Flashing Neon as a Stress Factor Promoting Aggression in the Rat

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Additive stress factors can lower the threshold for aggressive outbursts in a range of animals including humans. Flashing lights are known to affect aspects of behaviour and under certain conditions may lead to physiological and neurological disturbances (e.g. epileptic seizures). Experiments were undertaken to investigate whether 24 hour exposure to a flashing neon light would affect the behaviour of rats held in marginally stressful conditions. It was found that over the test period of 20 days the dominant rat in a pair significantly increased the number and severity of offensive interactions with its same-sex cage companion. Further, the reactions of the less-dominant rat to these encounters also changed away from full submission towards more active defensive behaviour. Inevitably this produced more frequent and more severe aggressive encounters and in 2 out of 10 pairs the test was terminated early to prevent serious injury to one or both members of the pair.

Keywords: *Aggression; offense; defense; submission; male behaviour; rat social interaction*

Aspects of the behaviour of both wild and caged rats have been studied extensively. In normal situations rats show 'aggressive' behaviour during competition for mates, space and resources and, in females, protection of the nest and young. Two classes of intraspecific aggressive behaviour have been described, *offense* and *defense*, originally in cats by Leyhausen (1956) and extended to rodents, including *Rattus norvegicus*, (Grant and Mackintosh, 1963; Adams, 1971, 1976; Lehman and Adams, 1977; Miczek, 1974; Blanchard et al., 1975). Intuitively, offense is a higher level of aggression than defense because it is produced in isolation whereas defense normally has to be provoked. In situations involving intraspecific physical conflict adaptive patterns of agonistic behaviour have evolved to limit the severity of the conflict (Scott, 1966). In rats, as in many other animals, *submission* is such an agonistic behaviour.

The Present Research

It is known that various modalities of continuous sensory stimulation increase the general level of activity in many animals, including man (Pentrover et al., 1996). The present research was undertaken to see if the threshold for the release of aggressive behaviour could be lowered by continuous visual stimulation by a flashing neon light.

METHOD

Background information

Experimental animals. It is known that inbred strains of rats show markedly different levels of aggressive and related behaviours (Broadbent, 1970) and for this reason the rats used in these trials were all from the same breeding colony and shared the same great grandparents. Two sets of twenty randomly selected young male rats (25 days old) were placed in cages, two per cage, and treated and maintained in an identical manner for the duration of the trials.

Housing and maintenance. A variation on the standard practice for cage rearing of rats was used (Logan et al., 1969). Water was supplied ad lib., food pellets (Rat Tucker®, Page Pet Supplies Ltd., 10gm per rat) were given at 08:00 and 15:00, cages were cleaned and bedding replenished on alternate days. Temperature 27°C approx. Pairs of male rats were housed in cages less voluminous than the recommended standard cage to impose a mild level of stress. The cages were of steel mesh (1.2 cm grid size) with a floor area and height slightly less than the minimum recommended dimensions (32 cm wide x 45 cm deep x 37.5 cm high). A plastic tray formed the internal floor of each cage. The cages were arranged in two-tiers of five cages individually screened from each other such that their occupants could not see into neighboring cages. The view from the fronts of the cages consisted of an off-white wall mounted with two, two meter long, neon light tubes (10 % Neon, lightning blue). Background lighting to mark daytime was provided by a, wall-mounted, daylight fluorescent light that was on from 06:00 to 18:00 (day) and a dim red light (night) for the rest of the 24 hours. Scent, other than that produced by the caged pair, was removed by an extractor fan that drew in air continuously through the cages, from front to back, and evacuated it to the outside remote from the experimental area.

Behaviour monitored

In this study three categories of behavior were identified for monitoring; *offense*, *defense*, and *submission*. These behaviors are formed from discrete, identifiable, elements and are expressed as part of a linked behavioral sequence. The sequence is initiated by an offensive act, normally by the dominant individual. This releases a defensive response in the other individual that may lead to a submissive response that normally serves to terminate the sequence. The intensity of the sequence, as manifested by the level of activity and duration of the individual actions that form it, is variable and depends primarily on the arousal state of one or both of the individuals involved in the exchange. The three behaviors monitored in this study are described in detail below:

Offense. Offense consists of an approach usually followed by the adoption of an offensive sideways posture followed by an offensive upright posture and a bite-and-kick attack. Approach has two stages, turn towards and locomotion towards and bite-and-kick involves three stages, a jump onto the opponent, lying across its back and biting its opposite flank. These responses have been recorded in both isolation-induced and competitive fighting in rats (Zook and Adams, 1975). The offensive sideways and upright postures have been described in detail by Grant and Mackintosh (1963). In the sideways posture the rats stand broadside on to each other whereas in the upright posture

they stand on their hind legs, facing each other and may push each other with their forepaws as if sparring. The offensive sideways posture is often accompanied by piloerection (Barnett, 1958a) and is believed to enhance the effectiveness of the threat. In the bite-and-kick (Banks, 1962) attack the attacker mounts the opponent across its back at right angles and bites its opposite flank whilst simultaneously kicking it with its hind legs. Teeth chattering may accompany offensive movements (Barnett, 1958a).

Defense. In defense the opponent may initially freeze, before turning and fleeing but if this proves difficult or impossible, sideways and upright postures similar to those evoked in the offensive behaviour may then be adopted. A lunge-and-bite may terminate the defense sequence. This may result in bites to the face of the attacker. Ewer gives detailed description of the flight sequence in *R. rattus* (Ewer, 1971). In that species flight may be preceded by an 'escape leap'. Defensive sideways posture mimics the sideways posture in offense but the defensive upright posture tends to be more vertical or backwardly-angled and the defender tends to make more locomotory movements (Lehman and Adams, 1977). The lunge-and-bite defense is initiated from either a crouching, quadrepedal, position or from the vertical defensive posture. It consists of the lunge (a rapid thrusting forward of the head and/ or the body with the fore-paws extended in front of the face) and the bite to any near and exposed part of the opponent. Withdrawal to the starting position or flight is aided by a pushing movement of the forepaws. Characteristically the lunge-and-bite defense results in injury to the opponent's face (Blanchard and Blanchard, 1977). Defense is often accompanied by vocalizations in the form of squeals or shrill squeaking (Ewer, 1971) and hissing (Hughes et al., 1976; Barnett, 1969). Non-vocal sounds may also be generated by thumping of the hind feet during defense (Eibl-Eibesfeldt, 1951).

Submission. When threatened by conspecifics, laboratory-reared rats normally adopt submissive postures and behaviors rather than attempting an aggressive defense. The full submissive posture is really an extension of defensive freezing in that the attacked rat rolls onto its back with its feet in the air and remains motionless (Grant and Mackintosh, 1963). This response may be accompanied by ultrasonic vocalizations known as 'piping' (Sales and Pye, 1975). Full submissive posture is not normally released until the back of the submissive animal is pressed on by the attacker. Rolling over results in maintained pressure on the submissive animal's back and can lead to the phenomenon of 'cataleptic' immobility (Grant and Mackintosh, 1963) in which the submissive animal remains still on its back for some time.

Assessment method

The rat's behavior was monitored intensively for 30 minute periods at three-hourly intervals over each of two days at the start (days 1 and 2), middle (days 10 and 11) and end (days 19 and 20) of the 20 day test period. During the 30 minute observation periods the presence and nature of any activity and social interaction between the two individuals in each cage was noted in detail. Activity was defined as locomotion, feeding and drinking, reorganization of bedding, social interaction such as grooming or aggression, etc.. Inactivity included resting and sleeping. The proportion of the 30 minute observation period spent in either active or inactive behaviour was recorded. Offensive,

defensive and submissive behaviors were broken down into elements (see Table 1) and assigned individual scores to facilitate subsequent recording and analysis. The data were recorded for each test pair as the number of occasions, in each of the 30 minute periods, each of the elements of offensive, defensive and submissive behaviour were observed for each pair of rats. Comparison of the summed scores for each behaviour (offensive, defensive and submissive) for each pair during each observation period gave an indication of the amount and nature of the interactions at each stage and how it changed over the period of the trial. The scores for all ten pairs (eight pairs in the last two day period in the neon trial) per 30 minute observation period were added together and averaged to give a single aggregate score for each of the eight observation periods.

Behavioural element	**Score**	**Repeats**	**Total score**	**Total duration (minutes) [Bout number; duration]**
Offense				
Turn towards opponent	1	3	3	1 ; 1.5
Move towards	1	3	3	2 ; 2.5
Sideways posture	2	3	5	3 ; 2.0
Upright posture	3	3	5	
Jump onto opponent	3	2	5	
Lying across back	3	2	5	
Bite flank of opponent	4	2	6	
Kick to escape	3	2	6	
Total	20	3	38	3 ; 6.0
Defense				
Freeze	1	2	2	1, 1.5
Turning to sideways	2	2	4	2, 2.0
Upright posture	3	2	5	
Lunge at attacker	4	1	5	
Bite attacker	4	0	4	
Flee	1	1	2	
Total	15	2	22	2, 3.5
Submission				
Freeze	2	1	2	3, 2.5
Roll onto back	4	1	4	
Total	6	1	6	1, 2.5

Table 1 Example of data recorded for one pair of rats during a session (30 minute observation period) at mid trial; neon group.

RESULTS

Total activity

Throughout the trial period the rats in both the control and experimental trials were consistently more active during the night (Figure 1).

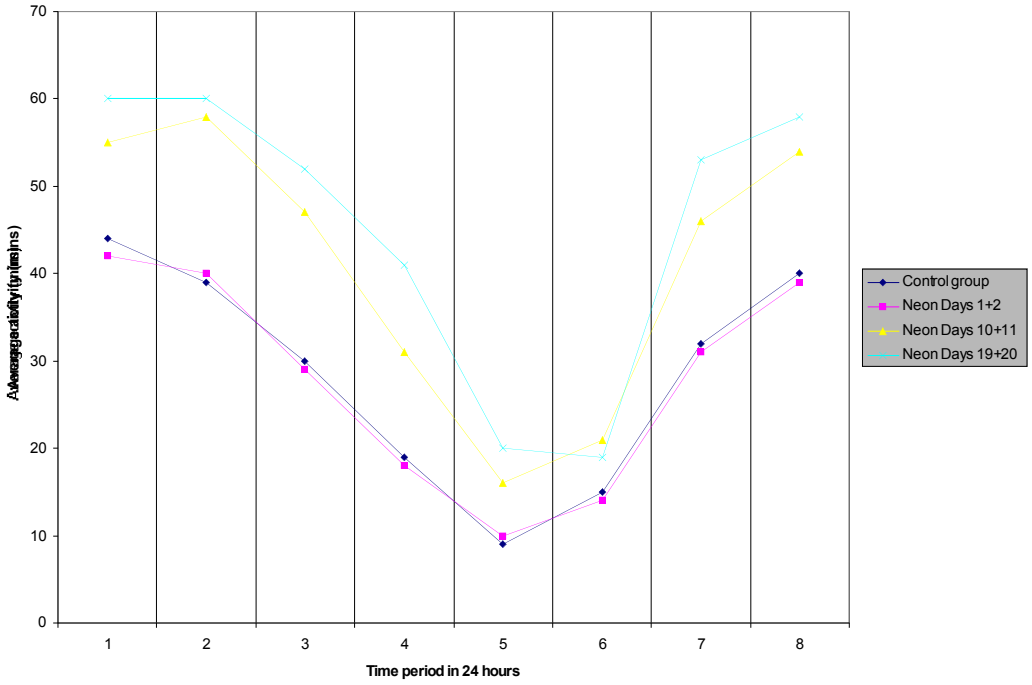

Figure 1 Patterning of average total activity recorded at eight 30 minute periods on each of 2 test days during a 24 hour day.
Day time = periods 3 – 6, night time = periods 1 – 2 + 7 – 8.

The pattern and total amount of activity in the control group on each of the three observation periods remained the same (p=0.001; Student's T test). In the group subjected to the continuously flashing neon light their overall level of daily activity increased over the period. In both groups the circadian pattern of offensive behaviour by the dominant individual in each pair, and the associated defensive and submissive behavior in the opponent, mirrored that of general activity, being more likely in the night than during the day. In the control group the level of aggressive behavior remained constant across the 20 day trial period but increased in the experimental group (see subsequent subsections, below, for details).

Offensive behavior

Control group. Offensive behavior in the control group was limited to occasional disputes about food, or access to water or bedding material. Such encounters were generally of low intensity, a defensive reaction was rarely elicited and submission was rapid and short-lived. This was the case at each of the three observation periods over the course of the trial.

Neon group.
Days 1+2. At the start of the trial the frequency, level and nature of offensive behaviour was similar (p = 0.001; Student's T-test) to that observed in the control group at each stage throughout the trial. Attacks were of short duration, confined to one or two attacks and terminated at an early stage. Attacks never proceeded further than the offensive upright posture.

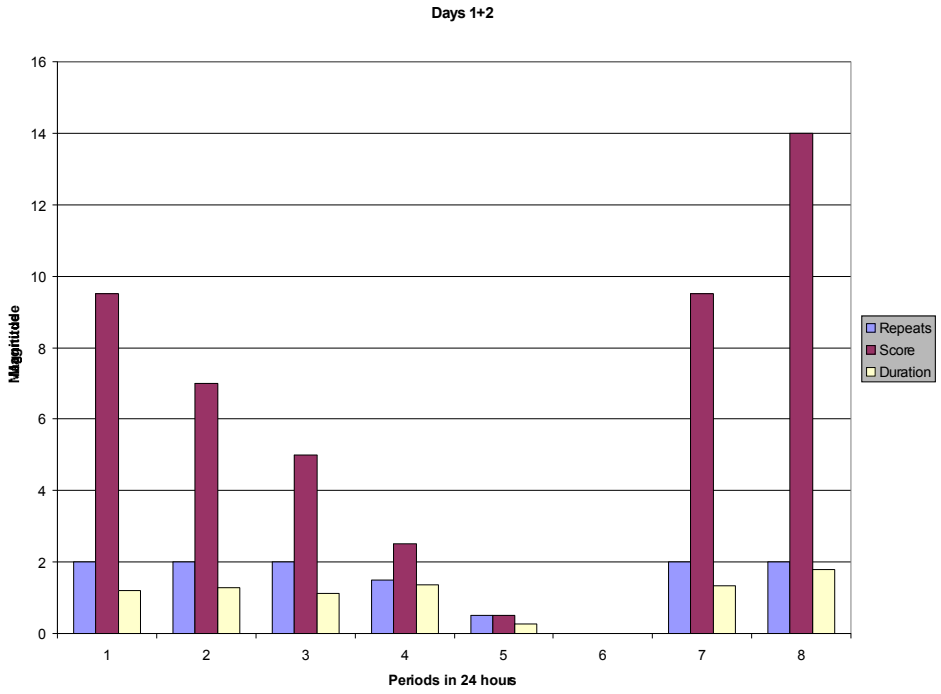

Figure 2 Analysis of offensive behaviour. Average for the two 30 minute observation periods over the first two days.

Days 10+11. By mid trial the number of attacks observed in each 30 minute period had increased to a maximum of six and the pattern of attack had extended to result in approximately 8% of the attacks reaching the bite-and-kick stage. This was correlated with an increase in the tendency for the opponent to offer defensive behavior prior to submission. There was also evidence of an increase in the general level of aggression in that individual bouts of offensive behavior including sequences involving repeated attacks. Three individuals, two opponent and one dominant rat, showed signs of injury due to bites on the flank and face, respectively.

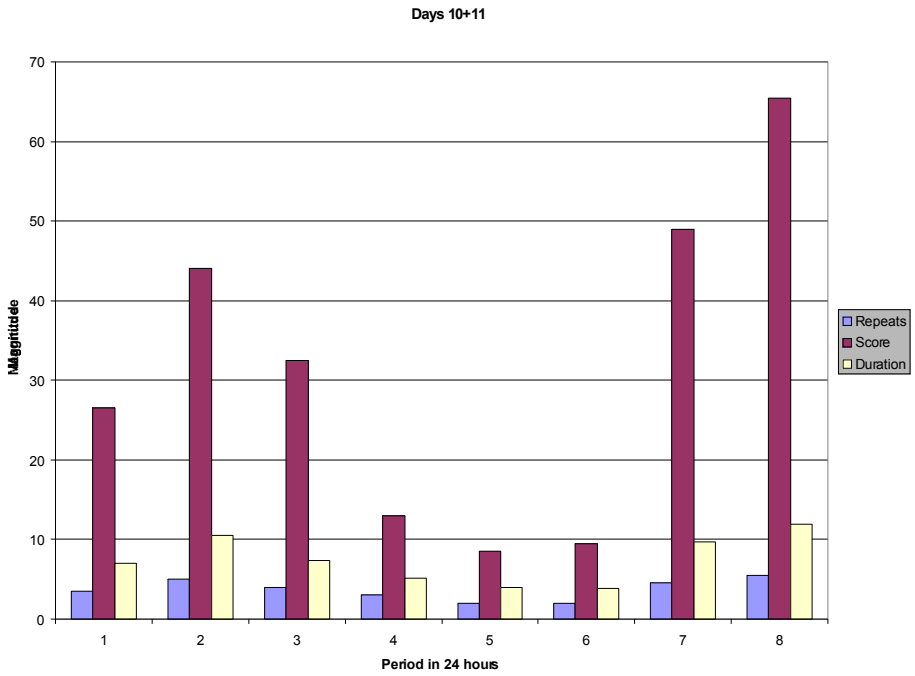

Figure 3 Analysis of offensive behaviour. Average for the two 30 minute observation periods over the middle two days.

Days 19+20. Between the mid and end of trial observations it was clear that two pairs of rats in the neon trial were dangerously aggressive towards each other so they were removed from the trial. By the end of trial observation periods the eight pairs remaining in the trial were more aggressive than at mid trial with eight times more offensive incidents and 38.6% of all such incidents reaching the bite-and-kick stage. It was also recorded that offensive rats were often vocal during their attacks.

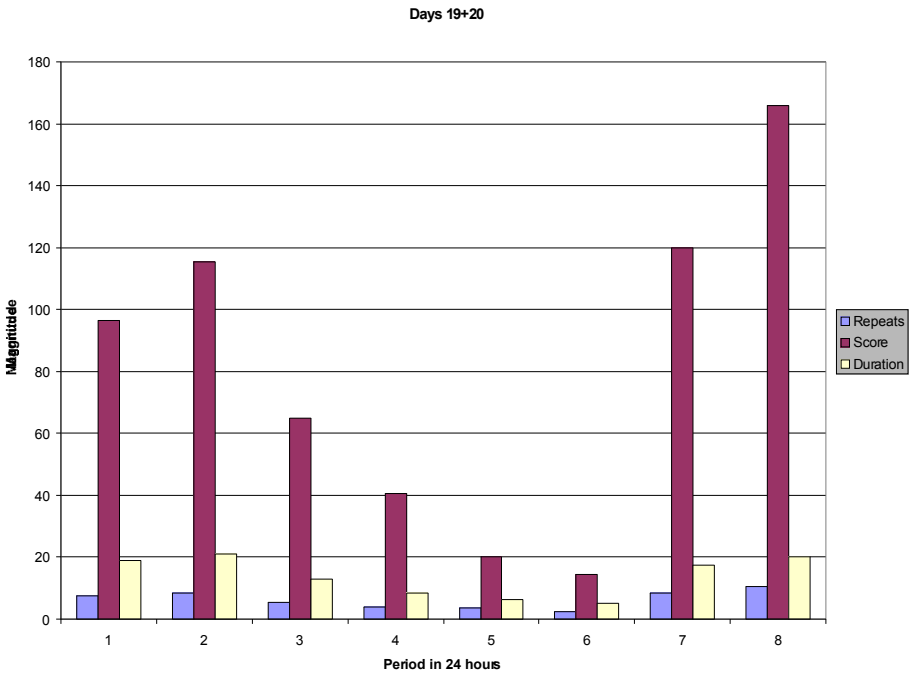

Figure 4 Analysis of offensive behaviour. Average for the two 30 minute observation periods over the last two days.

Defensive behavior

Control group. Defensive responses released by attack and followed similar pattern and intensity to offensive behaviour in the control group as described above.

Neon group.

Days 1+2. Occurrence of defensive behaviour was similar to that of the control group (p=001; Student's T-test). Most bouts consisted of a single response to an offensive move by the attacker and they usually ended in freezing or at most a sideways defensive posture. One attack was met by a lunge but no bite and roll-over submissions were not observed during the first two days observation period.

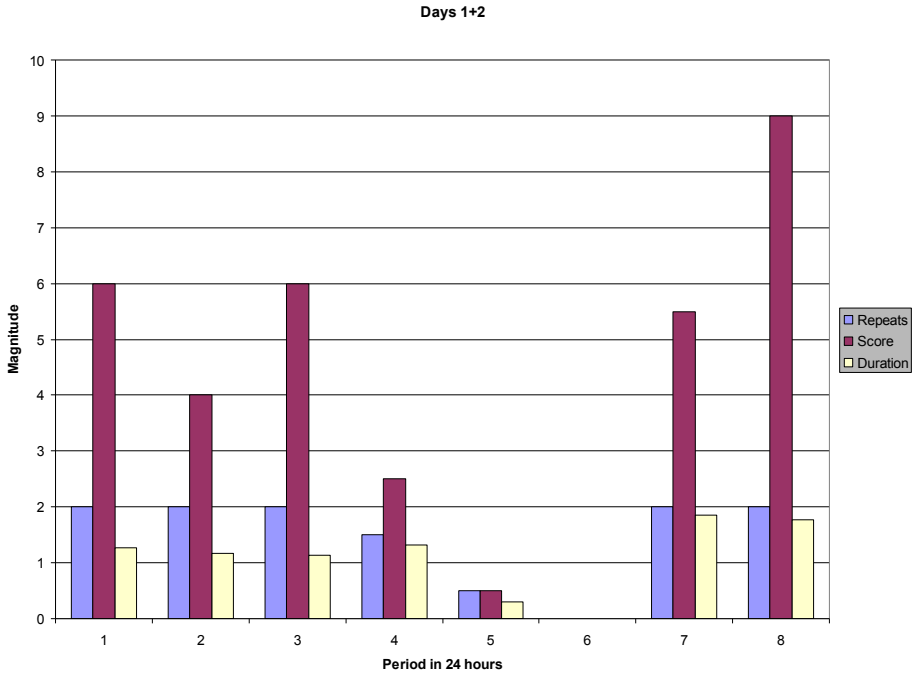

Figure 5 Analysis of defensive behaviour. Average for the two 30 minute observation periods over the first two days.

Days 10+11. The number of attacks met with aggressive defensive action increased by mid trial with 12% reaching the lunge and bite stage.

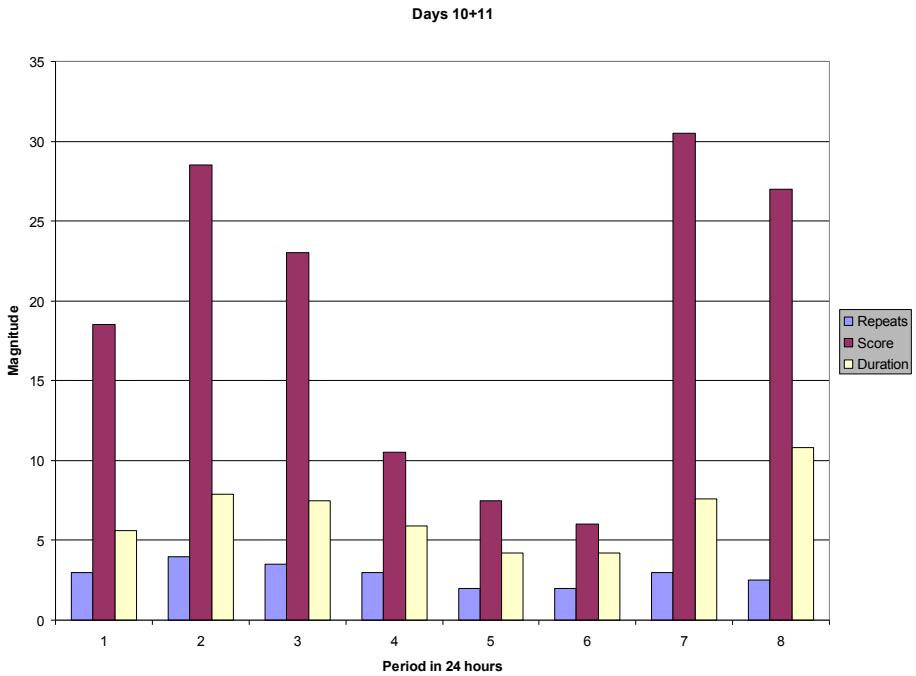

Figure 6 Analysis of defensive behaviour. Average for the two 30 minute observation periods over the middle two days.

Days 19+20. In the last two days of the trial 77% of defensive bouts reached to lunge and bite stage. In 50% of incidents offense was met with defensive action from the opponent, often at an intense level, with 77% of defensive bouts reaching the lunge-and-bite stage.

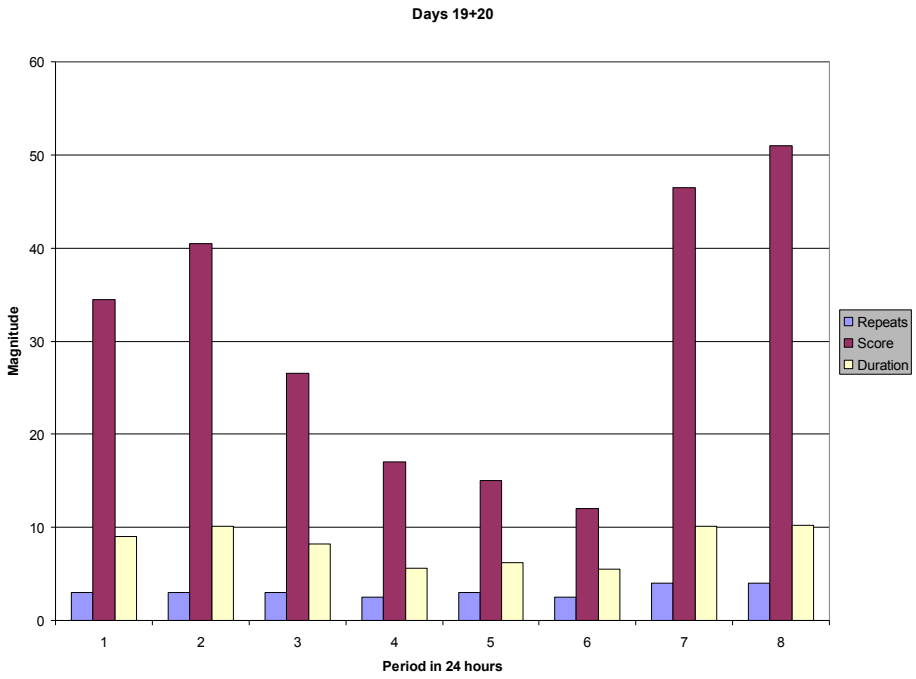

Figure 7 Analysis of defensive behaviour. Average for the two 30 minute observation periods over the last two days.

Submissive behavior

Control group.

Most aggressive encounters were mild and short-lived. Submission by freezing normally served to halt an attack.

Neon group.

Days 1+2. No full (roll-over) submissions were observed during the first two days of the trial.

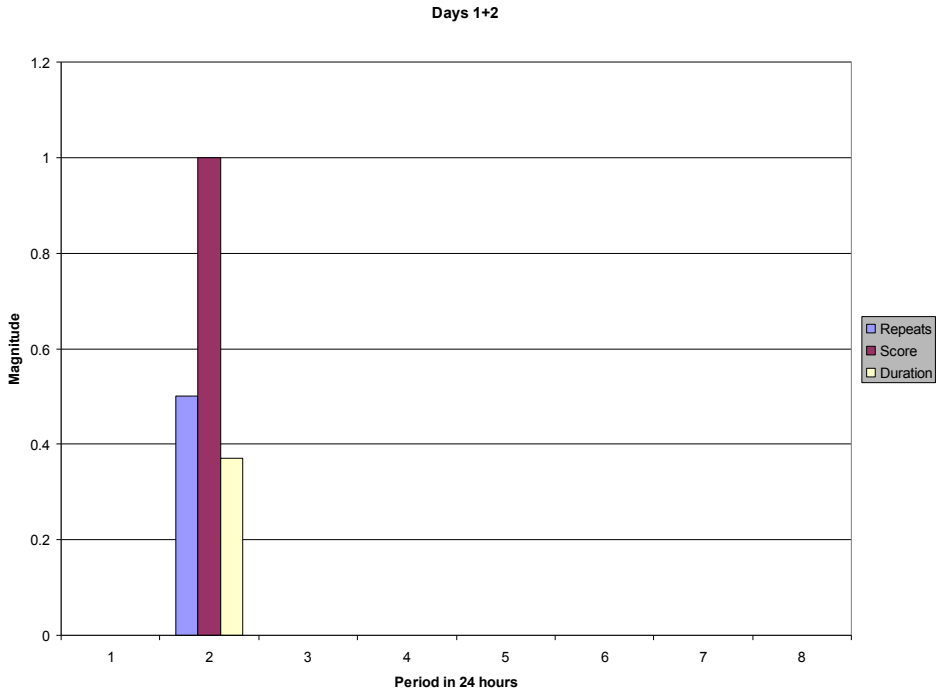

Figure 8 Analysis of submissive behaviour. Average for the two 30 minute observation periods over the first two days.

Days 10+11. Full submission was observed on 22% of occasions during the observation periods in the two day mid trial period.

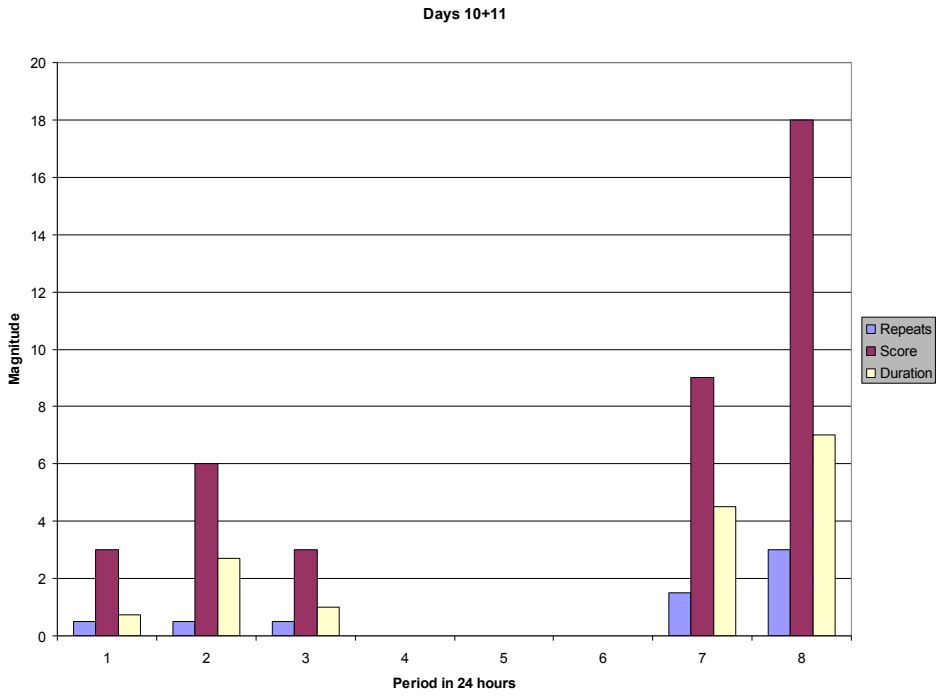

Figure 9 Analysis of submissive behaviour. Average for the two 30 minute observation periods over the middle two days.

Days 19+20.
The number of bouts leading to full submission was similar to that during the mid trial period, despite the marked increase in the number and severity of attacks, indicating that attack tended to be met by aggressive defense rather than by submission.

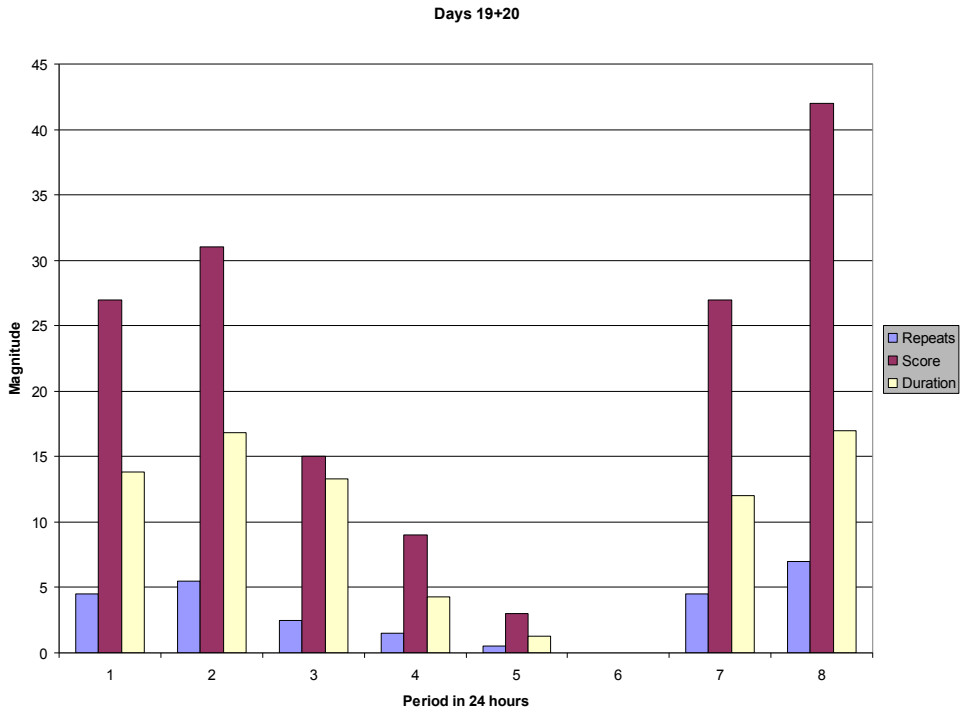

Figure 10 Analysis of submissive behaviour. Average for the two 30 minute observation periods over the last two days.

DISCUSSION

The behaviour of laboratory rats has become modified over time such that they are less territorial than wild rats. Scent marking is less pronounced and aggression between young males is focused on resource, food and bedding, acquisition and the establishment of a social hierarchy.

Defensive and submissive behaviour never occur in isolation and are always released by offensive behaviour by attack or threat of attack by a conspecific or, in the wild, a predator. In these experiments, therefore, the frequency and intensity of offensive behaviour set the pattern of aggression in the pairs of rats.

It is clear that the presence of a continually flashing neon light increased the level of general activity and aggression in both the dominant and opponent rats. Sheard (1973)

found that offensive behavior could be increased if activity-increasing drugs were administered to the opponent rat. A similar link appears to have operated in the experiments reported here.

A similar increase in 'irritability' has been induced in laboratory rats by damage to the olfactory system (Cain,1974). This indicates the importance of smell in the sensory repertoire of the rat. Pheromones and other volatile chemicals play an important role in modulating the social behaviour of rodents and for this reason every effort was taken to minimize the movement of air between cages.

By the end of the trial some of the neon group included vocalizations in their offense behaviour, this is a new finding and is not normal for the laboratory rat although it has been reported in *R. villosissimus* (Begg, 1975) and may be taken as an indication of increased agitation leading to increased aggression. It was not possible in the experiment reported here to monitor ultrasonic vocalizations but it is known that opponents can emit such sounds when attacked and they are believed to inhibit offensive behaviour in the attacker (Sales, 1972).

Normally rats restrict the expression of aggression to same-sexed individuals, mature male rats rarely attack females of the same species and are less aggressive towards juvenile males and castrated older males (Barfield et al., 1972; Thor & Flannelly, 1976). An exception to this generalization is that females will attack mature rats of either sex when they are rearing their young. There is, however, every reason to suppose that the boundaries for either sex would shift towards increased aggression if mixed-sex pairs had been used in the current trials.

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