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MATERNAL SEPARATION DURING A SPECIFIC POSTNATAL TIME WINDOW PREVENTS REINFORCEMENT OF HIPPOCAMPAL LONG-TERM POTENTIATION IN ADOLESCENT RATS

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Abstract—In an attempt to develop an animal model to study the etiology of brain dysfunction in relation to early life experience, we tested the hypothesis that early-life stress during specific postnatal time windows affects long-term potentiation (LTP) reinforcement in adolescence. Male Wistar rat pups were stressed by separation from their dams for 24 h at postnatal day (PND) 4, 9, or 18. The animals were tested for reinforcement of LTP at adolescence (9 weeks old) by exposing them to a 2-min swim-stress. Here, we show that maternal separation during (at PND9) but not at the beginning (at PND4) or after (at PND18) the stress-hyporesponsive-period of the hypothalamic–pituitary–adrenal-axis impairs emotional LTP-reinforcement in adolescent animals. Thus, this *in vivo* model allows the investigation of physiological and pathophysiological emotional information processing at the cellular level in freely behaving adolescent animals.

Key words: early stress, LTP-reinforcement, swim-stress, emotion, SHRP.

Disturbance of the infant–parent relationship early in life has been shown to affect cognitive function as well as emotionality, and represents a considerable risk factor for the development of behavioral disturbances and mental disorders (Sullivan and Brake, 2003; de Kloet et al., 2005; Pryce et al., 2005; Giachino et al., 2007). The maternal separation paradigm (MS), i.e. single or repeated separation of the newborn pups from their dam for a certain time period is a well-established animal model to analyze systematically the etiology of behavioral and brain dysfunctions. Most of these studies reveal the critical importance of specific time windows early in life for the behavioral, endocrine and neuroanatomical outcome of MS in adolescence and adulthood (Lehmann et al., 1999; Lehmann and Feldon, 2000; Cirulli et al., 2003; Bock et al., 2005; Holmes et al., 2005; Pryce et al., 2005). However, little is known with respect to molecular, cellular and physiological correlates. In particular, a single episode of 24-h MS at different time windows during the first 3 weeks of life provides a tool suited for the investigation of the ontogeny-dependent impact of early-life stress in rats (Lehmann and Feldon, 2000). Such stress paradigms trigger a cascade of events resulting in a complex outcome, involving a down-regulation of gluco- and mineralo-corticoid receptors (Sutanto et al., 1996) and modifications of the hippocampal serotonergic system (Vázquez et al., 2000).

On the cellular level, hippocampal long-term potentiation (LTP) serves as a model for memory formation. LTP, similar to the different phases of memory, can be divided into a protein synthesis–independent early-LTP lasting about 4–6 h, and a protein synthesis-dependent late-LTP, with a duration of more than 8 h. An associative mechanism ('synaptic tagging') has been suggested for the transformation of an early- into a late-LTP (Frey and Morris, 1997). In a weak, electrically-activated synaptic input S1 early-LTP can be induced which is characterized by the setting of a 'synaptic tag' being active for a distinct period of time. If in a second, temporally related but independent strong heterosynaptic input S2 the synthesis of plasticity-related-proteins is induced (which can be captured by the tag of the weakly stimulated synapse), a transformation of early-LTP into late-LTP in that particular input S2 can be obtained (LTP-reinforcement). In freely behaving adolescent rats, we have previously shown that early-LTP can be reinforced into a late-LTP by emotional challenges presented within an effective time-window (Korz and Frey, 2003, 2005). Such reinforcement or emotional tagging (Richter-Levin and Akirav, 2003; Korz and Frey, 2004) of LTP can be induced by brief acute swim-stress in context-naïve animals requiring the activation of mineralocorticoid receptors (MR) by corticosterone (Korz and Frey, 2003) and serotonergic activation (Ahmed et al., 2006). Along these lines the present study investigated the impact of a single exposure to 24-h MS on behavioral reinforcement in adolescent rats, predicting that this stressful experience during 'childhood' has a long-lasting effect on LTP-reinforcement, i.e. memory formation. Furthermore, we intended to identify distinct time windows of hypothalamic–pituitary–adrenal (HPA) axis development, during which the impact of exposure to 24 h MS on LTP reinforcement and memory formation is most pronounced. This was experimentally tested by exposing the animals to MS at the beginning (at postnatal day (PND) 4), during (at PND9) or after (at PND18) the stress-hyporesponsive-period (SHRP) of the HPA axis. The SHRP was proposed to protect the juvenile, developing brain against the deteriorating effects of high levels of stress

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Abbreviations: DG, dentate gyrus; DRN, dorsal raphe nucleus; fEPSP, field excitatory postsynaptic potentials; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; LTP, long-term potentiation; MR, mineralocorticoid receptor; MS, maternal separation; PND, postnatal day; PSA, population-spike amplitude; SHRP, stress-hyporesponsive-period.

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hormones (Levine, 2001), however, it has been demonstrated in rats that especially 24-h MS can increase the levels of circulating corticosterone, the predominant glucocorticoid, even during the SHRP of the HPA axis (Levine et al., 1992), thus overcoming the protective function of the SHRP. Therefore, we hypothesize that MS during the highly vulnerable period in brain development, i.e. at PND9 (during the SHRP), has the most tremendous impact on LTP-reinforcement.

EXPERIMENTAL PROCEDURES

Animals and housing conditions

Male Wistar rats (strain Schönwalde) from our breeding colony at the Leibniz Institute for Neurobiology, Magdeburg, Germany, were used for all experiments. Pregnant females were checked for litters daily, and at the day of birth (PND0) litters were standardized to five males/five females per dam. At PND21, male pups of all experimental groups (see below) were weaned and housed in sex-matched groups of five animals per cage (cage dimensions: 59×38×25 cm; length×depth×height) until the electrophysiological preparation, after which they were housed in single-cages (40×25×18 cm) for 1 week until the onset of the electrophysiological experiments. All animals were housed in translucent plastic cages under controlled laboratory conditions (temperature: 21±2 °C; humidity: 55±5%) with free access to food and water, and an artificial 12-h light/dark cycle (light on at 06:00 h). Husbandry, comprising cage cleaning done once a week, was the same for all animals.

All experiments were performed in accordance with the European Communities Council Directive of 24th Nov. 1986 (86/609/EEC), and according to the German guidelines for the care and use of animals in laboratory research and the experimental protocols were approved by the ethics committee of the land, Saxony-Anhalt. All efforts were made to reduce the number of rats used in this study and their suffering.

MS

MS was performed by removing the dam, while the litter as a whole remained in the home cage for the next 24 h (starting at 17:30 h) at either PND4 (MS4, i.e. at the beginning of SHRP), PND9 (MS9, i.e. during SHRP), or PND18 (MS18, i.e. after SHRP). Each litter was weighed as a whole, and the home cage was transferred to a separate, temperature-controlled (31±2 °C) room. Neither food nor water was available during MS. After 24 h, each litter was weighed again, and reunited with the dam. Control animals remained undisturbed with their dam.

Surgery and electrophysiological recording

Adolescent male rats (8 weeks old) were weighed and anesthetized with Nembutal (50 mg/kg, injected intraperitoneally; Sigma-Aldrich, St. Louis, MO, USA). A monopolar recording electrode was implanted under stereotaxic control (TSE Systems, Midland, MI, USA) into the granule cell layer of the dentate gyrus (DG; coordinates AP −2.8 from bregma, L 1.8, 2.8–3.2 ventral from dura) and a bipolar stimulation electrode into the perforant path (coordinates AP −6.9 from bregma, L 4.1, 2.2–2.5 ventral from dura) of the right hemisphere. The skulls of rats were mounted in the stereotaxic frame with an angle, so that bregma was 1 mm higher than lambda. Each electrode consisted of an insulated stainless steel wire 125 μm in diameter. During preparation, test pulses were delivered to optimize the population-spike amplitude (PSA). The animals were allowed to recover from surgery for at least 1 week.

For the experiments, rats were placed in a recording box (40×40×40 cm) and the electrodes connected to a swivel by a flexible cable. This allowed the freely moving animals *ad libitum* access to food and water. The evoked field potentials were amplified (differential amplifier, Science Products, Hochheim, Germany), transformed by an analog/digital interface (CED 1401+, Cambridge Electronic Design, Cambridge, UK) and stored on a computer. Biphasic constant current pulses (0.1 ms per half-wave) were applied (Isolated Pulse Stimulator, Model 2100; A-M Systems Inc., Carlsborg, WA, USA) to the perforant path in order to evoke DG field potentials of about 40% of the maximum PSA. After registering a stable baseline for 1 h, LTP was induced by weak tetanic bursts (three bursts of 15 pulses of 200 Hz with 0.1 ms duration of each stimulus and 10 s inter-burst interval, same stimulus intensity as for PSA testing). After 2 min, a test pulse was applied to control for a sufficient initial potentiation. Fifteen minutes after tetanus animals were transferred into the water tank for a 2-min swim-stress (see below). Thirty minutes and then every 15 min after returning into the recording box, five test stimuli (10 s interpulse interval) were delivered and the mean values of field potentials were stored for the next 8 h. In addition, a 24-h value was obtained the next day. After finishing the experiment, an 800 μA test stimulus was applied to check for the reliability of the maximum response of the PSA. For analysis and presentation, the 15 min recordings were averaged in groups of four to yield 1-h values.

Swim-stress

Animals of the four experimental groups (controls, MS4, MS9, MS18) were randomly assigned to two different conditions: whereas one half of the animals remained in their recording box during the entire experiment (no-stress), the other half of the animals after protecting the electrode connections from water contact by Vaseline were exposed to swim-stress in a circular water tank (1.82 m diameter; 58 cm height, filled with latex-stained (Sakret, Gieβen, Germany) opaque water up to a level of 38 cm with the temperature set to 25±2 °C). For minimizing litter effects, not more than two animals per litter were chosen for each experimental condition (i.e. no-stress, swim-stress). The animals' behavior was recorded using a video camera and fed to a video tracking (HVS image analyzer, VP200) and analysis system (Watermaze by Richard Morris and Roger Spooner V. 2.17a) located in an adjacent room. After swimming, animals were towel dried, and transferred back into the recording box. All experiments started between 08:00 h and 09:00 h, and the experimenter was unaware of the early-life experience of the animals. The whole experimental procedure is given in Fig. 1. Swimmers (χ^2=0.362, *df*=3, *P*=0.948; controls: 0.28±0.040 mA; MS4: 0.30±0.024 mA; MS9: 0.28±0.030 mA; MS18: 0.26±0.033 mA) and non-swimmers of the different groups (χ^2=0.893, *df*=3, *P*=0.893; controls: 0.31±0.043 mA; MS4: 0.33±0.031 mA; MS9: 0.29±0.026 mA; MS18: 0.31±0.036 mA) were stimulated for test pulses and tetani by comparable intensities. In addition, there were no differences between swimmers and non-swimmers within the groups (*P*>0.25, each).

Statistics

The general linear model (GLM) and subsequent least significant difference multiple comparisons tests (LSD) with main factor of MS (control, MS4, MS9, MS18) was used. Multiple group comparisons of stimulation intensities were done by Kruskal-Wallis-tests and for two group-comparisons of stimulation intensities and LTP a Mann-Whitney *U* test was applied. Litter weights were compared before and after MS using a Wilcoxon test. All tests were two-tailed and the level of significance was set at *P*<0.05. In all figures the mean±standard error of mean is given.

RESULTS

Basic physiological properties of DG granule cells

The samples for the different groups were tested for differences in DG cell excitability. Therefore, the field excita-