Social Stress in Hamsters: Defeat Activates Specific Neurocircuits within the Brain

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During an agonistic encounter, subordinate male hamsters display defensive and submissive postures and show increased secretion of glucocorticoids, whereas dominant males do not. To determine whether specific neuronal pathways are activated during the behavioral and neuroendocrine responses of subordinate males, expression of c-fos mRNA within the brains of subordinate males was compared with the pattern in dominant males after fighting. After 1 week of handling, pairs of hamsters were either swapped between cages (handled control males), or were allowed to interact for 30 min [dominant (DOM) males and subordinate (SUB) males]. A second group of control animals that received no handling or social stimulation (unhandled control males) were also included. After testing, all animals were killed by decapitation, their brains were removed for c-fos mRNA analysis in situ hybridization, and trunk blood was collected for analysis of plasma cortisol and corticosterone levels. Exposure of males to their partner’s cage for 30 min resulted in increased expression of c-fos mRNA in multiple brain regions. In addition, fighting increased c-fos expression in the medial amygdaloid nucleus of both DOM and SUB males as well as having more selective effects. In DOM males, c-fos expression was elevated within the supraoptic nucleus of the hypothalamus. In SUB males, c-fos expression increased within a multitude of brain areas, including cingulate cortex, lateral septum, bed nucleus of the stria terminalis, medial preoptic area, several hypothalamic nuclei, central amygdaloid nucleus, amygdaloidhippocampal area, dorsal periaqueductal gray, dorsal raphe, cuneiform nucleus, and locus coeruleus. These findings are discussed in relation to neurocircuits associated with behavioral arousal and stress.

Key words: c-fos; mapping activation; aggression; stress; fear; arousal; defense; hamster
within brain regions involved in fight or flight reactions (for review, see Graeff, 1994) or anxiety (for review, see Kuhar, 1986; Graeff, 1994).

MATERIALS AND METHODS

Behavioral testing. Twenty-four males were singly housed for at least 1 week. During this period of isolation, 18 animals were handled daily for 10–15 min by exposing one male to the odors and home cage environment of another male. “Cage-waxing” took place between the same pairs of males throughout the isolation period, and each dyad was selected randomly, being matched only by weight. The remaining six handled animals were simply swapped as before as controls (HC). The six unhandled controls (UHC) received no overt simulation. All tests were conducted within the first 4 hr of the animals’ dark period under dim illumination. The tests were 30 min in length and were videotaped for subsequent behavioral analyses.

Immediately after testing, each animal was rapidly transported to another room (within 1–2 min) and decapitated. Trunk blood was collected in heparinized vials on ice and stored at −20°C until samples could be analyzed for plasma levels of cortisol and corticosterone. Brains were removed, frozen in isopentane (−30 to −50°C), and then stored at −80°C until the tissue was processed for c-fos mRNA in situ hybridization histochemistry.

Radioimmunoassays. Cortisol was assayed using a kit obtained from Diagnostic Products Corp. (Los Angeles, CA; Coat-A-Count cortisol radioimmunoassay). The antisem is highly specific for cortisol, with very low cross-reactivities to other compounds (e.g., <1% cross-reactivity to corticosterone). The minimal detectable dose of this assay is 0.2 ng/ml. Intra-assay and interassay variations were <10%.

Corticosterone was assayed using a highly specific antibody developed in our laboratory and characterized by Dr. D. L. Helmreich (Mental Health Research Institute, University of Michigan). Cross-reactivities to related compounds (including cortisol) were <3%. Intra-assay and interassay variations were <10%.

In situ hybridization histochemistry. Each brain was sectioned on a cryostat at 10 μm, and a series of sections were mounted on poly-L-lysine-coated slides. Sections were taken at 250 μm intervals, except at the level of the paraventricular nucleus (PVN), in which the sections were collected at 100 μm intervals. The sections were then deproteinated with proteinase K (0.1 mg/ml in 0.1 M sodium phosphate buffer, pH 7.4, for 1 hr at room temperature. The sections were then deproteinized with proteinase K (0.1 mg/ml) for 5 min at 37°C, rinsed in distilled water, and treated for 10 min in 0.1 M triethanolamine (TEA) containing 0.25% acetic acid and 0.25% Triton X-100. The slides were then rinsed in distilled water and dehydrated in a series of alcohols.

35S-labeled cRNA probes were generated for c-fos from cDNA subclones in transcription vectors using standard in vitro transcription methods. The rat c-fos cDNA clone (courtesy of Dr. T. Curran, Roche) was subcloned in pGem 3Z and cut with HindIII to yield a 680 nucleotide cRNA probe. Probe was labeled in a transcription reaction that included 1 μg of linearized plasmid DNA, 1 μg each of the guanosine, cytosine, and adenine nucleotide bases (10 mM concentration), 1 μl of T7 polymerase, 1 μl of RNase inhibitor, 5 μl of transcription buffer, and 7.5 μl of 35S-uridine triphosphate (uracil nucleotide base). The reaction was incubated at 37°C for 2.5 hr, and the probe was then separated from free nucleotides over a Sephadex G50 column. Probes were diluted in hybridization buffer to yield 2.0 × 106 dpm/μl. The hybridization buffer consisted of 50% formamide, 3× SSC, 50 mM sodium phosphate buffer, pH 7.4, 1× Denhardt’s solution, and 0.1 mg/ml yeast tRNA. Diluted probe (70 μl) was applied to each slide and coverslipped. Slides were placed in sealed plastic boxes lined with moistened filter paper (50% formamide) and were allowed to incubate overnight at 55°C. Coverslips were then removed, and slides were rinsed several times in 2× SSC. Slides were then incubated in RNase A (200 μg/ml) for 60 min at 37°C and washed successively in 2× SSC and 1× SSC at room temperature for 5–10 min each, followed by incubating sections in 0.5× SSC at 60°C for 1 hr. Sections were then placed in fresh 0.5× SSC at room temperature for 5 min and dehydrated in a series of alcohols. All slides were exposed to Kodak (Rochester, NY) BioMax MR x-ray film for 1 week, with specific brain areas from each animal processed on the same film (e.g., septal region of all animals were processed on one film). The slides were subsequently dipped in Kodak NTB2 emulsion and stored in light-tight boxes for 8 weeks at 4°C. The emulsion-dipped slides were then developed in Kodak D-19 developer, counterstained with cresyl violet, dehydrated in a graded series of alcohols, cleared in xylene, and coverslipped with Permount.

During hybridization, several sections were either pretreated with RNase A (200 μg/ml at 37°C for 60 min) or hybridized with the sense c-fos mRNA probe to determine whether the cRNA probe reacted nonspecifically with tissue components other than mRNA in hamster brain.

Data analysis. As a way to standardize optical density measurements, a template or outline was developed for each brain nucleus or subnucleus based on the shape and size of the region. The location and relative size of each template are illustrated in Figure 1; the sections used in this illustration have been modified from the rat brain atlas of Paxinos and Watson (1997). Using these templates, optical density measurements were taken for each brain region from the left and right sides of the brain (where possible) or from rostral–caudal sections spaced ~200 μm apart. The optical density values were corrected for background, multiplied by the area sampled to produce an integrated optical density measurement, and then averaged to produce one data point for each brain region in each animal. These data points were averaged per group and compared statistically. Optical density measurements were quantified from x-ray film using National Institutes of Health Image software, with specific localization of signal confirmed from emulsion-dipped slides.

To determine the effect of handling, the mean level of c-fos mRNA within specific brain regions was compared between UHC and HC groups using a Student’s t test (p < 0.05). The effect of behavior was determined by comparing the mean level of c-fos mRNA within the same brain regions among HC, DOM, and SUB groups using a one-factor ANOVA (p < 0.05), with post hoc comparisons made between groups using the Tukey–Kramer multiple comparison (MC) test (p < 0.05). The Student’s t test and ANOVA require that the variance be comparable between or among the groups being compared. Although this requirement was met for many brain regions, it was not true of others, as determined by Bartlett’s test of the homogeneity of variance. If the variance differed across the groups, the data were first log-transformed, and then the appropriate test run. In most cases, log transformation resulted in equivalent variance among the groups being tested. However, in a couple of brain regions, the variance remained different. In this case, the Mann–Whitney nonparametric test (p < 0.05) was used for UHC–HC group comparisons, and the Kruskal–Wallis nonparametric ANOVA test (p < 0.05) was used for HC–DOM–SUB comparisons, with post hoc between-group comparisons made using the Mann–Whitney test (p < 0.015). All statistical comparisons were made using Instat 2.01 software.

RESULTS

Behavioral observations

Aggressive behavior was observed between each home cage male and intruder pair. The home cage male was dominant in two of the six behavioral tests, whereas the intruder was dominant in the remaining four tests. Dominant or subordinate status was determined quite rapidly on pairing the two males (19 sec on average), and once established, the relationship remained constant throughout the entire 30 min test. Dominant status was given to the male that chased and attacked the other male, which responded by fleeing and showing a variety of defensive responses (for review, see Lerwill and Makings, 1971). In addition to defensive postures and escape reactions, each SUB animal was extremely vigilant of the DOM male’s location throughout the 30 min test. Furthermore, on completion of testing, all SUB males showed high reactivity to handling by the experimenter.

Glucocorticoid secretion

The ANOVA revealed significant differences in glucocorticoid secretion among HC, DOM, and SUB groups (cortisol, F2,15 = 8.415; p < 0.01; corticosterone, F2,15 = 11.209; p < 0.01) (Fig.
Pairwise comparisons (Tukey–Kramer MC Test, \( p < 0.05 \)) showed that cortisol and corticosterone were significantly elevated in SUB males compared with the DOM and HC groups. A highly significant, positive correlation was observed between the levels of cortisol and corticosterone in all animals studied (Fig. 2B). No differences were observed in glucocorticoid secretion between the UHC and HC groups (cortisol, \( t_{(10)} = 1.307; p > 0.05 \); corticosterone, \( t_{(10)} = 1.005; p > 0.05 \)).

c-fos mRNA expression

Figure 3 illustrates the distribution of c-fos mRNA within select brain regions of UHC, HC, DOM, and SUB males. In general, low levels of c-fos mRNA were observed in the brains of UHC males. Placing a male in his partner’s cage on day 8 resulted in a significant elevation in c-fos expression throughout the neuraxis when compared with UHC group. In addition, allowing a pair of
males to interact in an agonistic encounter produced significantly higher numbers of activated cells within several brain nuclei above that observed in HC males, the most prominent increases occurring in SUB males. These differences in activation patterns are believed to reflect the transient expression of the c-fos proto-oncogene in hamster brain as pretreating sections with RNase A or hybridizing sections with sense strand probe resulted in an autoradiographic signal not different from background (data not shown).

The integrated optical density measurements for each brain area in UHC, HC, DOM, and SUB groups are provided in Table 1. Based on statistical comparisons between UHC and HC males (a measure of the effect of handling) and among HC, DOM, and SUB males (a measure of the effect of fighting), six distinct patterns of neuronal activation were noted: no change, handling, handling and behavior, handling and defense, defense, and offense (Fig. 4). Although most of these categories are self-explanatory, defense and offense patterns indicate that the greatest increase occurred in the SUB and DOM groups, respectively. However, in many instances, an increase in activation can be seen in both groups, with only a difference in the magnitude of activation.

No change
Although many brain regions showed an increase in c-fos mRNA after handling and/or fighting, the agranular insular (AI) and frontal (Fr) cortices were two areas that showed no change after either experimental procedure. A similar result was obtained for the CA3 and dentate gyrus (DG) of the hippocampus, although signal could be detected within these two areas in some males of the HC, DOM, and SUB groups.

Handling
After handling, c-fos expression increased within the accessory (AOB) and main (MOB) olfactory bulbs with grains present over cells within mitral cell layers. In addition, elevated levels of c-fos mRNA were observed in the lateral orbital cortex (LO), the amygdala [anterior (ACo), posterior medial (PMCo), and postero-lateral (PLCo) cortical nuclei, basolateral nucleus (BLa)], the piriform cortex (Pir), the hippocampus [subfields CA1 and CA2 and ventral subiculum (S)], and within several nuclei of the hypothalamus, including the suprachiasmatic nucleus (SCN), the posterior aspect of the paraventricular nucleus (PVN-post), the dorsomedial nucleus (DMH), and the ventral premammillary nucleus (PMV). In all of these brain areas, c-fos expression increased after handling alone, with no further increases after fighting [e.g., BLa (Fig. 5), DMH (Fig. 6), hippocampal subfields CA1 and CA2 (Fig. 7), ventral subiculum (Fig. 8), and posterior PVN (Fig. 9)].

Handling and behavior
The anterior subdivisions of the medial nucleus of the amygdala (MeA) showed increased c-fos labeling after handling, with further increases after fighting. The induction of c-fos mRNA by fighting was comparable between DOM and SUB males (Fig. 5).

Handling and defense
A number of brain regions with increased c-fos expression after handling also showed further elevations after defense. These areas included the cingulate cortex (Cg), the ventral (Lsv) and intermediate (Lsi) subdivisions of the lateral septum, the septo-hypothalamic nucleus (SHy), the anterior subdivisions of the bed nucleus of the stria terminalis (BNST), the amydalohippocampal area (AHi), the arcuate nucleus (ARC) of the hypothalamus, the dorsal periaqueductal gray (PAG), and the cuneiform nucleus (CnF) [Fig. 3; some areas shown at higher power: ARC (Fig. 6); AHi (Fig. 8); and SHy and BNST (Fig. 10)].

Offense
The supraoptic nucleus of the hypothalamus (SON) was the only brain region analyzed in this study that possessed the greatest increases after the display of offense (although circuits involved in offensive aggression were not the main focus of this study).

Defense
After defense, c-fos mRNA expression increased within the central nucleus of the amygdala (CeA), medial preoptic area (MPOA), anterior (AH), and ventromedial (VMH) hypothalamic nuclei, dorsal raphe (DR) and locus coeruleus (LC). In the PVN, induction of c-fos mRNA after defense was observed within the more anterior and intermediate aspects of this nucleus, whereas the handling alone elevated signal within the posterior PVN (Fig. 9). Interestingly, c-fos-positive cells in the SUB males were localized laterally within CeA (Fig. 5), BNST (Fig. 10), and VMH (Fig. 6).

In addition, in several areas selectively activated after defense, the level of c-fos mRNA tended to be lower in DOM males in comparison with both the HC and SUB groups. This trend reached significance for the dorsal subdivision of the lateral septum (LSd) and the supramammillary nucleus (SNM), in which SUB males had a significantly greater level of activation than DOM animals, whereas the HC group had values that were intermediate (Figs. 3C,D,G, 11).

DISCUSSION
Subordinate male hamsters possessed an increase in the frequency and intensity of activation of previously identified stress- and defense-related brain structures compared with dominant and handled control groups, and this pattern of neuronal activa-
Figure 3. Photomicrographs illustrating the distribution of c-fos mRNA within select brain regions from animals in UHC, HC, DOM, and SUB groups.
As we discuss the specific findings revealed by this experiment, it is important to bear in mind that c-fos expression may not provide a complete map of all neurons activated during a given stimulus (Robertson et al., 1989). Consequently, although induction of c-fos mRNA provides positive identification of brain areas activated during fighting, the absence of such labeling does not mean the lack of involvement. Additional studies with other “activation” markers may be neces-

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| Notes: The statistical relationship among HC–DOM–SUB groups is shown in columns three through five. *In brain regions showing an overall group effect, the letters indicate the result of pairwise comparisons among HC, DOM, and SUB groups, with a significant difference in integrated optical density measurements between any two groups indicated by different letters. Significant differences between UHC and HC groups. |
Repetitive exposure to partner’s cage

The purpose of placing each male in his partner’s cage daily was to reduce the effects of handling and exposure to another male’s odors on the responsiveness of the HPA axis. This goal was met, because plasma glucocorticoids and c-fos expression within the PVN were not significantly different between UHC and HC males.

Although the HC group did not show a stress response, these males did possess activation in numerous brain areas. Some of the most striking changes occurred in brain regions that process chemosensory cues including the mitral and granule cell layers of the AOB and MOB, and many of their central targets: amygdaloid nuclei (MeA, ACo, AH, PLC, and PMCo) and Pir cortex. Additional brain regions showed increased activation after handling including: Cg and LO cortices, lateral septum, anterior subdivisions of BNST, BLa, several hypothalamic nuclei (ARC, DMH, PMV, posterior PVN, and SCN), hippocampus, dorsal PAG, and cuneiform nucleus. It is likely that the specific functions mediated by activation within these various brain regions vary; however, these data indicate that behavioral arousal (e.g., locomotion and the investigation of conspecific odors by HC males) is associated with increased activation throughout the brain.

Acute exposure to intermale aggression

Fighting increased the activation of neurons within a number of brain regions. The MeA showed an equivalent level of c-fos labeling compared to the HC and DOM groups. This indicates that the MeA is involved in mediating the stress response associated with social defeat.
expression in DOM and SUB males above an initial response to handling. The MeA has been implicated in a variety of behavioral responses [e.g., mating (Lehman et al., 1980; Lehman and Winans, 1982; Kollack-Walker and Newman, 1995), aggression (Shibata et al., 1982; Luiten et al., 1985; Kollack-Walker and Newman, 1995; Potegal et al., 1996b), and affiliative behavior (Kirkpatrick et al., 1994)], and induction of c-fos mRNA after fighting is consistent with a role of this brain region in behavioral arousal (De Jonge et al., 1992; Kollack-Walker and Newman, 1995; Potegal et al., 1996b) and social memory (Bolhuis et al., 1984; Vochteloo and Koolhaas, 1987). The SON showed the greatest increase in DOM males. The significance of activation within this nucleus to offense or to related processes is presently unknown. Finally, a multitude of brain regions showed increased activation after defense. The relevance of this neuronal activation pattern in SUB males to the neuroendocrine and behavioral responses of stress is discussed below.

c-fos expression in PVN and glucocorticoid secretion

In comparison to the HC and DOM groups, SUB males showed significant elevations in circulating levels of cortisol and corticosterone at the end of a 30 min agonistic encounter. This rise in circulating glucocorticoids was accompanied by increased expression of c-fos mRNA within neurons of the paraventricular nucleus of the hypothalamus (PVN), a critical node in regulation of the HPA axis to stress (for review, see Herman et al., 1996).
Although SUB males showed the greatest increases in c-fos expression within the anterior region of the PVN, the DOM group had values that were intermediate. This finding suggests the possibility that some or all of the DOM males may have been stressed during the agonistic encounter, with an initial rise in plasma glucocorticoids that had declined to baseline by the end of testing. Indeed, studies in rats (Schuurman, 1980), mice (Bronson and Eleftheriou, 1964; Leshner, 1980), and swine (Fernandez et al., 1994) have reported elevated glucocorticoids in both the dominant and subordinate animals, with the subordinate males showing a larger response, as evidenced by higher peak levels and a slower decline to baseline.

**Stress neurocircuits: neuroendocrine regulation**

In addition to activation of the PVN, SUB males possessed a selective increase in c-fos labeling in brain regions previously implicated in stress regulation: Cg cortex, lateral septum, BNST, MPOA, CeA, hypothalamus (AH and ARC), and LC. This is one of the first studies to demonstrate that social stress activates neurons within distinct neurocircuits throughout the brain (also see Morton et al., 1984). The resultant pattern of neuronal activity parallels, in large part, the induction of immediate early genes after a variety of acute, nonsocial stressors (for review, see Cullinan et al., 1995a, and their illustrations).
The role of these various brain regions in regulation of the HPA axis has been the focus of several recent reviews and will be discussed here only briefly in light of our current findings. The activation of neurons within the CeA, anterior BNST, anterior MPOA, LS, DR, and LC is consistent with numerous studies showing that stimulation of these brain regions can increase levels of plasma glucocorticoids or adrenocorticotropin hormone (ACTH), which is released from corticotropes in the anterior pituitary, leading to the synthesis and release of adrenal steroids (for review, see Cullinan et al., 1995b; Herman et al., 1996). The role of several of these brain regions in facilitating adrenocortical activity is further supported by lesion studies showing that damage to cells within several of these areas can block or reduce glucocorticoid secretion in response to stress (for review, see Cullinan et al., 1995b; Herman et al., 1996).

The MeA has also been shown to stimulate glucocorticoid secretion (Dunn and Whitener, 1986). However, c-fos expression in this nucleus was elevated to comparable levels in DOM and SUB males, although cortisol and corticosterone were elevated only in the SUB group. This finding suggests that c-fos-positive neurons within the medial amygdala during fighting may not reflect excitation of the HPA axis but, rather, some other process, such as coupling sensory cues with behavioral arousal or social memory. Alternatively, activation within the medial amygdala could reflect an increased propensity for glucocorticoid secretion that is differentially regulated in DOM and SUB males at more central levels (e.g., blocked or inhibited in DOM males and stimulated in SUB males).

In addition to excitatory regulation, activation of neurons within the Cg cortex and ARC of SUB males is consistent with
previous reports showing that these two brain regions play an inhibitory role in regulation of the HPA axis. Lesions of the ARC increase basal glucocorticoid levels, and enhance adrenocortical activity in response to stress (for review, see Cullinan et al., 1995b; Herman et al., 1996). Lesions of the cingulate cortex (medial prefrontal cortex) also result in prolongation of ACTH and glucocorticoid release during exposure to stress (Diorio et al., 1993). The placement of steroid implants within the medial prefrontal cortex can reduce glucocorticoid secretion in response to stress, implicating this brain region in glucocorticoid negative feedback (Diorio et al., 1993).

Surprisingly, two brain regions shown to play a role in stress responsivity were not activated in SUB males in the present study. The hippocampus (CA1–3 and subiculum) has been implicated in the mechanism of glucocorticoid negative feedback (for review, see Jacobson and Sapolsky, 1991; Herman et al., 1995). The DMH has been implicated in mediating cardiovascular responses to stress (De Novellis et al., 1995; Stotz-Potter et al., 1996b), as well as a possible role in stress-induced release of ACTH (Stotz-Potter et al., 1996a). Both brain regions showed a significant increase in c-fos expression after handling with no further increases after fighting. Although one previous study did show a striking increase in c-fos expression after handling with no further increases after fighting, despite previous reports showing that these two brain regions play an inhibitory role in regulation of the HPA axis. Lesions of the ARC increase basal glucocorticoid levels, and enhance adrenocortical activity in response to stress (for review, see Cullinan et al., 1995b; Herman et al., 1996). Lesions of the cingulate cortex (medial prefrontal cortex) also result in prolongation of ACTH and glucocorticoid release during exposure to stress (Diorio et al., 1993). The placement of steroid implants within the medial prefrontal cortex can reduce glucocorticoid secretion in response to stress, implicating this brain region in glucocorticoid negative feedback (Diorio et al., 1993).

Stress neurocircuits: behavioral regulation

In addition to elevated levels of adrenal steroids, stressed animals show changes in behavior, such as freezing or flight, reactions that presumably reflect a state of increased fear or anxiety during stress (Maestripieri et al., 1991; Heinrichs et al., 1992). Of interest, most brain regions activated in SUB males and implicated in excitatory regulation of the HPA axis have also been shown to play a facilitatory role in stress-induced behavior: CeA (Jellestad et al., 1986), BNST (Shaikh et al., 1986; Casada and Dafny, 1991), MPOA (Gonzalez et al., 1996), lateral septum (Pesold and Treit, 1992), DR (for review, see Graeff, 1994), and LC (for review, see Bremner et al., 1996). Furthermore, the pattern of activation after defeat mirrors the distribution of Fos protein after electrical stimulation of brain regions that elicit aversion and defense (Silveira et al., 1993, 1995) or after behavioral or conditioning paradigms associated with increased anxiety or fear (Silveira et al., 1994; Beck and Fibiger, 1995). Thus, it is plausible that the neurocircuits activated in SUB males mediate both the neuroendocrine and behavioral responses to the stress of defeat.

The expression of c-fos mRNA within the LS of SUB males may reflect most closely an increased state of anxiety or fear. This conclusion is based on previous reports that increased anxiety or fear can inhibit offensive aggression (Blanchard et al., 1984; Maestripieri et al., 1991) and the observation that DOM males in the present study had the lowest levels of c-fos expression within the dorsal and, to a lesser degree, the intermediate subdivisions of the LS. The lateral septum has been implicated in behavioral inhibition, a process considered equivalent to anxiety (for review, see Graeff, 1994). The relatively sparse labeling of c-fos-positive cells within the lateral septum of DOM males may reflect a state of low anxiety and may be critical for the display of offense by these animals. Of interest, lesions to the septal region of hamsters increase aggression and social contacts (Sodetz and Bunnell, 1970; Potegal et al., 1981), a finding consistent with the proposed function of this brain region in behavioral inhibition.

In addition to neural circuits classically associated with stress, we found increased expression of c-fos mRNA within the ventrolateral aspect of the AH, lateral VMH, SMN, AH, dorsal PAG, and CnF in SUB males. Although the functional significance of activation within several of these brain regions is less clear, activation within the AH, VMH, and dorsal PAG play an impor-
tant role in mediating defensive behavior (for review, see Siegel and Pott, 1988). In the hamster, lesions of the AH increase the likelihood that females will show aggression against other females (Hammond and Rowe, 1976), which implies the presence of a neural circuit in the anterior hypothalamic area that either inhibits its aggression or stimulates a competing response like defense. Moreover, administration of norepinephrine into the MPOA–anterior hypothalamic region of dominant female hamsters results in a loss of their dominance and the display of submissive behaviors (Harmon et al., 1995). This latter study suggests a possible functional relationship between the activation observed within the AH and LC of SUB males. Interestingly, the unit activity of neurons within the LC of cats increases with the display of a defense reaction in response to threatening stimuli (Levine et al., 1990).

The ARC also displayed induction of c-fos mRNA selectively within SUB males. The ARC has been implicated in mediating the opioid form of stress-induced analgesia (Kelsey et al., 1987), a physiological response that may be critical for the display of defensive responses in situations that would normally elicit adaptive, more vegetative responses (e.g., licking a limb on injury). Opioid-mediated analgesia has been observed after defeat in mice (Miczek et al., 1982; Rodgers and Hendrie, 1983) and rats (Rodgers et al., 1983). The presence of analgesia in defeated male hamsters has not been reported.

Conclusions
Losing an agonistic encounter is clearly more stressful than winning. Our data provide evidence for the activation of specific neural circuits that may underlie the autonomic, neuroendocrine, and behavioral responses of socially defeated males. These findings lay the foundation for future experiments to identify the anatomical relationship among activated brain regions, the molecules involved in these specific brain circuits, and the regulation of these circuits after different types of stress.

APPENDIX

The following abbreviations are used: ac, anterior commissure; Acb, nucleus accumbens; ACo, anterior cortical nucleus of the amygdala; AH, anterior hypothalamic nucleus; AHi, amygdalo-hippocampal area; AI, agranular insular cortex; AOB, accessory olfactory bulb; AON, anterior olfactory nucleus; ARC, arcuate nucleus of the hypothalamus; BNST, bed nucleus of the stria terminalis; BNSTam, anteromedial subdivision, BNST; ANSTal, anterolateral subdivision, BNST; BNSTav, anteroventral subdivision, BNST; Bla, basolateral nucleus of the amygdala, anterior; C, core, Acb; CA1–3, field CA1–3 of Ammon’s horn, HIPP; CBL, cerebellum; cc, corpus callosum; CeA, central nucleus of the amygdala; CG, cingulate cortex; Cl, claustrum, CM, central medial thalamic nucleus; CnF, cuneiform nucleus; cp, cerebral peduncle; CPu, caudate putamen (striatum); CTF, central tegmental field; DEn, dorsal endopiriform nucleus; DG, dentate gyrus, HIPP; DMH, dorsomedial nucleus of the hypothalamus; DR, dorsal raphe; ec, external capsule; EPL, external plexiform layer of the olfactory bulb; f, fornix; fi, fimbria of the hippocampus; Fr, frontal cortex; Gl, glomerular layer of the olfactory bulb; GP, globus pallidus; Gra, granule cell layer, AOB; HIPP, hippocampus; Hb, habenula; ic, internal capsule; IC, inferior colliculus; IM, intercalated amygdaloid nucleus; IPL, internal plexiform layer of the olfactory bulb; La, lateral nucleus of the amygdala; LC, locus coeruleus; LH, lateral hypothalamus; LO, lateral orbital cortex; LOT, nucleus of the lateral olfactory tract; lot, lateral olfactory

Figure 11. Photomicrographs illustrating the distribution of c-fos mRNA within the rostral subdivisions of the LS in UHC (A), HC (B), DOM (C) and SUB (D) groups. Scale bar, 500 μm. Note the low levels of c-fos expression within L.Sd, L.Si, and L.Sv of the DOM male compared with the levels present in the HC and SUB animals; this activation profile contrasts with c-fos labeling in the adjacent CPu (striatum), which appeared elevated in all three groups relative to UHC males.
tract; LPOA, lateral preoptic area; LS, lateral septum; LSc, dorsal subdivision; LS, medial preoptic area; MO, medial orbital cortex; MOB, medial orbital bulb; MOA, medial cortex; AOB, medial geniculate nucleus; MFB, medial forebrain bundle; MPOA, medial preoptic area; MS, medial septum; mt, mammillothalamic tract; oc, optic chiasm; ot, optic tract; PAG, periaqueductal gray; PB, parabrachial nucleus; Pir, piriform cortex; PLCo, posterolateral nucleus of the amygdala; PMCo, posteromedial nucleus of the amygdala; PMd, ventral premammillary nucleus; PnR, pontine raphe nucleus; PT, paratenial thalamic nucleus; PVA, paraventricular thalamic nucleus; Post, posterior; PVN-post, PVN, posterior; PVP, paraventricular thalamic nucleus; posterior; py, pyramidal tract; S, subiculum; SC, superior colliculus; SCN, suprachiasmatic nucleus; Sh, shell, Acb; SHy, septohypothalamic nucleus; SMN, supramammillary nucleus; SN, substantia nigra; sm, stria medullaris; SOn, supraoptic nucleus of the hypothalamus; st, stria terminalis; Re, reuniens thalamic nucleus; Tu, olfactory tubercle; VEn, ventral endopiriform nucleus; VMH, ventromedial nucleus of the hypothalamus; ZI, zona incerta.

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