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

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which endings are temporary, we fire.

lay, and the speaker sits before a flames, he identifies himself most

"The ashes of his (the fire's) the fire will 'expire.' The word

right, sleep and death begun in once "nourished" the fire of

consumes" the passion, and quench embers.

some immediate audience, but in in the statement, "This thou more than the sense of sight.

line by using the word "behold"

ording to the Oxford English mean "to regard (with the

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ings. Death is inescapable: the capacity for love are still the decay of his body, there

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beside him. The sonnet also a real person—the reader. By

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his, our own. We all must

沉迷inds us of beginnings, would be to savor our memories

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

Ray Gensinger Jr.

Gensinger '86: A New Methodology for Determining Aggression Levels and Dominance

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Jos. Jane earned her Bachelor of 1986 and is currently living in technical work for Searle
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 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 maniculatus 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 maniculatus, a common inhabitant of old fields in Central Illinois. To more accurately quantify and compare aggression levels, six male P. maniculatus were used and numbered from 1-6. They were housed in laboratory cages and fed Purina chow indicating sexual maturity.

Animals 2, 3 and 4 were captured in September and October 1985 in Central Illinois, along a railroad right of way. Animal 5 was a pregnant wild female caught at the Pooles Fork Natural Area in April 1986. Animal 6 was captured in September and October 1985 in Central Illinois. A total of 60 trials were observed in the laboratory for five minutes (Lubeck, 1984). Tests were run between 1300-1530 hours on testing days.

Activities scored during each 5-minute trial included running or jumping, biting, scratching, fighting, hissing, and standing alert. A total of 60 trials were observed in the laboratory for five minutes. The exact behavior of the mouse was recorded every 30 seconds. After five minutes, the center barrier was removed and the mice were moved to the next trial. If the mice were not immediately following the first, five minutes, the center barrier was placed between the two mice. There was no difference in behavior of those who were and those who were not. Tests were run in simulated darkness.

Activities scored during each trial include running or jumping, biting, scratching, fighting, hissing, and standing alert.
A New Methodology for Determining Aggression Levels and Dominance

Six male *P. maniculatus* 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),
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 behaviors which followed or preceded attempts to avoid interaction with the other mouse.

After each trial, the mean aggression level was determined by summing their second intervals and averaging the aggression level for that trial with results for the entire testing period. After testing was complete, the overall mean aggression level was determined for each mouse. This was done for sixty 30-second periods each mouse.

Mice were ranked by three different criteria: one by mean initial aggression level; second, by mean overall aggression level; and third, by their overall aggressiveness.

The only exception to the normal testing procedures was in the first trial between Mouse 1 and Mouse 2. An injury caused Mouse 1 to be unable to roll across the arena, and Mouse 2 to be drowning while searching. Mouse 1 was given a defeat behavior for the first trial, Mouse 2 was given a searching behavior for the first trial. Mouse 1 recovered quickly from the injury, interacted freely for the remaining trials.

In 42 of the 60 trials, the difference in mean aggression levels was significant.

The aggression level of a mouse was compared to the mean aggression level on its first encounter with the other mouse. The probability of this difference being significant was determined by comparing the results to a normal distribution. In 10 out of 42 cases, the difference in aggression level was significant (Table 2).

Finally, the mice were tested for differences in their relative aggression levels. The second test for acclimation was given, this time with the mice being tested in pairs with increasing or decreasing in their aggression level during each successive trials of a set. Each
Healy (1967), and Sadlier of five categories from most (1962). The following terms describe contact with the other. Often, the are pressed together while the across the arena floor. Usually an animals displaying near equal aggression. attempting to catch the second. Often fight or aggressive grooming. chase (100) winner actively the fur of the other. Forepaws and in the manipulation. Only observed behavior between two animals -naso or naso-anal contact. Often or chase.往往 eyes open wide and ears animal often initiates a fight or chase. behavior where the animal may engage grooming, or wandering about the a rigid body stance with tail usually open to half closed. Ears mall back. Usually the response to an animal who is being groomed served to follow a fight or chase. of an animal to maintain a distance This often included hanging from top or sitting in one corner watching the activities of the interaction when approached by . A response to a chase (100) or who gets too close. actively seeking another of seeking behavior, (75) chase, (50) neutral behaviors

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...

In earlier observations I found a given mouse, regardless of the outcome of the encounter, would demonstrate this, I compared the first trial of each set, to the mean of all trials combined. In 22 out of 30 possible comparisons, a non-significant difference Between groups enabled the rejection of the second null hypothesis. It can be said that a mouse will adapt its behavior close to its mean level, upon introducing new individuals. Scott (1960) established such natural circumstances as to the establishment of a hierarchy. The hierarchy can be considered the survivorship of the animals within the group of mice.

When the hierarchical rank was tested, two aspects were looked at: (1) rank based on mean levels, (2) rank based on mean win-loss records based on each individual. In the case of the mice with rank 5 and 6 switched places, the order of the mice with rank 5 and 6 switched places in the hierarchy were equal in the entire population. Scott (1960) established such natural circumstances as to the establishment of a hierarchy. The hierarchy can be considered the survivorship of the animals within the group of mice.

Bronson (1963) concluded that once a hierarchy has been formed, it should be a decline in the interaction test for a similarity in *P. maniculatus*. The mean difference in aggression levels will change over time. This recognition of individuals who interact with such hierarchy is important for the survival of the animals.
The slope of those points per encounter. Had there been no number and mean aggression 
A comparison of the slopes though they were not significantly different. The value was not close to that value.

Hypothesis 1 was determined the order of their mean initial aggression level and individual win-loss records (Table 3). In the first and second, animal 4 second, animal 3 third, animal 5 sixth. In the second and fifth, animal 6 sixth. When the values were tested for significance, none was different. Animal 5 was considered the winner in head to head interaction (Table 3).

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 can be determined 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 affect breeding and survivorship of the animals within it.

When the hierarchical 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. maniculatus 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, 1969).
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. maniculatus. (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

This study would never have been completed had it not been for the time and patience of Dr. Louis Verner. Dr. Larry Stout was essential for the statistical analysis of the acclimation portion of this study. I am grateful to all others who have shown interest and support for this research. This study was supported by the Illinois Wesleyan University Biology Department and the local Tri-Beta chapter.
When a population has biological acclimation such as to decreased time and neighbors would simply waste with each other in the wild.

An increased aggression levels

advantages as to decreased time

and neighbors would simply waste

with each other in the wild.

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Dr. Larry Stout was essential for

portion of this study. I am

interest and support for this

the Illinois Wesleyan University

chapter.

Literature Cited


### 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)
- I (insignificant)

(df = 18; HS = $P < 0.01$, S = $P < 0.05$, I = $P > 0.05$, $t < 2.101$)

<table>
<thead>
<tr>
<th>Animal</th>
<th>1</th>
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<th>3</th>
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**Average**

| 37.5 | 62.5 | 42.2 | 47.8 | 23.1 | 24.1 |

### Table 2.

Chart comparing initial aggression levels for all first trials.
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<th>Set No.</th>
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<tr>
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<tr>
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Mouse 1

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<th>Mean Aggression Level</th>
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<tr>
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Mouse 2

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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Mouse 4</th>
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<td>4</td>
<td>5</td>
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<td>45.5</td>
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<td>2.55</td>
<td>0.83</td>
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</tr>
<tr>
<td>Sig</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
<td>P&gt;0.05</td>
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<table>
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<tr>
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<td>4</td>
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<td>P&gt;0.05</td>
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<td>P&gt;0.05</td>
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<table>
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</thead>
<tbody>
<tr>
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<td>3</td>
<td>4</td>
<td>5</td>
</tr>
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<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

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

---

<table>
<thead>
<tr>
<th>Mouse</th>
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</thead>
<tbody>
<tr>
<td>Initial Mean Aggression</td>
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<td>Rank</td>
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</tr>
<tr>
<td>Overall Mean Aggression</td>
<td>37.5</td>
</tr>
<tr>
<td>Rank</td>
<td>4</td>
</tr>
<tr>
<td>Win Loss Record</td>
<td>10-9-1</td>
</tr>
<tr>
<td>Rank</td>
<td>4</td>
</tr>
<tr>
<td>Final Rank</td>
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</tr>
</tbody>
</table>

Table 3. Rank order chart with 1 being the lowest.
<table>
<thead>
<tr>
<th>Mouse</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td><strong>Initial Mean Aggression</strong></td>
<td>28.5</td>
<td>67.5</td>
<td>38.5</td>
<td>45.5</td>
<td>23.0</td>
<td>21.0</td>
</tr>
<tr>
<td><strong>Rank</strong></td>
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<td>1</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>6</td>
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<tr>
<td><strong>Overall Mean Aggression</strong></td>
<td>37.5</td>
<td>62.5</td>
<td>42.4</td>
<td>47.8</td>
<td>23.1</td>
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</tr>
<tr>
<td><strong>Rank</strong></td>
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<td>1</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td><strong>Win Loss Record</strong></td>
<td>10-9-1</td>
<td>20-0</td>
<td>12-7-1</td>
<td>13-7</td>
<td>2-17-1</td>
<td>1-17-1</td>
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<tr>
<td><strong>Rank</strong></td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>6</td>
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<tr>
<td><strong>Final Rank</strong></td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

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

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

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:

\[
s_{y'x} = \sqrt{\frac{\sum(y_i - \bar{y})^2}{n-2}} \quad (1)
\]

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

\[
s_b = \frac{s_{y'x}}{\sqrt{\sum(x_i - \bar{x})^2}} \quad (2)
\]

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 then 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.

---

Ray Gensinger - grew up in Libertyville High School. Ray rec...
must be compared to a line of known data.

A graph was first plotted using a linear regression; the y-values were used to calculate the standard error of the sample

(1)

and to calculate the t value comparing the test with degrees of freedom $df = n - 2$.

If the t-value, there is a significant theoretical slope.

The one at right.

Solid Wood Barrier

Ray Gensinger - grew up in Libertyville, Illinois, and attended Libertyville High School. Ray received his Bachelor of Arts in Biology in May 1986 and is enrolled in Southern Illinois University Medical School, where he is currently working toward his M.D.