CHAPTER

6

The Effects of Stress on Measures of Alcohol Drinking in Rodents

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OUTLINE

INTRODUCTION

Alcohol-use disorders are a serious problem worldwide, with an estimated 3–8% of global deaths attributed to alcohol abuse (Rehm et al., 2009). Consumption of alcohol is the third most burdensome risk factor in worldwide disability/disease, and unhealthy use of alcohol is the number one risk factor for death in men aged 15–59 (World Health Organization, 2011).

Although the causes of alcohol abuse and alcoholism are complex and still not well understood, one important factor is stress. There is a high comorbidity between depression/anxiety disorders and alcohol abuse/alcoholism (Hasin, Stinson, Ogburn, & Grant, 2007; Kessler et al., 1997; Regier et al., 1990). Also, acute bouts of stress can trigger relapse to alcohol use and abuse in previously abstinent alcoholics, although there are major individual differences in whether stress will increase or decrease the likelihood of relapse (Brown, Vik, Patterson, Grant, & Schuckit, 1995; Cooper, Russell, Skinner, Frone, & Mudar, 1992; Hore, 1971). In addition, the abuse of, use of, and dependence on alcohol can generate significant stress through creating interpersonal conflict and legal/occupational difficulties (Caetano & Cunradi, 2002), through withdrawal symptoms (Becker, 2000; De Witte, Pinto, Ansseau, & Verbanck, 2003), and through long-lasting changes in "prostress" brain mechanisms (Koob & Le Moal, 1997; Roberts, Heyser, Cole, Griffin, & Koob, 2000). This could create a "vicious cycle" in which alcohol abuse leads to more stress, and more stress leads to greater alcohol abuse.

Stressors activate a wide array of neural circuits, including the hypothalamic-pituitary-adrenal (HPA) axis and "extra-hypothalamic" systems. Cells in the paraventricular nucleus (PVN) of the hypothalamus release corticotropin-releasing factor (CRF), which in turn stimulates the anterior pituitary to release adrenocorticotropic hormone (ACTH). ACTH circulates systemically and induces increased corticosterone (CORT) release from the adrenal cortex. In addition to the important roles the HPA axis and other hypothalamic regions—such as the dorsomedial and arcuate nuclei—play in stress regulation, limbic structures such as the nucleus of the solitary tract, bed nucleus of the stria terminalis (BNST), dorsal raphe, and nuclei of the amygdala are also involved. Certain neurochemicals contained within these structures have been associated with the stress response and with the effects of stress on alcohol-related

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behaviors. These include CRF, norepinephrine, neuropeptide Y (NPY), endogenous opioids, serotonin, cholecystokinin (CCK), substance P, and hypocretin/orexin, all of which will be discussed in greater detail ahead.

This chapter will review what is currently known from the rodent-based preclinical literature about how stress and neural stress systems interact with the seeking and consumption of alcohol. We have divided the discussion into five sections that will cover 1) genetic factors that are associated with both increased/decreased anxiety and increased/decreased alcohol consumption; 2) the role and neurobiology of alcohol-induced withdrawal stress in increased drinking behavior; 3) current knowledge of the neuronal mechanisms underlying increased drinking in response to stress; 4) what has been learned about the motivation to seek alcohol from the stress-induced reinstatement model, in which stress precipitates the resumption of a response that previously earned an ethanol reward; and 5) the effects that exposure to alcohol can have on brain stress systems, long beyond the acute withdrawal period.

GENETIC DIFFERENCES IN STRESS SYSTEMS CONTRIBUTE TO ALCOHOL CONSUMPTION

Genetic factors are estimated to be responsible for between 30–60% of variation in alcohol consumption in the human population (Goldman, Oroszi, & Ducci, 2005; Heath, 1995). There have been attempts to model the genetic component of alcohol drinking behaviors in rodents; early studies demonstrated the role of genetic variation in alcohol drinking through lines of rats and mice selectively bred for their levels of alcohol consumption or preference for alcohol-containing solutions over water. By selecting the highest and lowest drinkers from a heterogeneous population and breeding individuals with a similar phenotype, it was possible to produce a number of pairs of rodent lines in which one line has a high preference for alcohol and the other does not (for a full review, see Crabbe, 2002). Such lines include alcohol-preferring Alko Alcohol (AA) and nonpreferring Alko non-Alcohol (ANA) rats (Eriksson, 1968), Indiana University alcohol-preferring (P) and nonpreferring (NP) rats (Murphy et al., 2002), high-alcohol-drinking (HAD) and low-alcohol-drinking (LAD) rats (Li, Lumeng, & Doolittle, 1993), Sardinian alcohol-preferring (sP) and nonpreferring (sNP) rats (Colombo, Lobina, Carai, & Gessa, 2006), and high-alcohol preferring (HAP1 and 2) and low-alcohol preferring (LAP1 and 2) mice (Grahame, Li, & Lumeng, 1999).

A similar approach can be taken to investigate how individual genetic differences in stress reactivity and anxiety contribute to alcohol consumption. For example, selective breeding for high or low anxiety-related behavior in the elevated plus maze led to the development of the high anxiety-related behavior (HAB) and low anxiety-related behavior (LAB) lines, respectively (Landgraf, 2003). Testing alcohol consumption levels in these types of rodent lines could offer insight into how inherent differences in anxiety and stress behaviors can influence drinking behaviors.

Examination of stress responses in alcohol-preferring lines of rodents has been used to investigate the link between stress and a propensity for alcohol consumption, although results from these studies vary among lines. In P/NP and sP/sNP rats, anxiety and stress responses are generally increased in the alcohol-preferring line. For example, both P and sP rats exhibit increased anxiety-like behavior, relative to their NP and sNP counterparts, in the elevated plus maze (Colombo et al., 1995; Hwang, Stewart, Zhang, Lumeng, & Li, 2004; Leggio et al., 2003; Richter, Zorrilla, Basso, Koob, & Weiss, 2000; Stewart, Gatto, Lumeng, Li, & Murphy, 1993). P rats also show increased anxiety-like behavior in a passive avoidance task (Stewart et al., 1993) and exhibit increased fear-potentiated startle compared to NP rats (McKinzie et al., 2000), whereas sP rats are more anxious in an open field (Roman et al., 2012).

Increased anxiety-like behavior and stress reactivity is not common to all alcohol-preferring lines, however. No such phenotypic differences are observed between HAD and LAD rats (Hwang et al., 2004; Roman et al., 2012), and AA rats actually display less anxiety-like behavior than their ANA counterparts on the elevated plus maze, Vogel conflict test, resident intruder test, and defensive burying test (Möller, Wiklund, Thorsell, Hyytiá, & Heilig, 1997; Roman et al., 2012; Sandbak, Murison, Sarviharju, & Hyytia, 1998; Tuominen, Hilakivi, Paivarinta, & Korpi, 1990). It is difficult to conclude from these varied results how a genetic propensity to stress/anxiety-like behavior might influence alcohol drinking. It seems likely that there is a complex relationship between the two, which may differ across different rodent models.

Rodents bred or screened for high levels of anxiety have been observed to consume more or less ethanol than their less-anxious counterparts. Elevated levels of alcohol intake and preference have been demonstrated in Wistar and Long Evans rats screened for high anxiety-like behavior on the elevated plus maze (Primeaux, Wilson, Bray, York, & Wilson, 2006; Spanagel et al., 1995). On the other hand, lower levels of ethanol intake were observed in highly anxious Fischer rats as compared to less anxious Wistar rats (Langen & Fink, 2004). Amongst various lines selectively bred for anxiety-like behavior, increased ethanol consumption is observed in the Maudsley reactive (MR) rats, a line bred for open-field defecation that has been used as a model of increased emotionality (Drewek & Broadhurst, 1979; Satinder, 1972).

Conversely, Roman low-avoidance (RLA) rats, which display increased anxiety-like behavior under stressful conditions, consume less alcohol than Roman high-avoidance (RHA) rats (Drewek & Broadhurst, 1979; Fernandez-Teruel et al., 2002; Razafimanalina, Mormede, & Velley, 1996; Satinder, 1972). Similarly, HAB rats consume less alcohol than LAB rats (Henniger, Spanagel, Wigger, Landgraf, & Holter, 2002).

In addition to behavioral differences in selectively-bred lines, studies of these animals have revealed variations in certain stress neuropeptide systems implicated in stress and ethanol-related behaviors (Heilig & Koob, 2007). For example, brain levels of CRF are lower in the hypothalamus, amygdala, and prefrontal cortex of P rats (Ehlers et al., 1992; Hwang et al., 2004) and increased in the central amygdala of sP rats (Richter et al., 2000). Expression of the type 1 CRF receptor (CRF_1) is also upregulated in sP rats (Hansson et al., 2006). However, no differences in CRF levels are observed between HAD and LAD rats, (Hwang et al., 2004). Levels of neuropeptide Y (NPY), an anxiolytic peptide expressed in the limbic system, are lower in multiple alcohol-preferring lines, including P rats, AA rats, and HAD rats (Caberlotto et al., 2001; Ehlers et al., 1998; Hwang, Zhang, Enters, Lumeng, & Li, 1999), with the exception of the anxious and alcohol-preferring RHA rats (Yilmazer-Hanke, Faber-Zuschratter, Linke, & Schwegler, 2002).

Quantitative trait locus (QTL) analysis in both outbred and selectively-bred animals has also been helpful in identifying genetic targets that may influence alcohol consumption. QTL analyses, which serve to localize genomic regions associated with ethanol-related behaviors, have identified a region on chromosome 4 that influences alcohol drinking in NP and P rats (Carr et al., 1998). An overlapping QTL was found in a population derived from the low alcohol-drinking Wistar-Kyoto and high alcohol-drinking high-ethanol-preferring lines (Terenina-Rigaldie, Jones, & Mormede, 2003). Interestingly, two of the specific genes located within the identified region code for the type 2 CRF receptor (CRF_2) and NPY (Spence et al., 2009), further hinting at the role for these neuropeptide systems in alcohol consumption. Indeed, further study of the chromosome 4 QTL confirmed differential NPY gene expression in the amygdala of inbred P and NP rats (Suzuki, Lumeng, McBride, Li, & Hwang, 2004).

The stress-related mechanisms identified through selective breeding and QTL analysis have also been investigated using transgenic technology in mice. Targeted deletion of the CRF_1 receptor in mice results in reduced ethanol consumption, specifically at a high ethanol concentration (20%; Pastor et al., 2011). CRF_1 knockout mice also fail to escalate ethanol self-administration after induction of dependence (Chu, Koob, Cole, Zorrilla, & Roberts, 2007). Deletion of CRF_2, on the other hand, results in minimal changes in ethanol preference and consumption (Sharpe et al., 2005). Consistent with endogenous expression of the peptide in alcohol-preferring lines, genetic knockout of NPY increases ethanol consumption, whereas overexpression reduces drinking (Thiele, Marsh, Marie, Bernstein, & Palmiter, 1998; Thiele, Miura, Marsh, Bernstein, & Palmiter, 2000). Deletion of the NPY Y1 receptor also increases ethanol consumption, indicating a specific role for this receptor subtype (Thiele, Koh, & Pedrazzini, 2002).

In sum, although the results from selectively-bred lines remain somewhat ambiguous, approaches such as QTL analysis and the generation of transgenic mice support a role for the CRF and NPY systems in alcohol-related behavior. NPY in particular seems to be important in decreasing consumption, with higher endogenous levels of the peptide conferring protection against drinking. This effect may be related to NPY's ability to mitigate the anxiogenic-like effects of alcohol withdrawal, which, as will be discussed ahead, have an important role in promoting drinking.

RELATIONSHIP BETWEEN WITHDRAWAL AND ALCOHOL CONSUMPTION

Prolonged drinking leads to the development of neuroadaptations in the brain's reward and stress circuits, and abstinence from alcohol consequently triggers a withdrawal syndrome. In the first few days of abstinence, an individual may experience tremors, convulsions, restlessness, insomnia, sweating, and increased heart rate (Bayard, Mcintyre, Hill, & Woodside Jr, 2004). Affective dysregulation, including signs of anxiety, anhedonia, and irritability, also develops and can persist for long periods of time (Heilig, Egli, Crabbe, & Becker, 2010; Heilig & Koob, 2007). Both the acute and chronic affective consequences of withdrawal may contribute to relapse by triggering craving for the drug or providing negative reinforcement once relapse has occurred (Heilig et al., 2010; Koob & Volkow, 2009). Additionally, abstinence from alcohol is associated with long-term increases in stress sensitivity (Heilig & Koob, 2007; Sinha, 2007), which can contribute to phenomena such as stress-induced reinstatement (see "Stress-Induced Reinstatement" ahead).

Induction of alcohol withdrawal in rodents can be produced by removing access to alcohol after sufficient high levels of alcohol exposure. Exposure periods can range from acute (one or a few administrations) or chronic, which can be on the order of days to months. Modes of alcohol exposure vary, including injection, oral administration, or inhalation of ethanol vapor. Although alcohol withdrawal produces both somatic (e.g., convulsions, hypothermia, or body tremors) and affective signs of withdrawal, the latter have been implicated in

motivating drug intake and will therefore be the focus here. For example, the negative affective consequences of withdrawal can be assessed with standard rodent assays of anxiety, aversion, and depression. In rats, ethanol withdrawal causes both conditioned place aversion (Morse, Schulteis, Holloway, & Koob, 2000) and reductions in intracranial self-stimulation (ICSS) thresholds (Schulteis, Markou, Cole, & Koob, 1995), two common measures of the motivational consequences of withdrawal. Withdrawal-induced anxiety-like behavior has been demonstrated with the elevated plus-maze test (e.g., Baldwin, Rassnick, Rivier, Koob, & Britton, 1991), acoustic startle (e.g., Rassnick, Koob, & Geyer, 1992), ultrasonic vocalizations (e.g., Knapp, Duncan, Crews, & Breese, 1998), and the social interaction test (e.g., Overstreet, Knapp, & Breese, 2002). Depression-related behaviors have also been observed during ethanol withdrawal using endpoint measures such as the forced swim test (Walker et al., 2010).

In rats trained to drink ethanol, an increase in ethanol consumption and preference following periods of abstinence has been termed the alcohol-deprivation effect (e.g., Sinclair & Senter, 1967; Spanagel & Hölter, 1999). Following a period of deprivation, rodents consume more alcohol and at higher concentrations in a free-choice drinking paradigm (e.g., Spanagel & Hölter, 1999; Vengeliene et al., 2003), increase alcohol intake in an operant task, and increase breakpoints for alcohol in a progressive ratio test (e.g., Brown, Jackson, & Stephens, 1998; Hölter, Landgraf, Zieglgänsberger, & Spanagel, 1997). Passive administration of ethanol, for example, through exposure to ethanol vapor or intragastric infusions, can also induce dependence and promote consumption during withdrawal, and these effects are most robust when exposure is intermittent, resulting in repeated withdrawal episodes (e.g., Becker & Lopez, 2004; Cunningham, Fidler, Murphy, Mulgrew, & Smitasin, 2012; Griffin, Lopez, Yanke, Middaugh, & Becker, 2009; Lopez & Becker, 2005; O'Dell, 2004).

The alcohol-deprivation effect can be observed following deprivation periods ranging from three days to nine months (Spanagel & Hölter, 1999; Wolffgramm & Heyne, 1995), but it is typically more robust following prolonged periods of ethanol exposure (e.g., Heyser, Schulteis, & Koob, 1997). Acute, repeated deprivation exposure can also be effective; for example, rats provided with ethanol only every other day escalate their intake rapidly compared to periods in which ethanol is continuously available (Wayner & Greenberg, 1972). Repeated, daily withdrawal from chronic ethanol-vapor exposure also increases both home-cage drinking and self-administration of ethanol (Becker & Lopez, 2004; Griffin et al., 2009; Lopez, Anderson, & Becker, 2008; Lopez & Becker, 2005; Roberts, Cole, & Koob, 1996; Roberts et al., 2000). The effectiveness of long deprivation periods, during which somatic signs of withdrawal are no longer present, is particularly important because it suggests that long-lasting affective signs are likely driving escalated intake (Roberts et al., 2000).

Although a direct link between relief of anxiety and alcohol consumption has been difficult to establish, it has long been hypothesized that increased intake during withdrawal serves to relieve negative affect induced by abstinence. This idea is supported by the observation of affective signs of withdrawal in rodents following prolonged alcohol drinking or other forms of chronic exposure (e.g., Hölter et al., 1998; Rassnick, Heinrichs, Britton, & Koob, 1993; Rassnick et al., 1992; Schulteis et al., 1995), and the finding that escalated intake during abstinence is observed only when the animal has the opportunity to associate drinking with the relief of withdrawal (Hunter, Walker, & Riley, 1974; Roberts et al., 1996; Roberts et al., 2000).

The experience of negative affective symptoms during alcohol withdrawal may be the result of increased stress-system activation during abstinence. The case for increased CRF signaling as a mediator of withdrawal-induced anxiety-like behavior and ethanol consumption is particularly strong. Microdialysis studies have revealed increased release of CRF in the central amygdala and BNST during ethanol withdrawal (Olive, Koenig, Nannini, & Hodge, 2002; Pich et al., 1995), as have immunohistochemical investigations of CRF levels (Zorrilla, Valdez, & Weiss, 2001). Blockade of CRF receptors has been shown to block signs of ethanol withdrawal (Rassnick et al., 1993; Valdez, Zorrilla, Roberts, & Koob, 2003) and of increased drinking during ethanol deprivation (Chu et al., 2007; Finn et al., 2007; Hwa, DeBold, & Miczek, 2013; Roberto et al., 2010; Valdez et al., 2002). CRF_1 knockout mice also fail to show escalations in drinking during abstinence (Chu et al., 2007).

A number of studies also support a role of NPY in withdrawal-induced anxiety and ethanol drinking. NPY protein and mRNA is decreased during withdrawal (Bison & Crews, 2003; Olling et al., 2007; Roy & Pandey, 2002; Walker et al., 2010), which is consistent with studies demonstrating increased ethanol consumption following blockade of NPY signaling (Thiele et al., 1998; Thiele et al., 2000; Thiele, Harding, Juzytsch, Fletcher, & Shaham, 2002). Intracranial infusion or viral overexpression of NPY can also reduce signs of ethanol withdrawal (Woldbye, Ulrichsen, Haugbøl, & Bolwig, 2002) and reduce abstinence-induced intake (Gilpin, Stewart, Murphy, Li, & Badia-Elder, 2003; Thorsell et al., 2007).

In many ways, CRF and NPY have opposing influences on behavior, and their combined actions have been proposed to be an important regulator of alcoholic behavior. Koob and colleagues have proposed that CRF mediates anxiety and increases stress sensitivity during withdrawal and that compensatory ethanol consumption relieves this state through the actions of NPY (Valdez & Koob, 2004). Indeed, NPY and alcohol have similar

electrophysiological and behavioral effects in rodents (Badia-Elder et al., 2001; Ehlers, Somes, & Cloutier, 1998). Finally, it is interesting to note that these two peptides also mediate the individual differences in genetic vulnerability for alcohol consumption discussed previously. It therefore seems likely that genetic differences in alcohol intake are at least partially controlled by individual differences in withdrawal severity and the ability of alcohol to serve as a negative reinforcer.

EFFECTS OF STRESS ON ALCOHOL CONSUMPTION/INTAKE

Exogenous stressors affect drinking behavior or self-administration of ethanol (Becker, Lopez, & Doremus-Fitzwater, 2011; Boyce-Rustay, Janos, & Holmes, 2008). However, although the relationship between endogenous stress and ethanol consumption is strongly mediated by CRF and NPY, the effects discussed ahead recruit a much wider range of stress-related circuitry. We will use the term "stress-induced alcohol drinking" to refer to the increase in ethanol drinking above baseline that occurs after exposure to an exogenous stressor, although some baseline alcohol drinking is generally present. The types of stressors employed vary and include footshock, isolation housing, restraint, social defeat, and forced swim. An additional method of inducing stress is to give injections of the alpha-2 adrenoceptor antagonist yohimbine, which causes increased self-reports and behavioral signs of anxiety in humans (Charney, Heninger, & Redmond, 1983; Holmberg, Gershon, & Beck, 1962). In addition, because animals can often habituate to chronic stress, several chronic unpredictable stress (CUS) paradigms have been developed in which the animals are exposed to different stressors one at a time in an unpredictable order. Stressors employed in CUS protocols vary: for example, forced swim, a rocking/vibrating cage, open-field or novel-object exposure, injection stress, continuous light exposure, white noise exposure, and restraint (Sacharczuk et al., 2008).

A recent literature review by Becker and colleagues (Becker, Lopez, & Doremus-Fitzwater, 2011) estimates that acute or subchronic stress causes increases in drinking less than 50% of the time, with the remainder causing no change or a decrease in drinking. Across a wide variety of stressors, including footshock stress, restraint stress, social defeat, forced-swim stress, and isolation housing, there were no acute stressors that increased drinking in more than 50% of the studies reviewed. On the other hand, this literature review found that chronic stress was more likely to increase drinking, especially if given during adolescence, although there are still several examples of studies in which these increases were not seen (Becker et al., 2011).

The wide-ranging effects of stress on drinking behavior is surprising, but can most likely be attributed to a number of factors, including the genetic background of the animal, individual differences in ethanol preference, and the nature of the stressor. For example, footshock increased alcohol drinking over the next 23 hours in C57BL/6J mice but not in DBA/2J or A/J mice (Matthews et al., 2008), and social defeat increased drinking in C57BL/6J mice but not CBA/Lac mice (Kudryavtseva, Madorskaya, & Bakshtanovskaya, 1991). Rats bred for increased ethanol consumption generally demonstrate increases in drinking following stress (but see Chester, Blose, Zweifel, & Froehlich, 2004). Stressors that have been shown to increase drinking in the alcohol-preferring lines include footshock (HAD, P, and AA rats), immobilization stress (P rats), maternal separation from PND 1-21 (AA rats), and 16.5 weeks of isolation housing (P rats; Chester et al., 2004; Ehlers, Walker, Pian, Roth, & Slawecki, 2007; Roman, Gustafsson, Hyytia, & Nylander, 2005; Vengeliene et al., 2003). On the other hand, footshock stress increased alcohol preference in outbred rats with a low preference for ethanol and decreased it in rats with a high preference (Volpicelli, Ulm, & Hopson, 1990). Interestingly, forced-swim stress did not increase drinking in HAD, P, or AA rats or in C57BL/6J mice, though it did in outbred Wistars (Lowery, Sparrow, Breese, Knapp, & Thiele, 2008; Vengeliene et al., 2003; Boyce-Rustay, Cameron, & Holmes, 2007). Restraint stress also failed to increase drinking in C57BL/6J mice, though it did in 129/SvEv mice (Yang, Wang, Rice, Munro, & Wand, 2008). Finally, it is worth noting that stress has anhedonic effects that can oppose the motivational properties of ethanol and other reinforcers. Some of the variability in its effects is therefore likely due to nonspecific effects on the animals' behavior.

Although stress has variable effects on ethanol consumption, here we will focus on the neurobiological factors that enable stress to increase ethanol drinking, and those systems for which multiple studies (>1) have been conducted. Not surprisingly, the neural systems discussed ahead are all known for their involvement in the behavioral effects of stress. These systems include the stress-related neuropeptides CRF and cholecystokinin (CCK), the opioid and serotonergic systems, and benzodiazepines. Inhibition of any of these systems appears to attenuate to some extent the effects of stress exposure on ethanol consumption in rodents.

Generally, blockade of CRF_1 receptors prevents stress-induced alcohol drinking. This effect has been seen with receptor antagonists CP-154,526 (Lowery et al., 2008) and antalarmin for ethanol self-administration (Marinelli et al., 2007; but see Yang et al., 2008, for a lack of effect of antalarmin and R121919) as well as in CRF_1 knockout mice (Molander et al., 2012). Interestingly, decreases in drinking were only observed when CRF_1 knockout was

restricted to the CNS; global CRF_1 knockout either increased or had no effect on alcohol intake after stress (Molander et al., 2012; Sillaber et al., 2002). The effects of global CRF_1 knockout may be due to compensatory changes in the functioning of the HPA axis, as manipulations that increase ACTH and CORT can also cause an increase in baseline (Fahlke & Hansen, 1996; Fahlke & Hansen, 1999; Fahlke, Hård, Hansen, Eriksson, & Engel, 1995) and stress-induced drinking (Mutschler et al., 2010).

Another neuropeptide that, like CRF, is released in the limbic system in response to stress and promotes behavioral signs of anxiety in rodents is CCK (Bowers, Choi, & Ressler, 2012). Antagonism of the CCK_B receptor prevents increases in alcohol preference following both social defeat and injection stress (Croft, Brooks, Cole, & Little, 2005; Little et al., 1999). The dynorphin/kappa opioid system also facilitates stress-induced drinking, which is consistent with its effects on drinking in abstinent rats (Walker & Koob, 2007). Systemic injection of the kappa opioid receptor (KOR) agonist U-50,488H increased alcohol consumption following social defeat stress in C57BL/6J mice (Kudryavtseva, Gerrits, Avgustinovich, Tenditnik, & Van Ree, 2006), whereas the KOR antagonist nor-BNI and knockout of the prodynorphin-gene (Sperling, Gomes, Sypek, Carey, & McLaughlin, 2010) prevented the effects of stress on increased ethanol preference. Blockade of the serotonergic system has a similar effect. Decreasing brain serotonin levels with systemic injection of the tryptophan hydroxylase inhibitor p-chlorophenylalanine decreased alcohol drinking in rats after random shock exposure (Myers & Cicero, 1969). Stress-induced increases in ethanol self-administration are also decreased by the $5\text{-}HT_{1A}$-receptor antagonist WAY 100,635 (Le, Funk, Harding, Juzytsch, & Fletcher, 2009) and systemic injections of the antidepressant nefazodone (primarily a $5\text{-}HT_{2A}$ receptor antagonist; Nunez et al., 2002) decrease stress-induced drinking.

Finally, benzodiazepines can reduce stress-induced increases in drinking, although this effect is inconsistent. Both alprazolam and diazepam prevent increases in drinking following isolation stress in rats, and the latter was blocked by systemic injections of the GABA-A-receptor antagonist bicuculline (Mediratta, Mahajan, Sharma, Bhandari, & Dubey, 2003; Nunez et al., 1999). On the other hand, diazepam did not prevent stress-induced increases in alcohol preference following social defeat or injection stress in mice (Croft et al., 2005; Little et al., 1999).

In sum, investigations into the effects of stress on ethanol consumption have revealed that stress can increase intake, though it does not always do so consistently, and the mechanisms by which this is accomplished can be quite variable. Defining robust rodent models for the development of pharmacological interventions for alcoholism that mitigate stress therefore clearly presents a challenge.

STRESS-INDUCED REINSTATEMENT

Relapse is unfortunately a common occurrence in alcoholics, and stress is known to be a common contributing factor to this phenomenon (Sinha, 2007). Reinstatement of drug seeking has been used as an animal model of relapse for alcohol and other drugs of abuse (Shaham, Shalev, Lu, de Wit, & Stewart, 2003). In the rodent reinstatement model, the subject is trained to self-administer a drug or nondrug reward, usually by lever pressing. The response is then extinguished by allowing the subject to make the response without earning any drug, such that drug-seeking behavior is reduced to minimal levels. At this point, several manipulations can cause responding to return, including delivery of a small sample of the reward (drug or nondrug prime induced), environmental or discrete cues previously associated with drug availability (cue induced) or stress (stress induced; Epstein, Preston, Stewart, & Shaham, 2006; Nair, Adams-Deutsch, Epstein, & Shaham, 2009). The reinstatement model is widely considered to be a valid animal model of relapse to seek drug and nondrug rewards, because it causes relapse under similar circumstances to those that cause relapse in clinical populations, and reinstatement for palatable food is reduced by a pharmacological agent that is effective in human dieting (Epstein et al., 2006; Pickens et al., 2012; but see Katz & Higgins, 2003). Stress caused by footshock or the pharmacological stressor yohimbine are powerful stimuli for inducing reinstatement to ethanol seeking (Le, Harding, Juzytsch, Funk, & Shaham, 2005; Le et al., 1998).

Notably, the neurobiology of stress-induced reinstatement of alcohol seeking differs in many ways from the neurobiology that underlies reinstatement induced by other stimuli. For example, systemic injections of the μ-opioid-receptor antagonist naltrexone decrease reinstatement of alcohol seeking caused by oral injection of ethanol but have no effect on footshock-induced reinstatement, whereas injections of the selective serotonin reuptake-inhibitor fluoxetine decrease footshock-induced reinstatement but have no effect on alcohol prime-induced reinstatement (Le et al., 1999). Similarly, manipulations that affect stress-induced but not cue-induced reinstatement include antagonism of CRF receptors using the nonselective CRF receptor antagonist D-Phe-CRF, antagonism of neurokinin (NK) 1 receptors using the NK1 receptor antagonist L822429, and antagonism of the peroxisome proliferator-activated receptor-gamma (PPARγ) receptor with pioglitazone (Liu & Weiss, 2002; Schank et al., 2011; Stopponi et al., 2011).

Conversely, naltrexone, the KOR antagonist JDTic, and the melanin-concentrating hormone type 1-receptor antagonist GW803430 decrease cue-induced reinstatement but have no effect on footshock-induced reinstatement (Bienkowski, Kostowski, & Koros, 1999; Cippitelli et al., 2010; Liu & Weiss, 2002; Schank et al., 2012).

Investigations into the neurobiology of stress-induced reinstatement to seek alcohol have primarily focused on the role of stress neuropeptides and hormones, especially CRF. However, other neuropeptides, hormones, and neurotransmitters are also involved in stress-induced reinstatement. These include molecules involved in stress, such as the anxiogenic neuropeptide substance P and its preferred NK1 receptor, the glucocorticoid stress hormones, and the noradrenergic system as well as molecules more generally involved in reinstatement beyond that caused by stress, such as orexin/hypocretin (Shalev, Erb, & Shaham, 2010; Sinha, Shaham, & Heilig, 2011).

The effects of CRF in reinstatement appears to be CRF_1-receptor mediated, because nonspecific CRF-receptor antagonists (ventricular d-Phe-CRF; Le et al., 2000; Liu & Weiss, 2002) and CRF_1-receptor antagonists (systemic MTIP and CP-154,526; Gehlert et al., 2007; Le et al., 2000) as well as systemic injections of the CRF_1-receptor antagonist antalarmin in mSP rats but not Wistar rats (Hansson et al., 2006) decrease footshock-stress-induced reinstatement. Systemic injections of antalarmin also decrease yohimbine-stress-induced reinstatement (Marinelli et al., 2007). One site of CRF's action in stress-induced reinstatement is in the median raphe nucleus, as d-Phe-CRF injections into the median raphe block footshock-induced reinstatement (Le et al., 2002).

Systemic administration of the NK1 receptor antagonist L822429 decreases footshock-induced reinstatement while having no effect on cue-induced reinstatement (Schank et al., 2011). Yohimbine-induced reinstatement of alcohol seeking was dose-dependently decreased by systemic injections of the glucocorticoid-receptor antagonist mifepristone or by intra-CEA, but not intra-BLA, infusion of mifepristone (Simms, Haass-Koffler, Bito-Onon, Li, & Bartlett, 2012). In addition, ventricular injections of the anxiolytic neuropeptide nociceptin (orphanin FQ) decrease footshock-induced reinstatement of ethanol seeking (Martin-Fardon, Ciccocioppo, Massi, & Weiss, 2000), although it is unclear whether nociceptin plays a role in stress-induced reinstatement at normal physiological levels. Conversely, ventricular injections of JNJ-31020028, an antagonist of the NPY-Y2 presynaptic autoreceptor that would serve to disinhibit release of endogenous NPY, had no significant effect on footshock-induced reinstatement of ethanol seeking (Cippitelli et al., 2011). Finally, the hypocretin-1-receptor antagonist SB334867 decreased yohimbine-induced reinstatement of ethanol seeking (Richards et al., 2008). However, this drug also decreases reinstatement to alcohol seeking caused by a contextual (compound auditory/olfactory) cue, and the effects of SB334867 on stress-induced reinstatement for other rewards (e.g., food and oral sucrose) is mixed (Nair, Golden, & Shaham, 2008; Richards et al., 2008), suggesting that hypocretin-1-receptor activity is required more generally for reinstatement of ethanol seeking rather than specifically for stress (Martin-Fardon & Weiss, 2012).

The adrenergic system is also involved in stress-induced reinstatement. Systemic injections of the alpha-2-receptor agonist clonidine, the alpha-1 adrenoceptor-antagonist prazosin, and the alpha-2 adrenoceptor-agonist guanfacine decrease yohimbine-induced reinstatement of ethanol seeking (Le et al., 2009, 2011). In addition, systemic injections of the alpha-2 adrenoceptor-agonist lofexidine or the alpha-1 adrenoceptor-antagonist prazosin decrease footshock-induced reinstatement (Le et al., 2005, 2011), demonstrating that the effects of noradrenergic drugs on yohimbine-induced reinstatement are on stress mechanisms and not merely counteracting the adrenergic pharmacological effects of yohimbine.

It is interesting to note that the neural systems involved in stress-induced reinstatement do not completely overlap with those that mediate the effects of stress on levels of alcohol consumption (Figure 6.1). Although the former paradigm is based on resumption of behavior during a period of drug unavailability, the effects of stress on consumption are generally measured with bottle-drinking paradigms in which ethanol is made freely available. The observed differences may therefore represent differences in how stress affects ethanol seeking in the presence versus the absence of reinforcement or in effects of the various neural systems on the pharmacological effects of ethanol itself.

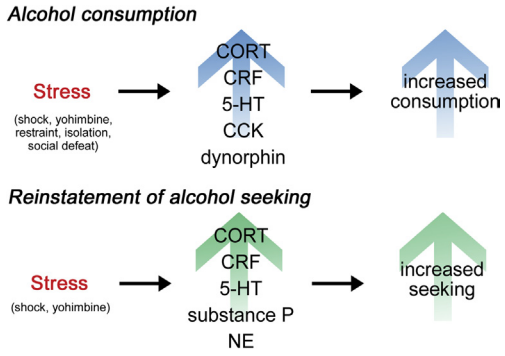

FIGURE 6.1 Neural stress systems involved in stress-induced consumption and reinstatement of alcohol seeking. Stress increases alcohol consumption and a previously extinguished alcohol-seeking response, but the neural systems mediating these effects do not completely overlap. This may represent differences in how stress affects ethanol-seeking in the presence vs. the absence of reinforcement or how various neural systems participate in the pharmacological effects of ethanol itself.

EFFECTS OF ETHANOL EXPOSURE ON STRESS-RELATED BEHAVIORS

As discussed previously, the ability of stress to influence drinking behavior is well documented. What have been less studied in animal models are the effects of ethanol on the functioning of stress systems and the expression of stress-related behaviors. The majority of the work that does exist on this topic has focused on the prenatal period, as fetal exposure to ethanol is a serious problem in human populations. Although work is limited, robust effects have been found, highlighting the need for additional research into how moderate to severe ethanol exposure, both *in utero* and during adulthood, may increase the risk for stress-related diseases (Hellemans, Sliwowska, Verma, & Weinberg, 2010; Kushner, Abrams, & Borchardt, 2000)

Acute and chronic ethanol exposure activates neural stress systems. Ethanol increases activity in the HPA axis, as demonstrated by increased blood levels of ACTH and CORT and increased synthesis of CRF and vasopressin by the paraventricular nucleus of the hypothalamus (Rivier, Imaki, & Vale, 1990). With repeated exposure, tolerance to these effects develops, resulting in normalized hormonal levels during exposure but impaired HPA-axis functioning during abstinence (Rasmussen et al., 2000; Richardson, Lee, O'Dell, Koob, & Rivier, 2008; Zhou et al., 2000; Zorrilla et al., 2001). Acute ethanol exposure during adulthood also activates noradrenergic and adrenergic nuclei in the brainstem, and this effect has been proposed to regulate the effects on HPA-axis functioning (Allen, Lee, Koob, & Rivier, 2011; Lee, Craddock, & Rivier, 2011). These effects on baseline HPA activity (CORT and CRF levels) have also been observed in adult rats exposed to ethanol during adolescence (Allen et al., 2011; Przybycien-Szymanska, Mott, Paul, Gillespie, & Pak, 2011). Prenatal alcohol exposure, on the other hand, results in increased HPA activity at baseline and hyper-responsiveness to stressors such as restraint and footshock (see Hellemans et al., 2010, for a full review).

In contrast to the blunted HPA sensitivity seen following chronic alcohol exposure, extra-hypothalamic systems are sensitized by these regimens. For example, increased levels of CRF and CRF release are observed in alcohol-dependent animals during abstinence (Olive et al., 2002; Pich et al., 1995; Zorrilla et al., 2001). Some of these effects may be mediated by interactions with the HPA axis, as initially high levels of CORT could induce upregulation of CRF mRNA in extended amygdala structures (Imaki, Nahan, Rivier, Sawchenko, & Vale, 1991; Makino, Gold, & Schulkin, 1994; Shepard, Barron, & Myers, 2000).

The effects of ethanol on stress-system functioning contribute to many of the behavioral effects discussed throughout this review, including the tendency for withdrawal and exogenous stressors to increase drinking behavior and induce reinstatement. But stress system sensitization also has long-lasting behavioral effects beyond ethanol drinking. For example, prenatal ethanol exposure increases anxiety behavior in adulthood (Brocardo et al., 2012; Cullen, Burne, Lavidis, & Moritz, 2013; Ohta, Sakata-Haga, & Fukui, 2012; Zhou, Wang, & Zhu, 2010). Recently, some of these behavioral effects have been associated with increased dendritic spine length and neuronal hyperexcitability in the basolateral amygdala (Cullen et al., 2013; Zhou et al., 2010). Decreases in the serotonergic cells in the midbrain raphe nuclei have also been observed in anxious, prenatally exposed rats (Ohta et al., 2012). Prenatal ethanol exposure was also found to decrease contextual fear conditioning in adult Long Evans rats, although this may be due to a more general deficit in hippocampal-dependent learning (Savage et al., 2010) rather than long-term effects on functioning of stress systems.

The effects of exposure on stress-system functioning outside of the prenatal period are just beginning to be explored. Binge-like alcohol drinking in adolescent rats reduced baseline anxiety on the elevated plus maze in adulthood (Gilpin, Karanikas, & Richardson, 2012). In adult mice, chronic intermittent ethanol exposure reduced extinction of fear through impairment of medial prefrontal cortex functioning (Holmes et al., 2012). The results of this latter study suggest that adult alcoholics may be more susceptible to stress-related diseases (e.g., posttraumatic stress disorder) than the general population. This hypothesis is consistent with the blunted cortisol response characteristic of both abstinent alcoholics and PTSD patients (Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000; Vescovi, DiGennaro, & Coiro, 1997; Yehuda, Kahana, Binder-Brynes, & Southwick, 1995; Yehuda et al., 1990). Continued work on this topic will be critical, especially since changes in the functioning of neural stress systems may have long-lasting consequences for the progression of and recovery from alcohol dependence.

CONCLUSIONS

Stress systems interact with ethanol exposure in a number of ways, but why does activation of these systems promote ethanol seeking and consumption in rodents? The answer may lie in ethanol's potent acute anxiolytic properties. It has long been hypothesized that individuals drink to relieve stress and anxiety brought on by external events, psychiatric disorders, or the experience of withdrawal (Heilig et al., 2010; Young, Oei, & Knight, 1990). This relief may come through ethanol's recruitment of anxiety-reducing systems, such as GABA and NPY. One scheme suggests that as dependence develops stress systems become more dysregulated, the ability of stress to promote drinking behavior is exacerbated, and

TABLE 6.1 Involvement of Neural Stress Systems in Alcohol Consumption and Seeking

Behavior Stress Systems		
Genetic factors	↑ or ↓ drinking	CRF, NPY
Stress-induced drinking*	↑ or ↓ drinking	CORT, CRF, 5-HT, CCK, DYN
Withdrawal-induced drinking	↑ drinking	CRF, NPY
Stress-induced reinstatement	↑ drinking	CORT, CRF, 5-HT, substance P, NE

Neural systems listed are involved in increased drinking following stress. See text for further discussion.
Abbreviations: cholecystokinin (CCK), corticosterone (CORT), corticotropin-releasing factor (CRF), dynorphin (DYN), neuropeptide Y (NPY), norepinephrine (NE), serotonin (5-HT).

increasing amounts of alcohol are necessary to achieve anxiolysis (Koob, 2003; Heilig et al., 2010).

It is not surprising that genetic differences in the functioning of stress systems as well as changes in these systems following exposure to stressful life events are both important in determining an individual's susceptibility to alcoholism (Enoch, 2006). Genetic differences in the CRF and NPY systems are involved in determining an individual's preference for ethanol and also play a large role in the emotional signs and motivational relevance of ethanol withdrawal. Individual differences in these two stress systems consequently play a large role in determining susceptibility to alcohol abuse.

The ability of exogenous stressors to increase consumption levels and promote relapse, on the other hand, appears to be less individualized and recruits a much broader range of stress-related mechanisms (Table 6.1). Stress-induced activation of CRF and the HPA axis as well as other stress-related neurotransmitters such as CCK, serotonin, and the kappa opioid system can all increase ethanol consumption. Similarly, CRF, glucocorticoids, serotonin, hypocretin/orexin, substance P, and norepinephrine all promote stress-induced reinstatement of ethanol seeking. This illustrates the daunting complexity of the relationship between stress and alcohol abuse but also offers hope that one or more of the multiple therapeutic target systems could potentially lead to the development of novel drugs that support the initiation and maintenance of abstinence in alcoholics.

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