PRENATAL EXPOSURE TO ALCOHOL: EFFECTS ON DISCRIMINATION LEARNING AND CONDITIONED INHIBITION IN THE CHICK

by

James G. Linakis

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(Professor in Charge of Thesis)

[redacted]

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Prenatal exposure to alcohol has been proposed as an endangerment to fetal growth and well-being, at least since the beginning of the twentieth century. Early reports in both the human and animal literature suggested that prenatal ethanol was responsible for reduced growth, and an increased incidence of miscarriages, infant mortality, epilepsy, idiocy, psychosis, and assorted deformities (cf. Roe, 1944; Sullivan, 1899). As Green (1974) points out, however, these early studies may be criticized on a variety of grounds, since many failed to control for such maternal factors as nutrition, and other drug use during pregnancy, and others failed to account for such postnatally relevant factors as socioeconomic background and maternal child-rearing behavior.

It was not until the late 1960s and early 1970s that the extent of the potential damage caused by prenatal exposure to alcohol became evident. Independent discoveries by Lemoine, Hareusseau, Borteryu, and Menuet (1968) in France, and Jones and Smith (1973) in the United States led to the description of a unique syndrome in children born to alcoholic mothers, the "fetal alcohol syndrome." The pattern of anomalies found to be present in children with the fetal alcohol syndrome includes: pre- and postnatal growth deficiencies, craniofacial abnormalities, cardiac defects, and histologic malformations resulting from neuronal and glial migratory failure. Additionally, one investigator has noted that "mental impairment is probably the most disabling aspect in children who actually do have the fetal alcohol syndrome" (Streissguth, 1976a, p. 266). Indeed, in a retrospective study, Streissguth found that, based on standard IQ tests, all of the child-

ren evaluated (in the clinics at the University of Washington) who clearly had the syndrome could be categorized as borderline to moderately retarded. Moreover, in a prospective study of offspring whose mothers had been identified as alcoholic during pregnancy (Streissguth, 1976b), the average IQ of children of alcoholics was found to be significantly lower (81) than that of children of nonalcoholic control mothers matched for maternal age, parity, education, race, and other factors (95).

While the importance of retrospective and prospective studies cannot be overlooked, these kinds of reports must generally be interpreted with caution, since accurate information about alcohol consumption and other risk factors during pregnancy is often difficult to obtain. There appear to be a number of factors other than (or in addition to) alcohol consumption that must be considered before attributing fetal abnormalities exclusively to alcohol: frequency of smoking (cf. Martin, Martin, Lund, & Streissguth, 1977), use of caffeine and other drugs, age, race, and socioeconomic status (cf. Streissguth, Martin & Martin, 1978). As a result of these difficulties and due to ethical concerns over manipulating fetal exposure to alcohol in human infants, several investigators have initiated animal experimentation to provide detailed information on the potential development-impairing effects of alcohol. Studies involving rats, mice and chick embryos have confirmed that increased mortality, growth deficiencies and dysmorphogenesis can be produced in animals by exposing them to alcohol during gestation or incubation (e.g., Chernoff, 1977; Kronick, 1976; Martin, Martin, Sigman, & Radow, 1977; Randall, 1977; Sandor & Elias,

1968). Warren (1977) has thoroughly reviewed the animal literature which deals with morphological and physiological effects of prenatal exposure to alcohol. However, in recent years, a considerable number of studies have been performed in which animal models were used to examine the behavioral consequences of pre- and perinatal exposure to ethanol.

Methodological Considerations

Unlike the research to be reported in the later sections of this thesis, the majority of investigations designed to study the behavioral consequences of prenatal exposure to alcohol in animals have utilized mammalian models. Although the methodological factors to be considered here are thus largely directed at such models, they include many elements which are important to prenatal alcohol studies regardless of the specific model employed.

Prenatal Factors

Behavioral scientists encounter a number of difficulties when studying the behavioral effects of embryonic exposure to alcohol. Many of these problems are common to most investigations within the broad area of teratology. Others, however, are unique to behavioral studies and require special consideration in the design of behaviorally oriented investigations. Although a comprehensive analysis of the proper control procedures for experiments in behavioral teratology is beyond the scope of this review, a number of issues are of sufficient relevance to deserve brief discussion.

Perhaps one of the more important considerations in the design of a study of prenatal drug effects on neonatal behavior is the potential

for postnatal effects of the drug on maternal behavior. It is well known that the behavior of offspring is readily influenced by the background and experiences of the parents which rear it (Joffe, 1969; Martin, Martin, Sigman, & Radow, 1978). Because alcohol, like many other drugs, clearly possesses the ability to disrupt normal maternal behavior, it is generally necessary to employ postnatal rearing controls in behavioral studies involving its prenatal administration. The control procedures most commonly employed in this regard have been described by Joffe (1969), and involve fostering/cross-fostering manipulations. Fostering describes the transfer of a litter to a mother "other than its biological mother, but from the same treatment group as its biological mother," whereas cross-fostering refers to the rearing of a litter by "a mother other than its biological mother, and from a different treatment group to that of its biological mother" (p.21). Although Joffe describes the numerous variations of fostering/cross-fostering procedures, acceptable control can usually be obtained by cross-fostering control and ethanol-treated offspring to untreated mothers. Unfortunately, a number of the studies designed to examine the behavioral effects of prenatal alcohol exposure are difficult to interpret because of the exclusion of such control procedures. These studies cannot be considered conclusive since they fail to distinguish between behavioral alterations resulting from prenatal effects of alcohol on the offspring and those resulting from the effects of drug treatment on postnatal maternal behavior.

Nutritional Factors

Another problem which deserves attention in studies of the consequences

of prenatal ethanol exposure is that of nutrition. Malnutrition or unbalanced dietary intake can have both direct effects on the fetus and indirect effects via disruption of the normal maternal physiology (Altman, Das & Sudarshan, 1970; Altman, Das, Sudarshan, & Anderson, 1971; Joffe, 1969). Consequently, it is essential that experimental and control mothers be matched as closely as possible for nutritional factors in studies of behavioral teratogenesis. Alcohol presents a special challenge in the development of nutritional controls for three basic reasons: (a) The dose range of alcohol most commonly used in behavioral investigations involves amounts of alcohol which contain a relatively large number of calories; (b) Because of its generally non-preferred taste and other aversive consequences (cf. Cunningham, 1979), animals which are provided with free access to alcohol solutions as their sole fluid source usually restrict their fluid intake and thus their food intake; and (c) Ethanol has been shown to slow gastric absorption of certain nutrients when administered orally (e.g., Arky, 1971), and to alter the metabolism of carbohydrates (Arky, 1971), proteins (Orten & Sardesai, 1971), vitamins (Vitale & Coffey, 1971), and minerals (Flink, 1971), especially during prolonged exposure to alcohol.

Since many of these latter effects are dose- and history-dependent, it is unlikely that precise control of the nutritional component is a reasonable goal (cf. Yanai & Ginsburg, 1977a). Nevertheless, due to the potential for behavioral effects produced by nutritional factors alone, it is clear that careful attention to nutritional controls in the design of prenatal alcohol experimentation is highly desirable for optimal interpretation of results. Perhaps the most prevalent means for

achieving nutritional control is through the inclusion of pair-fed dams as an explicit control group. Pair feeding is generally performed by restricting the caloric intake of the control animals to match that of the analogous experimental animals. It should be pointed out, however, that pair feeding simply assures that the diets of experimental and control mothers are calorically equivalent; the possibility remains, nonetheless, that offspring may be behaviorally impaired to some extent in both groups since malnutrition alone may act as a behavioral teratogen (cf. Altman, Sudarshan, Das, McCormick, & Barnes, 1971; Joffe, 1969; Mathura & Harper, 1979). The simplest control for this eventuality is the incorporation of untreated controls. This, of course, is far from a perfect control for evaluating the extent of nutritionally-induced impairments, since in many cases, such factors as treatment stress may be confounding elements. At the least, however, this procedure permits the separation of drug-induced alterations in offspring behavior from those produced by the nondrug aspects of treatment (e.g., handling, injection or intubation, dietary deficiencies, etc.).

Where pair-fed controls are employed, it is probably best to match groups on the basis of volume of ingestion (or injection) as well as caloric content. The primary reason for this is that large differences in liquid intake may produce differences in fluid balance in the mother and the fetus (Havlena & Werboff, 1963; Joffe, 1969) which alone could account for behavioral inequalities. Given the ready availability of commercially prepared ethanol and control diets, and the ease of preparation of isocaloric injections, there is little reason to neglect nutritional controls.

Mode of Ethanol Administration

Although the majority of the studies reviewed here employed an oral route of administration of the ethanol and control dosages, a number of investigators have used parenteral injection. This has prompted discussion regarding the relative importance of duplicating, in animal models, the pattern and mode of intake common to the human alcoholic. Proponents of this kind of duplication argue that it is necessary to mimic the mode and duration of human alcohol consumption in order to delineate fully the etiology, prevention, and cure of the disorder (e.g., Chernoff, 1977). Yet, there are clearly limitations to this approach, since no route of administration is expressly superior. Intravenous and intraperitoneal administration of solutions, while allowing relatively precise control over dosage, are generally stressful procedures for the recipient animals and have been eschewed on the basis of their dissimilarity to normal human modes of ingestion. Furthermore, intraperitoneal administration of ethanol to the gestating mother may represent an actual threat to the integrity of the uterus or the physical well-being of the fetus (Havlena & Werboff, 1963; Warren, 1977). Oral routes, on the other hand, while representing a greater degree of similarity to the human analog, present their own complications. Gastric intubation permits regulation of amounts ingested, but sacrifices control over effective dosages since gastric absorption of ethanol is relatively variable, and influenced by a number of extraneous factors (Kalant, 1971). Like parenteral administration, intubation is also generally a stressful procedure for most animals. Because maternal stress may, by itself, have deleterious effects on the subsequent be-

havior of offspring (Joffe, 1969), it is obviously best to minimize the amount of stress that dams are exposed to during pregnancy. On the other hand, allowing animals free access to liquids or liquid diets containing ethanol is often objectionable on the grounds that comparatively little experimenter control can be exercised over the amount of alcohol ingested, and often between-subject variablility in consumption is exceedingly large (cf. Abel, 1979). It would appear, then, that a precise animal analog to human alcohol consumption in studies of fetal alcohol's effects is not only extremely difficult to obtain, but may, in some cases, actually be undesirable. In any case, each mode of ethanol administration has both advantages and disadvantages, and therefore particular routes must be selected based upon the needs at hand.

A more immediate issue in attempts to duplicate human parameters of the fetal alcohol syndrome in animals is that of dosage. Warren (1977) has pointed out that comparisons between humans and other species are best made on the basis of blood alcohol concentration (BAC). It should be evident, however, that due to differences between species in such factors as rate of ethanol metabolism and adipose to lean body mass ratios (cf. Kalant, 1971), simple duplication of human BAC curves in animals is unlikely. Thus, for example, it may be possible to achieve peak levels in rats similar to those achieved by the human alcoholic, but without a complex dosing schedule, it is doubtful whether the duration of action will be comparable. Given the likelihood that both peak levels and duration of action may be important in producing adverse consequences in the fetus, extremely complicated dosing regimens may

be required to create a precise analogy. Again, however, a precise analogy may, in many cases, be unnecessary, since a number of animal studies have demonstrated alcohol-induced fetal abnormalities similar to those found in the fetal alcohol syndrome using relatively simple dosing schedules. It should be pointed out, however, that although corresponding time-response relationships in BAC may thus be unnecessary, careful monitoring of BAC values does permit a better evaluation of the actual differences between species.

Offspring Survival and Physical Status

A final point which is of particular relevance to behavioral studies concerns the survival rates of offspring treated prenatally with alcohol. In most cases, behavioral comparisons between experimental (alcohol-treated) and control animals are best made when survival rates (e.g., live birth rate) of the two groups are not appreciably different. If the drug treatment induces excessive prenatal mortality, the behavior patterns of the survivors may be more indicative of selection based on certain physical characteristics (e.g., "survival of the fittest") than of the drug's effect on the general functional abilities of the population.

Directly related to the issue of survival is the matter of the overall physical status of offspring. As Abel (1980) has pointed out, behavioral deficits in offspring with gross motor defects cannot easily be attributed to any one underlying construct, since behavioral tasks are often sensitive to differences in motivational conditions, emotional factors, and learning/memory function, as well as to disruption resulting from simple impairment of performance capabilities. Furthermore, less

dramatic physical effects of prenatal ethanol exposure could also have profound behavioral consequences. For example, reduced size and body weight may produce behavioral alterations via any one of several mechanisms (cf. Abel, 1980). Consequently, before behavioral differences can be attributed unambiguously to learning dysfunctions, sensory deficits, altered motivation, and so forth, it is generally necessary to conduct several investigations, each designed to test for the presumed causal factors.

Literature Review

Studies of the behavioral consequences of prenatal exposure to alcohol may loosely be divided into two categories: learned behaviors and unlearned behaviors. The latter category refers to those behaviors generally thought of as "innate," and includes unlearned emotional and social behaviors and simple behavioral indices of development. Learned behaviors, on the other hand, are those in which the animal demonstrates a change in responding based upon experience with a given set of contingencies. Typically, learned behaviors are studied using classical (Pavlovian) or operant conditioning techniques, whereas unlearned behaviors are simply observed in standardized settings, for example in an open field, or a social-interaction situation. While the distinction between learned and unlearned behaviors is clearly not perfect, this classification will adequately facilitate discussion of the behavioral studies to be reviewed here.

Unlearned Behaviors

Demers and Kirouac (1978) investigated the effects of ethanol administered prenatally on the subsequent development of a number of neuromotor responses in rat pups. Ethanol was administered to pregnant females through

an i.v. cannula in a dose of 1.2 g/kg/day on gestation days 5-7 and 1.5 g/kg/day on days 8-18 of the 21-day gestation period. Controls received an equivalent volume of physiological saline, or no treatment. Shortly after birth, offspring were cross-fostered to nontreated mothers and developmental measures were studied in a daily open field test until the pups were 21 days old. Pups treated prenatally with alcohol were found to reach a performance criterion at a significantly later mean day than saline or untreated controls on the following developmental measures: elevation of the head, elevation of the forelimbs and shoulders, elevation of the hindlimbs and pelvis, negative geotaxis, ascending a wire-mesh surface, and walking. Additionally, offspring of ethanol-treated dams were retarded in ability to right in mid-air when compared with the offspring of saline-treated mothers, but not when compared to the untreated controls. No between-group differences were found in measures of pivoting, crawling, head-pointing, cliff avoidance, righting on a surface, or rearing without support. According to the authors, these data support the contention that prenatal treatment with ethanol slows down the maturation of the central nervous system (CNS).

A problematic feature of the Demers and Kirouac (1978) study is the comparatively low survival rate of the ethanol-treated offspring. While none of the untreated offspring and 4 of 14 (29%) of the saline controls were found dead at birth, 13 of 23 (57%) of the alcohol-treated offspring were born dead. An additional experimental female spontaneously aborted on Day 11 of gestation. Although this high mortality rate is, in itself, suggestive of ethanol's teratogenic properties, as mentioned earlier, behavioral data must be interpreted cautiously when survival

rates of experimental and control groups differ significantly.

Yanai and Ginsburg (1977a), also studying developmental responses, allowed ad lib access to 10% (v/v) ethanol in a sweetened water solution to two strains of mice (DBA and C57) before, during, and for 14 days after pregnancy, as their sole drinking fluid. The solution, which was administered to both parents, was consumed in doses ranging from 10 to 15 g/kg of body weight per day at mating, and 12 to 38 g/kg/day during nursing. Control animals received sweetened water or tap water as their only liquid supply. Although litter size and survival rate were not significantly different for any of the groups, and there were no differences between ethanol and control offspring in the age at which hair appeared or in measures of startle and righting responses, the eyes of alcohol-treated DBA offspring opened reliably later than the controls'. The development of the ear was also reliably delayed in DBA offspring exposed to ethanol. These differences were not found in C57 offspring. Despite their careful monitoring of liquid consumption and maternal weight gain, Yanai and Ginsburg (1977a) made no attempt to restrict experimentals and controls to equivalent intakes of food and fluid in terms of calories or volume. Thus, as the authors point out, nutritional factors cannot be ruled out in evaluating the reported developmental differences between ethanol-treated offspring and controls.

Yanai and Ginsburg (1976) examined seizure susceptibility, administering ad lib mouse chow and water containing 10% (v/v) ethanol to parent mice of strains C57 and DBA up through Day 14 postpartum. During breeding

and pregnancy, the mean daily intake of ethanol was 15 g/kg for the C57 females, and 13 g/kg for DBA females. These amounts increased following delivery. A nutritional control group was fed a restricted amount of solid food designed to match the food intake of ethanol-treated mice, and a restricted amount of glucose solution equicaloric to the ethanol solution. A second control group was simply allowed ad lib access to mouse chow and tap water. When tested for susceptibility to audiogenic seizures starting at 29 days of age, a reliably higher percentage of C57 offspring whose parents drank alcohol exhibited seizures during the four day test period, when compared to either control group. Additional C57 alcohol offspring tested at 45 days of age also showed a higher incidence of seizures. For DBA mice, nearly all of the subjects in all groups experienced at least one seizure during the four day test phase, although the offspring of ethanol-treated parents had a reliably greater incidence of audiogenic seizures on the first day than either control group. The authors suggest that the increased susceptibility to seizures was due to direct effects of ethanol on the CNS, possibly the result of an ethanol-induced long-lasting hyperexcitability. Nevertheless, an indirect effect mediated by malnutrition cannot be ruled out entirely, since significantly more DBA nutritional control animals exhibited seizures on the second and third days of testing than untreated offspring (C57 nutritional controls did not differ from untreated controls).

Although Buckalew (1977) also reported an increased incidence of seizures in rats exposed to ethanol during development, a number of methodological difficulties make this and other findings of the study

difficult to interpret. Female rats were exposed to a 5% ethanol and water solution throughout pregnancy and up to weaning of the offspring. This resulted in a mean daily intake of approximately 40 ml of solution. While ethanol-treated offspring were reported to be growth retarded, hypersensitive to auditory stimuli, and limited in curiosity and exploratory behavior, it is not clear what control groups, if any, were used for comparisons. Indeed, no quantitative data or statistical analyses were offered in support of the observations and thus the results of the study must be considered cautiously.

Martin, Martin, Sigman, and Radow (1978) administered ethanol by intubation twice a day in a total dose of 8.5 g/kg/day to pregnant rats throughout the entire gestational period. Control dams were intubated with an equivalent volume of vehicle (saline controls) or were not intubated (no treatment controls) and were fed the mean amount of lab chow consumed on a given day by the alcohol animals. At birth, the litters of half of the ethanol-treated dams and half of the saline dams were cross-fostered to no treatment dams. There were no differences among the three groups in live litter size or weight at birth, although ethanol-treated mothers had reliably longer gestational periods. Developmental measures indicated no differences among treatment groups in righting response (Day 3), incisor eruption (Day 5), or distance travelled in a forward direction within a 5-sec time period (Days 7, 11, 15). However, ethanol offspring were found to be significantly delayed in ear-flap uncurling when the data for cross-fostered and mother-raised offspring were combined for comparison of the two treatment groups.

Moreover, alcohol offspring raised by their own mothers were significantly more retarded in eye opening than the alcohol cross-fostered or saline offspring. As the authors point out, this finding lends support to a hypothesis of developmental delay due to effects of ethanol on the mother. Nevertheless, a test of locomotor activity was interpreted as demonstrating a transplacental effect of alcohol in utero since offspring of ethanol--treated mothers were found to be significantly more active in an activity wheel than offspring of saline-treated mothers, and there was no effect of fostering.

Martin et al. (1978) have incorporated a number of important controls into their study. As is suggested by the results, the use of cross-fostering procedures is of utmost importance in behavioral studies, since pre- and postnatal factors are otherwise confounded. The nutritional control used by Martin et al. ensured that all groups received approximately the same number of lab chow-derived calories, although the extra calories provided by ethanol to the experimental animals were apparently not controlled for.

Perhaps the most pertinent question to arise in regard to the methodology of Martin et al. (1978) is in reference to the mode of ethanol administration. Presumably, the intubation procedure, like other forced administration methods, produces a significant amount of stress in the recipient animal. Since there is evidence suggesting an interaction of prenatal ethanol and stress (cf. Morra, 1969), the role of intubation-induced stress cannot be overlooked in the present study. It is likely that inclusion of behavioral data from untreated animals would help clarify the role of stress in studies utilizing forced administration procedures.

Hyperactivity in offspring exposed to alcohol prenatally has been reported relatively frequently in the animal literature, and it has been suggested that this hyperactivity may be analogous to the hyperactivity often reported in children of alcoholics (cf. Martin et al., 1978). Bond and DiGiusto (1976) used an open field test to monitor activity of rat pups whose mothers were exposed to ethanol. Specifically, pregnant females received a liquid diet consisting of 6.5% ethanol (95% v/v) and other sources of nutrition throughout their pregnancy, whereas control animals received lab chow. The average daily dose of ethanol consumed by experimental mothers was 14.01 g/kg. Following delivery, pups remained with their biological mothers until weaning (age = 25 days) at which time the offspring were housed individually. When rated for ambulation and rearing in the open field, starting 45-50 days after birth, the offspring of ethanol-treated mothers displayed significantly greater activity than the untreated controls on each of the 3 test days. In addition, in an alcohol preference test begun at 65-70 days of age, pups whose mothers had been exposed to alcohol consumed significantly more ethanol over the 6 experimental days than did the control animals. In a longitudinal extension of their open field findings, Bond and DiGiusto (1977b) treated experimental and control mothers in a manner identical to that of their 1976 study, and tested offspring in the open field at 28, 56 and 112 days of age. Although the average daily dose consumed by ethanol mothers was slightly less than in the earlier study (13.3 g/kg), the ambulation scores of the experimental groups were still found to be higher than those of controls. This result was apparent on Days 28 and 56, but not on Day 112. Because they found an effect of prenatal alcohol on

activity, but not on defecation in the open field, Bond and DiGiusto suggested that alcohol's impact was independent of changes in emotional responding, and speculated that alcohol might exert its influence by temporarily altering the development of neurochemical systems in the brain. It must be pointed out, however, that neither nutritional controls nor fostering procedures were employed in either of these studies, and thus the outcomes cannot be considered to be due unequivocally to alcohol.

Branchey and Friedhoff (1976) also studied the effect of prenatal exposure to alcohol on open field behavior. From the 10th day of pregnancy until delivery, experimental rats received a liquid diet of ethanol and liquid Metrecal, whereas nutritional controls received Metrecal and an isocaloric replacement of sucrose for ethanol. Both groups were restricted to equal volumes of their respective mixtures, and the daily ethanol dose taken by experimental subjects was approximately 12 g/kg. At birth, the pups remained with their biological mothers. Animals exposed prenatally to ethanol were reported to be significantly more active than controls in an open field test 23 days after birth. There were no differences between groups in number of risings on hind legs or groomings, or in measures of urination and defecation.

The results of open field testing in mice exposed to alcohol during early development reported by Ginsburg, Yanai and Sze (1975) are contrary to most of the other activity studies reviewed here. As in their other studies, Ginsburg et al. allowed DBA and C57 mice ad lib access to 10% v/v ethanol in water as the sole fluid supply throughout pregnancy and until

their litters were 14 days old. Control animals were given mouse chow and water ad lib. Although ethanol-treated pups were reported to be normal physically, when tested in the open field, offspring of C57-strain alcohol-treated parents had lower ambulation scores than controls on all four days of testing and a longer latency to leave the starting section on the final test day. Offspring of ethanol-treated DBA mice differed from controls only on the fourth day of testing, and then only on the ambulation score. No differences in number of defecations were found in either strain. The finding of decreased activity by experimental offspring is in striking contrast to the majority of studies in which open field activity was recorded. There are, however, at least two noteworthy differences between the Ginsburg et al. (1975) study and the other investigations. In those studies in which increased activity was reported (Bond & DiGiusto, 1976, 1977b; Branchey & Friedhoff, 1976; Martin et al., 1978), the subjects were rats, as opposed to mice used by Ginsburg et al. Additionally, Ginsburg et al. are apparently the only investigators of those studying activity to continue administering ethanol via the mother's milk (during lactation), that is, during early postnatal development. That residual effects of postnatal exposure to ethanol can be important in behavioral outcomes is made evident by the audiogenic seizure studies reported by Ginsburg et al. (1975). In these studies, mice which received alcohol prenatally responded with an increase in seizure incidence only if they also received alcohol postnatally via the mother's milk, even though they were tested at least 15 days after weaning. Thus, it is not entirely clear whether the differences reported as a result of this mode of exposure to ethanol reflect differences in species-specific

responses, differences in the timing of ethanol's effects on certain developing systems, differences in maternal response, or some combination of these.

Another study of prenatal alcohol's effect on activity in the open field was performed by Abel and York (1979) using much lower doses of ethanol than those employed in the previous studies. Groups of pregnant rats were intubated with either 1 g/kg or 2 g/kg of ethanol (as a 20% w/v solution in .9% NaCl) each day throughout pregnancy. Individual control groups were included for each of the ethanol--treated groups, and were intubated with a sucrose solution isocaloric to the solution administered to their ethanol-treated counterparts. The daily food allotment of controls was also matched to that of their paired experimental animals. At birth, pups were removed from their natural mothers and cross-fostered to untreated control mothers. In open field and free field tests begun when the pups were 75 days old, there were no differences between alcohol- and saline-treated offspring in latency to move, ambulation or rearing in the open field, or latency to contact food and latency to eat in the free field. Furthermore, at 5 months, there were no differences between groups in ethanol preference. The authors suggest that the lack of effect may have been due to the low doses of ethanol used.

A study of aggression was conducted by Elis and Krsiak (1975), who administered ethanol by oral intubation in a daily dose of 1 g/kg to pregnant mice throughout the entire gestational period, while tap

water was administered to controls. No differences were found between the groups in duration of pregnancy or in number of offspring delivered. The offspring were raised with their biological mothers for 5 weeks, and then separated according to sex. Approximately 5 months after birth, paired interactions of singly-housed male mice with group-housed male mice were observed in offspring of both alcohol- and water-treated animals. Frequency of occurrence of 12 motor acts and postures was recorded within the following categories: sociable activities, timid activities, aggressive activities, and individual activities. The offspring of ethanol-treated females were reported to exhibit significantly more aggressive behaviors than control animals, a finding which the authors speculate may have been due to ethanol-induced changes in activity of the catecholaminergic system of the brain. However, Krsiak, Elis, Poschlova, and Masek (1977), using the same procedure for ethanol administration, replicated the behavioral findings of Elis and Krsiak (1975), but found no differences between offspring of ethanol- and water-treated mothers in brain concentrations of norepinephrine and dopamine, although ethanol mice had significantly lower levels of brain serotonin.

Contrary to the results of Elis and Krsiak (1975) and Krsiak et al. (1977), Yanai and Ginsburg (1977b) reported <u>decreased</u> aggression in DBA and C57 mice following early exposure to alcohol. Experimental mothers were allowed ad lib access to 10% ethanol (v/v) in water, while controls were restricted in their intake of glucose solution and solid food to amounts equicaloric to that of experimentals. These diets were

maintained throughout pregnancy and until 14 days postparturition, with daily ethanol dosage for the experimental mothers reported as 10-15 g/kg at mating (resulting in peak blood ethanol concentrations of 45 mg% at night, dropping to zero during the day) and 12-28 g/kg during nursing. Other groups of mothers received the ethanol diet only during pregnancy or only after pregnancy. Male offspring were isolated in individual cages beginning on Day 28, and tested in a paired-fighting situation on Days 50, 51 and 52. Latency to first attack, number of tail rattles, number of attacks, and other measures of aggression were recorded. During testing, offspring treated with ethanol both pre- and postnatally demonstrated an increased latency to attack and a significant reduction in the number of observation periods in which fighting occurred when compared to the pair-fed nutritional controls. Interestingly, offspring which received ethanol only prenatally had scores similar to the controls, while postnatally treated offspring were more similar to the pre- and post ethanol group. The authors therefore conclude that the sensitive period for ethanol-induced decreases in aggression is postnatal, a suggestion which may partially explain the difference between the outcome of their study and that of Krsiak et al. (1977) in which ethanol was administered prenatally only. It is also important to note that the dose of ethanol administered by Elis and Krsiak (1975) and Krsiak et al. (1977) was considerably lower than that administered in the majority of studies reviewed here. Obviously, this difference prevents direct comparison of the studies, but could be partially responsible for the difference.

Ewart and Cutler (1979) administered ethanol in a 5% solution to male and female mice as the sole drinking fluid prior to mating, throughout pregnancy and lactation, and to the experimental offspring until adulthood. Control mice received tap water as the sole drinking fluid, and no attempt was made to match the groups nutritionally. While no congenital malformations were observed, at one day of age, the alcohol-treated pups weighed significantly less than controls, a difference which was still reliable at 18-24 weeks. Additionally, ethanol animals were significantly retarded in regard to the age at which fur appeared, although the age at which eye opening took place was unaffected by treatment. The pups were weaned at 21-30 days of age and their behavior was examined by ethological techniques when they were 3-4 weeks old, and again when they were 18-24 weeks of age. In paired interactions, the frequency and duration of social investigation was reported to be significantly lower in alcohol-treated juvenile mice than in untreated controls. As a consequence, the amount of time spent in nonsocial behaviors was increased in the alcohol-treated animals. There were no between-group differences in flight or aggression behaviors in the juvenile mice. In adulthood, the only difference between the alcohol-treated and control mice was an increased frequency of flight elements in males of the treated group. The authors caution, however, that postnatal behavioral effects of early exposure to alcohol such as they have reported may arise out of alterations in maternal behavior or an impairment of lactation as well as from direct effects on the fetus. In addition, the impact of this study is tempered by the lack of fostering and nutritional controls, and by a lack of information regarding levels of

ethanol intake. Furthermore, it is difficult to determine whether the social interaction differences were the result of prenatal exposure to alcohol or to a more 'acute' effect of alcohol, since experimental animals were given ethanol as their drinking fluid following weaning.

Summary of studies on unlearned behaviors. Although certain methodological insufficiencies preclude a definitive statement, the data do suggest that prenatal alcohol may exert a number of effects on subsequent unlearned behaviors. Developmental responses have been shown by various investigators to be retarded significantly by prenatal ethanol exposure (Demers & Kirouac, 1978; Martin et al., 1978; Yanai & Ginsburg, 1977a), although not all developmental responses are affected equally (e.g., Martin et al., 1978). Another commonly used assessment technique, activity level, has also proven to be sensitive to the effects of prenatal ethanol. At least four experiments with rats whose mothers were fed alcohol during gestation show that prenatal exposure to alcohol enhances activity levels during postweaning tests (Bond & DiGiusto, 1976, 1977b; Branchey & Friedhoff, 1976; Martin et al., 1978), although decreased activity has also been reported, in mice (Ginsburg et al., 1975). Tests of aggression and social interaction, while occasionally contradictory, appear also to be subject to the effects of prenatal alcohol. It should be noted, however, that the studies reviewed here differ from each other in a number of important respects, such as dose of ethanol, time of administration, mode of administration, etc., and thus any general statement must be made cautiously.

In summary, studies with animals have generally been found to confirm

the correlation observed in the case of human infants between prenatal exposure to alcohol and subsequent growth deficiency and malformation (cf. Warren, 1977). Furthermore, although the lack of necessary control groups in many studies makes general conclusions tentative, there are a number of studies which suggest a fetal alcohol effect on certain unlearned behaviors in animals which coincides with a similar general effect also noted for human offspring of drinking mothers (e.g., Landesman--Dwyer, Keller & Streissguth, 1978).

Learned Behaviors

The foregoing studies of effects on naturally occurring or 'unlearned' behaviors suggest that there may be important functional deficits attributable to prenatal alcohol exposure, even in the absence of obvious dysmorphology. Another class of studies has been concerned with the effects of prenatal exposure to alcohol on the ability of the organism to adapt appropriately to significant changes in its environment, that is, with effects on learning.

One of the few studies of the effects of prenatal alcohol exposure on learning to be published before the recent revival of interest in this topic involved the use of chicks as experimental subjects (Fletcher, Cowan & Arlitt, 1916). In these studies, five (or fewer) drops of 95% ethanol were injected into the air chamber of fertilized hen's eggs immediately prior to the onset of incubation. (Sandor and Elias, 1968, have since shown that this method of administration results in gradual penetration of ethanol into the egg.) Control eggs were either injected

with distilled water, simply pierced with a needle, or were not treated at all. At various times after hatching, the chicks were examined for effects on both "inherited and acquired reactions," with visual choice discrimination and maze learning included in the latter category. Although a number of behavioral differences were found between alcohol chicks and untreated chicks (e.g., alcohol chicks were slower to show visual choice discrimination learning), comparisons between alcohol chicks and distilled-water or pierced-shell chicks indicated no effects attributable to alcohol per se.

Studies performed more recently have used rats almost exclusively as experimental subjects, although modes of ethanol administration and learning tests have varied considerably. Phillips and Stainbrook (1976) allowed female rats access to chablis wine as their only source of fluid during mating, pregnancy and lactation, while control animals were given tap water. While there were no differences between groups in size of litter, pups whose mothers drank wine weighed significantly less at 25 days of age, but not at 50 days. It is not clear, of course, whether this difference was the result of direct prenatal or postnatal effects of alcohol on the pups, or whether it was due to effects on the mother (e.g., interference with lactation).

At 101 days of age, following 10 days of pretraining, the offspring began a visual discrimination task involving the formation of learning sets. In essence, the task required that the animal approach the one stimulus among four target stimuli which was different from the other three. Correct responses were rewarded with a food pellet. Over a series

of nine problems, the animals whose mothers drank wine were reported to perform at a significantly lower level than the control animals, although both groups improved over trials. It must be cautioned, however, that given the small magnitude of the effect, and the failure to specify dose levels or to include nutrition and fostering control procedures, these results should be interpreted with discretion.

Abel (1978) examined the effects of prenatal exposure to low doses of alcohol on later learning, using a number of different behavioral tasks. Throughout pregnancy, female rats were intubated daily with either 1 g/kg or 2 g/kg ethanol in a .9% NaCl solution (20% w/v ethanol), doses substantially lower than those used in most other studies. A control group was included for each of the two ethanol-treated groups, and was intubated daily with a sucrose solution which was isocaloric with the ethanol solution (pair-fed controls), while an additional control group was left untreated. Shortly after birth, pups were assigned to untreated surrogate mothers. Although the weights of ethanol-treated litters were not significantly different from their respective pair-fed control groups, reliably fewer live pups were born to ethanol-treated mothers and high-dose control mothers than to low-dose or untreated controls. Furthermore, in a test of maternal behavior conducted shortly after birth, dams treated with 2 g/kg of ethanol took significantly longer to retrieve a separated pup than their pair-fed controls. In behavioral tests beginning when the pups were 75 days of age, Abel found no differences between alcohol and control offspring in a one-way shock avoidance task, water-maze escape learning, or brightness discrimination learning in a T maze.

Abel (1979) investigated the effects of higher doses of ethanol than those used in his previous study, reasoning that the earlier doses may have been below the threshold necessary for demonstration of a behavioral effect. In a preliminary experiment, pregnant and nonpregnant rats were intubated with 2, 4, or 6 g/kg of ethanol (30% w/v), and peak blood alcohol levels and rate of blood alcohol disappearance were determined. The results indicated that following a low dose of ethanol, blood alcohol levels rise higher in nonpregnant rats than in pregnant rats, but at higher doses, this relationship is reversed. Furthermore, at the high dose, blood alcohol concentrations were found to remain at peak levels for nearly 6 hr in the pregnant rats compared to 2 hr for nonpregnant rats.

In a second study, designed to test the effects of prenatal alcohol on learning/memory performance, pregnant experimental animals were intubated daily with either 4 or 6 g/kg of ethanol (30% w/v) whereas pair-fed controls were fed in a manner analogous to that used by Abel (1978). A third control group received neither drug nor vehicle and had no dietary restrictions (untreated controls). At birth, pups were assigned to untreated surrogate mothers and were weaned at 21 days of age. Behavioral testing began when the offspring were 5 months of age. At that time, both male and female offspring of mothers treated with alcohol were found to weigh significantly less than pair-fed controls. Moreover, female, ethanol-treated offspring performed reliably worse than their pair-fed controls (also females) over the five days of a two-way shock avoidance task, exhibiting a significantly lower percentage of trials with an avoidance response and a significantly longer avoidance latency.

Abel suggests that these differences were not the result of differences in general motor activity since both experimentals and controls had an equally low number of intertrial interval responses. Not ruled out, however, was the possibility that experimental offspring, for whatever reason, were simply less responsive to the conditioned stimulus (e.g., due to sensory deficits). Unfortunately, this is a difficult possibility to control for in the context of learning paradigms such as those employed by Abel. Most likely separate experiments would have to be conducted to investigate individual hypotheses in this regard. In a final behavioral task, Abel found no group differences for either sex in a water-maze escape task.

A number of experiments performed by Riley, Lochry, Shapiro, and Baldwin (1979b) and Riley, Lochry and Shapiro (1979a) indicate that prenatal exposure to ethanol may disrupt a rat's ability to inhibit responding. In the first set of experiments (Riley et al., 1979b), pregnant rats consumed liquid diets containing 0, 17, or 35% of their total calories as ethanol from Day 5 of pregnancy to Day 20. The three liquid diets were isocaloric and consisted of ethanol and/or sucrose and other sources of nutrition. The animals were pair fed daily (based upon the intake of the 35% group), thus matching volume of intake as well as caloric content. Mothers on the 35% diet consumed an average of 12.96 g/kg of ethanol daily and those on the 17% diet consumed 6.66 g/kg/day. While there were no differences between groups in gestation length or litter size, offspring of mothers that consumed the 35% diet weighed significantly less than the 0% offspring from birth through 15 days of age. At birth,

pups remained with their biological mothers and behavioral testing was begun 20-21 days after birth. In a T-maze shock-escape paradigm in which only female offspring were tested, no differences were reported among the three treatment groups in first-trial latencies or trials to criterion during acquisition. However, the high-dose alcohol offspring made significantly more incorrect choices than the 0% controls during reversal learning. Additionally, in another study in this set of experiments, it was found that alcohol offspring required more trials before alternating in a test of "spontaneous alternation."

Riley et al. (1979a) provided pregnant rats with liquid diets similar to those of Riley et al. (1979b), with ethanol representing 0, 8, 19, or 32% of the total calories. The diet was given on Days 6-16 of gestation with animals pair fed to provide caloric equivalence, although again, no cross-fostering procedures were utilized. Daily maternal alcohol consumption averaged 14.30 g/kg for the 32% mothers, 8.49 g/kg for the 19% mothers, and 3.57 g/kg for the 8% females. No differences were observed between groups in length of gestation, litter size, weight of litters at birth and at 20 days of age, or in morphological appearance of the pups. A passive shock-avoidance test conducted with female offspring at 18 days of age disclosed a significant difference between the treatment groups in mean number of trials to criterion, with a reliable trend indicating a dose-related impairment in passive-avoidance learning. Additional male animals from the 32% group tested at 41-53 days of age were also found to be significantly impaired in passive-avoidance learning when compared with 0% animals tested

at the same time. In a second experiment, Riley et al. fed pregnant rats on Days 6-20 of gestation with liquid diets identical to those used by Riley et al. (1979b) and reported weight differences in the offspring similar to those described above. Taste aversion conditioning, in which female pups became ill after drinking a lithium chloride solution, took place when the offspring were 20-31 days of age. Although on the first test day the data indicated a linear trend with the 35% group demonstrating impaired taste aversion learning graphically, it is not clear whether the difference between the 35% and 0% groups was statistically significant. Thus on the basis of the information presented, it is inappropriate to conclude that prenatal alcohol had any effect on taste aversion learning.

In a study lacking in both nutritional and fostering/cross-fostering control procedures, Bond and DiGiusto (1977a) examined the effects of prenatal exposure to alcohol on active shock-avoidance learning. Pregnant rats were fed a liquid diet containing 35% ethanol-derived calories throughout pregnancy only, while control animals received lab chow and water. The average daily dose of ethanol taken by the experimental animals was reported as 13.3 g/kg. At birth, the pups were reared with their biological mothers until weaning, and at 112 days of age, they were tested for avoidance learning in a 2-way shuttle apparatus. In a measure of the number of trials with a successful avoidance response, alcohol offspring were found to be significantly impaired in avoidance learning with respect to the untreated controls, even though there were no differences between the groups in overall activity during a 5-min exploratory period. Again,

however, differences resulting from between-group differences in sensitivity to the buzzer conditioned stimulus (CS) cannot be ruled out, and thus the results may not be attributable to impairment of learning per se.

In another study of shock-avoidance conditioning in rats exposed prenatally to alcohol, Bond and DiGiusto (1978) confirmed and extended the findings of their earlier study, employing, in this case, fostering/cross-fostering controls. Experimental rats were fed a liquid diet containing 6.5% ethanol (95% v/v) throughout pregnancy, and consumed an average daily dose of 12.6 g/kg, whereas controls received lab chow. At birth, litters were either left with their biological mothers, fostered to mothers of the same group, or cross--fostered to mothers from the other group. Shock-avoidance testing in a two-way shuttle box and appetitively motivated Hebb-Williams maze testing began when the offspring were 150 days of age. Although there was no effect of postnatal rearing, and no effect of treatment on Hebb-Williams maze responding, offspring of alcohol-treated mothers avoided shock on significantly fewer trials than control offspring. Because the two groups reached different asymptotes of responding after 40-50 trials, the authors suggest that prenatal ethanol caused a distinct deficit in learning capability rather than a simple retardation or slowing of learning.

In interpreting results of learning studies such as those reported by Bond and DiGiusto, it is important to realize that many behavioral differences result from impaired motor coordination or physical develop-

ment. As Abel (1980) has pointed out, tests that measure speed of responding or that involve relatively complex motor skills are particularly susceptible to between-group differences in general motor capabilities which may be misinterpreted as differences in learning. Directly related to this is the possibility that ethanol-induced sensory impairments may mimic impairment in learning. As indicated earlier, these factors are difficult to control for, and thus the likelihood of their relative contributions to behavioral studies of prenatal alcohol's effects should always be assessed.

Martin, Martin, Sigman, and Radow (1977) examined the effect of exposure to alcohol during gestation and nursing or just during nursing on operant performance in both appetitive and aversive paradigms. Pregnant rats received a solution of 20% ethanol in a sweetened solution as their sole fluid during pregnancy and lactation. In addition, during pregnancy only, they received two .5-g/kg injections of 10% (w/v) ethanol in normal saline, subcutaneously. A separate group of rats was provided with ad lib food and water throughout pregnancy, but during lactation, received the sweetened ethanol solution as the sole fluid, and two daily injections of ethanol. Two control groups were pair fed the solution vehicle and sucrose pellets in amounts calculated to be isocaloric to the intakes of the analogous experimental groups. The controls were also injected with normal saline twice daily at the same times as the comparable experimental groups. For animals treated with alcohol during both pregnancy and lactation, the mean daily dosage of ethanol was 11.8 g/kg during pregnancy and 15.3 g/kg during lactation, and for those treated during lactation only, the dose was 14.2 g/kg/day. Pups which had received alcohol during gestation and nursing had a significantly longer gestation period than their pair-fed controls,

and were reliably growth retarded for over 2 months following birth, in spite of the fact that control mothers lost significantly more weight than experimental dams during the 23-day lactation period. There were, however, no differences in litter size or in righting ability and earflap uncurling on Day 4, and lower incisor eruption on Day 5.

In operant responding sessions begun when the offspring were 90 days old, rats that had been exposed to ethanol both in utero and during nursing, earned fewer reinforcements on continuous and fixed ratio schedules (barpressing for food reward) and more reinforcements on a DRL schedule, whereas rats exposed to alcohol just during nursing performed poorly only on fixed ratio schedules. These differences disappeared with continued training. Additionally, tests begun when the rats were 8 months of age revealed no consistent effect of treatment on barpress shock escape-avoidance or resistance to punishment, although offspring treated both pre- and postnatally were reported to be deficient in the ability to discriminate contingencies on punishment schedules. Martin et al. suggested that the occasionally less efficient performance of the alcohol offspring might have reflected a learning deficit, but conceded that alternative accounts were available (e.g., a general response inhibition due to enhanced emotionality---although this would not be consistent with the conclusions of Bond & DiGiusto, 1977b).

Finally, Harris and Case (1979) administered liquid diets containing 5.5% (v/v) ethanol or a calorically equivalent sucrose solution to pregnant rats before, during and after pregnancy until the pups were weaned. The ethanol-treated dams were given unlimited access to the liquid diet,

while sucrose controls were pair fed by giving each animal a volume of solution equal to the average volume consumed by ethanol animals on the preceding day. An untreated control group was also included. Because diets were continued after birth, no attempt was made to implement cross-fostering procedures. The approximate ethanol consumption of experimental dams was reported to be 13 g/kg/day. At 14 and 21 days of age, offspring of mothers treated with ethanol were found to weigh significantly less than their pair-fed counterparts, and offspring of sucrose-fed mothers weighed reliably less than untreated control offspring at birth and at ages 7, 14 and 21 days. When tested in a one-way shock-avoidance task at 21 days of age, pups from mothers consuming alcohol performed with a significantly shorter avoidance latency than pair-fed controls, but when retested at 28 days, however, there was no difference between the two groups. At 45 days of age, male pups were trained to respond on a fixed-ratio (FR) schedule, and rates of responding were recorded over 15 FR sessions and 4 extinction sessions. Although the response rates of the sucrose group were reliably higher than those of the untreated controls, there were no differences between ethanol- and sucrose-treated offspring. Furthermore, in a test of sensitivity to acute ethanol administration conducted when the pups were 45 to 50 days of age, females from each group were injected i.p. with ethanol and the duration of loss of righting reflex was measured. No between-group differences in response to ethanol were noted.

It is important to note that the only behavioral difference between the ethanol and pair-fed pups occurred (in the shock-avoidance paradigm) at a time when the lactating mothers were still permitted

access to the liquid diets. Since ethanol is readily secreted with the mother's milk (Kalant, 1971), it is certainly possible that the noted behavioral difference was the result of acute effects of alcohol on the experimental offspring rather than an actual prenatal effect of alcohol exposure.

Summary of studies on learned behaviors and general discussion. The majority of the published studies of prenatal alcohol's effects on learning may be divided into two general behavioral categories: discrimination studies and avoidance studies. Of the three investigations of embryonic alcohol's effect on visual discrimination, two reported no effect of alcohol per se (Abel, 1978; Fletcher et al., 1916), while the other reported mild effects (Phillips & Stainbrook, 1976). Similarly, in the avoidance studies, prenatal ethanol has been found to impair performance (Abel, 1979; Bond & DiGiusto, 1977a, 1978; Riley et al., 1979a, b) or to leave performance unaffected (Abel, 1978). While the contradictory nature of these findings is initially somewhat bothersome, it may be that parametric investigations would disclose the source of the discrepancies, since these studies differed in a number of factors: (a) species, (b) behavioral task, (c) mode and timing of ethanol administration, (d) dose, etc.

In any event, examination of the literature leads to the general conclusion that prenatal ethanol exposure may, in many cases, have the potential for disrupting normal behavioral development, even in the absence of any obvious dysmorphology. However, as mentioned above, conclusions in this regard must be drawn cautiously for a number of

reasons. Perhaps one of the most problematic features of those studies examining behavioral effects of prenatal alcohol exposure is the inconsistent use of, or basic disregard for, control groups. In mammalian models (which have been in the majority, by far), the fetus relies directly upon the mother for prenatal nutrition, and offspring are intimately influenced by maternal postparturition behavior (cf. Joffe, 1969). It is clear, therefore, that in studies utilizing mammalian models, pair-fed (nutrition) controls and postnatal fostering/cross-fostering procedures are highly desirable. It is notable that in only four of the studies reported here were both of these control procedures used (Abel, 1978, 1979; Abel & York, 1979; Martin et al., 1978), although a number of studies did include one or the other.

Those who have reported prenatally-induced behavioral effects of alcohol have found their results to be rather transient (e.g., Martin et al., 1977) and in very few cases have the effects represented large group differences. Nevertheless, various effects have been found consistently by a number of investigators (e.g., effects on shock-avoidance) and are at least suggestive of alcohol's behavioral teratogenic effects. The full nature and extent of prenatal ethanol's influence on behavior remains to be determined, however. For example, it is yet uncertain whether certain behavioral or learning processes are particularly susceptible to prenatal alcohol's effects. In addition, parametric studies in which dosage and timing of ethanol administration are systematically manipulated are of particular interest at the present time, since few investigators have yet examined these variables within the context of behavioral studies.

Perhaps one of the more intriguing generalities to arise from these studies has been the suggestion that prenatal exposure to alcohol may cause a deficit in response inhibition (cf. Riley et al., 1979a). According to Riley et al., such a deficit would not only account for their own results (Riley et al., 1979a, b), but also for those of others. Thus, for example, findings of hyperactivity (Bond & DiGiusto, 1976, 1977), impaired discrimination learning (Shaywitz, Klopper & Gordon, 1976), and increased aggression (Krsiak et al., 1977) caused by prenatal alcohol, might all be explained as a deficit in response inhibition. One way that ethanol might exert such an effect is suggested by a more general developmental approach. Since the increasing ability of an organism to inhibit responding has been hypothesized to correlate with the full development of the CNS (cf. Douglas, 1972), it is possible that prenatal alcohol exerts its disinhibitory effect by disrupting or retarding development of the CNS (cf. Bond & DiGiusto, 1977; Demers & Kirouac, 1978).

Rationale

The present series of experiments was designed with the intent of studying further the possibility that prenatal exposure to alcohol may cause a deficit in specific learning processes, particularly discrimination learning and conditioned inhibition. It is notable that this combination of procedures not only permits the investigation of prenatal alcohol's effects on different behavioral processes, but should also be sensitive to impairments in both excitatory and inhibitory responding.

The chick model. For several reasons, the domestic chicken was chosen as the subject for these experiments. The rationale for this choice is as follows: (a) There is a sizeable literature on the physical

and behavioral development of the chick, including its use for developmental studies of pharmacological agents (e.g., Sparber & Shideman, 1968); (b) There is clear evidence that administration of alcohol during incubation can retard growth and produce morphogenic aberrations (including CNS abnormalities) in embryonic chicks, a pattern generally consistent with the fetal alcohol syndrome (Sandor, 1968; Sandor & Elias, 1968); (c) The chick represents a "closed system" during the major portion of its development, that is, one that is relatively free of uncontrolled and potentially 'contaminating' maternal variables; (d) Because development occurs away from the mother, very precise control can be exerted over relevant environmental conditions (e.g., time of onset of incubation, temperature, humidity, etc.) and especially over the amount (dosage) and pattern of exposure to ethanol during any desired developmental stage; (e) Newly hatched chicks do not require the presence or care of the mother hen, and thus are raised communally; As a result, the postnatal maternal influences on behavior noted in the literature are largely avoided, and the posthatch physical environments of both treated and untreated chicks are fully equivalent; (f) The precocial nature of the chick permits early measurement of relatively complex behavior, thereby minimizing the influence of experience with the postnatal environment. Two objections that might be raised against a chick model are that (a) The mode of drug administration is very different from that which occurs in the alcoholic woman (cf. Randall, 1977), and (b) It may be difficult to extrapolate from development in an egg to development _in utero_ (cf. Warren, 1977). However, it is not the intended purpose of this research to provide a complete analogy to all features of the human fetal alcohol syndrome. On

the contrary, the question to be addressed here is of a more basic nature -- will contact of the developing organism with alcohol affect its subsequent ability to respond appropriately in discrimination and conditioned inhibition paradigms?

Conditioned inhibition. Because of the interpretational difficulties which still accompany theoretical considerations of the concept of conditioned inhibition, one particular theory of conditioned inhibition will be ascribed to in the present work (cf. Rescorla, 1975; Wagner & Rescorla, 1972). Although this theory need not be construed as the final word regarding the nature of conditioned inhibition, it has been detailed sufficiently to serve as a basis for the design of the current experimental paradigm. According to this theory, "a conditioned inhibitory stimulus is one that has become capable, through experience, of interfering with the production of a response by an excitor" (Rescorla, 1975, p. 20). Thus a conditioned inhibitor will attenuate the effects which a conditioned excitor would normally produce. While there are a number of different procedures for producing conditioned inhibition, it is generally believed that the necessary condition for the establishment of a stimulus as a conditioned inhibitor is that the stimulus (conditioned stimulus, CS) have a history of being negatively correlated with the unconditioned stimulus (US). In one sense, then, conditioned inhibition measures an organism's ability to learn that the CS and US are negatively related, or that the occurrence of the CS reliably predicts the absence of the US.

The primary purpose of this set of experiments was to investigate

differences in discrimination and conditioned inhibition learning between chicks treated prenatally with alcohol and those treated with a placebo. However, since the proposed experimental procedure had apparently never been used with chicks, an initial experiment was conducted with untreated chicks in order to establish acceptable parameters for conditioning.

EXPERIMENT 1

In this experiment, an autoshaping procedure was used to initiate and maintain responding to a lighted response key. Response acquisition was conducted under a discrimination contingency in which two key-light colors (referred to as A and B) were consistently followed by reinforcement, whereas a third color (referred to as Y) was never reinforced. In conditioned inhibition training, which followed acquisition, a procedure similar to that described by Pavlov (1927) was employed. Two stimuli were used: One stimulus (A) was consistently paired with the US, whereas the other stimulus (W) was presented in compound with the first stimulus, and that compound (AW) was not reinforced. Typically, with this type of procedure, responding will initially occur during both kinds of trials, but with repeated nonreinforcement of the compound (AW), a response decrement occurs on AW trials. As a result, A is said to have become an excitor, and W an inhibitor. In the current experiment, A was one of the two reinforced, colored

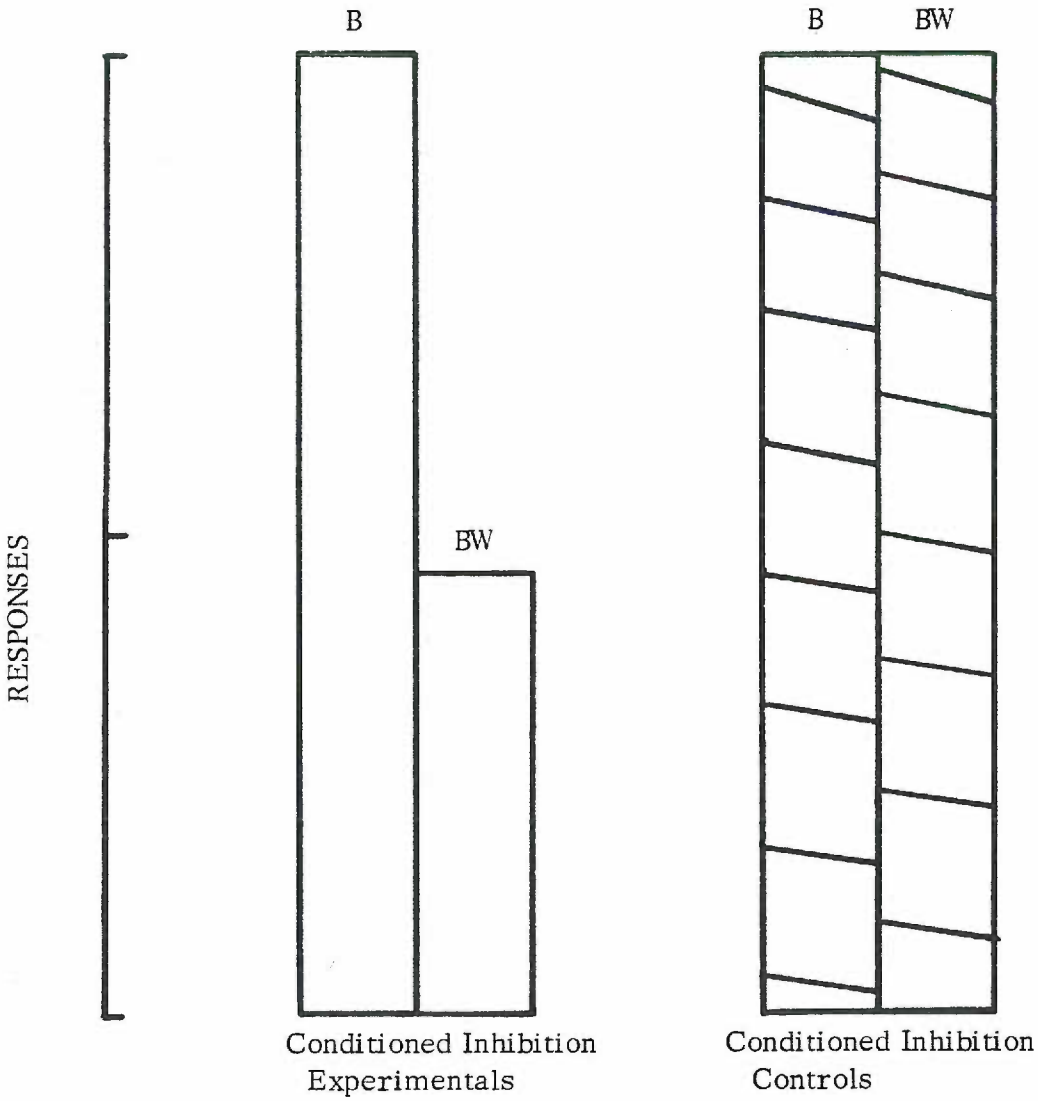
key lights used during acquisition, whereas W was an off-key white light. Thus, presentations of the key light (A) were consistently followed by reinforcement, whereas presentations of the key light compounded with the off-key light were never reinforced (AW). It was therefore expected that W would become a conditioned inhibitor. A third stimulus, B, the second original reinforced key light, was reliably reinforced throughout conditioned inhibition training with the sole intent of using it for a subsequent conditioned inhibition transfer test.

The control group during conditioned inhibition training was similar to one employed by Rescorla (1976), receiving A and B trials identical to those of the experimental group, plus nonreinforced presentations of the off-key light alone (W) instead of the compound (AW). It should be noted that this control group provides a conservative comparison, since the negative correlation between W and reinforcement may alone permit the conditioning of inhibition to W. However, as Rescorla (1976) has pointed out, there is both empirical and theoretical reason to believe that the presentation of W alone will leave W more neutral than presenting it as part of an AW compound. Indeed, Wagner and Rescorla (1972) have concluded that in order to make a neutral stimulus inhibitory as a result of nonreinforcement, it must be nonreinforced in compound with other excitatory stimuli. It is apparent that AW meets this criterion, whereas W alone does not, and thus significant inhibition should be conditioned to the off-key light (W) only in the experimental group.

The summation-transfer test was designed to test the inhibitory effect of the off-key light (W) when presented in compound with Stimulus B, which had never been presented as part of a compound to either group, but which had been consistently reinforced when presented alone. If W did indeed become a conditioned inhibitor during conditioned inhibition training, then responding to the BW compound should be at a considerably lower level than responding to B alone in the transfer test. Thus, the expected results for the transfer test were that responding to B would be the same for experimentals and controls, but that responding to the compound (BW) would be noticeably lower than responding to B in the experimental group, but not in the controls (see Figure 1).

Findings such as those depicted in Figure 1 are, however, explicable in terms other than those of conditioned inhibition. The most likely alternative explanation is that differences as plotted in Figure 1 are not the result of between-group differences in the effects of Stimulus W, but rather are due to differences in the strength of conditioning to, or associative value of B. The crux of this argument derives from the fact that during conditioned inhibition training, Stimulus A, when presented in compound with W, was nonreinforced on a number of trials for the experimental group. The control group, on the other hand, never received presentations of the compound, and thus never received nonreinforced presentations of A. It is possible that resulting differences in A's associative value could generalize to B. If such between-group differences in B's associative value did exist, it might be possible to explain the anticipated results of the transfer test in terms of B rather than referring to conditioned inhibition. Thus while responding

Figure 1. Idealized outcome of conditioned inhibition transfer test.


RESPONSES
B
BW
B
BW
Conditioned Inhibition
Experimentals
Conditioned Inhibition
Controls

to B might be at an asymptote for both groups, a smaller associative value of B for the experimentals might render it more susceptible to 'disruption' or 'weakening' by W. To assess such an explanation, additional chicks from the control and experimental groups were run on a series of extinction trials to Stimulus B. Any differences in associative value between controls and experimentals at the end of conditioned inhibition training should be evident in an extinction test. If, however, the anticipated outcome of the transfer test was confirmed and there were no differences in the extinction test, it could be reasonably assumed that W (the off-key light) had become a conditioned inhibitor.

Method

Subjects

Fertile eggs of Gallus domesticus (White Leghorn, Shaver Starcross) obtained from the Department of Poultry Science at Oregon State University (Corvallis, Oregon) were incubated and hatched in a commercial forced-air incubator (Marsh Roll-X) at 37-38° C and 55-62% relative humidity. During incubation, the eggs were turned automatically on an hourly basis from Day 1 to Day 18, and on Day 18 the eggs were set for hatching. Upon hatching, the chicks were weighed, banded for identification and placed in a communal brooder maintained at 37.8° C (± 3° C) during the first week posthatch, and 32.2° C (± 3° C) thereafter. The brooder was housed in a room maintained on a 12-hr light-dark cycle. The chicks were allowed continuous access to water throughout the experiment, but free access to food was permitted only during the first 3 days posthatch. Thirty-two of the hatched chicks were used in the present experiment.

Apparatus

Four conditioning chambers (30.5 x 31.3 x 35.2 cm, inside) were used throughout the experiment. The end panels of each conditioning chamber were constructed from aluminum sheet, the side walls and ceiling were made of clear Plexiglas and the floor consisted of a galvanized iron tray covered with wire mesh. A food hopper delivered chick starter mash through an aperture (5.1 x 5.1 cm) located in the center of one end panel, 2.9 cm above the floor. When the hopper was raised, the aperture was illuminated by two #1819 lamps (24 V, dc). A mirror (7.6 x 14.6 cm) was placed directly to the right of the aperture, facing left at an approximate angle of 30° with the end panel. A frosted BRS/LVE response key, 2.5 cm in diameter, was centered 6.8 cm above the floor, 6.7 cm to the left of the food hopper aperture, and an in-line mini-projector (Industrial Electronic Engineers, Series 0010-01, with lamp #44, powered by a 5-V dc power supply) was capable of projecting green, red or yellow lighting onto the key. A mask placed between the projector and key permitted the projection of a circular color patch, 1.6 cm in diameter onto the center of the key. A General Electric 7W white night-light bulb (with power source adjusted to 50 V, ac) was suspended in a downward direction, 5 cm above the response key, and served as an off-key stimulus when illuminated.

A houselight was located at the center of the junction of the ceiling and the end panel of the conditioning chamber directly opposite the key pecking panel (lamp #1820, 24-V dc power supply). The light was diffused by a thin piece of translucent, white plastic and was illuminated throughout the experimental session. In order to

reduce reflection, the end panel opposite the key pecking panel and the side wall to the left of the key were covered with black construction paper.

Each of the conditioning chambers was housed within a separate dual-compartment sound attenuating chamber constructed from plywood and Cellotex. Ventilation fans, mounted on the outside wall of the sound attenuating chambers, were in operation throughout the session, and provided a background noise level of 55 (± 4) dB (re: 20 $\mu N/m^2$) measured in the center of the conditioning chamber.

Stimulus presentations and response monitoring were controlled by a laboratory computer and electromechanical devices.

Procedure

The procedure for the first experiment is outlined in Table 1. Hopper training was initiated on the fourth day following Incubation Day 21 (the majority of chicks hatch on Day 21), approximately 24 hr after food was removed from the home brooder. On this and each subsequent day, the chicks were allowed 3.5 hr of access to food following the experimental session. On the first day of hopper training, the hopper was raised at the beginning of the session and each chick was allowed to eat for 20 sec. The food hopper was then lowered and raised several additional times until each chick ate consistently within 5 sec of hopper activation. On the following day, a total of 30 5-sec hopper presentations was given, with the interval between food presentations averaging 30 sec (± 15 sec).

Autoshaping acquisition. An autoshaping procedure was used to initiate and maintain responding to the illuminated key. Autoshaping

Table 1

Procedure for Experiment 1

Days	Treatment (trials/day)
1-2	Hopper Training
3-7	Acquisition 12: A+; 12: B+; 24: Y- ITI $\bar{X}$ = 30 sec
8-16	Conditioned Inhibition Group CI: 36: AW-; 12: A+; 6: B+ Group C: 36: W-; 12: A+; 6: B+ ITI $\bar{X}$ = 30 sec
17	Summation-Transfer Test or Extinction Transfer Test Groups: 6: AW- or W-; 2: A+; 1: B+ -------> 18: B-; 18: BW- Extinction Groups: 48: B- ITI $\bar{X}$ = 30 sec

+ = reinforced

- = nonreinforced

sessions were given over five consecutive days, and on each of these days, 5-sec illuminations of the key with either of two colors (Stimulus A and Stimulus B) were followed consistently by 5-sec periods of food accessibility. The illumination of the key with a third color (Stimulus Y) was never followed by food. The colors of Stimuli A and B were counterbalanced such that for half of the chicks, A was red, and B, green, while for the remaining half, A was green, and B, red. A yellow key light always served as Stimulus Y. Each session consisted of 12 A trials, 12 B trials, and 24 Y trials, with an average intertrial interval (ITI) of 30 sec (range = 15-45 sec). The number of responses during each stimulus presentation was recorded and from this, the response rate and percentage of trials on which at least one response was evoked were calculated for each stimulus.

Conditioned inhibition training. At the end of five days of discriminated autoshaping, the birds were divided into two groups (N = 16/group) matched for responding during acquisition. One group was randomly designated as the conditioned inhibition group (Group CI), whereas the other group served as a control (Group C). Conditioned inhibition training or control sessions were then conducted over the next nine days in the following manner. For Group CI, experimental sessions were comprised of 12 reinforced exposures to A, 6 reinforced exposures to B, and 36 nonreinforced presentations of a compound stimulus, AW, made up of A and the off-key light (W). Group C received reinforced A and B trials identical to those of Group CI, but the 36 nonreinforced trials consisted of presentations of the off-key light (W) alone. Again, for both groups, the colors of Stimuli A and B

were counterbalanced such that half of the animals in each group received red as Stimulus A, whereas the remaining half received green. As was mentioned earlier, presentations of Stimulus B during conditioned inhibition training were intended to maintain excitation to that stimulus, thus permitting the subsequent summation-transfer test of inhibition. All stimulus and reinforcement durations were 5 sec, and the mean ITI was 30 sec. During conditioned inhibition training or control sessions, responding to each of the three relevant stimuli was recorded and response rates and percentage of trials with at least one response were calculated.

Transfer test and extinction. At the end of the final day of conditioned inhibition training, the conditioned inhibition and control groups were each divided into two subgroups, matched for responding during the conditioned inhibition training phase. Each subgroup was comprised of eight chicks, with equal numbers from each of the previous stimulus counterbalancing conditions. One of the subgroups from Group CI and one from Group C were randomly assigned to the transfer test condition, while the remaining subgroups underwent extinction to Stimulus B. As mentioned earlier, the extinction test was performed to preclude alternative explanations for the results of the conditioned inhibition transfer test.

The transfer test was conducted following nine 'refresher' trials under the same stimulus and reinforcement conditions as during conditioned inhibition training (i.e., six nonreinforced AW or W trials, two reinforced A trials and one reinforced B presentation). The subsequent transfer test was then administered in extinction, and consisted

of 18 presentations of Stimulus B compounded with the off-key light (i.e., BW), and 18 presentations of B alone. It will be noted that until the transfer test, presentations of B had been followed consistently by reinforcement in both groups, and that B had never been presented as part of a compound. During the transfer test, responses to each of the two stimuli were monitored.

Subjects undergoing extinction received 48 nonreinforced presentations of Stimulus B alone, and the number of responses during each stimulus presentation was recorded. In both the transfer and extinction tests, the ITI averaged 30 sec (range = 15-45 sec).

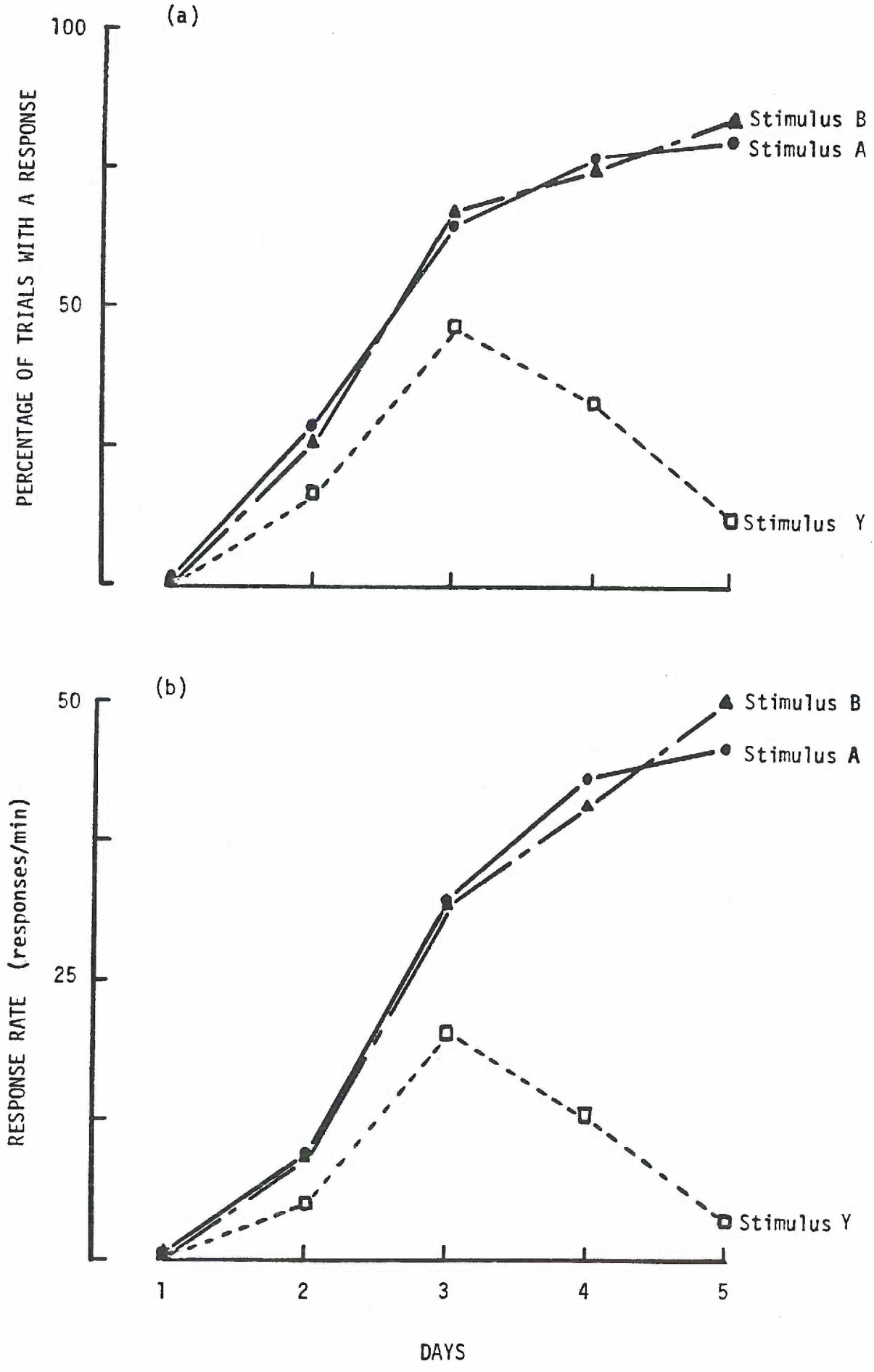
Results

Acquisition

Figure 2a depicts percentage of trials with at least one response for the various stimuli over the five days of acquisition. It can be seen from the graph that responding to the two reinforced stimuli (Stimuli A and B) increased over days, with little difference between the two stimuli. Responding to the nonreinforced stimulus (Y), however, increased only for the first three days of acquisition, decreasing markedly thereafter. These findings were indicated in a three-way analysis of variance by a reliable stimuli effect, F (2, 60) = 45.94, $p <$ .001, a reliable days effect, F (4, 120) = 45.55, $p < .001$, and an interaction of stimuli with days, F (8, 240) = 9.36, $p < .001$. Color counterbalancing had no effect on responding, and failed to interact with either of the other factors.

Figure 2b shows acquisition response rates for each of the three stimuli. Again, the analysis of these data disclosed no effect of

Figure 2. Response levels during autoshaped acquisition -- Experiment 1. Figure 2a represents percentage of trials with at least one response for the three stimuli, whereas Figure 2b depicts response rates.


(a)
100
50
PERCENTAGE OF TRIALS WITH A RESPONSE
Stimulus B
Stimulus A
Stimulus Y
(b)
50
25
RESPONSE RATE (responses/min)
Stimulus B
Stimulus A
Stimulus Y
1
2
3
4
5
DAYS

counterbalancing, while the stimuli and days effects and the stimuli x days interaction were all reliable (ps< .001).

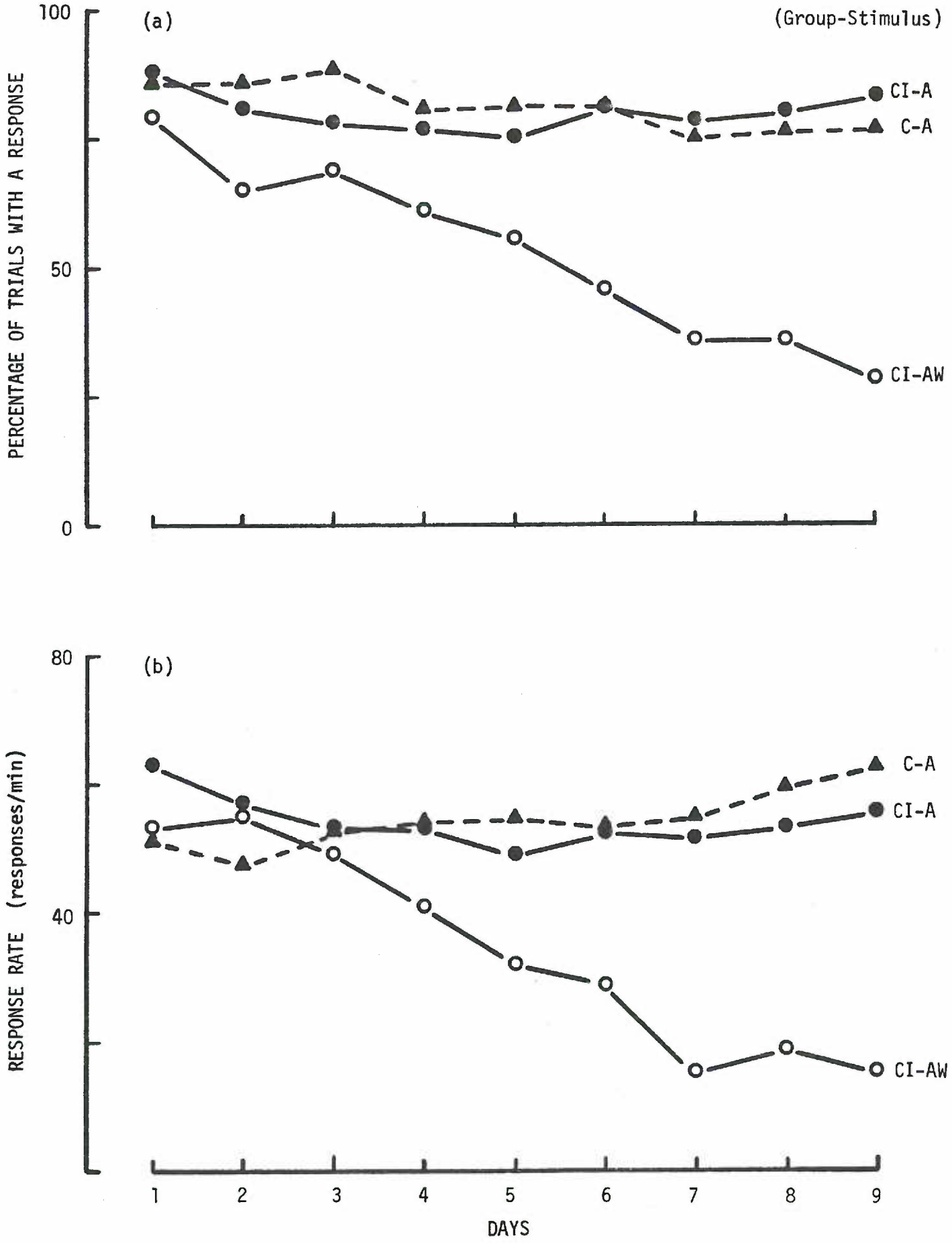
Conditioned Inhibition

Percentage of Stimulus A trials (solid symbols) and AW trials (open symbols) with at least one response are plotted over the nine days of conditioned inhibition training in Figure 3a. Responding during Stimulus W by the control group never exceeded .2% of the total responses in a single session, and is omitted from the figure. As can be seen from the figure, response levels to A remained relatively constant throughout conditioned inhibition training, but responding to the compound (AW) decreased steadily over days.

It is also apparent that the conditioned inhibition group responded more to the reinforced stimulus (A) than to the nonreinforced compound (AW), with the difference between the two stimuli increasing over days. A counterbalancing x stimuli (A vs. AW) x days analysis was run for Group CI to examine these response differences. The counterbalancing factor failed to attain significance, F (1, 14)< 1.0, and none of the interactions involving counterbalancing was reliable. There was a reliable effect of stimuli, F (1, 14) = 47.66, p < .001, a reliable days effect, F (8, 112) = 12.41, p< .001, and a significant stimuli x days interaction, F (8, 112) = 13.19, p < .001. Additional analyses indicated no differences between experimental and control animals in responding to either A or B during this phase of the experiment.

Conditioned inhibition response rates to the two stimuli are plotted in Figure 3b. The form of this graph and of the statistical analyses for this measure were very similar to those for the percentage measure

Figure 3. Responding by the conditioned inhibition group (CI) and the control group (C) to Stimuli A and AW over the nine days of conditioned inhibition training. Figure 3a depicts the percentage of trials with at least one response and Figure 3b depicts response rates. Response levels to Stimulus B are not shown.


(a)
(Group-Stimulus)
100
50
0
PERCENTAGE OF TRIALS WITH A RESPONSE
CI-A
C-A
CI-AW
(b)
80
40
RESPONSE RATE (responses/min)
C-A
CI-A
CI-AW
1
2
3
4
5
6
7
8
9
DAYS

and again, no between-group differences in responding to A or B were noted. As with the percentage measure, response rates to the AW compound decreased over conditioned inhibition sessions.

Summation-Transfer Test

The transfer test was comprised of 18 B and 18 BW presentations. For purposes of analysis, the 36 trials were divided into six blocks, with each block consisting of three trials with each stimulus. Percentage of transfer test trials with at least one response is plotted over blocks in Figure 4a. As is obvious from the figure, after the first block, Group CI responded considerably less to the compound stimulus (BW) than to Stimulus B. The control group, on the other hand, responded nondifferentially to the two stimuli throughout the session. These observations were supported statistically by a conditioning group (CI vs. C) x counterbalancing x stimuli x blocks analysis of variance in which a reliable conditioning group x stimuli x blocks interaction was disclosed, F (5, 60) = 3.50, $p < .01$. A follow-up analysis to this interaction indicated a significant stimuli x blocks interaction for Group CI, F (5, 35) = 3.48, $p < .05$, as well as a reliable main effect of stimuli, F (1, 7) = 32.16, $p < .01$. An analogous follow-up analysis for Group C disclosed no main effect due to stimuli, and no stimuli x blocks interaction. Other effects disclosed by the four-way analysis were reliable main effects of stimuli, F (1, 12) = 50.62, $p < .001$, and blocks, F (5, 60) = 22.20, $p < .001$, and an interaction of conditioning group with stimuli, F (1, 12) = 25.22, $p < .001$. In none of the analyses was the main effect of counterbalancing significant, and there were no interactions involving the counterbalancing factor.

Figure 4. Responding by the two groups to Stimuli B and BW over blocks of the summation-transfer test. Figure 4a depicts the percentage of trials with at least one response, while response rates are plotted in Figure 4b.

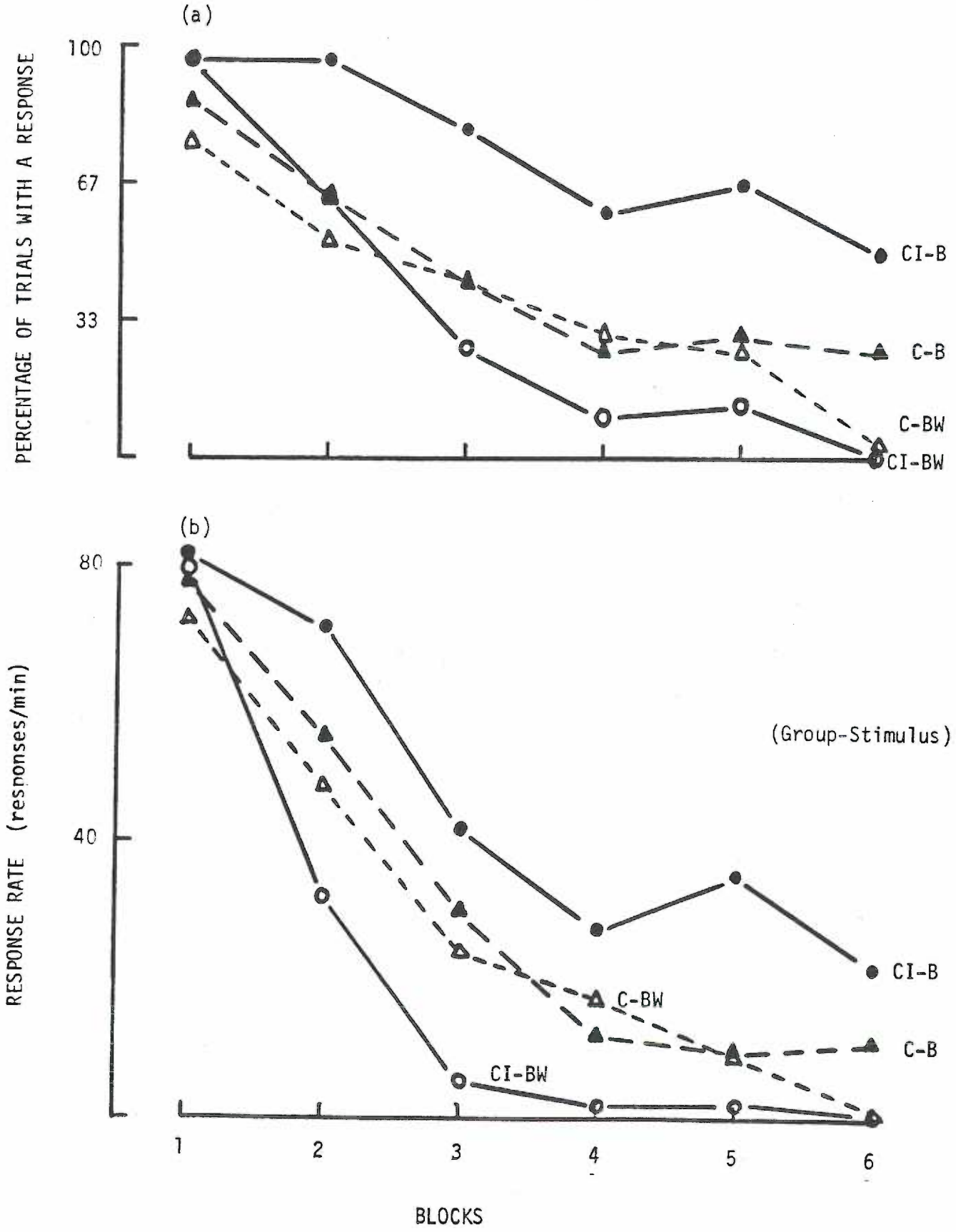

(a)
100
67
33
PERCENTAGE OF TRIALS WITH A RESPONSE
CI-B
C-B
C-BW
CI-BW
(b)
80
40
RESPONSE RATE (responses/min)
(Group-Stimulus)
CI-B
C-BW
C-B
CI-BW
1
2
3
4
5
6
BLOCKS

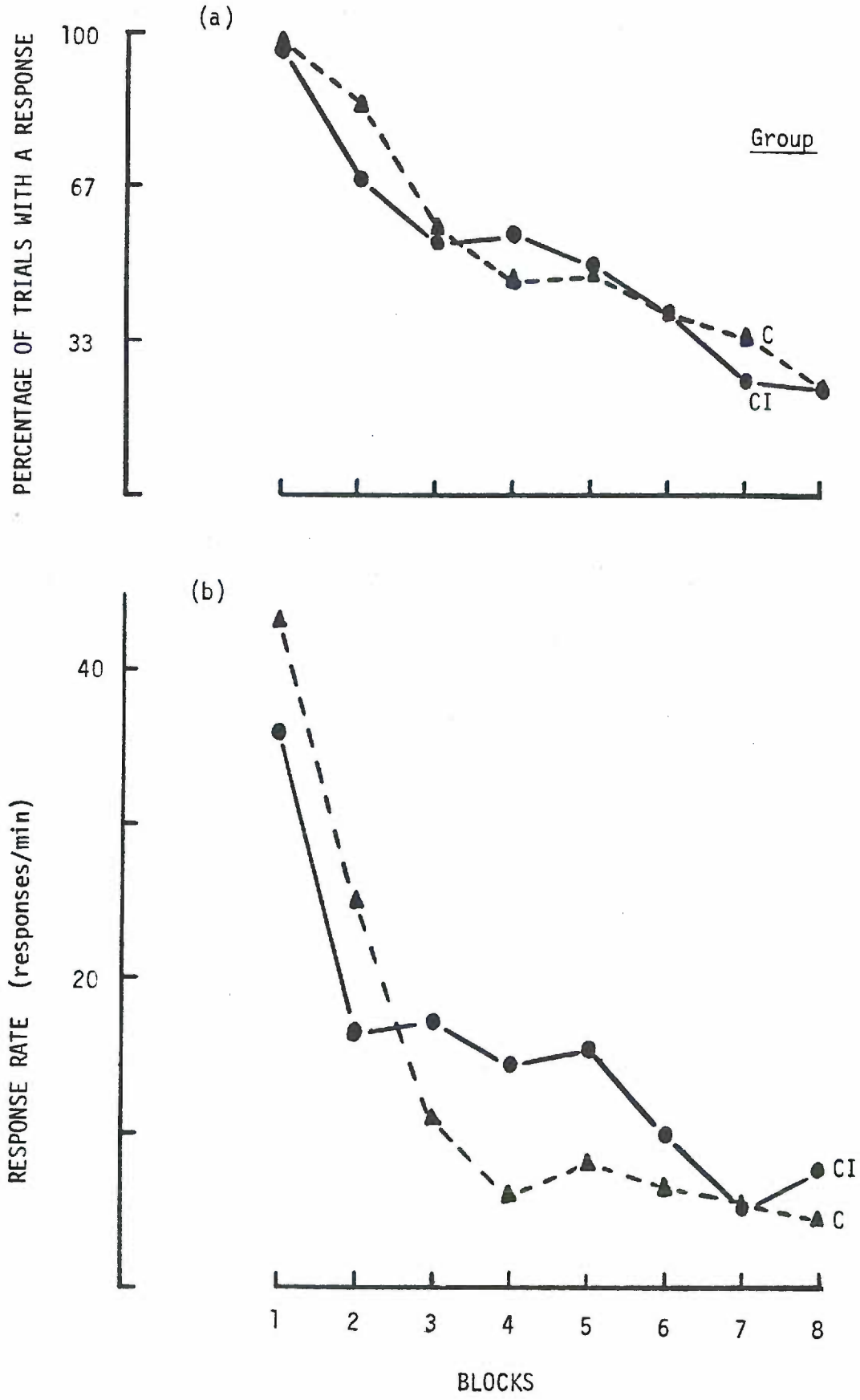
Figure 4b depicts transfer test response rates to the two stimuli across blocks. The statistical analyses for this measure were similar in result to those for the percentage measure, with the added reliable interaction of stimuli with blocks, F (5, 60) = 2.72, $p < .05$. All other main effects and interaction were the same as in the rate analyses.

Extinction

Subgroups of chicks from Groups C and CI were tested for rate of extinction to Stimulus B in order to determine whether a generalized weakening occurred to that stimulus in Group CI. The 48 presentations of B were divided into eight blocks of six stimulus presentations for analysis. Figure 5a depicts percentage of trials with a response for the two groups over blocks. It is apparent from the figure that overall responding decreased for both groups over blocks, with no large differences in rate of extinction. In fact, a conditioning group x counterbalancing x blocks analysis of variance disclosed a reliable blocks effect, F (7, 84) = 18.60, $p < .001$, but no effect of conditioning group and no conditioning group x blocks interaction. None of the other main effects or interactions was significant.

Response rates during extinction are plotted for each of the two groups in Figure 5b. The outcome of the analysis of variance for this measure was virtually identical to that of the percentage measure, except that the conditioning group x counterbalancing interaction was also reliable, F (1, 12) = 4.78, $p < .05$. This interaction was due to a higher level of responding by one of Group CI's subgroups than by the other, combined with no difference between subgroups of Group C. Follow-up analyses indicated that this effect was the result of mismatching of

Figure 5. Responding by subjects from Groups C and CI to Stimulus B over blocks of the extinction test. Percentage of trials with at least one response is plotted in Figure 5a, while Figure 5b depicts response rates. It should be noted that this figure represents subjects different from those represented by Figure 4 (transfer test).


(a)
100
67
33
PERCENTAGE OF TRIALS WITH A RESPONSE
Group
C
CI
(b)
40
20
RESPONSE RATE (responses/min)
CI
C
1
2
3
4
5
6
7
8
BLOCKS

subgroups at the end of conditioned inhibition training and that the difference existed prior to extinction testing. The effect was thus apparently not the result of treatment, and direct comparisons between like-subgroups of Groups CI and C indicated no main effects of subgroup.

Discussion

The outcome of the present experiment was consistent with the notion that the off-key light (W) had become a conditioned inhibitor for Group CI. Specifically, it was found that while responding by Group CI to Stimulus A alone remained unchanged during conditioned inhibition training, responding to the AW compound was markedly depressed at the end of that training. Furthermore, in the summation-transfer test, when W was presented in compound with a stimulus that had always been reinforced previously (i.e., was an excitor -- Stimulus B), the conditioned inhibition group responded considerably less than the controls. It is unlikely that these observed differences were the result of between--group differences in the associative value of B, since animals from the two groups did not differ in rate of extinction to B.

Also of interest is the finding that Group CI demonstrated what graphically appears to be a slower extinction rate to Stimulus B than Group C during the summation-transfer test (refer to Figure 4, solid symbols) but not during the extinction test (refer to Figure 5). This phenomenon has been noted in other studies of inhibition and has been labelled "protection from extinction" (cf. Wagner & Rescorla, 1972), since the decrement to Stimulus B is attenuated when B is nonreinforced in the presence of the inhibitory stimulus, W. The presence of the inhibitory stimulus is presumed to "protect" B from the usual reduction

in associative strength which occurs during extinction.

Finally, it should be mentioned that while the findings of this study are consonant with the idea that W became a conditioned inhibitor for Group CI, this deduction must be drawn cautiously. As Rescorla (1969) has pointed out, the most conclusive evidence of learned inhibition is provided by the use of a combination of the summation and retardation tests of inhibition. The summation-transfer test performed here offered strong support for the contention that W had become an inhibitor. A retardation test, which assesses whether the presumed inhibitory stimulus is slower than control stimuli in acquiring conditioned excitation, was not performed in this experiment. The primary reason for this was simply that it would be difficult to measure excitation to W, an off-key light. That is, there was no easy way to record responding to the off-key light. Nevertheless, given that this set of experiments was designed with the intent of studying the effects of ethanol on inhibition rather than inhibition per se, it was felt that the current demonstration of W's inhibitory properties was adequate for the present purposes. Since presentations of W were shown to result in an inhibition of responding in circumstances designed to produce and measure conditioned inhibition, it was concluded that the experimental design was satisfactory for use in the prenatal alcohol investigation.

EXPERIMENTS 2-4

In an attempt to examine the effects of exposure to ethanol during incubation on subsequent discrimination learning and conditioned inhibition, the paradigm described above was used with alcohol- and placebo--treated chicks. Specifically, fertile eggs were injected with alcohol or saline daily for five-day periods, and four days after hatching, behavioral testing was begun. Days 1-15 of incubation were selected as treatment days because they include the period of most active organogenesis and optimal brain growth (although the brain continues to grow even for a short time after hatching). It is also noteworthy that this time span includes two of the three "critical sensitivity periods" of development observed by Hamilton (1952), Days 4 and 12.

A total of three additional experiments was conducted, with each experiment utilizing a separate setting of eggs. The settings differed in terms of the time during development in which the eggs were treated, and each setting (i.e., each experiment) was comprised of two ethanol treatment groups and two corresponding saline (volume) control groups. The doses of alcohol administered varied across settings since the chick embryo is differentially sensitive to ethanol depending upon the stage of development. Dosages were determined based upon the results of pilot studies, and were selected primarily on the basis of their effects on hatchability. As discussed earlier, behavioral comparisons between experimental and control chicks are best made when the survival rates of the two groups are not greatly different.

Autoshaping acquisition and conditioned inhibition sessions were carried out using parameters similar to those described for Experiment 1.

However, because this series of experiments was concerned primarily with differences between treatment groups in discrimination learning and conditioned inhibition, and since the adequacy of the conditioned inhibition procedure was established in the first experiment, groups analogous to Group C of the first experiment (which received non-reinforced presentations of the off-key light alone as the control condition) were not included in these studies. Additionally, because the color counterbalancing factor affected neither acquisition nor conditioned inhibition in the experimental group of the above experiment (Group CI), counterbalancing was not included as a factor in this set of studies.

The present experiments permitted an evaluation of the relative effects of ethanol dose as well as effects due to treatment at various stages of development. It is conceivable that prenatal ethanol might affect the behavioral processes tested here in a number of ways. Accordingly, this paradigm should be sensitive to deficits in: (a) "excitatory conditioning (in which two events are positively correlated in time -- in the present case, the key light and food); (b) discrimination learning; (c) conditioned inhibition (testing the animal's ability to learn that two events are negatively correlated in time, or that the occurrence of one event reliably predicts the absence of the other event -- in the present case, the compound stimulus was negatively correlated with food); and (d) the transfer of inhibition.

Although three, separate experiments were performed to examine prenatal alcohol's effects, the three will be discussed together since

they differed only in dosage and timing of ethanol administration. It must be cautioned, however, that the three studies should be compared to each other on a tentative basis only, since they were performed sequentially, and the inherent characteristics of the eggs (and thus of the chicks) may have differed from one experiment to the next.

Method

Subjects and Apparatus

For each of Experiments 2, 3 and 4, 88 fertile hen's eggs (White Leghorn, Shaver Starcross) were obtained from the Department of Poultry Science at Oregon State University and treated identically to those described in Experiment 1, with the exception of the drug treatment described below in the Procedure section. Experimental sessions were conducted in the apparatus used in Experiment 1.

Procedure

Ethanol administration. Upon arrival in the laboratory, each egg was candled and the air space was outlined lightly in pencil. A hole large enough to accomodate a 25-gauge hypodermic needle was then drilled in the center of the outlined portion of the shell with a sterilized dental-type burr. The hole was covered immediately with plastic, water-proof tape (Scotch Patch and Repair Tape, Catalog 193) to prevent dessication. When all of the eggs had been drilled and covered, they were placed in the incubator and incubation was begun (at 37-38° C and 55-62% relative humidity). This was designated as the start of Day 1 of incubation.

Ethanol or saline treatment took place on Days 1-5 of incubation

in Experiment 2, Days 6-10 in Experiment 3, and Days 11-15 in Experiment 4. Injections were adminstered at the end of each relevant Incubation Day over a series of five days. Within each experiment, there were two alcohol-treated groups, each of which was associated with its own saline control group. The subgroups within each drug condition differed in terms of the injection volume. It will be noted that in the case of alcohol-treated eggs, the subgroups represent low- and high-dose treatments. The saline control solution consisted of 8.47 g/l NaCl in sterile water, whereas the ethanol solution was 25% (v/v) ethanol in 8.47 g/l saline. The osmolality of the saline solution was based on reports by Grabowski (1967) in which the above concentration was shown to be isotonic with the serum of embryonic chicks. The exact doses and volumes used for each of the experiments are presented in Table 2. In Experiments 3 and 4, eggs judged to be infertile by candling were discarded prior to the first treatment and the remaining eggs were then randomly assigned to treatment conditions. Because fertility cannot be determined by candling very early in incubation (i.e., before Day 4), all of the eggs in Experiment 2 were treated with either the alcohol or saline solution.

Immediately prior to its first injection, each egg was weighed and an identification code was pencilled on its side, indicating both the treatment group and a unique number within that group. Assignment to a treatment group was on a random basis, with the added stipulation that the four treatment groups were roughly matched on the basis of weight. Eggs from each of the treatment groups were distributed evenly throughout the incubator. On injection days, three eggs from each group

Table 2

Drug treatment protocol and hatch rates for Experiments 2-4

Experiment	Treatment Days	Daily injection volume	Daily dose g/kg/day	Total dose g/kg	% Hatched (# hatched)
2	1-5	Low Saline--0.30 ml/kg	0	0	67 (14)
2	1-5	Low Alcohol-0.30 ml/kg	.06	.30	64 (14)
2	1-5	High Saline--0.61 ml/kg	0	0	68 (15)
2	1-5	High Alcohol-0.61 ml/kg	.12	.60	73 (16)
3	6-10	Low Saline--0.51 ml/kg	0	0	75 (15)
3	6-10	Low Alcohol-0.51 ml/kg	.10	.50	50 (10)
3	6-10	High Saline--1.01 ml/kg	0	0	84 (16)
3	6-10	High Alcohol-1.01 ml/kg	.20	1.00	47 (9)
4	11-15	Low Saline--0.76 ml/kg	0	0	79 (11)
4	11-15	Low Alcohol-0.76 ml/kg	.15	.75	79 (11)
4	11-15	High Saline--1.52 ml/kg	0	0	86 (12)
4	11-15	High Alcohol-1.52 ml/kg	.30	1.50	64 (9)

were taken from the incubator at a time, the tape was removed briefly, and the appropriate solution injected into the air space. As mentioned earlier, this mode of alcohol administration has been reported to result in the gradual penetration of ethanol into the interior of the egg (Sandor & Elias, 1968). Sterile injection solutions were prepared daily, immediately prior to the administration of injections.

On the 19th day of incubation, all of the eggs were removed from the incubator and separated according to treatment group. The groups were then returned to the incubator, separated into four compartments by large mesh wire to prevent the chicks from intermixing as they hatched. When a chick hatched, its identification code and the approximate time of hatch were noted, and after at least 30 min, it was removed from the incubator, banded for identification, examined briefly for gross anomalies, and placed in a brooder. The chicks were allowed free access to chick starter mash and water for approximately 60 hr following Incubation Day 21. At that time, the chick starter was removed from the brooder, thus initiating food deprivation.

Acquisition, conditioned inhibition training and transfer testing. In Experiment 2, nine chicks were randomly selected from each treatment group to serve as subjects in the learning experiment. Eight chicks were selected from each group in Experiments 3 and 4. Autoshaping and conditioned inhibition sessions were carried out using parameters similar to those described in Experiment 1. Thus, after two days of magazine training, the chicks were autoshaped to respond to each of two colors of key light (red and green -- Stimuli A and B, respectively), while presentations of a third color were consistently non-

reinforced (yellow -- Stimulus Y). Then, during conditioned inhibition training, a white off-key light (W) was compounded with the previously reinforced red key light, and the compound was never followed by food (Stimulus AW). Red alone, however, was always reinforced, and presentations of green were also reinforced to maintain it as an excitor for the subsequent transfer test. After a number of these sessions, the ability of the off-key light to act as a conditioned inhibitor was examined in a summation-transfer test in which the off-key light was compounded with the green key light which had previously always been reinforced, and which had never been presented as part of a compound.

On each experimental day, the percentage of trials with at least one response, and the mean response rate were calculated for all animals for each of the stimuli.

Results

The results will be outlined in four sections: (a) Hatchability and Survival; (b) Body Weights; (c) Autoshaping Acquisition; and (d) Conditioned Inhibition Training and Summation-Transfer Testing. Within each of the two latter categories, descriptions of each of the three experiments will be dealt with sequentially.

Two measures were used to assess responding during behavioral testing: percentage of trials with at least one response, and response rate. In order to minimize the difficulty associated with the interpretation of massive quantities of data, only the percentage measure, which was subject to less variablility, will be discussed in the

main body of this text. Appendix A contains the analyses of the rate data.

Within each of the behavioral sections (acquisition, conditioned inhibition, and transfer test), the data were initially analyzed in four-way analyses of variance with factors of injection volume, drug treatment, stimuli, and days (or blocks). Parametric follow-up analyses were conducted as necessary, and the alpha level was set at .05. If the overall analysis indicated no contribution of a particular factor to an interaction which was to be investigated more fully, then follow-up analyses were conducted collapsed across that factor.

Hatchability and Survival

"Hatching" was defined as complete separation of the chick from its shell with no aid from the experimenter. In addition, only those chicks which successfully hatched within 42 hr after the end of Incubation Day 21 were included in the present experiments.

The percentage of chicks to hatch in each of the treatment groups is indicated in Table 2. As can be seen in the table, hatch rates were reasonably high in all of the saline groups (0 g/kg). The slightly lower hatch rates noted for Experiment 2 are probably attributable to the fact that unlike in Experiments 3 and 4, it was impossible to eliminate infertile eggs prior to treatment in Experiment 2. Reference to the table also suggests that the two alcohol doses given on Days 1-5 (Experiment 2) had little effect on hatch rate; that both doses on Days 6-10 (Experiment 3) lowered hatch rate; and that the high dose on Days 11-15 (Experiment 4) lowered hatch rate to a small extent. Chi-square tests were performed to compare hatch rates of each of the alcohol-treated groups

with hatch rates of their respective saline-treated controls. These tests indicated that only the difference between the high-volume alcohol and saline groups treated on Days 6-10 was statistically reliable, χ^2 (1) = 4.07, $p < .05$ (all other χ^2s (1) < 2.67).

It is important to note that although hatch rates for the saline control groups in Experiment 3 were reasonably high, nearly 30% of the chicks which hatched from these groups died within a week after hatching. There were no posthatch mortalities in either of the alcohol-treated groups in Experiment 3 or in any of the groups in Experiments 2 and 4. (A relatively high posthatch mortality in saline control chicks was also obtained, however, in one of three other studies of the Day 6-10 interval.) This finding is not readily explainable, but should be kept in mind during interpretation of the behavioral studies.

Body Weights

Within each of the three experiments, two tests were used to determine the effect of prenatal exposure to alcohol on body weight. The first consisted of a simple between-groups comparison of hatch weights for all of those animals which hatched successfully in a given experiment. In the second, the weights of animals that were randomly selected for inclusion in the behavioral studies were analyzed over the following days: Day 0 (the day of hatching), Day 3 (immediately prior to the beginning of food deprivation), Day 11 (the first day of conditioned inhibition training), and Day 20 (the summation-transfer test day).

The analysis in which the weights of all hatched animals were examined disclosed no differences among the groups in any of the three experiments. The data for the randomly selected animals are plotted

in Figures 6a (Experiment 2), 6b (Experiment 3), and 6c (Experiment 4). As can be seen in Panel a, birds treated on Incubation Days 1-5 showed little effect of volume or of drug treatment on weight. Indeed, a volume x drug treatment x days analysis revealed no effect of volume or treatment, and no interaction involving either factor. The days effect indicated a reliable increase in weight over days for all animals, F (3, 96) = 1,018.12, $p < .001$. Animals treated on Days 6-10 of incubation (Panel b) also showed a significant increase in weight over experimental days, F (3, 84) = 411.38, $p < .001$. Again, however, there was no effect of volume or of drug treatment and no interaction involving either factor.

Examination of Figure 6c reveals the expected weight increase over days for birds treated on Days 11-15 of incubation, F (3, 84) = 1,135.07, $p < .001$. However, although there is no readily apparent difference between groups in the figure, a volume x drug treatment x days analysis disclosed a reliable interaction of drug treatment with days, F (3, 84) = 4.63, $p < .01$, indicating a small but reliably faster weight gain by the alcohol-treated animals. This interaction is depicted more clearly in Figure 7, in which expanded axes are employed to illustrate weights of alcohol- and saline-treated animals (collapsed across the volume factor) over experimental days. It is notable that this weight difference did not appear to affect or to be reflected in any of the behavioral measures reported below.

Autoshaping Acquisition

During acquisition, the chicks were given five days of autoshaping in which presentations of Stimuli A and B (red and green) were consistently followed by food reinforcement, whereas presentations of Stimulus

Figure 6. Weight (in grams) at Day 0 (hatch), Day 3 (immediately prior to the onset of food deprivation), Day 11 (the first day of conditioned inhibition training), and Day 20 (the summation-transfer test day) of those animals which participated in the behavioral studies. Figure 6a depicts weights of birds from Experiment 2, whereas animals from Experiments 3 and 4 are represented in Panels b and c, respectively.

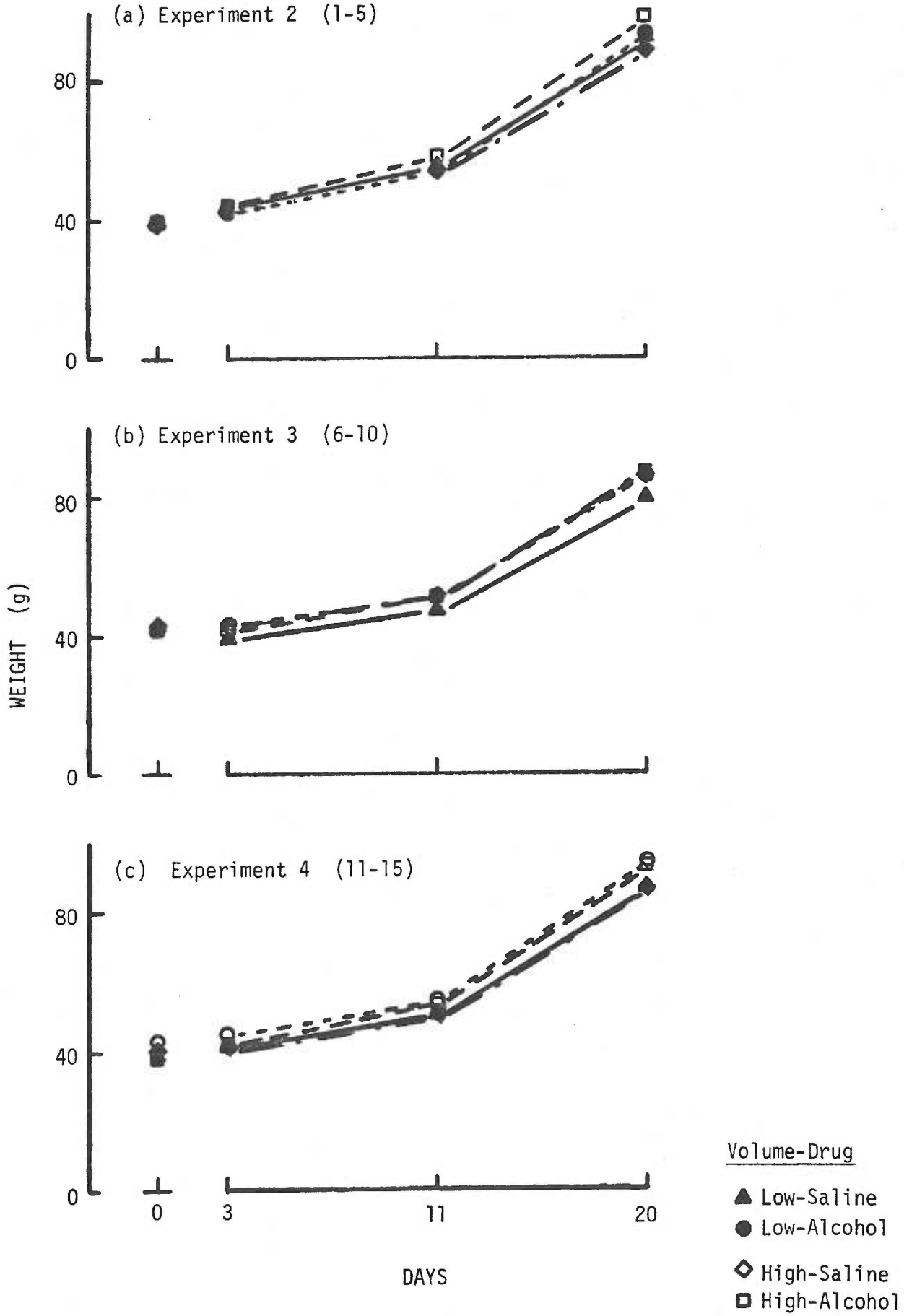
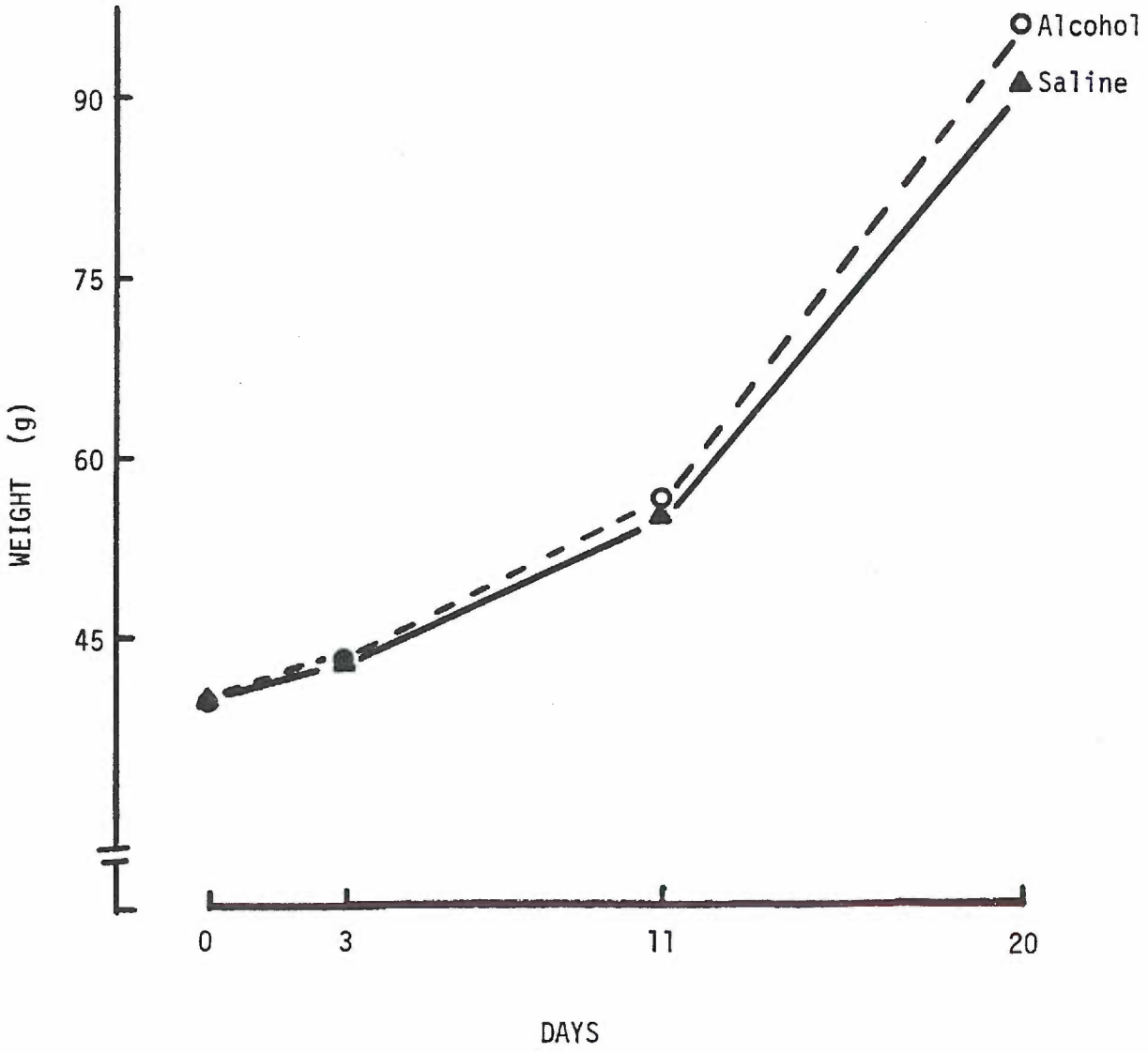

(a) Experiment 2 (1-5)
(b) Experiment 3 (6-10)
(c) Experiment 4 (11-15)
WEIGHT (g)
80
40
0
0
3
11
20
DAYS
Volume-Drug
Low-Saline
Low-Alcohol
High-Saline
High-Alcohol

Figure 7. Weight of alcohol- and saline-treated animals (collapsed across volume) from Experiment 4. The axes have been expanded to depict more clearly the drug treatment x days interaction.

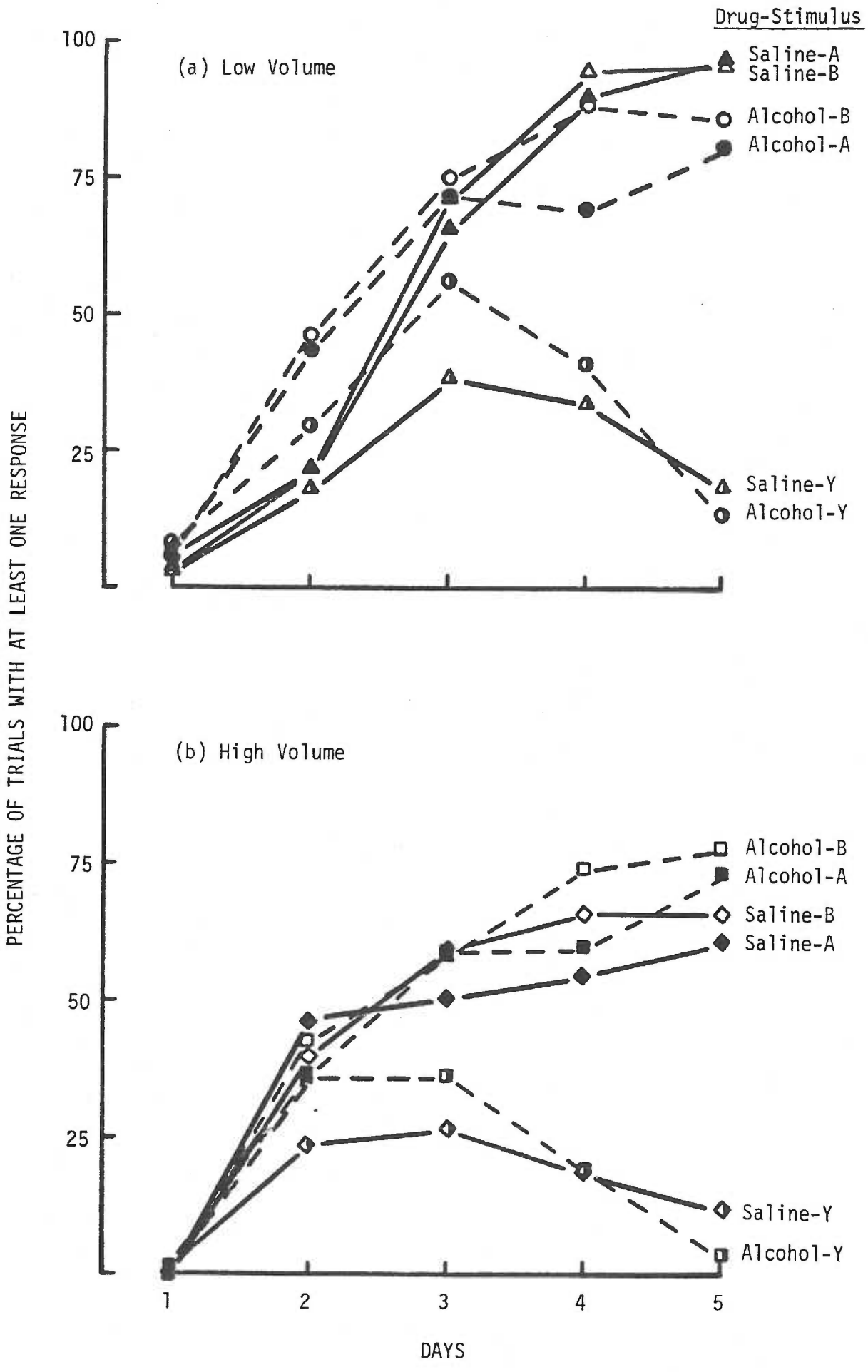

Alcohol
Saline
90
75
60
45
WEIGHT (g)
0
3
11
20
DAYS

Y (yellow) were never followed by food.

Experiment 2: Acquisition. Figure 8 depicts the percentage of acquisition trials with at least one response for the groups treated on Days 1-5 of incubation. Responding by the low-volume subgroups is plotted in Figure 8a, whereas responding by the high-volume birds is represented in Figure 8b. Solid symbols indicate response levels to Stimulus A (red), open symbols indicate Stimulus B (green), and half open, half solid symbols represent Stimulus Y (yellow). As can be seen in the figure, responding to the two reinforced stimuli (A and B) increased over days. Responding to the yellow, nonreinforced stimulus, on the other hand, increased for the first two to three days, and then decreased sharply. In addition, it is apparent that the terminal levels reached by low-volume subgroups in response to Stimuli A and B were higher that those reached by high-volume animals, and that response levels to Stimulus Y were also higher for the low-volume birds on the final three days of acquisition.

Statistically, all of these observations were supported by the outcome of a volume x drug treatment (alcohol vs. saline) x stimuli x days analysis of variance in which the main effect of days was reliable, F (4, 128) = 68.16, $p < .001$, as was the main effect of stimuli, F (2, 64) = 82.68, $p < .001$. Additionally, there was a reliable interaction of stimuli with days, F (8, 256) = 61.12, $p < .001$, and a significant volume x days interaction, F (4, 128) = 3.66, $p < .01$. The volume x treatment x stimuli x days interaction was also reliable, F (8, 256) = 2.60, $p < .01$, and thus follow-up analyses were performed for each of the three stimuli. Figure 9 portrays the percentage of

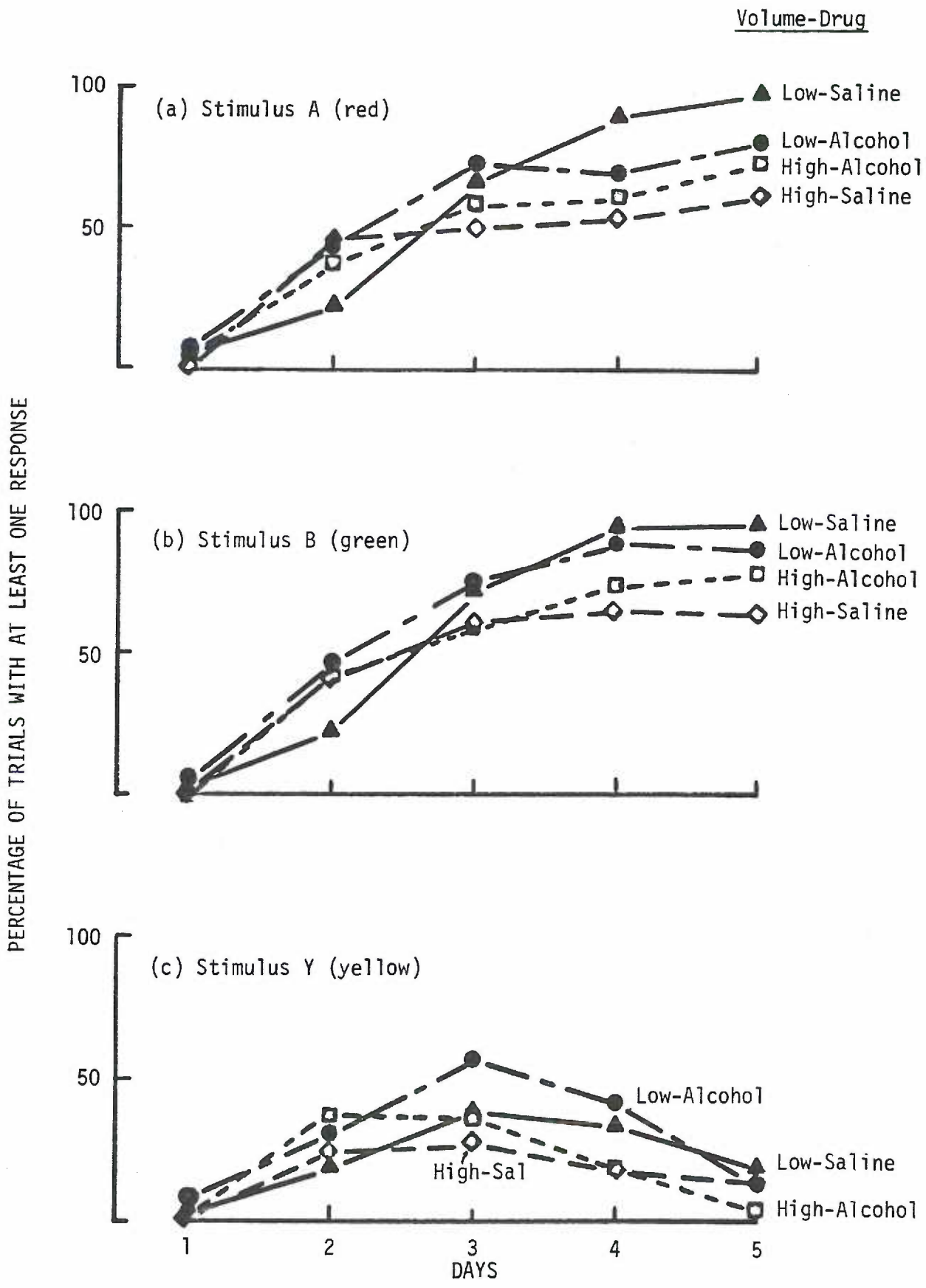
Figure 8. Percentage of autoshaping acquisition trials with at least one response for the various groups in Experiment 2 (Treatment Days 1-5 of incubation). Panel a depicts the low-volume birds, and Panel b, the high-volume birds.


Drug-Stimulus
(a) Low Volume
Saline-A
Saline-B
Alcohol-B
Alcohol-A
Saline-Y
Alcohol-Y
(b) High Volume
Alcohol-B
Alcohol-A
Saline-B
Saline-A
Saline-Y
Alcohol-Y
100
75
50
25
1
2
3
4
5
DAYS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

trials with at least one response for each of the acquisition stimuli over days. Response levels to Stimuli A, B and Y are plotted in Panels a, b and c, respectively, Open symbols represent high-volume subgroups, and solid symbols, low-volume subgroups. Reference to Panel a discloses two important points: First, by the end of acquisition training, the low-volume animals were responding on a larger percentage of trials to Stimulus A than high-volume animals. This was evidenced by a reliable volume x days interaction in the three-way analysis of variance for Stimulus A, F (4, 128) = 3.28, $p < .05$. Secondly, it can be seen that over days, the low-volume alcohol-treated animals achieved a lower level of responding to Stimulus A than low-volume placebo (saline)-treated animals, whereas for the high-volume subgroups, there was essentially no difference due to drug treatment. These effects were supported by a significant volume x treatment x days interaction, F (4, 128) = 2.78, $p < .05$, and by a reliable treatment x days interaction for the low-volume group in a follow-up two-way analysis, F (4, 64) = 3.44, $p < .05$. A comparable comparison between the high-volume subgroups indicated no differences, F (4, 64) < 1.0. For Stimulus B, the volume x drug treatment x days interaction was not significant, but the low-volume subgroups did achieve a higher response level to that stimulus than the high-volume animals, as evidenced by a reliable volume x days interaction, F (4, 128) = 3.16, $p < .05$.

From Figure 9c it can be seen that in the case of Stimulus Y, the low-volume animals again responded on a higher percentage of trials than the high-volume animals. This was supported statistically by a main effect of volume, F (1, 32) = 5.11, $p < .05$. There was,

Figure 9. Percentage of trials with at least one response for each of the three acquisition stimuli in Experiment 2. Response levels to Stimuli A, B and Y are plotted in Panels a, b and c, respectively.


Volume-Drug
100
50
(a) Stimulus A (red)
Low-Saline
Low-Alcohol
High-Alcohol
High-Saline
100
50
(b) Stimulus B (green)
Low-Saline
Low-Alcohol
High-Alcohol
High-Saline
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE
100
50
(c) Stimulus Y (yellow)
Low-Alcohol
High-Sal
Low-Saline
High-Alcohol
1
2
3
4
5
DAYS

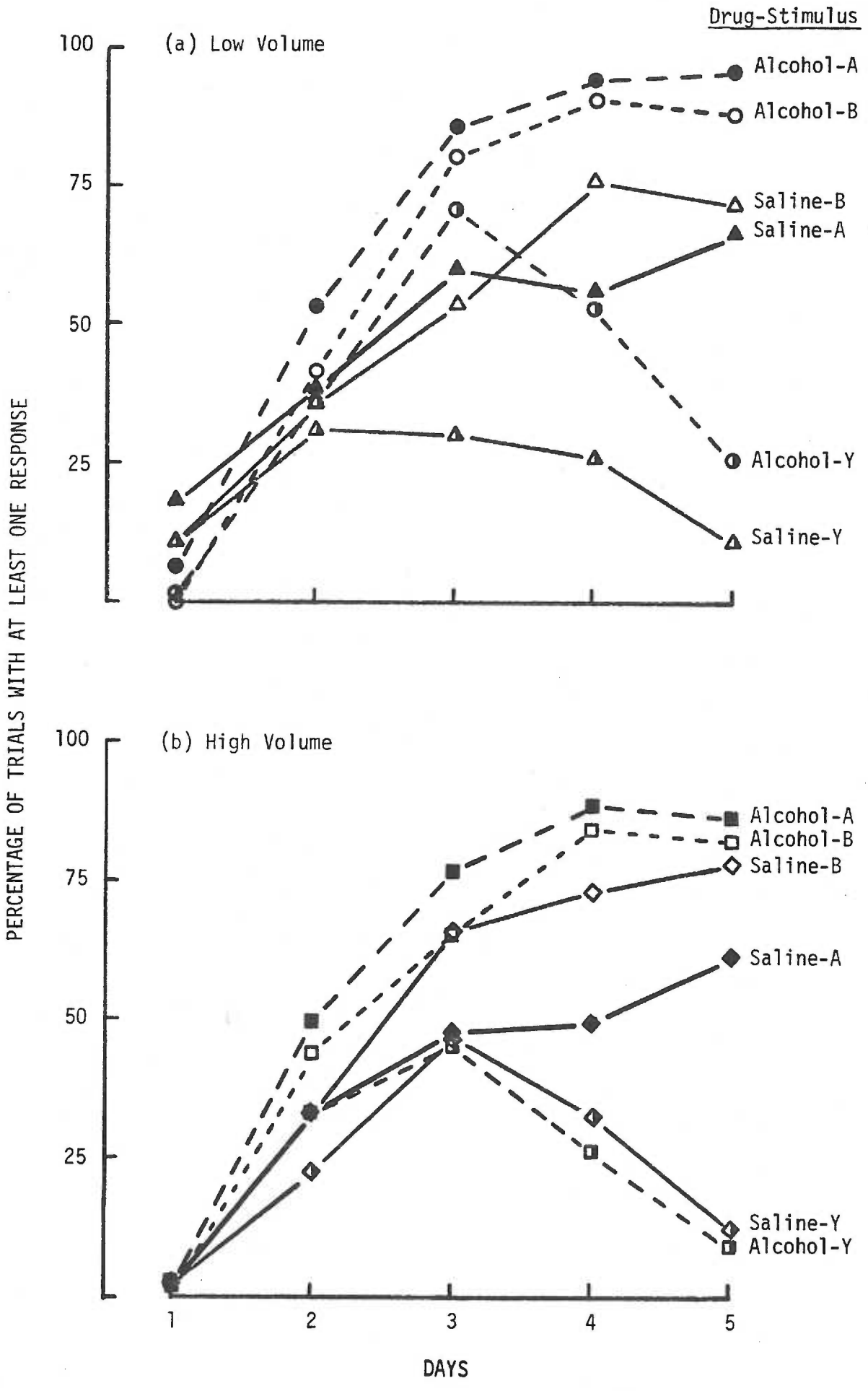
however, no difference due to alcohol treatment.

In summary, the four-way interaction observed in the overall analysis of Experiment 2 may be attributed to the following factors: (a) a higher terminal response level by low-volume animals than by high-volume animals for all three stimuli, and (b) the development, over days, of a higher level of responding to Stimulus A (red) by low-volume saline animals than by low-volume alcohol animals, combined with no difference between alcohol and saline animals in the high-volume subgroups. Alcohol had no effect on responding to the reinforced green (B) or nonreinforced yellow (Y) stimuli.

Experiment 3: Acquisition. Figure 10 portrays the percentage of acquisition trials with at least one response for those birds treated during Days 6-10 of embryonic development. Panel a shows responding by low-volume animals, Panel b shows responding by high--volume birds, and the drug treatments and stimuli are represented symbolically in the same manner as in Figure 8. As can be seen from the figure, responding to the two reinforced stimuli (A and B) increased over days for all groups. Responding to the nonreinforced stimulus (Y), on the other hand, initially increased and then decreased over the final three days of acquisition. This general pattern is similar to that described for subjects in Experiment 2. It is also apparent from the figures that while response levels were apparently unaffected by volume of injection, the ethanol-treated animals generally responded on a consistently higher percentage of trials than saline-treated birds.

These observations were supported statistically by a four-way

Figure 10. Percentage of autoshaping acquisition trials with at least one response for the various groups in Experiment 3 (Treatment Days 6-10 of incubation). Panel a depicts the low-volume birds, and Panel b, the high-volume birds.


Drug-Stimulus
(a) Low Volume
100
75
50
25
Alcohol-A
Alcohol-B
Saline-B
Saline-A
Alcohol-Y
Saline-Y
(b) High Volume
100
75
50
25
Alcohol-A
Alcohol-B
Saline-B
Saline-A
Saline-Y
Alcohol-Y
1
2
3
4
5
DAYS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

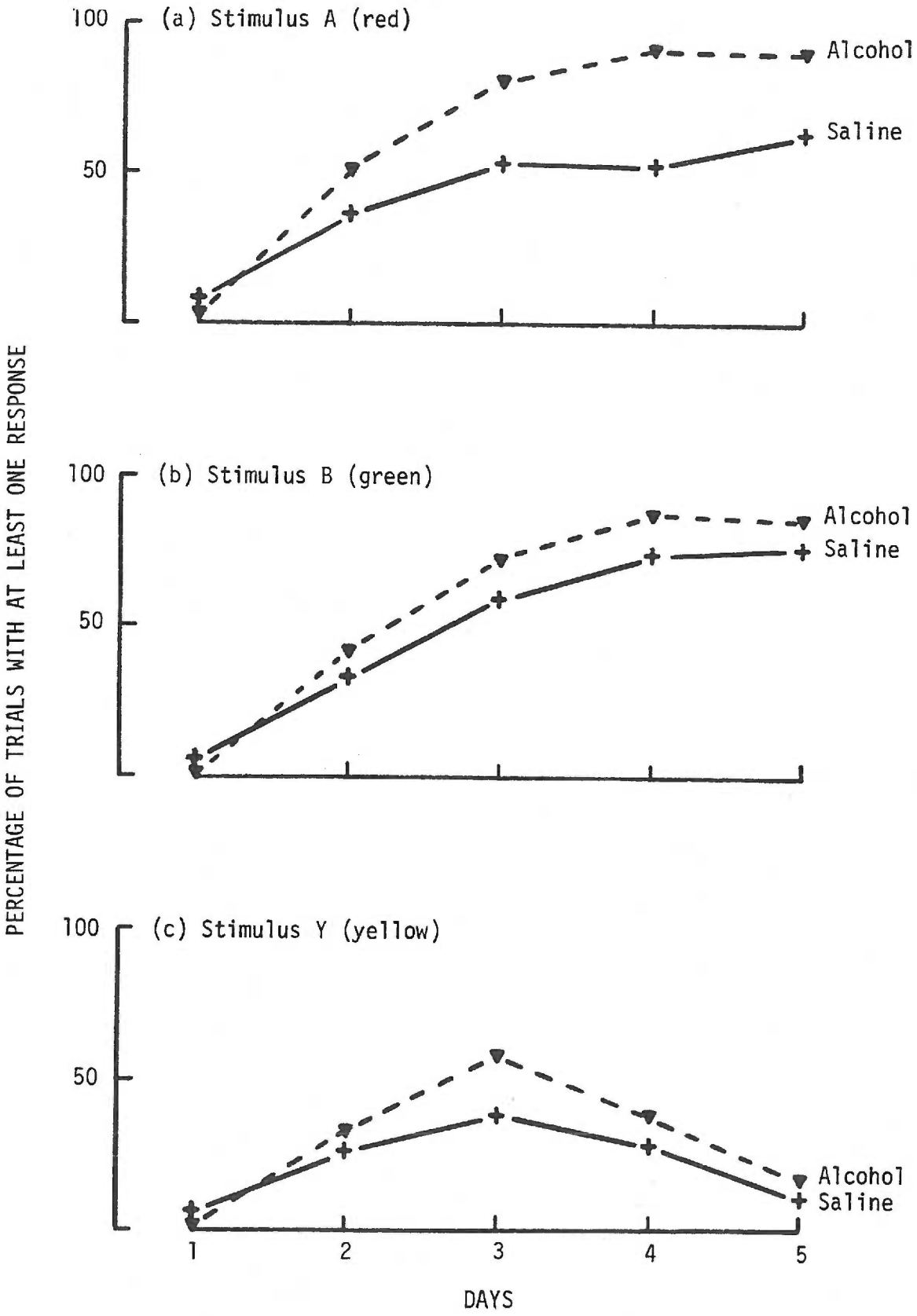
analysis of variance in which the main effect of stimuli was reliable, F (2, 56) = 90.29, $p < .001$, as were the main effect of days, F (4, 112) = 71.62, $p < .001$, and the stimuli x days interaction, F (8, 224) = 46.04, $p < .001$. There was no main effect of volume, and none of the interactions involving volume was significant. There was, however, a reliable treatment x stimuli interaction, F (2, 56) = 4.76, $p < .05$, a significant treatment x days interaction, F (4, 112) = 3.26, $p < .05$, and a reliable treatment x stimuli x days interaction, F (8, 224) = 2.30, $p < .05$.

In order to evaluate more fully the treatment x stimuli x days interaction, a two-way analysis was performed for each stimulus, with factors of treatment and days (collapsed across volume). Graphic representations of these data are presented in Figure 11. Although the main effect of days was reliable for all three stimuli ($ps < .001$), the main effect of treatment and the interaction of treatment with days failed to reach significance for either the green or the yellow stimulus (Stimuli B and Y). However, for the red key light (Stimulus A), the main effect of treatment was reliable, F (1, 30) = 5.82, $p < .05$, as was the treatment x days interaction, F (4, 120) = 6.92, $p < .001$.

In summary, acquisition responding was affected by treatment on Days 6-10 of incubation in only one way: Prenatal exposure to alcohol enhanced performance during the latter half of autoshaping acquisition, primarily as a result of increased responding to the reinforced red stimulus (A).

Experiment 4: Acquisition. Figure 12 depicts the percentage

Figure 11. Percentage of trials with at least one response for each of the three acquisition stimuli in Experiment 3, collapsed across the volume factor. Response levels to Stimuli A, B and Y are plotted in Panels a, b and c, respectively.


100
(a) Stimulus A (red)
Alcohol
Saline
50
100
(b) Stimulus B (green)
Alcohol
Saline
50
100
(c) Stimulus Y (yellow)
50
Alcohol
Saline
1
2
3
4
5
DAYS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

of acquisition trials with at least one response for those groups treated on Days 11-15 of incubation. Panel a portrays responding by low-volume animals, whereas Panel b portrays responding by high-volume birds. The symbols represent the same groupings as in Experiments 2 and 3. As in the previous experiments, responding to the two reinforced stimuli increased steadily over days, while responding to the nonreinforced stimulus increased initially and then decreased for all groups. The levels of responding of the high- and low-volume groups appear to be roughly comparable, although the high-volume saline chicks seem to have responded on a higher percentage of trials to all three stimuli on Days 2 and 3.

These observations were upheld statistically by the outcome of a volume x drug treatment x stimuli x days analysis of variance in which the days effect was reliable, F (4, 112) = 97.98, $p < .001$, as was the main effect of stimuli, F (2, 56) = 73.19, $p < .001$, and the interaction of stimuli with days, F (8, 224) = 57.12, $p < .001$. In addition, the volume x drug treatment x days interaction was significant, F (4, 112) = 3.66, $p < .01$. This latter interaction is best interpreted by reference to Figure 13 in which percentage of trials with a response is plotted for the four groups over acquisition days, collapsed across stimuli. It is evident from the figure that the high-volume saline group's responding was at a higher level than that of the remaining groups on the third day of acquisition. Reference back to Figure 12b suggests that this higher level of responding was present for all three stimuli, including the nonreinforced Stimulus Y. This overall increase in response level by the high-volume saline group on the third day of

Figure 12. Percentage of autoshaping acquisition trials with at least one response for the various groups in Experiment 4 (Treatment Days 11-15 of incubation). Panel a depicts the low-volume animals, and Panel b, the high-volume animals.

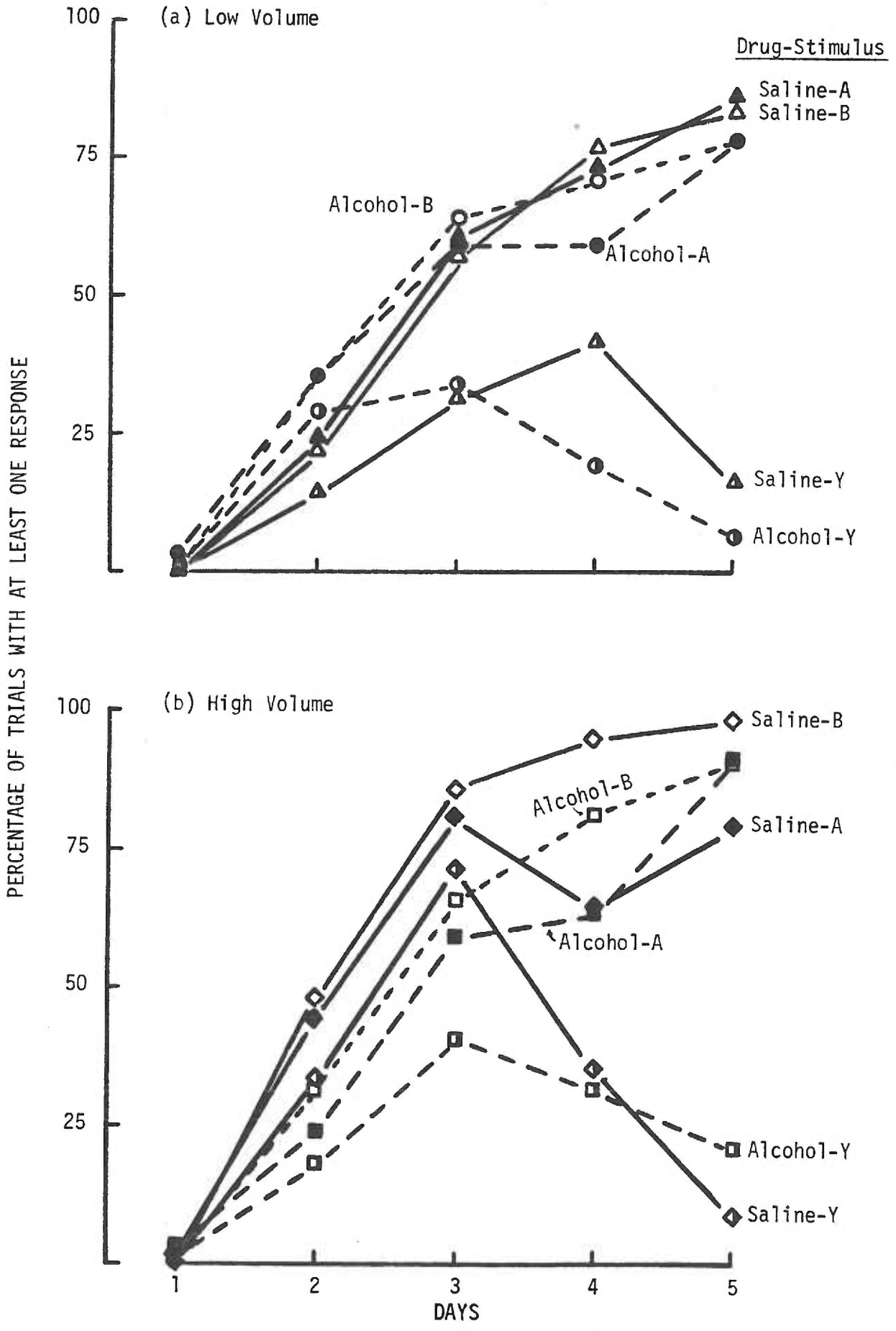
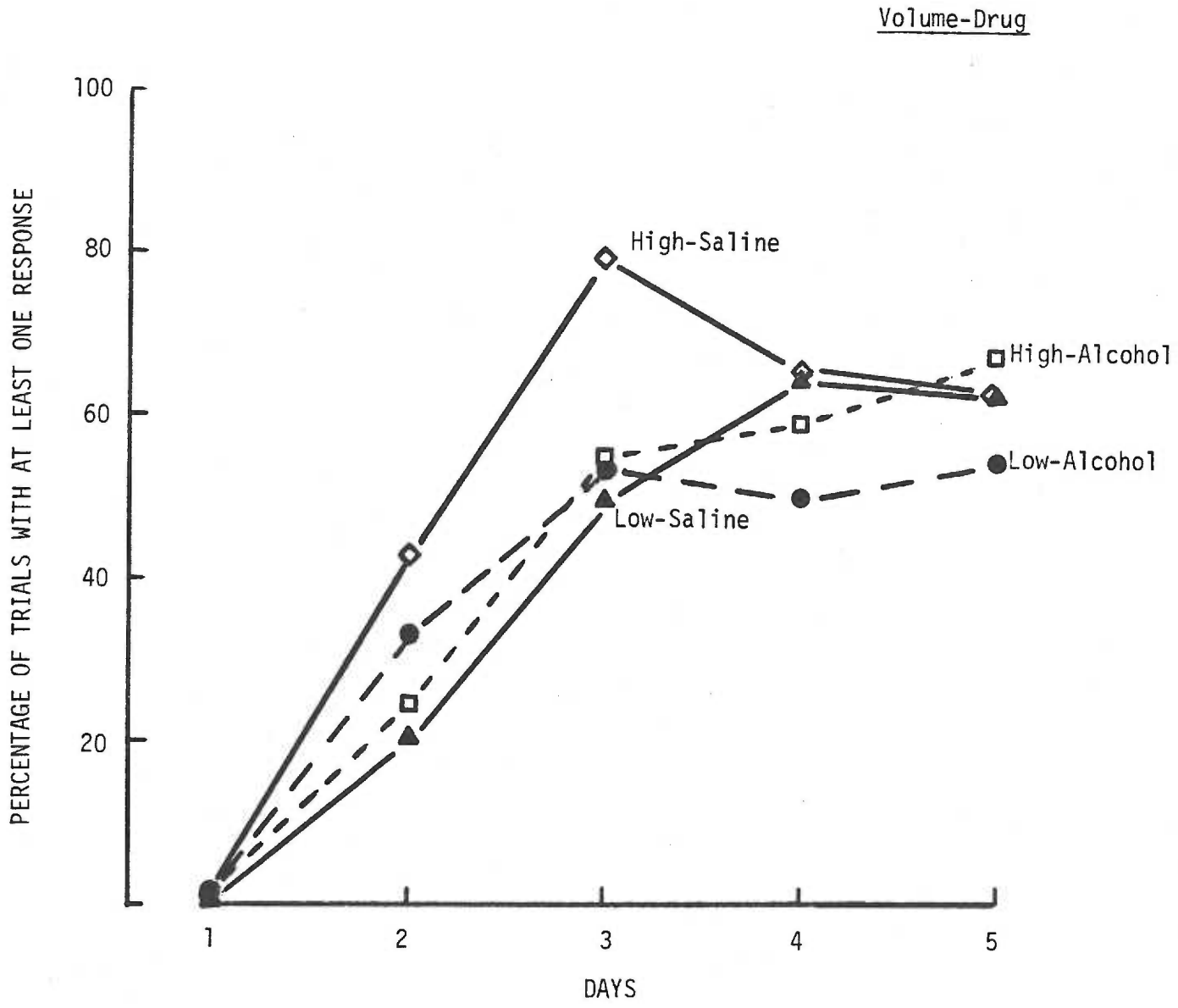

(a) Low Volume
Drug-Stimulus
Saline-A
Saline-B
Alcohol-B
Alcohol-A
Saline-Y
Alcohol-Y
100
75
50
25
(b) High Volume
Saline-B
Alcohol-B
Saline-A
Alcohol-A
Alcohol-Y
Saline-Y
1
2
3
4
5
DAYS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

Figure 13. Percentage of trials with at least one response for the four groups in Experiment 4, collapsed across stimuli.


Volume-Drug
100
80
60
40
20
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE
High-Saline
High-Alcohol
Low-Alcohol
Low-Saline
1
2
3
4
5
DAYS

training thus accounts for the volume x drug treatment x days interaction in the four-way analysis. It is important to note, however, that by the end of acquisition training, responding to the various stimuli was at approximately the same level for all groups (refer to Figures 12 and 13).

In summary, treatment on Days 11-15 of incubation resulted in an overall level of responding by the high-volume saline groups which was markedly higher on the third day of acquisition than responding by any of the other groups.

Overall summary: Acquisition. During discriminated autoshaping acquisition, responding was affected by experimental manipulation at each of the three developmental periods. Birds treated with a high--volume solution on Days 1-5 of incubation (Experiment 2) responded at lower levels to all stimuli, regardless of solution injected. Furthermore, alcohol produced a relatively lower terminal response level to the red stimulus in the low-volume condition, but did not affect responding to the green or yellow stimuli. In contrast, chicks treated with alcohol on Days 6-10 of incubation (Experiment 3) achieved a higher overall level of responding to the red stimulus, while responding to green and yellow was unaffected. Finally, animals treated with a high volume of saline on Days 11-15 of incubation (Experiment 4) demonstrated a level of responding which was markedly higher on the third day of acquisition than responding by any of the other groups. This effect was apparently the result of a random fluctuation in responding since it was not present on any other day of acquisition nor did it

occur during later phases of the experiment.

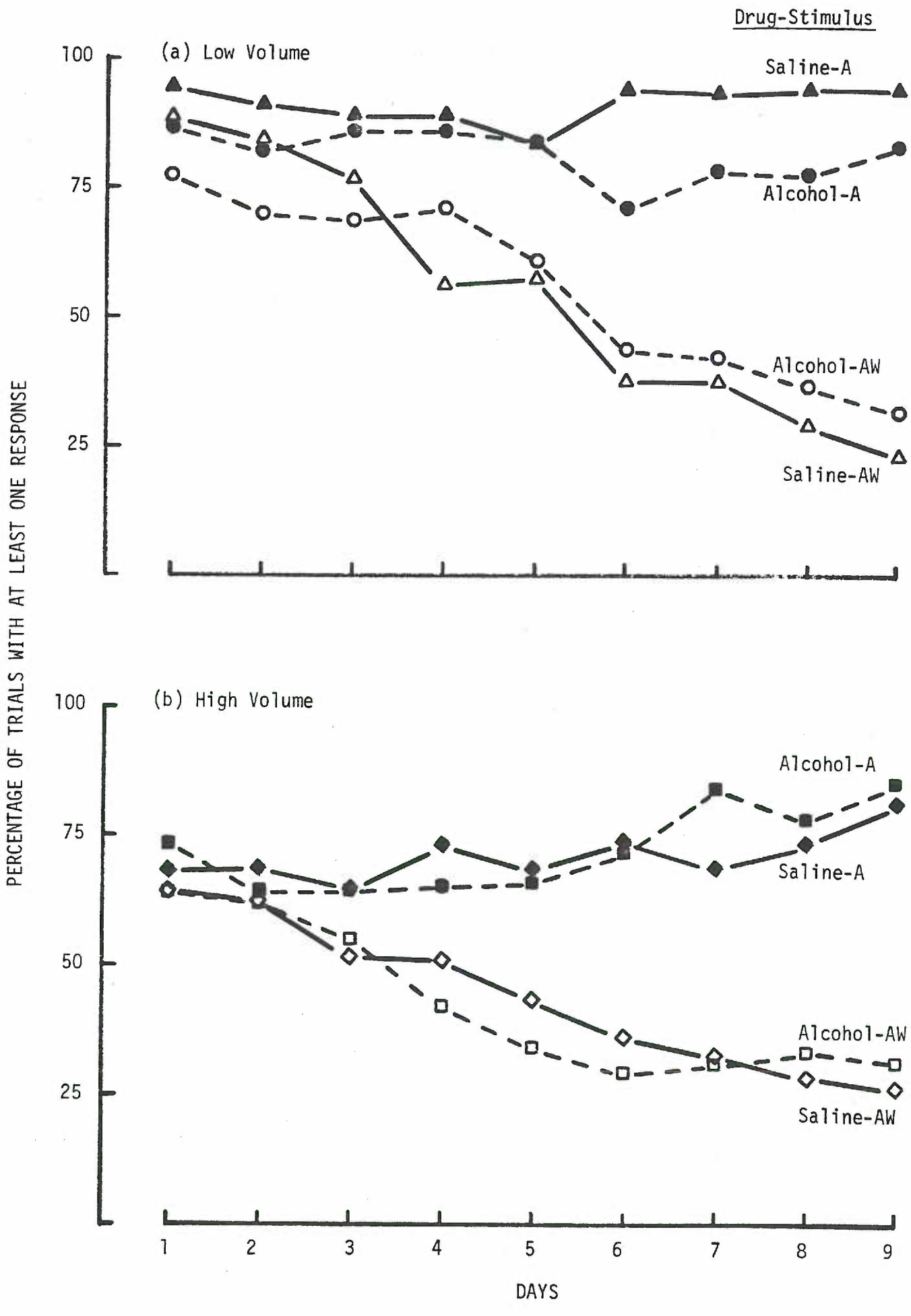
Conditioned Inhibition Training and Summation-Transfer Test

During the nine conditioned inhibition sessions, Stimulus A (red) was reliably followed by food presentations, while Stimulus AW (red key light plus white off-key light) was never followed by food. Throughout conditioned inhibition training, Stimulus B (green) was reinforced with the intent of maintaining it as an excitor for the later transfer test. Because this was the only purpose of B presentations, responding to B was only analyzed graphically during conditioned inhibition training (in order to ascertain that all animals continued to respond to it on a regular basis). In the subsequent summation-transfer test, the ability of the off-key light to act as a conditioned inhibitor was examined by compounding the off-key light with the green key light, which had previously always been reinforced, and which had never been presented as part of a compound.

Experiment 2: Conditioned inhibition training. Percentage of trials with at least one response over the nine days of conditioned inhibition training is plotted in Figure 14 for animals treated on Days 1-5 of incubation. Panel a portrays responding by the low-volume subgroups, whereas Panel b depicts responding by high-volume animals. The symbolic representation for this figure is the same as in previous figures. A four-way analysis of variance was performed on these data to compare responding to Stimuli A and AW (red and red-off key compound) over days of training.

As can be seen from the figure, responding to the nonreinforced

Figure 14. Percentage of A and AW trials with at least one response during conditioned inhibition training, Experiment 2. Panel a depicts low-volume birds, while Panel b represents high-volume animals.


Drug-Stimulus
(a) Low Volume
Saline-A
Alcohol-A
Alcohol-AW
Saline-AW
100
75
50
25
(b) High Volume
Alcohol-A
Saline-A
Alcohol-AW
Saline-AW
100
75
50
25
1
2
3
4
5
6
7
8
9
DAYS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

compound stimulus (AW) decreased steadily over days, indicating that the added off-key light acquired the ability to inhibit responding in all groups. Responding to Stimulus A, on the other hand, was relatively constant, with perhaps a small decrease for the first few days of conditioned inhibition training, and a subsequent return to near-original levels. Furthermore, it is apparent that the low-volume animals responded on a higher percentage of trials overall than did the high-volume subgroups.

These observations were supported statistically by a reliable volume effect, F (1, 32) = 4.39, $p < .05$, by a reliable effect of days, F (8, 256) = 10.61, $p < .001$, and by a reliable interaction of stimuli with days, F (8, 256) = 40.65, $p < .001$. In addition to the above-mentioned factors, the following items attained significance: the main effect of stimuli, F (1, 32) = 101.37, $p < .001$, the interaction of volume with days, F (8, 256) = 2.13, $p < .05$, and the volume x drug treatment x stimuli x days interaction, F (8, 256) = 2.11, $p < .05$.

The volume x days interaction is best interpreted by reference to Figure 14. As can be seen in the figure, the high-volume birds began conditioned inhibition training at a lower response level than low-volume birds. This is consistent with the pattern established during the acquisition phase. However, it is also apparent that over days of conditioned inhibition training, the high-volume animals achieved approximately the same terminal level to both stimuli as low-volume animals. The between-volume groups differences in initial levels of responding combined with the comparable terminal levels therefore accounts for the volume x days interaction.

In order to analyze further the four-way interaction, individual three-way analyses of variance with factors of volume, drug treatment and days were conducted for each of the two stimuli. None of the main effects or interactions was significant for Stimulus A, indicating that the difference between the low-volume alcohol and low--volume saline animals which occurred at the end of the acquisition phase was no longer present in the conditioned inhibition phase. In addition, only the days effect was significant for Stimulus AW, F (8, 256) = 32.67, $p < .001$, with none of the other main effects or interactions attaining significance.

Because neither volume nor drug treatment was shown to affect responding to A or AW in separate, follow-up analyses, a different approach was used to account for the four-way interaction: Three-way analyses, with factors of drug treatment, stimuli and days were performed for each of the volume groups. The analysis for the low-volume group disclosed no main effect of drug treatment, but there was a reliable drug treatment x stimuli x days interaction, F (8, 128) = 3.37, $p < .005$. For the high-volume group, however, there was no main effect of drug treatment, and none of the interactions involving the treatment factor was significant. A post hoc follow-up to the three-way interaction which was disclosed for the low-volume group indicated that the interaction was due primarily to a greater divergence in responding to A and AW by the low-volume saline-treated birds than by the low-volume alcohol chicks, F (1, 128) = 4.55, $p < .05$.

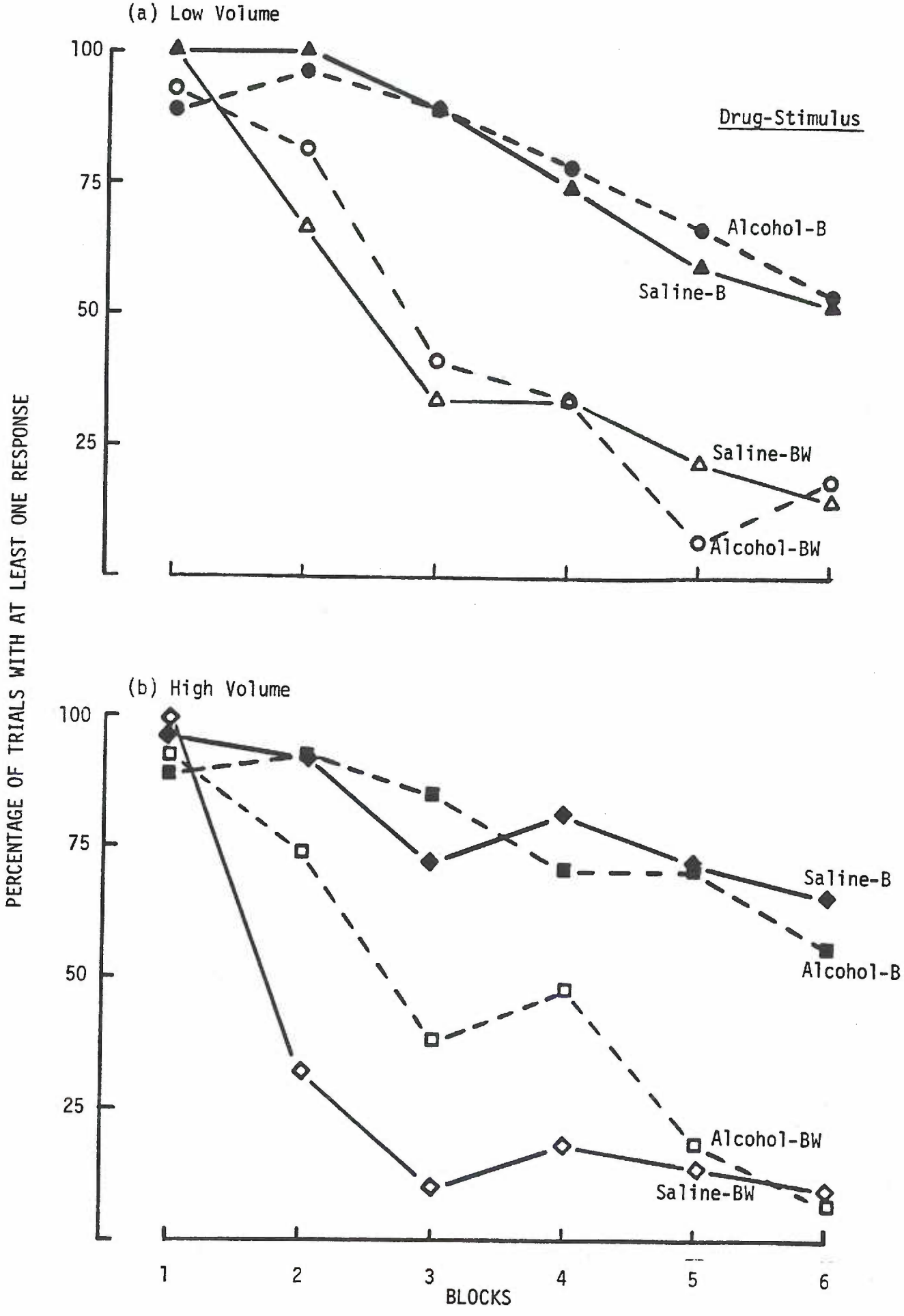
The four-way interaction in the overall analysis can thus be

accounted for as follows: (a) High-volume alcohol and saline birds responded approximately equivalently to Stimuli A and AW; and (b) Over days, low-volume saline animals showed a greater divergence (i.e., superior discriminated responding) to the two stimuli than the low-volume alcohol animals.

Finally, it should be noted that despite some minor fluctuations in responding to Stimulus B (green) over the days of conditioned inhibition training, responding to that stimulus was comparable for all groups by the end of training.

Experiment 2: Summation-transfer test. Figure 15 portrays the percentage of Stimulus B (solid symbols) and BW (open symbols) trials with at least one response over blocks of the transfer test. Responding by the low-volume birds is plotted in Panel a, whereas Panel b represents responding by the high-volume animals. It can be seen from the figure that the percentage of trials with a response decreased for all stimuli over blocks, although the decline in responding to the compound (BW) was markedly larger than the decline to Stimulus B alone. Furthermore, it is evident that responding to B was approximately the same for all groups, regardless of injection volume. Responding to the compound, however, appears to differ for the alcohol- and saline-treated animals, particularly in the high-volume (high-dose) birds. These observations were only partially supported by the outcome of a four-way analysis of variance with factors of volume, treatment, stimuli, and blocks. The analysis disclosed significant main effects of blocks, F (5, 160) = 67.45, $p < .001$, and stimuli, F (1, 32) = 190.69, $p < .001$, a significant interaction of

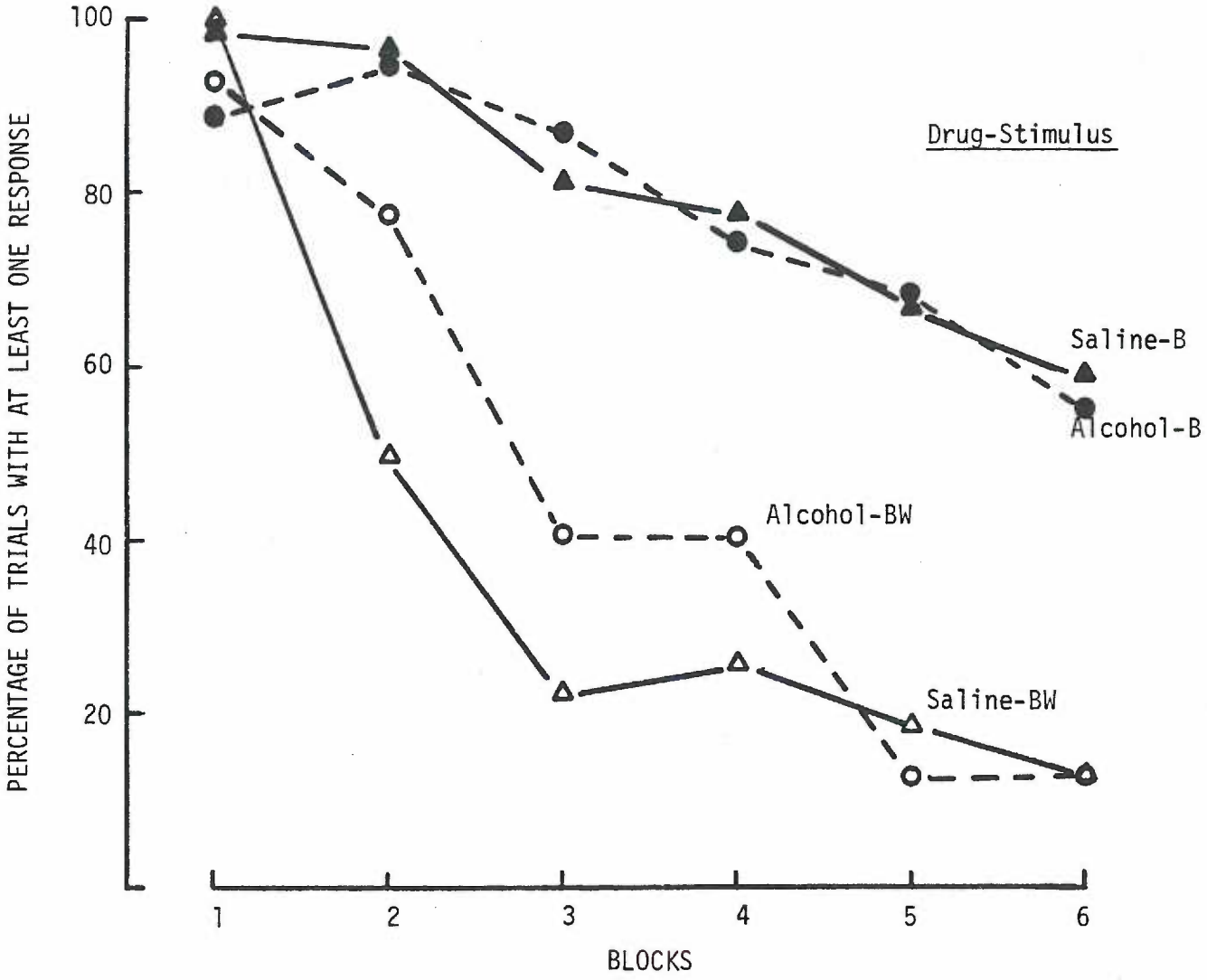
Figure 15. Percentage of B (solid symbol) and BW (open symbol) trials with at least one response over blocks of six trials during the summation-transfer test, Experiment 2. Panel a represents responding by low-volume birds, and Panel b represents responding by high-volume birds.


(a) Low Volume
100
75
50
25
Drug-Stimulus
Alcohol-B
Saline-B
Saline-BW
Alcohol-BW
(b) High Volume
100
75
50
25
Saline-B
Alcohol-B
Alcohol-BW
Saline-BW
1
2
3
4
5
6
BLOCKS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

stimuli with blocks, F (5, 160) = 15.54, $p < .001$, and a significant treatment x blocks interaction, F (5, 160) = 2.37, $p < .05$. All other interactions with treatment failed to reach significance, an outcome discrepant with the graphic interpretation given above.

In order to investigate this discrepancy more fully, and as a means of analyzing more succinctly the treatment x blocks interaction, the transfer test data were collapsed across the volume factor (since the four-way analysis disclosed no involvement of volume), and separate analyses of variance were performed for each stimulus (Figure 16). As is evident in Figure 16, the percentage of Stimulus B trials with a response was approximately the same for alcohol- and saline-treated animals, with a gradual decline over blocks. Responding to the compound (BW), on the other hand, declined much more rapidly, and appears to differ for the two treatment groups over blocks, with the alcohol--treated chicks responding to a greater extent than saline-treated birds in Blocks 2, 3 and 4. These observations were supported by the analyses, in which only the blocks effect was reliable for Stimulus B, F (5, 170) = 15.25, $p < .001$, whereas for Stimulus BW, the main effect of blocks was reliable, F (5, 170) = 70.58, $p < .001$, and the interaction of treatment with blocks was significant, F (5, 170) = 3.56, $p < .01$. It thus appears that the treatment x blocks interaction disclosed in the overall analysis is attributable primarily to a difference between the alcohol and saline animals in responding to BW. Although not indicated by statistical analysis, this difference graphically appears to be largely due to the high-volume animals (Figure 15b).

Figure 16. Experiment 2 summation-transfer test data, collapsed across the volume factor.


PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE
100
80
60
40
20
1
2
3
4
5
6
BLOCKS
Drug-Stimulus
Saline-B
Alcohol-B
Alcohol-BW
Saline-BW

Experiment 2: Summary of conditioned inhibition training and summation-transfer test. The difference between low-volume alcohol and saline animals in responding to Stimulus A at the end of acquisition was no longer reliable at the beginning of conditioned inhibition training. Nevertheless, the difference appears graphically to have been partially reinstated by the latter part of that training. Furthermore, a four-way interaction was found to be attributable to the following factors: (a) Over days, low-volume saline birds demonstrated a greater divergence to Stimuli A and AW than low-volume alcohol animals, and (b) High-volume alcohol and high-volume saline animals responded at comparable levels throughout training to Stimuli A and AW.

Although responding by high-volume birds was lower than responding by low-volume chicks at the beginning of conditioned inhibition training, the two volume groups were at comparable response levels by the end of that training. The initial difference was consistent with the pattern of responding established during the acquisition phase. In addition, responding to Stimulus B was found to be roughly equivalent for all groups by the final day of conditioned inhibition training.

In the summation-transfer test, alcohol- and saline-treated animals responded in a similar fashion to Stimulus B, an excitor. However, on trials in which B was compounded with a conditioned inhibitor, W, alcohol-treated birds responded on a greater percentage of trials during the second, third and fourth blocks of the transfer test than saline-treated chicks. Although both groups reached the same final low level of responding to the compound, the alcohol-treated animals

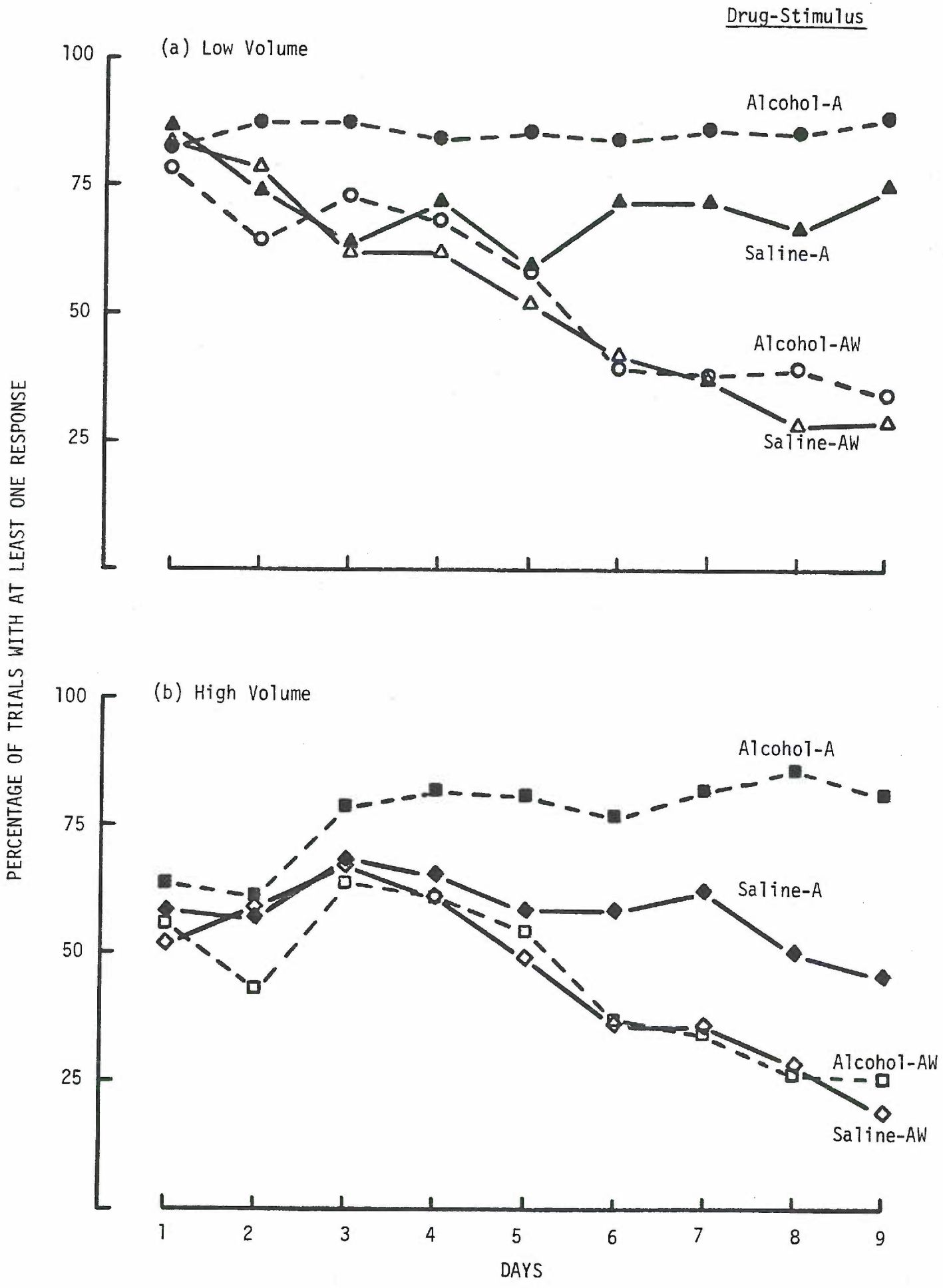
approached that level more gradually, suggesting that prenatal exposure to alcohol interfered with the transfer of inhibition.

Experiment 3: Conditioned inhibition training. Because of equipment failure, scores from two of the animals in each subgroup were lost on the second day of conditioned inhibition training. In the statistical analyses, group means were substituted for missing scores, and the degrees of freedom were reduced accordingly.

Percentage of conditioned inhibition training trials with at least one response is plotted for birds treated on Days 6-10 of incubation in Figure 17a (low-volume) and b (high-volume). The general features of this figure are similar to those of conditioned inhibition figures from the previous experiments. It can be seen that responding to the reinforced Stimulus A remained relatively constant throughout the nine days of training, although it will be noticed that alcohol--treated animals appear to have responded at a higher level than saline-treated animals. This pattern of responding is consistent with the trend established during the acquisition phase of this experiment. Responding to Stimulus AW was approximately equivalent for all groups through most of training, although it is apparent from Figure 17 that the high-volume animals started conditioned inhibition training at a lower level of responding to AW than the low--volume animals.

A four-way analysis was performed comparing response levels to Stimulus A and AW. The factors for the analysis were volume, drug treatment, stimuli, and days. The analysis disclosed reliable main

Figure 17. Percentage of A and AW trials with at least one response during conditioned inhibition training, Experiment 3. Panel a depicts low-volume birds, while Panel b represents high-volume animals.


Drug-Stimulus
(a) Low Volume
100
75
50
25
Alcohol-A
Saline-A
Alcohol-AW
Saline-AW
(b) High Volume
100
75
50
25
Alcohol-A
Saline-A
Alcohol-AW
Saline-AW
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE
1
2
3
4
5
6
7
8
9
DAYS

effects of stimuli, F (1, 28) = 61.43, $p < .001$, and days, F (8, 216) = 11.42, $p < .001$, and the following interactions were also significant: volume x days, F (8, 216) = 3.00, $p < .01$, stimuli x days, F (8, 208) = 31.02, $p < .001$, and treatment x stimuli, F (1, 28) = 7.00, $p < .05$.

Follow-up analyses for each stimulus indicated that the treatment x stimuli interaction was attributable to: (a) generally comparable levels of responding by alcohol- and saline-treated offspring to Stimulus AW, and (b) the achievement, over days, of a higher overall level of responding by alcohol animals to Stimulus A than by saline animals, F (8, 216) = 1.99, $p < .05$, for the treatment x days interaction. Thus, the treatment x stimuli interaction which was disclosed by the overall analysis was principally the result of differences in responding to A, not to AW.

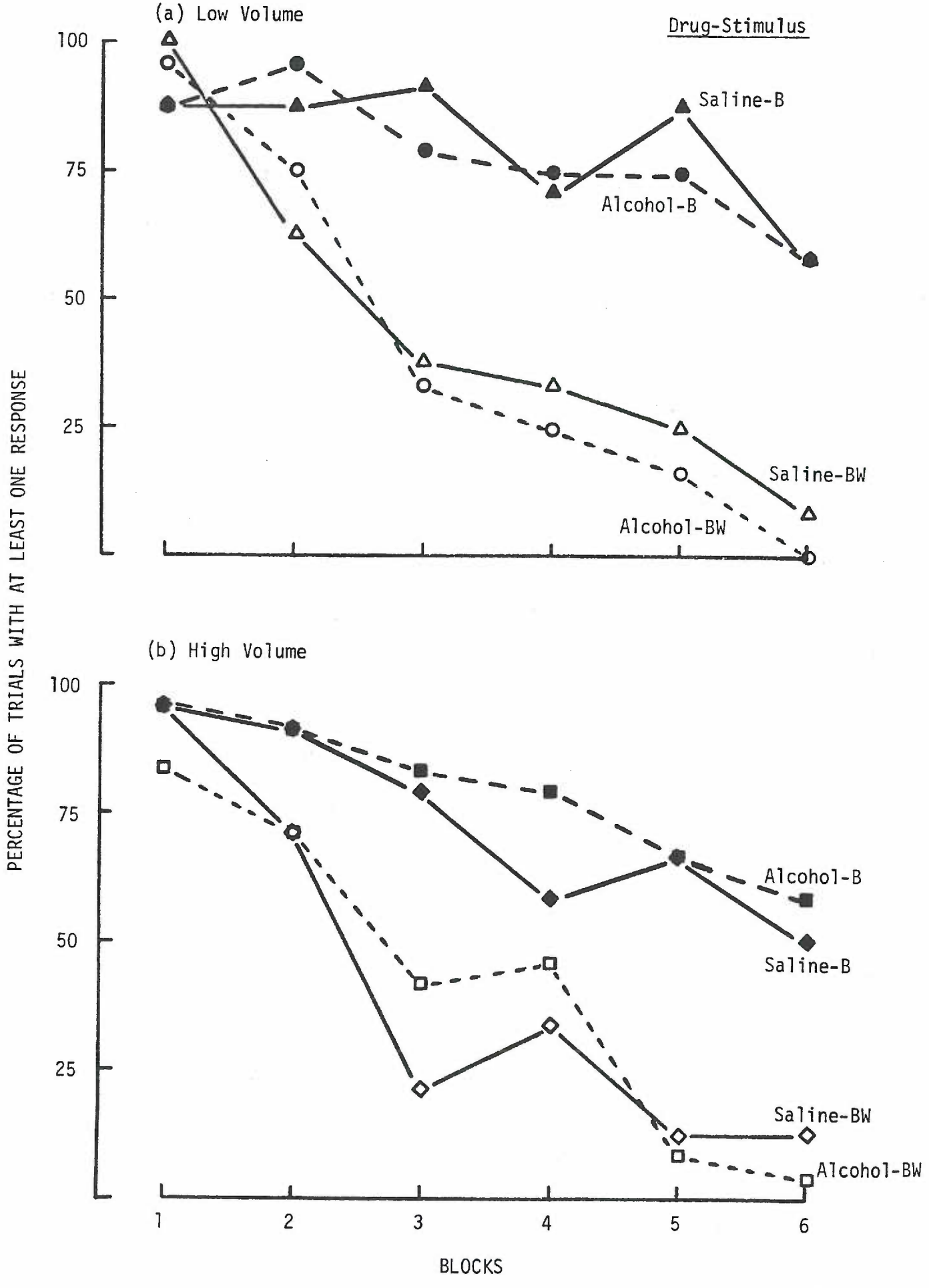
Reference to Figure 17 suggests that the volume x days interaction was the result of an initially lower response level for the high-volume animals than for the low-volume birds, combined with comparable terminal levels for the two volume groups. This pattern of responding was also found to account for a volume x days interaction in the previous experiment, although in that case, the initial difference appeared to have carried over from the acquisition phase. In the present experiment, this was not the case, since response levels of high- and low-volume animals were approximately equivalent at the end of acquisition training. Responding to Stimulus B (green) remained at approximately constant and equivalent levels for all groups throughout conditioned inhibition training.

Experiment 3: Summation-transfer test. Figure 18 depicts the percentage of transfer test trials with at least one response for the various groups in blocks of six trials. Panel a represents responding by the low-volume subgroups, whereas Panel b represents the high-volume subgroups. The solid symbols portray responding to Stimulus B and the open symbols, to Stimulus BW. It is apparent that responding to the green-off key compound (BW) was initially as high as responding to the green key light alone, but was reduced at a much greater rate over blocks. Overall, the response level to the compound can be seen to be markedly less than that to green alone. It is also evident that response levels were approximately the same for high- and low--volume animals, and that prenatal treatment with ethanol had little effect upon transfer test responding.

These observations were supported statistically by the outcome of a volume x drug treatment x stimuli x blocks analysis of variance in which there were main effects of stimuli, F (1, 28) = 136.96, $p < .001$, and of blocks, F (5, 140) = 48.64, $p < .001$, and an interaction of stimuli with blocks, F (5, 140) = 18.16, $p < .001$. None of the other main effects or interactions was significant. Thus while there was evidence of transfer of inhibition, there were no effects of prenatal treatment.

Experiment 3: Summary of conditioned inhibition training and summation-transfer test. For animals treated on Days 6-10 of incubation, the major difference disclosed in conditioned inhibition training was a higher response level by the alcohol-treated animals to the red key light (Stimulus A). This between-group difference carried over from

Figure 18. Percentage of B (solid symbols) and BW (open symbols) trials with at least one response over blocks of the summation-transfer test, Experiment 3. Panel a depicts responding by low-volume birds, and Panel b represents responding by high-volume birds.

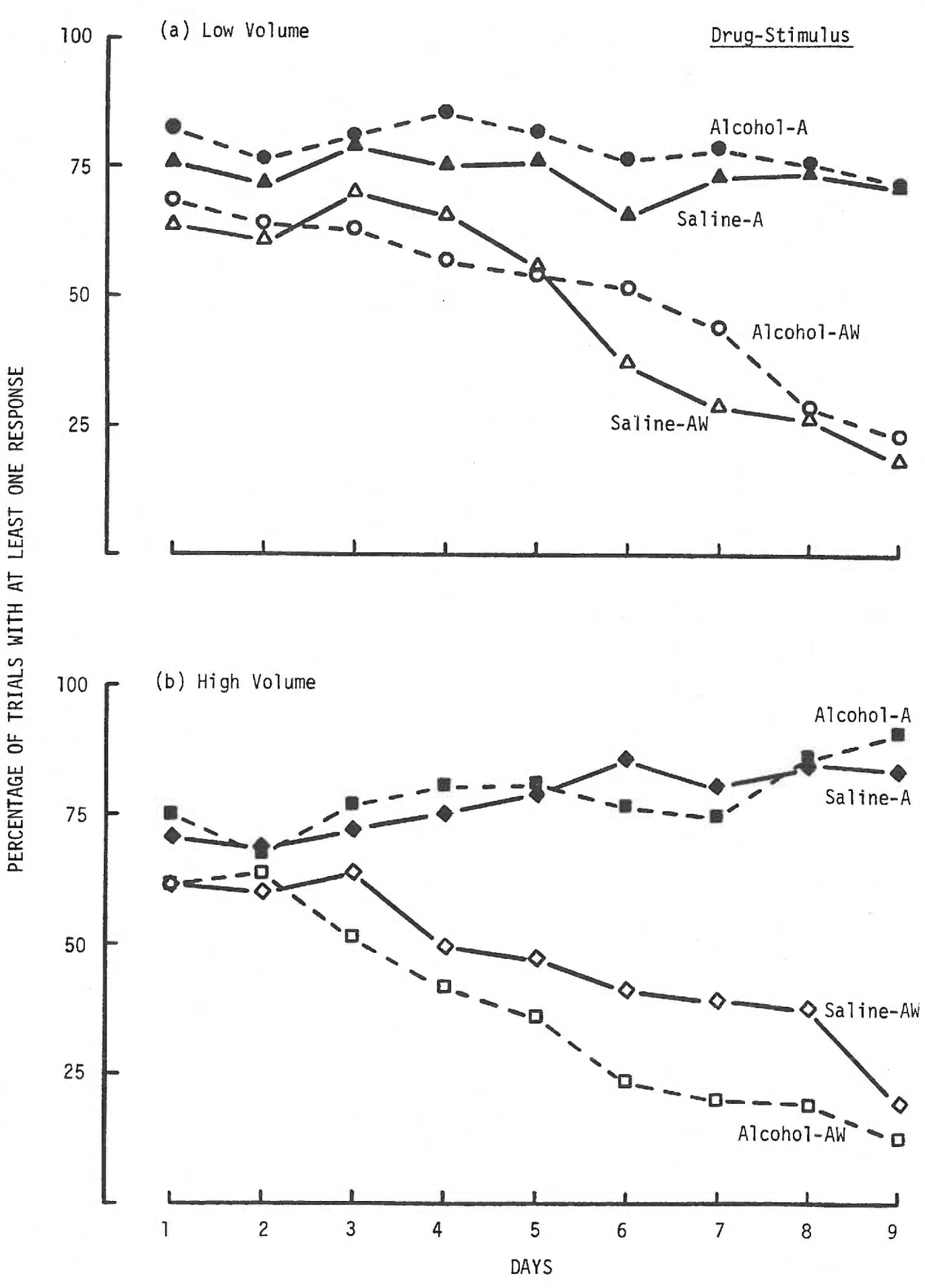

(a) Low Volume
Drug-Stimulus
100
75
50
25
Saline-B
Alcohol-B
Saline-BW
Alcohol-BW
(b) High Volume
100
75
50
25
Alcohol-B
Saline-B
Saline-BW
Alcohol-BW
1
2
3
4
5
6
BLOCKS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

the acquisition phase, and did not dissipate during conditioned inhibition training. Stimulus B (green) responding was equivalent for all groups throughout all nine of the conditioned inhibition sessions, and A and AW responding, although initially lower for the high-volume animals, became comparable for the high- and low-volume groups by the third day of training.

It is interesting to note that if responding to the red/off-key compound is compared to responding to the red key light alone, then the alcohol-treated birds actually performed at a relatively superior level (i.e., demonstrated better discrimination) to the saline-treated animals. Nevertheless, in a summation-transfer test of inhibition, there was no difference due to treatment.

Experiment 4: Conditioned inhibition training. Percentage of conditioned inhibition training trials with at least one response is plotted for animals treated on Days 11-15 of incubation in Figure 19. Responding by the low-volume subgroups is depicted in Panel a, and by the high-volume subgroups, in Panel b. It can be seen that responding to the compound stimulus decreased gradually over days with little difference between groups. In addition, it is apparent that responding to Stimulus A remained relatively constant throughout training, with perhaps a small increase over days for the high-volume animals and a slight decrease over days for the low-volume birds. Again, there appear to be no differences due to drug treatment. A four-way analysis of variance with factors of volume, drug treatment, stimuli, and days was performed to verify these observations. The analysis disclosed

Figure 19. Percentage of A and AW trials with at least one response during conditioned inhibition training, Experiment 4. Panel a depicts responding by low-volume animals, and Panel b represents high-volume animals.

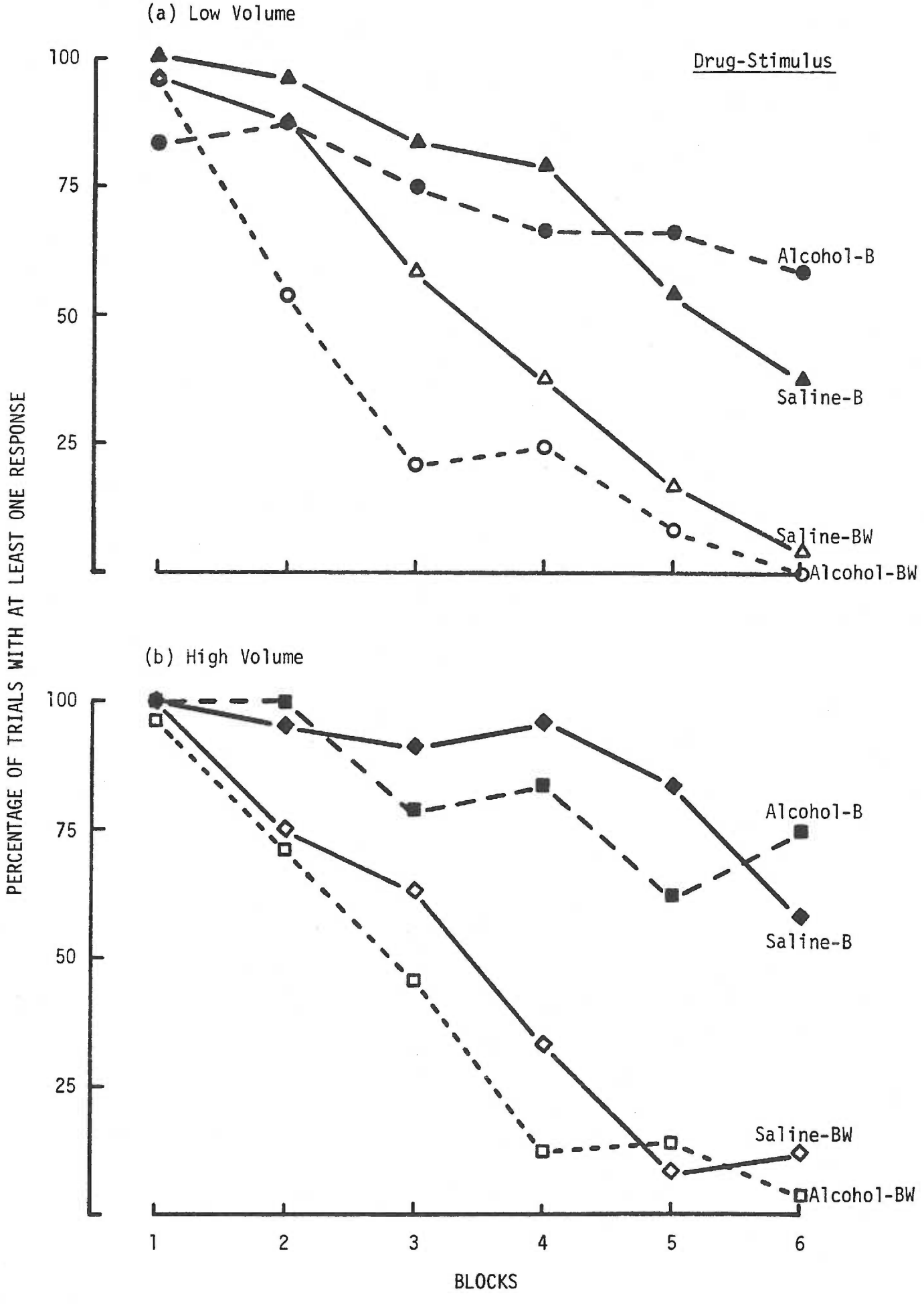

(a) Low Volume
Drug-Stimulus
100
75
50
25
Alcohol-A
Saline-A
Alcohol-AW
Saline-AW
(b) High Volume
100
75
50
25
Alcohol-A
Saline-A
Saline-AW
Alcohol-AW
1
2
3
4
5
6
7
8
9
DAYS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

a main effect of stimuli, F (1, 28) = 80.69, $p < .001$, and a main effect of days, F (8, 224) = 7.78, $p < .001$, and the stimuli x days interaction was also reliable, F (8, 224) = 3.85, $p < .01$. A significant interaction of volume x stimuli x days, F (8, 224) = 2.85, $p < .01$, was graphically interpreted to be the result of three factors: (a) an increase in responding to Stimulus A over days on the part of the high-volume birds, (b) a slight decrease in responding to Stimulus A over days by the low-volume birds, and (c) no difference between the volume groups in responding to Stimulus AW. There was no main effect of drug treatment and no interaction involving the treatment factor.

Responding to Stimulus B remained relatively constant throughout conditioned inhibition training, and at the end of that training, response levels were roughly comparable for all groups.

Experiment 4: Summation-transfer test. The percentage of trials with at least one response is plotted for each of the four groups over test blocks in Figure 20. As can be seen in the graphs, responding to the compound stimulus (BW) subsided at a much more rapid rate than responding to B alone. The apparent difference between low-volume ethanol- and saline-treated animals in response to the compound stimulus (BW) was accompanied by a relatively large variance, and thus it was not expected that the difference would achieve statistical significance. Indeed, in a volume x drug treatment x stimuli x blocks analysis of variance, there was no main effect of volume or of drug treatment, and no interaction invloving either factor. The main effect of stimuli was reliable, however, F (1, 28) = 113.83, $p < .001$, as was the blocks

Figure 20. Percentage of B (solid symbols) and BW (open symbols) trials with at least one response over blocks of the summation-transfer test, Experiment 4. Panel a represents responding by low-volume birds, whereas Panel b depicts responding by high-volume animals.


(a) Low Volume
Drug-Stimulus
100
75
50
25
Alcohol-B
Saline-B
Saline-BW
Alcohol-BW
(b) High Volume
100
75
50
25
Alcohol-B
Saline-B
Saline-BW
Alcohol-BW
1
2
3
4
5
6
BLOCKS
PERCENTAGE OF TRIALS WITH AT LEAST ONE RESPONSE

effect, F (5, 140) = 64.11, $p < .001$, and the stimuli x blocks interaction, F (5, 140) = 19.71, $p < .001$. Thus, while there was transfer of inhibition, there was no effect of prenatal treatment.

Experiment 4: Summary of conditioned inhibition training and summation-transfer test. Although low-volume chicks treated on Days 11-15 of incubation ended conditioned inhibition training with a slightly lower percentage of Stimulus A trials with a response than high-volume chicks, there were no significant differences between alcohol- and saline-treated animals' responding to any of the three stimuli. Furthermore, treatment with ethanol had no effect on the transfer of inhibition in a combined-cue test.

Overall summary: Conditioned inhibition training. Animals treated on Days 1-5 of incubation (Experiment 2) exhibited the following response patterns during conditioned inhibition training: (a) High-volume alcohol and saline animals responded at comparable levels to Stimuli A and AW throughout the nine days of training; (b) Over days, low-volume saline birds demonstrated a greater divergence to Stimuli A and AW (i.e., superior discrimination) than the low-volume alcohol animals; and (c) Although responding by the high-volume birds was lower than that of low-volume chicks at the beginning of conditioned inhibition training, the two volume groups were comparable by the second half of that training. The initial difference was consistent with the response pattern established during the acquisition phase.

Chicks treated with alcohol on Days 6-10 of incubation (Experiment 3) demonstrated higher response levels to Stimulus A than chicks which had

been treated with saline. This difference was the continuation of a trend which was initially established during the acquisition phase of the experiment. By the third day of training, A and AW responding, although initially lower for the high-volume animals, reached comparable levels for both volume groups.

Finally, there were no differences among chicks treated on Days 11-15 of incubation in conditioned inhibition responding (Experiment 4).

Overall summary: Summation-transfer test. During the summation--transfer test, alcohol- and saline-treated chicks in Experiment 2 (Treatment Days 1-5) responded similarly to Stimulus B, the excitor. However, on the second, third and fourth blocks of the transfer test, alcohol-treated birds responded on a greater percentage of BW (compound) trials than saline birds, suggesting that prenatal exposure to alcohol interfered with the transfer of inhibition.

In Experiments 3 and 4 (Treatment Days 6-10 and 11-15, respectively) there was no effect of drug treatment or volume on summation-transfer test responding.

Discussion

Experiment 2

Embryonic exposure of chicks to ethanol on Days 1-5 of incubation affected subsequent behavior in a variety of ways. First, during acquisition, birds which had been treated with a low dose of alcohol achieved a lower response level to the red stimulus than low-volume control birds, but there was no difference in responding to green or yellow. In addition, low-dose alcohol-treated animals displayed a reduced degree of divergence in responding to a reinforced red and nonreinforced compound stimulus over days of conditioned inhibition training. Finally, there was evidence that prenatal exposure to alcohol interfered with the chick's ability to demonstrate transfer of inhibition.

An effect of injection volume was also apparent in the present experiment: Birds that had been injected with a high-volume solution responded at a lower level, regardless of drug type. This volume effect was present through conditioned inhibition training, although it was no longer apparent in the summation-transfer test.

Because earlier pilot studies had indicated a possible effect of injection volume, the volume effect noted in the present study was not completely unexpected. Although this effect points to the sensitivity of these response measures to prenatal treatment, it also accentuates the degree of fragility of the embryo to extraneous substances (cf. Fletcher et al., 1916). In the present case, a supposedly innocuous agent (saline), when administered in sufficient volume, resulted

in altered responding in treated offspring. While the mechanism by which this volume effect was produced is uncertain, the most likely causes appear to be a disruption of fluid balance or pressure relationships within the egg. In any event, since the main effect produced by high-volume injections appeared to be an overall decrease in response levels, it is possible that responding was lowered sufficiently by volume alone to obscure any more subtle differences that might have been caused by ethanol injection.

The volume effect also suggests another issue which must be kept in mind throughout the interpretation of these results. Given the demonstration that high volumes of saline alone were shown to disrupt responding when compared to low volumes, it is possible that any injection whatsoever may result in behavioral alterations when injected animals are compared with untreated control. This possibility cannot be evaluated within the context of the present set of studies, since no untreated control groups were included. It must be cautioned, therefore, that the interpretation of these experiments must necessarily be limited to comparisons between ethanol- and placebo-treated subjects. Since it is possible, and indeed, likely, that even the saline-treated animals were subject to behavioral teratogenesis in some cases, deficits in alcohol animals can be explained as being <u>in addition to</u> those of saline animals. As will be seen in a later section, even this assumption must be interpreted with caution.

As has been mentioned, the volume-induced lowering of response levels may have obscured any between-group differences caused by alcohol injection in the high-volume animals. Nevertheless, there was a dif-

ference between the alcohol- and saline-treated birds in the low-volume group in terminal response levels to the red stimulus during acquisition (Figures 8 and 9). This difference is probably not best interpreted in terms of alterations in learning ability, since the groups did not vary in their response levels to the reinforced green stimulus or the nonreinforced yellow stimulus. Perhaps the most plausible alternative description is simply in terms of alterations in unlearned "approach tendencies" in which between-group differences in responding to a given stimulus cannot be attributed readily to differences in histories of reinforcement. Gray (1961), for example, has demonstrated that young chicks have unlearned "preferences" for specific colors, as measured by their readiness to imprint on objects of those colors. Furthermore, Gunther and Jones (1963) and Gunther (1965), by using pecking measures, found that the chick's tendency to approach certain colors can be modified by prenatal treatment -- in their case, with nonoptimal incubation temperatures. Although the basis for the chick's tendency to approach or respond differentially to certain colors is uncertain, the phenomenon appears to be reliable (see also Kovach, 1971; Kovach & Hickox, 1971).

Gunther and Jones (1963) have suggested two general mechanisms by which treatment-induced alterations in color approach tendencies may be produced. First, it is conceivable that embryonic treatment may result in structural changes in the peripheral visual receptors, thereby reducing (or enhancing) the sensitivity to certain colors. Alternatively, prenatal intervention might affect more central mechanisms, disrupting, for example, the "processing" of visual information. Both of these options are feasible in the current

studies since prenatal ethanol has been implicated in both opthalmic and CNS anomalies (cf. Chernoff, 1977; Randall, 1977). A final alternative explanation for treatment-induced disturbances in innate color approach tendencies refers to motivational mechanisms. As an example of this proposed mechanism, one might imagine that certain colors have associated with them a hedonic "value" (either positive or negative). Just as loud noises, for example, appear to have the ability to evoke fear in many organisms, so might certain colors evoke innate "positive" or "negative" reactions in chicks. Prenatal treatment with alcohol or other foreign agents might conceivably modulate the innate motivational "value" associated with a particular color. Obviously these hypothesized explanations are mere speculation at the present time, and the experiments reported here do not permit tests of their empirical validity. Nevertheless, the mechanisms described may provide direction for further studies which would furnish explanations for the ethanol-induced changes in the tendency to approach red noted here. For example, while it would be somewhat difficult to differentiate between the peripheral and the central neural hypotheses suggested by Gunther and Jones (1963), behavioral experimentation might be designed to evaluate the relative contribution of motivational mechanisms to treatment-induced changes in the tendency to approach certain hues.

Interestingly, the reduced responding to the red stimulus noted for the low-dose alcohol birds was no longer present at the beginning of conditioned inhibition training (Figure 14a), although by the final four days of conditioned inhibition training, the difference in responding to red had been partially reinstated. Nevertheless, analyses of responding

to red alone indicated that this difference was not, by itself, significant, although further analysis indicated that the pattern of responding to red and the red + white compound was different between the low-volume alcohol group and the low-volume saline group. This finding, that saline-treated birds demonstrated a greater response divergence to the reinforced red stimulus and the nonreinforced compound stimulus than alcohol-treated chicks, indicates inferior discriminated responding by the alcohol animals. It can be plausibly argued, however, that the alterations in discriminated responding observed during conditioned inhibition training for the low-volume alcohol animals were not the result of learning deficits in those animals, but rather were simply a further manifestation of the alcohol-induced reduction in the tendency to approach red which was first noted during acquisition. Kovach and Hickox (1971), for example, have shown that discrimination learning is hampered when the positive (reinforced) stimulus is a nonpreferred or less readily approached stimulus. This appeared to be the case in the present experiment. It must again be emphasized, however, that the difference in responding to red alone was not statistically reliable. Instead, the statistical analyses indicated that the divergence in responding to A (red) and AW (red + white compound) was not as great for the alcohol animals as it was for the saline animals. This implies that, relatively speaking, the low-volume alcohol birds demonstrated impaired inhibition to the compound when compared to low-volume saline birds. As indicated above, however, at least some of this impairment may have been the result of the alcohol animals' reduced tendency to approach red, rather than a disruption of the

learned inhibitory properties of the white off-key light per se.

In the summation-transfer test, there was evidence that treatment with alcohol on Days 1-5 of incubation resulted in an impairment in the transfer of inhibition (Figure 15). This impairment appeared graphically to be due principally to the high-volume animals, although this observation was not supported statistically. Because the difference between alcohol- and saline-treated animals was attributable only to BW (green + white compound) responding, and not to response differences to B (green) alone, it seems unlikely that the observed transfer test difference can be accounted for by appeals to alterations in unlearned tendencies to approach certain colors. It is uncertain, however, whether this between-group difference in inhibition was partially obscured during conditioned inhibition training due to the volume effect or if it was unique to the summation-transfer test (where there was no effect of volume).

In any event, Experiment 2 indicated that injection of chick embryos on Days 1-5 of incubation is capable of producing behavioral effects in a variety of ways: via volume effects on overall level of responding, alcohol effects on unlearned color approach tendencies, and alcohol effects on the transfer of conditioned inhibition.

Experiment 3

During the acquisition phase, birds treated with alcohol on Days 6-10 of incubation responded at a higher terminal level to the red stimulus (A) than saline-treated birds (Figure 11). This difference carried over to conditioned inhibition training (Figure 17) and did

not dissipate during that training, although responding to the compound stimulus (AW) was comparable for the two drug treatment groups. In addition, despite the fact that there were no differences in acquisition responding attributable to injection volume, the high-volume birds did display a lower response level to both stimuli for the first two days of conditioned inhibition training. Finally, during the summation--transfer test, there was no difference due to volume or drug treatment.

The outcomes of the acquisition and conditioned inhibition training phases of Experiment 3 were completely opposite those of Experiment 2 since in the present case, the alcohol-treated animals responded at a higher level to the red stimulus (A) than saline-treated birds. Reference to Figure 11 indicates that the alcohol- and saline-treated chicks differed markedly in their responding to red, but not in their responding to green (or yellow), suggesting that the difference was an unlearned one. Furthermore, as in Experiment 2, the between-group differences in discrimination performance exhibited during Experiment 3-conditioned inhibition training appear to be explicable solely in terms of the between-group differences in the tendency to approach red just noted (cf. Kovach & Hickox, 1971). This suggestion is supported by reference to Figure 17 where it can be seen that responding by alcohol- and saline-treated animals was the same for Stimulus AW and differed only to Stimulus A. However, because the relative difference between A and AW responding was different for the two drug treatment groups, differences in the effectiveness of the white light as an inhibitor cannot be entirely ruled out as a cause of the between-group differences during conditioned inhibition training.

The results of the summation-transfer test (Figure 20), on the

other hand, seem to indicate that the white off-key light was equally effective as an inhibitor for the alcohol and saline animals. Indeed, both groups responded at comparable levels to both the green stimulus alone and the green + white compound. This supplies additional support for the contention that the differences seen during conditioned inhibition training were simply the result of between-group differences in the unlearned tendency to approach red.

The pattern of differences noted during the acquisition and conditioned inhibition training phases of the present experiment may thus be described in terms of an increased tendency to approach and respond to the red stimulus on the part of the alcohol-treated birds, and in terms of superior discrimination by those same animals. These descriptions rely, of course, on the implicit assumption that the behavior of the saline-treated animals defines "normality." An alternative which seems plausible in the current situation is that the saline birds demonstrated altered performance, indicated by a decreased tendency to approach red and impaired discrimination.

Perhaps the strongest support for the suggestion that saline treatment on Days 6-10 of incubation disrupted normal development may be found in the survival data for Experiment 3. As was reported earlier, nearly 30% of the chicks to hatch in the saline groups in this experiment died within one week of hatching. This would seem to indicate, therefore, that saline, when administered on Days 6-10 of incubation, is capable of acting as a potent teratogen. Given the marked physical consequences of saline administration at this period of development, it does not seem unlikely that behavior, too, may be affected.

The actual mechanisms by which saline might produce teratogenic

effects are uncertain. Perhaps the most likely explanation is that the osmolality of the injected saline solution was amply different from that of the internal fluid environment of the egg to cause the proposed disorder in saline animals. Thus, despite Grabowski's (1967) report that the concentration of saline used here is isotonic with the embryonic chick's serum, an imbalance sufficient to cause teratogenesis may have occurred in the saline animals. To the author's knowledge, there are no other data to support or refute this hypothesis.

Experiment 4

Treatment on Days 11-15 of incubation resulted in an overall level of responding by the high-volume saline group which was markedly higher to all stimuli on the third day of acquisition than responding by any of the other groups in that experiment (Figure 13). By the end of acquisition training, however, responding to the various stimuli was at approximately the same level for all groups. Although high-volume chicks showed a slight increase in responding to red over days of conditioned inhibition training, there was no effect of drug treatment on any response measure during that training (Figure 19). In addition, prenatal treatment had no effect on the chicks' subsequent ability to demonstrate transfer in a combined-cue test of conditioned inhibition (Figure 20).

The finding that high-volume saline birds responded at an overall higher level on the third day of acquisition training is difficult to interpret. Although a general saline-induced hyperactivity would potentially explain the finding, it is not easy to explain why it only occurred on one day. Since the response level for the high-volume

saline group actually decreased after the third day of acquisition, it seems unlikely that a "ceiling effect" obscured any later differences. Given that the groups were comparable at the end of acquisition and throughout conditioned inhibition training and summation-transfer testing, it appears safe to conclude that treatment on Days 11-15 of incubation had little lasting effect on subsequent behavior within the context of the present experimental paradigm.

General Conclusions

Prenatal exposure to alcohol was found to affect later behavior in chicks treated on Days 1-5 of incubation (Experiment 2), and in chicks treated on Days 6-10 (Experiment 3), but not in those treated on Days 11-15 (Experiment 4). When compared with placebo-treated controls, birds treated on Days 1-5 demonstrated altered color approach tendencies with a decrease in responding to the red stimulus, whereas birds treated on Days 6-10 exhibited modified color approach tendencies with an increase in responding to red. In both instances, between-group differences in discrimination performance during conditioned inhibition training appeared to be best explained in terms of differences in the unlearned behaviors just described. Additionally, while there were no indications of an effect of drug treatment on the transfer of inhibition in birds treated on Days 6-10 (Experiment 3), prenatal treatment with alcohol on Days 1-5 of incubation was found to interfere with the transfer of conditioned inhibition.

Injection volume was also found to play a role in behavioral teratogenesis. The main effects of volume occurred: (a) in animals treated on Days 1-5, during both the acquisition and conditioned inhibition

phases; (b) in animals treated on Days 6-10, at the beginning of conditioned inhibition training only; and (c) in birds treated on Days 11-15, over days of conditioned inhibition training, but only to the red stimulus. Interestingly, at no point was there an effect of injection volume on responding in the summation-transfer test.

As with numerous other investigations of the behavioral consequences of prenatal exposure to alcohol, the results of the present set of experiments did not disclose large group differences. Furthermore, the behavioral teratogenic effects that were observed were not limited to treatment with ethanol: Under certain circumstances, saline injections and injection volume also appeared to play a role in the behavioral teratogenesis of the chick.

The specific set of behavioral procedures utilized in this set of studies appeared to be relatively well suited to tests of prenatal drug effects on later behavior. Nevertheless, the paradigms of greatest interest, discrimination learning and conditioned inhibition, did not seem to be exquisitely sensitive to embryonic alcohol effects as such. Indeed, only in animals treated on Days 1-5 of incubation (Experiment 2), and then only in the transfer test of conditioned inhibition, was there a relatively clear indication of an effect of prenatal ethanol on either of these processes. There are, of course, a number of possible explanations for this comparative lack of effect. The simplest of these is that in instances in which alcohol had no effect, the dose administered was not sufficient to produce behavioral teratogenesis. It is notable,

however, that the one study in which behavioral effects of prenatal alcohol were most evident was the one which was least susceptible to effects of ethanol on hatchability (Experiment 2). Since it is generally believed that the doses necessary to produce physical disturbances are greater than those needed to produce behavioral alterations (cf. Hutchings, 1978), and since hatchability was affected in both Experiments 3 and 4, it seems unlikely that the administered doses were insufficient.

Alternatively, it may be that behavioral processes different from those tested here are more subject to disruption by prenatal alcohol. For example, measures of aversive conditioning such as shock avoidance, or of behaviors more sensitive to changes in general activity may have disclosed greater ethanol-induced differences. However, given the relatively complex patterns of learning and behavior that were required of the animals in the present set of studies, it seems reasonable to assume that this paradigm should be sensitive to embryonic ethanol's effects, with certain restrictions. If, for instance, the alterations produced by prenatal alcohol are principally motivational, and if only aversively motivated tasks are affected, then the current procedure would probably not disclose such differences. Furthermore, if alcohol produced defects in sensory systems other than the visual system, there is little reason to believe that the methods employed here would have been sensitive to these kinds of defects. Nevertheless, as indicated above, the primary intent of these studies was to investigate the effects of prenatal alcohol on discrimination learning and conditioned inhibition, and within the confines of the restrictions just

noted, the paradigm should be reasonably sensitive to these effects. Consequently, a final alternative explanation for the lack of ethanol effects in animals treated on Days 6-10 and 11-15 (Experiments 3 and 4) appears viable. It seems plausible that despite the effect of alcohol on hatchability in both of these experiments, the chicks may have been insensitive to any behavioral effects during these periods of development. Whether this insensitivity represents a "survival of the fittest" or an actual insusceptibility of the relevant neurological structures to modification by ethanol at this stage of embryogenesis, cannot be determined from these studies. In any case, it is interesting to note that the period of development which appeared most sensitive to prenatal alcohol (the first five days of incubation) coincides roughly with the presumed period of highest teratogenic risk in the human embryo (cf. Streissguth et al., 1978).

SUMMARY AND CONCLUSIONS

A series of experiments was performed to examine the effects of embryonic exposure to alcohol on discrimination learning and conditioned inhibition in the chick. Following an initial study in which the experimental procedure was demonstrated to be an acceptable means for establishing conditioned inhibition, three additional experiments were conducted to investigate prenatal ethanol effects. Specifically, the experiments differed with regard to the time during incubation at which the developing chicks were treated with alcohol, and with regard to the dose of ethanol administered. Embryonic chicks were treated on Days 1-5, 6-10 or 11-15 of the 21-day incubation period, and within each experiment, two groups were treated with alcohol (high dose and low dose) and two with saline (high and low volume controls). Subsequently, the effects of both drug treatment and injection volume were examined in each study.

After hatching, chicks which had been treated with ethanol or saline were tested in tasks designed to measure discrimination learning and conditioned inhibition. Discrimination learning was assessed by monitoring autoshaped keypecking to two food-reinforced colors and one nonreinforced color, each of which was projected onto the response key. Conditioned inhibition training, which occurred after the discrimination phase, consisted of reinforced presentations of a key light (Stimulus A) and nonreinforced presentations of the same key light compounded with an off-key light (Stimulus AW). Typically, with this type of procedure, responding will initially occur to both stimuli, but with repeated nonreinforcement of

the compound (AW), a response decrement occurs on AW trials. As a result, A is said to have become an excitor, and W an inhibitor. Following conditioned inhibition training, a summation-transfer test of inhibition was performed in which the inhibitory effect of the off-key light (W) was tested by compounding it with a stimulus which had never been presented as part of a compound, but which had been consistently reinforced when presented alone (i.e., was an excitor -- Stimulus B).

The results of these studies may be summarized as follows:

(a) Chicks treated on Days 1-5 of incubation:

(1) Birds treated with a low dose of alcohol displayed a decreased tendency to approach and respond to the reinforced red stimulus, although responding to the reinforced green and nonreinforced yellow stimuli was unaffected.

(2) During conditioned inhibition training, chicks treated with a low dose of alcohol exhibited impaired discriminated responding; this impairment was postulated to be the result of alterations in unlearned rather than learned behaviors.

(3) Alcohol-treated chicks displayed an impairment in the transfer of conditioned inhibition.

(4) Animals treated embryonically with high volumes of solution (regardless of drug treatment) responded at lower levels during acquisition and conditioned inhibition training than birds treated with low volumes.

(b) Chicks treated on Days 6-10 of incubation:

(1) Animals treated with alcohol demonstrated an increased tendency to approach and respond to the reinforced red stimulus, although respond-

ing to the reinforced green and nonreinforced yellow stimuli was unaffected.

(2) There was no lasting effect of volume at any phase of the experiment, and no effect of drug treatment on the transfer of inhibition.

(c) Chicks treated on Days 11-15 of incubation:

(1) There were no lasting effects of drug treatment or injection volume during any phase of this experiment.

It was concluded that both drug treatment and injection volume are capable of producing behavioral teratogenesis. However, as with numerous other behavioral investigations of the consequences of prenatal exposure to alcohol, the present set of studies did not disclose large group differences. It is interesting to note, nonetheless, that the greatest effect of embryonic ethanol exposure appeared to occur as a result of treatment during early development, with progressively less disturbance caused by treatment during later periods.

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APPENDIX A

EXPERIMENTS 2-4: RESPONSE RATES

Autoshaping Acquisition

Experiment 2: Acquisition. Figure A1 depicts response rates of the various groups to the three stimuli during autoshaping acquisition in Experiment 2 (Treatment Days 1-5). Responding by the low-volume birds is plotted in Panel a, whereas responding by the high-volume animals is plotted in Panel b. Solid symbols represent response levels to Stimulus A (red), open symbols represent responding to Stimulus B (green) and half open, half solid symbols represent Stimulus Y (yellow) responding. As can be seen in the figure, response rates to Stimuli A and B gradually increased over days, while responding to Y first increased and then decreased over the final days of acquisition. By the end of the acquisition phase, response rates to yellow (Y) were markedly lower than those to red (A) or green (B). It is also apparent that responding by the low-volume birds attained a higher terminal level to Stimuli A and B than responding by the high-volume animals.

These observations were supported by the outcome of a volume x drug treatment x stimuli x days analysis of variance in which there were main effects of stimuli, F (2, 64) = 43.80, $p < .001$, and days, F (4, 128) = 45.25, $p < .001$, as well as a reliable interaction of stimuli with days, F (4, 128) = 2.78, $p < .05$. The volume x drug treatment x stimuli x days interaction was also significant, F (8, 256) = 2.14, $p < .05$, and accordingly, separate three-way analyses were performed for each stimulus to evaluate the interaction. Figure A2 depicts response rates to each of the individual stimuli plotted across days of acquisition. It is apparent that responding to yellow (Panel c) changed over days, but was generally equivalent for all groups. This was evidenced

Figure A1. Response rates (responses/min) for the various groups in Experiment 2 (Treatment Days 1-5) during acquisition. Panel a depicts responding by the low-volume birds, and Panel b, responding by the high-volume birds.

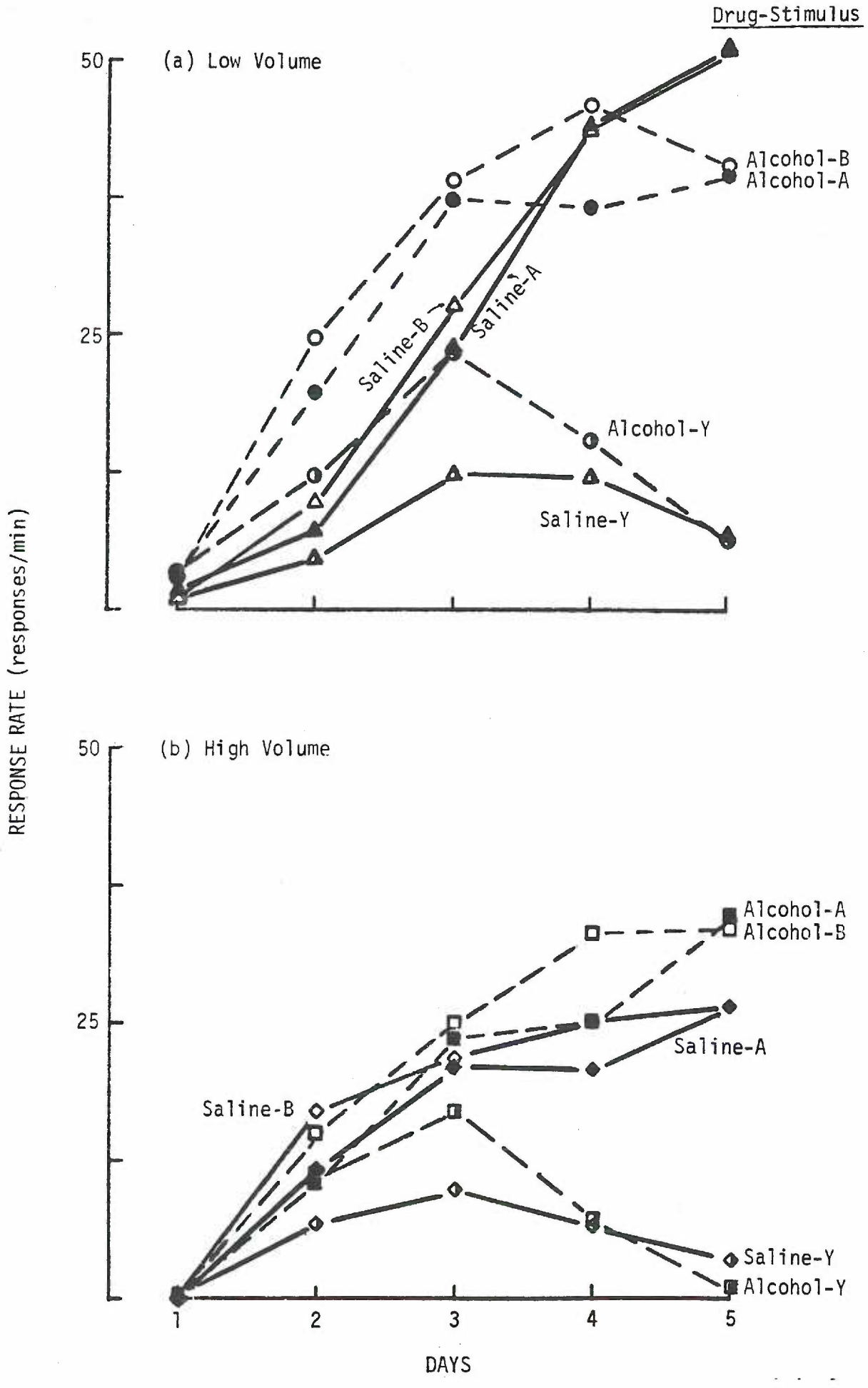
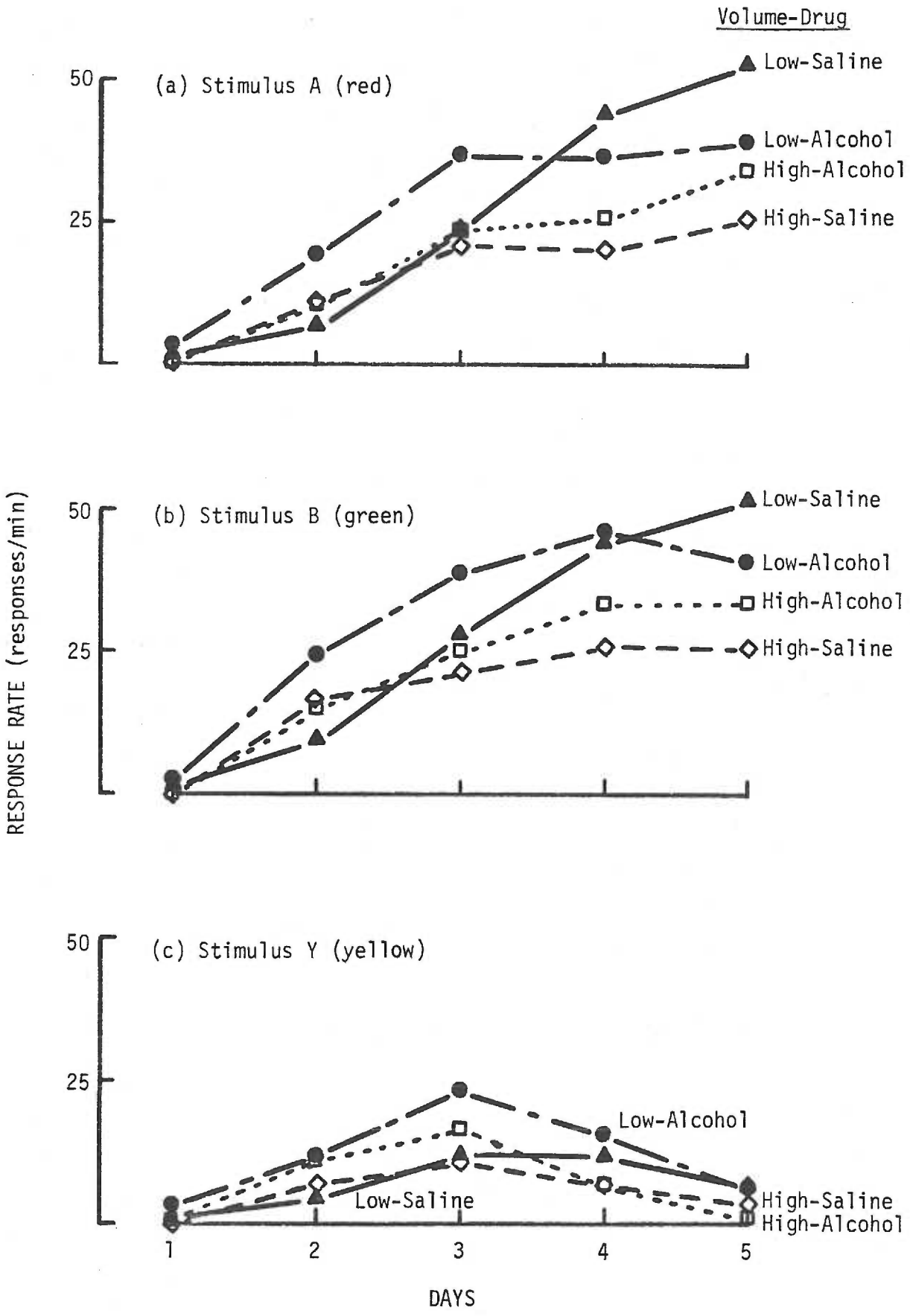

Drug-Stimulus
(a) Low Volume
50
25
Alcohol-B
Alcohol-A
Saline-B
Saline-A
Alcohol-Y
Saline-Y
(b) High Volume
50
25
Alcohol-A
Alcohol-B
Saline-A
Saline-B
Saline-Y
Alcohol-Y
1
2
3
4
5
DAYS
RESPONSE RATE (responses/min)

Figure A2. Response rates (responses/min) to each of the three acquisition stimuli in Experiment 2. Response levels to Stimuli A, B and Y are plotted in Panels a, b and c, respectively.


Volume-Drug
(a) Stimulus A (red)
50
25
Low-Saline
Low-Alcohol
High-Alcohol
High-Saline
(b) Stimulus B (green)
50
25
Low-Saline
Low-Alcohol
High-Alcohol
High-Saline
RESPONSE RATE (responses/min)
(c) Stimulus Y (yellow)
50
25
Low-Alcohol
Low-Saline
High-Saline
High-Alcohol
1
2
3
4
5
DAYS

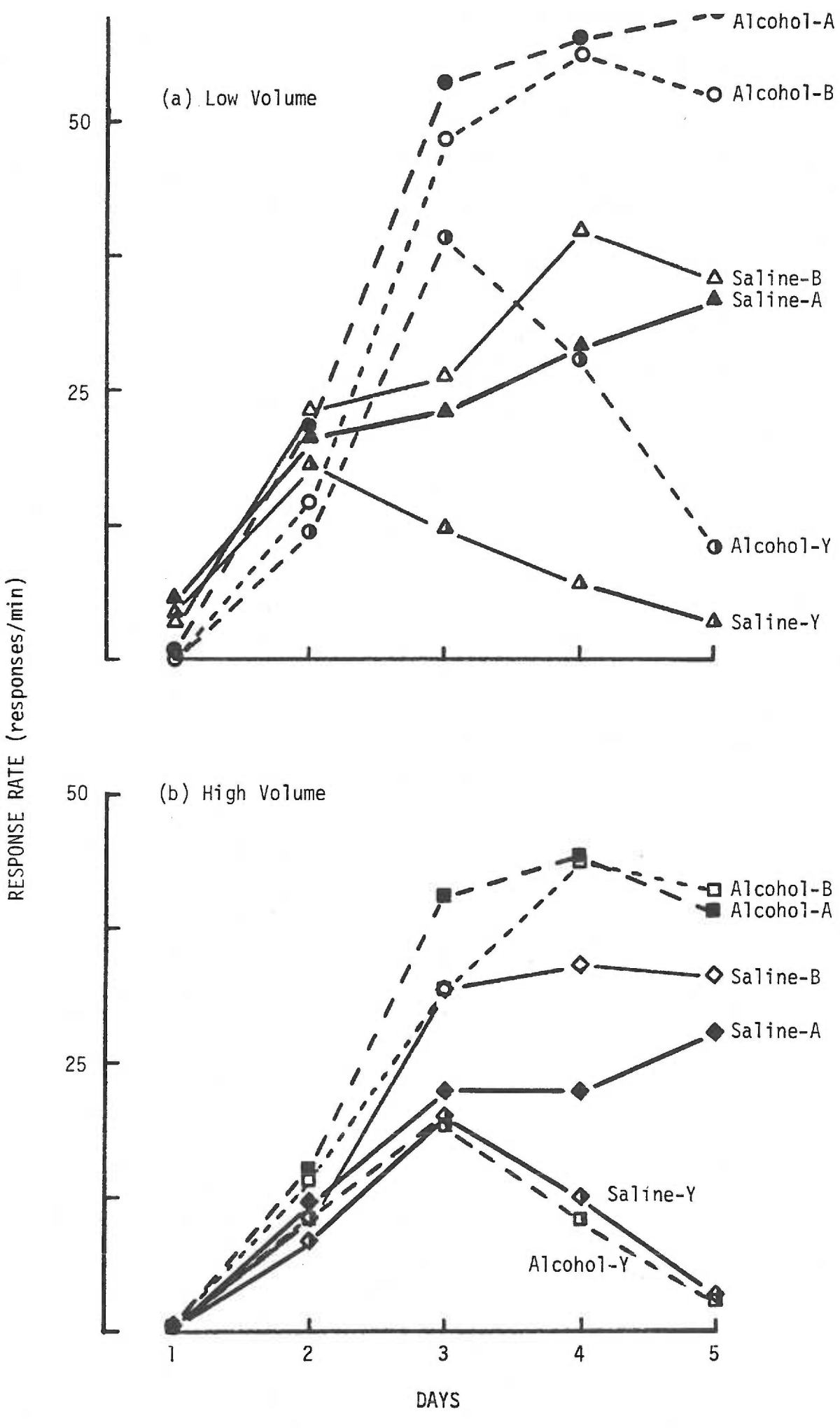
by a main effect of days in the three-way analysis for Stimulus Y, F (4, 128) = 15.60, $p < .001$. None of the other main effects or interactions was significant. Responding to green (Panel b) also showed a change over days, and it can further be seen that the low-volume animals reached a higher terminal response level to that stimulus than the high--volume birds. Indeed, the three-way analysis for green disclosed a main effect of days, F (4, 128) = 49.52, $p < .001$, as well as a reliable volume x days interaction, F (4, 128) = 2.58, $p < .05$.

Reference to the plot of responding to the red stimulus (Figure A2a) again indicates an increase in rate over days and a higher terminal level for the low-volume animals. In addition, however, it is apparent that while high-volume alcohol- and saline-treated birds had comparable rates to red over the entire acquisition phase, this was not the case for the low-volume birds. Instead, the low-volume alcohol animals responded at a higher level to red than low-volume saline animals for the first three days of acquisition. Yet on the final two acquisition days, the low-volume saline animals actually responded at a higher rate. These observations were indicated in the analysis of variance as a main effect of days, F (4, 128) = 44.33, $p < .001$, and a reliable volume x treatment x days interaction, F (4, 128) = 2.79, $p < .05$.

In summary, treatment on Days 1-5 had effects on the acquisition rate measure of responding which were nearly identical to those of the acquisition percentage measure.

Experiment 3: Acquisition. Figure A3 depicts response rates for the various groups treated on Days 6-10 of incubation to the three acquisition stimuli. Panel a shows responding by low-volume animals,

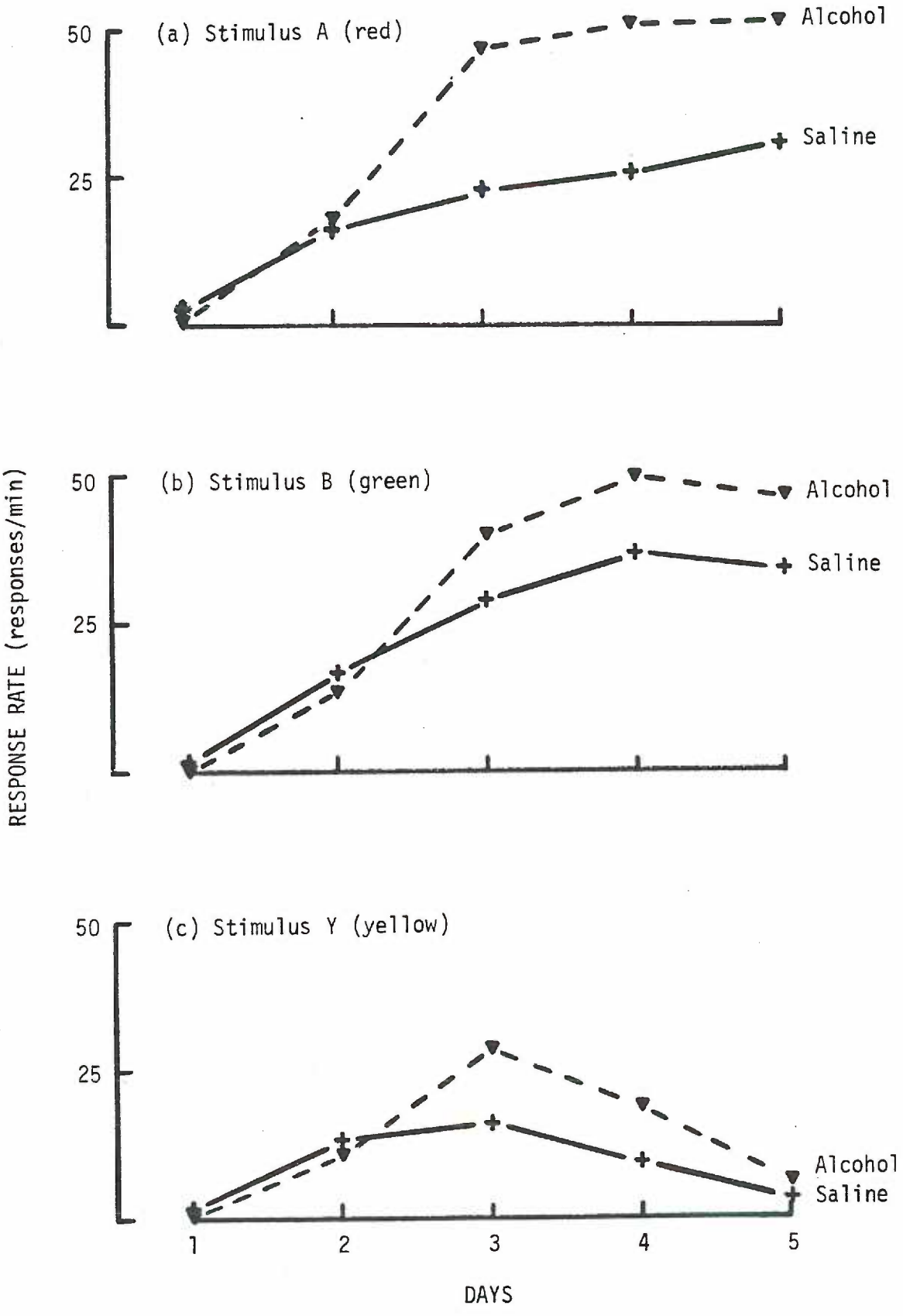
Figure A3. Response rates (responses/min) for the various groups in Experiment 3 (Treatment Days 6-10) during acquisition. Panel a depicts the low-volume birds, and Panel b, the high-volume birds.


(a) Low Volume
50
25
Alcohol-A
Alcohol-B
Saline-B
Saline-A
Alcohol-Y
Saline-Y
(b) High Volume
50
25
Alcohol-B
Alcohol-A
Saline-B
Saline-A
Saline-Y
Alcohol-Y
1
2
3
4
5
DAYS
RESPONSE RATE (responses/min)

whereas Panel b shows responding by high-volume birds. It can be seen that responding to Stimuli A and B increased over days and was approximately equivalent for low- and high-volume animals. The alcohol-treated animals, however, appear to have responded at a higher rate to all three stimuli during the final three acquisition sessions, ultimately reaching a higher terminal response level overall. These observations were supported in a four-way analysis by a reliable days effect, F (4, 112) = 45.23, $p < .001$, and a reliable interaction of drug treatment x days, F (4, 112) = 5.32, $p < .001$.

It is also apparent from the figure that responding to Stimulus Y first increased and then decreased over days, achieving a final level which was noticeably lower than responding to both Stimulus A and Stimulus B. Accordingly, the analysis disclosed a reliable effect of stimuli, F (2, 56) = 51.40, $p < .001$, and a reliable stimuli x days interaction, F (8, 224) = 27.57, $p < .001$. There was also a significant interaction of treatment with stimuli, F (2, 56) = 4.18, $p < .05$, and consequently, follow-up two-way analyses were performed for each stimulus, collapsed across the volume factor. Figure A4 depicts responding by the alcohol- and saline-treated animals to each of the acquisition stimuli. For each stimulus, the days effect was reliable ($ps < .001$). For Stimulus B (Panel b) there was no main effect or interaction involving drug treatment, but for Stimulus A (Panel a), the main effect of drug treatment was reliable, F (1, 30) = 5.43, $p < .05$, as was the treatment x days interaction, F (4, 120) = 7.72, $p < .001$. In addition, the treatment x days interaction for Stimulus Y (Panel c) was reliable, F (4, 120) = 2.57, $p < .05$.

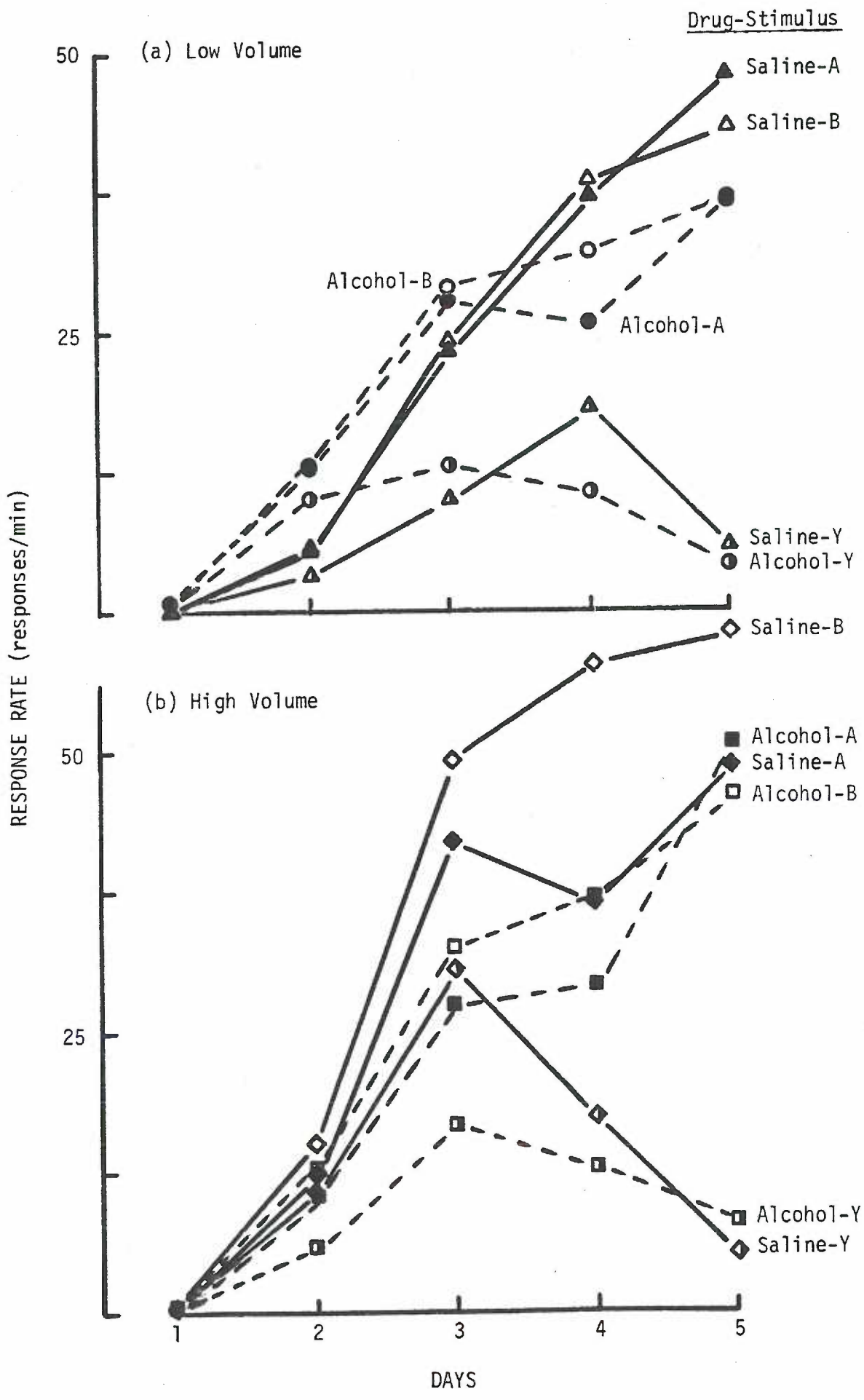
Figure A4. Response rates to each of the three acquisition stimuli in Experiment 3, collapsed across the volume factor. Response levels to Stimuli A, B and Y are plotted in Panels a, b and c, respectively.


(a) Stimulus A (red)
(b) Stimulus B (green)
(c) Stimulus Y (yellow)
Alcohol
Saline
50
25
RESPONSE RATE (responses/min)
1
2
3
4
5
DAYS

In summary, while there was no difference in responding due to volume, and no difference in response rates to green (Stimulus B), the alcohol-treated animals achieved a markedly higher response level to red by the end of acquisition training. Furthermore, alcohol-treated animals responded at a higher rate to Stimulus Y on the third and fourth days of acquisition, although this difference was no longer present on the final acquisition training day. These findings were generally consistent with those disclosed by the percentage response measure, although that measure failed to indicate the temporarily higher response level to yellow by the alcohol-treated animals.

Experiment 4: Acquisition. Figure A5 depicts response rates during acquisition for those groups treated on Days 11-15 of incubation. Panel a portrays responding by low-volume animals, whereas Panel b portrays responding by high-volume birds. It is apparent from the figure that responding increased over days to the two reinforced stimuli, but decreased over the final sessions of acquisition training for the nonreinforced stimulus (Y). The general similarity of responding among all of the treatment groups to the various stimuli is also evident. Indeed, as in other sections involving the rate measure, responding during this phase was accompanied by a relatively large between-subject variablility, and thus it was not expected that any of the apparent differences between groups would achieve significance. The volume x drug treatment x stimuli x days analysis disclosed a main effect of stimuli, F (2, 56) = 40.44, $p < .001$, a main effect of days, F (4, 112) = 72.96, $p < .001$, and an interaction of stimuli with days, F (8, 224) = 39.10, $p < .001$. None of the other main effects or interactions was significant.

Figure A5. Response rates (responses/min) for the various groups in Experiment 4 (Treatment Days 11-15) during acquisition. Panel a depicts the low-volume birds, and Panel b, the high-volume animals.

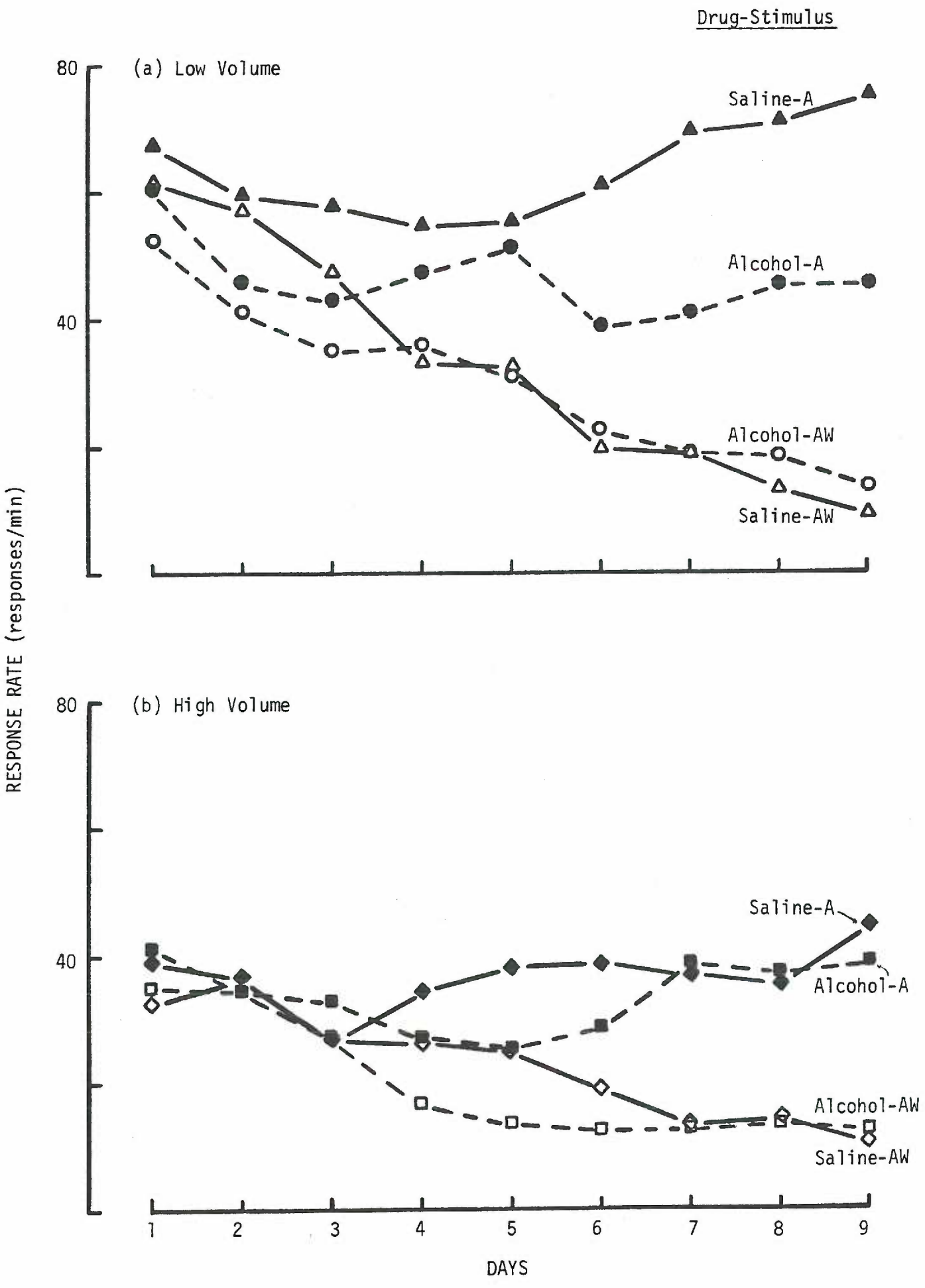

Drug-Stimulus
(a) Low Volume
50
25
Saline-A
Saline-B
Alcohol-B
Alcohol-A
Saline-Y
Alcohol-Y
Saline-B
(b) High Volume
50
25
Alcohol-A
Saline-A
Alcohol-B
Alcohol-Y
Saline-Y
1
2
3
4
5
DAYS
RESPONSE RATE (responses/min)

In summary, all groups displayed discriminated responding during the final days of acquisition training, raising their rates to the reinforced stimuli and reducing their rates to the nonreinforced stimulus. However, the analysis of variance disclosed no effect of either volume or drug treatment during the acquisition phase. This general pattern is similar to that disclosed by the percentage measure although that measure also indicated that the high-volume saline animals responded at a markedly higher level to the three stimuli on the third day of acquisition than the other groups. Despite the fact that this difference also appears to be present graphically with the rate measure of responding, the difference was not reliable -- apparently because of the large between-subject variability in response rates.

Conditioned Inhibition and Summation-Transfer

Experiment 2: Conditioned inhibition training. Response rates over the nine days of conditioned inhibition training are plotted in Figure A6 for animals treated on Days 1-5 of incubation. Panel a portrays responding by the low-volume birds, whereas Panel b depicts responding by high-volume animals. It is apparent from the figure that response rates to the two stimuli were markedly lower for the high-volume birds than for the low-volume subjects. As expected, however, responding to the nonreinforced compound (AW) declined gradually over days, while responding to Stimulus A remained considerably higher throughout conditioned inhibition training. It can also be seen that the difference between low-volume alcohol and saline animals in responding to Stimulus A which was established over the final days of acquisition training was

Figure A6. Response rates (responses/min) during conditioned inhibition training, Experiment 2. Panel a depicts low-volume birds, while Panel b represents high-volume animals.


Drug-Stimulus
(a) Low Volume
80
40
Saline-A
Alcohol-A
Alcohol-AW
Saline-AW
RESPONSE RATE (responses/min)
(b) High Volume
80
40
Saline-A
Alcohol-A
Alcohol-AW
Saline-AW
1
2
3
4
5
6
7
8
9
DAYS

maintained during conditioned inhibition training, and perhaps even widened.

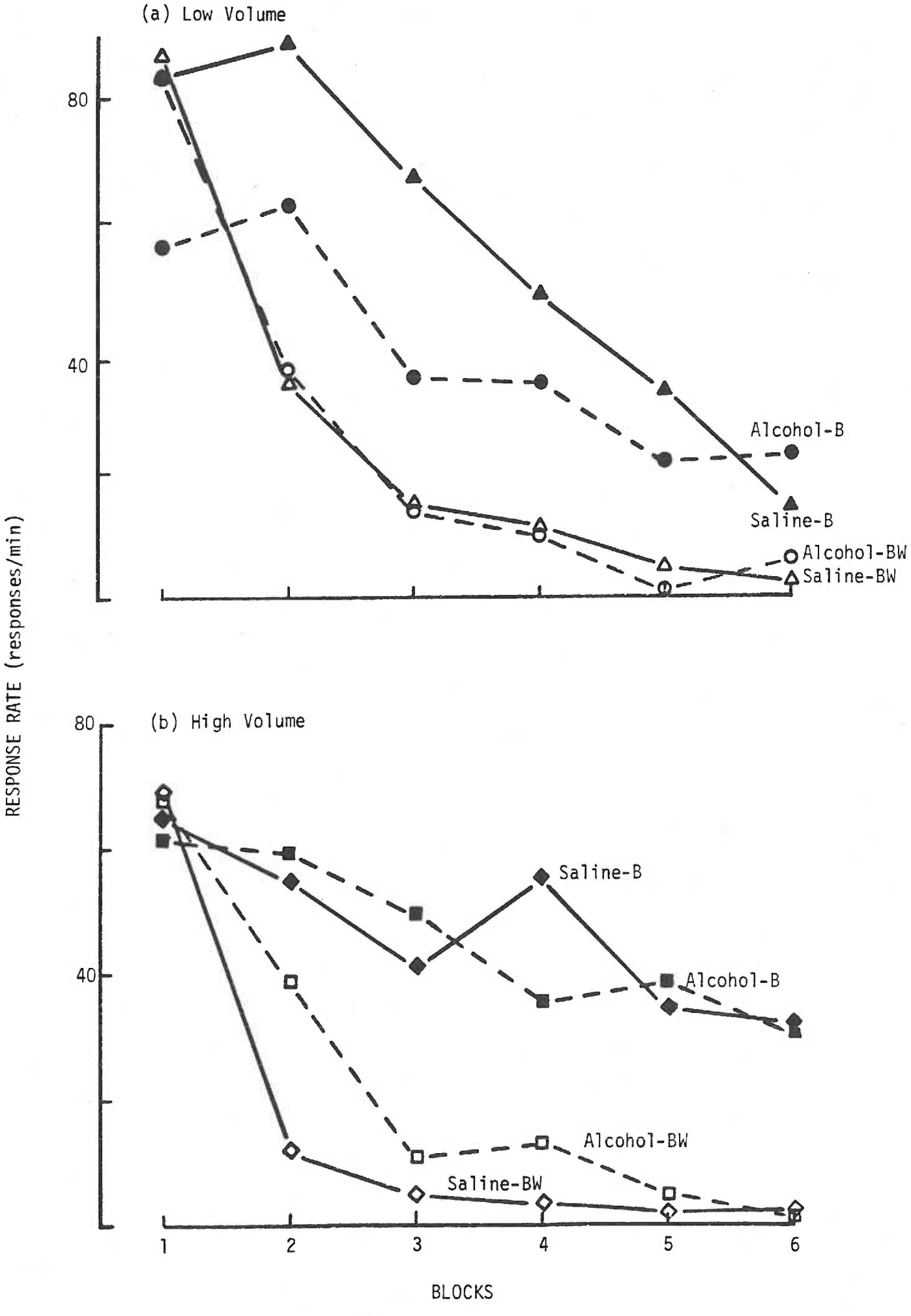
A four-way analysis of variance comparing responding to Stimuli A and AW disclosed a reliable volume effect, F (1, 32) = 7.19, $p < .05$, a reliable effect of days, F (8, 256) = 9.78, $p < .001$, a reliable effect of stimuli, F (1, 32) = 42.87, $p < .001$, and a reliable interaction of stimuli with days, F (8, 256) = 25.46, $p < .001$. The drug treatment x stimuli x days interaction was also significant, F (8, 256) = 2.47, $p < .05$, and thus follow-up analyses were conducted for each stimulus with factors of drug treatment and days (collapsed across volume). Because neither of these follow-up analyses disclosed a main effect or interaction involving the drug treatment factor, and since the low-volume group graphically appears to be responsible for any between-group differences, three-way analyses with factors of drug treatment, stimuli and days were performed for each of the volume groups. As with the percentage measure, the rate analysis for the low-volume group disclosed no main effect of drug treatment, but there was a reliable drug treatment x stimuli x days interaction, F (8, 128) = 3.78, $p < .001$. For the high-volume animals, however, there was no main effect of drug treatment, and none of the interactions involving the treatment factor was significant. A post hoc follow-up to the three-way interaction which was disclosed for the low-volume group indicated that the interaction was the result of a greater divergence in responding to A and AW by the low-volume saline birds than by the low-volume alcohol chicks, F (1, 128) = 10.72, $p < .005$.

Experiment 2: Summation-transfer test. Figure A7 depicts response

of the various groups to Stimuli B and BW over blocks of the summation--transfer test. It can be seen from the figure that response rates to both stimuli declined over blocks, and that the decline was much more rapid for Stimulus BW than for Stimulus B. In addition, it is apparent that the overall response rate for the low-volume birds was higher than that of the high-volume animals at the beginning of the transfer test, and that the low-volume birds' decrease showed a steeper slope over blocks. These observations were supported statistically by a main effect of stimuli, F (1, 32) = 70.52, $p < .001$, a main effect of blocks, F (5, 160) = 78.13, $p < .001$, a reliable interaction of stimuli with blocks, F (5, 160) = 22.31, $p < .001$, and a significant volume x blocks interaction, F (5, 160) = 2.58, $p < .05$. Unlike the percentage analysis, the rate analysis disclosed no involvement of the drug treatment factor. Part of this discrepancy between the two measures appears to be the result of the large degree of between-subject variability which accompanied the rate measure.

Experiment 2: Summary of conditioned inhibition training and summation-transfer test. Although the volume effect which developed during acquisition training was not evident in percentage measures of conditioned inhibition, there was a reliable main effect of volume for the rate measure during conditioned inhibition training, with high-volume birds responding significantly less to both stimuli. Furthermore, while statistical analyses failed to disclose unambiguously the source of a drug treatment x stimuli x days interaction, there was evidence that low-volume saline birds demonstrated a greater divergence to Stimuli A and AW than low-volume alcohol animals. During

Figure A7. Response rates to B (solid symbol) and BW (open symbol) over blocks of six trials during the summation-transfer test, Experiment 2. Panel a represents responding by low-volume birds, and Panel b represents responding by high-volume birds.

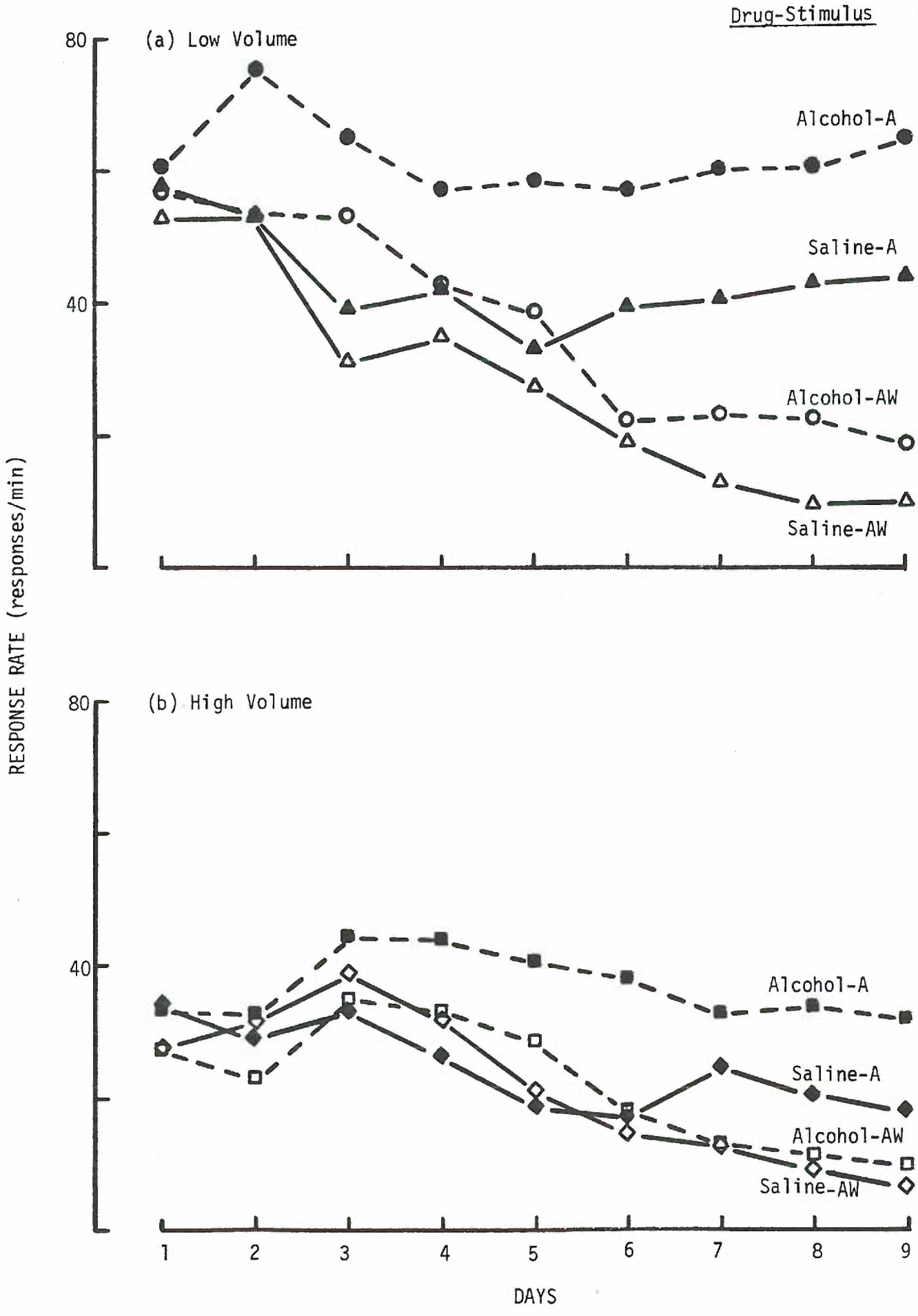

(a) Low Volume
80
40
Alcohol-B
Saline-B
Alcohol-BW
Saline-BW
RESPONSE RATE (responses/min)
80
(b) High Volume
40
Saline-B
Alcohol-B
Alcohol-BW
Saline-BW
1
2
3
4
5
6
BLOCKS

the summation-transfer test, there was no evidence of a drug treatment effect, although low-volume animals responded at a higher rate at the beginning of the transfer test than high-volume chicks.

Experiment 3: Conditioned inhibition training. Because of equipment malfunction, scores from two of the animals in each group were lost on the second day of training. In the statistical analyses, group means were substituted for lost scores and the degrees of freedom were adjusted accordingly.

Response rates to each of the two stimuli are plotted over the nine days of conditioned inhibition training in Figure A8. It is apparent from the figure that responding to the reinforced Stimulus A were somewhat variable over days, especially when compared with the percentage of A trials with at least one response, and that AW responding decreased gradually over days. It can also be seen that low-volume birds responded at a generally higher rate than high-volume birds to both stimuli. In addition, it appears that alcohol-treated animals responded at a higher level than saline-treated birds to Stimulus A, but not to AW. Responding to the compound (AW) was approximately equivalent for all groups by the latter half of conditioned inhibition training, although as with the percentage measure, the high-volume animals started training at a lower level of responding to AW than the low-volume animals. A four-way analysis was performed comparing response levels to Stimuli A and AW. The factors for the analysis were volume, drug treatment, stimuli, and days. The analysis disclosed reliable main effects of volume, F (1, 28) = 4.63, $p < .05$, stimuli,

Figure A8. Response rates (responses/min) during conditioned inhibition training, Experiment 3. Panel a depicts low-volume birds, while Panel b represents high-volume animals.

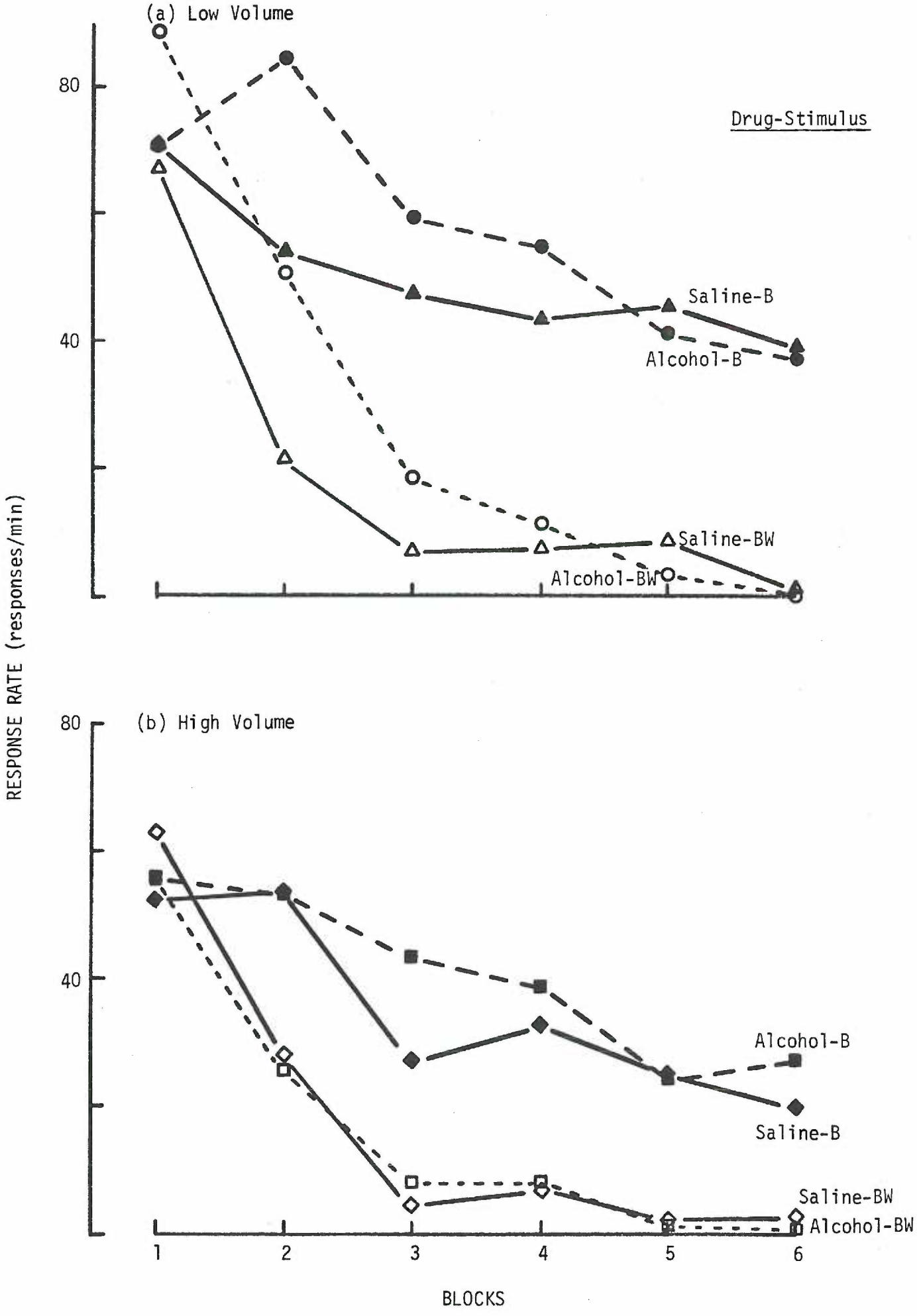

Drug-Stimulus
(a) Low Volume
80
40
Alcohol-A
Saline-A
Alcohol-AW
Saline-AW
RESPONSE RATE (responses/min)
(b) High Volume
80
40
Alcohol-A
Saline-A
Alcohol-AW
Saline-AW
1
2
3
4
5
6
7
8
9
DAYS

F (1, 28) = 19.81, $p < .001$, and days, F (8, 216) = 18.83, $p < .001$. The following interactions were also reliable: volume x days, F (8, 216) = 3.74, $p < .001$, stimuli x days, F (8, 208) = 15.48, $p < .001$, and volume x stimuli x days, F (8, 208) = 2.38, $p < .05$. This latter interaction was graphically interpreted to be the result of the following factors: (a) a steeper decline in responding to AW by the low-volume birds than by the high-volume birds, and (b) a greater divergence between A and AW responding over days for the low-volume animals, primarily as a result of the higher initial rates to both stimuli and subsequently steeper decline to Stimulus AW.

It is interesting to note that the apparent difference between alcohol- and saline-treated birds in responding to red (Stimulus A) did not achieve statistical significance. This is in contrast to the percentage measure, and was most likely the result of the large degree of variability in the rate data.

Experiment 3: Summation-transfer test. Figure A9 depicts response rates of the various groups to Stimuli B and BW over blocks of trials during the summation-transfer test. Panel a represents responding by the low-volume subgroups, whereas Panel b represents the high-volume subgroups. The solid symbols portray responding to Stimulus B (green), and the open symbols, to Stimulus BW (green + white compound). As can be seen in the figure, responding to both stimuli was initially high but decreased over blocks. Furthermore, response rates to the compound were reduced to a much greater extent than those to B alone. There was, however, no apparent difference due to drug treatment, and the volume subgroups, although graphically different at the beginning

Figure A9. Response rates to B (solid symbols) and BW (open symbols) over blocks of six trials during the summation-transfer test, Experiment 3. Panel a represents responding by low-volume birds, and Panel b represents responding by high-volume birds.


(a) Low Volume
80
40
Drug-Stimulus
Saline-B
Alcohol-B
Saline-BW
Alcohol-BW
RESPONSE RATE (responses/min)
(b) High Volume
80
40
Alcohol-B
Saline-B
Saline-BW
Alcohol-BW
1
2
3
4
5
6
BLOCKS

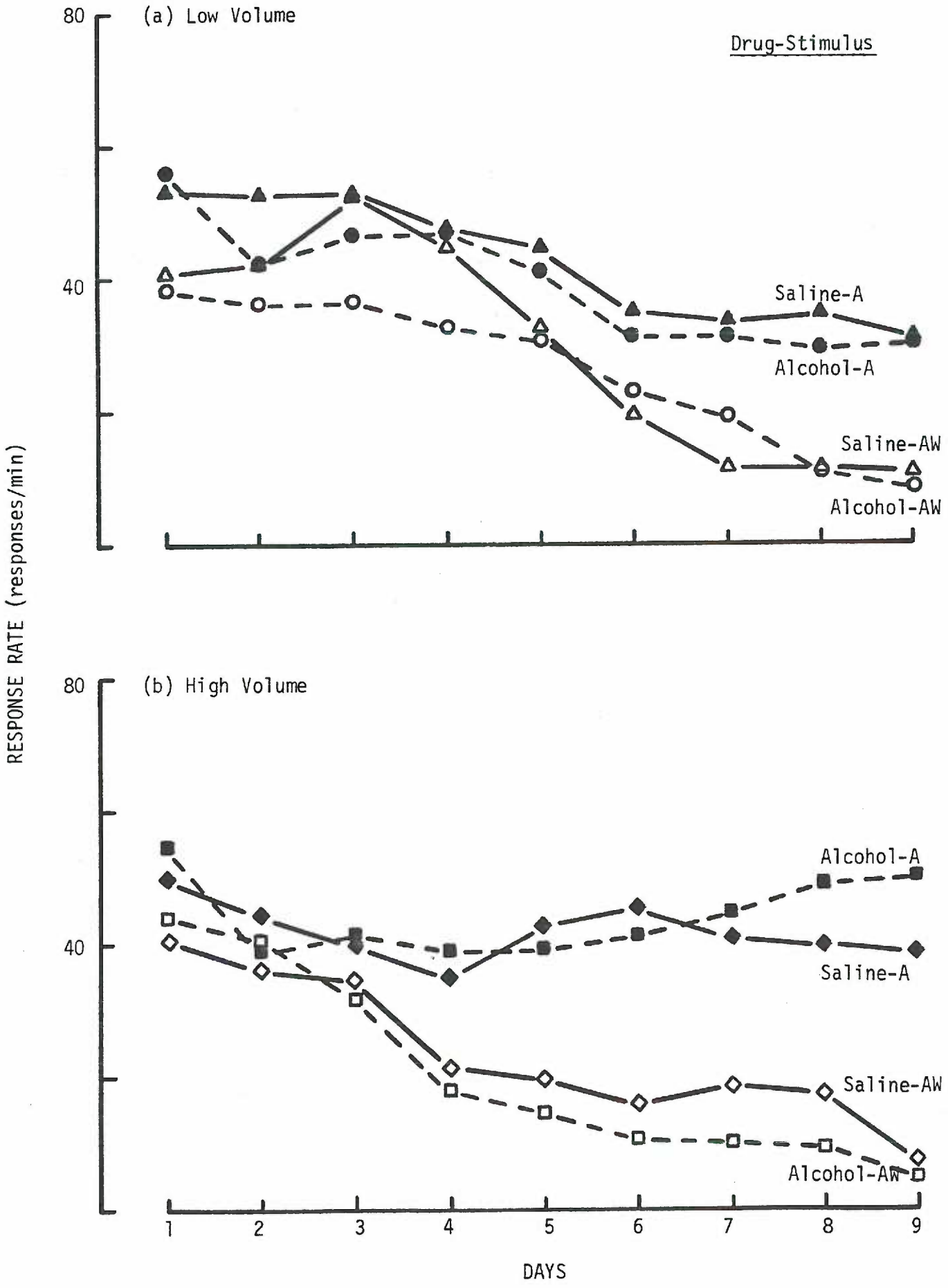
of the test, demonstrated large between-subject variability in their response rates and thus did not differ statistically.

These observations were indicated in the statistical analysis by main effects of stimuli, F (1, 28) = 51.94, $p < .001$, and blocks, F (5, 140) = 63.74, $p < .001$, and a significant interaction of stimuli with blocks, F (5, 140) = 17.06, $p < .001$. None of the other main effects or interactions was reliable. Thus, as with the percentage measure, the rate measure of responding indicated that there was transfer of inhibition, but no effect of prenatal treatment.

Experiment 3: Summary of conditioned inhibition training and summation-transfer test. The conditioned inhibition training data for animals treated on Days 6-10 of incubation were accompanied by a large degree of between-subject variability which may have obscured certain treatment effects. In any event, the statistical analyses indicated no effect of drug treatment on responding during conditioned inhibition training, although injection volume was found to affect the pattern of responding to the two stimuli over days. In the summation-transfer test of inhibition, however, there was no effect of drug treatment or of injection volume.

Experiment 4: Conditioned inhibition training. Figure A10 depicts response rates of the various groups treated on Days 11-15 of incubation to the two stimuli during conditioned inhibition training. Reference to the figure demonstrates that responding to the compound stimulus (AW) decreased steadily over days, with relatively little difference among the various groups. Responding to the reinforced red stimulus (A), on

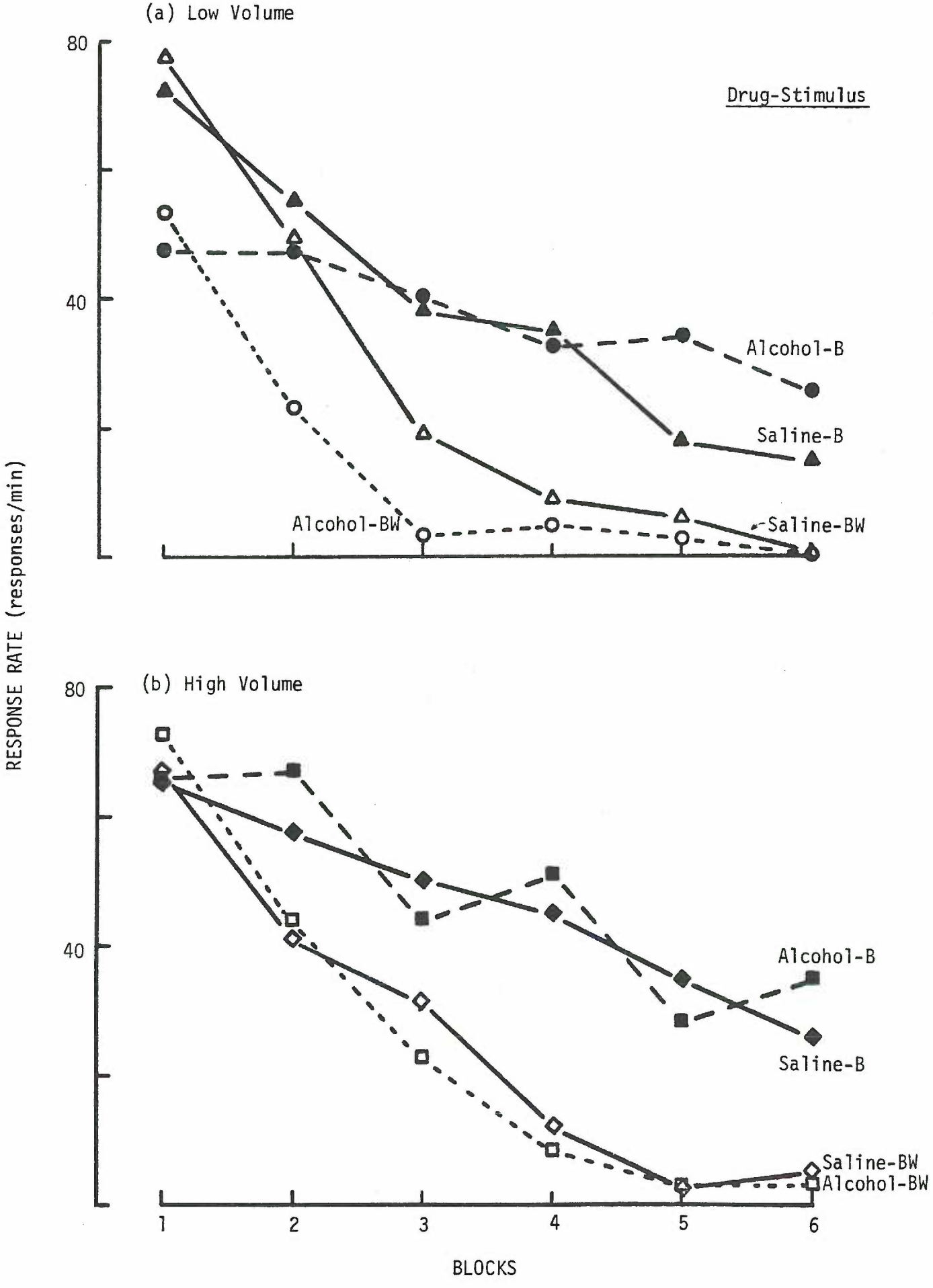
Figure A10. Response rates (responses/min) during conditioned inhibition training, Experiment 4. Panel a depicts low-volume birds, whereas Panel b represents high-volume animals.


(a) Low Volume
Drug-Stimulus
80
40
Saline-A
Alcohol-A
Saline-AW
Alcohol-AW
(b) High Volume
80
40
Alcohol-A
Saline-A
Saline-AW
Alcohol-AW
1
2
3
4
5
6
7
8
9
DAYS
RESPONSE RATE (responses/min)

the other hand, remained at a relatively higher level during the latter half of training, although it was subject to fluctuation, particularly for the low-volume animals. A four-way analysis of variance with factors of volume, drug treatment, stimuli, and days was performed to verify these observations. The analysis disclosed a significant effect of stimuli, F (1, 28) = 34.72, $p < .001$, a significant days effect, F (8, 224) = 13.80, $p < .001$, and reliable interactions of volume with days, F (8, 224) = 2.56, $p < .05$, and stimuli with days, F (8, 224) = 12.37, $p < .001$. The volume x stimuli x days interaction was also reliable, F (8, 224) = 3.36, $p < .01$, and, as with the percentage measure, was found to be the result of three factors: (a) a decrease in response rate to Stimulus A over days on the part of the low-volume animals, (b) a comparatively steady response rate to A over days for the high--volume birds, and (c) no difference between the volume groups in responding to Stimulus AW. There was no main effect of drug treatment and no interaction involving the treatment factor.

Experiment 4: Summation-transfer test. Response rates to the transfer test stimuli are plotted over blocks in Figure A11. From the figure it can be seen that responding to both stimuli decreased over blocks, with BW responding declining more rapidly and to a greater extent. Although there were no differences between saline- and alcohol--treated birds in the high-volume group, it is apparent that low-volume alcohol animals started the test session at lower rates to both stimuli than low-volume saline animals. This effect was not present for the percentage measure, but is readily explained by the observation that low-volume alcohol animals finished conditioned inhibition training at

Figure A11. Response rates to B (solid symbols) and BW (open symbols) over blocks of six trials during the summation-transfer test, Experiment 2. Panel a represents responding by low-volume birds, and Panel b represents responding by high-volume animals.


(a) Low Volume
80
40
Drug-Stimulus
Alcohol-B
Saline-B
Alcohol-BW
Saline-BW
(b) High Volume
80
40
Alcohol-B
Saline-B
Saline-BW
Alcohol-BW
1
2
3
4
5
6
BLOCKS
RESPONSE RATE (responses/min)

considerably lower response rates to green (Stimulus B) than the low--volume saline birds. Again, this Stimulus B effect was not present for the percentage measure. Nevertheless, the difference between low-volume alcohol and saline animals diminished over blocks, and for the final four blocks of the session, the rate of decrease in responding to the two stimuli was approximately comparable for the two treatment groups.

These observations were supported statistically by a volume x drug treatment x stimuli x blocks analysis of variance in which there was a reliable effect of stimuli, F (1, 28) = 41.73, $p < .001$, a reliable effect of blocks, F (5, 140) = 66.79, $p < .001$, a significant interaction of stimuli with blocks, F (5, 140) = 9.94, $p < .001$, and a reliable volume x drug treatment x blocks interaction, F (5, 140) = 2.29, $p < .05$.

Experiment 4: Summary of conditioned inhibition training and summation-transfer test. During conditioned inhibition training, animals treated on Days 11-15 of incubation exhibited the following response patterns: (a) Low-volume animals demonstrated a decrease in response rate to red (Stimulus A) over days; (b) High-volume animals maintained a comparatively stable response level to A over days; and (c) There were no differences between the volume groups in responding to AW. Furthermore, there were no differences due to drug treatment in A or AW responding, although the low-volume alcohol animals appeared to finish conditioned inhibition training at considerably lower response rates to green (Stimulus B) than low-volume saline animals. This difference was still present at the beginning of the

transfer test, but it diminished rapidly. Otherwise, there were no treatment-induced differences in responding during the summation-transfer test, although there was evidence for the transfer of inhibition in all groups.