

Exploration in outbred mice covaries with general learning abilities irrespective of stress reactivity, emotionality, and physical attributes

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Abstract

Across multiple learning tasks (that place different sensory, motor, and information processing demands on the animals), we have found that the performance of mice is commonly regulated by a single factor (“general learning”) that accounts for 30–40% of the variance across individuals and tasks. Furthermore, individuals’ general learning abilities were highly correlated with their propensity to engage in exploration in an open field, a behavior that is potentially stress-inducing. This relationship between exploration in the open field and general learning abilities suggests the possibility that variations in stress sensitivity/responsivity or related emotional responses might directly influence individuals’ general learning abilities. Here, the relationship of sensory/motor skills and stress sensitivity/emotionality to animals’ general learning abilities were assessed. Outbred (CD-1) mice were tested in a battery of six learning tasks as well as 21 tests of exploratory behavior, sensory/motor function and fitness, emotionality, and stress reactivity. The performances of individual mice were correlated across six learning tasks, and the performance measures of all learning tasks loaded heavily on a single factor (principal component analysis), accounting for 32% of the variability between animals and tasks. Open field exploration and seven additional exploratory behaviors (including those exhibited in an elevated plus maze) also loaded heavily on this same factor, although general activity, sensory/motor responses, physical characteristics, and direct measures of fear did not. In a separate experiment, serum corticosterone levels of mice were elevated in response to a mild environmental stressor (confinement on an elevated platform). Stress-induced corticosterone levels were correlated with behavioral fear responses, but were unsystematically related to individuals’ propensity for exploration. In total, these results suggest that although general learning abilities are strongly related to individuals’ propensity for exploration, this relationship is not attributable to variations in sensory/motor function or the individuals’ physiological or behavioral sensitivity to conditions that promote stress or fear.

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1. Introduction

A “general” influence on humans’ performance across diverse tests of cognitive abilities has been described as the most dominant cognitive trait ever identified (Plomin, 1999; Plomin & Spinath, 2002). While general cognitive abilities have been vigorously studied in humans (for reviews, see Jen-

sen, 1998; Mackintosh, 1998), comparable studies in nonhuman animals have been infrequent (but see Galsworthy, Paya-Cano, Monleón, & Plomin, 2002; LoCurto & Scanlon, 1998; LoCurto, Fortin, & Sullivan, 2003; Matzel et al., 2003). Nevertheless, the topic has begun to generate interest within the broader neuroscience community (e.g., Gray, Chabris, & Braver, 2003; Plomin, 1999, 2001), and animal studies may facilitate the elucidation of the brain substrates for individual differences in learning and intellect.

We have recently reported an analysis of the performance of 56 genetically diverse outbred mice (CD-1) on a

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battery of five learning tasks (Matzel et al., 2003). Tasks included associative fear conditioning, operant (passive) avoidance, path integration in a Lashley Maze, odor discrimination, and spatial navigation in a water maze, and the tasks were included and designed such that each made unique sensory, motor, motivational, and information processing demands on the animals. Indicative of a common source of variance, positive correlations were found between individuals' rate of learning across all tasks. A principal component factor analysis of individuals' acquisition rates on each task determined that a single factor accounted for 38% of the total variance across animals. In a separate analysis, several performance variables and physical characteristics (general activity, defecation [a potential index of emotionality], running speed in a novel open field, and body weights) of the mice loaded weakly and unsystematically on the same principal factor that accounted for general learning abilities. However, the propensity of animals to explore the open areas of a walled open field (i.e., the time spent in open quadrants *relative* to time spent adjacent to the fields walls) positively correlated with performance in all learning tasks, and furthermore, loaded strongly with performance on the five learning tasks in the principal factor. This relationship between the pattern of exploration in a novel environment is particularly striking given the testing procedures in the open field were limited to a single 4 min exposure, a test duration that we have found to support no appreciable habituation (i.e., learning). Thus, it is likely that innate (not learned) exploratory tendencies were responsible for the relationship between open field performance and individuals' general learning abilities.

The propensity to explore the open quadrants of a field is often interpreted as an index of an animal's proclivity for novelty seeking, and/or may reflect the degree to which an animal experiences stress in the unfamiliar open environment (Anderson, 1993; Kabbaj, Devine, Savage, & Akil, 2000). The relationship of novelty seeking and indices of maze reasoning has previously been observed in laboratory rats (Anderson, 1993). It is notable that among human infants, the degree of preference for novelty is positively correlated with later performance on standardized IQ test batteries (Bornstein & Sigman, 1986; Vietze & Coates, 1986), an observation which further suggests that the general learning factor that we observe in this population of mice might be analogous to the *g* Factor described in humans. While the nature of this relationship between novelty seeking and learning/intelligence is unknown, it is possible that animals more engaged by novelty are more likely to recognize (or attend to) those environmental relationships upon which learning depends. Relatedly, animals that are prone to novelty seeking may be less susceptible to the experience or physiological consequences of stress, which in many instances can impede learning (McEwen et al., 1997; for review, see McEwen (2003) and Shors (1998)). Our previous data, in which we observed a strong correlation between exploratory behavior in the open field and general

learning abilities (Matzel et al., 2003), do not allow us to distinguish between these (or other) possibilities.

The present experiments were conducted to ascertain the relationship between exploratory tendencies, sensitivity to stress, physical attributes (sensory/motor responses), and general learning abilities. This was accomplished by training and testing animals in a battery of six learning tasks (cf. five learning tasks; Matzel et al., 2003), after which the animals' strength, balance, activity, pain reactivity, fear/stress responsivity, and exploratory tendencies were evaluated. In total, six measures of learning and 21 measures of unlearned behaviors or physical characteristics were recorded. Using methods of factor analysis, we determined the extent to which different classes of unlearned behaviors or behavioral tendencies aggregated with performance on the six learning tasks.

2. Materials and methods

2.1. Subjects

A sample of 43 outbred male CD-1 mice (Harlan Sprague Dawley) were 78–92 days old at the start of experimentation. These animals served in an experiment wherein learning was assessed in combination with measures of unlearned behavioral tendencies and physical attributes. The CD-1 strain was particularly appropriate for these analyses as they exhibit considerably more between-animal behavioral variability than several inbred strains that we have tested with similar procedures. Animals were acclimated to our laboratory for 20–26 days prior to testing, and were handled (removed from the home cage and held in an experimenters' hands) for 90 s/day, 5 days/week during this period. This handling insured that differential stress responses to the experimenters, and any associated effects on learning, were minimized. Animals were individually housed in clear boxes with floors lined with wood shavings in a humidity- and temperature-controlled vivarium adjacent to testing rooms. A 12 h/12 h light/dark cycle was maintained. In a separate experiment, the relationship of exploratory behaviors to basal and stress-induced corticosterone levels was assessed. For this experiment, 27 additional animals served.

2.2. General behavioral training and testing methods

Animals were trained and tested in two separate replications ($ns = 19, 24$). In both replications, animals were tested on two learning tasks (passive avoidance and Lashley Maze) and 22 measures of unlearned behaviors and behavioral attributes (see below). A subset of these animals ($n = 24$) were trained and tested on six learning tasks (passive avoidance, Lashley Maze, fear conditioning, spatial water maze, odor discrimination, and spatial plus maze). For purposes of analysis, data obtained from the larger sample of 43 animals (which included two measures of learning) were subjected to a principal component factor analysis. For confirmatory purposes and to estimate the relationship of the Lashley Maze and passive avoidance tasks to the remaining four learning tasks, a separate analysis was performed on the subset of 24 animals that had been tested on all six learning tasks.

In our previous work (Matzel et al., 2003), we used five learning tasks (Lashley III Maze, odor discrimination, passive avoidance, associative fear conditioning, and a spatial navigation in a water maze) to assess the general learning abilities of individual animals. These tasks were chosen for inclusion and designed so as to impinge on diverse sensory, motor, motivational, and information processing systems. Here, animals were tested on those five tasks and a sixth task, spatial navigation in a plus maze.

All animals ($n = 43$) were first tested in an open field (to assess activity and exploratory tendencies), subsequently on either two ($n = 43$) or six ($n = 24$) tests of learning, and lastly, on other tests of exploratory

behaviors, sensory/motor function, stress, fear, and pain reactivity. Between each successive test (of learned and unlearned behaviors), animals received a day of rest. With 1–3 days required for each task, the entire test regimen was completed in 42 days or less. Different experimenters ($n = 7$) tested animals on different combinations of tasks, and no experimenter was aware of animals' performance on other tasks until after the completion of the entire battery of tests.

Prior to testing on any task, test chambers were "primed" by exposing two nonexperimental animals (ones not included in the data analysis) to the apparatus and procedures. This was intended to standardize the apparatus such that the first animals in a test cycle encountered a chamber that was nominally similar (e.g., in odor) to that experienced by subsequently tested animals. The surfaces of every piece of apparatus were cleaned with a mild alcohol solution following removal of every subject from the apparatus, or between successive trials on the Lashley Maze, odor discrimination, and spatial plus maze tasks.

For the three learning tasks that required food deprivation, ad lib food was removed from the animals' home cages at the end of the light cycle approximately 40 h prior to the start of training (and thus encompassing the "rest" day between successive tasks). During the deprivation period, animals were provided food in their home cages for 90 min/day during the last 2 h of the light cycle, and thus were approximately 16 h food-deprived at the time of training or testing. This deprivation schedule was deemed "mild" (animals typically lost less than 6% of their free-feeding body weight during this period), but sufficient to maintain stable performance on these tasks. In the one task that required water deprivation, the same schedule was followed except that free access to water was limited to 60 min per day.

So that the time of day did not differentially impact animals' performance, all animals were trained and tested during the middle 7 h of the light cycle, and procedures were administered to animals with as little temporal dispersion as possible. All animals were trained and tested under nominally identical conditions.

2.3. Tests of learning

All animals were tested in the Lashley Maze and Passive Avoidance Tasks. Twenty-four of the animals were tested in these two tasks plus (in this order): spatial water maze, odor discrimination, fear conditioning, and spatial plus maze.

2.3.1. Lashley III maze

A Lashley III maze was scaled for mice and constructed of black Plexiglas (see Matzel et al., 2003). The maze consisted of a start box, four interconnected alleys, and a goal box containing a food reward. A 2 cm wide \times 0.1 cm deep white cup was located in the rear portion of the goal box, and 45 mg BioServe (rodent grain) pellets served as reinforcers. Illumination was 80 lx at the floor of the maze. The maze was isolated behind a shield of white Plexiglas to mitigate against extramaze landmark cues.

On successive trials, the latency of food-deprived mice to locate the goal box decreases, as do their errors (i.e., wrong turns or retracing). For the present work, procedures were developed that supported rapid acquisition. Food-deprived animals were acclimated and trained on two successive days. On the day prior to acclimation, all animals were provided with three food pellets in their home cages to familiarize them with the novel reinforcer. On the acclimation day, each mouse was placed in the four alleys of the maze, but the openings between the alleys were blocked so that the animals could not navigate the maze. Each animal was confined to the start and subsequent two alleys for 4 min, and for 6 min in the last (goal) alley, where three food pellets were present in the food cup. This acclimation period promotes stable and high levels of activity on the subsequent training day. On the training day, each animal was placed in the start box and allowed to traverse the maze until it reached the goal box and consumed the single food pellet present in the cup. Upon consuming the food, the animal was returned to its home cage for a 20-min interval (ITI), after which it was returned to the start box to begin the next of five total trials. The apparatus was cleaned during each ITI, and the sequence

was repeated for five trials. Both the latency and errors (i.e., a turn in an incorrect direction, including those which result in path retracing) to enter the goal box were recorded on each trial.

Typically, on the first trial animals enter the goal box within 100–300 s, and make 15–25 "errors" prior to retrieving the food. On subsequent trials, performance markedly improves. For purposes of ranking animals, the average of performance on Trials 3 and 4 served as the index of learning for each animal. We have adopted the practice of averaging behavior over two trials so as to better represent animals' performance. Trials 3 and 4 were previously found to be a point during acquisition at which the greatest amount of variability between animals was apparent (i.e., was devoid of "floor" or "ceiling" constraints on performance), and was thus sensitive to variations in the rate of acquisition across animals.

2.3.2. One-trial passive avoidance

Animals learn to suppress specific movements to avoid contact with aversive stimuli. This "passive avoidance" response is exemplified in step-down avoidance procedures, where commonly, an animal is placed on a platform, whereupon stepping off of the platform the animal encounters a footshock. Following just a single encounter with shock, animals are subsequently reluctant to step off of the safe platform. The animals' reluctance to leave the platform is believed to *not* reflect fear, because typical fear responses are not expressed in animals engaged in the avoidance response (Bolles, 1969; Morris, 1974). So as to duplicate as few stimuli as possible between tasks in our battery, here we use a variant of the step-down avoidance task that does not rely on shock to motivate behavior (so as to not duplicate stimuli [i.e., footshock] used to support learning in our fear conditioning task). Upon stepping off the platform, animals were exposed to a compound of bright light and a loud oscillating tone (i.e., "siren"). Like more common procedures, our variant of this task supports learning after only a single trial (i.e., subsequent step-down latencies are markedly increased).

A chamber illuminated by dim (<52 lx) red light was used for training and testing. Animals were confined to circular ("safe") chamber (10 cm diameter, 8 cm high). The walls and floor of this chamber were white and the ceiling was translucent orange. The floor was comprised of plastic rods (2 mm diameter) arranged to form a pattern of 1 cm square grids. A clear exit door (3 cm square) was flush with the floor of the safe compartment, and the door could slide horizontally to open or close the compartment. The bottom of the exit door was located 4 cm above the floor of a second circular chamber (20 cm diameter, 12 cm high). This "unsafe" chamber had a clear ceiling and a floor comprised of 4 mm wide aluminum planks that formed a pattern of 1.5 cm square grids that were oriented at a 45° angle relative to the grids in the safe compartment. When an animal stepped from the safe compartment through the exit door onto the floor of the unsafe compartment, the compound aversive stimulus comprised of a bright (550 lx) white light and "siren" (a 4/s transition from 600 to 1200 Hz tones, 60 dB_c above the 50 dB_c background; Radio Shack Model 2730057) was initiated.

Animals were placed on the safe platform, the exit from which was blocked by the Plexiglas door. After 5 min of confinement, the door was retracted and the latency of the animal to leave the platform and make contact with the grid floor was recorded. Prior to training, step-down latencies typically range from 8 to 20 s. (This narrow range of baseline latencies reflects the 5 min of confinement of the animal on the platform, as determined by preliminary studies.) Upon contact with the floor, the door to the platform was lowered and the aversive stimulus (light and noise) was presented for 4 s, at which time the platform door was opened to allow animals to return to the platform, where they were again confined for 5 min. At the end of this interval, the door was opened and the latency of the animal to exit the platform and step onto the grid floor (with no aversive stimulation) was recorded, completing training and testing.

The ratio of post-training to pre-training step-down latencies was calculated for each animal and served to index learning. In pilot experiments, we determined that asymptotic performance was apparent in group averages following two to three training trials, thus performance after a single trial reflects (in most instances) subasymptotic learning.

2.3.3. Spatial water maze

For this task, animals are placed in a round pool of opaque water from which they can escape onto a hidden (i.e., submerged) platform. The latency for animals to find the platform decreases across successive trials. In this task, performance of animals can improve across trials despite the animals beginning each trial from a new start location. Such a procedure mitigates against egocentric navigation and promotes the animals' dependence on extramaze spatial landmarks. As demonstrated by Morris (1981), rats performance in the water maze need not rely on fixed motor patterns (i.e., performance improves despite the animal's irregular starting location) or the presence of discernable cues within the maze (e.g., visual, tactile, or olfactory signals). Instead, performance is dependent on the stability of extra-maze cues, or "landmarks," and is said to reflect the animals' representation of its environment as a "cognitive map."

We have developed a protocol in which mice exhibit significant reductions in their latency to locate the escape platform within six training trials. As this is unusually rapid learning in this task, several relevant modifications of the task should be emphasized. First, animals were confined in a clear Plexiglas cylinder on the safe platform for 5 min on the day prior to training. Second, a considerably longer ITI (10 min) was used than is typical (cf. 90 s). Lastly, the maze, surround, and water were black; visual cues were comprised of patterns of lights.

A round black pool (140 cm diameter, 56 cm deep) was filled to within 24 cm of the top with water made opaque by the addition of a nontoxic, water-soluble, black paint. A hidden 11 cm diameter perforated black platform was in a fixed location 1.5 cm below the surface of the water midway between the center and perimeter of the pool. The pool was enclosed in a ceiling-high black curtain on which five different shapes (landmark cues) were variously positioned at heights (relative to water surface) ranging from 24 to 150 cm. Four of these shapes were constructed of strings of white LEDs (spaced at 2.5 cm intervals) and included an "X" (66 cm arms crossing at angles 40° from the pool surface), a vertical "spiral" (80 cm long, 7 cm diameter, 11 cm revolutions), a vertical line (31 cm), and a horizontal line (31 cm). The fifth cue was constructed of two adjacent 7 W light bulbs (each 4 cm diameter). A video camera was mounted 180 cm above the center of the water surface. These cues provided the only illumination of the maze, totaling 172 lx at the water surface.

On the day prior to training, each animal was confined to the escape platform for 300 s. Training was conducted on the two subsequent days. On Day 1 of training, animals were started from a unique location on each of five trials. (The pool was conceptually divided into four quadrants, and two starting points were located in each of the three quadrants that did not contain the escape platform. The starting point on each trial alternated between the three available quadrants.) An animal was judged to have escaped from the water (i.e., located the platform) at the moment at which four paws were situated on the platform, provided that the animal remained on the platform for at least 5 s. Each animal was left on the platform for a total of 20 s, after which the trial was terminated. Trials were spaced at 10 min intervals, during which time the animals were held in a warmed (27.5°C) opaque (5 lx) box lined with wood shavings. On each trial, a 90 s limit on swimming was imposed, at which time any animal that had not located the escape platform was placed there by the experimenter, where it remained for 20 s. Animals were observed from a remote (outside of the pool's enclosure) video monitor, and animals' performance was recorded on video tape for subsequent analysis. Day 2 of training proceeded as did Day 1. However, after the last (fifth) training trial, a 90-min retention period was begun, after which animals were tested with a "probe" trial. On the probe test, the escape platform was removed from the pool, and all animals were started from the sixth position for that day. A 60-s test was conducted in which the animals' time searching in the target quadrant (that in which the escape platform was previously located) and nontarget quadrants were recorded.

2.3.4. Odor discrimination and choice

Rodents rapidly learn to use odors to guide appetitively reinforced behaviors. In a procedure based on one designed by Sara (Sara, Roulet, & Przybyslawski, 2001) for rats, mice learn to navigate a square field in which unique odor-marked (e.g., almond, lemon, and mint) food cups are

located in three corners. Although food is present in each cup, it is accessible to the animals in only one cup (e.g., that marked by mint odor). An animal is placed in the empty corner of the field, after which it will explore the field and eventually retrieve the single piece of available food. On subsequent trials, the location of the food cups are changed, but the accessible food is consistently marked by the same odor (i.e., mint). On successive trials, animals require less time to retrieve the food and make fewer approaches (i.e., "errors") to those food cups in which food is not available. We have adapted this procedure for use with mice, and typically observe errorless performance within three to four training trials. Control procedures (where the target odor is not consistent) indicate that odor is the principal determinant of animals' discrimination (i.e., performance does not improve under conditions for which the target odor is changed across trials).

A black Plexiglas 60 cm square field with 30 cm high walls was located in a dimly lit (108 lx) testing room with a high ventilation rate (3 min volume exchange). Three 4 × 4 × 2.0 cm (l, w, h) aluminum food cups were placed in three corners of the field. A food reinforcer (30 mg portions of chocolate flavored puffed rice) was placed in a 1.6 cm deep, 1 cm diameter depression in the center of each cup. The food in two of the cups was covered (1.0 cm below the surface of the cup) with a wire mesh so that it was not accessible to the animal, while in the third cup (the "target" cup), the food could be retrieved and consumed.

A cotton-tipped laboratory swab, located between the center and rear corner of each cup, extended vertically 3 cm from the cups' surface. Immediately prior to each trial, fresh swabs were loaded with 25 µl of either lemon, almond, or mint odorants (McCormick flavor extracts). The mint odor was always associated with the target food cup. (It should be noted that in pilot studies, the odor associated with food was counterbalanced across animals, and no discernible differences in performance could be detected in response to the different odors.)

On the acclimation day, each food-deprived animal was placed in the field for 20 min with no food cups present. At the end of that day's light cycle, three pieces of chocolate flavored puffed rice that would subsequently serve as the reinforcer were placed in each animal's home cage to acquaint them with the reinforcer. On the subsequent test day, animals received four training trials in the field with three food cups present. On each trial, an animal was placed in the empty corner of the field. On Trial 1, the reinforcing food (rice) was available to the animal in the cup marked by mint odor. On only this trial, an additional portion of food was placed on the top surface of the same cup. The trial continued until the animal retrieved and consumed the food from the target cup, after which the animal was left in the chamber for an additional 20 s and then returned to its home cage to begin a 6-min ITI. On Trials 2–4, the location of the food cups were re-arranged, but the baited cup remained consistently marked by the mint odor. Both the corner location of the mint odor and its position relative to the remaining odors were changed on each trial. On each trial, the latency to retrieve the food and errors were recorded. An error was recorded any time that an animal made contact with an incorrect cup, or its nose crossed a plane parallel to the perimeter of an incorrect cup. Similarly, an error was recorded when an animal sampled (as above) the target cup but did not retrieve the available food.

2.3.5. Associative fear conditioning

In such a procedure, animals are exposed to a stimulus (i.e., a CS; tone) that terminates in the onset of a mild footshock (i.e., a US). These tone–shock (CS–US) pairings come to elicit conditioned fear responses when animals are subsequently presented with the tone. This learned fear can be assessed in various ways. In the present studies, fear was indexed by CS-elicited suppression of ongoing drinking, as this measure is easily and precisely quantified. "Lick suppression" is conceptually analogous to the more commonly used measure of CS-elicited generalized "freezing" (i.e., during that time in which an animal freezes it necessarily is not capable of drinking from a lick tube). In our laboratory, lick suppression has proven to be of greater utility, given that the generalized freezing exhibited by mice is far less regular (and thus more ambiguous) than that which we have typically observed in rats. To avoid any interaction of the training context (which itself acquires an association with shock) with the CS at the

time of testing, training and testing were conducted in separate distinct contexts.

Two distinct experimental chambers (i.e., contexts; $32 \times 28 \times 28$ cm, $1 \times w \times h$) were used, each of which was contained in a sound- and light-attenuating enclosure. These boxes were designated as “training” and “testing” contexts, and differ as follows: The training context was brightly illuminated (100 lx), had clear Plexiglas walls, no lick tube, and parallel stainless-steel rods (5 mm, 10 mm spacing) forming the floor. The test context was dimly illuminated (6 lx), the walls covered with an opaque pattern of alternating black and white vertical stripes (3 cm wide), and the floor was formed from stainless 1.5 mm rods arranged at right angles to form a grid of 8 mm squares. A water-filled lick tube protruded through a small hole in one wall of the test chamber, such that the tube’s tip was flush with the interior surface of the wall at a point 3 cm above the floor. Upon contacting the tube, the animal completed a circuit such that the number of licks/s could be recorded. This circuit was designed so that if an animal made continuous contact with the tube (i.e., “mouthed” the tip), the circuit records 8 licks/s, a rate that approximates continuous licking.

In the training chamber, a 0.6 mA constant-current scrambled footshock (US) could be delivered through the grid floor. In both the training and test chambers, a 40 dB above background white noise (the CS) could be presented through speakers mounted at the center of the chambers ceiling.

Water-deprived animals were acclimated to the training and test chambers by placing them each in both contexts for 20 min on the day prior to training. Within several minutes of their first placement in the test context, water-deprived mice exhibit stable licking (for water). When subsequently placed in the chamber, these animals typically initiate licking within 5–10 s and lick at relatively stable rates for the subsequent 3–5 min. Training occurred in the training context in a single 30-min session during which each animal was administered a noise–shock pairing 10 and 20 min after entering the chamber. Each 10-s noise terminated with the onset of a 500-ms footshock. With our present parameters, we have observed that asymptotic performance (as evident in group means) is reached with 4–6 such pairings. Thus, two pairings (in most instances) support subasymptotic conditioned responding. At the end of the training session, animals were returned to their home cages for 60 min, after which they were re-acclimated to the test context for 20 min where they were allowed free access to the lick tubes. On the subsequent day (23–25 h post-training), animals were tested. Each animal was placed in the test context whereupon after making 50 licks, the noise CS was presented continuously until the animal completed an additional 25 licks. The latency to complete the last 25 licks during the pre-tone interval and in the presence of the tone was recorded, with a 600-s limit imposed on the second 25 licks (a limit not reached by any animal described here). With these measures, the latency to complete 25 licks in the presence of the tone CS serves as our index of learned fear, and the latency to complete 25 licks prior to CS onset served as an index of basal lick rates.

2.3.6. Spatial plus maze

An elevated maze in the form of a “+” was constructed of black Plexiglas. Each of the four arms measured 8×40 cm ($w \times l$). A 4-mm diameter food cup was located in the center of the arm 2 cm from its end. Food (a 14.5 mg Noyse pellet) was located in every cup, but was accessible to the animal in the arm designated as “west.” Twenty-four centimeters from the end of each arm and equidistant between successive arms were 18×18 cm visual cues, a black (240 pt) “X,” “O,” and “+.”

2.4. Tests of unlearned behaviors and fitness

A total of 43 animals were tested on 21 unlearned behaviors and/or measures of physical characteristics (including measures taken in an open field and an elevated plus maze). In a separate experiment, 27 animals provided behavioral data in an open field and an elevated plus maze, as well as measures of basal or stress-induced serum corticosterone levels.

Each of the following tests were administered with 1 day intervening between the completion of one test and the start of the subsequent test. Open field testing (Task 1) was conducted 2 days prior to the start of tests

of learning; all other tests were administered beginning 4 days after the completion of the tests of learning. (Testing animals in the open field prior to other training and testing served two purposes: (1) it is consistent with our previous work, and thus facilitates comparisons, and (2) by administering various tests of exploration both early and late in the testing sequence, we can ascertain if individuals’ pattern of exploration is impacted by intervening experience in our battery of tests).

In all but several instances, tests of unlearned behaviors and fitness were completed in a single day. Many tests yielded several different measures of performance such that 21 variables were assessed that were relevant to balance, strength, coordination, general activity, exploratory tendencies, fear and anxiety responses, response to novelty, and pain sensitivity. The apparatus and parameters that are described below had been chosen based on pilot work in which they were determined to be adequate to capture a wide range of variations in performance across individual animals.

2.4.1. Open field exploration

A square field (46×46 cm) with 13 cm high walls was constructed of white Plexiglas and was located in a brightly lit room (400 lx) with a background noise of 65 dB_r. The field was conceptually comprised of a 6×6 grid (7.65 cm quadrants), where 20 of the quadrants abutted the outer walls of the field (i.e., “wall” quadrants), and 16 quadrants were displaced from the walls and comprised the interior (i.e., “open” quadrants) of the field.

Animals were placed in the center of the field. After 20 s had elapsed (during which the animals self-selected a “starting” location), the animals’ behavior was monitored for 4 min. Throughout this time the animal’s entries into walled and open quadrants were recorded. An entry was recorded whenever both front paws crossed the border of a quadrant. Both total activity (i.e., quadrant entries regardless of category) was recorded (V1) as was the percentage of entries into unwalled (open) quadrants of the field (V2). Additionally, animals’ running speed was estimated (V3). In the open field, rodents often exhibit “bursts” of uninterrupted running, typically along the walls of the field. Here, running speed was calculated for those instances in which an animal ran continuously (i.e., without stopping, rearing, or overt head turning) along an outer wall (from corner to corner) of the field, but only on those instances in which the animal began from a stationary start in one corner. Four such episodes were recorded for each animal during the last 3 min of the test interval (such bursts are infrequent during the first minute of exposure), and the average of these four instances served as the index of each animal’s running speed (cm/s). (As rates varied between bursts of running, multiple instances were averaged to provide a more accurate estimate of each animal’s “typical” rate. Four such instances were averaged as it was determined that no animal in our sample made fewer than four bursts of running that satisfied our criterion for inclusion.) Lastly, the bolli deposited by each animals was recorded as a potential measure of emotionality (V4). It should be noted that a 4-min test was explicitly chosen (based on pilot work) because changes in *exploratory* behavior (not necessarily simple motor activity) were *not* detectable over time. Thus, we presume that open field performance was most sensitive to *unlearned* behavioral tendencies.

2.4.2. Balance beam

Animals were placed on a $40 \times 0.7 \times 2$ cm ($l \times w \times h$) beam suspended 30 cm above the ground. The beam was explicitly designed so that animals do not typically fall from it. Instead, movement along the beam was the variable of interest, as movement is presumed to interact with balance. In a 4-min test, mice exhibit wide variability in the amount of movement along its length (V5).

2.4.3. Rod suspension

Animals are hung from their front paws from a 4 mm rod coated with black rubber (shrink tubing). The rod was suspended 30 cm above ground. Latency to drop from the rod (an index of grip strength) was recorded (V6).

2.4.4. Elevated plus maze

The maze was constructed of grey Plexiglas in the form of a “plus.” Each arm of the maze was 6 cm wide, and the maze was suspended 30 cm

above a black surface. Two opposing arms of the maze were enclosed in 8 cm high, grey Plexiglas walls, and two of the arms were open. The maze was located in a 300 lx environment. Animals were placed in the center of the maze facing an open arm, and their behavior in the maze was recorded in 1-min blocks for 4 min. Of interest is the time to exit the first closed arm entered (V7), total number of arm entries (V8), percent of entries into open arms (V9), percent of re-entries into an arm previously exited (V10), latency to enter the first open arm (V11), and percent time in closed (V12) and open arms (V13). (It is noted that the percent time in open and closed arms might be reciprocals of a single measure, and could potentially inflate any factor score into which these two variables loaded. However, we use both measures in combination as they may reveal patterns of behavior not evident in either measure alone, e.g., if an animal were to spend time only in the closed arms and center compartment.) Generally, entries into open arms are considered to be stressful to animals, thus measures in the open arms provide other indices of exploratory tendencies similar in nature to that of exploration of the open quadrants of the open field.

2.4.5. Pain sensitivity

Upon being placed on a 52.6 °C aluminum plate, animals' latency to raise a hind paw and to either lick or shake the paw serves as the index of pain sensitivity (V14).

2.4.6. Screen hanging

Animals were placed in the underside of a wire mesh screen (7 mm grids) tilted 40° from vertical and suspended 24 cm from ground. The latency to drop from the screen (V15) and the distance moved prior to dropping from the screen (cm/s; 180 maximum test duration) was recorded (V16).

2.4.7. Post-shock freezing

Freezing after the offset on an unsignalled shock is often interpreted as a measure of fear. Animals were acclimated for 20 min to a 25 cm square chamber (60 lx illumination) with a stainless-steel grid floor. On the subsequent day, they were returned to the chamber, where after 10 min a 0.6 mA, 500 ms constant-current scrambled footshock was administered through the floor. The shock was delivered upon the command of the experimenter, who initiated the shock when each animal was located near the center of the chamber with all paws on the grid floor. Using this method, the actual delivery of the shock typically occurred between 10 and 10.5 min. During and for a brief time (500–1000 ms) following the shock, the animals exhibit a burst of activity, after which they exhibit "freezing," a presumed index of fear. The duration of freezing (the latency for each rear paw of the animal to move 2 cm [the span of three floor grids] after the initial point of freezing) served as the dependent variable (V17).

2.4.8. Exploration in a straight alley and magnitude of escape responding

A straight alley is used that was 30 cm above ground. The alley was 260 cm long and 7 cm wide with 3 cm high walls. The initial 29 cm of the alley (the "start" box) was enclosed in 12 cm high white walls with an orange acetate ceiling. The exit from this box could be blocked with sliding guillotine door made of clear Plexiglas. The interior of the start box is 4 lx, and the alley beyond the start box was 20 lx. A startle stimulus could be delivered in the start box. This stimulus was the compound of a bright light (400 lx) and a high-speed (3000 RPM) fan positioned so that its air flow was directed across the animal and down the alley. The fan raised background noise 50 dB_a.

Animals were placed in the start box with the exit blocked. After 60 s, the door was raised and animals were allowed to explore the alley for 4 min. The latency for each animal to cross a point in the alley 213 cm from the exit of the start box was recorded (V18) and served as an index of exploratory behavior, although in this instance, exploration might be confounded by running speed (i.e., it could not be taken as a ratio of exploration into other "safe" areas as in the open field or elevated plus maze). After 4 min, the animals were returned to the start box where they were again confined for 60 s, after which the door was raised. At the moment that each animal moved to within 2 cm of the exit and faced the open alley, the compound startle stimulus was presented for 800 ms. This stimulus

typically elicits rapid running (an "escape" response), which could serve as an index of fear. The distance that the animal ran prior to making a complete stop of forward movement for at least 500 ms was recorded (V19).

2.4.9. Pre-attentive auditory startle responses

A custom designed startle chamber was used. A 17 cm round platform (stainless-steel floor) was enclosed in a 5 cm high black wall with a screen mesh ceiling. The height of the walls prevented rearing during the test. The floor of the chamber was sensitive to deflections corresponding to as little as 1 mg of force. The chamber was dimly illuminated (2 lx) and maintained against a low background noise level (52 dB_a). A 200 ms, 60 dB_a above background burst of white noise was presented 6 and 12 min after the animal was placed in the chamber. The maximum deflection of the floor is computed during a 500 ms window beginning at the onset of the noise, and the two responses were averaged for each animal (V20).

2.4.10. Body weights

Body weights were recorded during a period of free feeding (V21).

2.4.11. Learning

All animals were tested on between 2 and 6 of the learning tasks described above. As all animals were tested in the Lashley Maze and Passive avoidance tasks, these two measure of learning (V22 and 23) were considered for purposes of factor analysis of the 43 animals that contributed to all 21 measures of unlearned behaviors.

2.4.12. Corticosterone elevation in response to an acute stressor

A separate sample of 27 animals were tested in the open field and elevated plus maze. Subsequently, these animals were divided into two groups, one of which was exposed to a mild stressor (confinement to an elevated platform in a bright, noisy room) and one of which was unstressed. Here, we intended to assess the relationship between basal and stress-related corticosterone levels and animals' pattern of exploration.

Serum corticosterone levels are sharply elevated in response to acute stressors and mediate many physiological responses to stress. Here, it was our intention to determine how both basal and stress (as might accompany exploration in a novel environment)-induced corticosterone responses were related to exploratory behaviors and general learning abilities. To that end, animals tested on the above procedures were divided into two groups (Stress or No Stress). Since we have previously found that exploratory tendencies in the open field are strongly correlated with general learning abilities, individuals were assigned to these groups counterbalancing for their percentage of time in the open relative to walled quadrants in the field.

Manipulations and blood collection were conducted 5 days after the last behavioral test, and blood was collected 10 min after the stress or no-stress manipulation. To inflict mild stress, animals were placed for 6 min on a 10-cm diameter platform elevated 120 cm above the floor in a brightly lit, unfamiliar room. (We have previously determined that this treatment typically induces a 2–4× increase in free corticosterone levels. This degree of corticosterone elevation is well below that induced by presumably severe stressors. Thus, not only is such a stressor more comparable to that which might accompany exploration in a novel environment, the mid-level corticosterone elevation that it induces is likely to be sensitive to a wide range of variation between individual animals.) The remaining animals remained in their home cages during this treatment. Ten minutes following the stress or no-stress treatment, animals were rapidly decapitated (in an isolated room under ventilation) for the collection of trunk blood.

Corticosterone levels were quantified using the mouse ImmuChem Double-Antibody kit (125/RIA) available from ICN Biomedicals. Blood was collected in centrifuge tubes coated with heparin and immediately spun to isolate serum. Samples were then frozen at –30 °C, and gamma counts were obtained within 14 days.

2.5. Analyses

It was our intention to determine how individual performance variables, including sensory and motor function, activity, fear and stress

responses, fitness, and exploratory tendencies, load together as factors, as well as which (if any) of the factor clusters load most heavily with performance in tests of learning (and open field performance, a reliable predictor of animals' learning capacity). Since exploratory behaviors are known to be modulated by such influences as stress and fear, it is imperative that we establish the degree to which indices of these different variables load together as factors.

A total of 43 animals were tested on two learning tasks (Lashley Maze and passive avoidance) and 21 tests of unlearned behaviors and physical characteristics. Performance data from this sample were subjected to an unrotated principal component factor analysis, the intent of which was to determine the clustering of variables into factors that could best describe the performance of individuals across all measures. Performance on six learning tasks was analyzed for a subsample of 24 animals. It is noted that a sample of this size may contribute to instability in the analysis. However, this analysis provides an opportunity to assess the generality of the conclusions based on the larger sample. Finally, correlations were computed between basal and stress-evoked serum corticosterone levels and various measures of exploration for an additional sample of 27 animals.

3. Results

3.1. Learning performance

For qualitative purposes and to provide a framework with which to interpret the broader results, first we will summarize data obtained from 24 of the animals (from the larger sample of 43) that were tested on six learning tasks (Lashley Maze, passive avoidance, odor discrimination, spatial water maze, fear conditioning, and spatial plus maze). These tasks impinge on different sensory, motor, motivational, and information processing systems. Animals exhibited a wide range of variability in performance across tasks, although subsamples of animals performed better or worse than the median performance on all tasks, a result consistent with our prior work (Matzel et al., 2003) and indicative of a conserved influence on performance across tasks. To characterize the "general" learning abilities of individual animals, each subject was assigned a rank for its performance on each task, where a rank of "1" indicated the best performance, and a rank of "24" indicated the worst performance. To characterize an animal's overall performance, its ranks from each of the five tasks were averaged. For illustrative purposes, the ranks of three animals on all tasks are illustrated in Fig. 1A. (These three animals were chosen for illustration because each exhibited a narrow range of performances across all tasks and represented distinct general performance patterns.) Also consistent with our previous work, we observed a positive correlation between individual animals' relative activity in the open quadrants of the open field and their aggregate performance across all learning tasks, i.e., exploration of presumably stress-evoking areas in the open field were predictive of general learning abilities, $r(23) = .46$, $p < .01$. This later relationship is illustrated in Fig. 1B.

The qualitative nature of the above observations was confirmed by an unrotated principal component analysis. Performance data from six learning tasks, total activity in the open field, and percent of activity in the open quadrants of the field were entered into this analysis. A principal

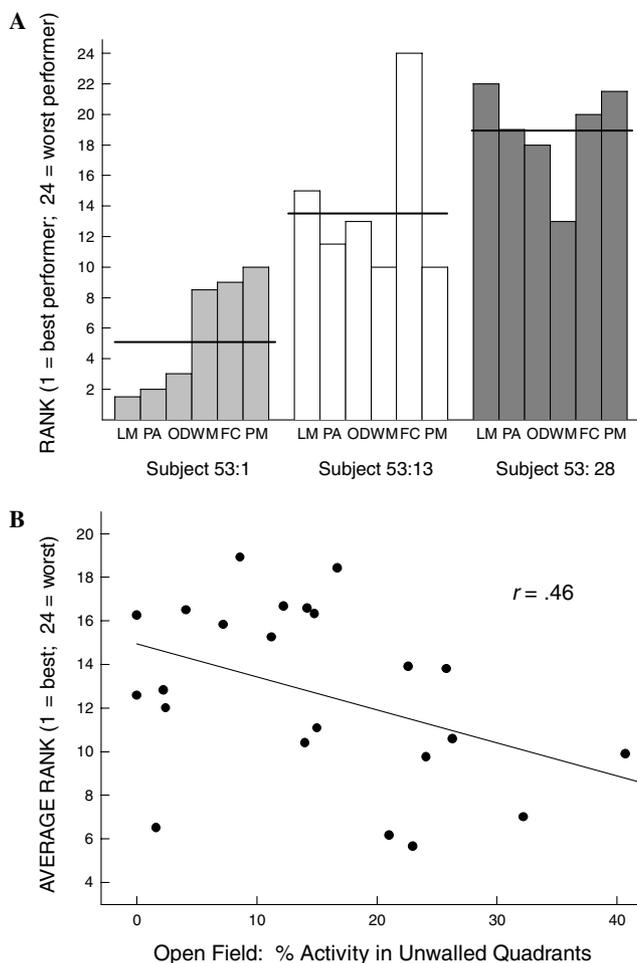


Fig. 1. (A) The ranks of three animals (Ss 1, 13, and 28) on each of six learning tasks (Lashley Maze, passive avoidance, odor discrimination, water maze, fear conditioning, and spatial plus maze). Ranks were computed relative to peers in a sample of 24 animals. Each subjects' average rank is indicated by the horizontal bar. Note that Subject 1 performed better than the expected median (12.5) on all six tasks, while Subject 28 was consistently worse than the expected median. (B) The percent of individual animals' activity in the open quadrants of a novel open field is plotted against the horizontal axis, and each animals' average rank across the six learning tasks is plotted on the vertical axis. A significant correlation was found between this exploratory tendency and animals' general learning ability (as characterized by their average ranks), such that the propensity to explore the open quadrants of the field was directly related to general learning abilities.

factor was extracted which accounted for 32% of the variability across animals and tasks; loadings on this factor are provided in Table 1. Notably, animals' preference for the open areas of the field loaded heavily on this factor, as did Lashley Maze, passive avoidance, and spatial plus maze performance. Performance on the water maze and fear conditioning tasks loaded consistently on this factor, although somewhat less strongly. In contrast to animals' tendency to enter open areas of the open field, overall activity in the field loaded weakly on this factor, suggesting that the *pattern* of exploratory behavior, not simple activity, accounted for the relationship of open field performance to general learning abilities. This pattern of variable loading on the

Table 1
Factor loadings, principal component analysis ($n = 24$)

	Factor 1	Factor 2	Factor 3
Open field: total activity ^a	-.128	.530	-.715
Open field: % open activity ^a	-.737	.302	-.052
Lashley maze: errors ^b	.726	.263	.120
Passive avoidance: –[step latencies] ^b	.667	-.285	-.065
Odor discrimination: errors ^b	.376	.467	-.376
Fear conditioning: –[lick latencies] ^b	.236	.637	.522
Water maze: latencies ^b	.542	-.392	-.422
Spatial plus maze: errors ^b	.755	.234	.097
Eigen value	2.59	1.358	1.136
Proportion of total variance	.324	.169	.142

Variables reflect performance of animals on six tests of learning tasks, as well as activity and exploratory patterns in a novel open field.

^a In the open field, *more* activity is reflected in *higher* values.

^b In the Lashley Maze, odor discrimination, spatial plus maze, and water maze tasks, better performance (faster acquisition) is reflected in *lower* values (e.g., fewer errors, shorter latencies). In their raw forms, better performance in the fear conditioning and passive avoidance tasks is reflected as *higher* values. To simplify interpretation of the variable loadings, passive avoidance and fear conditioning scores were converted to negative numbers such that for all learning measures, better learning would be reflected in lower values (and a similar impact of any factor on all measures of learning would be reflected in loadings of the same sign). Note that since *more* exploration is expressed as higher values and *better* learning is expressed as lower values, the pattern of loading on Factor 1 indicates that the amount of exploration is *positively* correlated with animals' actual learning performance (e.g., *more* exploration is associated with *better* learning).

primary factor is quite similar to data that we have previously reported (Matzel et al., 2003), although in the present case, six rather than five learning tasks were included for analysis.

3.2. Physical characteristics, exploratory/fear-related behaviors, and learning

Our principal interest in the present studies was to discern the impact of variations in unlearned sensory, motor, and emotional behaviors on exploratory tendencies and learning. To that end, we assessed animals' performance on a wide range of tests of sensory, motor, and emotional responses. As described above, data were obtained from 43 animals. All of these animals contributed data for 21 measures of unlearned behaviors and fitness and two measures of learning (the Lashley Maze and passive avoidance). (It should be noted that owing to the extended schedule necessary to administer all of these tests, it was not practical in each replication to test animals on all six measures of learning. In those instances, the Lashley Maze and Passive avoidance tasks were chosen for inclusion because (1) these two tasks consistently load heavily in the primary factor [see Table 1 here, and Matzel et al., 2003] that we have interpreted to account for general learning abilities, and thus are best representative of animals' general learning ability, (2) because these two tasks place such widely different sensory, motor, and information processing demands on the animals, and thus are not easily interpreted as being

representative of the same learning domain. Regardless, it should be reiterated that our goal here was to better characterize individual differences in exploratory behaviors [having demonstrated independently the relationship of these tendencies to general learning abilities], so it was not critical that all measures of learning be represented). However, it is possible that animals exposed to six learning tasks might respond differently on tests of unlearned behavior than would animals trained on only two tasks. To assess this concern, separate correlation matrixes were constructed for animals exposed to two or six learning tasks ($n_s = 24, 19$), and a comparison of these two matrixes found them to highly correlated (Mantel Test for Similarity of Two Matrixes, $r = .72$). This result suggests that our response measures are reliable (across two samples), but more importantly, that the relationship between unlearned responses was not impacted by the exposure of 24 animals to four additional tests of learning.

Twenty-one measures of sensory and motor function, exploration, fear, and physical attributes (see Table 2) were obtained in conjunction with measures of learning in the Lashley Maze and passive avoidance tasks. Performance

Table 2
Factor loadings, principal component analysis ($n = 43$)

	Factor 1	Factor 2	Factor 3
Open field: total activity	-.487	.212	.306
Open field: % open activity	-.713	.325	-.172
Open field: bolli count	.209	.534	-.398
Open field: running speed	-.081	.480	-.593
Balance beam: movement (cm/s)	.153	.444	.481
Rod hanging: latency to fall	-.093	.444	.634
Plus maze: total arm entries	-.659	-.487	.293
Plus maze: % open time	-.934	-.142	-.082
Plus maze: % closed time	.820	-.108	-.120
Plus maze: % open entries	-.934	-.113	-.086
Plus maze: % entries in new arm	-.633	-.430	-.435
Plus maze: time of first open entry	.861	.269	.030
Plus maze: time in first closed	.280	.542	-.380
Hot plate: latency to lick	.345	.073	-.188
Screen climbing: latency to fall	-.262	.207	.400
Screen climbing: movement (cm/s)	.209	-.559	-.312
Post-shock freezing: time	-.165	-.385	.199
Straight alley: latency to 213 cm	.126	-.700	-.182
Straight alley: escape distance	-.085	.405	-.304
Acoustic startle: force	-.151	.126	.139
Body weight	.223	-.654	-.355
Passive avoidance: –[step latencies] ^a	.566	-.478	.358
Lashley maze: errors ^a	.517	-.481	.284
Eigen value	5.853	3.968	2.534
Proportion of total variance	.254	.172	.110

Variables reflect fitness, sensory/motor responses, activity, exploratory behaviors, and learning.

^a Note that as described in Table 1, measures of learning are such that *lower* values are indicative of *better* performance. In considering the directionality of variable loadings, consider that in measures of exploration or activity, *more* exploration or activity is expressed as a *higher* value. Thus, for example, the negative loading of percent time in the open arms of the elevated plus maze reflects a positive correlation of the time spent in the open arms with animals' performance on learning tasks, whereas the positive loading of percent time in closed arms reflects an inverse relationship between this measure and learning performance.

data were entered into a principal component factor analysis. Three factors were extracted (see Table 2) that accounted for (respectively) 25, 17, and 11% of the variance of animals across all measures. Exploration of the open quadrants in the open field and performance on each of the learning tasks (Lashley Maze and passive avoidance) loaded most heavily on Factor 1, again indicating that exploration of the open quadrants of the field was related to animals' rate of learning on each of these tasks. Most interesting was the consistent pattern of loading on Factor 1 of other variables that are clearly indicative of exploratory behaviors (and/or novelty seeking) in environments that are typically associated with stress responses in rodents. In particular, heavy loadings were observed for the total number of arm entries in the elevated plus maze, the percent of those entries into open arms in the maze, the percent of time spent in open arms of the maze, the latency to make the first entry into an open arm in the maze, and the percent of entries into arms other than the arm most recently exited. Only one presumed measure of exploration (time to traverse 213 cm in a novel straight alley) did not load heavily on this factor, and as noted above, this measure might well be confounded by running speed. Equally important were those factors that *did not* load heavily on Factor 1. Again, running speed in the open field loaded weakly, as did movement on the elevated balance beam, suggesting that exploratory tendencies (indicative of stress-related behaviors) were not a reflection of simple activity (although in this instance, total activity in the open field did load at a moderate level). More importantly, there is no evidence that variations in "fear" (or related domains of emotionality) can account for the co-variations in exploration and learning abilities that we observe, as startle-induced escape responding, post-shock freezing, and bolli deposited in the novel open field loaded weakly and inconsistently on this factor. It is noted that post-shock freezing might additionally (or alternatively) reflect animals' pain sensitivity. However, the most direct measure of pain sensitivity, latency to lick a paw when placed on a hot surface, also loaded relatively weakly on this factor, suggesting that both pain sensitivity and fear responsiveness had little explanatory value in describing Factor 1.

It is extremely difficult to interpret secondary factors extracted by a principal component analysis, although Factor 2 is of some interest, given the moderate loadings on this factor of each of our measures of learning. However, only body weights and time to traverse 213 cm in a novel straight alley loaded heavily on this factor. As other measures of running speed and coordination (running speed, rod hanging, balance beam movement, and screen climbing) loaded moderately on this factor, it is possible that the loadings on this factor are a function of animals' weight, although it is not clear how performance on tests of learning can be accounted for in this manner. Given the relatively weak loading of learning variables on this factor and our previous failure to observe a relationship between body weight and general learning abilities or exploratory tenden-

cies (Matzel et al., 2003), it would be of little value to attempt to further characterize this factor. Similarly, there is no clear pattern to be discerned from Factor 3, although it is noted that learning variables and exploratory tendencies all loaded weakly on this factor.

Based on this and the analysis described above (in which open field exploration and six measures of learning were included), it is clear that the tendencies of mice to explore environments that are typically described as promoting stress (or "anxiety") are regulated in common with general learning abilities, although it is not yet possible to discern if any causal relationship exists between these two classes of behavior (learning and exploration). However, it is clear that this relationship is not mediated or co-regulated by variations in speed or amount of activity, or other physical characteristics such as coordination, strength, or body weight.

3.3. *Exploratory tendencies and stress responses*

The propensity to explore the open quadrants of a walled field or the open arms of an elevated plus maze is often interpreted as an index of an animal's proclivity for novelty seeking, and/or may reflect the degree to which an animal experiences stress in the unfamiliar open environment (Anderson, 1993; Kabbaj et al., 2000). Here and elsewhere, we have reported that individual animals' proclivity to explore such stress-evoking environments is predictive of their general learning abilities. Three interpretations of this relationship are immediately obvious. First, it is possible that animals more engaged by novel environments are more likely to recognize (or attend to) those environmental relationships upon which learning depends, i.e., exploration impacts learning. Inversely, it is possible that learning drives exploration, such that an animal's capacity for learning determines the rate at which it adapts to an environment, thus influencing the animal's pattern of exploration. Lastly (although possibly related to alternative one), animals that are prone to novelty seeking or exploration (of the open quadrants of an open field or the open arms in an elevated plus maze) may be less susceptible to the experience or physiological consequences of stress, which can in many instances impede learning (Shors, 1998). Given the later, it might be possible that variations in general learning abilities are a direct reflection of the variations in stress evoked by the conditions inherent to any episode of learning under laboratory conditions, e.g., handling by the experimenter, removal from the home cage, or confinement to new environments, as well as those conditions inherent to some learning tasks, e.g., deprivation or aversive stimulation.

The present experiment was designed to assess the later of the possibilities described above. A sample of 27 animals were first tested in an open field and an elevated plus maze, and nine performance measures (indicative of activity, exploratory tendencies, and emotionality) were obtained. (These measures included all of those described previously

for these two tasks, with the exception of running speed in the open field). Subsequently, the animals were divided into two groups. (Animals were assigned to these groups counterbalancing for their percent of time in open arms in the plus maze and their percent of activity in the open quadrants of the open field. Thus, these groups were nominally equivalent in their representation of variations between individuals in these crucial [and broadly indicative; see Table 2] measures of exploratory behavior.) Subsequently, animals in one of these groups (Group STRESS, $n = 14$) were confined for 6 min to a small elevated platform in a bright, noisy, and unfamiliar room. This was intended as a moderately stressful experience, similar in nature to that which might accompany exploration of the open field or elevated plus maze. Ten minutes after this stressful experience, animals were rapidly decapitated for the collection of trunk blood, which would later be assayed for serum corticosterone, a physiological indicator of stress (Conrad, LeDoux, Magarinos, & McEwen, 1999). The second group of animals (Group NO STRESS, $n = 13$) was treated identically, except the stressful experience was omitted, i.e., they remained in their home cages during the 6-min interval in which animals in Group STRESS were confined to the elevated platform. With this design, it was then possible to examine the correlations between innate exploratory tendencies (obtained in the open field and the elevated plus maze) and basal and stress-induced corticosterone levels.

Factor loadings from a principal component factor analysis are provided in Table 3. For this analysis, the performance data of all 27 animals were entered. Consistent with the data reported above (see Table 2), measure indicative of exploratory patterns loaded heavily on the primary factor, accounting for 46% of the variance across animals. It is notable that the percent of animals' activity in the open quadrants in the open field and their percent of time in the open arms of the elevated plus maze loaded strongly on this factor. As previously, overall activity in the open field and bolli counts exhibited relatively weak loading.

Of primary concern was the relationship between animals' sensitivity to stress (as reflected in serum corticoste-

rone levels) and their exploratory patterns. After the completion of all behavioral testing, animals were either untreated or placed for 6 min on an elevated platform in a bright, noisy room. As evident in the top panel of Fig. 2, confinement on the platform produced a significant eleva-

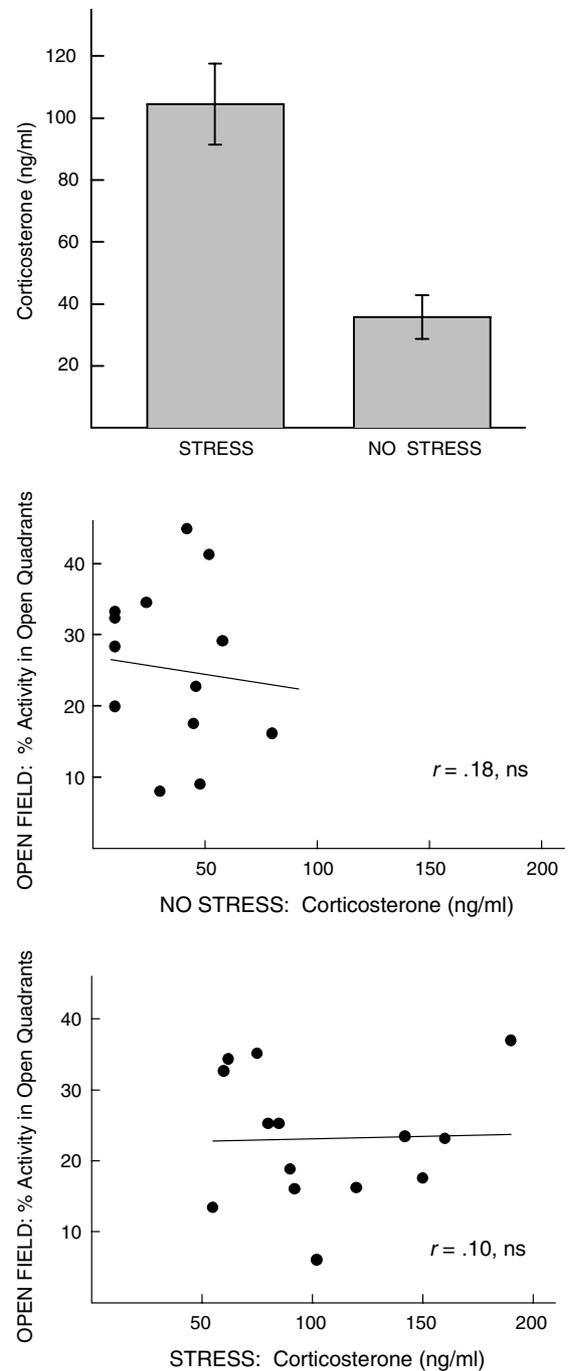


Fig. 2. (Top panel) Serum corticosterone levels were determined for two groups of animals that had been equated for their propensity to explore the open quadrants of a novel open field. Prior to blood collection, animals were either stressed by confinement for 6 min on an elevated platform or not stressed. Platform confinement resulted in a significant elevation of serum corticosterone. Brackets indicate standard error of the mean. (Lower panels) Neither basal (middle panel) nor stress-induced (lower panel) corticosterone levels were correlated with animals' propensity to explore the open quadrants of the novel open field.

Table 3
Factor loadings, principal component analysis of exploratory behaviors

	Factor 1	Factor 2
Open field: total activity	-.447	.513
Open field: % open activity	-.732	-.400
Open field: bolli count	-.132	.227
Plus maze: % open time	-.867	-.271
Plus maze: % open time	-.873	-.340
Plus maze: % entries in new arm	-.739	.180
Plus maze: total arm entries	-.550	.671
Plus maze: time in first closed	.675	-.515
Plus maze: time of first open entry	.791	.359
Eigen value	4.201	1.543
Proportion of total variance	.466	.171

These data were obtained from a subset of animals ($n = 27$) from which basal and stress-induced serum corticosterone levels were obtained.

Table 4
Correlations (r) of exploratory behaviors and basal serum corticosterone ($n = 13$)

	1	2	3	4	5	6	7	8	9	10
1. OF: total activity		-.01	-.25	-.38	-.10	.40	-.41	-.19	.28	-.18
2. OF: % open activity	-.01		.09	.73**	.71**	-.30	-.12	-.32	-.59*	-.19
3. OF: bolli count	-.25	.09		-.10	.05	.18	-.22	.23	.09	-.06
4. PM: % open time	-.38	.73**	-.10		.87**	-.58*	.39	-.47	-.69**	.25
5. PM: % open entries	-.10	.71**	.05	.87**		-.50	.09	-.40	-.76**	.37
6. PM: % new entries	-.40	.30	-.18	.58*	.50		.25	.02	-.45	.31
7. PM: total entries	-.41	-.12	-.22	.39	.09	-.25		-.51	.11	.38
8. PM: time in first closed	-.19	-.32	.23	-.47	-.40	-.02	-.51		-.06	-.19
9. PM: time of first open	.28	-.59*	.09	-.69**	-.76**	.45	.11	-.06		-.28
10. Corticosterone: basal	-.18	-.19	-.06	.25	.37	-.31	.38	-.19	-.28	

* $p < .05$.

** $p < .01$.

Table 5
Correlations (r) of exploratory behaviors and stress-induced serum corticosterone ($n = 14$)

	1	2	3	4	5	6	7	8	9	10
1. OF: total activity		.42	-.48	.51	.50	-.56*	.66**	-.57*	-.49	.36
2. OF: % open activity	.42		-.08	.62*	.66**	-.59*	.16	-.41	-.56*	.03
3. OF: bolli count	-.48	-.08		.29	.32	-.16	.42	-.36	-.19	.60*
4. PM: % open time	.51	.62*	.29		.93**	-.50	.45	-.53*	-.72**	.49
5. PM: % open entries	.50	.66**	.32	.93**		-.59*	.38	-.42	-.80**	.58*
6. PM: % new entries	.56*	.59*	.16	.50	.59*		.66**	-.55*	-.70**	.25
7. PM: total entries	.66**	.16	.42	.45	.38	.66**		-.71*	-.51	.39
8. PM: time in first closed	-.57*	-.41	-.36	-.53*	-.42	.55*	-.71**		.53*	-.21
9. PM: time of first open	-.49	-.56*	-.19	-.72**	-.80**	.70**	-.51	.53**		-.40
10. Corticosterone: STRESS	.36	.03	.60*	.49	.58*	-.25	.39	-.21	-.40	

* $p < .05$.

** $p < .01$.

tion of serum corticosterone, $t(25) = 5.28$, $p < .001$. Separate correlation matrices were constructed for stressed and unstressed animals, and all performance data and corticosterone levels were included for analysis. These matrices are presented in Tables 4 and 5. As can be seen in Table 4, basal (unstressed) corticosterone levels were not significantly correlated with any of the performance measures. Not surprisingly, we can conclude that basal corticosterone levels do not appreciably impact animals' activity, exploratory tendencies, or emotionality. The relationship between animals' sensitivity to the stress associated with confinement on an elevated platform and performance measures obtained during exploration is somewhat more complex. First, it is notable that stress-related corticosterone levels and bolli counts in the novel open field are positively correlated ($p < .02$), indicative of a relationship between animals' sensitivity to stress (corticosterone) and emotionality (as indicated by bolli) during exploration of a novel environment. Of more concern was the question of whether sensitivity to stress might mediate animals' exploratory patterns in the open field and elevated plus maze. The percent of activity in the open quadrants of the open field was unrelated to stress-induced corticosterone levels ($r = .03$; bottom panel, Fig. 2). In the plus maze, exploratory measures related to time or entries into open arms exhibited tendencies toward correlations with stress-induced corticosterone levels, with the correlation between corticosterone and percent of time in the

open arms approaching significance ($p < .06$). However, this tendency was in a direction that was entirely unexpected, i.e., *higher* stress-induced corticosterone levels were associated with *more* time spent in the open arms. This contrasts with our earlier suggestion that animals more susceptible to the impact of stress might exhibit *less* exploration of stress-provoking environments. Given that these correlations do not reach significance and given the absence of any correlation between corticosterone levels and exploration of the open quadrants in the open field, it appears that there is no systematic relationship between animals' sensitivity to stress and their exploratory patterns in these environments. In total, these results suggest that animals' sensitivity to stress cannot account for the relationship that we observe between general learning abilities and the propensity to explore stress-inducing environments.

4. General discussion

In this series of experiments, we determined the relationship between sensory/motor abilities, fitness, fear/stress sensitivity, and the general learning abilities of individual animals. In no instance were we able to detect a relationship between measures of simple fitness or sensory/motor function and general learning abilities. For instance, measures of balance, pain sensitivity or reactivity, running speed, and overall activity loaded weakly and inconsistently with fac-

tors on which performance on learning tasks loaded heavily in principal component factor analysis. However, animals' exploratory patterns in novel environments loaded heavily and consistently with performance on tests of learning. As we have previously reported, these particular exploratory tendencies were strongly indicative of individual animals' general learning abilities (Matzel et al., 2003). Specifically, the extent to which animals' engaged in exploration of areas of environments that are commonly asserted to promote stress responses was positively correlated with performance on learning tasks. Such performance measures as the percent of activity in open quadrants of the open field, percent of time and number of entries into open arms in the elevated plus maze, and latency to enter the first open arm in the plus maze were positively correlated and all loaded heavily with learning performance on a single factor extracted by principal component factor analysis. Relatedly, animals' proclivity to enter *new* arms in the elevated plus maze (as opposed to re-entering an arm just exited) loaded on this same principal factor.

Common measures of fear loaded weakly and inconsistently with measures of exploration or learning. In particular, bolli counts during exploration of the open field, shock-induced freezing, and startle-induced escape responding were seemingly unrelated to animals' exploratory patterns or their general learning abilities. This pattern of variable loading suggests that the relationship between exploration and learning was *not* attributable to variations in animals' expression of fear or their sensitivity to fear-evoking situations or stimuli. This is critical, as laboratory situations (e.g., handling, novel environments, and aversive stimulation) designed to assess learning in animals can reasonably be expected to promote fear, and these results mitigate the possibility that variations in individuals' sensitivity to fear might underlie the variations in general learning abilities that we observe in laboratory mice.

Exploration of the open quadrants of a novel open field or the open arms of an elevated plus maze is often interpreted as an indication of an animal's proclivity for novelty seeking, and/or may reflect the degree to which an animal experiences stress in the unfamiliar open environments (Anderson, 1993; Kabbaj et al., 2000). It is notable that the degree of preference for novelty in human infants is positively correlated with later performance on standardized IQ test batteries (Bornstein & Sigman, 1986; Vietze & Coates, 1986). While the underlying nature of this relationship between novelty seeking and learning/intelligence is unknown, it is possible that animals more engaged by novelty are more likely to recognize (or attend to) those environmental relationships upon which learning depends. Relatedly, animals that are prone to novelty seeking may be less susceptible to the experience or physiological consequences of stress, which in many instances can impede learning (Shors, 1998). This later possibility was assessed by examining the relationship between animals' serum corticosterone levels (a physiological index of stress). Of particular interest was the nature of this relationship under basal con-

ditions relative to the relationship under conditions in which animals were experiencing a level of stress comparable to that which might accompany exploration of a novel environment. Of note, no relationship was found between basal corticosterone levels and individual animals' propensity to explore the open quadrants of the open field or the open arms of an elevated plus maze. Similarly, the elevated corticosterone levels associated with a mild stressor (confinement on an elevated platform) were unrelated to animal's level of activity in the open quadrants of an open field. A tendency toward a relationship between stress-related corticosterone levels and time spent in the open arms of the elevated plus maze was observed, but in this case, the correlation was such that *higher* serum corticosterone was associated with a proclivity to spend *more* time in the open arms. The direct relationship between physiological stress reactivity and the propensity for exploration suggests that it is unlikely that *reduced* sensitivity to stress could underlie an enhancement of general learning abilities.

Upon initial consideration, it is somewhat surprising that fear and/or stress sensitivity are not inversely related to animals' exploratory tendencies in novel environments, particular when those tendencies are such that the exploratory pattern exposes animals to environments that are known to evoke stress responses (for results similar to those reported here, see Delu, Piazza, Mayo, Le Moal, & Simon, 1996; Piazza et al., 1991). However, using a similar factor analytic approach with different dependent measures of stress, a similar lack of covariance between these variables has been previously observed. For instance, Overmier, Murison, and Johnsen (2003) have reported that the initial propensity of rats to explore a novel environment was unrelated to the likelihood or extent of stress-induced ulceration. Similarly, Overmier et al. found no relationship between common measures of fear and animals' propensity for exploration or their sensitivity to stress-induced ulceration. It is notable that in the present studies, bolli counts in a novel open field *were* positively correlated with the level of serum corticosterone associated with mild stress. Given the absence of a relationship between bolli counts and exploratory patterns or learning abilities, this result further suggests that fear and/or stress sensitivity cannot account for variations in general learning abilities.

In total, these results provide further evidence for the existence of an influence on animals' learning abilities that transcends limited learning domains, and that is independent of the sensory, motor, motivational, and information processing demands of specific learning tasks (also see Galsworthy et al., 2002). Furthermore, variations in individuals' general learning abilities do not appear to be attributable to individual differences in fear responses or the sensitivity to (or physiological consequences of) stress. Nevertheless, we repeatedly observed a strong and consistent relationship between animals' tendency to explore stress-inducing novel environments and their general learning abilities. The underlying basis for this relationship has yet to be discerned. Of course, it is possible that these two

classes of behavior (learning and exploration/novelty seeking) are regulated in common but do not otherwise influence each other. A more intriguing possibility is that animals' proclivity for exploration predisposes them to encounter those contingencies upon which learning depends, and thus is a determinant of general learning abilities.

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