Neuroscience 150 (2007) 8–13

CORTICOTROPIN-RELEASING FACTOR PROJECTIONS FROM LIMBIC FOREBRAIN AND PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO THE REGION OF THE VENTRAL TEGMENTAL AREA

D. RODAROS, D. A. CARUANA, S. AMIR AND J. STEWART*

Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Room SP244, 7141 Sherbrooke Street West, Montreal, Quebec, Canada H4B 1R6

Abstract—Corticotropin-releasing factor (CRF) is a peptide neurotransmitter with high numbers of cell bodies found in limbic regions of the rat brain including the oval nucleus of the bed nucleus of the stria terminalis (BNSTov) and central nucleus of the amygdala (CeA) as well as in the paraventricular nucleus of the hypothalamus (PVN). CRF systems are activated in response to acute stressors and mediate a wide variety of physiological and behavioral responses to acute stress including aversive responses and responses that support appetitive behaviors. CRF is released in the ventral tegmental area (VTA), the cell body region of the mesocorticolimbic dopaminergic neurons, in response to acute stress and plays a role in stress-activation of appetitive behavior [Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You ZB (2005) Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. J Neurosci 25:5389–5396]. However, although it is known that the VTA region contains significant levels of CRF-immunoreactive fibers [Swanson LW, Sawchenko PE, Rivier J, Vale WW (1983) Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36:165–186], the source of CRF input to the region has not been identified. We used infusions of a fluorescent retrograde tracer, fluorogold, into the VTA region, combined with fluorescent immunocytochemistry for CRF to identify sources of this input. Double-labeled cells were found in BNSTov, CeA and PVN. The percent of fluorogold-labeled cells in each region that were CRF-positive was 30.8, 28.0 and 16.7% respectively. These data point to diffusely distributed sources of CRF-containing fibers in the VTA.

Key words: oval nucleus of the bed nucleus of the stria terminalis, central nucleus of the amygdala, paraventricular nucleus of the hypothalamus, fluorogold, stress, rat.

The behavioral functions of corticotrophin releasing factor (CRF) in the brain have been the subject of intense investigation in the last several years (Koob and Bloom, 1985; Koob et al., 1993; Merali et al., 1998; Makino et al., 1999; Sarnyai et al., 2001; Dunn et al., 2004; Heinrichs and Koob, 2004; Schulkin et al., 2005; Davis, 2006). CRF-containing cells are abundant in the central nucleus of the amygdala (CeA) and oval nucleus of the bed nucleus of the stria terminalis (BNSTov), as well as in the paraventricular nucleus of the hypothalamus (PVN, part of the hypothalamic–pituitary–adrenal stress axis). CRF systems are activated in response to stressors and mediate a wide variety of physiological and behavioral responses to stress including fear and anxiety (Schulkin et al., 2005; Davis, 2006), as well as responses that support appetitive behavior, such as increased locomotion (Kalivas et al., 1987; Cador et al., 1992, 1993) and facilitation of responses to incentive stimuli (Pecina et al., 2006). Although much is known about the role of CRF systems in fear and anxiety, less is known about the pathways through which activation of CRF systems facilitates appetitive behavior.

CRF plays an important role in stress-induced relapse to drug seeking (Shaham et al., 1997; Erb et al., 1998; Stewart, 2003; Spealman et al., 2004). In rats, infusions of CRF into the ventrolateral BNST (BNSTvl), a region of dense CRF fibers and terminals, can reinstate cocaine seeking in rats trained to self-administer cocaine, whereas infusions of a CRF receptor antagonist block foot-shock stress-induced reinstatement (Erb and Stewart, 1999; Erb et al., 2001). In addition, however, CRF is released directly in the ventral tegmental area (VTA) during foot-shock stress and in cocaine-experienced rats, infusions of a CRF receptor antagonist into the VTA block stress-induced reinstatement of drug seeking (Wang et al., 2005). These findings point to a more direct interaction between CRF-containing cell groups and the dopamine (DA) neurons in the VTA, providing a possible pathway for stress activation or modulation of appetitive behavior. Little is known, however, about the sources of CRF-containing fibers in the VTA. An understanding of the sources of the CRF innervation of the VTA would help to explain the role of stress and CRF in the modulation of appetitive behaviors. To address this issue, we combined infusions of a fluorescent retrograde tracer, fluorogold, into the VTA region, with fluorescence immunocytochemistry for CRF.

*Corresponding author. Tel: +1-514-848-2424x2193; fax: +1-514-848-2817.
E-mail address: jane.stewart@concordia.ca (J. Stewart).

Abbreviations: BNSTov, oval nucleus of the bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; CRF, corticotrophin releasing factor; DA, dopamine; DAB, 3,3′-diaminobenzidine; PB, phosphate buffer; PBS, phosphate-buffered saline; PVN, paraventricular nucleus of the hypothalamus; VTA, ventral tegmental area.

0306-4522/07$30.00+0.00 © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2007.09.043

EXPERIMENTAL PROCEDURES

Animals and housing

The experimental procedures followed the internationally recognized guidelines for the ethical use of laboratory animals of the Canadian Council on Animal Care and were approved by the Animal Care Committee, Concordia University. Every effort was made to minimize the number of animals used and their suffering. A total of eight adult male Wistar rats (Charles River, St. Constant, QC, Canada) weighing between 325 and 350 g were used. The rats were housed under normal 12-h light/dark cycle with food and water freely available at all times.

Tracers and antibodies

CRF. A CRF rabbit polyclonal antibody (1:8000), generously supplied by Dr. Wylie Vale (The Salk Institute for Biological Studies, San Diego, CA, USA), was used both for the standard immunocytochemistry and for the fluorescence immunocytochemistry. The secondary antibody for standard immunocytochemistry was a goat anti-rabbit IgG (1:200) (Vector, Burlingame, CA, USA). For the fluorescence immunocytochemistry an Alexa Fluor 555 goat anti-rabbit IgG (1:6000) (Molecular Probes, Eugene, OR, USA) was used.

Fluorogold. The retrograde tracer was a 2% fluorogold (Fluorochrome, Denver, CO, USA) solution prepared in distilled water. For standard immunocytochemistry of fluorogold, the primary antibody was a rabbit polyclonal antibody (1:6000; Chemicon, Temecula, CA, USA) and the secondary antibody was a goat anti-rabbit IgG (1:200) (Vector).

Surgery and histology

All rats received fluorogold infusions in the VTA. They were anesthetized with Nembutal (65 mg/kg, i.p., Sigma, St. Louis, MO, USA) and mounted on a stereotaxic instrument. The 2% fluorogold solution was infused unilaterally using a Hamilton microsyringe for a total volume of 0.15 μl over 10 min. Using a flat skull, the injector was inserted at a 15° angle from the midline using the coordinates: A/P −5.4 mm, M/L +2.6 mm from bregma and D/V −8.7 mm from skull surface (Paxinos and Watson, 1986). Cannulae were implanted on the contralateral side, 2 mm above the lateral ventral: A/P −1.0 mm. M/L +1.4 mm, D/L −3.0 mm from bregma, for future infusion of colchicine (Sigma-Aldrich, Oakville, ON; 2 μl at a concentration of 1 mg/ml) into the ventricle. One week later, infusions of colchicine were given to all rats 24 h before rats were anesthetized with Euthanyl (65 mg/kg, i.p.) and perfused transcardially with cold 0.1 M phosphate-buffered saline (PBS) (300 ml) followed by 4% paraformaldehyde in 0.1 M PBS (300 ml). The brains were post-fixed for 4 h in 4% paraformaldehyde and then transferred to 30% sucrose for 24 h. Serial coronal sections (30 μm) containing the BNST, PVN and CeA were collected. Alternate sections through each structure were used for fluorescence immunocytochemistry (CRF and fluorogold); the remaining sections were used for standard immunocytochemistry for either CRF or fluorogold.

Fluorescence immunocytochemistry

Free-floating sections were rinsed 3×10 min in 0.1 M phosphate buffer (PB) and then incubated for 1 h in 0.3% Triton PB with 5% normal goat serum. The sections were then incubated for 48 h at 4 °C in 0.3% Triton PB containing CRF rabbit polyclonal antibody (1:8000) and 2% normal goat serum. The sections were rinsed again with 0.1 M PB, 3×10 min and incubated with Alexa Fluor 555 goat anti- rabbit IgG (1:6000) for 1 h at room temperature. Following the incubation the sections were rinsed with 0.1 M PB and mounted onto gel-coated slides and coverslipped using ProLong Gold Antifade reagent (Molecular Probes).

Immunocytochemistry

CRF. Sections were rinsed 3×10 min in 0.1 M PB and incubated for 30 min in 3% hydrogen peroxide solution. Sections were again rinsed 3×10 min in 0.1 M PB followed by 1 h incubation in 0.3% Triton PB with 5% normal goat serum. The sections were then incubated for 48 h at 4 °C in 0.3% Triton PB containing CRF rabbit polyclonal antibody (1:8000) and 2% normal goat serum. Sections were rinsed 3×10 min in 0.1 M PB followed by

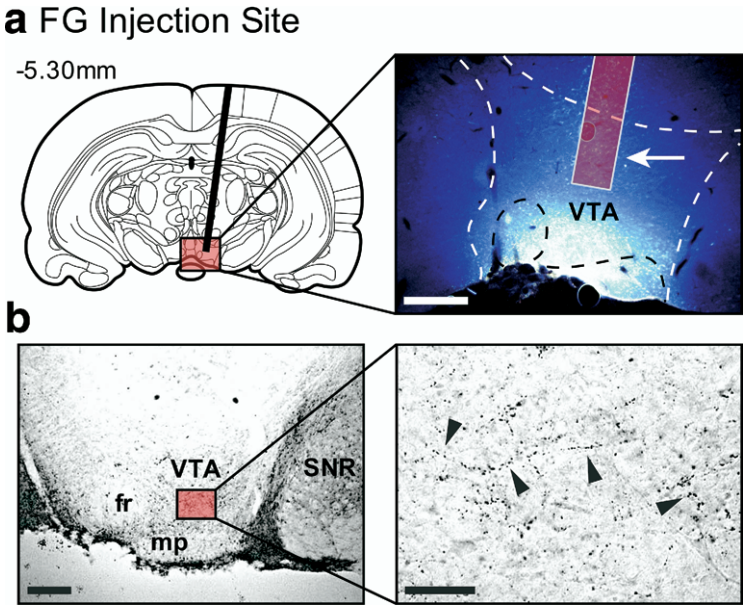

Fig. 1. (a) Coronal section (−5.30 mm from bregma) showing VTA region (Paxinos and Watson, 1986) and an example micrograph showing the site of a typical infusion of fluorogold in the VTA. Scale bar=250 μm. (b) Examples of CRF immunoreactive fibers in the region of the VTA shown in a. Scale bars=250 μm and 50 μm.

1 h incubation in the anti-rabbit IgG and then rinsed 3×10 min in 0.1 M PB after which they were placed for 30 min in a avidin–biotin–peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories). After rinsing in PB they were incubated for 10 min with 0.05% 3,3′-diaminobenzidine (DAB) in 0.1 M PB. Sections were then incubated in DAB/PB with 0.01% H_2O_2 and 8% $NiCl_2$. After rinsing the sections were mounted onto gel-coated slides, dehydrated through a series of alcohols, soaked in Citrisolv (Fisher Scientific, Ottawa, CA, USA), and coverslipped with Permount (Fisher Scientific).

Fluorogold. Sections from the second four rats were treated similarly except that the primary antibody for fluorogold was a rabbit polyclonal antibody (1:6000) (Chemicon).

Data analysis

Images containing fluorescent material were captured using a Leica DFC 480 camera at 20× magnification and Leica Firecam (1.7.1) software. DAB-stained sections were examined under a light microscope (Leica DM LB) and images were captured using a Hitachi (HV C20) camera, LG-3 frame-grabber (Scion, Frederick, MD, USA) and Image SXM (179-2C) software. Counts were done using software magnification for better identification of cells. Images for presentation were enhanced with Apple Aperture (1.5.2).

An estimate of the number of CRF-containing cells in brain sections through the BNST, CeA and PVN was obtained by counting cells in pairs of serial sections labeled by either fluorescence or DAB. One section from each pair had fluorescent labeling and the other DAB labeling for either CRF or fluorogold. The number of rats and sections included in the counts varied from structure to structure (BNSTov: four rats/six sections per rat, +0.2 to −0.4 mm from bregma; CeA: four rats/seven sections per rat, −1.88 to −3.3 mm from bregma; PVN: three rats/two sections per rat, −1.4 to −2.3 mm from bregma). Similarly fluorogold-labeled cells from each structure taken from a second group of rats were counted in serial sections labeled by either fluorescence or DAB (BNSTov: four rats/five sections per rat, +0.2 to −0.4 mm from bregma; CeA: four

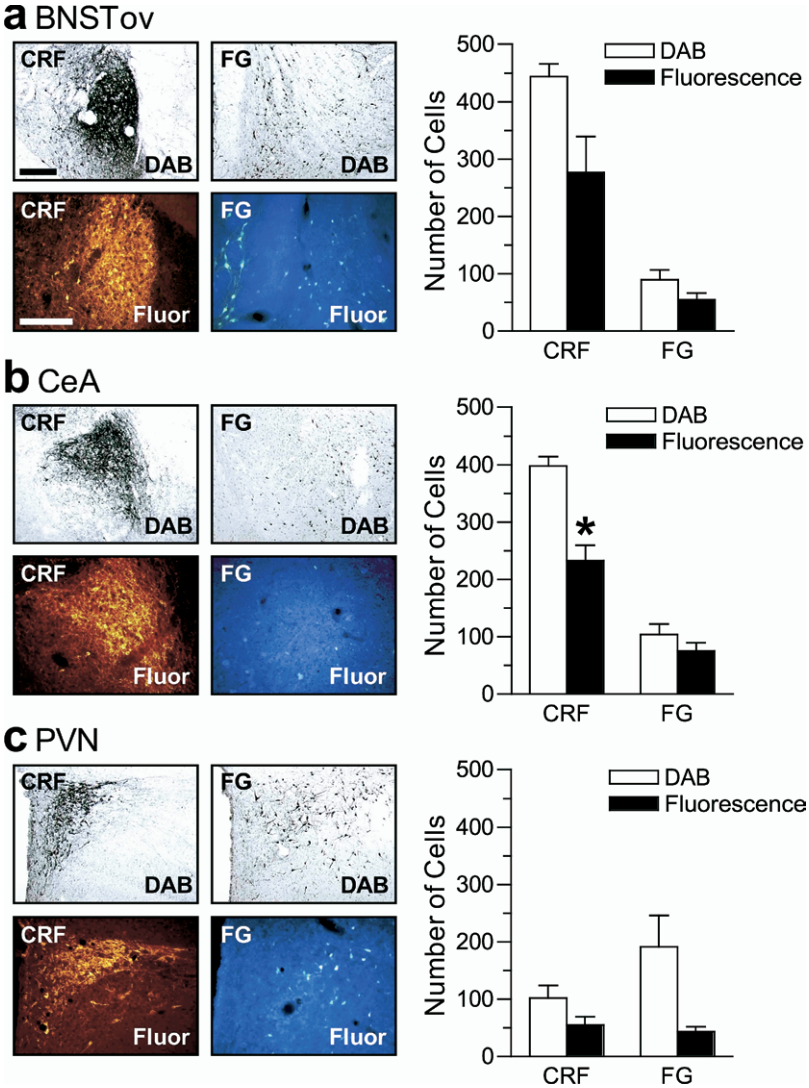

Fig. 2. Examples of DAB and fluorescent (fluor) labeling for both CRF and fluorogold (FG) in adjacent serial sections through the BNSTov (a), CeA (b), and PVN (c) taken from different groups of rats; scale bars=200 μm. Mean total number of CRF and fluorogold-labeled cells per rat in each region is shown in graphs on the right (see Experimental Procedures: Data analysis, for full description of how counts were made). * Paired *t*-test, $t(3)=6.87$, $P<0.01$.

rats/nine sections per rat, 1.88 to −3.3 mm from bregma; PVN: three rats/four sections per rat, −1.4 to −2.3 mm from bregma).

An estimate of the number of double-labeled fluorescent cells was obtained using the Leica DM 4000B fluorescence microscope (Leica filter A4 for fluorogold and N3 for CRF). Images were combined using Photomatix Pro (2.3). In order to determine the proportion of fluorogold-labeled cells projecting to the VTA from each structure that was CRF-containing, we compared the total number of double-labeled cells to the total number of fluorogold-labeled cells per rat for each structure (BNSTov: five rats/five sections per rat, +0.2 to −0.4 mm from bregma; CeA: five rats/10 sections per rat 1.88 to −3.3 mm from bregma; PVN: four rats/five sections per rat, −1.4 to −2.56 mm from bregma). Counts were verified by two individuals.

RESULTS

To determine the contribution of the BNSTov, CeA, and PVN cell groups to the CRF fibers in the VTA, we injected the retrograde tracer fluorogold into the VTA and looked for the presence of fluorogold/CRF double-labeled cells in BNSTov, CeA, and PVN. Fig. 1 shows the site of a typical fluorogold infusion in the VTA. It can be seen that the region contains CRF-labeled fibers. In five of the eight rats that received infusions, fluorogold fluorescence was clearly visible in the region shown and all had fluorogold-positive cells in BNSTov, CeA, and PVN ipsilateral to the injection.

Before trying to estimate the proportion of fluorogold-positive cells that were double-labeled for CRF, counts were made of fluorogold-labeled cells and CRF-labeled cells in pairs of alternate sections in each region common to all rats. Fig. 2a–c shows examples of CRF- and fluorogold-positive cells in each region labeled with DAB or fluorescence. As expected, all three regions contained numerous densely stained CRF cell bodies and fibers. Fluorogold-filled cells were also visible in each region. The graphs on the right of Fig. 2a–c show the mean total number of CRF and fluorogold cells per rat in each region in adjacent sections visualized using DAB or fluorescence. It can be seen that for both CRF and fluorogold, the counts for fluorescent-labeled cells are lower than those for the DAB-labeled cells. However, with the exception of CRF in the CeA where the density of cells makes counting fluorescent-labeled cells difficult, the differences are not statistically significant. Because both CRF and fluorogold labeling is densely cytoplasmic, estimates of the number of fluorogold/CRF double-labeled cells were made in the fluorescent-labeled sections. When we examined these sections for the presence of fluorogold/CRF double-labeled cells, we were able to detect a portion of fluorogold-positive cells in each rat that were also positive for CRF; 30.8±6.9% in BNSTov, 28.0±4.0% in CeA and 16.7±9.6% in PVN (see Figs. 3–5).

DISCUSSION

These results show that the VTA receives projections from each of the regions examined, BNSTov, CeA and PVN and that a significant proportion of the projections from each of these regions is CRF-positive. The greatest number of fluorogold/CRF double-labeled cells was seen in the BNSTov and CeA, whereas fewer double-labeled cells were found in the PVN. The finding that the CRF innervation of the VTA arises from multiple sources is consistent with evidence concerning the CRF afferents to noradrenergic and serotonergic cell regions (Moga and Gray, 1985; Moga et al., 1989; Gray, 1993; Tjoumakaris et al., 2003). In addition to the CRF afferents from other regions, CRF-containing cell bodies are found in monoamine-containing nuclei of the brainstem such as the locus coeruleus (LC) (Swanson et al., 1983). Furthermore, 5-HT cells in the dorsal raphe contain CRF (Commons et al., 2003), and DA cells in the VTA contain CRF mRNA (Liu and Morales, 2006).

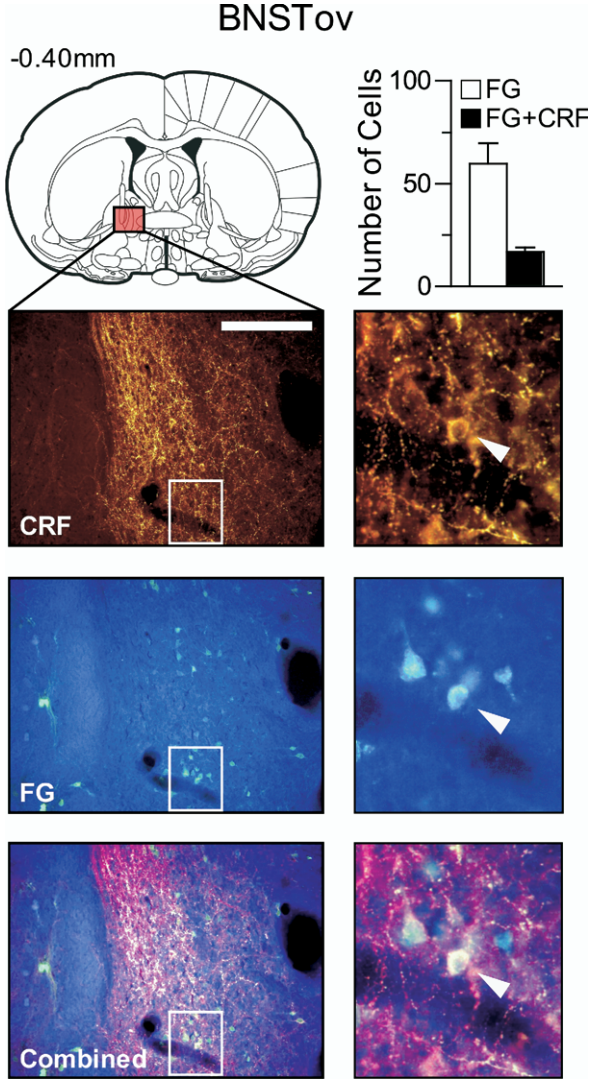

Fig. 3. Example photomicrographs showing CRF, fluorogold and fluorogold/CRF double-labeling in the BNSTov at the level indicated on the corresponding plate from Paxinos and Watson, 1986 (scale bar=200 μm). Boxes in the photomicrographs on the left show the expanded region on the right. White arrows point to a double-labeled cell. Graph in upper right shows the mean total number of double-labeled cells compared with the total number of fluorogold-labeled cells in BNSTov per rat (see Experimental Procedures: Data analysis, for full description of how counts were made).

Finally, it is well known that the BNST, CeA and PVN receive inputs from those monoamine cell groups underscoring the complexity of the CRF neuronal networks and of the interactions between CRF cells and monoamine systems of the brain. Interestingly, relapse to cocaine seeking induced by acute exposure to a stressor is blocked by the infusion of noradrenergic receptor antagonists into both the CeA and BNST (Leri et al., 2002), suggesting a way in which acute foot-shock stress may activate these CRF systems.

Our finding that the VTA receives CRF projections from the BNSTov, the CeA and PVN is consistent with the evidence that CRF is released in the VTA during exposure to acute stress (Wang et al., 2005). Stress activation of CRF systems, presumably via noradrenergic inputs (Freedman and Cassell, 1994; Leri et al., 2002), appears to facilitate glutamatergic synaptic transmission in DA neurons (Ungless et al., 2003; Wang et al., 2005, 2007), thereby providing a means whereby stress could engage appetitive motivational systems and lead to long-lasting facilitation within these systems.

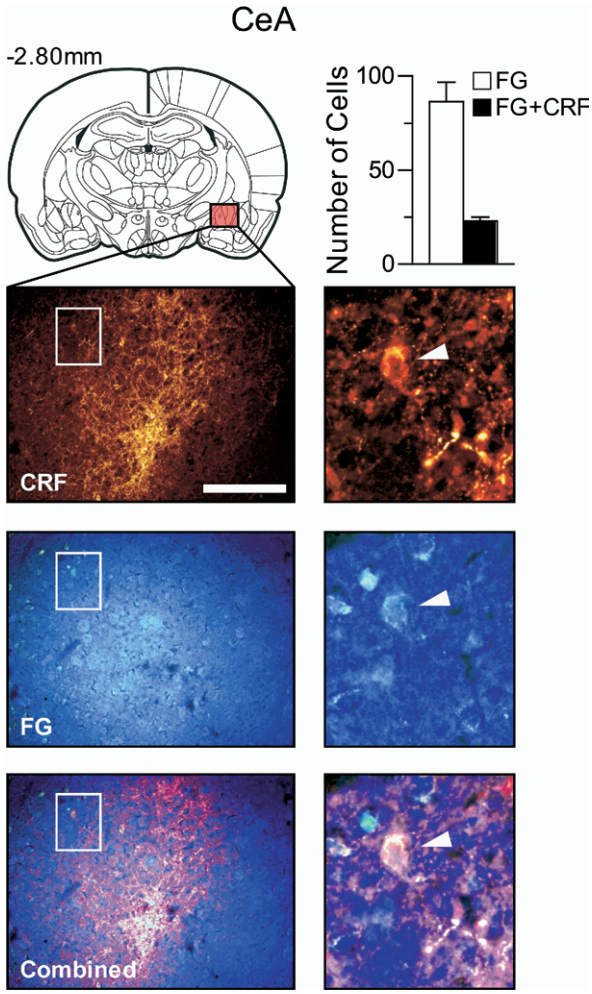

Fig. 4. Example photomicrographs showing CRF, fluorogold and fluorogold/CRF double-labeling in the CeA at the level indicated on the corresponding plate from Paxinos and Watson, 1986 (scale bar=200 μm). Boxes in the photomicrographs on the left show the expanded region on the right. White arrows point to a double-labeled cell. Graph in upper right shows the mean total number of double-labeled cells compared with the total number of fluorogold-labeled cells in CeA per rat (see Experimental Procedures: Data analysis, for full description of how counts were made).

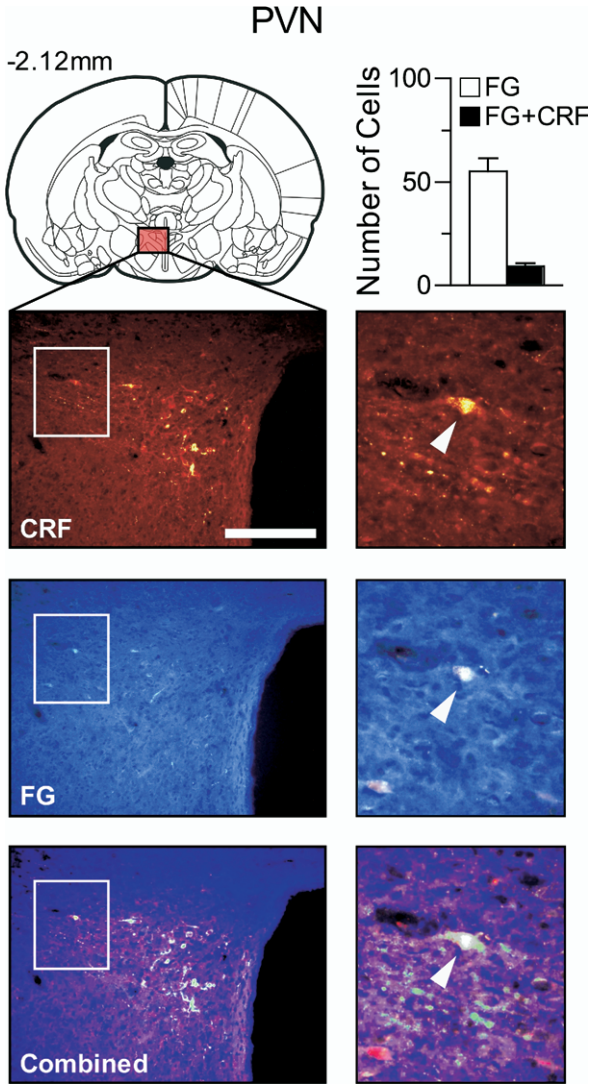

Fig. 5. Example photomicrographs showing CRF, fluorogold and fluorogold/CRF double-labeling in the PVN at the level indicated on the corresponding plate from Paxinos and Watson, 1986 (scale bar=200 μm). Boxes in the photomicrographs on the left show the expanded region on the right. White arrows point to a double-labeled cell. Graph in upper right shows the mean total number of double-labeled cells compared with the total number of fluorogold-labeled cells in PVN per rat (see Experimental Procedures: Data analysis, for full description of how counts were made).

Acknowledgments—Supported by a grant from the Canadian Institutes of Health Research (CIHR). We thank Dr. W. Vale for his generous gift of CRF antibody.

REFERENCES

Cador M, Cole BJ, Koob GF, Stinus L, Le Moal M (1993) Central administration of corticotropin releasing factor induces long-term sensitization to D-amphetamine. Brain Res 606:181–186.

Cador M, Dumas S, Cole BJ, Mallet J, Koob GF, Le Moal M, Stinus L (1992) Behavioral sensitization induced by psychostimulants or stress: search for a molecular basis and evidence for a CRF-dependent phenomenon. Ann N Y Acad Sci 654:416–420.

Commons KG, Connolley KR, Valentino RJ (2003) A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. Neuropsychopharmacology 28:206–215.

Davis M (2006) Neural systems involved in fear and anxiety measured with fear-potentiated startle. Am Psychol 61:741–756.

Dunn AJ, Swiergiel AH, Palamarchouk V (2004) Brain circuits involved in corticotropin-releasing factor-norepinephrine interactions during stress. Ann N Y Acad Sci 1018:25–34.

Erb S, Salmaso N, Rodaros D, Stewart J (2001) A role for the CRF-containing pathway from central nucleus of the amygdala to bed nucleus of the stria terminalis in the stress-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 158:360–365.

Erb S, Shaham Y, Stewart J (1998) The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. J Neurosci 18:5529–5536.

Erb S, Stewart J (1999) A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. J Neurosci 19:RC35.

Freedman LJ, Cassell MD (1994) Distribution of dopaminergic fibers in the central division of the extended amygdala of the rat. Brain Res 633:243–252.

Gray TS (1993) Amygdaloid CRF pathways. Role in autonomic, neuroendocrine, and behavioral responses to stress. Ann N Y Acad Sci 697:53–60.

Heinrichs SC, Koob GF (2004) Corticotropin-releasing factor in brain: a role in activation, arousal, and affect regulation. J Pharmacol Exp Ther 311:427–440.

Kalivas PW, Duffy P, Latimer G (1987) Neurochemical and behavioral effects of corticotropin-releasing factor in the ventral tegmental area of the rat. J Pharmacol Exp Ther 242:757–763.

Koob GF, Bloom FE (1985) Corticotropin-releasing factor and behavior. Fed Proc 44:259–263.

Koob GF, Heinrichs SC, Pich EM, Menzaghi F, Baldwin H, Miczek K, Britton KT (1993) The role of corticotropin-releasing factor in behavioural responses to stress. Ciba Found Symp 172:277–289; discussion 290–295.

Leri F, Flores J, Rodaros D, Stewart J (2002) Blockade of stress-induced, but not cocaine-induced reinstatement, by infusion of noradrenergic antagonists into the bed nucleus of the stria terminalis or the central nucleus of the amygdala. J Neurosci 22: 5713–5718.

Liu B, Morales M (2006) Neurons expressing corticotrophin-releasing factor are dopaminergic in the ventral tegmental area. Program No. 483.2. 2006 Neuroscience Meeting Planner. Atlanta, GA: Society for Neuroscience, online.

Makino S, Shibasaki T, Yamauchi N, Nishioka T, Mimoto T, Wakabayashi I, Gold PW, Hashimoto K (1999) Psychological stress increased corticotropin-releasing hormone mRNA and content in the central nucleus of the amygdala but not in the hypothalamic paraventricular nucleus in the rat. Brain Res 850:136–143.

Merali Z, McIntosh J, Kent P, Michaud D, Anisman H (1998) Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala. J Neurosci 18:4758–4766.

Moga MM, Gray TS (1985) Evidence for corticotropin-releasing factor, neurotensin, and somatostatin in the neural pathway from the central nucleus of the amygdala to the parabrachial nucleus. J Comp Neurol 241:275–284.

Moga MM, Saper CB, Gray TS (1989) Bed nucleus of the stria terminalis: cytoarchitecture, immunohistochemistry, and projection to the parabrachial nucleus in the rat. J Comp Neurol 283:315–332.

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York: Academic Press.

Pecina S, Schulkin J, Berridge KC (2006) Nucleus accumbens corticotropin-releasing factor increases cue-triggered motivation for sucrose reward: paradoxical positive incentive effects in stress? BMC Biol 4:8.

Sarnyai Z, Shaham Y, Heinrichs SC (2001) The role of corticotropin-releasing factor in drug addiction. Pharmacol Rev 53:209–243.

Schulkin J, Morgan MA, Rosen JB (2005) A neuroendocrine mechanism for sustaining fear. Trends Neurosci 28:629–635.

Shaham Y, Funk D, Erb S, Brown TJ, Walker CD, Stewart J (1997) Corticotropin-releasing factor, but not corticosterone, is involved in stress-induced relapse to heroin-seeking in rats. J Neurosci 17: 2605–2614.

Spealman RD, Lee B, Tiefenbacher S, Platt DM, Rowlett JK, Khroyan TV (2004) Triggers of relapse: nonhuman primate models of reinstated cocaine seeking. Nebr Symp Motiv 50:57–84.

Stewart J (2003) Stress and relapse to drug seeking: studies in laboratory animals shed light on mechanisms and sources of long-term vulnerability. Am J Addict 12:1–17.

Swanson LW, Sawchenko PE, Rivier J, Vale WW (1983) Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. Neuroendocrinology 36:165–186.

Tjoumakaris SI, Rudoy C, Peoples J, Valentino RJ, Van Bockstaele EJ (2003) Cellular interactions between axon terminals containing endogenous opioid peptides or corticotropin-releasing factor in the rat locus coeruleus and surrounding dorsal pontine tegmentum. J Comp Neurol 466:445–456.

Ungless MA, Singh V, Crowder TL, Yaka R, Ron D, Bonci A (2003) Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons. Neuron 39:401–407.

Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You ZB (2005) Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. J Neurosci 25:5389–5396.

Wang B, You ZB, Rice KC, Wise RA (2007) Stress-induced relapse to cocaine seeking: roles for the CRF(2) receptor and CRF-binding protein in the ventral tegmental area of the rat. Psychopharmacology (Berl) 193:283–294.

(Accepted 11 September 2007)
(Available online 21 September 2007)