and the highly specific inactivation of the two enzymes in a process that must compete with both reagent hydrolysis and with nonspecific reactions at other reactive amino acid residues (*10*). This proposed reaction sequence, together with the very schematic and oversimplified representations of the binding pockets of chymotrypsin and elastase given in Fig. 2, provides a reasonable model for the inactivation of the two enzymes by alkyl isocyanates. According to this model, OIC is a specific reagent for chymotrypsin because this enzyme-reagent complex I gives perfect alignment of the reactive groups and therefore a high probability of covalent bond formation. In the BIC-chymotrypsin complex I, on the other hand, the alignment and therefore covalent bond formation is a less probable event. The binding pocket of elastase is very similar to that of chymotrypsin, but because of the presence of threonine and valine in positions occupied by glycine in chymotrypsin (*3*), the elastase binding pocket is shallower, and the model proposes that in this case only BIC can bind to give proper alignment for covalent bond formation. The longer OIC probably also binds, but because of the poor alignment, no reaction can occur between the isocyanate and X in the elastase-OIC complex I. At this stage the model in Fig. 2 is rather speculative. The reactive residue X has been placed in the position of the active site serine (residue 195 in chymotrypsin and residue 188 in elastase), and in analogy with the many other reactions by which this reactive serine residue can be derivatized (*11*), it is certainly the most likely site for the isocyanate reaction as well. In direct support of this proposition, we have isolated three, short, overlapping, radioactively labeled peptides after oxidation and proteolytic digestion of ^{14}C-labeled BIC-chymotrypsin by a procedure similar to that used by Shaw *et al.* (*11*). These peptides, comprising residues 193 to 199, 192 to 195, and 187 to 195, respectively, have residues 193 to 195 (glycine-aspartate-serine) as their common sequence, strongly suggesting that serine 195 indeed is the reactive residue in chymotrypsin. It can furthermore be concluded from the yield of radioactivity in each step of the peptide purification that this serine 195 derivative represents essentially all the incorporated isocyanate. This eliminates another possible reaction site, namely, histidine (*12*) at position 57. The final conclusions on this point must, however, await the results of similar studies on elastase. The ideal test for the model would be direct observation by x-ray diffraction analysis of the actual position of the alkyl chain in the two inactive enzyme derivatives.

WILLIAM E. BROWN
FINN WOLD

Department of Biochemistry, Medical School, University of Minnesota, Minneapolis 55455

References and Notes

1. B. S. Hartley, *Nature* **201**, 1284 (1964); D. M. Shotton and B. S. Hartley, *ibid.* **225**, 802 (1970).
2. P. B. Sigler, D. M. Blow, B. W. Matthews, R. Henderson, *J. Mol. Biol.* **35**, 143 (1968); H. C. Watson, D. M. Shotton, J. M. Cox, H. Muirhead, *Nature* **225**, 806 (1970).
3. D. M. Shotton and H. C. Watson, *Nature* **225**, 811 (1970).
4. R. A. Oosterbaan and J. A. Cohen, in *Structure and Activity of Enzymes*, T. W. Goodwin, J. I. Harris, B. S. Hartley, Eds. (Academic Press, New York, 1964), p. 87.
5. T. A. Steitz, R. Henderson, D. M. Blow, *J. Mol. Biol.* **46**, 337 (1969).
6. G. R. Schonbaum, B. Zerner, M. L. Bender, *J. Biol. Chem.* **236**, 2930 (1961).
7. B. C. W. Hummel, *Can. J. Biochem. Physiol.* **37**, 1393 (1959).
8. A. Gertler and T. Hofman, *Can. J. Biochem.* **48**, 384 (1970).
9. We have found that the treatment of commercial trypsin samples with 1 to 5 molar equivalents (relative to trypsin) of OIC is a fast, simple, and inexpensive way in which traces of chymotryptic activity can be eliminated.
10. Alkyl isocyanates react readily with both amines, carboxylic acids, imidazoles, thiols, and alcohols and thus have many potential reaction sites in a protein.
11. A. J. J. Ooms, *Nature* **190**, 533 (1961); G. Schoellmann and E. Shaw, *Biochem. Biophys. Res. Commun.* **7**, 36 (1962); B. R. Erlanger and W. Cohen, *J. Amer. Chem. Soc.* **85**, 348 (1963); D. C. Shaw, W. H. Stein, S. Moore, *J. Biol. Chem.* **239**, PC671 (1964).
12. H. A. Staab and W. Benz, *Liebigs Ann. Chem.* **648**, 72 (1961).
13. Supported in part by a PHS research grant (GM 15053) and training grant (T1-GM-157).

6 July 1971; revised 16 August 1971

Signaled Reinforcing Brain Stimulation Facilitates Operant Behavior under Schedules of Intermittent Reinforcement

Abstract. *When single, rewarding brain stimulations were made predictable by preceding them with a brief warning signal, operant behavior was established and maintained under fixed ratio 200, variable ratio 30, fixed interval 3-minute, variable interval 2-minute, and differential reinforcement of low rate 20-second schedules of intermittent reinforcement. When the warning signal was removed, overall response rate declined in all but the fixed ratio schedules and then returned to the previous rate when the signal was reinstated.*

Brain stimulation reinforcement (BSR) has generally been less effective than food in establishing operant behavior under schedules of intermittent reinforcement. Sidman *et al.* (*1*) found that cats stopped responding when the ratio of responses to BSR's was greater than 8 : 1 [fixed ratio (FR) 8]. Similarly, behavior was not maintained when the value of a variable interval (VI) schedule exceeded 16 seconds, that is, when a response-contingent BSR was available only on the average of once per 16 seconds. The poor performance relative to that routinely maintained by conventional rewards such as food or water suggested to these authors that single BSR's were comparable to small amounts of food reward. Recently, Keesey and Goldstein (*2*) found that rats stopped responding when the schedule requirement of a progressive FR exceeded 30 : 1.

With the use of special procedures, performances like those usually obtained with food reinforcement have been obtained with schedules of intermittent BSR. Brown and Trowill (*3*), using rats, found that VI 1-minute and fixed interval (FI) 1-minute behavior could be maintained if the rat received not one but five response-contingent BSR's when a reinforcement was due. Pliskoff *et al.* (*4*) trained rats to perform the required schedule behavior (for example, VI or FR) on one lever to produce a second lever on which up to 20 BSR's were available on a continuous reinforcement schedule. In explanation of the typical performances that were obtained, the authors suggested (i) that the presentation of the second lever and the availability of continuous reinforcement simulated the early responses in the behavior chain leading to food, and (ii) that approximately ten BSR's were more similar to the amount of reinforcement in a standard food pellet than was one BSR.

The present data demonstrate that if a single BSR is made predictable by preceding it with a brief, exteroceptive warning signal, the establishment and maintenance of operant behavior under schedules of intermittent reinforcement is greatly facilitated.

Five male Sprague-Dawley rats, weighing approximately 300 g, were

maintained with food and water freely available throughout the experiment. They were implanted unilaterally with bipolar, stainless steel electrodes 2.0 mm lateral to the intersection of the bregma and the midline suture and 8.0 mm ventral to the leveled skull surface. Histology revealed that the tips of the electrodes were in the median forebrain bundle for all animals.

The apparatus consisted of a ventilated, sound-attenuated lever box that contained a dim light that was on continuously. A 5-watt signal light was mounted 12 cm above the end of the lever. Brain stimulation, delivered to the animal through a mercury commutator to prevent cord twisting, consisted of 0.5-second trains of 60 hertz sine wave current at 20 to 30 μa root-mean-square. Intensity was monitored by an a-c microammeter in series with the animal. Conventional operant conditioning apparatus was employed to program the various experimental contingencies and record responses.

The initial training sessions were the same for all animals. Analogous to "magazine training" with food reinforcement, 20 response-independent signal-BSR pairings were presented irregularly in a 10-minute period. The signal light above the bar was turned on for 1 second, and brain stimulation occurred in the last 0.5 second of this stimulus. Each animal was readily trained to press the lever to obtain the same signal-BSR pairing on a continuous reinforcement schedule. "Priming," the rapid presentation of response-independent BSR's commonly used to train rats to self-stimulate, was unnecessary. All rats pressed the lever approximately 1000 times in a half-hour test. The next day, animals were allowed to perform until they received 2000 BSR's on a continuous reinforcement schedule. Subsequently, individual rats were allowed to perform under different reinforcement schedules in daily 4-hour sessions.

In the course of seven sessions, the response requirement for rat 294 was increased to FR 100, that is, every 100th depression of the lever produced one signal-BSR pairing. Subsequently, a Multiple schedule, consisting of FR 100 and differential reinforcement of low rate 20-second schedules, was in effect. In the presence of a clicker having a frequency of 17 per second, the first response after a 20-second pause was reinforced by a signal-BSR pairing. When the clicker was off, the FR 100 schedule was in effect. The components of the schedule alternated every five reinforcements. The reinforcement contingencies came to control the rate of responding. A sample cumulative record of the 13th session of the Multiple schedule is presented in Fig. 1. A simple FR 100 schedule was then reinstated and, in the course of five sessions, raised to FR 200. At a BSR intensity of 30 μa the animal typically performed about 40 ratios in each of the five sessions of the FR 200. A sample cumulative response record of this performance is presented in Fig. 1.

The response requirement for rat 294 was subsequently lowered to FR 100 and the behavior was allowed to stabilize. Midway through a session, the signal-BSR pairing was altered so that the 0.5-second BSR and the 1-second signal light were initiated simultaneously. Fixed ratio performance was maintained as when the signal preceded the onset of BSR. By increasing the response requirement in small steps of five responses, behavior was maintained through a session of FR 130. The same results occurred when the signal light was removed entirely and the BSR was presented alone.

Rat 297 was trained on a VI 15-second schedule for a half session, a VI 1-minute schedule for two sessions, and then on a VI 2-minute schedule of signaled BSR. As indicated in record A of Fig. 1, local response rate showed considerable variability after 28 sessions under the VI 2-minute schedule. When the signal and BSR were initiated simultaneously or the signal was re-

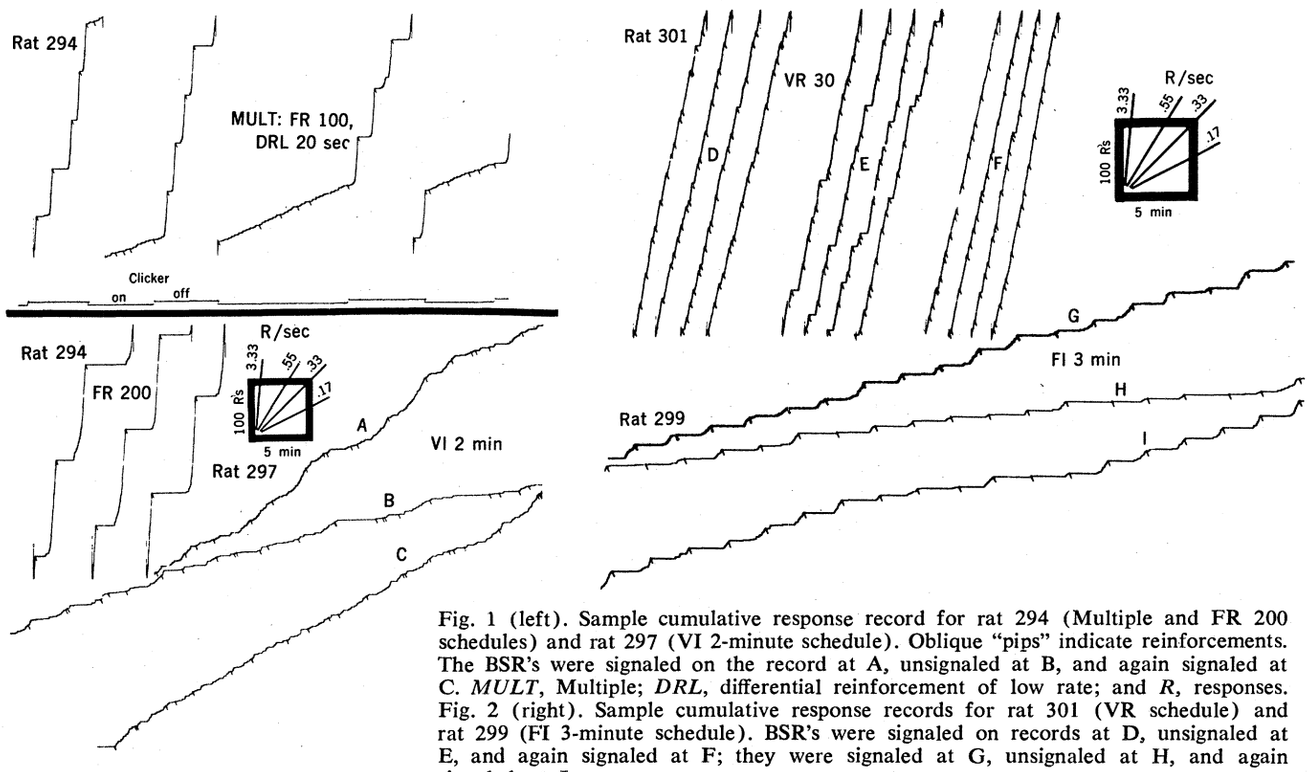

Fig. 1 (left). Sample cumulative response record for rat 294 (Multiple and FR 200 schedules) and rat 297 (VI 2-minute schedule). Oblique "pips" indicate reinforcements. The BSR's were signaled on the record at A, unsignaled at B, and again signaled at C. *MULT*, Multiple; *DRL*, differential reinforcement of low rate; and *R*, responses. Fig. 2 (right). Sample cumulative response records for rat 301 (VR schedule) and rat 299 (FI 3-minute schedule). BSR's were signaled on records at D, unsignaled at E, and again signaled at F; they were signaled at G, unsignaled at H, and again signaled at I.

moved entirely, response rate declined (Fig. 1, record B). When the signaled BSR was reinstated, the rate returned to the previously established level (Fig. 1, record C).

Rat 301 was trained successively on FR 5, FR 15, and FR 20 schedules and was then given two sessions on variable ratio (VR) 15 followed by seven sessions on VR 30. On the average, 30 responses were required to produce one response-contingent signal-BSR pairing. Fig. 2, record D, shows sample cumulative records of the VR behavior characterized by high response rates (1.5 per second) with no pauses after reinforcement. When the signal no longer predicted BSR, or no signal light was presented at all, local response rate remained about the same but occasional pauses after reinforcement were observed (Fig. 2, record E). Reinstatement of the signal produced a return to the previous baseline as shown in Fig. 2, record F.

Rat 299 was trained on an FI 15-second schedule for a half session, on FI 1-minute for two sessions, and terminally on an FI 3-minute schedule of signaled BSR; that is, a response-contingent signal-BSR pairing was made available every 3 minutes. A sample cumulative record of the behavior in the 25th session is presented in Fig. 2, record G. The typical FI postreinforcement pause followed by an accelerated response rate was demonstrated. Simultaneous initiation of the signal and BSR or the elimination of the signal entirely produced a decrease in response rate and numerous intervals that had only one response per reinforcement (Fig. 2, record H). Reinstatement of the signal-BSR pairing as a reinforcer produced a return to the previous baseline as shown in Fig. 2, record I.

In order to determine whether the decline in response rate upon the removal of the signal on the FI schedule and not the FR schedule was due to a difference between schedules or simply a difference between animals, a fifth rat, No. 318, was trained on a Multiple schedule (FR 100 and FI 2-minute schedule) where a clicker was correlated with the presence of the FI but not the FR contingency. When the BSR was signaled, performance in the FR component was the same as that of rat 294, and in the FI component, similar to that of rat 299. When the signal was removed, the response rate in the FI component declined as it did with rat 299; however, response rate stayed the same in the FR component as it did with rat 294. Occasionally, rat 318 would stop responding during the FR component but otherwise maintained his behavior as when the BSR was signaled. When the signal was reinstated, the response rate in the FI component returned to the previous rate.

These results can be understood in terms of the notion that predictable BSR is more reinforcing than unpredictable BSR (*5*). Steiner *et al.* (*6*) demonstrated that a temporal pattern of BSR which rats produced in a self-stimulation test was aversive for those same animals when presented independently of behavior, that is, when its onset was made uncertain. Cantor and Lo-Lordo (*7*) offered response-independent BSR on a VI 1-minute schedule on both sides of a shuttle box. On one side, the BSR was preceded by a warning signal and on the other it was not. Rats reliably preferred the signal-BSR side whether the interval between signal onset and BSR onset (the interstimulus interval) was 0.5 or 15.5 seconds. Using a similar procedure, Cantor (*5*) found that rats preferred brief, presumably easy-to-time interstimulus intervals in the range 0.5 to 3 seconds over longer interstimulus intervals and concluded that the reinforcing value of BSR is inversely related to the temporal uncertainty about its onset. This suggested that an animal's attention to BSR onset influences its effectiveness.

Accordingly, uncertainty about BSR onset seems to be at least one factor responsible for the typically poor performance seen when BSR is used to establish operant behavior under schedules of intermittent reinforcement. Once responding became asymptotic with signaled BSR, unsignaled BSR was differentially predictable under different schedules of reinforcement. Under FR 100, BSR was highly predictable since response rate and, thus, time to complete a ratio, was quite stable at about 30 seconds. Brain stimulation reinforcement was not predictable, however, when the ratio requirement was abruptly changed and, thus, the behavior broke down. Under the FI 3-minute schedule, the decline in response rate when the signal was removed could be explained by a decrement in reinforcement magnitude due to the long interval over which BSR had to be predicted. Stebbins *et al.* (*8*) found this same effect on an FI baseline when the magnitude of a sucrose reinforcer was reduced. A similar argument could explain the decline in response rate on the VI schedule when the signal was removed. Alternatively, the decreased response rate on both VI and FI schedules could be explained by the inherent increased probability, that is, predictability, of a response-produced reinforcement after a pause in responding. The maintained local rate and increased number of pauses after reinforcement observed when the signal was removed on the VR 30 schedule was probably due to a reduced magnitude of reinforcement. In summary, the effect upon schedule performance of removing the signal can be understood in terms of the decreased magnitude of reinforcement due to the unique loss of BSR predictability under each schedule. The occurrence of BSR was most predictable under the FR schedule and behavior on FR changed the least when the signal was removed.

This is not to imply that the reward value of one signaled BSR (as revealed by the highest FR schedule on which an animal's behavior could be maintained) is the same for all rats. In addition to those animals already mentioned, one rat did not maintain responding on schedules greater than FR 60 (although this animal's FI 3-minute performance was the same as that for rat 299), while another did not perform on schedules greater than FR 30. Both animals were tested on BSR intensities from 15 to 40 μa root-mean-square. Although adding a signal clearly increases the reinforcing value of BSR, the upper limit is determined by other factors.

Previous demonstrations of schedule control with BSR may be explainable in terms of BSR predictability also. Pliskoff *et al.* (*4*) presented a second lever on which predictable BSR could be obtained. Brown and Trowill (*3*) made BSR predictable by allowing the rat to receive five BSR's (continuous reinforcement) when a reinforcement was due. The first response-produced BSR signaled that the next four responses would produce BSR's.

In summary, single, signaled trains of BSR on a variety of reinforcement schedules established and maintained operant behavior comparable to that typically found with food reinforcement. When the signal was removed, changes in overall response rate were schedule-specific.

MICHAEL B. CANTOR
Department of Psychology, University of Pennsylvania, Philadelphia 19104

References and Notes

1. M. Sidman, J. V. Brady, J. J. Boren, D. G. Conrad, A. Schulman, *Science* **122**, 830 (1955).
2. R. E. Keesey and M. D. Goldstein, *J. Exp. Anal. Behav.* **11**, 293 (1968).
3. S. Brown and J. A. Trowill, *Psychol. Rep.* **26**, 699 (1970).
4. S. S. Pliskoff, J. E. Wright, D. T. Hawkins, *J. Exp. Anal. Behav.* **8**, 264 (1965).
5. M. B. Cantor, thesis, University of North Carolina, Chapel Hill (1970).
6. S. S. Steiner, B. Beer, M. M. Shaffer, *Science* **163**, 90 (1969).
7. M. B. Cantor and V. M. LoLordo, *J. Comp. Physiol. Psychol.* **71**, 183 (1970).
8. W. C. Stebbins, R. B. Mead, J. M. Martin, *J. Exp. Anal. Behav.* **2**, 351 (1959).
9. Portions of these data were the basis for experiment 3 of a doctoral dissertation [University of North Carolina (1970)]. This part of the research was supported by research grant MH 14121 from the National Institute of Mental Health to V. M. LoLordo and by a National Science Foundation traineeship held by M.B.C. The research was completed under a postdoctoral fellowship at the Institute of Neurological Sciences, University of Pennsylvania. I thank V. M. LoLordo and C. R. Gallistel for their criticism of an earlier version of the manuscript.

11 June 1971

Social Setting: Influence on the Physiological Response to Electric Shock in the Rat

Abstract. *A significant fall in tail blood pressure occurs in paired rats after shock-induced aggression. Pressure returns to baseline levels within 4 hours after fighting. Conversely, single rats subjected to jump threshold measurements or to shocks identical to those used in the aggression paradigm show significant elevations in tail blood pressure. The size of the pressure increase in rats shocked alone appears dependent on the intensity of the shocks, while the pressure fall in rats shocked in pairs occurs over a broad range of shock intensities.*

Shock-induced aggression is a social phenomenon. It will occur if two animals are placed together in an area that does not permit escape and an electric shock is applied to their feet. The animals will attack each other with species-specific aggressive and submissive motor patterns. Many aspects of this behavior have been defined, particularly in the laboratory rat (*1*), and the paradigm of shock-induced aggression has been used to study brain lesions and drug effects (*2*, *3*). If one of the rats is removed, the remaining animal's attack response is replaced by persistent escape attempts. It occurred to us that there might be different physiological correlates of these two different behavioral responses: attack and attempted escape. An analogous situation can be drawn from the human psycho-physiological experiments where anger and attention directed outward have been correlated with a norepinephrine-like physiological pattern, while anger and attention directed inward, anxiety, and fear have been associated with an epinephrine-like physiological pattern (*4*, *5*).

In experiment 1, 16 experimentally naive 90-day-old, male NIH Osborne-Mendel rats were randomly separated into eight fighting pairs, which were maintained for the duration of the study. The animals were housed separately and fed freely on rat chow. Shock-induced fighting rates and jump thresholds were determined as described (*2*). Briefly, shock-induced fighting involved the presentation of 50 footshocks of 2-ma intensity to paired rats. The shocks lasted 0.4 second and were presented every 7.5 seconds. An attack percentage for each rat pair was obtained by counting the number of shocks which initiated an attack response. Jump thresholds were determined by delivering a series of graded shocks to each rat alone. The intensity of the shocks ranged from 0.07 to 0.5 ma. A jump threshold for each rat was obtained, being that current intensity at which the rat jumped 50 percent of the time. Tail blood pressure (*6*, *7*) was measured by placing the rat in a warmed restrainer (*8*). Blood flow to the tail was then occluded by inflating a tail cuff to 200 mm-Hg. The cuff pressure was gradually released and the first pulsations were detected by impedance plethysmography (*9*). The impedance pulse was superimposed on the pressure tracing and recorded (*10*). By proper calibration, the pressure at which the first pulsations appeared distal to the cuff could be reproducibly determined and was interpreted as the tail blood pressure. Pulse rate was also determined from the plethysmographic tracing.

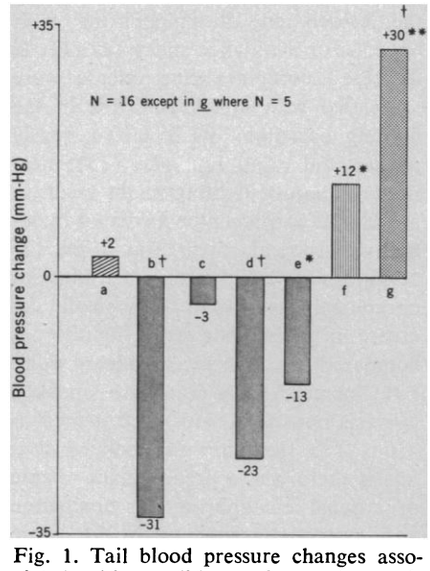

Fig. 1. Tail blood pressure changes associated with conditions of experiment 1 (a) Control, rats placed in cage between blood pressure measurements; (b to e) fighting paradigm on four consecutive days, rats paired; (f) jump threshold, rats alone; (g) fighting paradigm, rats alone; $*P < .05$ by paired t-test, two-tailed; † $P < .01$ by paired t-test, two-tailed.

Tail blood pressure and pulse were measured under the following conditions: (i) before and 3 to 5 minutes after shock-induced fighting on four successive days; (ii) 4 hours after shock-induced fighting on one of the aforementioned days; (iii) before and 3 to 5 minutes after jump threshold determination on one day; (iv) before and 3 to 5 minutes after a subsample of five rats received the fighting protocol of 50 2-ma shocks while alone in the box; and (v) before and after a control period in which the rat pairs were simply kept in cages together for 45 minutes.

Mature Osborne-Mendel rats are relatively aggressive, attacking after approximately 67 percent of their shocks. Their mean jump threshold for this experiment was 0.26 ma, identical to Sprague-Dawley males tested under similar conditions. The effects of the experimental conditions of experiment 1 on tail blood pressure are illustrated in Fig. 1. Shock-induced fighting was followed by a consistent fall in mean tail blood pressure. This fall reached statistically significant levels on 3 of the 4 days measured, with a mean drop over the 4 days of 17.5 mm-Hg. The failure to reach significance of day 2 remains unexplained. There was no change in pulse rate. The tail blood pressure had returned to baseline levels 4 hours after fighting. Conversely, when shock was delivered to single rats with either the parameters of intensity and timing identical to those of the fighting paradigm or the variable intensities of the jump threshold determination, there was a consistent rise both in tail blood pressure and in pulse rate.

Experiment 2 evaluated the role of differing shock intensities in relation to