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Review

Operant self-administration of ethanol in infant rats

Ricardo Marcos Pautassi [a,c,*], Roberto Sebastián Miranda-Morales [a,*], Michael Nizhnikov [b]

[a] Instituto de Investigación Médica M. y M. Ferreyra (INIMEC–CONICET-Universidad Nacional de Córdoba), Córdoba C.P 5000, Argentina
[b] Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, NY 13902-6000, USA
[c] Facultad de Psicología, Universidad Nacional de Córdoba, Córdoba C.P 5000, Argentina

HIGHLIGHTS

- The infant rat is highly sensitive to appetitive motivational effects of ethanol.
- Operant response to ethanol in infancy is enhanced by exposure to ethanol in utero.
- Operant response to ethanol in infancy is mediated by opioid transmission.
- Operant response to ethanol can facilitate subsequent ethanol intake.

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ABSTRACT

The review focuses on operant self-administration of ethanol in immature, infant rats. Several methods for the analysis of ethanol intake in infants are available, yet only oral self-administration models the typical pattern of ethanol consumption found in humans. The study of ethanol intake in infants is important for our understanding of how early alcohol experiences facilitate subsequent engagement with alcohol. It seems that sensitivity to ethanol-induced operant reinforcement is found very early in life, a few hours after birth, and throughout the first three weeks of life. Most of the studies reviewed complied with most, albeit not all, of the criteria for operant behavior (e.g., greater responding than yoked controls and persistence of this difference after withholding the reinforcer). Operant self-administration of ethanol in infant rats seems to be, at least partially, mediated by endogenous opioid transmission and can be enhanced by prior exposure to ethanol. Furthermore, acquisition of ethanol-mediated operant learning seems to facilitate drug self-administration during adolescence. Relative to older subjects, infants exhibit lower sensitivity to ethanol's sedative, hypnotic and motor impairing effects. On the other hand, they exhibit increased sensitivity to the motor stimulant and rewarding effects of ethanol. We suggest that this pattern of response to ethanol may favor the rapid acquisition of operant self-administration in infant rats.

Contents

* Corresponding author at: Instituto de Investigación Médica M. y M. Ferreyra (INIMEC–CONICET), Friuli 2434, Córdoba C.P 5000, Argentina. Tel.: +54 351 4681465; fax: +54 351 4695163.
E-mail address: rpautassi@gmail.com (R.S. Miranda-Morales).

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1. Introduction

The study of alcohol effects can be done at several levels. Cell cultures and brain slices provide a controlled environment in which the interaction of ethanol and specific neurons or their receptors can be carefully scrutinized. Behavioral studies, on the other hand, provide a benchmark to analyze mechanisms and treatment effects in vivo, as well as ethanol-related learning that can shape alcohol seeking and intake. Passive exposure to ethanol, via intraperitoneal, gavage administration or inhalation offers a method by which the researcher can carefully manipulate dose, concentration and interval of administration. These are key variables in the expression of phenomena such as ethanol-induced inflammation, or behavioral sensitization. Behavioral sensitization, the gradual increase in the stimulant effect of ethanol following chronic ethanol administration in mice [27], is more clearly observed after intermittent administration of low doses of ethanol, whereas continuous exposure to high ethanol doses is more likely to induce tolerance. This is just an example, but illustrates the usefulness of using a controlled, dose–response approach to analyze ethanol's effects.

As relevant as experimenter-administered studies are, ethanol oral self-administration is preferred in a wide-variety of situations, notably because the oral route is better to model the typical pattern of consumption found in humans. Moreover, oral self-administration procedures allow examining how ethanol's pharmacological, post-ingestive effects are modulated by the orosensory properties of ethanol (e.g., taste, smell). Several researchers have postulated that the bitterness of alcohol serves as a natural protection to prevent initial escalation into alcohol consumption. Kiefer [42] found significantly lower ingestive and higher aversive orofacial responses in rats given familiarization with alcohol than in naïve counterparts. Pautassi et al. [78] found avoidance of a texture that lined a chamber in which pups received intraoral infusion of 5% ethanol. Yet pups developed conditioned preference for the texture when it was paired with the delayed, post-ingestive effects of ethanol. This result suggests motivational dissociation between the aversive effects of ethanol odor and taste and the apparent reinforcing effects that take place following its ingestion. Two-bottle choice studies, in which animals are given access to water and ethanol [97] and taste reactivity studies (in which animals are subtly stimulated on the tongue with drops of liquid) also provide support for this "taste barrier" hypothesis [42]. Moreover, neurotransmitter release and utilization of glucose after administration of morphine or cocaine are different when using forced or self-administration procedures [91]. Other important phenomena, such as the usual peak in alcohol consumption after a period of abstinence (i.e., alcohol-deprivation effect) and its modulation by opioid antagonism [37] can only be analyzed through the use of self-administration models [10].

There are several examples of ethanol self-administration models, two of which are consummatory in which alcohol is readily available from tubes in forced or, more commonly, in two- or three bottle choice tests [111]; or operant self-administration models, in which animals have to execute an arbitrary behavioral response, such as pressing a lever or nose-poking, to obtain a small quantity of ethanol [48].

In the present review we will focus on operant self-administration studies of ethanol intake, and particularly in those conducted in immature, so called "developing" animals: infant rats [80]. It may seem illogical to study ethanol intake in infants, given that children usually rely on parental control to access food and liquids and, therefore, the possibilities for self-administration of alcohol would be scarce. Exposure to alcohol in infants, however, seems more common than usually thought, both due to accidental exposure, cultural practices such as use of cloths embedded with alcohol for analgesic purposes, and also due to maternal alcohol drinking during lactation in spite of scientific warning against it [109]. Perhaps more important, recent studies indicate that the onset of alcohol initiation is quickly descending worldwide. A birth cohort study, for instance, indicated that almost 20% of a sample of Brazilian children aged 11–12 years had already experimented with alcohol [76]. A more recent study conducted in Argentina [89] indicated alcohol sipping and tasting in 58% of its sample (n = 367) of 8–12 year old children. These early alcohol experiences could facilitate subsequent engagement with alcohol during adolescence, which in turn significantly enhances the possibilities of alcohol abuse and dependence later in life. Several works, notably a large state-wide Canadian study [20], have found greater ratio of alcohol-related problems in those who begin to drink before age 15, compared to those that delayed alcohol initiation till after age 15. These works may appear as a group of isolated studies, yet when taken together indicate the need for further analysis of drinking initiation during infancy and their impact on subsequent alcohol preference.

An added advantage of using an immature rat model is that the developing brain provides an opportunity to correlate normal, programmed changes in brain function with corresponding changes in learning and behaviors, or in sensitivity to or predisposition to ingest drugs. For instance, assessment of ethanol intake in infants through an independent feeding procedure revealed a sudden upward shift in ethanol acceptance by postnatal day 6, which coincides with the shift in function of the GABA system (from excitatory to inhibitory) around this age [98].

The review will provide a historical overview and in-depth discussion of studies analyzing operant self-administration of ethanol. The challenges and pitfalls of studies in adult, mature subjects will be discussed, yet the focus will then shift to studies conducted during early ontogeny. The main aim is to provide an updated and systematic review of studies on operant self-administration of ethanol during infancy. Particular emphasis will be put on how these studies shed light on the effects of early active exposure to alcohol on later alcohol preference at late adolescence and adulthood. Based on results obtained from more traditional, classical conditioning approaches [80] or from non-operant self-administration methods [98], the working hypotheses will be that operant self-administration of ethanol can be readily established in infants and that such ethanol-mediated learning is (a) enhanced by prior experience with alcohol odor, taste or post-ingestive effects, (b) comparable to that induced by non-drug reinforcers (e.g., sucrose), (c) dependent on the integrity of the endogenous opioid system, and (d) is associated with greater predisposition for later alcohol intake.

As we will find out, it is not always distinctively clear when a behavior falls under the umbrella of operant conditioning. When should we consider that operant behavior occurs in the context of ethanol self-administration in infancy? We propose that, to fully claim such a finding, any given study should exhibit several (and if possible all) of the following criteria: a) a seemingly arbitrary behavior is made contingent with alcohol access, b) after this arrangement the target behavior significantly grows in magnitude when compared to baseline, as well as vs vehicle and vs yoked, unpaired control groups; c) after withholding the reinforcer an extinction curve is observed and subsequently the behavior emerges from time to time without exposure to any explicit stimuli (i.e., spontaneous recovery). Last but not least, there should be evidence indicating that response is maintained by the post-ingestive, pharmacological effects of alcohol [97]. The use of yoked, unpaired controls should not be underestimated in studies assessing drug-mediated operant learning. Yoked animals are given the reinforcer each time the paired animal receives it, yet the delivery of this reinforcer is completely independent of the behavior. That is, yoked controls are exposed to equivalent amounts of the reinforcing stimulus as experimental animals but have no control over the relationship between operant behavior and reinforcement. The use of a yoked control provides similar advantages to those yielded by an unpaired control in classical conditioning studies. An unpaired control is exposed to both conditional and unconditional stimuli (CS and US, respectively) but in an unrelated manner. This reduces the possibility of pseudoconditioning and, in pharmacological studies, controls for unspecific (e.g., toxic) effects of drug exposure.

After discussing if and when infant rats establish operant self-administration, potential neural mechanisms underlying successful examples of this learning will be analyzed. In this search we will be guided by a conceptual framework that has been used to explain why adolescent rats drink twice as much alcohol than their adult counterparts [116]. It has been suggested that age-related differences in sensitivity to alcohol's postingestive effects may underlie the greater intake in adolescents [106,107]. Among other effects, it has been found that adolescents are more sensitive to the stimulating and rewarding effects of ethanol, but less sensitive to the sedative, aversive and hypnotic effects of the drug [108]. This pattern of response seems to put adolescents at risk for initiation of and escalation into alcohol consumption. Could a similar reasoning be used to explain rapid establishment of operant self-administration of ethanol in infant rats? The final section of this review will turn to examination of this issue.

2. Methods

A narrative review on studies assessing operant self-administration of ethanol in infant rats was conducted. Papers were searched from PubMed database, using the strings "operant ethanol infant rat", "ethanol self-administration infant rat", "ethanol operant infant rat" and combinations of these strings. The search returned around 20 unique hits. These were checked for accuracy and appropriateness to the subject matter. The studies by Johanson and Hall [40] and Arias et al. [9] were kept for the narrative review because they provided methodological basis for subsequent studies in the target matter, although they lack assessment of ethanol self-administration. A total of 11 papers were thus selected as the core studies to analyze, and those 9 that effectively assessed ethanol-induced reinforcement are summarized in Table 1. It should be noted that, for the sake of brevity, mice studies were omitted in the initial search and in the subsequent discussion, yet the reader is directed to the comprehensive review by Lopez and Becker [48].

3. Early studies and studies of ethanol-self administration in adult rats

The use of ethanol in self-administration studies has a long and rich history. The seminal work by Deneau et al. [19] indicated that animals (monkeys) would self-administer several drugs into the bloodstream, including ethanol. Similarly, ethanol was observed to decrease the level of central electrical stimulation required to maintain self-administration (a classic sign of addiction potential of a drug) [52] and rats were also found to self-administer ethanol directly into the brain. These developments allowed important discoveries on the mechanisms underlying ethanol reinforcement. For instance, the current ongoing discussion on the role of acetaldehyde on ethanol reinforcement [49,84] was greatly fueled by early discovery of acetaldehyde self-administration into the cerebral ventricles, an effect that was disrupted by a dopamine-beta-hydroxylase inhibitor [2].

Self-administration of ethanol into the bloodstream or brain indicates that the drug may be ingested for its central reinforcing properties and also provides a benchmark for assessing promising novel pharmacological compounds to reduce alcohol intake. Yet, humans predominantly employ the oral route to administer ethanol and this prompted several researchers to test if animals would perform a seemingly arbitrary behavior (e.g., lever pressing) to access a limited, fixed quantity of ethanol. These studies had to face similar problems as those faced by two-bottle choice studies. Animals would reluctantly self-administer ethanol, probably due to the apparent aversive properties of alcohol odor and taste. When they engaged in self-administration the blood alcohol levels achieved were low and it was disputable whether self-administration was driven by the pharmacological effects of the drug [96,97]. The delay between alcohol ingestion and onset of its pharmacological effects, significantly longer in the oral vs. the intraperitoneal or intravenous route, further complicates this issue [110]. Last but not least, even high levels of alcohol-self administration are confounded by the activating and sedative effects of the drug, that may by themselves increase or decrease the number of operant responses. The use of extinction phases, in which animals that achieve self-administration are suddenly non-reinforced, has been used to separate the acute effects of the drug from the underlying ethanol-mediated operant learning. Sometimes, animals exposed to operant extinction exhibit a response increase relative to baseline response levels during training. This spiked response has been suggested to be analogous to the frustration effect [112] observed in instrumental negative contrast paradigms [9].

A series of techniques have been developed to facilitate the initiation into alcohol oral self-administration. Most of them employ some sort of "acclimation" to the orosensory properties of alcohol. In one of the earliest, referred to as program-induced polydipsia, animals are deprived of food and water. Solid food is intermittently provided to these animals and is accompanied by access to alcohol and water. High consumption of ethanol quickly ensues and is usually maintained after animals are returned to an ad-libitum feeding program [53,55].

An alternative technique utilizes the progressive substitution of sucrose by alcohol in a mixed solution. Animals are given brief familiarization to 10% alcohol via forced access and then are trained to lever press in a fixed ratio 1 schedule (or lick a tube) for 10 or 20% sucrose. Once the response is established, typically following three or four sessions of variable length (see [94]), animals respond for 10% sucrose mixed with a diluted (e.g., 2%) alcohol solution. As training progresses, response requirements are increased and sucrose is progressively substituted for alcohol. This is, a fixed ratio 2 or 5 schedule is employed and animals lever press to have access to a mix of 10% sucrose and 5% ethanol, then to a mix of 10% sucrose and 10% ethanol, etc. In some studies, subjects that had begun responding for 20% sucrose end up in a few weeks accepting 10% alcohol [93]. The so-called sucrose-substitution procedure and the programmed induced polydipsia have received substantial criticism. It has been said that it is unknown if animals self-administer ethanol due its pharmacological effects or due to its caloric or orosensory effects [26]. Another possibility is that pairing of alcohol access with food or sucrose endows the flavor of alcohol with conditioned reinforcing properties that subsequently modulate its intake, regardless of its pharmacological effect. Alcohol's odor and taste may modulate initial acceptance of alcohol in humans, yet chronic, problematic drinking is maintained by the drug's pharmacological effects.

An elegant study [94] helped dispel some of these doubts. Rats self-administered alcohol plus sucrose and in later sessions only ethanol, with progressively increasingly demanding response requirements, until they reached a basal, constant level of responding. Specifically, after 16 sessions animals were required to execute 25 lever presses to have continuous access to alcohol for a limited amount of time (20 min) and achieved intake of around 1.3 g/kg/20 min. Although lacking unpaired or yoked control groups, this preparation (known as the "sipper" procedure) seems to comply with many of the most stringent criteria of ethanol-induced operant conditioning. This preparation undoubtedly separates alcohol seeking from alcohol intake, therefore controlling for the acute activating and depressing effects of the drug. Yet it could be asked if the post-ingestive effects of the drug are actually supporting the learned response. After session 16 half of the animals received an alcohol intubation (1.0 g/kg) or vehicle followed by intraperitoneal administration of the emetic agent lithium chloride. The aim was to devalue the pharmacological effect of alcohol to assess if this was followed by a consequent reduction in ethanol self-administration. Insensitivity of ethanol self-administration to devaluation of ethanol's pharmacological effects would indicate that self-administration was mainly regulated by orosensory effects of ethanol. The results obtained during extinction indicated that responding for ethanol was dramatically reduced in the devalued, but not in the control group, a result arguing in favor of pharmacological regulation of alcohol self-administration [94]. Moreover, although conditioned flavor aversions induced by alcohol have been reported far more often than conditioned preferences

Table 1
Studies assessing operant self-administration of ethanol in infant rats (i.e., postnatal days 1 to 21).

Reference	Test age (day)	Procedure associated with delivery of the reinforcer	Ethanol concentration and/or level of intoxication	Duration of operant self-administration sessions	Deprivation time before training	Rat strain	Outcome	Controls available	Additional details
[23]	3, 9 and 15	Upward or downward paddle pressing	Milk or 6% v/v ethanol + milk; 0.44, 4.72 and 6.17 g/kg, in 3, 9 and 15 day-old, respectively	12 consecutive hours.	12 h.	Wistar.	Significant responding for both reinforcers (greater for milk)	Yoked controls	Responding was greater in 3 day-old and 15 day old than in 9 day-old rats.
[11]	1	Newborns were placed in a supine position that allowed access to a touch-sensitive sensor, while strapped in spandex vest.	0.75, 1.5, 3.0, 5.0, 7.5 or 10% ethanol. BECs between 3 and 92 mg/dl, as a function of ethanol concentration.	15 min.	2 h.	Sprague–Dawley.	Significant Responding, better at intermediate concentrations and substantial responding at extinction	Untreated, vehicle, and yoked controls	Newborns were exposed to only 120 min of maternal separation before testing.
[78,83]	14–16	Operant chambers equipped with a touch-sensitive disk located on the floor.	3% or 5.0% ethanol. Consumption of ethanol decreased across training days. From 0.27 g/kg on day 14 to 0.12 g/kg on day 16.	20 min.	60 min.	Sprague–Dawley.	Paired pups performed fewer operant responses than controls and decreased their operant responses across sessions. Authors suggested that intraoral ethanol had an aversive hedonic value.	Yoked controls	Pre-exposure to ethanol on day 13 did not modify the decreased responding of Paired pups towards ethanol.
[90]	14–17	Nose-poking behavior	3.75 or 7.5% ethanol. BECs between 25–48 mg%.	15 min.	12 h.	Wistar.	Rapid and robust operant responding for both ethanol concentrations.	Yoked and vehicle-reinforced controls	During adolescence, animals reinforced with 3.75% ethanol exhibited greater ethanol intake than yoked control animals.
[50]	1	Newborn pups were placed in a supine position that allowed access to a touch-sensitive sensor, while strapped in spandex vest.	3.0, 6.0% or sucrose mixed with quinine.	15 min.	60 min.	Wistar.	Prenatal ethanol exposure facilitated responding for 3% ethanol and for sucrose mixed with quinine.	Yoked controls	Pups were prenatally exposed to ethanol (0, 1 or 2 g/kg) during gestational days 17 to 20
[58]	1	Newborn pups were placed in a supine position that allowed access to a touch-sensitive sensor, while strapped in spandex vest.	3.0% ethanol	10 min.	90 min.	Wistar.	Only pups prenatally exposed to ethanol responded for 3% ethanol. Prenatal naloxone before ethanol attenuated postnatal responding for ethanol.	Yoked controls	Pups were prenatally exposed to ethanol (0 or 2 g/kg) and naloxone (non-selective opioid antagonist) during gestational days 17 to 20
[59]	14–18	Nose-poking behavior	3.75% ethanol. Maximum BECs achieved were 20 mg/dl.	15 min.	6 h.	Wistar.	Ethanol promoted high levels of responding during training sessions and extinction. Re-exposure to ethanol preceded by naloxone attenuated later nose-poking for ethanol.	Yoked controls	During days 16–18, 6 h before operant training, animals were re-exposed to ethanol after naloxone injection.
[63]	14–18	Nose-poking behavior	5.0, 7.5, 10.0 or 15.0% ethanol. BECs were 12–15 mg/dl.	15 min.	3 h.	Sprague–Dawley.	7.5 and 10% ethanol promoted the highest levels of operant responding. Blockade of mu, delta or kappa opioid receptors (also stimulation of kappa receptors) attenuated ethanol responding.	Yoked controls	The specific participation of mu, delta, and kappa opioid receptors on ethanol reinforcement was analyzed.
[60,62]	5	Pups were placed in a semi-supine position and had access to a touch-sensitive sensor.	3.0 or 5.0% ethanol.	The training session lasted 15 min. The extinction session lasted 6 min in duration.	3 h.	Sprague–Dawley.	Operant behavior for 3% ethanol was facilitated by prenatal exposure to ethanol.	Yoked controls	Pups were prenatally exposed to ethanol (0 or 1 g/kg) during gestational days 17–20.

[80], under some circumstances the pharmacological effects of self-administered ethanol can enhance the hedonic value of flavors. These circumstances seem to involve distributed alcohol self-administration, resulting in moderate levels of drug ingestion, across several sessions. Cunningham and Niehus [17] made rats drink or bar press for access to 10% ethanol, in 30-min sessions. After rats successfully initiated to alcohol-self administration and ingested around 1 g/kg of alcohol per session, the drug was flavored with banana or an almond extract. Following termination of the self-administration protocol animals exhibited conditioned preference for the flavor paired with the pharmacological effects of alcohol.

The "sipper" procedure [95] has been used to assess the modulatory role of specific brain areas (e.g., lateral and basolateral amygdala [54]) and pharmacological treatments (e.g., neuropeptide Y [34]) on ethanol self-administration. The chained schedule of reinforcement (CSR) model provides an alternative to the "sipper". In this model, developed in baboons [41,119] animals are exposed to a sequence of contingencies of reinforcement. Successful fulfillment of each component is required to advance and ethanol is only present in the final component [41].

4. Assessment of ethanol self-administration in infant rats: intraoral cheek procedure, consumption-off-the-floor and surrogate nipple procedures

There are plenty of studies analyzing ethanol acceptance in preweanling animals. The most common route of administration involves infusion of alcohol through the use of an intraoral cannula connected to a polyethylene tubing which, in turn, is attached to an infusion pump [24]. Pups are isolated in cotton-lined square chambers (see Fig. 1) for 10 to 30 min, and receive varying concentrations of alcohol. Volume of infusion can be adjusted to achieve about 5 or 6% of the animals' body weight. Although alcohol is administered by the experimenter, it has been argued that pups can exert some control over its ingestion, by regulating taste reactivity responses [4,5]. Emission of mouthing and tongue protrusions helps ingest the fluid, whereas gaping, chin rubbing and passive drips allow for partial avoidance of the solution. Indeed, level of ethanol consumption in this task can be modulated by several factors, including response to novelty, previous ethanol experience due to maternal consumption during pregnancy [24] or breastfeeding [85] and by associating the taste of ethanol with aversive postingestive consequences [5]. A disadvantage of this technique is that it requires a minimal, yet invasive surgical preparation and involves some degree of forced exposure. Despite these drawbacks, the technique has allowed detection, among other effects, of the facilitative effects of prenatal ethanol on later alcohol intake [21], and the early differentiation and acceptance of sweet and bitter tastants in rats [45].

Another, more ecologically-sensitive preparation is the consumption-off-the-floor (COF) method [98]. In this preparation preweanlings are introduced in pairs into heated glass terrariums with slate bottoms. The terrarium is kept at 32 °C and lined with Kim wipes soaked with ethanol, water or other fluids [46]. This preparation allows pups to directly obtain ethanol by licking from the floor and consumption is measured by weighing the animals before and after a 15, 20 or 40 min test. COF is, however, subjected to several methodological caveats; the most prominent being that indirect alcohol dosing through inhalation or transdermal transmission can be high and account for one-third of blood alcohol levels. A common denominator of studies using intraoral cheek cannula and COF is that, unlike adult counterparts that require extensive initiation procedures or progressive alcohol substitution procedures, infant rats readily accept high concentrations of ethanol (e.g., 15%), prefer ethanol vs. water, and further increase this acceptance as a function of previous ethanol exposure. Ethanol intake is greater than water intake by postnatal day 6 (PD6), and increases gradually to reach a peak at PD12, when animals drink 2 g/kg in 15 min and achieve blood ethanol concentrations of 250 mg/dl [113]. Ethanol consumption, at least when assessed through COF, then declines more or less linearly as a function of age (see Fig. 2). These age-related differences are better observed at 5 or 10% ethanol than at 20 or 30% ethanol concentration [113,114] a result that highlights the role of orosensory factors in ethanol acceptance.

Regardless of the relevance of these paradigms, it is still uncertain whether they measure voluntary intake by the pup and it has been suggested that the rapid ethanol intoxication achieved in COF may sedate or stimulate subjects in a manner that interferes with subsequent consumption [113]. Classical and operant conditioning procedures may help untangle these confounding factors.

The surrogate nipple technique [70] represents another technique, amenable to be used shortly after birth. In this preparation newborn rat pups are given the opportunity to attach and withdraw a fluid from a surrogate nipple made out of soft vinyl or liquid latex [103, 104]. Rat pups readily attach to the nipple for extended periods of time and ingest significant amounts of appetitive fluids (such as milk or saccharin) through the nipple. Conversely, if no fluid or a neutral fluid is available through the nipple rat pups will not attach to or ingest substantial amounts of fluid from the nipple. Newborns that are first exposed to a pairing of intraoral milk infusions with a surrogate nipple later show opioid activation when the nipple is presented alone.

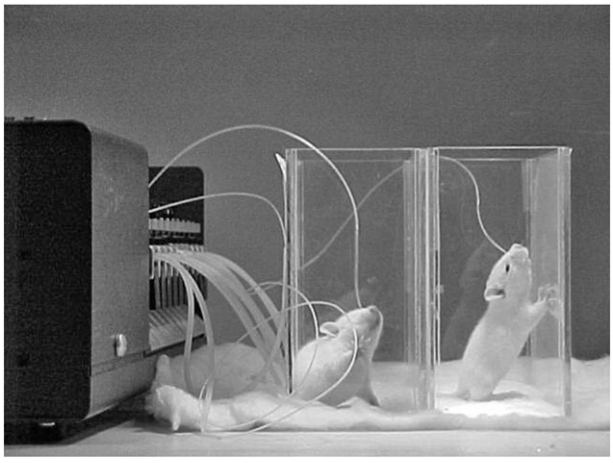

Fig. 1. Intraoral infusion procedure. Solutions (e.g., water, sucrose or ethanol) can be delivered in a continuous or pulsate pattern by means of an infusion pump connected to a cannula positioned in the cheek of the animal.
Reproduced from Pautassi et al. [78,83] with permission from the publisher.

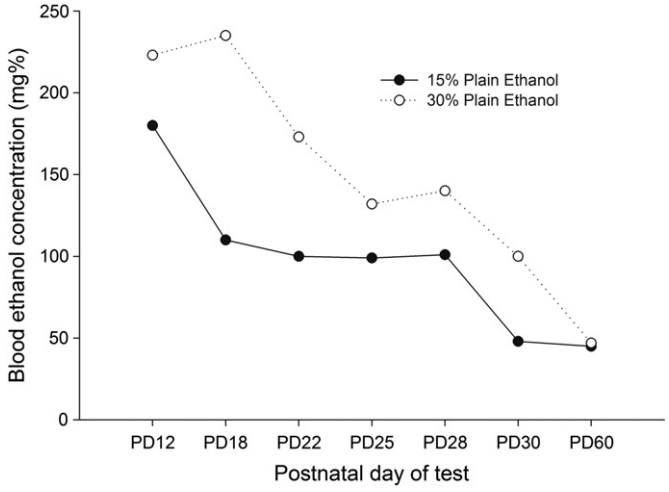

Fig. 2. Ethanol consumption, measured in blood ethanol concentration (mg%) assessed through consumption-off-the-floor (COF) sessions in rats of different ages that had access to 15 and 30% v/v plain ethanol.
Reproduced from Truxell et al. [114] with permission from the publisher.

Furthermore, they will attach to the empty nipple as if an appetitive fluid is available through it, in essence transforming short responding to the nipple into prolonged attachment. One of the first examples of newborn rat pups interacting with a surrogate nipple to obtain ethanol comes from the work of Varlinskaya and coworkers [86,115]. These experiments assessed the willingness of male and female 4-hour to 2-day-old rat pups to ingest a broad range of concentrations of ethanol fluids (2.0, 5.0, 10.0, or 15.0%). The findings of these seminal papers showed that rat pups of this age will readily attach to and ingest either 2.0 or 5.0% ethanol solution from a surrogate nipple. Therefore, newborn rat pups were shown to find these concentrations of ethanol appetitive and quickly learned to interact with the nipple to obtain the fluid.

While the previous study clearly showed that rat pups will drink alcohol from a nipple, whether ethanol was reinforcing was still a question to be answered. Cheslock et al. [14] answered this question by observing substantial attachment in 3-hour old rat pups to a surrogate nipple providing 5% ethanol, which was accompanied by blood alcohol levels of 20–30 mg/dl. Ethanol-mediated reinforcement was then confirmed by finding sustained attachment to an empty nipple an hour later. The finding of reliable ethanol-mediated intake through the use of the surrogate nipple in newborn rats has been proven to be an important tool in the search of mechanisms underlying ethanol reinforcement.

The findings of Cheslock et al. [14] lead to the question of whether it was the flavor or the pharmacological properties of ethanol rat pups find reinforcing. The effectiveness of an intraperitoneal (i.p.) route of ethanol administration was assessed for its effectiveness as an unconditioned stimulus. A surrogate nipple providing water (low levels of attachment) was used as a CS and was paired with i.p. ethanol injections of varying doses (0.125–0.75 g/kg). One hour following conditioning rat pups that received either 0.125 or 0.25 g/kg ethanol as the US showed significantly increased attachment to an empty surrogate nipple compared to controls. The higher doses had no effect on responding. Furthermore, ethanol injections or exposure to the surrogate nipple alone had no effect on future suckling behavior [88]. This result clearly indicates that the pharmacological properties of ethanol drive the reinforcing effects of the drug and that interaction with the surrogate nipple while under ethanol's pharmacological effects is necessary for changes in future responding.

Beyond shedding light on infant operant responding to ethanol this paradigm also stimulated the development of operant self-administration techniques for the analysis of ethanol reinforcement in infancy, which will be reviewed in the next section.

5. Assessment of ethanol self-administration in infant rats: operant procedures

The intraoral intake tests and the COF procedure, and to some extent the surrogate nipple intake test, are subjected to the obvious concern of confounding appetitive (i.e., "seeking") and consummatory (i.e., "intake") behaviors towards ethanol. To address this concern procedures that dissociate approach to and consumption of alcohol are needed.

An early, seminal study [40] showed that one-day-old rats learn to execute a seemingly arbitrary behavior to have access to a small quantity of milk into the mouth. Specifically, animals were deprived of suckling overnight prior to being placed for 12 h in small container equipped with one or two paddles. Upward probing one of the paddles provided access to milk, and this contingency – without the need of shaping or priming – made animals execute about 20 target behaviors per hour, much more than the 4–5 made by a corresponding yoked control. Many features of this elegant study helped conclude that this behavioral difference reflected acquisition of operant learning. Differences between paired and yoked controls exhibited a delayed onset, achieving significance after 3 h and responding in paired animals was not continuous but instead featured bursts of high-frequency responding punctuated by lack of responding. Moreover, differences between paired and yoked animals emerged during extinction trials and in follow-up discrimination studies in which an odor cue signaled which paddle was associated with reinforcement.

This preparation was subsequently adapted [23] to assess the reinforcing effect of intraorally-delivered ethanol. Rats of three age groups (3, 9 and 15 day-old) were given access to milk or 6% v/v ethanol mixed in milk following upward or downward paddle pressing, during a 12-h test. Ethanol reinforcement was robust – yet not as much as milk-induced reinforcement – and persisted throughout testing, inducing absolute ethanol intake of 2.44, 4.72 and 6.17 g/kg, in 3, 9 and 15 day-olds, respectively. Overall responding was generally constant across the test and lower for the intermediate age group than in the younger or older age group. An interesting feature was that a follow-up experiment assessed ingestion of the reinforcers through the more common intraoral intake test (5.5% of body weight in 30 min) using the cheek cannula. The results were fairly consistent with those obtained through the operant procedure. Ingestion was greater in 3 day-old and 15 day old than in 9 day-old rats and somewhat, although not significantly, greater for milk than for milk + ethanol.

Altogether, these results indicate early ethanol-mediated operant self-administration, albeit slightly weaker than that induced by more "natural" reinforcers such as milk. One important limitation was the prolonged maternal separation experienced by the pups, which could interact with the reinforcing effects of the tastants. The finding of ethanol-mediated reinforcement before day 8 is important given the relative scarcity of methods available during this ontogenetic stage to analyze drug-mediated appetitive reinforcement. Besides the nipple technique, the obvious perceptual and motor limitations of the infant rat hinder the use of more conventional tests, such as conditioned place preference. Taste conditioning, on the other hand, can be reliably established as early as PD1 [30] yet it is dramatically more sensitive to detect aversive [77] than appetitive conditioning (but see [73]) by ethanol.

Since Dominguez et al. [23] the literature on operant self-administration of ethanol in infant rats suffered a prolonged hiatus. A likely reason underlying this is that new preparations, mainly based on principles of classical conditioning, were developed to analyze ethanol-mediated reward [66,67] and anxiolysis. This steady state was changed by two studies that significantly updated the preparations put forward by Johanson and Hall [40] and Dominguez et al. [23].

The first study [9] analyzed the reinforcing effects of milk in 5-day old rats, an age in which the effectiveness and reliability of the surrogate nipple technique have significantly decreased. Infants, equipped with a cannula in the cheek, were positioned in a semi-supine position (angle of 40%) over a cotton surface and strapped and buckled into a vest fashioned out of spandex. Movement of the forelimbs and head was allowed. A metal rod equipped with a touch sensitive sensor was located 1.0 cm away from the pup's mouth. The set-up is depicted in Fig. 3.

Sensor-touching by the experimental paired animal triggered the intra-oral infusion of milk (1 μl). Animals were run in pairs, a paired animal and a yoked control that received milk whenever the paired animal emitted a target response. Essentially, the preparation relied on the normal motor repertoire of the infant. To motivate level of activity, and hence the likelihood of success in operant learning, animals were given 3 or 6 h of maternal deprivation and primed with 4 "free" reinforcements, at the beginning of the daily sessions conducted on PD5 and 6. Operant conditioning was mildly established during the first session, yet robust differences between paired and yoked controls were observed during the second session on PD6, and longer maternal deprivation seemed to promote single-trial learning. Perhaps more important, when the reinforcer was withheld (i.e., extinction phase) paired pups exhibited a transient but significant increase in responding that may be interpreted as a rebound effect, likely to reflect frustration due to the sudden omission of the reinforcer [3].

Shortly after the Arias et al. [9] study, Bordner et al. [11] adapted the preparation for assessment of operant reinforcement in 1-day old

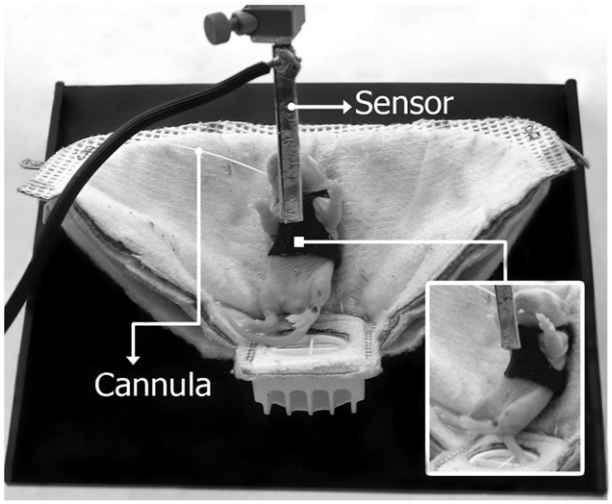

Fig. 3. Apparatus employed by Arias et al. [9] for assessment of operant conditioning in 5-day old rats. Infants are placed in a semi-supine position while resting in a 40 degree angle over a cotton surface. The animals are fixed in place through the use of vest fashioned out of spandex. The animals extend their forepaws through two holes and can touch the sensor, which is located about 1.0 cm from their mouth. Sensor touching activated an infusion pump which, in turn, delivered the milk reinforce through an intraoral cheek cannula. The photograph, which was kindly provided by Dr. Carlos Arias, was originally published in [9] and is used under permission from the publisher.

Sprague–Dawley rats. As depicted in Fig. 4, the animals were placed in a restrictor vest that fixed the pup in a complete supine position and allowed movement of the limbs and head. Slightly above the pup there was a touch-sensitive board. Contact with the sensor activated an infusion pump that delivered 1 μl of ethanol or milk in the oral cavity of the pup, by means of a cheek cannula. This preparation has the advantage of relying on a simple and relatively frequent target response, with a baseline level of approximately 4 contacts per minute in non-reinforced controls. Also, it employed only 120 min of food and maternal deprivation prior to sessions, which only lasted for 15 min. The ease of responding may be related to the fact that forelimb and head movements are spontaneous behaviors in the pup's repertoire that normally increase the probability of contact with the dam's nipple. Bordner et al. [11] observed successful responding for milk and for ethanol in paired pups, significantly more than in yoked or non-reinforced controls. Consistent with the "taste barrier" hypothesis [78], ethanol-self administration was dramatically dependent on ethanol concentration:

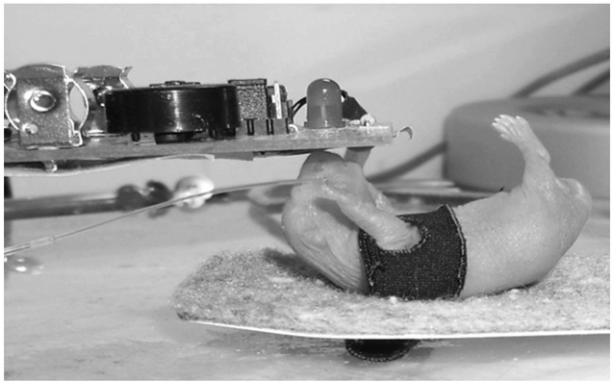

Fig. 4. Operant procedure employed in Bordner et al. [11] to train and test 1-day old rats in operant self-administration of ethanol. Pups, which are strapped in a vest fashioned out of spandex, can activate a sensor via head or forelimb movements. Sensor touching triggers intraoral delivery of ethanol in the paired but not in yoked controls. Yoked subjects received the reinforcer whenever the corresponding paired pup activated the sensor. Reproduced from Bordner et al. [11] with permission from the publisher.

significant differences between paired and yoked animals emerged at 1.5% v/v, became maximal at 3.0% v/v, were inconsistent at 5 and 7.5% and mostly disappear at 10% v/v. An interesting finding was that paired pups, particularly those reinforced with 3.0% v/v exhibited a peak of responding during extinction, which occurred immediately after the training session. Specifically, these pups escalated from 250 to 400 responses, an effect that can be accounted for by the frustration induced by the lack of reinforcement availability. Frustration can be measured in rats by assessing levels of consummatory or instrumental response towards a reinforcer that has been reduced in incentive value. Also important is that 1.5% and 3% ethanol induced greater reinforcement than water and milk and that pups administered around 0.2–0.4 g/kg/15 min achieved measurable blood ethanol concentrations (BECs) during the course of the operant procedure. Specifically, BEC in the paired pups reinforced with 3% ethanol – the concentration inducing maximal operant learning – was 28.20 +/− 2.29 mg/dl by the end of training, whereas those given the highest ethanol concentration of 10% v/v exhibited 92.00 +/− 18.17 mg/dl. Besides the aversive orosensory qualities of the odor of 10% alcohol, it is possible that motor stimulating effects induced by this high BEC inhibited the emergence of differences in responding between paired and yoked groups.

The preparation devised by Bordner et al. [11] was rapidly used [50] to analyze important questions: is ethanol-self administration in 1-day old neonates comparable to that induced by natural reinforcers (e.g., milk) or by an aversive, bitter taste (quinine) or by a combination of sweet and bitter (i.e., sucrose + quinine) that mimics the taste of ethanol, and is this drug-induced operant response enhanced by prenatal ethanol exposure? One-day old Wistar neonates exhibited greater sensor-touching than yoked controls when offered milk, but not when offered quinine, as reinforcer. In neonates without gestational drug exposure, sensor-touching for ethanol was no different between paired and yoked conditions. Yet pups that had been exposed to 2.0 g/kg ethanol on gestational days 17 to 20 exhibited reliable operant responding for ethanol and, notably, for the sucrose-quinine compound. Several studies (e.g., [43]) indicate that this tastant mimics the taste of ethanol. In other words, this study indicates that, under normal circumstances, ethanol's ability to support operant conditioning in neonates is weaker than that exerted by milk and that, similar to what had been observed through the artificial nipple procedure [71], prenatal ethanol significantly increases sensitivity to the rewarding effect of ethanol.

Contemporary to the Bordner et al. [11] experiment, two studies tried to replicate the successful ethanol operant self-administration found in 13–14 day-old rats [23]. These studies [83,90] employed fairly different approaches and yielded opposite results. An analysis of their results helps better understand the factors regulating operant self-administration in infant rats. In one study [83] animals were placed in a small box (10 × 10 × 12 cm) equipped with a dime-sized, touch sensitive disk that was slightly raised from the floor. Pups underwent daily sessions on PD14–16 (20 min) and each contact with the disk yielded a small (3 or 5 μl) intraoral infusion of ethanol (3 or 5% v/v). The underlying rationale was to employ a simple target behavior, thus minimizing length of training and allowing substantial ingestion of ethanol and perception of its postingestive effects. Baseline level of emission sensor-touching was around 3–6 per minute; yet ethanol-associated operant responding was lower for experimental, paired pups than for yoked controls. These results suggest that pups actively avoided touching the sensor leading to intraorally delivered ethanol. This apparent ethanol-mediated aversion, which was exacerbated during extinction procedures, was observed even after pups were pre-exposed to the taste and to the post-ingestive effects of ethanol. It seems that the combination of a high baseline response rate, leading to a small volume of low-concentrated alcohol allowed the orosensory, instead of the potentially reinforcing post-ingestive, effects of the drug to take over and control behavior. This study thus provides more evidence in favor of a "taste barrier" that precludes substantial initial engagement in operant self-administration.

Ponce et al. [90] also analyzed operant self-administration in 14–16 day old pups, but put forward a preparation in which the target response under training (nose poking a sensitive sensor located in a hole placed in the corner of a medium-size chamber) was very low under baseline circumstances. Unlike pups in Pautassi et al. [83], each target response yielded a sizeable (25 μl) infusion of 3.75% ethanol. Responding was low initially, yet by postnatal day 17 ethanol self-administration was significantly greater than water self-administration and than responding by yoked controls; and this effect was more pronounced, although statistically similar, in pups that had been passively pre-exposed to the orosensory and pharmacological effects of ethanol before training, on PD12–13 (see Fig. 5, lower panel). This result is in agreement with several (yet not all, see [18]) works indicating that ethanol pre-exposure can ameliorate ethanol's aversive effects and increase sensitivity to ethanol-induced reinforcement [12,29]. Across pre-exposure conditions and by the end of training, paired pups in this study self-administered around 0.5 g/kg/15 min.

A follow-up experiment replicated the successful acquisition of ethanol self-administration and kept the rats under normal rearing conditions until adolescence, when they were given two-bottle choice tests between alcohol and water. Those animals that, as infants, had actively worked for 3.75% v/v ethanol drank significantly more of the drug during the choice test than counterparts that had self-administered water

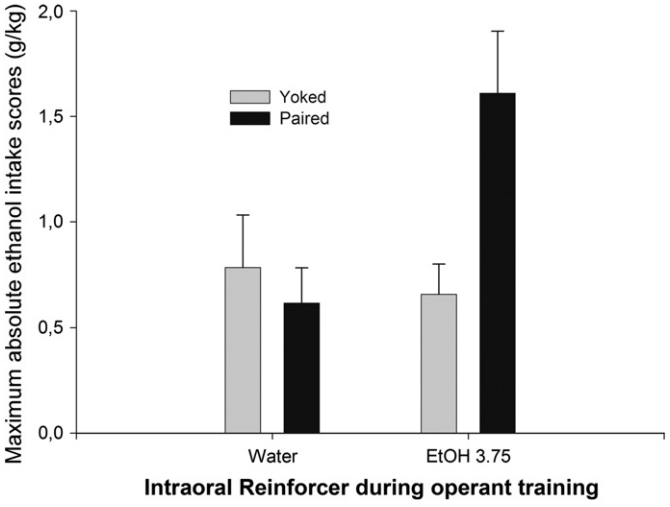

Fig. 6. Maximum absolute ethanol intake scores (g/kg) in a two bottle intake test as a function of prior infantile operant training defined by contingency (paired or yoked access to the reinforcer) and reinforcer received during infancy (water or 3.75 v/v ethanol). Vertical lines represent standard errors of the mean.
Reproduced from Ponce et al. [90] with permission from the publisher.

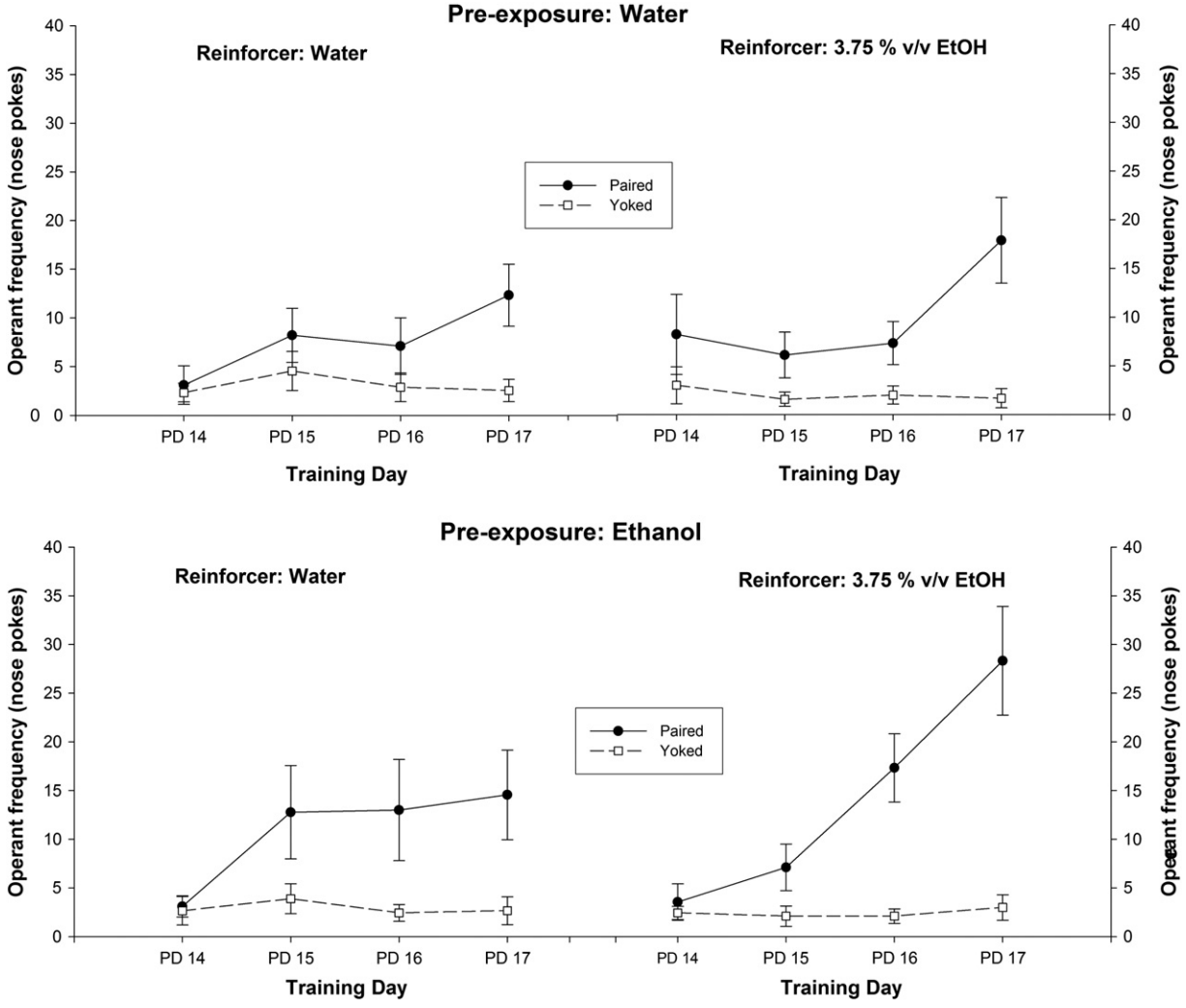

Fig. 5. Mean number of operant responses (nose-pokes) in infant rats as a function of reinforcer received during operant training (water or 3.75% v/v ethanol), contingency between behavioral emission and intraoral reinforcement delivery (paired or yoked) and training day (postnatal days 14–17, PD14–17). Animals were given passive pre-exposure treatment to water or ethanol, on PD13. Vertical lines represent standard errors of the mean.
Reproduced from Ponce et al. [90] with permission from the publisher.

or than animals that had been passively exposed to ethanol (i.e., yoked controls). This result, which is depicted in Fig. 6, is important because it meets the hypothesis, put forward by epidemiological studies [20], that early "debut" with alcohol can facilitate later approach and escalation into alcohol intake. Beyond this confirmation, the study suggests that active exposure to alcohol is more likely to induce later alcohol intake than passive, "experimenter-administered" ethanol.

Subsequent studies [50,58–60,63] built upon the preparations described in this section. These studies analyzed modulation of self-administration of ethanol during first or third-week of life, as a function of prior experience with ethanol in-utero and manipulation of the endogenous opioid system through general or specific (mu, delta or kappa) antagonism. These and other studies will be described in the next section.

6. Pharmacological manipulation of ethanol operant self-administration in infant rats

A few studies have addressed the neural underpinnings of operant self-administration of ethanol in infancy. As mentioned before and similar to adult rodents [31], ethanol reinforcement and acceptance in preweanling rats seem to be regulated, at least partially, by the opioid system.

At this time, four major classes of opioid receptors have been identified: mu, delta, kappa, and nociceptin/orphanin FQ peptide (NOP) receptors [69]. These subtypes are widely expressed in brain areas associated with the reinforcing effects of various drugs of abuse including ethanol [31]. In adult rats, the activation of mu and kappa receptors seems to exert opposite neurochemical and behavioral effects. Specifically, intracerebral administration of mu and kappa receptor agonists increases and decreases, respectively, dopamine release in the ventral tegmental area [51]. Administration of nociceptin/orphanin FQ, the endogenous ligand of NOP receptors, also suppresses the activity of the mesocorticolimbic/dopaminergic system [44]. Mu, delta, and kappa receptors follow different patterns of ontogenetic development; yet all three are functional by the second week of postnatal life [117]. The NOP receptor is expressed very early in life: it is detected as early as gestational day 12 in the rat and is observed at 16 weeks of gestation in humans [68]. After the first two weeks of postnatal life in the rat, NOP mRNA expression and distribution simulate those observed in the adult brain.

Research indicated that non-specific opioid antagonists (e.g., naloxone or naltrexone) co-administered with ethanol during gestation disrupt the facilitative effect of gestational ethanol in ethanol intake during infancy [15]. In newborn and infant rats, mu and kappa opioid receptors modulate ethanol-mediated appetitive reinforcement [74] with administration of selective mu and kappa antagonists inhibiting ethanol-induced reinforcement [72,75]. Ethanol-induced motor activation can also be reduced by mu or delta opioid antagonists or nociceptin (endogenous ligand for NOP receptor) during the preweanling period [7,61]. Given this evidence, it is not surprising that opioid receptor antagonists also proved effective to modulate ethanol operant self-administration. Miranda-Morales et al. [58] employed procedures similar to those of Bordner et al. [11] and found enhanced operant responding towards ethanol (and a high rate of responding during an extinction session) in 1-day old rats prenatally exposed to ethanol. This exaggerated response was completely blocked when a non-selective opioid antagonist (naloxone) preceded prenatal ethanol exposure. Milk-induced operant reinforcement was also found, which was greater than ethanol-induced reinforcement and insensitive to opioid manipulation. These results emphasize the implication of the opioid system on the acquisition of ethanol prenatal reinforcement. A more recent study went beyond and pointed out the importance of fetal mu opioid receptors. Prenatal blockade of the mu-opioid receptor system completely reversed ethanol effects on intake and palatability in infant rats, which suggests that the pharmacological effect of ethanol on the fetal mu opioid system is that of an appetitive reinforcer, which induces the prenatally conditioned preference (for ethanol) detected in the preweanling period [22]. Another study tested ethanol operant self-administration between postnatal days 14 and 18 [59]. As in previous work, infant rats exhibited reliable operant responding for ethanol (i.e., responding was greater in paired than yoked controls given ethanol), which proved to be higher than responding for water but significantly lower than that observed for 5% sucrose. As in some of the previous studies, pups kept responding after the reinforcer was withheld. Of major importance, brief non-reinforced re-exposure to ethanol preceded by administration of naloxone reduced subsequent ethanol operant responding. Mu, delta, and kappa opioid mediation of ethanol self-administration during infancy was tested in a following study [63]. Blockade of mu and delta opioid receptor as well as kappa blockade or stimulation induced a sharp reduction in ingestion and nose-poking for ethanol. These pharmacological manipulations also attenuated ethanol seeking during extinction.

These results are consistent with studies conducted in adult rats. Central injection of mu or delta opioid antagonists suppressed ethanol responding in alcohol-preferring and genetically heterogeneous adult rats [35,39]. The endogenous peptides for mu and delta receptors can act as positive reinforcers and are released after ethanol consumption [32]. There is also evidence that kappa antagonists increase ethanol intake in adult rats [120]. Kappa agonists activate stress systems and blockade of the kappa system has an anxiolytic effect [13]. The reduced responding for ethanol in infant rats given kappa antagonism [63] could be explained by a reduction in the background level of anxiety, which in turn diminished the anxiolytic effects of self-administered ethanol. It was also observed that pups given a high dose of a kappa antagonist exhibit, after several days of training, greater ethanol responding than control peers [63]. This delayed, yet significant enhancement of responding was not unexpected. Mitchell et al. [65] found enhanced ethanol self-administration in adult rats treated with kappa antagonists, but only four days after the administration of the kappa blocker. It should be noted that kappa antagonists in ethanol-dependent animals reduce ethanol self-administration, an effect probably related to hyperactivity of the kappa system after chronic drinking [118].

When taken together, these studies emphasize the relevance of the opioid transmitter system for modulation of ethanol reinforcement during early ontogeny. It seems that mu opioid receptors are implicated in ethanol's pharmacological effects during gestation [4]. Later, during the first postnatal week, both mu and kappa receptors are able to modulate ethanol reinforcement. Pharmacological manipulation of any of the four opioid receptors produced a change in ethanol acceptance during the third postnatal week.

7. Mechanisms underlying sensitivity to operant self-administration of ethanol in infant rats

A preliminary conclusion and common denominator among the studies analyzed is the relative ease with which successful operant self-administration of ethanol was obtained in infant rats. Unlike studies conducted in adults neither shaping, initiation procedures nor progressive alcohol substitution procedures were required for infant rats to readily accept and work for ethanol. One question that we asked ourselves was whether we could find age-related differences between infants and adults, much like the idiosyncratic pattern of response described in adolescents, which could explain this apparent higher propensity to engage in ethanol operant self-administration [105].

Following moderate and high ethanol doses PD16 infant rats exhibit significantly shorter sleep time than adults and, similar to adolescents and unlike adults, displayed acute tolerance [100]. In fact, acute (but not chronic, see [101]) tolerance was significantly more pronounced at PD16 than at any other age. These ethanol-related resistances are particularly striking when considering that, due to their immature liver processing, rates of ethanol metabolism in blood and brain are dramatically lower in PD16 infants than in adolescents or adults [102]. Unlike

mice, adult rats usually exhibit motor sedation (i.e., less ethanol-induced distance traveled) after moderate and high ethanol doses [1] and conditioned place aversion by ethanol [92]. This is not the case in two-week old rats, which exhibit ethanol-induced motor stimulation at doses ≥1.25 g/kg [6] and first- and second order conditioned place preference by 0.5–2.0 g/kg ethanol [67,72]. Consistent with the findings of self-administration studies, these effects of ethanol can be blocked by general and mu opioid antagonism [7,72]. The hypothermic effect of ethanol seems to be an important component of the aversive properties of ethanol. Infant rats are much less sensitive to ethanol-induced hypothermia than adults. Young adult (PD56) rats exhibit similar hypothermia (a reduction of 1.6 to 2 °C) after 1.5 or 4.5 g/kg ethanol. Infant (PD16) rats exhibited very mild hypothermia (less than 0.5 °C) after 1.5 g/kg and reduced hypothermia than adults after the highest dose [102].

The extent and direction of age-related differences are not universal across measures of ethanol reinforcement. Infant and adult rats readily exhibit conditioned taste aversion by ethanol and, at least when using high ethanol doses, ethanol's aversive effects in infants are similar to those induced by the prototypical emetic agent lithium chloride [8]. Two-week old rats also exhibit considerably lower basal and ethanol-induced corticosterone release than adolescents or adults [102]. This result is not unexpected. Until postnatal days 15–20 the levels of hypothalamic corticotrophin-releasing hormone are low [99].

8. Discussion

As summarized in Table 1, ethanol (as well as milk and sucrose [9,16], respectively) self-administration was successfully achieved across studies. Our hypothesis of a similar reinforcing value of ethanol and non-drug reinforcers, however, was not corroborated. Consistently, responding for ethanol was lower than responding for sucrose or milk [50], or the addition of ethanol to these solutions lessened their reinforcing value [23].

An important exception to the otherwise reliable support of operant learning by ethanol is the study by Pautassi et al. [83]. In this work a target behavior with high probability of execution at baseline was made contingent with access to small quantities of ethanol. This combination, which resulted in paired pups actually exhibiting less target behavior than yoked controls, likely favored an association with the apparent aversive effects of the odor and taste of alcohol and preclude perception of the rewarding, postingestive effects of ethanol. Yet the rest of the studies discussed indicate that pups readily and quickly learn to nose-poke or paddle for ethanol. This is in sharp contrast with studies in adults, in which prolonged training is required to achieve stable responding. Intriguingly, this age-related discrepancy parallels results obtained through conditioned place preference preparations and ethanol-induced motor activation tests. Unlike adult rats, infant rats readily acquire CPP by ethanol and exhibit ethanol-induced motor activation [79].

The sensory limitations concerning sensory development represent critical factors when trying to adapt unconditioned or conditioned techniques to the developing organism. The review, however, helped highlight several advantages of using operant conditioning protocols in infant and neonate rats. Training and testing are usually much shorter than those used in adults, and these "developing" organisms readily accepted uncontaminated alcohol. Moreover, an immature rat model features the obvious advantage of its logistical ease (i.e., easy handling and short housing leading to lower costs). Yet, do any of the studies reviewed completely fulfill the criteria for operant responding set forth in the introduction? Among most of the studies, pups that achieved self-administration ingested around 0.25–0.5 g/kg ethanol in about 15–20 min. Although relatively low, these levels yielded rewarding effects [66,82] and a recent study [62] found that 0.5 g/kg ethanol exerted anxiolytic effects across different tests in preweanling rats. Moreover, it has been observed [16] that sucrose-induced reinforcement can be enhanced by pairings of sucrose and 0.5 g/kg ethanol. Specifically, pups given sucrose followed by 0.5 g/kg ethanol subsequently exhibited heightened operant responding for the sweetener. What is lacking to confirm that postingestive effects of alcohol are driving these instrumental responses, however, is a devaluation study, similar to that employed in [94], in which pups that acquired a stable level of responding are then exposed to ethanol-malaise parings. Future work should tackle this important issue. It should be noted, though, that in one study [63] pups that had acquired operant responding for ethanol were given naloxone injections paired with intraoral infusion of ethanol. This manipulation reduced subsequent responding for ethanol. Also lacking are studies in which ethanol was directly self-administered in the brain, or aimed at analyzing activation of brain areas (e.g., through the use of immediate early genes like c-fos) or alterations of brain transcription factors after acquisition of ethanol self-administration. Moreover, it is still unknown if it is possible to implement the sipper model [95] in infant rats, although the short time span of infancy may play against its implementation. The sipper model offers the advantage of separating ethanol seeking and drinking behaviors.

One common denominator across studies is that there were no manipulations of rate of response: all employed a fixed ratio program in which every target response was reinforced (FR1). Moreover, unlike studies in which the target response and the reinforcer are geographically separated (i.e., in pigeons key pecking in one corner of the cage may provide access to a feeder located in the opposite corner), all the studies reviewed used intraorally infused alcohol. This lack of separation introduces an important caveat: it could be the case that animals in these studies did not learn an association between a given behavior and access to the reinforcer, but instead learn that the place in which the target behavior was made (i.e., hole, sensor, etc.) was the actual source of the fluid, much alike to what happens with a drinking tube in a two-bottle intake test. Noticeably, across studies the target behavior was part of the complex fixed action pattern associated with nursing. Under this framework, the studies may have conflated, at least partially, appetitive and consummatory behavior. Also, many of the studies reviewed employed substantial deprivation of food, liquid and maternal care – e.g., 12 h [23,90] – prior to commencement of training. Maternal deprivation is a significant stressor that can, by itself, alter reactivity to alcohol [38]. It is noteworthy that an association was apparently observed between age and the level of deprivation employed. Across studies, those using neonatal rats imposed only 1 or 2 h of deprivation before training. In contrast, the studies that employed two-week old rats had significantly greater deprivation time, up to 12 h [23,90]. Interestingly, the only study with 14-day old pups that employed short deprivation (i.e., 60 min) reported decrease responding in paired than in yoked pups [78,83]. Only two rat strains were used (Wistar and Sprague–Dawley) and both exhibited fairly similar sensibility to the reinforcing effects of ethanol.

Another caveat in alcohol self-administration is that intoxication may interfere with performance. Therefore, it could be the level of intoxication and not the flavor of the ethanol solution that contributed to the lower intake found in studies that employed high alcohol concentrations [11] or exposed animals to lengthy training sessions yielding ethanol ingestion levels ≥4.00 g/kg [23]. To our knowledge, none of the studies reviewed made independent motor assessments to control for this issue.

On the other hand, several studies comply with the requirement of significant emission of target responding after withholding the reinforcer. Among those reviewed, the study by Bordner et al. [11] is perhaps the clearest. In this study the target behavior significantly grew in magnitude relative to baseline, as well as in comparison to vehicle, yoked, and untreated controls and the response during extinction was three to four times higher in paired than in yoked controls. It should be noted, however, that extinction trials were relatively short and repeated extinction trials were not conducted in the studies reviewed, therefore preventing the assessment of spontaneous recovery effects or dependence of extinction on environmental cues.

At this point an important issue should be discussed. Several of the studies reviewed [23,50] assume, and their results suggest, that the ability to discriminate between different tastes emerges very early in ontogeny. This seems to clash with early studies indicating that most of the taste buds are not functionally mature (i.e., do not exhibit pores) until about PD10–12 [64]. Subsequent work [33] reported that, although some degree of taste differentiation was observed during the first week of life, the typical pattern of responsiveness to sucrose and quinine emerged by PD12. When substances such as ethanol or highly sweetened solutions are used it can be claimed that most of the stimulation is attributable to olfaction, yet this cannot be applicable to bitter substances such as quinine. Another study [36] indicated that, in rats as young as two days of age, the chorda tympani (the nerve carrying taste messages from the taste buds to the brain) exhibits concentration-dependent changes in electrical activity to ammonium chloride and differential response to stimulation with sodium chloride and lithium chloride, although a progressive increase in the efficacy of these responses was observed and taste buds seem to mature around PD15. Despite this apparent immaturity, differential responsiveness to sweet and bitter tastes has been reported through the use of the artificial nipple in 1-day old rats [87]; and as early as 3 h after birth when tested through an independent feeding procedure that mimics adult-like ingestive conditions. Specifically, Kozlov et al. [45] compared consumption of sweet (saccharin), bitter (quinine), and neutral (water) tastants in rat neonates exposed to a continuous flow of liquid that enters and exits the mouth through check cannulas. Animals sense the fluid and can accept or reject it. This procedure yielded similar results as those found with the artificial nipple: the neonates drank less quinine than water and also attached less to the nipple providing this bitter tastant, whereas the opposite profile was found with saccharin. Moreover, these taste responses seem to be plastic and dependent on experience. One-day old pups exhibit selective association in aversive learning, with taste cues being associated with internal malaise but not with exteroceptive nociceptive stimulation [30]. Conditioned taste aversion can also be observed in-utero, following in-utero injection of 0.3% saccharin closely paired with lithium chloride administration [56]. When these and the studies discussed in the present review are taken together it seems that the relative immaturity of the rat's gustatory system does not preclude early detection, discrimination and differentiation among tastants [28].

Studies by Miranda-Morales et al. [58,59,61,63] indicated the sensitivity of ethanol-mediated operant responding to pharmacological manipulation. Collectively, these studies indicate that operant self-administration of ethanol during early ontogeny is dependent on the integrity of the endogenous opioid system. A fully functional opioid system seems to be needed for ethanol to sustain operant reinforcement during very early stages of development. This is similar to what has been consistently found in adult rats [35,39,47] and meets the hypothesis that one of the neural underpinnings of ethanol-induced reinforcement is an opioid-dependent disinhibition of dopamine release in mesocorticolimbic pathways [121]. The comparisons made between the outcomes of the studies with infants and the studies conducted with adult subjects suffer, however, from the limitation that the methods employed in each age are not the same.

In summary, it seems that the findings gathered through the use of operant techniques strengthen the notion that the infant, developing rat, is highly sensitive to appetitive motivational effects of ethanol. It is important to highlight that the magnitude of this response can be enhanced by prior exposure to ethanol in utero [50,71], an effect that cements the idea that prenatal ethanol increases the rewarding effect of ethanol later in life [81]. In turn, self-administration of ethanol in infancy can facilitate subsequent ingestion of the drug during adolescence [90]. This could create a vicious cycle (the so called alcohol generator, see [57]) because it has been shown [25] that adolescent, but not adult, rats given brief exposure to alcohol then peak in their alcohol acceptance, when compared to peers that did not undergo alcohol exposure.

Acknowledgments

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