The expression of inducible transcription factors was studied following repetitive electroconvulsive seizures (ECS). c-Fos, c-Jun, J unB, and J unD immunoreactivities were investigated following a single (1× ECS) or repetitive ECS evoked once per day for 4, 5, or 10 days (4× ECS, 5× ECS, or 10× ECS). Animals were killed 3 or 12 h following the last ECS. Three hours after 1× ECS, c-Fos was expressed throughout the cortex and hippocampus. After 5× ECS and 10× ECS, c-Fos was reexpressed in the CA4 area, but was completely absent in the other hippocampal areas and cortex. In these areas, c-Fos became only reinducible when the time lag between two ECS stimuli was 5 days. In contrast to c-Fos, intense J unB expression was inducible in the cortex and hippocampus, but not CA4 subfield, after 1× ECS, 5× ECS, and 10× ECS. Repetitive ECS did not effect c-Jun and J unD expression. In a second model of systemic excitation of the brain, repetitive daily injection of kainic acid for 4 days completely failed to express c-Fos, c-Jun, and J unB after the last application whereas injection of kainic acid once per week did not alter the strong expressions compared to a single application of kainic acid. In order to study the maintenance of c-Fos expression during repetitive seizures, brain-derived neurotrophic factor (BDNF) was applied in parallel for 5 or 10 days via miniosmotic pumps and permanent cannula targeted at the hippocampus or the parietal cortex. Infusion of BDNF completely reinduced c-Fos expression during 5× ECS or 10× ECS in the cortex ipsilaterally to the cannula and, to a less extent, also increased the expression of c-Jun and J unB when compared to saline-treated controls. BDNF had no effect on the expression patterns in the hippocampus. ECS with or without BDNF infusion did not change the expression patterns of the constitutive transcription factors ATF-2, CREB, and SRF. These data demonstrate that various transcription factors substantially differ in their response to acute and chronic neural stimulation. Repetitive pathophysiological excitation decreases the transcriptional actions of neurons over days in the adult brain, and this decrement can be prevented by BDNF restoring the neuroplasticity at the level of gene transcription.

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INTRODUCTION

In the nervous system, expression of inducible transcription factors (ITFs) such as J un, Fos, or Krox proteins is considered to be indicative of plasticity at the level of gene expression (17, 51, 52). Their rapid expression has profound consequences for gene transcription finally resulting in alterations of behavior, morphology, survival, or degeneration of neurons following pathophysiological stimuli (24, 25, 32, 35, 60, 69, 72). However, only a few studies have addressed the effect of chronic or repetitive stimuli on ITF expression. For example, ITF expression is reduced following chronic transynaptic stimulation paradigms such as chronic peripheral inflammation (43), electroconvulsive seizures (71), immobilization stress (66, 67), and visual or acoustic stimulation during postnatal development (40, 55). It remains to be elucidated whether this refractory behavior in the chain of stimulus–transcription–coupling is due to the decreased novelty of the chronic input or concerns only distinct second messenger cascades or presents a mechanism of transcriptional stabilization protecting the neuron from transcriptional overactivation. On the other hand, down-

ABBREVIATIONS

Abbreviations used: AP-1, activator protein 1; ATF-2, activating transcription factor 2; BDNF, brain-derived neurotrophic factor; CREB, calcium/cAMP response element binding protein; ECS, electroconvulsive seizures; IR, immunoreactivities; ITFs, inducible transcription factors; SRF, serum response element binding factor.
regulation of ITFs could result in loss of plasticity with stabilization at a putatively pathophysiological level. This stabilization might account for therapeutic resistance or fixation of syndromes ultimately leading to lasting irreversible alterations such as recurrent seizure activity or endogenous pain syndromes. Therefore, it is an important issue to study whether the decreased inducibility of transcription factors can be restored during ongoing stimulation, e.g., by the application of compounds such as the brain-derived neurotrophic factor (BDNF) that have strong effects on neuroplasticity and c-Fos expression in vitro (22, 36).

BDNF is a member of the neurotrophin family that includes NGF, NT-3, and NT-4/5. Originally, the role of BDNF was defined as potent survival factor for otherwise dying neurons during the pre- and postnatal development as well as following nerve fiber damage (3, 37). BDNF also supports the differentiation of embryonic neurons and formation of cytoarchitecture in the cortex and the synaptic plasticity (22, 47, 65). Moreover, BDNF and its high-affinity receptor trkB are rapidly expressed following seizure activity, kindling, and long-term potentiation (13–16, 38, 73). Recent experiments on targeted disruption of the BDNF locus have indicated a role of the BDNF protein for neuronal hyperexcitation (41, 42).

In the present study we have investigated whether infusions of BDNF can modulate the expression of ITFs following repetitive electroconvulsive seizures. Particular attention was paid to the effect of BDNF on c-Fos expression that is down-regulated with repetition of seizure events.

MATERIAL AND METHODS

Animal Experiments

Electroconvulsive seizures (ECS). All experiments were performed with male Wistar rats (250–300 g body wt). For application of ECS, rats were stimulated via saline-soaked electrode earclips from an electroshock seizure apparatus (Ugo Basile, Italy) by electric impulses at 60 mA (50 Hz and 0.8 s in duration), and this stimulus was given once daily. ECS elicited a full generalized tonic–clonic convulsion with hindlimb extension and terminated by a period of immobility and exhaustion. Recovery occurred within a few minutes. The body weight, duration, and intensity of the convulsions as well as the motor behavior were visually monitored.

ECS were applied as a single daily stimulus for 1 day (1 × ECS), for 5 days (5 × ECS), or for 10 days (10 × ECS). The rats were allowed to survive the last ECS for 3 or 12 h (for each group and each survival time n = 4) and were killed by intracardial perfusion (4% paraformaldehyde in phosphate buffer) under deep anesthesia (100 mg/kg pentobarbital, ip). For controls, untreated rats (n = 4) were perfused within 20 min following removal from the animal house. For sham treatment, the electrodes were clipped onto the ears for 2 min without application of current (n = 2).

In a further experimental set, the fifth ECS was applied either 2 or 5 days after the fourth ECS (each n = 3), and the rats were killed 3 h after the last ECS. Table 1 summarizes the stimulation protocols.

Application of brain-derived neurotrophic factor. For application of BDNF, miniosmotic pumps (Alzet 2002, flow rate 0.5 µl/h) were implanted under the skin of the neck in anesthetized rats (60 mg/kg pentobarbital, ip). A 27-gauge cannula was implanted at bregma −3.8 and 2.7 mm lateral targeted to the upper blade of the dentate gyrus (4.0 mm in depth) or to the cortical layer V (1.8 mm in depth). The rats were infused for 6 or 11 days with recombinant human BDNF (total amount 14 µg/day, diluted with 0.9% saline; Amgen-Regeneron Partners, U.S.A.) or with 0.9% saline alone. The day after the implantation, the rats were stimulated by the ECS for 5 or 10 days (for each implantation site and treatment n = 4, total of 32 rats). Saline- and BDNF-treated rats were implanted, ECS stimulated, and perfused, and the brains were immunocytochemically processed under identical conditions. A second control group was infused with BDNF for 10 days without any further treatment. The infusion protocols are summarized in Table 1.

Application of kainic acid. KA was applied (10 mg/kg body wt, ip) as single injection, daily for 5 days, or one time per week for 5 weeks (each n = 4). The seizures were terminated after 2 h following injection of diazepam (10 µg/100 g, ip). The rats were killed 3 h after the last injection of KA. Within each group, each two brains were used for immunocytochemistry and for Western blotting analysis.

Western Blotting Analysis

Rats were rapidly anesthetized (100 mg/kg body wt pentobarbital, ip) and decapitated after decay of the

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**TABLE 1**

<table>
<thead>
<tr>
<th>Infusions</th>
<th>Basal</th>
<th>1 × ECS</th>
<th>5 × ECS</th>
<th>10 × ECS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECS alone</td>
<td>4 4 4</td>
<td>3h 12h</td>
<td>3h 12h</td>
<td>3h 12h</td>
</tr>
<tr>
<td>ECS + saline in cortex</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>ECS + saline in hippocampus</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>ECS + BDNF in cortex</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>ECS + BDNF in hippocampus</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Note: The numbers are given of animals and the time points investigated following ECS alone and parallel application of BDNF or saline into the cortex or hippocampus.
nociceptive reflexes. The brain was rapidly removed, the cortexes with the underlying hippocampus of two rats were pooled and homogenized, and then nuclear extracts were prepared as described previously (34). Equal amounts of protein (30 µg/lane) were separated on 12% SDS–polyacrylamide gels. Following electrophoretic transfer to nitrocellulose filters, the reactions of primary antisera were visualized by ECL Western blotting detection system (Amersham Corporation, U.S.A.). The dilutions were anti-c-j un 1:15,000, anti-J unB 1:5,000, anti-c-Fos 1:15,000, and anti-Krox-24 1:6,000.

Immunocytochemistry

After perfusion, the removed brain was postfixed overnight in the same fixative followed by cryoprotection in 30% sucrose. Coronal 40-µm sections were processed free-floating for immunocytochemistry using conventional visualization by avidin–biotin complex and diaminobenzidine (Vectastain, Vector Laboratories) as described in detail previously (25). The primary antibodies were incubated for 48 h and were diluted as follows: c-Fos 1:15,000, c-j un 1:25,000, J unB 1:7,000, J unD 1:6,000 (generous gift from Dr. R. Bravo, Bristol-Myers Squibb Research Institute, Princeton, NJ), GFAP 1:5,000 (Boehringer Mannheim), somatostatin 1:2,000 (Cambridge Research), ATF-2 1:3,000 (UBI), CREB 1:30,000 (generous gift from Dr. W. Schmid, DKFZ Heidelberg), and SRF 1:1,500 (generous gift from Dr. D. Ginty, Harvard Medical School). Antisera against NPY and somatostatin (Cambridge Biological Research, UK) were each diluted 1:1,500.

Counting Analysis

From each rat, the immunoreactive nuclei of two sections were counted in a blind fashion by the first and last author within a 1-mm² area each in the somatosensory cortex between bregma −3.50 and −2.00 (Fig. 5A). The counts comprised all those nuclear immunoreactive signals that could be clearly distinguished from the background. Means and standard deviations were calculated, and significance was determined by the Student t test with P < 0.05. Counting analysis was performed only for the stainings in the cortex since BDNF affected only this compartment.

RESULTS

Body Weight and Seizure Activities

In controls that were infused with saline during repetitive seizures, the body weight increased after 5×ECS and 10×ECS by 3.2 ± 4.1 and 4.4 ± 4.9%, respectively, when compared to the body weight measured before the first ECS. Following infusion of BDNF, the body weight decreased after 5×ECS by 1.8 ± 7.3% and increased after 10×ECS by 2.7 ± 4.1%. These changes in body weight were not significantly different between the two experimental groups.

The seizure activity was carefully monitored for each rat. The convulsions, including tonic flexion and extension followed by clonic episodes, had median durations of 20 s (15–45 s) after the first ECS, 30 s (19–60 s) after 5×ECS, and 30 s (18–60 s) after 10×ECS. These differences between the groups were not significant. The tendency for increase in duration following repetitive ECS compared to acute ECS is most likely due to some kindling effect.

Expression in Untreated Rats and Sham Controls

In the cortical layers, hippocampus, and medial thalamus, the basal expression of ITFs did not differ from that pattern that was recently published (27, 28). Briefly, c-Fos immunoreactivity (IR) was found in single scattered neurons of the superficial cortical layers and in the entorhinal and retrosplenial cortex (Fig. 1A). In the hippocampus, scattered neurons were found in the CA1 and CA3 area, but not in the dentate gyrus and CA4 subfield (Fig. 1B). c-j un was expressed at high intensity in numerous neurons of the dentate gyrus, and a moderate c-j un-IR was found in scattered neurons mostly in the superficial cortical layers (Figs. 2A and 2B). Expression of J unB in the cortex and dentate gyrus was restricted to few labeled neuronal nuclei (Figs. 3A and 3B) whereas a moderate J unB-IR was visible in numerous neurons of the CA1 hippocampal subfield. A clear labeling of J unD was seen in all cortical layers and the hippocampal subfields.

ATF-2 showed an intense nuclear labeling of apparently all neuronal nuclei throughout the brain including the cortex and the hippocampal CA1–4 areas, but was only weakly expressed in the dentate gyrus (28, 64). SRF and CREB were heavily expressed in the vast majority of hippocampal and cortical neurons as described recently (27, 29).

In the nucleus periventricularis of thalamus that processes stress-related stimulation, c-Fos, c-j un, and J unB were absent, whereas J unD-IR was distributed throughout this compartment at a prominent intensity (28).

In sham-treated controls which received the ear dips without application of electrical current 1×, 5×, or 10×, all the cortical and hippocampal immunoreactivities did not differ from basal labelings.

Single Electroconvulsive Seizures

The changes following repetitive ECS are summarized in the Table 2 (quantitative changes) and Table 3 (quantification of labeled neurons in the cortex).

c-Fos. Three hours following 1×ECS, expression of c-Fos was dramatically enhanced throughout all corti-
cal layers and subfields as well as in the dentate gyrus (DG) and all hippocampal areas. A particular intense c-Fos-IR was seen in the superficial layer of the entorhinal cortex and the hilar interneurons of CA4 (Figs. 1C and 1D; Tables 2A and 3).

c-Jun. 1 × ECS was ineffective at changing the moderate basal c-Jun-IR in the cortex and hippocampus (Tables 2B and 3).

JunB. Similar to c-Fos, JunB was strongly expressed in the cortex and hippocampus following 1 × ECS (Figs. 3C and 3D; Tables 2C and 3). Maximal levels of expression were found in the entorhinal cortex and dentate gyrus, but JunB was not induced in the CA4 subfield.

JunD. Similar to c-Jun, the expression of JunD protein did not change following 1 × ECS (data not shown).

**FIG. 1.** Expression of c-Fos in the parietal cortex (left column, A,C,E,G,I) and dentate gyrus (right column, B,D,F) of untreated rats (A,B), following 1 × ECS with saline (C,D), and following 1 × ECS with BDNF (E,F). Magnification, A,C,E, 300×; B,D,F, 600×.

**FIG. 2.** Expression of c-Jun in the parietal cortex (left column, A,C,E) and upper blade of dentate gyrus (right column, B,D,F) of untreated rats (A,B), following 10 × ECS with saline (C,D), and following 10 × ECS with BDNF (E,F). Magnification, A,C,E, 300×; B,D,F, 600×.
TABLE 2
Summary of Results: Altersations of Immunoreactivities Compared to Basal Levels and Effect of BDNF or Saline

<table>
<thead>
<tr>
<th></th>
<th>1 × ECS</th>
<th>5 × ECS</th>
<th>10 × ECS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
<td>12 h</td>
<td>3 h</td>
</tr>
<tr>
<td>A. c-Fos</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>++ b</td>
<td>+ b</td>
<td>b b b</td>
</tr>
<tr>
<td>DG, hippocampus CA1-3</td>
<td>++ b</td>
<td>+ b</td>
<td>b b b</td>
</tr>
<tr>
<td>Hippocampus CA4</td>
<td>++ b</td>
<td>+ b</td>
<td>b b b</td>
</tr>
<tr>
<td>Saline-cortex</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Saline-DG, hippocampus CA1-3</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Saline-hippocampus CA4</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BDNF-cortex</td>
<td>–</td>
<td>–</td>
<td>*</td>
</tr>
<tr>
<td>B. c-Jun</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>b b b</td>
<td>b b b</td>
<td>b b b</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>b b b</td>
<td>b b b</td>
<td>b b b</td>
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<tr>
<td>Saline-cortex</td>
<td>–</td>
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<tr>
<td>Saline-hippocampus</td>
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<td>–</td>
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<tr>
<td>BDNF-cortex</td>
<td>–</td>
<td>–</td>
<td>*</td>
</tr>
<tr>
<td>BDNF-hippocampus</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B. c-Jun</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td>+ + –</td>
<td>+ + –</td>
<td>+ + –</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>+ + –</td>
<td>+ + –</td>
<td>+ + –</td>
</tr>
<tr>
<td>Saline-cortex</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Saline-hippocampus</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BDNF-cortex</td>
<td>(†)</td>
<td>(†)</td>
<td>(†)</td>
</tr>
<tr>
<td>BDNF-hippocampus</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Note. The alteration of immunoreactivities following ECS with or without BDNF or saline infusion in the parietal cortex or dorsal hippocampus is given. ++, strong increase; +, moderate increase compared to basal levels; b, basal, i.e., no changes compared to basal expression. *Significant increase; (†), non-significant increase following BDNF infusion compared to saline infusion. –, no changes following BDNF or saline infusion compared to ECS alone.

TABLE 3
Means and Standard Deviations of Nuclei Labeled for c-Fos, c-Jun, and J unB in a 1-mm² Area that is Marked in Fig. 5A and that is Considered to be Representative for the Expression Levels in the Cortex

<table>
<thead>
<tr>
<th></th>
<th>c-Fos</th>
<th>c-Jun</th>
<th>J unB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>32 ± 19</td>
<td>88 ± 34</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>10 × sham</td>
<td>53 ± 24</td>
<td>101 ± 25</td>
<td>30 ± 12</td>
</tr>
<tr>
<td>1 × ECS</td>
<td>319 ± 43</td>
<td>72 ± 26</td>
<td>197 ± 36</td>
</tr>
<tr>
<td>5 × ECS</td>
<td>45 ± 25</td>
<td>78 ± 33</td>
<td>151 ± 19</td>
</tr>
<tr>
<td>10 × ECS</td>
<td>38 ± 15</td>
<td>108 ± 40</td>
<td>186 ± 43</td>
</tr>
<tr>
<td>NaCl + 5 × ECS</td>
<td>62 ± 23</td>
<td>116 ± 21</td>
<td>181 ± 45</td>
</tr>
<tr>
<td>NaCl + 10 × ECS</td>
<td>74 ± 18</td>
<td>123 ± 34</td>
<td>202 ± 38</td>
</tr>
<tr>
<td>BDNF + 5 × ECS</td>
<td>427 ± 51*</td>
<td>155 ± 36</td>
<td>254 ± 67</td>
</tr>
<tr>
<td>BDNF + 10 × ECS</td>
<td>408 ± 49*</td>
<td>208 ± 32*</td>
<td>287 ± 54</td>
</tr>
</tbody>
</table>

*Significance (P < 0.05) following BDNF treatment compared with saline application.

Twelve hours after 1 × ECS, all those immunoreactivities that were enhanced after 3 h had returned to basal levels. In the nucleus paraventricularis of thalamus, 1 × ECS evoked c-Fos, J unB, and J unD expression in around 50 neurons/section, whereas c-Jun was visible in only 5–10 nuclei/section.

5 × ECS and 10 × ECS

- c-Fos. Three hours after the fifth ECS (5 × ECS), suprabasal c-Fos-IR was barely detectable (n = 2) or virtually absent (n = 2) in the neocortical layers and the hippocampal CA1–CA3 areas (Table 2). In contrast, intense c-Fos-IR was still present in the CA4 interneurons that did not differ from that evoked by 1 × ECS. After 10 × ECS, c-Fos was only present in few weakly labeled scattered neurons in the cortex and were virtually absent in all rats investigated (Figs. 1E and 1F; Tables 2A and 3).

- c-Jun. In the cortex and hippocampus, the pattern of c-Jun-IR did not differ from that of untreated rats following either 5 × ECS or 10 × ECS (Tables 2B and 3).

- J unB. Of all IIFs investigated, only the expression of J unB reflected the repetitive seizure activities (Figs. 3E and 3F; Tables 2C and 3). After 5 × ECS and 10 × ECS, J unB-IR was as intense as following 1 × ECS in the cortex and hippocampus. J unB remained completely absent in the hilar CA4 interneurons at any time point investigated.

- J unD. Similar to c-Jun, J unD-IR was not altered by repetitive ECS in the cortex and hippocampus (data not shown). As with 1 × ECS, 12 hours after 5 × ECS or 10 × ECS, the immunoreactivities had disappeared or returned to basal levels in all areas of interest. In the nucleus paraventricularis of thalamus, the expression of c-Fos and J un proteins remained reinducible at the level that was seen following 1 × ECS.

Reinduction of c-Fos

In order to determine the latency for c-Fos reexpression following repeated ECS, rats underwent daily ECS for 4 days with the fifth ECS applied after a 48-h or 5-day interval free of ECS. An interval of 48 h was not effective at reinducing c-Fos whereas a temporal lag of 5 days before the last ECS resulted in strong c-Fos reexpression which reached the levels of that labeling evoked by 1 × ECS (Fig. 4).

Fig. 3. Expression of J unB in the parietal cortex (left column, A–E, G, I), dentate gyrus (right column, B, D, F), and CA1 area (H, J) of untreated rats (A, B), following 1 × ECS (C, D), 10 × ECS (E, F), 10 × ECS with infusion of saline (G, H), and 10 × ECS with infusion of BDNF (I, J). Original magnification, A–G, I, 300×; H, J, 600×.
Effect of Chronic BDNF Infusion on ITF Expression Following Repetitive ECS

Implantation of the Cannula in Otherwise Untreated Rats

Ten days following the implantation of the cannula in the cortex or hippocampus, numerous neurons and glial cells labeled by c-Fos or J un were visible within 150 µm along the implanted cannula and in the superficial layer of the ipsilateral cortex. Apart from this site of injury, the expression patterns of all the proteins studied did not differ from that of untreated rats.

Infusion of BDNF in Otherwise Untreated Rats

Chronic infusion of BDNF (in 0.9% saline) into the cortex or hippocampus of otherwise untreated rats for 10 days did not alter the basal immunoreactivity of c-Fos, c-Jun, J unB, and J unD (see “Expression in Untreated Rats and Sham Controls” under Results) when the rats were killed at day 10.

Infusion of Saline (Controls) during 5×ECS and 10×ECS

Infusion of 0.9% saline during 5×ECS or 10×ECS evoked a slightly increased expression of c-Fos and J un proteins in the area immediately adjacent to the cannula. In the more remote areas of the cortex and hippocampus, c-Fos, c-Jun, and J unB were visible in a slightly increased number of neurons that did not significantly differ from those provoked by repetitive seizures without cannula and NaCl infusion (Table 3; Figs. 1G and 1H, 2C and 2D, 3G and 3H, and 6A).

Infusion of BDNF during 5×ECS and 10×ECS

C-Fos. Infusion of BDNF into the cortex substantially altered the expression of c-Fos in the cortex following 5×ECS and 10×ECS when compared with mere 5×ECS or 10×ECS (see “5×ECS and 10×ECS” under Results) or with the controls that received saline. c-Fos was expressed at high levels in all layers throughout the ipsilateral cortex (Table 3). Particularly intense reexpression was seen in the transition region of the parietal and entorhinal cortex, and c-Fos was also visible in the transition region of the contralateral cortex (Figs. 1C and 5B). c-Fos-IR was not altered by BDNF in other brain structures.

In contrast, BDNF infusion into the hippocampus did not raise the level of c-Fos in this compartment nor in any other brain areas compared to 5×ECS or 10×ECS alone (Fig. 1J) (see “5×ECS and 10×ECS” under Results). Consequently, BDNF neither enhanced nor reduced the labeling in the hilar CA4 neurons.

C-Jun. BDNF significantly increased the number of c-Jun in the cortex after 10×ECS, but not after 5×ECS,
when compared to the saline controls and to the mere ECS stimulation. The majority of the neurons also exhibited an enhanced intensity of nuclear labeling (Figs. 2E and 2F; Table 2B).

JunB. Infusion of BDNF did not significantly change the expression of JunB in the cortex after 5×ECS and 10×ECS (Table 3; Fig. 3I) when compared with controls (Fig. 3G) or ECS alone (Fig. 3C), and JunB remained in the CA4 subfield (Fig. 3J; Table 3).

Expression of ATF-2, CREB, and SRF

In addition to the inducible transcription factors, we studied the expression of the preexisting constitutive transcription factors ATF-2, CREB, and SRF. These
proteins display a strong immunoreactivity in the cortical layers and hippocampal areas. Following repetitive ECS with or without BDNF, the immunoreactivities of ATF-2, CREB, and SRF did not change in these compartments compared to untreated rats, and this was also true for the moderate basal ATF-2 labeling in the dentate gyrus.

Expression of ITFs Following Repetitive Application of Kainic Acid

This experimental paradigm addressed the question whether the habituation of c-Fos expression after repeated exposure to seizures is a general neuronal property following repeated seizure activity. Daily provocation of 3-h limbic seizures by kainic acid for 4 days resulted in complete disappearance of c-Fos including the CA4 interneurons when analyzed 3 h after the last application of kainic acid by Western blotting analysis and immunocytochemistry. Similar as with c-Fos and in contrast to ECS, c-Jun, JunB, and Krox-24 completely disappeared. When kainic acid was injected once per week for 4 weeks, the expression of c-Fos, JunB, and Krox-24 did not differ from that following a single injection (Figs. 6A and 6B).

DISCUSSION

The present study has investigated the effect of repetitive ECS on the expression of the ITFs c-Fos, c-Jun, JunB, and JunD. These proteins represent the earliest alterations in gene expression following neuronal stimulation and, more importantly, mediate the transcription of those genes that are partially or predominantly controlled by AP-1 or CRE response elements (20, 51, 52).

Application of acute or repetitive ECS represents a model for treatment of depression, seizure activity, or development of kindling, respectively, that is highly reproducible in terms of both the applied stimulation and the animals' behavioral response. In contrast, chemical convulsants such as kainic acid, bicuculline, or pentylenetetrazole produce generalized seizures that are more variable in duration, intensity, and behavioral expressions.

Neither ECS alone nor ECS in combination with BDNF are able to induce the expression of the constitutive transcription factors (CTFs) ATF-2, CREB, or SRF. Subsequently, this operative standby confers to ITFs such as Jun and Fos proteins a fundamental role in gene transcription since AP-1 and in part CRE-controlled effector genes are dependent on the de novo synthesis of ITFs (2, 17).

Single ECS

1×ECS evokes a strong expression of c-Fos and JunB proteins but not of c-Jun and JunD, in the cortex and hippocampus. This expression pattern clearly differs from that following application of the chemoconvulsants kainic acid (12, 19, 28), bicuculline (18), or pentylenetetrazole (62) characterized by induction of all these ITFs. The absence of c-Jun and JunD following ECS can be explained by the inability of membrane depolarization to induce c-Jun and JunD as has been observed following application of KCl (4), cortical spreading depression (27), and following cerebellar long-term depression (56). Previously, induction of c-jun mRNA was observed after 1×ECS but it was provoked at stimulation intensities substantially stronger as used in the present study (10). Finally, the selective expression patterns demonstrate the specificity of the antisera used.

The c-Fos expression following acute ECS is characterized by a high de novo synthesis in the cortex and hippocampus including a strong nuclear signal in the hilar interneurons. The JunB expression parallels that of c-Fos apart from its complete absence in the hilar interneurons demonstrating an independent regulation of c-Fos and JunB even following intense stimuli such as ECS. The JunB protein, a major partner of c-Fos in AP-1 transcription complexes, can support the survival of cultured perinatal hippocampal neurons (60). Thus, the vulnerability of hilar neurons to seizure activity could be related to the absence of JunB and the subsequent reduced transactivation of AP-1-driven promoters.

Desensitization of c-Fos Following Repetitive ECS: Loss of Plasticity at the Genetic Level?

With increasing numbers of applied ECS, c-Fos becomes progressively less inducible in the cortex and hippocampal CA1-3 areas, but, in contrast, remains fully inducible in the vulnerable hilar interneurons. The decrease in c-Fos expression is not due to a decrease in transsynaptic impulse transfer since JunB remains fully inducible and the behavioral symptoms are not attenuated. Both the loss of c-Fos expression and the reinduction of JunB following ECS occur at the...
transcriptional level (50, 53, 71), and in addition to c-Fos, FosB have also been found to habituate following ECS (9).

The c-Fos protein becomes only reinducible after a latency of more than 2 days following the preceding ECS. Such a temporal lag deserves a strong interest demonstrating that short-lasting neuronal excitation can result in lasting suppression of gene induction. In contrast, junB mRNA is reinducible by a second ECS even when applied only 2 h following the first ECS (8).

The refractoriness of c-Fos to repetitive stimulation has been observed under both physiological and pathophysiological conditions such as repetitive visual stimuli (55), mechanical brain injury (39), repetitive acoustic stimuli (40), chronic application of apomorphine (58), daily immobilization stress (67), or chronic peripheral inflammation (43).

What might be the meaning of down-regulated expression of c-Fos and other ITFs? The absence of c-Fos might result in substantial decrease of AP-1-mediated gene transcription, the more so as c-jun and JunD are not elevated from their basal levels. Consequently, absence of c-Fos or other ITFs, e.g., following chronic arthritis (43), results in reduction of gene transcription. This loss of plasticity at the level of gene expression might severely restrict the ability of stimulated neurons to respond to pathophysiological stimuli with an adaptive regulation of effector genes. Subsequently, decrease in ITF-regulated transcription will result in “fixation” of the neuronal genetic programs and, consequently, could contribute to chronic establishment of acute harmful events such as epileptic seizures and pain. The idea of “fixation” at pathophysiological levels is supported by the finding that repetitive ECS evoked long-lasting, perhaps permanent, changes in evoked potentials (6). On the other hand, it cannot be ruled out that the lasting expression of Fos-related proteins (Fra’s) (9) compensate for the habituation of c-Fos.

The desensitization of the c-fos induction must also be discussed in view of protection against transcriptional overactivation. Repetitive ECS result in lasting expression and DNA binding of Fra proteins (Fos-related antigens) that surpasses the last ECS for several days (9, 33). The rapid down-regulation of c-Fos might prevent neurons for further prolongation of Fra expression, since fra-1 induction is under control of c-Fos as shown in cells with targeted disruption of the c-fos locus (5).

In contrast to ECS, repetitive application of kainic acid abolishes also the induction of c-jun, junB, and Krox-24. Kainic acid provokes much stronger and longer seizure activities including status epilepticus. Thus, the extent of refraction of ITF induction parallels the increase in stimulation intensity, and lack of ITF-mediated transcriptional operations is one aspect of excitotoxic insults in the adult brain.

Reinduction of c-Fos by BDNF

Considering the preceding hypothesis, it is an intriguing and relevant question whether the refractory expression of ITFs can be alleviated. We tested the effect of the neurotrophin BDNF on ECS-triggered down-regulation of c-Fos. BDNF substantially interferes with the pathogenesis and pathophysiology of seizure activity by several mechanisms. (i) BDNF regulates the differentiation and survival of embryonic cortical neurons and is a potent inducer of c-Fos in this paradigm (1, 21, 22, 41, 68). (ii) Infusion of BDNF protects the rat brain from development of kindling (44). In contradistinction, BDNF can also enhance the efficiency of synaptic transmission in vitro (45), and targeted disruption of the BDNF gene blocks the development of kindling and hippocampal long-term potentiation (40, 41) suggesting the involvement of BDNF in the hyperexcitation of mammalian neurons. (iii) ECS increase the synthesis of BDNF and induction of its receptor trkB (the full-length and truncated form) in the cortex and hippocampus (13, 15, 46, 57).

Here, we have demonstrated that BDNF restores the induction of c-Fos following repetitive ECS in the rat cortex. c-Fos is reexpressed in the ipsilateral and to some extent in those compartments of the contralateral cortex that are homologous to the site of maximal ipsilateral c-Fos expression. A similar pattern has been observed for neuropeptides that are moderately expressed in the contralateral cortex following unilateral BDNF infusion (11). Since BDNF has only a restricted diffusion (11), this widespread reexpression of c-Fos might be triggered by an indirect mechanism, e.g., release of diffusible factor or, more likely, by transsynaptic stimulation via reciprocally interhemispheric connections. Inter- and intrahemispheric transport of BDNF seem also unlikely since immunocytochemistry showed a restricted distribution of BDNF (11) and since diffused BDNF should evoke a widespread distribution of c-Fos-IR. The effect of reexpression of c-Fos by nonspecific factors such as the implantation of the cannula can be excluded: (i) Controls with saline infusion do not show reexpression of c-Fos. (ii) Insertion of the cannula into the hippocampus is not followed by expression of c-Fos after 10 days, and (iii) insertion of a cannula with chronic infusion can also be considered as an ongoing stimulus, and, as discussed above, ongoing stimuli lead to the desensitization of c-Fos.

The discussion of the c-Fos reinduction must also consider the putative epileptogenic effect of BDNF. Such an effect should provoke c-Fos expression throughout the limbic system and a permanent seizure activity during the entire infusion period of BDNF. However, the postseizure behavior of the rats did not reveal any motoric disturbances. BDNF induction during ECS that per se prevents c-Fos expression suggests that BDNF transactivates the c-fos promoter by second
messenger cascades different from those activated by the ECS. This is supported by the finding that BDNF evokes gene induction even during inhibition of action potential formation (48).

The question arises as to why BDNF restores c-fos expression only in the cortex, but not in the hippocampus. Thus, BDNF might selectively act on cortical versus hippocampal neurons. Assuming that BDNF is involved in hyperexcitation of hippocampal neurons (41, 42, 45), BDNF infusion could reinforce the desensitization of c-fos expression and this process might involve down-regulation of trkB receptors (57). Furthermore, BDNF might act on cortical neurons predominantly as trophic factor using specific signal cascades terminating onto the c-fos gene (61).

Besides a putative tissue-specific control of c-Fos expression by BDNF, the nature of BDNF action on c-fos expression in adult brain must still be elucidated. Thus, BDNF and its full-length receptor trkB are expressed in the hippocampus of the untreated rat in which c-fos is virtually absent. The induction of c-fos mRNA in neonatal hippocampal neurons in vitro (36, 48) does not simply point at the existence of a similar induction mechanism in adult neurons in vivo. In another experimental paradigm, transection of nerve fibers induces trkB receptor and, subsequently, renders the axotomized neurons sensitive to BDNF supplement (3, 37), but c-Fos remains absent in axotomized neurons (17).

Conclusions

Repetitive stimulation such as neuronal excitation by ECS provokes an intricate pattern of ITF expression and habituation. Application of trophic factors such as BDNF provokes an intricate pattern of ITF expression and this process might involve down-regulation of trkB receptors (57). Furthermore, BDNF might act on cortical neurons predominantly as trophic factor using specific signal cascades terminating onto the c-fos gene (61).

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