Ph.D. Psychology

Gilles Kirouac

# Alcohol Drinking in the Rat as a Function of Constitution and Experience.

Voluntary alcohol consumption as a joint function of experience with alcohol initiated in infancy and the constitutional variables of age, sex and strain was systematically investigated in 189 rats.

In Experiment I, the effects of experience initiated at two different ages in infancy, in free or forced choice presentations were studied in male and female Hooded rats. Experiment II was a control study to show that experience with alcohol initiated after maturity did not have the same effects as initial experience in infancy.

In Experiment III, Wistar rats were used to make a comparison between strains, and in Experiment IV a selective breeding study was undertaken with Hooded rats in order to obtain two lines of subjects that might diverge in their rejection thresholds.

These experiments supported the suggestion that early experience with alcohol influences adult intake in a way that reflects complex interactions with age, sex and strain.

Ph.D. Psychologie

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# L'influence de facteurs constitutionnels et expérientiels sur la consommation d'alcool chez le Rat.

Il s'agit d'une étude systématique de l'effet combiné de facteurs expérientiels et constitutionnels sur la consommation d'alcool de 189 Rats. La recherche a surtout porté sur l'effet de l'expérience avec l'alcool commençant en bas âge sur la consommation à l'âge adulte tout en tenant compte de l'âge, du sexe et de la souche des animaux utilisés.

Une première expérience porta sur l'effet de deux techniques de présentation de l'alcool (consommation libre ou consommation forcée) et de deux âges au début de la période d'expérience en bas âge. Des Rats mâles et femelles de souche Hooded furent utilisés dans cette expérience. La seconde expérience avait pour but de montrer que l'expérience avec l'alcool commençant à l'âge adulte n'avait pas des effets semblables à ceux observés chez des sujets ayant eu leur expérience préalable en bas âge.

Pour la troisième expérience, la souche Wistar fut utilisée pour fins de comparaisons entre deux souches. Enfin, dans la quatrième expérience, un programme de sélection artificielle fut entrepris chez la souche Hooded en vue d'obtenir deux lignées d'animaux ayant des niveaux de consommation divergents.

Ces expériences sont en accord avec l'hypothèse voulant que l'expérience avec l'alcool donnée en bas âge a un effet sur la consommation à l'âge adulte qui dépend de façon complexe de l'âge, du sexe et de la souche des animaux.

# ALCOHOL DRINKING IN THE RAT
# AS A FUNCTION OF
# CONSTITUTION AND EXPERIENCE

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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L'auteur désire exprimer sa reconnaissance à son directeur de thèse, le Dr. Muriel H. Stern, pour son constant encouragement durant les diverses phases de l'expérimentation et pour sa patiente collaboration tout ou long de la rédaction de cette thèse.

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# INTRODUCTION

A variety of experiential (Veale and Myers, 1969; Cicero, Snider, Perez and Swanson, 1971) and constitutional factors, like age (Paricella and Pritham, 1964), sex (Clay, 1964) and strain (McEwen, 1965) have been shown to be involved in the determination of alcohol intake behavior of laboratory rats.

However, in behavior research it generally appears that whenever either experience (Hymovitch, 1952) or constitution (Thompson, 1954) is allowed to vary, while the other factor remains constant, subsequent behavior can be attributed wholly to the factor that is varied. There is, therefore, a need for studies in which constitutional and experiential factors are varied systematically within the same experiment, so that a better understanding can emerge of the ways in which these complex factors interact in determining observed behavior. The purpose of the present thesis is to use this interactionist approach in the measurement of voluntary alcohol intake.

The following review of the literature will selectively report on data dealing separately with various factors that are relevant to the experimentation which follows. This will serve to illustrate the need for the kind of research design suggested above. Also the usefulness of a method which takes account of individual differences, both during treatment and testing, will

be emphasized.

## Review of the Literature

The following sections will review the effects of selected experimental manipulations on voluntary alcohol intake. Because most systematic studies of such manipulations have used mice and rats, only experiments using these species will be described. The effects of exposure to alcohol on subsequent intake will be discussed first. Because the present investigation is concerned exclusively with studying the effects on intake of prior exposure to alcohol, only that experiential variable will be considered in detail. Age and sex will be dealt with first; another section will describe studies of relations between individual differences and constitutional factors underlying alcohol intake. Finally a review will be made of studies stressing the role of genetic factors in alcohol consumption.

### Effects of Exposure on Voluntary Alcohol Intake.

Since the ultimate objective of experimental studies of alcohol intake is an understanding of the mechanisms underlying human alcoholism, many experimenters have assumed that experience with alcohol would enhance alcohol consumption and ultimately result in addiction. Therefore, long term exposure to alcoholic beverages has been of particular interest. Secondly, an animal

analog of human alcohol dependence would require that the experimental animals consume significant quantities of alcohol. Since many mouse strains and most rat strains have shown a marked aversion to alcohol, a variety of exposure studies have been designed to increase alcohol consumption in these animals. The second part of this section describes some of these studies.

Long-term exposure studies. A classic study referred to in the literature of alcohol self-administration in the rat is that of Richter (1956) who reported on the effects of long-term restriction to alcohol solutions. After 3-4 months of forced drinking (20% ethanol), a few wild Norway rats drastically changed their fluid intake patterns. When a free choice between the alcohol solution and water was available, the Ss selected the alcohol in increasing amounts, while progressively decreasing their water and food intake. They finally died after losing a significant proportion of their body weight. The same treatment administered to laboratory rats did not effect a permanent change in their alcohol intake pattern.

Restricting the fluid intake of mice to a 5% alcohol solution as the only drinking fluid available from weaning till adulthood, Mirone (1952, 1957) found that the animals drank more alcohol than water-reared controls when given free-choice access to the 5% ethanol. Using a factorial design and a 10% ethanol solution Mirone (1958) later compared alcohol-fed and water-fed offspring

of alcohol-fed and water-fed parents for successive generations. In general, as adults, the alcohol-fed mice drank more alcohol than water-fed Ss when given free access to both fluids, although, in a few cases, feeding the parents with alcohol was not followed by higher intake in the alcohol-fed progeny.

Except for the higher intake of the female Ss in one study (Mirone, 1952), no sex differences were reported. The variations in strains ($CF_1$, DBA, C57, Swiss albino) from study to study, in the length of exposure, and in concentrations used preclude reliable comparisons between the studies. However, feeding weanling mice with alcohol during the growth period appeared to enhance adult alcohol intake.

Clay (1964) initiated earlier (day 19) and later (day 60) alcohol experience in male and female rats. She used Ss that had different genetic backgrounds after selective breeding for certain specific behavioral characteristics. The free choice exposure lasted until day 180 and the alcohol concentrations were increased from 2% at day 19, to 5% at day 60 to 15% at day 154. The earlier exposure condition was more effective than the later exposure one. The general exposure effect, however, was the least important variable determining alcohol intake. The constitutional characteristics of the Ss, particularly sex, were more important than exposure alone.

Cicero, Snider, Perez, and Swanson (1971) maintained 12 rats

(7 males and 5 females) of the Holtzman strain on forced access to 7% ethanol from weaning (21 days) to 154 days of age. The Ss drank large volumes of ethanol throughout the developmental period. Using a series of specific tests, the authors suggested that they established alcohol dependence in seven out of their nine surviving experimental Ss. These animals had also shown a particular drinking pattern in the last 25 days of the forced exposure period: they steadily increased their alcohol intake, whereas there were no parallel fluid intake increases in the 6 male and 6 female control Ss and the two experimental rats which did not become dependent on alcohol. The "dependent" Ss also had different patterns of intake in other tests. When given access to three alcohol solutions (3%, 7% and 14%) as their only drinking fluids, the dependent Ss drank more alcohol and distributed their intake over the three concentrations whereas the other Ss drank mostly from the weakest solution. Similar differences emerged when the rats were offered free choice between water and a series of daily increasing concentrations of ethanol (3% to 30%). Finally the alcohol dependent rats were not affected by the presence of saccharin as a third choice while other animals drank virtually no alcohol in the presence of saccharin. No differences in the data, attributable to sex of the subjects, were reported.

In summary, it appears that long-term exposure can affect

subsequent alcohol drinking. But the results differ from study to study; and in most successful experiments, not all experimental Ss change their alcohol intake significantly. Methodological differences as well as constitutional factors appear to account for many of these discrepancies.

Methodological studies of alcohol exposure. Many methods of alcohol exposure have been used to modify voluntary alcohol intake in laboratory animals. This section will report some of these studies.

Using four groups of four male Hooded rats, Myers and Carey (1961) first restricted the Ss to either a 5% or a 20% ethanol solution for a period of either 30 days or 120 days. The animals were then tested with free choice access to water and alcohol solutions that were varied daily in concentration between 3% and 15%. An ascending series was offered to half the animals in each prior exposure condition and a descending series was used for the other half. The results showed that the concentration used for prior exposure was of no significance, but that the longest prior exposure condition (120 days) was more effective than the shorter one (30 days) in enhancing alcohol intake. Also, testing with an increasingly concentrated series of solutions produced a shift from alcohol to water preference at a higher concentration than testing with a decreasing series. A long period of exposure seems necessary to modify voluntary alcohol intake and mild solutions

seem to facilitate the further choice of stronger solutions. The possibility exists that had they used concentrations between the extremes used for the prior restriction period the experimenters might have produced different effects.

Withdrawing Hooded rats from alcohol for a week after six months of free choice access to a 6% ethanol solution resulted in a significantly higher intake of a 20% solution than in Ss shifted to the 20% solution without the intervening withdrawal (Senter and Richman, 1969). But this differential effect was not found if 6% ethanol was used in the post-withdrawal period. So long-term alcohol exposure can be effective under appropriate conditions and it is clear that the solution used for testing is important.

Rick and Wilson (1966) studied the alcohol intake of groups of Wistar rats over a six month period. Each group was maintained in a forced-exposure situation with access to 2%, 4%, 8% or 16% concentrations respectively. From time to time, each group underwent a free choice for a one-day period. A significant gradual increase in total fluid consumption occurred over the whole exposure period, the effect being greatest for the 8% group. With the exception of the 16% group, which consumed less alcohol, the animals consumed approximately the same quantities of absolute alcohol when tested in a free choice situation. However, when the free choice followed a 24-hour fluid deprivation period, the animals selected more alcohol at all concentrations than when they

were in the non-deprived condition. So forced choice effectiveness was related to the concentration used; also free choice testing depended on the concentration and the method used. Unfortunately the particular experimental design used made it difficult to partial out the effects of many long prior exposure periods.

Powell, Kamano, and Martin (1966) compared the intake of 10% ethanol of shocked and unshocked male and female Wistar rats. Various exposure conditions were used over a 52 day period: two periods of free choice lasting 14 days and 8 days respectively; an intervening 8 day period of forced access on alternate days, and two more free choice periods of 8 days and 14 days respectively. Shock was applied to the experimental group during the three 8 day sessions. Because the present review deals mainly with the effects of exposure to alcohol solutions, the control group (non-shocked) is particularly relevant here. Despite an absence of initial sex differences in alcohol intake, male Ss increased their alcohol consumption to a greater extent than female Ss during the five experimental sessions. Interestingly enough, the Ss could be classified as high and low drinkers depending on their initial alcohol intake in the first free choice period. In the following experimental periods, they showed differential drinking patterns: the high drinkers showed a linear increase throughout the experiment whereas the low drinkers increased intake only after

the forced intake session. So effectiveness of exposure seemed dependent on some individual predispositions. And, although there was a general exposure effect, males responded to a greater extent than females.

McEwen (1965) studied the drinking patterns of rats given free access to alcohol and water for 25-day periods. A first group underwent constant exposure to 10% alcohol and another group was given intervening experience with 5% and 15% solutions between two tests with 10% ethanol. The author used male and female Ss from three strains of rats in each condition (Wistar, Sprague-Dawley and Hooded). Exposure produced overall increases in alcohol intake, but the patterns were different in the two situations and also different depending on the strain and the sex of the Ss. The exposure conditions could not be evaluated without accounting systematically for constitutional variation (e.g. the Wistar Ss responded to a greater extent to the second condition and the Hooded Ss, to the first).

Using ethanol and whisky ranging in concentration from 5% to 25%, Mendelson and Mello (1964) compared the drinking patterns of male Hooded rats as affected by forced and free choice presentation. They found that forced choice seemed to influence later free choice intake. The animals in the 20% ethanol condition even preferred alcohol over water. These effects cannot be attributed completely to forced exposure since the animals could also have

been affected by the long free choice periods they underwent. Also the utilization of whisky during the forced choice sessions might have produced some effects.

Veale and Myers (1969) restricted male Sprague-Dawley rats, housed in small group cages, to 12% alcohol for 10 days; these animals drank small amounts of alcohol at all concentrations when offered forced choice between water and a series of systematically increasing ethanol concentrations (3% to 30%). But Ss without the forced pre-exposure had significantly higher intakes than pre-experienced Ss when tested with the same series. They also preferred alcohol solutions up to 12% concentration. Using individually caged Hooded male Ss the authors found that a different intervening experience between two series of increasing alcohol concentrations produced different effects. If the identical series was repeated, very significant increases in alcohol intake were observed whereas water drinking control Ss increased alcohol intake to a lesser extent. An intervening forced exposure to 15% ethanol, however, precluded the appearance of increases in alcohol intake. Those two experiments indicate that forced exposure to alcohol can decrease alcohol intake in subsequent testing.

Finally, in individually caged male Hooded rats, Veale and Myers (1969) produced an "acclimation" to alcohol by repeating free choice testing with a daily gradually increasing series of concentrations from 3% to 30% (with between session intervals

ranging from one day to five months). The concentration at which the alcohol solution constituted half of the fluid intake increased from 3% to 30% over the series of presentations. The rats selected more alcohol from all the concentrations from series to series despite the fact that total fluid intake remained constant. In view of the long intervals between some of the sessions, the effect appeared to be permanent. So free choice of many concentrations appears to be a potent way of inducing significant modifications in alcohol intake. An important feature of the testing procedure consisted in the free access to a gradually increasing series of ethanol concentrations. This ascending property was certainly relevant in view of the results already reported by Myers and Carey (1961), where an ascending presentation of alcohol solutions was more effective than a descending one.

Conclusion. In general, the studies reviewed above provide data suggesting that prior exposure to alcohol solutions modifies subsequent alcohol intake. However, the parameters responsible for the trends in the data are not always clear because the exposure conditions vary from study to study and sometimes involve the utilization of many different methods within the same experiment. Also, the non-systematic variation in the constitutional backgrounds of the animals used (strain, age, sex) makes the results attributable to a variety of possible factors. It is accordingly difficult in many cases to partial out the sources of variance.

Individual differences appear to be related to constitutional predispositions of the animals. Powell et al. (1966) found that Ss with high or low initial responses to 10% alcohol solutions showed differential susceptibility to later exposure effects. Cicero et al. (1971) had deviant animals in their alcohol dependence study which indicates that exposure effects are difficult to separate from individual constitutional (and genetic) variables. The next sections will be concerned with studies addressed to this problem.

Constitutional Variables and Alcohol Intake.

The preceding section on exposure effects revealed that constitutional factors had an effect on the patterns of results. The following sections will review relevant literature that deals systematically with constitutional factors affecting alcohol intake. After firstly considering the separate contributions of sex and age of subjects to the variance in alcohol intake, individual differences will be considered as they relate to some predispositions to consume alcohol. Finally experiments devoted to the genetic analysis of voluntary alcohol intake will be reviewed.

Age and alcohol intake. The study of Clay (1964) and that of Cicero, Snider, Perez and Swanson (1971) showed that the age at which exposure to alcohol was initiated could be relevant for later intake. In a study designed to demonstrate the effect of age on alcohol intake, Kakihana and McClearn (1963) showed that

younger BALB male and female mice (between three and nine weeks) drank more 10% alcohol than adults. Animals older than nine weeks showed low intake. Exposure during the high acceptance period did not enhance intake in adulthood.

Parisella and Pritham (1964) compared groups of male Wistar rats of different ages (1 to 2 months, 3 to 4, 10 to 15, 20 to 24 months). Half the animals in each group were given forced exposure to alcohol solutions increased weekly by 2% from a 2% solution to an 8% solution over a four week period. Then all the animals were offered a three-bottle free choice between 8% alcohol, 5% sugar and water. In general, prior exposure to alcohol enhanced intake of the 8% alcohol concentration, although the animals generally preferred sugar to alcohol. The post-pubertal Ss (3 months to 4 months), however, preferred the alcohol over water and sugar. This preference occurred in Ss with or without prior forced exposure to alcohol. This indicates that age was a more important factor of variance in determining alcohol intake than prior exposure to alcohol. Since the phenomenon was observed in animals around the age of puberty, it suggests a possible hormonal effect. Unfortunately, no female Ss were used in the experiment.

Using a free choice situation, Goodrick (1967) tested male Sprague-Dawley rats of various ages at the initiation of testing (1 month, 3, 5, 10, 15 and 24 months). The four-week experimental

session consisted in the presentation of four successive sequences of alcohol solutions increasing during each week from 2% to 8% in 2% steps followed by three days of water intake. Alcohol intake increased with increasing age (between the groups) up to 5 months and decreased at 10 and 15 months. Two-year old Ss drank more alcohol than the 15-month old ones. This result was interpreted as due to sensory deficits in the older animals. The possibility exists, however, that the result is a function of a strain difference. Parisella and Pritham (1964) using Wistar rats did not observe such a phenomenon. Despite differences in methodology, the two experiments described above both observed the highest intake in the animals in the post-pubertal period.

Wallgren and Forsander (1963), however, reported that 18-month old Ss drank more alcohol than 3-month old ones in a free choice between water and 10% alcohol. This finding is inconsistent with that of the previously mentioned studies. Unfortunately, the authors do not mention the strain of rats they used. Also they found an age by exposure interaction when they compared younger and older Ss that were exposed to alcohol for long periods. Indeed, long term exposure to alcohol enhanced later ethanol selection to a great extent in older than in younger subjects.

In summary, age of exposure of the subjects is an important factor in the variance in patterns of alcohol intake. However, differences in strains from study to study make any conclusions

tentative. Also, the existence of age by experience interactions (e.g. Wallgren and Forsander, 1963) implicates the importance of the age at which exposure is initiated in studies of the effects of initial exposure on subsequent intake. Finally, the particular responses of Ss at about puberty makes hormonal effects plausible and the absence of female Ss in these experiments deplorable. Accordingly, it indicates the necessity of looking at sex as a possibly relevant source of variance in voluntary alcohol intake.

Sex differences. Although sex is commonly cited as a constitutional factor affecting alcohol intake, all the studies using male and female Ss do not report sex differences. In inbred strains of mice some investigators have found that sex accounts for less than 3% of the variance in alcohol intake (e.g. McClearn and Rodgers, 1959). In rats, because of no apparent sex differences, data are frequently pooled together (e.g. Kahn and Stellar, 1960; Wallgren and Forsander, 1963). But some clear-cut sex differences have been reported in other studies and Wallgren (1959) found a greater ethanol tolerance in female than in male rats.

Eriksson and Pikkarainen (1968) compared males and females of the C57BL and CBA mouse strains in free choice behavior between water and 10% ethanol. C57BL females had greater preference ratios and drank greater amounts of absolute alcohol than males per unit of body weight; the difference was more clear cut using

the latter measure. Also a significant difference between the sexes existed in ADH (alcohol-dehydrogenase) activity; it was in the same direction as the other differences, but smaller. On the other hand, no significant differences between males and females appeared in the data for the CBA mice. In Wistar rats, Eriksson and Malmström (1967) also found that females had higher intake than males in a free choice test between water and 10% ethanol. Once again, the statistical difference was greater if the amount of absolute alcohol per unit of body weight was used in calculation instead of the preference ratio ($p < .001$ vs $p < .02$). The experimenters also found that alcohol was eliminated at a quicker rate in female Ss than in male Ss. Moreover, in a selection study also using Wistar animals, Eriksson (1968) demonstrated a greater heritability of voluntary alcohol intake proneness in female Ss than in male Ss and, in both lines, females had higher free choice intake of the 10% solution.

However, not all studies report higher alcohol intakes for female than for male Ss. Using a heterogeneous stock of rats, Clay (1964) found that male Ss generally had higher alcohol intake than female Ss. Within her complex experimental design, sex emerged as the most important factor contributing to variance. However, the excess of intakes in one sex over another can depend on the strain used (reported above by Eriksson and Pikkarainen, 1968). For instance, Russell (1971) compared the alcohol re-

jection thresholds of males and females of the $S_1$ and $S_3$ lines of the Tryon bright and dull rats: females of the bright line had higher rejection levels than the males, whereas the males of the dull line had higher levels than the females. As cited above, McEwen (1965) found that her exposure effects were related to certain constitutional factors of her Ss: with three strains of rats (Wistar, Sprague-Dawley, Hooded), males responded to a greater extent to constant exposure to 10% alcohol than did females, but the effect was significant in the Hooded males only. On the other hand, intervening experience with other concentrations produced higher intake levels in males than in females, but especially in those of the Wistar strain. Interestingly enough, no consistent sex differences appeared when the same 3 strains were used for selective breeding for low and high preference levels.

Hormonal influences were suggested above to explain changes in voluntary alcohol intake with age. Besides these within-subject differences a comparable role of hormones can be assumed to account for differences in alcohol intake between subjects of different sex.

Female hormones were found to reduce alcohol intake. In gonadectomized male and female rats, Aschkenasy-Lelu (1960) reported that injections of oestradiol benzoate decreased alcohol intake in a free choice situation between water and 5%

ethanol. Also, in intact female rats in oestreous(ascertained by daily vaginal smears) a decrease in alcohol intake was usually observed.

Clearly, sex dependent processes can produce differences in voluntary alcohol intake. However, the size and direction of such differences vary from study to study. A primary source of variation appears to be the strain of the animals, since some strains show significant sex differences while under similar conditions others do not. The experimental procedure can also be an important source of variation. In one study, selection was followed by the disappearance of prior sex differences in alcohol intake (McEwen, 1965). Moreover testing procedures vary from study to study; some experiments use long-term exposure and others a much shorter testing period. Finally, age may be a factor since Eriksson and Pikkarainen (1968) found a sex difference with four-month old C57BL mice and McClearn and Rodgers (1959) did not report such a difference with Ss of the same strain, but much older (9½ - 12 months).

Individual differences and constitutional background. In animals under constant alcohol exposure conditions, large individual differences are commonly found. Richter (1956) reported that, in rats, the variations between animals in alcohol intake were much greater than for other substances like salt or sugar. Senter, Eimer, and Rickman (1968) found that 63% of the total variance

in alcohol intake of Hooded rats exposed to free choice between 6% alcohol and water for 26 weeks, was due to individual differences. Even in highly inbred strains of laboratory animals there were widespread individual differences in alcohol intake (Rodgers and McClearn, 1962; Reed, 1961). The present section will review experiments in which factors involved in individual variability in alcohol intake were studied.

Gustatory and olfactory variables. The sensory properties of alcohol solutions (i.e. their olfactory and gustatory qualities) can be related to the tendency of animals to find them more or less aversive. For example, Amit and Stern (1969) found that Hooded rats ingested much more alcohol if the oropharyngeal sensations generally attributable to the alcohol were eliminated by direct infusion. In general, alcohol intake can be enhanced by sweetening the solutions (Rodgers and McClearn, 1962). Olfactory cues were found to be involved in the alcohol preference of rats for low concentrations and in their aversion for high ones (Kahn and Stellar, 1960). By contrast, anosmic rats did not prefer low concentrations as intact rats did and they ingested ethanol at much higher concentrations. Similar increases in alcohol rejection levels were found in anosmic mice by McClearn and Rodgers (1962) and in rats by Richter (1956). Therefore, variation in alcohol intake might be correlated with differences in taste and smell.

After bulbectomy, BALB mice (alcohol rejecting strain) drank significantly greater amounts of alcohol, even if they had rejected it before the intervention (Nachman, Larue and Le Magnen, 1971). With the same treatment, C57BL mice (high preferring strain) showed an immediate high preference whereas intact Ss usually took 3-4 days before reaching their high levels. Olfaction then has an important function in the particular drinking pattern usually shown by these different strains.

LeMagnen and Marfaing-Jallat (1961) designated rats as "drinkers" and "non-drinkers" on the basis of individual free selection of 6% ethanol. They found that the high drinking Ss rejected quinine solutions at a concentration 2½ times stronger than the one that was aversive to the "non-drinkers". Similar results were found when a series of concentrations, increasing from 1% to 31.6% was used instead of the 6% solution. So the differences in alcohol consumption seemed to be related to a different sensitivity to bitter substances in general. But drinker and non-drinker rats also differed in their intake of alcohol concentrations below the gustatory threshold (Marfaing-Jallat and LeMagnen, 1964). Since rats have a very high olfactory acuity, it appears that the non-drinkers showed a stronger aversion to solutions when the discrimination was based not on taste but on olfaction. In conclusion, gustatory and olfactory cues appear to be highly relevant to individual variations in

in alcohol intake.

Role of behavioral predispostions. Among the constitutional factors underlying individual differences in alcohol intake, some behavioral tendencies have been found to be particularly relevant. Tobach (1957) correlated individual differences in certain behavioral tests with alcohol intake in Sprague-Dawley rats. Alcohol drinking was more related to timidity (timid Ss drinking less) than to automonic reactivity. Since concentrations around 3% are often preferred by a variety of rats, an ethanol concentration higher than the 3.4% one used in the experiment might have yielded clearer results. Clay (1964) found that the factor that contributed the most important source of variation in her study was sex of the subjects. Other constitutional factors as well, were important contributors. Indeed, Ss bred for a greater flexibility drank more than fixation prone animals. But audiogenic seizure proneness had no correlation with alcohol drinking. Duveau, Dahan and Cosnier (1966) came to a similar conclusion for audiogenic seizure susceptibility in mice.

Brewster (1969) compared the voluntary alcohol intake of Wistar rats bred for reactivity and non-reactivity in the open-field test (Mausdley rats, MR and MNR). Six-month old rats were offered free choice between water and ethanol increasing in concentration daily from .001% to 10%. The reactive line had higher preference ratios (particularly at 5% and 10%), but there was

no difference in intake of absolute alcohol per unit of body weight (except at 5% and 10%). Four-month old Ss were compared using only 5% ethanol. Surprisingly, the non-reactive line had greater intake using both measures.

This second set of results is particularly interesting. Indeed, high activity and low emotional reactivity in the open field were correlated with greater alcohol intake, as was lesser timidity in Tobach's results. This is consistent with the observations of Thompson (1953) that C57BL mice (high drinking strain) show open-field behavior similar to that of the Mausdley non-reactive rats. But the low drinking BALB mice resemble the Mausdley reactive line in their open-field responses. The consistent correlation between the tendency to explore and the tendency to ingest greater amounts of alcohol strongly suggests a common constitutional factor.

The Roman strains, which were selected on the basis of high and low avoidance performance, were also tested by Brewster. In a free choice test with daily increasing concentrations (.01% to 10%), the lines differed at 10%; the high avoidance line had greater intake than the low and the control strains which did not differ among themselves. Finally, females had higher intake than males in all Brewster's experiments.

Conclusion. When groups of Ss differ in their voluntary alcohol intake, a multiplicity of factors appears to contribute to

the variance in the data. Sex and development have already been shown to be important factors in the patterns of intake. Experiments summarized above indicated that gustatory and olfactory variables should not be ignored in accounting for at least a part of the differences. Moreover, behavioral tendencies also affect alcohol intake. Since all these factors appear to be under the control of some underlying genetic mechanisms, heredity must be important in alcohol intake. The next section reports on genetic studies of alcohol intake in animals.

Genetics and Voluntary Alcohol Intake.

The demonstration of constitutional differences in voluntary alcohol intake suggests that the behavior may have a genetic basis. In the literature on voluntary alcohol intake, many experimental reports are concerned with genetic variables. In the following review, there will be a section for the two classical methods used in behavior-genetic analysis, strain comparisons and selective breeding.

Strain comparisons. Many studies have compared alcohol intake in animals that are genetically different, but not selected for any specific traits.

Reed (1951) compared the alcohol consumption of six strains of rats with different degrees of inbreeding (from 9 to 101 generations of brother x sister mating). The free choice in-

take of 10% ethanol (ml/unit of body weight) varied significantly from strain to strain over the 30-day testing period. Wide individual differences were found within each strain and, curiously, the most inbred strains(i.e. those assumed to be the most homogeneous genetically) showed more individual variation than the less inbred ones. This was interpreted by Reed as an indication that the behavior was under polygenic control. Myers (1962) compared groups of male rats of two strains with different degrees of inbreeding: a Wistar stock (9 generations) and the G-4 strain (20 generations). The various groups were given free access to water and one of five alcohol concentrations (1.5% to 20%). Two different room temperatures were also used. In all the experimental conditions, the G-4 Ss showed greater alcohol intake than the Wistar Ss. The author suggests that the fact that the G-4 rats look more emotional than the Wistar Ss could account for the strain difference in alcohol intake. However, as seen above (Tobach, 1957; Brewster, 1969) differences in emotionality and in alcohol intake are not necessarily directly related. Also the greater alcohol selection of the G-4 strain is not attributable to an olfactory deficit since tests showed that the animals had good olfactory acuity. This does not exclude the possibility that olfactory differences between the strains are responsible for at least a part of the difference.

Van Steenkiste (1964) compared Wistar and London Black rats

in their free choice intake of water and either 10% ethanol or white wine. The Wistar Ss drank more ethanol than the Black strain, but the strains did not differ in their white wine intake. The nature of the solution therefore seems important in the manifestation of strain differences. With a Wistar domestic stock, Richter (1956) could not produce the apparent addiction he found in wild Norway rats after restricting their fluid intake to alcohol. Moreover, Eimer and Senter (1968) could not replicate Richter's original finding with domestic (Hooded) rats nor with wild rats. However, as pointed out by Boice and Aspey (1968), they used a different genus of wild rats. This could account for the difference in result (Senter and Eimer, 1968).

Richter (1956) mentioned two important factors that could explain the failure of the domestic rats in his experiments to consume significant amounts of alcohol: the greater fluid requirements of the wild rats which made them consume greater amounts of ethanol during forced exposure; and the constant stress they experienced in captivity. Disagreements (Boice and Aspey, 1968, Senter and Eimer, 1968) about physiological differences between wild and domestic rats make difficulty for an evaluation of Richter's explanation in terms of fluid requirements. Also the failure by Eimer and Senter (1968) to replicate Richter's original data raises questions about the explanation of the re-

sults in terms of a stress response. The wild rats used by the latter authors also experienced severe stress. However, McEwen (1965) showed some genotype by experience interactions in studies on the effects of stress on alcohol intake.

McEwen (1965) compared Hooded, Wistar and Sprague-Dawley strains in two free choice situations. In general, exposure to alcohol enhanced further intake. But the Hooded strain responded to a greater extent to a constant exposure situation (10% solution) whereas the Wistar Ss were more affected by intervening experience with other solutions (5% and 15%).

In mice, the main body of research was established by McClearn, Rodgers and their associates. They found that, in a two-week session with free choice access to 10% ethanol, strains of mice differed in their alcohol intake (McClearn and Rodgers, 1959). The C57BL strain showed high alcohol preference levels whereas A/2, DBA, and BALB mice showed a general aversion to ethanol. The C3H strain, while generally a low preference strain, showed more variation in intake than the three other low drinking strains. Subsequent experiments indicated that AKR and I/S mice were low drinkers whereas the RIII strain had intermediate preference. (McClearn and Rodgers, 1959; McClearn, 1968). Genetic mechanisms involved in some of these strain differences were studied in experiments reported in several review papers (Rodgers and McClearn, 1962; Rodgers, 1966; McClearn, 1968).

In brief, crosses between strains resulted in intermediate inheritance when one of the parents was a C57BL mouse. Crosses between 2 low-preferring strains yielded low preference; so heterozigozity by itself did not produce intermediate preference. Double crosses showed that the preference level of the offspring depended on the presence or the absence of C57BL genes. Reciprocal crosses and cross fostering studies excluded any significant intervention of maternal effects in the strain differences. Analysis of $F_1$, $F_2$, and backcross generations did not permit any precise estimation of the various genetic and environmental components of variance, but it indicated that a multiple gene system best accounted for the data. The extensive individual differences in the various experiments and the existence of deviant animals also supported that interpretation.

Instead of the standard 10% solution, McClearn and Rodgers offered various strains a choice among water and six ethanol concentrations simultaneously (2.5% to 15%). The peak alcohol intake was at 12.5% for the C57BL Ss. BALB, A/2 and A/3 animals did not drink any significant amount at any concentration. However, the C3H strain (originally a more variable one) drank the weaker solutions and gradually shifted to higher ones so that, by the end of the study, alcohol was selected mostly from the 10% ethanol instead of the 5% solution. That interesting finding was interpreted by the investigators as a confirmation of the

polygenic nature of the control of alcohol selection. In order to compare A/J, C3H, C57BL, DBA mice and their six hybrids, Fuller (1964) restricted them to six alcohol solutions. The concentrations, increasing by a factor of 2 from .5% to 16%, were offered simultaneously. The author computed as a preference measure the ethanol concentration below which the animal obtained 50% of its fluid intake. The DBA and C57BL strains were the most extreme phenotypes and appeared dominant over the more intermediate C3H and A/J, when the data of the various crosses were compared. When crossed, however, the DBA and C57BL strains gave offspring with intake levels intermediate between the parent strains. The intake measure used by Fuller permitted a genetic analysis of the data yielded by hybrids which confirmed the more potent effect of C57BL and DBA strains in increasing or reducing alcohol selection. The fact that the 6 crosses yielded different modes of inheritance suggested the important proposition that the physiological basis for voluntary alcohol intake can vary from strain to strain.

Thomas (1969) did a systematic comparison of the C57BL and DBA strains, giving free choice between water and one of 13 ethanol concentrations ranging from 30% to $10^{-10}$% offered in a decreasing or increasing series. In both procedures, the C57BL mice had much higher intake, although, in the descending series, pre-exposure to 30% ethanol produced a decrease in the usual high

alcohol intake at 10%. A further comparison of the 2 strains was performed using $F_1$, $F_2$ crosses and backcrosses of $F_1$ to both strains; free choice between water and 10 concentrations ranging from .00001% to 10% was given to the various groups. The greater alcohol intake of the C57BL over the DBA strain which was observed with 10% ethanol was maintained when concentrations were lower: DBA rejected ethanol solutions as dilute as .05% and C57BL appeared indifferent to concentrations of 2% and below. Analysis of the hybrid data did not permit a precise evaluation of the genetic variance. However the data of the $F_1$ Ss revealed incomplete dominance of the DBA over the C57BL strain (not found by Fuller with a different testing method). The magnitude of the preference ratios was in direct relation to the proportion of C57BL genes for a given cross. According to Thomas, the order of preference levels of the various hybrid groups can be explained either by a single gene model or a polygenic model. The suggestion of a single explanation might be based on the fact that Thomas utilized the DBA strain which Fuller (1964) found to be at the extreme of low-preference strains.

The possible involvement of few genes in the control of alcohol intake was also supported by Henry and Schlesinger (1967). They compared normal C57BL mice with single-gene mutants at the albino locus, and normal DBA mice and mutants at the dilute locus. The non-pigmented C57BL mutants drank significantly less 10%

ethanol than the pigmented ones whereas the low preference levels of the mutant and non-mutant DBA Ss were not different. However the C57BL mutants still drank significantly more alcohol than the DBA mice. While one gene mutation can affect the between-strain difference in alcohol intake, there are still unexplained intake differences. Fuller and Collins (1967) found that a two-unit model could fit the intake data expressed in mean preference scores or in absolute alcohol when they compared C57BL and DBA strains as well as their various reciprocal hybrids. The testing consisted in an ethanol exposure technique similar to the one used by Fuller (1964).

These last few papers support a limited unit model for explaining the inheritance of voluntary alcohol intake. However they were based on two inbred strains, C57BL and DBA. A two-unit model is attractive since it is consistent with recent findings in which two enzymes appear related to alcohol consumption (Lindzey, Loehlin, Manosevitz, and Thiessen, 1971), although as de Fries (1967) pointed out, such correlations can be fortuitious and may disappear after appropriate cross-breeding. It is interesting to note that the biochemical studies have usually utilized comparisons between DBA and C57BL mice (McClearn, Bennett, Hebert, Kakihana and Schlesinger, 1964; Sheppard, Albersheim, and McClearn, 1968), which strains represent the extremes of Fuller's comparative study (1964). Moreover

there is only one mouse strain (C57BL) in which high preference for alcohol is consistently reported, whereas many strains exhibiting low preference or aversion have been found. Accordingly it looks premature to extend the limited-unit model to other strains. Indeed Fuller (1964) suggested that different physiological mechanisms may control voluntary alcohol intake in various mouse strains. Moreover, other studies, in which many strains were compared, generally favored a polygenic interpretation.

Interestingly enough, despite the great differences in alcohol intake between the same DBA and C57BL strains, Schlesinger, Bennett and Hébert, (1967) in their assays of alcohol metabolism differences between these two strains, found considerable overlap between them. But when voluntary alcohol intake was measured, the two strains differed to a greater extent without any overlapping. The authors concluded that alcohol intake could not be controlled by the few genes associated with alcohol metabolism and they proposed a polygenic interpretation.

Even in rats an interpretation based on the differences in alcohol intake limited to the metabolic evidence does not seem to fit the phenotypic data. Segovia-Riquelme, Vitale, Hegsted and Mardones (1956) found no difference in alcohol metabolism between Ss classified as drinkers and non-drinkers on the basis of their absolute alcohol intake in a free choice situation be-

tween water and 10% ethanol.

In summary, differences in voluntary alcohol intake between strains of rats and mice may be related to genetic mechanisms. In some mouse studies, 97% of the variance in alcohol intake has been attributed to genetic factors. However, the mode of inheritance of voluntary alcohol intake in inbred strains of mice still remains controversial, although a polygenic explanation appears plausible. In rats, hypotheses about genetic mechanisms are more difficult to derive. But in view of the numerous factors that appear to underlie alcohol intake, a multi-factor model looks more promising.

Selective Breeding.

A response to selection clearly indicates that a behavior has a genetic basis (De Fries, 1967). In the area of drinking behavior, rats have been efficiently selected for adipsia and polydipsia (Roubicek and Ray, 1969), for saccharin preference (Nachman, 1959), and for morphine addiction (Nichols and Hsiao, 1967). Nichols and Hsiao reported that the lines also differed in alcohol intake in the same direction as that of response to morphine. Since selection has been effective in other aspects of drinking behavior, it is reasonable to expect a similar success in voluntary alcohol intake. Studies have shown that selective breeding has also been effective in that area.

Mardones (1960) found that with a diet deficient in the factor $N_1$ of the vitamin B complex, rats would increase their alcohol consumption; but there was great individual variability. When extreme animals were selectively inbred, there was a significant parent/offspring correlation in alcohol intake for the 3rd to the 7th generations of the study. Rodgers and McClearn (1962) undertook a selection experiment with high and low extremes of a heterogeneous base group resulting from the crosses of C57BL, BALB, DBA and C3H mouse strains. In the $F_1$ generation, there was a significant difference between the high drinking and the low drinking lines. The high line Ss covered a whole range of low, intermediate and high preferers of 10% ethanol, whereas the low-preference line essentially consisted in low preference Ss. A more direct response in the low line is a common finding in selection research (Falconer, 1960). Also the complexity of the results (particularly in the high line) suggested to the authors that several genes were involved in the control of the behavior. The selection study was terminated at that point because of a lack of fertility (McClearn, 1968). McEwen (1965) got straightforward results after one generation of selection in two experiments where interesting strain differences emerged. She first bred for high and low baseline ethanol preference (10%). The Wistar and Sprague-Dawley strains yielded significant differences which were greater in the Wistar strain. In the Hooded

rats, the difference was in the predicted direction, but it failed to reach significance. Also all the $F_1$ data did not reveal the sex differences which had been observed in the parent groups. Finally, breeding for increased and decreased ethanol consumption associated with environmental stress resulted in a significant separation of the $F_1$ groups only in the Hooded strain.

Using a 10% solution, Eriksson (1968) initiated a selection program with Wistar rats. His purpose was to develop two lines drinking as much and as little absolute alcohol per unit of body weight as possible. His breeding system, where inbreeding and outbreeding were counterbalanced, favored good selection without reducing fertility and variability. By the 8th generation, marked differences were obtained between the high and low lines. Moreover, the sexes responded differentially to the selection. There was a general tendency for female Ss to drink more alcohol per unit of body weight than male Ss. So the females had the highest intake levels in the high line and the males, the lowest levels in the low line. But the sex difference was greater in the high line. Also the lines differed markedly in body weight, the low animals being significantly heavier: Eriksson attributed that to a difference in metabolism resulting from the selection procedure.

A constant solution technique, where absolute alcohol per

unit of body weight was measured, should be related to metabolic variables. Despite the fact that the significant difference between the two lines explained 65% of the variance, there remained wide individual variations and the coefficient of heritability was low. This supports the idea that metabolism does not account for the whole phenotype variance in alcohol intake. Accordingly, selection focusing on metabolic differences might not partial out all the variance.

Conclusion. Selective breeding of extreme phenotypes in rats and mice can produce divergent groups of Ss with high and low alcohol intake. This is a most clear demonstration that the behavior has some genetic basis. The testing and breeding procedures have been varied from experiment to experiment, and the fact that some studies had more immediate results may be due to these methodological differences. Strain differences in one study and heterogeneity in the majority of the data of the experiments favor a multiple unit interpretation of the results. Eriksson's selection study appeared particularly related to metabolic processes and the clear separation of the lines was achieved after a long breeding program. A significant proportion of the variance remains unexplained. The constitutional factor of sex was important in the results and heritability was found to be low. In sum, these observations are indicative that a polyfactorial interpretation remains most appropriate.

General Summary.

A review of the relevant literature shows that giving laboratory animals exposure to alcohol solutions can either enhance or reduce their subsequent intake. The experiential effects on alcohol intake however, cannot be evaluated easily because of the existence of many extraneous factors. First, testing procedures, periods of exposure, alcohol solutions used have all served as important methodological differences in the various exposure experiments. Moreover the sex, the strain and the age of the animals have not been systematically controlled. Finally individual differences in voluntary alcohol intake have emerged as an uncommonly common finding.

Studies of the effects of sex and age on voluntary alcohol intake were reviewed. They showed that constitution was an important factor of variance and, consequently, that it should be taken account of where possible. The intervention of strain differences in the manifestation of constitutional factors and the importance of individual differences indicated the existence of genetic contribution. An overview of the research done on the genetic mechanisms affecting alcohol intake suggests that a pertinent approach to these variables should be in terms of a multiple-unit system. This belief was strengthened by the constant observation of individual differences in most of the reports.

In view of the complexity and the variability of the factors

affecting voluntary alcohol intake, it would be most relevant to investigate alcohol selection with an experimental design that takes account of the multiplicity of these important components. Accordingly, the following investigation will consist of experiments in which age, sex, strain and method of presentation will be varied systematically. The possibility that the effects of infantile exposure to alcohol are different than those after adult experience will be explored.

Finally, the method of measuring the phenotypical expression of voluntary alcohol intake should be sensitive to various properties of that behavior, i.e. a technique that takes account of the multiple factors controlling alcohol selection and the crucial role played by individual differences. In consequence, it seems that a single solution more or less arbitrarily selected would be limited in its possibilities to reflect all these relevant variables. So, it appears appropriate to let an animal select its own rejection concentration by presenting an ascending series of increasing concentrations of ethanol and using a constant rejection criterion. That threshold screening technique would permit the manifestation of individual differences, since different concentrations do not have the same gustatory and olfactory qualities. A change in threshold following prior screening would reflect a modification of an animal's response to alcohol. Accordingly in the following experiments a modification of the pro-

cedure used by Cicero and Myers (1968) will be utilized to obtain, for each animal, a particular rejection concentration.

## The Present Investigation

The following experiments were carried out to study systematically the relations between certain experiential and constitutional variables that may affect alcohol aversion thresholds of laboratory rats. The basic experimental procedure consisted first in ascertaining the concentration of alcohol solution an individual rat would reject and then repeating the procedure a few weeks later. The purpose was to see if the earlier exposure would influence a later threshold. Two methods of presentation of alcohol were compared. To evaluate the relevance of constitutional factors in the manifestation of an alcohol exposure effect, the groups of rats differed in sex, strain and in the age of first exposure. The method of determining rejection thresholds was designed to account for individual differences in initial and final self-selection response. Finally an attempt was made to explore an effect of selective breeding among animals with high and low rejection thresholds, after infantile exposure to alcohol.

### Experiment I

In order to explore ways in which early experience parameters and constitution might interact in determining intake thresholds, the effect of infantile exposure on adult threshold levels was studied in the Hooded strain of laboratory rats. Two

methods of exposure to alcohol were used: a forced choice and a free choice technique. Also the effects of the age of first exposure was studied by initiating the early exposure at two different ages, at day 21 and at day 35. Male and female animals were compared in all experimental conditions.

Subjects. For this experiment, the Ss were 48 Hooded rats, bred in our own laboratory to multiparous females from the Québec Breeding Farm. They were distributed from six litters to four experimental groups so as to distribute both male and female Ss from all litters throughout the groups. Deaths of Ss resulted in the following distribution by treatment: forced-choice earlier - 12 Ss; forced-choice later - 9 Ss; free-choice earlier - 10 Ss; free-choice later - 11 Ss, or a total of 42 Ss.

Procedure. All Ss were weaned at day 18 and placed by litter in group cages for three days after which they were housed individually for the balance of the experiment with ad libitum access to food. The cages were Plexiglas with covers of stainless steel tubing through which drinking tubes could be inserted. The two groups undergoing earlier exposure were offered alcohol, starting at day 21 and the two undergoing later exposure at day 35. Adult testing began for all animals on day 91.

Solutions. Alcohol solutions were mixed volume by volume (v/v) by adding tap water to 95% ethanol. For instance, 100 ml

of a 10% alcohol solution contains 10.56 ml of 95% ethanol and 89.44 ml of water. Other alcohol concentrations were prepared in the same way.

Determination of intake thresholds. A modification of the procedure used by Cicero and Myers (1968) was utilized. That multiple-solution technique had the animal select for itself its particular rejection concentration, thus allowing for individual differences and the multiple factors affecting voluntary alcohol intake within the same animal.

Method. For all the Ss during treatment and testing, liquids were offered in graduated polyethylene tubes with long drinking spouts, the distance of which from the floor of the cage was adjusted systematically to keep pace with the growth of the animals. The general procedure was to offer the Ss alcohol solutions in gradually increasing concentrations until an individual rejection level was reached by each animal.

Forced vs free-choice conditions. In the forced-choice condition, the rats had access to one water tube, alternated every other day with an alcohol solution tube. For the free-choice procedure, the animals were offered two tubes daily, one containing water and the other alcohol. The position of the tubes was alternated daily. Since the techniques of free choice and forced choice have different effects on intake (Veale and Myers, 1968), the method of determining the rejection

threshold differed for the two conditions. So with forced choice, the rejection level was defined as that concentration of alcohol for which the individual animal's intake was equal to or less than one-half the water intake of the preceding day, over a four-day period. In the free-choice condition, the rejection level was defined as the concentration at which the preference level was .15 or less over a four-day period. Preference level here refers to the ratio: $\frac{\text{alcohol intake}}{\text{total fluid intake}}$. The defined rejection levels were the same for infant and for adult testing.

Concentrations series. During infantile experience, starting with a 3% solution, the daily increase in concentration was 1% until a concentration of 10% was reached, after which increases were 2% daily, until the rejection level was reached. The same procedure was used for these animals tested at maturity except that the initial concentration was 4% in all cases, and daily increases in concentration were 2%.

Results. The data of this experiment represent the end results of the two testing sessions for the Ss of each early experience condition: the first initiated at either day 21 or day 35, and the second initiated at day 91. They are expressed as means and ranges of rejection level concentrations and are shown in Table 1, for the free choice situation, and in Table 2 for the forced choice situation. Overall inspection of these

Table 1

Means and Ranges of Rejection Concentrations (% volume/volume) Attained by Male and Female Rats after the First Session Initiated either at Day 21 or Day 35, and the Second Session Initiated at Day 91, for the Free Choice Situation in Experiment I.

Means

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 13.60 | Day 91 | 28.40 |
| Day 21 | Females | 13.20 | Day 91 | 19.20 |
| Day 35 | Males | 20.66 | Day 91 | 22.33 |
| Day 35 | Females | 16.40 | Day 91 | 32.40 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 6-24 | Day 91 | 14-42 |
| Day 21 | Females | 8-18 | Day 91 | 16-26 |
| Day 35 | Males | 14-32 | Day 91 | 12-46 |
| Day 35 | Females | 10-20 | Day 91 | 16-56 |

Table 2

Means and Ranges of Rejection Concentrations (% volume/volume) Attained by Male and Female Rats after the First Session Initiated either at Day 21 or Day 35, and the Second Session Initiated at Day 91, for the Forced Choice Situation in Experiment I.

Means

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 18.33 | Day 91 | 18.66 |
| Day 21 | Females | 13.66 | Day 91 | 16.66 |
| Day 35 | Males | 18.00 | Day 91 | 16.80 |
| Day 35 | Females | 14.00 | Day 91 | 14.50 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 12-26 | Day 91 | 12-28 |
| Day 21 | Females | 10-16 | Day 91 | 14-22 |
| Day 35 | Males | 14-26 | Day 91 | 10-24 |
| Day 35 | Females | 10-16 | Day 91 | 8-20 |

data reveals a wide range of individual differences in the rejection levels. It also reveals that, on the whole, the subjects of this experiment rejected alcohol in concentrations much higher than those usually reported in the literature. As the ranges of rejection levels show, a significant proportion of Ss rejected alcohol solutions only after they were increased well beyond the 10% that has classically been used as a test solution. In fact, a few of the animals in the free choice situation, established their rejection levels at concentrations above 42%.

In the free choice situation, a comparison of day 21 and day 35 conditions shows that, in infancy, mean thresholds increased with increasing age. On the other hand, the resultant adult rejection thresholds were higher than the infant ones for the same Ss, suggesting either an age effect or a treatment effect. It appears too that the results are not simple functions of the individual variables, (age and sex), but complex functions of the interactions between these variables. Indeed, age and sex reveal their importance by their interaction: on the average, at maturity, males with exposure initiated at day 21 had higher rejection levels than those given exposure initiated at day 35, whereas, at maturity, females with exposure initiated at day 35 had the higher rejection threholds. Inspection of the forced choice data shows that unlike the free choice situation

data a difference between adult and infant thresholds did not emerge. However, male Ss overall had higher rejection levels than the female Ss. Finally, the data for the forced choice condition did not suggest complex interactions among the variables.

Analyses of the data. To evaluate the relevance of the factors mentioned in the description of the data of the free choice situation, a Three-Way Analysis of Variance (age X sex X session) with repeated measures on one variable (session) was applied to the data of the free choice condition; another similar analysis was performed for the forced choice data, (Winer, 1962).* In the latter (Appendix 1) there were no significant differences between infant and adult rejection levels, supporting the suggestion of the absence of a treatment or an age effect. The significant sex effect ($p < .01$) was due to the consistency in males' higher rejection levels. In the free choice situation (Appendix 2), the age effect ($p < .01$) reflected the increases with increasing age that were observed in the infant data when day 21 and day 35 conditions were compared. Also the overall increases in adult over infant rejection levels just reached significance at the .05 level. This supports the existence of an age or treatment effect suggested from the inspection of the data. The analysis also confirms the complex age by sex interaction pointed out above ($p < .01$): male Ss showed higher rejection levels when first exposed to alcohol at day 21, whereas

* see Appendix 17-1.

females showed higher levels when first exposure was at day 35. So, for the Hooded animals in the free choice alcohol drinking condition, there is a good indication of an interaction with experience of two constitutional variables, age and sex, in influencing adult rejection thresholds.

Comparisons between various groups. Because of the absence of session effects and of the much smaller contribution of constitutional variables to the forced choice data, in the remainder of the analysis of Experiment I, only the free choice condition will be considered. The conditions for the two groups for which first experience was initiated at day 21 and day 35 respectively will be referred to as Day 21 and Day 35 conditions and the sets of thresholds that were obtained from their second testing initiated at day 91, will be labelled Day 91 data.

In order to have a stronger confirmation of the session effect revealed in the overall analysis of variance further analyses were performed on the data of infant and adult final rejection levels separately for the Day 21 and Day 35 conditions. First, two-way analyses of variance (sex X session) with repeated measures on session (Appendices 3 and 4) revealed a session effect significant at the .05 level for both conditions; no sex effect and no interaction emerged from the two analyses. Wilcoxon related samples tests also compared the data of the

two sessions separately for the two conditions.* For Day 21 condition, the test revealed a significant difference in the final rejection levels of the two sessions at the .01 level; but for Day 35 condition, the difference was significant only at the .05 level: the higher infant thresholds of Day 35 condition presumably contributed in lessening the difference between infant and adult thresholds in that experimental condition (this is also reflected by the smaller F ratio for session yielded in the analysis of variance of Day 35 condition).

The influence of a developmental factor was tested by comparing infant rejection levels in Day 21 and Day 35 conditions, using the Mann-Whitney U test. Infant rejection levels were significantly higher in the Day 35 condition than in the Day 21 condition ($U=30$, $p \leq .05$). This confirms the suggestion that in infancy rejection levels increased with increasing age.

To investigate further the sex X age interaction revealed by the analysis of variance, Day 91 rejection levels of females of the Day 21 condition were compared with Day 91 data of females of the Day 35 condition: the latter levels were significantly higher than the former ($U=4$, $p \leq .05$). But there was no significant difference between the Day 91 data of males of the Day 21 condition and those of males of the Day 35 condition ($U=11$, $p > .05$). However, the Day 91 data of males of the Day 21 condition pooled with those of females of the Day 35 condition

see Appendix 17-2.

were significantly higher (U=31, $p < .05$) than the pool of Day 91 data of males of the Day 35 condition and of the females of the Day 21 condition. So the sex by age interaction is confirmed and it appears to be due particularly to the intake levels of the female Ss.

Discussion of Experiment I. The results of the present experiment showed that responses of Hooded rats to early experience with alcohol solutions are the resultant of several factors. Firstly, the method used for early exposure and adult testing appears to be important since the forced choice procedure did not reveal any significant increases from the first testing session to the second, whereas the free choice procedure did. Moreover, constitutional variables, sex and age, interacted in producing quite different Day 91 data in the sub-groups of the free choice situation. Finally, in the free choice situation, evidence for a developmental variable suggests that at least a part of the increase in thresholds shown in Day 91 data could be due simply to an age factor. To investigate this question and to see if the results of early experience differ from the results of adult exposure, an adult control study was undertaken.

## Experiment II

Because of the restricted nature of the results, using the forced choice technique, a decision was made at this point

to confine further investigation to the free choice situation.

Subjects. The Ss of this experiment were 15 Hooded rats bred in our own laboratory to multiparous females. They were randomly selected from two litters so that there were three male and four female subjects from one litter, and four males and four females from the other. All Ss underwent the same experimental treatment. They were weaned and housed in the same way as the Ss in Experiment I. Rejection thresholds were ascertained in the same free choice manner as in Experiment I. First exposure to alcohol solutions was initiated for all Ss at day 91, with a concentration of 4%, increasing daily by 2% steps until rejection levels were reached. The second session, initiated on day 161, followed the same procedure. In the following analysis, the results of the two sessions will be labelled Day 91 and Day 161 Control data respectively.

Results. The data of this experiment, presented in Table 3, are expressed as means and ranges of rejection thresholds. They represent the concentrations at which alcohol was rejected for each subject at the end of the two testing sessions: the first initiated at day 91 and the second initiated at day 161. Inspection of these data reveals a wide range of individual differences of high rejection levels: one male was particularly deviant producing a threshold of 58% at both testing sessions. Overall there appeared to be no great change from the first to

Table 3

Means and Ranges of Rejection Concentrations (% volume/volume) Attained by Male and Female Rats after the First Session Initiated at Day 91, and the Second Session Initiated at Day 161, for Experiment II.

Means

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 91 | Males | 20.86 | Day 161 | 25.71 |
| Day 91 | Females | 22.25 | Day 161 | 20.50 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 91 | Males | 10-58 | Day 161 | 10-58 |
| Day 91 | Females | 12-44 | Day 161 | 10-38 |

the second exposure session indicating an absence of significant experience or age effect. Also sex did not appear to be a major factor determining differences in intake levels at any phase of this experiment.

Statistical analysis. To test the apparent absence of significant components of variance, a two-way analysis of variance (sex X session) with repeated measures on session was performed (Appendix 5). It revealed no significant difference in threshold levels between the final data of the two testing sessions. There was no sex effect. To confirm the absence of a session effect in the control data, a Wilcoxon related samples test compared the rejection concentrations, for males and females combined; it also revealed no significant difference. Therefore the data revealed that adult experience did not produce changes in rejection levels comparable with those seen after infantile exposure and that, after maturity, sex, as a constitutional variable influencing alcohol intake, was of less importance.

Comparative analysis of sets of data in Experiments I and II. To ascertain if the results of Experiment I were attributable to an effect of age or of experience, a comparison of Day 91 Control data of Experiment II (Ss without pre-exposure to alcohol) was made with the Day 91 data of Experiment I (Ss with pre-exposure to alcohol). It revealed that the rejection thresholds of the latter were significantly higher than those of the former

(Mann-Whitney, $z=1.64$, $p < .05$). So it appears that, at maturity, rats with previous early exposure to alcohol have significantly higher rejection levels than naive rats. The early exposure effect was evident only when the final rejection levels derived from the Day 91 data of Day 21 and Day 35 conditions were combined. The separate comparisons of the Day 91 data of the two conditions with the Day 91 Control data did not reveal significant differences ($U=52$ and 54 respectively, $p > .05$). Moreover there was no significant difference between the second testing thresholds of Experiments I and II, i.e. between Day 91 data of Experiment I and Day 161 Control data of Experiment II ($z=0.73$, $p=.23$). Two paradoxical findings emerge from the preceding statistical analyses. Firstly, a treatment effect following early exposure to alcohol, was apparent only when both conditions of early exposure were included in the analysis. Secondly, Day 91 data of Experiment I did not remain higher than the data of the control Ss when the latter were given equivalent exposure at maturity (Day 161).

To investigate the nature of these paradoxical results, the data of the two conditions of Experiment I were compared with the data of the adult control study separately for each sex. Accordingly, four two-way analyses of variance (age X session) with repeated measures on session were performed (Appendices 6, 7, 8, 9). All the significant effects which were

revealed were in parallel with the sex X age interaction that emerged from the analysis of Experiment I. In the comparisons between Day 21 condition and the adult control study, the analyses revealed a significant session effect ($p < .05$) for the males, but not for the females. The comparison of the Day 35 condition with the control study yielded a significant age X session interaction ($p < .05$) for the females, but not for the males. So partialling out the constitutional variable of sex, some session effects were apparent even when the control data were included in the analysis. But this effect was clearer in the Day 21 condition since the males yielded a session effect whereas, in Day 35 condition, only an age X session interaction was revealed by the females. The absence of an experience effect when Day 91 data of Day 21 and Day 35 conditions were compared separately with Day 91 Control data is therefore presumably due to the presence of the data of the two sexes in each comparison. Finally, the existence of some session effect when adult control data were included in certain comparisons, might be partly responsible for the observation, reported above, that there was no significant difference between the second testing thresholds of Experiments I and II, that is between Day 91 data of Experiment I and Day 161 Control data of Experiment II.

Discussion of the Hooded experiments. Exposing rats to

alcohol solutions during two different periods in infancy indicated it was possible to modify alcohol rejection thresholds of Hooded rats at maturity. It was also shown that exposure to alcohol solutions at maturity did not produce any changes in later thresholds. This implies that, in voluntary alcohol drinking, there is a difference between infant and adult exposure. However, it is not possible to interpret the results as due uniquely to an experiential factor. The situation is greatly complicated by the constitutional variables systematically introduced into the experimental design. Indeed, the existence and the pattern of the experience effects were affected by age and sex in a complex manner. It was particularly evident when a combined analysis of the early and adult studies was performed. Also the method of letting each animal select its own rejection concentration resulted in a wide range of individual differences; which could be due to specific attributes of the particular strain employed in this study. For all these reasons, further investigations were undertaken. First the testing of another strain of rats to see if the results would indicate similar general and particular effects. Secondly, a special breeding program utilizing animals at the high and low ends of the distribution as parental stock was undertaken to help partial out the factors contributing to the variability.

## Experiment III

The purpose of this experiment was to investigate the alcohol drinking patterns of animals of the Wistar strain and to compare the intake of this albino strain with that of the Hooded animals. An early exposure study was carried out using the same two ages of initial experience as those of Experiment I; at the same time, adult control animals were utilized in the same manner as in Experiment II.

Subjects. The Ss were 48 Wistar rats born in our own laboratory to multiparous females obtained from the Canadian Breeding Laboratories. Thirty-two animals were selected on a random basis from three litters and were used for the infant experience study. They were distributed as follows: earlier experience - 8 males and 8 females; later experience - 8 males and 8 females. Sixteen animals, 8 males and 8 females, came from two litters and were used for the adult control study. The procedure for weaning, housing, solutions series and rejection levels, was identical to the one used in the first two experiments. Again, for the early experience study, the two groups which corresponded to the two ages when first exposure was initiated, will be referred to as Day 21 and Day 35 conditions and their respective sets of thresholds which were obtained after maturity, in a session initiated at day 91, will labelled Day 91 data. The two sessions of the control study, initiated at day 91

and at day 161, will be called Day 91 and Day 161 Control data respectively.

Results. In general the results of this study indicate that experience with alcohol drinking initiated in infancy influences later rejection thresholds to a greater extent than alcohol experience initiated after maturity. That this effect is not, however, a simple age or development factor but is the result of complex interactions of experience with sex and age can be seen in Table 4. This table represents means and ranges of the alcohol concentrations of the initial and final testing sessions for the Day 21 and Day 35 groups, and the Day 91 Control group. Examination of Table 4 shows great variability and relatively high rejection levels in all the groups, which is comparable with the data of Experiments I and II. For subjects of both the Day 21 and Day 35 conditions, there was an increase in concentration of rejection levels between initial and final testing sessions. This was a consistent finding. The increase for males of the Day 35 group, however, was the greatest, and for the females of the same group the increase was the smallest. In contrast with these findings, there were no increases from initial to final testing sessions for either male or female subjects of the Day 91 Control group. The rejection levels for the male subjects of the adult control group were higher at the end of initial testing than those of the female control

Table 4

Means and Ranges of Rejection Concentrations (% volume/volume) Attained by Male and Female Rats after the First Session Initiated at Day 21, Day 35 or Day 91, and the Second Session Initiated either at Day 91 or Day 161, for Experiment III.

Means

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 12.75 | Day 91 | 27.75 |
| Day 21 | Females | 11.13 | Day 91 | 25.25 |
| Day 35 | Males | 12.75 | Day 91 | 34.00 |
| Day 35 | Females | 10.75 | Day 91 | 17.00 |
| Day 91 | Males | 23.50 | Day 161 | 23.25 |
| Day 91 | Females | 15.00 | Day 161 | 13.50 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 8-20 | Day 91 | 12-50 |
| Day 21 | Females | 7-24 | Day 91 | 12-50 |
| Day 35 | Males | 9-20 | Day 91 | 14-62 |
| Day 35 | Females | 7-20 | Day 91 | 12-30 |
| Day 91 | Males | 10-60 | Day 161 | 10-50 |
| Day 91 | Females | 12-18 | Day 161 | 10-22 |

subjects.

Statistical analysis of the infant study. The trends observed in the results of the infant study were statistically evaluated by a three-way analysis of variance (age X sex X session) with repeated measures on session. (Appendix 10). A highly significant ($p < .001$) session effect was found, indicating a clear difference between first and second testing data in the study. There was also a significant sex effect ($p < .05$), due to the significantly higher thresholds for males than for females, particularly for day 91 data in Day 35 Condition. (Mann-Whitney, U=8, p=.005).

To further verify the session effect found in the infant study, some Wilcoxon tests compared the data of the two sessions in both conditions for early exposure; and, for each group, the comparison was made for each sex together and separately. For Day 21 condition, there was a significant difference between thresholds of initial and second sessions (.005) when the data for males and females were combined; but, for males and females separately, the difference was greater for females than for males (.01 vs .05). On the other hand, for Day 35 condition, the difference was significant at the .005 level when the data of the two sexes were combined. For males and females separately, the difference for males (.005) was more highly significant than for the females (.01). This supports a sex X age interaction

resembling the one found in Experiment I. In the present experiment, however, it was in the reverse direction. For confirmation, the appropriate pools of data were compared: contrary to the Hooded data, the day 91 rejection levels of males in the Day 21 condition pooled with those of females in Day 35 condition were significantly lower (Mann-Whitney, U=79, $p < .05$) than the pool of day 91 data of Day 35 condition males and females of the Day 21 condition.

Discussion of the Wistar infant study. Early experience with alcohol was followed by obvious increases in thresholds in the second testing session initiated at day 91. But again constitutional factors, age and sex, influenced the results in a complex way. The interactions, however, were different than the ones observed in the data of the Hooded animals. Moreover, age as a developmental factor was not clearly reflected in the infant data as it was in the data for the Hooded Ss and this possibly had a role in the greater session effect which was observed in the data of the Wistar Ss.

Analysis of the Wistar control data. For a statistical evaluation of the trends observed in the Wistar adult control data, a two-way analysis of variance (sex X session) with repeated measures on session was performed (Appendix 11). It revealed no significant difference between the two testing sessions indicating an absence of effect of either experience

or age with exposure initiated after maturity. The analysis of variance yielded no sex effect despite the observed general tendency of adult males to have higher thresholds than adult females in both the initial and second sessions.

To further verify the absence of a session effect, Wilcoxon tests were performed on the control data: they revealed no significant differences between the sessions using males and females together and separetely. Despite the fact that the analysis of variance did not reveal a significant sex effect the possibility of a sex difference was further evaluated by a comparison of males and females using Days 91 and 161 Control data separately (Mann-Whitneys): males had significantly higher rejection levels than females ($U=12$, $p=.02$) only for the Day 161 Control data. The slight (non-significant) decrease of the females from the first to the second session seems responsible for this result.

Discussion of the Wistar control data. The data of the control study revealed no indication of an effect of initial adult exposure on later thresholds. This indicates that the effect was different when first exposure was initiated in infancy than when it was initiated after maturity. The less influential role played by the constitutional factor of sex in the adult data also indicates that the effects of early and adult exposure were different.

Comparative analysis of infant and control studies. To compare rejection thresholds of naive and experienced animals after maturity, a Mann-Whitney U test was performed comparing the Day 91 data yielded by both conditions of the infant experiment with Day 91 Control data; it revealed that Day 91 rejection levels of the infant study were significantly higher than those of the Day 91 Control condition ($z=1.88$, $p=.03$). This indicates that previous exposure, and not age only, contributed to the highly significant session effect found in the analysis of the data of the early experience study. Day 91 data of Day 21 and Day 35 conditions of the infant study were then separately compared with Day 91 Control data: the comparison revealed that the exposure effect was particularly due to the Day 35 exposure conditions since only the day 91 data of the condition were significantly different from the Day 91 Control data ($U=82$, $p=.05$). Also the combined day 91 data of the two conditions of the early experience study were significantly higher than Day 161 Control data ($z=2.02$, $p=.02$). So, at maturity, Ss having undergone early experience with alcohol had higher alcohol rejection levels after day 91 testing than naive animals with first testing initiated at that age; and they remained significantly higher even after those previously naive Ss had been retested in a session initiated at day 161.

At the end of the initial testing sessions the final

rejection levels of the infant Ss, in both conditions of the early experience study, were lower than the final rejection levels of adult Ss of the Day 91 Control session, indicating an increase with increasing age. To evaluate this apparent age effect, a two-way analysis of variance using sex and age of first testing in the early and adult studies (i.e. Day 21 and Day 35 infant data of the early experience study and Day 91 Control data) was performed (Appendix 12). The analysis revealed an age effect significant at the .05 level which reflects the higher levels of the Day 91 Control data. For a detailed examination of the age effect, separate Mann-Whitney U tests were made to compare the various sub-groups. The infant rejection levels of the Day 21 condition were significantly lower than those of the Day 91 Control Ss, for males and females combined ($U=61$, $p<.01$). But, comparing the data for the sexes separately, only the females differed significantly ($U=10$, $p<.01$). For the Day 35 condition, infant levels of males and females combined ($U=54$, $p<.01$), males ($U=16$, $p<.05$), females ($U=10$, $p<.01$) were significantly lower than Day 91 Control levels.

General discussion of the Wistar data. The results obtained with the Wistar strain confirmed some of the basic conclusions derived from the analysis of the Hooded data. Again, early experience was an important variable which enhanced rejection

levels in voluntary alcohol drinking. The control data showed that mere experience did not induce threshold changes: early and adult exposures were really different conditions. But the variance was not totally explicable in terms of experiential factors. The results were influenced by age as a developmental variable and also, in interaction with sex, as an experiential parameter. They both modified and regulated the direction and the strength of the experimental treatments. Interestingly enough, the Day 35 condition which was the more potent in modifying later intake, was also the condition where constitution played its greater role. Finally, as an indication that the range of rejection thresholds observed in the Hooded Ss, was not an experimental artifact, it is important to note that the Wistar subjects, permitted to select their own rejection thresholds revealed a similar broad range of individual differences.

## Strain Comparisons

The purpose behind the utilization of two strains in the present investigation was to see if they would respond to identical experimental conditions with different alcohol rejection levels. Despite different patterns of response following early exposure, at maturity, the two strains from which the samples of animals came, were not different in voluntary

alcohol intake. The rejection levels of naive Ss in the Day 91 Control data of the two adult studies did not differ significantly (Mann-Whitney, U=120, $p > .05$). Also the rejection levels of the Hooded and Wistar Ss which were the end results of testing sessions initiated after previous exposure to ethanol in adulthood or in infancy, were not different for the two strains (Mann-Whitney tests: Day 161 Control data of the two strains, U=95.5, $p > .05$; Day 91 data of the infant studies of the two strains, z=.30, p=.38): this indicates that neither the Wistar rats nor the Hooded rats responded more consistently to exposure to alcohol. The separate statistical analyses of the data of the Hooded and Wistar infant studies however, did indicate that the animals of the two strains differed in their response to the infant treatments with alcohol.

Statistical comparisons of the Hooded and Wistar infant studies. To compare the results of the early exposure studies between the strains, Day 21 and Day 35 conditions were compared separately in two 3-way analyses of variance (strain X sex X session) with repeated measures on session. In the analysis using Day 21 condition (Appendix 13), no strain effect emerged; but there were a session ($p < .01$) and a sex ($p < .01$) effect. But for Day 35 condition (Appendix 14), the analysis revealed a strain effect ($p < .05$) and a sex by strain interaction ($p < .01$). Also the analysis yielded a session effect ($p < .05$) and a sex effect just significant at the .05 level. The Day 35 exposure

condition appears then to be the experimental situation particularly responsible for the differences between the strains. The differential responses of the sexes for the two strains in that condition seems the factor underlying the differences.

Discussion of the strain comparisons. The two strains used in the present investigation differed in their results when given early exposure to ethanol solutions. The session effect was clearer in the Wistar study. Age effects were not evident in the Wistar early experience study as they were in the Hooded experiment. Also the comparison between early and adult control studies did not reveal, in the Wistar Ss, the paradoxical results which were found in the Hooded Ss. This indicates that the strain differences were the resultant of complex factors. This suggestion is further supported by the reverse sex by age interaction which was found in the two early experience studies. The Day 35 condition was the situation where the reverse interaction was the most obvious: this explains why that condition was found to be the one which best accounted for the strain differences in the infant studies.

## Experiment IV

This study was a preliminary attempt to discover a possible trend in response to selection. The purpose was to see if the tendencies to respond to early exposure that were found in the

Ss of the Hooded strain could be attributed to some heritable characteristics. The high individual variability which was found in the data of the infant studies of both the Hooded and the Wistar strains suggested some genetic basis. Because of the more complex responses of the Hooded rats to early experience with alcohol, this strain was used in the selection study. The original idea was to breed selectively for high and low day 91 thresholds following both Day 21 and Day 35 exposure conditions; but breeding difficulties precluded the use of a Day 35 condition group. The experiment lasted for three generations and a limited proportion of animals was used for breeding: therefore some brother X sister matings resulted. The small base group did not permit a large scale breeding program with the usual outbred population.

General procedure. For the $F_1$ generation, the parents were selected from the Ss of the Day 21 condition in Experiment I; the breeding pairs were the male and the female showing the highest, and the male and the female showing the lowest day 91 alcohol rejection thresholds respectively. Using the respective $F_1$ groups, the breeding pairs for the $F_2$ generations were selected in the same way. The third generation of the high line was the offspring of the crosses of the two males and two females producing the highest rejection thresholds following infantile treatment in the $F_2$ high group. Because of lesser fertility, only

one breeding pair, selected in the same way as for the other low line generations, was used from the $F_2$ low animals for the third generation. During the entire selection study, the general treatment was essentially the same as for the previous Day 21 exposure groups. There was however a difference in the Day 91 testing of the third generation Ss of both high and low lines. In order to perform a blind test of the intake of the animals, the Ss were identified by a number and placed in individual wire mesh cages into which Richter-type drinking tubes were inserted. After a few days of habituation to the cages and tubes, the data collection for the Day 91 session was made by another experimenter who did not know to which group the rats belonged.* In the following analysis the data yielded by the two testing sessions will be called Day 21 and Day 91 data respectively and the Ss in the Day 21 condition of Experiment I, the Base Group.

Results. Table 5 gives the means and ranges of rejection levels for the groups of the first generation of Experiment IV. Tables 6 and 7 present the data of the second and third generations respectively. The thresholds are the end results of the two sessions for each subject: one initiated at Day 21 and the other at Day 91. Inspection of these data reveals that individual variations still remained fairly broad and also that

* Thanks to Miss Deborah Levitan for testing the animals.

Table 5

Means and Ranges of Rejection Concentrations (% volume/volume) Attained by Male and Female Rats after the First Session Initiated at Day 21 and the Second Session Initiated at Day 91, for the First Generation of Experiment IV.

High Line
Means

| First Session Initiated | | | Second Session Initiated | | |
|---|---|---|---|---|---|
| Day 21 | Males | 10.72 | Day 91 | 16.18 | n=11 |
| Day 21 | Females | 11.33 | Day 91 | 17.06 | n=15 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 7-18 | Day 91 | 12-40 |
| Day 21 | Females | 8-34 | Day 91 | 12-34 |

Low Line
Means

| First Session Initiated | | | Second Session Initiated | | |
|---|---|---|---|---|---|
| Day 21 | Males | 10.50 | Day 91 | 17.75 | n=8 |
| Day 21 | Females | 7.66 | Day 91 | 19.66 | n=6 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 7-20 | Day 91 | 12-30 |
| Day 21 | Females | 6-10 | Day 91 | 16-26 |

Table 6

Means and Ranges of Rejection Concentrations (% volume/volume) Attained by Male and Female Rats after the First Session Initiated at Day 21 and the Second Session Initiated at Day 91, for the Second Generation of Experiment IV.

High Line
Means

| First Session Initiated | | | Second Session Initiated | | |
|---|---|---|---|---|---|
| Day 21 | Males | 12.27 | Day 91 | 20.00 | n=11 |
| Day 21 | Females | 14.85 | Day 91 | 28.85 | n=7 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 8-22 | Day 91 | 12-44 |
| Day 21 | Females | 10-24 | Day 91 | 12-54 |

Low Line
Means

| First Session Initiated | | | Second Session Initiated | | |
|---|---|---|---|---|---|
| Day 21 | Males | 10.40 | Day 91 | 17.60 | n=5 |
| Day 21 | Females | 12.50 | Day 91 | 19.00 | n=4 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 7-20 | Day 91 | 12-34 |
| Day 21 | Females | 9-20 | Day 91 | 14-24 |

Table 7

Means and Ranges of Rejection Concentrations (% volume/volume) Attained by Male and Female Rats after the First Session Initiated at Day 21 and the Second Session Initiated at Day 91, for the Third Generation of Experiment IV.

High Line
Means

| First Session Initiated | | | Second Session Initiated | | |
|---|---|---|---|---|---|
| Day 21 | Males | 12.50 | Day 91 | 24.00 | n=4 |
| Day 21 | Females | 17.00 | Day 91 | 37.00 | n=4 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 6-18 | Day 91 | 16-32 |
| Day 21 | Females | 10-26 | Day 91 | 24-48 |

Low Line
Means

| First Session Initiated | | | Second Session Initiated | | |
|---|---|---|---|---|---|
| Day 21 | Males | 8.71 | Day 91 | 17.14 | n=7 |
| Day 21 | Females | 14.00 | Day 91 | 26.00 | n=2 |

Ranges

| First Session Initiated | | | Second Session Initiated | |
|---|---|---|---|---|
| Day 21 | Males | 7-12 | Day 91 | 10-24 |
| Day 21 | Females | 12-16 | Day 91 | 20-32 |

from generation to generation the Day 21 thresholds neither increased nor decreased in any systematic way. They were, however, much higher than those usually reported in the alcohol literature. For all the generations, there were increases in final thresholds from the session initiated at day 21 to the one initiated at day 91.

Table 8 shows the means of Day 91 thresholds for the various selection groups and for the base group, and compares the two lines for males and females together and separately. For males and females together, after a decrease in the first generation, the high line increased gradually in the following generations and reached a much higher mean level in the third generation than that of the Base Group. On the other hand, after an initial drop, particularly in males' levels, the low line remained essentially stable. By the third generation, there was evidence that the thresholds of the two lines were diverging, indicating possible effects of selection.

In the high line, the selection affected the two sexes differently: the females almost doubled their mean rejection threshold by the third generation as compared with the Base Group results: the males decreased their mean level at the first generation and, despite a gradual increase, remained lower than the mean level of the Base Group. In the low line, the males had, in general, lower mean levels than the Base Group,

Table 8

Means of Rejection Concentrations (% volume/volume) Attained by Male and Female Rats after the Second Session Initiated at Day 91, for the Three Generations of Experiment IV and for the Base Group.

Males and Females

| | | $F_1$ | $F_2$ | $F_3$ |
|---|---|---|---|---|
| Base Group: 23.80 | High Line: | 16.69 | 23.44 | 30.50 |
| | Low Line: | 18.57 | 18.22 | 19.11 |

Females

| | | $F_1$ | $F_2$ | $F_3$ |
|---|---|---|---|---|
| Base Group: 19.20 | High Line: | 17.06 | 28.85 | 37.00 |
| | Low Line: | 19.66 | 19.00 | 26.00 |

Males

| | | $F_1$ | $F_2$ | $F_3$ |
|---|---|---|---|---|
| Base Group: 28.40 | High Line: | 16.18 | 20.00 | 24.00 |
| | Low Line: | 17.75 | 17.60 | 17.14 |

whereas the females remained at about the original level. There was a surprising rise in the mean level of the females in the third generation of the low line: there were, however only two females in that group. In summary, females appeared to have responded to selection for high thresholds following day 21 exposure. But the males, after an initial decrease in response to selection in the first generation showed a tendency in the two subsequent generations of the high line to increase threshold levels. This increase was not seen in the males of the low line.

Analysis of the selection data. As a statistical evaluation of the effectiveness of the selection procedure, the Day 91 data of the two $F_3$ generations were compared for males and females combined. The two lines reached a significant separation at that third generation (Mann-Whitney, U=13, p=.03). But neither differed significantly from the Base Group indicating that response to selection was not complete. To verify the apparent gradual increase in the high line and the suggested different responsiveness of males and females to selection in the increasing direction, Linear Trend Analyses (Ferguson, 1971) were performed for three generations using the data of the two sexes together and separately. (Appendix 15). For both sexes together, there was a linear regression at the .01 level of significance. For the females the linear regression was highly significant ($p < .001$)

whereas, for the males, no significant linear regression emerged.

In order to confirm the observed reduction in mean Day 91 threshold levels of males in both lines in response to selection, Day 91 data of the Base Group were compared with $F_1$ high and $F_1$ low groups Day 91 data. The males of the $F_1$ high level group differed from the Base Group males at the .03 level of significance (Mann-Whitney, U=7.5). However, males of the $F_1$ and $F_3$ low level groups were not significantly different from the Base Group males. (U=9, p=.06; U=8, p=.07). So, the apparent decreases in the low line failed to reach significance.

An increase in rejection thresholds from Day 21 session to Day 91 session was apparent from an inspection of the data of all groups for each generation. To determine if these increases were significant, two-way analyses of variance (sex X session) with repeated measures on session were performed on the data for the high and low lines at each generation. The Anovas revealed highly significant session effects for all the groups at least at the .01 level of significance (Appendix 16). Also to evaluate if these session effects were due to an age or an experience factor, the Day 91 data of all the groups of Experiment IV were compared with Day 91 Control data of Experiment II. The only significant difference in Day 91 levels was found when using the Day 91 data of the high line group in the $F_3$ generation (U=29, p=.03). Only that group indicated a

significant experience effect.

Discussion. In the discussion of the results of the experiments with the Hooded Ss, it was suggested that a program of selective breeding might help to isolate some of the components contributing to the complex responses of these animals, following early experience with alcohol. The selective breeding was, indeed, somewhat effective in separating the age and experiential components that were jointly responsible for the significant session effect in the Day 21 condition of Experiment I.

The females were particularly responsive to selection in the high line. This sex difference was a reversal of the results of Experiment I, where, in the Day 21 condition, the males produced the higher Day 91 levels. This reversal in sex difference indicates that some interesting new interactions between sex and age might have emerged if it had been possible to include a Day 35 condition in the selection study. Presumably, the significant decreases observed in the threshold levels of the males of the $F_1$ generation of the high line, precluded the possibility of their reaching a significant trend in response to selection because the selection study did not last long enough. Finally, except for the decrease in threshold levels of the males in the first generation, the low line remained essentially stable suggesting that the tendency to increase following early treatment had reached a minimal level.

## General Discussion

The purpose of this investigation was to study the role of both constitution and experience in voluntary alcohol intake in the laboratory rat. The experimental design consisted in comparing the effects of early and adult exposure to alcohol while systematically taking account of the constitutional factors of age, sex, and strain. The results of the studies indicated that the experience effects were not independent of the constitutional variables.

The first question arising from this investigation is why the free-choice early exposure conditions used in the experiments produced significant changes in alcohol intake at maturity whereas the same procedure initiated at maturity did not produce significant changes in intake at the second adult session.

Early treatment triggered the manifestation of complex constitutional factors and significant constitutional/experiential interactions. After adult experience, the only evidence for such interaction was a reduction in final rejection levels of female Ss of the Wistar strain. These results appear to reflect the fact that a single short-term intervention at maturity following critical development of the CNS is no longer efficacious in modifying voluntary alcohol intake.

In other investigations, Veale and Myers (1969), for example, got their "acclimation" effect in adult rats after many presenta-

tions of a series of increasing alcohol concentrations, the first three of which were repeated after only one-day intervals. Russell (1971) induced changes in voluntary alcohol intake of adult rats after a long-term exposure procedure. But she could not alter the alcohol intake of adult rats of four strains with short-term exposure followed by similar testing many weeks later.

Cicero et al. (1971) have suggested that the alcohol dependence they induced in rats was related to the Ss' exposure to ethanol during the critical period of CNS development (21-60) days. The possible importance of that critical period has also been supported by the finding of Clay (1964), who reported that exposure to alcohol was more effective when initiated at day 19 than at day 60.

In the present experiments, the Ss were also exposed to alcohol in sessions initiated during the critical period of brain development. But exposure was initiated at two different times within this period, at the very beginning and in the middle of it. Inspection of individual records of subjects shows that some drank large amounts of alcohol and had high rejection levels despite their small body size. Two factors might be involved. As compared to adult animals, weanling rats have a greater propensity to drink bitter substances (e.g. quinine: Cicala and McMichael, 1964). This propensity may have facilitated voluntary alcohol drinking in young rats during the critical

period of brain development. In addition, since ADH reaches adult levels about 18 days after birth (Raïhä, Koshinen and Pikkarainen, 1967), early alcohol consumption does not appear to be noxious.

The results of the present study clearly showed that early exposure to alcohol initiated at different ages corresponding to two distinct periods during the development of the CNS, led to different intake patterns when the Ss were mature. These differences were dependent on the sex and the strain of the animals, and suggest that a sensitizing effect of hormones might be involved.

There is evidence that sex hormones are present in significant amounts at different ages in young male and female rats and that testosterone has an organizing effect very early in the life of the male rat (Harris, 1964). The different effects of sex hormones on the two sexes, at the two ages of initial alcohol exposure may be reponsible for the different intake patterns of male and female Ss at maturity. Since sex differences in alcohol intake have generally not been observed in studies using a single age of initial early exposure (e.g. Cicero et al., 1971) whereas, with two ages of initial exposure, sex differences have emerged (Clay, 1964), such hormonal involvement as exists may be evident only when there is a comparison possible in the results of Ss at two different ages of initial exposure.

The important age effects found in the results also suggest an influence of growth hormones on alcohol rejection levels particularly since, in infancy, there were some increases in rejection thresholds with increasing age whereas, after maturity, such age dependent processes were less apparent. Furthermore, it has been shown that the effective age of an early treatment can be different in different strains. Henry and Bowman (1970) demonstrated this in an experiment on audiogenic seizures in inbred strains of mice. In the present studies, different patterns of interactions were produced at different ages in the two strains employed. Assuming that hormones had some role, the strain differences imply that genotype modified the patterns of these hormonal effects and could have had an influence on age dependent processes.

Finally, the suggestion of possible hormonal influences is supported by the fact that the Day 35 early exposure condition produced the more complex results. Indeed, this condition was the main source of the sex X age interaction in the Wistar infant study and the one in which the difference between the two strains was the more clear cut. The experimental intervention at a relatively later stage of the critical CNS development period coincided with the beginning of the puberty period where hormonal stimulation should be particularly significant. Usually, in rats, the duration of the period of puberty is assumed to last from approximately day 37 to day 67 (Farris,1950; Robinson, 1965).

Therefore the Ss that were under treatment in a session initiated at day 35 had their alcohol experience during the first half of the puberty period. In the Wistar Ss, following alcohol exposure initiated at day 35, females showed lower rejection levels than males. This may be attributed to the prior inhibitory effects of oestrogenic hormones, which would have been present in significant amounts in the early pubertal females. On the other hand, following alcohol exposure initiated at day 35, females of the Hooded strain had higher rejection levels at maturity than the males. This cannot be explained by a simple effect of oestrogenic hormones and leads once again to the speculation that a different genotype contributes to different hormone-related processes.

The selection study of the present investigation, although limited in scope, showed that, with an appropriate breeding program, the separation of Ss showing greater and lesser increases following early exposure to alcohol was possible. The procedure was designed to produce higher alcohol intake following early exposure, and it was expected that the selection for greater increases following early experience with alcohol would be more efficient than selection for lesser increases, since the Ss showing the lesser increases were already at a low level of responsiveness to exposure.

In the low line, except for the initial reduction in the rejection levels of males of the $F_1$ generation, the data look

more or less like a flat line. This might mean that the factors responsible for the increases from one session to the next could not be diminished further. Selection in the low direction is often quicker to respond (Falconer, 1960) and Rodgers and McClearn (1962) reported that the low intake bred progeny were more homogeneous (mainly low drinkers) than the high intake line, after one generation of selection.

In the high line, a significant linear increase was observed over the three generations and depended mainly on the females. When a trait is controlled by few genetic factors, response to selection for extremes of the trait is usually quick and efficient. The moderate response to selection which was observed in the present study probably indicates that the factors underlying the tendencies for voluntary alcohol drinking are quite complex. Since a behavior trait which is controlled by many factors is usually characterized by wide individual variability, the wide ranges in rejection levels which were observed with the threshold technique, provide further support for this assumption.

The changes in genotypes induced by the selection modified some constitution/experience interactions that were observed in the Hooded Ss after initial early experience. First, the sex difference in response to Day 21 exposure still emerged but in a reverse direction. Males were more responsive to selection for lesser increases than were females presumably because, as can

be seen from the data of Experiment I, females were already quite low. As a consequence, females responded to a greater extent than males to selection for higher thresholds. Both the surprising decline observed in the male Ss in the $F_1$ generation of the high line and the slower rate of increase of the males are difficult to explain. These paradoxical findings are in line with the assumed complexity of the behavior. Secondly, the selection permitted the partialling out of the contribution of age and experience in the session-to-session increases in the infant experiment. This might be another instance where different genotypes produced different constitution related processes. But also this result could be attributed to the particular nature of genotype distribution that is induced by a selective breeding program.

Because of the small size of the Base Group some inbreeding occurred. Presumably, inbreeding affected the results of the selection, the direction and extent of which are difficult to ascertain. In the present study, the role played by inbreeding could have been either to mask or to enhance the effectiveness of selection and remains a question for further investigation. Whatever the effects of inbreeding, the moderate success in separating the two lines from each other and from the Base Group and the wide ranges of individual differences within the separated groups, support the idea of multiple-unit control of voluntary

alcohol intake. The wide range of individual differences also reflects the fact that the testing method which was used was sensitive to the various factors underlying individual propensity to consume alcohol solutions.

The establishment of individual rejection thresholds appears to be a particularly relevant aspect of the experimental procedures utilized in the present investigation, as it influenced the patterns of the results described above. The threshold technique was designed so that each animal selected for itself its own rejection concentration in accordance with its own propensity for voluntary alcohol drinking. The ranges of rejection levels in most sub-groups indicate that these individual propensities differed from S to S. In fact, the technique induced young and adult rats voluntarily to ingest ethanol in concentrations much higher than those usually reported in the alcohol literature and appears to be a powerful method for the manifestation of phenotypical drinking patterns. These observations lead to the conclusion that the technique could be sensitive to the various neuro-hormonal mechanisms that were postulated above (e.g. hormonal sensitization inducing changes in olfactory or gustatory acuity) and to all the variability resulting from the genetic background of the individual Ss. A single solution technique would not have permitted the observation of such variation.

Despite the fact that the objective of the present investigation was the manipulation of the factors involved in voluntary alcohol intake, a forced-choice technique of presentation of alcohol solutions was compared with the free-choice method in Experiment I. The forced choice method, however, did not affect later alcohol intake and was not pursued. This is consistent with the findings of a number of other investigators (e.g. Veale and Myers, 1969). The failure of this technique to influence later behavior, however, may have been due in part to the fact that both initial and later testing were carried out under forced-choice conditions, since in a pilot study with Hooded rats where free-choice adult testing followed earlier and later initial infantile forced exposure, Ma (1969) found adult threshold levels not unsimilar to the results obtained in the free-choice situation of Experiment I.

The forced-choice situation of the present study did not produce the increases in rejection levels observed in the free-choice paradigm. However, unlike the free-choice condition, the male Ss in all the forced-choice conditions had higher rejection thresholds than the female Ss. This may indicate either the potency of sex as a variable in early experience studies, or some aspect of the forced-choice situation which still remains unclear. Further experimentation is required before a definitive conclusion can be reached about the effectiveness of the forced-

choice technique in inducing significant changes after early experience.

In summary, the present investigation showed the effectiveness in adult Ss of a short-term alcohol exposure initiated in infancy. The results particularly suggested that the enhancement of alcohol rejection thresholds was related to complex neuro-hormonal processes. However, the patterns of the results did not reveal any apparent "dependence" on alcohol comparable with the findings reported by Cicero et al. (1971). It is reasonable to suppose that such a permanent change in animals' behavior toward alcohol needs a long "consolidation" period such as the one provided by Cicero et al. (1971). This can be compared with the results reported by Russell (1971) where long-term free-choice exposure initiated in adulthood induced strong increases in voluntary alcohol drinking. The particular feature of her experimental design consisted in giving long-term free access to an alcohol concentration which was equal to 80% of the initial rejection concentration of each subject. It is hypothesized that a similar procedure undertaken during the time period intervening between first and second testing in the early exposure paradigm of the present investigation could trigger some further interesting modifications in voluntary alcohol intake.

Although it was not designed for a direct application to human alcoholism, the present investigation supports the idea

that a combination of constitutional and experiential factors could make an organism more or less prone to voluntary alcohol consumption. Such particular interrelations could very well be the keystone for the understanding of the reasons certain individuals become alcoholic and others do not under apparently similar environmental conditions.

## Summary

The experiments reported in this thesis were performed to investigate systematically the rôle of constitutional and experiential factors in voluntary alcohol intake in the laboratory rat. Effects of experience initiated in infancy were studied in particular, and the constitutional variables of age, sex, and strain were systematically introduced into the experiments.

Because of the importance of individual variations observed even in highly inbred stocks of animals, a testing method was used whereby the experimental Ss could establish for themselves their alcohol rejection concentration. Overall, this testing technique permitted the manifestation of a wide range of individual differences and the voluntary ingestion of ethanol solutions in concentrations usually not reported in the literature.

In Experiment I, the effects of experience initiated at two different ages in infancy were studied in male and female Hooded rats. Free and forced choice presentations of alcohol solutions were compared. It was found that only the free choice technique yielded a significant session effect. However, in addition to the revealed session effect, there was a complex sex by age interaction: male and female Ss responded differently to the treatment depending on the age at which early exposure was initiated. Also the results suggested that a significant age or developmental factor was influencing the results. Further

studies utilized only the free-choice technique.

Experiment II was a control study to ascertain whether the session effect found in Experiment I was merely an age or developmental artifact. It was found that experience with alcohol initiated after maturity did not enhance later rejection levels in male and female Hooded rats. The comparative analysis of the results of these two experiments showed, therefore, that the results of Experiment I were not simply due to an experiential or developmental factor, but were the complex end result of the interaction of experience, age, and sex.

In Experiment III, the Wistar strain of rats was used as a comparison with infant and adult exposure studies of the Hooded strain. Some of the results were similar for the two strains. For both, exposure initiated in infancy enhanced later intake whereas experience initiated after maturity did not. Also constitutional variables significantly affected the results. However, a difference in genotype produced a difference in the pattern of the effects of constitutional factors. This was particularly shown by the reversal of the age by sex interaction observed in the infant exposure study using Wistar rats.

In Experiment IV, a selective breeding study was undertaken in order to obtain two diverging lines with Ss having high and low rejection thresholds as adults after infantile exposure to alcohol. Despite the small base group that was used and the few

generations the experiment lasted, a significant separation between the lines was obtained. The selection study also permitted a partialling out of age and experiential factors as they are related to the effects of early exposure to alcohol.

The particular nature of the early experience effects found in the present investigation was discussed in relation to possible neuro-hormonal mechanisms that could be involved and also as a function of the properties of testing techniques used.

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## Appendix 1

Results of the three-way analysis of variance (age X sex X session); with repeated measures on session, for the final rejection levels of Ss in the forced-choice condition in Experiment I.

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 20 | | | |
| Age (A) | 1 | 10.73 | 1.64 | ns |
| Sex (B) | 1 | 106.28 | 16.30 | $<.01$ |
| A X B | 1 | .05 | .01 | ns |
| Ss within cells | 17 | 6.52 | | |
| Within Subjects | 21 | | | |
| Session (C) | 1 | 4.65 | .10 | ns |
| A X C | 1 | 10.73 | .24 | ns |
| B X C | 1 | 11.80 | .26 | ns |
| A X B X C | 1 | .56 | .01 | ns |
| C X Ss within cells | 17 | 44.37 | | |

## Appendix 2

Results of the three-way analysis of variance (age X sex X session), with repeated measures on session, for the final rejection levels of Ss in the free-choice condition in Experiment I.

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 20 | | | |
| Age (A) | 1 | 197.51 | 20.83 | $<.01$ |
| Sex (B) | 1 | 9.40 | .99 | ns |
| A X B | 1 | 154.77 | 16.32 | $<.01$ |
| Ss within cells | 17 | 9.48 | | |
| Within Subjects | 21 | | | |
| Session (C) | 1 | 962.15 | 4.41 | $= .05$ |
| A X C | 1 | 6.68 | .03 | ns |
| B X C | 1 | 20.46 | .09 | ns |
| A X B X C | 1 | 351.20 | 1.61 | ns |
| C X Ss within cells | 17 | 217.84 | | |

## Appendix 3

Results of the two-way analysis of variance (sex X session), with repeated measures on session, for the data of the infant and adult final rejection levels in the Day 21 condition.

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 9 | | | |
| Sex (A) | 1 | 115.60 | 1.27 | ns |
| Ss within cells | 8 | 90.70 | | |
| Within Subjects | 10 | | | |
| Session (B) | 1 | 540.80 | 10.75 | $<.05$ |
| A X B | 1 | 96.80 | 1.92 | ns |
| B X Ss within cells | 8 | 50.30 | | |

Appendix 4

Results of the two-way analysis of variance (sex X session), with repeated measures on session, for the data of the infant and adult final rejection levels in the Day 35 condition.

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 10 | | | |
| Sex (A) | 1 | 45.99 | .31 | ns |
| Ss within cells | 9 | 147.26 | | |
| Within Subjects | 11 | | | |
| Session (B) | 1 | 425.37 | 5.68 | <.05 |
| A X B | 1 | 279.74 | 3.73 | ns |
| B X Ss within cells | 9 | 74.85 | | |

## Appendix 5

Results of the two-way analysis of variance (sex X session), with repeated measures on session, for the data of the infant and adult final rejection levels in the adult control condition.

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 14 | | | |
| Sex (A) | 1 | 27.25 | .08 | ns |
| Ss within cells | 13 | 339.89 | | |
| Within Subjects | 15 | | | |
| Session (B) | 1 | 17.95 | .28 | ns |
| A X B | 1 | 81.35 | 1.26 | ns |
| B X Ss within cells | 13 | 64.09 | | |

## Appendix 6

Results of the two-way analysis of variance (age X session), with repeated measures on session, for the data of the final rejection levels of male Ss in the Day 21 condition of Experiment I and final rejection levels of male Ss in the control condition (Experiment II).

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 11 | | | |
| Age (A) | 1 | 30.84 | .07 | ns |
| Ss within cells | 10 | 405.89 | | |
| Within Subjects | 12 | | | |
| Session (B) | 1 | 559.91 | 8.36 | $<.05$ |
| A X B | 1 | 145.75 | 2.17 | ns |
| B X Ss within cells | 10 | 66.94 | | |

## Appendix 7

Results of the two-way analysis of variance (age X session), with repeated measures on session, for the data of the final rejection levels of female Ss in the Day 21 condition of Experiment I and final rejection levels of female Ss in the control condition (Experiment II).

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 12 | | | |
| Age (A) | 1 | 163.11 | 1.65 | ns |
| Ss within cells | 11 | 98.67 | | |
| Within Subjects | 13 | | | |
| Session (B) | 1 | 28.43 | 0.55 | ns |
| A X B | 1 | 91.16 | 1.77 | ns |
| B X Ss within cells | 11 | 51.43 | | |

## Appendix 8

Results of the two-way analysis of variance (age X session), with repeated measures on session, for the data of the final rejection levels of male Ss in the Day 35 condition of Experiment I and final rejection levels of male Ss in the control condition (Experiment II).

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 12 | | | |
| Age (A) | 1 | 20.93 | .05 | ns |
| Ss within cells | 11 | 371.80 | | |
| Within Subjects | 13 | | | |
| Session (B) | 1 | 66.15 | 1.16 | ns |
| A X B | 1 | 16.54 | .29 | ns |
| B X Ss within cells | 11 | 56.82 | | |

## Appendix 9

Results of the two-way analysis of variance (age X session), with repeated measures on session, for the data of the final rejection levels of female Ss in the Day 35 condition of Experiment I and final rejection levels of female Ss in the control condition (Experiment II).

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 12 | | | |
| Age (A) | 1 | 57.19 | 0.38 | ns |
| Ss within cells | 11 | 150.37 | | |
| Within Subjects | 13 | | | |
| Session (B) | 1 | 314.38 | 3.92 | ns |
| A X B | 1 | 481.72 | 6.01 | $<.05$ |
| B X Ss within cells | 11 | 80.15 | | |

## Appendix 10

Results of the three-way analysis of variance (age X sex X session), with repeated measures on session, for the final rejection levels of Ss in the free-choice condition in Experiment III.

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 31 | | | |
| Age (A) | 1 | 5.64 | .05 | ns |
| Sex (B) | 1 | 534.77 | 4.94 | $<.05$ |
| A X B | 1 | 221.26 | 2.04 | ns |
| Ss within cells | 28 | 108.09 | | |
| Within Subjects | 32 | | | |
| Session (C) | 1 | 3,206.40 | 28.61 | $<.001$ |
| A X C | 1 | 2.63 | .02 | ns |
| B X C | 1 | 252.00 | 2.24 | ns |
| A X B X C | 1 | 199.54 | 1.78 | ns |
| C X Ss within cells | 28 | 112.07 | | |

## Appendix 11

Results of the two-way analysis of variance (sex X session), with repeated measures on session, for the data of the infant and adult final rejection levels in the adult control condition in Experiment III.

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 15 | | | |
| Sex (A) | 1 | 666.12 | 3.26 | ns |
| Ss within cells | 14 | 204.48 | | |
| Within Subjects | 16 | | | |
| Session (B) | 1 | 6.12 | .15 | ns |
| A X B | 1 | 3.13 | .07 | ns |
| B X Ss within cells | 14 | 39.77 | | |

## Appendix 12

Results of the two-way analysis of variance (sex X age of first testing) for the final rejection levels of the Ss of the Day 21 and Day 35 conditions and of the Day 91 Control condition in Experiment III.

| Sources | df | MS | F | p |
|---|---|---|---|---|
| Sex (A) | 1 | 196.02 | 2.81 | ns |
| Age (B) | 2 | 292.72 | 4.20 | $<.05$ |
| A X B | 2 | 59.74 | 0.86 | ns |
| Within cells | 42 | 69.70 | | |

Appendix 13

Results of the three-way analysis of variance (strain X sex X session), with repeated measures on session, for the final rejection levels of Ss in the Day 21 free-choice condition of Experiments I and III.

| Sources | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 25 | | | |
| Strain (A) | 1 | 4.73 | 0.70 | ns |
| Sex (B) | 1 | 144.70 | 21.34 | $<.01$ |
| A X B | 1 | 23.06 | 3.40 | ns |
| Ss within cells | 22 | 6.78 | | |
| Within Subjects | 26 | | | |
| Session (C) | 1 | 1,915.72 | 8.30 | $<.01$ |
| A X C | 1 | 53.19 | 0.23 | ns |
| B X C | 1 | 72.01 | 0.31 | ns |
| A X B X C | 1 | 48.21 | 0.21 | ns |
| C X Ss within cells | 22 | 230.81 | | |

## Appendix 14

Results of the three-way analysis of variance (strain X sex X session), with repeated measures on session, for the final rejection levels of Ss in the Day 35 free-choice condition of Experiments I and III.

| Source | df | MS | F | p |
|---|---|---|---|---|
| Between Subjects | 26 | | | |
| Strain (A) | 1 | 242.79 | 7.27 | $< .05$ |
| Sex (B) | 1 | 141.35 | 4.23 | $= .05$ |
| A X B | 1 | 498.95 | 14.94 | $< .01$ |
| Ss within cells | 23 | 33.38 | | |
| Within Subjects | 27 | | | |
| Session (C) | 1 | 1,551.69 | 6.57 | $< .05$ |
| A X C | 1 | 181.33 | 0.76 | ns |
| B X C | 1 | 103.06 | 0.43 | ns |
| A X B X C | 1 | 595.52 | 2.52 | ns |
| C X Ss within cells | 23 | 236.10 | | |

## Appendix 15

Results of the linear trend analyses performed on the data of the three high-line generations of the selection study (Experiment IV).

Males and females

| Source | df | MS | F | p |
|---|---|---|---|---|
| Linear regression | 1 | 1,310.38 | 10.44 | <.01 |
| Deviation | 1 | 0.41 | .003 | ns |
| Within | 49 | 125.46 | | |
| | 51 | | | |

Females

| Source | df | MS | F | p |
|---|---|---|---|---|
| Linear regression | 1 | 1,534.26 | 11.39 | <.01 |
| Deviation | 1 | 15.49 | 0.12 | ns |
| Within | 23 | 134.69 | | |
| | 25 | | | |

Males

| Source | df | MS | F | p |
|---|---|---|---|---|
| Linear regression | 1 | 198.24 | 1.92 | ns |
| Deviation | 1 | .12 | .001 | ns |
| Within | 23 | 103.11 | | |
| | 25 | | | |

Appendix 16

Table of F ratios for the session effects found in the two-way analyses of variance (sex X session), with repeated measures on session, for all the groups in Experiment IV.

| Group | df | MS | F | p |
|---|---|---|---|---|
| $F_1$ High | 1 | 397.19 | 17.69 | $<.01$ |
| $F_1$ Low | 1 | 635.51 | 23.70 | $<.001$ |
| $F_2$ High | 1 | 1,010.42 | 11.14 | $<.01$ |
| $F_2$ Low | 1 | 208.32 | 29.84 | $<.01$ |
| $F_3$ High | 1 | 992.25 | 47. 45 | $<.001$ |
| $F_3$ Low | 1 | 324.49 | 18.48 | $<.01$ |

## Appendix 17

## Statistical Procedures

1. The analyses of variance applied to the data of the experiments described in the body of this thesis utilized a formula that permitted a statistical correction for unequal group size, wherever this was relevant. The procedure was based on one suggested by Winer (1962), p. 374.

2. Since the assumption of normality of the distributions was not verified, non-parametric tests were used to verify some of the main effects that emerged from the analyses of variance applied to the data of Experiments I, II, and III.

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