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Exploratory Behavior in Mice Selectively Bred for Developmental Differences in Aggressive Behavior

ABSTRACT: *The development and expression of exploratory behavior was assessed in the Cairns lines of Institute for Cancer Research (ICR) mice that were selectively bred for differences in aggressive behavior, with a high-aggressive 900 line, low-aggressive 100 line, and control 500 line. Four paradigms were employed. Developmental changes were evident in the complex novel arena, with older males faster to contact a novel object, and ambulating more than young males. Within the control 500 line, older males showed longer latency to emerge from the home cage, and shorter latency to contact novel objects. In the 900 line, younger males showed this same pattern. R. B. Cairns proposed that line differences in aggressive behavior arise through alterations in developmental timing [Cairns et al. [1983] Life-span developmental psychology (Vol. 5). New York: Academic Press; Gariépy et al. [2001] Animal Behaviour 61: 933–947]. The early appearance of mature patterns of exploratory behavior in 900 line males supports this interpretation. The 900 line males also appear to be behaviorally inhibited in novel settings such as the light–dark box and the neohypophagia paradigm, compared to the 500 and 100 lines (Experiments 1, 2, and 4). Moreover, in the most complex apparatus, the novel arena, 900 line males were slowest to exit the home cage, and fastest to contact a novel object. The apparent contrast in these parameters of exploratory behavior is discussed in relation to T. C. Schneirla's [1965 Advances in the study of behavior (Vol. 1). New York: PN Academic] approach–withdrawal theory. © 2007 Wiley Periodicals, Inc.* Dev Psychobiol 50: 32–47, 2008.

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INTRODUCTION

Understanding the development and social ecology of exploratory and social behaviors is an enduring objective of behavioral science. These behaviors are embedded in coordinated behavioral systems for effective movement, exploration, and resource defense (food, mates, nest sites, offspring) as well as social interactions with conspecifics. Relationships among these behavioral systems may be clarified through analyses of the differential emergence and expression of social and exploratory behaviors in early and later life. An additional goal is to characterize the processes that support social-behavioral development as well as exploratory behaviors. Such an account may yield a more integrative understanding of factors related

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to aggressive expression (Archer, 1976; Cairns & Hood, 1983; Gray, 1987).

To consider these issues, exploratory behaviors are examined in three lines of mice that have been selectively bred for differences in aggressive behavior, with a high-aggressive line (the 900 line), a low-aggressive line (the 100 line), and a control line bred without selection (the 500 line). In a developmental-genetic analysis of selective breeding effects, alterations in the timing of social development produced significant line differences in behaviors, with a prolonged delay in the onset of aggressive behavior over generations in the low-aggressive line (neoteny), and an accelerated onset of short-latency attacks over generations in the high-aggressive line (Cairns, Gariépy, & Hood, 1990; Gariépy, Bauer, & Cairns, 2001).

Two dimensions of behavioral adjustment are useful in considering the origins of aggressive behavior: first, a dimension of *degree* related to the subject's level of reactivity or arousal in response to novelty, and a related dimension of *direction* through which reactivity is expressed as an approach to novelty or withdrawal from novelty (Cairns, MacCombie, & Hood, 1983; Hood, 1995; Tobach & Schneirla, 1968). These selectively bred mice exemplify the two dimensions, with the high-aggressive line more likely to approach and attack, while low-aggressive line mice withdraw and avoid the novel social partner. Control line animals show lower levels of reactivity, switching between approach and withdrawal in a moderated form of social exploration. The expectation is that in a nonsocial novel arena, levels of arousal will be observable through patterns of activity and approach or withdrawal tendencies in exploratory behaviors. Whether different kinds of assessments evoke similar responses in individuals is a key question. The present studies use four broadly characterized and well-established models for assessing exploratory behavior, aimed to characterize the range of responses to novelty that are manifest in these selectively bred subjects.

Developmental Changes in Exploratory Behavior

Developmental changes in the exploration of the natural setting can be considered in relation to the social ecology of the deme and the typical dispersal of young males from the natal colony (Crowcroft, 1966). Expulsion into novel arenas follows for most young males, so that the active exploration of new settings may be critical for establishing favorable adaptations. In laboratory studies, both very young (peripubertal) and very old (100–140 days) mice and rats show lower levels of locomotor and exploratory behaviors in unfamiliar settings, compared to young adults (Andrade, Tomé, Santiago, Lucia-Santos, & Spera de Andrade, 2003; Macri, Adriani, Chiarotti, & Laviola, 2002; Masur, Schutz, & Boerngen, 1980; Rowe, Spreekmeester, Meaney, Quiron, & Rochford, 1998; but see Hefner & Holmes, 2007; Renner, Bennett, & White, 1992). In particular, Nadel, Wilson and Kurz (1993) found that individual rat pups acquire exploratory behavior abruptly, with eightfold increases from 1 day to the next in object exploration (with a novel object placed into the home cage). This transition occurs between 17 and 25 days of age, when the processes of weaning and obtaining novel foods also may begin (also see Goodwin & Yacko, 2004). By contrast, withdrawal responses to social stimulation (odors from neonates) appear in juveniles at about 25 days (Rosenblatt & Mayer, 1995). Object exploration in Institute for Cancer Research (ICR; CD-1) mice peaks at young adulthood, age 48 days, while locomotor activity in a novel arena peaks at age 90 days (Ricceri, Colozza, & Calamandrei, 2000). In the ICR foundation strain from which the selectively bred lines are derived, aggressive behavior peaks at age 90 days (Cairns, Hood, & Midlam, 1985).

Assessment of Exploratory Behaviors

To consider a range of exploratory behaviors, multiple methods of assessment are employed, using a large and complex open field with novel objects and climbing structures, a two-chambered light–dark box, a plus maze with four elevated runways, and an opportunity to consume a preferred food in a novel arena. Each of these paradigms offers a distinctive perspective on novelty and exploration. Assessments of free and spontaneous exploratory behavior are most compatible with observation in a large and complex novel arena. An additional step in the assessment of free exploration is to add an ''emergence'' component. This component assesses the reluctance or eagerness of animals to emerge from a protected location, such as their home cage. Fuller (1967) proposed that this initial stage of emergence yields the most sensitive measure of differences in emotion and temperament. By placing the home cage containing the subject into a novel arena, measures of latency to emerge from the home cage become informative, as well as measures of exploratory behavior in the novel arena (Hall, 1941; King 1970; Whitshaw, Gharbawie, Clark, & Lehman, 2006). Another extension of the open field procedure is the addition of novel objects to yield a measure of object investigation. Placing a novel object into the home cage also has been employed, using latency to contact the object as a measure of exploratory behavior (Bateson & D'Undine, 1968; Berlyne, 1950; Nadel et al., 1993; Renner, 1990; Renner et al., 1992; in primates, Champoux et al., 2002).

A different ethological approach is based on the diurnal patterns of rodents, with the observation that burrow-dwelling, nocturnally active species find very bright lighting to be aversive. In the light–dark box, mice are placed into a small, enclosed dark compartment and are free to move into a brightly lighted area. Entering and remaining in the brightly lighted box is interpreted as exploratory behavior (Ramos, Berton, Mormede, & Chaouloff, 1997).

The novel arena of the elevated plus maze offers burrow and trail-like features for exploration, with four runways set on a tall stand, two with walled (closed) runways and two open runways without walls. When the apparatus is built of transparent Plexiglas, the plus maze offers no cover from conditions of bright lighting. When built with opaque materials, the plus maze resembles the light–dark box, with two runways arms resembling a dark burrow system. Exploratory behavior is recorded when the subject enters the open arms without walls, presumably risking a fall to the floor (Green & Hodges, 1991; Hogg, 1996; Lister, 1987).

An additional protocol for the assessment of adjustment to novelty is the novelty-induced hypophagia paradigm, with a preferred food made available in the home cage, followed by access to the preferred food in a novel setting. Reactivity in the novel setting is indicated by longer latency to begin eating and smaller amounts consumed, compared to the familiar arena (Dulawa & Hen, 2005).

Strain and Line Differences in Exploratory Behaviors

Inbred strains of mice display remarkable diversity in behavioral features. In a comprehensive assessment of correlated behaviors among 10 inbred strains of mice, Guillot and Chapouthier (1996) found that aggressive behaviors and exploratory behaviors were strongly associated. With 20 males assessed from each strain, the rank-order correlation is −0.86 for percent of males attacking and time in the lighted chamber of the light–dark box. Males from the high-aggressive strains were found to be significantly *less* exploratory than males from the low-aggressive strains. However, measures of activity (the number of transitions between the light and the dark boxes) showed no relation to aggressive tendencies. These results offer a comparison to the findings from comprehensive multi-laboratory assessments of Crabbe, Wahlsten, and Dudek (1999), who employed a battery of assessments including the elevated plus maze to provide a broad portrayal of the characteristic behavioral propensities of three inbred strains that were tested in both studies (DBA, BALB, and C57; see Table 1). Of the three strains, DBA mice were highest in aggressive behavior and lowest in exploratory behaviors in 4 independent samples and 11 sets of behavioral assessments. These convergent

Table 1. Rank Order of Mouse Strains on Assessments of Aggressive Behavior and Exploratory Behavior

Mouse Strain	Aggressive Behavior[1]	Light–Dark Box[1]	Plus Maze[2]	Plus Maze[3]	Plus Maze[4]	Open Field Activity[2]	Open Field Activity[3]	Open Field Activity[4]	Plus Maze Arm Entries[2]	Plus Maze Arm Entries[3]	Plus Maze Arm Entries[4]
DBA	1	1	1	1	1.5	1	1	1	1	1	1.5
BALB	2	2	3	3	1.5	2	2	3	2.5	2	1.5
C57	3	3	2	2	3	3	3	2	2.5	3	3

[1]Guillot and Chapouthier, 1996. Proportion of males attacking: 1 = highest proportion. Light–dark box, 1 = least time in the light box.
[2]Crabbe et al. (1999). Portland, Figures 1, 2; 1 = least amount of time in the open arms or least activity (horizontal activity in the open-field or number of arm entries in the plus maze).
[3]Crabbe et al. (1999). Edmonton, Figures 1, 2; 1 = least amount of time in the open arms or least activity.
[4]Crabbe et al. (1999). Albany, Figures 1, 2; 1 = least amount of time in the open arms, or least activity.

results suggest that aggressive behaviors and exploratory behaviors are inversely related.

Selective breeding studies of aggressive behavior have assessed line differences in other behavioral domains. The lines of mice selectively bred for differences in aggressive behavior by van Oortmerssen and colleagues showed no line difference in the light–dark box, while the high-aggressive line males displayed less exploratory behavior in an exposed area of a tunnel maze (Hogg et al., 2000). Tests were administered at age 140 days after a series of location changes. By contrast, in the selectively bred Lagerspetz lines, high-aggressive line males were more active in open areas of the plus maze and in the light compartment of the light–dark box (Nyberg, Vekovischeva, & Sandnabba, 2003). These animals were subjected to a battery of assessments with repeated measures starting at age 110 days.

A large program of selective breeding of both mice and rats for differences in exploratory behavior utilizes the elevated plus maze as the core assessment (for a review, see Landgraf et al., 2007). Using ICR mice, line differences in activity in the elevated plus maze were replicated across assessment paradigms: in the light–dark box, and in additional contexts for the assessment of locomotor activity. However, another recent result demonstrates the specificity of selective breeding procedures. Mice that were selectively bred for thigmotaxis (wall-hugging) in the open field over 23 generations showed consistent differences in thigmotaxis, but did not show differences in ambulation scores in the open field (Leppanen, Ravaja, & Ewalds-Kvist, 2006; also see Paterson, Whiting, Gray, Flint, & Dawson, 2001).

In selectively bred Wistar rats (Landgraf et al., 2007), the low-exploration line showed marked passivity and freezing in social interactions, with some evidence for higher levels of stress reactivity. In earlier studies, rats that were selectively bred for low levels of activity also showed lower levels of aggressive behavior than the high-activity line (Hall & Klein, 1942; Kitaoka & Fujita, 1991). The Maudsley reactive and non-reactive rats were selectively bred for low or high levels of exploratory behavior in the open field, but more recently have been found to behave inconsistently in the elevated plus maze and other assessments (Paterson et al., 2001).

Preliminary Studies

To produce replicated lines of mice that reliably differ in aggressive behavior, Robert B. Cairns initiated a program of selective breeding for high or low levels of aggressive behavior based on a 10 min dyadic test of social interactions involving an isolation-reared subject and a group-reared test partner from the ICR Swiss Webster albino outbred strain. After 30 generations of selective breeding, within-line breeding was instituted in the Penn State colony to maintain the high-aggressive or 900 line, the low-aggressive or 100 line, and the control or 500 line which was bred without selection for behavior. Line differences in aggressive behavior were significant in the third generation of selection and in every generation thereafter, with a correlated character in the 100 line of increased freezing (prolonged immobility after social contact) in the dyadic test. Developmental analyses revealed that line differences in social behaviors at the criterion age of assessment (age 45 days) were achieved by a process of neoteny in the low-aggressive 100 line, whereby the development of aggressive behavior occurs later in ontogeny, and by the acceleration of development in the high-aggressive 900 line, with earlier onset of attacks compared with the 500 line (Cairns et al., 1983; Gariépy et al., 2001). Notably, the behavioral components of aggressive behavior are identical in all three lines, although they vary significantly in frequency and latency.

An initial puzzle was posed by observed high levels of freezing (prolonged immobility) in the 100 line mice, with continuing increases in the level of freezing behavior over generations of selective breeding. This occurred despite the criterion for selection, which was for low levels of aggressive behavior (Gariépy, Hood, & Cairns, 1988). However, when low-aggressive and 900 line males were treated with an anxiolytic drug (Weerts, Miller, Hood, & Miczek, 1992), the expectation was that the low-aggressive line males would begin to show more normal social behaviors in a novel social setting. However, after treatment with chlordiazepoxide, the low-aggressive line males did not change; rather, the 900 line males showed reduced levels of aggressive behaviors and increased levels of exploratory prosocial behaviors with a novel social partner. Among untreated mice, those from the low-aggressive line had the highest levels of endogenous activity in the GABA system (at the benzodiazepine receptor site on the $GABA_A$ receptor complex, assessed by chloride flux in specific brain areas), with lower levels in control line mice, and lowest levels of GABA system activity in high-aggressive line males. A replication of this result by Nehrenberg, Gariépy, Cyr, and Wetsel (in preparation) used related ICR male mice from the Cairns selectively bred lines maintained at the University of North Carolina. An extension of these findings of high levels of anxiety-like behaviors supports the hypothesis that *900 line males will show the lowest levels of exploratory behavior, with exploration inhibited by anxiety-like behaviors*.

An alternative hypothesis is based on differences in a behavioral inhibition system, as ''executive control'' or as Pavlov's concept of ''strength of inhibition'' (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001; Fox, Henderson, Marshall, Nichols, & Ghera, 2005; Gray,

1964). From this perspective, the 900 line males are hypothesized to have less inhibitory control of aggressive behaviors and exploratory behaviors. Supporting this possibility are differences in behavioral and neural reactivity after isolation housing in the Cairns lines, with increased social approach and attacks by 900 line males in reaction to social contact, whereas low-aggressive line males are more likely to show freezing (tonic immobility) or withdrawal in response to social stimulation. Isolation-housed 900 line males have significantly increased dopamine receptor densities in the nucleus accumbens and caudate nucleus, compared to group-housed 900 line males and compared to low-aggressive line males (Gariépy, Gendreau, Mailman, Tancer, & Lewis, 1995; Lewis, Gariépy, Gendreau, Nichols, & Mailman, 1994; also see Cardinal et al., 2001). These findings support an interpretation of high inhibitory tone in the 100 line, manifested as tonic immobility (freezing), with low inhibitory tone in the high-aggression line. The hypothesis follows that *900 line males will show the highest levels of "impulsive" exploratory behaviors, due to low levels of inhibitory control*.

Preliminary studies of exploratory behavior addressed these issues with males from the Cairns lines with two paradigms in four independent replications: ambulation and defecation in an open field, and exploratory behavior in a complex novel arena (Hood, unpublished data). During 10-min observations, there were no line differences in the activity or defecation. However, two measures of exploratory behavior did point to line differences: latency to emerge from a start box (the home cage) into the novel enclosure, and latency to contact a novel object in the enclosure. In a subsequent study, observations were carried out under dim illumination in a large novel enclosure constructed from a galvanized watering tank (described in Experiment 1). Each subject in his home cage was placed into the tank. To begin observations, the cage top was removed and replaced with a wire mesh ramp, bridging the wall of the home cage and descending into the large enclosure. Compared to 100 line males, males from the 900 line seemed to be slower to emerge from the home cage but were not significantly different. Nine hundred line males did tend to more quickly contact a novel object after emerging from the home cage ($t\ (14) = 2.01,\ p = .06$). Other measures (ambulation, rearing) showed no line differences.

These observations suggested that a rigorous investigation of spontaneous exploratory behaviors in a variety of assessment situations would clarify the relationship of social and aggressive reactions to novel conspecifics, and exploratory behaviors in novel arenas. Moreover, developmental changes in exploratory behavior within each line may reveal key factors that mediate the obtained line differences in social and aggressive behaviors (Cairns et al., 1990).

In summary, four hypotheses are proposed. Old male mice (>100 days) will be less likely to explore novel settings than young adult males, while young adult males may be more likely to explore novel objects and settings than older mature mice (Ricceri et al., 2000). The expectation from pilot studies is that latency measures in the 900 line males will show delayed entry into novel settings and reduced exploratory activity in novel settings such as the light box (in the light–dark box setting) or the large novel arena. This may be the result of interference from anxiety-like or withdrawal behaviors (Weerts et al., 1992; Guillot & Chapouthier, 1996). At the same time, after voluntarily emerging from the home cage into the novel arena, 900 line males are expected to show faster approach to novel objects with relatively higher levels of exploratory activity, due to generalized disinhibition of behavior in the 900 line (Gariépy et al., 1995; Lewis et al., 1994). Control 500 line males are expected to show lower levels of reactivity to new settings and lower levels of exploratory behaviors.

Methods

To assess a wide range of situations for exploration, the protocols are deliberately varied, including a quite large open field with novel objects, a light–dark box, an elevated plus maze, and an opportunity to consume a preferred food in a novel setting (neohypophagia). In each assessment situation, measures of latency and frequency of movements, together with exploratory behaviors that are unique to each situation, will be of interest. All studies were carried out according to the *Guide for the Care and Use of Laboratory Animals* and approved in advance by the Institutional Animal Care and Use Committee of the Pennsylvania State University. For all studies, subjects were individually housed in transparent plastic mouse compartments ($28 \times 18 \times 13$ cm) after weaning at age 21–23 days, until testing, and had free access to Purina 5001 lab chow and water except during testing. Subjects were maintained on a reversed light–dark cycle and were tested during the first half of the dark phase under dim red illumination. However, the light–dark box and the brightly lighted plus maze trials included bright lighting as a condition of the assessment. Each animal was tested one time only. Observers were blind with regard to the line designation of subjects.

In statistical tests, degrees of freedom for t tests are adjusted for unequal variance between groups. Two-way comparisons are two-tailed unless indicated otherwise. Latency scores were subjected to a log transformation as needed to reduce skewness and kurtosis.

EXPERIMENT 1

Developmental Change and Line Differences in Exploratory Behavior

To evaluate the extent of developmental changes and line differences in a variety of exploratory behaviors, a series

of observations was carried out using a complex novel arena. To realize a key objective of the protocol—assessing spontaneous exploration—mice in their own home cages were placed into the novel arena, where they were free to emerge from the home cage at will to explore the surroundings and objects in the arena. Line differences in aggression were maintained with continued selective breeding. For example, mean scores for brothers of subjects in the 24th generation, tested at age 45 (±2 days), showed an average of 40.57 attacks and 0 freezing events for 900 line males, compared to 0 attacks and 12.73 freezing events on average for 100 line males. The lines in this generation are significantly different ($p < .001$) for attack frequency, attack latency, and freeze frequency (Cairns et al., 1990). Scores for 500 line males should be intermediate between 900 line and 100 line males, for all measures.

Subjects. The sample was composed of 78 male mice from the 24th, 28th, and 29th generations of three selectively bred lines. To determine the extent of developmental change in exploratory behaviors, 47 males were assessed at young adulthood (age 43–47 days), and an independent group of 31 males was assessed at maturity (age 105–120 days). Line differences in exploratory behavior were evaluated with 34 male subjects in the 100 line (12 from the 24th generation, 9 from the 28th generation, and 13 from the 29th generation), 30 males from the 900 line (12 from the 24th generation, 7 from the 28th generation, and 11 from the 29th generation), and 14 males from the 500 line (5 from the 28th generation and 9 from the 29th generation).

Apparatus. For assessing exploratory behavior, a large enclosure (75.5 × 59 × 59 cm) was constructed using an oval galvanized metal watering tank. In the enclosure were three novel objects: an arched wire mesh climbing structure 17 cm high, placed 20 cm from the end of the enclosure, a plastic elbow pipe 6 cm in diameter and 23 cm long, and a wadded ball of paper. The two smaller objects were placed equidistant from the start box and the wire structure, 20 cm apart. Bedding material (wood chips) identical to the home cage bedding was spread on the floor of the apparatus and fresh bedding was replaced for each observation.

Procedure. Subjects were housed for 1 week prior to testing in plastic shoebox cages that had a round hole 5 cm in diameter cut into one end, and a removable hole cover attached to close the hole. Observations were carried out under dim red illumination during the dark phase of the light cycle. For each observation, one cage containing a subject was placed into a marked location 20 cm from the opposite end of the apparatus. The wire cage top with food and water was left in place, and the hole cover was removed at the start of the observation. All subjects exited from the home cage during the 20-min trial. After subjects' first emergence from the home cage, the experimenter recorded the occurrence of exploratory behaviors for 20 min. A tone sounded at 15 s intervals, allowing reasonably accurate estimations of duration as well as frequency.

Measures. Exploratory behaviors were scored with acceptable reliability among three independent observers, including *exit latency* (the number of elapsed seconds before the subject's first emergence from the home cage: inter-rater reliability, $r = .99$); *contact latency* (rater $r = .85$); *ambulation* (the number of half-circumambulations of the enclosure: inter-rater $r = .96$). Additional measures of interest include the *number of contact bouts* initiated with novel objects, the total *duration of contacts* with objects, returning to *contact the home cage*, *reentries into the home cage*, *rearing frequency*, and *climbing* on the wire structure. Reliability for these measures with three different observers exceeds $r = .93$ ($p = .05$). Timed measures are accurate within 15 s of error.

Results. To consider the combined effects of age and line, a multivariate ANOVA was executed with exit latency and contact latency as dependent measures. The 2 (age) by 3 (line) design yielded a multivariate interaction (Hotelling's $F = 1.40$, $p = .05$), a main effect of age for contact latency, ($F(1, 72) = 12.17$, $p = .001$), a main effect of line for exit latency ($F(2, 72) = 4.49$, $p = .02$), and a trend for the interaction of age and line ($F(2, 72) = 2.88$, $p = .06$; Figure 1). Developmental differences in contact latency consist of shorter latencies to contact a novel object (Fig. 1) and higher levels of ambulation (Table 2) in old males, age 105–120 days.

With a multivariate trend for interactions of line and age ($p = .05$), within-age line comparisons are of interest. Age-related changes are significant only in the 500 line, with longer exit latency ($t(12) = 3.23$, $p = .007$) and shorter contact latency ($t(12) = 3.34$, $p = .02$) in older males.

Line differences also are revealed in a priori tests, based on our pilot studies. These expectations were confirmed for the 900 line versus 100 line males, with significantly longer exit latency in the 900 line ($t(62) = 2.24$, $p = .03$). Additional findings are related to contacting a novel object after the first emergence from the home cage: 900 line males had a shorter latency to first contact with a novel object, compared to 100 line males: contact latency, ($t(62) = 1.84$, $p = .03$, one-tailed), and 900 males also showed a trend for shorter latency compared to 500 line males ($t(42) = 1.39$, $p = .09$, one-tailed; Fig. 1).

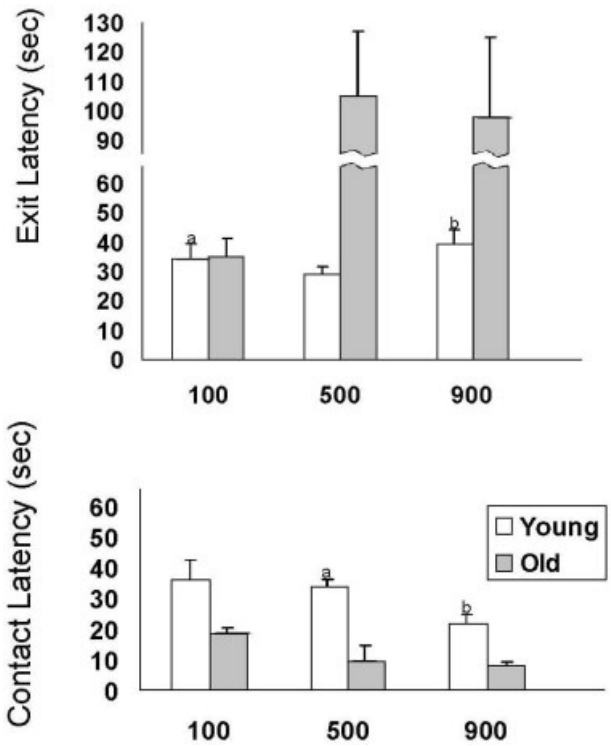

FIGURE 1 Exploratory behavior in the complex novel arena with young (∼45 days) and old (∼100 days) male mice from three selectively bred lines: 900 (high aggressive), 500 (control), and 100 (low-aggressive line). Measures include latency to emerge from the home cage (exit latency) and latency to contact a novel object after emerging from the home cage.

In young males, at about age 45 days, the line difference is confirmed with a significantly longer exit latency for the 900 line compared to the 100 line ($t\,(36) = 1.75$, $p = .05$, one-tailed). The young 900 line males also are faster to contact a novel object than young 500 line males: for contact latency ($t\,(25) = 2.60$, $p = .01$).

For older males, the 100 and 500 lines are significantly different for exit latency ($t\ (17) = 3.22$, $p = .005$) with longer latency in the 500 line, and for contact latency ($t\ (17) = 2.18$, $p = .04$), with shorter latency in the 500 line.

Additional measures of exploratory behaviors were examined to provide a description of line differences in exploratory behavior among young and old males. From a MANOVA with two factors: 2 (age) × 3 (line) for 10 observed behaviors, differences are reported in Table 2. For all effects of age or line; $p < .02$ (Table 2). In general, age differences consist of increased ambulation in older males. The most salient line differences were related to behaviors directed at the home cage, which was placed in the novel arena (with the subject inside) to begin observations of exploratory behavior. After each male's first emergence from the home cage, observations were made of spontaneous returns to the home cage, with males making contact through sniffing, rearing or walking on the exterior of the cage and cage top, or reentering the home cage through the open hole. Significantly more returns to the home cage were evident for 900 line males than for males from the 100 and 500 lines. Nine hundred line males also made more reentries into the home cage, compared to 100 line males (Table 2). An examination of the two latency scores (exit latency, contact latency) shows a correlation only for young males from the 100 line: $r\,(20) = .95$, $p = .001$, suggesting that a

Table 2. Exploratory Behaviors in the Novel Arena with a Subset of Males from Three Lines (Mean and Standard Error)

	Age		Line		
	45 Days	120 Days	100	500	900
Climb frequency	4.24 (0.90)	1.19 (0.57)	5.04 (1.27)	1.57 (0.81)	1.78 (0.56)
Climb duration (s)	9.00 (2.01)	2.28 (1.09)	NS	NS	NS
Ambulation	17.42 (2.64)	43.09 (6.75)	NS	NS	NS
Rears	NS	NS	41.91 (4.20)	16.78 (4.30)	28.11 (3.98)
Contact home cage (frequency)	NS	NS	26.68 (1.81)	31.71 (3.70)	39.66 (3.61)
Enter home cage (frequency)	NS	NS	3.81 (0.56)	3.21 (0.80)	7.28 (1.21)
N	33	21	22	14	18

Note. From an exploratory 2 (age) × 3 (line) MANOVA for 10 observed behaviors: for all effects of age or line; $p < .02$. "NS" indicates no significant difference between groups for that measure.

different process may underlie exploratory behavior in this line.

Discussion. Developmental changes are revealed in the control line, whereby older mature males in the 500 line show the same pattern as young 900 males: slower to emerge from the home cage and faster to contact a novel object after emerging. Counter to expectation, the older males were more active in ambulating about the novel arena and faster to contact novel objects, compared to younger males. Otherwise, predictions about line differences in exploratory behaviors from preliminary studies are confirmed: young 900 line males—but not old—show longer latency to exit the home cage than 100 line males, and also are faster to contact novel objects compared to 500 line males. The three lines are not different in activity. In the 900 line, males more often returned to the home cage rather than exploring the novel arena.

EXPERIMENT 2

Line Differences in Exploratory Behavior in the Light–Dark Box

Opportunities for exploratory behavior in the light–dark box are similar in some dimensions to those in the novel arena employed in Experiment 1. Both constitute a novel arena for subjects, and both allow the animal to freely explore the arena, or to retreat. Other features of the light–dark box are distinctive: the subject is placed into the dark side of the apparatus. The start box itself is novel, and both chambers of the box are empty of objects, differing only in the level of illumination. An assumption in this procedure is that the brightly lighted box is more aversive to nocturnal rodents, especially for albino rodents. The expectations from the results of Experiment 1 are that 900 line males will be slower to first enter the light box, and will spend less time exploring the light box compared to 100 line males and 500 line males. A third expectation is that there will be no line differences in activity, here measured as transitions between chambers.

Subjects. Mice from the 32nd generation of the two selectively bred lines and the control line, age 42–56 days, were assigned to the assessment of exploratory behavior in the light–dark box, with 16 males from the 900 line, 15 males from the 100 line, and 17 males from the 500 control line.

Apparatus. The light–dark box consists of a dual-chambered container with the walls and floor of the light box of opaque white Plexiglas with a transparent Plexiglas lid, while the walls, floor, and lid of the dark box are made of opaque black Plexiglas. For each chamber, the floor is 20×20 cm square; walls are 14 cm high. A wall of black Plexiglas separates the two chambers and includes an opening (4×4 cm) cut at the bottom to permit the animal to move between the two chambers.

Procedure. For behavioral assessments, the light–dark box was placed on a platform and was brightly lighted from above by a lamp (890 lux from a 100 W bulb placed 39 cm above the platform floor). Clean wood chip bedding identical to that in the home cage was replaced in both chambers of the light–dark box prior to each animal's test. All other lights in the room were extinguished.Immediately prior to testing, the animal's home cage was brought to the dimly lighted testing room adjacent to the colony room. The subject was removed from the home cage by hand, using the base of the tail, and placed in the center of the dark box. The lid was quickly closed and the 100 W bulb was turned on to begin the trial. In a 5-min observation period, records indicate when the subject enters the light box or enters the dark box.

Measures. From records of transitions between the dark box and the light box, three measures were derived: *latency to first entry into the light box* (s), *total amount of time in the light box* (s), and the number of *transitions* between the dark box and the light box. Entry into a box (transition) was scored when the animal first placed all four paws into the adjacent compartment. Timed measures are accurate within 5 s of error. High levels of inter-rater reliability were obtained for latency to enter the light box ($r\,(8) = .99, p = .01$) and for the number of 5 s periods spent in the light box ($r\,(8) = .97, p = .01$).

Results. The expectations from Experiment 1 are substantially supported in a priori *t*-tests. The general expectation that 900 line males would show less exploratory behavior than the two other lines was supported. The 900 line males showed a significantly longer latency to first enter the light box, compared to the 100 line ($t\,(29) = 2.12,\ p = .04$) and the 500 line ($t\,(31) = 1.97, p = .05$). The 900 line males spent less time in the light box than 100 line males ($t\,(29) = 3.78, p = .001$) and made fewer transitions than 100 line males ($t\,(29) = 2.21,\ p = .03$) and 500 line males ($t\,(31) = 2.3,\ p = .01$). In addition, 500 line males spent less time in the light box than the 100 line males ($t\,(30) = 2.03, p = .05$) (Table 3).

Discussion. Exploratory behavior in the light–dark box is consonant with line differences observed in the novel arena, with 900 line males showing less exploratory behavior—longer latency to enter the light box and

Table 3. Exploratory Behavior in the Light–Dark Box (Mean and Standard Error)

	Line		
	100	500	900
Exploratory behaviors			
Latency to enter light box (s)	18.33*	19.41*	37.50*
	(4.13)	(3.05)	(8.88)
Time in the light box (s)	193.16*	161.02*	141.87*
	(11.20)	(11.26)	(7.86)
Transitions	30.40*	31.11*	22.38*
	(3.41)	(2.99)	(1.42)
N	15	17	16

*Significant differences ($p < .05$).

less time in the light box—as well as fewer transitions between the two chambers of the light–dark box, compared to100 or 500 line males. In this assessment, activity (transitions between the light and dark boxes) shows line differences with the 900 line less likely to transition between boxes.

EXPERIMENT 3

Exploratory Behavior in the Elevated Plus Maze

Based on the consistent line differences in exploratory behavior observed in two distinctive procedures, the expectation was that parallel line differences would be observed in a widely employed assessment protocol, the elevated plus maze. The 900 line males were expected to spend less time in the open arms of the plus maze (the arms without walls), and to show fewer entries into the open arms, compared to 100 line males. Control line males were expected to produce scores intermediate to the two selectively bred lines. Lower levels of activity (the total number of arms entered) were expected in the 900 line.

Two additional factors were taken into account: the effect of the level of illumination of the plus maze and whether the location and orientation of the placement of the male in the plus maze affected subjects' choice of arm entry. We also proposed that 900 line males would be less likely to foray to the end of the open arms, and would show fewer exploratory head dips over the edge of the maze while on the open arms. Finally, we speculated that self-grooming behaviors might show line differences if they are functioning as displacement behaviors in an uncertain and novel arena, with more self-grooming behaviors by 900 line males.

Subjects. Subjects were males drawn from the 37th, 38th, and 39th generations of the selectively bred lines of mice (900 line, $n = 63$; 100 line, $n = 62$; 500 line: $n = 20$). Testing occurred at ages 82–110 days.

Apparatus. A murine plus maze was constructed following the guidelines first set forth by Lister (1987) and more specifically by Crabbe et al., 1999. Each of the four arms of the maze are 5 cm wide and 30 cm long with a 5×5 cm square in the center of the four arms. Closed arms made of clear Plexiglas are enclosed by 15.2 cm high transparent walls on three sides, while the open arms have only a 5 mm high lip to demarcate the edges of the arm. The floor of the maze is made of opaque black Plexiglas and is raised 48.7 cm from the floor on a wooden plus-shaped platform. The transparent walls fit onto the closed arms so that the walls can be removed for cleaning of the arms and the walls between assessments.

Procedure. Plus maze tests were conducted under two lighting conditions, dim (24.4 lux) and bright (899.6 lux). The maze was lighted from directly above the center square by a 15 W bulb placed 99 cm above the maze floor (dim condition) or a 100 W bulb placed 63 cm above the maze floor (bright condition). A video camera was set up between two adjacent arms so that all four arms were clearly visible. Objects in the room were masked with white sheets, and were placed in symmetry about the plus maze.

Prior to testing, the experimenter lifted the subject by the base of the tail to place it onto the center square of the maze. A 5 min test was video recorded for later coding of behaviors. Following the test, the experimenter recorded the number of fecal boli in the maze and locations on the arms with urine on the maze floor. The maze was cleaned with a 10% alcohol solution between tests.

Measures. Behaviors were recorded in 5-s intervals from videotaped records. Time sampled behaviors included *location in the open arms*; *closed arms or center square*

(all four paws in one location; assessed in 5 s intervals); and *self-grooming bouts*. Frequency counts were recorded for *open arm entries* (when an animal moved all four paws into an open arm); *closed arm entries; open arm forays* (an animal moved to the end of an open arm); *head dips* in the open arm (the nose was dipped over the edge of the maze with the eyes at or below the level of the floor); *transitions* (from one arm into another arm with all four paws); *rears* (where both forepaws had to be on a wall). Inter-rater reliability for two observers coding from videotapes of 10 subjects was acceptable for time in the open arms ($r = .90$), time in the closed arms ($r = .90$), time in the center square ($r = .93$), total number of head dips ($r = .99$), grooming bouts ($r = 1.0$), and total number of rears ($r = .98$).

In analyses, a priori *t* tests and MANOVA with three levels (three lines of subjects) were employed. Significant effects were examined using post-hoc tests (Tukey's HSD) to compare exploratory behaviors in the three lines. In addition, a set of analyses was completed that examined the effects of maze lighting conditions (bright or dim) and the effect of placement into the center square of the maze facing either an open or a closed arm on subsequent arm entry into an open or closed arm.

Results. Expected outcomes were not obtained in the plus maze. The 900 and 100 lines were not different, and the 500 line was not intermediate in behavioral outcomes. The exception is with regard to urination, which was more likely in the 900 line than the 100 line ($t\ (118) = 2.44$, $p = .02$). The frequency of self-grooming bouts ($t\ (118) = 3.53$, $p = .001$) shows a difference between 100 and 900 lines, with more in the 100 line than the 900 line. Of 30 observed grooming bouts, 29 occurred in the closed arms of the maze (Table 4).

However, line differences in the plus maze are significant, with new findings that vary from patterns observed in the novel arena or the light–dark box. In the plus maze, the 500 line has distinctly lower scores for exploratory behaviors compared to the two selectively bred lines. Both 100 and 900 line males spent more time on the open arms, and did not differ from each other, compared to males from the 500 control line. In a MANOVA, the measure of latency to first exit from the center square shows no line differences, while other variables do show significant line differences (F (2, 113) = 3.27 to 7.33, $p =$ s range from .04 to .001) for total time in the open arms, total number of arms entered (activity), number of fecal boli, presence of urination, and number of grooming bouts. In general, the control 500 line is different from the 100 and 900 lines, (Tukey's HSD; $p =$ s range from .04 to .001), whereas the 100 and 900 lines do not differ in post-hoc tests. The exceptions are urination (500 and 900 lines are different, $p = .03$, with a post-hoc trend for 100 and 900 to differ, $p = .08$) and grooming bouts (100 and 900 lines are different, $p = .04$; Table 4).

Of the remaining issues, only differences in lighting for the plus maze (bright or dim) showed an effect on behaviors in these albino males. In bright light, scores for total number of arms entered (activity) are lower than in dim light (for bright condition, $x = 13.29$, for dim condition, $x = 18.94$; $t\ (124) = 3.73$, $p < .001$). That effect does not interact with line (Goodrick, 1973). Other comparisons showed no line differences for head dips, rears, or forays to the end of arms, and no effects or

Table 4. Exploratory Behavior in the Plus Maze (Mean and Standard Error)

	Line		
	100	500	900
Time in the open arms (sec.)	77.05[a]	39.40[b]	77.71[a]
	(7.99)	(8.45)	(8.86)
Number of entries to open arms	6.71**	4.14[a]	7.35[b]
	0.75	0.89	0.65
Self grooming bouts	1.03[a]	0.66	0.63[b]
	(0.08)	(0.12)	(0.07)
Total number of arm entries	16.66[a]	11.90[b]	16.57[a]
	(1.14)	(1.68)	(1.14)
Fecal boli	2.00[a]	0[b]	2.04[a]
	(0.31)	0	(0.36)
Urine spots	0.32	0.14[a]	0.54[b]
	(0.06)	(0.08)	(0.06)
N	62	21	63

Significantly different by post-hoc MANOVA tests (Tukey's HSD) $p < .05$.
*Indicates a trend ($p = .07$) for the 100 versus 500 line.
Note: Superscripts a and b are.

interactions with line for the outcome measures of interest (time in the open arms, total number of arms entered) or for aspects of test procedures: initial arm entered, direction facing when placed in the center square, and initially starting from the closed arm.

Discussion. The line differences that are obtained in the plus maze are distinct from previous findings in the novel arena or the light–dark box. The 500 line shows less exploratory behavior: less time in the open arms, fewer entries to the open arms, and less activity (total arm entries). Latency scores in this paradigm are not informative due to the forced placement of the animal into the small center square of the apparatus to begin the test. A number of subjects were observed to run indiscriminately through the apparatus without regard to open or closed arms, in what seemed to be frantic disarray (e.g., Matzel et al., 2006, p. 232). Fewer excretions of fecal boli and urine by the 500 line may be related to the pattern of 500 line males spending less time in the unrestricted open arms. The 100 and 900 lines are not different in this assessment.

EXPERIMENT 4

Neohypophagia: Inhibition of Consummatory Behavior in a Novel Arena

The inhibition of normal behaviors in a novel setting includes the inhibition of eating. By comparing food consumption in a familiar setting and in a novel setting, a reduced level of consumption in the novel setting suggests that novelty-related behavioral adjustments may compete or interfere with food consumption (e.g., Dulawa & Hen, 2005).

Subjects. Mice from the 52nd generation of breeding were subjects, with 12 males from the 100 line and 12 males from the 900 line, weaned at age 23 days and individually housed until assessments at age 56–70 days.

Procedure. On four consecutive days, male mice in their home cages were given the opportunity to a novel food, a sweetened milk solution (1:3 parts canned sweetened condensed milk (Carnation) to warm water). Graduated cylinders with sipper tubes were used to measure the amount of solution consumed in a 30 min exposure. Water bottles were not available for the 30 min test period.

On the 5th consecutive day, animals were assessed in a novel setting, an adjacent room with cages containing no bedding, but rather warm water 2 cm deep. Each male was placed into the novel watery cage, a bare cage top was replaced on the cage, and the milk solution was made available. Bright lights were turned on to start the trial. Measures obtained are *latency to first consume the solution*, and *the amount consumed* in 30 min., with a critical comparison between consumatory behaviors on Day 4 in the home cage, and consumatory behaviors in the novel arena on Day 5. Inter-rater reliability for readings of the amount of milk in the graduated tube was acceptable (r (6) = .97, p = .001) with two observers.

Results. Significant line differences were obtained in the Day 4 (home cage) consumption patterns and again on Day 5, with a strong reversal of effects over 2 days of assessment. In Day 4 observations, 900 line males showed significantly shorter latency to first drink (t (23) = 1.76, $p = 0.04$, one-tailed), and significantly larger amounts of solution were consumed by 900 line mice, compared to 100 line mice (t (23) = 2.80, $p = 0.01$). On Day 5 in the novel arena, these results were up-ended. Nine hundred line mice showed a significant reduction in the amount consumed, compared to 100 line mice: for change scores (Day 4–5), t (23) = 3.00, $p = 0.01$ (Table 5).

Discussion. The expectation of decreased consumption in the novel arena was amply realized in the 900 line. Males reduced consumption in the novel setting by almost half compared to the amount consumed in the familiar setting on Day 4. For the 100 line, consumption *increased* by half in the novel arena. The pattern of fast approach and large amounts consumed on Day 4 arguably shows an impulsive style which is disrupted on Day 5 by the need to provide behavioral adjustments in a novel arena. The

Table 5. Neohypophagia in S_{52} Males: Consumption in Familiar (Day 4) and Novel (Day 5) Arenas (Mean and Standard Error)

	100 Line	900 Line
Day 4 amount	0.21	0.61*
	0.06	0.13
Day 4 latency	48.31	22.50**
	13.05	5.79
Day 5 amount	0.41	0.36
	0.08	0.10
Day 5 latency	526.92	540.08
	169.58	142.29
Change amount	0.19	−.25***
	0.08	0.13
Change latency	478.61	517.58
	169.77	146.2

Note. 100 line is the low-aggressive line ($N = 13$). 900 line is the high-aggressive line ($N = 12$). Amount in mL. Latency is seconds. Change scores are Days 4–5.

*$p = .01$.

**$p = .09$.

***$p = .007$.

increase in consumption in the 100 line in the novel arena may represent a high level of reactivity or activity in response to the novel arena. If this finding is replicated in future experiments, comparisons with control 500 line mice will be important for understanding the foundational aspects of this phenomenon.

DISCUSSION

In this examination of dimensions of exploratory behavior, two questions were raised about developmental change in exploratory behaviors and the differential expression of exploratory behaviors in lines of mice selectively bred for differences in aggressive behavior. Three of the present findings support the negative relationship between aggressive behavior and exploratory behavior reported by Guillot and Chapouthier (1996), Hogg et al. (2000), and Crabbe et al., 1999. High-aggressive line males were slower to leave the home cage (Experiment 1) and the dark box (Experiment 2), and drank less in a novel setting (Experiment 4). These varied forms of experimental assessment offer distinctive perspectives on the development and underpinnings of exploratory behaviors.

Developmental Change in Exploratory Behavior in the Novel Arena

Alterations of developmental timing through selective breeding of the 100 and 900 lines produced changes in aggressive expression over generations (Cairns et al., 1983; Gariépy et al., 2001). Developmental processes are highlighted in the consideration of the control 500 line, which was bred without regard for social-interactional behavior. The 500 line showed evidence of developmental changes in exploratory behavior, as assessed in the most complex setting for free and spontaneous exploration, the novel arena. Old males, age 105–120 days, were more active than young males in ambulating about the novel arena. Other investigators have reported similar findings, with higher rates of ambulation in older animals; Renner et al., 1992 with Long-Evans rats at 30, 60, and 90 days of age, and Hefner and Holmes, 2007, who used C57BL/6J mice at 28, 42, and 56 days (but see different results from Macri et al. (2002) using CD-1 mice in a plus maze at 35, 48, and 60 days of age; Stansfield & Kirstein (2005) using Sprague–Dawley rats with a novel object at ages 30 and 60 days of age). Few studies continue to include subjects which are 100–200 days old.

Old males in the control 500 line show a process of aging in which they become slower to emerge from the home cage and faster to contact a novel object, a pattern which resembles that of young 900 males. In these young males, additional aspects of accelerated maturation are associated with the early onset of aggressive behavior: relatively long latency to exit the home cage and short latency to contact objects. By contrast, 100 line males manifest the opposite trend, a prolonged retention of juvenile characteristics (neoteny), manifested in old 100 line males as exit latencies equivalent to young 100 line males. For comparison, the control 500 line males represent more normative age changes (Cairns et al., 1983; Gariépy et al., 2001).

Line and Age Differences in the Novel Arena

The selectively bred lines showed a range of outcomes in the relationships of aggressive and exploratory behaviors in the novel arena. In Experiments 1, 2, and 4, the 900 line males showed slower approach or lower levels of exploratory behaviors. However, the pattern found in young 900 line and older 500 line males' behaviors seems paradoxical, with slowest exit from the home cage, and fastest approach to contact a novel object, together with high levels of returns and re-entries to the home cage by 900 line males (Table 2). (An interpretation of these behaviors in relation to approach, withdrawal and reactivity to novelty is discussed below.) Relationships between exit latency and contact latency in the novel arena show a positive correlation only for young 100 line males, suggesting that approach to novel stimuli is a predominant response for the 100 line males.

Line Differences in Exploration: Other Paradigms

Three additional environments for exploration used in these studies offered different parameters of novelty, one paradigm with extreme differences in lighting (the light–dark box), one with elevated runways (and the possibility of falling), and a novel environment for consumption of a favorite food. Age was not a variable in these designs. It turns out that the simplest apparatus provides the clearest outcome. In the light–dark box, line differences are consistent with findings in the novel arena and in the neohypophagia test: 900 line males are slowest to enter the light box, remain longer in the dark box, and drink less milk in the novel environment.

While the consistency of findings among selectively bred lines and strains is remarkable (Guillot & Chapouthier, 1996; Hogg et al., 2000), the failure to confirm patterns with one set of selectively bred lines is also notable (the Lagerspetz lines of mice selectively bred for differences in aggressive behavior: Nyberg et al., 2003). This different outcome may be partly related to experimental factors: use of a plus maze using opaque walls, testing during the bright portion of nocturnal animals' diurnal cycle or the

use of dim lighting in the behavioral assessment (350 vs. 900 lux in the present assessments). Also of interest is the possibility that the Cairns and Lagerspetz lines are fundamentally different. Considering only the first occasion of testing, the key measures (time in the open arms, time in the light box) were equivalent for the Lagerspetz high-aggressive and control lines, with lower scores for the low-aggressive line. Another interpretation is that the Lagerspetz lines present a model of ''proactive'' or instrumental aggression, while the Cairns lines represent ''reactive'' or anxious/impulsive aggression (Raine et al., 2006; Vitaro, Brendgren, & Barker, 2006).

It is noteworthy that some subjects in the present study showed hyperactive behaviors in the plus maze, running at speeds that appeared to be inconsistent with exploratory behavior. These ''runners'' may account for the pattern of results in the plus maze. Hyperactivity also is observed in the open field (Lester, 1968; Matzel et al., 2006; Nadel et al., 1993; Tobach & Schneirla, 1968). High levels of ambulation are proposed by Tobach and Schneirla (1962) as dialectical forms: either exploration and stimulus seeking or, alternatively, anxiety, escape behaviors, and fear-induced agitation. Along with these ambiguities of interpretation, the plus maze is viewed as ''the most intrinsically unstable task'' (Crabbe et al., 1999, p. 1671; also see Do-Rego et al., 2006; Mineur, Belzung, & Crusio, 2006).

Approach and Withdrawal

The structure of exploratory behavior by young 900 line males and older 500 line males in the novel arena consists of long latency to emerge from the home cage, suggesting a reluctance to experience novel settings, followed by short latency to contact a novel object, suggesting impulsivity or low inhibitory control. These two aspects can be represented in a dialectical analysis with an emphasis on higher level processes that unite seemingly contradictory outcomes. Considered in the context of high levels of returns and re-entries to the home cage by 900 line males (Table 2), an interpretation of opposed tendencies (approach, withdraw) under high reactivity to novelty suggests that in the novel arena, the opposed tendencies are expressed in turn. High levels of stimulation from the novel arena first engender withdrawal responses, which delay the departure from the home cage. When these subside, approach tendencies to objects can be manifested (for a discussion, see Hood, 1995).

T. C. Schneirla's (1965) discussion of approach–withdrawal theory includes an analysis of the ''apparently paradoxical character of aggression as both approach and withdrawal'' (Tobach & Schneirla, 1968; Hood, 1995). By placing two behavioral forms, aggression and exploration, into relation within a more general behavioral system of response to stimulation, the interplay of inhibition, impulse, and stimulus-seeking might well be drawn as approach–withdrawal gradients (e.g., Brown, 1948; Hull, 1938; Miller, 1944; Ulrich, 1966). For example, to better understand the emergence of maternal behavior in rats, Rosenblatt and Mayer (1995) proposed that complex behaviors appear in the context of approach–withdrawal biphasic processes. For nonpregnant females, intense stimulation provided by pups evokes withdrawal responses from the female, which decline over time, transitioning through a phase of ambivalence and resolving with approach behaviors to pups and the eventual appearance of complex forms of maternal behaviors. This analysis offers a fresh look at the foundations of behavioral expression, suggesting that ''both approach and withdrawal responses can be aroused simultaneously'' (Hood, 1995; Rosenblatt & Mayer, 1995).

Developmental questions about individual differences in aggression and exploratory behaviors are at the heart of the issue. In an early review, Archer (1973; also see 1976) concluded that the hypothesis of a central or constitutional state of fearfulness that strongly organizes behavior across situations is not well supported, proposing instead that exploratory behaviors are best studied without assumptions about underlying motivational bases. Moreover, he critiqued the use of a battery of test situations with group scores rather analyses of individual response patterns (Lister, 1987; Pellow, Chopin, File, & Briley, 1985; Rogers, Cao, Dalvi, & Holmes, 1997; Trullas & Skolnick, 1993; also see Barrett, 2006). Support for that perspective is evident in a developmental study of ''emotionality.'' Using rats that had been selectively bred for high or low levels of ultrasonic vocalization in early life, Dichter, Brunelli, and Hofer (1996) implemented a longitudinal design using heterotypic assessments: pup ultrasonic vocalizations after separation from the dam and litter, and adult exploratory behavior in the plus maze. In general, the low-ultrasound line of rats showed more exploration of the plus maze. However, a closer look revealed scant evidence of developmental continuity: very few animals were highly emotional at both ages and in both assessments. Exploratory and social behaviors can be conceived as individual and group differences, as in the present report, and on trajectories and developmental patterns, as in previous reports (e.g., Hood, Dreschel, & Granger, 2003). The additional consideration of opposed tendencies—approach and withdrawal in dynamic tension—may provide a useful framework for understanding the emergence of complex behaviors.

NOTES

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