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A New Methodology for Determining Aggression Levels and Dominance Hierarchies in Deermice, Peromyscus Maniculatis

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Introduction

When animals of the same species are placed in close proximity to one another, an aggressive interaction is likely to occur. The aggression level that is displayed by *Peromyscus leucopus* varies inversely with the proximity of the individuals (Vestal and Hellack, 1977). Interspecific aggression in mice and other small mammals has been investigated with respect to juvenile populations (King, 1968), population density (Hoffman et al, 1982), and territorial invaders (Sadlier, 1965; Healy, 1967).

Using descriptions of aggressive behavior by Eisenberg (1962), methods for analyzing aggressive behavior in mice were first described by Sadlier (1965). Sadlier's method was later tested by Healy (1967) and his conclusions reconfirmed. Both Sadlier and Healy ran dyadic interactions recording different aggressive behaviors in each 10-second interval of a trial. No distinction was made between aggressive and submissive behaviors. Only aggressive behaviors were used in evaluating and mouse's aggression. Each aggressive behavior was considered equal and no attempt was made at determining relative differences in these behaviors.

Aggressiveness for each mouse was rated by the total number of aggressive acts for each 5-minute (Healy, 1967) or 10-minute (Sadlier, 1965) bout. Both authors used this aggression index to monitor seasonal changes in aggressive behavior. They independently demonstrated a seasonal rise and fall in aggressiveness that coincided with the beginning and end of the breeding season, respectively. Sadlier postulated that aggression may be linked to a stable dominance hierarchy during the breeding season.

A dominance hierarchy is the relationship between individuals in a population where the animals are organized or classified into a rank based on their aggression levels. For example, a population of mice establishes a hierarchy where the most aggressive individual is the highest ranking and may have the largest home territory (Stickel and Warbach, 1960). The least aggressive individual is the lowest ranking and may have a small territory or none at all.

In preliminary observations I observed a hierarchy in *Peromyscus maniculatis* which I had housed in the school's lab. By ranking both aggressive and submissive behaviors, an aggression level for each mouse was determined. The purpose of the observations was to test for an innate aggression level. This has led to my present research. The purpose of the current research was to study aggressive interactions in white-footed deer mice, *Peromyscus maniculatis*, a common inhabitant of old fields in Central Illinois. To more accurately quantify and compare aggression levels, however, a new indexing method was developed by testing mice in dyadic encounters. For each individual tested, I determined a mean level of aggression. On the basis of mean aggression levels, a dominance hierarchy among tested individuals was constructed. In addition, the following null hypotheses were tested: (1) The difference in aggression level of two interacting mice will be insignificant. (2) The initial aggression level of a mouse, for the first trial of a set, will differ significantly from its mean aggression of all first encounters. (3) The mean difference in aggression levels for two mice will not change over time.

Methods

Six male *P. maniculatis* were used. Each was fur-clipped for identification and numbered from 1-6. They were kept in separate 25 x 25 x 35 cm laboratory cages and fed Purina Lab Chow. All mice tested were scrotal indicating sexual maturity.

Animals 2, 3 and 4 were captured two miles northeast of Normal, Illinois, along a railroad right of way. Animal 5 was a male born to a pregnant wild female caught at the same site. These animals were captured in September and October 1985. Animal 1 was captured at the same time of year at the Parklands Foundation in northeastern McLean County, Illinois. Animal 6 was captured at Parklands in April 1986. Tests were run in April 1986.

Tests were run between 1300-1800 hours in glass arenas 75 x 30.5 x 30.5 cm (Fig. 1). Each animal was run between one and three sets per testing day. Sawdust was used to cover the arena floor. Each side contained a water dish and supply of sunflower seeds.The mice were put in competition in a round robin fashion. Each pair of mice met in four dyadic encounters or "trials," constituting a "set" for those two individuals. A total of 60 trials were observed for the 15 possible pairings.

Animals were moved from their nest cages to the arena and allowed to acclimate for a minimum of five minutes (Healy, 1967). After acclimation, the center barrier was removed and the animals were allowed to interact for five minutes. The exact behavior and position of each mouse was recorded every 30 seconds. After the last recording, the center barrier was replaced. If the mice were moved to a new cage or had another trial immediately following the first, they were allowed to reacclimate for five minutes (Lubeck, suggestion through personal communication). There was no difference in behavior between animals tested back to back and those who were not. Tests were run under a 25 watt red light to simulate darkness.

Activities scored during each trial were adapted from Scott (1966), Vestal and Hellack (1977), Eisenberg (1962), Healy (1967), and Sadlier (1965). Behaviors were ranked in one of five categories from most aggressive (100) to most submissive (0). The following terms describe observed behaviors:

Fight	100 —	Each mouse in contact with the other. Often, the ventral surfaces are pressed together while the animals roll across the arena floor. Usually an activity of animals displaying near equal aggression.
Chasing	100 —	One animal attempting to catch the second. Often ends with a fight or aggressive grooming.
Aggressive Grooming	100 —	Fight (100) or chase (100) winner actively manipulating the fur of the other. Forepaws and teeth are used in the manipulation. Only observed as the outcome of a fight (100) or chase (100).
Contact	75 —	A reciprocating behavior between two animals involving naso-naso or naso-anal contact. Often precedes a fight or chase.
Upright	75 —	Standing position with eyes open wide and ears upright. This animal often initiates a fight or chase.
Searching	50 —	A neutral behavior where the animal may engage in feeding, self-grooming, or wandering about the cage.
Elongate	25 —	The animal is in a rigid body stance with tail erect. Eyes are usually open to half closed. Ears tend to be held back. Usually the response to an upright posture.
Submission	25 —	Behavior of an animal who is being groomed (100). Only observed to follow a fight or chase.
Avoidance	0 —	Any attempt of an animal to maintain a distance from the other. This often included hanging from the arena's screen top or sitting in one corner while continually watching the activities of the other mouse.
Flight	0 —	Avoidance of all interaction when approached by another mouse. A response to a chase (100) or searcher (50) who gets too close.

Behaviors were ranked as follows: (100) actively seeking another individual or the aggressive outcome of seeking behavior, (75) behaviors which instigated a fight or chase, (50) neutral behaviors where an animal seemed to ignore any others present, (25) submissive behaviors which followed or preceded a fight or chase, and (0) any attempts to avoid interaction with another mouse.

After each trial, the mean aggression level for the mice was determined by summing their aggression scores at each of the ten 30 second intervals and averaging. The mouse with the higher mean aggression level for that trial was considered the winner. After all testing was complete, the overall mean aggression level was calculated for each mouse. This was done by summing aggression scores for all sixty 30-second periods each mouse interacted and then averaging.

Mice were ranked by three different sets of criteria: First, by their mean initial aggression level; second, by their overall mean aggression level; and third, by their overall win-loss record in 20 trials.

The only exception to the methods presented here was during the first trial between 1 and 2. Animal 1 was removed after running into the side of the arena and falling into his water dish. Animal 1 seemed to be drowning while 2 searched the arena. Animal 1 was removed and given a defeat behavior for the rest of that trial's 30 second periods. Mouse 2 was given a searching behavior for the rest of that trail's time periods. Mouse 1 recovered quickly and, showing no signs of permanent injury, interacted freely for the rest of his trials.

Results

In 42 of the 60 trials, the difference between mean aggression levels of the two mice was significant. In 34 of those 42, the difference in the aggression levels was highly significant (Table 1).

The aggression level of a mouse, for the first trial of each set, was compared to the mean aggression level for all first trials combined. This was used to determine if a mouse would exhibit a greater than average aggression level on its first encounter with a new mouse. When these results were compared, 22 of 30 comparisons were found to be non-significant (Table 2).

Finally, the mice were tested for acclimation towards each other by two different methods. The first test for acclimation was to analyze each trial within a set. In 10 of the 30 sets, the mice showed a stepwise increase or decrease in their aggression level for each successive trial in that set. The probability of 4 events occurring in any specific order in 10 of 30 sets was significantly unlikely ($P < 0.001$, binomial expansion).

The second test for acclimation was the analysis of the mean differences between relative aggression levels of a pair of mice through successive trials of a set. Each of the 15 differences was plotted for each successive encounter within a set (Fig. 2). The slope of those points was calculated to be -4.58 aggression units per encounter. Had there been no relationship between encounter number and mean aggression level difference, the slope would be zero. A comparison of the slopes (Kachigan, 1986: appendix 1) show that they were not significantly different ($t = 1.574$, $P < 0.07$, $df = 58$). Though this value was not significant at the 0.05, it did fall very close to that value.

When the six mice were ranked by order of their mean initial aggression level, overall aggression level, and individual win-loss record, the following results were observed (Table 3): In the first and third categories animal 2 placed first, animal 4 second, animal 3 third, animal 1 fourth, animal 5 fifth, and animal 6 sixth. In the second category, animal 6 was fifth and animal 5 was sixth. When the aggression level between 5 and 6 was tested for significance, none was found ($t = 0.105$, $P > 0.05$, $df = 18$). Animal 5 was considered the more aggressive based on a 2-1-1 record in head to head interaction with 6.

Discussion

Through observation of previously described *Peromyscus* behaviors, I was able to rank order these behaviors with regard to their aggression level. Though it may be impossible to rank these behaviors completely and discretely, they can be lumped together in groups of similar aggression level. Using this ranking system I was able to determine a mean overall aggression level for each mouse. This is an improvement over the method of determining aggression levels used by Sadlier (1965) and Healy (1967). Their method used a rank developed from the total number of aggressive acts where mine was developed by assigning discrete values to those aggressive acts. I believe that my method is more accurate because not all aggressive behaviors are of equal intensity and submissive behaviors are just as important for ranking as are aggressive behaviors. It is entirely possible that one mouse will be more aggressive than another yet not demonstrate a truly aggressive behavior.

The first null hypothesis tested was that the difference in aggression levels of two interacting mice would be insignificant. I found that in 42 of 60 trials there were significant differences ($P < 0.05$), and in 34 of those 42 trials the difference was highly significant ($P < 0.01$) (Table 1). This was enough to reject the null hypothesis and accept the alternate: The difference in aggression levels of two mice did differ significantly. Such a large number of highly significant aggression level differences makes the integrity of the ranking system seem sound.

In earlier observations I found a consistent aggression level for any given mouse, regardless of the other mouse's aggression level. To re-demonstrate this, I compared the aggression level of a mouse, for the first trial of each set, to the mean aggression level for all first trials combined. In 22 out of 30 possible comparisons, I found that there was a non-significant difference between these values (Table 2). This enabled the rejection of the second null hypothesis. By rejecting this, it can be said that a mouse will demonstrate a natural aggression level, close to its mean level, upon initial contact with a strange mouse. The advantage to this kind of natural aggression level would be that an animal could quickly determine its ability to compete against another individual. It should be noted that 5 of the 8 significant were for mouse 6, the one most recently caught. This erratic behavior may have been caused by his failure to adjust to the laboratory.

Analysis of agonistic interactions between individuals should provide insight into a population's social behavior. If animals differ in their natural aggression, a dominance hierarchy should be determinable for the entire population. Scott (1966) showed that in *Mus musculus*, the house mouse, there was only one dominant individual while all others were equal subordinates. My data show differences in the "subordinate" individuals. The hierarchy, once established, can have an effect on such natural circumstances as territory size (Stickel and Warbach, 1960). The establishment of a hierarchy can also effect breeding and survivorship of the animals within it.

When the hierarchial rank was determined (Table 3), three separate aspects were looked at. (1) rank based on mean initial aggression levels, (2) rank based on mean overall aggression levels, and (3) win-loss records based on each individual encounter. In the first and third aspects, the order of the mice was identical. In the second, however, animals 5 and 6 switched places for the fifth and sixth spot. Five was ranked higher than 6 by virtue of their head to head interaction. The consistency of this ranking makes a hierarchy seem natural for this group of mice.

Bronson (1963) concluded that, in a population of woodchucks, once a hierarchy has been formed and organized by its members, there should be a decline in the interaction rate between the individuals. To test for a similarity in *P. maniculatis* the final null hypothesis tested: The mean difference in aggression levels, for two mice in a set, will not change over time. This recognition could save both time and energy for individuals who interact with some frequency. Other authors have also reported observing acclimation of animals toward one another with regard to a social hierarchy (Eisenberg, 1962; Healy, 1967; King, 1968). Acclimation between animals in a population has biological significance. The natural advantages to such an acclimation are many. Energy can be conserved through decreased aggression levels (Eisenberg, 1962). There could also be advantages as to decreased time spent marking territorial boundaries and neighbors would simply waste less time upon subsequent encounters with each other in the wild. Energy saved could then be available for seeking mates and foraging.

Though only one test for acclimation gave significant results, the other was still close enough to merit consideration. The binomial expansion showed a 0.001 probability that the sets would randomly distribute in any kind of order. The probability of them lining up in ascending or descending order would be far less. The t test value for significance of the slope only differed by 0.102. When comparing the slopes it is possible that the high variance in tests 2 and 4 may have led to the non-significance of the results. Such low probabilities in both cases do not allow us to reject the null hypothesis but strongly suggests that there is an acclimation of the mice towards each other over time.

These data appear to justify the following conclusions. (1) Ranking behaviors seems to be a reliable method for measuring aggression levels. (2) There tends to be a significant difference in aggression levels of interacting *P. maniculatis*. (3) The initial aggression level of a mouse, in the first trial of a set, remains consistent with that animal's mean aggression level for all initial trials. (4) A hierarchy within a *Peromyscus* population can be determined. (5) There appears to be a negative correlation between the subsequent trials in a set and the difference of aggression levels between the two interacting mice.

The next step in this study would be to take these experiments and extend them to natural populations. If results in the wild are consistent with those in the lab, then a natural hierarchy could be established. Another aspect to be looked at would be the results of aggressive interactions in other than dyadic encounters. King (1957) has shown a difference in interspecific aggression levels when tested in dyadic versus group encounters.

Acknowledgements

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Animal		1	2	3	4	5	6
1	I		75.0	40.0	55.0	7.5	5.0
	II		70.0	47.5	57.5	5.0	7.5
	III		60.0	42.5	42.5	0.0	10.0
	IV		50.0	47.5	50.0	15.0	5.0
2	I	12.5-HS		2.5	17.5	25.0	5.0
	II	22.5-HS		10.0	22.5	0.0	15.0
	III	30.0-S		25.0	22.5	10.0	35.0
	IV	40.0-I		50.0	30.0	30.0	10.0
3	I	20.0-S	62.5-HS		55.0	20.0	45.0
	II	17.5-S	75.0-HS		17.5	30.0	35.0
	III	42.5-I	55.0-HS		75.0	30.0	50.0
	IV	37.5-I	67.5-I		100.0	42.5	15.0
4	I	25.0-HS	70.0-HS	40.0-I		12.5	10.0
	II	42.5-S	65.0-HS	40.0-S		5.0	5.0
	III	47.5-I	42.5-I	35.0-HS		15.0	10.0
	IV	57.5-I	62.5-HS	25.0-HS		25.0	40.0
5	I	45.0-HS	65.0-HS	55.0-HS	50.0-HS		40.0
	II	45.0-HS	82.5-HS	60.0-S	50.0-HS		45.0
	III	35.0-HS	70.0-HS	60.0-S	55.0-HS		45.0
	IV	40.0-S	62.5-HS	57.5-I	50.0-HS		50.0
6	I	40.0-HS	65.0-HS	55.0-I	50.0-HS	50.0-I	
	II	50.0-HS	50.0-HS	50.0-I	50.0-HS	50.0-I	
	III	50.0-HS	50.0-I	50.0-I	55.0-HS	40.0-I	
	IV	50.0-HS	50.0-HS	50.0-HS	50.0-I	50.0-I	
Average		37.5	62.5	42.2	47.8	23.1	24.1

Table 1. Data table containing individual aggression levels for all mice during all encounters. Mouse number is read across the top while set number (Arabic) and trial number (Roman) are read down the left side. Symbols represent the relative difference in aggression levels and whether they are
HS (highly significant), S (significant), or I (insignificant).
(df= 18: HS= P< 0.01, S = P< 0.05, I = P> 0.05, t< 2.101)

Mouse 1

Set No.	1	2	3	4	5
Aggression Level	12.5	20.0	25.0	45.0	40.0
Mean Aggression Level	32.5	32.5	32.5	32.5	32.5
t Value	2.75	1.23	0.73	1.51	0.72
Sig	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Mouse 2

Set No.	1	2	3	4	5
Aggression Level	75.0	62.5	70.0	65.0	65.0
Mean Aggression Level	67.5	67.5	67.5	67.5	67.5
t Value	0.87	0.63	0.32	0.31	0.31
Sig	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Mouse 3

Set No.	1	2	3	4	5
Aggression Level	40.0	2.5	40.0	55.0	55.0
Mean Aggression Level	34.5	34.5	34.5	34.5	34.5
t Value	0.60	3.03	0.52	2.04	2.04
Sig	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Table 2. Chart comparing initial aggression levels, of the first trial in a set, to mean aggression levels for all first trials. (more)

Mouse 4

Set No.	1	2	3	4	5
Aggression Level	55.0	17.5	55.0	50.0	50.0
Mean Aggression Level	45.5	45.5	45.5	45.5	45.5
t Value	1.10	2.55	0.83	0.64	0.64
Sig	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Mouse 5

Set No.	1	2	3	4	5
Aggression Level	7.5	25.0	20.0	12.5	50.0
Mean Aggression Level	23.0	23.0	23.0	23.0	23.0
t Value	1.69	0.15	0.23	0.96	3.65
Sig	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Mouse 6

Set No.	1	2	3	4	5
Aggression Level	5.0	5.0	45.0	10.0	40.0
Mean Aggression Level	21.0	21.0	21.0	21.0	21.0
t Value	2.23	2.23	2.23	1.36	2.73
Sig	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

Table 2. Chart comparing initial aggression levels, of the first trial in a set, to mean aggression levels for all first trials.

Mouse	1	2	3	4	5	6
Initial Mean Aggression	28.5	67.5	38.5	45.5	23.0	21.0
Rank	4	1	3	2	5	6
Overall Mean Aggression	37.5	62.5	42.4	47.8	23.1	24.1
Rank	4	1	3	2	6	5
Win Loss Record	10-9-1	20-0	12-7-1	13-7	2-17-1	1-17-1
Rank	4	1	3	2	5	6
Final Rank	4	1	3	2	5	6

Table 3. Rank order chart with 1 being the highest rank and 6 being the lowest.

Appendix

To test for the significance of a line's slope, it must be compared to a line of known slope, zero. The following variables are needed for this comparison:

b = slope of the best fit line through actual data.
B = the hypothetical slope, zero.
$s_{y \cdot x}$ = standard error of estimate for y values.
s_b = standard error of the sample slope.

The raw data of the difference in the aggression levels was first plotted using a linear regression analysis. Both actual and calculated y values were used to calculate the standard error of estimate:

$$S_{y \cdot x} = \sqrt{\frac{\Sigma(y_i - y_i')^2}{n - 2}} \qquad (1)$$

The standard error estimate is then used to calculate the standard error of the sample slope:

$$s_b = \frac{s_{y.x}}{\sqrt{\Sigma(x_i - \bar{x})^2}} \qquad (2)$$

The standard error of the sample slope is used to calculate the t value comparing the actual slope to the hypothetical slope:

$$t = \frac{b - B}{s_b}$$

This value is than compared with a one tailed t test with degrees of freedom df = n − 2. If the calculated t value is greater than the table value, there is a significant difference between the actual slope and hypothetical slope.

Fig. 1. The encounter test arena was similar to the one drawn below.

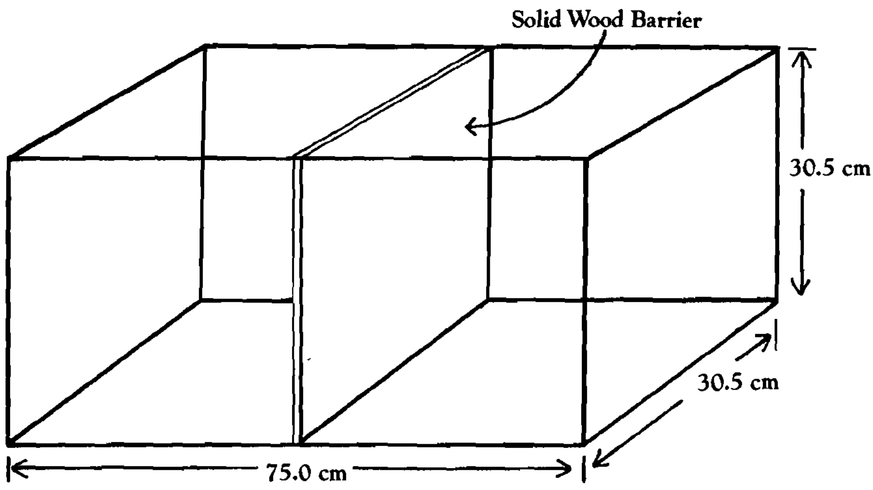
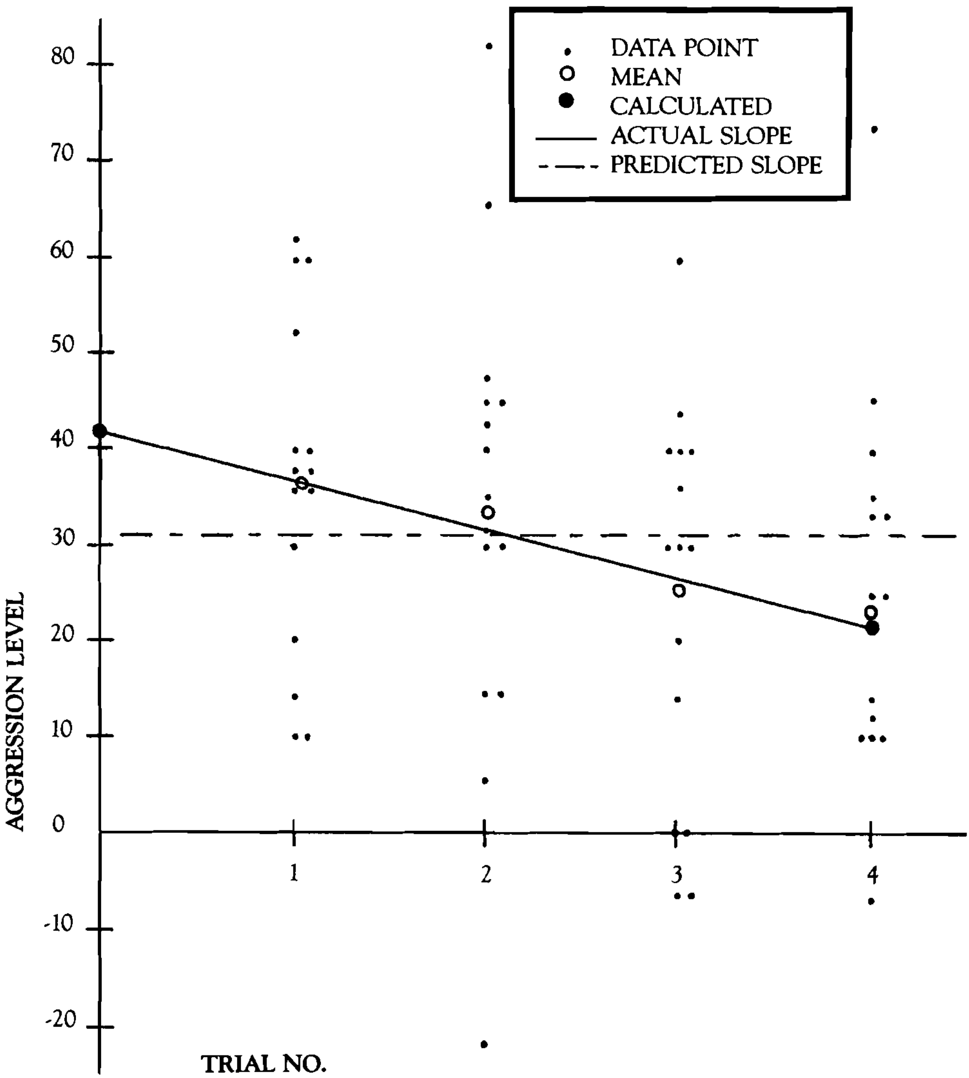


Fig. 2

Aggression Level vs. Trial No.

SCATTER PLOT



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