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Induction of Inducible cAMP Early Repressor Expression in Nucleus Accumbens by Stress or Amphetamine Increases Behavioral Responses to Emotional Stimuli

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Previous research has shown that cAMP response element (CRE)-mediated transcription is activated in the nucleus accumbens, a major brain reward region, by a variety of environmental stimuli and contributes to neuroadaptations to these stimuli. CRE-binding protein (CREB) is the most studied activator of CRE transcription and has been implicated in this brain region as a gating mechanism for behavioral responses to emotional stimuli. Little attention, however, has been given to naturally occurring inhibitors of CRE-mediated transcription, such as the inducible cAMP early repressor (ICER), an inhibitory product of the CRE modulator gene. In the present study, we investigated the extent to which ICER is induced in the nucleus accumbens by two types of environmental stimuli, stress and amphetamine, and characterized how induction of ICER in this region affects complex behavior. We show that stress and amphetamine each induces ICER expression and that overexpression of ICER in the nucleus accumbens, using viral-mediated gene transfer, increases behavioral responses to both rewarding and aversive emotional stimuli. For example, ICER overexpression increases sensitivity to amphetamine-stimulated locomotor activity as well as to natural rewards such as sucrose and social grooming. However, ICER overexpression also increases measures of anxiety in the elevated plus maze and neophobia to novel tastes. Finally, ICER produces an antidepressant-like effect in the forced swim test, further indication of an enhanced active response to stress. These results suggest that ICER is an important mechanism for modulating CRE-mediated transcription in the nucleus accumbens.

Key words: depression; addiction; anxiety; drugs of abuse; stimulants; CREB; CREM; striatum; cocaine; sucrose preference

Introduction

Gene transcription is one mechanism by which an organism can adapt to changing environmental conditions. The CAMP response element (CRE) is a promoter/enhancer element present in many genes (Impey et al., 2004; Zhang et al., 2005) that has proven sensitive in many brain areas to diverse environmental stimuli ranging from psychotropic drugs to psychological stress to natural reward (for review, see Konradi et al., 1994; Conti and Blendy, 2004; Carlezon et al., 2005). CRE-mediated transcription is increased when CRE-binding protein (CREB) or several related family members dimerize, bind to DNA, and are activated by phosphorylation, although not necessarily in that order (for review, see Mayr and Montminy, 2001). Previous work has shown that increases in CREB-mediated transcription in the shell region of the nucleus accumbens (ventral striatum), induced by drugs of abuse or stress, decrease behavioral responses to both positive and negative emotional stimuli, whereas blocking CRE transcription, via overexpression of a dominant-negative mutant CREB or via genetic knockdown of CREB, generally does the opposite (Carlezon et al., 1998; Pliakas et al., 2001; Walters and Blendy, 2001; Barrot et al., 2002; Newton et al., 2002; Walters et al., 2003). Although much attention has been directed at activation of CRE-mediated transcription by CREB itself, relatively little attention has been directed at naturally occurring inhibitors of CRE-mediated transcription.

The inducible cAMP early repressor (ICER) protein is a highly inducible and potent endogenous repressor of CRE-mediated transcription (Mioduszewska et al., 2003). ICER is an alternative product of the CRE modulator (CREM) gene driven by a second promoter (P2) located in an intron region of the gene. The full-length CREM protein is very similar to CREB in structure and function; the N terminal consists of glutamine-rich activation domains and a kinase-inducible domain also important for activation of transcription. The C terminal consists of a basic region of charged residues for binding DNA at CRE sites and a nearby leucine zipper allowing for dimerization. ICER, because it is missing the N terminus of the CREM gene, dimerizes and binds DNA but cannot be activated because of a lack of activation and kinase-inducible domains. Thus, ICER is an endogenous...
dominant-negative inhibitor of CRE-mediated transcription. The intrinsic promoter driving ICER has two pairs of CRE sequences in close proximity, which renders this protein highly inducible by stimuli that activate CRE transcription (Molina et al., 1993; Conti et al., 2004). Accordingly, ICER has been implicated in several biological systems as an important negative-feedback mechanism for shutting off CREB-induced transcription (Serrillo et al., 2002; Shepard et al., 2005).

In the present study, we characterized the induction of ICER in the nucleus accumbens by stress or amphetamine and studied the implications of its induction on complex behavior. Our findings demonstrate an important role for ICER in opposing the actions of CREB on regulation of emotional behavior and thereby helping an individual adapt to a range of aversive and rewarding stimuli.

Materials and Methods

Animals. Male Sprague Dawley rats (Harlan, Houston, TX), 250–350 g, were used for all experiments. Rats were pair-housed in an American Association for the Accreditation of Laboratory Animal Care-approved colony, and all experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1995). Rats were housed on a 12 h light/dark cycle with lights on at 7:00 A.M. All procedures were conducted during the light phase of the cycle.

Quantification of mRNA. Rats were killed by rapid decapitation, the entire striatum was dissected, and mRNA was isolated using the RNA Stat 60 reagent (Teltest, Houston, TX) according to the manufacturer’s directions. Contaminating DNA was removed with DNase treatment (Servillo et al., 2002; Shepard et al., 2005). ICER transcript was quantified using quantitative real-time PCR (SYBR Green; Applied Biosystems, Foster City, CA) on a Stratagene (La Jolla, CA) Mx5000 96-well thermal cycler using the Superscript III first-strand kit (Invitrogen). Levels of CREM transcript were normalized to GAPDH, which was not itself affected by ICER.

In initial experiments, the HSV–ICER vector was injected into rat nucleus accumbens shell, and expression was verified using fluorescent immunohistochemistry. Polyclonal rabbit antibody to the full-length human CREM protein (sc-440; Santa Cruz Biotechnology, Santa Cruz, CA) was used at 1:2000 to visualize viral-mediated transgene expression. These conditions do not label endogenous CREM or ICER proteins. The secondary antibody (donkey anti-rabbit; Jackson ImmunoResearch, West Grove, PA) was conjugated to CY2 fluorophore and used at a concentration of 1:200. To further verify expression, PC12 cells were infected with HSV–LacZ or HSV–ICER, and protein was harvested 12 h after infection. ICER protein was identified via Western blot using the CREM antibody at a concentration of 1:1000. To verify the ability of HSV–ICER to decrease expression of CRE–target genes, HSV–ICER and HSV–LacZ were each mixed with a small amount (10%) of HSV–GFP, and the two mixtures were injected into the nucleus accumbens of separate groups of rats. At 48 h after injection, rats were given injections of 4 mg/kg amphetamine. Brains were harvested 3 h later and frozen on dry ice. Brains were later sliced on a cryostat (8 μm), and slices were mounted on polyethylene naphthalate-membrane slides for laser microdissection. Using GFP as a marker for the site of infection, infected tissue was microdissected by laser capture, and RNA was purified using the PicoPure RNA isolation kit (Arcturus, Mountain View, CA). RNA was then amplified using the RibOamp OA kit (Arcturus) according to the manufacturer’s instructions. RNA was subsequently reverse-transcribed using the Superscript III first-strand kit (Invitrogen). Levels of brain-derived neurotrophic factor (BDNF) mRNA, a well-characterized CREB target, were measured using real-time PCR. All PCR data are normalized to GAPDH, which was not itself affected by ICER.

To further verify the ability of HSV–ICER to decrease CRE-driven transcription, viral vectors were also injected into the nucleus accumbens of CRE–LacZ reporter mice. These transgenic mice express β-galactosidase driven by a synthetic promoter consisting of seven tandem repeats of the consensus CRE sequence upstream of a minimal promoter (Barrot et al., 2002; Shaw-Luttmann et al., 2003). For double-labeling experiments, a goat anti-β-galactosidase primary antibody (catalog #4600-1409; Biogenesis, Kingston, NH) was used at a concentration of 1:5000, with a CY3-conjugated secondary antibody. Brains were fixed with 4% paraformaldehyde before sectioning and staining. A laser-scanning confocal microscope was used for imaging. The Z-stack images were analyzed for pixel intensity using the Volocity (Improvision, Lexington, MA) computer program. Mean pixel intensity of uninfected LacZ-positive cells (n = 51) was compared with that of infected LacZ-positive cells (n = 21 in three mice).

Chromatin immunoprecipitation (ChIP) assays were performed exactly as described by Kumar et al. (2005) to study the binding of endogenous ICER to target gene promoters. Briefly, chromatin was isolated from formalin-fixed rat nucleus accumbens, sheared, and immunopre-
ipated with the anti- CREM antibody or with non-immune IgG. Levels of the BDNF gene present in the immunoprecipitates were then analyzed by real-time PCR. In contrast to the CREM antibody, non-immune IgG did not precipitate the BDNF gene.

**Locomotor activity.** Activity was measured in a circular corridor (10 cm wide × 60 cm in diameter × 30 cm high; Med Associates, St. Albans, VT) via four photoelectric cells located every 90° along the circle as described previously (Rahman et al., 2003). For spontaneous locomotor activity, rats were placed in circular corridor chambers and total activity was measured for 120 min. For amphetamine-stimulated locomotor activity, rats were given two habituation sessions (60 min each day, beginning 48 h after surgery) before testing. On the test day (day 4 after surgery), rats were placed in corridors for 60 min before they were given intraperitoneal injections of 2 or 4 mg/kg amphetamine hemi-sulfate. Locomotor activity was assessed for an additional 120 min.

**Sucrose preference and neophobia.** For sucrose preference, water was removed and rats were pre-exposed to a 1% (w/v) sucrose solution for 3 d ending at least 48 h before surgery. For testing, rats were first isolated at 5:00 P.M. with food but no water. When the lights went out at 7:00 P.M., preweighed water and 1% sucrose bottles were placed on the home cage and rats were allowed to drink for 10 min. The procedure was similar for sucrose neophobia, except that rats were not pre-exposed to sucrose before surgery and the duration of the test session was 30 min.

**Social behavior.** To investigate social interaction, viral vectors were injected into the nucleus accumbens and social behavior was measured on day 4 after surgery. Rats were isolated for 24 h before testing. For testing, cage mates were reunited in novel square chambers (50 × 50 cm) and behavior was videotaped and later scored for 30 min. Pairs of rats were scored together using an observer-based scoring system (Noldus Observer; Noldus Information Technology, Wageningen, The Netherlands). Durations of social grooming (one rat grooming the other), exploring, playing, and rearing were coded as state variables. This paradigm of short-term isolation before measuring social behavior has been shown to be sensitive to dopamine and opioid manipulations (Niesink and Van Ree, 1989).

**Forced swim test.** The forced swim test was performed according to published procedures (Eisch et al., 2003). Rats were placed in 25° water in tubs (60 cm tall filled to 45 cm) for 15 min for the initial session and 5 min for the test session. The latency to immobility is defined as no struggling for a full 1 s and has been validated as an antidepressant-sensitive measure (Pliakas et al., 2001; Eisch et al., 2003).

**Elevated plus maze.** Anxiety-like behavior in the elevated plus maze was measured according to published procedures (Barrot et al., 2002). The arms of the elevated plus maze measured 12 × 50 cm, and the maze was 1 m in the floor. Time on open arms was measured for 5 min (expressed as a percentage of total time). Behavior was scored using an automated video-tracking system (Noldus Ethovision; Noldus Information Technology).

**Peanut butter chip neophobia.** Rats were isolated with access to food and water for 16 h before testing. A preweighed amount (−15 g) of Reese’s peanut butter chips was placed in a cup in the home cage for 2 h during the light phase of the light/dark cycle, and the amount of chips consumed was recorded.

### Results

#### Effect of amphetamine on ICER mRNA expression

We first examined whether amphetamine induces ICER expression in the striatum including the nucleus accumbens. We chose amphetamine because it has been shown previously to dramatically induce CRE-mediated transcription within this brain region (Shaw-Lutchman et al., 2003). ICER mRNA levels were measured by real-time PCR. Acute amphetamine administration increased ICER mRNA levels in the striatum, an effect that peaked at 3 h and returned to control within 12 h (F<sub>5,22</sub> = 6.28; p < 0.005) (Fig. 1a). Amphetamine regulation of ICER expression was dose dependent (F<sub>3,14</sub> = 34.3; p < 0.001) (Fig. 1b). Interestingly, however, the induction of ICER mRNA diminishes progressively with repeated amphetamine administration (F<sub>4,15</sub> = 20.77; p < 0.001) (Fig. 1c–e). Thus, whereas the first amphetamine injection causes a maximal approximate threefold increase in ICER expression, only an ∼50% increase is seen after seven daily doses of the drug (Fig. 1d).

The CREM gene encodes inhibitory products, in addition to ICER, via a distinct promoter, P1. These products have been proposed, like ICER, to mediate negative-feedback control over CREM transcription (Fitzgerald et al., 1996; Borsook et al., 1999; Servillo et al., 2002; Mioduszewska et al., 2003); however, virtually nothing is known about their function in brain. We found that the inhibitory P1-driven CREM isoforms (αCREM, βCREM, and γCREM) were not regulated in the nucleus accumbens by amphetamine (data not shown). mRNA levels of full-length τCREM isoforms were also not affected by amphetamine administration.

#### Effect of stress on ICER expression

To study the effect of aversive environmental stimuli on ICER expression in the striatum, we subjected rats to acute restraint stress. An ANOVA showed that this stress treatment produced a moderate but significant (∼75%) increase in ICER mRNA levels, which peaked 1–4 h after the stress and reverted to control levels.
within 12 h ($F_{(6,20)} = 4.97; p < 0.005$) (Fig. 2a). Although this effect was smaller in magnitude compared with that seen with amphetamine, the duration of peak ICER induction was longer in response to restraint stress (compare Figs. 1a, 2a). In contrast to acute restraint stress, chronic (7 d) restraint stress failed to induce ICER mRNA levels in the striatum (data not shown). One limitation of this latter experiment is that habituation is known to occur to many effects of restraint stress. Therefore, it was important to study whether desensitization of ICER induction also occurs after a different type of chronic stress, chronic unpredictable stress, which shows much less habituation (Ortiz et al., 1996). Chronic unpredictable stress, like chronic restraint stress, failed to induce ICER mRNA levels in the striatum ($F_{(2,11)} = 11.7; p < 0.005$) (Fig. 2b). In contrast, both the major inhibitory P1-driven CREM isoforms ($\alpha$CREM, $\beta$CREM, and $\gamma$CREM) as well as the excitatory isoform ($\tau$CREM) were not regulated in the nucleus accumbens by stress (data not shown).

Other forms of acute stress, including anxiogenic stimuli, induce ICER expression in the striatum. Ten minutes of forced swimming, or being confined to the open arm of the elevated plus maze, significantly increased ICER mRNA levels ($F_{(2,11)} = 42.5; p < 0.001$) 4 h after the stressor (Fig. 2c).

**Validation of HSV–ICER vector**

To study the functional role played by ICER induction in the striatum in response to amphetamine or stress, we constructed an HSV vector that expresses ICER under the control of a viral promoter. In cell culture, the vector induced the expression of a protein of the correct $M_r$ recognized by an anti-ICER/CREM antibody as determined by Western blotting (Fig. 3a). HSV–ICER was next injected into the nucleus accumbens shell of rat brain. Viral-mediated expression of ICER protein was easily visualized using immunohistochemistry (Fig. 3b). To study the functional activity of HSV–ICER in rat brain, mRNA levels of BDNF, a well established CRE target gene (Finkbeiner et al., 1997; McClung and Nestler, 2003), was compared in nucleus accumbens tissue infected with HSV–ICER or HSV–LacZ. In animals examined 3 h after a single dose of amphetamine (4 mg/kg) to boost BDNF mRNA expression, we found that ICER robustly decreased BDNF mRNA levels by 73 ± 21% ($p < 0.05$). To further demonstrate an in vivo connection between ICER and BDNF, a ChIP experiment was performed on striatal tissue. We found significant binding of CREM/ICER to the P3 rat BDNF promoter, which contains a well characterized CRE sequence, but no significant binding to the P2 promoter ($F_{(2,20)} = 22.6; p < 0.001$; Dunnet post hoc: P2, $p = 0.98$; P3, $p < 0.001$). This binding is likely attributable to ICER, because it was induced by acute amphetamine, conditions under which ICER is the only CREM gene product induced (2.3 ± 0.4-fold amphetamine vs saline; $n = 3–4; p < 0.05$).

To further study the functional activity of virally encoded ICER on CRE-mediated transcription, we injected HSV–ICER into the nucleus accumbens of CRE–LacZ reporter mice and 3 d later, when HSV expression is maximal, administered a moderate dose of amphetamine (1 mg/kg) to boost the normally low levels of LacZ expression in the nucleus accumbens of these mice (Shaw-Lutchman et al., 2003). Animals were analyzed 4 h later, a time point that provides a stable and reliable measure of LacZ induction (Barrot et al., 2002; Shaw-Lutchman et al., 2002, 2003). ICER expression significantly decreased the mean intensity of $\beta$-galactosidase immunofluorescence ($F_{(1,20)} = 52.75; p < 0.001$) (Fig. 3c–f), an effect not seen after viral-mediated expression of a control protein (e.g., GFP) (Fig. 3g,h). These results show that viral infection per se does not affect CRE activity and confirm the
biological activity of viral-encoded ICER as an inhibitor of CRE transcription in the nucleus accumbens in vivo.

As observed with many other HSV vectors (Carlezon et al., 1998; Barrot et al., 2002), viral expression was seen in neurons only and was not associated with any toxicity beyond that observed with vehicle injections alone (data not shown).

**Effect of ICER overexpression on locomotor activity**

Using this HSV–ICER vector, we wanted to study the influence of ICER overexpression in the nucleus accumbens on an animal’s responses to a wide range of emotional stimuli, both aversive and rewarding. The first behavior examined was locomotor activity. We found that ICER overexpression in the nucleus accumbens decreased spontaneous activity compared with an HSV–LacZ vector that overexpresses β-galactosidase as a control protein. A two-factor ANOVA (virus × time) revealed a main effect of virus ($F_{(1,77)} = 18.3; p < 0.005$) (Fig. 4a) and a virus × time interaction ($F_{(1,77)} = 2.69; p < 0.05$). Contrasts revealed a significant quadratic component of the interaction, meaning that ICER decreased spontaneous locomotor activity only during the middle part of the test session ($F_{(1,77)} = 27.0; p < 0.001$). When challenged with a 2 mg/kg dose of amphetamine, however, ICER overexpression in the nucleus accumbens strongly potentiated amphetamine-induced locomotor activity throughout the session compared with LacZ-overexpressing animals ($F_{(1,110)} = 11.6; p < 0.01$) (Fig. 4b). The time × virus interaction also yielded significant results ($F_{(1,110)} = 2.69, p < 0.005$), meaning that most of the potentiation occurred from 20 to 60 min after amphetamine injection. There was a nonsignificant trend for ICER to potentiate locomotor activity at a higher amphetamine dose (4 mg/kg; $F_{(1,110)} = 2.15; p = 0.17$) (Fig. 4c). The lack of significant influence of ICER was likely attributable to a ceiling effect and the appearance of stereotypy, which is known to limit locomotor activity at higher amphetamine doses.

**Effect of ICER overexpression on responses to natural rewards**

To determine whether levels of ICER in the nucleus accumbens also affect an animal’s responses to natural rewards, we studied the animals in a sucrose preference paradigm. ICER overexpression in this brain region produced an increase in sucrose preference in animals that were already familiar with sucrose compared with LacZ overexpression ($F_{(1,14)} = 7.88; p < 0.05$) (Fig. 5a).

As with humans, rats find nonaggressive social contact to be rewarding and prefer social contact (e.g., grooming) to isolation (Calcagnotto and Schechter 1992). ICER overexpression increased social reward as indicated by an increased amount of time rats groomed each other in a test of social interaction ($F_{(1,10)} = 21.85; p < 0.005$) (Fig. 5b). In contrast, the full-length CREM protein (τCREM), in comparison, had no effect on social grooming and was indistinguishable from HSV–LacZ-injected animals.

**Effect of ICER overexpression on depression-related behavior**

One hallmark of depression is subsensitivity to hedonic stimuli. As described above, ICER overexpression in the nucleus accumbens increases social reward (grooming) and sucrose reward. It was therefore of interest to directly study the influence of ICER in a test of depression-related behavior. We found that ICER overexpression in the nucleus accumbens lengthened the latency to immobility in the forced swim test compared with LacZ overexpression ($F_{(1,12)} = 4.64; p < 0.05$) (Fig. 5c). This is an antidepressant-like effect that is seen in normal animals after administration of any of several chemical antidepressants (Lucki, 2001; Pliakas et al., 2001). An HSV–mCREB virus, which overexpresses a synthetic dominant-negative mutant of CREB, was used as a positive control. This vector caused a similar increase in latency to immobility as seen for HSV–ICER and thereby replicates the findings of Pliakas et al. (2001) with mCREB ($F_{(1,10)} = 4.34; p < 0.05$; one-tailed test). In contrast, the full-length CREM protein (τCREM) had no antidepressant effect (Fig. 5c).

**Effect of ICER overexpression on anxiety-related behavior**

To further understand the functional relevance of ICER induction in the nucleus accumbens by emotional stimuli, we examined the influence of HSV–ICER in several tests of anxiety-like behavior. Viral-mediated overexpression of ICER in the nucleus accumbens produced a robust anxiogenic-like effect. Specifically, it decreased the time an animal spent in the open arms of an elevated plus maze ($F_{(1,72)} = 3.14; p < 0.05$) (Fig. 6a). ICER overexpression also produced an anxiogenic-like effect in increasing an animal’s avoidance of novel tastes (a phenomenon known as “neophobia”), including sucrose ($F_{(1,6)} = 23.5; p < 0.005$) (Fig. 6b) and peanut butter chips ($F_{(1,32)} = 6.21; p < 0.05$) (Fig. 6c). This increased aversion to sucrose as a novel taste is
interesting in light of the enhanced preference for sucrose when it is a familiar taste (compare Figs. 5a, 6b). Thus, although rats find sucrose and other sweets (like peanut butter chips) rewarding once they are familiar with the taste, they, like many other species, exhibit a strong aversion to novel tastes. This neophobia has been shown to have a strong anxiety component (Hall et al., 1997) and is regulated by the nucleus accumbens (Burns et al., 1996).

**Discussion**

The major findings of the present study are that emotionally salient environmental stimuli, both rewarding and aversive, increase ICER expression in the striatum and that mimicking this effect within the nucleus accumbens, the ventral subregion of the striatum, by use of viral-mediated gene transfer affects a wide range of behavior domains, from anxiety to depression to reward.

The results show that aversive or rewarding environmental factors, including stress on the one hand or amphetamine on the other, induce expression of ICER mRNA in the striatum. ICER mRNA peaks within 2–4 h after the inducing stimulus and returns to baseline within 12 h. In agreement with other stimuli in other brain regions, ICER mRNA induction is slower than that of other inducible transcription factors such as c-Fos (Konopka et al., 1998; T. A. Green and E. J. Nestler, unpublished data). The somewhat delayed time course of ICER induction is consistent with the hypothesized role of ICER as a negative-feedback mechanism for CREB-mediated transcription (Servillo et al., 2002; Mioduszewska et al., 2003; Shepard et al., 2005). According to this view, the delay in ICER induction allows CREB to induce transcription of its myriad of target genes; as ICER (one of those target genes) is translated, it subsequently impedes additional CREB-induced transcription. Consequently, ICER is hypothesized to function as an endogenous dominant-negative regulator of CREB function. A previous study showed CREM induction in the striatum by dopaminergic agonists, but it is unclear which CREM products (excitatory and/or inhibitory) were identified in the analysis (Berke et al., 1998).

The loss of induction of ICER mRNA after repeated amphetamine or stress exposure parallels effects seen with other inducible transcription factors after these and other repeated treatments, such as c-Fos, c-Jun, and JunB (Hope et al., 1992; Daunais et al., 1993; Persico et al., 1993; Hiroi and Graybiel, 1996; O’Donovan et al., 1999; Perrotti et al., 2004). Interestingly, all of these genes (including ICER) have now been identified as part of the CREB regulon (Impoy et al., 2004) (i.e., they are all targets of CREB). To the extent that ICER binds the same CRE or CRE-like sites as CREB, all of these genes should be targets of ICER regulation as well, a possibility that now requires direct investigation. It is unlikely, however, that ICER itself is responsible for the desensitization of induction of these genes seen after repeated administration of amphetamine or stress, because ICER, like other immediate-early genes, shows this desensitization. Hence, it would seem that another factor, yet to be identified, is responsible for this form of tolerance.

To understand the functional relevance of ICER induction in the nucleus accumbens by amphetamine or stress, we used HSV-mediated gene transfer to overexpress ICER selectively within the nucleus accumbens and then examined the effect on an animal’s responses to a wide range of emotional stimuli, both aversive and rewarding. One observation is that ICER overexpression in the nucleus accumbens increases behavioral responses to amphetamine. This finding raises the interesting possibility that ICER induction contributes to the mechanisms by which a previous exposure to amphetamine or to stress increases an animal’s sensitivity to subsequent amphetamine exposure, a phenomenon that has been well established (Covington and Miczek, 2001; Robinson and Berridge, 2001). Our findings with amphetamine-induced locomotor activity are also in agreement with previous research, which showed that inhibition of CREB function in the nucleus accumbens, achieved via overexpression of the dominant-negative mutant mCREB, made animals more sensitive to the behavioral effects of cocaine and morphine (Carlezon et al., 1998; Barrot et al., 2002). In contrast, overexpression of CREB blunted responses to these drugs of abuse.

Furthermore, the current behavioral findings are consistent with the results of previous work, which demonstrated that CREB, acting in the nucleus accumbens, blunts behavioral responses to a wide range of emotional stimuli in addition to drugs of abuse (Barrot et al., 2002). In those previous studies, overexpression of CREB in the nucleus accumbens decreased responses to a range of aversive and rewarding stimuli and induced certain features of depression-like behavior, including symptoms of anhedonia and increased immobility in the forced swim test. However, these animals also show reduced anxiety-like behavior, consistent with a general numbing in behavioral responsivity. In these previous experiments, overexpression of mCREB, which is a synthetic dominant-negative mutant that lacks the serine 133 required for CREB phosphorylation and activation, in the nucleus accumbens caused the opposite behavioral effects: animals showed increased responsiveness to aversive and rewarding stimuli. In the present study, overexpression of ICER in the nucleus accumbens has similar effects as mCREB: increased ICER levels enhance an animal’s responses to anxiogenic and rewarding stimuli and also decrease depression-like behavior in the forced swim test. However, unlike mCREB, which is an artificial mutant, ICER is a naturally occurring protein, which we show is induced in the nucleus accumbens in response to aversive and rewarding stimuli. Thus, our data demonstrate that emotional stimuli induce both CREB and ICER, which produce opposing effects on behavioral responses to subsequent emotional stimuli.

Induction of ICER protein in the nucleus accumbens produced a clear antidepressant phenotype in the forced swim test and in experiments measuring natural reward. However, overexpression of ICER also increases anxiety-like behavior in the elevated plus maze and in taste neophobia experiments. The ICER protein itself does not affect behavior directly; rather, it affects expression of other proteins that then affect neuronal function-
ing. As an example, we show that ICER repressed BDNF expression in the nucleus accumbens, and previous studies have shown that reduced levels of BDNF in this brain region cause antidepressant-like responses in animal models (Eisch et al., 2003; Berton et al., 2006). One caveat of the present studies is that they rely on overexpression of ICER, and exogenous ICER may depend on CREB–ICER competition at CRE sites. Therefore, a complete understanding of the role of CRE-mediated transcription in the nucleus accumbens, or any other brain region of interest, on complex behavior will require an integrated analysis of CREB and ICER and their functional interactions.

References


