synaptic reorganization and rewiring of the brain. Such actions may be especially important in immature animals who are in the process of developing cognitive and emotional functions.

This study unambiguously demonstrated that early-life SE leads to long-lasting dysfunction in various behavioral dimensions, interpretable in terms of emotional cognitive and social comorbidities present in such conditions as early-onset dementia or ASD. Discovering the critical network alterations and mechanisms responsible for network remodeling would provide putative therapeutic targets for comorbidities of epilepsy in later life.

Acknowledgments

We gratefully acknowledge the expert technical help by Mrs. Blanka Čejková and Mrs. Irinka Nešev. This study was supported by grant 16-04726S of the Czech Science Foundation and by the Czech Health Research Council (AZV) grant 16-29857A and support for long-term conceptual development of research organization RVO: 67985823. Ales Stuchlík was supported by the Czech Science Foundation grant 17-04047S. Institutional support was provided by Research Project RVO: 67985823.

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