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Intravenous self-administration of alcohol in rats – Problems with translation to humans

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Abstract

Alcohol is consumed orally by humans, and oral self-administration has been successfully modeled in laboratory animals. Over the last several years, attempts have been made to develop a procedure for the reliable intravenous (IV) self-administration of alcohol in rodents. IV self-administration would provide a better tool for investigating neurobiological mechanisms of alcohol reinforcement and dependence because confounding factors associated with oral self-administration, such as variations in orosensory sensitivity to alcohol and/or its absorption are avoided. A review of the literature shows that rats, mice and non-human primates can initiate and maintain IV self-administration of alcohol. However, there are 50–100 fold interspecies differences in the reported alcohol infusion doses required. Most surprising is that the infusion dose (1–2 mg/kg) that reliably maintains IV alcohol self-administration in rats results in total alcohol intakes of only 20–25 mg/kg/hour, which are unlikely to have significant pharmacological effects. The evidence to support IV self-administration of such low doses of alcohol in rats as well as the potential biological mechanisms underlying such self-administration are discussed. The minute amounts of alcohol shown to reliably maintain IV self-administration behavior in rats challenge the relationship between their blood alcohol levels and the rewarding and reinforcing effects of alcohol.

Introduction

Since the first report of operant alcohol self-administration (SA) in non-human primates (NHP) by Deneau and colleagues in 1969, numerous studies have extended the procedure to opioids, psychostimulants, barbiturates and nicotine in NHP and rodents. The successful adaptation of the technique to rodents and its application to numerous classes of drugs of abuse has been instrumental in developing our knowledge about the behavioral and neurobiological mechanisms underlying drug dependence.

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In these studies, psychostimulants, opioids and barbiturates were self-administered via the intravenous (IV) route, by which humans frequently take such drugs, and which shares the rapid onset of drug action associated with certain other routes of administration, such as inhalation. In contrast, oral SA has been the most commonly used route of administration of alcohol in laboratory animals, as this is the usual way humans consume alcohol. Reinforcing effects of oral alcohol have also been clearly demonstrated in rodents. From a neurobiological perspective, however, there are a number of limitations associated with oral SA of alcohol. Two of the most important are: 1) potential problems associated with orosensory aspects of alcohol in that its taste might be aversive to some animals at certain concentrations and 2) individual variation in absorption rate and Cmax attained or the impact of stomach conditions on alcohol absorption.

To address these issues, the IV SA of alcohol in rodents has been studied over the past several decades. Theoretically, IV SA offers an attractive alternative to investigate the neurobiological underpinnings of alcohol reinforcement without the complication of orosensory influences or variations in absorption, since the alcohol is delivered directly into the blood stream and rapidly enters the brain. While there are a number of reports detailing negative findings, many other studies demonstrate that alcohol can function as a positive reinforcer when administered intravenously via operant means. However, in most of the work demonstrating a positive reinforcing effect of IV alcohol in rodents, the unit dose (amount of alcohol delivered per reinforcement) is very low, especially in rats. This raises the issue of whether such observations might be accounted for by something other than a direct pharmacological reinforcing action of alcohol itself in the brain under these experimental conditions, since the effective concentrations are far lower than would be observed after a reinforcing oral dose. Conversely, if direct reinforcement by such low doses of alcohol cannot be ruled out, these data may serve to challenge traditional notions about the rewarding and reinforcing effects of alcohol.

In this paper we review the literature on IV SA of alcohol in experimental animals. We first provide a brief review and summary of the early work on IV SA of alcohol in NHP and then a more detailed review of the published data on IV SA of alcohol in rodents. This sets the background for subsequent discussion and interpretation of the findings dealing with IV SA of alcohol in rats.

IV SA of alcohol in NHP

Deneau et al. (1969) were the first to report IV SA of alcohol in NHP. Using an infusion dose of 200 mg/kg, they found that 4 out of 5 monkeys readily acquired alcohol SA on an FR-1 schedule with daily maximum intakes as high as 8.6 g/kg. The monkeys also displayed obvious signs of motor incoordination and even light anesthesia, and showed signs of withdrawal (tremor and convulsions) at 6 hours after the last dose of alcohol. Since then, the IV SA of alcohol has been extensively studied by the Woods laboratory in Michigan. Winger and Woods (1973) reported that six out of 14 rhesus monkeys readily acquired IV SA of alcohol at an infusion dose of 100 mg/kg, and another 2 acquired alcohol SA when the infusion dose was increased to 200 mg/kg and subsequently were maintained on 100 mg/kg per infusion. The remaining monkeys acquired alcohol SA following initiation with cocaine

or methohexital self-administration. Under 24 hr access conditions, the alcohol intake was as high as 8 g/kg/day and some of the monkeys displayed self-initiated termination for a few days, resuming alcohol SA afterwards. When access to alcohol was limited to 3 hrs per day, intake was quite stable, the animals did not self-terminate alcohol SA, and the number of infusions was significantly reduced when saline was substituted for alcohol solution (extinction). The average number of alcohol infusions obtained per session was about 30 (intake of about 3 g/kg).

In follow-up studies with these monkeys (Carney et al., 1976, Karoly et al 1978), the patterns of alcohol SA under different experimental conditions were examined. Using a VI-2 min schedule with a session duration of 1 h, and employing infusion doses from 32 to 560 mg/kg, they reported that responding for alcohol displayed a bitonic function in that low to moderate doses increased responding, while higher doses decreased responding. This resulted in an inverted U-shaped dose response function with a maximum response rate occurring at an infusion dose of 180 mg/kg (Carney et al 1976). While intake was minimal at the 32 mg/kg infusion dose, intakes in the range of 3.0 to 4.5 g/kg occurred at doses from 180–560 mg/kg, with ataxia and impairment of corneal reflexes observed at the 180 and 320 mg/kg infusion doses and with hypnosis occurring at the highest infusion doses. The amount of alcohol self-administered by these monkeys increased modestly when the session duration was extended to 3 or 6 h, yielding mean intakes of 4.0 to 4.9 g/kg respectively, accompanied by blood alcohol levels of 405 to 439 mg/dl. Other important findings from this study (Karoly et al 1978) are that 1) the numbers of self-infusions were reduced substantially when saline was substituted for alcohol solution and 2) when monkeys were preloaded with 2 g/kg of alcohol, the numbers of self-infusions of alcohol were reduced by an amount comparable to that needed to produce an intake of 2 g/kg, showing that the monkeys were capable of regulating their intake.

IV SA of alcohol has also been demonstrated by others (DeNoble and Begleiter 1978; Mello et al 1986) in male and female macaques or in male rhesus monkeys. A summary of the various studies is provided in Table 1. For the present paper, the most important information from these studies is that in NHPs, the typical training dose needed for the initiation and maintenance of IV alcohol SA is about 100 mg/kg/infusion.

IV SA of alcohol in rodents

Mice

There are three reports on the IV SA of alcohol in mice. They all employed a nose poking operant response rather than lever pressing to obtain alcohol infusions (Table 2). On an FR-3 schedule, and with infusion doses of 60, 75 and 90 mg/kg per infusion, C57BL/6 mice self-administered 1.0, 2.0 and 2.37 g/kg of alcohol respectively, as assessed on the last 2 days of the SA period. Under the same conditions, DBA/2N mice also self-administered 1.27 to 1.52 g/kg of alcohol with these infusion doses (Grahame and Cunningham 1997). In a subset of animals, alcohol SA was demonstrated with infusion doses ranging from 25 to 125 mg/kg; responding was robust at 25 mg/kg in both strains and responding for all doses of alcohol was significantly higher than for saline. During the SA sessions, a positive relationship between alcohol intake and locomotor activity was observed, indicating that the self-

administered alcohol resulted in behavioral effects (Grahame and Cunningham 1997). In a subsequent study using an infusion dose of 75 mg/kg, these workers investigated the rate of acquisition of alcohol SA in β-endorphin deficient and WT mice over 9 daily sessions. β-Endorphin-deficient mice self-administered about 1.5 g/kg over the 2 hr sessions on the last 3–4 training days, whereas WT mice self-administered only about 0.5 g/kg over the same period (Grahame et al 1998). The fact that β-endorphin deficient mice still self-administered alcohol appears inconsistent with the hypothesis that endorphins play an important role in ethanol reinforcement (Gianoulakis 1993, O'Malley et al; 1992). However, it is possible that compensatory changes in other endogenous opioid peptides such as Met- and Leu-Enkephalin might also play a more important role in the rewarding effect of alcohol in these mice.

These data therefore show that IV infusion doses of 60 to 90 mg/kg of alcohol are reinforcing in naïve mice. Of particular interest is that while the amounts of alcohol self-administered by DBA mice were slightly lower than those of C57BL/6 mice, the differences between the two strains under conditions of IV SA are much smaller than the differences observed with oral consumption either under 24 hr (McClearn and Rodgers 1959, Belknap et al 1978) or 1 hr (Le et al. 1994) 2-bottle choice access conditions. This finding suggests that when taste factors are excluded, alcohol is actually rewarding in DBA mice, which is consistent with findings from studies employing conditioned place preference (Cunningham et al 1992). IV SA of alcohol in Swiss and DBA/2 mice at infusion doses of about 90 mg/kg was also studied by Blokhina et al (2004). A significantly higher nose poking rate was observed in the mice which received alcohol infusion upon nose poking than in yoked control mice. Neither alcohol intake nor the actual number of nose pokes was reported for either group.

In summary, these three reports with mice (Graham and Cunningham 1997, Grahame et al 1998, Blokhina et al 2004), show that IV alcohol SA can be established using infusion doses ranging from 25–90 mg/kg with the amount of alcohol self-administered ranging from 1.3 to 2.5 g/kg over 2 hr sessions.

Rats

The earliest report concerning IV SA of alcohol in rats was made by Smith and Davis (1974). Using operant chambers equipped with a single lever, and with alcohol infusions available on a continuous reinforcement schedule, they reported that 8 out of 11 rats would make an average of 300 responses over a 12 hr session following 6 days of training. Each infusion consisted of 0.12 mg/kg of alcohol delivered in a volume of 0.018 ml over 0.2 sec and was accompanied by a 0.2 s buzzer presentation. Thus, the total intake was about 36 mg /kg over the12 hr session. The response pattern over the 12 hr sessions was not reported. The amount of alcohol self-administered over this period was therefore minuscule. In a subsequent study, the authors examined IV and intragastric (IG) SA of alcohol over a wide range of infusion doses (0.03, 0.1, 0.3, 1 and 3 mg/kg) under similar experimental conditions, with the exception that the session duration was 10 rather than 12 hr (Smith et al. 1976). While there was minimal responding for saline or the dose of 0.03 mg/kg alcohol/infusion, an average of about 200 presses over the 10 hr sessions was reported for the

infusion doses of 0.1 and 0.3 mg and about 120 presses for both 1 and 3 mg/kg infusion doses. The numbers of infusions reported produced a maximum intake of about 400 mg/kg and 100 mg/kg for the 3 and 1 mg /kg infusion doses respectively. Surprisingly, the number of responses made for IG SA of the 3 mg/kg dose and the total intake of alcohol were essentially the same as for the IV route.

Since these initial reports by Smith and Davis, there have been a number of positive (Numan 1981), Sinden and LeMagnen 1982, Lyness and Smith 1992, Kuzmin et al 1999, Gass and Olive 2007, Gass et al 2011, Polston et al 2013) as well as negative (Oei and Singer 1979, Grupp 1981, Grupp and Perlanski 1982, Numan et al 1984, DeNoble et al 1985, Windisch et al 2014) reports concerning IV SA of alcohol in naive rats. These studies are summarized in Table 3. In naïve rats, the majority of studies demonstrating a reinforcing effect of alcohol utilized infusion doses of 1–2 mg/kg/delivery, while those that reported negative results (with the exception of Numan et al. 1984) employed much higher infusion doses. In the studies that showed reinforcing effects of IV alcohol in rats, an FR-1 schedule was employed and an average of 50 to 75 responses on the active lever was reported for 1 or 2 mg/kg infusion doses in sessions of 8–24 hrs (Sinden and LeMagnen 1982, Lyness and Smith, 1992). While these studies suggested that alcohol infusion doses of 1–2 mg/kg might be reinforcing to rats, it is difficult to draw any conclusion regarding the significance of such SA given: 1) the long session durations employed and 2) the small amounts of alcohol self-administered by the animals and the absence of data on the patterns of SA within the sessions.

Gass and Olive (2007) and Gass et al (2011) found that rats would make roughly 30 responses on the active lever to obtain infusions about 1 mg/kg of alcohol on an FR-1 schedule during 1-hour sessions; importantly, they showed that such responding was extinguishable by saline substitution. A recent study by Polston et al. (2013) showed that rats subjected to sham or Roux-en-Y Gastric Bypass (RYGB) also obtained about 15–25 reinforcements of IV alcohol at an infusion dose of about 1 mg/kg under an FR-5 schedule, and 10–17 reinforcements under a progressive ratio schedule. Similarly, Kuzmin et al. (1999) reported that rats obtained approximately 60 and 45 infusions per 2 h session for, respectively, 1 and 2 mg/kg infusion doses of alcohol. Assuming that there were no wasted responses (lever presses during time out periods), these animals would have self-administered a maximum of 30 mg/kg over 1 hr (Gass and Olive 2007, Gass et al 2011) or 60 to 90 mg/kg of alcohol over a 2 hour period. Employing a dose range of 1–4 mg/kg of alcohol per infusion, Hyytia et al. (1996) also found that high alcohol preferring rats (AA rats), previously trained to self-administer heroin, would self-administer about 22 to 50 mg/kg of alcohol/ 3 hour session when heroin was replaced with alcohol. ANA rats, bred for low alcohol consumption, self-administered about half as much alcohol as AA rats. Importantly, the AA and ANA rats did not differ in heroin SA.

In these studies on alcohol-naïve rats, very small IV infusion doses of alcohol (1–2 mg/kg per infusion) were shown to be reinforcing, but such doses result in only minuscule amounts of alcohol being self-administered over the session. A different pattern of results was observed in IV SA of alcohol in rats that had been exposed to large amounts of alcohol by passive infusion, or by concurrent exposure to cocaine. In one study, Numan (1981) reported

that in naïve rats, an infusion dose of approximately 100–110 mg/kg (0.2 ml of 20%v/v of alcohol per infusion) was aversive. However, in rats rendered dependent on alcohol by multiple cycles of exposure to alcohol via passive IV infusion (9–16 g/kg for 4–6 days in each cycle), rats would self-administer 7.0–13.38 g/kg/day on an FR-1 schedule and 4 out of 8 rats still self-administered 8–12 g/kg per day at FR-2 using similar infusion parameters. Seven out of 8 rats displayed withdrawal signs during the alcohol withdrawal phase. Ikegami et al (2002) used a fading technique in which rats were first trained to self-administer cocaine +125 mg/kg alcohol in each IV infusion during daily 1 hr operant sessions for 7 weeks during which the amount of cocaine was reduced on a weekly basis (starting from 0.75 mg/kg per infusion and being reduced to 0.1). This was followed by 5 weeks of evaluation of IV SA of alcohol alone in the animals. During the fading period, rats would self-administer approximately 2 g/kg of alcohol in combination with cocaine during their daily 1 hr operant sessions. When tested with alcohol alone (infusion dose range 62.5 mg to 500 mg/kg per infusion), rats that had been trained to self-administer cocaine + alcohol previously would self-administer 1–1.5 g/kg alcohol during 1 h sessions at infusion doses of 125, 250 and 500 mg/kg. In rats that were trained to self-administer cocaine alone, no significant SA of alcohol was observed when cocaine was replaced by alcohol. Thus, the exposure to alcohol during the fading procedure is the most likely contributor to the enhanced IV SA of alcohol. Table 3a and 3b summarize these studies on IV SA of alcohol in rats.

Summary of the IV SA literature

It is clear from this brief review of the literature that the IV SA of alcohol can be initiated and maintained in NHP and in rodents. In NHP, although individual differences have been demonstrated, IV SA of alcohol is readily acquired, either spontaneously or following training with other drugs such as cocaine or methohexital. Initiation and maintenance of IV SA of alcohol can be achieved with infusion doses ranging from 32 to 100 mg/kg in NHP resulting in intakes of 1–8 g/kg depending on the duration of the operant session. Reports on IV SA in mice indicate that a minimum infusion dose of 25 mg/kg is necessary to maintain responding for IV alcohol. Depending on the strain employed, mice can self-administer 1.3 to 2.3 g/kg of alcohol over a 2 h period.

The data from the rats, however, are rather complex with the majority of the studies demonstrating that significant responding for alcohol occurs at infusion doses in the range of 0.12–4 mg/kg with 1–2 mg/infusion eliciting the highest rate of responding. On the other hand, in rats rendered dependent on alcohol or otherwise previously exposed to large amounts of alcohol, the infusion dose required to maintain IV SA of alcohol ranges from 62–100 mg/infusion, resulting in intakes ranging from 1.5 to 9 g/kg over, respectively, 1 or 24 h.

These findings with IV SA in rats are the most challenging to interpret in light of the extremely small infusion doses and amounts of alcohol self-administered. They raise a number of questions that will be the focus of the remainder of this paper:

I. Based on the small effective infusion doses (1–2 mg) required to initiate and maintain SA in rats and the small amounts of alcohol self-administered over a session, does such SA result in blood and/or brain alcohol levels relevant to reinforcement? Could such responding for alcohol be mediated by other factors besides the direct pharmacological effects of alcohol in the brain?

II. If responding for alcohol cannot be explained by other factors, how can reinforcing effects of alcohol occur at such low doses?

III. Why are the infusion doses needed to maintain IV SA of alcohol so much higher in rats that have a history of exposure to alcohol?

IV. Why is there such a large variation across species in the effective doses of IV alcohol required to maintain responding?

I. Does IV SA result in blood or brain alcohol levels relevant to reinforcement?

In most studies involving oral SA of alcohol, rats generally take an average of 500 to 1200 mg/kg of alcohol in 30 or 60 minute sessions, attaining BALs ranging from 15 to 70 mg/dl, as determined at the end of the SA sessions (Weiss et al. 1993, Marinelli et al. 2007, Simms et al. 2010, Augier et al. 2014). Each reinforcement is commonly about 0.1–0.2 ml of 8–12% w/v alcohol which is calculated to be about 26.7 to 80 mg/kg for a rat with a weight of 300 g. On the other hand, under conditions of IV SA of alcohol with infusion doses of 1–2 mg/kg, rats generally obtain about 20–30 infusions over 1 or 2 h sessions, providing a total of only 20–60 mg/kg.

The total body water in the adult rat is approximately 60–70% of body weight, or about 600–700 ml per kg of body weight. If the infusion dose is 1 mg/kg, and assuming complete distribution without any elimination, the resulting blood alcohol level would be roughly 0.123 mg/dl if adjusted to reflect the fact that about 80% of the blood is water. Without taking elimination into account, if rats obtain 30 infusions in a 60 minute session, the maximum BAL that could be achieved would be around 4 mg/dl. Based on the average blood alcohol disappearance rate of 30 mg/100 ml per hour (Khanna et al 1985) there would be no alcohol present in the blood if metabolism is taken into account, especially if most of the alcohol intake occurs within the first 10–15 minutes of the session as is commonly observed.

Following IV administration through the jugular vein, alcohol will first enter the pulmonary circulation and within a matter of seconds, the brain. The main factor most relevant to a potential explanation of SA of low doses is rapid redistribution of alcohol. Specifically, it is highly likely that in the seconds after IV infusion, blood and brain alcohol levels would be much higher than after redistribution. Sunahara et al. (1978) reported a rapid equilibration between arterial and hippocampal tissue levels of alcohol that occurred within 1 minute after cessation of IV infusion of different doses of alcohol. To our knowledge, there is only one study that has examined blood and brain alcohol levels following IV infusion of low doses of alcohol (2 mg/kg administered over 30 s). This is a dose comparable to those that support IV

SA by rats (Asin et al 1985) yet the rate of infusion was much slower. These authors reported brain alcohol levels of 5.2, 3 and 1.2 μg/g of tissue obtained at 1, 2 and 5 minutes after infusion. Trunk blood collected at the same time points showed BALs of approximately 0.35, 0.28 and 0.2 mg/dL or roughly about 1 to 3 times higher than a projected equilibrated BAL of 0.123 mg for a 1 mg infusion dose, assessed at 1 minute. These results suggest that the low infusion doses of ethanol (1–2 mg/kg) employed in various studies would produce detectable alcohol levels in the brain that might contribute to its reinforcing effects.

Under conditions of operant SA, most of the responding for alcohol occurs within the first 10–15 minutes of the session. If many infusions are obtained and consumed during a short period of time, this may lead to sufficiently high brain/blood alcohol levels to exert pharmacological actions. Unfortunately, most of the studies on IV alcohol SA did not present the within-session pattern of IV SA, with the exception of the study by Hyytia et al (1996) that used AA and ANA rats. Responding for the 1 mg/kg infusion dose of alcohol by AA rats was characterized by a burst of approximately 4–10 infusions (4 to 10 mg/kg of alcohol) in the first 10–20 minutes of the sessions. Even then, 10 infusions (10 mg/kg) of alcohol taken over a period of 10 minutes would not result in detectable BALs if one takes into account the redistribution of alcohol from the blood. Another issue is whether brain alcohol levels of 5–10 μg/g, that would be projected from such SA would produce reinforcing effects.

a. Responding for low infusion doses of IV alcohol: is it a false positive, unrelated to reinforcement produced by direct pharmacological action in the brain?

Non-pharmacological variables involved in operant procedures, particularly the use of light, tone or light+tone stimuli associated with drug delivery, have been shown to play an important role in maintaining or facilitating responding for drugs. This is especially important for nicotine SA as first demonstrated by Caggiula et al (2002). This work showed that rodents do respond for the presentation of light and/or tone alone and that nicotine enhances such responding. Most interesting is the study by Olsen and Winder (2009) showing that C57/BL mice respond at high rates for a flashing light on fixed-ratio (FR) as well as on progressive ratio (PR) schedules and that such behavior can be extinguished by removing the cue light. This phenomenon has been referred to as “operant sensation seeking” (OSS). Nicotine has been shown to act as a primary reinforcer as well as to enhance responding for other reinforcing stimuli including stimuli typically associated with nicotine SA procedures (Donny et al 2003, Chaudhri et al 2006, Palmatier et al 2006). Neither the possibility that a low infusion dose of alcohol might have reinforcing effects of its own, nor that it enhances the reinforcing effect of non-pharmacological stimuli (light / tone) associated with alcohol delivery or both can be ruled out. The possibility that these factors contribute to the reported acquisition and maintenance of IV SA of alcohol needs to be evaluated.

An examination of the work demonstrating IV SA of alcohol at low infusion doses (1–4 mg/kg/infusion) in rats suggests that OSS alone likely does not account for the reported SA of low doses of alcohol. With the exception of studies utilizing a compound visual and auditory cue (Gass and Olive 2007 and Gass et al 2010) or an auditory cue alone associated

with alcohol delivery (Smith and Davis, 1974, Smith et al 1976), the remaining studies on IV alcohol SA did not employ or describe any stimulus associated with alcohol infusion (Smith and Davis 1974, Sinden and Le Magnen 1982, Kuzmin et al 1999).

b. Evidence that IV SA of low doses of alcohol is reinforcing due to direct pharmacological action in the brain

i. Responding for alcohol occurs on high demand schedules and can be extinguished and reinstated—One of the most critical tests used to evaluate the reinforcing efficacy of a drug is to determine if responding for the drug occurs on high demand schedules. Polston et al. (2013) demonstrated that rats self-administer alcohol at infusion dose of 1mg/kg under both FR-5 and PR-2 schedules and that such SA can be modified by Roux-En-Y-Gastric Bypass (RYGB) surgery. In this study, licking was used as the operant response; licking an active empty tube would lead to an infusion of 1mg/kg of IV alcohol whereas licking another distinct empty tube (inactive) or a water tube had no consequences. The sham operated rats would make about 90 licks at the active tube and obtain about 18 infusions of alcohol under an FR-5 schedule. Under a PR2 schedule, in which the demand to obtain each subsequent reinforcement is increased by 2 licks, these rats would make about 150 licks and receive about 12 infusions. Rats that had been subjected to RYGB worked harder to obtain IV infusions of alcohol, making respectively 130 and 350 licks on the FR-5 and PR-2 schedules and received 26 and 17 IV infusions of alcohol. While the mechanisms underlying the enhanced reinforcing effects in the RYBG rats relative to the sham operated rats are unknown, with respect to the present discussion the important fact is that greater response rate for alcohol was seen when higher demand schedules were employed.

Another critical test of whether operant responding to obtain a drug is due to its pharmacologically-based reinforcing effects is to see if such behavior is extinguished following removal of the drug. This is commonly done by substituting saline for the drug, or inactivating the infusion pump. There are four such studies of IV SA of alcohol in rats. Smith and Davis (1974), using an infusion dose of 0.12 mg/kg and 12 hr daily sessions, reported that rats made about 300 responses on the lever by the sixth session. Saline was substituted for alcohol beginning on the 7^{th} session, and this produced a marked reduction to about 10 lever presses by the 8^{th} session. Lyness and Smith (1992) reported that following 9 days of IV SA of alcohol at doses ranging from 1–4 mg/kg per infusion, substituting saline for alcohol on day 10 resulted in an extinction burst on that day in all rats that previously displayed alcohol SA. On day 11, one would expect to see a reduction in response rate but unfortunately this was not measured. When the alcohol solution was reintroduced, responding returned to pre-extinction levels.

Probably the clearest and most systematic data suggesting that lever pressing for low doses of IV alcohol is maintained by reinforcing pharmacological effects of ethanol come from Gass and coworkers. Gass and Olive (2007) reported that the number of active lever responses made by rats pressing for IV alcohol (1 mg/kg) was about 28. This was reduced to about 6–7 presses after 10 or more extinction sessions. Importantly, re-exposure to cues previously associated with alcohol delivery, priming with a 0.5 g/kg dose of alcohol or

exposure to the pharmacological stressor yohimbine reinstated alcohol seeking (Gass et al 2011).

ii. Responding for IV alcohol in rats can be influenced by genetics—Additional evidence that supports reinforcing effects of IV SA of low infusion doses of alcohol in rats is derived from a study by Hyytia et al. (1996). They showed that AA and ANA rats which were bred for, respectively, high and low voluntary oral intake of alcohol consumption did not differ in IV SA of heroin. However, when heroin was replaced by IV alcohol (1–4 mg/kg/infusion), AA rats self-administered about 2.5 times as much alcohol as ANA rats.

iii. Other evidence of reinforcement being produced by direct pharmacological action of alcohol in the brain—A number of studies using a variety of different techniques have evaluated the rewarding or reinforcing effects of low doses of IV alcohol or intracranial administration of alcohol. The results of these studies may help to shed light on the question of whether rats self-administer low doses of IV alcohol for its pharmacological effects in the brain. The following section will discuss and interpret these data. Finally, the results of this work and that previously discussed will be used to evaluate a possible mechanism by which rats self-administer low doses of IV alcohol.

Place conditioning: Based on the evidence that IV SA of alcohol in rats occurs at low infusion doses, Asin et al (1985) examined the possibility that these doses have positive motivational effects in the conditioned place preference paradigm. No CPP to alcohol was observed after several training trials with doses of 1–8 mg/kg of alcohol given IV. However, in the same study the authors also reported that IP alcohol (0.05 to 1 g/kg) did not affect preference; the lack of a positive result with IP alcohol makes the data with IV infusions difficult to evaluate. Alcohol doses of 1g/kg or higher, given by either IV or intragastric infusion, have been reported to produce conditioned place aversion (Van der Kooy et al 1983). On the other hand, Walker and Ettenberg (2007) found that ICV infusion of 180 nmol (8.3 μg) of alcohol/2 μl, induced a significant CPP, while other doses (60, 120 and 240 nmol) did not. These data suggest that rewarding effects of alcohol can be elicited by central administration of a low and narrow dose range of ethanol.

Runway task: Steffensen et al. (2009) assessed the rewarding effects of IV infusion of a 10 mg/kg dose of alcohol in a runway paradigm in which rats received the infusions upon reaching a goal box. Rats that had been treated twice daily with alcohol (2 g/kg IP) for 14 days, had much shorter running times for the IV alcohol infusions (10 mg/kg) than did saline treated animals. Furthermore, animals receiving the IV alcohol in the goal box had shorter running times than those receiving saline. These data indicate that IV infusion of 10 mg/kg of alcohol has rewarding effects. However, they also reported that IV infusion of 10 or 30 mg/kg alcohol enhanced both the firing rate and evoked synaptic activity of 50% of VTA neurons identified as GABAergic. How these neuronal effects relate to the behavioral changes remain to be explained.

Intracranial SA: Intracranial SA of alcohol (IC SA) in a 100 nl infusion volume at concentrations of 100 to 200 mg/dl into the posterior ventral tegmental area (VTA) or into the shell of the nucleus accumbens (NACs) has also been demonstrated (Rodd et al 2004,

2005; Engleman et al 2009). An infusion of 100 nl of 100 mg/dl alcohol would be equivalent to about 0.1 μg of alcohol per infusion. At concentrations of 75 to 200 mg/dl, Wistar and P rats obtained about 30 to 50 intracranial infusions over a 4 hr session. While the pattern of the intracranial SA of alcohol into the VTA and NACs during the 4 h session was not presented, it is clear that these low doses of alcohol, infused into these discrete brain areas can maintain operant responding.

Taken together, these studies using CPP, runway task and IC SA demonstrate that low doses of alcohol administered IV or intracranially can exert rewarding effects and elicit neuronal activation in reward-relevant brain regions.

c. Potential mechanisms underlying IV SA of low doses of alcohol

i. Infusion doses of 1–2 mg/kg of IV alcohol result in sufficient brain alcohol levels to exert rewarding or reinforcing effects—As mentioned earlier, IV infusion of 2 mg/kg of alcohol over a duration of 30 s results in detectable levels of blood and brain alcohol 1 and 5 min after administration (Asin et al 1985) with 5 μg/g of alcohol being detected in brain tissue 1 min after completion of the infusion. This establishes that alcohol is detectable in the brain after IV administration of doses of alcohol that support SA. Rats tend to show a “burst” pattern of responding for IV alcohol (1–2 mg/kg/infusion), particularly early in the SA sessions (Hyytia et al 1996). Given this high rate of responding early in the session, it can be calculated that at IV infusion doses of 1–2 mg/kg, rats could obtain 3–6 mg of alcohol within a matter of seconds or minutes. It is possible that these amounts of alcohol would be sufficient to activate neuronal circuits involved in the rewarding or reinforcing effects of alcohol. In the studies of IC SA into the VTA or the NACs mentioned above, infusion doses of 100 nl at concentrations of 100–200 mg/dl supported the initiation and maintenance of responding; these doses are equivalent to about 0.1–0.2 μg of alcohol per infusion. The average weight of the NAC in rat brain is about 70 mg. Therefore infusion of 0.2 μg of alcohol in this area would lead to a maximum alcohol concentration of about 2.8 μg/g tissue, an amount comparable to the 3–5 μg/g of alcohol measured in brain tissue following IV infusion of a 2 mg/kg dose of alcohol (Asin et al 1985). It is therefore possible that sufficient amounts of alcohol can reach brain areas such as the VTA and NAC that are critical to the reinforcing effects of alcohol, and thus maintain responding for IV infusions of these doses.

ii. Acetaldehyde—A possible mechanism underlying the reinforcing effects of low infusion doses of alcohol may be its metabolism to acetaldehyde (AcH). The potential euphoric action of AcH was proposed by Truitt and Walsh (1971). Alcohol is metabolized primarily in the liver to AcH by alcohol dehydrogenase (ADH) and then to acetate by aldehyde dehydrogenase-2 (ALDH2) with cytochrome P450 and catalase playing more minor roles. In the brain, catalase is responsible for about 60% of alcohol metabolism, CYP 2E1 for about 20% and the rest by ADH and some other unknown factors (Zimatkin et al 2006). It should be noted that the partial metabolism of alcohol by ADH is consistent with the high level of detection of ADH1 mRNA in the CNS epithelial and vascular tissues (Martinez et al 2001). Catalase activity is readily detectable in rat brain areas such as the VTA, NAC and ventral pallidum (Moreno et al 1995), as well as in neurons containing DA,

noradrenaline and serotonin (Zimatkin and Lindros 1996) that are thought to be involved in the mediation and regulation of drug reward. AcH generated in the periphery does not cross the blood brain barrier. AcH has been shown to have dual actions on motivation with a well documented aversive effect mediated by peripheral AcH and a rewarding effect mediated through central AcH (see Quertemont et al 2005, Deng and Deitrich 2008; Israel et al 2013). Earlier work by Amit's group demonstrated that rats self-administer AcH into the lateral ventricle (Brown et al 1979) and that ICV administration of AcH can produce CPP (Amit and Smith 1985). P rats also self-administered AcH into the posterior VTA at concentrations 1000 times less than those required for alcohol (Rodd et al 2005). The central reinforcing effect of AcH has also been demonstrated recently in a series of elegant studies in which administration into the VTA of an anti-catalase gene linked to a viral vector, that reduced catalase activity by 70–80%, suppressed alcohol consumption under normal or alcohol deprivation conditions in high alcohol drinking UChB rats (Karahanian et al 2011, Tampier et al 2013). Conversely, increasing AcH in the VTA by administering a gene coding for ADH enhances alcohol consumption.

As pointed out earlier, detectable alcohol concentrations in the brain are found following IV infusion of 2 mg/kg of alcohol. It is possible that with IV SA of alcohol in such a low dose, there may be a shift in the balance between the aversive and rewarding effects of AcH. With IV administration, the amount of AcH that can be produced via metabolism in the liver would be minimal, therefore minimizing the induction of aversive effects of AcH. It is therefore possible that the resulting levels of AcH in the brain with such doses of IV alcohol would be reinforcing. Conversely, the absence of reinforcing effect of higher doses of IV alcohol could be produced by relatively higher levels of peripheral AcH, outweighing the reinforcing effects in the brain.

Independent of the differences or similarities in catalase activity between rats, mice and NHPs, one potential approach to determine the role of AcH in IV SA of low doses of alcohol in rats is to examine whether inhibition of brain catalase by intracerebral injection of anti-catalase gene linked to a viral vector or a catalase inhibitor would attenuate or block responding for low doses of IV alcohol. This is a question that merits investigation.

iii. Is responding for low doses of alcohol mediated peripherally?—The differential effects of alcohol on the rewarding effects induced by peripheral and central administration might be related to the balance between the opposing effects (aversive and rewarding) of acetaldehyde in the periphery and in the brain. Rewarding effects of drugs can also be mediated by their peripheral actions. Bechara and Van der Kooy (1985) have shown that IP and SC administration of methyl naltrexone, which does not cross the blood-brain barrier, can induce CPP in rats. On the other hand, administration of naltrexone via the same routes produced conditioned place aversion. Under both conditions, the IP route of administration produced a greater degree of preference and aversion than the SC route for methyl naltrexone and naltrexone, respectively. The authors suggested that opioids produce positive reinforcing effects in the brain but aversive effects in the periphery.

It is possible that responding for low doses of IV alcohol might also be mediated through such a mechanism. For example, one might consider the possibility that local activation of

sensory receptors in the jugular vein could be induced by a low concentration of alcohol or by the volume of infusion and thus might induce afferent stimuli carried to the brain via the vagus nerve and arriving a few seconds before ethanol itself. This would be analogous to the role of environmental cues such as light or sound in operant sensation seeking mentioned above. Such a possibility can be examined neurophysiologically but these studies have not yet been done. Alternatively, one can argue that IV administration of 1–2 mg/kg of alcohol can produce intravascular effects that influence mechanisms underlying taste perception, that might be reinforcing to the rats. Although alcohol delivery via the IV route can minimize or reduce the oral perception of alcohol taste, it does not, however, completely eliminate the factor of taste, as a conditioned taste aversion to IV saccharin paired with gamma irradiation has been demonstrated (Bradley and Mistretta 1971). The possibility that such a small amount of intravascular alcohol affects taste can be tested by examining whether IV alcohol functions as an effective CS in an alcohol taste aversion procedure.

Alcohol also has significant caloric content, with 1 g of alcohol generating 7000 cal. As mentioned above, the total amount of alcohol intake under IVSA is about 30–50 mg/kg over a 1 hr session which would yield about 350 cal/kg of body weight or about 140 cal for a 400 g rat. This caloric value, however, is unlikely to play a significant role in the IV SA of alcohol as the energy derived from this amount of alcohol is slightly lower than is contained in a single 45 mg food pellet (158 cal) (Bioserv). Rats with unlimited access to food in the home cage can obtain about 80 of these pellets in a 1 hr session when pressing on an FR-10 schedule (Rudski et al 1994). A final argument against caloric mediation of alcohol IV SA is that IV SA alcohol intakes under slight food restriction (20 g per day) and under ad lib conditions are comparable (Gass and Olive 2007, Polston et al 2013).

III. Why are the infusion doses needed to maintain IVSA of alcohol so much higher in rats that have a history of exposure to alcohol?

As noted earlier in this review, IV infusion doses of alcohol in the 100 mg/kg range are aversive in alcohol-naïve rats, whereas rats rendered dependent by multiple cycles of exposure to alcohol self-administer alcohol at infusion doses 40–55 times higher than naïve rats, and receive total amounts of alcohol 200–300 fold higher than naïve rats (see table 2). There are two possible interrelated factors that might account for such high levels of IV SA in dependent rats. The first factor might be the development of tolerance to alcohol induced by chronic exposure. Tolerance has been regarded as an important factor in the regulation of alcohol intake, and can do so in two different ways. First, alcohol has both rewarding and aversive properties and with repeated exposure, tolerance to the aversive effect of alcohol may develop, therefore unmasking or amplifying its rewarding effects. Thus, an initially aversive infusion dose of alcohol might become reinforcing following repeated exposure (Numan 1981). In addition, as tolerance develops, it would enable the animals to self-administer much higher amounts of alcohol. It is possible that such high levels of intake in dependent rats might be governed by different mechanisms than in naïve rats. One could argue that the major factor in driving such levels of SA in alcohol-dependent rats is the negative reinforcing effects of alcohol, due to alleviation of withdrawal distress.

Such a phenomenon, however, was not observed in NHP as Winger (1988) reported that responding for IV alcohol was reduced or suppressed in the presence of withdrawal signs. Similarly, Myers et al (1972) reported no changes in volitional alcohol consumption (oral) following induction of dependence in monkeys. The absence of dependence-induced changes in IV SA of alcohol in monkeys might be related to differences in experimental design. In the study with monkeys, the subjects were exposed to withdrawal and then received access to alcohol once, whereas in the Numan study with rats, animals were exposed to multiple withdrawal-SA cycles. Under such circumstances, the animals would have an opportunity to learn that consumption of alcohol would alleviate withdrawal symptoms.

In one of the earliest studies (Hunter et al 1974), increases in alcohol consumption were not observed following one cycle of exposure and withdrawal in the rat, but were observed after multiple cycles. A number of recent studies have also found that consumption in mice (Becker and Lopez 2004, Lopez et al 2012) or rats (O'Dell et al 2004, Vendruscolo et al 2012) increased only after animals were exposed to multiple withdrawal episodes. Recent work by Vendruscolo et al (2012) is of interest in relation to the strength of the negative reinforcing effect of alcohol under these conditions. The alcohol-dependent rats, but not non-dependent ones, still self-administered alcohol orally despite the aversive effects of quinine that was added to the alcohol solution.

It is noteworthy that in the studies of oral alcohol SA mentioned above, induction of dependence through multiple cycles of withdrawal increased total alcohol intake by rats and mice by about 25% relative to controls. In contrast, in the IV SA study, the dependent animals obtained roughly 200 times as much alcohol as the non-dependent controls. The oral studies, however, mainly assessed alcohol SA in 30 min to 2 hr SA sessions, whereas in the Numan study with IV SA, alcohol intake was assessed over 24 hrs. To resolve this issue, the extent to which dependence induces changes in alcohol intake by the two routes needs to be compared over the same time frames.

IV. Differences in the infusion dose required to maintain IV SA among rats, mice and NHP

As pointed out earlier, there are 25–30 fold differences in the alcohol infusion doses required to initiate and maintain responding for alcohol in rats vs. mice and NHP. While an IV infusion dose of 1–2 mg/kg is required for rat IV SA, a minimum dose of 25–30 mg/kg is needed for mice and NHP. An obvious question is whether such differences might be related to differential sensitivity to alcohol between these species. There are some data that allow such inter-species comparison. For example, IP doses of alcohol in the range of 0.5 to 2 g/kg in rats (Baldwin et al 1991) or 1–2 g/kg in mice (Vogel et al 1980) have been shown to increase punished responding in a conflict paradigm. Oral administration of 1.5 to 2.5 g/kg of alcohol increases punished responding in squirrel monkeys (Glowa and Barrett 1976; Barrett et al 1985). While the effects of alcohol on aggression show large individual variation, a dose range of 0.1–0.6 g/kg given orally has been suggested to apply to rats, mice and monkeys (Miczek et al 1997). Similarly, alcohol doses of 1.0 to 2.0 g/kg have been

shown to function as effective interoceptive stimuli, as studied with the drug discrimination procedure in these species (Middaugh et al 1991, Helms et al 2007, Besheer et al 2012). It is therefore unlikely that interspecies differences in the infusion dose needed to maintain IV SA is due to differential sensitivity to alcohol.

A related but different possibility is that the species differences in infusion doses might simply reflect phenotypic differences in patterns of responses to alcohol across species. For example, the stimulatory effect of alcohol on locomotor activity, that has been related to the rewarding effect of alcohol, can be readily demonstrated in mice, but not in rats (Frye and Breese 1981). Similarly, conditioned place preference to alcohol can be established in mice (Cunningham et al 1992) but not in rats (Asin et al 1985, Van der Kooy et al. 1983, Bormann and Cunningham 1998). In addition, doses of alcohol 0.75 g/kg or higher can produce conditioned place aversion in rats (Van der Kooy et al. 1983, Bormann and Cunningham 1998, Ciccocioppo et al 1999) but no conditioned place aversion was seen in mice at doses at high as 4 g/kg (Cunningham et al 1992). Thus rats appear much more sensitive to the aversive effects of alcohol than mice. If they are, it could mean that only very low doses are reinforcing for them because higher doses produce aversive effects that over-ride the reinforcing effects.

Another potential explanation for the differences between rats, mice and NHP in the IV infusion doses of alcohol that maintain responding might be related to AcH. One could argue that such differences might be related to species differences in brain catalase activity and/or the capacity to metabolize alcohol and generate AcH in the brain. As pointed out earlier, the doses of IV alcohol effective in maintaining responding in rats are 1–2 mg/kg/infusion whereas in mice it is about 25 mg/kg/infusion. A large difference in catalase activity would be required to account for such differences, but it appears that catalase activity in the brains of rats and mice is comparable. For example, the catalase activity in the caudate putamen and hippocampus is about 52 and 50 U/mg protein respectively in mice (Przedborski et al 1992) and about 58 and 49 U/mg protein in rats (Vertechy et al 1993). Catalase is also present in NHP brains (Shukla et al 1995), but to our knowledge, there are no data allowing valid comparison of brain catalase activity between rodents and NHPs.

It is important to note that the large species difference in infusion doses for alcohol IV SA does not occur with other drugs of abuse such as cocaine and nicotine. In the case of nicotine, infusion doses in the range of 10–60 μg/kg have been been used to initiate and maintain self-administration in mice (Fowler and Kenny 2011), rats (Corrigall and Coen 1989) and monkeys (LeFoll et al 2007). In the case of cocaine, self-administration can be initiated and maintained in monkeys in the dose range of 0.02 to 0.3 mg/kg (Howell et al 2007), from 0.03 to 0.75 mg kg in mice (Kmiotek et al 2012) and from 0.08 to 0.7 mg/kg in rats (Sizemore et al 1997).

Summary and conclusions

The development of the IV SA paradigm overcomes a number of confounding factors encountered in the use of oral SA, such as individual variation in the absorption and metabolism of alcohol, and taste factors, which can complicate or limit the interpretation of

mechanistic studies related to the actions of alcohol and its abuse. Over the last few years, using computer assisted self-infusion of ethanol (CASE) methodology, a number of studies have established that humans do self-administer alcohol intravenously with an average intake of about 0.6 g/kg over a 2 hour period (Zimmerman et al 2008, 2009; Hendershot et al 2016) with resulting BALs of 80–100 mg/dl, consistent with many other indications that IV alcohol is reinforcing in humans.

The literature reviewed above shows that NHPs and mice also self-administer alcohol intravenously in amounts exceeding 1.0 g/kg per 1–2 hr session, which is comparable to observations in humans. In rats, however, the amount of intake by IV SA is very much smaller, ranging from 0.02 to 0.045 g/kg over a 1–2 hr session. Therefore, the translational value or relevance of such IV SA of alcohol to alcohol-taking by humans is uncertain. However, the initiation and maintenance of operant responding for such small amounts of alcohol by rats has been observed by a number of laboratories, and raises a number of interesting issues regarding the mechanisms underlying this behavior. One study of IV SA suggests that the reinforcing effects of alcohol can be produced by very small amounts of alcohol and correspondingly low levels of alcohol in the brain. However, there are very few data on the concentrations of alcohol in brain regions implicated in the rewarding effects of the IV SA of alcohol. Microdialysis techniques have been used successfully to measure alcohol concentrations in the NAC during oral alcohol SA. Perhaps this technique can be employed to measure alcohol levels in VTA and NAC during IV SA of these low doses of alcohol.

The possibility that responding for these low IV doses of alcohol is mediated by a peripheral mechanism involving local activation of sensory receptors in the jugular vein cannot be ruled out. Given that such operant responding occurs only for infusion doses of 1–2 mg/kg of alcohol, but neither for saline nor higher infusion doses of alcohol suggests that the potential reinforcing effects induced by peripheral sensory activation in the jugular vein occur within a narrow, low alcohol dose range in a fixed volume of saline. While a direct experimental approach to address this issue is rather difficult to design, one possible way is to examine whether such operant responding for a 1 mg/kg infusion dose of alcohol can be maintained under different alcohol concentrations and hence with different infusion volumes. If self-administration induced by 1 mg/kg infusion of alcohol is still maintained under these conditions, it would be likely that the responding is mediated by the actions of alcohol in the brain. The development of other experimental approaches to answer this question will require a great deal of ingenuity.

There is only one study examining the role of physical dependence on IV SA of alcohol in rats, but the findings are of particular interest due to the marked changes in the infusion dose required and the amount of alcohol self-administered in dependent rats. The findings also have potential translational value to understanding human alcohol-taking. It is clear that more work is required to confirm these limited findings and to further investigate this phenomenon. Similarly, more work must be done to further our understanding of the role of negative reinforcement and the development of tolerance in the production of such pronounced changes in the IV SA of alcohol.

Finally, the inter-species differences in animal studies indicate that caution is required in extrapolating from rats to humans with respect to the neurobiological mechanisms involved in the positive and negative reinforcing effects of alcohol. The fact that these species respond to various other effects of alcohol in comparable doses and that there is little interspecies difference in the reinforcing or rewarding effects of other drugs of abuse raises some challenges as to whether IV SA of such low doses of alcohol in rats reflects a direct effect of alcohol on the brain.

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Table 1

IV self-administration of alcohol in NHP

Reference	History	Schedule and Time Out (TO)	Infusion dose (mg/kg)	Session duration	Vol. concentration	Maximum intake	Behavioral signs	Note
Deneau et al 1968	naïve	FR1; TO: NA	200	24 hr/day over 22 weeks	0.25ml in 20%	8.5 g/kg	Motor impairment, stupor-withdrawal	4 out of 5 acquired
Winger and Wood 1973	14 naïve	FR1; TO: NA	100–200	3–24 hr/day	15–30% w/v	3–8 g/kg in monkeys that acquired (8 out of 14)	NA	8 acquired SA; 4 and 2 monkeys acquired SA after training with cocaine or methohexital
DeNoble and Begleiter 1978	2 naïve and 2 treated with alcohol for 4 months	FR-10; 1 m TO	100	20 hr/day: 4 hr food-4hr-E and -4 hr Sucrose	15% w/v 0.5 ml/s	Aver 6.9 g/kg/day	NA	Alcohol maintains responding but not isocaloric sucrose
Carney et al 1976	Macaca Mullata	VI 2-min; TO :NA	100 training/ DRC 32–560	1 hr	NA	Dose related 1–4.8 g/kg	Ataxic to hypnotic	No significant SA at 32 mg/kg infusion dose
Karoly et al 1978	13 from Winger and Wood	FR-1 and FR30; TO : NA	50–200	3 and 6 hr	0.66 ml of 15% w/v	Dose related 3,9--4.7	NA	BAL of 405
Altshuler et al 1980	22-	CRF; TO: NA	100	24 hr and then 4 hr	2ml in 15–20% w/v	3g/kg over 4 hr	NA	only 13 acquired and maintain responding
Mello et al 1986	6 female	VI food and alcohol on second order schedule; TO: NA	120	4 1hr-session /day	12% w/v	1.5–4.5 g/kg/day	NA	Lower SA during menstruation.
Williams et al 1998	3 (with history of methohexital SA)	FR-10; TO: NA	10–100	130 m twice daily	NA	Dose related 1.9 to 2.5 g/kg	NA	Difference in response rate from saline was detected only at 32 mg/kg dose
Broadbear et al 2005	53 (cocaine and methohexital)	FR-30; 10s TO	100 training/ DRC 32–560	130 min	30% w/v	Dose related 1.8–2.4 g/kg	NA	Difference in response rate from saline was detected only at 30 mg/kg

Abbreviations: SA: Self-administration; BAL: Blood Alcohol Level; CRF: Continuous reinforcement; FR: Fixed ratio; VI: Variable Interval; VR: Variable ratio; NA: Not available

TABLE 2

IV self-administration of alcohol in mice

Reference	Strain	Schedule	Session duration	Infusion dose (mg/kg)	Vol/ rate of delivery	Cue and Time Out (TO)	Intake g/kg	Note
Grahame and Cunningham 1997	C57BL/6 (16) and DBA/6j (22)	FR-3	2hrs	25–125	0.005 in 1 s	Light; 2s TO	1.0–2.4 (C57) and 1.3–15 (DBA) for infusion doses range 60–90 mg/kg	DBA do self-administer alcohol
Graham et al 1998	β-end deficient mice	FR-3	2 hrs	75	0.005ml in 1 s	Light; 2s TO	1.5 for β-end deficient and 0.6 for WT	129/Sv × C56BL/6 hybrid background
Blokhina et al 2004	Swiss and DBA	FR1	0.5	0.9 in 2% v/v	0.0019 ml in 1s	NA/ 1s TO	NA	No raw data was presented

Abbreviations: FR; fixed ratio; NA: not available

Table 3

A: A summary of Positive studies of IV alcohol self-administration in rats								
Reference	**Strain**	**Schedule**	**Session Duration/ number of sessions**	**Infusion dose (mg/kg)**	**Infusion rate Vol/time**	**Cue and Time Out (TO) in second**	**Maximum intake g/kg**	**Note**
Smith and Davis 1974	Holtzman	CRF	12 h	0.12	0.018 ml over 0.2s	Buzzer; NA	300 infusions or 0.036	Rapid extinction
Smith et al 1976	Sprague Dawley (SD)	CRF	10 h	0.03 to 3	0.023 ml in 0.25 s	Buzzer; NA	0.05 to 0.4 at 0.3 to 3 dose	100–120 infusion at 1 and 3 mg/kg dose
Numan 1981	Long Evans	FR1- FR-3	24 h	80–110	0.2 ml/ 1s	None; 1s TO	7.9–13.4	SA initiated after rats rendered dependent.
Sinden and LeMagnen 1982	Wistar	FR1	24 h	0.5–5	0.1 ml/ 3s	None; 3s TO	about 0.065	2–3 responses/ 5 min interval at 1 mg. No SA at 0.5 or 5 mg
Lynes and Smith 1992	SD	CRF	8 h	0.5–8	NA	None; NA	0.075–0.1	SA at 0.5– 4 mg/kg infusion dose.
Hyytia et al 1996	AA/ANA (Heroin experience)	FR-1	3h	1–4		Light; 10s TO	0.02 -0.05 g/kg for AA rats and lower for ANA	Bursting responses within first 10 min for 1 mg/kg dose
Kuzmin et al 1999	Wistar	FR-1 (nose poke)	2 h	0.5–4 and saline	0.03ml/3s	None; 3 s TO	45–60 infusion for 2 and 1 mg dose or total of 0.045 and 0.06 g/kg	Similar responding for saline, 0.5 and 4 mg doses.
Ikegami et al 2002	SD (Cocaine fading)	FR-1	75 m	62.5–500	0.1 ml/6s	Light; 20 s TO	0.75–1.4 at 125–500 mg/kg infusion dose	BAL:44–221 mg/dl; self-infused 7–11 g/kg/week over 7 weeks of cocaine fading-
Gass and Olive 2007	Wistar	FR-1	1 h 2 sessions/day	1mg	0.03ml in 1 s	Light /tone; 4s TO	0.02	-extinction; cue, priming and stress-induced reinstatement
Gass et al 2011	Wistar	FR1	1hr	1mg	0.03 ml in 1s	Light+tone; 4s TO	0.02	-decrease responding with saline substitution)
Polston et al 2013	Sprague-Dawley, obese sham and RYGB	FR-5 and PR-2	1 hr	0.6–1.0 mg	0.03 ml in 2s	None; 10s TO	18 and 25 infusions at FR5 and 12 and 17 at PR for sham and RYGB rats.	RYGB increase IVSA on FR-5 and PR-2.

B: A summary of Negative studies of IV alcohol self-administration in rats								
Reference	**Strain**	**Schedule**	**Session Duration/ number of sessions**	**Infusion dose (mg/kg)**	**Infusion rate/Vol**	**Cue and Time out (TO)**	**Maximum intake g/kg**	**Note**
Oei and Singer 1979	Wistar	FT-1; Schedule induced injection	1 hr	8	0.05 ml	None; 5s TO	0.08–0.12	Food restriction 80% FFBW;↑ intake with contingent food delivery
Grupp 1981	Wistar	FR-3 to FR8	1.5 hrs	1–180	0.1 ml of 0.4–52% w/v over 60 s	Tone; 3s TO	0.5 at 30 mg dose (estimated)	No relationship with FR No SA
Grupp and Perlansky 1982	Wistar	Concurrent VI 60 and FI 600 s for food and E	75 min	200–400	0.6 ml/60 s	Clicking noise		No SA
Numan et al 1984	Long Evans	FR-1	24	1–16 mg; glucose	0.06–0.1 ml / 0.5–1.2 s	NA; NA	5–20 responses/day; no difference from glucose	Absence of SA at doses of 1–16 mg/kg
DeNoble et al 1985	Hooded	FR-1	24	30–360	0.2–0.5 ml over 4–8 s	Blinking light; 4s TO	No differences from saline	
Windisch et al 2014	P rats	FR-4 FR4 sequential schedule with sucrose (S) and S+ alcohol	30 min	25 mg/kg	20% v/v	Light; 7s TO	480 mg; alcohol infusions were paired with S deliveries	IV 25 mg/kg is aversive. It is S that drives responding.

Abbreviations: FR; fixed ratio; CRF: continuous reinforcement; NA not available; RYGB: Roux-en-Y gastric bypass.

Abbreviations: FI: Fixed Time; FI Fixed Interval; FFBW Free feeding Body Weight; NA: Not available