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Corticotropin-Releasing Factor and Schedule-Induced Polydipsia

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COLE, B. J. AND G. F. KOOB. *Corticotropin-releasing factor and schedule-induced polydipsia.* PHARMACOL BIOCHEM BEHAV 47(3) 393-398, 1994.—Two experiments examined the effects of ICV-administered corticotropin-releasing factor (CRF) and α-helical CRF (9-41), a CRF antagonist, on the performance of schedule-induced polydipsia (SIP). Infusions of CRF into the lateral ventricle dose-dependently (0.02, 0.1, and 0.5 μg) attenuated both the volume of water consumed and licking on a fixed-time 60-s schedule. This effect of CRF on schedule-induced drinking was accompanied by a reduction in the number of nose pokes made into the food tray, suggesting that CRF may attenuate SIP through an action on appetitive motivation. Neither the temporal distribution of responding nor the locomotor activity induced by the schedule was affected by CRF. In marked contrast to these effects of exogenous CRF on the performance of SIP, infusions of α-helical CRF (1, 5, and 25 μg) into the lateral ventricle did not affect the performance of schedule-induced polydipsia. The implications of these results for the hypothesis that SIP is a coping response to stress are discussed.

Corticotropin-releasing factor α-Helical CRF Schedule-induced polydipsia Rat Stress

CORTICOTROPIN-RELEASING FACTOR (CRF) is a 41-amino-acid polypeptide, originally isolated and characterized on the basis of its ability to stimulate the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary (59). In addition to its critical role in the activation of the hypothalamic pituitary adrenal axis during stress (39,42,43), several lines of evidence suggest that CRF may also act in the central nervous system as a neurotransmitter. For example, neuroanatomical studies have shown CRF-immunoreactive neurons and fibres outside of the hypothalamus (53), and receptor-binding studies have shown CRF-binding sites heterogeneously distributed throughout the neuraxis (15,16). In addition, electrophysiological studies have shown that CRF produces a profound depolarization and excitation of hippocampal neurons (1) and increases the firing rate of norepinephrine (NE)-containing neurons in the locus coeruleus (60).

At the functional level, ICV administration of CRF has been shown to produce a wide range of behavioural effects that are similar to those seen in stressful or fearful situations. These include decreased locomotion in a novel open field (7), decreased exploratory behavior (3), decreased responding in an operant conflict paradigm (8) and on a conditioned suppression schedule (13), and an increase in the acoustic startle reflex (33). These results have led to the suggestion that CRF plays a critical role in initiating behavioural responses to stressful stimuli (28).

Further evidence for a role of CRF in the response to stress comes from the observations that CRF immunoreactivity and mRNA are altered in discrete brain regions following exposure to stressors (11,25), and the evidence showing that a CRF antagonist, α-helical CRF, can attenuate many stress-induced behavioural alterations. These include stress-induced anorexia (29), stress-induced reductions in exploratory behaviour (4), foot shock-induced freezing (27), stress-induced sensitization of amphetamine-induced stereotypy (12), alcohol withdrawal-induced anxiety (2), and novelty-induced defensive-withdrawal behaviour (54,55).

The purpose of the present series of experiments was to examine the effects of CRF and the CRF antagonist α-helical CRF on the performance of schedule-induced polydipsia. This behavior develops when a food-deprived (but not water-deprived) rat is exposed to a schedule in which small amounts of food are delivered intermittently. Such schedules result in the development of excessive drinking during the intervals between food delivery (20). The amount of drinking is critically dependent upon the degree of food deprivation and the inter-

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food pellet interval (21). Although several hypotheses have been proposed to explain the occurrence of SIP, including dryness of the mouth following the ingestion of the food pellet (51) or adventitious reinforcement of licking (48), Falk has convincingly argued that such hypotheses, based on operant or homeostatic principles, cannot adequately explain the characteristics of SIP (22). Instead, Falk has argued that SIP may be an experimental analogue of the displacement behaviours described by ethologists (57).

Both displacement activities and SIP (5) have been hypothesized to serve a stress- or arousal-reduction function. In the case of SIP, this theory is supported by the demonstration of lower levels of corticosterone in the plasma of rats that are allowed to drink, compared to those not given access to water during an intermittent food delivery schedule (5). However, this reduction in peripheral indices of stress (or arousal) appears to be specific to plasma corticosterone, since other peripheral indices of stress, such as the levels of prolactin, norepinephrine, and epinephrine, are not lowered by drinking (14).

Given the central role of CRF in initiating behavioural and endocrine responses to stress [see (19) for review], the effects of this peptide and the CRF antagonist on SIP have obvious relevance for the hypothesis that SIP is a coping response to stress. For example, if SIP serves to reduce an aversive state of high arousal, as indexed by high levels of plasma corticosterone, then CRF, which increases corticosterone levels, should enhance SIP.

GENERAL METHODS

Subjects

Twenty-four male Wistar rats (Charles River strain, Kingston, NJ) were used. They were housed three per cage in a temperature-controlled room with water freely available and the lights on from 0400 to 1600.

Apparatus

Four operant chambers (Colbourn Instruments, LeHigh Valley, PA) controlled on-line by a Control Universal System microcomputer (Paul Fray Ltd, Cambridge, UK) programmed in ONLIBASIC were used. Each operant chamber was enclosed in a sound-attenuating box and equipped with a loudspeaker which provided background white noise (70 dB). In the center of the front wall of each chamber was a food tray into which food reinforcement (45-mg food pellets, P. J. Noyes, Lancaster, NH) could be delivered. A photocell beam was located across the food tray so that nose pokes into the tray could be recorded. Situated 7 cm to the left of the food tray and 5 cm above the grid floor was a metal drinking spout, connected to a 50 ml glass burette, and a lickometer. The glass burette was filled with tap water, and the volume of water consumed could be measured to an accuracy of 0.1 ml. Situated on the sides of the boxes were two photocell beams. They were located 1 cm above the grid floor and 8 and 20 cm from the front wall.

Surgery

To enable ICV administration of peptides, the rats were implanted with a stainless steel guide cannula aimed at the lateral ventricle. Rats were anaesthetized with sodium pentobarbital (50 mg/kg) and placed in a Kopf stereotaxic instrument fitted with atraumatic earbars. A 7-mm stainless steel guide cannula (23 gauge) was secured to the skull with three stainless steel screws and dental cement. The coordinates were anterior-posterior, -0.6 mm from bregma; lateral, ± 2.0 mm from the midline; and dorsal-ventral, -3.2 mm from the skull surface, with the incisor bar set 5 mm above the interaural line (40). A 7-mm stylet was placed into the cannula, and the rats were allowed at least four days to recover from surgery before behavioural testing.

Peptides and Injection Procedure

Rat CRF and α-helical CRF (9-41) were synthesized as previously described using preparative purification techniques (43,44). CRF was dissolved in isotonic saline and α-helical CRF (9-41) was dissolved in distilled water (pH 6.7). For ICV injections of CRF an 8-mm injector connected to approximately 70 cm of calibrated PE 10 tubing was inserted. One microliter of CRF was then infused under gravity, by lifting the tubing above the head of the rat. For ICV injections of α-helical CRF the stylet was removed from the guide cannula, and an 8-mm (30 gauge) stainless steel injector connected by PE 10 tubing to a 10-ml Hamilton syringe was inserted. Five milliliters of α-helical CRF (9-41) was then infused by hand over an approximately 60-s period. These different methods of injections were used because of the viscosity of α-helical CRF. In both cases the injector was left in place for 30 s after the injection to prevent backflow before the stylet was replaced in the guide cannula.

To verify cannula placements the rats were overdosed with sodium pentobarbital at the end of the experiment and injected ICV with 5 ml methylene blue dye. The brains were then removed and examined to check that the blue dye had spread bilaterally through the ventricular system.

EXPERIMENT 1: EFFECTS OF CRF ON THE PERFORMANCE OF SCHEDULE-INDUCED POLYDIPSIA

The first experiment investigated the effects of ICV administration of CRF on the performance of SIP. It could be predicted that like other drugs which can induce "anxiogenic-like" behavioural effects, such as the β-carboline FG 7142 (36), CRF would cause a reduction in SIP. Alternatively, it could be hypothesized that CRF would actually enhance SIP, since it produces an aversive state of high arousal which can be reduced by excessive drinking (5).

Procedure

Twelve male Wistar rats weighing 220–240 g at the beginning of the experiment were used. The rats were briefly handled by the experimenter (5 min) and then food-deprived to 80% of their free-feeding weight over a seven-day period. The rats were then each fed 12 g of Purina Lab Chow per day for the duration of the experiment.

On the first day of behavioural training the rats were placed into the operant chambers with the food trays containing 30 food pellets. No behavioural data were recorded. On all subsequent sessions a single food pellet was delivered into the food tray every 60 s (fixed-time [FT] 60-s schedule). The following behavioural measures were recorded:

1. The number of licks.
2. The number of nose pokes into the food tray.
3. The number of crossovers (a crossover was defined as breaking the two photocell beams located along the sides of the operant chambers consecutively).
4. The volume of water consumed.

Each 60-s inter-food pellet interval was divided into six 10-s time bins, and the number of behavioural responses that occurred in each of these time bins was recorded and summed for the whole session.

The rats were tested on this schedule for 10 sessions. The rats which attained a criteria of drinking at least 10 ml/30-min session were then implanted with ICV cannulae, as described above (n = 8). They were then left for a 5-day postoperative recovery period before testing for a further 5 sessions on the FT 60-s schedule. Over the next 10 days the rats received a sequence of ICV infusions of CRF 30 min before testing. The doses used were 0, 0.02, 0.1, and 0.5 μg, and the order of doses was based on a 4×4 Latin Square. A Latin Square design, in which an equal number of animals receive each drug treatment on each test day, was used in these studies to control for possible tolerance or sensitization to the effects of the peptide. All ICV infusions were separated by two baseline sessions.

Data analysis. The number of licks, nose pokes, and crossovers were analysed with a two-way analysis of variance (ANOVA) with repeated measures on both time bin and dose, and the volume consumed was analysed with a one-way ANOVA with repeated measures on the factor dose. All analyses were performed both on the raw data and following a logarithmic (natural) transformation to control for possible skewed distribution (63). However, since the transformation did not affect the significance of any analysis, only the analyses performed on the raw data are reported. If a significant main effect was found in the ANOVA, specific comparisons of the means were made with Newman-Keuls post hoc tests ($p < .05$). Following a significant interaction, further analysis was made using analysis of simple main effects (63).

Results

As shown in panels B, C, and D of Fig. 1, the temporal distribution of licking, panel pushing, and activity in the saline-treated animals of the present experiment was similar to that previously reported [e.g., (36)]. Specifically, licking was predominantly confined to the time bins immediately following the presentation of the food reinforcer, reaching a maximum in the second time bin. This temporal pattern of responding is a characteristic of all schedule-induced behavior (21). In contrast, nose pokes into the food tray were at their lowest level during the second 10-s interval, and then gradually rose throughout the remaining time bins. Crossovers also showed a characteristic temporal profile that was different from those found for licking and nose pokes. Specifically, crossovers were minimal during the first two time bins, then rose to a maximum in the fifth time bin, and then dropped during the last 10 s.

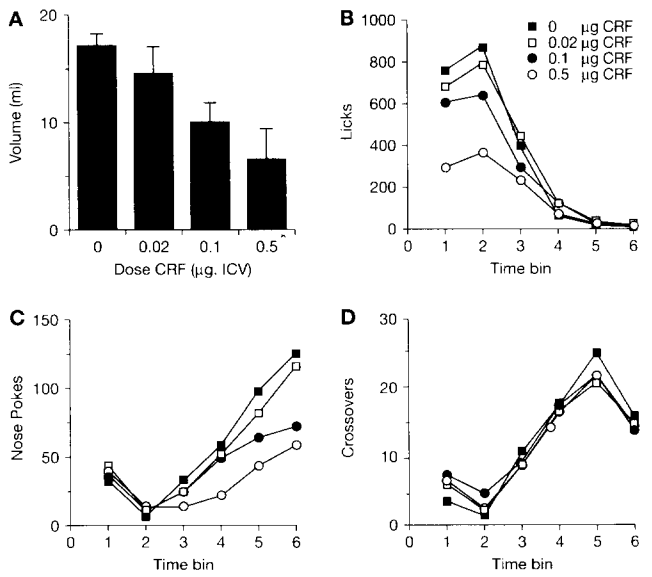

FIG. 1. (A) The effects of CRF on total water consumption during the 30-min session. The error bars represent the SE. (B–D) The effects of CRF on the temporal distribution of licking (B), nose pokes (C), and crossovers (D) during the 60-s interpellet interval.

As shown in panel A of Fig. 1, ICV infusions of CRF caused a dose-dependent reduction in the volume of water consumed during the 30-min session. ANOVA revealed a significant main effect of dose, $F(3, 21) = 9.61$, $p < .001$, and post hoc Newman-Keuls tests showed that both 0.1 and 0.5 μg CRF significantly reduced the volume of water consumed. Panel B shows that CRF also dose-dependently reduced the number of licks. ANOVA revealed a significant main effect of time bin, $F(5, 35) = 9.19$, $p < .001$, and a significant Dose × Time Bin interaction, $F(15, 105) = 4.43$, $p < .001$. Analysis of simple main effects revealed that 0.1 and 0.5 μg CRF significantly reduced licking in the first two time bins, when the response occurs at its maximum rate (analysis of simple main effects and Newman-Keuls tests).

Panel C of Fig. 1 shows that ICV infusions of CRF also caused a reduction in the number of nose pokes into the food tray. ANOVA revealed a significant main effect of Time Bin, $F(5, 35) = 22.59$, $p < .001$, and a significant Dose × Time Bin interaction, $F(15, 105) = 4.25$, $p < .001$. Further analysis revealed that in the last three time bins 0.5 μg CRF caused a significant reduction in nose pokes in the food tray (analysis of simple main effects and Newman-Keuls tests). Panel D of Fig. 1 shows that ICV infusions of CRF did not affect either the temporal distribution or the number of crossovers. ANOVA revealed a significant main effect of time bin, $F(5, 35) = 6.76$, $p < .001$, but no significant main effect of dose or Dose × Time Bin interaction (both F ratios < 1). All of the food pellets were consumed by all of the rats in every session.

In summary, this experiment has shown that ICV infusions of CRF cause a dose-dependent reduction in both the volume of water consumed and the number of licks. At the highest dose studied (0.5 μg), CRF also causes a reduction in the number of nose pokes into the food tray. Previous studies have shown that ICV infusions of CRF produce both stimulant (52) and "anxiogenic-like" (28) behavioural effects. The present results are therefore compatible with previous results, since drugs with purported "anxiogenic-like" behavioural effects, such as the β-carboline FG 7142 (36), and stimulants, such as amphetamine and caffeine [(24,34,41,46,47,62); but see (61)], have previously been shown to attenuate the performance of SIP.

EXPERIMENT 2: EFFECTS OF α-HELICAL CRF (9-41) ON PERFORMANCE OF SCHEDULE-INDUCED POLYDIPSIA

The previous experiment has shown that ICV administration of CRF dose-dependently attenuates the performance of SIP. Consequently, in experiment 2 the effects of the CRF antagonist α-helical CRF (9-41) on the performance of SIP were studied to examine whether endogenous CRF is involved in the maintenance of this behavior.

Procedure

A separate group of 12 male Wistar rats weighing 220–240 g at the beginning of the experiment were used. They were food-deprived and maintained under the conditions described for experiment 1. Following 10 acquisition sessions on the FT 60-s schedule, the rats which consumed more than 10 ml/30-min session were implanted with ICV cannulae (n = 8) and then returned to the polydipsia schedule for 5 further sessions after a 5-day postoperative recovery period. Over the next 10 days the rats received a sequence of ICV injections of α-helical CRF (9-41) 30 min before testing. The doses used were 0, 1, 5, and 25 μg/5 μl, and the order of doses was based on a 4 × 4 Latin Square. All ICV injections were separated by two baseline sessions.

RESULTS

As in the previous experiment, all of the rats showed strong temporal patterns of responding (data not shown). However, as shown in Table 1, α-helical CRF did not affect the volume of water consumed, the number of licks, the number of nose pokes in the food tray, or the number of crossovers (all F ratios including interactions < 1).

In summary, this experiment has shown that ICV administration of the CRF antagonist does not affect the performance of SIP. These results therefore suggest that endogenous CRF is not involved in the maintenance of this behavior.

DISCUSSION

These experiments have shown that ICV administration of CRF causes a dose-dependent reduction in both the volume of water consumed and the amount of licking on a FT 60-s schedule. The attenuation of SIP by CRF does not appear to be a nonspecific disruption of behavior, since the characteristic temporal profile of adjunctive behaviour was not altered. Central administration of CRF also caused a reduction in the number of nose pokes made into the food tray. This effect was apparent following a dose of 0.1 μg CRF, but was only statistically significant following 0.5 μg CRF. A certain degree of behavioural specificity in the effects of CRF on this schedule was demonstrated, since the number of crossovers was unaffected by any dose of CRF. In marked contrast to these effects of CRF, central administration of the CRF antagonist did not affect the performance of schedule-induced drinking, panel-pressing, or locomotor activity.

Performance of SIP is critically dependent upon level of food deprivation (22), with greater levels of food deprivation resulting in higher levels of SIP. Because CRF has been shown to reduce deprivation-induced food consumption (31,37), it could be hypothesized that CRF attenuates the performance of SIP through an effect on appetitive motivation. Relatively high doses of CRF (minimum 1 μg) reduce deprivation-induced eating, although as this effect is accompanied by the induction of displacement behaviours, it may not be caused by an effect of CRF on hunger. Although the doses of CRF used in the present study have previously been demonstrated not to affect feeding behavior (31,37), some of the data in the present experiment appear to be consistent with an attenuating effect of CRF on appetitive motivation. For example, CRF dose-dependently decreased the number of nose pokes made into the food tray, although this behavioural measure may not reflect the level of hunger, particularly as all of the food pellets were eaten.

Central administration of CRF has been shown to induce both behavioural activation and "anxiogenic-like" behavioural effects [reviewed in (19)]. Previous studies have also shown that other drugs which have stimulant or anxiogenic properties can also attenuate SIP. These include the benzodiazepine receptor partial inverse agonist FG7142 (36); both direct and indirect dopamine agonists, such as amphetamine and apomorphine (46,50,62); and caffeine (34). Similarly, behavioural manipulations that alter emotionality can attenuate SIP, although the effects of such manipulations have been examined primarily on the acquisition of SIP. Specifically, isolation rearing (26) and prior exposure to uncontrollable foot shock (6) have both been shown to attenuate the acquisition of SIP, although it should be noted that mild foot shock presented during a SIP session enhances drinking (49).

There are several potential neurochemical mechanisms that could underlie the effects of CRF on SIP. Central administration of CRF has been shown to activate both central dopaminergic and noradrenergic systems (18), the autonomic nervous system (9,23), and the hypothalamic pituitary adrenal axis (18). All of these systems have been implicated in schedule-induced polydipsia (17,32,35,45,58,64).

Although CRF had marked effects on the performance of SIP, endogenous CRF appears not to be involved in the maintenance of this behaviour, as central administration of the CRF antagonist (1–25 μg) had no effects on SIP. These doses of centrally administered α-helical CRF attenuate many stress-induced behaviours and physiological responses to stress, including stress-induced sensitization (12), stress-induced reductions in exploratory behaviour (4), stress-induced anorexia (29), stress-induced release of plasma epinephrine (10), and gastric acid secretion (30). Therefore, the present results do not support the hypothesis (5) that the maintenance of SIP serves as a coping response to reduce an aversive state of high arousal.

This hypothesis was developed from the observation that rats given the opportunity to drink have lower plasma corticosterone levels than rats exposed to the schedule but with no access to water (5,56). However, as other peripheral indices of arousal such as prolactin, norepinephrine, and epinephrine

TABLE 1

EFFECTS OF α-HELICAL CRF (9-41) ON THE PERFORMANCE OF SCHEDULE-INDUCED POLYDIPSIA

Dose α-helical CRF (μg)	0	1	5	25
Volume consumed (ml)	15.2 ± 2.5	15.4 ± 2.3	16.2 ± 1.9	15.8 ± 1.8
Number of licks	1859 ± 699	1733 ± 634	1940 ± 746	1698 ± 568
Number of nose pokes	422 ± 69	371 ± 47	383 ± 59	432 ± 64
Number of crossovers	126 ± 43	101 ± 16	132 ± 25	120 ± 28

are not altered by SIP (14), this effect appears to be specific to plasma corticosterone. The data from experiments in which corticosterone levels have been pharmacologically manipulated have also not always provided support for this hypothesis. For example, systemic injections of corticosterone do not reliably increase the performance of SIP (35), and as shown in the present experiments, ICV administration of CRF at doses that have previously been shown to increase plasma corticosterone (18,38) results in a decrease in SIP.

These data therefore create an apparent paradox in that scheduled food delivery causes an increase in plasma corticosterone (5), which is thought to induce SIP, whereas infusions of CRF, which increase plasma corticosterone (18,38), decrease SIP. These apparently conflicting data could be explained by hypothesizing that there is an inverted-U-shaped function between plasma corticosterone and the expression of SIP. Thus there is an optimal level of plasma corticosterone for inducing SIP, and either increases or decreases from this level attenuate SIP.

In summary, these experiments have shown that ICV administration of CRF attenuates the performance of SIP, whereas ICV administration of a CRF antagonist, α-helical CRF, has no effect on the performance of SIP. These results add to a growing body of evidence that is hard to incorporate within the hypothesis that SIP is a coping response to stress.

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REFERENCES

1. Aldenhoff, J. B.; Groul, D.; Rivier, J.; Vale, W.; Siggins, G. Corticotropin-releasing factor decreases postburst hyperpolarizations and excites hippocampal neurons in vitro. Science 221:875–877; 1983.
2. Baldwin, H. A.; Rassnick, S.; Rivier, J.; Koob, G. F.; Britton, K. T. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. Psychopharmacology 103:227–232; 1991.
3. Berridge, C. W.; Dunn, A. J. Corticotropin-releasing factor elicits naloxone sensitive stress-like alterations in exploratory behavior in mice. Regul. Pept. 16:83–93; 1986.
4. Berridge, C. W.; Dunn, A. J. A corticotropin-releasing factor antagonist reverses the stress-induced changes of exploratory behavior in mice. Horm. Behav. 21:393–401; 1987.
5. Brett, L. P.; Levine, S. Schedule-induced polydipsia suppresses pituitary-adrenal activity in rat. J. Comp. Physiol. Psychol. 93: 946–956; 1979.
6. Brett, L. P.; Patterson, J.; Levine, S. Adjunctive drinking and the pituitary-adrenal response: Effects of prior aversive stimulation (preshock). Physiol. Behav. 29:219–223; 1982.
7. Britton, D. R.; Koob, G. F.; Rivier, J. Intraventricular corticotropin-releasing factor enhances behavioural effects of novelty. Life Sci. 31:363–367; 1982.
8. Britton, K. T.; Morgan, J.; Rivier, J.; Vale, W.; Koob, G. F. Chlordiazepoxide attenuates response suppression induced by corticotropin releasing factor in the conflict test. Psychopharmacology 86:170–174; 1985.
9. Brown, M. R.; Fisher, L. A. Central nervous system effects of corticotropin-releasing factor in the dog. Brain Res. 280:75–79; 1983.
10. Brown, M. R.; Fisher, L. A.; Webb, V.; Vale, W.; Rivier, J. Corticotropin-releasing factor: A physiological regulator of adrenal epinephrine secretion. Brain Res. 328:355–357; 1985.
11. Chappell, P. B.; Smith, M. A.; Kilts, C. D.; Bissette, G.; Ritchie, J.; Anderson, C.; Nemeroff, C. B. Alterations in corticotropin-releasing factor-like immunoreactivity in discrete rat brain regions after acute and chronic stress. J. Neurosci. 6:2908–2914; 1986.
12. Cole, B. J.; Cador, M.; Stinus, L.; Rivier, J.; Vale, W.; Koob, G. F.; LeMoal, M. Central administration of a CRF antagonist blocks the development of stress-induced behavioural sensitization. Brain Res. 512:343–346; 1990.
13. Cole, B. J.; Koob, G. F. Propranolol antagonizes the enhanced conditioned fear produced by corticotropin releasing factor. J. Pharmacol. Exp. Ther. 247:902–910; 1988.
14. Dantzer, R.; Terlouw, C.; Mormede, P.; LeMoal, M. Schedule-induced polydipsia experience decreases plasma corticosterone levels but increases plasma prolactin levels. Physiol. Behav. 43: 275–279; 1988.
15. DeSouza, E. B.; Insel, T. R.; Perrin, M. H.; Rivier, J.; Vale, W.; Kuhar, M. J. Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: An autoradiographic study. J. Neurosci. 5:3189–3203; 1985.
16. DeSouza, E. B.; Perrin, M. H.; Insel, T. R.; Rivier, J.; Vale, W.; Kuhar, M. J. Corticotropin-releasing factor receptors in rat forebrain: Autoradiographic identification. Science 224:1449–1451; 1984.
17. Devenport, L. D. Schedule-induced polydipsia in rats: Adrenocortical and hippocampal modulation. J. Comp. Physiol. Psychol. 92:651–660; 1978.
18. Dunn, A. J.; Berridge, C. W. Corticotropin-releasing factor administration elicits a stress-like activation of cerebral catecholamine systems. Pharmacol. Biochem. Behav. 27:685–691; 1987.
19. Dunn, A. J.; Berridge, C. W. Physiological and behavioural responses to corticotropin-releasing factor administration: Is CRF a mediator of anxiety or stress responses? Brain Res. Rev. 15:71–100; 1990.
20. Falk, J. L. Production of polydipsia in normal rats by an intermittent food schedule. Science 133:195–196; 1961.
21. Falk, J. L. The nature and determinants of adjunctive behavior. Physiol. Behav. 6:577–588; 1971.
22. Falk, J. L. The origin and functions of adjunctive behaviours. Anim. Learn. Behav. 5:325–335; 1977.
23. Fisher, L. A.; Rivier, J.; Rivier, C.; Spiess, J.; Vale, W.; Brown, M. R. Corticotropin-releasing factor (CRF): Central effects on mean arterial pressure and heart rate in rats. Endocrinology 110: 2222–2224; 1982.
24. Harris, A. D.; Snell, D.; Loh, H. H. Effects of stimulants, anorectics, and related drugs on schedule-controlled behavior. Psychopharmacology 56:49–55; 1978.
25. Imaki, T.; Nahan, J. L.; Rivier, C.; Sawchenko, P. E.; Vale, W. Differential regulation of corticotropin-releasing factor mRNA in rat brain regions by glucocorticoids and stress. J. Neurosci. 11: 585–599; 1991.
26. Jones, G. H.; Robbins, T. W.; Marsden, C. A. Isolation-rearing retards the acquisition of schedule-induced polydipsia in rats. Physiol. Behav. 45:71–77; 1989.
27. Kalin, N. H.; Sherman, J. E.; Takahashi, L. K. Antagonism of endogenous CRH systems attenuates stress-induced freezing behavior in rats. Brain Res. 457:130–135; 1988.
28. Koob, G. F.; Bloom, F. E. Corticotropin-releasing factor and behavior. Fed. Proc. 44:259–263; 1985.
29. Krahn, D. D.; Gosnell, B. A.; Grace, M.; Levine, A. S. CRF antagonist partially reverses CRF- and stress-induced effects on feeding. Brain Res. Bull. 17:285–289; 1986.
30. Lenz, H. J.; Raedler, A.; Greten, H.; Vale, W. W.; Rivier, J. E. Stress-induced gastrointestinal secretory and motor responses in

rats are mediated by endogenous corticotropin-releasing factor. Gastroenterology 95:1510–1517; 1988.
31. Levine, A. S.; Rogers, B.; Kneip, J.; Grace, M.; Morley, J. E. Effect of centrally administered corticotropin releasing factor (CRF) on multiple feeding paradigms. Neuropharmacology 22: 337–339; 1983.
32. Levine, R.; Levine, S. Role of pituitary-adrenal hormones in the acquisition of schedule-induced polydipsia. Behav. Neurosci. 103: 621–637; 1989.
33. Liang, K. C.; Melia, K. R.; Miserendino, M. J. D.; Falls, W. A.; Campeau, S.; Davis, M. Corticotropin-releasing factor: Long-lasting facilitation of the acoustic startle reflex. J. Neurosci. 12: 2303–2312; 1992.
34. McMillan, D. E. Effects of d-amphetamine and caffeine on schedule-controlled and schedule-induced responding. J. Exp. Anal. Behav. 32:445–456; 1979.
35. Mittleman, G.; Blaha, C. D.; Phillips, A. G. Pituitary-adrenal and dopaminergic modulation of schedule-induced polydipsia: Behavioural and neurochemical evidence. Behav. Neurosci. 106: 408–420; 1992.
36. Mittleman, G.; Jones, G. H.; Robbins, T. W. Effects of diazepam, FG 7142, and RO 15-1788 on schedule-induced polydipsia and the temporal control of behavior. Psychopharmacology 94: 103–109; 1988.
37. Morley, J. E.; Levine, A. S. Corticotropin releasing factor, grooming and ingestive behavior. Life Sci. 31:1459–1464; 1982.
38. Ono, N.; De Castro, J. C. B.; McCann, S. M. Ultrashort loop positive feedback of corticotropin (ACTH)-releasing factor to enhance ACTH in stress. Proc. Natl. Acad. Sci. U. S. A. 82:3528–3531; 1985.
39. Ono, N.; Samson, W. K.; McDonald, J. K.; Lumpkin, M.; Bedran de Castro, J. C.; McCann, S. M. Effects of intravenous and intraventricular injection of antisera directed against corticotropin-releasing factor on the secretion of anterior pituitary hormones. Proc. Natl. Acad. Sci. U. S. A. 82:7787–7790; 1985.
40. Pelligrino, L. J.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Century-Crofts; 1967.
41. Pellon, R.; Blackman, D. E. Effects of drugs on the temporal distribution of schedule-induced polydipsia in rats. Pharmacol. Biochem. Behav. 43:689–695; 1992.
42. Rivier, C.; Rivier, J.; Vale, W. Inhibition of adrenocorticotropic hormone secretion in the rat by immunoneutralization of corticotropin releasing factor. Science 218:377–378; 1982.
43. Rivier, J.; Rivier, C.; Vale, W. Synthetic competitive antagonists of corticotropin-releasing factor: Effect on ACTH secretion in the rat. Science 224:889–891; 1984.
44. Rivier, J.; Spiess, J.; Rivier, C.; Galyean, R.; Vale, W.; Lederis, K. Solid phase synthesis of amunine (CRF), sauvegine, and two urotensin I. In: Blaha, K.; Malon, P., eds. Peptides 1982. Berlin: de Gruyter; 1983:597–602.
45. Robbins, T. W.; Koob, G. F. Selective disruption of displacement behavior by lesions of the mesolimbic dopamine system. Nature 285:409–412; 1980.
46. Robbins, T. W.; Roberts, D. C. S.; Koob, G. F. Effects of d-amphetamine and apomorphine upon operant behavior and schedule-induced licking in rats with 6-hydroxydopamine-induced lesions of the nucleus accumbens. J. Pharmacol. Exp. Ther. 224: 662–673; 1983.
47. Sanger, D. J. d-Amphetamine and adjunctive drinking in rats. Psychopharmacology 54:273–276; 1977.
48. Segal, E. F. The development of water drinking on a dry food free-reinforcement schedule. Psychonom. Sci. 3:101–102; 1965.
49. Segal, E. F.; Oden, D. L. Effects of drinkometer current and of foot shock on psychogenic polydipsia. Psychonom. Sci. 14:13–15; 1969.
50. Snodgrass, S. H.; Allen, J. D. Effect of dopamine agents on schedule- and deprivation-induced drinking in rats. Pharmacol. Biochem. Behav. 27:463–475; 1987.
51. Stein, L. Excessive drinking in the rat: Superstition or thirst? J. Comp. Physiol. Psychol. 58:237–242; 1964.
52. Sutton, R. E.; Koob, G. F.; Le Moal, M.; Rivier, J.; Vale, W. Corticotropin-releasing factor produces behavioural activation in rats. Nature 297:331–333; 1982.
53. Swanson, L. W.; Sawchenko. P. E.; Rivier, J.; Vale, W. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibres in the rat brain: An immunohistochemical study. Neuroendocrinology 36:165–186; 1983.
54. Takahashi, L. K.; Kalin, N. H.; Baker, E. W. Corticotropin-releasing factor antagonist attenuates defensive-withdrawal behavior elicited by odors of stressed conspecifics. Behav. Neurosci. 104:386–389; 1990.
55. Takahashi, L. K.; Kalin, N. H.; VandenBurgt, J. A.; Sherman, J. E. Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. Behav. Neurosci. 103:648–654; 1989.
56. Tazi, A.; Dantzer, R.; Mormede, P.; LeMoal, M. Pituitary-adrenal correlates of schedule-induced polydipsia and wheel running in rats. Behav. Brain Res. 19:249–256; 1986.
57. Tinbergen, N. "Derived" activities: Their causation, biological significance, origin, and emancipation during evolution. Q. Rev. Biol. 27:1–32; 1952.
58. Tung, C.-S.; Yin, T.-H. Clonidine suppression and its adrenoceptor mediation in schedule-induced polydipsia. Physiol. Behav. 40: 317–322; 1987.
59. Vale, W.; Spiess, J.; Rivier, C.; Rivier, C. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213:1394–1397; 1981.
60. Valentino, R. J.; Foote, S. L.; Aston-Jones, G. Corticotropin-releasing factor activates noradrenergic neurons of the locus coeruleus. Brain Res. 270:363–367; 1983.
61. Wayner, M. J.; Greenberg, I.; Towbridge, J. Effects of d-amphetamine on schedule induced polydipsia. Pharmacol. Biochem. Behav. 1:109–111; 1973.
62. Williams, J. L.; White, J. M. The effects of amphetamine and scopolamine on adjunctive drinking and wheel-running in rats. Psychopharmacology 82:360–367; 1984.
63. Winer, B. J. Statistical principles in experimental design, 2nd ed. New York: McGraw Hill; 1971.
64. Wright, J. W.; Kelso, S. C. Adrenal demedullation suppresses schedule-induced polydipsia in rats. Physiol. Behav. 26:1–5; 1981.