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BRIEF REPORT

CINP

Remodelling by early-life stress of NMDA receptor-dependent synaptic plasticity in a gene–environment rat model of depression

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Abstract

An animal model of depression combining genetic vulnerability and early-life stress (ELS) was prepared by submitting the Flinders Sensitive Line (FSL) rats to a standard paradigm of maternal separation. We analysed hippocampal synaptic transmission and plasticity in vivo and ionotropic receptors for glutamate in FSL rats, in their controls Flinders Resistant Line (FRL) rats, and in both lines subjected to ELS. A strong inhibition of long-term potentiation (LTP) and lower synaptic expression of NR1 subunit of the NMDA receptor were found in FSL rats. Remarkably, ELS induced a remodelling of synaptic plasticity only in FSL rats, reducing inhibition of LTP; this was accompanied by marked increase of synaptic NR1 subunit and GluR2/3 subunits of AMPA receptors. Chronic treatment with escitalopram inhibited LTP in FRL rats, but this effect was attenuated by prior ELS. The present results suggest that early gene–environment interactions cause lifelong synaptic changes affecting functional and molecular aspects of plasticity, partly reversed by antidepressant treatments.

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Key words: Depression, gene–environment, NMDA receptor, stress, synaptic plasticity.

Introduction

Although several animal models of depression have been developed, a model faithfully replicating the aetiological factors in humans is lacking (Nestler et al., 2002). Stress factors, such as early-life adverse events, have been shown to interact with a variable background of genetic vulnerability (Caspi and Moffit, 2006), markedly increasing the risk for development of depression in adult life (Caspi et al., 2003). Moreover, stress profoundly affects cognitive functions and hippocampal synaptic plasticity (Kim and Diamond, 2002). Here we attempted to reproduce the interaction between environmental adverse events and genetic vulnerability by setting up an animal model combining early-life stress (ELS) with an inherited trait of vulnerability. We employed Flinders Sensitive Line (FSL) rats, a well-validated model of depression carrying genetic vulnerability associated with distinct features of pathology. This rat line showed face, construct and predictive validity and exhibited several behavioural features of human depression, such as psychomotor retardation, increased amount and reduced latency of REM sleep, reduced appetite and weight, as well as several neurochemical abnormalities (Overstreet et al., 2005). To reproduce ELS events we subjected FSL rats and their controls, Flinders Resistant Line (FRL) rats, to a standard maternal separation (MS) protocol, in a new model of gene–environment ($G \times E$) interaction (El Khoury et al., 2006; Plotsky and Meaney, 1993). Finally, the rats were chronically treated with escitalopram (Esc), a selective serotonin reuptake inhibitor. We have previously shown that MS worsens depressive-like behaviour of

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FSL, while Esc partly reverses this change (El Khoury et al., 2006).

To investigate how genetic vulnerability and ELS affect synaptic plasticity, the ability of high-frequency stimulation (HFS) to induce long-term potentiation (LTP) in vivo was assessed in CA1 area of hippocampus (HC). Short-term plasticity measured as magnitude of paired pulse facilitation (PPF), baseline excitability and synaptic strength were also monitored. Furthermore, to identify molecular correlates of changes in synaptic plasticity, we purified synaptic terminals (synaptosomes) from HC and analysed changes in ionotropic glutamate receptors.

Methods and materials

Animals, maternal separation and drug treatment

Male FSL and FRL rats from the Karolinska Institutet were used. Experimental schedule and rat groups are summarized in Supplementary Tables S1 and S2 (available in the online Appendix); MS (3 h/d, postnatal days 2–14), drug treatment (Esc, ~25 mg/kg.d) and other experimental procedures are illustrated in detail in the online Appendix and are similar to those described in El Khoury et al. (2006).

Electrophysiology

Electrophysiological methods used to record excitatory synaptic transmission and plasticity of transmission in the CA1 area of the dorsal HC in vivo were essentially those described previously (Shakesby et al., 2002). Following transport from the Karolinska Institutet, animals were rehoused for 1 wk before commencing treatment with Esc. Recordings were made under urethane (2.1 g/kg i.p.) anaesthesia. The test stimuli evoked responses of 50–55% maximum field excitatory post-synaptic potential (EPSP) amplitude. The HFS protocol comprised 10 trains of 20 pulses, an interpulse interval of 5 ms (200 Hz) and an intertrain interval of 2 s, at an intensity that evoked a 75% maximum EPSP.

Preparation of synaptosomes

Animals were sacrificed and HC was quickly excised on ice. Purified synaptic terminals (synaptosomes) were prepared essentially as in Dunkley et al. (1986), with minor modifications (Bonanno et al., 2005).

Western blot analysis

Western blots were carried out as previously described (Bonanno et al., 2005).

Statistical analysis

Paired two-tailed Student's t tests were used to analyse LTP induction compared to baseline within each group. Pre-planned unpaired two-tailed Student's t tests were used to analyse PPF, baseline excitability and EPSP size between specific groups. To analyse LTP magnitude and Western blot results between different groups two-way analysis of variance (ANOVA) was performed for the variables rat line (FRL/FSL) and treatment (MS/Esc). If the ANOVA revealed significant group differences, post-hoc Bonferroni tests were carried out. Significance was assumed at $p<0.05$. Statistical analysis of the findings was carried out using GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA, USA).

Results

Synaptic plasticity in basal conditions and following ELS or Esc treatment

Basal FSL vs FRL rats

In basal FRL rats (Figure 1a) HFS induced robust non-decremental LTP [EPSP amplitude at 10, 30 and 60 min post-HFS: 123.2±2.9%, 127.9±4.4% and 124.6±4.9% respectively (mean±S.E.M.); % pre-HFS baseline, $p<0.001$ compared to baseline, 101.3±1.0%, $n=12$, paired Student's t test]. In contrast, induction of LTP was strongly inhibited in basal FSL rats (Figure 1e): application of HFS failed to induce LTP or even transient potentiation (102.4±2.5%, 104.4±4.8% and 103.4±4.1% at 10, 30 and 60 min, respectively, $n=14$, $p>0.05$ vs. baseline; paired Student's t test). According to two-way ANOVA (hereafter 2WA) the only significant effect for all results reported in Figure 1 was that of rat line and of treatment ($p<0.05$), but not of line × treatment interaction. The Bonferroni post-hoc tests (hereafter BPHT) showed that the inhibition of LTP in FSL was significant relative to basal FRL rats ($p<0.01$). There were no differences between FSL and FRL groups in PPF, baseline excitability or EPSP size.

Effect of maternal separation in FRL and FSL rats

MS had a different pattern of effects in FRL vs. FSL rats. In FRL-MS animals (Figure 1c) HFS induced a relatively small but statistically significant persistent increase in synaptic transmission (122.4±4.8% and 117.9±4.2% at 30 min and 60 min, respectively, $n=12$, $p<0.05$, paired Student's t test), lower than in basal FRL rats, although not significantly different. There were no differences in PPF, baseline excitability or EPSP size relative to basal FRL rats. Remarkably, in

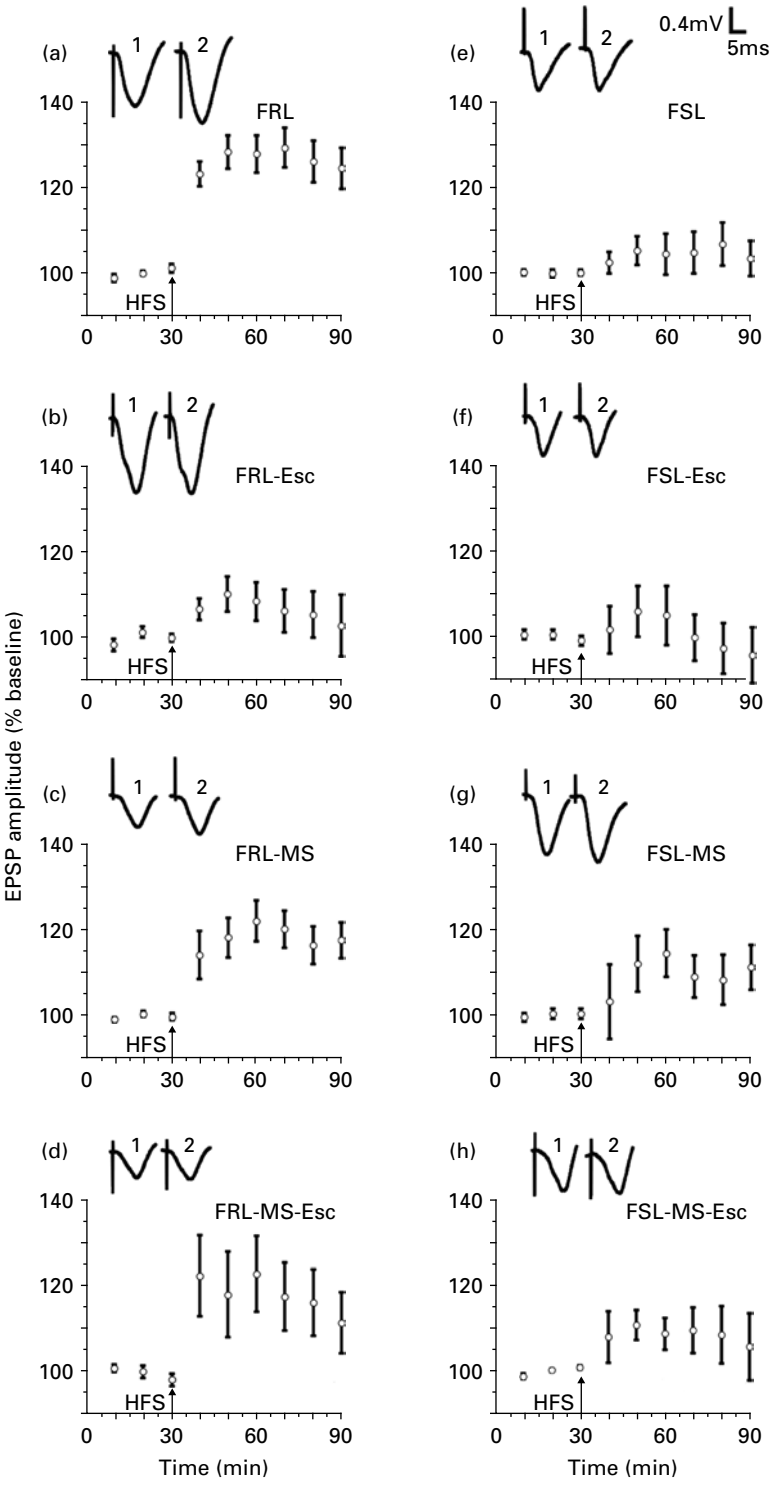

Figure 1. Effects of high-frequency stimulation (HFS) on the amplitude of the field excitatory post-synaptic potential (EPSP), monitored in the CA1 area of the dorsal hippocampus of anaesthetized rats in vivo. HFS induced stable long-term potentiation in basal FRL (a) and FRL-MS (c) animals; transient potentiation in FRL-Esc (b), FRL-MS-ESC (d), FSL-MS (g) and FSL-MS-Esc (h) animals; and failed to induce significant potentiation in basal FSL (e) or FSL-Esc (f) animals. Insets show EPSP recordings ~10 min before (1) and 60 min after (2) HFS. Basal FRL ($n=12$), FRL-Esc ($n=6$), FRL-MS ($n=6$), FRL-MS-EscC ($n=8$); basal FSL ($n=14$), FSL-Esc ($n=6$), FSL-MS ($n=11$), FSL-MS-Esc ($n=7$).

FSL-MS rats (Figure 1g) HFS induced a decremental LTP lasting over 30 min (114.4 ± 5.5% and 111.2 ± 5.3% at 30 min and 60 min, $p < 0.05$ and $p = 0.06$ vs. baseline, respectively, $n = 11$, paired Student's t test). This LTP increase was intermediate between basal FSL (no significant potentiation) and basal FRL animals. There were no differences in EPSP size, although baseline excitability was reduced in FSL-MS rats (6.9 ± 0.4 mA, $p < 0.05$, unpaired Student's t test) and PPF increased (at 40 ms but not 80 ms or 120 ms interpulse interval) relative to basal FSL rats.

Effect of Esc in FRL and FSL rats

Chronic Esc treatment inhibited LTP in basal FRL rats, HFS now only inducing a small transient potentiation (106.7 ± 2.5% at 10 min, $n = 6$, $p < 0.05$ vs. baseline; paired Student's t test) (Figure 1b), and no LTP (108.5 ± 4.5% and 102.8 ± 7.2% at 30 min and 60 min, respectively, $p > 0.05$ vs. baseline, paired Student's t test; $p < 0.05$ vs. basal FRL group, 2WA, BPHT). No difference from basal FRL rats was found in PPF or maximum EPSP size, but baseline excitability was reduced in FRL-Esc rats (6.8 ± 0.3 mA, $p < 0.05$, unpaired Student's t test). Similar to basal FSL, in FSL-Esc rats HFS failed to induce any significant potentiation (101.5 ± 5.5%, 104.8 ± 6.9% and 95.4 ± 6.5% at 10, 30 and 60 min, respectively, $n = 6$, $p > 0.05$ vs. baseline, paired Student's t test) (Figure 1f). However, baseline excitability (6.9 ± 0.5 mA) and maximum EPSP size (1.3 ± 0.2 mV) were significantly reduced in FSL-Esc rats ($p < 0.05$ vs. basal FSL, unpaired Student's t test).

Effect of Esc in MS-FRL and FSL rats

The inhibitory effects of Esc in FRL rats appeared to be attenuated by prior MS. In FRL-MS-Esc rats (Figure 1d) HFS induced a decremental LTP lasting over 30 min (123 ± 8.8% and 111.6 ± 7.1% at 30 min and 60 min, $p < 0.05$ and $p > 0.05$ vs. baseline, respectively, $n = 6$, paired Student's t test). There were no significant differences in LTP, PPF, baseline excitability or EPSP size compared to FRL-MS rats. This contrasts with the strong inhibition of any form of potentiation observed in FRL-Esc rats. In FSL-MS-Esc rats (Figure 1h) HFS induced a transient potentiation lasting only 20 min (110.8 ± 3.6% and 105.8 ± 7.8% at 20 min and 60 min, $p < 0.05$ and $p > 0.05$, respectively, $n = 7$, paired Student's t test) that, however, was not significantly different from the decremental LTP induced in FSL-MS rats ($p > 0.05$, 2WA). Furthermore, there were no significant differences between untreated FSL-MS and FSL-MS-Esc rats in PPF, baseline excitability or EPSP size.

In summary, the level of LTP was significantly higher in FRL vs. FSL rats; MS partially prevented inhibition of LTP in FSL; Esc reduced LTP in FRL, but this effect was attenuated by prior MS.

Expression of NR1-NMDA, GluR1 and GluR2/3 AMPA receptors in basal conditions and following ELS or Esc treatment

With regard to the synaptic level of NR1, the core subunit of NMDAR, 2WA demonstrated significant effect of treatment (MS and Esc) ($p < 0.001$) as well as treatment × line interaction ($p < 0.01$). Interestingly, although 2WA showed no significant effect of rat line, in HC synaptosomes from basal FSL rats the level of NR1 was significantly lower relative to basal FRL rats (−28.50 ± 9.29%, $p < 0.05$, BPHT) (Figure 2a), consistent with the lack of LTP observed in the former rats. Post-hoc tests also showed that, in line with chronic Esc inducing significant decrease in LTP, the drug also reduced NR1 in basal FRL (−27.04 ± 3.42%, $p < 0.01$, BPHT) but had no effect on FRL-MS (−0.65 ± 5.26%, $p > 0.05$, BPHT) animals. Furthermore, MS dramatically up-regulated NR1 in FSL (45.20 ± 4.44%, $p < 0.001$, BPHT) and Esc treatment partly reversed this change (−31.38 ± 5.27% vs. FSL-MS, $p < 0.01$, BPHT), in line with the partial recovery of LTP in FSL-MS rats and its reversal by Esc. In contrast with synaptic levels, in all rat groups total HC NR1 expression was unchanged (see Supplementary Figure S1, online Appendix).

There is strong evidence that LTP involves a postsynaptic process, which selectively enhances AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor (AMPAR)-mediated transmission (Malenka and Nicoll, 1999), in particular by adding AMPAR to silent synapses. In order to assess if AMPAR were differentially regulated in FRL/FSL rats by Esc and MS, we measured expression levels of AMPAR subunits GluR1 and GluR2/3 in HC synaptosomes. 2WA showed significant effects of rat line ($p < 0.001$), treatment ($p < 0.001$) and rat line × treatment interaction ($p < 0.05$). In particular, in line with Esc inducing a significant decrease of LTP/NR1 expression, Esc also reduced GluR1 in basal FRL (−26.51 ± 3.12%, $p < 0.001$, BPHT), and in FRL-MS (−10.05 ± 0.95% vs. FRL, $p < 0.01$, BPHT) rats (Figure 2b). In basal FSL rats, Esc reduced GluR1 (−14.21 ± 1.39%, $p < 0.001$, BPHT) but not after MS (+2.40 ± 1.98%, $p > 0.05$, BPHT).

Analysis of GluR2/3 expression showed significant effect of treatment only ($p < 0.001$, 2WA), with similar effects of MS and Esc in both FRL/FSL rats (Figure 2c). Post-hoc tests showed that MS induced a significant

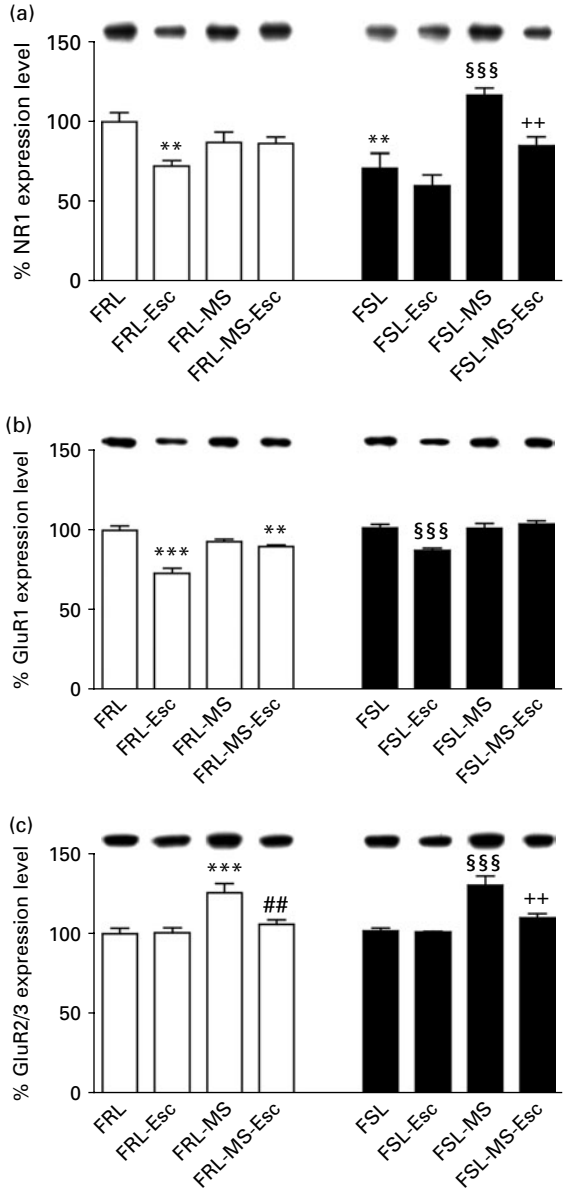

Figure 2. Expression levels of NMDA receptor subunit 1 (NR1) (a), AMPA receptor subunits GluR1 (b) and GluR2/3 (c) in hippocampal synaptosomes. Data are expressed as % intensity units/mm² (mean ± S.E.M.). Insets: representative immunoreactive bands from Western blots. (a) ** $p<0.01$ vs. FRL; §§§ $p<0.001$, vs. FSL; ++ $p<0.01$ vs. FSL-MS. (b) *** $p<0.001$ vs. FRL; ** $p<0.01$ vs. FRL; §§§ $p<0.001$ vs. FSL. (c) *** $p<0.001$ vs. FRL; ## $p<0.01$ vs. FRL-MS; §§§ $p<0.001$ vs. FSL; ++ $p<0.01$ vs. FSL-MS; $n=4$ in duplicate.

increase of GluR2/3 expression ($+25.64\pm5.82\%$, $p<0.01$ for FRL and $+28.73\pm2.88\%$, $p<0.01$ for FSL, BPHT), reversed by chronic Esc ($-19.79\pm5.72\%$, $p<0.01$ vs. FRL-MS rats and $-20.41\pm2.46\%$, $p<0.01$ vs. FSL-MS rats, BPHT).

Discussion

Remodelling by ELS of synaptic plasticity in genetically vulnerable FSL rats

With regard to behaviour, the rat groups employed in this work were similar to those previously subjected to the Porsolt forced swim test (El Khoury et al., 2006). The effect of Esc was more marked in FSL relative to FRL rats and immobility in FSL was exacerbated by MS. Esc partly reduced the increased immobility after MS. Synaptic plasticity is different in FSL compared to FRL rats, because HFS failed to induce LTP in basal FSL. Chronic Esc strongly reduced LTP in FRL, in line with previous findings showing that antidepressants block LTP after acute (Kojima et al., 2003; Mnie-Filali et al., 2006; Shakesby et al., 2002) or chronic (Ohashi et al., 2002; Stewart and Reid, 2000) administration, and reduce baseline excitatory synaptic transmission in the CA1 area in vivo. Remarkably MS, while partially preventing inhibition of LTP and reducing excitability in FSL, had no significant effect on LTP in FRL, suggesting a qualitative difference in the response to ELS. ELS protocols have previously been shown to increase magnitude of LTP in dentate gyrus (Kehoe et al., 1995; Kehoe and Bronzino, 1999; Stewart et al., 2004), and to accelerate LTP development in CA1 area (Huang et al., 2005). Indeed, the present results suggest that ELS remodels synaptic plasticity in genetically vulnerable rats and attenuates the typical inhibitory effect of antidepressants in the CA1 area. This change is accompanied at synaptic level by lifelong up-regulation of NMDAR. The first 2–3 postnatal weeks are a critical period for NMDAR expression and establishment of synaptic connections (Babb et al., 2005); therefore ELS in this same period may permanently alter synaptic plasticity in FSL rats. ELS also induced in both rat lines up-regulation of GluR2/3 AMPAR subunits, a change that (as for NR1) was reversed by Esc treatment, suggesting that the ratio NMDAR-/AMPAR-mediated trasmission could be permanently altered by Esc in FSL rats.

It could be speculated that remodelling of NMDAR-dependent plasticity represents a maladaptive change induced in these vulnerable rats by ELS. Several studies have previously suggested up-regulation of NMDAR-mediated neurotransmission in the pathophysiology of depression (Agid et al., 2007; Kim and Diamond, 2002; Zarate et al., 2003). An alternative explanation could be that early adverse events may prime the HC for a better coping with plasticity-requiring tasks in adult life. Two recent studies on the outcome of ELS or low maternal care in rodents point to this direction (Champagne et al., 2008; Nair et al., 2007).

Interestingly, we recently found that basal FSL rats also display higher HC cytogenesis relative to FRL, with further increase by ELS (Petersen et al., 2008), a feature that may contribute to the observed remodelling of plasticity.

Note

Supplementary material accompanies this paper on the Journal's website (http://journals.cambridge.org).

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Statement of Interest

None.

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