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Relationship of negative contrast to animal models of fear and anxiety

CHARLES F. FLAHERTY, ANNA GREENWOOD, JOSEPH MARTIN, and MARY LESZCZUK
Rutgers University, Piscataway, New Jersey

The relatedness of behavior elicited by reward reduction (successive negative contrast procedure) and behaviors produced by three animal models of anxiety (open-field emergence, elevated plus-maze, and context-shock fear conditioning) was examined by correlational and factor analytic procedures. Factor analysis (oblique rotation) indicated substantial independence among the tests: Trials 1 and 2 of the plus-maze loaded on two different factors unaccompanied by any other test; open-field emergence and context-shock fear loaded on the same factor; and negative contrast loaded on a fourth factor. However, negative contrast proved to be a dynamic process, with factor loadings changing across a 4-day postshift period—moving from an independent loading on the 1st postshift day to being clustered with context-shock fear and open-field emergence on the 2nd and 3rd postshift days to being clustered with just context-shock fear on the last postshift day. These latter data support a multistage theory of successive negative contrast.

Rats shifted from a preferred reward to a less preferred reward show an abrupt decrement in goal-directed behavior and an increase in exploratory behavior (e.g., Crespi, 1942, 1944; Elliott, 1928; Flaherty, 1996). The goal-directed performance of the shifted rats decreases to a level below that of unshifted control animals given the same level of reward—an outcome termed a *successive negative contrast* (SNC) effect.

One enduring interpretation of successive negative contrast is that it is caused by an emotional reaction to the reward reduction. Terms such as *anger*, *depression*, *disappointment*, and *frustration* have often been used in referring to a rat's or a monkey's reaction to reduced reward (e.g., Amsel, 1992; Crespi, 1942, 1944; Flaherty, Krauss, Rowan, & Grigson, 1994; Gray, 1982, 1987; Spence, 1956). Evidence supporting the involvement of some form of stress in negative contrast includes the success of antianxiety agents (e.g., benzodiazepines, ethanol, barbiturates, and morphine) in alleviating aspects of contrast (Becker & Flaherty, 1982; Flaherty, 1991b, 1996), the elevation of corticosteroids as a consequence of reward reduction (Flaherty, Becker, & Pohorecky, 1985; Goldman, Coover, & Levine, 1973; Mitchell & Flaherty, 1998), covariation of contrast and other forms of emotional reactivity in rats selectively bred for performance in avoidance learning (Flaherty & Rowan, 1989), and the role of amygdala lesions in reducing contrast (Becker, Jarvis, Wagner, & Flaherty, 1984).

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We have recently proposed a multistage theory of SNC that suggests that perceptual and cognitive stages (detection that the new reward is different from the memory of the preshift reward; hedonic evaluation of the new reward; search for the "missing" reward if the new reward is of lesser value) precede the activation of an emotional response to reward reduction (Flaherty, 1996). In the present paper, we are concerned with an investigation of this hypothesis, and we compare daily postshift performance of rats downshifted in reward with their performance in three procedures frequently used to assess anxiety or fear in rats: the elevated plus-maze, context-shock fear conditioning, and emergence from the home cage to an open field.

Correlational and factor analytic approaches to animal models of anxiety have been used in a number of recent papers. Belzung and Le Pape (1994) examined the correlations in behavior among five different novelty related tests: light/dark choice, elevated plus-maze, holeboard, free exposure to a novel space, and confrontation with a novel object. The authors concluded the five different tests of novelty induced anxiety were not measuring the same psychological state. In a factor analytic study of defensive behaviors in mice, Griebel, Blanchard, and Blanchard (1996) concluded that there were five different aspects of anxiety measured by their 17 variables. Other studies have also raised the issue that different tasks or test procedures may be measuring different aspects of anxiety (File, 1991, 1995; Handley, McBlane, Critchley, & Njung'e, 1993; Rodgers, Cole, Aboualfa, & Stephenson, 1995). In some cases, a single test may measure multiple components of anxiety. Exposure of mice to a single 5-min trial in an elevated plus-maze may yield from two to six factors, depending on the number and nature of dependent variables scored (Rodgers & Johnson, 1995). However, the substantial effectiveness of the benzodiazepine tranquilizers in many animal models of anxiety suggests that there must

also be some degree of commonality (Davis, 1991; Flaherty, 1991a, 1991b; Howard & Pollard, 1991; Lister, 1991).

In comparing negative contrast with other models of anxiety, we used a negative contrast procedure that involved the consumption of sugar solutions. Rats were given access to a 32% sucrose solution for 5 min a day for 10 days, and, on the 11th day, shifted to a 4% sucrose solution. Typically, the "shifted" rats lick substantially less of the 4% solution than do "unshifted" controls, but the shifted rats recover from contrast after approximately 4 days of access to the new solution (Flaherty, Krauss, Rowan, & Grigson, 1994). The involvement of some aspect of anxiety or stress in negative contrast is indicated by the fact that corticosterone levels are elevated after the 2nd, but not the 1st, postshift day (Flaherty et al., 1985; Mitchell & Flaherty, 1998) and that chlordiazepoxide and ethanol reduce contrast when administered acutely on the 2nd postshift day, although they are not effective on the 1st postshift day (Flaherty, 1996; Flaherty, Grigson, & Lind, 1990).

The test battery selected for comparison with contrast in the present experiment included a measure of novelty-induced anxiety (emergence from the home cage to an open field), conditioned fear (context-shock pairings), and a test that involved both generalized anxiety and a specific fear of open places (elevated plus-maze). Previous studies have investigated novelty-induced anxiety by measuring latency to emerge from a familiar area to an unfamiliar area (Corey, 1978; Hughes, 1968; Maren, Patel, Thompson, & Mitchell, 1993), such as from the animal's cage to a table top (Henck, Mattsson, Rezabek, Carlson, & Rech, 1994), runway (King, 1968), or open field (Jellestad, Markowska, Bakke, & Walther, 1986). The evidence that exposure to novel context induces anxiety in laboratory animals includes increased heart rate, increased defecation, and increased corticosterone release (Blanchard, Kelley, & Blanchard, 1974; Crawley, 1985; Misslin & Cigrang, 1986). In these tests, antianxiety drugs increase ambulation (Treit, 1985), increase exploration (Crawley, 1985), and decrease emergence latencies into a novel environment (Einon & Tye, 1975).

An alternative way to measure novelty-related anxiety is through the use of an elevated plus-maze (Fernandes & File, 1996; File, 1996; Hogg, 1996; Lister, 1987; Pellow, Chopin, File, & Briley, 1985). In this apparatus, rodents show fewer entries into open arms (no walls) than into closed arms (walls)—an outcome that primarily reflects rodents' aversion to open spaces (Pellow et al., 1985; Treit, Menard, & Royan, 1993). Rats show typical stress reactions when placed in an elevated plus-maze, including increased corticosterone levels, increased freezing, and increased defecations—more so in open arms than in closed arms (File, Zangrossi, Sanders, & Mabbutt, 1994; Pellow et al., 1985). Anxiolytic drugs administered on the first trial increase entries into, and time in, open arms (Pellow et al., 1985). Anxiogenic compounds, such as benzodiazepine inverse agonists and yohimbine, suppress open-arm entries (Handley et al., 1993; Pellow & File, 1986; Rodgers et al., 1995).

Behavior in a second trial in the elevated plus-maze differs from that seen on the first trial. Benzodiazepines have been found to be ineffective in the second trial, regardless of whether they were administered on the first trial, leading to the suggestion that the second trial measures a different type of anxiety than the first trial (File, 1990). This has been supported by factor analytic studies that found that the measures of Trial 1 load on a different factor from those of Trial 2 (e.g., Fernandes & File, 1996; File, Zangrossi, Viana, & Graeff, 1993).

Context-shock fear conditioning differs from the above models in that it involves a physically aversive event. Corticosterone levels have been shown to be elevated after exposure to the context in which shocks had been given (Urban, Van de Kar, Lorens, & Bethea, 1986), and benzodiazepines disrupt conditioned fear (Fanselow & Helmstetter, 1988; Sanger & Joly, 1985), whereas anxiogenics enhance conditioned fear (Fanselow, Helmstetter, & Calcagnetti, 1991).

The principal purpose of this experiment was to determine whether behavior at different stages of the contrast recovery cycle was differentially correlated with these tests. A secondary purpose was to determine the relatedness of the other three tests themselves.

METHOD

Subjects

Sixty male Sprague-Dawley rats purchased from Harlan were used as subjects. The rats were approximately 90 days old, weighing 300–350 g at the beginning of the experiment. The animals were individually housed under 14:10-h light:dark conditions. The rats were given free access to water and were maintained at 82% of their free-fed weight by once-daily feedings. The deprivation regimen is standard for negative contrast experiments.

The rats were run in two consecutive groups of 30 each, approximately 1½ months apart. Thirty of the subjects were subsequently used in an anticipatory contrast study and then underwent fear conditioning. The fear conditioning, but not the anticipatory contrast, data are included in this report.

One rat died prior to the beginning of testing.

Apparatus

Negative contrast procedure. All testing was done in six Plexiglas chambers measuring 29 × 28.5 × 32 cm. Sucrose was presented in Nalgene test tubes with metal ball-bearing spouts. The tubes were advanced by motors into holes centered on one wall, 6 cm above a hardware cloth floor. Licks were measured through a contact relay circuit and were recorded by microprocessors.

Elevated plus-maze. The maze consisted of two open arms and two enclosed arms, each measuring 49.5 × 10 cm, with black Plexiglas floors. The open arms were bounded by 1-cm-high ledges on the sides, but there were no ledges at the ends of the arms. The closed arms had 39.5-cm-high transparent Plexiglas walls. The maze was elevated 50.5 cm from the ground. The rats were carried to the room in a clear Plexiglas box measuring 20 × 26 × 19.5 cm, which was covered in black paper. The room was brightly lit, and white noise was on in the background during testing. A camera was mounted directly above the maze (WAT-902A, Edmund Scientific) and was connected to a monitor in an adjacent room.

Home-cage emergence. Emergence was tested from the subject's home cage into an open field. Each home cage measured 24.5 × 20.5 × 18.5 cm and had a wire-mesh bottom and front and solid stainless steel sides and back. Cages were placed into a circular white Plexiglas open field 76 cm in diameter, with walls 31.5 cm tall. The experimental room was brightly lit, with white noise in the background.

Fear conditioning. The training and testing was conducted in a 23 × 22 × 25.5 cm operant chamber, with clear Plexiglas walls and a floor consisting of metal rods. The chamber was outfitted with shock presentation capabilities and was enclosed in a sound-attenuating chamber. A houselight was on during training and testing. The foot shock was a 0.8-mA scrambled alternating current presented for 2 sec.

Procedure

The rats were divided into six groups (for five of the six, $n = 10$; for the remaining group, $n = 9$) for purposes of counterbalancing the order of administration of the first three tests (successive negative contrast, elevated plus-maze, open-field emergence). Thus, all possible sequences of these tests were administered. There were 2 days between each test, during which the subjects were weighed and fed.

Fear conditioning, which was administered to a subset of 30 subjects, occurred approximately 60 days after the first three tests had been completed. Thus, fear conditioning was not included in the counterbalancing, which applied only to the first three tests.

Negative contrast procedure. The animals received 10 days of acquisition training, during which all subjects received access to 32% sucrose solution for 5 min daily. On the 11th through 14th days, all subjects received 4% sucrose.

No unshifted 4% control group was included, since the interest was in degree of decrement in consummatory behavior in shifted rats as a variable to correlate with other measures of anxiety. Lick frequency was presented in order to provide a comparison with the many earlier studies that used this variable and also included unshifted control groups.

Negative contrast was reported as shift percentage on the first postshift day (100− [first postshift day lick frequency/terminal preshift day lick frequency]). This measure was used as the index of the initial reaction to reward reduction. For purposes of assessing the possible changing nature of contrast across the postshift period, shift percentage was also calculated for each of the 4 postshift days and entered into a factor analysis with measures from the other tests. The slope of the recovery of lick frequency across the 4 postshift days was used as an index of rate of recovery from contrast.

Sucrose solutions were mixed from commercial grade sugar on a weight basis (sugar/[sugar + water]) approximately 24 h before each experimental session.

Elevated plus-maze. The rats were carried individually to the experimental room, where they were kept in the carrying box for 5 min prior to the start of the test. Subsequently, the rats were placed in the center of the maze facing an open arm to begin a 5-min trial. Video recordings of the session were later scored in terms of time spent in open and closed arms and number of entries into open and closed arms. An entry consisted of all four paws in a particular arm. Each subject was given two 5-min trials spaced 72 h apart.

Home-cage emergence. Each rat was carried individually in its home cage to the experimental room, where the cage was turned on its side (the rat was held by the experimenter during the turning) and then placed in the center of the open field, and a timer was started. Time for the subjects to emerge with two paws and then with four paws from the home cage was recorded by an experimenter seated outside of the testing room. Maximum time allowed was 16 min.

Fear conditioning. The animals were on a free-feeding schedule during the 60-day period following the completion of the other three tests and were reduced to 82% of their new free-feeding weight prior to the start of fear conditioning. During acquisition training, the rats were transported individually to the testing apparatus and given six trials of 2-sec shocks presented 4 min apart. Approximately 30 sec after the last shock presentation, the rats were removed and returned to their home cages. The apparatus was cleaned thoroughly after each subject. For 20 subjects, training occurred on the 6th day after deprivation; for the remaining 10 subjects, training occurred on the 11th day after deprivation.

Testing for fear of the context occurred on the day after training. The rats were again brought individually into the testing room and were placed into the same apparatus as was used for training. They were given a 5-min test period, during which no shocks were presented. Freezing episodes (as described in File, 1990) were recorded every 5 sec in a time-sampling procedure, and proportion of episodes during which freezing occurred was calculated. The number of defecations was also noted.

Data Analysis

Initial analyses performed included an analysis of variance, followed by Fisher's LSD post hoc tests, paired comparison t test, and normal curve analysis using the Shapiro-Wilk W test. Subsequent analyses included Pearson's correlation coefficient (r) on z scores and a factor analysis using the SAS statistical package with a promax rotation method.

RESULTS

The results will be presented first in terms of performance on each of the four tests (negative contrast procedure, elevated plus-maze, home-cage emergence, and conditioned fear) and then in terms of relationships among the tests as determined by correlational analyses and factor analysis.

Negative Contrast Procedure

Descriptive statistics for this, and all other tests, are presented in Table 1. The degree of reduction in lick frequency in individual animals following the reduction in sucrose concentration ranged from 44% to 89%. The distribution did not differ reliably from a normal distribution (Shapiro-Wilk W test, $p = .064$). Although an unshifted

Table 1
Descriptive Statistics for Selected Variables of the Tests of Anxiety

Variable	*M*	*SD*	Range
Negative Contrast Test			
Percent shift (%)	68	12	44–89
Home-Cage Emergence Test			
Two-paw latency (sec)	124.7	145.1	1–960
Four-paw latency (sec)	319.6	224.6	10–960
Plus-Maze Trial 1			
Proportion of time in open arms	.29	.15	0–.65
Proportion open-arm entries	.36	.13	0–.67
Total entries	14.9	3.9	6–22
Plus-Maze Trial 2			
Proportion of time in open arms	.17	.13	0–.52
Proportion open-arm entries	.24	.14	0–.48
Total entries	16.4	4.1	1–26
Conditioned Fear Test			
Proportion of time freezing	.45	.17	.15–.85

control group was not included in this experiment, the shift ratio (1 − [shift percent / 100]) obtained (.32) was approximately equivalent to that obtained in standard contrast experiments (e.g., Flaherty, Krauss, & Hill, 1994), indicating a typical negative contrast effect.

Although there were no differences in initial reaction to reward reduction among the counterbalancing conditions ($F < 1.0$), recovery from contrast was affected by prior experience in that the two groups exposed to contrast first were slower to recover than were groups that were exposed to contrast second or third [group × day, $F(10,94) = 3.88, p < .01$]. Simple effects tests indicated that the groups that had other experiences prior to contrast did not differ from each other. These data are illustrated in Figure 1, with the two groups that received contrast first combined and the four groups that experienced contrast second or third also combined. Whether this difference in rate of recovery was due specifically to experience in the other test procedures or was simply due to the increased amount of handling that the animals received cannot be determined from this study.

Elevated Plus-Maze

In Trial 1, time in open arms ($M = 73.2$ sec) was reliably less than time in closed arms ($M = 181.6$ sec) [$t(57) = 10.5, p < .01$], entries into open arms ($M = 5.5$) were fewer than entries into closed arms ($M = 9.4$) [$t(57) = 8.25, p < .01$], proportion of time in open arms was less than proportion of time in closed arms [$t(57) = 19.17, p < .001$], and proportion of entries into open arms was less than proportion of entries into closed arms [$t(57) = 14.58, p < .001$]. These data are presented in Figure 2.

The same measures also differed reliably in Trial 2. Time in open arms ($M = 43.5$ sec) was less than time in closed arms ($M = 215.4$ sec) [$t(57) = 17.18, p < .01$], open entries ($M = 4.1$) were fewer than closed entries ($M = 12.3$) [$t(57) = 10.22, p < .01$], proportion of time in open arms was less than proportion of time in closed arms [$t(57) = 10.87, p < .001$], and proportion of entries into open arms was less than proportion of entries into closed arms [$t(57) = 8.13, p < .001$].

Although Trials 1 and 2 were spaced 72 h apart, there were substantial changes in the direction of further avoidance of the open arms on Trial 2. Thus, time in open arms and number of open arm entries were reliably lower on Trial 2 than on Trial 1 [$F(1,53) = 40.59, p < .01$, and $F(1,53) = 14.64, p < .01$, respectively]. Higher on Trial 2 than on Trial 1 were time in closed arms [$F(1,53) = 34.57, p < .01$], entries into closed arms [$F(1,53) = 46.54, p < .01$], and total entries [$F(1,53) = 11.88, p < .01$]. Lower on Trial 2 than on Trial 1 were proportion of time spent in open arms [$F(1,53) = 39.54, p < .01$] and proportion of entries made into open arms [$F(1,53) = 40.08, p < .01$].

There were no overall effects across trials due to prior experience with other tests [number of open entries, $F(5,53) = 1.10, p > .36$; number of closed entries, $F(5,53) = 1.07, p > .39$; time spent in open arms, $F(5,53) = 1.07, p > .39$; time spent in closed arms, $F(5,53) = 1.58, p > .18$; proportion of time spent in open arms, $F(5,53) = 1.18, p > .33$; and proportion of entries that were open, $F < 1.00$].

However, there were some group × trial interactions that showed that changes in behavior that occurred from the first to the second trial in the plus-maze were influenced by prior test experience. The reliable effects were on time in open arms [$F(5,53) = 3.92, p < .01$], time in closed arms [$F(5,53) = 3.36, p < .01$], and open-arm entries [$F(5,53) = 2.61, p < .03$]. In general, these interactions indicated that Trial 1 versus Trial 2 differences were greater in the rats that experienced the plus-maze first in the sequence of tests. Thus, these interactions indicated a tendency of prior test experience (or perhaps handling) to reduce the psychological difference between the two trials in the plus-maze (see Hogg, 1996).

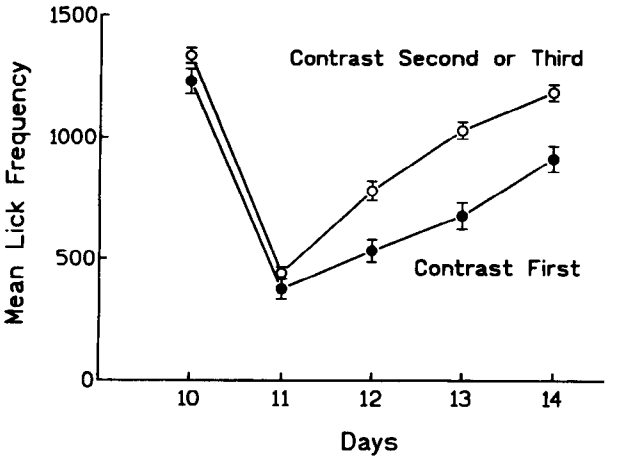

Figure 1. Lick frequency of rats shifted from 32% sucrose (Days 1–10) to 4% sucrose (Days 11–14). The function labeled *contrast first* represents the data of the 20 rats that received contrast testing prior to any other test. The other function represents the 40 rats that received contrast after one or both of the open-field emergence and elevated plus-maze tests.

Home-Cage Emergence

Emergence from the home cage into an open field showed an extensive range of individual differences for both two-paw latency (range = 1–960 sec) and four-paw latency (range = 10–960 sec). Sixteen minutes (960 sec) was the maximum time allowed; 3 rats failed to emerge with four paws, and 1 of these rats also failed to emerge with two paws. The distributions differed from normal and were skewed toward the lower times to emerge (Shapiro-Wilk W test, $p < .01$). There were no significant differences in latency to emerge due to previous experience (Fs < 1.00).

Fear Conditioning

There was a range of individual differences in percent of time spent freezing in the shock context (15%–85%), and the distribution of these data was not significantly different from a normal distribution (Shapiro-Wilk W test,

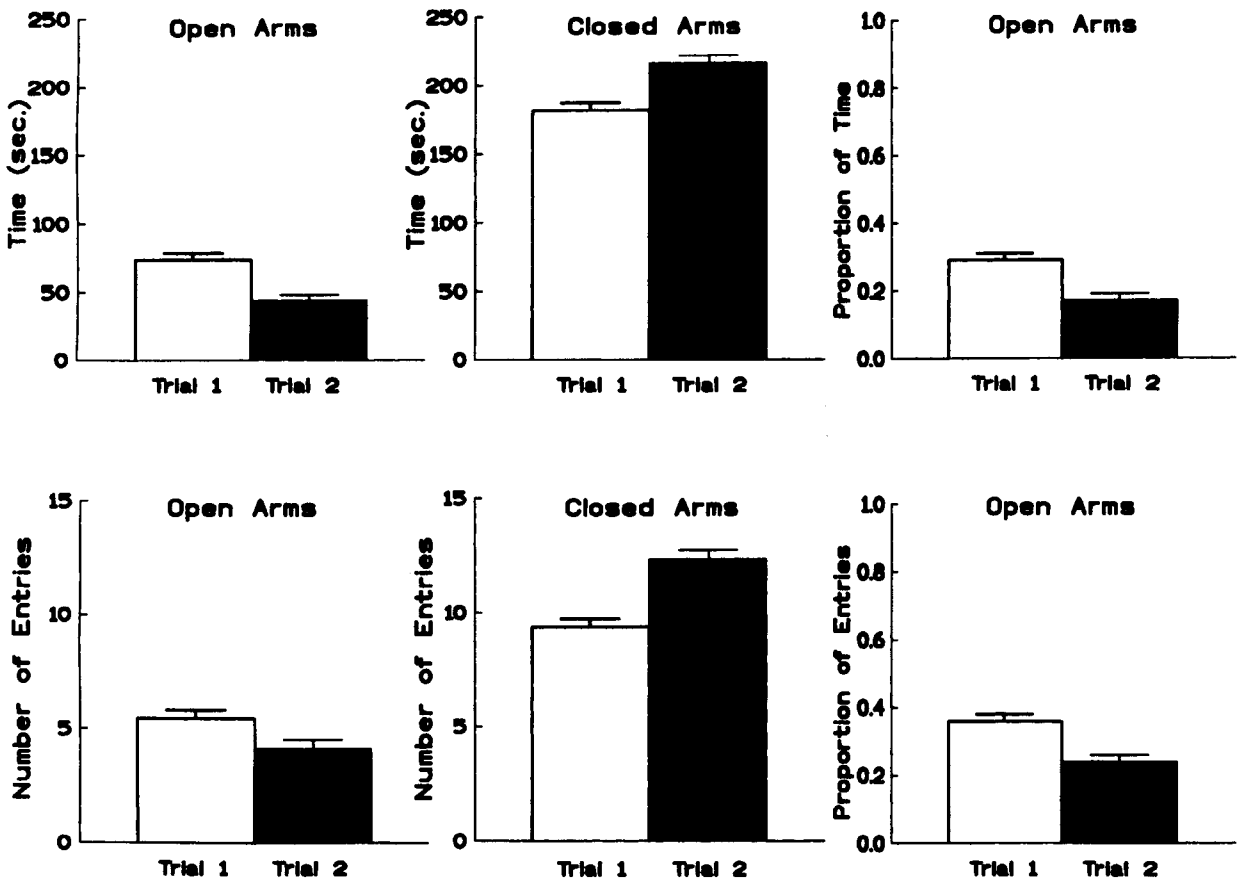

Figure 2. Total time, total entries, proportion of time, and proportion of entries into open and closed arms on Trials 1 and 2 of the elevated plus-maze.

$p > .53$). There were no test order effects because fear conditioning was given last.

Correlations Between Variables

For purposes of correlational analyses, all variables were converted to z scores, and Pearson correlation coefficients were computed. The resultant correlation matrix is shown in Table 2. The data will be presented and discussed in terms of within-test correlations, then between-test correlations, and, finally, factor analysis.

Within-Test Correlations

Several additional variables were used in the correlation analysis that were not described above. For negative contrast, Day 1 licks and Day 1 latency were used to model initial responsivity to the sucrose solution. To portray recovery from contrast, the slope of the recovery line (lick frequency) from Day 11 to Day 14 was computed. Variables for plus-maze, emergence, and fear conditioning remained the same as described above.

Negative contrast procedure. Variables from the negative contrast procedure were interrelated. Slope of recovery was significantly correlated with percent shift on the 1st postshift day ($r = .46$, $p < .01$). This correlation probably reflects the fact that shifted rats tend to return to the level of an unshifted control group, which represents performance (intake) due to the absolute reward value of 4% sucrose. Given that this is a stable parameter of the population, all rats would tend to return to the same approximate level, and, thus, the rats with a greater shift would tend to have a steeper recovery slope.

No reliable correlations were found with Day 1 licks and Day 1 latency to first lick. This failure to find a correlation is informative in that it suggests that whatever neophobic reactions might have been involved with the initial exposure to sucrose, this neophobia was not related to the degree of decrement when the rats were shifted from 32% to 4% sucrose.

Elevated plus-maze. Variables from the elevated plus-maze were highly interrelated within a trial. For instance, time in open arms for Trial 1 and open arm entries for Trial 1 were highly correlated ($r = .86$, $p < .01$), and the same measures were also highly correlated for Trial 2 ($r = .93$, $p < .01$). Measures between trials were significantly correlated, but not as highly as within-trial measures. For instance, time in open arms in Trials 1 and 2 ($r =$

Table 2
Correlation Matrix for All Variables

					Trial 1					Trial 2					
	Percent shift	Slope	Two-paw latency	Four-paw latency	Time in open arms	Open-arm entries	Proportion of time in open arms	Proportion of open-arm entries	Total entries	Time in open arms	Open-arm entries	Proportion of time in open arms	Proportion of open-arm entries	Total entries	Proportion of time freezing
Percent shift															
Slope	.47*														
Two-paw latency	−.04	−.26													
Four-paw latency	−.33*	−.22	.55*												
Trial 1															
Time in open arms	.18	.11	.03	−.26*											
Open-arm entries	.31*	.25	−.11	−.35*	.86*										
Proportion of time in open arms	.20	.14	.02	−.26*	.99*	.85*									
Proportion of open-arm entries	.16	.15	.05	−.04	.79*	.81*	.79*								
Total entries	.35*	.20	−.18	−.52*	.50*	.71*	.49*	.25							
Trial 2															
Time in open arms	.02	.08	−.08	−.09	.37*	.45*	.38*	.25*	.42*						
Open-arm entries	.11	.18	−.13	−.15	.42*	.50*	.42*	.32*	.44*	.93*					
Proportion of time in open arms	.05	.07	−.08	−.10	.35*	.43*	.36*	.23	.42*	.99*	.93*				
Proportion of open-arm entries	.06	.14	−.05	−.03	.40*	.44*	.41*	.33*	.31*	.91*	.94*	.91*			
Total entries	.16	−.03	−.14	−.27*	.31*	.39*	.31*	.16	.57*	.49*	.61*	.48*	.42*		
Proportion of time freezing	.06	.15	.38	.32	−.03	−.17	−.07	.02	−.19	−.18	−.23	−.22	−.21	−.15	

*$p < .05$.

.37, $p < .01$) were correlated, as was time in open arms in Trial 1 and open-arm entries in Trial 2 ($r = .42, p < .01$).

Although there were some reliable between-trial correlations, percent of variance explained by the correlations was higher for within-trial variables than for between-trial variables. This finding is consistent with earlier reports that Trial 1 and Trial 2 reflect different types of fear (File et al., 1993).

Emergence. Latency to emerge with two paws was positively correlated with latency to emerge with four paws ($r = .55$, $p < .01$). However, there was substantial variance unexplained by this correlation ($r^2 = .30$), which suggests that there is something quite different about these two measures of emergence.

Between-Test Correlations

Negative contrast procedure and elevated plus-maze. Percent shift correlated positively with Trial 1 open-arm entries ($r = .31, p < .02$) and Trial 1 total entries ($r = .35, p < .01$). The positive correlation between degree of shift and frequency of arm entries may be taken as support for the hypothesis that an early stage of contrast involves search for the "missing" preshift reward (Flaherty et al., 1990) and that rats that show larger contrast may be the ones less inhibited in regard to leaving the familiar and exploring the novel.

Given the relationship between degree of contrast and Trial 1 behavior, the lack of a correlation between contrast and Trial 2 open-arm entries ($r = .11, p > .41$) as well as total Trial 2 entries ($r = .16, p > .24$) is consistent with the hypotheses that different psychological processes are involved in Trial 1 and Trial 2 behavior in the elevated plus-maze.

The failure of the proportion measures of Trial 1 performance to correlate with shift behavior (proportion of time spent in open arms, $r = -.20, p > .10$; proportion of entries into open arms, $r = -.16, p > .23$) may indicate that the important factor related to contrast is activity or exploration in this apparatus rather than a specific lack of fear of the open arms.

The failure of the slope of the recovery function to correlate with any measures from the plus-maze suggests that it is the initial occurrence of contrast, rather than the recovery process, that is related to behavior in the elevated plus-maze.

Negative contrast procedure and emergence. Greater degrees of contrast were related to relatively rapid emergence into the open field (four-paw latency)—a result consistent with the relationship between contrast and Trial 1 elevated plus-maze performance in suggesting that larger degrees of contrast are related with a tendency to enter novel environments.

Negative contrast procedure and conditioned fear. The correlational data indicated no relationship between these tests on the 1st postshift day.

Elevated plus-maze and conditioned fear. No variables from the plus-maze were correlated with conditioned fear.

Elevated plus-maze and emergence. There was a substantial relationship between four-paw emergence latency and Trial 1 performance in the elevated plus-maze. Generally, long emergence times were related to hesitancy to enter the open arms in the plus-maze (latency and number of open arm entries on Trial 1, $r = -.35, p < .01$; latency and time spent in open arms on Trial 1, $r = -.26$, $p < .05$; latency and proportion of time spent in open arms on Trial 1, $r = -.26, p < .04$; latency and total entries on Trial 1, $r = -.51, p < .01$).

Total number of entries was the only Trial 2 variable correlated with four-paw emergence ($r = -.27, p < .05$). The separation seen between Trial 1 and Trial 2 behavior in this test supports the previous data and File's earlier statement that Trials 1 and 2 measure different psychological phenomena (File et al., 1993). The finding that number of total entries was the only Trial 2 variable to correlate with emergence suggests that a factor related to general activity might be something in common between the two trials in the plus-maze and the emergence test.

Emergence and conditioned fear. There was a significant positive correlation found between proportion of time spent freezing and two-paw latency ($r = .36$, $p < .05$). The correlation between four-paw latency and time spent freezing came close to reliability ($r = .32, p < .08$).

Factor Analysis

The data were submitted to a factor analysis, using a principal component solution with an oblique rotation of the factor matrix (promax). An oblique rotation was selected on the assumption that the factors underlying the various tests of emotionality employed here would have some degree of correlation, as suggested by the finding that benzodiazepine tranquilizers are effective in

Table 3
Final Rotated (Oblique) Factor Pattern

	Factor			
	1	2	3	4
Percent shift	−.162	−.087	.040	.847*
Recovery slope	−.084	.180	−.336	.921*
Two-paw latency	−.044	−.025	.910*	−.221
Four-paw latency	.007	−.099	.896*	−.148
Trial 1				
Time in open arms	.332	.943*	−.140	−.017
Open-arm entries	.270	.943*	−.378	.127
Proportion of time in open arms	.334	.952*	−.172	.044
Proportion of open-arm entries	.107	.884*	.012	.032
Total entries	.342	.557*	−.719*	.249
Trial 2				
Time in open arms	.969*	.226	−.097	−.188
Open-arm entries	.967*	.367	−.233	−.088
Proportion of time in open arms	.958*	.163	−.064	−.179
Proportion of open-arm entries	.937*	.332	−.028	−.026
Total entries	.577*	.021	−.562*	−.022
Proportion of time freezing	−.113	−.140	.478*	.298

*Factor loadings greater than ±.45.

aspects of all of the tests (Davis, 1991; File, 1991; Flaherty, 1991b).

Four factors were extracted using the default criterion of an eigenvalue greater than one. These four factors were found to account for 91% of the variance. The final rotated factor pattern is shown in Table 3. Only loadings greater than ±.45 were considered meaningful (Floyd & Widaman, 1995), and these are highlighted in the table.

Because Day 1 licks and Day 1 latency were not significantly correlated with any measures, these variables were dropped from the factor analysis. As seen in Table 4, plus-maze variables were loaded on the first two factors: Trial 2 variables loaded on Factor 1 (accounting for the most variance in the set), whereas Trial 1 variables loaded on Factor 2. Emergence and fear conditioning variables loaded on Factor 3, whereas negative contrast loaded alone on Factor 4.

The final factor intercorrelations are presented in Table 4. Factor 1 and Factor 2 (the two plus-maze factors) were more highly correlated than any other pair of factors. Factors 3 and 4 shared relatively little common variance with the other factors.

An interpretation of the factors is shown in Table 5. Factor 1 loaded plus-maze Trial 2 variables only. Because rats show an increased avoidance of the open arms on Trial 2 relative to on Trial 1, and because chlordiazepoxide is generally ineffective on Trial 2, File (1995) has considered Trial 2 performance to be based on learned fear of the open arms—a form of "phobia" rather than generalized anxiety or fear of novel places.

Factor 2 consisted entirely of Trial 1 behaviors in the elevated plus-maze. The greater amount of time that rats spend in the closed arms represents a fear of openness (Treit et al., 1993), which could be related to fear of predators or fear of intraspecific encounters (Pellow et al., 1985; Rodgers & Dalvi, 1997). However, the fact that the animals do enter the open arms suggests a conflict between exploratory tendencies and fear of open spaces. Exposure to this apparatus activates various physiological indices of stress, including increased extracellular cortical 5-HT (Rex, Marsden, & Fink, 1994), and corticosterone (File et al., 1994; Pellow & File, 1986). Also, open-arm entries on this trial may be increased by the administration of benzodiazepines (Pellow & File, 1986). File's interpretation of Trial 1 in the elevated plus-maze is that the avoidance of the open arms represents a generalized anxiety state (File et al., 1993).

Table 4
Correlations Between the Four Factors

	Factor			
Factor	1	2	3	4
1				
2	.289			
3	−.143	−.203		
4	−.118	.068	−.173	

Note—See Table 3 for the variables that loaded onto each factor.

Table 5
Interpretation of the Factor Pattern

Factor 1: Plus-Maze Trial 2 (Learned Fear of the Open Arms)
Time in open arms
Number of open entries
Proportion of time spent in open arms
Proportion of open entries
Total entries
Factor 2: Plus-Maze Trial 1 (Generalized Anxiety State)
Time in open arms
Number of open entries
Proportion of time spent in open arms
Proportion of open entries
Total entries
Factor 3: Emergence, Context-Shock Conditioning (Timidity/Fear)
Two-paw latency
Four-paw latency
Proportion of time spent freezing
Total entries, Trial 1*
Total entries, Trial 2*
Factor 4: Successive Negative Contrast (Disappointment)
Percent shift
Slope of recovery function

*Negative loading.

Factor 3 loaded latency to emerge from a familiar to an unfamiliar environment and proportion of time spent freezing in a shock-paired context. This constellation suggests a commonality in the emotional state (or behavioral repertoire) involved in an apparent innate fear of emerging from a familiar place into a novel environment/open space and learned fear of an environment paired with shock. The finding that total entries measures from Trial 1 and Trial 2 of the plus-maze loaded negatively on this factor suggests a role for general activity level in the sense that a tendency to be active detracts from inhibition of behavior in both innate and conditioned fear tasks (as measured by these two tests, but apparently not the elevated plus-maze). Perhaps there is an underlying dimension of activity/inhibition that modifies performance in these tasks—a dimension related to "timidity" or response inhibition (cf. Gray's, 1987, view of introversion vs. extroversion or neuroticism).

Negative contrast variables loaded exclusively on Factor 4. This finding suggests that initial responsivity to reward reduction is distinct from the reactions involved in the other three tests—a result consistent with interpretations of initial degree of contrast in terms of detection, evaluation, and search processes rather than emotionality per se (Flaherty, 1996).

Postshift Data

Because evidence from drug studies and from measurement of corticosterone has indicated that the nature of negative contrast changes across the postshift period, further factor analyses were conducted with percent shift calculated for each of the last 3 postshift days. Table 6 illustrates the dynamic aspect of negative contrast as the rats recover from reward reduction across the postshift period. The loading of percent shift on Factor 4 declines across

Table 6
The Changing Factor Structure Across the Postshift Period

Variable	Day 1	2	3	4
	Factor 1			
Trial 2				
Time in open arms	.97	.97	.97	.97
Open-arm entries	.97	.96	.96	.97
Proportion of time in open arms	.96	.96	.96	.96
Proportion of open-arm entries	.94	.93	.92	.93
Total entries	.58	.59	.59	.58
	Factor 2			
Trial 1				
Time in open arms	.94	.94	.94	.94
Open-arm entries	.94	.94	.94	.94
Proportion of time in open arms	.95	.95	.95	.95
Proportion of open-arm entries	.88	.89	.89	.88
Total entries	.56	.55	.55	.55
	Factor 3			
Two-paw latency	.91	.91	.91	.88
Four-paw latency	.90	.87	.86	.88
Total entries				
Trial 1	−.71	−.73	−.72	−.69
Trial 2	−.56	−.53	−.49	−.56
Conditioned freezing	.48	.31	.27	.58
Percent shift	.04	.16	.26	.45
Slope	−.34	−.53	−.58	−.24
	Factor 4			
Percent shift	.85	.75	.52	−.80
Slope	.92	.46	.52	.81
Conditioned freezing	.30	.72	.79	.42
Two-paw latency	−.22	.31	.27	−.54

Note—Conditioned freezing and percent shift factor loadings below ±.45 are included to illustrate the changing nature of the factors across the postshift period.

the postshift period, becoming strongly negative on the last postshift day. Of greater interest is the fact that conditioned fear and contrast load on the same factors on the 2nd and 3rd postshift days, when contrast may involve an emotional reaction, but not on the 1st postshift day, when corticosterone is not elevated and when chlordiazepoxide and ethanol are not effective in reducing contrast. On the last postshift day, the factor structure is different still: contrast now loads with fear on a different factor (Factor 3), along with emergence latency and plus-maze activity measures. Behaviorally, contrast has typically dissipated by the 4th postshift day. The factor loadings seen here may suggest that there is still an underlying emotional reaction to the shift even though the shifted animals have typically recovered by the last postshift day.

DISCUSSION

Two aspects of the data will be considered here: the relevance of the results to animal models of anxiety in general, and the implications of the results for interpretations of SNC.

Animal Models of Anxiety

Emergence, the elevated plus-maze, negative contrast, and fear conditioning are all considered animal models of emotion; however, procedurally and empirically, there would seem to be many differences among these models. Home-cage emergence and the first trial on the elevated plus-maze could reflect unconditioned behaviors. Context-shock fear is associative, and negative contrast may also be associative in that the animals learn to expect a given level of reward as a function of prior experience.

Factor analytic data of plus-maze performance obtained in this experiment and in other experiments suggest that the nature of the anxiety involved in this test changes with experience in the apparatus (Fernandes & File, 1996; File, 1996; File et al., 1993; Hogg, 1996; Rodgers et al., 1996). The first trial may measure generalized anxiety, a condition that may be alleviated by benzodiazepines and other anxiolytic agents. The experience gained on the first trial may lead to the development of a specific fear of the open arms, as evidenced by the decline in open-arm entries from Trial 1 to Trial 2 seen in the present study and in earlier studies. Support for the hypothesis that Trial 2 represents a different type of anxiety is the finding that benzodiazepine anxiolytics are effective on Trial 1 but are ineffective on Trial 2 (e.g., File, 1995).

The data suggest still more facets of emotional shading or behavioral repertoire measured by these tests. For instance, the separate aspects of anxiety/fear apparently measured in Trials 1 and 2 of the elevated plus-maze and conditioned fear established by context-shock pairings loaded on three distinct factors. A priori, it would seem that there should be a strong relationship between context-conditioned fear and performance on Trial 2 in the plus-maze, given File's interpretation that Trial 2 represents a learned fear. However, Trial 2 in the plus-maze and shock-conditioned fear loaded on two different factors. Also, there were no reliable correlations between context-shock-conditioned fear and elevated plus-maze performance. Perhaps different unconditioned stimuli (shock vs. openness) condition different behaviors/emotions. Because of the design of this experiment, it is not known whether plus-maze performance would have been altered if context-shock fear conditioning was given prior to the plus-maze experience.

Successive negative contrast has long been attributed to an emotional reaction to the reward reduction, but different labels have been applied to the postulated emotion involved: anger (Crespi, 1944; Tinklepaugh, 1928), depression (Crespi, 1942), frustration (Amsel, 1992; Spence, 1956), and disappointment (Flaherty, 1996). Interpretation of the correlational and the factor analytic data indicate some relationships between negative contrast and other tests of emotionality, but the more striking outcome is the degree of independence of the four tests employed here.

The relationship between the plus-maze and the emergence test is complicated. Although the two tests loaded on distinct factors, there were several reliable correlations between four-paw latency to emerge and Trial 1 behavior in the plus-maze. Part of this relationship may be due to general activity level, since there were negative corre-

lations between emergence and total entries on Trials 1 and 2. A part may also be specifically fear-related, because there was a negative correlation between emergence latency and total open entries on Trial 1 and also between emergence latency and proportion of open entries on Trial 1. This relationship of emergence to Trial 1 elevated plus-maze behavior, but not Trial 2, further supports the distinct emotion-eliciting conditions of the two experiences in the plus-maze.

Successive Negative Contrast Procedure

The factor analysis was particularly informative in regard to negative contrast. Although degree of initial negative contrast loaded exclusively on a single factor, the correlation analysis suggested a positive relationship between degree of initial contrast and tendency to enter novel environments. This may suggest that rats prone to large contrast effects are the ones less inhibited in regard to exploration, perhaps reflecting a propensity, in contrast-prone rats, to leave a depleted reward source to search for the missing (preferred) reward.

The absence of a relationship of the initial degree of contrast to the other models of anxiety or to fear-inducing situations could be surprising in the context of the historical preference for interpreting negative contrast in terms of an emotional reaction to reward reduction. However, data showing that chlordiazepoxide and ethanol are ineffective in moderating the initial response to reward reduction (Becker & Flaherty, 1982, 1983; Flaherty et al., 1990; Flaherty, Lombardi, Wrightson, & Deptula, 1980) and the failure to find an elevation in corticosterone on the initial day of reward reduction (Flaherty et al., 1985) are consistent with the absence of relationships to animal tests of anxiety in the initial reaction to reward reduction.

The relationship of initial degree of contrast to exploratory tendency supports the contention that an early aspect of the reaction to reward reduction is searching (Elliott, 1928; Flaherty, 1996) and that the emotional components of contrast occur later. Thus, the changing factor structure across the postshift period (Days 1–4) seen in the behavioral data may well reflect a change in dominant psychological status of the rats from search (1st postshift day, when contrast is isolated in its own factor) to anxiety/frustration/conflict (2nd and 3rd postshift days, the point at which benzodiazepines become effective and corticosterone is elevated) to recovery, but with "reluctant" acceptance of the postshift solution (we say "reluctant" because of the loading with conditioned fear on the last postshift day; see Flaherty, 1996).

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