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Review

Adolescence, glucocorticoids and alcohol

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Abstract

This review examines the evidence that glucocorticoids are involved, during both adolescence and adulthood, in the cognitive deficits caused by long-term alcohol consumption and in the mechanism(s) of alcohol dependence. During adolescence, the hypothalamopituitary–adrenal (HPA) axis undergoes well-characterized changes in basal activity and many of these are influenced by alcohol consumption. While the former have been fairly well studied, there is little information about whether alcohol effects on the HPA in adolescents differ from those in adults. The means by which glucocorticoids may influence alcohol-related neurotoxicity are presented, and potential differences between adolescence and adults in this regard noted. The substantial evidence for involvement of glucocorticoids in alcohol-induced cognitive deficits is described, with particular reference to the consequences of alcohol withdrawal. The use of immature organotypic cultures of rodent brain in the study of alcohol neurotoxicity is considered in detail, and the information obtained from this methodology concerning the role of glucocorticoid receptors and excitable membrane proteins in this neurotoxicity. The influence of glucocorticoids on alcohol consumption and possible contributions to alcohol dependence are then considered. In conclusion, more information concerning the effects of glucocorticoids on plasticity and alcohol neurotoxicity during the adolescent period is needed.
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Keywords: Alcohol; Glucocorticoids; Adolescence; Withdrawal; Neurotoxicity

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There is now considerable information suggesting that glucocorticoid hormones play an important role in the adverse effects of prolonged excess alcohol consumption. The evidence for involvement of these hormones in alcohol-induced neurotoxicity is strong, and there is also increasing support for their contribution to the development of alcohol dependence. Adolescent alcohol use is a significant risk factor for prolonged alcohol dependence and emerging evidence suggests that adolescents respond differently than adults to alcohol. This review will present evidence that glucocorticoid hormones and their receptors may be involved in both the neurotoxic and

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dependence-producing effects of alcohol. Although studies on effects of alcohol during adolescence are now ongoing, there is not a large amount of information about possible differences in glucocorticoid effects over this time period, so this review will summarize available information on alcohol–glucocorticoid interactions in both the adolescent and adult phases. Adolescence in humans is regarded as the transition phase between childhood and adulthood, lasting from approximately 12 to 18 years of age and including the periods prior to and after puberty (Spear, 2000). Definitions of adolescence in rats and mice are not totally consistent, but it has been defined as between postnatal 28 and 42 days (Spear, 2000), and also as from 7 to 10 days before puberty (about 40 days postnatal) to approximately one week after puberty (Laviola et al., 2003). However some authorities have used a wider timescale and have applied the phrase "early adolescence" to describe the juvenile period between weaning and puberty, postnatal days 21 and 34 (Laviola et al., 2003).

1. Stress reactivity during adolescence

HPA function and reactivity to stress during adolescence have been covered in the excellent review by Spear (2000), so the present discussion will be confined to the glucocorticoids and aspects specifically relevant to alcohol neurotoxicity and alcohol consumption. Under normal circumstances, glucocorticoid release (cortisol in humans, corticosterone in rodents) follows a circadian rhythm, with high circulating concentrations prior to and after awakening, falling to lower levels during the remainder of the active period and the sleep phase. During stressful situations, release of glucocorticoids is elicited via adrenocorticotrophic hormone (ACTH) from the pituitary. The cellular effects of glucocorticoids are produced via action on two types of receptors, Type I, also known as mineralocorticoid receptors (MR) that have a high affinity for the ligands, and Type II or glucocorticoid receptors (GR) that are activated at the high glucocorticoid levels seen during the circadian peak and during stress. The CNS contains both types, with different regional distributions (Joels and De Kloet, 1994), and activation of these receptors mediates not only feedback control of glucocorticoid release but also important effects of glucocorticoids on many aspects of brain function.

In humans, evidence on differences between absolute plasma glucocorticoid levels in adolescents and adults is somewhat conflicting, some studies show a gradual increase in cortisol levels during puberty (Kiess et al., 1995; Walker et al., 2001) while others found no correlation with age, the individual differences being maintained from childhood to adulthood (Knutsson et al., 1997). It has been suggested that lower cortisol levels in adolescents are associated with conduct disorders, including low self-control, low harm avoidance and higher aggression and antisocial behavior (Shoal et al., 2003; Ramirez, 2003). However, the situation is complicated by the fact that many studies have not examined the whole circadian period, and raised cortisol levels at one time of day may be accompanied by smaller than normal levels at a different time. The increase in cortisol levels seen during the 30 min after awakening, for example, was reported by Rosmalen et al. (2005) to be lower in children than adults. In rodents, most studies have concentrated on the immediately postnatal or juvenile (from weaning until adolescence) phases, rather than the adolescent period. Basal plasma corticosterone concentrations in naive adolescent mice were found to be two-fold higher than those in corresponding adults, but this difference was seen only males, not females (Laviola et al., 2002).

During the first postnatal days, rodents undergo a period of decreased hormonal reactivity to stressful situations, known as the "stress hyporesponsive period", that lasts in rats from postnatal days 4 to 14. This period is controlled by Type II glucocorticoid receptor feedback, as well as maternal signals (Levine, 2001; Schmidt et al., 2005). It appears to have a functional analogue in humans aged 1–2 years (Gunnar and Donzella, 2002). During this time the release of glucocorticoids in response to many external stimuli is considerably lower than at any other time, although some responses, such as cytokine-induced glucocorticoid release, still occur (Furukawa et al., 1998). After this period and through the adolescent phase, glucocorticoid responsivity gradually increases until adulthood. The switch to a mature pattern of stress responding is accompanied by the appearance of the polyamine stress response, a transient increase in brain polyamine metabolism that is seen in the adult state (Gilad and Gilad, 2003). Some experimental studies have found glucocorticoid release in response to stressful stimuli to be lower but more prolonged during the adolescent phase than in adulthood (Laviola et al., 1999), but other studies indicated that such differences seen in juveniles are resolved by the adolescent phase (Choi and Kellogg, 1996). In addition, some evidence does suggest that a dissociation between basal corticosterone levels and corticosterone responses to stress may exist. For example, Laviola et al. (2002) demonstrated that periadolescent rats (30–45-day old) had higher basal corticosterone levels than adults suggesting higher basal HPA activation in younger animals but lesser corticosterone release than adults in response to a social stress. Given the qualitative differences in the nature of different stress models, it is clear that significant expansion of this area of work is needed.

The situation is not simply a matter of total glucocorticoid concentrations, as glucocorticoids in plasma are bound to protein (primarily corticosterone binding globulin, CBG, with some albumen binding), and they act via specific receptor sites. Only approximately 10% of the total plasma concentration remains unbound and thus available for action on tissues. Salivary cortisol levels reflect the free glucocorticoid component, but many studies in both humans and rodents have measured only total plasma concentrations. The free concentration of glucocorticoid is the proportion which is available to enter the brain, since the bound form does not pass the blood brain barrier in normal circumstances although the albumen bound component may dissociate rapidly enough to also be available to the CNS (Pardridge et al., 1983). The binding capacity of CBG is not constant and in stressful situations can,

for example, decrease considerably to as low as 30% of control values, (Fleschner et al., 1995; Spencer et al., 1996; Tennebaum et al., 1997). There is also circadian variation, with higher CBG during the quiescent phase (Meaney et al., 1992). During the postnatal stress hyporesponsive period CBG binding is very low, as little as 3% of adult values, so the availability and hence the effects particularly on the CNS of circulating glucocorticoid will be considerably greater than that of corresponding total circulating concentrations in adult life (Viau et al., 1996). By postnatal day 15, the CBG levels in rats had risen to 25% of adult levels (Viau et al., 1996), but a detailed analysis of changes during the adolescent period does not appear to be available.

The consequences of glucocorticoid release are also determined by the receptor levels, in addition to the free concentration of glucocorticoids. Data published by multiple laboratories, including those of the authors, strongly suggests that Type II glucocorticoid receptor occupation is a key factor in glucocorticoid regulation of both substance use and neuronal injury (Cho and Little, 1999; Packan and Sapolsky, 1990; Mulholland et al., 2005). Densities of both Type I and II receptors are low at birth, except in the pituitary, but Type I density rises to adult levels within a week after birth and Type II density within three weeks (in rats), after which levels do not change extensively under normal circumstances (Joels and De Kloet, 1994; Vazques, 1998). Stressful situations can, however, alter the density and affinity of both glucocorticoid receptor subtypes (e.g. Sapolsky and McEwen, 1985; Meaney et al., 1992).

2. Alcohol effects on glucocorticoids

Blood concentrations of glucocorticoids are increased by alcohol and the circadian rhythm is lost during long-term consumption (Tabakoff et al., 1978; Sipp et al., 1993). During the acute phase of alcohol withdrawal, the circulating concentrations of corticosterone are greatly raised, in both humans and animals (Adinoff et al., 1991; Tabakoff et al., 1978; Roberts et al., 1992; Lamblin et al., 1996). During abstinence these levels usually return to normal once the acute phase of withdrawal is over, although Farren et al. (2004) found increased serum cortisol in abstinent alcoholics of Type 2 but not Type 1 drinkers. The data of Keedwell et al. (2001) illustrates the importance of measuring free, rather than total, concentrations of glucocorticoid, as they found a five-fold rise in salivary (free) cortisol in alcoholics, while previous studies over the corresponding period showed increase in total plasma cortisol of only one half to two-fold. The normal circadian rhythm often remains attenuated for some time after drinking ceases. The hormonal responses to stress, however, are blunted for a considerable time during the abstinence period both in humans (Ehrenreich et al., 1997; Vescovi et al., 1997) and rodents (Sipp et al., 1993; Lee and Rivier, 1995; Rivier, 1995), suggesting a prolonged disturbance of feedback control. Lack of dexamethasone suppression of cortisol release is reported in 20–65% of alcohol dependent individuals during abstinence (Hundt et al., 2001).

Differences are seen in HPA responses to alcohol during adolescence. Silveri and Spear (2004) found the acute effect of alcohol in raising total plasma corticosterone was less marked in young rats compared with adults, both when alcohol administration was matched for dose and when doses were matched for motor impairment to take into account the differential functional effects of alcohol in adolescents. This pattern resembles that reported for other drugs of dependence (e.g. Adriani and Laviola, 2000). Apart from this study, however, there has been little investigation of alcohol effects on HPA function during adolescence. It is possible that HPA changes during or after chronic alcohol consumption differ in the adolescent phase from the adult but such information is not, as yet, available.

3. Alcohol-induced cognitive deficits, neurotoxicity and adolescence

Cognitive deficits are seen in 50 to 80% of alcoholics (Bates et al., 2002) and currently there is no effective therapeutic treatment. Cognitive deficits not only affect the quality of life of alcoholics and the amount of health care they need, but are also considered to have a detrimental effect on treatment programs and on the ability of alcoholics to refrain from drinking (Ihara et al., 2000; Bowdon et al., 2001). Partial recovery of cognitive function occurs after long-term (months or years) abstinence from alcohol, but some residual memory problems remain (Johnson-Greene et al., 1997).

The acute actions of alcohol on cognitive function appear to be greater during the adolescence phase than in adulthood (Brown and Tapert, 2004). Young adults aged 21 to 24 years were more impaired in several measures of memory after consumption of 0.6 g/kg alcohol than those aged 25 to 29 years (Acheson et al., 1998). In rats, spatial memory at postnatal day 30 was more affected by acute alcohol administration than in adults (Markwiese et al., 1998) and performance in an odor discrimination test after a range of doses of alcohol was more impaired in adolescents than adult animals (Land and Spear, 2004a). Learning of fear conditioning, however, was more greatly affected by acute alcohol in adult than adolescent rats (Land and Spear, 2004b). The mechanism(s) of the acute action of alcohol on memory processes, however, are likely to differ from the mechanism(s) by which chronic alcohol and withdrawal affect memory.

A binge pattern of alcohol drinking, with very high intermittent tissue alcohol concentrations, is frequent in adolescents and there is substantial evidence that it is a precursor to the development of alcohol dependence later in life (Grant and Dawson, 1997). This drinking pattern has been suggested to be more liable to cause brain damage than a continuous alcohol intake with lower maximal tissue alcohol concentrations (Hunt, 1993; Collins et al., 1996; Zou et al., 1996; Crews et al., 2000). The neuronal damage may be due to a direct effect of alcohol rather than to alcohol withdrawal, suggesting the mechanisms of neurotoxicity caused by binge drinking differ from those after long-term alcohol consumption (see below). Neuronal degeneration has been studied in two models of binge drinking in rats, both lasted four days and used the Majchrowicz (1975) method of dose titration according to behavioral effects. In both studies, the damage was maximal

before the time of peak abstinence signs (Collins et al., 1996), was greatest during the last dose and was less evident after alcohol withdrawal and during subsequent abstinence (Crews et al., 2000; Obernier et al., 2002). White et al. (2000) studied memory impairment in the 8-arm radial maze, twenty days after cessation of administration of alcohol in a "binge-drinking" pattern by injections of high doses at 48 h intervals for twenty days. Greater impairment was seen when the treatment was started at postnatal day 30 than when it was commenced at postnatal day 70. There were no differences in either acquisition of the task or in anxiety-related behavior. There does not appear to have been any investigation of the potential role of glucocorticoid hormones in binge drinking and the later consequences of this pattern of alcohol consumption.

Direct examination of the influence of age of commencement of excessive drinking on the subsequent development of cognitive problems has not been carried out in humans and only a small amount of information is available as yet from experimental studies comparing long-term alcohol consumption during the adolescent and adult phases. Sircar and Sircar (2005), compared the effects of five days of injections of 2 g/kg alcohol on adolescent (postnatal days 30 to 34) and adult (postnatal days 60 to 64) rats. When the animals were tested at intervals after the alcohol treatment, deficits in memory for the Morris water maze were seen in the adolescents that lasted into the adult phase, but the rats given alcohol as adults did not demonstrate such deficits. This important study demonstrates that further investigation is needed of the prolonged effects of chronic alcohol intake during adolescence.

During adolescence maturation of brain regions takes place with changes particularly in the prefrontal cortex and hippocampus (Spear, 2000; Monti et al., 2005). These are the areas particularly damaged by long-term alcohol consumption (Harper, 1998) and that are known to be crucially involved in memory processes. Some evidence suggests alcohol has greater acute effects on neuronal plasticity during the adolescent period than in adulthood. The effects of alcohol in depressing long-term potentiation (LTP) were found to be more pronounced in tissues from adolescent rats than those from adults (Swartzwelder et al., 1995; Pyapali et al., 1999). This evidence, taken with that demonstrating the resistance of adolescent brain compared with adult to alcohol-potentiated $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (Li et al., 2006), suggests age-dependent effects primarily on *N*-methyl-D-aspartate receptor (NMDAr) function. It is not clear, though, how these findings can be reconciled with those demonstrating a role for $GABA_A$ receptor function in ethanol's inhibition of LTP (Izumi et al., 2006). Glucocorticoids also affect neuronal plasticity and the Type II glucocorticoid receptor has been found to mediate depression of LTP and increases in long-term depression (Xu et al., 1998). Alteration in calcium flux and calcium handling, resulting in raised intracellular calcium concentrations, is thought to be involved in the neurotoxic effects of high concentrations of glucocorticoids (Kim and Yoon, 1998), but information is not available on possible differential effects during the adolescent phase. Given the high circulating glucocorticoid concentrations that occur during alcohol consumption and withdrawal, further information is needed regarding potential interactions between alcohol and glucocorticoids on neuronal plasticity in both adolescents and adults.

4. The importance of the alcohol withdrawal syndrome in neurotoxicity and cognitive deficits

The acute alcohol withdrawal syndrome is thought to be causally involved in the cognitive deficits seen after long-term alcohol intake. Neuronal hyperexcitability during the alcohol withdrawal syndrome has been shown to contribute to the neuronal degeneration caused by chronic alcohol intake, although some neuronal damage can occur without withdrawal (Hunt, 1993). Greater deficits in memory (Lukoyanov et al., 1999; Farr et al., 2005) and more neuronal degeneration (Phillips and Cragg, 1984; Cadete-Leite, 1990) were seen after cessation of chronic alcohol intake than during its consumption. Multiple withdrawal episodes also cause greater learning impairment in rats than a single withdrawal episode (Bond, 1979; Lundqvist et al., 1994). Repeated alcohol withdrawal increases the severity of withdrawal signs, and this effect is prevented by nifedipine but not by diazepam (Veatch and Gonzales, 2000; Mhatre et al., 2001). In humans, the severity of the cognitive deficits in alcoholics was found to be related to the number of detoxification episodes they had undergone (Duka et al., 2003).

During the acute phase of alcohol withdrawal, we have previously demonstrated that the neuronal hyperexcitability in hippocampal neurons involves increases in excitatory amino acid-mediated synaptic transmission and greater conductance of dihydropyridine sensitive calcium channels (Whittington and Little, 1993; Whittington et al., 1995; Molleman and Little, 1995). Changes were not, however, seen in $GABA_A$-receptor or $GABA_B$-receptor mediated inhibitory synaptic potentials in these neurons (Whittington et al., 1995; Molleman and Little, 1995). These changes could be related to the substantial increases in glucocorticoid release during the acute phase of alcohol withdrawal (Adinoff et al., 1991; Tabakoff et al., 1978). The regional distribution of Type II glucocorticoid receptors through the CNS shows a high density in regions known to be involved in memory formation such as the frontal cerebrocortical regions and, in rodents, in the hippocampus (McEwen and Wallach, 1973; Reul and De Kloet, 1985), brain areas that are particularly affected by long-term alcohol consumption.

The hippocampus in particular is a critical mediator of HPA axis function, providing glucocorticoid receptor-dependent negative feedback (for review, see Brown et al., 1999). The hippocampus has been widely, though not uniformly (Harding et al., 1997), reported to suffer volume loss during alcohol dependence in both adults and adolescents with an alcohol use disorder, that may be both hemisphere- and sex-dependent (Agartz et al., 1999; Laakso et al., 2000; Harper, 1998; Nagel et al., 2005; Sullivan et al., 1995). The greater hippocampal damage seen in rodents, compared with primates, after chronic alcohol consumption may be related to the higher concentration of Type II glucocorticoid receptors in the rodent hippocampus

(Mar Sanchez et al., 2000). Thus dysregulation of this negative feedback subsequent to alcohol-induced hippocampal injury may well contribute to the altered HPA responsivity observed with alcohol dependence.

It is well established that prolonged excess plasma glucocorticoid concentrations per se can cause neurotoxicity and cognitive deficits (Sapolsky, 1986, 1993, 1996a, 2000; Packan and Sapolsky, 1990; Seckl, 2000; Newcomer et al., 1999). The neurotoxicity involves increased vulnerability to other potential neurological insults, such as raised excitatory amino acid activity, rather than a direct neurotoxic effect of glucocorticoids (Sapolsky, 1996b). The Type II glucocorticoid receptors are thought to be involved in the glucocorticoid-induced neuronal damage, while the higher affinity Type I receptors may mediate neuroprotection (Abraham et al., 2001). During the acute alcohol phase of alcohol withdrawal, the increased activity at excitatory amino acid receptors (Whittington et al., 1995; Molleman and Little, 1995) and raised calcium conductance (Whittington and Little, 1993) plus the high circulating corticosterone concentrations therefore make this period one where neurotoxicity is highly likely to occur. Glucocorticoids have been found to increase the severity of the behavioral aspects of the alcohol withdrawal syndrome. Roberts et al. (1991) showed administration of corticosterone to alcohol withdrawal seizure-prone mice considerably increased the severity of the behavioral withdrawal signs, and the glucocorticoid synthesis inhibitor aminoglutethimide decreased the hyperexcitability. In two mouse strains, higher blood total corticosterone concentrations were associated with a greater severity of behavioral withdrawal signs and administration of corticosterone to alcohol naive mice caused convulsive behavior similar to that during alcohol withdrawal, that could be measured by the tremor and convulsive movements induced by gentle handling (Roberts et al., 1992). Studies in our laboratory have shown that administration of RU38486 (also known as mifepristone) in mice significantly decreased the severity of the alcohol withdrawal syndrome, as measured by the responses to handling (unpublished results).

The adverse effects of excessive circulating glucocorticoid concentrations on cognition and memory are well established, with functions involving the hippocampus and prefrontal cerebral cortex being particularly affected (Belanoff et al., 2001; Erickson et al., 2003). A study in psychotic depressives found that administration of mifepristone to patients alleviated both the depressive symptoms and the memory problems suffered by these patients (Young et al., 2004). Recent reports have indicated that glucocorticoids are involved in the cognitive deficits in alcoholics. Errico et al. (2002) found that 4–5 weeks after detoxification, alcoholics who had higher plasma cortisol levels during their most recent acute withdrawal phase demonstrated more severe cognitive deficits. Cognitive impairment was correlated with the number of previous withdrawal episodes and heavier alcohol consumption, suggesting these predispose to greater cortisol release during each withdrawal episode. High cognitive impairment was also associated with attenuation of the cortisol response to stress, indicating a greater dysfunction of HPA feedback control.

Although direct comparisons have not been made and would be problematic to carry out, reports indicate that the alcohol withdrawal syndrome is less severe during the adolescent phase than in adulthood (Martin and Winter, 1998). Doremus et al. (1998) found measures of anxiety-related behavior in adolescent rats in the period following a single high dose of alcohol were significantly less than the corresponding behavior in adult rats. Salimov et al. (1996) showed that oral consumption of alcohol by alcohol-preferring rats from 3 to 8 weeks of age decreased anxiety-related behavior measured after eight days of abstinence. While a less severe withdrawal syndrome would tend to encourage greater alcohol consumption, information is not yet available on the contribution of glucocorticoids in adolescents to either alcohol withdrawal symptoms or the adverse consequences of alcohol withdrawal.

5. Alcohol withdrawal neurotoxicity and glucocorticoids in organotypic cultures

A wealth of evidence has been published previously demonstrating the compensatory upregulation of NMDAr (Devaud and Morrow, 1999; Hu and Ticku 1995; Rudolph et al., 1997; Whittington et al., 1995) and L-type Ca^{2+} channels (Dolin et al., 1987; Brennan et al., 1989), as well as reduced expression of $GABA_A$ receptors (Devaud et al., 1999) in response to alcohol. These studies typically employed adult rodents or dissociated cell cultures from fetal rodents. The organotypic immature brain slice culture model, first described by Bosquet and Meunier (1962) and later refined by Stoppini et al. (1991), has been used extensively by the authors, and others (Belmadani et al., 2004; Thomas et al., 1998), to investigate effects of alcohol that promote alcohol withdrawal-induced neuronal excitability and neurotoxicity (i.e. Gibson et al., 2003; Harris et al., 2003; Mayer et al., 2002; Mulholland et al., 2005; Prendergast et al., 2004). It is of importance to note that, while organotypic slice cultures are typically obtained in P8–P9 rat pups, ethanol exposure often does not begin until cultures are at an age commensurate with that considered to be closely preadolescent or periadolescent (15–38 days). These ex vivo ages extend well into the preadolescent (Carlezon et al., 2003) and periadolescent periods (Spear, 2000). A recent paper showed the distribution of AMPA, kainate, and NMDA receptors in the CA1 region of P30 organotypic hippocampal slices and acute hippocampal slices taken from P30 adolescent rats was “identical”, though modest differences in the distribution of MK-801 and AMPA binding sites in CA3 were observed (Martens and Wree, 2001). Thus, the relevance of this model to understanding possible effects of alcohol and corticosteroids on the adolescent hippocampus may be considerable. Before such a definitive conclusion can be made, though, comparative studies using this model system and adolescent rodents in vivo must be completed.

These studies cumulatively have delineated a cascade of events induced by prolonged ethanol exposure that promote neuronal excitability and Ca^{2+}-dependent neuronal injury during acute ethanol withdrawal. Such changes include reduced expression of the Ca^{2+}-buffering protein calbindin-D28K and

increased expression of NR1 and NR2 subunits during ethanol exposure, followed by release of high concentrations of glutamate and the polyamine spermidine, which function as a positive allosteric modulator of NMDAr open time via actions at NR2 subunits. Thus, the ability to buffer intracellular Ca^{2+} is reduced during ethanol exposure and accumulation of intracellular Ca^{2+} is markedly increased during ethanol withdrawal, producing neuronal excitability and "classical" NMDAr-mediated excitotoxicity (Choi, 1994).

Evidence of a role for NMDAr in this form of neuronal excitability and toxicity leads consideration of the impact of corticosteroids on this form of neuronal hyperactivity. The primary rodent glucocorticoid corticosterone, in a Type II receptor-dependent manner, can: (1) enhance expression of specific NR2 subunits (Takahashi et al., 2002; Weiland et al., 1997); (2) delay extrusion of accumulated intracellular Ca^{2+}, likely via effects on Ca^{2+}-ATPase activity (Elliott and Sapolsky 1993); (3) promote accumulation of extracellular glutamate, possibly by inhibiting glial transport of glutamate (Jacobsson et al., 2006); and increase the synthesis of the NMDAr modulating polyamines, via increases in expression of the synthetic enzyme ornithine decarboxylase (Ientile et al., 1998; Orti et al., 1987). In a recent publication (Mulholland et al., 2005), organotypic hippocampus slice cultures were exposed for 10 days to a moderate concentration of ethanol (50 mM) with or without the addition of physiologically relevant concentrations of corticosterone. Though no neuronal injury was observed when cultures were withdrawn in the absence of exogenous corticosterone, co-exposure to this hormone with ethanol for 10 days and during ethanol withdrawal produced marked neuronal injury that was both Type II receptor- and NMDAr-dependent (Fig. 1). Thus, this model of alcohol-induced hypercortisolemia demonstrates significant neuronal excitability and neurotoxicity and suggests a genomic effect of corticosterone in altering NMDAr activity. However, it must be noted that evidence exists demonstrating the ability of short-term (24-h) corticosterone exposure to potentiate NMDAr-mediated neuronal injury in a Type II receptor- and Type I receptor-independent manner (Mulholland et al., 2006). Hypercortisolemia may, then, promote NMDAr function via multiple mechanisms. However, corticosterone is also known to regulate the expression and/or function of ion channels (for example L-type Ca^{2+} channels (Zhou et al., 2000 and inwardly rectifying potassium channels (Muma and Beck, 1999), thus, its role in promoting neuronal excitability is likely to be quite complex.

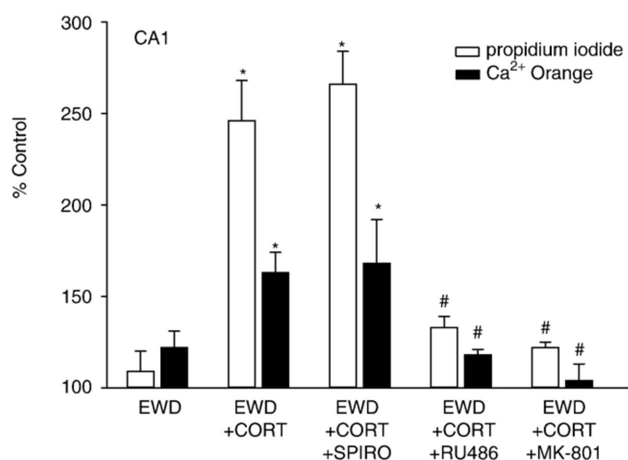

Fig. 1. Exposure of organotypic hippocampal slice cultures to corticosterone (CORT; 1 μM) with ethanol (50 mM) for 10 days, followed by 1 day of ethanol withdrawal (EWD) produced marked neuronal calcium accumulation (Ca^{2+} Orange) and injury (propidium iodide) in the CA1 pyramidal layer. Co-exposure to the Type I receptor antagonist sprironolactone did not attenuate these effects. Co-exposure to the Type II receptor antagonist RU486 (0.1–1.0 μM) or MK-801 (20 μM) abolished these effects. $^{*}P<0.05$ vs EWD; $^{\#}P<0.05$ vs EWD+CORT (after Mulholland et al., 2005).

In a general sense, the organotypic model described may best be characterized as a model of NMDAr over-activity during acute alcohol withdrawal, rather than a specific model of neurotoxicity. This characterization has important implications when interpreting the finding that exposure to high concentrations of corticosterone potentiates the ethanol withdrawal phenomena. In addition to neuronal injury, NMDAr activation during acute or prolonged abstinence may contribute to anxiety (Gatch et al., 1999), cognitive impairment (Thomas et al., 2004); conditioned abstinence effects (Cole et al., 2000) and even the alcohol deprivation effect (Vengeliene et al., 2005), though the latter findings may simply represent partial substitution of NMDAr antagonists for ethanol. Thus, evidence that corticosteroids may alter the function or expression of some NMDAr during ethanol exposure or withdrawal may be highly relevant to understanding the consequences of alcohol-associated hypercortisolemia for myriad abstinence-related phenomena. It will be of significant interest to examine the extent to which alcohol promotes corticosteroid release in adolescents, as compared with adults, as this may markedly impact progression to dependence and the development of untoward consequences associated with alcohol use. Further, it is tempting to regard Type II receptors as potential therapeutic targets in the maintenance of abstinence to alcohol intake, independent of age.

6. Involvement of glucocorticoids in alcohol consumption and dependence

The possible role of glucocorticoids in alcohol consumption and dependence is currently supported by less evidence than the importance of these hormones in the neurotoxicity and cognitive deficits produced by long-term alcohol consumption, but the evidence for the influence of stressful experiences at all stages of the development of alcohol dependence is considerable and the neuronal mechanisms involved need to be understood.

Both clinical and preclinical evidence indicate the importance of stress in alcohol dependence. Moncrieff et al. (1996) found that in people with alcohol drinking problems, 54% of women and 24% of men were victims of sexual abuse or assault, percentages substantially higher than the general population; in the majority of cases the trauma was experienced at an early age. In a national (US) survey of adolescents ages 12 to 18 (Grades 7 to 12), high risk factors for

regular alcohol consumption were found to be exposure to childhood abuse and stressful life events in boys and these factors plus violence within the family and depressive symptoms in girls (Simantov et al., 2000). Gorman and Brown (1992) found a higher incidence of stressful major life events in patients diagnosed with alcohol dependence than in a control population. Employment in occupations that provided high strain and low control was shown by Crum et al. (1995) to be associated with an increased risk of alcohol abuse. Furthermore, the relapse rate in abstinent alcoholics was higher in individuals who had experienced severe psychosocial stress (Brown et al., 1995). More recently, an impaired cortisol response to a psychosocial test in detoxified alcoholics was shown to be a predictor of early relapse drinking (Junghanns et al., 2003).

Clearly, stressful situations involve activation of the whole HPA, with release of corticotrophin releasing factor and adrenocorticotrophin in addition to glucocorticoids, and long-term changes may involve alterations in hormone receptors and signaling mechanisms. Little investigation has been made so far in humans of the direct effects of alterations in circulating glucocorticoids on alcohol consumption. Although a small investigation in social drinkers did not demonstrate any effect of the cortisol synthesis inhibitor, metyrapone, on alcohol consumption (Eriksson et al., 2001), the metyrapone dose used did not significantly alter the circulating cortisol concentrations and the study was carried out on social drinkers rather than alcoholics.

Preclinical evidence, however, shows that alcohol consumption can be directly affected by the plasma glucocorticoid concentrations. Corticosterone administration was found to increase voluntary alcohol drinking in rats, while lowering glucocorticoid concentrations with the glucocorticoid synthesis inhibitor metyrapone or by adrenalectomy, decreased ethanol intake (Fahlke et al., 1994a,b, 1995; Fahlke and Hanson, 1999). We have found that administration of the Type II glucocorticoid receptor antagonist, mifepristone, decreased the slowly developing stress-induced increases in voluntary alcohol consumption in mice, while spironolactone, a Type I glucocorticoid receptor antagonist, had no effect (O'Callaghan et al., 2005). Koenig and Olive (2004) found an effect of mifepristone on limited access alcohol drinking. Pasad and Pasad (1995) showed that in a variety of experimental conditions, high basal corticosterone levels plus attenuated stress-induced cortisol release were associated with greater voluntary alcohol consumption in rodents. Acute severe stress, such as footshock, can decrease alcohol consumption. This effect was found to be more pronounced in adolescent rats than in adults, and it did not correlate with the total plasma corticosterone concentrations (Brunell and Spear, 2005). Mice and rats with subordinate social status have high circulating plasma corticosterone concentrations and a consistent finding has been that subordinate animals have higher voluntary consumption of alcohol than those of more dominant status (Hilakaivi-Clarke and Lister, 1992; Blanchard et al., 1993).

Further evidence comes from the extensive work of Piazza and colleagues, who have demonstrated that rodents with higher circulating glucocorticoid concentrations have greater tendencies to self-administer a variety of dependence-producing drugs (Piazza and La Moal, 1996, 1998; Marinelli and Piazza, 2002). This research group also demonstrated that rats will even self-administer corticosterone itself, in amounts that result in blood concentrations in the range found during stressful situations (DeRoche et al., 1993; Piazza et al., 1993). All drugs that cause dependence, with the possible exception of benzodiazepines, increase glucocorticoid release, although it is important in this context to distinguish between corticosterone release caused by experience of an unfamiliar situation and a specific pharmacological effect of increasing glucocorticoid release. This

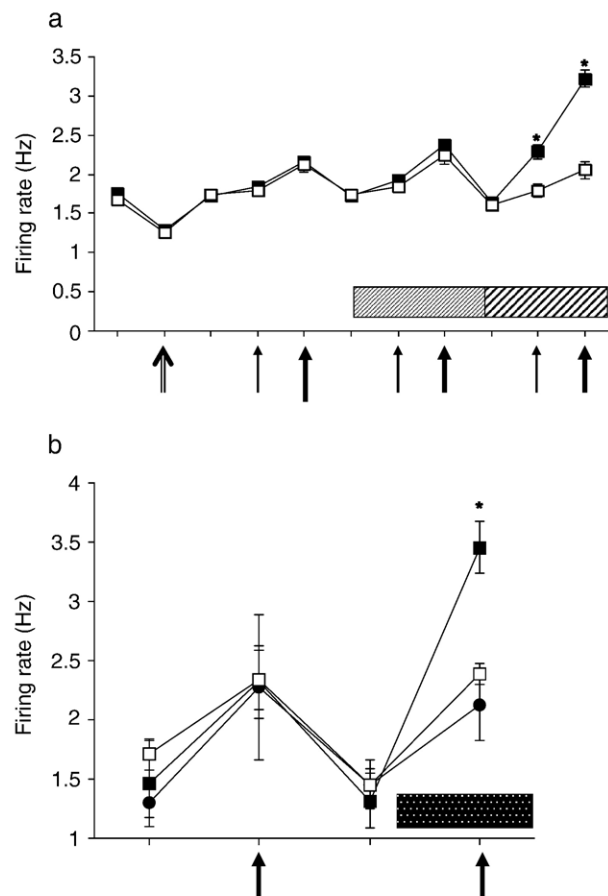

Fig. 2. The effect of corticosterone on the activity of single unit VTA neurons in isolated midbrain slices from adult rats, basal firing and responses to NMDA. Values are mean ± s.e.m. for the frequency of spontaneous firing of dopamine-sensitive neurons. The small solid arrows indicate applications of 5 μM NMDA and the large solid arrows applications of 15 μM NMDA; tissue contact time for NMDA was 5 min, after which the preparations were washed with artificial cerebrospinal fluid. The open arrow indicates the application of dopamine, 15 μM, for 5 min. The bars show the duration of the bath application of the following concentrations of corticosterone: a) fine hatched bar 50 nM, coarse hatched bar 100 nM; b) shaded bar 500 nM. a) shows the effects of corticosterone concentrations of 50 nM and 100 nM and b) the effects of 500 nM, in the presence and absence of RU38486 500 nM. a) and b): open squares = frequency of spontaneous firing in the absence of corticosterone; closed squares = frequency of spontaneous firing in the presence of corticosterone; b) closed circles = frequency of spontaneous firing in the presence of corticosterone plus RU38486 (RU38486 had no effect on firing frequency when added alone). *$P<0.05$ in a) compared with parallel recordings in the absence of corticosterone. Reproduced with permission from Cho and Little (1999).

glucocorticoid releasing effect may be involved in the development of dependence, and corticosterone has been shown to increase the saliency of rewarding stimuli in rodents (Abrahamsen and Carr, 1996; Bhatnagar et al., 2000) and also the palatability of alcohol (Soderpalm and Hansen, 1999).

At first sight, the established beneficial effects of opiate antagonists in decreasing relapse drinking in alcoholics argue against an action of glucocorticoids in increasing alcohol consumption, since this type of drug has a specific pharmacological action in stimulating glucocorticoid release via the pituitary. However, this action is seen on acute administration of an opiate antagonist and most of the studies have demonstrated it following a single intravenous infusion of naltrexone or naloxone. In contrast, the therapeutic effects of naltrexone in alcoholics are apparent over weeks and months of treatment. Repeated administration of opiates does not appear to cause HPA stimulation, as demonstrated in both experimental and human studies (Na and Lee, 2002; Lee et al., 2005; McCaul et al., 2001), although there is one report of raised cortisol after six days of naltrexone (O'Malley et al., 2002). The situation is complicated by the fact that alcohol has been found to decrease the HPA stimulant effects of naltrexone (McCaul et al., 2001; Williams et al., 2001), but has also been reported to increase this effect (O'Malley et al., 2002).

With regard to possible neuronal mechanisms of the involvement of glucocorticoids in dependence on alcohol and other drugs, these are likely to lie in the actions of glucocorticoids on the limbic system. Corticosterone had a specific action on dopaminergic neurons in the ventral tegmental area (VTA), potentiating synaptic activity mediated by NMDA, AMPA or kainate receptor activation, but no effect on the basal firing of these neurons (Fig. 2). The effect was shown to involve influx of calcium ions (Cho and Little, 1999). Although the action of corticosterone in potentiating glutamate transmission was suggested by Overton et al. (1996) to be via Type I glucocorticoid receptors, we demonstrated antagonism of the effect in vitro by the Type II glucocorticoid receptor antagonist mifepristone (Cho and Little, 1999). The important descending projection from the prefrontal cortex to the VTA involves glutaminergic transmission and the activation of VTA neurons by this pathway would therefore be potentiated by Type II receptor activation. Such an interaction would be likely to take place during times of stress, intake of drugs of abuse or alcohol withdrawal, when the glucocorticoid concentrations are sufficiently high to activate the Type II receptors.

The above studies show that glucocorticoids may play an important role in alcohol dependence and that early stressful experiences contribute to the development of such dependence, but the precise role of glucocorticoids in adolescent drinking and alcohol abuse has yet to be clarified.

7. Summary

Given the wide range of signaling systems affected by glucocorticoid receptor activation, it will be critical to characterize further the pathway(s) most relevant to understanding glucocorticoid effects on alcohol use and its consequences. Doing so may be of significance in identifying potential targets that may be exploited pharmacologically in the treatment of a wide range of disorders, including alcohol dependence. Perhaps one of the key initial issues needing attention is the response of the adolescent HPA axis to alcohol, both acutely, during binge use, and with prolonged use. Compelling work has recently been published and suggests that indeed the adolescent HPA axis responds to stressors quite differently than that of the adult. The consequences of this with regard to the development of alcohol dependence and organ injury remain to be clarified.

Examining glucocorticoid effects on alcohol and its consequences is complicated by the relative lack of specificity of available receptor antagonists. Mifepristone, a widely-used glucocorticoid (Type II) receptor antagonist, also modulates the function of structurally similar neurosteroid receptors. However, newer, more selective Type II receptor antagonists are the target of current drug development (i.e. Peeters et al., 2004). The evidence from preclinical and clinical studies, described above, indicates that such drugs could decrease alcohol withdrawal signs, decrease alcohol consumption, alleviate depression and prevent neurotoxicity and the development of cognitive deficits in alcoholics. Development of selective agents for these receptor sites will be critical with regard to their use as investigative tools and, potentially, therapeutic agents.

References

Abrahamsen GC, Carr KD. Effects of corticosteroid synthesis inhibitors on the sensitization of reward by food restriction. Brain Res 1996;726:39–48.

Abraham IM, Harkany T, Horvath KM, Luiten PGM. Action of glucocorticoids on survival of nerve cells: promoting neurodegeneration or neuroprotection? J Neuroendocrinol 2001;13:749–60.

Acheson SK, Stein RM, Swartzwelder HS. Impairment of semantic and figural memory by acute ethanol: age-dependent effects. Alcohol Clin Exp Res 1998;22:333–42.

Adinoff B, Richer-Flowers D, De Jong J, Ravitz B, Bone GHA, Nutt DJ, et al. Disturbances of hypothalamo–pituitary–adrenal axis functioning during ethanol withdrawal in six men. Am J Psychiatry 1991;148:1023–5.

Adriani W, Laviola G. A unique hormonal and behavioral hyporesponsivity to both forced novelty and d-amphetamine in periadolescent mice. Neuropharmacology 2000;39:334–46.

Agartz I, Momenan R, Rawling RR, Kerich MJ, Hommer DW. Hippocampal volume in patients with alcohol dependence. Arch Gen Psychiatry 1999;56:356–63.

Bates ME, Bowdon SC, Barry D. Neurocognitive impairment associated with alcohol use disorders: implications for treatment. Exp Clin Psychopharmacol 2002;10:193–212.

Belanoff JK, Gross K, Yager A, Schatzberg AF. Corticosteroids and cognition. J Psychiatr Res 2001;35:127–45.

Belmadani A, Kumar S, Schipma M, Collins MA, Neafsey EJ. Inhibition of amyloid-induced neurotoxicity and apoptosis by moderate ethanol preconditioning. Neuroreport 2004;15:2093–6.

Bhatnagar S, Bell ME, Liang J, Soriano L, Nagy TR, Dallman MF. Corticosterone facilitates saccharin intake in adrenalectomized rats; does corticosterone increase stimulus salience? J Neuroendocrinol 2000;12:453–60.

Blanchard RJ, Yudho EB, Blanchard DC. Alcohol, aggression and the stress of subordination. J Stud Alcohol Suppl 1993;11:146–55.

Bond NW. Impairment of shuttlebox avoidance learning following repeated alcohol withdrawal episodes in rats. Pharmacol Biochem Behav 1979;11:589–91.

Bowdon SC, Crews FT, Bates ME, Fals-Stewart W, Ambrose ML. Neurotoxicity and neurocognitive impairments with alcohol and drug-use disorders: potential roles in addiction and recovery. Alcohol Clin Exp Res 2001;25:317–21.
Bosquet J, Meunier JM. Organotypic culture, on natural and artificial media, of fragments of the adult rat hypophysis. C.R. Seances Soc. Boil. 1962;156: 65–7.
Brennan CH, Lewis A, Littleton JM. Membrane receptors involved in upregulation of calcium channels in bovine adrenal chromaffin cells chronically exposed to ethanol. Neuropharmacology 1989;26:275–9.
Brown SA, Tapert SF. Adolescence and the trajectory of alcohol use: basic to clinical studies. Ann N Y Acad Sci 2004;1021:234–44.
Brown SA, Vik PW, Patterson T, Grant I. Stress, vulnerability and adult alcohol relapse. J Stud Alcohol 1995;56:538–45.
Brown EW, Rush AJ, McEwen BS. Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. Neuropsychopharmacology 1999;21:474–84.
Brunell SC, Spear LP. Effect of stress on the voluntary intake of a sweetened ethanol solution in pair-housed adolescent and adult rats. Alcohol Clin Exp Res 2005;29:1641–53.
Cadete-Leite A. Structural changes in the hippocampal formation and frontal cortex after long-term alcohol consumption and withdrawal in the rat. In: Palmer TN, editor. Alcoholism: a molecular perspective. London: Plenum Press, Inc; 1990.
Carlezon Jr WA, Mague SD, Andersen SL. Enduring behavioral effects of early exposure to methylphenidate in rats. Biol Psychiatry 2003;54:1330–7.
Cho K, Little HJ. Corticosterone increases excitatory amino acid responses of ventral tegmental neurons. Neuroscience 1999;88:837–45.
Choi DW. Calcium and excitotoxic neuronal injury. Ann NY Acad Sci 1994;747:162–71.
Choi S, Kellogg CK. Adolescent development influences functional responsiveness of noradrenergic projections of the hippocampus. Dev Brain Res 1996;94:144–51.
Cole J, Littleton JM, Little HJ. Acamprosate, but not naltrexone, inhibits conditioned abstinence behaviour associated with repeated ethanol administration and exposure to a plus maze. Psychopharmacology 2000;147: 403–11.
Collins MA, Corso TD, Neafsey EJ. Neuronal degeneration in rat cerebrocortical and olfactory regions during subchronic "binge" intoxication with ethanol: possible explanation for olfactory deficits in alcoholics. Alcohol Clin Exp Res 1996;20:284–92.
Crews FT, Braun CJ, Hoplight B, Switzer RC, Knapp DJ. Binge ethanol consumption causes differential brain damage in young adolescent rats compared with adult rats. Alcohol Clin Exp Res 2000;24:1712–23.
Crum RM, Muntarne C, Eaton WW, Anthony JC. Occupational stress and the risk of alcohol abuse and dependence. Alcohol Clin Exp Res 1995; 19:647–55.
DeRoche V, Piazza PV, Deminiere J-M, Le Moal M, Simon H. Rats orally self-administer corticosterone. Brain Res 1993;622:315–20.
Devaud LL, Morrow AL. Gender-selective effects of ethanol dependence on NMDA receptor subunit expression in cerebral cortex, hippocampus, and hypothalamus. Eur J Pharmacol 1999;369:331–4.
Devaud LL, Mathews DB, Morrow AL. Gender impacts behavioral and neurochemical adaptations in ethanol-dependent rats. Pharmacol Biochem Behav 1999;64:841–9.
Dolin SJ, Little HJ, Hudspith MJ, Pagonis C, Littleton JM. Increased dihydropyridine-sensitive calcium channels in rat brain may underlie ethanol physical dependence. Neuropharmacology 1987;26:275–9.
Doremus TL, Brunell SC, Varlinskaya EL, Spear LP. Anxiogenic effects during withdrawal from acute alcohol in adolescent and adult rats. Pharmacol Biochem Behav 1998;75:411–8.
Duka T, Gentry R, Ripley TL, Borlokova G, Stephens DN, Veatch LM, et al. Consequences of multiple withdrawals from alcohol. Alcohol Clin Exp Res 2003;28:233–46.
Ehrenreich H, Schuck N, Pitz J, Gefeller O, Schilling L, Poser W, et al. Endocrine and hemodynamic effects of stress versus systemic CRF in alcoholics during early and medium term abstinence. Alcohol Clin Exp Res 1997;21:1285–93.
Elliott EM, Sapolsky RM. Corticosterone impairs hippocampal neuronal calcium regulation-possible mediating mechanisms. Brain Res 1993;602: 84–90.
Erickson K, Drevets W, Schulkin J. Glucocorticoid regulation of diverse cognitive functions in normal and pathological emotional states. Neurosci Biobehav Rev 2003;27:233–46.
Eriksson M, Fahlke C, Hansen S, Berggren U, Marin P, Balldin J. No effect of the cortisol-synthesis inhibitor metyrapone on alcohol drinking: a pilot study. Alcohol 2001;25:115–22.
Errico AL, King AC, Lovallo WR, Parsons OA. Cortisol dysregulation and cognitive impairment in abstinent male alcoholics. Alcohol Clin Exp Res 2002;26:1198–204.
Fahlke C, Hanson S. Effect of local intracerebral corticosterone implants on alcohol consumption. Neurosci Abst 1999;25:440.13.
Fahlke C, Engel JA, Eriksson CPJ, Hard E, Soderpalm B. Involvement of corticosterone in the modulation of ethanol consumption in the rat. Alcohol 1994a;3:195–202.
Fahlke C, Hard E, Thomasson R, Engel JA, Hansen S. Metyrapone-induced suppression of corticosterone synthesis reduces ethanol consumption in high-preferring rats. Pharmacol Biochem Behav 1994b;48:977–81.
Fahlke C, Hard E, Eriksson CPJ, Engel JA, Hansen S. Consequence of long-term exposure to corticosterone or dexamethasone on ethanol consumption in the adrenalectomized rat, and the effect of type I and type II corticosteroid receptor antagonists. Psychopharmacology 1995;117: 216–24.
Farr SA, Scherrer JF, Banks WA, Flood JF, Morley JE. Chronic ethanol consumption impairs learning and memory after cessation of ethanol. Alcohol Clin Exp Res 2005;29:971–82.
Farren CK, Clare AW, Dinan TG. Basal serum cortisol and dexamethasone-induced growth hormone release in the alcohol dependence syndrome. Hum Psychopharmacol 2004;10:207–13.
Fleschner M, Deak T, Spencer RL, Laudenslager ML, Watkins LR, Maier SF. A long term increase in basal levels of corticosterone and a decrease in corticosterone binding globulin after acute stressor exposure. Endocrinology 1995;136:5336–43.
Furukawa H, Del Rey A, Monge-Arditi G, Besedovsky HO. Interleukin-1, but not stress, stimulates glucocorticoid output during early postnatal life in mice. Neuroimmunomodulation 1998;840:117–22.
Gatch MB, Wallis CJ, Lal H. Effects of NMDA antagonists on ethanol-withdrawal induced "anxiety" in the elevated plus maze. Alcohol 1999;19: 207–11.
Gibson DA, Harris BR, Prendergast MA, Hart SR, Blanchard JA, Holley RC, et al. Polyamines contribute to ethanol withdrawal-induced neurotoxicity in rat hippocampal slice cultures through interactions with the NMDA receptor. Alcohol Clin Exp Res 2003;27:1099–106.
Gilad GM, Gilad VH. Overview of the brain polyamine-stress-response: regulation, development, and modulation by lithium and role in cell survival. Cell Mol Neurobiol 2003;23:637–49.
Gorman DM, Brown GW. Recent developments in life-event research and their relevance for the study of addictions. Br J Addict 1992;87:837–49.
Grant BF, Dawson DA. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiological Survey. J Subst Abuse 1997;9: 103–10.
Gunnar MR, Donzella B. Social regulation of the cortisol levels in early human development. Psychoneuroendocrinology 2002;27:199–220.
Harding AJ, Wong A, Svoboda M, Kril JJ, Halliday GM. Chronic alcohol comsumption does not cause hippocampal neuron loss in humans. Hippocampus 1997;7:78–87.
Harper C. The neuropathology of alcohol-specific brain damage, or does alcohol damage the brain? J Neuropathol Exp Neurol 1998;57:101–10.
Harris BR, Gibson DA, Prendergast MA, Blanchard JA, Holley RC, Hart SR, et al. The neurotoxicity induced by ethanol withdrawal in mature organotypic hippocampal slices might involve cross-talk between mGluR5s and NMDARs. Alcohol Clin Exp Res 2003;27:1724–35.
Hilakaivi-Clarke L, Lister RG. Social status and voluntary alcohol consumption in mice: interaction with stress. Psychopharmacology 1992;108: 276–82.

Hu XJ, Ticku MK. Chronic ethanol treatment upregulates the NMDA receptor function and binding in mammalian cortical neurons. Mol Br Res 1995;30: 347–56.

Hundt W, Zimmermann U, Pottig M, Spring K, Holsboer F. The combined dexamethasone-suppression/CRH-stimulation test in alcoholics during and after acute withdrawal. Alcohol Clin Exp Res 2001;25:687–91.

Hunt WA. Are binge drinkers more at risk of developing brain damage? Alcohol 1993;10:559–61.

Ientile R, De Luca G, Di Girogio RM, Macaione S. Glucocorticoid regulation of spermidine acetylation in the rat brain. J Neurochem 1998;51: 677–82.

Ihara H, Berrios GE, London M. Group and case study of the dysexecutive syndrome in alcoholism without amnesia. J Neurosurg Psychiatry 2000;68:731–7.

Izumi Y, Nagashima K, Murayama K, Zorumski CG. Acute effects of ethanol on hippocampal long-term potentiation and long-term depression are mediated by different mechanisms. Neuroscience 2006;136:509–17.

Jacobsson J, Persson M, Hansson E, Ronnback L. Corticosterone inhibits expression of the microglial glutamate transporter GLT-1 in vitro. Neuroscience 2006;139:475–83 [Electronic publication ahead of print].

Joels M, De Kloet ER. Mineralocorticoid and glucocorticoid receptors in the brain. Implications for ion permeability and transmitter systems. Prog Neurobiol 1994;43:1–36.

Johnson-Greene D, Adams KM, Gilman S, Koeppe RA, Junck L, Kluin KJ, et al. Effects of abstinence and relapse upon neuropsychological function and cerebral glucose metabolism in severe chronic alcoholism. J Clin Exp Neurol 1997;19:378–85.

Junghanns K, Backhaus J, Tietz U, Lange W, Bernzen J, Wetterling T, et al. Impaired cortisol stress response is a predictor of early relapse. Alcohol Alcohol 2003;38:189–93.

Keedwell PA, Poon L, Papadopolous AS, Marshall J, Checkley SA. Salivary cortisol measurements during a medically assisted withdrawal. Addict Biol 2001;6:247–57.

Kiess W, Meidert A, Dressendorfer Ra, Schriever K, Kessler U, Konig A, et al. Salivary cortisol-levels throughout childhood and adolescence — relation with age, pubertal stage, and weight. Pediatr Res 1995;37:502–6.

Kim JJ, Yoon KS. Stress: metaplastic effects in the hippocampus. Trends Neurosci 1998;21:505–9.

Knutsson U, Dahlgren J, Marcus C, Rosberg S, Bronnegard M, Stierna P, et al. Circadian cortisol rhythms in healthy boys and girls: relationship with age, growth, body composition, and pubertal development. J Clin Endocrinol Metab 1997;82:536–40.

Koenig HN, Olive MF. The glucocorticoid receptor antagonist mifepristone reduces ethanol intake in rats under limited access conditions. Psychoneuroendocrinology 2004;29:99–1003.

Laakso MP, Vaurio O, Savalainen L, Repo E, Soininen H, Aronen HJ, et al. A volumetric MRI study of the hippocampus in type 1 and 2 alcoholism. Behav Brain Res 2000;109:177–86.

Lamblin F, Meert TF, De Witte P. Adrenalectomy protects ethanol-withdrawn rats from harmine-induced tremor. Alcohol Alcohol 1996;12:75–181.

Land C, Spear NE. Ethanol impairs learning of a simple discrimination in adolescent rats at doses that leave adult memory unaffected. Neurobiol Learn Mem 2004a;81:75–81.

Land C, Spear NE. Fear conditioning is impaired in adult rats by ethanol doses that do not affect periadolescents. Int J Dev Neurosci 2004b;22: 355–62.

Laviola G, Adriani W, Terranova ML, Gerra G. Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. Neurosci Biobehav Rev 1999;23:993–1010.

Laviola G, Adriani W, Morley-Fletcher S, Terranova M. Peculiar response of adolescent mice to acute and chronic stress and to amphetamine: evidence of sex differences. Behavioral Brain Research 2002;130:117–25.

Laviola G, Macri S, Morley-Fletcher S, Adriani W. Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenic influence. Neurosci Biobehav Rev 2003;27:19–31.

Lee S, Rivier C. Altered ACTH and corticosterone responses to interleukin-1β in male rats exposed to an alcohol diet: possible role of vasopressin and testosterone. Alcohol Clin Exp Res 1995;19:200–8.

Lee YS, Joe KH, Sohn IK, Na C, Kee BS, Chae SL. Changes of smoking behavior and serum adrenocorticotropic hormone, cortisol, prolactin and endogenous opioids levels in nicotine dependence after naltrexone treatment. Prog Neuropsychopharmacol Biol Psychiatry 2005;269: 639–47.

Levine S. Primary social relationships influence the development of the hypothalamic–pituitary–adrenal axis in the rat. Physiol Behav 2001;73: 255–60.

Li Q, Wilson WA, Swartzwelder HS. Develomental differences in the sensitivity of spontaneous and miniature IPSCs to ethanol. Alcohol Clin Exp Res 2006;30:119–26.

Lukoyanov NV, Madeira MD, PaulaBarbosa MM. Behavioral and neuroanatomical consequences of chronic ethanol intake and withdrawal. Physiol Behav 1999;66:337–46.

Lundqvist C, Volk B, Knoth R, Alling C. Long-term effects of intermittent versus continuous ethanol exposure on hippocampal synapses of the rat. Acta Neuropathol 1994;87:242–9.

Majchrowicz E. Induction of physical dependence upon alcohol and the associated behavioral changes in rats. Psychopharmacologia 1975;43: 245–54.

Mar Sanchez M, Young LJ, Plotsky PM, Insel TR. Distribution of corticosteroid receptors in the rhesus brain: relative absence of glucocorticoid receptors in the hippocampal formation. J Neurosci 2000;20:4657–68.

Marinelli M, Piazza PV. Interaction between glucocorticoid hormones, stress and psychostimulant. Eur J Neurosci 2002;16:387–94.

Markwiese BJ, Acheson SK, Levin ED, Wilson WA, Swartzwelder HS. Differential effects of ethanol on memory in adolescent and adult rats. Alcohol Clin Exp Res 1998;22:416–21.

Martens U, Wree A. Distribution of [3H]MK-801, [3H]AMPA and [3H]Kainate binding sites in rat hippocampal long-term slice cultures isolated from external afferents. Anat Embryol 2001;203:491–500.

Martin CS, Winter KC. Diagnosis and assessment of alcohol use disorders among adolescents. Alcohol Res Health 1998;22:95–105.

Mayer S, Harris BR, Gibson DA, Blanchard J, Prendergast MA, Littleton JM. Acamprosate has no effect on NMDA-induced toxicity but reduces toxicity induced by spermidine or by changing medium in organotypic hippocampal slice cultures from rat. Alcohol Clin Exp Res 2002;26:655–61.

McCaul ME, Wand GS, Stauffer R, Shing ML, Rohde CA. Naltrexone dampens ethanol-induced cardiovascular and hypothalamic–pituitary–adrenal axis activation. Neuropsychopharmacology 2001;25:537–47.

McEwen BS, Wallach G. Corticosterone binding to hippocampus: nuclear and cytosol binding in vitro. Brain Res 1973;57:373–86.

Meaney MJ, Aitkin DH, Sharma S, Viau V. Basal ACTH, corticosterone and corticosterone binding globulin levels over the diurnal cycle and age-related changes in hippocampal Type I and Type II corticosteroid receptor binding capacity in young and aged handled and nonhandled rats. Neuroendocrinology 1992;55:204–13.

Mhatre MC, Mckenzie SE, Gonzalez LP. Diazepam during prior withdrawals does not alter seizure susceptibility during a subsequent withdrawal. Pharmacol Biochem Behav 2001;69:39–346.

Molleman A, Little HJ. Increases in non-NMDA glutaminergic transmission, but no change in GABAB transmission, during withdrawal from chronic ethanol treatment. J Pharmacol Exp Ther 1995;274:1035–41.

Moncrieff J, Drummond DC, Candy B, Chechinski K, Farmer R. Sexual abuse in people with alcohol problems: a study of the prevalence of sexual abuse and its relationship to alcohol drinking behavior. Br J Psychiatry 1996;169:355–60.

Monti PM, Miranda R, Nixon KJ, Sher KJ, Swartzwelder HS, Tabert SF, et al. Adolescence, booze, brains and behavior. Alcohol Clin Exp Res 2005;29: 207–20.

Mulholland PJ, Self RL, Harris BR, Little HJ, Littleton JM, Prendergast MA. Corticosterone increases damage and cytosolic calcium accumulation associated with ethanol withdrawal in rat hippocampal slice cultures. Alcohol Clin Exp Res 2005;29:871–81.

Mulholland PJ, Self RL, Hensley AK, Little HJ, Littleton JM, Prendergast MA. A 24 h corticosterone exposure exacerbates excitotoxic insult in rat hippocampal slice cultures independently of glucocorticoid receptor activation or protein synthesis. Brain Res 2006;1082:165–72.

Muma NA, Beck SG. Corticosteroids alter G protein inwardly rectifying potassium channels protein levels in hippocampal subfields. Brain Res 1999;839:331–5.

Na C, Lee Y-S. Alcohol urge and plasma beta-endorphin change after alcohol challenge with naltrexone pretreatment in social drinkers. Prog Neuropsychopharmacol Biol Psychiatry 2002;26:663–70.

Nagel BJ, Schweinsburg AD, PHan V, Tapert SF. Reduced hippocampal volume among adolescents with alcohol use disorders without psychiatric comorbidity. Psychiatry Res 2005;139:181–90.

Newcomer JW, Selke G, Melson AK, Hershey T, Craft S, Richards K, et al. Decreased memory performance in healthy humans induced by stress level cortisol treatment. Arch Gen Psychiatry 1999;56:527–33.

Obernier JA, White AM, Swartzwelder S, Crews FT. Cognitive deficits and CNS damage after a 4-day binge ethanol exposure in rats. Pharmacol Biochem Behav 2002;72:521–32.

O'Callaghan MJ, Croft AP, Jacquot C, Little HJ. The hypothalamopituitary–adrenal axis and alcohol preference. Brain Res Bull 2005;68:171–8.

O'Malley SS, Krishnan-Sarin S, Farren C, Sinha R, Kreek MJ. Naltrexone decreases craving and alcohol self-administration in alcohol-dependent subjects and activates the hypothalamo–pituitary–adrenocortical axis. Psychopharmacology 2002;160:19–29.

Orti E, Moses DF, Grillo C, De Nicola AF. Glucocorticoid regulation of glycerol phosphate dehydrogenase and ornithine decarboxylase activities in the spinal cord of the rat. J Neurochem 1987;48:425–31.

Overton P, Tong Z-Y, Brain PF, Clark D. Preferential occupation of mineralocorticoid receptors by corticosterone enhances glutamate-induced burst firing in rat midbrain dopaminergic neurons. Brain Res 1996;737: 146–54.

Packan DR, Sapolsky RM. Glucocorticoid endangerment of the hippocampus: tissue, steroid and receptor specificity. Neuroendocrinology 1990;51: 613–8.

Pardridge WM, Sakiyama R, Judd HL. Protein-bound corticosteroid in human serum is selectively transported into rat brain and liver in vivo. J Clin Endocrinol Metab 1983;57:160–5.

Pasad C, Pasad A. A relationship between voluntary alcohol preference and basal hypercortisolemia associated with attenuated rise in corticosterone output during stress. Alcohol 1995;12:59–63.

Peeters BW, Tonnaer JA, Groen MB, Broekkamp CL, van der Voort HA, Schoonen WG, et al. Glucocorticoid receptor antagonists: new tools to investigate disorders characterized by cortisol hypersecretion. Stress 2004;7: 233–41.

Phillips SC, Cragg BG. Alcohol withdrawal causes a loss of cerebellar Purkinje cells in mice. J Stud Alcohol 1984;45:475–80.

Piazza PV, La Moal M. Pathophysiology of vulnerability to drug abuse. Annu Rev Pharmacol 1996;36:359–78.

Piazza PV, La Moal M. The role of stress in drug self-administration. Trends Pharmacol Sci 1998;19:67–74.

Piazza PV, DeRoche V, Deminiere J-M, Maccari S, Le Moal N, Simon H. Corticosterone in the range of stress-induced levels possesses reinforcing properties: implications for sensation-seeking behaviors. Proc Natl Acad Sci 1993;90:11738–42.

Prendergast MA, Harris BR, Mulholland PJ, Blanchard II JA, Gibson DA, Holley RC, et al. Hippocampal CA1 region neurodegeneration produced by ethanol withdrawal requires activation of intrinsic polysynaptic hippocampal pathways and function of *N*-methyl-D-asparate receptors. Neuroscience 2004;124:869–77.

Pyapali GK, Turner DA, Wilson WA, Swartzwelder HA. Age and dose-dependent effects of ethanol on the induction of hippocampal long term potentiation. Alcohol 1999;19:107–11.

Ramirez JM. Hormones and aggression in childhood and adolescence. Aggress Violent Behav 2003;8:621–44.

Reul JM, De Kloet ER. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. Endocrinology 1985;117: 2505–11.

Rivier C. Adult male rats exposed to an alcohol diet exhibit a blunted adrenocorticotrophic hormone response to immune or physical stress: possible role for nitric oxide. Alcohol Clin Exp Res 1995;19: 1474–9.

Roberts AJ, Chu HP, Crabbe JC, Keith LD. Differential modulation by the stress axis of ethanol withdrawal seizure expression in WSP and WSR mice. Alcohol Clin Exp Res 1991;15:412–7.

Roberts AJ, Crabbe JC, Keith LD. Genetic differences in hypothalamic–pituitary–adrenal axis responsiveness to acute ethanol and acute ethanol withdrawal. Brain Res 1992;597:296–302.

Rosmalen JGM, Oldehinkel AJ, Ormel J, de Winter AF, Buitelaar JK, Verhulst FC. Determinants of salivary cortisol levels in 10–12 year old children; a population-based study of individual differences. Psychoneuroendocrinology 2005;30:483–95.

Rudolph JG, Walker DW, Iimuro Y, Thurman RG, Crews FT. NMDA receptor binding in adult rat brain after several chronic ethanol treatment protocols. Alcohol Clin Exp Res 1997;21:1508–19.

Salimov RM, McBride WJ, Sinclair JD, Lumeng L, Li TK. Performance in the cross-maze and slip funnel tests of four pairs of rat lines selectively bred for divergent alcohol drinking behavior. Addict Biol 1996;1:273–80.

Sapolsky RM. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocrinol Rev 1986;7:284–301.

Sapolsky RM. Potential behavioral modification of glucocorticoid damage to the hippocampus. Behav Brain Res 1993;57:175–82.

Sapolsky RM. Why stress is bad for your brain. Science 1996a;273:749–50.

Sapolsky RM. Stress, glucocorticoids and damage to the nervous system: the current state of confusion. Stress 1996b;1:1–19.

Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Arch Gen Psychiatry 2000;57:925–35.

Sapolsky RM, McEwen BS. Down-regulation of neural corticosterone receptors by corticosterone and dexamethasone. Brain Res 1985;339:161–5.

Schmidt M, Levine S, Oitzl MS, van der Mark M, Muller MB, Holsboer F, et al. Glucocorticoid receptor blockade disinhibits pituitary–adrenal activity during the stress hyporesponsive period of the mouse. Endocrinology 2005;146:1458–64.

Seckl JR. Glucocorticoids, ageing and nerve cell damage. J Neuroendocrinol 2000;12:711–4.

Shoal GD, Giancola PR, Kirillova GP. Salivary cortisol, personality, and aggressive behavior in adolescent boys: a 5-year longitudinal study. J Am Acad Child Adolesc Psychiatry 2003;42:1101–7.

Silveri MM, Spear LP. Characterizing the ontogeny of ethanol-associated increases in corticosterone. Alcohol 2004;32:145–55.

Simantov E, Schoen C, Klein JD. Health-compromising behaviors: why do adolescents smoke or drink? Identifying underlying risk and protective factors. Arch Pediatr Adolesc Med 2000;154:1025–33.

Sipp TL, Blank SE, Lee EG, Meadows GG. Plasma corticosterone response to chronic ethanol consumption and exercise stress. Proc Soc Exp Biol Med 1993;204:184–90.

Sircar R, Sircar D. Adolescent rats exposed to repeated ethanol treatment show lingering behavioral impairments. Alcohol Clin Exp Res 2005;29:1402–10.

Soderpalm AHV, Hansen S. Alcohol alliesthesia: food restriction increases the palatability of alcohol through a corticosterone-dependent mechanism. Physiol Behav 1999;67:409–15.

Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.

Spencer RL, Miller AH, McEwen BS, Blanchard RJ, Blanchard DC, Sakai RR. Chronic social stress produces reductions in available splenic Type II corticosteroid receptor plasma binding and corticosterone binding globulin levels. Psychoneuroendocrinology 1996;21:95–109.

Stoppini L, Buchs PA, Muller D. A simple method for organotypic cultures of nervous tissue. J Neurosci Meth 1991;57:173–82.

Sullivan EV, Marsh L, Mathalon DH, Lim KO, Pfefferbaum A. Anterior hippocampal volume deficits in nonamnesic, aging chronic alcoholics. Alcohol Clin Exp Res 1995;19:110–22.

Swartzwelder HS, Wilson WA, Tayyeb MI. Age-dependent inhibition of long term potentiation by ethanol in immature versus mature hippocampus. Alcohol Clin Exp Res 1995;19:320–3.

Tabakoff B, Jaffe RC, Ritzmann RF. Corticosterone concentrations in mice during ethanol drinking and withdrawal. J Pharm Pharmacol 1978;30: 371–4.

Takahashi T, Kimoto T, Tanabe N, Hattori TA, Yasumatsu N, Kawato S. Corticosterone acutely prolonged N-methyl-d-aspartate receptor-mediated

Ca2+ elevation in cultured rat hippocampal neurons. J Neurochem 2002;83: 1441–51.

Tennebaum B, Rowe W, Sharma S, Diorio J, Steverman A, Walker M, et al. Dynamic variations in plasma corticosteroid binding globulin and basal HPA activity following acute stress in adult rats. J Neuroendocrinol 1997;9: 163–8.

Thomas MP, Monaghan DT, Morrisett RA. Evidence for a causative role of N-methyl-D-aspartate receptors in an in vitro model of alcohol withdrawal hyperexcitability. J Pharmacol Exp Ther 1998;287:87–97.

Thomas JD, Garcia GG, Dominguez HD, Riley EP. Administration of eliprodil during ethanol withdrawal in the neonatal rat attenuates ethanol-induced learning deficits. Psychopharmacology 2004;175:189–95.

Vazques DM. Stress and the developing limbic–hypothalamic–pituitary adrenal axis. Psychoneuroendocrinology 1998;23:663–700.

Veatch LM, Gonzales LP. Nifedipine alleviates alterations in hippocampal kindling after repeated ethanol withdrawal. Alcohol Clin Exp Res 2000;24: 484–91.

Vengeliene V, Bachteler D, Danysz W, Spanagel R. The role of the NMDA receptor in alcohol relapse: a pharmacological mapping study using the alcohol deprivation effect. Neuropharmacology 2005;48:822–9.

Vescovi PP, DiGennaro C, Coiro V. Hormonal (ACTH, cortisol, β-endorphin and met-enkephalin) and cardiovascular responses to hyperthermic stress in alcoholics. Alcohol Clin Exp Res 1997;21:1195–8.

Viau V, Sharma S, Meaney MJ. Changes in plasma adrenocorticotrophin, corticosterone, corticosterone-binding globulin and hippocampal glucocorticoid receptor occupancy translocation in response to stress. J Neuroendocrinol 1996;8:1–8.

Walker EF, Walder DJ, Reynolds F. Developmental changes in cortisol secretion in normal and at-risk youth. Dev Psychopathol 2001;13:721–32.

Weiland NG, Orchinik M, Tanapat P. Chronic corticosterone treatment induces parallel changes in N-methyl-D-aspartate receptor subunit messenger RNA levels and antagonist binding sites in the hippocampus. Neuroscience 1997;78:653–62.

White AM, Ghia AJ, Levin ED, Schartzwelder HC. Binge pattern ethanol exposure in adolescent and adult rats: differential impact on subsequent responsiveness to alcohol. Alcohol Clin Exp Res 2000;24:1251–6.

Whittington MA, Little HJ. Changes in voltage-operated calcium channels modify ethanol withdrawal hyperexcitability in mouse hippocampal slices. Exp Physiol 1993;78:347–70.

Whittington MA, Lambert JDC, Little HJ. Increased NMDA-receptor and calcium channel activity during ethanol withdrawal hyperexcitability. Alcohol Alcohol 1995;30:105–14.

Williams KL, Broadbear JH, Woods JH. Noncontigent and response-contingent intravenous ethanol attenuates the effect of naltrexone on hypothalamic–pituitary–adrenal activity in rhesus monkeys. Alcohol Clin Exp Res 2001;28:566–71.

Xu L, Holscher C, Anwyl R, Rowan MJ. Glucocorticoid receptor and protein synthesis-dependent mechanisms underlie the control of synaptic plasticity by stress. Proc Natl Acad Sci 1998;95:3204–8.

Young AH, Gallagher P, Watson S, Del-Estal D, Owen BM, Ferrier IN. Improvements in neurocognitive function and mood following adjunctive treatment with mifepristone (RU-486) in bipolar disorder. Neuropsychopharmacology 2004;29:1538–45.

Zhou JZ, Zheng JQ, Zhang YX, Zhou JH. Corticosterone impaires cultured hippocampal neurons and facilitates Ca^{2+} influx through voltage-dependent Ca^{2+} channel. Acta Pharmacol Sin 2000;21:156–60.

Zou J-Y, Martinez DB, Neafsey EJ, Collins MC. Binge ethanol-induced brain damage in rats: effect of inhibitors of nitric oxide synthase. Alcohol Clin Exp Res 1996;20:1406–11.