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Diurnal pituitary-adrenal activity during schedule-induced polydipsia of water and ethanol in cynomolgus monkeys (*Macaca fascicularis*)

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Abstract

Rationale—Intermittent delivery of an important commodity (e.g., food pellets) generates excessive behaviors as an adjunct to the schedule of reinforcement (adjunctive behaviors) that are hypothesized to be due to conflict between engaging and escaping a situation where reinforcement is delivered, but at sub-optimal rates.

Objectives—This study characterized the endocrine correlates during schedule-induced polydipsia (SIP) of water and ethanol using a longitudinal approach in non-human primates.

Methods—Plasma adrenocorticotrophic hormone (ACTH) and cortisol were measured in samples from awake cynomolgus monkeys (*Macaca fascicularis*, 11 adult males) obtained at the onset, midday and offset of their 12-h light cycle. The monkeys were induced to drink water and ethanol (4% w/v, in water) using a fixed time (FT) 300-s interval schedule of pellet delivery. The induction fluid changed every 30 sessions in the following order: water, 0.5 g/kg ethanol, 1.0 g/kg ethanol, and 1.5 g/kg ethanol. Following induction, ethanol and water were concurrently available for 22 h/d.

Results—The FT300-s schedule gradually increased ACTH, but not cortisol, during water induction to a plateau sustained throughout ethanol induction in every monkey. Upon termination of the schedule, ACTH decreased to baseline and cortisol below baseline. Diurnal ACTH and cortisol were unrelated to the dose of ethanol, but ACTH rhythm flattened at 0.5 g/kg/d and remained flattened.

Conclusions—The coincidence of elevated ACTH with the initial experience of drinking to intoxication may have altered the mechanisms involved in the transition to heavy drinking.

Keywords

monkeys; self-administration; ethanol; schedule-induced polydipsia; cortisol; adrenocorticotrophic hormone

Introduction

Intermittent schedules of reinforcement may produce excessive behaviors not instrumental to reinforcement, but are an adjunct to the schedule. These are known as adjunctive behaviors. An example of adjunctive behavior is excessive drinking (polydipsia) when small rations of food are delivered intermittently (Falk 1961). The amount of adjunctive behavior generated is an inverted U-shaped function of the inter-reinforcer interval (Flory 1971;

Shelton et al. 2001). Another characteristic of adjunctive behavior is excessiveness. For example, during only three hours of a variable interval (VI) 60-s schedule of food delivery, rats drank 2-8 times the volume of water consumed in the previous 24 hours (Falk 1961). The behavior that is generated depends on the reinforcer properties and the environmental opportunities, e.g., polydipsia, aggression or wheel running (Roper 1978; Grant and Johanson 1990; Nader and Woolverton 1992). Further, the quantity of the behavior generated is modified by food deprivation (Lamas and Pellón 1995), reinforcer magnitude (Pitts and Malagodi 1996; Samson and Falk 1974) and by punishing the adjunctive behavior (Flores and Pellón 2000). Thus, adjunctive behavior is regulated by both the properties of the schedule of reinforcement, the nature of the scheduled reinforcer, the environmental opportunities and the consequences of the adjunctive behavior. Understanding the generation of adjunctive behavior can inform the etiology of excessive behaviors such as addiction (Falk 1998) and obsessive-compulsive disorders (Platt et al. 2008).

Intermittent schedules may produce adjunctive behavior because of conflict between engaging in and escaping a situation associated with a suboptimal rate of reinforcement (Falk 1977; Grant and Johanson 1988). Interval schedules can be aversive in proportion to the duration of the interval, which appears to be characteristic of intervals that induce adjunctive behavior. Given the opportunity, animals commonly escape intermittent schedules that produce adjunctive behavior (Brown and Flory 1972; Lydersen et al. 1980).

Consistent with aversive aspects of interval schedules is heightened pituitary-adrenal activity as measured by circulating corticosterone during SIP in rats (Brett and Levine 1979; Wallace et al. 1983; Mittleman et al. 1988; López-Grancha et al. 2006). Brett and Levine (1979) suggested that regulation of the endocrine consequences of induction schedules is causally related to adjunctive behaviors when escape is not an option or is costly. For example, adjunctive behavior can be suppressed by hypophysectomy (Lin et al. 1990) and adrenalectomy (Lin et al. 1988; Levine and Levine 1989; Mittleman et al. 1992) and restored in adrenalectomized rats by corticosterone administration, but not dexamethasone (Cirulli et al. 1994; Levine and Levine 1989). Further, natural variation in HPA axis activity (e.g., strain differences) correlates with SIP. Specifically, rat strains with greater basal diurnal adrenocortical activity as measured by corticosterone (Stöhr et al. 2000) had greater schedule-induced polydipsia (DeCarolis et al. 2003). Consistent with the hypothesis that regulation of corticosterone is a main function of adjunctive behavior (Brett and Levine 1979), adjunctive behavior in rats is associated with suppressed pituitary-adrenal activity (i.e., lowers circulating corticosterone), which is elevated under intermittent schedules when adjunctive behavior is blocked (Brett and Levine 1979; Tazi et al. 1986; Dantzer et al. 1988).

On the other hand, Cirulli et al. (1994) noted that some rats acquired SIP despite lacking corticosterone, suggesting a modulatory rather than a causal role for glucocorticoids, similar to the modulation of attention, learning and memory by pituitary and adrenal hormones (van Wimersma Greidanus et al. 2000). Overall, the available data suggest that interval schedules that induce adjunctive behaviors have both an appetitive component (delivery of an important commodity) and an aversive component (no control over the rate of reinforcement; Grant and Johanson 1988). Studies suggesting that adjunctive behavior co-occurs with an increase in HPA axis activity all used food-restricted rats, superimposing metabolic stress on stress due to uncontrollable food delivery, with a limited number of blood samples that did not characterize diurnal effects. Indeed, most rat studies measured corticosterone from plasma obtained once, immediately after several days of intermittent food delivery during sessions of fixed duration. No studies to date have determined the time course of changes in pituitary-adrenal activity relative to the onset of intermittent food delivery, or challenged the assumption that these measures temporally coincide.

The current study characterized the pituitary-adrenocortical activity of cynomolgus monkeys under an interval schedule (fixed time 300-s) that optimally induces SIP of water and 4% (w/v) ethanol in monkeys that are not food deprived (Shelton et al. 2001). Characteristics of the drinking topography during the SIP of 1.5 g/kg ethanol strongly predicted future voluntary ethanol intake after the induction schedule was terminated and water was concurrently available with ethanol (Grant et al. 2008). However, it is not known whether pituitary-adrenal activity during adjunctive drinking is also a risk factor for future heavy ethanol drinking in monkeys. To address this gap, the current study characterized the diurnal regulation of the pituitary-adrenal hormones ACTH and cortisol during SIP in monkeys.

Materials and methods

Animals

A detailed description of the monkeys in this study has been published (Grant et al. 2008). Briefly, adult male cynomolgus monkeys ($n = 11$, *Macaca fascicularis*, 50-62 months of age, 3.84-5.74 kg; World Wide Primates, Miami, FL) lived in the laboratory in with visual, auditory, and olfactory contact with other monkeys. The monkeys were housed individually in quadrant cages (1.6 m $\times$ 0.8 m $\times$ 0.8 m) under constant temperature (20-22° C), humidity (65%) and a 12-hour light cycle (lights on, 7:00 am). Directly prior to the present study, these monkeys were studied for the effects of social housing and social status on hypothalamic-pituitary-adrenal axis activity (details in Helms et al. 2012a). The current data set follows about 5 months of stable, individual housing of all monkeys in the same housing room. The monkeys were trained to provide their leg through an opening in the cage front for blood collection without anesthesia via saphenous or femoral venipuncture (for details about the training, see Grant et al. 2008). The monkeys were weighed weekly and body weight increased steadily throughout the experiment (percentage of baseline weight: $105 \pm 6\%$). All primate handling procedures were performed in accordance with the NIH and were approved by Wake Forest University ACUC and with the Commission on Life Sciences, National Research Council (1996) *Guide for the Care and Use of Laboratory Animals* (National Academy Press, Washington).

Hormone and blood-ethanol concentration assays

Femoral blood samples (3 ml, 22-g $\times$ 2.54-cm Vacutainer needle and hematology tube; Becton Dickinson) were obtained from non-sedated monkeys on Friday morning (7:00 am), Wednesday evening (5:30 pm, onset of dark cycle) and Monday at noon (12:00 pm) for tri-weekly assays of cortisol and ACTH. All blood samples were stored on ice for approximately 5 minutes until centrifuged for 15 minutes at 4°C (Beckman Coulter, Model Allegra 21R). Plasma samples were stored at -20°C and -80°C, respectively, until assayed for cortisol (bound and unbound) and ACTH at the Yerkes Endocrine Core Laboratory (Atlanta, GA) using commercially available kits. ACTH was assayed in duplicate using a DiaSorin kit #24130 (Stillwater, MN), with sensitivity 6.8-436.0 pg/ml. Cortisol was assayed by radioimmunoassay using DSL Kit #2000-06142 (Webster, TX), with an inter-assay coefficient of variation (CV) 9.8-15.4% and intra-assay CV 2.9-8.6% and sensitivity of 1.25-150 μ g/dl. These assays were used to generate data for previous publications (e.g., Shively et al. 1998). Circulating corticotropin-releasing hormone (CRH) was not measured in this study because it is low or absent due to an abundance of CRH binding protein in the plasma of macaques and apes (Woods et al. 1994; Bowman et al. 2001). Blood-ethanol concentration was assayed every five days 7 hours after session onset as described by Grant et al. (2008).

Apparatus

The operant conditioning panel that allowed access to all fluid and food has been described in detail (Vivian et al. 2001; Grant et al. 2008). Briefly, the panels contained two drinking spouts each set below three horizontally parallel stimulus lights, one retractable lever, and a centrally-positioned recessed dowel, all controlled by a computerized system (Macintosh G4, Apple Computer, Inc. Cupertino, CA) using custom hardware and programming (Biotic Micro, Clemmons, NC). Each spout was connected via tubing to a 1-liter reservoir set on a digital scale (Ohaus Navigator Balances N1B110, Ohaus Corporation, Pine Brook, NJ) to obtain drinking data.

Experimental Phases

The phases of the experiment are listed in Table 1. During baseline, the monkeys were trained to operate the panel in daily 60-min sessions. Training was complete once the monkey regularly drank from the spout, and earned their daily allowance of 1-g banana-flavored (Research Diets Incorporated, New Brunswick, NJ) by responding on the push panel. Next, the monkeys were induced to drink water and ethanol (4% w/v in water) as described in Grant et al. (2008). All sessions began at 11:00 am. Briefly, monkeys received a banana pellet every 300 seconds (fixed time 300-s) until the pre-set volume of fluid was consumed or the daily ration of pellets was delivered. After drinking the pre-set fluid volume under the FT300-s schedule, only water was available to drink and remaining food pellets were available as a meal after two hours. During induction, the pre-set volume of water was equal to the volume of 1.5 g/kg ethanol (150-227 ml). Following water induction, the monkeys were induced to drink 0.5 g/kg/day (52-74 ml), 1.0 g/kg/day (110-147 ml), then 1.5 g/kg/day (170-223 ml).

Following all four induction phases (water, 0.5, 1.0, and 1.5 g/kg ethanol), the fixed-time 300 s schedule was terminated. Ethanol (4%, w/v) and water were concurrently available 22 h/d, 7 d/week (see Vivian et al. 2001; Grant et al. 2008), allowing 2 h/d for technicians to download data, refill the fluid reservoirs (ethanol was made fresh daily), pellet dispensers, provide fresh fruit, and to clean cages and panels or replace tubing if needed. Food pellets were available according to a fixed-ratio 1 schedule in at least three daily meals at 2-h intervals starting with onset of the drinking session.

Data Analyses

The data were analyzed using linear mixed models (Krueger and Tian 2004) in which monkey was the subject variable. The dependent variables analyzed included ACTH and cortisol concentrations (both log-transformed to meet distributional assumptions), time to consume the pre-set fluid volume, index of curvature (IOC, more negative values reflecting drinking early in inter-pellet interval, i.e., after pellet delivery; Fry et al. 1960), and percentage of intervals with drinking. The independent variables were experimental phase (six levels, see Table 1) time of day, and where appropriate, week. Results are reported from models with the best-fit covariance structure according to Schwarz's Bayesian Information Criteria. Bonferroni-corrected planned comparisons were used to evaluate main effects. For all analyses, $\alpha < 0.05$. Analyses were conducted using SAS 9.2 (Cary, NC).

Results

The FT300-s induction schedule had a robust effect on circulating ACTH independent of volume induced to drink, whether the fluid was water or 4% ethanol, or the amount of time taken to consume this volume. As shown in Figure 1, ACTH increased steadily throughout the 30 sessions of water induction, with an overall mean ($\pm$ SD) increase of $286 \pm 136\%$ from the last sample during baseline to the last sample obtained during water induction.

From baseline to water induction, ACTH increased [$F(5, 50) = 71.5, p < 0.0001$] at all times of day, $F(10, 100) = 3.8, p = 0.0003$ [am, $t(170) = -5.7, p = 0.001$; noon, $t(170) = -3.9, p = 0.001$; pm, $t(170) = -5.4, p = 0.001$]. Within and across monkeys, ACTH levels during water induction were greater than baseline from the same times of day in 83/110 (75%) matched samples, indicating incremental increases in ACTH across the days of water induction (Figure 2). In contrast to the effect of the induction schedule on ACTH, circulating cortisol was very stable during water induction, at all times of day, with the last sample during water induction being $102 \pm 39\%$ of the last sample during baseline (Figure 3). Induction of 0.5 g/kg ethanol was associated with a further increase in ACTH by 12.6 ± 5.1 pg/ml above water induction, but after 0.5 g/kg induction, average concentrations of ACTH and cortisol were stable throughout the remaining phases, irrespective of the dose of ethanol consumed (Figure 1). Stable ACTH during ethanol induction occurred despite an increase in average BEC with ethanol dose (0.5 g/kg, 21 ± 13 mg/dl; 1.0 g/kg, 57 ± 31 mg/dl; 1.5 g/kg, 83 ± 41 mg/dl).

The strong diurnal rhythm of cortisol, $F(2, 20) = 668.4, p < 0.0001$, was maintained during all phases of induction and upon termination of the induction schedule, in which morning cortisol was significantly greater than evening cortisol [$t(170) = 34.4, p = 0.004$]. The diurnal rhythm of ACTH was significant, $F(2, 20) = 31.9, p < 0.0001$, although it had a lower difference between peak and nadir across diurnal samples compared to cortisol. Specifically, at baseline compared to water induction, ACTH concentrations were slightly but significantly greater at noon (baseline, 38.6 ± 12.8 pg/ml; water induction, 55.6 ± 22.8 pg/ml) compared to morning (baseline, 29.6 ± 11.9 pg/ml; water induction, 47.2 ± 20.4 pg/ml) [$t(170) = -4.0, p = 0.002$] or evening (baseline, 25.5 ± 11.0 pg/ml; water induction, 37.9 ± 16.6 pg/ml) [$t(170) = -7.0, p = 0.002$]. Also in contrast to cortisol diurnal rhythm, beginning with 0.5 g/kg ethanol, there was a flattening of the diurnal rhythm as there were no significant differences between concentrations of ACTH across the times of day (Figures 1 and 2).

Concentrations of ACTH sharply and immediately decreased to baseline levels upon termination of the induction schedule at all times of day [$F(10, 100) = 3.8, p = 0.003$; am, $t(170) = 4.4, p = 0.002$; noon, $t(170) = 5.3, p = 0.002$; pm, $t(170) = 3.4, p = 0.01$]. In 10 of the 11 monkeys, ACTH was lower on the first day of 22 h/d access (35.6 ± 12.8 pg/ml) compared to the last day of 1.5 g/kg induction (62.7 ± 12.1 pg/ml). Although plasma cortisol was not significantly increased during induction, on average, cortisol also decreased when 1.5 g/kg induction was terminated [$F(5, 50) = 94.4, p < 0.0001$; am, $t(170) = 4.4, p = 0.001$; noon, $t(170) = 5.3, p = 0.001$; pm, $t(170) = 9.34, p = 0.001$], but the decrease was observed in only 3 of the 11 monkeys. A weak correlation was observed between ACTH and cortisol only in samples obtained in the evening ($r = 0.20, p = 0.006$).

Beginning with the first induction session, all monkeys consumed the pre-set volume of fluid in greater than 99% of induction sessions. However, requiring the monkeys to drink progressively larger volumes of ethanol increased the duration of the induction schedule (i.e., the time needed to drink the ethanol dose and terminate the schedule) for all monkeys (Table 2), and thereby increased the time spent under the FT 300-s schedule, $F(3, 30) = 185.8, p < 0.0001$, with each of the four phases of induction differing significantly from every other phase (0.5 g/kg, 50 ± 76 min; 1.0 g/kg, 67 ± 63 min; 1.5 g/kg, 127 ± 91 min). As expected most monkeys drank after, rather than before, each pellet was delivered as indicated by negative IOC. Neither the time to drink the required fluid volume, nor the timing of water drinking relative to pellet delivery (IOC) differed between the beginning and the end of water induction (Table 2). The percentage of intervals in which drinking occurred decreased between week 1 and week 4 of water induction, $F(1, 10) = 5.9, p = 0.04$, but not consistently across monkeys, all of whom showed incremental increases in ACTH

throughout water induction. Therefore, changes in the main variables of drinking patterns during water induction therefore did not account for the increases in ACTH over sessions.

Discussion

The current study provides the most extensive characterization of the endocrine consequences of an intermittent schedule of reinforcement that induces adjunctive behavior in any species to date, and is the first characterization using non-human primates. Daily sessions of schedule-induced polydipsia induced by a FT 300-s interval schedule of flavored-pellet delivery had a prolonged effect on the pituitary activity of cynomolgus macaques as measured by circulating ACTH. All monkeys, at all times of day, showed increased ACTH on days when the FT 300-s schedule was presented even though the sessions were in the morning and usually < 4 hours. Because the half-life of ACTH is very short (< 20 min; Gallagher et al. 1973), pituitary stimulation must have remained increased each day of induction after termination of the FT 300-s schedule. It is unlikely that increased ACTH during induction was due to food consumption because the daily food ration was constant and body weights increased steadily. . The mechanism by which ACTH increased is unknown, but possibilities include decreased sensitivity of ACTH to negative feedback by glucocorticoids, or increased hypothalamic corticotropin releasing hormone and vasopressin pulses (Levine 2000).

It may seem surprising that cortisol levels were not also elevated during induction. Proximity to the FT 300-s schedule (i.e., the stressor) was not a factor because the absence of correlation between ACTH and cortisol occurred even among 12:00 pm samples that were obtained one hour after the onset of the FT 300-s schedule (11:00 am). Pituitary ACTH stimulates the adrenal cortisol (corticosterone in rodents) sometimes due to multiple sequential pulses (Gallagher et al. 1973) and cortisol exerts negative feedback on the HPA axis to decrease ACTH (Chrousos 2009). However, cortisol diurnal rhythms are largely independent of pituitary ACTH (Meier 1976), driven by the thoracic splanchnic nerve (Ulrich-Lai et al. 2006), visceral afferents (Kuramoto et al. 1987), sympathetic innervation (Holbert et al. 1995), and intrinsic adrenal innervation (Holbert et al. 1998). Given the strong diurnal rhythm of cortisol it appears that these latter mechanisms of operated independently of increased ACTH in this study. Suppressed plasma cortisol during chronic ethanol self-administration in the current study is consistent with our previous reports in cynomolgus monkeys (Cuzon Carlson et al. 2011; Helms et al. 2012a), and in human alcoholics (Wand and Dobs 1991).

The flattening of diurnal ACTH beginning with consumption of 0.5 g/kg/d was previously reported for this same cohort of monkeys, during open access of ethanol 22 h/d on a set light-dark schedule (Helms et al. 2012a). The current study suggests that flattening of ACTH diurnal rhythms was due to ethanol and not the induction schedule because it was not seen during water induction. The disruption of diurnal ACTH by a low dose of ethanol (two-drink equivalent) suggests sensitive pituitary modulation by ethanol. Although circulating ACTH is primarily influenced by CRH released from parvocellular neurons of the PVN into portal vessels draining into the adenohypophysis (anterior pituitary), magnocellular PVN neurons projecting to the neurohypophysis (posterior pituitary) also influence ACTH release (de Wied 1961). Voltage-gated currents that are sensitive to ethanol (Knott et al. 2002) mediate suprachiasmatic nucleus (SCN) neuron activity important for circadian rhythm of pituitary hormones (Colwell et al. 2011) and project to the PVN (Watts et al. 1987; Hermes et al. 1996). Interestingly, a low concentration (20 mM) of ethanol acting at GABA_A receptors containing δ subunits has been shown to block glutamate regulation of SCN circadian rhythms (McElroy et al. 2009). The endogenous neurosteroid pregnanolone positively modulates GABA_A receptor responses in acutely dissociated SCN neurons (Shimura et al.

1996), suggesting that ethanol interaction with neurosteroids (Helms et al. 2012b) could account for potent regulation of diurnal pituitary activity.

Heightened ACTH in the current study is likely to have increased circulating neuroactive steroids, which are derived from cholesterol and influence sensitivity to the endocrine and behavioral effects of ethanol (Helms et al. 2012b). In the adrenal cortex, ACTH stimulates cholesterol side-chain cleavage and increases serum adrenal steroids (Pederson et al. 1980). Ethanol increases plasma neuroactive steroids in rodents via ACTH release and facilitation of cholesterol transfer (Boyd et al. 2010). Additional studies can determine whether the coincidence of increased neuroactive steroids due to heightened ACTH and drinking to intoxication during 1.5 g/kg induction to promote the transition to heavy drinking (Grant et al. 2008).

Previous reports of endocrine activity during induction schedules are not entirely consistent, possibly because of variable studies in rodents used 80-85% food deprivation. Most studies found that intermittent food delivery was associated with increased corticosterone (Brett and Levine 1981; Levine and Levine 1989; Mittleman et al. 1988). In contrast, a negative correlation between plasma corticosterone and water intake was interpreted to indicate that adjunctive drinking is anxiolytic and decreases adrenocortical activity (Mittleman et al. 1988). There is also at least one report of a positive correlation between corticosterone and adjunctive water intake (Cirulli et al. 1994). Extensive food deprivation may superimpose a glucocorticoid response to food ingestion under the interval schedules. None of the studies of rats and SIP that show heightened corticosterone also measured ACTH.

In the current study, the monkeys maintained or gained weight from baseline across the experiment. Any initial glucocorticoid (cortisol) response to the FT 300-s schedule quickly adapted, but ACTH increased and remained heightened throughout induction (90 sessions). The concentration of ACTH at plateau was similar to concentrations after exposure to a conditioned punisher (catch gloves, Herod et al. 2011). Increments in ACTH and maximum concentration were consistent across individuals despite wide individual differences in exposure to the induction schedule due to drinking rates. The lack of correlation between ACTH and SIP agrees with the finding in rats that engaging in adjunctive drinking, per se, does not decrease HPA axis activation (Cirulli et al. 1994).

Heightened ACTH in the current study may reflect conflict produced by the FT 300-s schedule (Grant and Johanson 1987) and vigilance about pellet delivery. When the induction schedule was terminated and food was available under a fixed-ratio 1 schedule, ACTH fell markedly. In terms of behavioral regulation, ACTH (Wolthuis and de Wied 1976) along with other peptides and hormones (e.g., vasopressin, androgens; van Wimersma Greidanus et al. 2000) modulates vigilance (e.g., Born et al. 1984), learning and memory (van Wimersma Greidanus et al. 2000). Previously, we reported that daily consumption of 0.50 g/kg ethanol increased vigilance toward other social groups even several hours after the daily dose had been eliminated (Shively et al. 2002). Increased vigilance may also account for the prolonged elevation of ACTH during social housing of cynomolgus monkeys in the absence of an induction schedule (Helms et al. 2012a).

Consistently elevated ACTH may have modulated the effects of drinking to intoxication during induction of 1.5 g/kg ethanol, providing a mechanism by which monkeys that drank to intoxication during this phase became heavy drinkers (Grant et al. 2008). Specifically, heightened ACTH may have disrupted adaptation to repeated, intoxicating doses of ethanol, which were consumed only by a subset of monkeys (Grant et al. 2008). For example, treatment with N-methyl-D-aspartate (NMDA), a specific agonist of the NMDA-type glutamate receptor, and an NMDA receptor antagonist, respectively, increased locomotor

activity and impaired performance in the Morris water maze, but these effects were attenuated by treatment with the peptide fragment ACTH₄₋₉ (Spruijt et al. 1994). Heightened ACTH coincident with ethanol intoxication in the current study may have altered glutamatergic processes that are key mechanisms of ethanol intoxication (Krystal et al. 2003), and that we previously showed were upregulated in the putamen of macaques after chronic ethanol self-administration and repeated withdrawal (Cuzon Carlson et al. 2011). We also showed in monkeys that the expression of NMDA receptor subunit mRNA is significantly and positively correlated with lifetime ethanol intake (orbitofrontal cortex, Acosta et al. 2010). To determine whether ACTH may causally effect the transition to heavy drinking, or is simply a marker of brain and hypothalamic activity induced by intermittent food, future studies could evaluate the effects of ACTH on NMDA receptor mechanisms mediating the discriminative stimulus effects of ethanol in monkeys (Vivian et al. 2002). Monkeys treated with ACTH may be less sensitive to ethanol's glutamatergic mechanisms, increasing the risk of heavy drinking. Additional studies are also needed to determine whether ACTH or its upstream regulators (e.g., CRH) can explain individual differences in subsequent heavy drinking after SIP (Grant et al. 2008).

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Figure 1. Diurnal variation in ACTH and cortisol across the experimental phases. Data are mean ($\pm$ SD) ACTH and cortisol at 7:00 (AM), noon (12) and 5:30 (PM) at baseline and under fixed-time-300 s presentation of banana pellets to induce water or ethanol drinking and when the induction schedule was terminated such that the monkeys had 22 h/d concurrent access to water and ethanol (#, $p < 0.001$).

Figure 2.

ACTH concentrations [7:00 am, 12:00 pm, 5:30 pm (ticks)] for individual monkeys during consecutive sessions of the six experimental phases split by gaps (baseline, water induction, 0.5 g/kg ethanol induction, 1.0 g/kg ethanol induction, 1.5 g/kg ethanol induction, 22 h/d concurrent access to water and ethanol). Monkeys 88, 87, 89 and 93 were subsequently shown to be heavy drinkers (mean, > 3.0 g/kg/d) when the induction schedule was terminated, whereas the other monkeys were non-heavy drinkers (Grant et al. 2008).

Figure 3.

Cortisol concentrations [7:00 am, 12:00 pm, 5:30 pm (ticks)] for individual monkeys during consecutive sessions of the six experimental phases split by gaps (baseline, water induction, 0.5 g/kg ethanol induction, 1.0 g/kg ethanol induction, 1.5 g/kg ethanol induction, 22 h/d concurrent access to water and ethanol). Monkeys 88, 87, 89 and 93 were subsequently shown to be heavy drinkers (mean, > 3.0 g/kg/d) when the induction schedule was terminated, whereas the other monkeys were non-heavy drinkers (Grant et al. 2008).

Table 1

Timeline and duration of experimental phases and number of ACTH and Cortisol assays per monkey included in the current study. Tri-weekly blood draws for the assays occurred at 7:00 am, 12:00 pm and 5:30 pm, approximately equally within each experimental phase.

Phase	Duration	Number of hormone assays/hormone
Baseline	3 weeks	9
Water induction	1 month	11-13 ^a
0.5 g/kg ethanol induction	1 month	11-12 ^a
1.0 g/kg ethanol induction	1 month	12 ^a
1.5 g/kg ethanol induction	1 month	13 ^a
22 h/d concurrent access to water and ethanol	2 weeks	6 ^b

^afixed-time 300-s schedule of pellet delivery

^bfixed-ratio 1 schedule of pellet delivery

Table 2

Mean ($\pm$ SD) time (minutes) to consume the pre-set fluid volume, index of curvature (more negative values indicate drinking earlier in the inter-pellet interval), and the percentage of intervals during which drinking occurred. Averages from the first and last weeks of water induction indicate that the incremental increases in ACTH were not related to drinking pattern.

Monkey	Time to drink pre-set fluid volume (min)		Index of curvature (IOC)		Intervals with drinking (%)	
	Week 1	Week 4	Week 1	Week 4	Week 1	Week 4
88	19 $\pm$ 6	21 $\pm$ 4	-0.52 $\pm$ 0.15	-0.62 $\pm$ 0.08	92 $\pm$ 14	85 $\pm$ 14
87	29 $\pm$ 16	30 $\pm$ 8	-0.37 $\pm$ 0.26	-0.52 $\pm$ 0.20	92 $\pm$ 14	84 $\pm$ 13
93	16 $\pm$ 10	11 $\pm$ 1	-0.41 $\pm$ 0.20	-0.58 $\pm$ 0.05	90 $\pm$ 17	100 $\pm$ 0
89	17 $\pm$ 6	17 $\pm$ 3	-0.41 $\pm$ 0.18	-0.45 $\pm$ 0.17	100 $\pm$ 0	83 $\pm$ 16
94	12 $\pm$ 6	12 $\pm$ 5	-0.15 $\pm$ 0.40	-0.24 $\pm$ 0.35	88 $\pm$ 21	55 $\pm$ 42
92	14 $\pm$ 13	11 $\pm$ 4	-0.43 $\pm$ 0.13	-0.36 $\pm$ 0.27	90 $\pm$ 16	40 $\pm$ 35
95	58 $\pm$ 50	60 $\pm$ 80	-0.55 $\pm$ 0.15	-0.67 $\pm$ 0.09	65 $\pm$ 30	83 $\pm$ 28
86	255 $\pm$ 45	274 $\pm$ 57	-0.44 $\pm$ 0.16	-0.30 $\pm$ 0.21	50 $\pm$ 5	26 $\pm$ 4
91	61 $\pm$ 41	111 $\pm$ 50	-0.49 $\pm$ 0.15	-0.60 $\pm$ 0.13	95 $\pm$ 9	35 $\pm$ 13
96	13 $\pm$ 7	13 $\pm$ 3	-0.25 $\pm$ 0.37	-0.51 $\pm$ 0.30	100 $\pm$ 0	100 $\pm$ 0
90	137 $\pm$ 51	173 $\pm$ 32	0.04 $\pm$ 0.28	0.41 $\pm$ 0.09	85 $\pm$ 9	66 $\pm$ 8