SOCIAL INDUCTION OF ETHANOL CONSUMPTION IN ADOLESCENT RATS, *RATTUS NORVEGICUS*

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

SOCIAL INDUCTION OF ETHANOL CONSUMPTION IN ADOLESCENT RATS

DOCTOR OF PHILOSOPHY (2002) McMaster University

(Psychology) Hamilton, Ontario

TITLE: Social Induction of Ethanol Consumption in Adolescent Rats, *Rattus norvegicus*

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NUMBER OF PAGES: x, 114

Abstract

Based on results of human and animal studies, it has been suggested that exposure to ethanol during early development leads to an increased likelihood of ethanol consumption by exposed individuals. Unfortunately, those results are sometimes conflicting and often incomparable due to substantial differences in methodology. Additionally, it is difficult to determine whether there is a critical period or mechanism for the development of affinity for ethanol. In both human and animal studies, social exposure to ethanol has been confounded with other forms of exposure. In sum, no systematic evaluation of effects of exposure to ethanol during early development on subsequent ethanol affinity has been conducted to date.

This thesis represents such a systematic evaluation, using a rodent model. In a series of seven experiments I assessed the relative and interactive effects of exposure to moderate doses of ethanol during three developmental stages (gestation, lactation and weaning) on subsequent voluntary ethanol consumption in adolescent Long-Evans rats. Results of experiments described in this thesis indicate: (1) Exposure to ethanol throughout gestation and lactation does not enhance voluntary ethanol consumption by adolescents unless such passive exposure is followed by opportunity to ingest ethanol during weaning. (2) Social

exposure to an ethanol-consuming adult female during weaning is sufficient to enhance voluntary ethanol consumption by adolescent rats. (3) Neither direct access to ethanol during weaning nor access to ethanol in mother's milk is necessary for enhanced ethanol consumption by adolescents after social exposure during weaning. (4) Any ethanol-consuming adult female can induce enhanced affinity for ethanol in adolescent rats, but weanling rats are more affected by cues from their dam than from another adult female when both are present. In summary, social exposure to an ethanol-consuming adult female during the weaning period is a sufficient condition for induction of ethanol consumption by adolescent rats.

Acknowledgments

I have been privileged to have had the support of so many wonderful people throughout the course of my graduate studies, and indeed throughout my life. It's not often that we have the opportunity to thank such people in print, so I will take this opportunity to do so.

For countless reasons I thank Alan, my husband, my best friend, and my most stalwart supporter. It would not even be possible to list all the things that you have done for me and still have this book fit in the library, but I hope that over the rest of our lives I can thank you for them.

Zane, my inspiration for wanting to be the best person I can be, you are my most important contribution to this world. Thanks for coming to work with me in the lab, for tolerating my absences, and for turning into such a great kid.

My family, otherwise known as my cheerleading squad, has been encouraging me to do whatever I had my heart set on since before I can remember. Nothing was ever out of my reach, and I never saw obstacles because you told me that I was capable of anything. Good thing I didn't want to become a ballerina, eh?

I have grown up, over the last few years, in the Galef lab. Thank you, Doc, for letting me make my own mistakes and do things my way (even when I could see you cringing and biting your tongue). Thank you, Elaine, for being my friend, working alongside me, and for all the trips to Timmy's and Taco Hell. Dave, you showed me the way. Alex, I can't imagine the last few years without you, in and out of the lab. Thanks guys.

I have been most fortunate in my life to have great friends. Some of them have been there since before I knew what grad school was. The Girls from Echo loved me whether I was ditching highschool, tending bar, driving cab, or teaching in a university. There were some, jealous of all the fun we were having, who said that none of us would do anything with our lives. We proved them so wrong.

Some of my friends I didn't know until I got here, but I can't imagine not having them around me forever. The NASA contingent, forged in a fire of stats assignments, CP critiques and Phoenix sing-alongs, made grad school survivable. I've got Friends in Low Places, baby. I couldn't be more proud of them, more awed by their talents and their hearts, and more thankful that they're mine.

Finally, the research conducted for this thesis would not have been possible without the efforts of duct tape, hot glue, Tim Horton's coffee, and my wonderful rats. Good times.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

The first steps in development of an addiction to alcohol are exposure to alcohol and initiation of alcohol consumption. If initial responses to alcohol exposure are positive, then the likelihood of subsequent alcohol seeking behaviour is increased. If, on the other hand, alcohol is experienced as an aversive substance, then the likelihood for maintenance of alcohol consumption, and of appetitive behaviour toward alcohol, is reduced. In fact, although alcoholism is often defined in terms of dependence and tolerance, both Alcohol Abuse and Alcohol Dependence are characterized by intense alcohol seeking behaviour (APA, 1994).

What makes one individual more likely than another to seek out and respond positively to alcohol? There is a great deal of evidence that some individuals inherit a propensity for alcohol abuse (Cloninger, Sigvardsson, Gilligan, von Knorring, Reich & Bohman, 1988). Specifically, children of alcoholics are at greater risk for alcoholism than are other members of the population, suggesting heritability of a predisposition for alcoholism, although, human population-genetics studies are confounded by social effects of parental alcoholism.

There is direct evidence of heritability of alcohol affinity to be found in animal models. Rodent strains have been bred for extreme alcohol preference and avoidance (see reviews by Fuller, 1985; McKinzie, McBride, Murphy, Lumeng & Li, 1999), as well as for differential responses to pharmacological effects of alcohol (Kurtz, Stewart, Zweifel, Li & Froehlich, 1996).

Although genetic models of alcoholism predisposition are invaluable, they do not explain all aspects of alcoholism (McKinzie, et al., 1998). The social environment of the individual also plays a role in initiation and maintenance of alcohol consumption. There are multiple opportunities for exposure to alcohol and observance of alcohol consumption by the people around us. A fetus may experience ethanol in amniotic fluid (Chotro & Molina, 1990). An infant may consume breast milk containing alcohol (Mennella & Beauchamp, 1997), or may be given alcohol as medicine. Children observe alcohol consumption by their elders and may sample alcohol at the instigation of their families. Adolescents continue to observe alcohol consumption by both elders and peers, and may experience pressure to drink large quantities of alcohol. Social exposure continues into adulthood, and alcohol consumption is an integral part of many social events, both religious and secular. The potential for learning, socially, about alcohol and alcohol consumption is enormous.

Social Learning

To survive, any young animal has to acquire information about the world around it. Because learning trials are expensive in terms of time, energy and risk of error (Boyd & Richerson, 1985), some lessons are not best learned by individual trial and error. Consequently, strategies that reduce the time, energy and risk associated with learning are expected to have evolved. Observation of conspecifics and adoption of behaviours they exhibit is a potentially useful way to acquire adaptive patterns of behaviour from choosing safe food (Galef & Clark, 1971), speaking a local language and dialect (Maratzos, 1989) and determining suitable mates (White & Galef, 1999) to learning species- or sex-specific behaviours (Marler, 1970). Social biasing of phenotypic plasticity can help organisms to develop into successful, and surviving adult members of their species.

Social learning of various behavioural strategies can explain some of the variance in behavioural repertoires seen in a randomly selected group of individuals. For example, Bandura and his colleagues (1963) demonstrated that aggressive behaviour performed by an adult, and observed by children, will be imitated, even though neither the model nor the observing children receive any overt reinforcement of such behaviour. Cross culturally, the variability of expression of many social behaviours including aggression, parenting style,

courtship rituals, and other 'customs' or 'traditions' can be most easily explained in terms of social learning (Bandura, 1977).

Social Learning of Food Preference

Both eating and drinking are highly social behaviours, and most adults are keenly aware of the social expectations surrounding preparation, presentation and consumption of food and drink (Rozin, 1996). Social learning, transmission of information from individual to individual, is the mechanism by which we learn rules surrounding food. A consequence of learning various traditions surrounding food and drink is that individuals develop preference, affinity or level of acceptance for various flavours. Such individual traits are influenced by information provided by contact with others.

Prenatal Exposure

Flavours present in amniotic fluid can be detected by the fetus (Menella & Beauchamp, 1997) and exposure to food cues from a mother's diet can impact subsequent food choice, or acceptance, by her offspring (Galef & Clark, 1971; Hepper, 1988; Altbacker, Hudson & Bilko, 1995). It is also possible that the fetus can detect olfactory stimuli in the amniotic fluid, but this has not yet been determined. Hepper (1988) fed garlic to a group of pregnant rats late in gestation, and examined the behaviour of those garlic-exposed pups at 12 days of age. Pups

were offered a choice between a dish containing garlic-flavoured food and one containing onion-flavoured food. Pups that had experienced garlic *in utero* stayed near the dish containing garlic. A control group of pups that had not experienced garlic *in utero* showed no preference between the locations of garlic or onion dishes.

In an experiment similar to, but more extensive than, that of Hepper, pregnant chinchilla rabbits (*Oryctolagus cuniculus*) were fed either lab chow or lab chow flavoured with juniper or thyme. At weaning, pups were given the opportunity to choose among the three diets. The first food eaten by juveniles in each group was consistent with the diet consumed by their dams; the 'juniper' pups chose the juniper flavoured diet first, the 'thyme' pups chose the thyme flavoured diet first, and the 'plain' pups chose the plain chow as their first food to sample (Altbäcker, Hudson and Bilkó, 1995). The nature of the substances for which the pups in this experiment developed an affinity is particularly interesting. Both juniper berries and thyme leaves contain aromatic chemicals that are generally considered toxic (Altbäcker, et al., 1995), yet pups that were exposed to juniper and thyme *in utero* developed a preference for, not an aversion to, those flavours. When given a choice, pups chose the food that they had experience with, not the food that was the 'safest'. In fact, the growth rate of pups in both the juniper- and thyme-exposed groups was slower than that of pups in the control

group, and the pup mortality rate in the thyme group was higher than that in the other two groups (Altbäcker, et al., 1995).

Similarly, in a study involving human mothers and infants, prenatal exposure to carrot flavour led to an increased acceptance of carrot flavour at 6 months of age (Mennella, Jagnow & Beauchamp, 2001). Infants whose mothers had consumed carrot juice during the last trimester of pregnancy produced fewer negative facial expressions in response to being fed an unfamiliar carrot-flavoured cereal than did infants who had not been exposed prenatally to carrot flavour. Infants exposed to carrots prenatally ate more of the carrot-flavoured cereal than did control infants that had no prior exposure to carrots, and maternal ratings of their infants' enjoyment of the carrot-flavoured cereal were higher for carrot-exposed infants than for control infants. There was no difference in the two groups' responses to plain cereal, suggesting that the difference between carrot-exposed infants' and control infants' response to carrot-flavoured cereal was not due to an overall increase in food acceptance.

The previously described experiments indicate that: (a) the fetus can detect information about flavours, (b) the young animal uses information that it received about flavours *in utero* to make decisions, (c) the relative 'safety' of a flavour experienced *in utero* does not influence food preference, and (d) exposure to a particular flavour will cause food containing that flavour to be treated as palatable and, perhaps, preferred to other novel foods.

Exposure During Nursing

Like amniotic fluid, mothers' milk is a vehicle for exposure of young to food flavours and food odours (Galef & Sherry, 1973). It has been well documented that weanling animals actively seek out foods containing flavours that they experienced in milk, and prefer those foods to novel foods. Galef and Henderson (1972) examined food choices of weanling rat pups that had been exposed to distinctive flavours in milk. Weanling rats preferentially sought out and ingested the food that had previously been experienced in milk, even if that food was relatively unpalatable. This preference was exhibited without any prior experience with the solid food. Galef and Henderson ruled out the possibilities that the demonstrated preference was due to experience with the flavour in feces or from experience with particles of food clinging to the fur and vibrissae of the mother. Thus, exposure to flavours during nursing is sufficient to guide food choices by weaning animals.

The human infant is also sensitive to a change in mothers' milk and responds to it. When garlic or vanilla are added to a mother's previously bland diet, those flavours are transmitted to the infant through breast milk and the infant responds by feeding for a longer period of time than usual (Mennella & Beauchamp, 1997).

Although the influence of exposure to flavours present in mothers' milk on food choices of weaning animals is clearly established, the long term response to flavour present in mothers' milk is not known. Consequently, it cannot be stated that familiarity with flavours in milk determines food choices at a later age. There is recent evidence, however, that experience with a flavour in breast milk may determine the degree of acceptance of a solid food many months later. As described previously, Mennella and her colleagues (2001) examined the acceptance of a carrot-flavoured cereal by infants whose mothers had consumed carrot juice throughout the third trimester of pregnancy. In that same study, acceptance of carrot-flavoured cereal by 6-month-old infants whose mothers had consumed carrot juice throughout nursing was also examined. Those infants with experience with carrot flavour in milk responded with fewer negative facial expressions when fed carrot-flavoured cereal, than when fed plain cereal. While this result is intriguing, it does not predict whether a child will actively seek out (rather than merely accept) carrot-flavoured food, or other previously experienced flavours, to the exclusion of novel foods.

Exposure During Weaning

As described above, young animals receive information about the food their mothers have eaten throughout gestation and lactation. Odour-bearing chemicals present in food enter the mother's bloodstream and affect the smell and

flavour of both amniotic fluid and milk. These signals about foods eaten by the mother, often redundant due to selection of the same foods during both gestation and lactation, increase the likelihood that her young will select those same foods when it is time for them to forage for themselves.

During weaning and beyond, young animals are also exposed to a great variety of cues about food. Mother, father, siblings, and other conspecifics can all provide information about food and food locations, and such postnatal sources of information can exert considerable influence on food selection by the weanling.

One piece of information that weanling rats use to guide their food selection is the physical location of adult rats. The simple presence of an adult rat at a feeding site makes that site attractive to young rats, and increases the likelihood that the young rat will eat the food at that site (Galef & Clark, 1971). In fact, the adult rat need not even be conscious; the presence of an anesthetised rat at a feeding site makes that site more attractive than sites without an adult present (Galef, 1981). This simple strategy, eating where an adult eats, is useful for weanling rats that are venturing away from the nest to feed on solid food for the first time.

Of course, an adult is not always present at a feeding site. In that case the young rat might rely on residual olfactory cues left by adults at feeding sites and on the food that the adults were eating. These olfactory remnants are attractive,

and lead young rats to feed more at scent-marked sites than at unmarked ones (Galef & Heiber, 1976).

Young rats use cues about food sites, but also about the flavours of food that are at a distance. While information gathered during gestation and lactation may still be exerting an effect on the food choice of the young rat, recently acquired information is also utilized. Galef and his colleagues have demonstrated, repeatedly, that olfactory cues about recently eaten food are transmitted from a demonstrator to an observer rat during social interaction (for a review see Galef, 1996). Such interaction leads to a preference, by the observer, for food containing the flavour or flavours of the food that its demonstrator had eaten. Preference for a flavour, experienced through interaction with a demonstrator, is exhibited even when the demonstrated diet is relatively unpalatable and not normally accepted by naïve rats, such as chow containing cayenne pepper (Galef, 1989). Further, an established taste aversion can be reversed by allowing a rat with a learned taste aversion to interact with a demonstrator that has eaten that avoided flavour (Galef, Whiskin & Bielavska, 1997). The effect of social demonstration is very robust, and is seen in a variety of situations, in rats of both sexes and all post-weaning ages (Galef, 1996).

The mechanism by which transmission of food preference occurs involves respiration of food odours mixed with carbon disulfide in the breath of the demonstrator. Detection by an observer of the combination of carbon disulfide

and food odour leads to a preference for food bearing that odour, but detection of the food odour alone is not sufficient to induce a preference (Galef, Mason, Preti & Bean, 1988).

Summary

Social learning of flavour preference is a robust phenomenon that has been well documented in animals, especially rodents. Early experience with a flavour or odour, in amniotic fluid, mothers' milk or through social interaction with conspecifics, leads to a preference for food containing that flavour or odour. The strategy of attending to cues provided by chemosensory contact with one's mother or other conspecifics is an elegant means of reducing costs associated with learning to choose the 'right' foods. Redundant messages about readily available, and presumably safe, foods in the environment will be transmitted to the young animal throughout development, by the mother and others. One recurrent point that is of particular interest in the context of the present discussion is that the nature of the demonstrated diet seems to be somewhat irrelevant: relatively unpalatable, and indeed even toxic, foods can come to be preferred, if flavour cues associated with those foods have been experienced in social contexts of the sort described. From toxic thyme leaves to piquant cayenne pepper, the importance of social learning for food preference appears often to outweigh the qualities of a food in determining ingestion.

Early Experience With Ethanol

In theory, the same factors that are important for development of food preference should affect acquisition of an affinity for ethanol. Ethanol has readily detectable flavour and odour and passes intact into both amniotic fluid and mothers' milk (Mennella & Beauchamp, 1997). Ethanol is also readily detected in the breath of its consumers (McKim, 2000). Therefore, if ethanol is consumed by the mother during gestation and/or lactation, it would be expected that her young would be more accepting of ethanol than would naïve controls. Likewise, we would expect that demonstration of ethanol consumption by adult conspecifics would induce an affinity for ethanol in weanlings. These hypotheses have been tested in both human and animal models and indeed exposure to ethanol, during early development, may lead to an affinity for this relatively unpalatable, and toxic, substance.

It should be noted that the literature about early exposure to ethanol is not as straightforward or conclusive as is the literature on social learning of food preferences. There are widely discrepant interpretations of results, as well as methods used to achieve those results using various animal models. One explanation for inconsistency in interpretation is that researchers using animal models have not always intended to test effects of social learning on ethanol consumption, but discovered such effects serendipitously. For example, Phillips

and Stainbrook (1976) set out to examine effects of physiologic exposure to ethanol on the learning ability and taste preferences of young rats. Although the young rats were exposed to social demonstration of ethanol consumption throughout their development, the researchers did not comment on the potential impact of this form of exposure, and discussed instead effects of exposure prenatally and during lactation.

Even when researchers explicitly examine effects of social exposure to ethanol on later response to ethanol, methods used to measure such effects are not consistent. After social exposure to ethanol, some animals have been tested for their preferences for locations associated with ethanol or for their preferences for ethanol odour (e.g., Chotro & Molina, 1990), while others have had ethanol administered directly into their mouths and their passive acceptance of ethanol assessed (e.g., Hunt, Lant & Carroll, 2000). A few studies have tested for voluntary consumption of ethanol in a choice situation (e.g., Phillips & Stainbrook, 1976), but the results across those studies are not necessarily comparable due to differences in form of exposure or ethanol concentration tested.

Social Learning about Ethanol

Prenatal Exposure

The vast majority of studies examining the impact of prenatal exposure to ethanol have focused on the teratogenic effects of ethanol on the physiology and behaviour of the developing organism. Fetal Alcohol Syndrome (FAS) and Fetal Alcohol Effects (FAE) have been examined in both humans and animals. This research provides clear evidence that exposure to large, chronic doses of ethanol during gestation results in detrimental morphological, cognitive and behavioural effects (Streissguth, Landesman-Dwyer, Martin & Smith, 1980). Prenatal exposure to moderate or mild doses of ethanol does not result in morphological changes to the developing organism (Abel, 1984; Hannigan, 1996), but does have behavioural consequences (Abel, 1979; McGivern, Clancy, Hill & Noble, 1984; Ness & Franchina, 1990), one of which is an altered response to ethanol. In particular, prenatal exposure to ethanol results in long-term alterations in an organisms' response to the pharmacological effects of ethanol.

Abel, Bush and Dintcheff (1981) exposed pregnant rats to substantial doses of ethanol throughout gestation (3 g/kg, twice daily) and examined the physiological responses of affected offspring beginning at 6 months of age. Control rats experienced no ethanol during gestation. After a challenge dose of ethanol, pre-exposed rats had a smaller drop in body temperature than did control rats, indicating that some measure of tolerance remained, even 6 months after

exposure to ethanol. Adult rats exposed to a teratogenic dose of ethanol during gestation displayed less ataxia to a challenge dose of ethanol (Reyes, Duran & Switzer, 1993) and similarly-treated mouse pups took longer than naïve controls to lose the righting reflex after an ethanol challenge (Perez, Gonzalez & Smith, 1983). Although ethanol exposure during gestation does not effect changes in an organism's ability to metabolize ethanol (Perez, et al., 1983; Reyes, et al., 1993), ethanol exposure during gestation does alter an individual's response to ethanol upon initiation of ethanol consumption in adolescence or adulthood.

Exposure to ethanol during gestation also results in changes in later responsiveness to the chemosensory properties of ethanol. Eight days after birth, rat pups exposed to ethanol prior to birth spent more time near an ethanol odour than did control pups, and they spent more time near an ethanol odour than near a lemon odour (Chotro & Molina, 1990). Eleven-day-old rat pups avoided an ethanol odour, if ethanol intoxication had been paired with foot-shock, unless those pups had prenatal experience with ethanol, in which case their preference for an ethanol odour was no different from that of pups whose experience with ethanol was unpaired with shock (Chotro, Cordoba & Molina, 1991). The fetus perceives and responds to the chemosensory characteristics of ethanol in amniotic fluid (Chotro & Spear, 1997). After birth, the organism recognises the smell and taste of ethanol, and this recognition directs behaviour (Chotro et al., 1991).

Learning about ethanol during gestation occurs and, based on the longevity of effects of that learning, appears to be robust. However, little is known about whether learning leads to enhanced appetitive behaviour toward ethanol. Results of experiments designed to address that issue are mixed. Bond and Di Giusto (1976) fed ethanol in a liquid diet to pregnant rats and assessed the ethanol intake of female offspring of their litters, as well as the ethanol intake of control subjects that had no prior ethanol exposure. At 65 days of age, ethanol-exposed rats drank more ethanol than did controls, but only if the ethanol concentration provided to subjects at test was 6% or lower. At higher concentrations, ethanol intake did not differ between the two groups.

Bond and Di Giusto's study can be criticized for its small sample (3 litters), and researchers who replicated Bond and Di Giusto's methods failed to find enhanced ethanol intake in ethanol-exposed rats (McGivern, Clancy, Mousa, Couri & Noble, 1984). In addition to increasing the sample size, McGivern and his colleagues tested ethanol-exposed rats at 120 days of age, rather than 65 days of age as in Bond and DiGiusto's (1976) study. This delay in testing is not likely to account for the failure to find an effect of prenatal ethanol exposure, however, as another study found that maternal ethanol consumption during gestation did not significantly influence the ethanol consumption of 45 day old offspring (Reyes, Garcia & Jones, 1985). We cannot yet conclude that prenatal exposure to ethanol

effects subsequent ethanol affinity until mixed results like those described are clarified.

Exposure During Nursing

Not only is information about ethanol transmitted through mothers' milk (Galef & Sherry, 1973; Mennella & Beauchamp, 1997), but the act of suckling is itself reinforcing (Hunt, Kraebel, Rabine, Spear & Spear, 1993). In fact, nursing pups are unable to learn an aversion to flavours experienced while suckling, even if those flavours predict illness (Martin & Alberts, 1979). Exposure to flavour in a nursing context is rewarded by the act of suckling and this positive association can lead to enhanced affinity for that flavour.

Hunt and her colleagues (1993) found that both 12- and 16-day-old rat pups that had experience with ethanol while suckling accepted 5.6% ethanol administered directly into their mouths more readily than did controls. Mennella (1999) indicates that human infants do not reject low concentrations of ethanol present in mother's milk, and will suck frequently when drinking ethanol-flavoured milk either from the breast or from a bottle. However, other research suggests that interaction with an intoxicated mother results in an aversion to ethanol cues (Molina, Pepino, Johnson & Spear, 2000). Rat pups that interacted with an intoxicated dam later demonstrated an aversion for a texture associated

with the odour of ethanol, and accepted less ethanol via intraoral cannula than did pups that had interacted with a sober dam.

Possibly, the effect on pups of ethanol exposure during lactation is dose dependent. Ethanol-naïve newborn rats will readily accept 2 or 5% ethanol, a low to moderate concentration, from a surrogate nipple, but will reject 10 or 15% ethanol solutions (Varlinskaya, Petrov, Cheslock & Spear, 1999). Small doses may be accepted readily, and may lead to learning of positive associations with ethanol, while larger doses may result in aversion.

Although the evidence described above suggests that there is considerable opportunity for social learning about ethanol during nursing, there have been no direct tests of effects of exposure to ethanol during lactation on voluntary ethanol consumption or ethanol seeking after weaning. That ethanol exposed 12- and 16-day-old rat pups will ingest ethanol more readily when it is administered directly into their mouths (Hunt, et al., 1993) suggests that the consumptive behaviour is enhanced, but does not demonstrate that appetitive behaviour directed toward ethanol is also increased. The requisite studies on humans are ethically impossible, but suitable animal studies might determine whether enhancement of voluntary consumption of ethanol results from exposure to ethanol during lactation.

Weaning and Beyond

The periadolescent period brings more sources of social information about ethanol to the developing organism. During gestation and lactation, the mother is the primary source for information. However, as the young animal becomes increasingly independent, other conspecifics can provide information as well. Rat pups as young as 8 days of age that interact with an intoxicated sibling demonstrate enhanced ethanol acceptance, via intraoral cannula, relative to controls that lack such experience (Hunt et al., 2000). Such enhancement of passive ethanol acceptance is long-lasting, as evidenced by enhanced acceptance of ethanol at 22 days of age by pups that interacted with an intoxicated sibling 6-10 days earlier (Hunt et al., 2000).

Randall and Lester (1975b) provided a dramatic example of the impact of adolescent rodents' social environment on their later ethanol consumption. Strain-specific ethanol consumption patterns of both C57BL and DBA mice have been well documented: C57BL mice have an affinity for ethanol, whereas DBA mice tend to avoid ethanol. Randall and Lester removed weanling mice from their mothers at 21 days of age and housed 2 pups from each litter with a group of adults of the same strain, and 2 pups from each litter with a group of adults of the other strain. After 7 weeks of group housing, the ethanol consumption of all pups was assessed. The ethanol consumption of DBA mice housed with "drinker" C57BL mice was twice that of DBA mice housed with other "non-drinker" DBA

mice. Ethanol consumption by C57BL mice housed with "non-drinker" DBA mice was half that of C57BL mice housed with other "drinker" C57BL mice. Strain-typical ethanol consumption patterns, assumed to be under genetic control, were not reversed, but were modified by social experience with the ethanol consumption pattern of the foster strain.

Such social exposure around the time of weaning has been overlooked as a potential confound or contributing factor in some studies. For example, Randall and Lester (1975a) report that weanling DBA mice (that normally avoid ethanol) reared from birth to Day 25 postpartum by a foster dam of the C57Bl strain (that voluntarily ingests ethanol) drank more ethanol than did DBA mice reared by dams of their own strain. Randall and Lester attributed this alteration in strain-typical ethanol consumption to experience with ethanol in mother's milk. However, pups in Randall and Lester's (1975a) experiment were exposed to ethanol not only through their foster dam's milk, but also through contact with their foster dam and ethanol-ingesting siblings and, possibly, as a result of local enhancement effects (Thorpe, 1963), with the ethanol their dam was drinking.

In a creative experiment that avoided many of the difficulties inherent in determining the opinions of small children, Mennella and Garcia (2000) assessed the hedonic response to alcohol odour of 4 to 6 year old children, using familiar television character dolls. The experimenters asked children to name an odour presented from a squeeze bottle, and then give that bottle either to a Big Bird™

doll (if they liked the odour) or to an Oscar the Grouch™ doll (if they did not like the odour). Odours included beer, bubble gum, pyridine (which the authors state has a "rotten egg" odour), and a neutral odour derived from a squeeze bottle filled with mineral oil. While bubble gum was nearly always given to Big Bird™, and pyridine was nearly always given to Oscar the Grouch™, the decision about beer was split. Upon further analysis, the authors determined that children who did not like the smell of beer were more likely than other children to live in a house where at least one parent frequently drank alcohol "to escape". (Regarding this study, it should be noted that pyridine is toxic, and human exposure to pyridine should be avoided.)

Human children who, like rats or mice, live with alcohol consuming parents experience social cues about alcohol on a regular basis. Infants with extensive social exposure to ethanol are more likely than are less-exposed infants to mouth an ethanol-scented toy (Mennella & Beauchamp, 1998). Children's ability, by age 2, to identify alcohol by odour is positively related to frequency of parental drinking (Noll, Zucker & Greenberg, 1990).

Given that rat pups that interact with an intoxicated dam develop an aversive reaction to ethanol cues (Molina et al., 2000), high concentrations of ethanol are rejected by suckling rats (Varlinskaya et al., 1999), and children whose parents drink frequently do not like the smell of beer (Mennella & Garcia, 2000), one might expect that children of alcoholics would not be at risk for

developing problems with alcohol themselves. This is not the case. Children of alcoholics drink more than children of non-alcoholics, and are at greater risk for development of alcoholism (Cloninger, et al. 1988). Apparent aversion to alcohol in experimental situations during early development has not yet been reconciled with increased risk for alcoholism in later life by children of alcoholics.

Multiple Sources of Exposure

Outside the laboratory, exposure to ethanol by the developing child or adolescent is rarely confined to discrete periods during development. There are multiple opportunities for exposure to alcohol and people who consume alcohol. Mothers who drink during pregnancy may also drink during lactation, and throughout the life of her child. Even if mother does not drink, the child's father, older siblings, grandparents or role models may drink while the child observes, and this social observation may continue for years. Several studies have measured ethanol consumption of humans and other animals exposed to multiple sources of social learning opportunities. While these studies make it difficult to determine the critical factors associated with enhanced ethanol consumption, each suggests that exposure to ethanol during early development results in enhanced affinity for ethanol later in life.

Phillips and Stainbrook (1976) gave female rats wine as their sole drinking fluid. The rats became pregnant, delivered, nursed and weaned their offspring,

drinking wine throughout. At 170 days of age, wine consumption by offspring exposed in this manner was compared to that of unexposed controls. Wine-exposed rats drank the same amount of water as did controls, but drank more wine in a two-bottle choice test. This study, like that of Bond and Di Giusto (1976) used very small samples (n = 3 in each group), so the results, while intriguing, are not particularly persuasive.

As mentioned previously, humans are rarely exposed to only one source of social information about alcohol. In animal models it is possible to isolate certain periods or forms of exposure, but there are few comparable "natural experiments" in a human population. Population based studies rely on statistical regression methods to tease out critical factors, but some factors are often inextricably linked. For example, in a population of women who drank alcohol during pregnancy, it is unlikely that a large proportion of those women began to abstain from alcohol immediately after childbirth (e.g., Little & Streissguth, 1978). The ability to separate effects of prenatal exposure from postnatal exposure is therefore compromised.

The Seattle Longitudinal Study on Alcohol and Pregnancy has yielded a wealth of information regarding the impact of maternal alcohol consumption on fetal and childhood development. Five hundred children born in 1974 and 1975 have been assessed periodically on various neuropsychological, psychosocial and lifestyle measures (for a review, see Streissguth, Barr, Bookstein, Sampson &

Carmichael Olson, 1999). In addition to information about the role of ethanol as a neuropsychological teratogen (including its role in FAS and FAE), researchers associated with the project have suggested that prenatal alcohol exposure is a better predictor than family history of indices of early alcohol use by adolescents including age at first intoxication and frequency of alcohol consumption (Baer, Barr, Bookstein, Sampson & Streissguth, 1998). While the authors stated that "indicators of the postnatal environment thought to be directly related to child development" (Carmichael Olson et al., 1997) were measured and used as covariates in the regression analyses, it is not evident that childhood observation of parental alcohol consumption was included as a factor.

In addition to animal models, one method of assessing the contribution of parental environmental influences on offspring, independent of genetic influences, is through the use of adoption studies. A recent adoptive and step-family study indicates that the drinking behaviour of adoptive and step-parents may increase the likelihood of abuse of alcohol or other drugs by adoptive or step-children (Newlin, Miles, van den Bree, Gupman & Pickens, 2000). Having an adoptive or step-parent with an alcohol use disorder is a risk factor for the development of a substance use disorder in their adolescent and adult children, independent of physiological exposure to ethanol or genetic predisposition toward alcohol affinity.

Rationale

The results of the research reviewed above suggest that there are multiple pathways leading to enhanced acceptance or affinity for alcohol, and possibly development of an alcohol-use disorder. The wealth of information regarding the relative influences of prenatal exposure, exposure in mothers' milk, and exposure due to social interaction indicates that each of those avenues may contribute to affinity for alcohol. Unfortunately, discrepant methods, including ethanol concentration used, route of administration, and method of testing, make it difficult to integrate the results of one experiment with another.

In this thesis, I describe a series of experiments designed to systematically examine the relative importance of ethanol exposure during each of three developmental periods on voluntary ethanol consumption by adolescent rats, as well as the effects of exposure during more than one of those periods. Young rats exposed to ethanol during gestation, lactation, and weaning, provided through passive, physiological exposure (amniotic fluid and mothers' milk) as well as active social exposure (through interaction with conspecifics) and the opportunity to examine and consume ethanol prior to testing, were subsequently assessed for their ethanol affinity relative to the appropriate controls. I began with an experiment designed to replicate earlier studies (Bond & Di Giusto, 1976; Phillips

& Stainbrook, 1976) but with a larger sample size, consistent concentrations of ethanol, consistent ages for both exposure and testing, and additional relevant control groups. Each subsequent experiment was designed to isolate critical periods and factors associated with enhanced ethanol intake.

Each experiment is based on a modified version of Galef's food choice paradigm (see Galef, 1996). In this modified paradigm, a demonstrator (usually the mother) consumed ethanol and provided information about ethanol to observers (usually her offspring). These observers were then tested, after weaning, in a two-bottle choice between an ethanol solution and water. The g/kg ethanol intake of observers was compared with that of other adolescent rats that had experienced a different type of demonstrator.

For the purpose of the experiments described in this thesis, I chose to use methods that minimize the stress experienced by both demonstrator and observer rats. Ethanol consumption is enhanced in physiologically or psychologically stressed animals, and stress is often cited as a critical factor in development of human alcoholism (Mello, 1973). For this reason, I implemented the following procedures. Each litter was culled within a few days of birth to eight pups to reduce stress on the dam and ensure adequate suckling for each pup. Handling of rats was limited to cage changing (prior to testing), culling, and weighing (during the testing period). Pups were never deprived of maternal care, or care by a foster dam, prior to postnatal day 26. None of the rats in the studies were food deprived

at any time. As a final precaution, any animal that appeared to be unhealthy, stressed (based on an assessment of excessive vocalization) or was failing to gain weight was removed from the testing procedure.

I wished to maintain a consistent age at testing across experiments, and chose to test during the periadolescent stage of development. This age was chosen for several reasons. First, initiation of alcohol consumption during early adolescence is often cited as a risk factor for human alcoholism (Fergusson, Lynskey & Norwood, 1994), so assessment of alcohol affinity during a comparable developmental stage in an animal model is of some importance. Second, assessment of ethanol affinity beginning at 27 days of age in the rat allows for selective manipulation of exposure to ethanol during different developmental stages, while maintaining comparable measures of intake. Third, while ethanol acceptance prior to weaning is an important measure of hedonic response to ethanol, such measures do not provide information on appetitive behaviour directed toward ethanol. Waiting until the animal is old enough to actively select ethanol when given a choice between ethanol and water, allows the researcher to observe appetitive behaviour toward ethanol. Finally, assessing ethanol consumption during the periadolescent period does not preclude later assessment of ethanol consumption in adulthood, which makes possible other interesting comparisons in the future.

This thesis represents a significant original contribution to the field of social learning about ethanol. The experiments described here represent a systematic evaluation of the relative importance of critical developmental periods, and the importance of social learning opportunities, for the initiation of ethanol consumption in the adolescent rat. The results of these experiments are substantial evidence of the importance of experience during early development for alcohol seeking behaviour later in life.

Chapter 2: Social Induction of Ethanol Consumption

EXPERIMENT 1: Early Exposure to Ethanol

I designed Experiment 1 as a starting point to examine the impact on adolescents' ethanol consumption of social exposure to ethanol during gestation, lactation and weaning. Dams of pups socially exposed to ethanol drank 4% ethanol in tap water. Four-percent ethanol solution is generally accepted by naïve rats (Samson, Pfeffer & Tolliver, 1988), and its ad libitum consumption by rat dams during gestation does not cause birth defects in their offspring (Abel, 1984).

During both the weaning period and testing, I provided some pups and/or dams with access to an 8% ethanol solution, a concentration not readily accepted by naïve rats (Samson et al., 1988). I compared ingestion of 8% ethanol by adolescent rats exposed to ethanol throughout early development, with ethanol intake of rats exposed to ethanol, either during specific portions of their early development, or not at all.

Method

Subjects

One hundred and twenty four adolescent rats, born to 62 female Long-Evans rats acquired from Charles River Breeding Farms (St. Constant, Quebec)

and maintained in the vivarium of the McMaster University Psychology Department served as subjects. Within 48 hr of birth (Day 0), I culled each litter to 8 pups (where possible, four pups of each sex) and randomly assigned the litter to one of the five treatment conditions described below.

Apparatus

Until pups were 14 days old, I housed each dam and her litter in a transparent polypropylene cage measuring 36 x 31 x 17 cm. The top of each cage was covered with a wire lid that held food (PMI Rodent Diet 5001, Brentwood, MO) and a bottle. The bottle held either tap water, or a solution of 4% ethanol in tap water (all ethanol solutions were prepared volume/volume, and changed twice weekly). The floor of the cage was covered with wood-chip bedding and, for environmental enrichment, each cage contained a length of polyvinylchloride (PVC) conduit approximately 15 cm long and 10 cm in diameter.

From Day 14 to Day 26, each dam and litter occupied a large floor enclosure constructed of galvanized metal frame and wire mesh, measuring 184 x 92 x 31 cm (Figure 1). The floor of this enclosure was carpeted with wood shavings to a depth of approximately 4 cm, and I provided each enclosure with two 30 x 30 x 18 cm wooden nest boxes, two food containers, and two 30 cm^3 Plexiglas drinking boxes.

One of the two drinking boxes in each enclosure had a 5-cm^2 entrance that allowed both dam and pups to enter, while the other box had a round entrance, 2.5

cm in diameter, that permitted only pups to enter. Each box contained a bowl filled with either tap water or 8% ethanol in tap water.

I monitored each litter by closed-circuit camera, monitor and video-cassette recorder (Panasonic Color CCTV Camera WV-CP610, Osaka, Japan; Panasonic Color Video Monitor CT 1386 Y; Panasonic Time-Lapse Video-Cassette Recorder AG 6720, Osaka, Japan) in order to determine that pups from each litter sampled both ethanol and water during the weaning period.

On Day 26, I moved adolescent rats from the large enclosure to individual transparent polypropylene cages, like those described above, for ethanol-choice testing. During a choice test, all adolescents chose between two 50-ml test tubes, one containing tap water and the other 8% ethanol in tap water. Each test tube was closed with a rubber stopper and a stainless steel sipper tube.

Procedure (See Table 1)

Gestation, Lactation and Weaning (GLW) Condition (n = 14 litters): I gave pregnant rats assigned to the GLW Condition 4% ethanol (all ethanol solutions were prepared volume/volume in tap water) as their sole source of fluids during the last 2 weeks of gestation and the first 2 weeks postpartum. From Day 14 until Day 26, I housed each GLW litter in one of the large floor enclosures described above.

I had observed in a pilot study that pups housed in these floor enclosures began to drink from fluid containers on or about Day 18. Consequently, by placing each litter in a floor enclosure from Day 14 to 26, I allowed approximately 4 days for litters to become acclimatized to the enclosure, and approximately 1 week for pups to experience access to both 8% ethanol solution and water.

While dams and young resided in large enclosures, dams' fluid intake was restricted to 8% ethanol by placing a container of 8% ethanol in the drinking box with the large opening. Pups in this condition had access to both 8% ethanol from the same source as their dam and water in the drinking box with the smaller opening.

Gestation and Lactation (GL) Condition (n = 8 litters): I treated litters assigned to the GL condition just as I treated litters assigned to the GLW condition, until Day 14. From Day 14 to Day 26, dams assigned to the GL condition, had access to water rather than 8% ethanol in the drinking box with the larger opening, while pups could access both water in the drinking box with the larger opening, and 8% ethanol in the drinking box with the smaller opening.

Weaning (W) Condition (n = 12 litters): Litters assigned to the W Condition were not exposed to ethanol before Day 14. On Day 14, I moved each litter assigned to the W Condition to a floor enclosure and treated them just as I had treated litters assigned to the GLW condition, i.e., I restricted dams to

drinking 8% ethanol from the drinking box with the larger opening, while pups could access both 8% ethanol from the drinking box with the larger opening and water from the drinking box with the smaller opening.

Access (A) Condition (n = 13 litters): Litters in the A Condition were not exposed to ethanol before Day 14. On Day 14, I moved each litter to a floor enclosure, where I restricted dams to drinking water from the drinking box with the larger opening while pups had access both to water from the same source as their dam, and 8% ethanol from the drinking box with the smaller opening.

Control (C) Condition (n = 15 litters): Litters assigned to the C Condition were not exposed to ethanol before testing. On Day 14, I moved each litter to a large enclosure, where I restricted dams to drinking water from the drinking box with the larger opening. Pups could access water from the same source as their dam and also had access to another bowl of water in the drinking box with the smaller opening.

Testing: On Day 26 postpartum, I selected one male and one female adolescent at random from each litter, and housed each of these subjects individually. Each rat had access to both water and 8% ethanol for 2 hr/day for each of 7 consecutive days of testing. I determined the weight of each fluid container before and after each 2-hr drinking session and determined the weight of each subject every second day.

In a pilot study, I had found that drinking tubes leaked approximately 0.1 g of fluid in each 2 hr session. I, therefore, subtracted this amount from each drinking tube weight each day before undertaking further calculations. I used g/kg intake as the dependent measure to both compensate for body weight differences among adolescents and to estimate levels of intoxication.

If a subject drank no water during a drinking session, data for that subject for that day was discarded (27 of 434 data points) because lack of water intake was generally cause by an air bubble blocking a drinking spout. We also removed a subject from testing if it lost more than 10% of its body weight as a result of restricted fluid intake (2 of 124 pups). We averaged scores for males and females in each litter (after checking for an effect of sex) so that only one score from each litter entered into statistical comparisons.

Results and Discussion

The main results of Experiment 1 are presented in Figure 2. Although each subject was tested for 7 days, there was no effect of day of testing in this or any of the subsequent experiments. Also, there were no effects of sex on ethanol intake in any experiment in this thesis. Each mean score that is reported is the mean daily ethanol intake for a litter, across the entire week of testing. As can be seen in Figure 2, exposure to an ethanol-consuming dam during development resulted in enhanced voluntary intake of ethanol by adolescent rats, one-way

ANOVA: F (4, 57) = 7.07, $p < 0.001$. Planned orthogonal comparisons revealed that ethanol consumption by adolescents raised by an ethanol-consuming dam (Conditions GLW, GL and W) was significantly greater than ethanol consumption by adolescents from groups A and C whose mothers did not drink ethanol ($t_{\Psi 1}$ = 3.26, $p < .001$). There were no differences in ethanol consumption among groups exposed to ethanol drinking dams (GLW vs. GL and W, $t_{\Psi 2}$ = .21, ns; GLW and GL vs. W, $t_{\Psi 3}$ = .47, ns). Further, there was no effect of mere access to ethanol during the weaning period on adolescent ethanol intake, as adolescents assigned to the Access Condition did not drink more ethanol than did adolescents assigned to the Control Condition ($t_{\Psi 4}$ = .20, ns).

For subjects raised with an ethanol consuming dam, the mean g/kg ethanol intake in 2 hr was greater than 1.5 g/kg (Group GLW = 1.88 ± 0.21 g/kg, Group GL = 1.74 ± 0.16, Group W = 1.74 ± 0.17). When ethanol is injected intraperitoneally, 1.5 g/kg produces intoxication (see Larson & Siegel, 1998, or Wenger, Tiffany, Bombardier, Nicholls & Woods, 1981 for examples of the ataxic effects of a 1.5-g/kg dose of ethanol).

When subjects in this experiment were given 2 hr to consume fluids voluntarily, most animals completed drinking within 30 min of fluid presentation, so subjects that consumed a dose greater than 1.5 g/kg within a session might have experienced some intoxication. In fact, informal behavioral observations were consistent with the view that many subjects in Groups GLW, GL and W

were intoxicated following test sessions. In particular, on days when individuals consumed more than 3.0 g/kg of 8- percent ethanol, they showed loss of locomotory co-ordination or appeared sedated. We attempted to formally measure ataxia in intoxicated 27- to 33-day-old rats using the procedures of Larson and Siegel (1998). Unfortunately, adolescent rats were so light that often, even when unconscious, they did not slide down the increasingly elevated inclined plane used to measure ataxia any sooner than did control subjects not suffering from alcohol intoxication.

Subjects raised by water drinking dams consumed smaller doses of ethanol (Group A = 1.08 ± 0.12 g/kg, Group C = 1.00 ± 0.09 g/kg), and no animals assigned to Groups A or C ever exhibited signs of intoxication.

It is interesting to note that adolescent rats exposed to an ethanol-consuming dam were not universally affected by their exposure. As can be seen in Figure 3, many litters assigned to the GLW, GL and W Conditions drank very little ethanol. The variance among litters assigned to those 3 ethanol-exposed conditions was greater than the variance among litters assigned to the Access and Control Conditions, suggesting that there may be individual differences in vulnerability to exposure to an ethanol-consuming dam.

In addition to confirming that exposure to ethanol during early development can result in enhanced affinity for ethanol in adolescent rats, the results of the present experiment suggest that asocial exposure to ethanol through

simple access to a source of ethanol is not sufficient in itself to enhance adolescent rats' ethanol consumption. Pups assigned to the Access Condition had opportunity to drink ethanol throughout the weaning period (Day 14 - 26), unlike pups assigned to the Control Condition that had no opportunity to ingest ethanol. In fact, pups assigned to the Access Condition also had the opportunity to interact socially with siblings that had consumed ethanol. However, there was no difference in ethanol consumption between adolescents assigned to the two groups. Access to ethanol and demonstration of ethanol consumption by siblings appears to be insufficient to enhance voluntary ethanol intake by adolescent rats. Consumption of substantial quantities of ethanol by subjects in this experiment must therefore have been due to something other than exposure to ethanol or opportunity to consume ethanol during the weaning period.

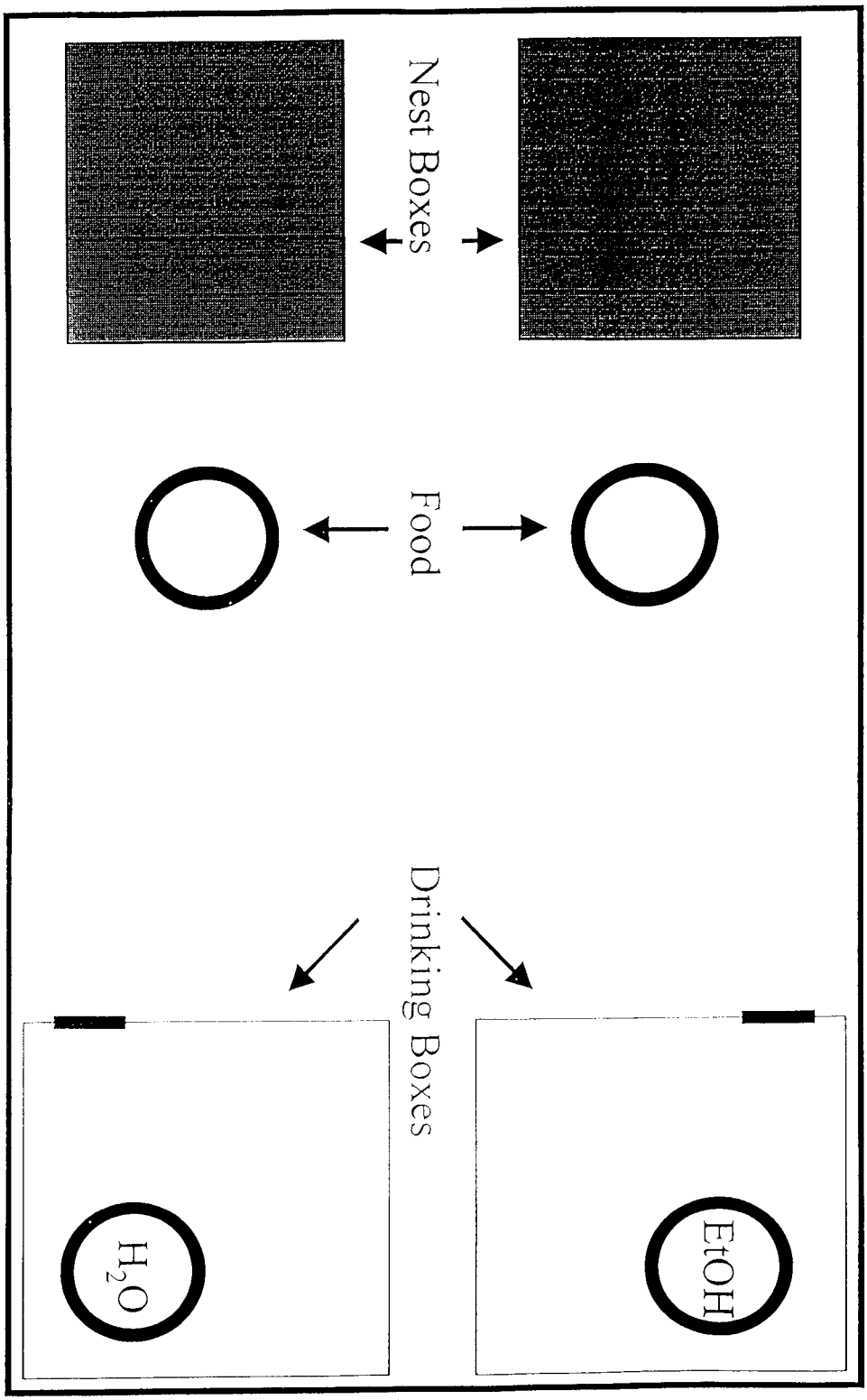
The lack of differentiation among ethanol exposed groups could have been due to a general effect of maternally-derived ethanol exposure on ethanol consumption levels, such that any form of early social exposure to ethanol will result in enhanced ethanol affinity later in life. On the other hand, the degree of ethanol exposure, both social and asocial, that I administered to each exposed group in the present experiment was extensive. Pups lived with an ethanol consuming dam, and had access to ethanol, just prior to testing. Such access to ethanol during or after the experience of social exposure and immediately before testing may have increased levels of ethanol consumption seen in the testing

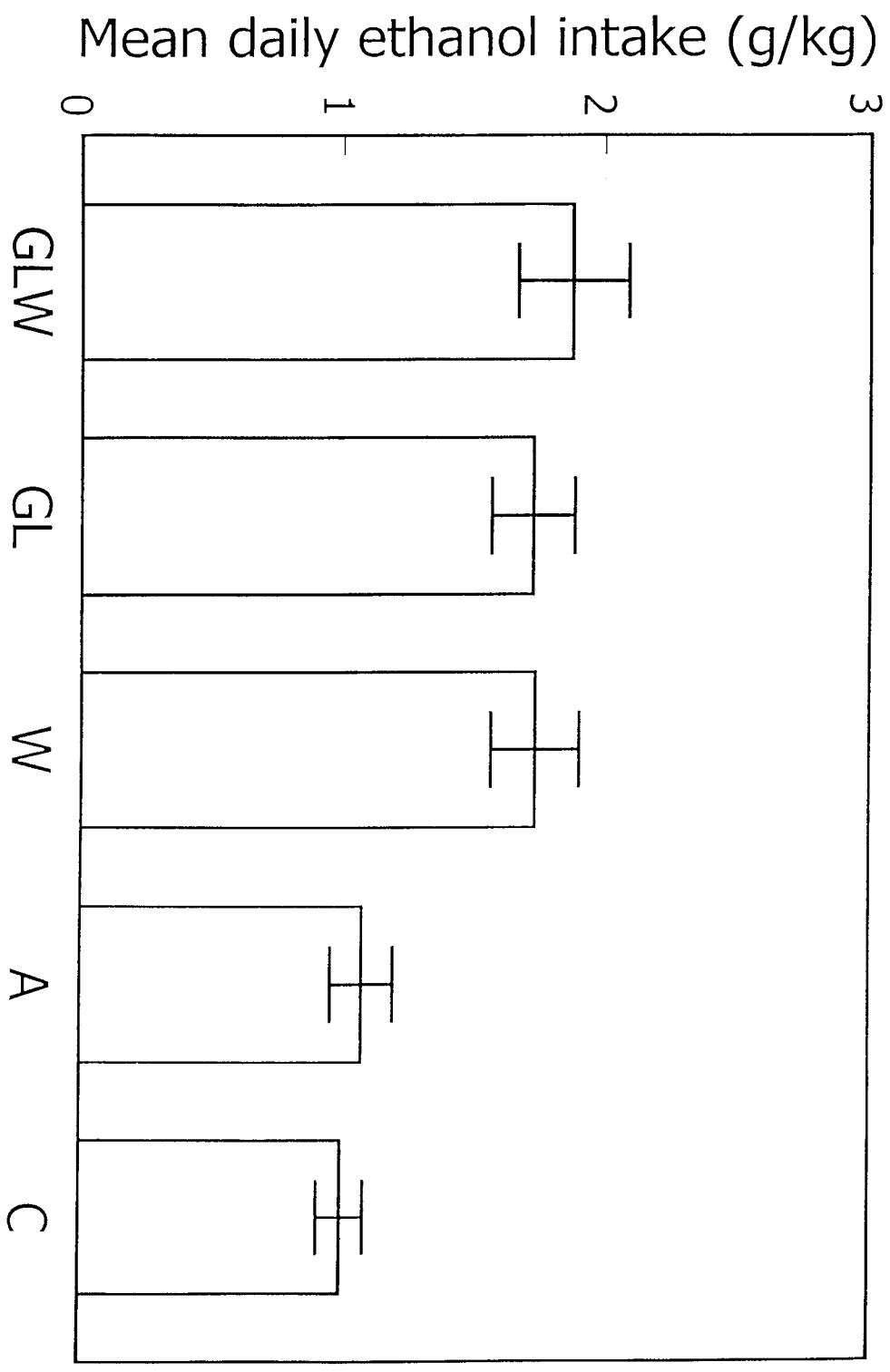
period. Experiment 2 was designed to isolate effects of maternal exposure during gestation and lactation, without interference from effects of asocial exposure of pups to ethanol during the weaning period and any potential effects of exposure to ethanol-consuming littermates during that time (Days 14 to 26).

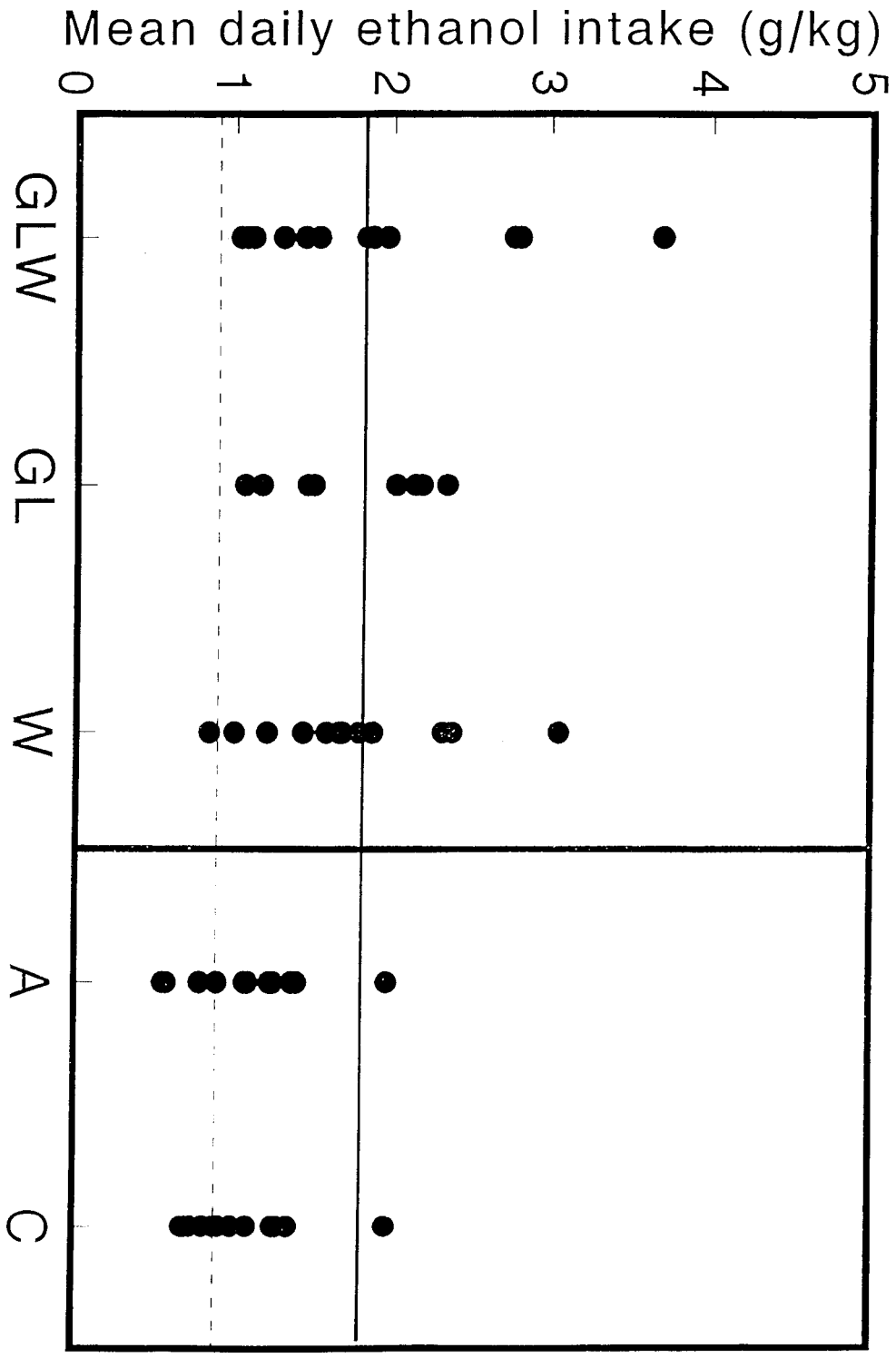
Table 1

Procedure for Experiment 1

	Dam Access		Pup Access
Condition	Prior to Day 14	Day 14 – Day 26	Day 14 – Day 26
GLW	Ethanol	Ethanol	Ethanol and Water
GL	Ethanol	Water	Ethanol and Water
W	Water	Ethanol	Ethanol and Water
A	Water	Water	Ethanol and Water
C	Water	Water	Water


Nest Boxes
Food
Drinking Boxes
H_2O
EtOH


Mean daily ethanol intake (g/kg)
0
1
2
3
GLW
GL
W
A
C


Mean daily ethanol intake (g/kg)
0
1
2
3
4
5
GLW
GL
W
A
C

EXPERIMENT 2: Gestation versus Lactation

Experiment 2 was designed with two main purposes: First, to isolate effects of exposure to ethanol during gestation from effects of exposure to ethanol during lactation, and second, to remove effects of asocial exposure to ethanol during the weaning period in order to examine only the effects of social exposure during gestation and lactation. Although asocial exposure to ethanol, alone, did not have an effect on post-weaning ethanol intake by adolescent rats in Experiment 1, such asocial exposure to ethanol during weaning may have been experienced differently by weanlings that had experienced ethanol during gestation and lactation than by weanlings that had not.

Method

Subjects

I randomly assigned each of 37 pregnant female Long-Evans rats to one of five conditions described in Procedure. Within 48 hours of birth (Day 0), I recorded the number of live births, and culled each litter to eight pups (where possible, four pups of each sex). I documented the mass of each litter on Day 7,

and on Day 26 randomly selected one adolescent rat of each sex from each litter for testing.

Apparatus

Until pups were 26 days of age, I housed each dam and her litter in a transparent polypropylene cage, equipped as described in Apparatus of Experiment 1. At 26 days of age, each tested adolescent moved to its own cage for 7 days of testing. During a choice test, each adolescent chose between two 50-ml test tubes, described in Apparatus of Experiment 1.

Procedure (See Table 2)

Gestation and Lactation (GL2) Condition (n = 8 litters): I gave pregnant rats assigned to the GL2 condition 4% ethanol in tap water as the sole source of fluid during the last 2 weeks of gestation and the first 2 weeks postpartum. From Day 14 to Day 26 dams and their litters in Group GL2, unlike dams and litters assigned to the GL Condition in Experiment 1, had access only to tap water.

Gestation (G) Condition (n = 8 litters): I gave pregnant rats assigned to the G Condition 4% ethanol as the sole source of fluid during the last 2 weeks of gestation and tap water from Day 0 until Day 26.

Lactation (L) Condition (n = 8 litters): I gave pregnant rats assigned to this condition tap water throughout gestation. From Day 0 to Day 14, they had 4% ethanol as the sole source of fluid, and tap water from Day 14 to Day 26.

Control (C) Condition (n = 8 litters): Dams and their litters assigned to the Control Condition drank tap water throughout gestation, lactation and until Day 26.

Blood-Ethanol Concentration (BEC) Condition (n = 5 litters): Pregnant rats assigned to the BEC Condition drank 4% ethanol during the last 2 weeks of gestation. On or about Prenatal Day 16, I collected approximately .25 ml of blood from each rat via .5 mm tail amputation. Blood was collected into heparinized tubes and analyzed for blood ethanol concentration (YSI Model 2700 Biochemistry Analyzer, Yellow Springs Industries Inc., Yellow Springs, OH). All blood ethanol concentrations are presented as mg per 100 ml of blood (mg%). No litters from this condition were used for ethanol choice testing.

Testing: We tested subjects assigned to GL2, G, L and C Conditions just as we had tested subjects in Experiment 1

Results

The main results of Experiment 2 are presented in Figure 4. A one-way ANOVA revealed no effect of group assignment on mean daily g/kg intake of ethanol F (3, 28) = 0.83, ns). Levels of ethanol consumption by adolescent rats in

this experiment did not appear to be affected by prior exposure to ethanol during either gestation or lactation, or throughout both of those stages of development.

Failure in the present experiment to find an effect of early exposure to ethanol on adolescent ethanol consumption cannot be attributed to a lack of exposure during gestation. Dams assigned to the BEC condition consumed sufficient ethanol to result in a mean blood-ethanol concentration of 165.6 mg% (± 21.7), and we can reasonably assume that dams assigned to GL2 and G conditions consumed similar amounts of ethanol. Blood-ethanol concentrations in this range are known to produce neurobehavioural changes, but not physiological anomalies, in exposed offspring (for a review, see Driscoll, Streissguth & Riley, 1990).

Dams' fluid consumption during lactation was similar to that during gestation, so pups were exposed to moderate levels of ethanol during that developmental stage, as well. Exposure to ethanol did not affect litter size, or pup size, as both litter size and weight were similar across groups (Table 3).

Discussion

Unlike Experiment 1, the results of Experiment 2 indicate that exposure to a moderate dose of ethanol during gestation, lactation, or throughout both gestation and lactation, does not lead to enhanced ethanol affinity after weaning. The main methodological difference between the two studies that could account

for such a difference in outcome was the removal of asocial exposure to ethanol during the weaning period.

I interpret the results of Experiment 2 as providing evidence that moderate exposure *in utero* or through mothers' milk did not effect ethanol intake in adolescence unless such exposure was followed by an opportunity to ingest and experience ethanol during weaning. Recall that in Experiment 1 opportunity to sample ethanol during weaning (without prior exposure from the dam) did not enhance ethanol intake in adolescent rats. Exposure to an ethanol-consuming dam during some portion of development, together with opportunity to sample ethanol during the weaning period, may be necessary to enhance ethanol intake.

Another possible explanation for the discrepancy in results between Experiments 1 and 2 is related to the concentrations of ethanol that I used during exposure and testing. Pups were exposed to 4% ethanol during gestation and lactation in both Experiments 1 and 2. In Experiment 1, pups could also sample 8% ethanol (either with or without their dams) during weaning. During the test phase of both experiments, I offered adolescents a choice between water and 8% ethanol. Four and eight-percent ethanol differ in both palatability and potential pharmacological effects. If pups' social exposure to ethanol during gestation and lactation had come from dams drinking 8% ethanol, perhaps exposure during gestation or lactation would have been sufficient to enhance ethanol consumption after weaning.

To test this hypothesis and clarify the results of Experiment 2, I replicated the conditions experienced by rats assigned to the GL2 Condition in Experiment 2 and compared the ethanol choices of adolescent rats that had lived with dams that consumed 8% ethanol throughout gestation and lactation (n = 10 litters) to the ethanol choice of adolescents that had lived with dams that had consumed 4% ethanol during the same period (n = 10 litters). I also documented the blood-ethanol concentration of six additional pregnant rats that drank 4% ethanol, and six that drank 8% ethanol. There was no effect of ethanol concentration fed to dams on the intake of 8% ethanol by adolescent rats. Mean ethanol intake by adolescents exposed to 4% ethanol was 0.94 g/kg ± .19, while mean ethanol intake by adolescents exposed to 8% ethanol was 1.02 g/kg ± .12 (t = 0.39, ns).

Failure to find an effect of ethanol concentration was not due to a lack of difference in blood-ethanol concentration between dams in the two conditions. Mean blood-ethanol concentration of pregnant females that drank 4% ethanol was 111.7 mg% ± 20.1, while mean blood-ethanol concentration achieved by pregnant rats that drank 8% ethanol was 239.3 mg% ± 37.0 (t = 3.03, $p < .05$). Thus, exposure to either 4% ethanol or 8% ethanol throughout gestation and lactation does not lead to enhanced voluntary consumption of 8% ethanol by adolescent rats.

Although the concentration of ethanol experienced during early development did not appear to affect ethanol consumption by adolescent rats, there may have

been an effect of delay between exposure to ethanol and testing for ethanol intake. None of the adolescent rats in Experiment 2 had any access to ethanol from Day 14 until Day 26, when testing began. In Experiment 1, juveniles that had early exposure to ethanol also had the opportunity to sample ethanol from Day 14 to Day 26, and this continuous exposure to ethanol may have facilitated ethanol consumption after weaning was complete.

Table 2

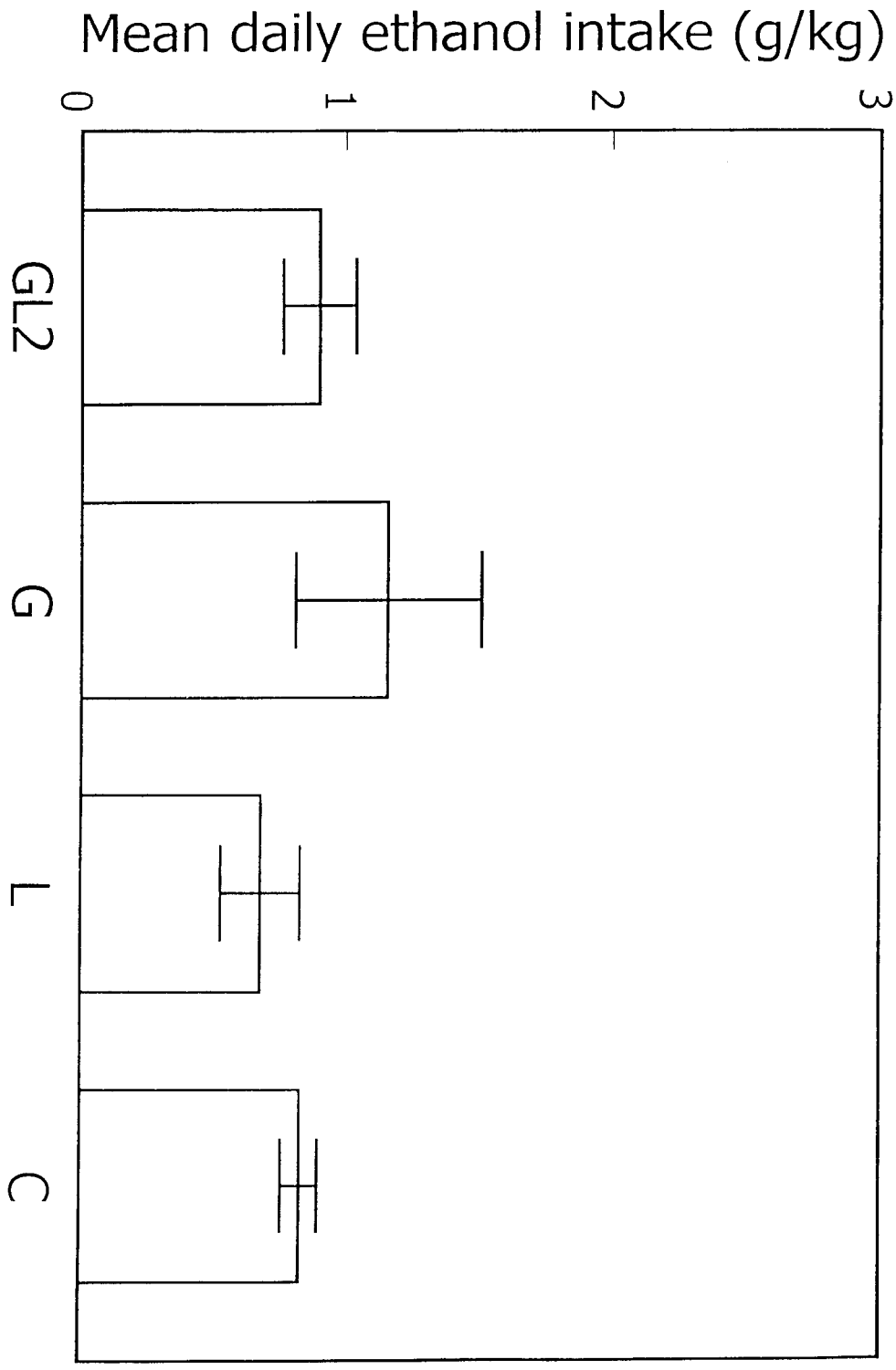
Procedure for Experiment 2

	Dam Access		Dam and Pup Access
Condition	Prior to Day 0	Day 0 – Day 14	Day 14 – Day 26
GL2	Ethanol	Ethanol	Water
G	Ethanol	Water	Water
L	Water	Ethanol	Water
C	Water	Water	Water
BEC	Ethanol	Water	Water

Table 3

Mean Litter Sizes and Litter Weights in Experiment 2

Condition	Mean Litter Size	Mean Litter Weight (Day 7)
GL2	14.5 pups	141.1 g
G	14.0 pups	137.3 g
L	15.9 pups	133.5 g
C	14.4 pups	138.5 g
BEC	15.0 pups	143.2 g


Mean daily ethanol intake (g/kg)
0
1
2
3
GL2
G
L
C

EXPERIMENT 3: Is Access to Ethanol Necessary?

I undertook Experiment 3 to examine effects of social exposure to ethanol during the weaning period, independent of effects of opportunity to sample ethanol during that period, on subsequent voluntary ethanol consumption during adolescence. I found in Experiment 1 (Group W) that social exposure to ethanol during the weaning period enhanced subsequent voluntary ethanol intake by adolescent rats. However, the social exposure employed in Experiment 1 allowed weaning pups direct access to ethanol. Although I found no effects of asocial access to ethanol, alone, during weaning (Group A in Experiment 1), I did find that adolescents that had experienced prenatal and nursing exposure to ethanol demonstrated enhanced ethanol intake only if they later experienced asocial access to ethanol (Group GL in Experiment 1 compared with Group GL2 in Experiment 2). Thus, effects of social exposure to ethanol during weaning may also depend upon concurrent access to ethanol. In the present experiment, I assessed effects of social exposure independent of effects of opportunity to sample ethanol by physically isolating a dam's source of ethanol so that pups could not sample it.

Method

Subjects

Sixty-six adolescent rats, born to 33 female Long-Evans rats acquired from Charles River Breeding Farms (St. Constant, Quebec) and maintained in the vivarium of the McMaster University Psychology Department served as subjects. Within 48 hours of birth (Day 0), I culled each litter to 8 pups (where possible, four pups of each sex) and randomly assigned the litter to one of three treatment conditions described in Procedure.

Apparatus

Until pups were 14 days of age, I housed each dam and her litter in a transparent polypropylene cage, equipped as described in Apparatus of Experiment 1. From Day 14 to Day 26, each dam and litter occupied a large floor enclosure measuring 92 x 92 x 31 cm constructed of galvanized metal frame and wire-mesh. The floor of this enclosure was carpeted with wood shavings to a depth of approximately 4 cm, and I provided each enclosure with a 30 x 30 x 18 cm wooden nest box, a food container and a 30 cm^3 Plexiglas drinking box. The drinking box in each enclosure had a circular entrance 2.5-cm in diameter that permitted pups, but not dams, to enter. Each drinking box contained a bowl filled with tap water.

In addition to water provided for all pups, dams and pups assigned to the Access condition (described below) had access to 8% ethanol from a second bowl in the enclosure (Figure 5a). Dams assigned to both No Access and Control conditions (described below) drank either 8% ethanol or tap water dispensed through a drinking spout mounted on the lid of the large enclosure, approximately 25cm above the cage floor (Figure 5b). This drinking spout entered the enclosure through a small hole drilled in a $20cm^2$ Plexiglas plate mounted on the underside of the lid of the floor enclosure. The Plexiglas plate prevented pups from climbing along the wire mesh lid to drink from the spout that their dam could easily access. To collect drips and thus prevent pups from sampling ethanol in that fashion, I mounted a small glass container (filled with absorbent wood shavings and closed with a wire mesh lid) directly beneath the drinking spout.

On Day 26, I moved adolescent rats from the large floor enclosure to individual transparent polypropylene cages, like those described above, for ethanol-choice testing. During a choice test, all adolescents chose between two 50-ml test tubes, one containing tap water and the other 8% ethanol. Each test tube was closed with a rubber stopper and a stainless steel sipper tube.

Procedure (see Table 4)

From Day 0 to Day 14, each dam and her litter occupied a transparent polypropylene cage, as described above, with ad libitum access to both food and water.

Access Condition (n = 11 litters): From Day 14 until Day 26, I housed each litter assigned to the Access Condition in a large floor enclosure like that illustrated in Figure 5a. Dams' fluid intake was restricted to 8% ethanol. Pups had access both to 8% ethanol, from the same source as their dam, and to water in the drinking box which their dam could not enter.

No Access Condition (n = 11 litters): I treated litters assigned to the No Access Condition just as I treated litters assigned to the Access condition except that pups assigned to the No Access Condition did not have access to their dams' source of ethanol. Pups in the No Access Condition could drink water within the drinking box (as pups did in the Access Condition), while their dams drank 8% ethanol dispensed from a drinking spout mounted on the lid of the enclosure (See Figure 5b).

Control Condition (n = 11 litters): I treated litters assigned to the Control Condition as I treated those assigned to the No Access Condition, except that Control dams drank tap water dispensed from a drinking spout mounted on the lid of their enclosure. Neither dams nor pups in this condition drank ethanol before testing of adolescents' ethanol choice.

Testing: Testing began on Day 26 and was conducted exactly as in previous experiments.

Results

The main results of Experiment 3 are presented in Figure 6. As can be seen in Figure 6, exposure to an ethanol consuming dam during the weaning period (Day 14 to Day 26) resulted in enhanced voluntary intake of ethanol by adolescent rats, one-way ANOVA: $\underline{F}$ (2, 32) = 13.34, $\underline{p} < 0.001$. Planned orthogonal comparisons revealed that adolescents that lived with an ethanol-consuming dam (Access and No Access Conditions) consumed significantly more ethanol than did adolescents in the Control condition, whose mothers did not drink ethanol ($\underline{t}_{\Psi 1} = 2.97$, $\underline{p} < 0.01$). There was no difference in ethanol consumption between the two groups of adolescents that had lived with an ethanol-consuming dam (Access vs. No Access, $\underline{t}_{\Psi 2} = .32$, $\underline{ns}$). As in Experiment 1, for adolescents raised with an ethanol-consuming dam, the mean ethanol intake in 2 hr was greater than 1.5 g/kg (Access = 1.79 ± 0.19, No Access = 1.91 ± 0.19 g/kg).

As in Experiment 1, not all adolescents that had lived with an ethanol-consuming dam were affected by their exposure, but pups from many litters drank substantial quantities of ethanol. As can be seen in Figure 7, the ranges of ethanol intake among subjects assigned to the Access and No Access Conditions was

much greater than the range of ethanol intake by subjects assigned to the Control Condition.

Discussion

The results of Experiment 3 replicate the result found in Experiment 1 that adolescent rats that had lived with an ethanol-consuming dam throughout a 12-day period drank more ethanol than did adolescents that lived with a water-consuming dam. In addition, I found here that enhanced voluntary consumption of ethanol did not depend on direct access to ethanol during the weaning period. Thus, social exposure to ethanol during the weaning period was sufficient to enhance voluntary ethanol consumption by adolescents.

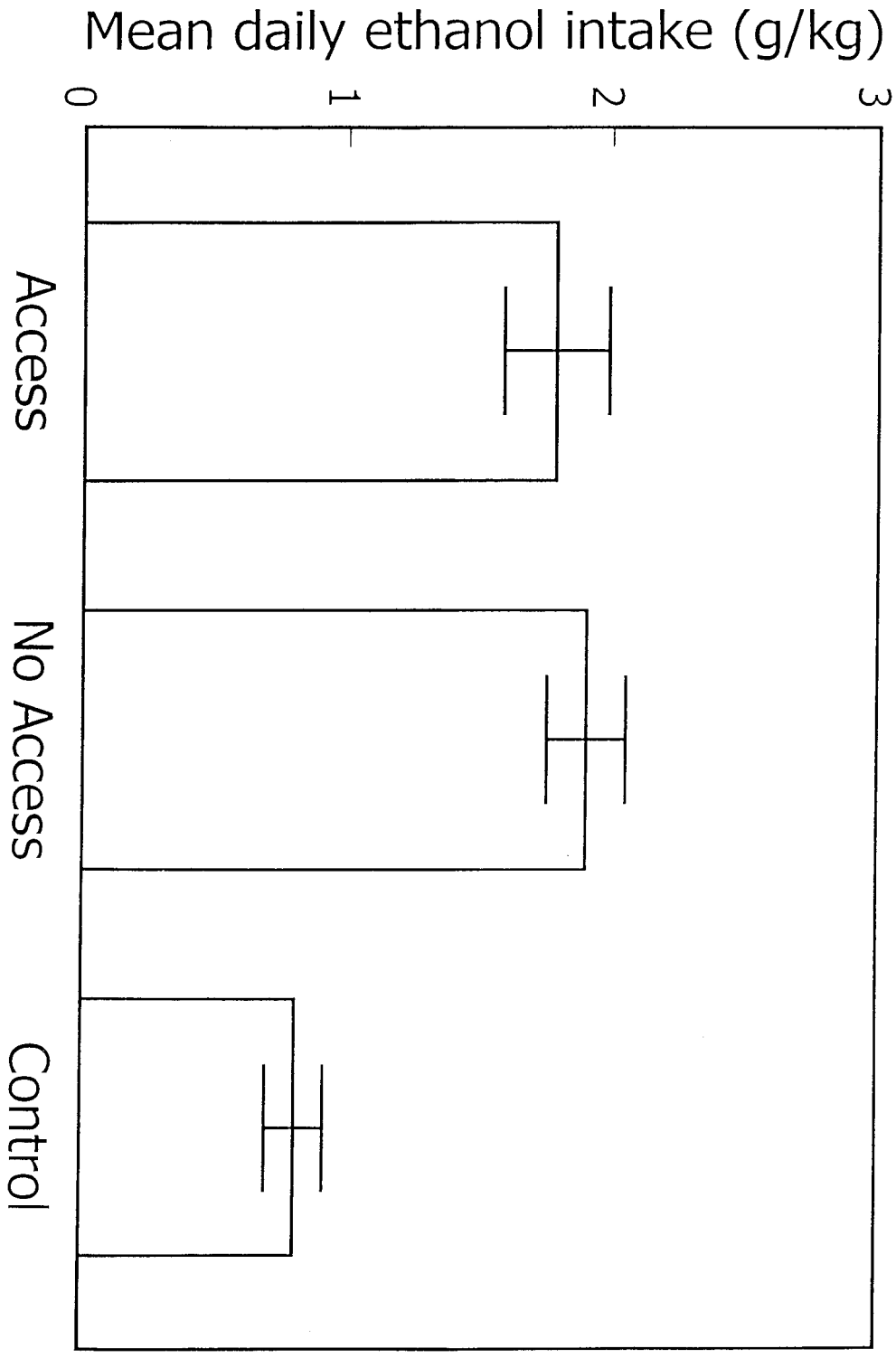
Although adolescents that had lived with an ethanol-consuming dam in Experiment 3 were not able to sample ethanol independent of their dam, they were still able to experience ethanol while nursing. Consequently, although social exposure to ethanol during the weaning period is sufficient to induce ethanol affinity, exposure to ethanol while nursing may be critical for development of affinity for ethanol after weaning. This issue will be addressed in Experiment 5.

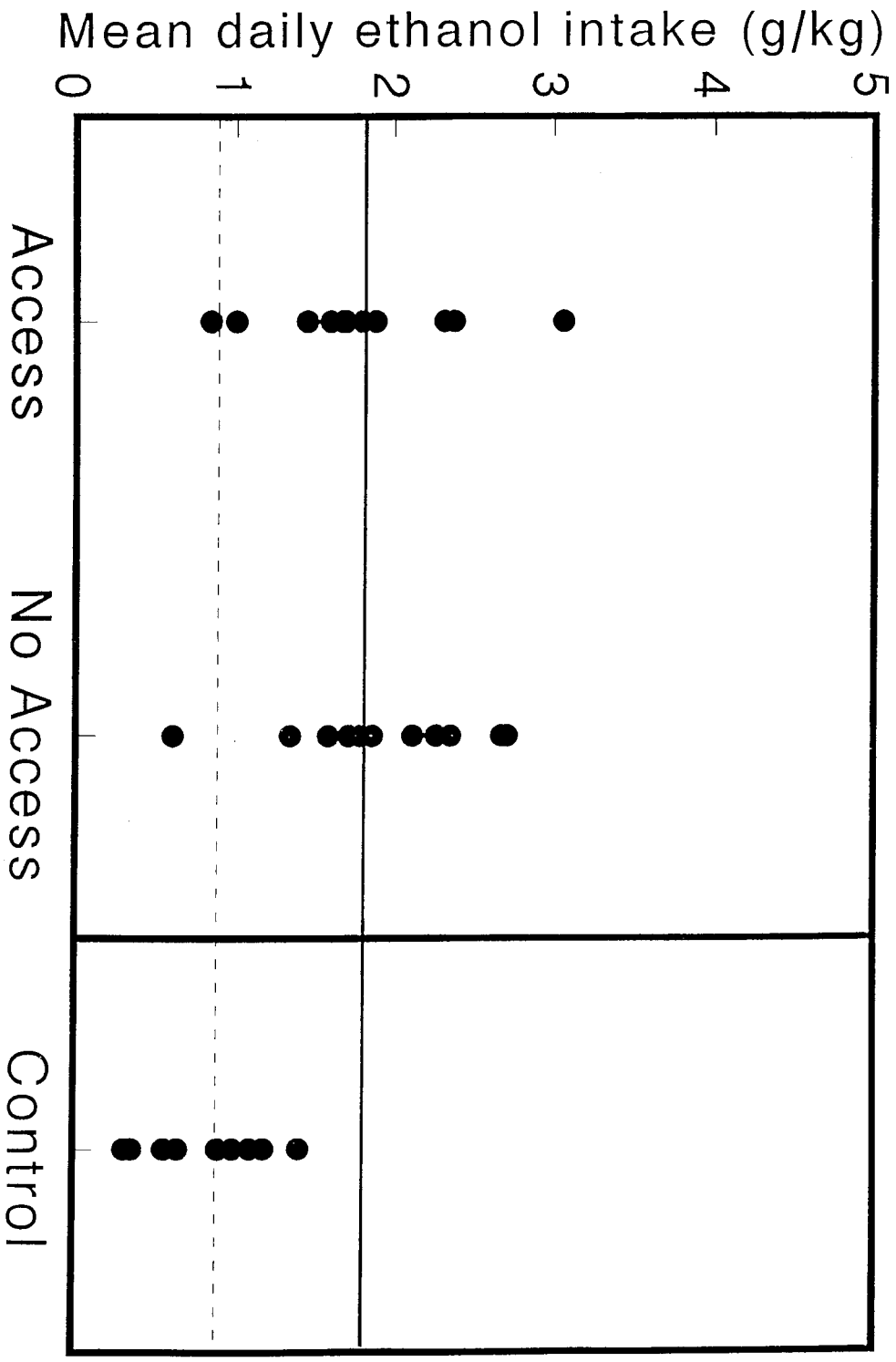
Table 4

Procedure for Experiment 3

Group	Dam Access	Pup Access
Access	Ethanol	Ethanol and Water
No Access	Ethanol	Water
Control	Water	Water

Nest Box
EtOH
Drinking Box
H_2O
Entrance
Food
Access Condition
Nest Box
Drinking Box
H_2O
Entrance
Food
No Access and Control Conditions


Mean daily ethanol intake (g/kg)
0
1
2
3
Access
No Access
Control


Mean daily ethanol intake (g/kg)
0
1
2
3
4
5
Access
No Access
Control

EXPERIMENT 4: Reduction of Exposure and Delay to Test

Adolescents in Experiments 1 and 3 experienced 12 days of social exposure to ethanol while weaning, and proceeded directly from exposure to ethanol-choice testing. Adolescents in Experiment 2 experienced a delay of at least 12 days between maternally-mediated ethanol exposure and ethanol-choice testing. I undertook Experiment 4 to determine both whether a shorter period of social exposure to ethanol would result in enhanced voluntary ethanol consumption by adolescents, and, if so, whether such affinity for ethanol would be expressed after a delay between exposure to ethanol and ethanol-choice testing.

Exposure to an ethanol-consuming dam throughout a 12-day weaning period reliably results in enhanced ethanol affinity in adolescent rats. Reducing exposure time during the weaning period allows me to determine whether one portion of that developmental stage is more critical than another for development of ethanol affinity. For example, if exposure to ethanol around the time of eye-opening results in greater intake of ethanol by adolescent rats than does exposure to an ethanol consuming dam near the conclusion of the weaning period, then I would suggest that a small critical period exists within the weaning period.

Method

Subjects

Fifty-four adolescent rats, born to 27 female Long-Evans rats acquired from Charles River Breeding Farms (St. Constant, Quebec) and maintained in the vivarium of the McMaster University Psychology Department served as subjects. Within 48 hours of birth (Day 0), I culled each litter to 8 pups (where possible, four pups of each sex) and randomly assigned the litter to one of the three treatment conditions described below in Procedure.

Apparatus

I used the apparatus described in Experiment 3, and illustrated in Figure 5b.

Procedure (see Table 5)

Until pups were 14 days of age, I housed each dam and her litter in a transparent polypropylene cage, equipped as described in Apparatus of Experiment 1. Each dam and her litter had ad libitum access to food and water.

Early Condition (n = 10 litters): From Day 14 until Day 26, I housed each litter assigned to the Early Condition in one of the large floor enclosures (Figure 5b). From Day 14 to Day 20, I restricted dams' fluid intake to 8% ethanol, dispensed from a drinking spout mounted on the lid of the enclosure. From Day 21 to Day 26, dams assigned to the Early Condition drank tap water

from the drinking spout. Pups in this condition could not reach their dam's source of ethanol, but had ad libitum access to water in the drinking box (which their dams could not enter).

Late Condition (n = 10 litters): I treated litters assigned to the Late Condition as I treated those assigned to the Early Condition, except that dams in the Late Condition drank water from Day 14 to Day 20, then drank 8% ethanol from Day 21 to Day 26.

Control Condition (n = 7 litters): I treated litters assigned to the Control condition as I treated litters assigned to the other two conditions, except that dams in the Control condition drank only tap water from Day 14 to Day 26.

Testing: I tested all pups starting on Day 26, as in previous experiments.

Results and Discussion

The main results of Experiment 4 are presented in Figure 8. As is evident in Figure 8, a 6-day exposure during the weaning period resulted in enhanced voluntary intake of ethanol by adolescent rats, one-way ANOVA; F (2, 26) = 4.06, $p = 0.03$. Planned orthogonal comparisons revealed that ethanol consumption by adolescents whose dam had consumed ethanol during the weaning period (both Early and Late Conditions) was significantly greater than ethanol consumption by adolescents assigned to the Control Condition, whose dam did not drink ethanol ($t_{\psi 1} = 1.73$, $p < 0.05$). There was no difference in

ethanol consumption between the two groups of adolescents that had lived with an ethanol-consuming dam ($t_{\Psi 2} = .02$, ns).

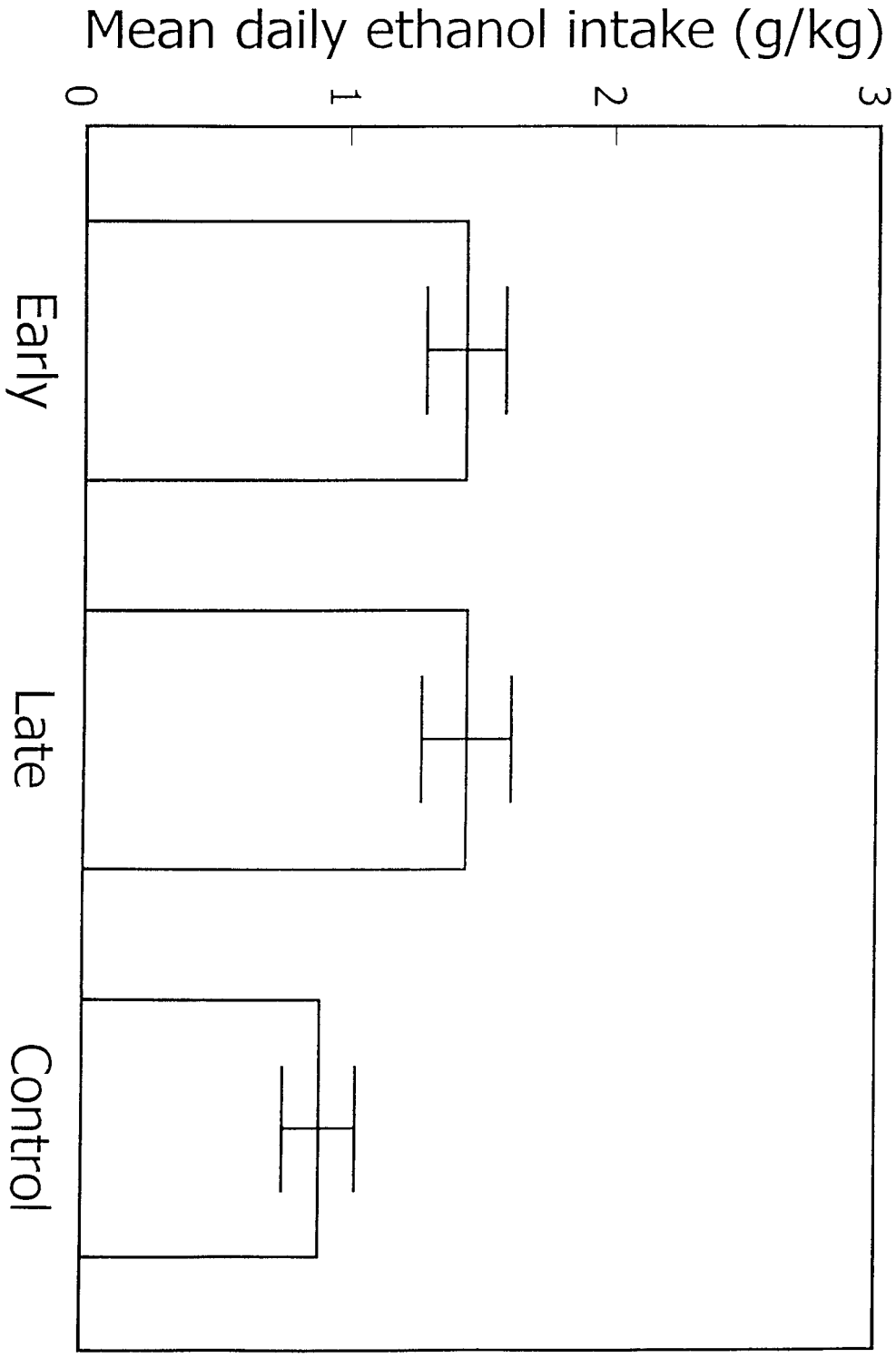
Ethanol-exposed adolescents in Experiment 4 did not consume as much ethanol in an average drinking session as did ethanol-exposed adolescents in Experiments 1 and 3, but mean ethanol consumption by such adolescents in Experiment 4 did approach 1.5 g/kg. Ethanol consumption by Control adolescents was again low. As in Experiments 1 and 3, ranges of ethanol intake by adolescent rats exposed to an ethanol-consuming dam were greater than ranges of ethanol intake by Control rats (Figure 9).

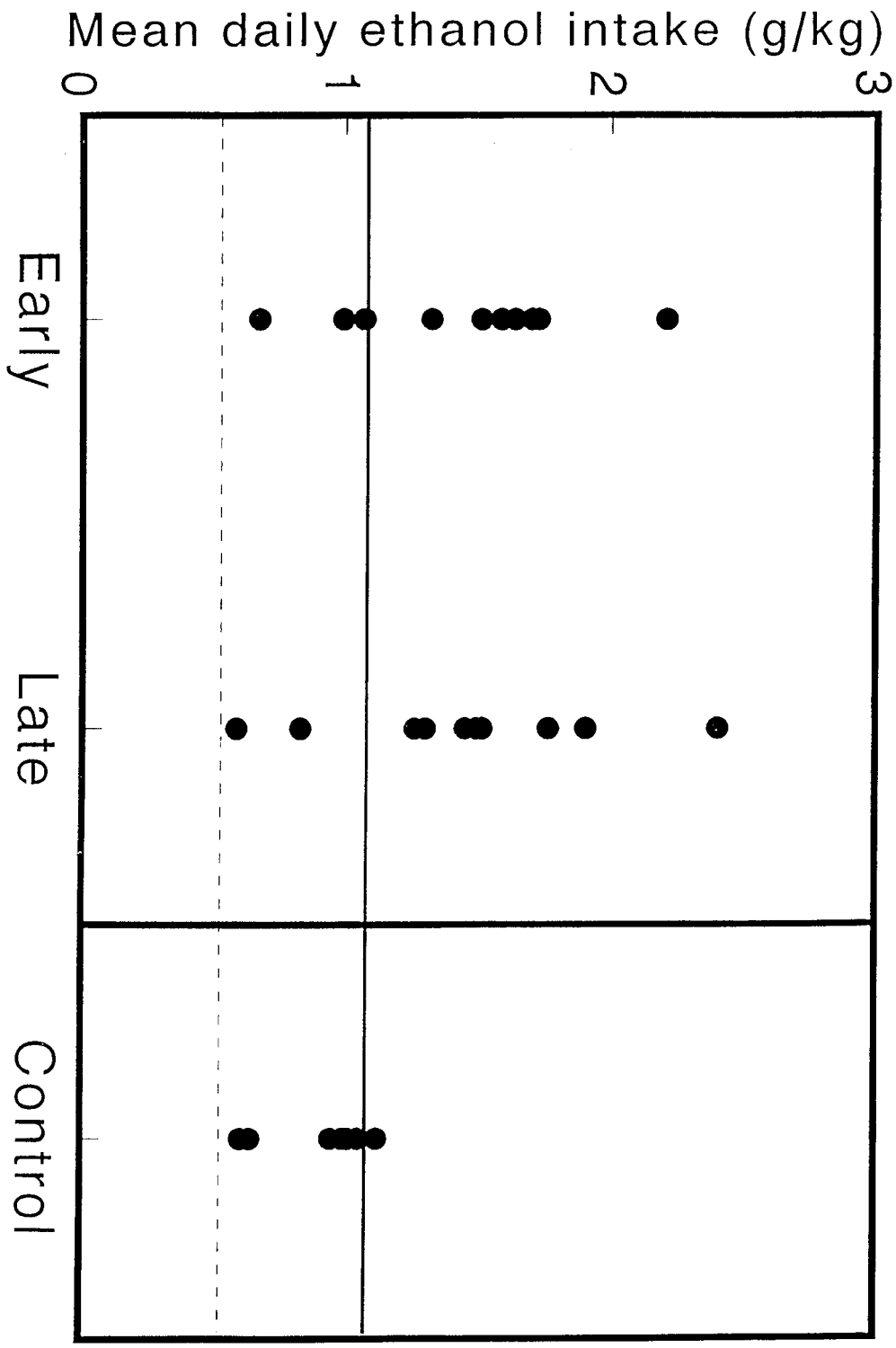
After 6 days of social exposure to ethanol during the weaning period, adolescent rats voluntarily consumed more ethanol than did adolescents without social exposure to ethanol. Exposure throughout Days 14 – 20 results in the same level of voluntary ethanol consumption as exposure throughout Days 21 – 26, suggesting that a 6-day delay between exposure and ethanol-choice testing does not weaken effects of social exposure to ethanol, and that exposure during one portion of the weaning period is not more effective than another portion in inducing ethanol affinity in adolescent rats.

Table 5

Procedure for Experiment 4

	Days 14-20		Days 21-26	
Group	Dam Access	Pup Access	Dam Access	Pup Access
Early	Ethanol	Water	Ethanol	Water
Late	Water	Water	Ethanol	Water
Control	Water	Water	Water	Water


Mean daily ethanol intake (g/kg)
0
1
2
3
Early
Late
Control


Mean daily ethanol intake (g/kg)
0
1
2
3
Early
Late
Control

EXPERIMENT 5: Is Exposure to Ethanol in Milk Necessary?

I found in Experiments 1, 3 and 4 that, after weaning, young rats reared by ethanol-consuming dams consumed more ethanol than did pups reared by water-consuming dams. I undertook Experiment 5 to determine whether consumption of milk from a lactating rat ingesting ethanol is necessary for development of enhanced affinity for ethanol.

It was found in Experiment 2 that exposure to ethanol in mothers' milk from birth to Day 14 postpartum does not enhance ethanol affinity in adolescent rats. However, it remains possible that exposure to ethanol in milk together with social exposure to an ethanol-consuming dam during the weaning period does lead to enhanced ethanol affinity. In order to determine whether social exposure to an ethanol-consuming adult is sufficient to induce enhanced ethanol consumption by adolescent rats, it is necessary to evaluate the effects of social exposure in the absence of exposure to ethanol in milk.

In the present experiment, I removed rat dams from their litters on Day 18 postpartum and replaced the dams with either a lactating or non-lactating female rat that had access to only a single fluid, either tap water or 8% ethanol. I removed dams and introduced novel females on Day 18 because 18-day-old pups

are ready to be weaned. Most of my previous experiments employed 12 days of exposure to an ethanol-consuming dam during the weaning period. However, to test adolescents at an age consistent with previous experiments, I chose to expose pups in this experiment for only 8 days. I found in Experiment 4 that exposure to an ethanol-consuming dam for 6 days during the weaning period resulted in enhanced ethanol intake by adolescents, so any effects of social or nursing exposure during weaning should be expressed after 8 days of exposure as well.

Method

Subjects

Forty-eight pups, born to 24 female Long-Evans rats acquired from Charles River Breeding Farms (St. Constant, Quebec) and maintained in the vivarium of the McMaster University Psychology Department served as subjects. I used 12 dams as well as 12 adult virgin female rats as foster "mothers". Virgin adult females were approximately the same age as dams (14-16 weeks old). Within 48 hours of birth of a litter (Day 0), I culled it to 8 pups (where possible, four pups of each sex), and randomly assigned the litter to one of four treatment conditions described in Procedure. Two subjects from each litter were tested for voluntary ethanol consumption starting when they were 26 days old.

Apparatus

Until pups were 14 days of age, I housed each dam and her litter in a transparent polypropylene cage, equipped as described in Apparatus of Experiment 1. From Day 14 to Day 18, each dam and litter occupied a large floor enclosure, measuring 92 x 92 x 31 cm. Each enclosure contained a nest box, as well as food and water.

Just before I removed mothers and replaced them with other females on Day 18, I isolated adults' source of fluid from that of pups (Figure 10). I enclosed the sipper tube of a water bottle attached to one side of each floor enclosure with a 30-cm^3 Plexiglas drinking box. The drinking box had a circular entrance 2.5-cm in diameter that permitted pups, but not adults, to enter. I mounted a second bottle on the lid of each enclosure. Its sipper tube protruded into the enclosure through a 20 cm^2 Plexiglas plate attached to the underside of the lid. That plate prevented pups from climbing along the wire-mesh lid to drink.

Although adults could easily drink from the sipper tube suspended approximately 25 cm above the floor of the enclosure, pups could not reach it. To collect drips and thus prevent pups from sampling fluid from under the drinking spout, I mounted a small glass container (filled with absorbent wood shavings and closed with a wire mesh lid) directly beneath the spout.

On Day 26, I moved subjects from the large floor enclosure to individual transparent polypropylene cages for ethanol-choice testing. During the choice

test, each subject chose between two 50-ml test tubes, as described in Apparatus of Experiment 1.

Procedure (see Table 6)

Lactating-Female/Ethanol Condition (n = 6 litters): From Day 14 until Day 26, I housed each litter assigned to the Lactating-Female/Ethanol Condition in a large floor enclosure like that illustrated in Figure 10. From Day 14 until Day 18, each dam remained with her litter. On Day 18, I removed each dam and replaced her with a lactating female that I had just removed from her own litter. I then restricted each foster dam's fluid intake to 8% ethanol by placing ethanol in the water bottle mounted on the lid of the floor enclosure. Pups had access to water in the drinking box that their foster-dam could not enter.

Lactating-Female/Water Condition (n = 6 litters): I treated litters assigned to the Lactating-Female/Water Condition just as I treated litters assigned to the Lactating-Female/Ethanol Condition except that foster-dams drank water from the sipper tube on the lid of the enclosure.

Virgin-Female/Ethanol Condition (n = 6 litters): I treated litters assigned to the Virgin-Female/Ethanol Condition as I treated those assigned to the Lactating-Female/Ethanol Condition, except that foster-dams introduced into the cages were virgin, rather than lactating, adult females.

Virgin-Female/Water Condition (n = 6 litters): I treated litters assigned to the Virgin-Female/Water Condition as I treated those assigned to the Lactating-Female/Water Condition, except that foster dams were virgin, rather than lactating, adult females.

Testing: Testing began on Day 26 postpartum and was conducted as described in previous experiments.

Results and Discussion

The main results of Experiment 5 are presented in Figure 11. As can be seen in the figure, exposure during the weaning period (Day 18 to Day 26) to an ethanol-consuming foster-dam, whether lactating or virgin, resulted in enhanced voluntary intake of ethanol by adolescent rats. A two-way ANOVA revealed a main effect of the fluid consumed by foster females (either ethanol or water) ($F(1, 20) = 18.26$, $p < 0.001$), but no main effect of whether a foster female was lactating or virgin ($F(1, 20) = 0.54$, ns), and no interaction between main effects ($F(1, 20) = 2.23$, ns).

Mean ethanol intake in 2 hr by adolescents that had lived for 8 days with an ethanol consuming foster female was greater than 1.5 g/kg (Virgin Female Ethanol = 1.89 ± 0.27 g/kg, Lactating Female Ethanol = 1.53 ± .21 g/kg). As in previous experiments, not all ethanol-exposed adolescents drank more ethanol than did water-exposed adolescents, and the ranges of ethanol intake among

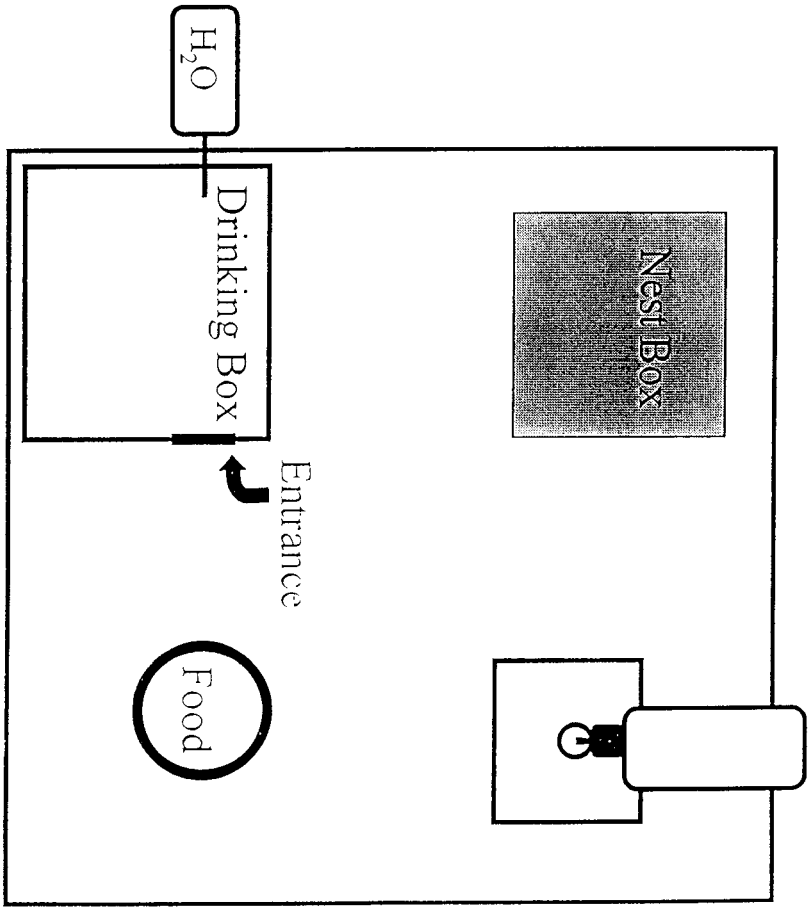
ethanol-exposed litters were greater than the ranges of intake among unexposed litters (Figure 12).

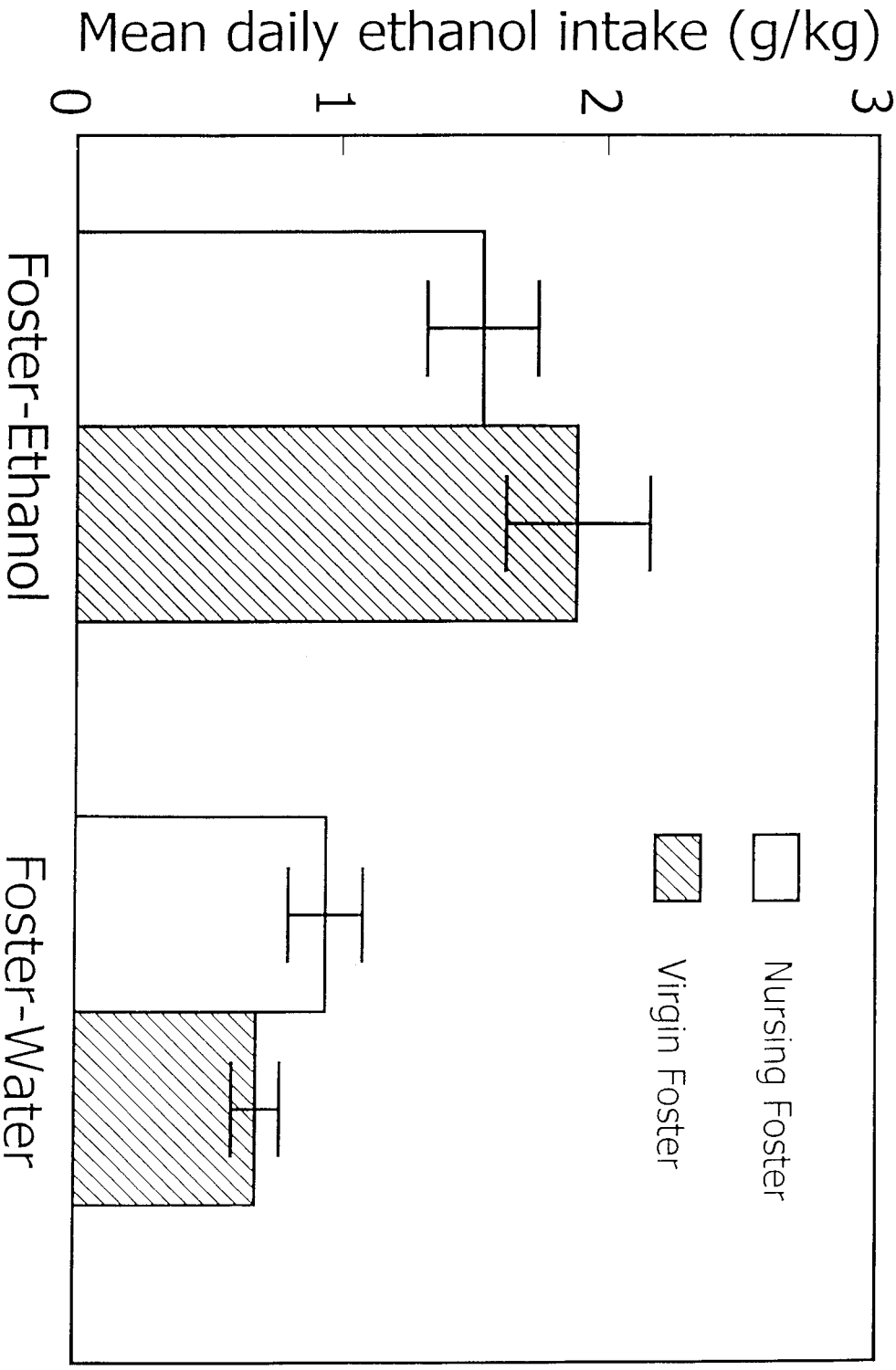
The results of Experiment 5 both replicate my previous finding that adolescent rats that had lived with an ethanol-consuming adult during weaning drank more ethanol than did adolescents that had lived with a water-consuming adult and showed that enhanced voluntary consumption of ethanol in adolescence did not depend on experience with ethanol in mothers' milk. Social exposure to an ethanol-consuming adult female during the weaning period is sufficient to enhance voluntary ethanol intake by adolescent rats.

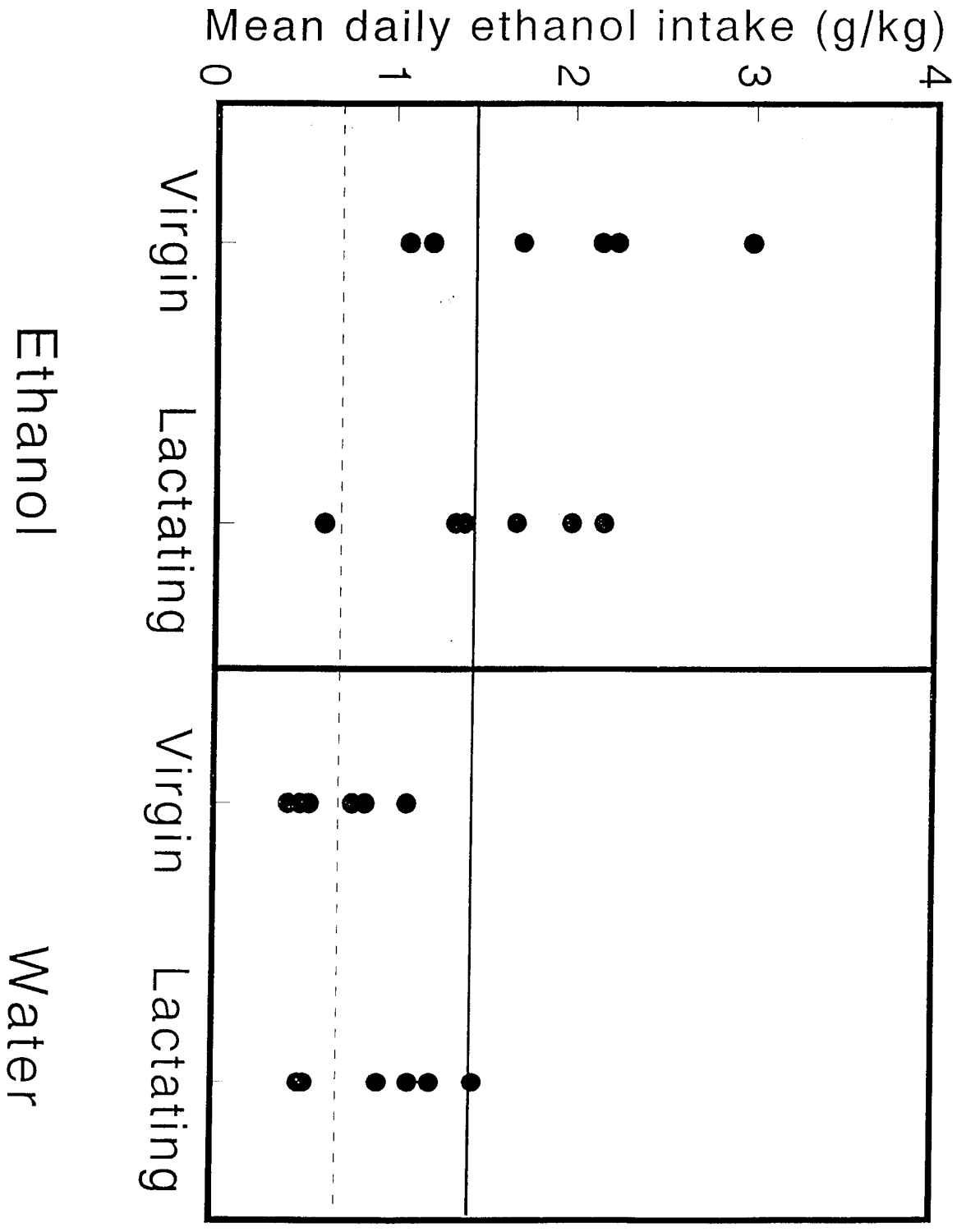
Table 6

Procedure for Experiment 5

Group	Foster-Dam Type	Foster-Dam Access
Lactating-Female/Ethanol	Lactating	Ethanol
Lactating-Female/Water	Lactating	Water
Virgin-Female/Ethanol	Virgin	Ethanol
Virgin-Female/Water	Virgin	Water


H_2O
Drinking Box
Nest Box
Entrance
Food


Mean daily ethanol intake (g/kg)
0
1
2
3
Foster-Ethanol
Foster-Water
Nursing Foster
Virgin Foster


Mean daily ethanol intake (g/kg)
0
1
2
3
4
Virgin
Lactating
Virgin
Lactating
Ethanol
Water

EXPERIMENT 6: Sober Dams and Virgin Females

Adolescents in Experiment 5 that, as pups, had lived with an ethanol-consuming foster female drank more ethanol than did adolescents that had lived with a water-consuming foster female. In fact, levels of ethanol consumption among adolescents that had lived with an ethanol-consuming foster-dam in Experiment 5 are comparable to levels of ethanol consumption among adolescents that learned socially about ethanol from their own dams in Experiment 1 (Group W), Experiment 3 (Groups Access and No Access) and Experiment 4 (Groups Early and Late).

Results like those described suggest that any adult female is capable of influencing ethanol affinity in young rats. We do not know, however, whether all adult females are equally effective in inducing ethanol affinity when more than one adult is present. In each experiment that I have described so far in this thesis, I have employed a single adult demonstrator for each litter to interact with. Possibly, when more than one demonstrator is present pups will attend preferentially to one over another.

In Experiment 6, I determined the relative effectiveness of a familiar, non-lactating female in inducing voluntary ethanol consumption in adolescent rats when a pup's own dam was also present.

Method

Subjects

Forty adolescent rats, born to 20 female Long-Evans rats acquired from Charles River Breeding Farms (St. Constant, Quebec), maintained in the vivarium of the McMaster University Psychology Department, served as subjects. I used an additional 20 adult virgin females as foster-dams. Each foster female was approximately the same age as the dam with which she was paired. Within 48 hr of birth, I culled each litter to 8 pups (where possible, four pups of each sex) and randomly assigned the litter to one of two treatment conditions described in Procedure.

Apparatus

Until pups were 14 days old, I housed each dam and her litter in a transparent polypropylene cage. From Day 14 to Day 18, each dam and her litter, along with a virgin female (introduced on Day 14), resided in a large floor enclosure outfitted with a nest box, food and a water bottle. On Day 18, I added two 30 cm^3 Plexiglas holding cages to each large floor enclosure (Figure 13). The

2.5-cm entrance to each box permitted entry by pups, but prevented adults from exiting. I placed inside each holding cage a food container, a 15-cm length of PVC conduit and mounted a bottle on the lid. The sipper tube closing the bottle protruded into the holding cage, and was suspended approximately 25 cm from the floor of the enclosure. I placed the dam in one holding cage, and the virgin female in the other.

Dams and virgin females remained in their respective holding cages from Day 18 to Day 26. For two litters assigned to each condition, I monitored and recorded activity in the large enclosures using a closed-circuit video camera, monitor and time-lapse video recorder, as described in Apparatus of Experiment 1. On Day 26, I removed adolescents from floor enclosures and began testing.

Procedure (see Table 7)

Pups in both conditions had ad libitum access to both food and water throughout the experiment and could interact with both adult females in their holding cages but could not reach the sipper tubes in those holding cages.

Virgin – Water Condition (n = 10 litters): From Day 18 to 26, I restricted both dams' and virgin females' fluid intake to water dispensed through the sipper tube mounted on the lid of each holding cage.

Virgin – Ethanol Condition (n = 10 litters): I treated litters assigned to the Virgin – Ethanol Condition as I treated litters assigned to the Virgin – Water

Condition, except that while dams assigned to the Virgin – Ethanol Condition drank water from Day 18 to Day 26, virgin females drank 8% ethanol.

Videotape Review: I sampled the activity of 4 litters (two assigned to each condition) for 4 h on each of 3 days for a total of 48 hours of activity. I recorded the number of visits by pups to each holding cage, and compared the number of times a pup moved its entire body into the holding cage containing its dam with the number of visits to virgin females.

Testing: I tested all pups as I had tested pups in previous experiments.

Results

The main results of Experiment 6 are presented in Figure 14. A two-sample *t*-test revealed that adolescents that had interacted with an ethanol-consuming virgin female during weaning drank more ethanol than did adolescents that had interacted with a water-consuming virgin female ($\underline{t}$ (17) = 2.02, $\underline{p}$ = 0.03). Thus, even in the presence of a water-consuming dam, an adult virgin female had a significant effect on the ethanol choice of pups that interacted with her.

Upon review of videotapes, I found that pups in both conditions visited their dam's holding cage more often than the cage containing the virgin female. In fact, in no litter did pups visit the virgin female more often than they did their dam (Mann-Whitney $\underline{U}$ = 0, $\underline{p}$ = 0.014). On average, pups visited their dams 5.1 times per hour, and visited virgin females 3 times per hour.

Discussion

Although pups that interacted with an ethanol-consuming virgin female drank more ethanol than did pups that interacted with a water-consuming virgin female, the amount of ethanol consumed by exposed pups was less than I had seen in previous experiments. In previous experiments, pups exposed to an ethanol-consuming adult for at least 8 days during the weaning period consume at least 1.5 g/kg during an average test session. Exposed pups in Experiment 6 consumed approximately 1.3 g/kg during an average test session.

It is possible that ethanol consumption by exposed adolescents in Experiment 6 was lower than expected due to effects of divided attention between two models. On such a hypothesis, modeling of ethanol consumption by a virgin female is not as potent, if pups interact with her less often than they would if she were the only adult present. It is also possible that modeling of ethanol consumption by a virgin female, while potent in isolation, is weakened by modeling of water consumption by another adult, in this case a pup's dam.

All demonstrators may not be created equal. Boyd and Richerson (1985) suggest that the effectiveness of a demonstrator may depend on some form of bias on the part of the observer. For example, direct bias on the part of the observer would result in the observer attending preferentially to a demonstrator that displays an innately-preferred behaviour. In the case of ethanol and water, water

is the more palatable fluid (Samson et al, 1988) so observing pups influenced by direct bias would be expected to attend more to demonstration by water-consuming adults. On the other hand, if observing pups were influenced by indirect bias then some attribute of the demonstrator itself would be critical. It is reasonable to assume that rat pups would prefer their dam over a relatively unfamiliar adult female, and that fluid consumption by their dam (regardless of whether that fluid were relatively palatable or unpalatable) would wield a greater influence over pups' subsequent fluid choices (Boyd & Richerson, 1985).

Although Chou and Richerson (1992) showed that Long-Evans rats, ranging in age from 31- to 77-days old, do not appear to be either directly or indirectly biased when attending to multiple demonstrators, the results of the present experiment suggest that weanling rats may in fact express indirect bias when observing two adult female demonstrators, when one demonstrator is their dam. Weanling rats interacted more with their dam than with the virgin female, and such interaction may be related to ethanol consumption rates observed in the present experiment.

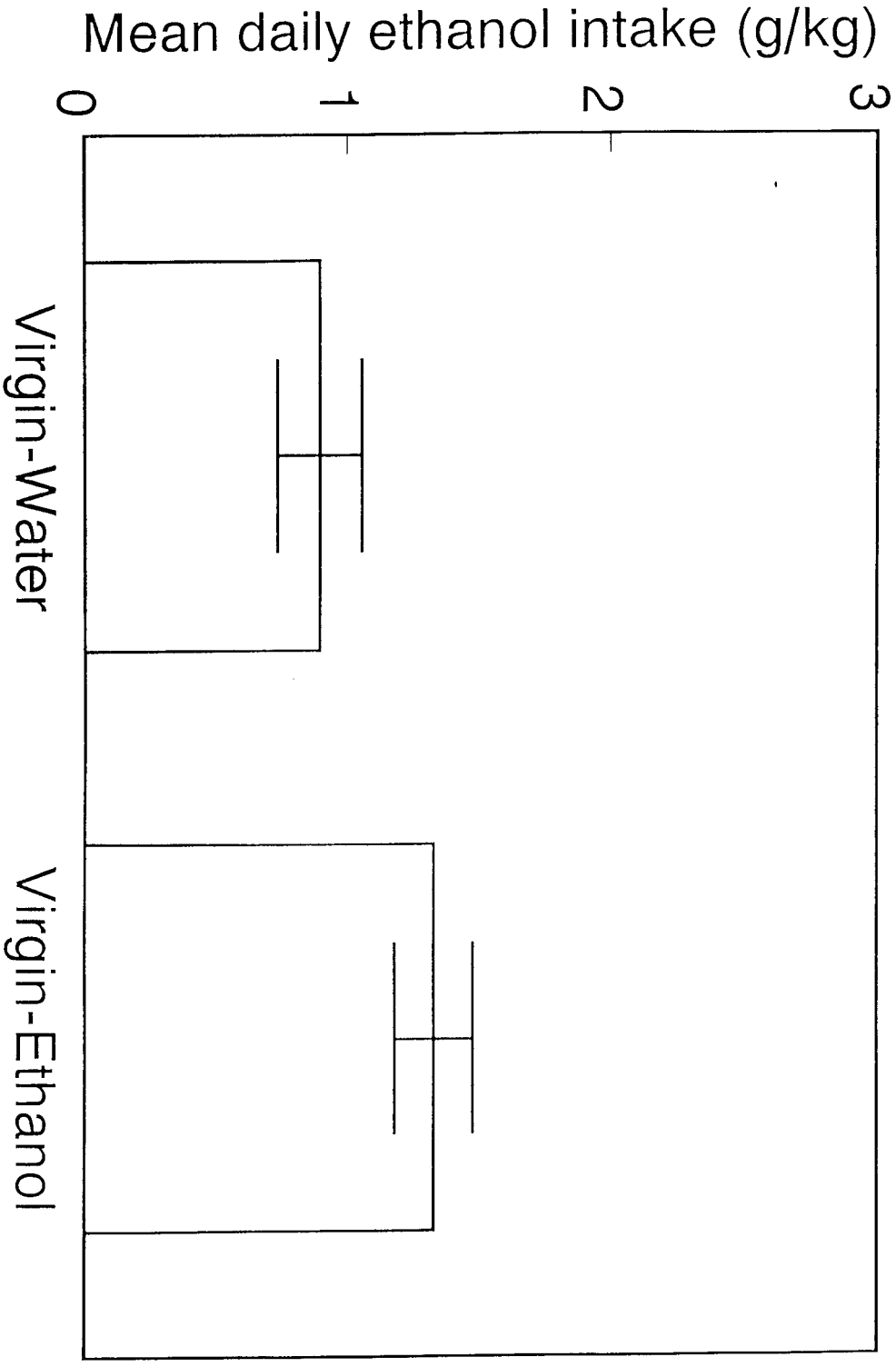
It is also possible that observing pups exhibited direct bias. Dams in Experiment 6 each consumed water throughout their pups' weaning. Pups may have attended preferentially to dams not merely because they prefer dams, but because they prefer water.

Table 7

Procedure for Experiment 6

Group	Dam Access	Virgin Female Access
Virgin-Water	Water	Water
Virgin-Ethanol	Water	Ethanol

Holding Cage
Nest Box
Food
H_2O
Holding Cage


Mean daily ethanol intake (g/kg)
0
1
2
3
Virgin-Water
Virgin-Ethanol

EXPERIMENT 7: Ethanol-Consuming Dams and Virgin Females

In Experiment 6, adolescents that had lived with an ethanol consuming adult during weaning drank more ethanol after weaning than did adolescents that had not interacted with an ethanol consuming adult, but drank less ethanol than was expected based on results of previous experiments. It appears that weanling pups are expressing some form of bias when presented with two demonstrators. Results from experiment 6 are not sufficient to determine what form of bias is inherent in pup-demonstrator interactions.

Competition for pups' attention resulted in more pup visits to each dams holding cage than to the cages of virgin females. Thus, each virgin female was a less-attended model than each dam. Pups may be more influenced by a preferred model (their dam) and less influenced by a non-preferred model (an unrelated female). This hypothesis conforms to Boyd and Richerson's (1985) model of indirect bias, wherein an attribute of a model is used as a cue for acquisition of information. Thus, behaviour by a pup's dam would be more likely to influence that pup's later behaviour than would behaviour by another conspecific, in this case a virgin adult female.

Although divided attention between two models may be sufficient to reduce the effectiveness of an adult's modeling of ethanol consumption, it is also possible that effects of modelled ethanol consumption are in competition with effects of modelled water consumption. Ethanol is less palatable than water, and so pups might experience what Boyd and Richerson (1985) describe as direct bias, wherein transmission of information about flavours that are innately more preferred (in this case water) is more potent than transmission of information about less preferred flavours (in this case ethanol).

I undertook Experiment 7 to determine whether the presence of either an ethanol- or water-consuming virgin female would alter effects of ethanol consumption by pups' own dams. If pups are influenced by direct bias, then even when dams model ethanol consumption, modelling of water consumption by a virgin female should result in ethanol consumption by adolescents that is less than 1.5 g/kg (a level consistent with intake by adolescents had been exposed to an ethanol-consuming dam in previous experiments). It might also be reasonable to expect that pups would spend more time with a water-consuming virgin than with an ethanol-consuming dam. If, however, pups are influenced by indirect bias then water-consumption modelled by a virgin female should have not reduce the impact of modelled ethanol consumption by pups' own dam, and levels of ethanol consumption by adolescents should be comparable to those seen in previous

experiments (at least 1.5 g/kg). Additionally, pups would be expected to interact with their dam more than with a virgin female, as was observed in Experiment 6.

Methods

Subjects

Forty pups, born to 20 female Long-Evans rats acquired from Charles River Breeding Farms (St. Constant, Quebec), maintained in the vivarium of the McMaster University Psychology Department, served as subjects. I used an additional 20 adult virgin females as foster-dams. Within 48 h of birth, I culled each litter to 8 pups (where possible, four pups of each sex) and randomly assigned the litter to one of two treatment conditions described in Procedure.

Apparatus

I used the same apparatus as those described for Experiment 6 and illustrated in Figure 13.

Procedure (see Table 8)

Pups in both conditions had ad libitum access to both food and water throughout the experiment and could interact with both adult females in their respective holding cages but could not reach the sipper tubes in those holding cages.

Virgin – Water Condition (n = 10 litters): From Day 18 to 26, I restricted dams' fluid intake to 8% ethanol dispensed through the sipper tube mounted on the lid of each holding cage, while virgin females were restricted to tap water.

Virgin – Ethanol Condition (n = 10 litters): I treated litters assigned to the Virgin – Ethanol Condition as I treated litters assigned to the Virgin – Water Condition, except that both dams and virgin females assigned to the Virgin – Ethanol Condition drank 8% ethanol from Day 18 to Day 26.

Videotape Review: As I had done in Experiment 6, I sampled the activity of 4 litters (two assigned to each condition) for 4 h on each of 3 days for a total of 48 hours of activity.

Testing: I tested all pups starting on Day 26 as I had tested pups in previous experiments.

Results and Discussion

The main results of Experiment 7 are presented in Figure 15. A two-sample *t*-test revealed that ethanol consumption by adolescents that had interacted with an ethanol-consuming virgin female during weaning did not differ from that of adolescents that had interacted with a water-consuming virgin female ($t(12) = 0.02$, ns). Adolescents in each condition drank at least 1.5 g/kg in an average test session, an amount comparable with the results of our previous experiments.

Modeling of either water consumption or ethanol consumption by virgin females neither weakened nor enhanced effects of modeling of ethanol consumption by pups' own dams.

As in Experiment 6, pups visited their dams more often than they visited virgin females (Mann-Whitney $\underline{U} = 0$, $\underline{p} = 0.014$). Pups visited their dams approximately 6 times/hr and visited virgin females approximately 2.6 times/hr. Pups do not avoid virgin females, but do spend a great deal more time visiting their dams.

These results suggest that a pup's own dam is a more potent model than is a virgin adult female. Although a virgin female can have a profound effect on the ethanol choice of an adolescent rat (as seen in Experiment 5), those effects are weakened if the dam is also present. Pups visit their dams more and thus may attend preferentially to cues about food and fluid choice from their dams. Although water consumption by dams may have weakened effects of ethanol consumption by virgin females in Experiment 6, water consumption by virgin females did not reduce effects of ethanol consumption by dams in Experiment 7.

The combined results of Experiments 6 and 7 suggest that weanling rats experience indirect bias about information presented by conflicting demonstrators. Such bias reflects the amount of time spent with each demonstrator in the present experiments. Although Chou and Richerson (1992) found no evidence of bias (indirect or otherwise) in Long Evans rats that were

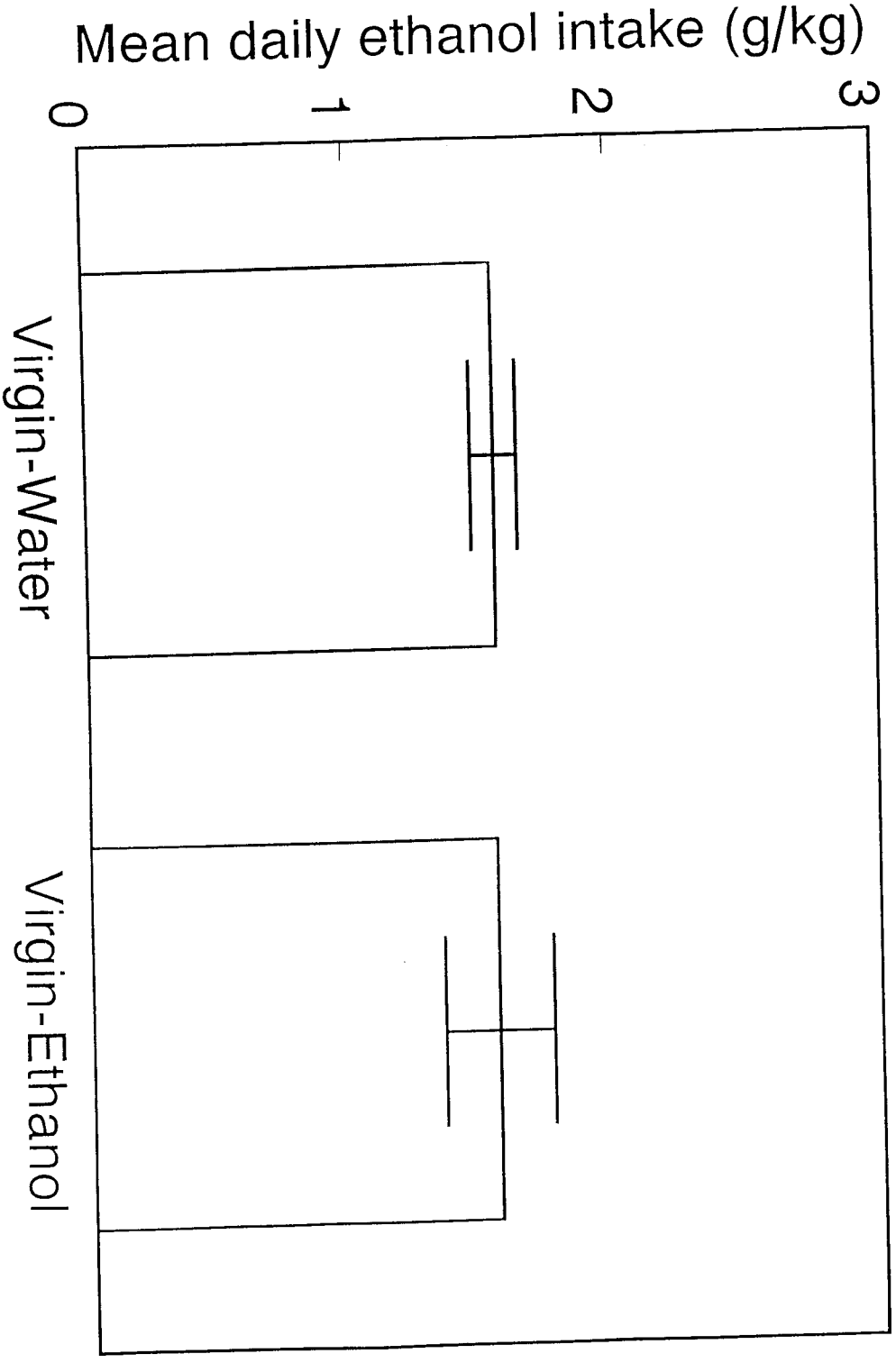
exposed to multiple, conflicting, demonstrators of information about food flavours, results of Experiments 6 and 7 indicate that Long Evans rats may in fact be biased toward attending to their dams' behaviour more than to the behaviour of an unrelated conspecific.

Any adult female is an effective demonstrator of ethanol consumption, but her efficacy is probably contingent upon the nature of her relationship with pups with whom she interacts. Pups that experience social exposure to ethanol through their dam develop an affinity for ethanol despite the presence of another, sober, adult. Conversely, pups that live with both an unrelated ethanol-consuming adult female and sober dam, and spend more time with the latter, may not be as vulnerable to effects of social exposure to the former.

Table 8

Procedure for Experiment 7

Group	Dam Access	Virgin Female Access
Virgin-Water	Ethanol	Water
Virgin-Ethanol	Ethanol	Ethanol


Mean daily ethanol intake (g/kg)
0
1
2
3
Virgin-Water
Virgin-Ethanol

Chapter 3: Conclusion

Collectively, results of the seven experiments described in this thesis provide strong evidence that social exposure to an ethanol-consuming adult female during the weaning period is sufficient to induce enhanced ethanol consumption by adolescent rats. Through systematic assessment of the relative impact of varying forms of exposure during varying portions of early development, I have determined that prenatal exposure, exposure in mother's milk and direct access to ethanol during weaning are not necessary for young rats to learn about ethanol consumption. Although prenatal exposure, nursing exposure and access to ethanol during weaning are not necessary for enhancement of ethanol affinity, I did find that those three forms of exposure interact to induce enhanced ethanol consumption by adolescent rats. Thus, there is more than one way for a young animal to learn socially about ethanol.

Prenatal Exposure

Prenatal exposure to either 4% or 8% ethanol does not, in itself, lead to enhanced ethanol affinity in adolescent rats. In Experiment 2, ad libitum consumption of either concentration of ethanol solution led to substantial blood-

ethanol concentrations in pregnant rats, but exposed offspring were not more likely than controls to consume substantial amounts of ethanol.

Exposure During Nursing

In Experiment 2, exposure to 4% ethanol in mother's milk did not enhance voluntary ethanol intake by adolescent rats. Additionally, exposure to ethanol during nursing even in addition to exposure during gestation was not sufficient to induce ethanol affinity, neither when the concentration experienced was 4%, nor 8%. Exposure to 8% ethanol in mother's milk while weaning is not necessary to enhance adolescent affinity for ethanol and does not enhance effects of social exposure to an ethanol-consuming adult female (Experiment 5). In short, exposure to moderate concentrations of ethanol in mother's milk does not appear to affect voluntary ethanol consumption by adolescent rats.

Exposure During Weaning

In Experiments 1, 3, 4, 5, 6 and 7, adolescents that had interacted with an ethanol-consuming dam for 6, 8 or 12 days during the weaning period drank more ethanol than did rats without such exposure. Effects of interacting with an ethanol-consuming adult female were robust, even when: (1) exposed pups had no direct access to ethanol (Experiment 3 – 7), (2) there was a delay between exposure and ethanol choice testing (Experiment 4), (3) pups had no access to

ethanol in mother's milk (Experiments 5 – 7), and (4) the model was either a novel lactating or virgin adult female (Experiments 5 and 6). Simply interacting with an ethanol-consuming adult model for as little as 6 days during the weaning period was sufficient to induce ethanol affinity in adolescent rats.

Multiple Sources of Exposure

In Experiment 1, adolescent rats that had experienced prolonged exposure to ethanol (throughout gestation, lactation and weaning) drank much more ethanol than did controls that interacted with dams that drank only water. Results of subsequent experiments indicated that, in the case of rats assigned to the GLW Condition, interaction with a dam that consumed ethanol during the weaning period could have been sufficient to induce such levels of ethanol consumption by exposed subjects.

Adolescents assigned to the GL Condition in Experiment 1, however, experienced a series of exposures each of which was subsequently found to be ineffective in inducing ethanol affinity. Direct access to ethanol during the weaning period did not, in itself, enhance ethanol consumption (A Condition in Experiment 1), and neither exposure to 4% ethanol nor exposure to 8% ethanol throughout gestation and the first 2 weeks of lactation led to enhanced ethanol intake in exposed rats, even though dams that consumed either ethanol solution during gestation achieved substantial blood-ethanol concentrations (GL2

Condition in Experiment 2). However, gestation and lactation exposure combined with weaning access to ethanol (GL Condition in Experiment 1) did result in substantial voluntary ethanol consumption by adolescent rats.

Integration of Present Results with the Existing Literature

In Experiment 1, I demonstrated that adolescent rats that had lived with ethanol-consuming dams drank more ethanol than did adolescent rats that had lived with water-consuming dams. This increased ethanol consumption by adolescents following prolonged exposure to an ethanol-consuming dam is consistent with results obtained by other researchers both in rats (Phillips & Stainbrook, 1976) and in humans (Streissguth et al., 1999).

I also found in Experiment 1 that asocial exposure of pups to ethanol during the weaning period was not sufficient to enhance voluntary ethanol consumption after weaning. Thus, asocial exposure to ethanol odour and opportunity to consume ethanol during early development did not increase the probability that an adolescent rat would drink copious amounts of ethanol after weaning, whereas social exposure to ethanol by an ethanol-consuming dam did increase adolescents' ethanol consumption. This result corresponds with those of Randall and Lester (1975b) who found that prolonged social exposure to ethanol preferring C57bl mice increased the strain typical ethanol preference of young DBA mice.

Although some results of my experiments may appear to conflict with results obtained by other researchers who have used rodent models to examine effects of exposure to ethanol during gestation and lactation on response to ethanol, the conflict is more apparent than real. Those who have found effects of prenatal or nursing exposure to ethanol on rodents' response to ethanol have not generally measured voluntary ethanol consumption. For example, Chotro and Molina (1990) found that a brief ethanol exposure during late gestation resulted in increased preference for ethanol odour, and increased ethanol acceptance by 8- and 9-day-old rat pups when ethanol was directly introduced into the mouth via cannula. Similarly, Hunt and colleagues (1993) demonstrated that experience with ethanol in a nursing context resulted in enhanced acceptance by 12- and 16-day-old rats of ethanol introduced directly into the oral cavity. Taken together, these reports suggest that prenatal and early postnatal experience with ethanol alters rat pups' responsiveness to ethanol.

I did not find enhanced voluntary intake of ethanol after exposure to ethanol during gestation and/or nursing. Similarly, it does not appear that interaction with intoxicated littermates is necessary to enhance voluntary ethanol consumption by adolescent rats (there would have been no opportunity for such interaction for pups assigned to several conditions in Experiments 5 through 7), although such interaction has been found sufficient to enhance passive ethanol intake by adolescents in other experiments (Hunt et al, 2000).

Possibly, pups in my experiments would have demonstrated enhanced acceptance of ethanol, or demonstrated enhanced preference for ethanol odour, but I did not examine those dependent variables. Also, I tested subjects for the first time when they were 26 days old. I do not know how subjects would have responded to ethanol at earlier ages. Conversely, it is not known whether pups in Chotro and Molina's (1990), or Hunt's (1993) experiments would have consumed ethanol voluntarily, or if the effects those researchers described would have lasted until after weaning. Such questions regarding the impact of moderate doses of ethanol on voluntary ethanol consumption, by both animals and humans, remain to be answered.

I did find that prenatal and nursing exposure followed by direct access to ethanol or an ethanol consuming dam enhanced ethanol affinity in adolescent rats. By analogy, results such as those found in the Seattle Longitudinal Study (Streissguth, et al. 1999), in which the offspring of women who drank during pregnancy were found to be at enhanced risk for adolescent alcohol abuse, may reflect both exposure to alcohol during pregnancy, and continued access to alcohol and exposure to alcohol-consuming adults in the home environment throughout childhood and early adolescence.

The interaction of early, social exposure and asocial access to ethanol during the weaning period suggests that the developmental trajectory leading to

ethanol affinity is malleable. While young organisms that experience ethanol *in utero* may be at risk for development of ethanol abuse or dependence, there may be an opportunity for intervention during early postnatal life to reduce that risk. If gestational exposure is followed by exposure during lactation and through social demonstration of ethanol consumption by caregivers, then the risk of developing an alcohol use disorder increases.

Implications for Studies of Human Risk Factors

Researchers should take into consideration results of animal models of ethanol consumption when designing human epidemiological studies. Effects of family history of alcoholism, genetic predispositions and prenatal exposure are inextricably linked with children's social exposure to alcohol-consuming adults. Genetic transmission of predisposition toward alcoholism is not the only mechanism by which alcohol affinity could be passed from one generation to another. As with other aspects of culture, young members of a society are particularly vulnerable to cues about appropriate behaviour toward alcohol. This may be especially true of cues provided by parents, if children are influenced by indirect bias (as in Boyd & Richerson, 1985) toward information provided by parents rather than other role models.

As indicated by the results of experiments described in this thesis, social learning about alcohol consumption by observing alcohol consumption by adults

may be a powerful determinant of alcohol consumption by adolescents. Awareness of factors that predict adolescent alcohol consumption is particularly important because initiation of alcohol consumption by early adolescence is already considered a risk factor for subsequent alcohol-use disorders (Fergusson, et al, 1994). Streissguth and colleagues have indicated that prenatal exposure to alcohol is a better predictor of adolescent problems with alcohol than is family history, and merits further study (1999). The present studies suggest that exposure to alcohol-consuming adults predicts future levels of alcohol consumption and should be studied as a potential precursor to later alcohol abuse or dependence.

Although the results of experiments described here suggest that adolescent ethanol intake is increased following exposure to ethanol-consuming adults, I have not determined that such enhanced affinity in adolescence leads to a condition of alcohol abuse and dependence. There are multiple developmental trajectories open to an ethanol-consuming adolescent. Possibly, adult modeling of "responsible" alcohol consumption could lead to adoption of those behaviours by observing young, and that adult modeling of "dangerous" alcohol consumption leads to more deleterious consequences for the observer.

It is also possible that individual differences among members of an exposed population modify the experience of exposure to an alcohol-consuming model. Throughout several experiments in this thesis I found that variance in

level of ethanol intake among ethanol-exposed adolescents was greater than ethanol-intake variance among non-exposed adolescents. Some adolescents drank very little ethanol even though many others of the same experience, age, sex and even parentage routinely drank to intoxication. Non-exposed adolescents rarely drank to intoxication, and were consistent in amounts consumed across experiments. Exploration of individual differences in vulnerability to social learning about ethanol consumption could provide information leading to identification of those most at risk for alcohol-use disorders, allowing for targeted intervention.

Chapter 4

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