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Recent advances in animal models of alcohol craving and relapse

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Abstract

Animal models designed to examine different facets of alcohol-related behaviors have been developed to study genetic and neurobiological factors underlying alcoholism and alcohol abuse. One goal has been to develop valid, congruent, complementary animal models of alcohol craving and relapse, with the ultimate objective of assessing the effectiveness of pharmacological agents with these models. Animal models of alcohol craving include drug-induced responding (drug reinstatement), cue-induced responding, Pavlovian Spontaneous Recovery (PSR), and appetitive/consummatory responding. A primary experimental approach to study alcohol relapse has been through expression of the Alcohol Deprivation Effect (ADE) following a single deprivation or multiple deprivations. To date, five selectively bred lines of rats have been developed to study alcohol-drinking behavior. These are the ALKO/Alcohol (AA), alcohol-preferring (P), high alcohol-drinking (HAD-1 and HAD-2 replicates), and the Sardinian alcohol-preferring (sP) lines of rats. Findings thus far indicate that only the P line of rats meets all the criteria established for a valid animal model of alcoholism, with progress having been made in characterizing the AA, HAD and sP lines of rats. The focus of the current review will be to analyze the various models of alcohol craving, emphasizing the use of the Indiana University selected rat lines (P and HADs). Overall, the findings indicate substantial progress has been made in developing animal models of alcohol abuse, relapse and craving using these selectively bred rat lines, as well as outbred rats.

Keywords: Alcohol-preferring P rats; High-alcohol drinking (HAD); Alcohol deprivation effect; Reinforcement; Reinstatement

1. Introduction

The intent of this review is to highlight recent developments in the field of alcohol research that have focused on animal models of alcohol relapse and craving. The review is divided into three sections. The first section discusses the high alcohol-consuming, selectively bred lines of rats as animal models of alcoholism. The second section discusses animal models of relapse, with specific focus on research using high alcohol-consuming selectively bred lines of rats. The third section discusses animal models of craving, again, with specific focus on research using high alcohol-consuming selectively bred lines of rats. Specifically, the animal models of relapse drinking will focus on the Alcohol Deprivation Effect (ADE) following multiple deprivation cycles. The review will also examine animal models developed to assess the presence of craving-like behaviors including drug-induced responding (drug reinstatement), Cue-Induced Responding, Pavlovian Spontaneous Recovery (PSR) and Appetitive/Consummatory Dissociation Model.

2. Animal models of alcoholism

Animal models attempt to parallel various aspects of human conditions, but most animal models are limited by the fact that animals do not express the plethora of behaviors that humans produce. However, a number of advancements

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in understanding human alcoholism have been predicated upon animal research using various models. Cicero (1979) proposed the following criteria for an animal model of alcoholism: (1) the animal should orally self-administer EtOH; (2) the amount of EtOH consumed should result in pharmacologically relevant blood EtOH levels; (3) EtOH should be consumed for its post-ingestive pharmacological effects, and not strictly for its caloric value or taste; (4) EtOH should be positively reinforcing, or in other words, the animals must be willing to work for EtOH; (5) chronic EtOH consumption should lead to the expression of metabolic and functional tolerance; and (6) chronic consumption of EtOH should lead to dependence, as indicated by withdrawal symptoms after access to EtOH is terminated. More recently, a 7th criterion has been added that states an animal model of alcoholism should also display characteristics associated with relapse (McBride and Li, 1998) because alcoholics generally go through episodes of abstinence and relapse.

The alcohol-preferring (P) line of rat has been well characterized both behaviorally and neurobiologically (e.g., Li and Lumeng, 1977; Li et al., 1993; Lumeng et al., 1995; McBride and Li, 1998; Murphy et al., 2002) and satisfies criteria proposed as essential for an animal model of alcoholism (Lester and Freed, 1973; Cicero, 1979). P rats were selected for preference for 10% (v/v) EtOH compared to water under 24 h free-choice drinking conditions. The selection criterion was for EtOH to comprise greater than 66% of total fluid intake. Currently, the P rat is in the 55th generation, and breeders are still selected for alcohol drinking aptitude. Simultaneously, there was selection for alcohol non-preferring (NP) rats. The NP rats were selected for low preference for EtOH (10% v/v), and are also in the 55th generation (for a more thorough review see Li et al., 1993; Murphy et al., 2002). *First*, P rats readily self-administer greater than 5 g/kg of EtOH per day (Lumeng et al., 1977; Rodd-Henricks et al., 2000a). After adjusting for the fourfold greater alcohol metabolism rate in rats, compared with humans, the drinking of 6 g/kg/day of EtOH is equivalent to consuming nine standard size alcoholic drinks per 70 kg person per day (cf., Li et al., 1993). *Second*, P rats can achieve blood ethanol concentrations (BECs) of 200 mg% or greater during 24-h free-choice EtOH self-administration, although typically these rats maintain BECs in the range of 50–70 mg% (Murphy et al., 1986; Waller et al., 1982a). *Third*, the alcohol preference of P rats appears to be stable even in the presence of dietary changes (Li et al., 1987). These authors reported that despite varying the carbohydrate content of the solid food (22%, 59%, or 78%), the P rats maintained high EtOH intake and preference over water. In another study, when a chocolate or saccharin solution was presented as a third-choice, P rats maintained a high level of EtOH self-administration (greater than 7 g/kg/day) (Lankford et al., 1991). *Fourth*, P rats will self-administer EtOH under operant conditions. Using a one-lever design procedure, free-fed P rats will exceed 1000 bar presses for EtOH in a 24-h period (Penn et al., 1978). In a two-lever procedure (EtOH and water) with ad lib food, P rats will operantly self-administer greater than 9 g/kg/day of 15 or 20% EtOH, whereas NP rats show a preference for water when the EtOH concentration exceeds 5% (Murphy et al., 1989). Ethanol naïve P rats do not require fluid deprivation, food restriction, or sucrose substitution procedures to acquire EtOH self-administration under operant conditions. In fact, acquisition of operant oral self-administration occurs within the 4th–6th daily 1-h operant session in P rats (Rodd-Henricks et al., 2002a,b). Further, P rats will self-administer EtOH intragastrically, which precludes the influence of taste (Waller et al., 1984). These authors reported that some animals achieved BECs greater than 300 mg% when infusing 20% or 40% EtOH. P rats will also self-administer nanoliter quantities of EtOH directly into the ventral tegmental area (Gatto et al., 1994; Rodd et al., 2004a). *Fifth*, P rats develop metabolic and neuronal tolerance under 24-h free-choice alcohol drinking conditions (Lumeng and Li, 1986). After chronic free-choice EtOH drinking, P rats display tolerance to the motor impairing (Gatto et al., 1987) and aversive effects of EtOH (Stewart et al., 1991). *Sixth*, P rats develop dependence after chronic free-choice EtOH drinking (Kampov-Polevy et al., 2000; Waller et al., 1982b).

Similar to P rats, the high-alcohol drinking (HAD-1 and HAD-2) replicate lines of rats have been selectively bred on the basis of their preference for a 10% (v/v) EtOH solution with water and food concurrently available (Li et al., 1993). Although the replicate lines of HAD rats are not as well characterized as the P line, HAD rats voluntarily consume similar amounts of EtOH as the P line during adolescence (McKinzie et al., 1998) and adulthood (Li et al., 1993), and will emit an operant response for oral EtOH self-administration (Ritz et al., 1994; Samson et al., 1998). Additionally, both P and HAD rats display an EtOH-induced enhancement of locomotor activity, which is not observed in their companion low alcohol-preferring selected lines (NP and LAD; Rodd et al., 2004b). However, the HAD replicate lines have not been as well characterized as the more established P line of rats.

The other two selectively bred rat lines have also not been as well characterized as the P rat. The alcohol-preferring AA and alcohol-avoiding ANA rats were developed from a Wistar foundational stock in Helsinki, Finland (Eriksson, 1968). Regarding criteria for an animal model of alcoholism, under free-choice conditions, AA rats readily self-administer appreciable levels of ethanol (>5.0 g of ethanol/kg body weight/day), whereas ANA rats avoid ethanol (e.g., Ritz et al., 1986). The AA rats operantly self-administer ethanol (Files et al., 1998; Samson et al., 1998). Free access to ethanol results in increased ethanol elimination rate (metabolic tolerance) for AA, but not ANA, rats (Forsander and Sinclair, 1992), and AA rats develop and display greater functional tolerance to the motor-impairing, hypothermic and hypnotic effects of ethanol than ANA rats with repeated ethanol injections (Le and Kiianmaa, 1988).

With regard to sensitivity to ethanol, AA rats are less sensitive than ANA rats to the hypnotic and ataxic effects of ethanol (Nikander and Pekkanen, 1977; Rusi et al., 1977) and AA rats display locomotor activation after limited access to ethanol (Paivarinta and Korpi, 1993). Thus, the AA line satisfies some of the criteria for an animal model but lack certain key data. For example, no information has been published on the contribution of taste and caloric factors, whether these rats can attain pharmacologically relevant blood alcohol concentrations, and if AA rats develop tolerance and dependence with free-choice drinking.

The sP and sNP rats were also developed from a Wistar foundational stock at the University of Cagliari, Italy (cf., Colombo, 1997). Regarding criteria for an animal model of alcoholism, under free-choice conditions, sP rats readily self-administer appreciable levels of ethanol (>5.0 g of ethanol/kg body weight/day) (e.g., Fadda et al., 1989), whereas sNP rats avoid ethanol. The sP rat will also operantly self-administer ethanol, indicating their willingness to work for access to ethanol (Vacca et al., 2002). Furthermore, sP rats achieve pharmacologically relevant blood ethanol levels during nocturnal free-choice bouts (cf., Colombo, 1997). Additionally, sP rats find ethanol reinforcing as indicated by a conditioned place preference (Colombo et al., 1990), and display locomotor activation after low dose ethanol, whereas sNP rats do not (Agabio et al., 2001). Also, sP rats display locomotor activation after limited access to ethanol (Colombo et al., 1998). These findings indicate that the sP line shows promise in meeting the criteria for an animal model of alcoholism. Findings thus far indicate that some of the criteria are satisfied but that additional studies need to be conducted to satisfy the criteria of involvement of taste and caloric factors, and the development of tolerance and dependence with free-choice drinking.

3. Animal models of alcohol relapse

3.1. Alcohol Deprivation Effect (ADE)

Relapse behavior is a ubiquitous problem for individuals "recovering" from alcoholism, since at least 60–80% of abstinent alcoholics will relapse during their lifetime (cf., Barrick and Connors, 2002; Chiauzzi, 1991; Jaffe, 2002; Weiss et al., 2001). Although a number of criteria for relapse have been put forth (cf., Chiauzzi, 1991), the primary criterion holds that a return to levels of ethanol consumption equal to or greater than that observed prior to "abstinence" constitutes a relapse. Because alcohol abuse has been difficult to model in non-human species, it is not surprising that there are a limited number of animal models purported to mimic relapse to high alcohol drinking behavior.

The ADE is defined as a temporary increase in the ratio of alcohol/total fluid intake and voluntary intake of EtOH solutions over baseline drinking conditions when EtOH is reinstated following a period of alcohol deprivation (Sinclair and Senter, 1967). The ADE is a phenomenon that has been observed in rats (McKinzie et al., 1998; Rodd-Henricks et al., 2000a; Sinclair and Senter, 1967), mice (Salimov et al., 1993), monkeys (Kornet et al., 1990; Sinclair, 1971), and humans (Burish et al., 1981; Mello and Mendelson, 1972). An ADE can be observed following a short (12-h or less; Sinclair and Li, 1989) or long (up to 75 days; Sinclair et al., 1973) deprivation intervals. The ADE phenomenon is not an obvious consequence of physiological withdrawal because it is evident long after observable withdrawal symptoms, which usually dissipate within 1 week (Cicero et al., 1971; Waller et al., 1982b). Furthermore, the ADE occurs in animals in which the duration of exposure to EtOH is not sufficient to result in physical dependence (McKinzie et al., 1998; Sinclair and Senter, 1967; Rodd-Henricks et al., 2000a, 2001).

The study of ADE in outbred rats has included 24-h free-choice drinking and operant oral EtOH self-administration. In the experimental paradigm of Wolffgramm and Heyne (1995), non-selected rats are concurrently presented with 5%, 10%, and 20% (v/v) EtOH over an extended time that is segmented by a long period of abstinence. Under this paradigm, some of the animals will gradually progress from a 'controlled' level to a 'high' level (2.0–3.5 g/kg/day) of intake that is thought to reflect a 'loss of control' or an 'addicted' state (Wolffgramm and Heyne, 1995). Wistar rats display a modest ADE under operant conditions (increasing 40% to ~50% total EtOH responses, Heyser et al., 1997, 2003). Both naltrexone and acamprosate are effective at reducing the expression of an ADE under operant conditions in Wistar rats (Heyser et al., 1997, 2003). In Lister rats, which had repeated training with progressive ratio testing, withdrawal from an ethanol diet (11 g/kg/day) increased the breakpoint determinant for EtOH (Brown et al., 1998). Additionally, an ADE-like phenomenon was observed in EtOH vapor-exposed rats that were abstinent for 2 or 4 weeks, and repeatedly withdrawn under operant conditions (Roberts et al., 2000). However, a shortcoming of the ADE research conducted in outbred rats is that their EtOH intake resembles those of light social drinkers at baseline and only modest increases in EtOH self-administration are observed.

The ADE phenomenon has been examined in the P rat. Adult male P rats exposed to 10% (v/v) ethanol for approximately 1 month of continuous access display an increase in ethanol consumption following deprivation intervals of either 12 h or 1 week (Sinclair and Li, 1989). Weanling male and female P rats exposed to ethanol for 7 weeks of continuous access and deprived for 4 weeks, displayed a pronounced ADE upon re-exposure (McKinzie et al., 1998). Under a 4-h operant paradigm, adult male P rats exhibited an increase in responding for ethanol compared to baseline responding after 2 weeks of EtOH deprivation (McKinzie et al., 1998). Additionally, the ADE is a long-lasting phenomenon because P rats readily express an ADE following 8 weeks of deprivation (Rodd-Henricks

et al., 2000a). The expression of the initial ADE can be prolonged if P rats are given concurrent access to 10%, 20% and 30% EtOH (Rodd-Henricks et al., 2001). Under operant conditions (1-h session length), P rats display an ADE following 5 weeks of deprivation (Rodd et al., 2003a).

In contrast to the research demonstrating that the P rat readily expresses an ADE, the other rat lines selectively bred for high alcohol preference do not readily exhibit an ADE. The Alko Alcohol accepting (AA) line of rats, prior to and after revitalization, did not express an ADE following a long-term deprivation interval (Hilakivi et al., 1984; Sinclair and Li, 1989; Sinclair and Tiihonen, 1988). In fact, the AA line of rats displays a maximal ADE between 12 and 24 h after alcohol deprivation (Sinclair and Li, 1989), but does not show an ADE with deprivation intervals exceeding 5 days (Sinclair and Li, 1989; Sinclair and Tiihonen, 1988). In addition, the Sardinian alcohol-preferring (sP) rat did not display an ADE during the initial 24-h period of EtOH re-exposure after being deprived for periods between 3 and 30 days (Agabio et al., 2000). The sP rats show a modest ADE when EtOH intake is measured during the first 3 h of re-exposure (Serra et al., 2003). The expression of an ADE in the HAD lines of rats appears to be influenced by environmental manipulations. When given access to only 10% EtOH and water, HAD rats fail to express an initial ADE (Rodd-Henricks et al., 2000b). However, given concurrent access to 10%, 20% and 30% EtOH concentrations, HAD rats express a robust ADE following the initial deprivation period (unpublished data). On the other hand, sP rats given concurrent access to 10%, 20%, and 30% EtOH do not display a robust ADE, although a modest increase in EtOH intake was observed in the first hour of re-exposure. Furthermore, a direct comparative study examining the expression of an ADE in AA, P, HAD, and Wistar rats indicated that a period of forced abstinence only increased EtOH intake in P and Wistar rats (Vengeliene et al., 2003). Overall, the data derived from the selectively bred lines do not indicate a consistent association between selection for high alcohol preference and expression of a robust ADE.

3.2. ADE following multiple deprivation–consumption cycles

Although most animal studies examining the ADE have employed a single deprivation period, research has shown that the drinking patterns of human alcoholics are segmented by multiple periods of abstinence and intake (Burish et al., 1981; Hilbrom, 1990; McMillen, 1997). This cyclic pattern of consumption and deprivation may have severe consequences in humans since multiple previous detoxifications are associated with a reduction in the response to treatment of withdrawal symptoms and heavier drinking during outpatient detoxification (Malcolm et al., 2000).

Holter et al. (1998) reported that repeated short-term deprivations (3 days every 4 weeks) produced a significant increase in consumption of 20% EtOH compared to pre-deprivation levels after 26 weeks of drinking, but there was no expression of an ADE. The increased preference for the 20% EtOH solution following repeated cycles of availability and deprivation suggested that alterations in the rewarding and/or aversive effects of EtOH might have occurred. Additionally, exposure to repeated periods of alcohol deprivation increase 'anxiety'-like behavior (Holter et al., 1998). Recently, a metabotropic glutamate receptor 5 (mGlu5) antagonist, MPEP, reduced relapse drinking following repeated alcohol deprivations (Backstrom et al., 2004). To date, the effects of repeated deprivation on operant responding for EtOH in outbred rats have not been tested.

An animal model of excessive alcohol intake has been developed with repeated deprivations in some selectively bred rats. Repeated cycles of EtOH access and forced abstinence to a single concentration (10%) of EtOH resulted in EtOH intakes of greater than 10 g/kg/day and more prolonged expression (4 consecutive days of increase intake) of an ADE in P rats (Rodd-Henricks et al., 2000a). The expression of an ADE in HAD rats given a single concentration of EtOH (10%) is dependent upon exposure to repeated cycles of deprivation and EtOH access (Rodd-Henricks et al., 2000b). In the HAD lines, there was a stepwise progression in the duration of the ADE as the number of deprivations–EtOH access cycles was increased (Rodd-Henricks et al., 2000b). However, in P and HAD rats, concurrent access to multiple concentrations of EtOH (10%, 20%, and 30%) and exposure to repeated cycles of deprivation and EtOH access markedly increased the amount of alcohol consumed (Rodd-Henricks et al., 2001; Rodd et al., 2004a,b, this volume). In P rats, exposure to repeated alcohol deprivation–access cycles results in rats consuming greater than 16 g/kg (~equivalent of a human consuming 27–32 standard drinks; cf., Li et al., 1993) during the first 24-h re-exposure period and prolongs the increased EtOH consumption for 6 consecutive days (intakes>12 g/kg/day; Rodd-Henricks et al., 2001). Additionally, exposure to repeated EtOH deprivation–access cycles altered the preference for the four solutions. Prior to any alcohol deprivations, approximately 50% of total fluid intake was from water, 25% from 10% EtOH, 15% from 20% EtOH, and 10% from 30% EtOH. Following the initial deprivation period, during the initial 24 h of re-exposure, there was a shift toward higher consumption of the 20% and 30% EtOH solutions, such that intake from these two solutions comprised over 45% of total fluid intake. Moreover, following the 4th deprivation period, during the initial 24-h of re-exposure, 40% of the total fluid intake was 30% EtOH and approximately 30% was from 20% EtOH (Rodd-Henricks et al., 2001). In fact, P rats exposed to four cycles of EtOH abstinence–access preferred the 30% EtOH solution (>40% of total fluid intake) for 4 consecutive days (Rodd-Henricks et al., 2001). Additionally, exposure to repeated alcohol deprivations and concurrent exposure to multiple EtOH concentrations produced

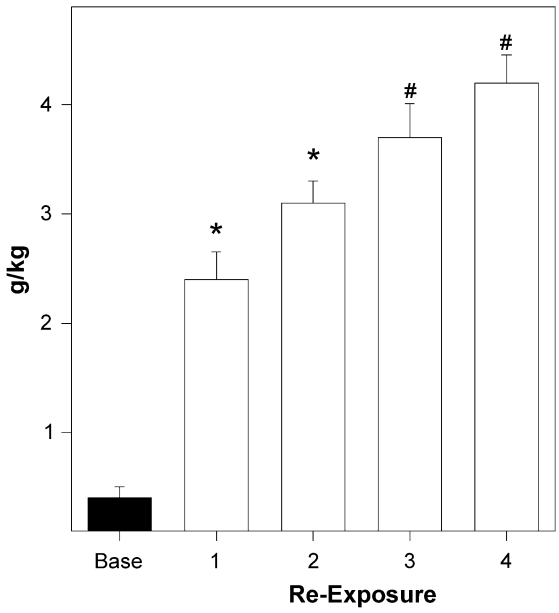

Fig. 1. Effects of repeated cycles of alcohol deprivation–access on ethanol intake during the first 1-h period of each re-exposure following 2-week deprivation periods by P rats (n=10). Baseline EtOH intake were measured during the 1-h period on the last EtOH drinking day immediately prior to the initial deprivation period. * Indicate significantly higher EtOH intake compared to baseline levels. # Indicate significantly higher EtOH intake compared to baseline levels and the first re-exposure. Data are the means±S.E.M.

a pronounced 'binge' drinking episode during the initial hour of re-exposure. During the first hour of re-exposure of the initial deprivation period, P rats consumed approximately 2.5 g/kg (Fig. 1). With each subsequent deprivation period, the amount of EtOH consumed in the initial hour of re-exposure increased until, following the 4th deprivation period, P rats consumed over 4 g/kg in that time period (Fig. 1). BECs determined by sampling blood from the tail vein 2 h after a 5th re-exposure period revealed that P rats readily consumed EtOH to obtain a BEC of over 150 mg% (Rodd-Henricks et al., 2001).

The effects of repeated deprivation–access cycles on oral operant EtOH self-administration of P rats have also been examined. Similar to 24-h free-choice consumption, exposure to repeated deprivations increased the magnitude and prolonged the expression of the ADE (Rodd et al., 2003a). Following a 3rd EtOH deprivation period, P rats were responding for 15% EtOH more than 700 times during a 1-h session (Fig. 2; Rodd et al., 2003a). Furthermore, repeated alcohol deprivation–access cycles increased the reinforcing properties of EtOH as indicated by the repeatedly cycled rats displaying a higher break-point ratio than non-deprived rats when tested under a modified progressive ratio procedure (Rodd et al., 2003a).

4. Animal models of alcohol craving

The first three models of alcohol craving are comprised of three general separate parts. Animals are trained to self-administer a specific drug, responding for drug is extinguished, and then the subject is placed into the self-administration environment in the absence of the trained reinforcer. The dependent measure for all three tests is the number of responses on a lever previously paired with the delivery of the drug, but at the time of craving testing

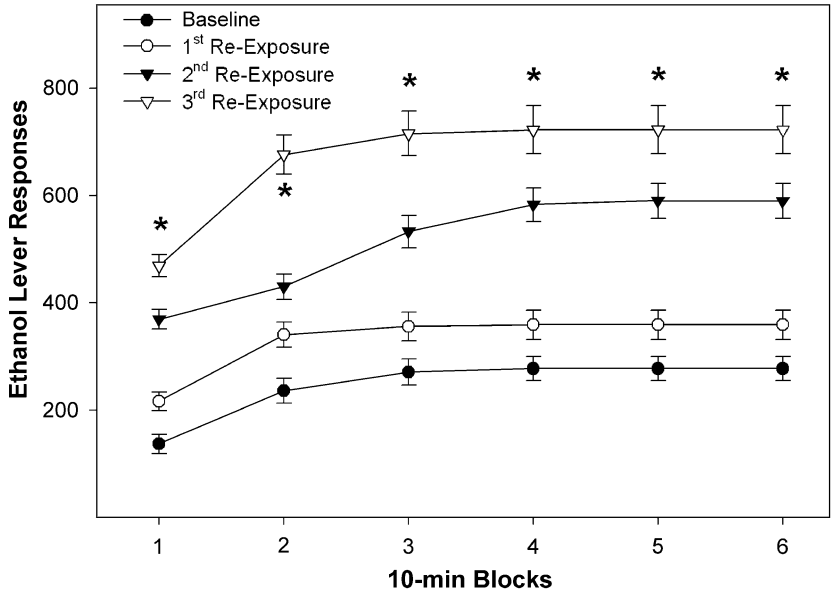

Fig. 2. The means (±S.E.M.) cumulative responses (in 10-min blocks) on the EtOH (15%) lever in the first session after each re-exposure for P rats initially deprived for 2 weeks (n=10/group), and then subjected to two cycles of 2 weeks of ethanol access and 2 weeks of deprivation (2nd and 3rd re-exposures). Baseline values are the average responses for each rat on the last 3 days prior to the initial deprivation. *p<0.05 values for all three re-exposures greater than baseline and values for each re-exposure period significantly different from the others.

responding on the operant lever is not reinforced by administration of the drug. It is the re-initiation of lever responding that is supposed to indicate drug seeking or drug craving. These models have been classified as 'reinstatement models' or more appropriately 'reinstatement of drug seeking' models (Stewart and de Wit, 1987; Shaham et al., 2003), since in the learning field, reinstatement specifically refers to a situation in which a subject regains access to a reinforcer in a certain environment (Macintosh, 1977). The models differ in the manner in which responding on the lever previously associated with drug delivery is elicited.

4.1. Drug-induced responding (drug reinstatement)

Given that operant training is generally thought to consist of both classical conditioning and instrumental learning aspects (Shaham et al., 2003; Macintosh, 1977; Bouton and Swartzentruber, 1991) it is not surprising to note that the 'fathers' of both classical and instrumental conditioning reported on the drug reinstatement model. Pavlov (1927) indicated that the ability of a conditioned stimuli (CS) to invoke a conditioned response (CR) after extinction training could be reinitiated after re-exposure to the unconditioned stimulus (US). Similarly, Skinner (1938) reported that reinstatement of lever pressing could be obtained following extinction training by non-contingent access to food or water. The ability of non-contingent priming injections of previously self-administered drugs (cocaine and amphetamine) to re-initiate lever responding after extinction training was reported by Stretch and Gerber (Stretch et al., 1971; Gerber and Stretch, 1975). Priming injections of a number of drugs of abuse (i.e., opioids, cocaine, and nicotine) are effective at promoting the performance of operant behaviors that had previously been associated with the delivery of a reinforcer (de Wit and Stewart, 1981, 1983; Shaham et al., 1997). The drug reinstatement model has been postulated as a 'drug-induced craving' model (Grimm et al., 2001) and may parallel the human studies that indicate that priming doses of cocaine can increase ratings of craving (Jaffe et al., 1989; Haney et al., 2001; Shalev et al., 2002). General criticisms of the model include that drug-induced reinstatement is associated with sensitized locomotor responses to the drug, therefore it is difficult to dissociate between craving and increase in activity (De Vries et al., 1999). Additional criticisms of the drug reinstatement model include that lack of pharmacological validity of the model (drugs that block drug reinstatement fail in the clinic to block relapse/craving) and poor ecological validity (cf., Katz and Higgins, 2003).

Le et al. (1998, 2000, 2003) have employed the drug-elicited responding model to examine alcohol-seeking behavior. In Wistar rats, priming injections of EtOH (0.5 g/kg) and exposure to footshock significantly increased responding on a lever previously associated with the delivery of EtOH (Le et al., 1998, 1999). Prior to extinction training, Wistar rats exhibit modest levels of EtOH self-administration and modest levels of drug-induced responding (Le et al., 1998, 2000, 2003). However, electric shock can induce a reinstatement of lever responding (cocaine and heroin) only if it is delivered in the same environment as drug self-administration and extinction occurred (Shalev et al., 2000). To date, the contextual specificity of the footshock induced EtOH lever reinstatement of responding has not been assessed.

4.2. Cue-induced responding

Conditioned reinforcement refers to the fact that stimuli can act as a reinforcer after previous pairings with a primary reinforcer, and this phenomenon has been postulated to be an important factor motivating relapse and drug-seeking behavior (for review, see O'Brien et al., 1998). Alcohol-associated conditioned reinforcement has been modeled in animals typically through the use of discriminative stimuli. Briefly, the experimental procedure is as follows: (a) animals are typically trained to self-administer EtOH through use of a sucrose/saccharin fade procedure (Samson, 1986), (b) animals have a CS paired (CS^+) or unpaired (CS^-) with access to EtOH, (c) EtOH responding is extinguished in the absence of the two CSs, and (d) testing occurs following extinction, during which time the CS^+ and CS^- are presented in the absence of access to EtOH. In Wistar rats following extinction training (<3 responses), olfactory cues previously paired with EtOH delivery, but not extinguished with EtOH, elicited a small increase in the recovery of responding (approximately 10 responses/session) during the operant session immediately after the last extinction session (Katner et al., 1999; Katner and Weiss, 1999). Additionally, the cue-elicited model appears to be modality dependent, with olfactory cues conditioning a response, while auditory stimuli do not (Katner et al., 1999; Katner and Weiss, 1999; Ciccocioppo et al., 2002; 2004). The amount of conditioning observed may be reduced from the optimal level as the result of blocking (presentation of the US without the CS) and from second-order conditioning effects (EtOH has a noticeable odor; Domjan and Burkhard, 1982; Macintosh, 1977).

Recently, combining vapor chamber-induced physical dependency with cue-elicited responding has advanced this model. A history of physical dependency after the acquisition of self-administration but prior to extinction training increased responding during conditioned reinforcement testing from approximately 10 to 20 responses (Liu and Weiss, 2002a,b). Exposure to footshock (10 min) can augment the effect of prior history of physical dependence on cue-elicited responding in an additive manner (~50 responses; Liu and Weiss, 2002a,b). However, a concern of the vapor chamber/footshock cue-induced reinstatment paradigm is that exposure to a vapor chamber is a stressor, thus the effects of the footshock procedure could be the result of an application of an acute stressor on an organism primed from a previous prolonged stress exposure. However, the advantage of this model over the drug-induced

model is that animals are drug-free during testing, and responding cannot be attributed to general locomotor activation of the test drug.

4.3. Pavlovian spontaneous recovery

Pavlov was the first researcher to report on the phenomenon he termed spontaneous recovery. In his early studies, Pavlov (1927) noted that while extinguishing a behavior across a number of days, there would be a small, significant increase in responding at the beginning of each new extinction session. As part of his classic bell-salivation association experiment, Pavlov (1927) indicated that following a series of extinction trials sufficient enough to eliminate the early session responding, the test subjects would salivate in response to the ringing bell if there was a sufficient time period from the last extinction session. Pavlov termed this phenomenon spontaneous recovery and defined it as reinstatement of responding (goal seeking) or a conditional response in the absence of the previously trained reward following a period of rest after extinction. However, because the term spontaneous recovery may have different connotations in the alcohol/addiction field (a recovery from alcoholism in the absence of any therapeutic treatment), the term Pavlovian Spontaneous Recovery (PSR) will be used in lieu of spontaneous recovery.

Unlike the cue-induced reinstatement model which uses discriminative stimuli to re-initiate lever responding, the PSR model is a contextual/environmental stimuli model of craving. Environmental or contextual stimuli associated with drug use is an important mitigating factor in relapse to drug use following a prolong period of abstinence (Childress et al., 1992; Crombag et al., 2002). Additionally, contextual cues have been shown to modulate various effects of drugs of abuse (Siegel, 1989; Robinson et al., 1998; Crombag et al., 2002). Recently, Shaham and colleagues have shown that reinstatement of cocaine seeking can be observed when rats are re-exposed to an environment previously associated with drug self-administration following extinction training in another environment (Crombag et al., 2002; Crombag and Shaham, 2002).

The application of the PSR phenomenon to study alcoholism and drug abuse has a number of beneficial aspects. First, spontaneous responding procedures assess operant behavior in the absence of passive drug administration within the environment previously associated with drug availability. Therefore, all responses are thought to be intrinsically motivated (Pavlov, 1927) and are not the result of drug-induced actions. Thus, spontaneous responding can be conceived as a suitable paradigm to assess ‘drug-craving’ or ‘drug-seeking’ in animals. Further, the persistence of responding in the absence of reward may parallel the compulsive nature of drug abuse in humans (Anton, 1999).

Another benefit of the PSR paradigm, and all of the reinstatement models, is that animals are processed through an extinction period. Extinction training has been postulated to assess conditioning of the reinforcing properties of stimuli to environmental cues of the operant chamber (Macintosh, 1977; Katner et al., 1999), general reward saliency of stimuli (Macintosh, 1977), and tolerance to the emotional effects of the extinction paradigm (i.e., frustration; Arzin et al., 1966). Specifically, the rate of extinction is greater if extinction training occurs in a novel environment or in an environment in which contextual cues have been removed (Macintosh, 1977). The rate of extinction is negatively correlated with reward saliency of the reinforcer as indicated by breakpoint testing (Macintosh, 1977). Arzin et al. (1966) showed that animals which gnawed less on a chew stick during extinction (a measure of frustration) displayed a reduction in the rate of extinction. An additional strength of the PSR model compared to the other animal models of craving is that the expression of an EtOH PSR is time dependent. Preliminary data have indicated that there must be a significant time period away from the operant chamber to observe an EtOH PSR (>7 days, Rodd et al., 2003b). Thus, unlike the other models, the expression of an EtOH PSR is associated with presumed neuroadaptations during the homecage period that elicit responding when re-introduced into the environment, and not the result of drug administration or learning associated cues.

The PSR phenomenon was used to determine the long-term effects of periadolescent EtOH drinking in P rats on alcohol-seeking in adulthood (Rodd-Henricks et al., 2002a). Briefly, P rats were given access to 15% EtOH and water or water only during periadolescence (post-natal days 30–60), followed by 2 weeks of water only for both groups. Both groups were then self-trained for oral administration of 15% EtOH on a concurrent FR1–FR1 schedule of reinforcement (EtOH vs. water). The FR schedule for EtOH was increased to FR5 prior to undergoing extinction training. All rats were maintained in their homecage for 2 weeks without access to EtOH, followed by re-introduction into the operant chamber in the absence of EtOH and water reinforcers. The results indicated that compared to the periadolescent alcohol-naïve group, periadolescent EtOH drinking enhanced the expression of an EtOH PSR in P rats (Rodd-Henricks et al., 2002a). Specifically, responding on the lever previously associated with the delivery of EtOH increased from an average of approximately 20 responses during extinction, to over 150 responses during PSR testing, which was a twofold higher increase than the responses for the adolescent group given only water (Rodd-Henricks et al., 2002a). In humans, adolescent alcohol drinking may have enduring consequences as suggested by the association of early onset of alcohol and drug abuse with increased risk for later drug-related problems, including alcoholism (Anthony and Pertonis, 1995; Chou and Pickering, 1992). Furthermore, priming amounts of EtOH further enhanced the expression of the PSR (Rodd-Henricks et al., 2002a) suggesting that activation of pathways mediating alcohol reinforcement could mediate alcohol-seeking behavior in the PSR model.

To determine if the effects of prior EtOH consumption on the expression of an EtOH PSR were specific to EtOH consumption during peri-adolescence, the effects of comparable adult access to EtOH prior to operant training for EtOH self-administration (30 days) on the expression of a PSR were determined (Rodd-Henricks et al., 2002b). The experimental procedures were identical to the periadolescent experiment (Rodd-Henricks et al., 2002a) except that alcohol access occurred during adulthood (Rodd-Henricks et al., 2002b). In contrast to periadolescent access to EtOH, adult EtOH drinking prior to the operant sessions did not alter the expression of an EtOH PSR (~70 responses on the EtOH lever during the initial PSR test session, threefold increase over extinction levels). Additionally, the responses on the EtOH lever increased when an EtOH odor cue was present (~120 responses in the initial PSR test session, sixfold increase compared to extinction levels). Therefore, the expression of an EtOH PSR in P rats can be enhanced with exposure to the natural odor of EtOH that may stimulate the neurocircuitry associated with the learned appetitive/approach behavior the rats had acquired during EtOH self-administration.

The effects of naltrexone on the expression of an EtOH PSR in P rats have been examined. Briefly, adult inbred alcohol-preferring (iP) rats were given 8 weeks of operant access to EtOH and water with concurrent FR5–FR1 schedule reinforcement prior to extinction training. EtOH responding was subsequently extinguished (no water or EtOH available in operant chambers), and then the rats were maintained in their homecages for 14 days. Naltrexone (0, 5, 10, or 15 mg/kg; i.p.) was administered 15 min prior to the initial PSR test session. The expression of an EtOH PSR was reduced by the 5 mg/kg dose and completely blocked by the 10 and 15 mg/kg dose of naltrexone (Fig. 3). Thus, it appears that activity within the endogenous opioid system is needed for the expression of alcohol craving within the PSR model.

In summary, the initial series of experiments exploring the expression of an EtOH PSR in P rats demonstrate that the expression of an EtOH PSR is enhanced by EtOH priming (drug reinstatement), cue-induced responding (EtOH odor cue), and environmental factors that impact drug use/abuse (periadolescent consumption of EtOH). The EtOH PSR model is also pharmacologically validated by the fact that administration of an FDA approved pharmacological agent for the treatment of alcoholism (naltrexone) decreased the expression of an EtOH PSR. Overall, the PSR data indicate this experimental approach could be important for determining mechanisms underlying alcohol craving and relapse, which could lead to the development of novel pharmaco-therapies for the treatment of alcoholism and alcohol abuse.

4.4. Appetitive/consummatory dissociation model

While the previous three models of alcohol craving examined appetitive/approach behavior to a previously obtainable reinforcer, the current model attempts to separate the performance of operants to obtain a reinforcer (appetitive) from the self-administration of the reinforcer (consummatory) at the onset of the experimental procedure. Typically, operant experiments employ a fixed-ratio (FR) schedule of reinforcement in which an animal performs a set number of responses (e.g., lever pressing) to obtain a single reinforcer. In this model, the subject performs a required number of responses and then has free-access to the reinforcer (Czachowski and Samson, 1999; Czachowski et al., 2001a,b). The FR requirement is increased across sessions until the subject fails to perform the required number of responses. Raclopride reduced responding during the appetitive phase, but acamprosate reduced only the consummatory phase without reducing responding during the appetitive phase (Czachowski et al., 2001a,b). Of

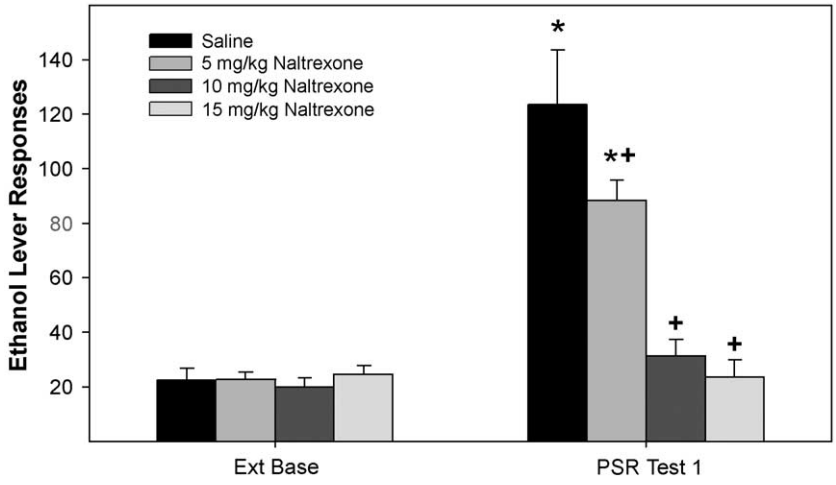

Fig. 3. The mean (±S.E.M.) responses on the lever previously associated with the delivery of EtOH during the initial 60-min PSR test session by P rats given an initial 8-week access to operant oral EtOH (15%) self-administration, extinguished for 10 sessions, and then maintained alcohol-free in their homecage for 2 weeks. Naltrexone (0, 5, 10, or 15 mg/kg; n=6/group) was given 15 min prior to PSR testing. Ext. Baseline values are the average responses for each rat on the last 3 days of extinction training. *p<0.05 significantly increased responding on the lever vs. Ext. Baseline. +p<0.05 vs. saline injected group.

particular interest, the P and HAD lines have been tested under this procedure. Both the HAD-1 and HAD-2 lines displayed a significantly higher break-point ratio (~200 and 150, respectively) than outbred rats (Czachowski and Samson, 2002). Moreover, in the P rat, the optimal break-point ratio approached 1000 responses, and the P rats consumed a greater amount of EtOH during the consummatory period. In general, these researchers concluded that both the HAD and P line of rats model the human condition of 'loss of control' that is perhaps absent in Wistar rats, and that the P rat may provide a model of 'high alcohol craving and drinking' (Czachowski and Samson, 2002).

5. Conclusion

Various animal models can produce 'craving-like' behaviors under operant conditions in both outbred and selectively bred rats. However, outbred rats typically show less evidence for alcohol craving than rats selectively bred for high alcohol preference, and an EtOH PSR has not thus far been demonstrated for outbred rats. In contrast, the selectively bred P rat exhibit significant PSR behavior, consume excessive amounts of alcohol over several days (Rodd-Henricks et al., 2001), which produced elevated BECs (>150 mg%), and have very high levels of responding in the Appetitive/Consummatory Dissociation Model (Czachowski and Samson, 2002). Therefore, genetic factors apparently underlie high levels of expression of alcohol craving/relapse. Currently, there is a need to assess the other selectively bred lines in these measures of craving and relapse. In addition, there is a need to combine models of relapse (repeated cycles of deprivation–access) and experimental induced dependency (vapor chamber exposure) with measures of craving (i.e., PSR, Appetitive/Consummatory, Cue-Elicited Responding) in outbred rats. Overall, the results indicate that there are current valid animal models of both relapse drinking/excessive alcohol intake and alcohol craving, that the P line of rats may be unique in its predisposition to expressing pronounced alcohol craving/relapse, and that the PSR procedure may be a potentially valid, important measure for studying alcohol-craving behavior.

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