

Effects of individual stressors used in a battery of “chronic unpredictable stress” on long-term plasticity in the hippocampus of juvenile rats

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We have studied alterations in the properties of long-term potentiation (LTP) in hippocampal slices of juvenile rats induced by the exposure of animals to different individual stressors usually used in batteries of chronic unpredictable stress (CUS), a widely used model of depression. Social isolation for 16 h did substantially affect neither the magnitude and nor the development of LTP. The effects of stroboscopic illumination and water deprivation appeared most severe, though opposite: the first stressor had activating effect, whereas the second one inhibited the development of LTP. In addition to the effects of these factors on the LTP magnitude, they also affected the patterns of LTP development. In this study weak tetanization with different probability of maintenance was used, and most of stressors, in spite of the similar LTP magnitude, influenced significantly on the process of consolidation. In hippocampal slices from rats maintained on wet bedding for 16 h, the time course but not magnitude of LTP significantly differed from that observed in the control or socially isolated rats. The weakest effect on LTP was observed in hippocampal slices of the rats exposed to food deprivation. In these animals, only some differences were observed in the development of LTP as compared to socially isolated rats. These data allow ranging stressors used in CUS paradigms according to the severity of their potential effects on neuronal function and animal behavior.

Key words: long-term potentiation, hippocampus, chronic unpredictable stress, social isolation, food and water deprivation, stroboscopic illumination

INTRODUCTION

Depression is a common mental disorder, characterized by sadness, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, feelings of tiredness, and poor concentration (WHO Depression, 2016). It is considered that mood disorders, including depression, may be a consequence of a complex interaction of social, psychological and biological factors, chronic action of various daily stressors and life threatening events being very important (Grigoryan et al. 2014, Maccari et al. 2014, Peterlik et al. 2016). Persistent depressed mood and loss of pleasure as a feature of major depressive disorder pathology are also associated with cognitive impairments and a set of emotional and behavioral alterations. In spite of great efforts to understand the relationships between exposure to chronic stress and development of depression, the pathogenesis of this disease remains unclear.

In experimental studies exposure to chronic unpredictable stress (CUS) is considered one of the most suitable models for production of depressive-like conditions in laboratory rodents (Katz 1981, Papp et al. 2003, Willner 1997, Willner 2005, Willner et al. 1987). CUS protocols typically include repeated exposures to inescapable stressors for one to eight weeks. Initially, a protocol suggested by Katz et al. (1981) included a list of relatively strong factors, such as electric foot shock, swimming in cold water, heating and other. The rats exposed to this protocol exhibited anhedonia, an important symptom of depressive disorders. Later on, this protocol was modified by Willner et al. (1987) who exposed animals to a battery of stressful factors, specifically food and water deprivation, stroboscopic illumination, inclined cage, wet bedding, and some other so called mild stressors in order to induce anhedonia-like behavior. This model was successfully adopted in several laboratories (Holderbach et al. 2007, Luo et al. 2008, Wang et al. 2008), including our lab (Stepanichev et al., 2016). The primary

advantage of this model is its similarity to chronic stressful conditions of human life in terms of high level of unpredictability of presentation time, duration of exposure, and modality of stressors (Armario and Nadal 2013). In addition to anhedonia, multiple studies have shown that CUS is followed by decreased locomotor and exploratory activity (Wang et al., 2008) and impaired learning and memory in water maze (Han et al. 2015). Studies on brain morphology and biochemistry in CUS exposed animals also revealed significant modifications in structural and functional properties in several brain structures (Hollis et al. 2013, Qiao et al. 2016). The hippocampus is particularly vulnerable to damaging effects of stress, in particular, due to high expression of glucocorticoid receptors in this brain region (Joëls et al. 2004, Suri and Vaidya 2015).

Recent clinical studies have demonstrated the antidepressant efficacy of ketamine, a N-methyl-D-aspartate receptor (NMDAR) antagonist, in treatment of patients with drug-resistant depression (Berman et al. 2000, Zarate et al. 2012) indicating the importance of glutamatergic neurotransmission in the mechanisms of depression pathogenesis (Castren 2013, Musazzi et al. 2011, Popoli et al. 2011, Wang et al., 2015). The important attribute of stress-induced modifications is the modulation of long-term plasticity (Christoffel et al. 2011, de Kloet et al. 2005, Huang et al. 2005, Lupien et al. 2009), in particular in the hippocampus (Howland and Wang 2008, Kim et al. 2006, Radahmadi et al. 2014) including CA1 area (Artola et al. 2006, Hiraide et al. 2012, Kallarackal et al. 2013). Such modifications may be related to synaptic metaplasticity (Hirata et al. 2009, Schmidt et al. 2013), possibly underlying emotional memory (Segal et al. 2010, Grigoryan et al. 2015). One of the factors of metaplasticity is the activation of different receptors in response to glucocorticoids in a specific for brain area manner (Krugers et al. 2005, Maggio and Segal 2007, Sharvit et al. 2015). However, several groups found some aspects of LTP disturbance (Jin et al. 2015, Park et al. 2015). CUS exposure was shown to impair the development of long-term potentiation (LTP), a well-known NMDA-dependent phenomenon of long-term plasticity, in the hippocampus and prefrontal cortex (Alvarez et al. 2003, Burgdorf et al. 2015, Qiao et al. 2014). In most studies, the consequences of chronic stress exposure (effects of cumulative stressors action) were revealed (Li et al. 2012, Yu et al. 2016). Nevertheless, the effects of each specific stressor used in the protocols of CUS on hippocampal plasticity and their contribution to the general outcome, remain obscure. However, these individual effects are very important to understand the development of pathological processes in the brain induced by CUS exposure (Armario and Nadal 2013).

In the present study we have examined LTP properties in hippocampal slices from rats acutely exposed to individual stressors typically included in the CUS protocols, spe-

cifically: social isolation, food and water deprivation, wet bedding, and stroboscopic illumination.

MATERIALS AND METHODS

Animals

Thirty-six 1–1.5-month-old Wistar rats were used in the study. The animals were supplied by “Stolbovaya” animal farm (Moscow region, Russia) or born in vivarium of the Institute of Higher Nervous Activity and Neurophysiology RAS. The animals were housed 5–8 per a cage under 12/12-h light/dark cycle (light on at 8.00 a.m.) and had free access to food and water. The experiments were carried out in accordance with the EU Directive 2010/63/EU for animal experiments. The experimental protocol was approved by the Ethical Commission of the Institute of Higher Nervous Activity and Neurophysiology RAS.

Exposure to stressors

The animals were divided into six groups. The animals of the control group were maintained in their home cages prior to the experiment. The animals of the other group were isolated for 16 h but not exposed to any other stressors and used as an “active control” (AC). Cages with isolated rats were located in the same room so the isolated rats could sense the smell of their sibs without physical or visual contacts to them. The animals of the other four groups were isolated prior to the start of the experiment and additionally exposed for 16 h to one of the following stressors: food deprivation (FD), water deprivation (WT), wet bedding produced by adding of 250 ml of water to an individual cage (WB) or stroboscopic illumination with the frequency of 120 pulses/min (SI).

Electrophysiological studies

In order to study the effects of exposure to various stressors on interneuronal interactions, we evaluated long-term potentiation (LTP) in the CA1 field of the hippocampus in slice preparations as described previously (Gulyaeva et al. 2003, Tishkina et al. 2016). One-to four 300–400- μ m hippocampal slices were prepared from each brain. The perfusion medium consisted of (mM): NaCl, 124; KCl, 5; MgSO₄·7H₂O, 1.3; CaCl₂, 2.5; NaH₂PO₄, 1; NaHCO₃, 26; D-glucose, 10; carbogen, 95% O₂ and 5% CO₂; pH 7.3–7.4, (all chemicals were from Sigma-Aldrich, USA). The slices were maintained at the temperature of 32 °C during the experiment. Prior to the start of recording, the slices were allowed to stabilize in an experi-

mental chamber for at least 1 h. Field potentials were recorded in the CA1 field of the pyramidal layer using glass microelectrodes (1–3 mOhm) filled with 0.33 M sodium chloride. Stimulating bipolar electrodes were located in the Schaffer collaterals of the radial layer. Prior to the main experiment, we estimated a stimulation threshold and dependence of the response amplitude on the stimulation intensity. Then, we used intensity of test stimulation, which induced the responses with the amplitudes not higher than 40–45% of the maximum amplitude. LTP was induced with high frequency stimulation (HFS) of the Schaffer collaterals (1 s, 100 Hz). Prior to and during 1 h after tetanization the electrophysiological indices were tested every 30 s. Twenty responses prior to and 120 responses after the induction of LTP were recorded. LTP was estimated as population spike amplitude in percent of mean baseline value. The experiments were performed using a CED Micro1401-3 Data Acquisition Unit (A-M Systems, USA) and Spike2 Version 8 Software Suit (CED, Great Britain).

Statistical analysis

The animals were randomly assigned for the groups and each group consisted of at least 5 animals from different litters. Recording and statistical analysis were performed by researchers (AAG and IVK) who were blinded to group assignment and outcome assignment. Repeated measures analysis of variances (ANOVA) was applied for electrophysiological data with within-subject factor “time after stimulation” and between-subject factor “group”. Samples for comparison consisted of either averaged data from all slices of a rat, or all individual slices (to control possible non-physiological factors). Group effect was determined by all variants of ANOVA test including 2 (a stress group and each control), 3 (a stress group and both controls), or all 6 groups with *post hoc* analysis (Tukey test). Individual z-scores were calculated using mean and standard deviation of general population including all 6 groups. In addition, we compared different phases of LTP separately. Period of 5–20 min after HFS was considered as early potentiation, and the period 25–60 min after HFS were referred to as late LTP. Since weak tetanization in our experiments was followed sometimes by short-term post-tetanic depression, modifications during first 5 minutes were compared separately. The differences were considered as significant at $P < 0.05$. Data are presented as mean \pm SEM. z scores were calculated as the distance from the sample mean (m) to the population mean (M) in units of the standard deviation (SD): $(m-M)/SD$, using mean and standard deviation of general population including all 6 groups. All calculations were performed using STATIS-

TICA data analysis software system, version 8.0 (StatSoft Inc., Tulsa, OK, USA).

RESULTS

Short-term social isolation does not influence LTP induction but modifies LTP maintenance

In order to evaluate the effect of short-term social isolation, the LTP properties were studied in the control rats and rats isolated for 16 h. Fifteen hippocampal slices from isolated rats and 18 slices from control animals were studied. In all slices, LTP reached maximal values within first 15–20 min after stimulation; however, the LTP magnitude varied substantially from slice to slice. In the control group, the magnitude increased by 113–208% of the baseline observed prior to tetanization, while in the isolated group, the LTP magnitudes varied within 136–296% of the baseline level.

Data from the slices of each rat were averaged for the following analysis. Comparison of the data from control ($n=9$) and AC ($n=5$) using repeated measures ANOVA did not reveal substantial differences in LTP development in the CA1 field of the hippocampus ($p=0.96$). It should be noted that LTP magnitude was not always identical in different slices from the same hippocampus, probably due to some non-physiological factors such as even small differences in slice preparation and maintenance, its different position and time of recording, etc. Since these factors may nonspecifically increase variability, we have also compared between-group differences taking into consideration all individual slices without averaging. Likewise, there was no significant either main “group” effect ($F_{1,31}=0.98$, $P=0.33$) or “group” \times “time after stimulation” interaction ($F_{119,3689}=1.04$, $P=0.36$), indicating the similarity in the LTP value and development in the control and AC groups. However, when LTP maintenance phase was analyzed separately for the last 40 min of recording, i.e. 21–60 min after stimulation, a significant “group” \times “time after stimulation” interaction ($F_{79,2449}=1.44$, $P=0.007$) was revealed. These data suggested that LTP maintenance substantially differed in these two groups. In particular, in the socially isolated rats of the AC group, LTP was higher at the initial stage and then it slightly declined, whereas in the control group, LTP gradually increased during the observation period.

Social isolation did not significantly influenced other phases of LTP, such as the level and pattern of short-term post-tetanic depression (main “group” effect $F_{1,31}=0.08$, $P=0.78$ and “group” \times “time after stimulation” interaction $F_{9,179}=1.02$, $P=0.42$). The development of the early phase of potentiation was also similar in the

control and AC groups (main “group” effect $F_{1,31}=2.26$, $P=0.14$ and “group” \times “time after stimulation” interaction $F_{29,899}=0.65$, $P=0.92$).

Water deprivation (WD) significantly decreases LTP in hippocampal slices

The effect of 16-h WD on LTP in the hippocampus individually housed rats was most significant. LTP development was evaluated in 13 slices from 5 rats individually housed without access to water, whereas food was available with no limitation. Characteristics of neuronal responses in slices from the WD rats exhibited high consistency. Thus, variability of the LTP magnitudes in different slices from the same animal as well as in slices from different animals was considerably lower in this group as compared to the other groups exposed to other stressors. Maximal increase in the LTP amplitude in WD exposed rats was within the range of 108–155% as compared to the baseline level.

Fig. 1A demonstrates LTP profiles in hippocampal slices of WD and AC rats. ANOVA applied to the data from the whole period of observation revealed a significant main “group” effect ($F_{1,8}=9.14$, $P=0.016$ comparing animals and $F_{1,26}=7.44$, $P=0.01$ comparing slices) and “group” \times “time after stimulation” interaction ($F_{119,952}=5.39$, $P<0.0001$ comparing animals and $F_{119,3094}=3.74$, $P<0.001$ comparing slices) when LTP features were compared between AC and WD groups. Similarly to the other stressor, WD affected the post-tetanic depression phase, which was less expressed in WD-exposed animals. LTP amplitudes were significantly lower during other phases including both early potentiation phase (main “group” effect ($F_{1,26}=16.26$, $P<0.001$) and LTP maintenance phase (main “group” effect $F_{1,26}=6.37$, $P<0.018$). In addition to the effects of WD on LTP amplitudes in different phases (Figs 2A, B), it also modified the time course of LTP development. In the WD-exposed animals, the amplitudes of responses increased during 21–60 min after the tetanization, whereas in the rats of the AC group, the amplitudes of responses remained unchanged during this period of observation (“group” \times “time after stimulation” interaction $F_{79,2054}=1.43$, $P=0.009$).

The analysis of data from the hippocampal slices of WD-exposed rats and control animals revealed similar differences. Thus, a trend to the main “group” effect ($F_{1,12}=1.77$, $P=0.2$ comparing animals and $F_{1,29}=3.55$, $P=0.07$ comparing slices) and significant “group” \times “time after stimulation” interaction ($F_{119,1428}=1.92$, $P=0.0001$ comparing animals and $F_{119,3451}=4.08$, $P<0.001$ comparing slices) were found for the whole observation period. In the WD-exposed animals, the LTP amplitudes were lower in both the early phase and maintenance phase (Fig. 2, main “group” effects $F_{1,29}=4.79$, $P=0.04$ and $F_{1,29}=3.78$, $P=0.06$, respectively).

Similar results were obtained by ANOVA test including all 3 groups (WD and both controls). Group effect ($F_{2,43}=3.51$, $P=0.03$) and “group” \times “time after stimulation” interaction ($F_{238,5117}=2.57$, $P=0.0001$) were significant, and *post hoc* test (Turkey) confirmed significant difference ($P=0.03$) between WD and AC groups. Thus, WD is a severe stressor, which modifies the development of LTP in the hippocampus.

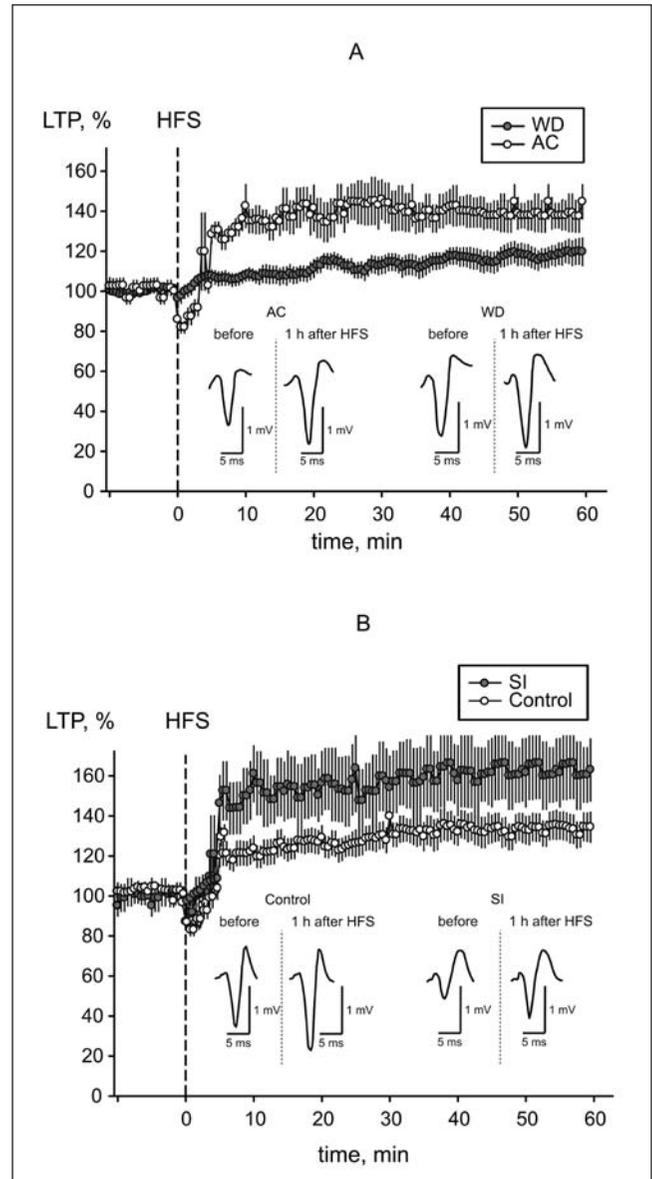


Fig. 1. Development of LTP in the CA1 field of hippocampal slices of rats subjected to water deprivation (WD) or stroboscopic illumination (SI) as compared to “active” (AC, socially isolated rats) and passive controls. Black circles – mean LTP in WD (A) and in SI (B) groups; white circles – mean LTP in the slices of “active” (A) and passive (B) control groups. Data are presented as $M \pm$ s.e.m. Dotted line indicates time point of HFS. Ordinate axis – LTP magnitude, expressed as percentage of population spike (PS) amplitude, relative to baseline before HFS; abscissa axis – time after tetanization. Typical examples of PSs recorded in WD (A) and SI (B) slices prior to HFS are presented in the insert.

Stroboscopic illumination (SI) stimulates LTP development in the early phase

To study the effect of SI on LTP development in the hippocampus, 11 slices from 5 rats were used. In this group, the LTP amplitude varied substantially with maximum level within the range of 136–367%. In the SI exposed rats, an increase in the amplitudes of responses to the testing stimuli after LTP induction was more expressed as compared to the control value (Fig. 1B), indicating the activating effect of this stressor. ANOVA a significant main “group” effect ($F_{1,12}=6.49$, $P=0.02$) comparing animals, and an important trend ($F_{1,27}=3.83$, $P=0.06$) comparing slices. A significant “group” × “time after stimulation” interaction ($F_{119,1428}=1.97$,

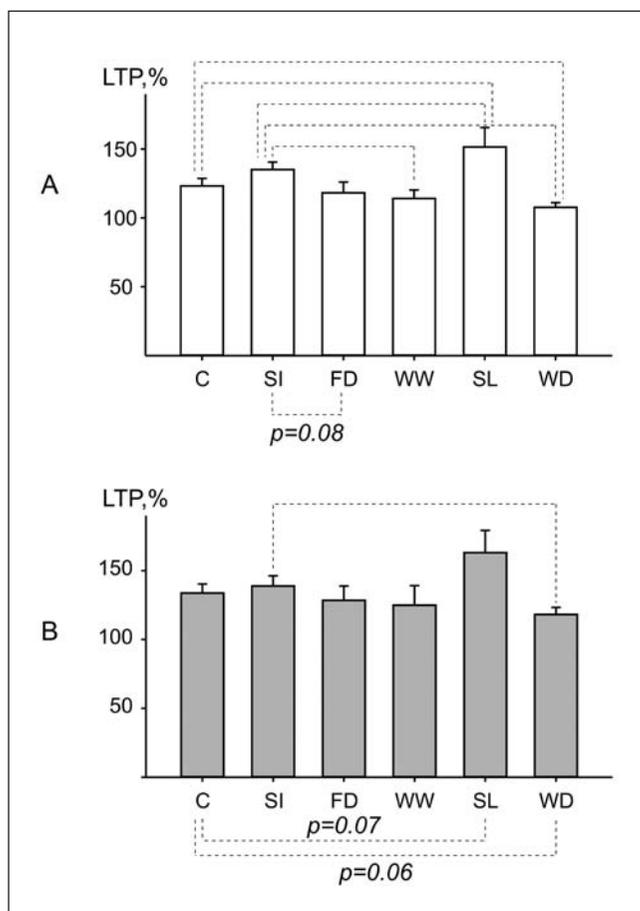


Fig. 2. Effects of individual stressors on the specific LTP phases in the CA1 field of hippocampal slices.

A – early phase of potentiation (6–20 min after HFS); B – maintenance phase of potentiation (21–60 min after HFS). Data are presented as $M \pm$ s.e.m. from all slices of each experimental group. Ordinate axis – LTP magnitude, expressed as percentage of PS amplitude, relative to baseline before HFS. C – control group; AC – “active” control; FD – food deprivation; WB – wet bedding; SI – stroboscopic illumination; WD – water deprivation. Significant differences between the groups are indicated with dotted lines upside ($P<0.05$); important trends are indicated with dotted lines bottom side ($P<0.1$).

$P=0.0001$ comparing animals and $F_{119,3213}=1.54$, $P<0.001$ comparing slices) was related to the specific difference of LTP development in the phase of post-tetanic depression and immediately after it. A significant difference of the response amplitudes in the control and SI exposed groups was observed in the phase of early potentiation (main “group” effect $F_{1,27}=4.7$, $P=0.04$), and the averaged LTP amplitude in the SI group was 150% whereas in the control group, it was 127% as compared to the baseline level (Fig. 2A). In the phase of LTP maintenance, a trend to higher values of LTP level was also found in the SI group as compared to the control animals (main “group” effect $F_{1,27}=3.57$, $P=0.07$; Fig. 2B).

There were no significant differences between the magnitude of LTP development in the SI exposed and AC rats. Main “group” effect was $F_{1,8}=2.09$, $p=0.18$ comparing animals and $F_{1,24}=1.42$, $P=0.25$ comparing slices, “group” × “time after stimulation” interaction $F_{119,2856}=1.05$, $P=0.35$ comparing slices, while $F_{119,952}=1.54$, $P=0.0003$ comparing animals. Similar results were obtained by ANOVA test including all 3 groups (WD and both controls). “Group” × “time after stimulation” interaction ($F_{238,4879}=1.17$, $P=0.04$) but not group effect ($F_{2,41}=2.26$, $P=0.11$) were significant. Post hoc test (Turkey) revealed an important trend to difference ($P=0.09$) between SI and AC groups.

Exposure to wet bedding (WB) induces highly variable responses in hippocampal slices

To study the effects of 16-h WB exposure, we have compared LTP induced in 9 slices from the animals exposed to WB to that, observed in slices from the rats of the AC group (since each rat was placed into a cage with wet bedding individually), and from the control group. In slices from the WB group, LTP was most variable as compared to other groups studied. The slices with depression to 71% of the baseline level 1 h after the tetanization, and the slices with significant potentiation up to 276% were observed. In the records, a high variability of neuronal responses to stimuli was found and their amplitudes and patterns changed non-monotonously.

The development of LTP in the WB group substantially differed from that observed in the control and AC groups. Significant “group” × “time after stimulation” interactions were revealed for both control and WB ($F_{119,2975}=1.5$, $P<0.001$) and AC and WB ($F_{119,2618}=1.87$, $P<0.001$) group comparisons, while mean LTP magnitude was practically identical with passive control, especially comparing animals ($P=0.98$). These differences in the time courses of LTP development were due to the absence of the phase of post-tetanic depression in most slices from the rats of the WB group. Analysis of specific LTP stages also supported this suggestion. In the first 5 min after the tetanization, the responses were higher in the control as compared to the WB group (main

“group” effect $F_{1,25}=6.24$, $P=0.02$); however, the difference between the AC and WB groups was not significant (main “group” effect $F_{1,22}=1.81$, $P=0.19$). During the phase of early potentiation, the magnitudes of neuronal responses to stimuli increased significantly up to 140% in average as compared to the baseline level in the AC group, whereas in the WB group, only a slight and insignificant growth of the neuronal response magnitudes was observed (Fig. 2A). ANOVA applied to the data recorded within 6–20 min after the tetanization also revealed a significant main “group” effect ($F_{1,22}=5.85$, $P=0.02$) during this period.

Food deprivation (FD) affects only early phase of LTP in hippocampal slices

The effects of 16-h FD on LTP were studied in 13 hippocampal slices from rats housed individually without food access. Drinking water was available without limitations within the period of FD. LTP magnitude in this group was also near the same to that of both controls. However ANOVA applied to the data from the AC and FD groups revealed a significant “group” × “time after stimulation” interaction ($F_{119,3094}=1.31$, $P=0.016$), indicating the differences in the time course of LTP. Similar to the records from the WB group, the phase of post-tetanic depression was not noticeably expressed in the LTP curves recorded in the slices from the FD group. In the first 20 min after the tetanization, the response amplitudes increased monotonously, although they remained lower as compared to the response magnitudes observed of the AC group, and ANOVA applied to the data within this period of LTP development revealed an important trend to the main “group” effect ($F_{1,26}=3.18$, $P=0.086$). The development of other phases of LTP was similar in the AC and FD groups (Figs 2A, B). Comparisons of LTP-related processes in the control and FD animals did not reveal significant differences either in the LTP amplitudes or development features (main “group” effect $F_{1,29}=0.07$, $P=0.79$ and “group” × “time after stimulation” interaction $F_{119,3451}=0.89$, $P=0.79$). Thus, short-term exposure to FD in the CUS model is not sufficient to induce gross modifications in long-term plasticity in the rat hippocampus.

Evaluation of severity of the effects of individual stressors

In the present study we also tried to evaluate quantitatively the severity and/or efficacy of the influences of individual stressors using LTP paradigm. The data in Fig. 3A demonstrate the differences in the development of LTP in hippocampal slices from rats of different experimental groups. Only in some of these groups LTP development significantly differed from control. We performed ANOVA including all 6 groups. Group effect was significant ($F_{5,73}=2.63$,

$P=0.03$) and *post hoc* test confirmed main contribution of SI and WD groups ($P=0.016$). Considering all factors studied as possible stressors in CUS paradigm, we looked for a method for the ranging factors based on quantitative estimations. For this purpose we performed z-normalization. Individual z-scores were calculated using mean and standard deviation of general population including all 6 groups. The values of z for all individual stressors used in this study are presented in Fig. 3B. According to these data, SI and WD had the strongest impact, although their influences were opposite: SI had the activating effect on the development of LTP whereas WD, on the contrary, suppressed this process. Our data show that FD is the weakest stressor in the context of its influence on LTP.

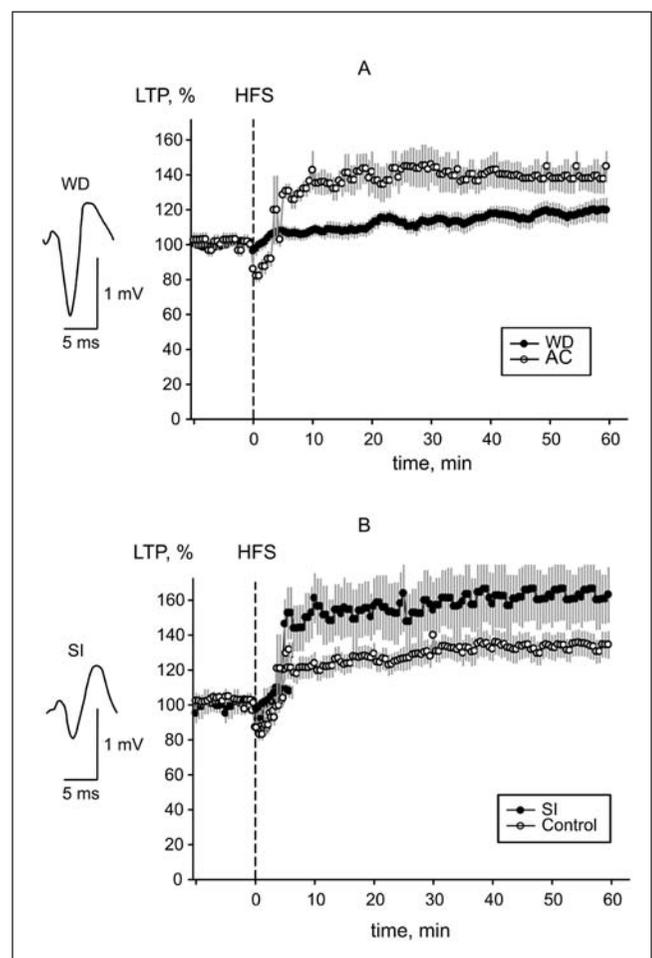


Fig. 3. Effects of individual stressors on the development of LTP.

A – mean LTP profiles from all slices of the rats exposed to different stressors. Ordinate axis – LTP magnitude, expressed as percentage of PS amplitude, relative to baseline before HFS; abscissa axis – time of recording. In order to simplify the profiles standard errors of means are not presented. Dotted line indicates time point of HFS. B – normalized LTP 1 h after HFS (Z) calculated using mean and standard deviation of general population including all 6 groups Control – group of rats maintained in the home cage; AC – “active” control (socially isolated rats); FD – food deprivation; WB – wet bedding; SI – stroboscopic illumination; WD – water deprivation.

DISCUSSION

In the present study, we evaluated the effects of specific stressors, which are most often included in the CUS protocols, on the development of LTP in the rat hippocampus. CUS model (eight-week exposure of individually housed rats to a set of randomly repeated stressors) was successfully employed in our laboratory in adult rats. It resulted in the development of anhedonia, tested in the sucrose preference test, increased anxiety, and higher locomotor activity in the open field test (Stepanichev et al. 2016). Thus, we could expect that individual stressors used in our experimental conditions were effective to induce significant alterations in animal behavior similar to those reported by other groups. However, we did not study long-term plasticity in those animals, although several groups reported impaired LTP in the hippocampus or prefrontal cortex after CUS exposure (Alvarez et al. 2003, Burgdorf et al. 2015, Qiao et al. 2014). Since we are interested, first of all, how each stressor may contribute to the initiation of stress-induced cognitive impairment, the present study was focused on hippocampal LTP.

The effect of WD on LTP development was most severe. In the rats exposed to 16-h WD, both early phase of potentiation and phase of LTP maintenance were impaired. In contrast to WD, SI had an activating effect and increased the LTP magnitude. The exposure of rats to WB influenced the time course of LTP development without strong effect on the magnitudes of the responses to testing stimuli. The weakest of all stressors studied was FD: it was not able to significantly modify long-term plasticity. Significant effects of SI or WD may be related to the changes of excitability and background input-output function, for example, synaptic depression or facilitation. For more detailed analysis further experiments should be performed, e.g. additional slices of each effectual group are necessary for multiple correlation analysis to study EPSP-spike coupling.

Several groups reported impaired LTP in the hippocampus or prefrontal cortex after CUS exposure (Alvarez et al. 2003, Burgdorf et al. 2015, Qiao et al. 2014). However, a consequence of events resulting in a deficit of LTP, and the role of each individual stressor included in a CUS protocol in this deficit was not studied making difficult possible comparison of results from different groups using various protocols of CUS, as well as optimization of these protocols (Armario and Nadal 2013). Qiao et al. (2014) have studied synaptic plasticity in the hippocampus of rats exposed to CUS of various durations. They have reported that three-week exposure to CUS significantly impaired LTP induction in CA1-CA3 synapses. In contrast to this, two-week exposure to CUS enhanced observed LTP induction, whereas one-week exposure did not significantly influence LTP. In our study activating or inhibiting influences of individual stressors of a CUS battery were observed. It is possible that

the absence of visible modifications in LTP development reported by Qiao et al. (2014) may be a result of cumulative effect of these influences. It should be taken into account that these authors analyzed LTP development within the whole period of observation and did not studied functionally different phases of LTP separately. Our data also show that most of individual stressors, except WD, did not affect LTP on the whole. At the same time detailed analysis of specific phases of LTP revealed more “delicate” effects of the stressors. Furthermore, in the present study less strong tetanization was used to induce LTP as compared to that applied by Qiao et al. (2014). It is possible that using of a protocol of “stronger” tetanization would mask weaker alterations in LTP development and did not allow finding any modifications of LTP after exposure to CUS of shorter duration. Obviously, apparently subtle differences in the CUS paradigm also contribute to incomparability of results reported by different groups. Thus, it has been shown that exposure to CUS facilitated LTD and had no effect on LTP (Holderbach et al. 2007).

Stress-induced bidirectional plasticity was described by many authors (Joëls and Krugers 2007, Akirav and Richter-Levin 1999, Diamond et al. 1992) that was region-specific for brain area (Kavushansky et al. 2006). However LTP activation was usually observed in response to acute stress (Ahmed et al. 2006, Spyrka et al. 2011). Bidirectional plasticity was observed also after corticosterone treatment (Avital et al. 2006, Joëls 2006, Groc et al. 2008, Martin et al. 2009). Experiments with specific agonists and antagonists showed that LTP enhancement is related to activation of mineralocorticoid receptors (Joëls et al. 2008, Maggio and Segal 2012, Olijslagers et al. 2008), while glucocorticoid receptors suppress LTP in most structures (Alvarez et al. 2002, Cazakoff and Howland 2010, Joëls et al. 2009, Kamal et al. 2014). Possible influence of SI may be additionally mediated by the involvement of noradrenergic inputs as a component of a stress response, but also of arousal and attention (Grigoryan et al. 2015, Inoue et al. 2013, McReynolds et al. 2010, Wong et al. 2012). Likewise, it is not clear whether LTP suppression during WD is mediated purely by activation of glucocorticoid receptors. In fact, drinking after WD immediately prolonged LTP induced by weak tetanization, at least in the dentate gyrus in vivo (Seidenbecher et al. 1995). We cannot exclude that regulation of a water balance and, in particular, the renin-angiotensin system may contribute to LTP inhibition under the WD condition (Wright et al. 2002, 2008).

At present, we do not know how “stressors” with different effects on long-term plasticity may contribute to pathological modifications in the hippocampus and what may be the role of neutral or activating stressors in these impairments. A battery of most efficient stressors was formed and optimized in behavioral studies aiming to induce an anhedonia condition in animals (Katz et al. 1981, Luo et al. 2008,

Wang et al. 2008, Willner et al. 1987). Our data show that short-term exposure of a rat to each of stressors from this battery may result in different acute plasticity response in the hippocampus. It is possible that the response to activating or neutral stimuli may be altered if plasticity of synapses is weakened by previous stimuli or on the other hand, the stressogenic potential of the stimuli may additionally enhance the detrimental effects of the other signals. Unexpected destabilizing of metaplasticity may have negative consequences exceeding the adaptive capacities of the system (Marsden 2013). In any case, a deficit of LTP after CUS exposure cannot be considered as a simple summation of negative modifications in response to each stressor (Spirka and Hess 2010). Complexity of alterations found in our experiments and studies of other authors support the hypothesis of stress-induced modifications in the brain as a part of a general adaptive response (Qiao et al. 2014, Armario and Nadal 2013).

Although the consequences of short-term exposure to individual stressors of a CUS protocol on most neurochemical systems of the hippocampus remain poorly studied, the effects of short-term mild stress on neurotrophin-linked processes related to both LTP and depression have been investigated. The development of LTP in the hippocampus requires a strong balance in the neurotrophin systems, primarily of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF; Conner et al. 2009, Leal et al. 2015), and neuroinflammatory mediators, such as interleukins (see Lynch 1998). Moreover, impairment in the balance of neurotrophic factors and proinflammatory cytokines is associated with the development of depression (Grigoryan et al. 2014, Stepanichev et al. 2014). In the present study, we did not evaluate the contents of neurotrophins or cytokines after short-term exposure to the stressors. However, Remus et al. (2015) have reported that overnight food and water deprivation significantly decreased sucrose preference even in the animals, which were resilient to CUS, and this effect was associated with elevated content of interleukin-1 β in the hippocampus. In experimental studies, 15–20-min exposure of rats to a brightly lighted open field arena resulted in a significant increase in the number of neurons, expressing NGF in the CA1 and CA3 fields of the hippocampi of adult and old animals (Badowska-Szalewska et al. 2015). This effect was not found after acute forced swim stress of same duration. A single exposure of rats to the forced swim stress decreased BDNF mRNA level in the hippocampus 2 h and in the cortex 24 h after the stress (Berezova et al. 2011, Shishkina et al. 2010), whereas repeated exposure to this stressor caused the elevation of hippocampal BDNF mRNA level assessed 24 h after the second exposure (Berezova et al. 2011). It is also known that WD for at least 24 h may induce BDNF expression in the subfornical organ (Hindmarch et al. 2008, Saito et al. 2003). Though the effect of short-term WD or SI on the expression of neurotrophins and interleukins in the hippo-

campus are not described, we can suppose that, in addition to corticosteroids, these important systems may be involved in LTP alterations found after exposure to these stressors in the present study.

CONCLUSIONS

In the present study the effects of individual stressors composing a battery underlying CUS protocol were studied in juvenile rats using an LTP model of synaptic plasticity in hippocampal slices. Short-term exposure to the stressors induced highly variable responses in rats exposed to different stressors. Most prominent and opposite effects were found in the animals exposed to stroboscopic illumination (stimulation of LTP) or water deprivation (inhibition of LTP). These data indicate unequal contribution of individual stressors to the development of neuroplasticity impairments and probably depressive-like behavior in models of CUS. So far the important information is lacking – how sequential application of different stressors with specific effects on hippocampal plasticity can induce stable and prolonged changes reflected in behavioral disturbances. Does the sequence and multiplicity of stressors matter? To complete this puzzle lots of additional experiments are needed. However, we believe that our relatively simple approach may help to categorize different types of stress to evaluate how factors of different nature and intensity influence neuronal functions and, consequently, animal behavior.

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