Published in final edited form as:

Alcohol Clin Exp Res. 2016 June ; 40(6): 1202–1214. doi:10.1111/acer.13075.

Adolescent Social Isolation as a Model of Heightened Vulnerability to Comorbid Alcoholism and Anxiety Disorders

Tracy R. Butler, Anushree N. Karkhanis, Sara R. Jones, and Jeffrey L. Weiner

Department of Psychology (TRB), University of Dayton, Dayton, Ohio; and Department of Physiology and Pharmacology (ANK, SRJ, JLW), Wake Forest School of Medicine, Winston Salem, North Carolina

Abstract

Individuals diagnosed with anxiety-related illnesses are at increased risk of developing alcoholism, exhibit a telescoped progression of this disease and fare worse in recovery, relative to alcoholics that do not suffer from a comorbid anxiety disorder. Similarly, preclinical evidence supports the notion that stress and anxiety represent major risk factors for the development of alcohol use disorder (AUD). Despite the importance of understanding the link between anxiety and alcoholism, much remains unknown about the neurobiological substrates underlying this relationship. One stumbling block has been the lack of animal models that reliably reproduce the spectrum of behaviors associated with increased vulnerability to these diseases. Here, we review the literature that has examined the behavioral and neurobiological outcomes of a simple rodent adolescent social isolation procedure and discuss its validity as a model of vulnerability to comorbid anxiety disorders and alcoholism. Recent studies have provided strong evidence that adolescent social isolation of male rats leads to the expression of a variety of behaviors linked with increased vulnerability to anxiety and/or AUD, including deficits in sensory gating and fear extinction, and increases in anxiety measures and ethanol drinking. Neurobiological studies are beginning to identify mesolimbic adaptations that may contribute to the behavioral phenotype engendered by this model. Some of these changes include increased excitability of ventral tegmental area dopamine neurons and pyramidal cells in the basolateral amygdala and significant alterations in baseline and stimulated catecholamine signaling. A growing body of evidence suggests that adolescent social isolation may represent a reliable rodent model of heightened vulnerability to anxiety disorders and alcoholism in male rats. These studies provide initial support for the face, construct, and predictive validity of this model and highlight its utility in identifying neurobiological adaptations associated with increased risk of developing these disorders.

Keywords

Alcohol Use Disorder; Comorbidity; Early Life Stress; Dopamine; Norepinephrine

Although the recreational consumption of alcohol has been an integral element of human culture for thousands of years, alcohol use and abuse remain a major socioeconomic and

health concern around the world. The most recent status report on alcohol and health from the World Health Organization (2014) revealed that almost 6% of all global deaths can be attributed to alcohol. In the United States, alcohol use disorder (AUD) is associated with 3% of all deaths, 5% of all years of life lost to premature mortality (Rehm et al., 2014), and costs the U.S. economy an estimated \$249 billion dollars annually (Debell et al., 2014).

Despite the gravity of these numbers, some populations of individuals are impacted to an even greater extent by alcohol-related problems. One such group is individuals diagnosed with a comorbid affective disorder. For example, recent data from the 2012 to 2013 U.S. National Epidemiologic Survey on Alcohol and Related Conditions, which randomly surveyed over 36,000 U.S. noninstitutionalized civilians, found that the prevalence of DSM-5 “12 month” and “lifetime” prevalence of AUD were 13.9 and 29.1%, respectively (Grant et al., 2016). However, recent and historic data have shown that these numbers were significantly higher in individuals diagnosed with diseases such as major depressive disorder or trauma and stressor-related disorders and these populations tended to be less responsive to treatment (Grant et al., 2016; Kranzler and Rosenthal, 2003). These findings are consistent with a large body of literature suggesting a high degree of comorbidity between AUD and a wide range of affective disorders (Brenhouse and Andersen, 2011; Brodie et al., 1999; Buckley, 2006; Buckley and Brown, 2006; Kessler, 2004; Kushner, 2014).

A considerable body of evidence suggests that the development of AUD involves adaptive changes in some of the same neural circuits that underlie negative affective states, such as anxiety and depression (Koob, 2013). These changes gradually shift motivation for alcohol away from positive reinforcement (e.g., euphoria) toward negative reinforcement (e.g., relief from the negative affective state that manifests during withdrawal). If this framework is correct, then it may not be surprising that AUD and mood disorders are highly comorbid. In individuals with a preexisting anxiety disorder, the negative affective state may likely be present during early drinking episodes, thus obviating the need for the transition from positive to negative reinforcement (Fig. 1). Indeed, other epidemiological data suggest that, relative to healthy controls, individuals with anxiety disorders are diagnosed with AUD 5 years earlier and transition faster from milestones like “age at first regular drinking” to the “onset of alcohol dependence” (Kushner et al., 2011). Notably, individuals with comorbid AUD and anxiety disorders have a poorer prognosis in recovery, with significantly higher relapse rates than individuals diagnosed solely with AUD (Kranzler and Rosenthal, 2003; Kushner et al., 2005).

Despite compelling evidence that affective disorders are common in alcoholics and may exacerbate the progression and treatment of both disorders, relatively little is known about the neurobiological substrates that may contribute to the strong association between these diseases. One challenge has been a lack of animal models that generate a behavioral profile reflecting heightened risk of AUD and anxiety disorders. Brain maturation continues throughout adolescence into early adulthood (Brenhouse and Andersen, 2011; Eiland and Romeo, 2013) and human studies have shown that exposure to a wide range of stressors during this critical developmental period is associated with increased vulnerability to AUD and mood disorders in adulthood (April et al., 2015; Enoch, 2011; Saalfeld and Spear, 2015; Spear, 2015). Several recent studies have reexamined a well-established rodent adolescent

social isolation model to highlight that many of the behaviors that manifest in adulthood following exposure to this early life stressor may reflect an increased vulnerability to AUD and anxiety disorders. The goal of this focused review is to summarize recent studies that support the face, predictive, and construct validity of adolescent social isolation (aSI) as a rodent model of vulnerability to comorbid AUD and anxiety disorders as well as initial neurobiological evidence of mesolimbic adaptations that may contribute to the behavioral phenotype associated with this model. For more comprehensive treatment of other important and related topics, including the historic development of social isolation models, other animal models of addiction vulnerability and dependence, and the influence of sex differences in addiction, the reader is directed to these excellent reviews: Becker and Koob (2016), Butler and Weiner (2016), Hall (1998), McBride and colleagues (2014), Meinhardt and Sommer (2015), Nylander and Roman (2013), and Sánchez and colleagues (2001).

CHRONIC EARLY LIFE STRESS: ADOLESCENT SOCIAL ISOLATION MODEL

Wild rats are inherently social animals, living in large, mixed-sex groups that can contain hundreds of animals (McEwen et al., 2015). Many laboratories have demonstrated that depriving rats of social interaction, particularly during adolescence, lead to numerous behavioral alterations that manifest in adulthood (Marsden et al., 2011; Ratajczak et al., 2013; Toth and Neumann, 2013). Importantly, as discussed below, many of these behavioral changes may represent risk factors of excessive ethanol (EtOH) drinking and/or develop in animal models of AUD. Although several early studies reported that rodent adolescent social isolation promoted increases in alcohol drinking behaviors (Deatherage, 1972; Hall et al., 1998b; Schenk et al., 1990; Wolffgramm, 1990), until recently, few studies have sought to employ this model to delve into the neural substrates of AUD vulnerability or comorbidity with anxiety disorders. In our model, male Long–Evans rats are procured from a commercial supplier (Envigo, Indianapolis, IN) immediately postweaning (near postnatal day [PD] 21) and housed in groups of 4 in large, opaque plastic cages for 1 week. On PD 28, near the onset of rodent adolescence (Brenhouse and Andersen, 2011; Spear, 2015), subjects are randomly divided into 2 cohorts. Group housed rats remain in the same large cages for at least 6 weeks, through the late phase of adolescence and into early adulthood (adolescent group housed [aGH]), while aSI rats are housed individually in smaller cages for this same period (Chappell et al., 2013; McCool and Chappell, 2009). aSI rats reside in the same room as their group housed counterparts and are exposed to similar olfactory, auditory, and visual stimuli. The primary experimental variable is that aSI rats are deprived of tactile contact with their peers. Experimenter interaction during this housing manipulation is also identical for both groups and is restricted to a single weekly weighing. Importantly, aSI does not lead to any obvious differences in general health such as alterations in weight gain (McCool and Chappell, 2009). Following the housing manipulation, subjects are then used for behavioral and/or neurobiological assessments. One notable feature of this model is that many of the behavioral and neurobiological phenotypes that manifest immediately after aSI persist for weeks and even months into adulthood, even when comparisons are made with aGH rats that are then isolated in adulthood (Skelly et al., 2015; Yorgason et al., 2013). Thus, a critical feature of this model is the exposure to chronic stress during adolescence.

HYPERLOCOMOTION AND RESPONSE TO NOVELTY

Impulsivity and sensation seeking are 2 personality traits that are positively correlated with greater alcohol intake and abuse vulnerability (Cyders et al., 2009; Woicik et al., 2009). Rats bred as low or high responders to novelty show differential susceptibility to addiction-related behaviors (Flagel et al., 2014), suggesting a relationship between this behavior and addiction-like phenotypes. Additionally, hyperactivity in a novel environment is positively correlated with acquisition of EtOH intake (Nadal et al., 2002). In contrast, decreases in measures of spontaneous locomotion have been reported in rodent models of EtOH dependence (Zhao et al., 2007) and enduring deficits in measures of motoric activity have been reported in recovering alcoholics (Kwon et al., 1997; Rosenbloom et al., 2004; Sullivan et al., 2002).

Fabricius and colleagues (2011) compared locomotor activity in 45 cohort replications using aGH and aSI Lister hooded rats and showed that aSI subjects were hyperactive in 44 of these cohorts (Fabricius et al., 2011). This phenotype has also been reported in aSI Long–Evans (Butler et al., 2014a; Chappell et al., 2013) and Sprague Dawley (Ishikawa et al., 2014) strains.

SENSORY GATING

Prepulse inhibition (PPI) is a highly translational measure of sensorimotor gating, in which delivery of an auditory pre-pulse inhibits the startle response to a second closely timed auditory pulse. Inability to inhibit the response to the second pulse is interpreted as impaired sensorimotor gating, which is associated, at least in part, with overactivity of dopaminergic signaling (Geyer, 2008; Geyer et al., 2001). PPI deficits are commonly reported in schizophrenic patients (Javitt and Freedman, 2015; Powell et al., 2012) as well as in individuals with obsessive–compulsive and panic disorder (Kohl et al., 2013). Impaired PPI has also been described in children of alcoholics (Grillon et al., 1997, 2000), heavy drinkers (Hutchison et al., 2003), and abstinent alcoholics (Marin et al., 2012). In aSI rats, PPI deficits have been observed immediately postisolation (Ko and Liu, 2015; Liu et al., 2011; Powell et al., 2015) and well into adulthood, even when aGH rats were then isolated as adults (McCool and Chappell, 2009).

MEASURES OF UNCONDITIONED ANXIETY-LIKE BEHAVIOR

Unconditioned anxiety-like behaviors are observed in threat-provoking paradigms, such as the elevated plus maze, which takes advantage of a rodent's desire to avoid exposed, well-lit environments. The elevated plus maze is possibly the most widely used assay of unconditioned anxiety-like behavior with time spent on the open arms of the maze, and the number of entries into the open arms being the most common measures of anxiety-like behavior (Campos et al., 2013; Griebel and Holmes, 2013; Holmes and Rodgers, 1999). The number of closed arm entries is typically used as a measure of nonspecific locomotor activity (Holmes and Rodgers, 1999). Several lines of rats selectively bred for high levels of EtOH preference also exhibit enhanced measures of anxiety-like behavior on this assay (Colombo et al., 1995; Stewart et al., 1993), and a positive correlation has been reported

between anxiety measures on the plus maze and EtOH drinking in outbred Wistar rats (Spanagel et al., 1995). Moreover, numerous studies have reported that models of EtOH dependence that promote robust escalations in voluntary EtOH intake also lead to increased measures of anxiety-like behavior on the plus maze (Morales et al., 2015; Valdez et al., 2002, 2003; Zhao et al., 2007).

Decreased open-arm time and/or open-arm entries represent some of the most robust behavioral phenotypes observed in aSI rats (Chappell et al., 2013; Hall et al., 1998a; Hellemans et al., 2004; McCool and Chappell, 2009; Wright et al., 1991). We have conducted a plus maze assessment on over a dozen cohorts of aGH and aSI rats over the past 4 years and have observed a significant increase in at least 1 measure of anxiety-like behavior in all of these experiments.

FEAR LEARNING

Fear learning is a simple experimental paradigm in which subjects are first trained to associate a neutral stimulus (e.g., bright light), with an aversive unconditioned stimulus (UCS; e.g., mild shock). Once this association is formed, the neutral stimulus becomes a conditioned stimulus (CS) and elicits a fear response when presented alone (e.g., increased freezing behavior or a heightened startle response; Bowers and Ressler, 2015; Maren et al., 2013). Subjects can also be exposed repeatedly to the CS in the absence of the UCS to extinguish the association between these stimuli (Milad and Quirk, 2012). Fear learning paradigms are highly translational assays that are used in humans and research animals (Borghans and Homberg, 2015; VanElzakker et al., 2014). In fact, patients with trauma and stressor-related disorders often exhibit perturbations in fear learning, particularly deficits in extinction (Milad and Quirk, 2012; VanElzakker et al., 2014). Moreover, a number of human imaging and animal studies have extensively examined the neural basis of fear learning and many of the mesolimbic circuits implicated in addiction appear to be dysregulated in these disorders (e.g., basolateral amygdala [BLA], prefrontal cortex; Jovanovic and Ressler, 2010; Mahan and Ressler, 2012).

We recently reported that aSI and aGH rats do not differ in the acquisition of fear learning but aSI animals exhibit a significant deficit in extinction learning. Using a protocol that completely abolished the CS–UCS association in aGH rats, aSI rats still exhibited a fear response to the CS (Skelly et al., 2015). Similar deficits in fear extinction have also been reported in a mouse model of EtOH dependence (Holmes et al., 2012). These findings suggest that disruption of the neurocircuitry that mediates fear extinction may represent a common phenotype associated with AUD vulnerability, the development of an AUD and anxiety disorders, and further underscores the idea that both diseases may share common neural substrates.

DEPRESSIVE-LIKE BEHAVIOR

Anxiety and depressive disorders frequently co-occur, and their symptoms fall along a spectrum that often overlaps. In fact, antidepressants are now a first-line treatment for many types of anxiety disorders (Reinhold and Rickels, 2015). Although we have not examined

the depressive phenotype of aSI rats, a few studies have assessed the influence of aSI on the forced swim test (FST). Immobility during this assay is commonly used as a measure of depressive-like behavior in rodents, and aSI is associated with reduced swim time and increased immobility in the FST (Brenes et al., 2008; Das et al., 2015). Desipramine, an antidepressant, has also been shown to reduce immobility selectively in SI animals (Simpson and Kelly, 2012).

ETOH DRINKING PHENOTYPE

Given that aSI alters a wide range of behaviors that may be associated with increased vulnerability to AUD, a number of studies have examined the impact of this early life stress model on EtOH drinking-related behaviors. Several early studies reported that aSI promotes increases in EtOH intake and/or preference (Deatherage, 1972; Hall et al., 1998b; Lodge and Lawrence, 2003; Schenk et al., 1990; Wolffgramm, 1990; although this phenotype may not occur in Sprague Dawley rats: Gill et al., 2014; Pisu et al., 2011; Rockman et al., 1989). Recently, we and others have reexamined the influence of aSI on EtOH-related measures in outbred rats to determine whether this model can be used to identify potential neural substrates associated with heightened vulnerability to excessive EtOH drinking behavior. In our first studies, using a standard 1-week, home-cage 2-bottle choice procedure, aSI significantly increased intake and preference for a 10% EtOH solution (Chappell et al., 2013; McCool and Chappell, 2009). We have also employed the intermittent variation of this drinking paradigm to examine the influence of aSI on EtOH consumption over much longer time frames. In the intermittent procedure, a relatively high EtOH concentration (20% v/v) is used and EtOH is only available on Monday, Wednesday, and Friday. This paradigm promotes an escalation in EtOH intake in many strains of rats (for review, see Carnicella et al., 2014). Using this model, we have found that aSI leads to a significant increase in EtOH drinking measures which endure for at least 8 weeks (Chappell et al., 2013; Skelly et al., 2015), even when aGH rats are single housed in adulthood throughout these studies.

Another feature of the intermittent model is that rats typically consume ~25% of their daily EtOH intake in the first 30 minutes of the sessions in which EtOH is available. We took advantage of this feature to confirm that aSI rats achieve significantly higher blood EtOH levels than group housed subjects, with many reaching binge-like blood levels of EtOH (80 mg%; Skelly et al., 2015). In a separate study, we also demonstrated that aSI had no significant effects on the absorption, distribution or elimination of EtOH (Karkhanis et al., 2014).

To our knowledge, only 2 studies have examined the pharmacology of EtOH drinking in aSI and aGH rats. These studies employed the intermittent model discussed above and found that systemic administration of 3 anxiolytic medications that modulate noradrenergic signaling (prazosin, propranolol, and duloxetine) and NOR-BNI, a kappa opioid receptor (KOR) antagonist that also has anxiolytic properties, all significantly decreased the binge-like EtOH drinking promoted by the intermittent model in aSI rats (Karkhanis et al., 2016; Skelly et al., 2015). In contrast, none of these medications significantly influenced EtOH drinking in aGH subjects.

The effects of aSI have also been examined using an operant EtOH drinking procedure (McCool and Chappell, 2009). In this study, rats were trained to complete 30 lever presses to obtain 20 minutes access to a sipper tube containing 10% EtOH (v/v). This paradigm affords the opportunity to discretely assess appetitive (e.g., latency to first lever press) and consummatory (e.g., EtOH intake) measures. These behaviors are not correlated with each other (Samson and Czachowski, 2003) and are likely mediated by distinct neurobiological substrates (Butler et al., 2013; Samson and Chappell, 2004). Nevertheless, aSI was associated with increases in both appetitive (lever press response rate) and consummatory (number of licks, EtOH intake) measures of EtOH drinking. Notably, in a separate cohort of aSI and aGH rats trained to self-administer a 3% sucrose solution using the same operant procedure, no differences in any appetitive or consummatory measures were observed (McCool and Chappell, 2009).

Finally, a study in outbred Sprague Dawley rats used a similar, albeit shorter, aSI procedure (PD 21 to 42) and examined the acquisition of conditioned place preference (CPP) for EtOH and amphetamine (Whitaker et al., 2013). A single day of conditioning with EtOH (0.5 g/kg, intraperitoneal [i.p.], 15% v/v) or amphetamine (5 mg/kg, i.p.) versus 0.9% saline engendered significantly greater CPP in aSI rats relative to animals housed in groups of 3 during early adolescence. This study went on to show that, while the difference in the magnitude of amphetamine CPP between these groups disappeared after 7 days of conditioning, aSI rats exhibited a significant delay in the extinction of this form of contextual drug memory.

ADOLESCENT SOCIAL ISOLATION DOES NOT PROMOTE AN ADDICTION VULNERABLE PHENOTYPE IN FEMALE RATS

One important caveat is that all of the preceding studies were conducted in male rats. We recently tested female Long–Evans rats using an aSI protocol identical to the one used in males. While aSI increased EtOH drinking in female rats, this effect was transient and was not associated with significant changes in anxiety-like behaviors (Butler et al., 2014b). Thus, although early life stress leads to increases in AUD vulnerability in both men and women (Enoch, 2011), and aSI promotes many behaviors linked with increased addiction vulnerability in male rats, this form of early life stress does not engender an “addiction vulnerable” phenotype in females. Several other studies have noted equivocal effects of aSI on anxiety-like behaviors in female rodents (Kinzig and Hargrave, 2010; Mathews et al., 2008; McCormick et al., 2008) and, while other forms of stress have been shown to promote increases in anxiety-like behaviors in female rats (e.g., activity-induced anorexia: Kinzig and Hargrave, 2010; social instability: McCormick et al., 2008), no studies to date have examined whether these stress protocols engender long-lasting increases in EtOH drinking behaviors.

MESOLIMBIC ADAPTATIONS THAT MAY CONTRIBUTE TO THE ADDICTION VULNERABLE PHENOTYPE ENGENDERED BY ADOLESCENT SOCIAL ISOLATION

Numerous studies have examined neurobiological adaptations that may contribute to the complex behavioral phenotypes that manifest following aSI (for reviews, see Fone and Porkess, 2008; Lapiz et al., 2001; Lukkes et al., 2009). Here, we focus on a few recent studies, by us and others, that have tried to relate these adaptations to the “addiction vulnerable” phenotype engendered by this model. Not surprisingly, these initial studies have focused on the mesolimbic circuitry that is thought to play an integral role in the pathophysiology of AUD.

VENTRAL TEGMENTAL AREA

The ventral tegmental area (VTA) dopamine system plays a critical role in motivation and reward learning and is also likely involved in the development and progression of AUD (Koob and Volkow, 2010). Fabricius and colleagues (2010) reported that aSI Lister hooded rats exhibit increases in the in vivo firing rate and burst-like activity of putative dopamine neurons, relative to recordings from rats housed in groups of 5 during adolescence. Whitaker and colleagues (2013) demonstrated that the same aSI procedure that led to increased EtOH and amphetamine CPP also increased long-term potentiation of NMDA receptor-mediated glutamatergic transmission in putative VTA dopamine neurons. This increase in excitatory plasticity was driven by an enhancement of mGluR/IP3-mediated Ca^{2+} signaling in these cells. Notably, this adaptation was only observed in animals isolated for at least 3 weeks during early adolescence. A similar 3-week isolation during late adolescence (PD 42 to 63) had no effect on NMDA plasticity in VTA cells. Moreover, resocialization during late adolescence did not reverse these adaptive changes.

NUCLEUS ACCUMBENS

While the VTA projects to many cortical and subcortical structures, the dopaminergic projection from the VTA to the nucleus accumbens (NAc) has received the most attention in the alcohol research field. This circuit plays an integral role in motivated and reward seeking behaviors (for review, see Sulzer, 2011) and acute and chronic EtOH exposure profoundly influence this pathway (Brodie, 2002; Brodie and Appel, 1998; Budygin et al., 2007; Ding et al., 2009; Rose et al., 2015; Yim and Gonzales, 2000; Yim et al., 1998). Early evidence demonstrated that the accumbal dopamine system is also significantly impacted by aSI (for review, see Lapiz et al., 2001). We recently employed ex vivo fast scan cyclic voltammetry to characterize the effect of aSI on NAc core dopamine signaling (Yorgason et al., 2013). Two separate experiments were conducted in this study. The first examined the effect of aSI early in adulthood, within 2 to 3 weeks following the adolescent housing manipulation, while the second was conducted on aSI and aGH rats that were all subsequently isolated for at least 3 months in adulthood. Behavioral assessments confirmed that aSI rats exhibit increased measures of anxiety-like behavior immediately following the adolescent housing manipulation and demonstrated, for the first time, that this anxiogenic phenotype persists

well into adulthood, despite the fact that both experimental groups were isolated in adulthood. Neurochemical studies revealed significant increases in both electrically stimulated dopamine release and the rate of dopamine uptake in accumbal slices from aSI rats, and these alterations were not associated with a change in D2 autoreceptor function. These perturbations in accumbal dopamine release dynamics also persisted well into adulthood. Subsequent studies have now confirmed that aSI also leads to increased dopamine release and uptake in the dorsal striatum (Yorgason et al., 2016). This study also reported higher dopamine transporter levels in ventral and dorsal striatal tissue extracts from aSI rats, providing a possible mechanism that may contribute to the increased rate of dopamine uptake. In addition to the dopamine transporter, the KOR plays an integral role in regulating dopamine transmission. Activation of KORs reduces dopamine release in the NAc (Ebner et al., 2010; Werling et al., 1988). In another recent study, we showed that KOR-mediated inhibition of dopamine release was enhanced in aSI compared to aGH rats (Karkhanis et al., 2016). These data suggest that aSI results in increased functional responsiveness of KORs in the NAc which may contribute to the observed increase in EtOH intake.

In a separate study, in vivo microdialysis was used to examine NAc dopamine levels in freely moving aSI and aGH rats (Karkhanis et al., 2014). As this brain region also receives substantial noradrenergic input, and noradrenergic signaling is profoundly impacted by chronic stress in animal and human studies (Brenes et al., 2008; Goddard et al., 2010), NAc norepinephrine levels were also assessed. No difference in baseline dopamine or norepinephrine levels was observed between aSI and aGH rats. However, adolescent housing profoundly altered both the dopamine and norepinephrine response to an acute EtOH injection. While EtOH increased accumbal dopamine levels in both groups of rats, the magnitude of the dopamine response to the highest EtOH dose tested (2 g/kg, i.p.) was significantly greater in aSI subjects (Fig. 2). Although the relationship between EtOH-stimulated dopamine release in the NAc and alcohol drinking is complex (Yim et al., 2000), this measure has been shown to correlate with EtOH preference in lines of rats selectively bred for high and low EtOH intake (Katner and Weiss, 2001).

EtOH had no effect on accumbal norepinephrine release in aGH rats, even at the highest dose tested, but significantly increased norepinephrine levels in aSI rats (Karkhanis et al., 2014). Vehicle injections had no effect on norepinephrine (or dopamine) levels, suggesting that the increase in response to EtOH was not due to a generalized enhancement in the stress responsivity of the noradrenergic system. Rather, these data suggest that aSI confers an EtOH sensitivity to the noradrenergic system that is otherwise absent in animals that have not been exposed to this early life stressor (Fig. 2). These data may help to explain our findings that 3 different medications that suppress noradrenergic signaling selectively decreased binge-like EtOH drinking in aSI rats (Skelly et al., 2015). It will be of interest in future studies to determine whether aSI alters catecholamine signaling following contingent EtOH exposure as opposed to the noncontingent treatments employed in these initial studies.

BASOLATERAL AMYGDALA

The BLA plays a major role in the regulation of anxiety and stress as well as reward-related behaviors (for review, see Janak and Tye, 2015). This region is comprised largely of glutamatergic pyramidal cells which project to other amygdala nuclei and many other limbic and cortical structures (Janak and Tye, 2015; McDonald, 1998). The NAc receives strong excitatory input from the BLA and optogenetic activation of BLA glutamatergic synapses in the NAc is sufficient to maintain reinforced behavior (Stuber et al., 2011).

Dopamine (Pape, 2005; Rosenkranz and Grace, 1999) and norepinephrine (Buffalari and Grace, 2007; Silberman et al., 2010; Southwick et al., 1999) play a vital role in modulating these glutamatergic efferents from the BLA. These modulatory effects are complex as dopamine (Diaz et al., 2011; Marowsky et al., 2005) and norepinephrine (Buffalari and Grace, 2007; Silberman et al., 2010; Southwick et al., 1999) both exert excitatory and inhibitory effects on neuronal communication. However, exposure to chronic stress can affect the excitation–inhibition balance mediated via dopamine (Maxwell, 2013) and norepinephrine (Braga et al., 2004; Buffalari and Grace, 2009). Therefore, *in vivo* microdialysis was used to first examine the effects of aSI on dopamine and norepinephrine and then investigate the responses of these 2 neurotransmitters when the animals were exposed to an acute EtOH challenge (Karkhanis et al., 2015a). aSI rats showed lower baseline dopamine levels compared to aGH subjects. Moreover, Western hybridization assays showed significantly greater dopamine transporter levels in BLA tissue extracts from aSI rats. This aSI-associated increase in dopamine transporter protein levels may contribute to the reduction in baseline dopamine, creating a hypodopaminergic state that promotes the increased EtOH self-administration observed in aSI animals. Furthermore, an acute EtOH (2 g/kg) challenge significantly increased dopamine levels in both cohorts; however, this elevation in dopamine was potentiated in aSI rats. With respect to norepinephrine, neither the baseline levels nor the norepinephrine transporter protein levels were significantly different between aGH and aSI rats. However, as observed in the NAc, an acute EtOH challenge selectively increased extracellular levels of norepinephrine in the BLA of aSI subjects (Fig. 3).

Many animal studies have also documented that a variety of stressors promote increased excitability of BLA pyramidal neurons (Correll et al., 2005; Padival et al., 2013; Rosenkranz et al., 2010; Suvrathan et al., 2014), and there is compelling clinical evidence that heightened BLA excitability is associated with both anxiety disorders and AUD (Jovanovic and Ressler, 2010; Koob and Volkow, 2010; Mahan and Ressler, 2012). We recently employed perforated patch electrophysiological recordings to examine the impact of aSI on the intrinsic excitability of BLA pyramidal neurons (Rau et al., 2015). A significant increase in the number of action potentials elicited by depolarizing current injections was observed in aSI recordings. This change was observed in the absence and presence of GABA and glutamate receptor antagonists, confirming that aSI led to an increase in the intrinsic excitability of these cells (Rau et al., 2015). Additional studies revealed significant deficits in the slow and medium afterhyperpolarizations (AHPs) in recordings from aSI rats. These responses play an integral role in regulating intrinsic excitability (Adelman et al., 2012). Although the ion channels that underlie the slow AHP are not conclusively known, small

conductance (SK) calcium-activated potassium channels mediate the medium AHP (Adelman et al., 2012). Using Western blots, we found lower expression of the 2 predominant SK isoforms expressed in aSI BLA tissue extracts (SK2 and SK3). We also showed that allosteric potentiation of SK channels with 1-EBIO restored measures of intrinsic excitability in recordings from aSI rats to levels seen in recordings from aGH animals and that 1-EBIO had no effect on intrinsic excitability in recordings from aGH rats (Rau et al., 2015). In contrast, apamin, a toxin that blocks SK channels, had greater effects in recordings from aGH relative to aSI rats. Finally, in vivo microinjection of 1-EBIO reduced anxiety-like behaviors in the plus maze and open field assay.

CONCLUSIONS

As reviewed in the Introduction, epidemiological evidence suggests that the diagnosis of an anxiety disorder represents a major risk factor for the development of AUD. Moreover, pathological anxiety accelerates the progression of AUD and is associated with a much poorer prognosis in recovery. Although there is considerable preclinical interest in identifying the neural substrates responsible for these relationships, a reliable animal model that engenders enduring increases in anxiety-like behaviors and measures of EtOH drinking has proven elusive. In general, a good animal model of complex psychiatric disorders should have face and predictive validity (Belzung and Lemoine, 2011). In other words, the model needs to generate the central behavioral phenotypes that characterize the human condition and medications that treat the human condition should alleviate these behaviors. If the model is going to be employed to study the neural underpinnings of the human condition, it should also have construct validity. The pathophysiological alterations that result in the human condition should also generate the relevant phenotypes in the animal model. Although additional work is needed, the studies reviewed here provide initial support that rodent aSI fulfills each of these criteria, at least in male rats.

A series of recent studies have confirmed and extended earlier work demonstrating that aSI leads to the expression of a wide range of behaviors that have either been suggested as measures of AUD vulnerability and/or are observed in animal models of alcohol dependence. These include increases in locomotor activity in a novel environment (Chappell et al., 2013), elevated measures of unconditioned anxiety-like behavior (primarily using the elevated plus maze; Chappell et al., 2013; McCool and Chappell, 2009), deficits in PPI (McCool and Chappell, 2009), and impaired fear extinction (Skelly et al., 2015). Importantly, several of these changes persist for months into adulthood even when comparisons are made with aGH rats that were then isolated in adulthood. Importantly, aSI also leads to robust, long-lasting increases in multiple measures of voluntary EtOH drinking (Chappell et al., 2013; McCool and Chappell, 2009; Skelly et al., 2015) and enhances EtOH CPP (Whitaker et al., 2013). In addition, 2 studies have examined the pharmacology of EtOH drinking in aSI rats and reported that anxiolytic medications targeting the noradrenergic system and KORs significantly attenuated binge-like EtOH drinking in aSI rats at doses that had no significant effect in aGH subjects (Karkhanis et al., 2016; Skelly et al., 2015). Collectively, these findings provide promising initial evidence that this model has both face and predictive validity.

With respect to construct validity, human imaging studies have underscored the importance of mesolimbic adaptations in the pathophysiology of anxiety disorders and AUD. For example, amygdala activity is significantly elevated in patients with anxiety disorders as well as trauma and stressor-related disorders (Fredrikson and Faria, 2013; Jovanovic and Ressler, 2010), and alcohol dependence is associated with enhanced cue-associated amygdala activity (Heinz et al., 2009). The mesolimbic dopamine system is also dys-regulated in patients with anxiety disorders and AUD. For example, affective disorders and AUD may be associated with low baseline levels of dopamine (Gardner, 2011; Koob and Volkow, 2010; Koob et al., 2014) and both disorders are linked with hyper responsivity of central noradrenergic signaling (Kalk et al., 2011; Patkar et al., 2004; Pervanidou, 2008). Experiments using rodent aSI models have identified neuroadaptive changes that are consistent with all of these neural adaptations. For example, aSI leads to a decrease in BLA SK channel expression and function and the associated increase in intrinsic excitability of BLA pyramidal neurons may contribute to the amygdala hyperactivity observed in patients with anxiety disorders and AUD. Moreover, the increase in dopamine uptake in ex vivo accumbal slices (Yorgason et al., 2013, 2016) along with the increased expression of the dopamine transporter in BLA and accumbens tissue (Karkhanis et al., 2015a; Yorgason et al., 2016), and the low levels of extracellular dopamine in the BLA (Karkhanis et al., 2015a), may all promote a hypodopaminergic state that contributes to the behavioral phenotype of aSI rats.

Interestingly, while many of the adaptive changes associated with aSI are similar to those seen in animal models of EtOH dependence, others may be unique to this procedure. One notable example is the increase in stimulated catecholamine release observed in aSI animals. Several studies have noted that the magnitude of electrically stimulated dopamine transients is greater in ex vivo accumbal recordings from aSI rats, and aSI is associated with augmented EtOH-evoked dopamine and norepinephrine release in the NAc and BLA (Karkhanis et al., 2014, 2015a; Yorgason et al., 2013, 2016). These findings contrast with the majority of human (Gardner, 2011; Koob and Volkow, 2010) and animal (Bailey et al., 2001; Diana et al., 1993; Karkhanis et al., 2015b; Rose et al., 2015; Shen et al., 2007; Zapata and Shippenberg, 2006; Zapata et al., 2006) studies which suggest that chronic EtOH exposure may promote tolerance to the acute stimulatory effects of EtOH on catecholamine signaling. These findings may indicate that sensitization of these catecholamine systems represents an adaptation that is particularly associated with vulnerability, a finding consistent with the observation that EtOH-stimulated dopamine release is positively correlated with initial EtOH preference in lines of rats bred for differences in this drinking phenotype (Katner and Weiss, 2001).

One potential concern with the aSI model may be its apparent lack of specificity. While we have outlined evidence in support of this procedure as a model of alcohol addiction vulnerability, others have made similar arguments for the use of this model to study the neurobiology of other psychiatric disorders such as schizophrenia and attention deficit hyperactivity disorder (ADHD: Russell, 2011; Yates et al., 2012; schizophrenia: Ratajczak et al., 2013). It should however be noted that early life stress is also linked with increased risk for these disorders (Abel et al., 2014; Debost et al., 2015; Humphreys and Zeanah, 2015; Martins and Gorelik, 2011). Moreover, while there is a high degree of comorbidity between

anxiety disorders and AUD, other psychiatric conditions, including schizophrenia and ADHD, are also associated with heightened vulnerability to alcohol-related problems (Maxwell, 2013; Thoma and Daum, 2013). Therefore, the apparent lack of specificity of this model may actually further validate its construct validity and underscore the idea that there may well be common neurobiological perturbations that increase vulnerability to a broad spectrum of neuropsychiatric disorders.

In summary, rodent aSI is a simple model that engenders enduring and reproducible alterations in a number of behaviors that may be associated with increased vulnerability to anxiety disorders and AUD. A number of neurobiological adaptations have recently been uncovered that point toward potential new avenues for pharmacotherapies. Future studies will be needed to examine, in greater detail, the circuit-specific alterations associated with aSI. For example, recent data suggest that distinct subpopulations of BLA pyramidal cells project to the central amygdala and NAc and mediate negative and positive behaviors, respectively (Namburi et al., 2015). It will be of great interest to employ opto- and chemogenetic approaches to identify the specific downstream BLA projections that give rise to the behavioral phenotypes associated with this model. Collectively, it is hoped that these studies may lead to novel and more effective pharmacotherapies for the large number of patients suffering from AUD and comorbid mood disorders.

Acknowledgments

This research was funded by grants from P01 AA21099 (JLW, SRJ), R37 AA17531 (JLW), R37 AA10442 (JLW), U01 AA014091 (SRJ), T32 AA00756 (TRB, ANK). The authors have no conflict of interests.

References

- Abel KM, Heuvelman HP, Jorgensen L, Magnusson C, Wicks S, Susser E, Hallkvist J, Dalman C. Severe bereavement stress during the prenatal and childhood periods and risk of psychosis in later life: population based cohort study. *BMJ*. 2014; 348:f7679. [PubMed: 24449616]
- Adelman JP, Maylie J, Sah P. Small-conductance Ca^{2+} -activated K^{+} channels: form and function. *Annu Rev Physiol*. 2012; 74:245–269. [PubMed: 21942705]
- April MD, Fossum K, Hounshell C, Stolper K, Spear L, Semelrath K. A sinister cause of anterograde amnesia: painless aortic dissection. *Am J Emerg Med*. 2015; 33:989 e985–987.
- Bailey CP, O'Callaghan MJ, Croft AP, Manley SJ, Little HJ. Alterations in mesolimbic dopamine function during the abstinence period following chronic ethanol consumption. *Neuropharmacology*. 2001; 41:989–999. [PubMed: 11747903]
- Becker JB, Koob GF. Sex differences in animal models: focus on addiction. *Pharmacol Rev*. 2016; 68:242–263. [PubMed: 26772794]
- Belzung C, Lemoine M. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biol Mood Anxiety Disord*. 2011; 1:9. [PubMed: 22738250]
- Borghans B, Homberg JR. Animal models for posttraumatic stress disorder: an overview of what is used in research. *World J Psychiatry*. 2015; 5:387–396. [PubMed: 26740930]
- Bowers ME, Ressler KJ. An overview of translationally informed treatments for posttraumatic stress disorder: animal models of pavlovian fear conditioning to human clinical trials. *Biol Psychiatry*. 2015; 78:E15–E27. [PubMed: 26238379]
- Braga MF, Aroniadou-Anderjaska V, Manion ST, Hough CJ, Li H. Stress impairs $\alpha(1A)$ adrenoceptor-mediated noradrenergic facilitation of GABAergic transmission in the basolateral amygdala. *Neuropsychopharmacology*. 2004; 29:45–58. [PubMed: 14532911]

- Brenes JC, Rodriguez O, Fornaguera J. Differential effect of environment enrichment and social isolation on depressive-like behavior, spontaneous activity and serotonin and norepinephrine concentration in prefrontal cortex and ventral striatum. *Pharmacol Biochem Behav.* 2008; 89:85–93. [PubMed: 18096212]
- Brenhouse HC, Andersen SL. Developmental trajectories during adolescence in males and females: a cross-species understanding of underlying brain changes. *Neurosci Biobehav Rev.* 2011; 35:1687–1703. [PubMed: 21600919]
- Brodie MS. Increased ethanol excitation of dopaminergic neurons of the ventral tegmental area after chronic ethanol treatment. *Alcohol Clin Exp Res.* 2002; 26:1024–1030. [PubMed: 12170113]
- Brodie MS, Appel SB. The effects of ethanol on dopaminergic neurons of the ventral tegmental area studied with intracellular recording in brain slices. *Alcohol Clin Exp Res.* 1998; 22:236–244. [PubMed: 9514313]
- Brodie MS, McElvain MA, Bunney EB, Appel SB. Pharmacological reduction of small conductance calcium-activated potassium current (SK) potentiates the excitatory effect of ethanol on ventral tegmental area dopamine neurons. *J Pharmacol Exp Ther.* 1999; 290:325–333. [PubMed: 10381795]
- Buckley PF. Prevalence and consequences of the dual diagnosis of substance abuse and severe mental illness. *J Clin Psychiatry.* 2006; 67(Suppl 7):5–9.
- Buckley PF, Brown ES. Prevalence and consequences of dual diagnosis. *J Clin Psychiatry.* 2006; 67:e01. [PubMed: 17107226]
- Budygin EA, Oleson EB, Mathews TA, Lack AK, Diaz MR, McCool BA, Jones SR. Effects of chronic alcohol exposure on dopamine uptake in rat nucleus accumbens and caudate putamen. *Psychopharmacology.* 2007; 193:495–501. [PubMed: 17492432]
- Buffalari DM, Grace AA. Noradrenergic modulation of basolateral amygdala neuronal activity: opposing influences of alpha-2 and beta receptor activation. *J Neurosci.* 2007; 27:12358–12366. [PubMed: 17989300]
- Buffalari DM, Grace AA. Chronic cold stress increases excitatory effects of norepinephrine on spontaneous and evoked activity of basolateral amygdala neurons. *Int J Neuropsychopharmacol.* 2009; 12:95–107. [PubMed: 18647435]
- Butler TR, Ariwodola OJ, Weiner JL. The impact of social isolation on HPA axis function, anxiety-like behaviors, and ethanol drinking. *Front Integr Neurosci.* 2014a; 7:102. [PubMed: 24427122]
- Butler TR, Carter E, Weiner JL. Adolescent social isolation does not lead to persistent increases in anxiety-like behavior or ethanol intake in Female Long–Evans rats. *Alcohol Clin Exp Res.* 2014b; 38:2199–2207. [PubMed: 25092210]
- Butler TR, Chappell AM, Weiner JL. Effect of beta3 adrenoceptor activation in the basolateral amygdala on ethanol seeking behaviors. *Psychopharmacology.* 2013; 231:293–303. [PubMed: 23955701]
- Butler TR, Weiner JL. Social isolation and ethanol drinking: a preclinical model of addiction vulnerability in males, but not females. *Neuropathol Drug Addict Subst Misuse.* 2016; 59:637–648.
- Campos AC, Fogaca MV, Aguiar DC, Guimaraes FS. Animal models of anxiety disorders and stress. *Rev Bras Psiquiatr.* 2013; 35(Suppl 2):S101–S111. [PubMed: 24271222]
- Carnicella S, Ron D, Barak S. Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. *Alcohol.* 2014; 48:243–252. [PubMed: 24721195]
- Chappell AM, Carter E, McCool BA, Weiner JL. Adolescent rearing conditions influence the relationship between initial anxiety-like behavior and ethanol drinking in male Long Evans rats. *Alcohol Clin Exp Res.* 2013; 37(Suppl 1):E394–E403. [PubMed: 22924742]
- Colombo G, Agabio R, Lobina C, Reali R, Zocchi A, Fadda F, Gessa GL. Sardinian alcohol-preferring rats: a genetic animal model of anxiety. *Physiol Behav.* 1995; 57:1181–1185. [PubMed: 7652041]
- Correll CM, Rosenkranz JA, Grace AA. Chronic cold stress alters prefrontal cortical modulation of amygdala neuronal activity in rats. *Biol Psychiatry.* 2005; 58:382–391. [PubMed: 16023619]
- Cyders MA, Flory K, Rainer S, Smith GT. The role of personality dispositions to risky behavior in predicting first-year college drinking. *Addiction.* 2009; 104:193–202. [PubMed: 19149813]

- Das SK, Barhwal K, Hota SK, Thakur MK, Srivastava RB. Disrupting monotony during social isolation stress prevents early development of anxiety and depression like traits in male rats. *BMC Neurosci.* 2015; 16:2. [PubMed: 25880744]
- Deatherage G. Effects of housing density on alcohol intake in the rat. *Physiol Behav.* 1972; 9:55–57. [PubMed: 4673096]
- Debell F, Fear NT, Head M, Batt-Rawden S, Greenberg N, Wessely S, Goodwin L. A systematic review of the comorbidity between PTSD and alcohol misuse. *Soc Psychiatry Psychiatr Epidemiol.* 2014; 49:1401–1425. [PubMed: 24643298]
- Debost JC, Petersen L, Grove J, Hedemand A, Khashan A, Henriksen T, Mors O, Hollegaard M, Hougaard D, Nyegaard M, Borglum A, Mortensen PB. Investigating interactions between early life stress and two single nucleotide polymorphisms in HSD11B2 on the risk of schizophrenia. *Psychoneuroendocrinology.* 2015; 60:18–27. [PubMed: 26115144]
- Diana M, Pistis M, Carboni S, Gessa GL, Rossetti ZL. Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence. *Proc Natl Acad Sci USA.* 1993; 90:7966–7969. [PubMed: 8367449]
- Diaz MR, Chappell AM, Christian DT, Anderson NJ, McCool BA. Dopamine D3-like receptors modulate anxiety-like behavior and regulate GABAergic transmission in the rat lateral/basolateral amygdala. *Neuropsychopharmacology.* 2011; 36:1090–1103. [PubMed: 21270771]
- Ding ZM, Rodd ZA, Engleman EA, McBride WJ. Sensitization of ventral tegmental area dopamine neurons to the stimulating effects of ethanol. *Alcohol Clin Exp Res.* 2009; 33:1571–1581. [PubMed: 19485970]
- Ebner SR, Roitman MF, Potter DN, Rachlin AB, Chartoff EH. Depressive-like effects of the kappa opioid receptor agonist salvinorin A are associated with decreased phasic dopamine release in the nucleus accumbens. *Psychopharmacology.* 2010; 210:241–252. [PubMed: 20372879]
- Eiland L, Romeo RD. Stress and the developing adolescent brain. *Neuroscience.* 2013; 249:162–171. [PubMed: 23123920]
- Enoch MA. The role of early life stress as a predictor for alcohol and drug dependence. *Psychopharmacology.* 2011; 214:17–31. [PubMed: 20596857]
- Fabricius K, Helboe L, Fink-Jensen A, Wortwein G, Steiniger-Brach B. Pharmacological characterization of social isolation-induced hyper-activity. *Psychopharmacology.* 2011; 215:257–266. [PubMed: 21193984]
- Fabricius K, Helboe L, Fink-Jensen A, Wortwein G, Steiniger-Brach B, Sotty F. Increased dopaminergic activity in socially isolated rats: an electrophysiological study. *Neurosci Lett.* 2010; 482:117–122. [PubMed: 20637831]
- Flagel SB, Waselus M, Clinton SM, Watson SJ, Akil H. Antecedents and consequences of drug abuse in rats selectively bred for high and low response to novelty. *Neuropharmacology.* 2014; 76(Pt B): 425–436. [PubMed: 23639434]
- Fone KC, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. *Neurosci Biobehav Rev.* 2008; 32:1087–1102. [PubMed: 18423591]
- Fredrikson M, Faria V. Neuroimaging in anxiety disorders. *Mod Trends Pharmacopsychiatry.* 2013; 29:47–66.
- Gardner EL. Addiction and brain reward and antireward pathways. *Adv Psychosom Med.* 2011; 30:22–60. [PubMed: 21508625]
- Geyer MA. Developing translational animal models for symptoms of schizophrenia or bipolar mania. *Neurotox Res.* 2008; 14:71–78. [PubMed: 18790726]
- Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology.* 2001; 156:117–154. [PubMed: 11549216]
- Gill KE, Chappell AM, Beveridge TJ, Porrino LJ, Weiner JL. Chronic methylphenidate treatment during early life is associated with greater ethanol intake in socially isolated rats. *Alcohol Clin Exp Res.* 2014; 38:2260–2268. [PubMed: 25156616]

- Goddard AW, Ball SG, Martinez J, Robinson MJ, Yang CR, Russell JM, Shekhar A. Current perspectives of the roles of the central norepinephrine system in anxiety and depression. *Depress Anxiety*. 2010; 27:339–350. [PubMed: 19960531]
- Grant BF, Goldstein RB, Saha TD, Chou P, Jung J, Zhang H, Pickering RP, Ruan WJ, Smith SM, Huang B, Hasin DS. Epidemiology of DSM-5 alcohol use disorder: results from the National Epidemiologic Survey on Alcohol and Related Conditions III. *JAMA Psychiatry*. 2016; 72:757–766.
- Griebel G, Holmes A. 50 years of hurdles and hope in anxiolytic drug discovery. *Nat Rev Drug Discovery*. 2013; 12:667–687. [PubMed: 23989795]
- Grillon C, Dierker L, Merikangas KR. Startle modulation in children at risk for anxiety disorders and/or alcoholism. *J Am Acad Child Adolesc Psychiatry*. 1997; 36:925–932. [PubMed: 9204670]
- Grillon C, Sinha R, Ameli R, O'Malley SS. Effects of alcohol on baseline startle and prepulse inhibition in young men at risk for alcoholism and/or anxiety disorders. *J Stud Alcohol*. 2000; 61:46–54. [PubMed: 10627096]
- Hall FS. Social deprivation of neonatal, adolescent, and adult rats has distinct neurochemical and behavioral consequences. *Crit Rev Neurobiol*. 1998; 12:129–162. [PubMed: 9444483]
- Hall FS, Huang S, Fong GW, Pert A, Linnoila M. Effects of isolation-rearing on locomotion, anxiety and responses to ethanol in Fawn Hooded and Wistar rats. *Psychopharmacology*. 1998a; 139:203–209. [PubMed: 9784074]
- Hall FS, Huang S, Fong GW, Pert A, Linnoila M. Effects of isolation-rearing on voluntary consumption of ethanol, sucrose and saccharin solutions in Fawn Hooded and Wistar rats. *Psychopharmacology*. 1998b; 139:210–216. [PubMed: 9784075]
- Heinz A, Beck A, Grusser SM, Grace AA, Wrase J. Identifying the neural circuitry of alcohol craving and relapse vulnerability. *Addict Biol*. 2009; 14:108–118. [PubMed: 18855799]
- Hellemans KG, Benge LC, Olmstead MC. Adolescent enrichment partially reverses the social isolation syndrome. *Brain Res Dev Brain Res*. 2004; 150:103–115. [PubMed: 15158074]
- Holmes A, Fitzgerald PJ, MacPherson KP, DeBrouse L, Colacicco G, Flynn SM, Masneuf S, Pleil KE, Li C, Marcinkiewicz CA, Kash TL, Gunduz-Cinar O, Camp M. Chronic alcohol remodels prefrontal neurons and disrupts NMDAR-mediated fear extinction encoding. *Nat Neurosci*. 2012; 15:1359–1361. [PubMed: 22941108]
- Holmes A, Rodgers RJ. Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. *Neurosci Biobehav Rev*. 1999; 23:971–980. [PubMed: 10580311]
- Humphreys KL, Zeanah CH. Deviations from the expectable environment in early childhood and emerging psychopathology. *Neuropsychopharmacology*. 2015; 40:154–170. [PubMed: 24998622]
- Hutchison KE, McGeary J, Wooden A, Blumenthal T, Ito T. Startle magnitude and prepulse inhibition: effects of alcohol and attention. *Psychopharmacology*. 2003; 167:235–241. [PubMed: 12677352]
- Ishikawa J, Ogawa Y, Owada Y, Ishikawa A. Hyperlocomotor activity and stress vulnerability during adulthood induced by social isolation after early weaning are prevented by voluntary running exercise before normal weaning period. *Behav Brain Res*. 2014; 264:197–206. [PubMed: 24534713]
- Janak PH, Tye KM. From circuits to behaviour in the amygdala. *Nature*. 2015; 517:284–292. [PubMed: 25592533]
- Javitt DC, Freedman R. Sensory processing dysfunction in the personal experience and neuronal machinery of schizophrenia. *Am J Psychiatry*. 2015; 172:17–31. [PubMed: 25553496]
- Jovanovic T, Ressler KJ. How the neurocircuitry and genetics of fear inhibition may inform our understanding of PTSD. *Am J Psychiatry*. 2010; 167:648–662. [PubMed: 20231322]
- Kalk NJ, Nutt DJ, Lingford-Hughes AR. The role of central noradrenergic dysregulation in anxiety disorders: evidence from clinical studies. *J Psychopharmacol*. 2011; 25:3–16. [PubMed: 20530586]
- Karkhanis AN, Alexander NJ, McCool BA, Weiner JL, Jones SR. Chronic social isolation during adolescence augments catecholamine response to acute ethanol in the basolateral amygdala. *Synapse*. 2015a; 69:385–395. [PubMed: 25963724]

- Karkhanis AN, Locke JL, McCool BA, Weiner JL, Jones SR. Social isolation rearing increases nucleus accumbens dopamine and norepinephrine responses to acute ethanol in adulthood. *Alcohol Clin Exp Res*. 2014; 38:2770–2779. [PubMed: 25421514]
- Karkhanis AN, Rose JH, Huggins KN, Konstantopoulos JK, Jones SR. Chronic intermittent ethanol exposure reduces presynaptic dopamine neurotransmission in the mouse nucleus accumbens. *Drug Alcohol Depend*. 2015b; 150:24–30. [PubMed: 25765483]
- Karkhanis AN, Rose JH, Weiner JL, Jones SR. Early-life social isolation stress increases kappa opioid receptor responsiveness and downregulates the dopamine system. *Neuropsychopharmacology*. 2016; Epub ahead of print. doi: 10.1038/npp.2016.21
- Katner SN, Weiss F. Neurochemical characteristics associated with ethanol preference in selected alcohol-preferring and nonpreferring rats: a quantitative microdialysis study. *Alcohol Clin Exp Res*. 2001; 25:198–205. [PubMed: 11236833]
- Kessler RC. The epidemiology of dual diagnosis. *Biol Psychiatry*. 2004; 56:730–737. [PubMed: 15556117]
- Kinzig KP, Hargrave SL. Adolescent activity-based anorexia increases anxiety-like behavior in adulthood. *Physiol Behav*. 2010; 101:269–276. [PubMed: 20566408]
- Ko CY, Liu YP. Isolation rearing impaired sensorimotor gating but increased pro-inflammatory cytokines and disrupted metabolic parameters in both sexes of rats. *Psychoneuroendocrinology*. 2015; 55:173–183. [PubMed: 25770703]
- Kohl S, Heekeren K, Klosterkötter J, Kuhn J. Prepulse inhibition in psychiatric disorders—apart from schizophrenia. *J Psychiatr Res*. 2013; 47:445–452. [PubMed: 23287742]
- Koob GF. Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. *Curr Top Behav Neurosci*. 2013; 13:3–30. [PubMed: 21744309]
- Koob GF, Buck CL, Cohen A, Edwards S, Park PE, Schlosburg JE, Schmeichel B, Vendruscolo LF, Wade CL, Whitfield TW Jr, George O. Addiction as a stress surfeit disorder. *Neuropharmacology*. 2014; 76(Pt B):370–382. [PubMed: 23747571]
- Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology*. 2010; 35:217–238. [PubMed: 19710631]
- Kranzler HR, Rosenthal RN. Dual diagnosis: alcoholism and comorbid psychiatric disorders. *Am J Addict*. 2003; 12(Suppl 1):S26–S40. [PubMed: 14972778]
- Kushner MG. Seventy-five years of comorbidity research. *J Stud Alcohol Drugs Suppl*. 2014; 75(Suppl 17):50–58. [PubMed: 24565311]
- Kushner MG, Abrams K, Thuras P, Hanson KL, Brekke M, Sletten S. Follow-up study of anxiety disorder and alcohol dependence in comorbid alcoholism treatment patients. *Alcohol Clin Exp Res*. 2005; 29:1432–1443. [PubMed: 16131851]
- Kushner MG, Maurer E, Menary K, Thuras P. Vulnerability to the rapid (“telescoped”) development of alcohol dependence in individuals with anxiety disorder. *J Stud Alcohol Drugs*. 2011; 72:1019–1027. [PubMed: 22051216]
- Kwon LM, Rourke SB, Grant I. Intermanual differences on motor and psychomotor tests in alcoholics: no evidence for selective right-hemisphere dysfunction. *Percept Mot Skills*. 1997; 84:403–414. [PubMed: 9106827]
- Lapiz MD, Fulford A, Muchimapura S, Mason R, Parker T, Marsden CA. Influence of postweaning social isolation in the rat on brain development, conditioned behaviour and neurotransmission. *Russ Fiziol Zh Im I M Sechenova*. 2001; 87:730–751. [PubMed: 11534200]
- Liu YP, Kao YC, Tung CS. Critical period exists in the effects of isolation rearing on sensorimotor gating function but not locomotor activity in rat. *Prog Neuropsychopharmacol Biol Psychiatry*. 2011; 35:1068–1073. [PubMed: 21396422]
- Lodge DJ, Lawrence AJ. The effect of isolation rearing on volitional ethanol consumption and central CCK/dopamine systems in Fawn-Hooded rats. *Behav Brain Res*. 2003; 141:113–122. [PubMed: 12742247]
- Lukkes JL, Watt MJ, Lowry CA, Forster GL. Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. *Front Behav Neurosci*. 2009; 3:18. [PubMed: 19738931]

- Mahan AL, Ressler KJ. Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends Neurosci.* 2012; 35:24–35. [PubMed: 21798604]
- Maren S, Phan KL, Liberzon I. The contextual brain: implications for fear conditioning, extinction and psychopathology. *Nat Rev Neurosci.* 2013; 14:417–428. [PubMed: 23635870]
- Marin M, Ponce G, Martinez-Gras I, Koenke A, Curivil P, Jimenez-Arriero MA, Rubio G. Impairments of prepulse inhibition of the startle response in abstinent alcoholic male patients. *Alcohol Alcohol.* 2012; 47:545–551. [PubMed: 22582183]
- Marowsky A, Yanagawa Y, Obata K, Vogt KE. A specialized subclass of interneurons mediates dopaminergic facilitation of amygdala function. *Neuron.* 2005; 48:1025–1037. [PubMed: 16364905]
- Marsden CA, King MV, Fone KC. Influence of social isolation in the rat on serotonergic function and memory—relevance to models of schizophrenia and the role of 5-HT(6) receptors. *Neuropharmacology.* 2011; 61:400–407. [PubMed: 21414329]
- Martins SS, Gorelick DA. Conditional substance abuse and dependence by diagnosis of mood or anxiety disorder or schizophrenia in the U.S. population. *Drug Alcohol Depend.* 2011; 119:28–36. [PubMed: 21641123]
- Mathews IZ, Mills RG, McCormick CM. Chronic social stress in adolescence influenced both amphetamine conditioned place preference and locomotor sensitization. *Dev Psychobiol.* 2008; 50:451–459. [PubMed: 18551462]
- Maxwell A. Are some individuals diagnosed with ADHD prone to alcohol abuse? Consideration of two possible mediating factors for this susceptibility. *J Atten Disord.* 2013; 17:98–101. [PubMed: 22100688]
- McBride WJ, Rodd ZA, Bell RL, Lumeng L, Li TK. The alcohol-preferring (P) and high-alcohol-drinking (HAD) rats: animal models of alcoholism. *Alcohol.* 2014; 48:209–215. [PubMed: 24268381]
- McCool BA, Chappell AM. Early social isolation in male Long–Evans rats alters both appetitive and consummatory behaviors expressed during operant ethanol self-administration. *Alcohol Clin Exp Res.* 2009; 33:273–282. [PubMed: 19032581]
- McCormick CM, Smith C, Mathews IZ. Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. *Behav Brain Res.* 2008; 187:228–238. [PubMed: 17945360]
- McDonald AJ. Cortical pathways to the mammalian amygdala. *Prog Neurobiol.* 1998; 55:257–332. [PubMed: 9643556]
- McEwen BS, McKittrick CR, Tamashiro KL, Sakai RR. The brain on stress: insight from studies using the Visible Burrow System. *Physiol Behav.* 2015; 146:47–56. [PubMed: 26066722]
- Meinhardt MW, Sommer WH. Postdependent state in rats as a model for medication development in alcoholism. *Addict Biol.* 2015; 20:1–21. [PubMed: 25403107]
- Milad MR, Quirk GJ. Fear extinction as a model for translational neuroscience: ten years of progress. *Annu Rev Psychol.* 2012; 63:129–151. [PubMed: 22129456]
- Morales M, McGinnis MM, McCool BA. Chronic ethanol exposure increases voluntary home cage intake in adult male, but not female, Long–Evans rats. *Pharmacol Biochem Behav.* 2015; 139:67–76. [PubMed: 26515190]
- Nadal R, Armario A, Janak PH. Positive relationship between activity in a novel environment and operant ethanol self-administration in rats. *Psychopharmacology.* 2002; 162:333–338. [PubMed: 12122492]
- Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, Holden SS, Mertens KL, Anahtar M, Felix-Ortiz AC, Wickersham IR, Gray JM, Tye KM. A circuit mechanism for differentiating positive and negative associations. *Nature.* 2015; 520:675–678. [PubMed: 25925480]
- Nylander I, Roman E. Is the rodent maternal separation model a valid and effective model for studies on the early-life impact on ethanol consumption? *Psychopharmacology.* 2013; 229:555–569. [PubMed: 23982922]
- Padival M, Quinette D, Rosenkranz JA. Effects of repeated stress on excitatory drive of basal amygdala neurons in vivo. *Neuropsychopharmacology.* 2013; 38:1748–1762. [PubMed: 23535779]

- Pape HC. GABAergic neurons: gate masters of the amygdala, mastered by dopamine. *Neuron*. 2005; 48:877–879. [PubMed: 16364892]
- Patkar AA, Marsden CA, Naik PC, Kendall DA, Gopalakrishnan R, Vergare MJ, Weinstein SP. Differences in peripheral noradrenergic function among actively drinking and abstinent alcohol-dependent individuals. *Am J Addict*. 2004; 13:225–235. [PubMed: 15370942]
- Pervanidou P. Biology of post-traumatic stress disorder in childhood and adolescence. *J Neuroendocrinol*. 2008; 20:632–638. [PubMed: 18363804]
- Pisu MG, Mostallino MC, Dore R, Maciocco E, Secci PP, Serra M. Effects of voluntary ethanol consumption on emotional state and stress responsiveness in socially isolated rats. *Eur Neuropsychopharmacol*. 2011; 21:414–425. [PubMed: 21067904]
- Powell SB, Khan A, Young JW, Scott CN, Buell MR, Caldwell S, Tsan E, de Jong LA, Acheson DT, Lucero J, Geyer MA, Behrens MM. Early adolescent emergence of reversal learning impairments in isolation-reared rats. *Dev Neurosci*. 2015; 37:253–262. [PubMed: 26022788]
- Powell SB, Weber M, Geyer MA. Genetic models of sensorimotor gating: relevance to neuropsychiatric disorders. *Curr Top Behav Neurosci*. 2012; 12:251–318. [PubMed: 22367921]
- Ratajczak P, Wozniak A, Nowakowska E. Animal models of schizophrenia: developmental preparation in rats. *Acta Neurobiol Exp*. 2013; 73:472–484.
- Rau AR, Chappell AM, Butler TR, Ariwodola OJ, Weiner JL. Increased basolateral amygdala pyramidal cell excitability may contribute to the anxiogenic phenotype induced by chronic early-life stress. *J Neurosci*. 2015; 35:9730–9740. [PubMed: 26134655]
- Rehm J, Dawson D, Frick U, Gmel G, Roerecke M, Shield KD, Grant B. Burden of disease associated with alcohol use disorders in the United States. *Alcohol Clin Exp Res*. 2014; 38:1068–1077. [PubMed: 24428196]
- Reinhold JA, Rickels K. Pharmacological treatment for generalized anxiety disorder in adults: an update. *Expert Opin Pharmacother*. 2015; 16:1669–1681. [PubMed: 26159446]
- Rockman GE, Gibson JE, Benarroch A. Effects of environmental enrichment on voluntary ethanol intake in rats. *Pharmacol Biochem Behav*. 1989; 34:487–490. [PubMed: 2623006]
- Rose JH, Karkhanis AN, Chen R, Gioia D, Lopez MF, Becker HC, McCool BA, Jones SR. Supersensitive kappa opioid receptors promotes ethanol withdrawal-related behaviors and reduce dopamine signaling in the nucleus accumbens. *Int J Neuropsychopharmacol*. 2015; Epub ahead of print. doi: 10.1093/ijnp/pyv127
- Rosenbloom MJ, Pfefferbaum A, Sullivan EV. Recovery of short-term memory and psychomotor speed but not postural stability with long-term sobriety in alcoholic women. *Neuropsychology*. 2004; 18:589–597. [PubMed: 15291737]
- Rosenkranz JA, Grace AA. Modulation of basolateral amygdala neuronal firing and afferent drive by dopamine receptor activation in vivo. *J Neurosci*. 1999; 19:11027–11039. [PubMed: 10594083]
- Rosenkranz JA, Venheim ER, Padival M. Chronic stress causes amygdala hyperexcitability in rodents. *Biol Psychiatry*. 2010; 67:1128–1136. [PubMed: 20378100]
- Russell, VA. Overview of Animal Models of Attention Deficit Hyper-activity Disorder (ADHD). In: Crawley, Jacqueline N., et al., editors. *Current protocols in Neuroscience*. Vol. Chapter 9. 2011. p. 35
- Saalfeld J, Spear L. Consequences of repeated ethanol exposure during early or late adolescence on conditioned taste aversions in rats. *Dev Cogn Neurosci*. 2015; 16:174–182. [PubMed: 25698309]
- Samson HH, Chappell AM. Effects of raclopride in the core of the nucleus accumbens on ethanol seeking and consumption: the use of extinction trials to measure seeking. *Alcohol Clin Exp Res*. 2004; 28:544–549. [PubMed: 15100604]
- Samson HH, Czachowski CL. Behavioral measures of alcohol self-administration and intake control: rodent models. *Int Rev Neurobiol*. 2003; 54:107–143. [PubMed: 12785286]
- Sánchez MM, Ladd CO, Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev Psychopathol*. 2001; 13:419–449. [PubMed: 11523842]
- Schenk S, Gorman K, Amit Z. Age-dependent effects of isolation housing on the self-administration of ethanol in laboratory rats. *Alcohol*. 1990; 7:321–326. [PubMed: 2390208]

- Shen RY, Choong KC, Thompson AC. Long-term reduction in ventral tegmental area dopamine neuron population activity following repeated stimulant or ethanol treatment. *Biol Psychiatry*. 2007; 61:93–100. [PubMed: 16697354]
- Silberman Y, Ariwodola OJ, Chappell AM, Yorgason JT, Weiner JL. Lateral paracapsular GABAergic synapses in the basolateral amygdala contribute to the anxiolytic effects of beta 3 adrenoceptor activation. *Neuropsychopharmacology*. 2010; 35:1886–1896. [PubMed: 20410872]
- Simpson J, Kelly JP. The effects of isolated and enriched housing conditions on baseline and drug-induced behavioural responses in the male rat. *Behav Brain Res*. 2012; 234:175–183. [PubMed: 22732260]
- Skelly MJ, Chappell AE, Carter E, Weiner JL. Adolescent social isolation increases anxiety-like behavior and ethanol intake and impairs fear extinction in adulthood: Possible role of disrupted noradrenergic signaling. *Neuropharmacology*. 2015; 97:149–159. [PubMed: 26044636]
- Southwick SM, Bremner JD, Rasmusson A, Morgan CA 3rd, Arnsten A, Charney DS. Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiatry*. 1999; 46:1192–1204. [PubMed: 10560025]
- Spanagel R, Montkowski A, Allingham K, Stohr T, Shoaib M, Holsboer F, Landgraf R. Anxiety: a potential predictor of vulnerability to the initiation of ethanol self-administration in rats. *Psychopharmacology*. 1995; 122:369–373. [PubMed: 8657835]
- Spear LP. Adolescent alcohol exposure: are there separable vulnerable periods within adolescence? *Physiol Behav*. 2015; 148:122–130. [PubMed: 25624108]
- Stewart RB, Gatto GJ, Lumeng L, Li TK, Murphy JM. Comparison of alcohol-preferring (P) and nonpreferring (NP) rats on tests of anxiety and for the anxiolytic effects of ethanol. *Alcohol*. 1993; 10:1–10. [PubMed: 8095393]
- Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM, Kempadoo KA, Zhang F, Deisseroth K, Bonci A. Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature*. 2011; 475:377–380. [PubMed: 21716290]
- Sullivan EV, Desmond JE, Lim KO, Pfefferbaum A. Speed and efficiency but not accuracy or timing deficits of limb movements in alcoholic men and women. *Alcohol Clin Exp Res*. 2002; 26:705–713. [PubMed: 12045480]
- Sulzer D. How addictive drugs disrupt presynaptic dopamine neurotransmission. *Neuron*. 2011; 69:628–649. [PubMed: 21338876]
- Suvrathan A, Bennur S, Ghosh S, Tomar A, Anilkumar S, Chattarji S. Stress enhances fear by forming new synapses with greater capacity for long-term potentiation in the amygdala. *Philos Trans R Soc Lond B Biol Sci*. 2014; 369:20130151. [PubMed: 24298153]
- Thoma P, Daum I. Comorbid substance use disorder in schizophrenia: a selective overview of neurobiological and cognitive underpinnings. *Psychiatry Clin Neurosci*. 2013; 67:367–383. [PubMed: 23890122]
- Toth I, Neumann ID. Animal models of social avoidance and social fear. *Cell Tissue Res*. 2013; 354:107–118. [PubMed: 23760888]
- Valdez GR, Roberts AJ, Chan K, Davis H, Brennan M, Zorrilla EP, Koob GF. Increased ethanol self-administration and anxiety-like behavior during acute ethanol withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. *Alcohol Clin Exp Res*. 2002; 26:1494–1501. [PubMed: 12394282]
- Valdez GR, Zorrilla EP, Roberts AJ, Koob GF. Antagonism of corticotropin-releasing factor attenuates the enhanced responsiveness to stress observed during protracted ethanol abstinence. *Alcohol*. 2003; 29:55–60. [PubMed: 12782246]
- VanElzakker MB, Dahlgren MK, Davis FC, Dubois S, Shin LM. From Pavlov to PTSD: the extinction of conditioned fear in rodents, humans, and anxiety disorders. *Neurobiol Learn Mem*. 2014; 113:3–18. [PubMed: 24321650]
- Werling LL, Frattali A, Portoghesi PS, Takemori AE, Cox BM. Kappa receptor regulation of dopamine release from striatum and cortex of rats and guinea pigs. *J Pharmacol Exp Ther*. 1988; 246:282–286. [PubMed: 2839666]
- Whitaker LR, Degoulet M, Morikawa H. Social deprivation enhances VTA synaptic plasticity and drug-induced contextual learning. *Neuron*. 2013; 77:335–345. [PubMed: 23352169]

- Woicik PA, Stewart SH, Pihl RO, Conrod PJ. The substance use risk profile scale: a scale measuring traits linked to reinforcement-specific substance use profiles. *Addict Behav.* 2009; 34:1042–1055. [PubMed: 19683400]
- Wolffgramm J. Free choice ethanol intake of laboratory rats under different social conditions. *Psychopharmacology.* 1990; 101:233–239. [PubMed: 2349365]
- World Health Organization. [Accessed April 7, 2016] Global Status Report on Alcohol and Health 2014. 2014. Available at: http://apps.who.int/iris/bitstream/10665/112736/1/9789240692763_eng.pdf
- Wright IK, Upton N, Marsden CA. Resocialisation of isolation-reared rats does not alter their anxiogenic profile on the elevated X-maze model of anxiety. *Physiol Behav.* 1991; 50:1129–1132. [PubMed: 1798767]
- Yates JR, Darna M, Gipson CD, Dwoskin LP, Bardo MT. Isolation rearing as a preclinical model of attention/deficit-hyperactivity disorder. *Behav Brain Res.* 2012; 234:292–298. [PubMed: 22580232]
- Yim HJ, Gonzales RA. Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. *Alcohol.* 2000; 22:107–115. [PubMed: 11113625]
- Yim HJ, Robinson DL, White ML, Jaworski JN, Randall PK, Lancaster FE, Gonzales RA. Dissociation between the time course of ethanol and extracellular dopamine concentrations in the nucleus accumbens after a single intraperitoneal injection. *Alcohol Clin Exp Res.* 2000; 24:781–788. [PubMed: 10888065]
- Yim HJ, Schallert T, Randall PK, Gonzales RA. Comparison of local and systemic ethanol effects on extracellular dopamine concentration in rat nucleus accumbens by microdialysis. *Alcohol Clin Exp Res.* 1998; 22:367–374. [PubMed: 9581642]
- Yorgason JT, Calipari ES, Ferris MJ, Karkhanis AN, Fordahl SC, Weiner JL, Jones SR. Social isolation rearing increases dopamine uptake and psychostimulant potency in the striatum. *Neuropharmacology.* 2016; 101:471–479. [PubMed: 26525189]
- Yorgason JT, Espana RA, Konstantopoulos JK, Weiner JL, Jones SR. Enduring increases in anxiety-like behavior and rapid nucleus accumbens dopamine signaling in socially isolated rats. *Eur J Neurosci.* 2013; 37:1022–1031.
- Zapata A, Gonzales RA, Shippenberg TS. Repeated ethanol intoxication induces behavioral sensitization in the absence of a sensitized accumbens dopamine response in C57BL/6J and DBA/2J mice. *Neuropsychopharmacology.* 2006; 31:396–405. [PubMed: 16034441]
- Zapata A, Shippenberg TS. Endogenous kappa opioid receptor systems modulate the responsiveness of mesoaccumbal dopamine neurons to ethanol. *Alcohol Clin Exp Res.* 2006; 30:592–597. [PubMed: 16573576]
- Zhao Y, Weiss F, Zorrilla EP. Remission and resurgence of anxiety-like behavior across protracted withdrawal stages in ethanol-dependent rats. *Alcohol Clin Exp Res.* 2007; 31:1505–1515. [PubMed: 17760785]

Fig. 1.

The diagnosis of an anxiety disorder is associated with increased risk of developing alcohol use disorder, a telescoped progression of the disease, and a poorer prognosis in recovery. One possible explanation for these deleterious associations is that antecedent anxiety may accelerate or even eliminate the transition from positive to negative reinforcement that is thought to occur during the progression from recreational drinking to the development of alcohol use disorder. Blue: positive reinforcement; Red: negative reinforcement; Bold: increase in strength of associations.

Nucleus Accumbens

Fig. 2.

Summary of the effects of adolescent social isolation on catecholamine signaling in the nucleus accumbens (NAc). No baseline differences in extracellular levels of dopamine and norepinephrine were found in the NAc of socially isolated and group housed rats. However, higher dopamine transporter protein levels were observed in socially isolated accumbal tissue. Furthermore, social isolation was associated with increased electrically stimulated dopamine release and uptake (not illustrated) as well as a sensitization of both the dopaminergic and noradrenergic systems following an acute ethanol challenge.

Basolateral Amygdala

Fig. 3.

Summary of the effects of adolescent social isolation on catecholamine signaling in the basolateral amygdala (BLA). Lower extracellular dopamine levels and greater dopamine transporter protein levels were observed in the BLA of socially isolated rats compared to group housed subjects. No differences in these measures were observed in the norepinephrine system across the 2 groups. However, adolescent social isolation resulted in a sensitization of the dopaminergic and noradrenergic systems in response to an acute ethanol challenge.