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Brain Regional Neuropeptide Changes Resulting From Social Defeat

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Past work has demonstrated robust brain changes in cholecystokinin (CCK-8) following social defeat. Here the authors analyzed brain regional, CCK-8, substance P, corticotropin releasing factor (CRF), and neuropeptide Y levels in adult male Long–Evans rats defeated in a resident–intruder social aggression paradigm, as indexed by elevated bites received, freezing, and emission of 20-kHz calls. Brains harvested 6 hr after social defeat were dissected into 12 regions (olfactory bulbs, 3 cortical regions [frontal cortex, cortex above the basal ganglia, cortex above the diencephalon], caudate–putamen, basal forebrain, hypothalamus, hippocampus, thalamus, tectum, tegmentum, and lower brain stem). Neuropeptide radioimmunoassays demonstrated the following statistically significant regional changes in defeated rats as compared with nondefeated rats: CCK-8 was reduced in frontal cortex and cortex overlying diencephalon, the olfactory bulbs, caudate–putamen, hippocampus, tectum, and lower brainstem. Neuropeptide Y was elevated in the caudate–putamen. Substance P was elevated in the cortex over the basal ganglia and decreased in basal forebrain. CRF was diminished in the hippocampus. The results highlight more robust CCK modulation by social defeat as compared with 3 other neuropeptide systems involved in brain emotional regulation.

Keywords: aggression, social dominance, neuropeptides, cholecystokinin, depression

Neuropeptides have been increasingly implicated in a wide variety of psychiatric disorders accompanied by negative affective states such as panic, anxiety, and depression (Beinfeld, 2001; McLean, 2005; Panksepp, 1998; Panksepp, Burgdorf, Beinfeld, Kroes, & Moskal, 2004). In particular, cholecystokinin (CCK), neuropeptide Y (NPY), substance P, corticotropin releasing factor (CRF), and β-endorphin have recently received attention as potential modulators of stress and negative affective states associated with psychiatric disorders. Intravenous injections of CCK-4 have been shown to induce panic and anxiety in humans with a history of panic attacks (Beinfeld, 2001; Rehfeld, 2000). Antagonists for substance P and CRF have recently been shown to have efficacy in reducing depressive symptoms in unipolar depressives (McLean, 2005). Last, NPY has been shown recently to be a potent anxiolytic in rodent models of anxiety (Heilig, 2004).

These neuropeptides bind primarily to specific G-protein receptors in the brain (Foord et al., 2005). Direct injections of these neuropeptides into the brain modulate negative emotional behaviors in various animal models. NPY injections into lateral ventricles have strong anxiolytic effects, whereas CCK and substance P injections increase anxiety levels (Duarte, Testolin, & De Lima, 2004; Kask, Nguyen, Pabst, & Von Horsten, 2001; Zanoveli, Netto, Guimaraes, & Zangrossi, 2004). Beta-endorphin injections into the periaqueductal gray can markedly decrease nociceptive behaviors (Pavlovic, Cooper, & Bodnar, 1996).

In laboratory rats, social-defeat-induced submissive behavior in resident–intruder aggression has been suggested to model aspects of human anxiety and depression (Miczek, Weerts, Vivian, & Barros, 1995; Ruis et al., 1999). Freezing and 20-kHz calls are exhibited by defeated rats during aggressive social defeat encounters, and these behaviors may reflect negative affective states in rats, because they are sensitive to anxiolytic and antidepressant pretreatment (Knutson, Burgdorf, & Panksepp, 2002; Miczek et al., 1995). Also, social defeat has been found to induce physiological and hormonal changes similar to those evident in human depression (Blanchard, Sakai, McEwen, Weiss, & Blanchard, 1993; Buwalda et al., 2005; Ruis et al., 1999).

In the current study we sought to explore the brain changes in CCK-8, NPY, substance P, and CRF in response to social defeat;

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all of these peptides have been strongly implicated in negative affective states (Beinfeld, 2001; McLean, 2005; Panksepp, 1998). Negative affective states were monitored by measurement of 20-kHz ultrasonic calls. Also, in contrast to previous work, where effects on CCK-8 of a single bout of isolation-induced aggression were evaluated (Panksepp et al., 2004), regional CCK-8 and several additional neuropeptides were monitored in this study after more severe as well as several social defeats by more consistently aggressive residents (whose heightened aggression was due to social housing with a female companion as opposed to mere social isolation housing, used in the previous report). Because social defeat reflects social loss, which is a major vector in the onset of depression, we anticipate that monitoring regional neuropeptide changes, along with the study of gene-expression patterns (Kroes, Panksepp, Burgdorf, Otto, & Moskal, 2006), may lead to the identification of brain neurochemical changes that regulate the onset of depression.

Method

Subjects and Housing

Subjects were 16 adult male Long–Evans rats (body weight [$M \pm SEM$] = 480.8 ± 7.3 g at death) born and raised in the Bowling Green State University animal facility. Eight control rats were allowed social interaction with a nonaggressive conspecific male for 0.5 hr, and 8 experimental rats received identical social encounters with a male previously shown to be aggressive. Since weaning (at 21 day of age), all test rats were housed socially (2–4 per cage) in 20 × 40 × 20–cm translucent polycarbonate cages with corncob bedding, with continuous free access to food and water. Intruder rats remained in group housing through testing. Temperature was kept constant at about 21 °C, and lighting was on a 12-hr light–dark cycle with lights on at 8:00 a.m. All behavioral testing occurred during the light part of the cycle (as in Kroes et al., 2006; Panksepp et al., 2004). This work was approved by the Bowling Green State University Animal Care and Use Committee.

To evoke aggression, we used 4 prescreened "resident" males that had, during prior screening, exhibited consistent strong aggression toward intruders and 4 control residents that had exhibited essentially no aggression. Aggressive resident Long–Evans rats were pair housed with tubally ligated Long–Evans females (to sustain sexual receptivity without reproduction), which were removed approximately 0.5 hr before social defeat testing. In contrast, the control residents were nonaggressive, isolate-housed Long–Evans rats that were of the same age and weight as the defeated and nondefeated rats. All defeated rats were obtained from paired encounters with aggressive residents, and all rats in this defeated group exhibited clear submissive behavior (e.g., 20-kHz calls and freezing; Figure 1). The nondefeated rats were tested with nonaggressive residents, and none of the rats in the nondefeated group showed any submissive behavior (i.e., there was no freezing accompanied by 20-kHz ultrasonic vocalization; Figure 1).

Behavioral Testing

All testing was conducted in a separate test room, so that the social activities would not be disruptive to the rest of the colony. Social defeat testing consisted of placing the intruder rat into the home cage of an aggressive male resident (consistently yielding defeated rats); nondefeat testing consisted of placing the intruder into the home cage of a nonaggressive male resident (consistently yielding nondefeated rats). All testing lasted 30 min under dim (~2 lx) light. Just prior to testing, a plastic lid was placed on top of each resident's home cage, with a small hole (6 × 6.5 cm) to position the ultrasonic sound-sensitive microphone. The height of

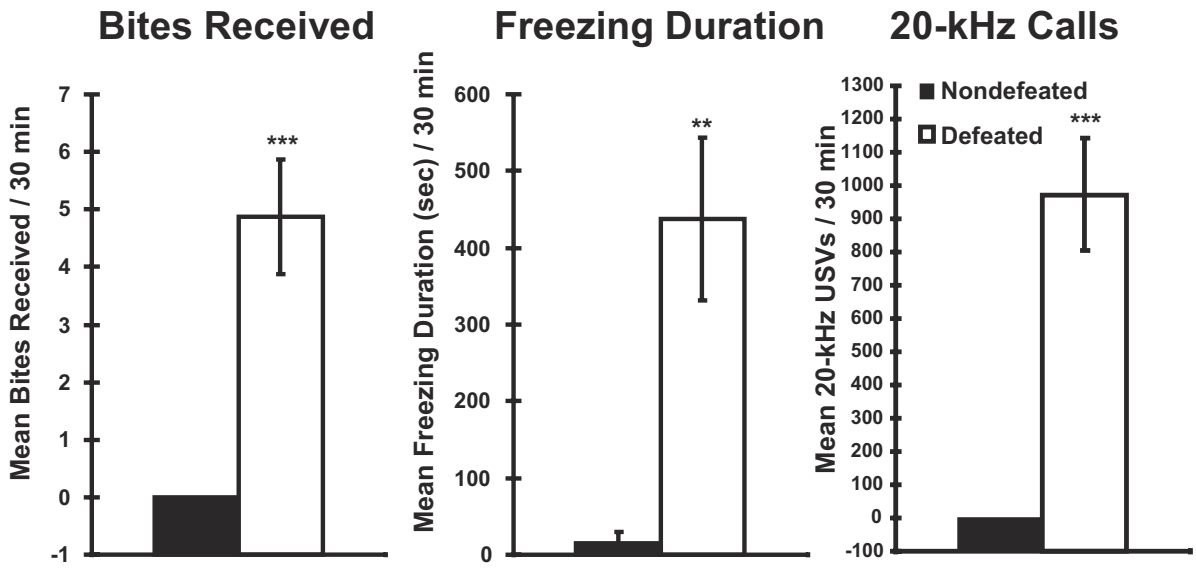

Figure 1. Mean (± *SEM*) bites received by the intruder rat from the resident rat, duration of freezing by the intruder rat, and 20-kHz ultrasonic vocalizations (USVs) during 30-min resident–intruder interactions in experimental rats (defeated group) or control rats (nondefeated group). $^{**}p < .01$, independent-samples t test (two-tailed). $^{***}p < .001$, Mann–Whitney U test (two-tailed).

the testing cage (20 cm) used in this experiment limited some aggressive behavior (i.e., offensive upright posture), but for comparability, it was identical to housing and testing apparatus used in previous studies of this series (Panksepp et al., 2004). Half of the experimental rats ($n = 4$) received a second aggressive encounter and half of the controls received a second nonaggressive encounter 24 hr after the initial test to evaluate for any additional neurochemical changes that may result from multiple defeats. Because neither the behavioral nor the neurochemical results differed from the 4 experimental and control rats that received only a single test, their data were combined to yield a single experimental and control group for statistical analysis.

Social interactions of both experimental (defeated intruders) and control rats (nondefeated intruders) were archived onto DVDs for subsequent scoring of social behaviors. During these recordings, ultrasonic vocalizations (USVs) were monitored using a Pettersson D980 ultrasonic detector (Uppsala, Sweden). Both the frequency division (1/10 division) and heterodyne channel (tuned to 55 kHz, range ±5 kHz) were recorded onto DVD via separate audio channels. Additionally, high-frequency direct recordings were also obtained via a digital field recorder (Fostex FR2; Fostex, Boonton, NJ), which yielded the highest quality recordings used for data analysis.

Behavioral Coding

Behavioral data were analyzed by blind scoring, independent of any knowledge of the biochemical data. Videotape records were hand scored for bites (most accompanied by audible squealing by the resident) and freezing behavior individually for each rat in testing pairs. A freezing bout was counted after approximately 5 consecutive seconds without movement (except sniffing). Biting was scored as frequency of occurrence for each rat. All USVs from the high-frequency recordings were also scored by a trained experimenter who was blind to experimental conditions of the rats, the video scored behavior (i.e., bites, freezing, and dorsal contacts), and the biochemical data. USVs were hand scored from computer-generated sonograms (SASLab Pro; Avisoft Bioacoustics, Berlin, Germany) in a blind manner. Given that the freezing behavior by intruders was primarily coincident with emissions of 20-kHz USVs, the freezing behavior clearly reflected submissive behavior.

Brain Tissue Sampling

Approximately 6 hr after testing ($M \pm SEM = 5.93 \pm 0.05$ hr), rats were rendered unconscious with ambient carbon dioxide and decapitated, and their brains as well as pituitary were rapidly removed. Initial dissection of areas was first achieved by obtaining approximately 2–3-mm coronal sections with a calibrated tissue block, followed by manual dissection of brain subregions from the brain slabs. All work was conducted on an ice-cold platform using microdissection tools. The following brain areas were harvested: olfactory bulb; hypothalamus; frontal neocortex, consisting of the frontal pole just anterior to the caudate–putamen and rostral edge of diencephalon; anterior neocortex over basal ganglia; posterior neocortex above the diencephalon; caudate–putamen, which included the septum; all basal forebrain ventral to the caudate–putamen, including nucleus accumbens; entire thalamus; temporal cortex ventral to rhinal fissure, which included the amygdala; body of the hippocampus; substantia nigra and ventral tegmental area; tectum, including colliculi; dorsal half of the periaqueductal gray and surrounding mesencephalic tissue, including some posterior thalamus and hypothalamus; tegmentum, consisting of the ventral midbrain above substantia nigra and ventral tegmental area, with mesencephalon up through the ventral periaqueductal gray along with some pontine tissue; cerebellum; and lower brainstem, consisting of remaining pons and medulla. Tissue samples were promptly placed in Eppendorf tubes and frozen on dry ice. Samples were stored at −80 °C until assayed. In statistical comparisons, degrees of freedom may vary slightly owing to loss of a few brain samples during processing.

Peptide Radioimmunoassay

Biochemical procedures were essentially the same as previously described (Beinfeld, Meyer, Eskay, Jensen, & Brownstein, 1981). The brain samples were homogenized in 0.1 N HCl; an aliquot was taken for protein determination (Bradford method), and another for radioimmunoassays of CCK. The utilized method detects mainly CCK-8, including both sulfated and unsulfated varieties, with a moderate preference for the sulfated form. It has little affinity for CCK-4 or −5, of which there is, in fact, very little in rat brain. Iodinated peptide and antibodies for NPY, substance P, and CRF were obtained from Phoenix Pharmaceuticals (Burlingame, CA). Radioimmunoassays for β-endorphin NPY, substance P, and CRF were performed in an identical manner to CCK-8. Data are expressed in nanograms of peptide per milligram of total protein for each brain sample.

Statistical Analysis

Behavioral data were analyzed using either JMP (SAS Institute, Cary, NC) or InStat (GraphPad Software, San Diego, CA) with between-subjects *t* tests except for data that were not normally distributed (i.e., failed a Kolmogorov–Smirnov test of normality), in which case a between-subjects Mann–Whitney *U* test was performed. Neurochemical data were analyzed with analysis of variance (ANOVA). Between-subjects *t* tests were used as post hoc tests, and the false discovery rate (probability that a significant neurochemical change in a specific brain region represents a Type II error) was calculated for each post hoc comparison by comparing the observed data versus 5,000 iterations of randomly permuted data using Excel (Microsoft). All *p* values reported are from two-tailed tests.

Results

Behavioral

In all social defeat pairings with aggressive residents, the aggressive residents exhibited clear aggression (i.e., bites), and the defeated rats all exhibited clear submissive behaviors (i.e., freezing with 20-kHz USVs). No clear aggressive behaviors were exhibited by the defeated rats or by the nondefeated and nonaggressive residents. Defeated rats received significantly more bites than the nondefeated rats, which received no bites (Mann–Whitney U, $p < .001$; Figure 1). The defeated rats also exhibited considerably more freezing behavior than nondefeated rats, $t(14) = 4.0$, $p < .005$

(Figure 1). Defeated rats exhibited abundant 20-kHz calls, whereas nondefeated rats exhibited none (Mann–Whitney U, $Z = 3.6$, $p < .001$; Figure 1). Examination of audio–video recordings revealed that in all instances in which both the resident and the intruder were clearly visible, the 20-kHz calls were emitted by the defeated rats (i.e., they were always temporally associated with thoracic compressions, which is consistent with previous findings; Panksepp et al., 2004; Thomas, Takahashi, & Barfield, 1983). Indeed, levels of freezing in the defeated experimental intruder rats were positively correlated with 20-kHz USVs (Spearman $r = .92$, $p < .001$).

Neurochemical

Across all brain regions in which the peptide levels were above the threshold of detection of the radioimmunoassay, CCK-8 content was reduced in defeated rats compared with controls, $F(1, 13) = 27.41$, $p < .0005$ (Figure 2). Overall, there was only a nonsignificant trend for NPY content to be elevated in defeated rats compared with nondefeated rats, $F(1, 11) = 1.26$, $p < .10$ (Figure 3). When specific brain regions were compared, CCK-8 content was reduced in defeated experimental rats as compared with nondefeated control rats in the frontal cortex, $t(14) = 2.8$, $p < .05$; posterior neocortex above the diencephalon, $t(14) = 4.4$, $p < .001$; olfactory bulbs, $t(14) = 3.8$, $p < .005$; caudate–putamen, $t(14) = 3.7$, $p < .005$; hippocampus, $t(14) = 3.0$, $p < .01$; tectum, $t(13) = 2.7$, $p < .05$; and lower brainstem, $t(14) = 3.2$, $p < .01$ (Figure 2). NPY content was increased in defeated experimental rats when compared with control rats in the caudate–putamen, $t(14) = 3.0$, $p < .01$ (Figure 3). The false discovery rate (probability that a significant change represents a Type II error) for each of the significant changes in CCK or NPY in the above brain regions was less than 1% for each comparison. Substance P and CRF content did not vary consistently across brain regions in experimental and control rats (ANOVAs, $p > .05$). Substance P levels were increased in the experimental rats in the anterior neocortex over the basal ganglia, $t(14) = 4.9$, $p < .001$; and decreased in the basal forebrain samples, $t(13) = 2.8$, $p < .05$, compared with control rats (Figure 4). CRF levels were decreased in the hippocampus of experimental rats as compared with controls, $t(14) = 3.0$, $p < .01$ (Figure 5). Each of the significant neuropeptide level changes had a false discovery rate of less than 5%. Beta-endorphin levels in most brain regions were below the limit of quantification (data not shown).

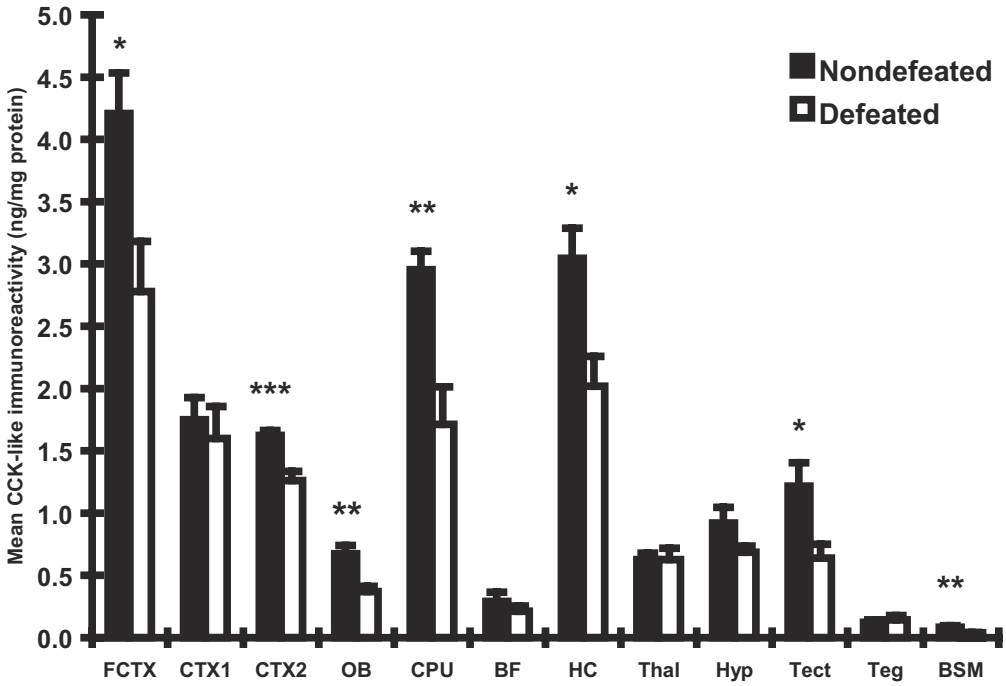

Figure 2. Mean (± *SEM*) content of cholecystokinin (CCK-8) in tissue homogenates of various brain regions in intruder rats sacrificed 6 hr after 30-min resident–intruder encounters with a nonaggressive male resident (nondefeated group) or an aggressive male resident (defeated group). FCTX = frontal neocortex, consisting of the frontal pole just anterior to the caudate–putamen and rostral edge of diencephalon; CTX1 = anterior neocortex over basal ganglia; CTX2 = posterior neocortex above the diencephalon; OB = olfactory bulb; CPU = caudate–putamen, which included the septum; BF = all basal forebrain ventral to the CPU, including nucleus accumbens; HC = body of the hippocampus; Thal = entire thalamus; Hyp = hypothalamus; Tect = tectum, including colliculi, dorsal half of the periaqueductal gray, and surrounding mesencephalic tissue, including some posterior thalamus and hypothalamus; Teg = tegmentum, consisting of the ventral midbrain above substantia nigra and ventral tegmental area, with mesencephalon up through the ventral periaqueductal gray along with some pontine tissue; BSM = lower brainstem, consisting of remaining pons and medulla. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, independent-samples t test (two-tailed).

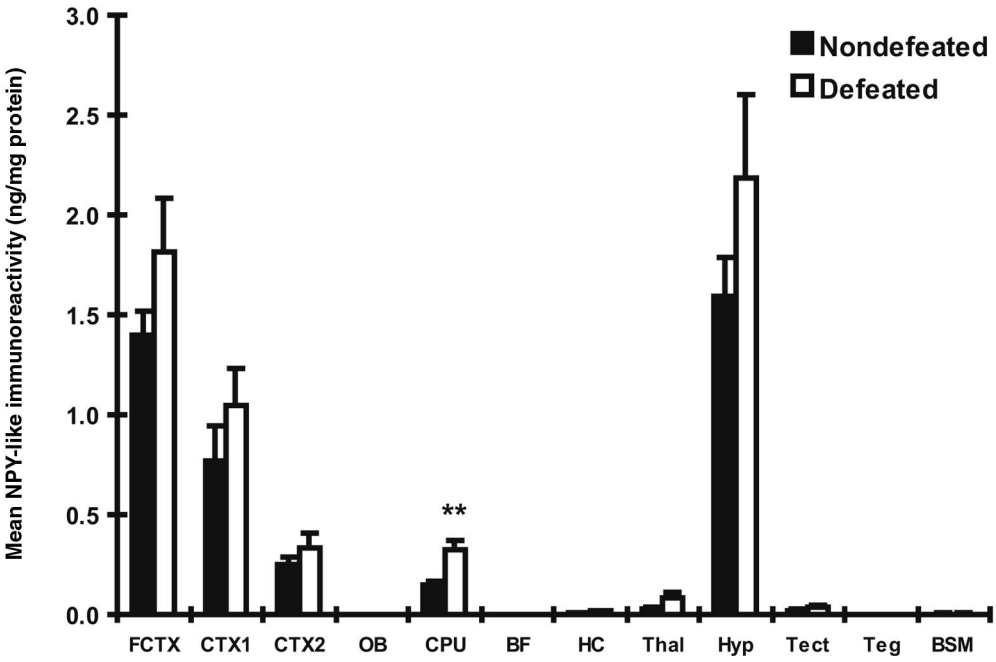

Figure 3. Mean (± *SEM*) content of neuropeptide Y (NPY) in tissue homogenates of various brain regions in intruder rats sacrificed 6 hr after 30-min resident–intruder encounters with a nonaggressive male resident (nondefeated group) or an aggressive male resident (defeated group). FCTX = frontal neocortex, consisting of the frontal pole just anterior to the caudate–putamen and rostral edge of diencephalon; CTX1 = anterior neocortex over basal ganglia; CTX2 = posterior neocortex above the diencephalon; OB = olfactory bulb; CPU = caudate–putamen, which included the septum; BF = all basal forebrain ventral to the CPU, including nucleus accumbens; HC = body of the hippocampus; Thal = entire thalamus; Hyp = hypothalamus; Tect = tectum, including colliculi, dorsal half of the periaqueductal gray, and surrounding mesencephalic tissue, including some posterior thalamus and hypothalamus; Teg = tegmentum, consisting of the ventral midbrain above substantia nigra and ventral tegmental area, with mesencephalon up through the ventral periaqueductal gray along with some pontine tissue; BSM = lower brainstem, consisting of remaining pons and medulla. $^{**}p < .01$, independent-samples *t* test (two-tailed).

Discussion

Previously it was determined that social defeat and aversive aspects of play behavior alter levels of CCK-8 in the posterior cortex in experimental rats as compared with controls, and that CCK-8 levels in this brain region were correlated with aversive behaviors: pin duration in play and 20-kHz USVs in aggression (Burgdorf, Panksepp, Beinfeld, Kroes, & Moskal, 2006; Panksepp et al., 2004). However, in the present study we observed decreases in CCK-8 levels of the posterior cortex in response to social defeat, whereas in our previous work, increases were seen. It is possible that the decreases in CCK-8 levels reported in this study were due to a greater level of release than the rate of resynthesis of active CCK-8 peptide. Compared with the previous study on CCK-8 levels in the brain in response to aggression using isolate-housed residents (Panksepp et al., 2004), in the present study, which used resident rats paired with females, there were approximately twice as many 20-kHz calls and twice as many bites received by the defeated rats (but similar levels of freezing behavior). These findings suggest that the overall level of emotional arousal and submissive behavior was much greater in the present study. Therefore, if submissive behavior is positively related to CCK-8 release, then it is possible that greater release in this study could have outstripped the resynthesis, leading to lower content of CCK-8. Indeed, in our previous study (Panksepp et al., 2004), defeated rats had higher CCK content in several brain regions compared with controls, whereas the opposite pattern was seen in the current study. We suspect that the stronger levels of aggression and consequent emotional arousal seen in this study led to the expected increased utilization of CCK-8 in defeated rats.

In this study, CCK levels in 8 of the 12 brain regions reported were decreased in defeated rats as compared with controls. This widespread decrease may reflect a global increase in the rate of release of CCK or a decrease in synthesis as a result of submissive behavior. It has been shown that release of CCK in the frontal cortex is increased following social defeat and that CCKB receptor antagonists reduce submissive behavior in intruders (Becker et al., 2001). The results of the current study are consistent with that finding. Also, it has been shown that both systemic and direct injections of CCK-8 into the periaqueductal gray produce a state of panic in rats (Zanoveli et al., 2004). Given that 20-kHz calls in rats reflect in part an anxiety-like state (e.g., reduced by anxiolytic drugs; Miczek et al., 1995), it is possible that they were being modulated by CCK release.

20-kHz calls have been shown to be strongly modulated by acetylcholine input into the basal forebrain from hindbrain cholin-

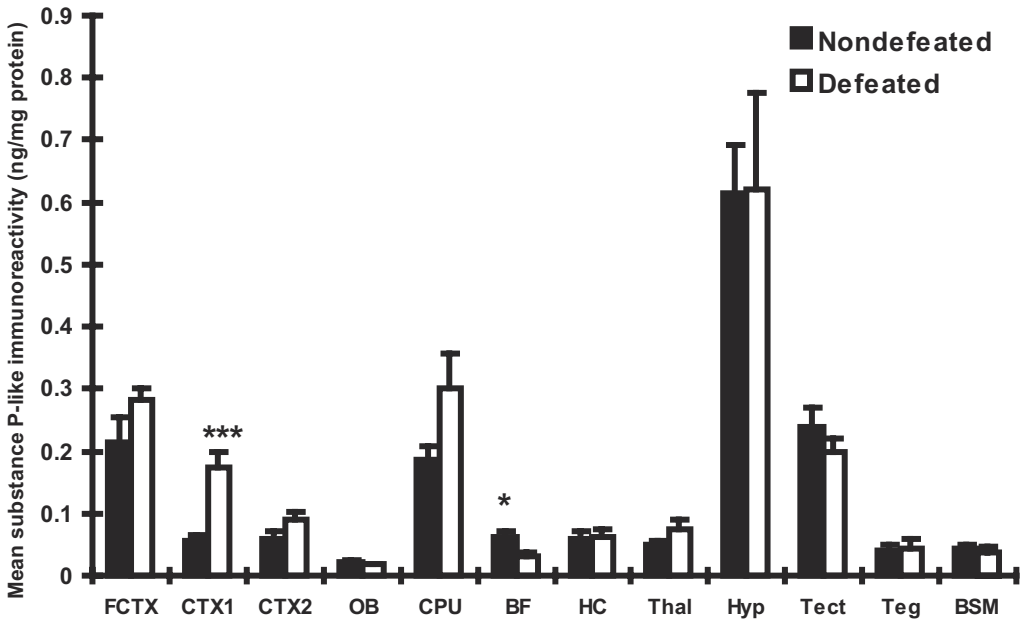

Figure 4. Mean (± *SEM*) content of substance P in tissue homogenates of various brain regions in intruder rats sacrificed 6 hr after 30-min resident–intruder encounters with a nonaggressive male resident (nondefeated group) or an aggressive male resident (defeated group). FCTX = frontal neocortex, consisting of the frontal pole just anterior to the caudate–putamen and rostral edge of diencephalon; CTX1 = anterior neocortex over basal ganglia; CTX2 = posterior neocortex above the diencephalon; OB = olfactory bulb; CPU = caudate–putamen, which included the septum; BF = all basal forebrain ventral to the CPU, including nucleus accumbens; HC = body of the hippocampus; Thal = entire thalamus; Hyp = hypothalamus; Tect = tectum, including colliculi, dorsal half of the periaqueductal gray, and surrounding mesencephalic tissue, including some posterior thalamus and hypothalamus; Teg = tegmentum, consisting of the ventral midbrain above substantia nigra and ventral tegmental area, with mesencephalon up through the ventral periaqueductal gray along with some pontine tissue; BSM = lower brainstem, consisting of remaining pons and medulla. $^{*}p < .05$, $^{***}p < .001$, independent-samples *t* test (two-tailed).

ergic cells that colocalize with substance P (Bihari, Hrycyshyn, & Brudzynski, 2003; Sutin & Jacobowitz, 1990). In this study, we found that substance P levels in the basal forebrain were also altered by social defeat with concomitant elevation in 20-kHz ultrasonic calls. Therefore, it is possible that this could reflect increases in substance P release in combination with acetylcholine in the basal forebrain during submissive behavior.

NPY, which has long been known to elicit robust feeding behavior when injected directly into the brain (Kalra & Kalra, 2003), has recently been shown to also have strong anxiolytic properties when injected into the lateral ventricles (Badia-Elder, Stewart, Powrozek, Murphy, & Li, 2003), whereas injection of NPY antagonists into the periaqueductal gray is anxiogenic (Kask, Rago, & Harro, 1998). Both CCK and NPY currently remain prime targets for anxiolytic drug development despite various potential pitfalls (Harro, 2006). In the present study, NPY content was increased in the caudate–putamen in the defeated rats compared with the nondefeated rats. Given that the caudate–putamen is adjacent to the lateral ventricle, it is possible that it may relate to the anxiolytic effects of NPY and may represent a decrease in release of NPY, resulting in an increase in anxiety in the defeated rats compared with the nondefeated rats.

CRF has long been implicated in aversive affective states such as stress, anxiety, and depression (Reul & Holsboer, 2002). Most recently, abnormality in CRF activity in the hippocampus has been implemented in depression, with increased activity in the hippocampus leading to hippocampal atrophy, a hallmark of depression in humans (Brown, Varghese, & McEwen, 2004). In the present study, we found changes in CRF in response to social defeat only in the hippocampus. Therefore, this change may reflect the depressogenic effects of submissive behavior, which has been recently been shown to mimic behavioral and physiological symptoms of depression in rats (Blanchard et al., 1993; Buwalda et al., 2005; Ruis et al., 1999).

To clarify what these neurochemical changes mean, future studies will need to explore the in vivo release and metabolism of the above neuropeptides during and after single and multiple social defeats. The meaning of such postdefeat neurochemical changes cannot be unambiguously related to specific phases of the present experimental paradigm (problems of interpretation are more extensively discussed in Panksepp et al., 2004). Also, in the present work, it is possible that the nonaggressive social interactions had effects on some of these neuropeptides, and different conclusions would be warranted had neuropeptide levels also been measured in animals that had been handled but remained in social isolation. Indeed, rough-and-tumble play behavior has yielded more modest increases and decreases of CCK in a few of the brain regions sampled in this study (Burgdorf et al., 2006). However, within the

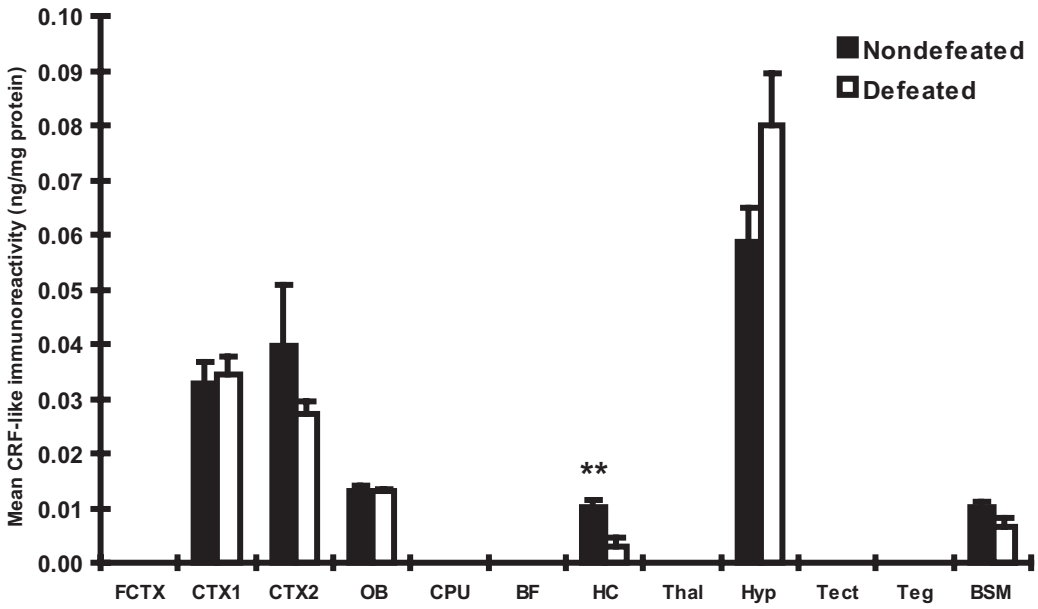

Figure 5. Mean (± *SEM*) content of corticotropin releasing factor (CRF) in tissue homogenates of various brain regions in intruder rats sacrificed 6 hr after 30-min resident intruder encounters with a nonaggressive male resident (nondefeated group) or an aggressive male resident (defeated group). FCTX = frontal neocortex, consisting of the frontal pole just anterior to the caudate–putamen and rostral edge of diencephalon; CTX1 = anterior neocortex over basal ganglia; CTX2 = posterior neocortex above the diencephalon; OB = olfactory bulb; CPU = caudate–putamen, which included the septum; BF = all basal forebrain ventral to the CPU, including nucleus accumbens; HC = body of the hippocampus; Thal = entire thalamus; Hyp = hypothalamus; Tect = tectum, including colliculi, dorsal half of the periaqueductal gray, and surrounding mesencephalic tissue, including some posterior thalamus and hypothalamus; Teg = tegmentum, consisting of the ventral midbrain above substantia nigra and ventral tegmental area, with mesencephalon up through the ventral periaqueductal gray along with some pontine tissue; BSM = lower brainstem, consisting of remaining pons and medulla. $^{**}p <$ .01, independent-samples *t* test (two tailed).

constraints of interpretation, our overall conclusion is that regional brain CCK-8 decreases are more strongly associated with social defeat than changes in a variety of other neuropeptides that also participate in stress regulation. Clearly, more work is needed to establish functional links between the neuropeptide changes associated with defeat in the animal model used here and the cascade of events that establish a defeat-induced depressive tone within the brain.

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