

AN ABSTRACT OF THE THESIS OF

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Title: WHEN A RAT THINKS HE SMELLS A RAT HE LETS THE EXPERIMENTER

KNOW: AN EXPANDED STUDY OF THE EFFECTS OF ODOR CUES ON RUNWAY

PERFORMANCE IN RATS

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A three-phase experiment was conducted throughout which rats received a double-alternation schedule of reward and nonreward. During Phase 1, the baseline period, double-alternation behavior (patterning) developed in all three measures.

During Phase 2, a subject-rotation procedure was initiated. Each day, the last subject in the previous day's running sequence was moved to the first position. The results showed that shifting a subject to the first position disrupted that subject's patterning in all measures.

During Phase 3, two naive rats were inserted at the beginning of the squad, and two additional naive rats were inserted at the end of the squad. The results showed that the naive animals placed at the end of the squad acquired patterning more quickly than the animals at the beginning positions.

WHEN A RAT THINKS HE SMELLS A RAT
HE LETS THE EXPERIMENTER KNOW:
AN EXPANDED STUDY OF
THE EFFECTS OF ODOR ON RUNWAY PERFORMANCE IN RATS

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CHAPTER I

INTRODUCTION

Animals apparently are not radical behaviorists. In both laboratory and nonlaboratory species, behaviors can be observed which appear to violate reinforcement contingencies. In a classic paper entitled "The Misbehavior of Organisms," Breland and Breland (1961), pointed out that only more comprehensive physiological, genetic and ecological models could adequately explain certain animal behaviors. Shettleworth (1972) has described what she calls biological constraints on learning, and Seligman and Haber (1972) speak of biological boundaries of learning in approaches to behavior theories that emphasize an organism's genetic characteristics and evolutionary history.

The specific aspect of the "biological constraints" model to be addressed in this study is the nature and function of odor communication in rats. In independent studies, McHose and Ludvigson (1966) and Spear and Spitzner (1966) found that the runway performance of rats in a control group was being affected by the reinforcement contingencies presented to the experimental group preceding it. The experimenters proposed that the rats in the experimental group were leaving odor traces behind which, in turn, were affecting the control subjects. They discovered that as unconditioned stimuli, these odors had a weak but measurable effect on runway performance. This was an important finding, particularly since one would intuitively assume that the performance of one subject in a runway or maze should have no effect on the performance of subsequent subjects.

A year later, Ludvigson and Sytsma (1967) conducted a study showing that rats could "learn" double-alternation patterning if, and only if, they followed animals in the apparatus which were on the same reinforcement schedule. Most of the patterning was evident in the goal section, which further confirmed the odor hypothesis, since the goal section is where reinforcement or nonreinforcement took place and where the odors given off by previously reinforced subjects would remain. The odor associated with nonreward apparently became a conditioned or discriminative stimulus in that situation, and as such its effects were much stronger than they were as an unconditioned stimulus.

The discovery that rats apparently use odor as a form of communication was important, since the design of many previous experiments involving rats did not even consider the possible contaminating effects of inter-subject olfactory communication. Prime examples of such designs would include extinction studies and studies of the ability of rats to learn single- and double-alternation reinforcement schedules where runway speed is the dependent variable. In such cases, the effects of accumulated odor as either a conditioned or unconditioned stimulus could significantly affect the performance of the animals.

A logical extension of previous studies was done by Seago, Ludvigson, and Remley (1970). They showed that anosmic rats (i.e., those with their olfactory bulbs removed) were incapable of acquiring double-alternation patterning under conditions where odor was the only possible discriminative stimulus, whereas sham-operated rats did pattern. That finding strongly supports the odor hypothesis. Pratt and Ludvigson (1970) demonstrated that a latent extinction effect is greater if the animal-produced odor of non-reward is left in the goalbox. Those

findings point to a possible source of contamination of the results of similar extinction studies where the effects of odor were not controlled. Certain observations from previous studies where the effects of odor were not considered appear to fit the odor hypothesis. For example, Seward and Levy (1949) observed that their subjects visibly slowed down as they approached the goal platform. This kind of behavior is characteristic of rats responding to accumulated non-reward goalbox odors. Dyal (1961) has reported the goal time to be the most sensitive measure of latent extinction. In such cases, much of what has been interpreted as latent extinction effect may be artifact due to the accumulation of the odor of non-reward in the goal section of the apparatus used. Related to extinction is the "depression effect" which is reflected by a temporary reduction in runway speed when the reward received is drastically reduced. Davis and Ludvigson (1969) showed that carefully swabbing the apparatus between trials can reduce this effect.

Another situation where odor seems to play an important role is partial reinforcement in a runway. Prytula, Bridges and Anderson (1972) showed that rats ran acquisition trials on a PRE (partial reinforcement) schedule faster if there was an exhaust fan operating in the apparatus. The fan made no difference if the rats were on a continuous reinforcement schedule (CRF). This finding supports the odor hypothesis since rats on a CRF schedule would have no reason to emit any "odor of non-reward" and therefore the speeds would remain the same whether any odors were eliminated or not. In contrast, subjects on a PRE schedule would be exposed to odors of nonreward left in the apparatus from previous subjects experiencing nonreward. Exhausting those odors on a PRE schedule would lead to faster acquisition and slower extinction than for a control group on a PRE schedule, which is what was observed.

As we have seen, odor can be an important factor in various experimental situations. For this reason, the results of "odor susceptible" experiments need to be re-examined in light of what we are now discovering about odor communication in rats. Some of the spinoffs of the work in progress in this area are effective methods to control for odor as a factor in certain experimental designs.

Since odor communication itself seems to be such an important factor in rat behavior, it deserves to be studied as a key element in rat behavior under the broader heading of "biological constraints in learning." Much remains to be learned about the actual chemical composition of these odors, the glands that emit them, and the various ways rats use them. Ludvigson (1977) has written a paper in which some tentative explanations are offered as to the biochemical nature of these odors. It is, however, beyond the scope of the present study to explore the biochemical specifics of odor communication in rats. Hence, we shall concern ourselves with some of the key studies that are beginning to reveal the nature of odor cues as conditioned and unconditioned stimuli.

While the majority of investigations in the area of odor cues have been concerned with the nature of odor cues as conditioned stimuli, Mellgren, Fouts and Martin (1973) reported an interesting study on odors of reward and nonreward as intrinsically attractive or aversive (i.e., unconditioned) stimuli. The investigators found that unconditioned, naive rats would slowly approach but quickly escape from the middle compartment of a runway in which there was an odor of nonreward left by a frustrated "donor" animal. Conversely, other naive rats would approach the center section about twice as quickly and leave twice as slowly when an odor of reward had been left there by a rewarded "donor" animal.

These results tentatively support the hypothesis that there is something inherently attractive about the "odor of reward" and that the "odor of nonreward" is inherently aversive. In one control group, the odor in the middle section was left by a rat which was simply allowed to explore the apparatus. In that group, approach and escape times were quite close. An interesting and somewhat puzzling finding was that the fastest times of all were logged by rats who ran in a clean apparatus. Why rats exposed to a "no odor" situation should run several times faster than rats in a "neutral odor" situation is not clearly understood. Mellgren et al. (1973), however, do seem to offer us data which support the idea of separate odors of reward and nonreward which can act as unconditioned (i.e., intrinsically attractive or aversive) stimuli.

As discriminative (conditioned) stimuli, odors appear to play an important role in the determination of runway behavior in rats. It seems that the odor of nonreward is a stronger and more predictable stimulus than the odor of reward, however. Morrison and Ludvigson (1970) were able to show that a donor-produced odor of nonreward at the choice point of a T-maze could serve as a cue for a left or right turn. This was not true for the odor of reward. Prytula and Davis (1976) have demonstrated that placing an odor donor animal in the start or run section of a straight runway affected running speed in that section, as well as subsequent sections. This change in speed, incidentally, is typically observed as a depression in speeds on nonreward (N) trials, and not an elevation in speeds on reward (R) trials. It should be noted, however, that these studies of odors as discriminative stimuli are looking at the repeated-measures variance between R and N trials. When Prytula and Davis (1976) reversed the correlation between the donor and run animals, they noted several things. First, patterning was temporarily disrupted in all

sections of the runway. Then the animals apparently quit using the odors left by the donors in the startbox as discriminative stimuli since they had lost their predictive value. Finally, patterning was quickly reestablished in the goal section as the rats began using the odor cues left there by previous run subjects. In a second experiment, the donors were placed in the run section. There, reversing the correlation between donor and run subject reinforcement schedules had similar results; patterning was disrupted in the run and goal sections and very quickly reestablished in the goal section. The rapidity with which the run subjects reestablished patterning in the goal measure suggests that reward and nonreward odors may be different for different animals. Also seen in the results of the Prytula and Davis (1976) study was evidence that, to a limited extent, "memory" is a determinant of some aspects of runway performance. Specifically, performance on the first trial of the day seemed to be controlled by cues associated with the first trial of the day and not odor cues when those cues were reversed in different phases of this experiment. Similarly, the next to the last N trial of the day apparently became a cue signaling "time out" from reinforcement. This resulted in slower N speeds on the last trial of the day than would be expected on the basis of odor cues alone.

Thus, several interesting hypotheses are tentatively supported by the Prytula and Davis (1976) study. For one, there is probably a "memory" factor involved in rat performance. Also, there is quite probably significant inter-subject specificity of odors. That is, each individual rat's reward or nonreward odor has that individual animal's chemical "signature" on it that other rats can distinguish. Rats can apparently learn to ignore consistently incorrect odor cues, and "adopt" previously little-used

redundant odor cues left by other run subjects in the goal section of a straight runway. Finally, rats are probably very good at using odor cues and that use may have some very subtle aspects.

In the context of the specificity of odors used for inter-subject communication, Davis, Prytula, Noble, and Mollenhour (1976) have studied the motivational specificity of odor cues. They used water-deprived donor subjects and food-deprived run subjects. Interestingly, patterning did not develop (except for goal-section patterning due to odor buildup from previous run subjects) until the run subjects were also placed on water deprivation. This means that either R and N odors serve as discriminative cues under one deprivation state but not another, or that the donor animals were actually excreting odors that carried a rather specific message regarding the deprivation state of the donor animal. The former interpretation could be called the "cue interpretation," while the latter could be called the "pheromone interpretation." The central issue in accepting the pheromone interpretation is whether or not scent marking by rats during learning tasks can actually communicate rather specific information to other rats. Ideally, the investigation of the pheromone hypothesis would involve a sophisticated chemical analysis of the substances involved along with a detailed study of the source, sites of reception, and specific behaviors mediated by these chemicals. This topic is further discussed by Reynierse (1974).

If any doubt remains in the mind of the reader that odor is the key to a laboratory rat's ability to "remember" a single- or double-alternation reinforcement schedule, then it would be well to briefly review an experiment reported by Davis, Prytula and Voorhees (1979). In this study, rats were administered eight trials per day, four reward and four nonreward, with the odors from "donor" animals corresponding to an R and N situation

experienced by the run subjects actually traversing the runway. As would be expected, significant patterning developed in all measures after about seven days. When the donors were taken away, patterning remained in the goal section as would be expected from previous studies, since the run animals act as their own "serial odor donors" in the goal section. What has not been mentioned until now for rhetorical purposes, however, is that the schedule was neither a single- or double-alternation schedule, but rather a random schedule. The only restrictions on this random schedule were that the animals would receive four R and four N trials each day, and that the same sequence would not occur two days in a row. Unless one would wish to pursue the idea of a metaphysical explanation to the phenomenon of random-sequence patterning, it would probably be best to recognize that such otherwise inexplicable behavior is best explained by some kind of chemical communication hypothesis.

In summary, there does not remain much of a question of whether or not odors are important in mediating behaviors in rats under certain circumstances. What now remains to be done is to follow up some of the pioneering work that has been done in this interesting field with studies involving both replications and new design refinements. Let us look then at some elements of experimental designs that could yield additional information concerning the manner by which odors mediate rat behaviors. It should be noted that designs requiring sophisticated chemical analysis and applied physiology are being excluded from the scope of the present investigation. Hence, the present research will be concerned with the manner(s) by which the rats use odors "in vivo" in situations that will permit us to make meaningful inferences about how rats use odors.

Following the pattern of Ludvigson and Sytsma's (1967) early study,

virtually all of the studies assessing runway performance have used small squads of rats (typically seven), often run on a double-alternation schedule. The dependent measure has typically been time (or speed as a function of time and distance) and it is typically considered as a within-groups (i.e., repeated measure) variable. Such designs have certainly yielded considerable information about the differences in behavior when such a small group of rats is alternately exposed to the odors of reward and nonreward. One question such studies have not answered however, is what are the effects of the accumulation of odor where a large squad of animals is used? In such a design, the R or N condition would remain the repeated measure, but another independent variable, level of odor buildup, could be employed. As recent studies (e.g., Ludvigson, 1977; Davis, Prytula, & Voorhees, 1979) offer some tentative evidence that odors may be cumulative, the "large squad" design should yield additional, otherwise unobtainable data on the effects of odor accumulation. With the squad arbitrarily divided into "low buildup" (first) and "high buildup" (last) segments, the between groups variable of odor buildup could easily be examined.

Another question that the "large squad" design would answer is whether or not odors of reward or nonreward can eventually diffuse from the goal section back to the run or even the start sections of the apparatus. In the studies conducted to date, patterning due to run subjects acting as their own "odor donors" has only been observed in the goal section of the apparatus. Possibly with a larger squad, and potentially greater odor accumulation, the odors would disseminate to earlier segments of the instrumental response chain.

In addition to running a "large squad" of rats, two other design

features will be incorporated in the present design which should shed some light on some other aspects of the use of odors. First, the effects of squad rotation have yet to be studied. Hence, it is not known what will happen if the last rat in the squad is rotated to the first or "donor" position. If the Sequential Theory (Capaldi, 1966, 1967, 1971) is correct, there should be no change in R/N patterning when the rats are rotated to a "no odor cue" position in the squad, since according to the Sequential Theory, the observed speed difference between R and N trials is due to internal "memory" cues. On the other hand, the elimination of patterning would, according to the odor hypothesis, be expected when the terminal animal in the daily sequence is rotated to the initial or "no donor cue" position. Also, if patterning had not developed in the first animal (it should develop, according to the Sequential Theory), one would expect to see it develop in the original "donor" animal as this subject is rotated deeper into the squad order. Hence, the results of this rotation should tell us a good deal about the relative importance of memory and odor in mediating runway speeds on R and N trials. While Prytula and Davis (1976) cite evidence that "memory" cannot be completely ruled out as a factor in runway performance, it is expected that "memory" will be relatively unimportant in comparison to the effects of changing odor conditions for each animal.

A final condition to be set up in the present experiment is the introduction of naive animals into the larger squad of "experienced" rats. There are three types of effects that might be predicted in this previously untried experimental situation. First, will there be any disruption of patterning in the animals immediately following the first naive subjects? This will tell us something about if and how the subjects "learn" to transmit as well as receive odors. An alternative interpretation to such

a disruption would be an "inter-animal specificity" hypothesis. If, as Prytula and Davis (1976) have suggested, the R or N odors of any given animal have any recognizable individuality, it might take the animals following the naive subjects some time to become accustomed to their new "donors." In either case, a disruption would be significant. Since the first naive animals will be placed right after the first or "donor" animal, they will be exposed to relatively weak odors, whereas, the other naive animals will be placed at the end of the squad where they will be exposed to the accumulated odors of a total of fifteen subjects. This will tell us if such an accumulated odor can act as a significant unconditioned stimulus. If so, a weak effect would be observed right away. If the naive subjects at the end of the squad acquire the strong conditioned stimulus effect before the naive subjects placed at the beginning of the squad, it would give us additional evidence that such patterning is odor-mediated and that the odors are cumulative.

In summary, the proposed experiment represents an expansion of past studies. By using a squad approximately twice the size of that employed in previous odor studies, and by setting up experimental conditions that remain untried, this experiment was designed to replicate and confirm the results of past studies and to begin to answer additional questions about the manner in which odors mediate behavior in the ubiquitous albino rat.

CHAPTER II

METHOD

Subjects

The subjects were 17 experimentally naive male, albino rats which were purchased from the Holtzman Company, Madison, Wisconsin. They were approximately 90 days old at the beginning of the experiment. The subjects were individually caged with water available on an ad libitum basis. The vivarium lights were left on 24 hours a day before and during the experiment. Ten days prior to the pretraining, all the subjects were placed on a food-deprivation regimen that maintained them at 85% of their free-feeding weight. They remained on that deprivation schedule for the duration of the experiment. Four of the 17 subjects which were not run until Phase 3 of the experiment were not placed on food deprivation, nor did they receive pretraining until ten days prior to the start of Phase 3.

Apparatus

The apparatus consisted of a single straight runway (11.4 cm wide x 12.7 cm high), with a gray startbox (28.1 cm long), a black run section (91.4 cm long), and a black goalbox (30.5 cm long). Masonite guillotine doors separated the startbox and the goalbox from the run section. Start, run, and goal times were produced by the activation of a micro-switch located on the startbox door, and three photoelectric cells located 15.2, 92.4, and 116.8 cm from the startbox door. The resulting times were registered on three electronic stopclocks, and recorded for each trial. A plastic receptacle recessed into the end wall of the goalbox served as the goal cup. A pellet dispenser was used to feed 45 mg Noyes food-pellets into the goal cup via a flexible plastic tube. The loaded dispenser was left in place during all trials on that the

food-pellet odor remained constant. A thin plastic sheet was used to cover the runway-access doors to slow the dissipation of odors from the apparatus.

Procedure

Thirteen rats were randomly assigned permanent positions in the squad for Phase 1 of the experiment. Six days before pretraining, they were placed on food deprivation. On Days 1 and 2 of pretraining, each animal was handled and tamed for one minute. On Days 3 and 4 of pretraining, each animal received five minutes of exploration time in the clean experimental apparatus with both doors raised, all photoelectric equipment operative, and five 45 mg Noyes food-pellets in the goal cup. On all four pretraining days, each subject received twelve 45 mg Noyes food-pellets in the home cage to habituate them to the pellets. Ten days before the start of Phase 3 of the experiment, the four naive subjects underwent the same deprivation and pretraining regimen.

Experimental testing was divided into three phases. During all three phases of the experiment, all subjects received four reward (R) and four nonreward (N) trials per day in an RRNNRRNN sequence. To preclude odor contamination from trial to trial, the apparatus was opened and swabbed with a damp sponge after the squad completed each trial. All the animals received their daily ration after all trials had been administered. During Phase 1 which was 14 days, the order for running subjects within the squad remained constant from day to day.

During Phase 2 which was eight days, the run order was rotated so that Subject 13 from the Phase 1 sequence occupied the first (donor) position on Day 1, Subject 12 from the Phase 1 sequence occupied the first position on Day 2, and so on, until Day 8 when Subject 6 from the Phase 1

sequence was run first and Subject 7 from Phase 1 was run last.

During Phase 3, the order of running was left unchanged from Day 8 of Phase 2. Two naive subjects were then added after the first subject (i.e., they occupied positions 2 and 3 in the run order). The other two naive subjects were added at the end, i.e., in run positions 16 and 17.

On all days, all subjects received Trial 1 before Trial 2, and so on. To run a trial, the appropriate subject was removed from the home cage and placed in the startbox of the apparatus. After a 10-second confinement, the startbox and goalbox doors were opened allowing the subject to traverse the runway. On R trials, the subject was removed from the goalbox as soon as the twelve 45 mg food-pellets were consumed. On N trials, the subject was removed after a 30-second confinement.

CHAPTER III

RESULTS

The raw data consisted of latencies in each section of the apparatus for each trial. The times for each pair of like-condition trials (RR or NN) were reciprocated, averaged, and multiplied by the appropriate constant to obtain an average speed in meters per second for the pair of trials. These transformed scores were used as the data for the graphs, and selected scores were subjected to analysis of variance.

Data from the last nine days of Phase 1 were subjected to a three-factor mixed-design ("split-plot") analysis of variance with one between-groups factor (low vs. high odor buildup) and two within-groups factors (reward vs. nonreward, and trials). The first four subjects in the run order constituted the low-buildup group, and the last four subjects were the high-buildup group. Phase 1 data are presented graphically in Appendix A.

Since Phase 2 involved a daily rotation of the run order of the subjects, statistical tests involving orthogonal comparisons were not appropriate. For practical purposes, Phase 2 could be looked at as a series of 8 single-subject experiments. The data for both single-subject and group performance during Phase 2 are presented graphically (see Appendix A).

The data for the eight days of Phase 3 were also subject to a three-factor mixed-design analysis of variance. Data for the first and last four experienced subjects and for the naive subjects were analysed separately. These data are also presented graphically in Appendix A.

Since 63 F statistics with their associated probability levels were generated by the analyses of variance, an alpha level of .01 was chosen to limit the possibility of false positive (Type 1) errors. Complete

summaries of the ANOVAs can be found in Appendix B. The following table summarizes the statistically significant results:

PHASE	SECTION	SOURCE	PROBABILITY
1	Start	R-N x Trials	.00021
1	Run	R-N	.00358
1	Run	Trials	.00000
1	Run	L-H x Trials	.00534
1	Run	R-N x Trials	.00000
1	Run	L-H x R-N x Trials	.00017
1	Goal	R-N	.00002
1	Goal	Trials	.00030
1	Goal	L-H x R-N	.00495
1	Goal	R-N x Trials	.00000
3	Start	R-N	.00010
3	Start	Trials*	.00049
3	Run	R-N	.00002
3	Run	R-N x Trials	.00066
3	Run	Trials*	.00226
3	Run	L-H x R-N*	.00614
3	Goal	R-N	.00000
3	Goal	Trials	.00919
3	Goal	R-N x Trials	.00482
3	Goal	R-N x Trials*	.00000

*Naive Subjects

CHAPTER IV

DISCUSSION

Phase 1:

The results of Phase 1 suggest that the effects of odor as an unconditioned stimulus are relatively insignificant in comparison to the effects of odor as a conditioned stimulus. The single statistically significant result observed in the start measure of Phase 1 was the R-N x Trials interaction, which implies that the animals eventually became conditioned to respond to the weak odors that reach the start box. Notably, the between-groups factor of low vs. high odor buildup was not significant as an independent factor, even though the graphical impression would suggest that it had some effect.

A significant R-N x Trials interaction was also observed in the run section, along with an L-H x Trials interaction and an L-H x R-N x Trials interaction. This would suggest that performance improved with "practice" and that learning the R vs. N discrimination took place faster when the concentration of odor was higher. The R-N and Trials factors were also significant. These results suggest again that the R-N discrimination is conditioned and that conditioning takes place somewhat faster when the concentration of odors is higher.

In the goal section, the R-N and trials factors were significant along with the L-H x R-N interaction and the R-N x Trials interaction. Once again the results suggest that odor is primarily a conditioned stimulus, and that conditioning is acquired across trials. The L-H x R-N interaction would suggest that a higher odor concentration facilitates R vs. N discrimination.

Two of the questions raised in Chapter I concerning the "large-squad"

design are answered by the Phase 1 data. First, odor buildup alone does not appear to be a particularly significant factor affecting runway speed. However, odor buildup does appear to interact with other factors, such as the number of trials administered, to enhance conditioning. Although the graphs (see Appendix A) would suggest that greater buildup of odors led to greater differences in R vs. N performance, those differences were not statistically significant.

The second question that the Phase 1 data answer concerns the possibility of the dissemination of odors from the goal section back to the run and perhaps even the start section. The present data suggest that the odors do diffuse back through the apparatus, and that the animals eventually learn to discriminate those odors. Looking at the graphs of the Phase 1 data, we can see that goalbox patterning appears to start on Day 5 in the high buildup group and on Day 7 for the low buildup group. In the run section, patterning does not develop in either group until Day 7, and in the start section, patterning does not develop in either group until Day 10.

Phase 2:

The Phase 2 data shed light on two areas. First, what role does "memory" play in mediating double-alternation patterning in the rat, and second, what are the effects of squad rotation. The top portion of the first set of figures for Phase 2 (see Appendix A) show the R and N trial speeds of a single subject as the last animals in the squad (Day N). The bottom of each figure shows the same subject's trial speeds on the day that subject is rotated to the first or "donor" position (Day N + 1).

An examination of these figures will reveal that on Day N (with the exception of subject 1 in the start section), the R trial speeds are

faster than N trial speeds for any given animal. In fact, in the run and goal sections where odor effects are stronger, the slowest R speeds are faster than the fastest N speeds, individual differences in speed notwithstanding. Collectively, 96% of the data points conform to the "R faster than N" rule for any given pair of "R" and "N" trials. In the run and goal sections, 100% of the data points conform to the "R faster than N" rule.

In contrast, an examination of the graphs for Day N + 1, where the subject has been rotated to the first or "donor" (no odor) position, reveals considerable confounding of R vs. N patterning. Collectively, only 52% of the data points conform to the "R faster than N" rule for any given pair of "R" and "N" trials. In the run and goal measures, the amount of "inversion" of R and N speeds was exactly 50%. This would seem to indicate that when the animal is deprived of odor cues, performance on a double-alternation schedule drops to chance. This would seem to rule out "memory" as a mediator of double-alternation patterning in the rat.

The interpretation of the data for the first and last four subjects is complicated by the fact that since the squad was being rotated daily, orthogonal comparisons between the groups are not appropriate. However, the graphical impression of the instrumental performance of the first four animals is that considerable confounding of R vs. N discrimination took place. This effect is especially strong in the start and run sections, and less pronounced in the goal section.

This would seem to support the idea of inter-animal specificity of odor cues. The last four animals would not be affected under such conditions since they are still responding to "familiar cues." The relative

lack of confounding in the goal section can probably be attributed to the strength of the odors which are present in that section.

A tentative conclusion would be that the odor cue from a familiar animal (or series of animals) is a more potent discriminative stimulus by virtue of being more conditioned than is the odor from a nonfamiliar animal (or series of animals). From what we observe, once R-N patterning is established, the R or N odor from any animal may serve as a conditioned stimulus. However, since part of what the receiving animal is conditioned to is a "chemical signature" given off by a particular animal, we observe a decrement in response in the receiver animal since it is to a degree generalizing a response from a similar but not identical stimulus.

Phase 3:

The results of Phase 3 help to answer several questions concerning the manner in which odor communication in rats functions. The relative absence of any disruption in patterning in the four animals immediately following the first pair of naive animals would suggest that rats do not "learn" to excrete R or N odors. This result lends some support, albeit indirect, that the odors in question are the result of reward and nonreward rather than "frustration." If "frustration" were the cause of odor secretion, one would expect the animal not to secrete such an odor until it had been conditioned to expect a reward. Since no more disturbance in patterning was observed for the rotation of experienced subjects in Phase 2, it would appear that rats do not have to "learn" to secrete odors, but that they do become conditioned to those odors depending on the reinforcement contingencies the animals is subjected to.

The last pair of naive rats which followed a total of 15 subjects

in the daily sequence, and thus were exposed to quite strong odor cues, learned to pattern after five days. This is the same number of days it took for the Phase 1 high-odor group to start patterning. Again, this result implies that a particular response pattern may well be conditioned to odor cues, but the exuding of such odors is unconditioned. The fact that the naive animals in the high-odor condition learned to pattern first indicates that odors are indeed cumulative, and this result effectively replicates Phase 1.

No immediate patterning was observed in either pair of naive rats. This result suggests that if odor does function as an unconditioned stimuli, the effects are too weak to be picked up by the methods used in the present experiment.

In summary, the results of the naive-subject manipulations of Phase 3 suggest that rats do not "learn" to transmit odor cues. The results also suggest that odor cues are cumulative, and that high odor conditions facilitate the process of becoming conditioned to odor cues. Finally, these results also virtually rule out the possibility that odor cues function as unconditioned stimuli in the type of experiment performed here.

Turning to the performance of the experienced subjects in Phase 3, an examination of the analyses of variance confirms the graphical impression of a significant difference between R and N speeds in all sections of the runway. An examination of the significant interactions for the experienced subjects shows that there was an R-N x Trials interaction in the run and goal sections. This would suggest that patterning improved over trials. This result is compatible with the concept of inter-subject specificity of odor cues, because the squad was most likely still becoming

conditioned to the changes in run order that took place in Phase 2.

The statistical significance of the R-N and Trials factors in the goal section indicates that while performance varied across trials, the difference between R and N trials was statistically significant. The significant R-N x Trials interaction indicates that performance improved somewhat over trials. This could be due to the fact that the squad was in the process of accustoming itself to two disrupting factors: the introduction of two naive subjects at the beginning and the reordering that took place during Phase 2. An examination of the results for the naive subjects shows that no patterning developed in the start section of the apparatus. The typical pattern of an overall increase in speed for the first several trials is observed, and is reflected by the statistical significance of the Trials factor.

In the run section, the Trials factor is again significant. The significant L-H x R-N interaction confirms the graphical impression that little or no patterning developed in the first pair of naive subjects (which were exposed to low odor) but that patterning did eventually develop in the second pair of naive subjects (which were exposed to high odor). This result reconfirms the idea that the presence of strong odor cues facilitates the acquisition of R-N patterning as a conditioned response.

In the start section, the R-N and L-H x R-N factors approached statistical significance, but the only result that met criterion for this study was the R-N x Trials interaction. This result again confirms the graphical impression that patterning was finally acquired across trials. The graphical impression suggests that high odor conditions facilitated acquisition of patterning, and that there were observable but not

significant differences between the speeds of the low-buildup group and the high-buildup group.

Summary and Conclusions:

By looking at the various results as inter-related pieces of contributory information about the nature of odor communication in rats, several general conclusions can be made about the results of the present study. One such conclusion is that odor seems to function as a conditioned rather than as an unconditioned stimulus.

Another general conclusion is that these "special" odor stimuli behave in much the same way as one would expect any airborne stimuli to behave. The stimuli diffuse, as is evidenced by eventual run- and start-section patterning, and they apparently build up, as is evidenced by the interaction between odor buildup and various other factors.

Another conclusion which is supported by the graphical impressions is that it is principally the "N" odor that is responsible for R-N patterning. Some very tentative evidence that "R" odor may increase runway speeds is found in the goal speeds of Phase 1. This result, however, is not borne out in either Phase 2 or Phase 3, so it must be concluded that if "R" odor does have an effect as a conditioned stimulus, that effect is weak.

It does not appear that the introjection of naive animals has any more disruptive effect on the patterning of the animals that immediately follow than does the rotation of run order in an experienced squad. The present study does not yield data which permit us to make any definitive conclusions about the "conditioned secretion" of "R" or "N" odors. However, under the strongest odor conditions in the study, namely, the last four animals in the goal section of Phase 1 and the last two naive animals

in the goal section of Phase 3, both groups of animals took five days to develop significant patterning. This would seem to indicate that the degree of "odor donor" experience does not influence either the quality or quantity of odor secreted in such a way that the R-N conditioning process is affected. The minimal effect of introjecting naive animals in the experienced squad did not appear to exceed the effect of rotating an experienced squad. Both of these effects can probably be explained most parsimoniously by the inter-animal specificity hypotheses.

The secretion of odor appears to be an automatic, unconditioned response to the stimulus of "Reward" and "Nonreward," and does not appear to have a significant conditioned component. This finding lends some indirect support to the idea that the secretion of the odors in question is the direct result of the immediate reinforcement (positive or negative) of a response (in this case, goal-seeking behavior). In contrast, there does not appear to be any "frustration" (which implies expectation) involved in the secretion of "R" or "N" odors.

Finally, it might be interesting to offer some speculation regarding possible roles that odor cues might play in the daily routine of the average rat. Even non-laboratory species of rats have relatively poor vision. Yet they must search for food almost constantly to keep up with their fast metabolisms. At the same time, they are fair game for a wide variety of predators, including owls, hawks, cats, coyotes, snakes, and other common predators. From previous experiments, it is known that rats have an exceptionally sensitive sense of smell, and that they can detect the presence of both food and water at some distance. It is however, impossible to smell many of the potential hazards that a rat might encounter. It is also dangerous for a rat to explore nonproductive (i.e., non food-yielding) areas, since at such times it is typically more vulnerable to

predators. From an evolutionary and ecological standpoint then, it would be highly adaptive if a colony of rats could utilize some form of odor communication to put up "chemical warning signs" so that other rats would avoid unproductive or dangerous areas. By using chemical communication, a colony of rats could reduce its average exposure time to predators, something that should, and probably does, have a good deal of survival value.

As experimenters, we need to start thinking of rats as animals with extraordinary olfactory skills, and not as miniature humans. A better understanding of the laboratory rat should lead to a better utilization of that animal in behavioral experiments.

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APPENDIX A
FIGURES

Figure 1. Mean start speeds for low odor buildup (first 4) and high odor buildup (last 4) subjects -- Phase 1.

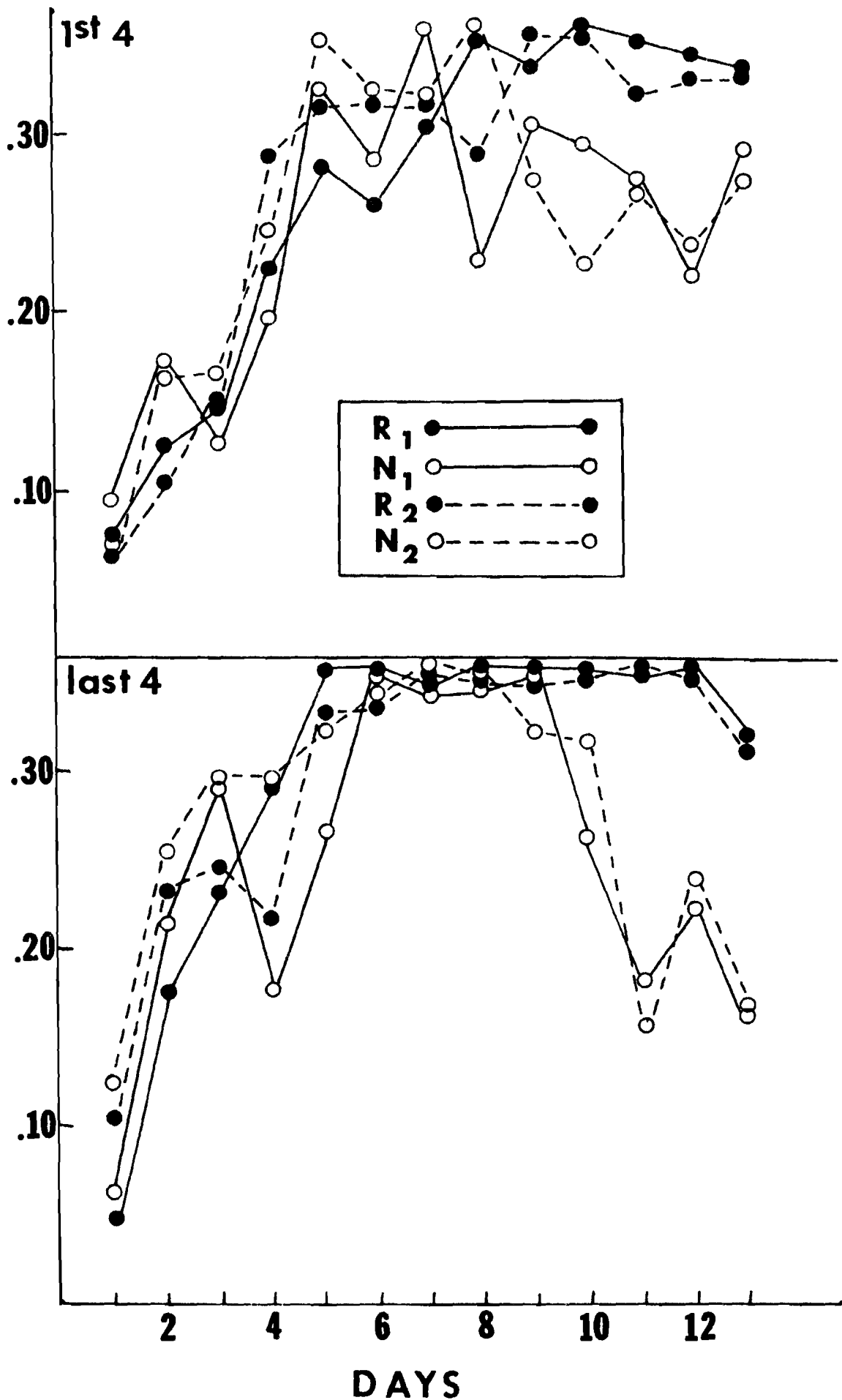
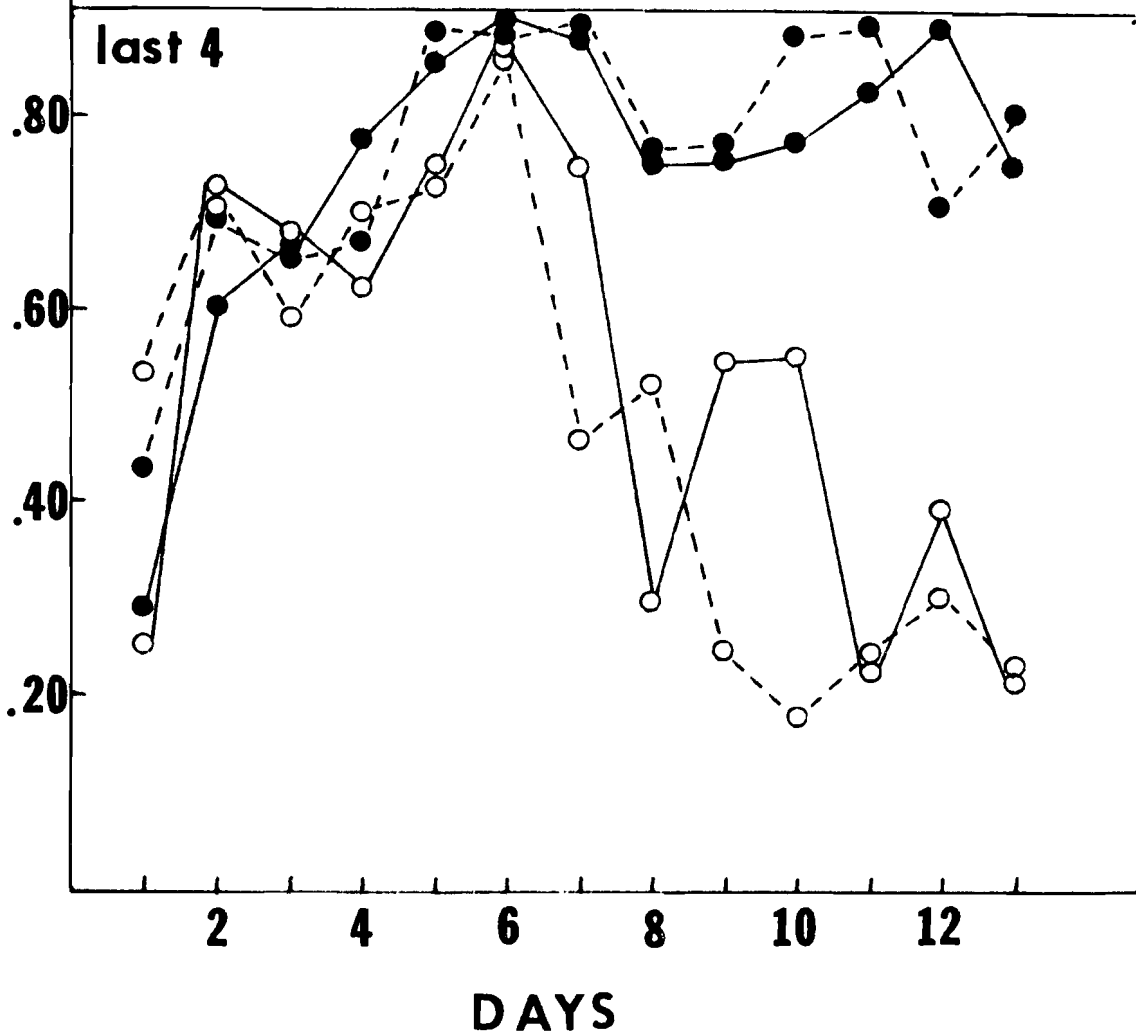
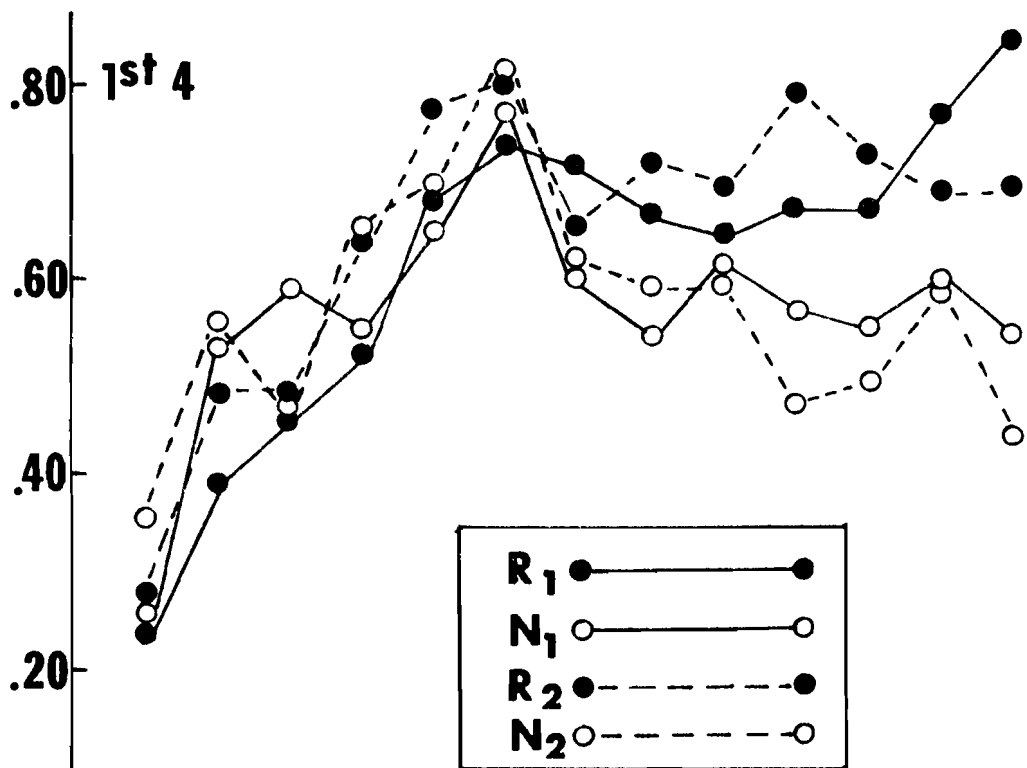


Figure 2. Mean run speeds for low odor buildup (first 4) and high odor buildup (last 4) subjects -- Phase 1.

MEAN SPEED (METERS/SEC.)



DAYS

Figure 3. Mean goal speeds for low odor buildup (first 4) and high odor buildup (last 4) subjects -- Phase 1.

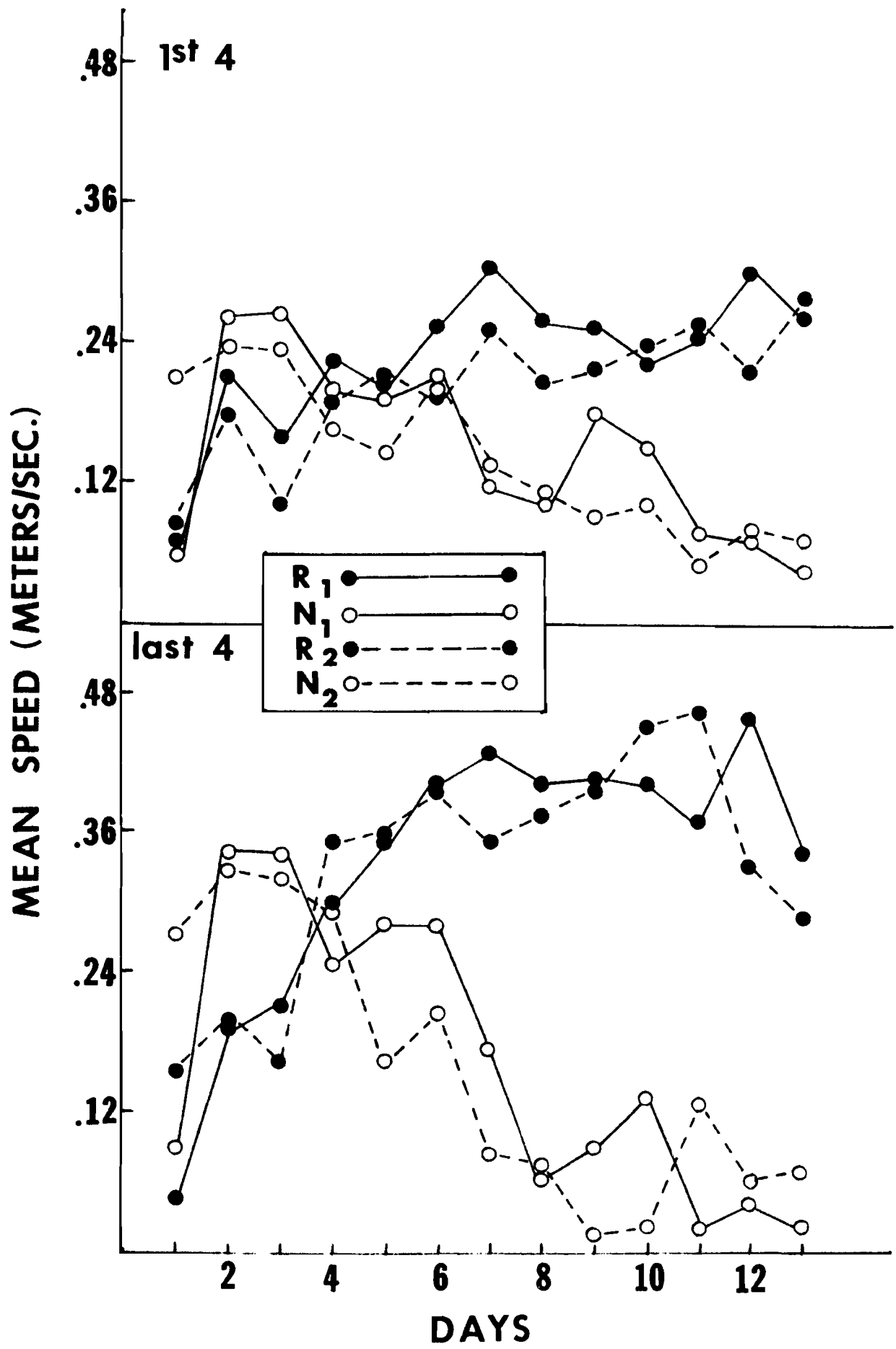


Figure 4. Mean start speeds for individual subjects as a function of being last (Day N - top panel) or first (Day N + 1 - bottom panel) in the squad rotation -- Phase 2.

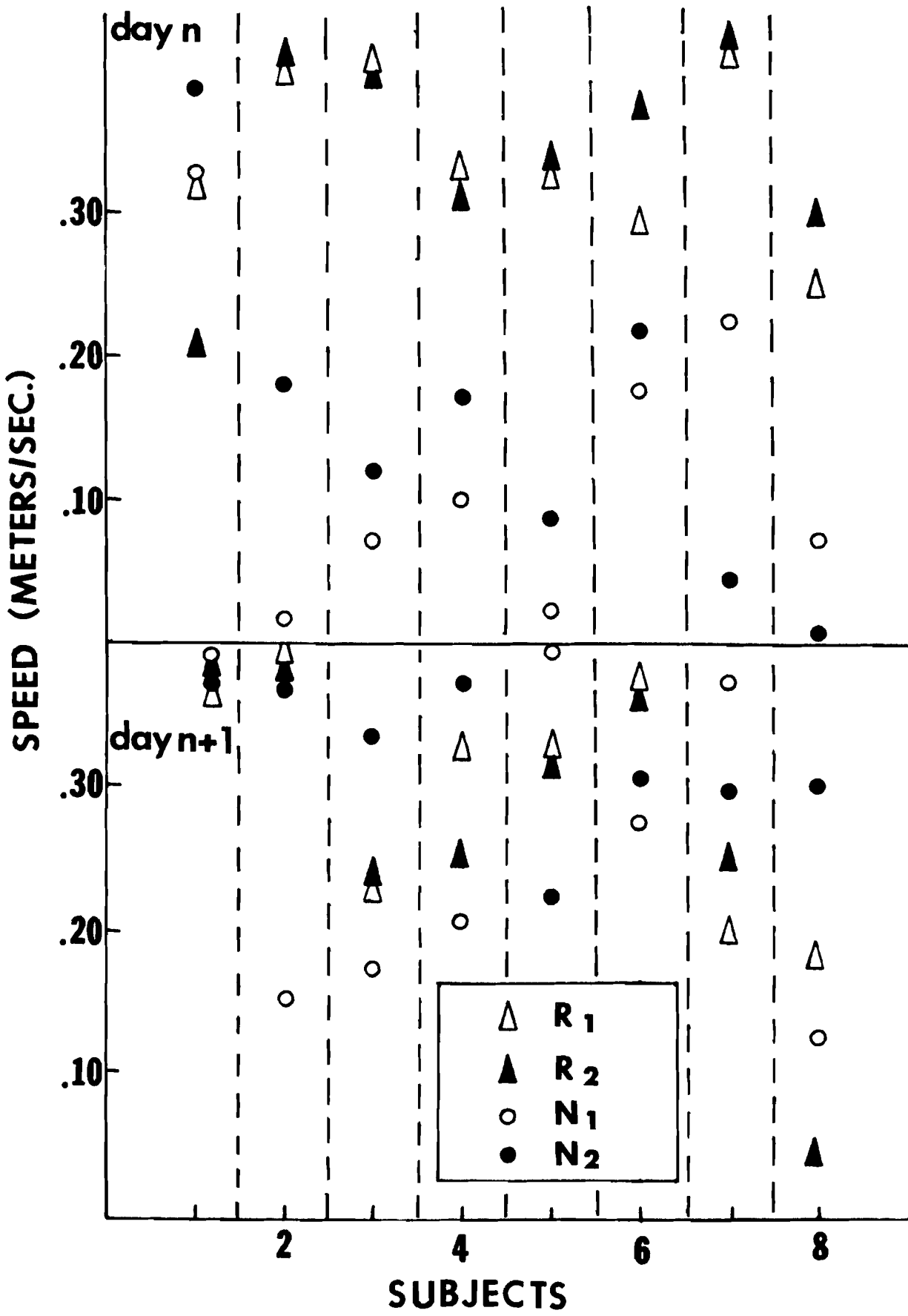


Figure 5. Mean run speeds for individual subjects as a function of being last (Day N - top panel) or first (Day N + 1 - bottom panel) in the squad rotation -- Phase 2.

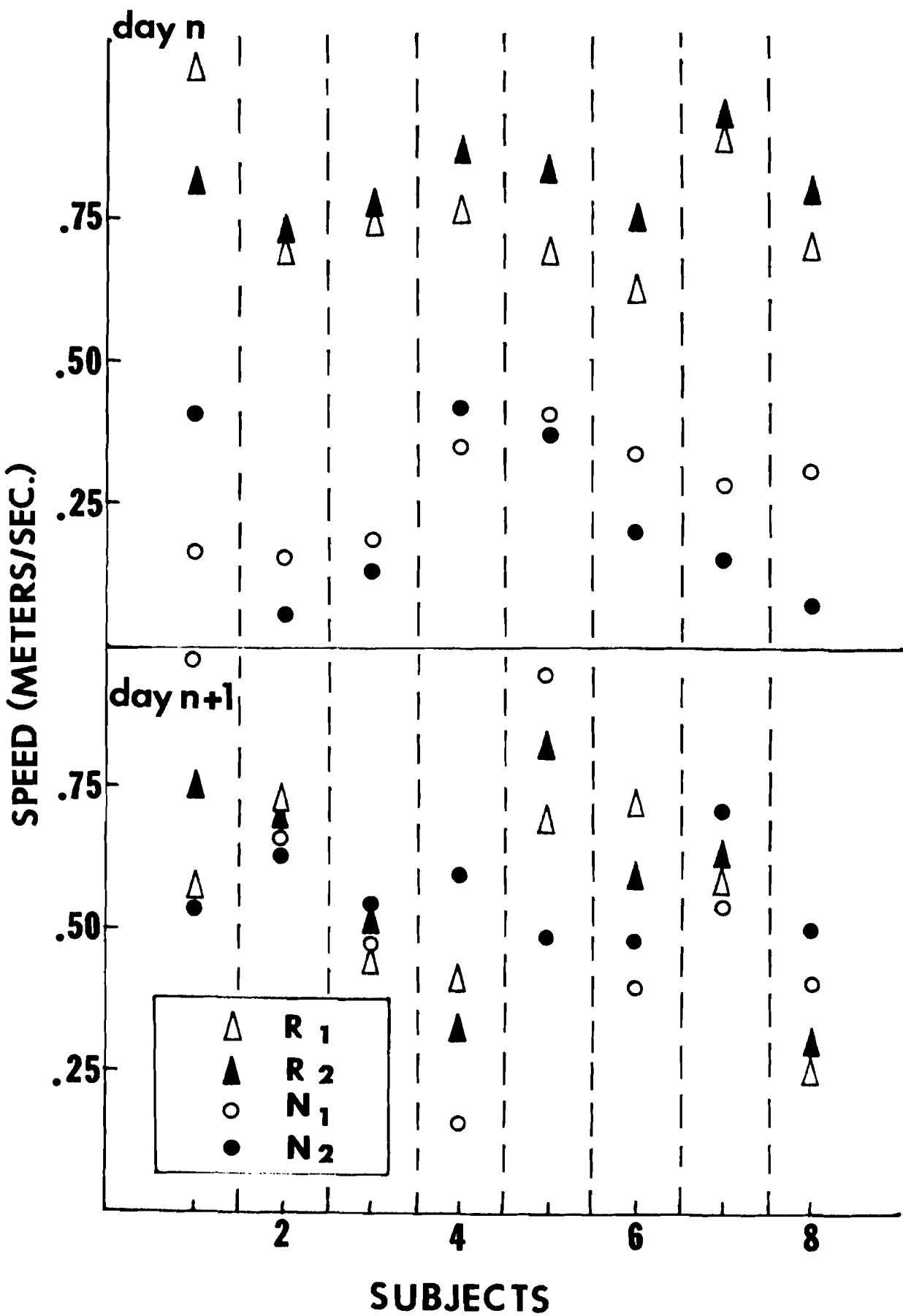


Figure 6. Mean goal speeds for individual subjects as a function of being last (Day N - top panel) or first (Day N + 1 - bottom panel) in the squad rotation -- Phase 2.

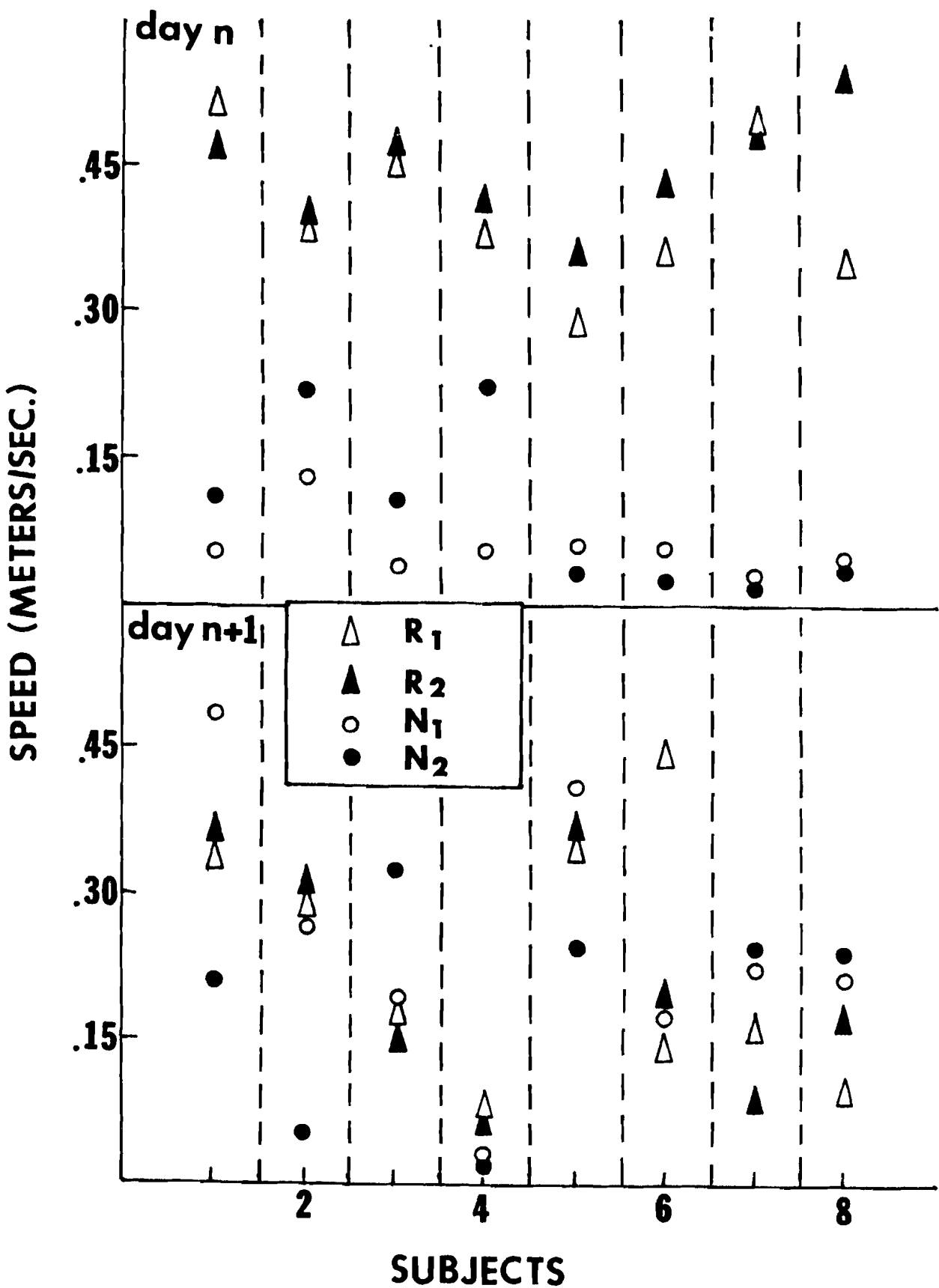


Figure 7. Mean start speeds for low odor buildup (first 4) and high odor buildup (last 4) subjects -- Phase 3.

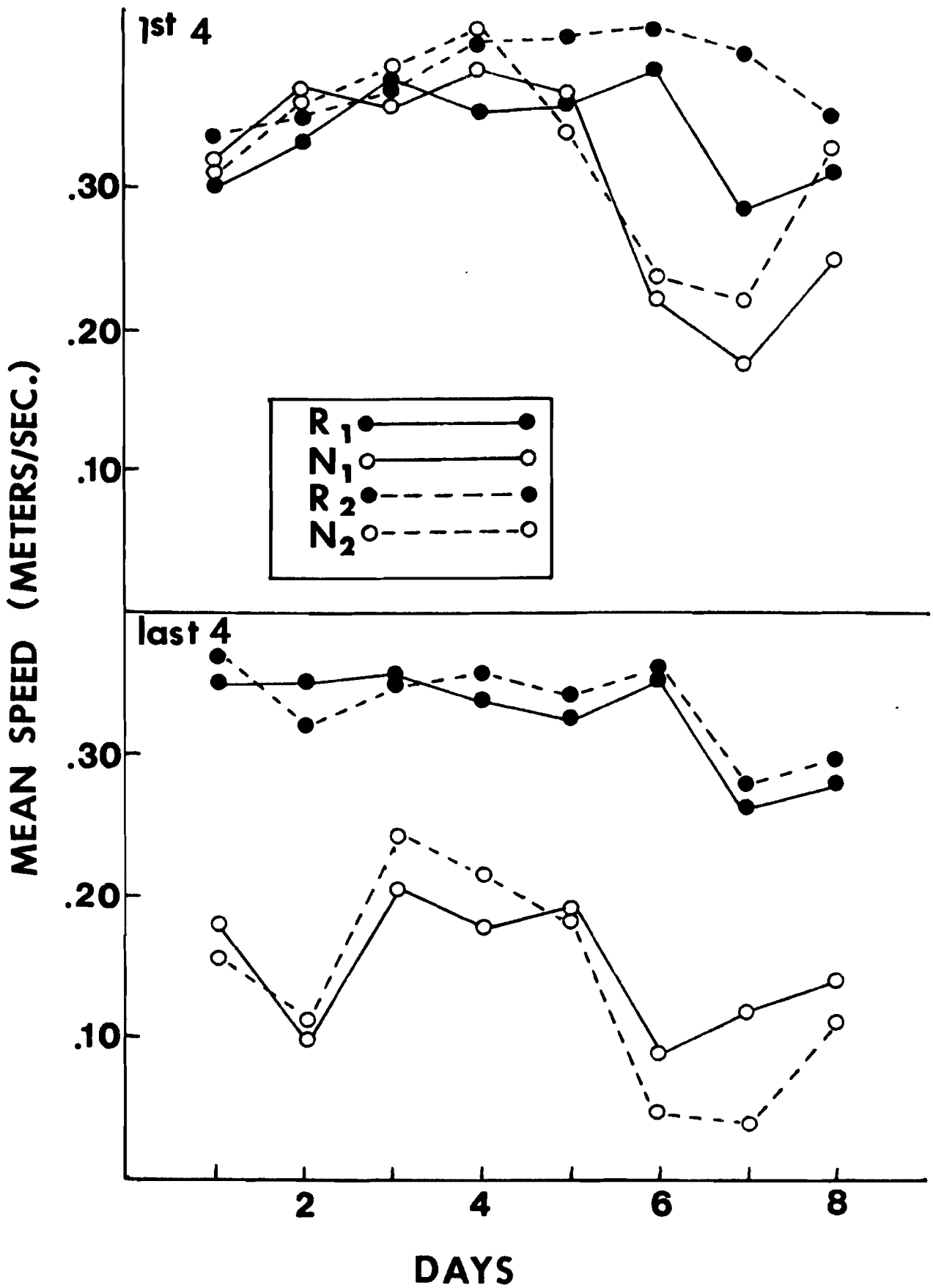


Figure 8. Mean run speeds for low odor buildup (first 4) and high odor buildup (last 4) subjects -- Phase 3.

MEAN SPEED (METERS/SEC.)

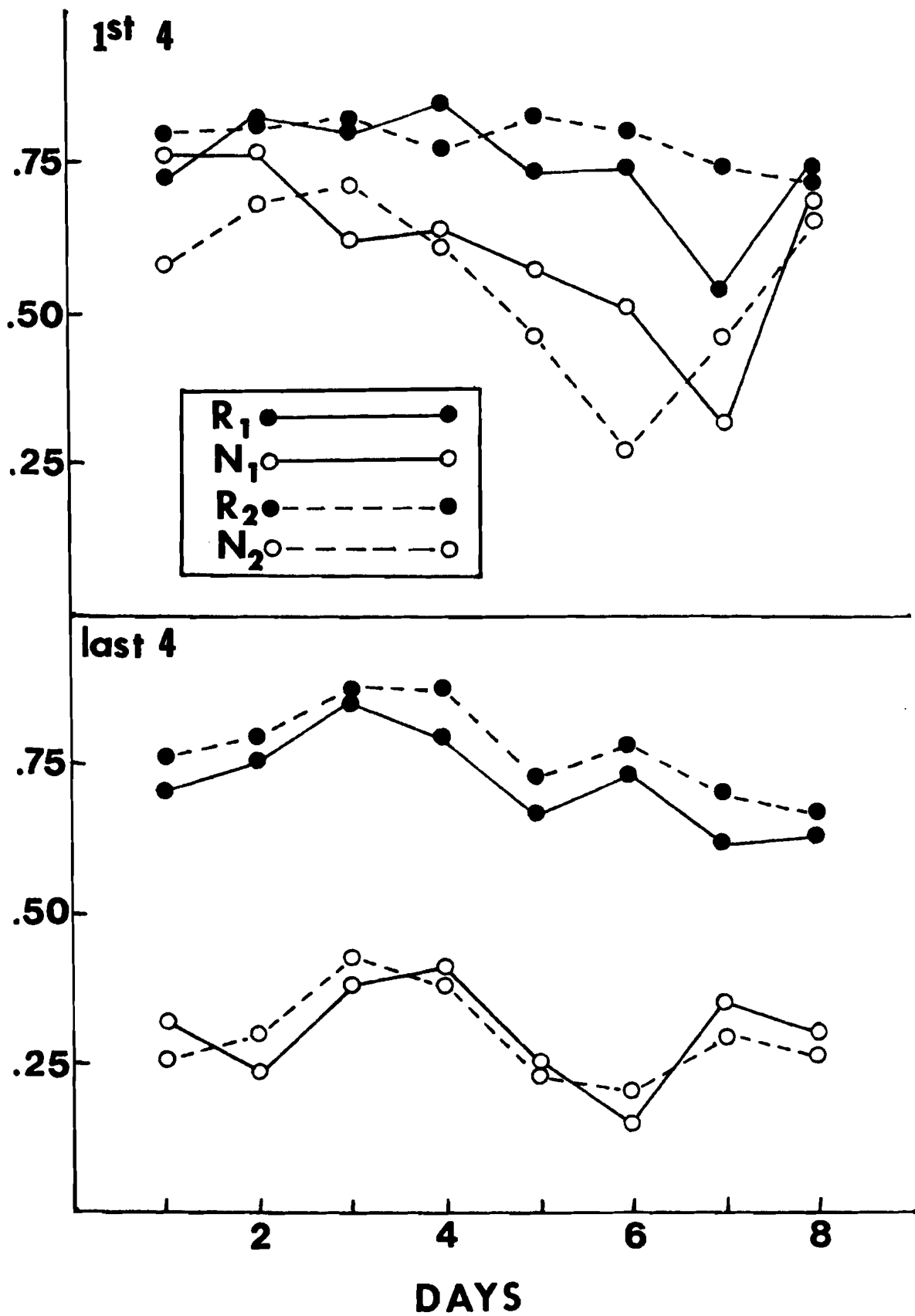


Figure 9. Mean goal speeds for low odor buildup (first 4) and high odor buildup (last 4) subjects -- Phase 3.

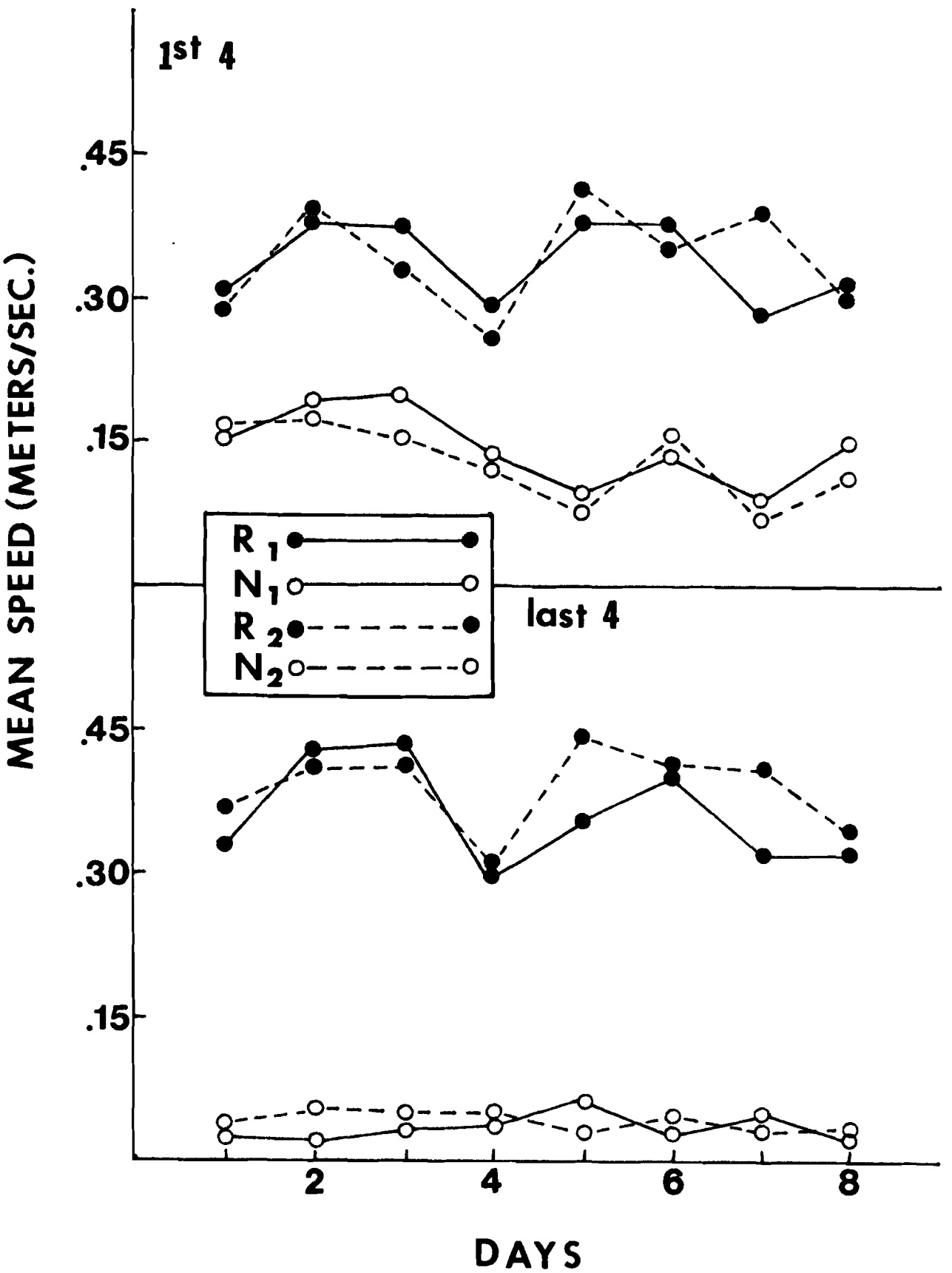
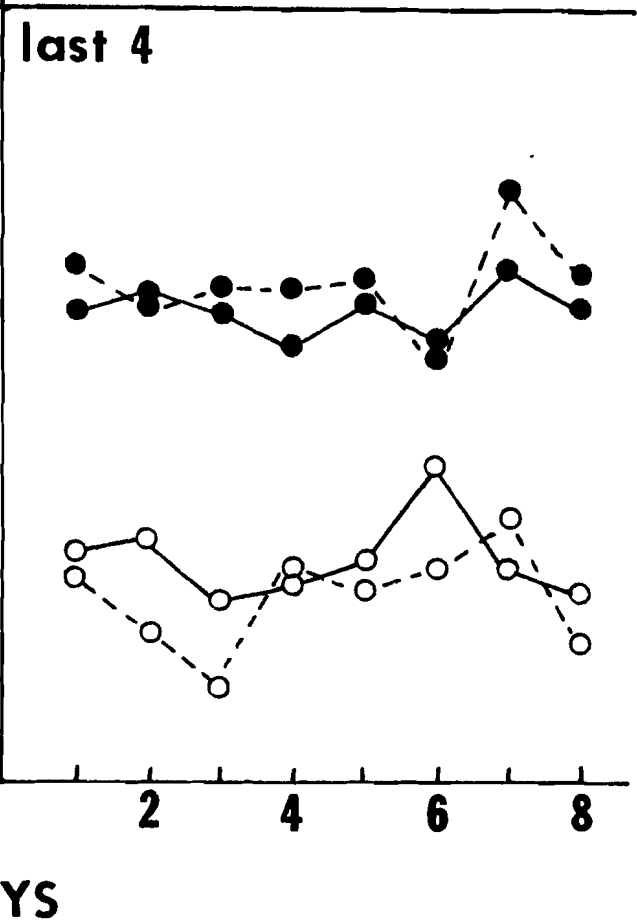
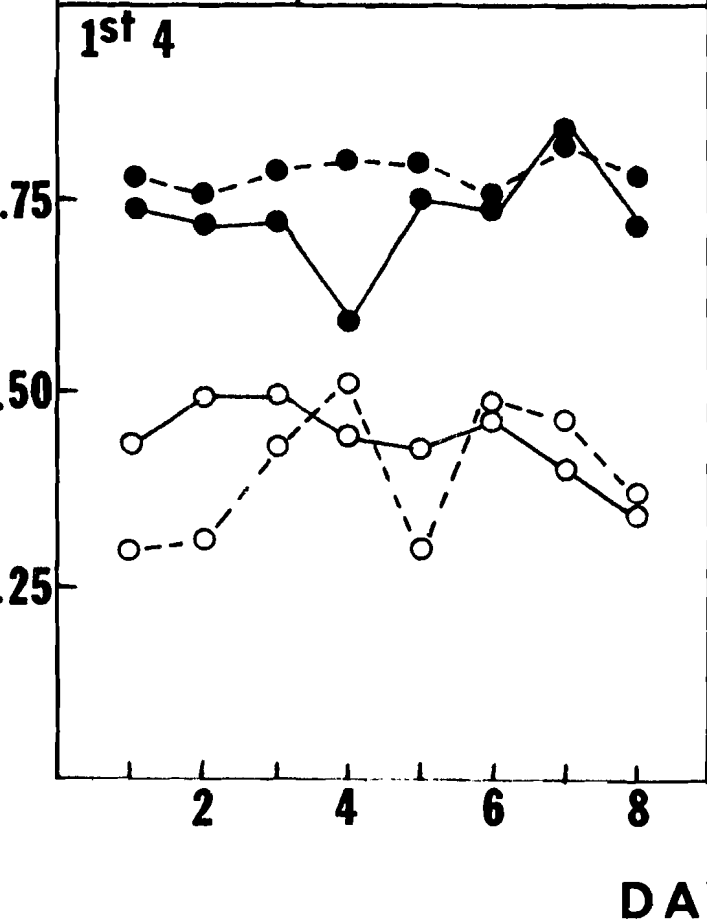
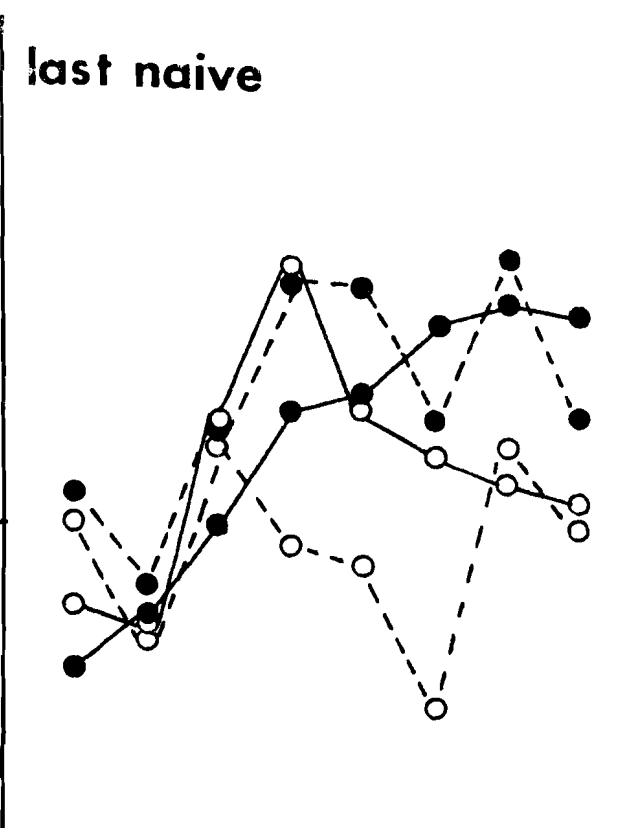
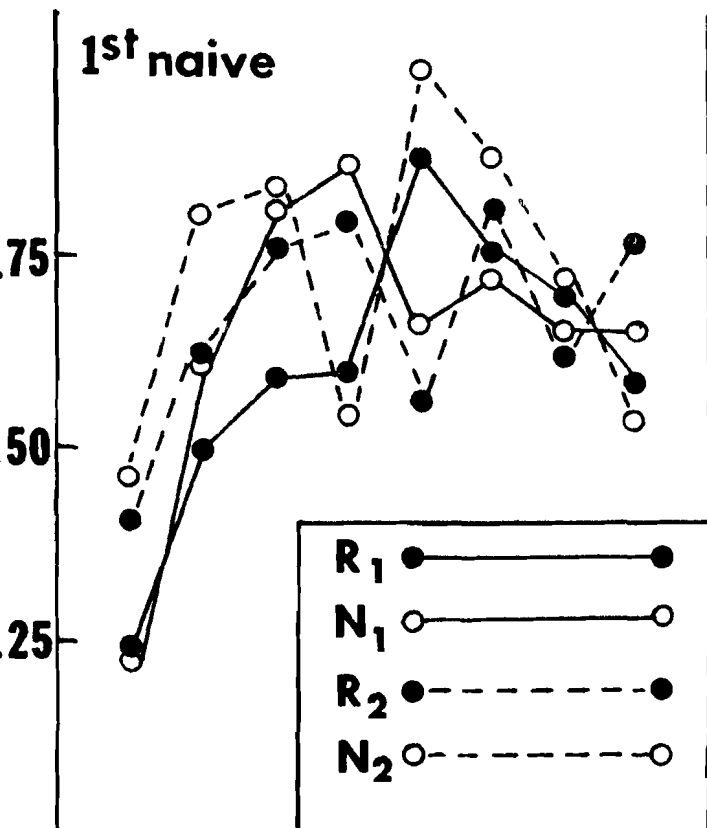


Figure 10. Mean start speeds for first and last naive pairs (top panel and low (first 4) and high (last 4) odor buildup subjects (bottom panel) during Phase 3.



DAYS

Figure 11. Mean run speeds for first and last naive pairs (top panel), and low (first 4) and high (last 4) odor buildup subjects (bottom panel) during Phase 3.

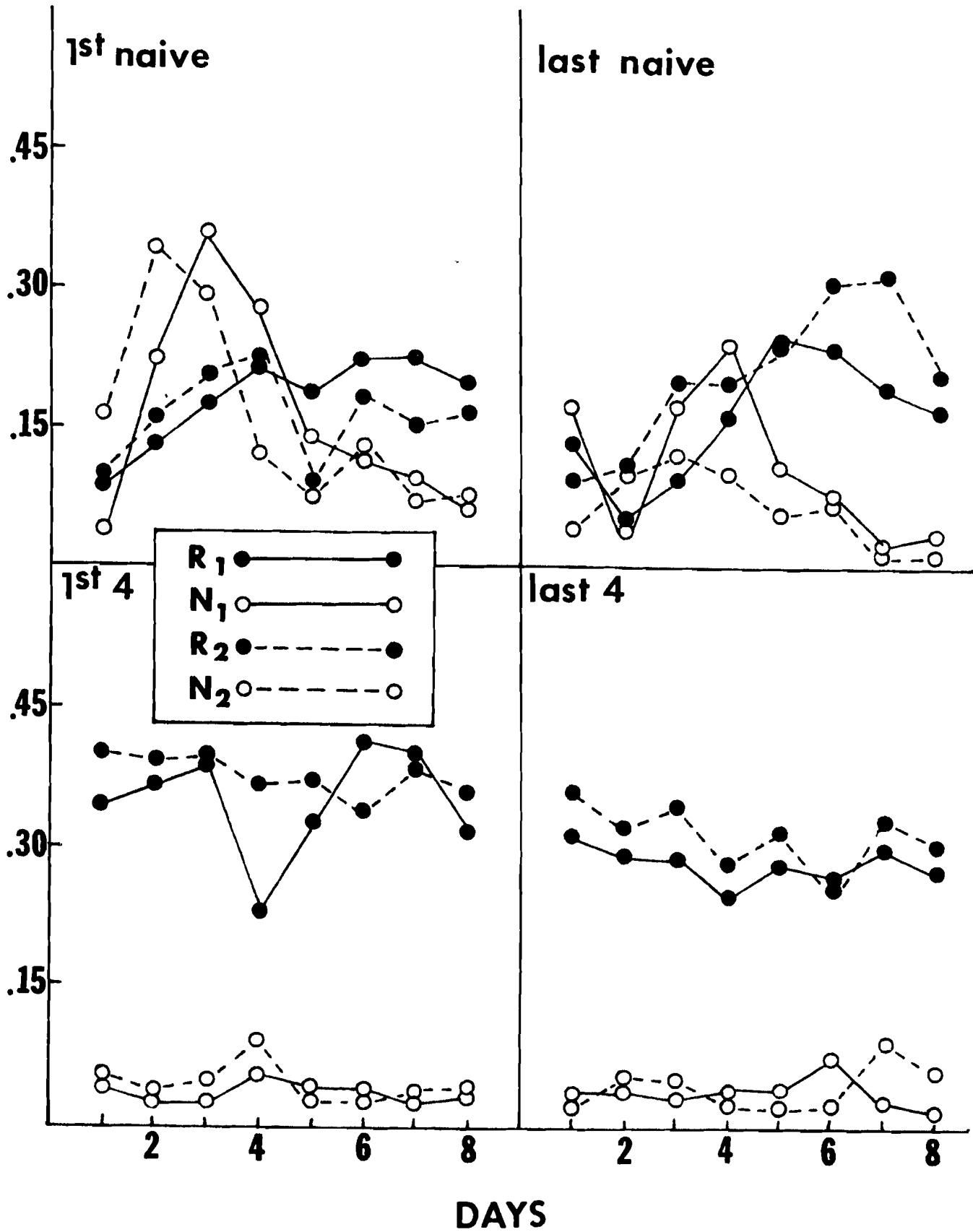
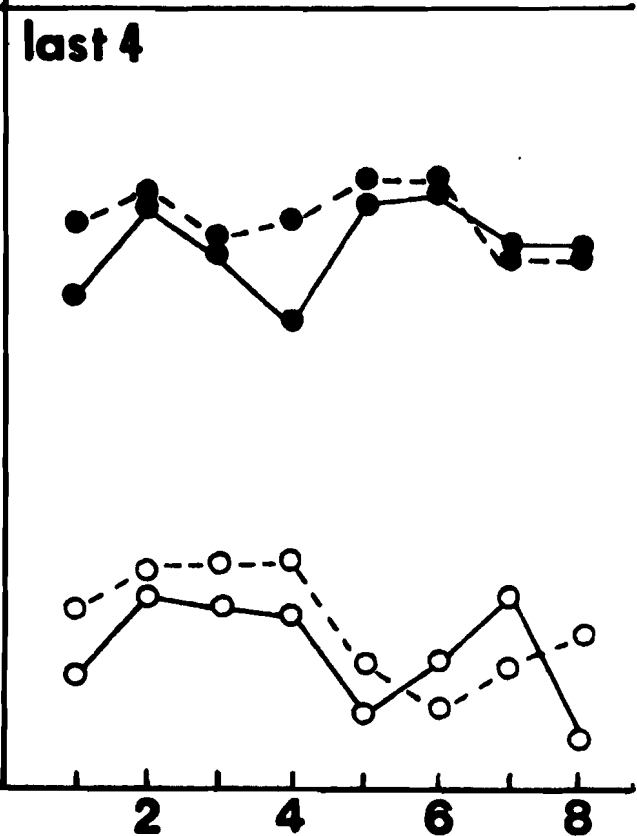
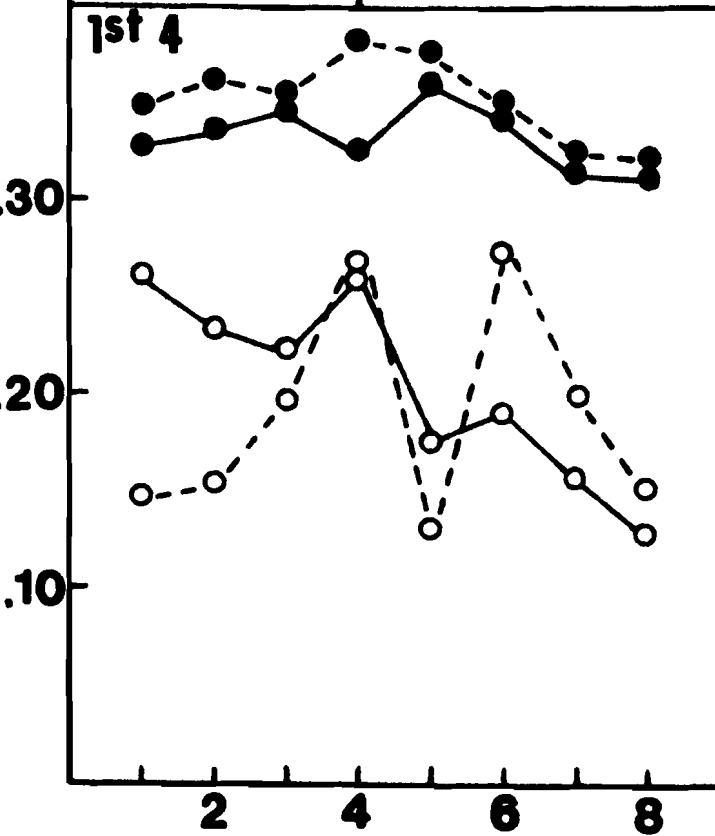
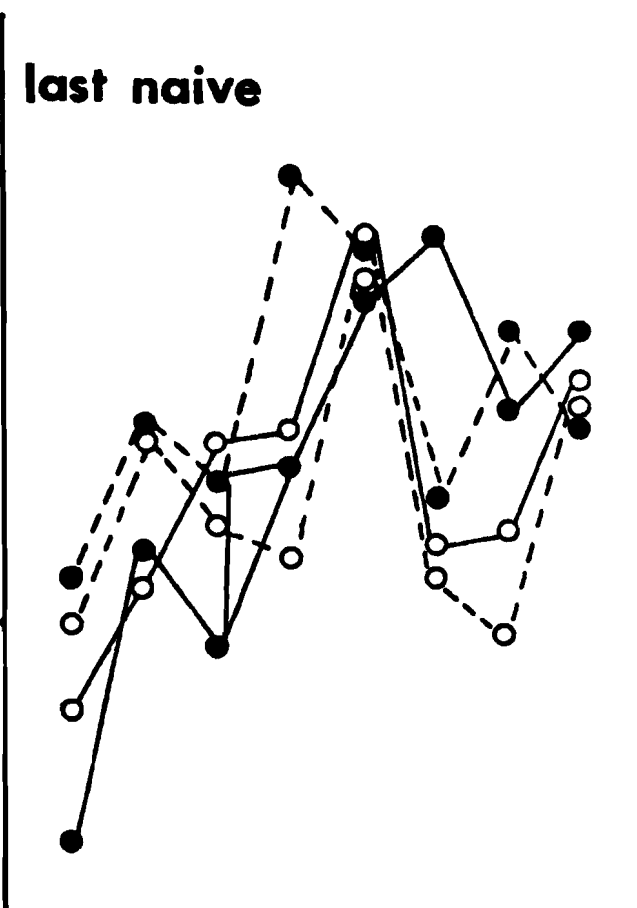
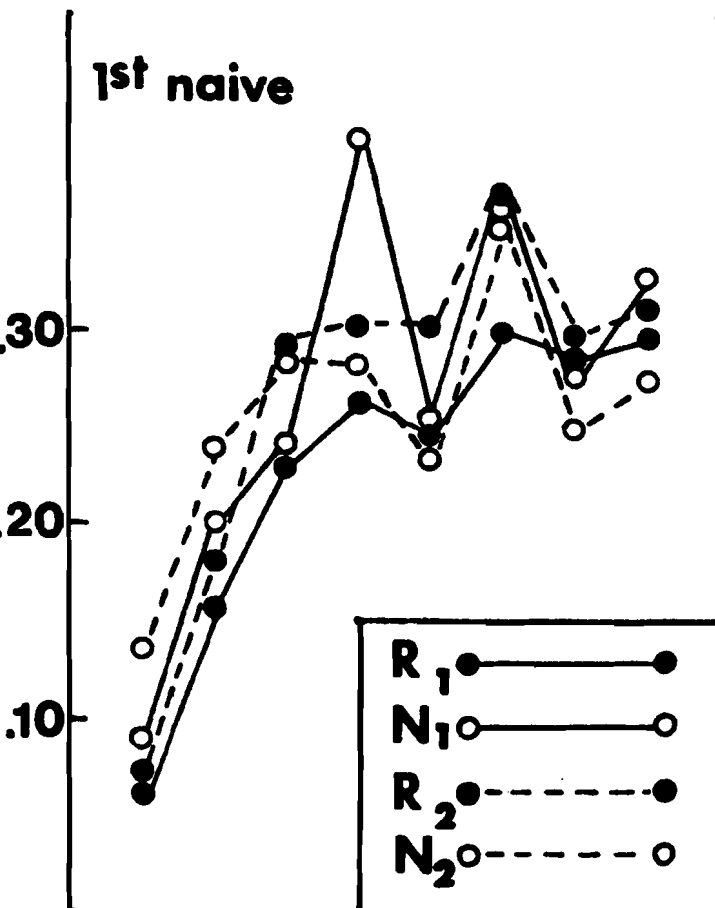


Figure 12. Mean goal speeds for first and last naive pairs (top panel), and low (first 4) and high (last 4) odor buildup subjects (bottom panel) for Phase 3.



DAYS

APPENDIX B

TABLES

TABLE 1
SUMMARY OF PHASE 1 START SECTION ANALYSIS OF VARIANCE

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	434.1	1	434.1	.2858	.61211
Within Groups ₁ (R-N)	2599.9	1	2599.9	3.9304	.09469
Within Groups ₂ (Trials)	1825.8	17	107.4	1.1069	.35776
L-H x R-N	385.9	1	385.9	.5834	.47392
L-H x Trials	1783.2	17	104.9	1.0811	.38216
R-N x Trials	3116.7	17	183.3	3.0946	.00021
L-H x R-N x Trials	1312.4	17	77.2	1.3031	.20571

TABLE 2
SUMMARY OF PHASE 1 RUN SECTION ANALYSIS OF VARIANCE

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	152.0	1	152.0	.0143	.90854
Within Groups ₁ (R-N)	46161.0	1	46161.0	21.4188	.00358
Within Groups ₂ (Trials)	29808.0	17	1753.4	5.6259	.00000
L-H x R-N	12489.0	1	12489.0	5.7949	.05277
L-H x Trials	12215.0	17	718.5	2.3054	.00534
R-N x Trials	19191.0	17	1128.9	6.7176	.00000
L-H x R-N x Trials	8988.0	17	528.7	3.1461	.00017

TABLE 3
SUMMARY OF PHASE 1 GOAL SECTION ANALYSIS OF VARIANCE

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	3213.7	1	3213.7	1.2908	.29923
Within Groups ₁ (R-N)	29367.2	1	29367.2	134.6350	.00002
Within Groups ₂ (Trials)	3767.9	17	221.6	3.0068	.00030
L-H x R-N	4082.4	1	4082.4	18.7160	.00495
L-H x Trials	930.2	17	54.7	.7422	.75269
R-N x Trials	3846.0	17	226.2	6.0200	.00000
L-H x R-N x Trials	1073.9	17	63.2	1.6810	.05835

TABLE 4

SUMMARY OF PHASE 3 START SECTION ANALYSIS OF VARIANCE

Experienced Subjects:

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	5016.8	1	5016.8	5.6858	.05443
Within Groups ₁ (R-N)	19488.5	1	19488.5	82.7537	.00010
Within Groups ₂ (Trials)	771.6	15	51.4	1.6560	.07476
L-H x R-N	713.3	1	713.3	3.0289	.13244
L-H x Trials	570.5	15	38.0	1.2244	.26883
R-N x Trials	876.8	15	58.4	2.1002	.01664
L-H x R-N x Trials	492.0	15	32.8	1.1786	.30288

Naive Subjects:

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	162.1	1	162.1	.0324	.87362
Within Groups ₁ (R-N)	9.9	1	9.9	.1886	.70640
Within Groups ₂ (Trials)	5297.8	15	353.2	4.1061	.00049
L-H x R-N	155.8	1	155.8	2.9755	.22667
L-H x Trials	1237.9	15	84.9	.9873	.49127
R-N x Trials	822.7	15	54.8	1.5185	.16064
L-H x R-N x Trials	597.0	15	39.8	1.1019	.39526

TABLE 5

SUMMARY OF PHASE 3 RUN SECTION ANALYSIS OF VARIANCE

Experienced Subjects:

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	12455.6	1	12455.6	1.3031	.29714
Within Groups ₁ (R-N)	79404.1	1	79404.1	154.1550	.00002
Within Groups ₂ (Trials)	3250.5	15	216.7	1.7626	.05279
L-H x R-N	55.0	1	55.0	.1068	.75494
L-H x Trials	1308.5	15	87.2	.7096	.76873
R-N x Trials	4415.2	15	294.4	3.2124	.00066
L-H x R-N x Trials	1316.8	15	87.8	.9580	.50521

Naive Subjects:

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	13039.5	1	13039.5	3.3491	.20873
Within Groups ₁ (R-N)	706.0	1	706.0	32.6003	.02933
Within Groups ₂ (Trials)	16725.4	15	1115.0	3.3662	.00226
L-H x R-N	3495.4	1	3495.4	161.4050	.00614
L-H x Trials	5810.1	15	387.3	1.1694	.34508
R-N x Trials	7267.1	15	484.5	2.3148	.02455
L-H x R-N x Trials	4922.7	15	328.2	1.5680	.14333

TABLE 6

SUMMARY OF PHASE 3 GOAL SECTION ANALYSIS OF VARIANCE

Experienced Subjects:

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	792.5	1	792.5	1.6614	.24487
Within Groups ₁ (R-N)	54186.4	1	54186.4	458.3170	.00000
Within Groups ₂ (Trials)	870.1	15	58.0	2.2679	.00919
L-H x R-N	735.4	1	735.4	6.2199	.04690
L-H x Trials	211.4	15	14.1	.5511	.90366
R-N x Trials	814.7	15	54.3	2.4474	.00482
L-H x R-N x Trials	473.8	15	31.6	1.4234	.15368

Naive Subjects:

SOURCE	SS	df	MS	F	P
Between Groups (L-H)	448.8	1	448.8	1.2699	.37682
Within Groups ₁ (R-N)	1112.1	1	1112.1	41.3409	.02334
Within Groups ₂ (Trials)	1746.3	15	116.4	1.5322	.15565
L-H x R-N	872.7	1	872.7	32.4406	.02947
L-H x Trials	1335.9	15	89.0	1.1721	.34311
R-N x Trials	3430.6	15	288.7	9.2077	.00000
L-H x R-N x Trials	642.6	15	42.8	1.7248	.09941