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Pharmacological Modulation of Stress Reactivity Dissociates General Learning Ability From the Propensity for Exploration

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It has previously been reported that general learning ability (GLA) correlates positively with exploratory tendencies in individual outbred mice. This finding suggests the possibility that variations in stress reactivity modulate GLA and thus its relationship to exploratory tendencies. Here, the authors investigated the potential role of stress reactivity in regulating this relationship by assessing the effects of the anxiolytic chlorodiazepoxide (CDP; 10 mg/kg) on subjects' performance in a battery of diverse learning tasks as well as exploratory behaviors and stress reactivity. CDP-treated mice exhibited reductions in stress-induced corticosterone levels and behavioral reactivity to mild stressors and a corresponding increase in exploration. However, CDP-treated mice did not exhibit facilitated acquisition of any of the learning tasks and expressed GLA comparable to controls. Results indicate that although reduced stress reactivity promotes exploration, this does not translate into an up-regulation of GLA, suggesting that the relationship between GLA and exploration is not mediated by stress reactivity. The authors propose that variations in GLA reflect individuals' propensity for novelty seeking, whereas exploration reflects both stress reactivity and novelty seeking, the latter of which may underlie the relationship between exploration and GLA.

Keywords: learning, exploration, novelty seeking, stress, anxiolytics

Studies of intelligence have demonstrated the existence of positive correlations among seemingly distinct cognitive abilities such that the performance of individuals tends to covary across diverse tests of learning (Carroll, 1993; Kolligian & Sternberg, 1987). Evidence derived from a growing body of research in humans suggests that 25%–50% of the variance between individuals across cognitive tasks can be accounted for by a single factor (Plomin, 1999; Plomin & Spinath, 2002). This factor influences performance on each particular test and accounts for the patterns of consistent, domain-independent variations between individuals (Sternberg & Kaufman, 1998). Thus, it is postulated that every test of cognitive ability measures both domain-specific abilities as well as general cognitive ability, or *g*.

Although principally studied in humans, animals pose a valuable opportunity for studies of *g* by allowing for more controlled study of the underlying mechanisms of general cognitive ability. Furthermore, this approach affords a collateral benefit in attempts to evaluate the effects of presumed cognition-influencing manipulations (e.g., pharmacological, transgenic, or environmental) by providing means for dissociating effects of a manipulation on specific cognitive domains from its impact on cognition as a whole. To address these needs, researchers have recently developed a battery of diverse learning tasks that measures a range of learning abilities in mice and thus can assess individual differences in general learning ability in this common laboratory species (e.g., Galsworthy, Payo-Cano, Monleon, & Plomin, 2002; Kolata, Light, Grossman, Hale, & Matzel, 2007; Kolata, Light, Townsend, Hale, Grossman, & Matzel, 2005; Locurto, Fortin, & Sullivan, 2003; Matzel et al., 2003, 2006). A typical test battery is composed of tasks that each engage unique combinations of sensory, motor, motivational, and processing systems. This design maximizes sensitivity to individual differences in performance (across all tasks) that can be accounted for by general abilities as opposed to domain-specific abilities or performance-related variables. In a previous study (Matzel et al., 2003) using a sample of 56 genetically diverse outbred mice (CD-1), positive correlations were found between subjects' performance on all tasks. A principal-components factor analysis of subjects' performance on the learning tasks extracted a single factor that accounted for 38% of the variance between individuals across tasks. These results suggest that this learning battery is sensitive to a factor analogous to human *g*, that is, a general learning ability.

In addition to their descriptions of general learning abilities in mice, Matzel et al. (2003, 2006) also reported that the tendencies of mice to explore the open regions of a walled open field and the open arms of an elevated plus-maze were positively correlated with performance on all learning tasks and loaded strongly with performance on the learning tasks in the principal (i.e., general learning) factor. Exploration in both the open field and elevated plus-maze was limited to a single 4-min exposure because it was

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believed from prior work that no appreciable habituation occurred during such a short duration of exposure. Thus, it is likely that this measure of exploratory behavior is not merely a reflection of learning and, as such, that innate exploratory tendencies per se are correlated with individual subjects' general learning abilities. This result is consistent with findings in humans (Bornstein & Sigman, 1986; Vietze & Coates, 1986), in which the degree of preference for novelty in infants is positively correlated with later performance on standardized intelligence tests.

The above pattern of results suggests the possibility that an animal's sensitivity to stress might underlie or modulate its learning ability. Because of conditions such as the novelty of the learning environments and stimuli, handling by experimenters, and the organic deprivation or aversive reinforcement that some tasks rely on, testing in all of the learning tasks is associated with some degree of stress. A wealth of research has yielded demonstrable effects of both acute and chronic stress on learning in animals (McEwen, 2003; McEwen et al., 1997; Shors, 2004). In view of these effects and the level of stress potentially associated with testing in our test battery, it could be argued that the factor interpreted as an analogue of *g* alternatively represents, or reflects, stress reactivity. Moreover, this interpretation of stress reactivity as a determinant of general learning abilities is particularly compelling in light of the relationship between animals' exploratory tendencies and learning abilities. Given the sensitivity of exploratory behavior to anxiety or stress (File, 1985; Montgomery & Monkman, 1955), this relationship further highlights the possibility that variations in stress reactivity account for differences in animals' learning abilities.

The expression of exploratory behavior in animals is said to be regulated by at least two opposing biological functions, the tendency to seek novelty and neophobia, or anxiety (Montgomery, 1955; Whimbey & Denenberg, 1967). Although rodents have been shown to prefer exploration of novel places or objects in lieu of those that are familiar, they also typically exhibit anxiety and stress responses on exposure to novel objects or environments (Denenberg, 1969; File, 1985). These two opposing responses to novelty combine to determine the course of exploratory activity: Exploration is enhanced by preference for novelty seeking and reduced by anxiety or stress. Accordingly, increased exploration may result either from greater levels of the former or lower levels of the latter and consequently may, in varying situations, reflect either function.

In light of the complexity of factors that influence exploratory behavior, the relationship between exploration and learning is not straightforward. One possibility, consistent with the novelty-seeking basis of exploratory behavior, is that mice that tend to explore are more engaged by novelty and thus have more opportunities to recognize and attend to environmental and stimulus relationships on which learning depends. Another possibility is that mice that tend to explore to a greater degree may be less prone to the anxiety or stress that typically accompanies exposure to novelty in rodents. In turn, this state would likely allow for more active exploration of novelty and concurrently render these mice less susceptible to the aversive impact of stress on learning performance. As such, individual differences in levels of anxiety or stress reactivity might underlie both exploratory tendencies and general learning abilities, yielding the particular combination of results found with this set of behavioral tests.

In a previous study (Matzel et al., 2006) conducted to address the latter possibility, the relationship of exploratory behavior to stress sensitivity, fear, and general learning abilities was examined. A sample of CD-1 mice was tested in a variant of the learning test battery and assessed on various measures of fear and exploratory tendencies to investigate the relationship between individual subjects' learning performance, exploratory behavior, and expression of fear. In a second experiment, a sample of CD-1 mice was evaluated on measures of exploratory behavior as well as physiological stress indexes under basal or stressed conditions to determine the relationship between their exploratory behaviors and physiological responses to environmental stressors comparable with learning tasks. Common measures used to quantify fear or emotionality in mice were unrelated to individual subjects' exploratory patterns or general learning abilities (i.e., they loaded weakly and inconsistently with measures of exploration and learning on a principal-component factor analysis). Further, no consistent relationship was found between basal or stressed corticosterone levels and subjects' tendency to enter the open quadrants of an open field and the open arms of an elevated plus-maze, measures of exploratory behavior (although on some measures, *higher* stress reactivity was associated with *more* exploration). These results, taken in total, suggest that differences in individual mouse sensitivity to stress or expression of fear are unlikely to account for variations in exploratory tendency and their relationship to general learning abilities.

Although suggestive that stress reactivity does not mediate the relationship between the propensity for exploration and general learning ability, the above correlational results are not conclusive. Here, the role of anxiety and stress reactivity in regulating general learning ability and its relation to exploratory behavior was directly assessed by examining the effects of an anxiolytic on subjects' general learning performance as well as on stress-sensitive exploratory behaviors and the physiological stress response. A sample of CD-1 mice, consisting of two groups, was trained and tested in a variant of the learning battery composed of seven learning tasks. One of the two groups was administered the anxiolytic chlorodiazepoxide (CDP) prior to each session of training or testing, and the second group was administered a vehicle injection of saline. In addition to the learning tasks, both groups of mice were tested on several measures of exploration designed to be sensitive to stress and thus comparable with measures of exploration previously found to correlate with general learning abilities. This dimension of exploration was explicitly emphasized in these measures so as to elucidate its role in the relationship between general learning abilities and exploratory behavior. Mice were also assayed for blood levels of free corticosterone following stress exposure to assess physiological responses to stress. To ensure that the impact of CDP on learning and exploration was due to its effects on anxiety–stress rather than to potential secondary effects on sensory–motor function, mice were also assessed on unlearned measures of sensory–motor function, anxiety, and stress reactivity on completion of the battery of learning tasks.

Method

Subjects

A sample of 30 male outbred CD-1 mice (Harlan Sprague Dawley; Indianapolis, IN) was 84–92 days old at the start of

experimentation. Fifteen mice were assigned to each of two groups, one administered CDP and the other a vehicle injection of saline. Mice were trained and tested in three independent replications separated by 2 days, and each of these consisted of 5 mice from each group. For some tests, the number of mice was reduced because of 2 mortalities and, in several instances, apparatus malfunction. Mice were acclimated to our laboratory for 14–20 days prior to testing and were handled for 90 s/day 5 days/week during this period. This handling ensured that differential stress responses to the experimenters and any associated effects on learning were minimized. Mice 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-hr light–dark cycle was maintained.

Drug Treatment

After acclimation to our colony, mice were acclimated to injection procedures and drugs for 3 consecutive days, during which they were administered the injection treatment appropriate for their group. This acclimation, followed by a day of rest prior to testing, was implemented to minimize any stress evoked by injection procedures and to adapt the mice to the effects of the injections before the onset of critical behavioral tests. Mice in the treatment group were administered CDP (10 mg/kg, 10 mL/kg) suspended in 0.9% saline, and mice in the control group received 0.9% saline at an equivalent volume (10 mL/kg). This dose of CDP was selected on the basis of pilot work by our laboratory in which this concentration was ascertained to produce an effect on stress reactivity (but to have only marginal effects on locomotor behaviors). Injections were administered at 2.5–4.5 hr into the light cycle 30–45 min prior to behavioral training or assessment for each mouse.

General Behavioral Training and Testing

All mice were tested on seven learning tasks (Lashley III maze, passive avoidance, reinforced alternation, fear conditioning, odor discrimination, spatial plus-maze, and water maze) and 21 measures of unlearned performance and fitness. These learning tasks were explicitly designed and included in the test battery so as to impinge on different sensory, motor, motivational, and information-processing systems. In addition, these tasks measure learning during acquisition and thus are sensitive to differences in learning between mice that can potentially be obscured by measures of asymptotic performance. Mice were first tested in an open field to assess activity and exploratory tendencies, followed by testing in seven learning tasks, and, finally, on all remaining tasks designed to assess activity and exploratory tendencies, as well as sensory and motor function, stress and pain reactivity, and fear. Between each successive test (of learned and unlearned behaviors), mice received a day of rest. With 1–3 days required for each task, the entire test regimen was completed in 50 days. Different experimenters trained or tested mice in different tasks, and no experimenter was aware of mice's performance on other tasks until after the completion of the entire battery of tests.

Prior to testing on any task, the test chambers were primed by exposing two nonexperimental mice to the apparatus and procedures. This was intended to standardize the apparatus such that the first mice in a test cycle encountered a chamber that was nominally similar (e.g., in odor) to that experienced by subsequently tested mice. 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 when intertrial intervals (ITIs) involved removing subjects from the apparatus.

For the four learning tasks that required food deprivation, ad lib food was removed from the subjects' home cages at the end of the light cycle approximately 40 hr prior to the start of training (and thus encompassing the rest day between successive tasks). During the deprivation, mice were provided food in their home cages for 90 min per day during the last 2 hr of the light cycle and thus were approximately 16-hr food deprived at the time of training or testing. This deprivation schedule was deemed mild (mice typically lost less than 6% of their free-feeding body weight during this period) but was 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 subjects' performance, all mice were trained and tested during the middle 7 hr of the light cycle and procedures were administered to mice with as little temporal dispersion as possible. All mice were trained and tested under nominally identical conditions.

Tests of Learning

The order of testing was designed so as to provide separation between any two tasks that were motivated by either food or water deprivation (to prevent excessive physical strain and to minimize any potential cross-task influences due to motivational factors). All mice were tested in the following order: Lashley III maze, passive avoidance, reinforced alternation, fear conditioning, odor discrimination, spatial plus-maze, and water maze.

Lashley III Maze

The Lashley III maze consists of a start box, four interconnected alleys, and a goal box containing a food reward. Over trials, the latency of rats to locate the goal box decreases, as do their errors (i.e., wrong turns or retracing). Here, the Lashley III maze was scaled for mice, and parameters were developed (Matzel et al., 2003) that supported rapid acquisition. The maze was constructed of black Plexiglas. A 2 cm wide $\times$ 0.1 cm deep white cup was located in the rear portion of the goal box, and 45-mg Bio-Serve (rodent grain; Frenchtown, NJ) pellets served as reinforcers. Illumination was 80 Lux at the floor of the maze. The maze was isolated behind a shield of white Plexiglas to mitigate against extramaze landmark cues.

Food-deprived mice were acclimated and trained on 2 successive days. On the day prior to acclimation, all mice 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 mice could not navigate the maze. Each mouse was confined to the start box and subsequent two alleys for 4 min and to the last (goal) alley for 6 min, 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 mouse 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. On consuming the food, the mouse was returned to its home cage for a 25-min ITI, after which it was returned to the start box to begin the next trial. This 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 and used to index learning.

One-Trial Passive Avoidance

Animals learn to suppress movement 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 it encounters a footshock. To not duplicate the motivating stimuli (i.e., shock) used to support associative learning (fear conditioning; see below) between tasks, here we used a variant of the step-down avoidance task that does not rely on shock to motivate behavior. On stepping off the platform, mice were exposed to a compound of bright light and loud oscillating noise. Like more common procedures, our variant of this task has been shown to support learning after only a single trial (Matzel et al., 2003), following which subsequent step-down latencies are increased.

A chamber illuminated by dim (40 Lux) red light was used for training and testing. Mice 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 composed 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 comprising 4-mm-wide aluminum planks that formed a pattern of 1.5-cm square grids, which were oriented at a 45° angle relative to the grids in the safe compartment. When a mouse stepped from the safe compartment through the exit door onto the floor of the unsafe compartment, the compound aversive stimulus composed of a bright (550 Lux) white light and siren (60 dB above the 50-dB background) was initiated.

Subjects were placed in the safe compartment, behind the exit blocked by the Plexiglas door. After 4 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. On contact with the floor, the door to the platform was lowered and the aversive stimulus (light, noise, and vibration) was presented for 4 s, at which time the platform door was opened to allow mice 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 subject to exit the platform and step onto the grid floor (with no aversive stimulation) was recorded, completing training and testing. The ratio of posttraining to pretraining step-down latencies was calculated for each animal and served to index learning. In previous research (Matzel et al., 2003), we determined that asymptotic performance was apparent in group averages following two–three training trials; thus performance after a single trial reflects (in most instances) subasymptotic learning.

Reinforced Alternation

In this task, animals learn to alternate between two arms of a maze to obtain food on each trial. An elevated maze in the form of a T was constructed of black Plexiglas. Each of the two cross-arms measured 36 cm in length and 4 cm in width and had 10 cm walls. A 4 mm in diameter food cup was located in each cross-arm, 2 cm from its end. The base of the T consisted of a 14-cm start box and a 16-cm central compartment from which the cross-arms connected. The portion designated as the start box could be blocked with a sliding guillotine door, as could the intersection between each cross-arm and the central compartment. The maze was diffusely lit from above (80 Lux).

On the first day of training, food-deprived mice were adapted to the maze, in which one eighth of a fruit loop (General Mills, Inc., Minneapolis, MN) was available at the end of each choice arm. Over four more trials, mice were forced to alternate arm entry by closing the opposite arm's guillotine door. In the forced-choice arm, subjects obtained a reinforcer. On the subsequent day, mice were placed in the start compartment (at the base of the T), held behind the closed guillotine door for 60 s, and then, after the door was opened, allowed to choose one arm for entry, wherein the reward was available. On subsequent trials (30-s ITI), the mouse could choose either arm but food was available only in the arm opposite the arm reinforced on the prior trial. Incorrect choices terminated the trial. On ensuing trials, food was available in the same arm until a correct choice was made and the food was retrieved. With our adaptation and training procedures, young adult mice often begin to perform without error after 8–10 training trials. Training proceeded for 14 trials.

Associative Fear Conditioning

In such a procedure, animals are exposed to a stimulus (i.e., a conditioned stimulus [CS]; tone) that terminates in the onset of a mild footshock (i.e., an unconditioned stimulus [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. To avoid any interaction of the training context (which itself acquires an association with shock) with the CS at the time of testing, we conducted training and testing in two separate distinct contexts.

Two distinct experimental chambers (i.e., contexts; 32 cm long $\times$ 28 cm wide $\times$ 28 cm high) were used, each of which was contained in a sound- and light-attenuating enclosure. These boxes were designated as training and testing contexts and differed as follows. The training context was brightly illuminated (100 Lux), 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 Lux), had walls covered with an opaque pattern of alternating black and white vertical stripes (3 cm wide), and had a floor 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. On contacting the tube, the mice completed a circuit such that the number of licks per second

could be recorded. This circuit was designed so that if a mouse made continuous contact with the tube (i.e., "mouthed" the tip), the circuit recorded 8 licks/s for the duration of the continuous contact, 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 chamber ceiling.

Water-deprived mice 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 exhibited stable licking (for water). When subsequently placed in the chamber, these mice typically initiated licking within 5–10 s and licked at relatively stable rates for the subsequent 2–4 min. Training occurred in the training context in a single 40-min session, during which each mouse was administered a noise–shock pairing 14 and 28 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 three–five such pairings. Thus, two pairings (in most instances) support subasymptotic conditioned responding. At the end of the training session, subjects were returned to their home cages for 60 min, after which they were reacclimated to the test context for 20 min and were allowed free access to the lick tubes. On the subsequent day (23–25 hr posttraining), mice were tested. Each mouse was placed in the test context, whereupon after making 50 licks the noise CS was presented continuously until the subject completed an additional 25 licks. The latency to complete the last 25 licks during the pretone 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 subject described here). With these measures, the ratio of the latency to complete 25 licks prior to CS onset over the latency to complete 25 licks in the presence of the tone CS served as our index of learned fear.

Odor Discrimination and Choice

Rodents rapidly learn to use odors to guide appetitively reinforced behaviors. In a procedure based on one designed by Sara, Roullet, and Przybyslawski (1999), rats learn to navigate a square field in which unique odor-marked (e.g., almond, lemon, mint) food cups are located in three corners. Although food is present in each cup, it is accessible to the rats in only one cup (e.g., that marked by mint odor). A rat 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 cup is changed, but the accessible food is consistently marked by the same odor (i.e., mint). On successive trials, rats 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 have typically observed errorless performance within three–four training trials (Matzel et al., 2003).

A black Plexiglas 60-cm square field with 30 cm high walls was located in a dimly lit (90 Lux) testing room with a high ventilation rate (3 min volume exchange). Three 4 × 4 × 2 cm (length × width × height) 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 mouse, whereas 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 ul of lemon, almond, or mint odorants (McCormick flavor extracts; Sparks, MD). The mint odor was always associated with the target food cup.

On the acclimation day, each food-deprived mouse 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 mouse's home cage to acquaint them with the reinforcer. On the subsequent test day, mice received four training trials in the field with three food cups present. On each trial, a mouse was placed in the empty corner of the field. On Trial 1, the reinforcing food (rice) was available to the mouse 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 mouse retrieved and consumed the food from the target cup, after which the mouse was left in the chamber for an additional 20 s and then returned to its home cage to begin a 5-min ITI. On Trials 2–4, the location of the food cups were rearranged 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 was changed on each trial. On each trial, the latency to retrieve the food and errors were recorded. An error was recorded any time that a subject 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 a subject sampled (as above) the target cup but did not retrieve the available food.

Spatial Plus-Maze

In this task, mice must learn to consistently enter the west arm of a maze to obtain food, despite alternating on each trial between east, north, and south starting positions. An elevated maze in the form of a plus sign was constructed of black Plexiglas, each of the four arms measuring 8 cm wide × 40 cm long. 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; Lancaster, NH) was located in every cup but was accessible to the mouse only in the arm designated as west. The maze was surrounded by a dark blue field marked by a light in the north quadrant and a white placard in the south quadrant. The 7-W light was suspended 16 inches (40.64 cm) over and 3 inches (7.62 cm) behind the north start position and the 6-inch (15.24-cm) white polygon was positioned 6 inches (15.24 cm) over and 3 inches (7.62 cm) behind the south start position. The area was dark other than illumination provided by the cue light.

Training and testing occurred in the same session. On the day prior to training, mice were given a sample reinforcer in their home cage. On the subsequent day, mice were trained and a food reinforcer was present in the west arm on each trial. On the first trial, each subject was placed in the east start box for 60 s and was

then released and allowed to explore the maze until it entered all arms, explored all food cups, and collected the food. All mice were kept in the maze for a minimum of 3 min. On the second trial, each subject was placed in the north start box for 10 s and allowed to explore the maze until it entered the west arm and retrieved the food, at which point it was removed. On subsequent trials, mice were started in the south and then east arms, and for the following trials, this order of starting arms (north, south, east) was subsequently repeated for every set of three trials until a total of 10 trials (Trials 1–10) was completed. During each of these trials, an entry into an incorrect arm terminated the trial (at which time the exit was blocked and the subject was removed after 5 s). If subjects chose the correct arm, then they were allowed to consume the food, following which they were removed. If a subject began to exit the arm before checking the food cup, then this was considered an error and it was removed. Trials were separated by a 60-s ITI, during which the apparatus was cleaned, the food cup baited, and the arm choice recorded.

Morris Water Maze

For this task, animals are immersed in a round pool of opaque water from which they can escape onto a hidden (i.e., submerged) platform. In this task, performance of animals can improve across trials despite the animals beginning each trial from a new start location. Such a procedure discourages the use of egocentric navigation and promotes animals' dependence on extramaze spatial landmarks (Morris, 1981). 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, mice 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). Finally, the maze, surround, and water were black; visual cues were constructed 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 perforated black platform 11 cm in diameter 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 24–150 cm. Four of these shapes were constructed of strings of white light-emitting diodes (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 in 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 in 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 140 Lux at the water surface.

On the day prior to training, each subject was confined to the escape platform for 360 s. Training was conducted on 2 consecutive days, with five trials administered each day. On Day 1 of training, mice 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.) A subject 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 subject remained on the platform for at least 3 s. Each mouse 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 mice were held in a warmed (27.5 °C) opaque (5 Lux) box lined with wood shavings. On each trial, a 90-s limit on swimming was imposed, at which time any mouse that had not located the escape platform was placed by the experimenter onto the platform, where it remained for 20 s. Mice were observed from a remote (outside of the pool's enclosure) video monitor, and their performance was recorded on videotape for subsequent analysis. Day 2 of training proceeded in the same manner as Day 1. The latency to reach the platform was recorded on each trial as an index of learning.

Tests of Unlearned Behaviors and Sensory–Motor Function

Each of the following tests was administered with 1 day intervening between the completion of one test and the start of the subsequent test. Open-field testing was conducted 2 days prior to the start of tests of learning; all other tests were administered beginning 2 days after the completion of tests of learning.

In all but several instances, tests of unlearned behaviors and sensory–motor function were completed in a single day. Many of the tests yielded several different measures of performance such that 20 variables were assessed that are relevant to balance, strength, coordination, general activity, pain sensitivity, anxiety, stress reactivity, and exploratory tendencies. The apparatus and parameters that are described below have been chosen on the basis of pilot work (e.g., Matzel et al., 2006) in which they were determined to be adequate to capture wide variations in performance across mice. The tests were conducted in the following order: rod suspension, pain sensitivity, screen hanging, balance beam, dark–light test, startle response, shock-induced freezing, and elevated plus-maze.

Measures of General Activity and Exploration

Open-field exploration. A square field (46 × 46 cm) with 13 cm high walls was constructed of white Plexiglas and located in a brightly lit room (400 Lux) with a background noise of 65 dB. The field was conceptually divided into a grid comprising 6 × 6 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. Mice were placed in the center of the field. After 20 s had elapsed (during which the mice self-selected a starting location), the subjects' behavior was monitored for 4 min. Throughout this time, subjects' entries into walled and open quadrants were recorded. An entry was recorded whenever both front paws crossed the border of a quadrant. Total activity (i.e., quadrant entries regardless of category) was recorded, as was the percentage of entries into unwalled (open) quadrants of the field. It should be noted that a 4-min test was explicitly chosen (on the basis of pilot work) because changes in behavior (e.g., that which accompanies habituation) were not detectable over time. Thus, we presume that

open-field performance was most sensitive to unlearned behavioral tendencies.

Elevated plus-maze. The maze was constructed of gray 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 gray Plexiglas walls, and two of the arms were open. The maze was located in a 300-Lux environment. Mice were placed in the center of the maze facing an open arm, and their behavior in the maze was recorded for 4 min. Of interest was the number of entries into closed arms as well as the total number of arm entries, considered to be indexes of general locomotor activity (Cruz, Frei, & Graeff, 1994; Ramos, Berton, Mormede, & Chauloff, 1997). In addition, the ratio of number of open-arm entries to number of closed-arm entries and of time spent in open arms compared with time spent in closed arms were recorded. As additional measures of exploration, the percentage (of total entries) of open-arm entries and the percentage (of total time) of time spent in open arms were recorded. Generally, entries into open arms are considered to be stressful to mice; thus, measures in the elevated plus-maze provide other indexes of exploratory tendencies similar in nature to that of exploration of the open quadrants of the open field.

Dark–light test. A 10 × 36 cm chamber divided along its length into two equal-sized compartments was used. One compartment was moderately lit (100 Lux), with a clear plastic top and white-lined bottom, and the other compartment was dim (10 Lux), with a dark plastic top and black-lined bottom. The two compartments were divided by a center wall with a 3-cm square opening that joined the dark and light compartments. Mice were placed in the dark compartment of the chamber and allowed to explore for 4 min. Generally, entries into the brightly lit compartment are considered aversive and stressful to mice, thus measures in this test provide indexes of exploratory behavior similar to exploratory measures in the open field and elevated plus-maze. The number of visits into the light compartment (i.e., transitions from the dark compartment into the light compartment) and, to a lesser degree, the percentage of time spent in this compartment, have been shown to be the most reliable indexes of anxiety-dependent exploration behavior in this apparatus (Crawley, 1985; Ramos et al., 1997).

Measures of Anxiety and Stress Reactivity

Shock-induced freezing. Freezing after the offset of an unsignaled shock is often interpreted as a measure of fear. Mice were acclimated for 20 min to a 25-cm square chamber (60 Lux) 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 on the command of the experimenter, who initiated the shock when each subject 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 10–10.5 min. During, and for a brief time (500 ms) following the shock, the mice exhibit a burst of activity, after which they exhibit freezing, a presumed index of fear. Mice were assessed for the amount of movement (number of grid squares crossed) exhibited in a period of 20 s following the shock as a function of the amount of movement (number of grid squares crossed) exhibited in the 20 s prior to the shock. In addition, the duration of freezing (the latency for the rear paws of the mice to move 20 cm) served as a dependent variable.

Preattentive 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 Lux) and maintained against a low background noise level (52 dB). A 200-ms burst of white noise, 60 dB above background, was presented 6 and 12 min after the subject was placed in the chamber. The maximum deflection of the floor was computed during a 500-ms window beginning at the onset of the noise, and the two responses were averaged for each subject.

Corticosterone elevation in response to an acute stressor. Serum corticosterone levels are sharply elevated in response to acute stressors and mediate many physiological responses to stress. Following all behavioral testing, we took blood samples of mice that were exposed to either a mild stressor (injection—to which subjects are presumably acclimated to some extent at this phase of testing) or a moderate stressor (injection followed by confinement for 6 min to an elevated platform in a bright, novel room).

Here, it was our intention to determine whether both mild (as might accompany any behavioral test) and more moderate (as might accompany exploration in a novel environment) stress-induced corticosterone responses were attenuated by administration of the anxiolytic CDP and how this presumed stress attenuation related to exploratory behaviors and general learning abilities. To that end, mice tested in each of the treatment groups were divided into two groups, counterbalancing (within treatment group) them for the percentage of time previously spent in the open relative to walled quadrants in the open field.

Stress manipulations and blood collection were conducted 5 days after the last behavioral test, and blood was collected 10 min after the moderate or mild stress manipulation. Half of the mice in each treatment group were moderately stressed by placing them for 6 min on a 10-cm diameter platform elevated 120 cm above the floor placed directly facing a blowing fan to which paper streamers were attached, in a brightly lit, unfamiliar room. (We have previously determined that this treatment induces a two–four times increase in free corticosterone levels. Although elevated, this level of corticosterone is well below that induced by presumably severe stressors. Thus, such a stressor is more comparable to that which might accompany exploration in a novel environment.) Ten minutes following the moderate or mild stress treatment, mice 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 Biochemicals (Costa Mesa, CA). 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.

Measure of Pain Sensitivity

Upon being placed on a 52.6 °C aluminum plate, subjects' latency to raise a hind paw and to either lick or shake the paw was measured as an index of pain sensitivity.

Measures of Motor Strength–Coordination

Balance beam. Mice were placed on a 40 × 0.7 × 2 cm (length × width × height) beam suspended 30 cm above the ground. Movement along the beam was the variable of interest, in addition to latency to fall, 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.

Rod suspension. Mice were 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.

Screen hanging. Mice were placed on 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 and the distance moved prior to dropping from the screen (cm/second; 180 maximum test duration) were recorded.

Analyses

Two types of analyses were performed, including a comparison of groups (CDP and control) on each task and an assessment of the general learning abilities of the two groups. For tasks in which a single performance measure was obtained, the two groups (CDP- and saline-treated) were compared using a *t* test for independent samples. Where the performance measure was obtained over multiple trials, a two-way analysis of variance (ANOVA; Group × Trial) was used, with one variable (trial) treated as a repeated measure. Of more direct relevance to the present study, we were interested in the expression of general learning abilities under baseline and reduced levels of stress reactivity (control and CDP treatment). To assess this difference, we subjected the learning data from CDP and control mice to a single unrotated principal-components analysis (i.e., the data from both groups contributed to this analysis). This analysis provides an estimate of the variance accounted for by any general learning factor. On the basis of this overall analysis, it was then possible to assign factor scores (derived from the general learning factor) to each mouse that contributed to the analysis. A factor score is analogous to an average *z* score for each subject, obtained from the performance of each mouse on each task, weighted by the extent to which that task contributed to the general learning factor. Thus factor scores served to rank the general learning ability of each mouse relative to others in the same sample. Having determined each subject's factor score, the general learning abilities of CDP-treated and control mice could then be compared with a *t* test for independent samples. A second principal-components analysis was conducted on data obtained on learning tasks and on tests of exploration. The purpose of this analysis was to establish the relationship of exploration to performance on the learning tasks.

Results

Exploration Behaviors

In the elevated plus-maze, mice treated with CDP exhibited a greater ratio of entries into open arms compared with entries into closed arms, $t(25) = 2.23$, $p < .05$ (see Figure 1A) and tended toward a greater ratio of time spent in open arms compared with closed arms, although this did not reach significance, $t(25) =$

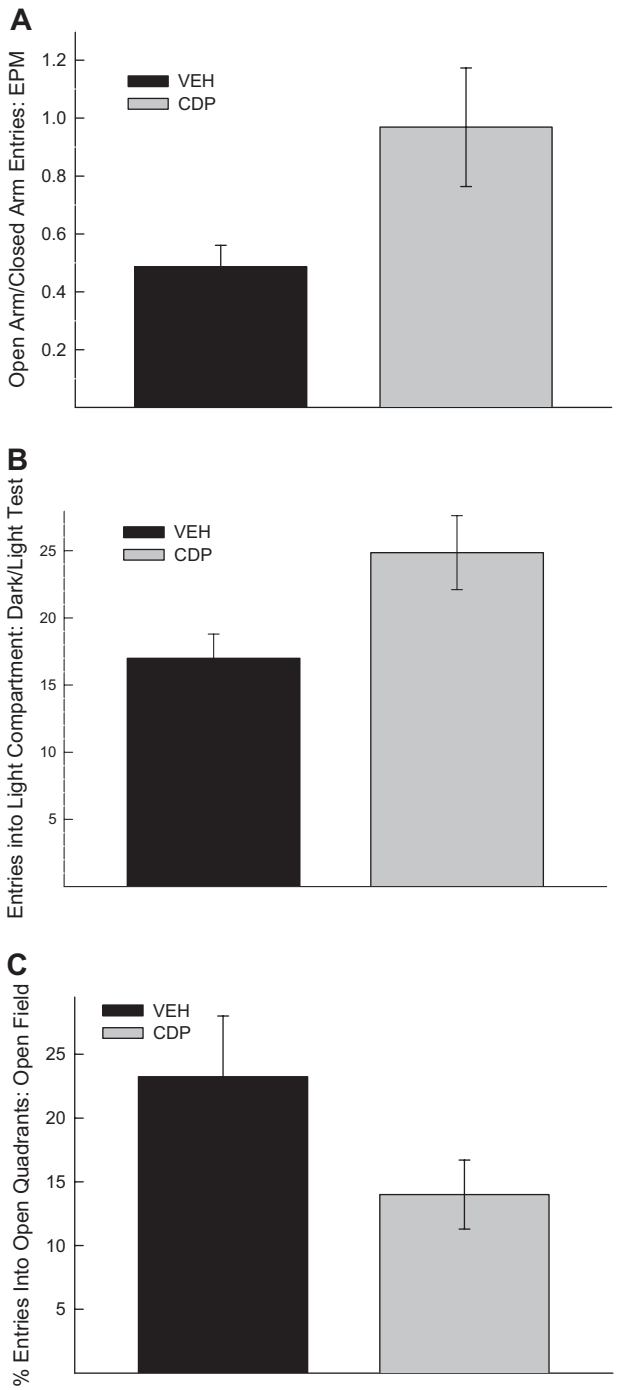

Figure 1. Behavioral assays of exploration demonstrated a pattern of largely increased exploration for chlorodiazepoxide- (CDP) treated mice, with a greater ratio of open-arm compared with closed-arm entries in the elevated plus-maze (EPM; A) and a greater number of entries into the light compartment of a box with light and dark compartments (B). Similar patterns were observed in other tests of exploration (see the text for details). However, CDP-administered mice exhibited a reduced percentage of entries into open quadrants of the open field (C), suggesting a tendency for perseverative behavior along the walls of the field. Error bars indicate standard error of the mean. VEH = vehicle.

-1.61, $p = .12$. In the dark–light preference test, mice administered CDP exhibited an increase in entries into the light compartment, $t(25) = 2.60$, $p < .05$ (see Figure 1B). It is noted that this test is considered to be the most reliable assay of anxiety-sensitive exploratory behaviors for purposes of assessing benzodiazepine effects (Chaouloff, Durand, & Mormede, 1997; Crawley, 1981, 1985). CDP-administered mice also tended to spend more time in the light compartment, although this was not significant, $t(25) = 1.61$, $p = .12$. In contrast to the pattern of results suggested by tests in the elevated plus-maze and the light–dark test, CDP-treated mice exhibited a reduction in percentage of entries into open (unwalled) quadrants of the open field as compared with saline-injected mice, $t(27) = 2.47$, $p < .05$ (see Figure 1C). This pattern appeared to reflect a tendency to perseverate along the walls of the open field, an effect that was further exemplified in some behaviors associated with measures of learning (see below).

Learning Performance

In most of the learning tasks, mice treated with CDP did not exhibit significant changes in performance compared with untreated mice. As illustrated in Figure 2A, there was no difference between mice in their ability to acquire a conditioned fear response, assessed by the ratio of latency to 25 licks following the tone CS as compared with their latency to 25 licks prior to the tone CS. Similarly, CDP-treated mice appeared to have learned the passive avoidance response in a manner comparable with saline-injected mice, $t(23) = -.167$, *ns* (see Figure 2B).

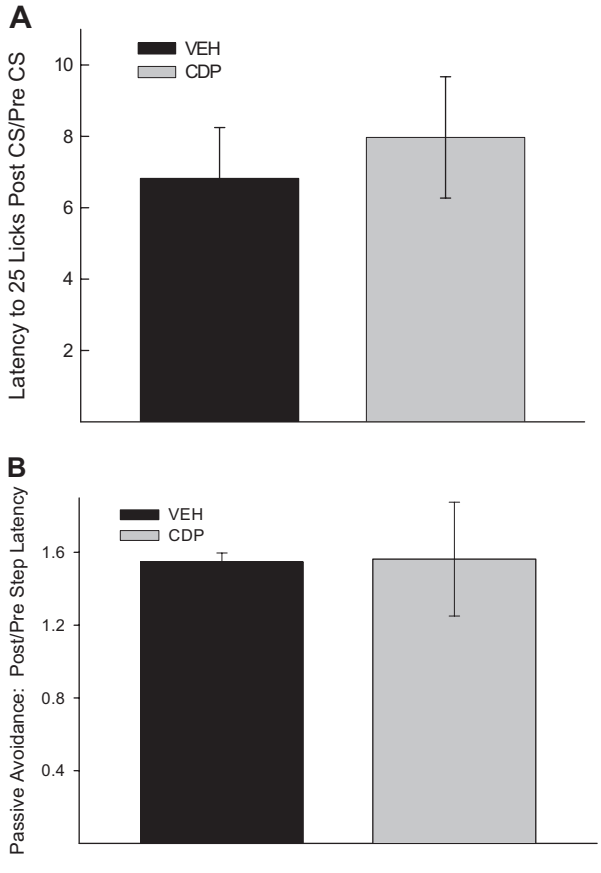

Figure 2. Mice treated with chlorodiazepoxide (CDP) exhibited a pattern of learning similar to controls treated with saline in both fear conditioning (A) and passive avoidance (B) tests. Error bars indicate standard error of the mean. VEH = vehicle; CS = conditioned stimulus.

In the Lashley III maze, mice administered CDP learned how to navigate the maze in a manner comparable to mice injected with saline, with no significant difference between groups in the amount of time that it took them to reach the goal across trials, $F(1, 25) = 0.64$, *ns*, or the number of errors committed prior to reaching the goal across trials, $F(1, 25) = 1.60$, *ns* (see Figure 3A).

Mice in both groups exhibited similar rates of learning in the odor discrimination task. Despite largely comparable overall rates of learning in this task, there was a significant effect for both the number of errors, $F(3, 75) = 3.67$, $p < .03$ (see Figure 3B) and the latency for mice to locate the food, $F(3, 75) = 3.52$, $p < .03$, because of differences in performance on the first trial (prior to any learning having occurred), on which saline-injected mice performed significantly worse, $F(2, 50) = 4.02$ and 4.54, $p < .03$ (errors and latency, respectively). As this difference occurred on the first trial, it is not a reflection of subjects' ability to learn. Typically, high error rates and longer latencies on the first trial of this task principally reflect subjects' hesitation (i.e., neophobia) to eat the novel food that serves as a reinforcer, causing the mice to repeatedly approach the target food cup prior to consuming the food, a behavior that increases latencies and errors before actually consuming the food. Consequently, their superior performance on the first trial may reflect a reduction in neophobia in mice treated with CDP. In this regard, it is notable that mice treated with CDP commit fewer errors on Trial 1 than has been evident in any of our prior reports (e.g., Kolata et al., 2005, 2007; Matzel et al., 2003, 2006) in which mice were not administered CDP.

In the water maze, a significant difference between groups was observed in their performance across trials, $F(9, 225) = 2.42$, $p < .02$ (see Figure 3C). However, inspection of the acquisition curves suggests that rate of acquisition was in fact similar across the CDP- and saline-injected groups, although latencies were generally shorter in the saline-injected mice (including on the first trial, before any learning was possible). A significant difference between groups was detected only on Trial 6, $F(2, 50) = 10.13$, $p < .001$, on which a 24-hr retention interval had been imposed (i.e., the interval between Days 1 and 2 of training). It is noted that these mice exhibited rapid reacquisition, that is, performance returned to Trial 5 levels after only a single trial. This result is consistent with prior observations of an amnestic effect of CDP in long-term measures of retention, particularly in tasks relying on spatial processing (File, 1985; McNamara & Skelton 1992; Thiebot, 1985). Furthermore, in this case, the rapid reacquisition suggests that this amnesia reflects a retrieval deficit rather than a storage failure.

A clear performance decrement was observed in CDP-treated mice in both the reinforced alternation and spatial plus-maze tasks. In reinforced alternation, performance of mice in the CDP-treated group remained at or below chance levels across 14 trials (see Figure 4A), suggesting that they favored responding in one direction, a behavior pattern that would impede efficient performance. This contrasts with the performance of saline-injected mice, whose performance was well above chance after four trials, $\chi^2(1, N = 13) = 4.94$, $p < .03$. In the spatial plus-maze, mice treated with

CDP exhibited nominally inferior performance relative to mice treated with saline (see Figure 4B). Although as a group they appeared to have performed well above chance on select trials, overall, they exhibited a marked pattern of instability, ranging across trials from greater than 80% errors to less than 20% errors (even after eight training trials). This oscillating pattern across trials reflected the tendency of CDP-treated mice to navigate in a straight line across the maze, regardless of the starting location (a pattern which would yield errorless performance on every third trial, a pattern evident in Figure 4B). In contrast, the performance of saline-injected mice suggests stable acquisition of the learned response. Thus, the deficits exhibited by CDP-treated mice reflect their tendency to make a fixed motor response (as was their tendency in the reinforced alternation task). This tendency is consistent with prior findings of perseverative response patterns (Hodges & Green, 1986) and impaired reversal learning (Galizio, Miller, Ferguson, McKinney, & Pitts, 2006) in animals treated with CDP.

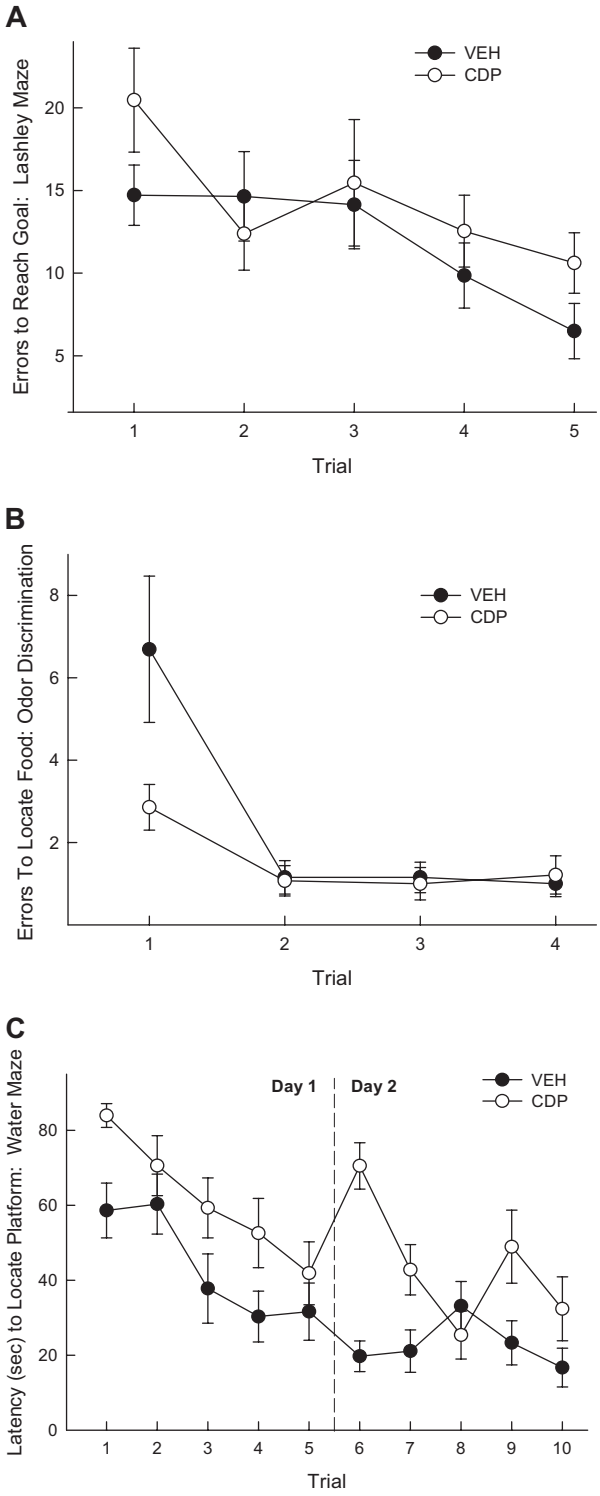

Figure 3. Chlorodiazepoxide- (CDP) and saline-treated mice exhibited similar rates of learning in the Lashley III maze, indexed by the number of errors to reach the goal box (A), and in an odor discrimination task, again indexed by the number of errors before retrieving the reward (B). CDP-treated mice tended to require longer to reach the safe platform in the spatial water maze than did saline-treated controls (C), although the rates of acquisition were similar. A significant impairment in CDP-treated mice was observed only on Trial 6, the first trial on the second day of training (i.e., after a 24-hr retention interval). Rapid reacquisition after Trial 6 suggests that the Trial 6 deficit reflected a CDP-induced retrieval failure rather than a learning deficit. Error bars indicate standard error of the mean. VEH = vehicle.

General Learning Ability

To determine whether CDP affected the expression of general learning abilities, we assessed aggregate performance across learning tasks in both CDP- and saline-treated groups. The learning data from both CDP and control mice were subjected to a single unrotated principal-component factor analysis. Performance on the Lashley III maze, odor discrimination, water maze, fear conditioning, and reinforced alternation were included in this analysis, whereas performance in the passive avoidance task was excluded because of data loss for several subjects that resulted from apparatus malfunction. A single factor accounted for approximately 28% of the variance in the performance of individuals across learning tasks (see Table 1), a degree of explanatory variance comparable with prior studies (Kolata et al., 2005, 2007; Matzel et al., 2003, 2006) with larger sample sizes and different combinations of learning tasks. To assess general learning abilities, we assigned each subject a factor score derived from this principal-component analysis conducted on learning tasks. (A factor score is analogous to an average z score of a subject's performance across all tasks, weighted by the contribution of that task to the principal factor.) The factor scores of CDP-treated or control mice were then compared. Crucially, a t test conducted on these factor scores (indicative of a subject's general learning ability) revealed no significant difference between the two groups, $t(24) = 0.30$, *ns*. This latter result suggests that CDP does not impact the overall expression of general learning abilities.

To determine the relationship of exploration to performance on the various learning tasks, we conducted a second principal-component analysis that included learning variables and three measures of exploration (spatial plus-maze, light–dark preference test, and open field). All learning tasks were found to load posi-

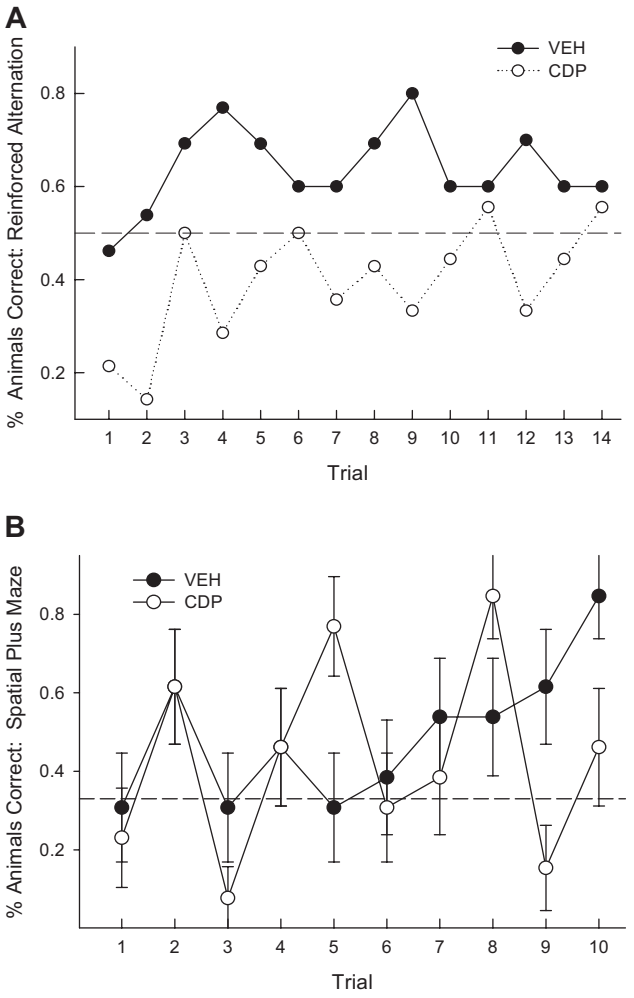

Figure 4. Chlorodiazepoxide- (CDP) treated mice exhibited clear deficits in learning performance on only two tasks: reinforced alternation (A) and the spatial plus-maze (B). In the reinforced alternation task, CDP-administered mice failed to demonstrate a stable pattern of learning across trials and, in contrast to control mice, did not perform with greater than chance accuracy over the latter half of the training trials. Similarly, mice treated with CDP did not show stable learning across trials in the spatial plus-maze. CDP-treated mice tended to navigate straight across the maze regardless of their starting position (which could be in one of three locations), resulting in a behavior pattern where every third trial was performed correctly. Error bars indicate standard error of the mean. VEH = vehicle.

tively on this principal factor. Consistent with results of previous studies, exploration in the elevated plus-maze (assessed by the percentage of entries into open arms) loaded at −.66 with performance on the learning tasks in the same principal factor (see Table 2), indicative of more exploration in the elevated plus-maze being associated with better performance on learning tasks. This pattern was paralleled by exploration in the dark–light test, wherein the percentage of time spent in the light compartment of the dark–light chamber during a 4-min period loaded at −.59 with learning performance in the same principal factor (see Table 2). In contrast, the percentage of entries into open quadrants of a walled open field during a 4-min period loaded weakly and positively on the principal factor, suggesting that more exploration in the open field was associated with worse performance on learning tasks. This pattern is aberrant in the context of previous findings and the overall trend of exploratory assays in the present study, likely reflecting the perseverative effects of CDP on open-field behavior that were observed in this study.

Measures of Anxiety–Stress and Pain Reactivity

CDP-treated mice exhibited a marked decrease in the magnitude of their startle response in reaction to a fear-provoking stimulus, $t(25) = 4.13$, $p < .001$ (see Figure 5A). Likewise, they were less impacted in their reactions to shock: Relative to control mice, CDP-treated mice demonstrated a greater amount of movement in a 20-s period following an unsignaled shock compared with movement in the 20 s preceding the shock, indicating that they were less immobilized by fear, $t(25) = 2.42$, $p < .05$ (see Figure 5B).

Mice administered CDP demonstrated no significant difference from control mice in latency to lick their hind paws during placement on a hot plate, $t(25) = 1.25$, *ns* (see Figure 5C). Therefore, it appears that their reduction in reactions to fearful (and potentially painful) stimuli cannot be attributed to reduced pain sensitivity.

Consistent with their behaviors indicative of stress and/or anxiety, there was a significant reduction in the amount of serum corticosterone present in mice treated with CDP as compared with mice injected with saline. This reduction was observed at both mild and moderate levels of stress, although the difference between groups was significant only at the lower stress level, $F(1, 22) = 5.19$, $p < .05$ (see Figure 6).

Measures of General Locomotor Activity

Mice treated with CDP exhibited an equivalent number of total quadrant entries in the open field, $t(27) = 0.36$, *ns*, suggesting that

Table 1
Factor Loadings From the Principal-Components Analysis (n = 26) for Performance on the Six Learning Tasks

Variable	Factor 1
Lashley III maze: Errors	0.57
Odor discrimination: Errors	0.62
Fear conditioning: Post- and pre-CS lick latencies	0.38
Spatial plus-maze: Errors	0.66
Water maze: Latencies	0.21
Reinforced alternation: Errors	0.56
Eigenvalue	1.67
Percentage of variance	0.28

Note. In the Lashley III maze, odor discrimination, spatial plus-maze, reinforced alternation, and water maze tasks, better performance (faster acquisition) is reflected in lower values (e.g., fewer errors, shorter latencies). In the raw form, better performance in the fear conditioning task is expressed as higher values. To simplify interpretation of the variable loadings, we converted fear conditioning scores 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). CS = conditioned stimulus.

Table 2
Factor Loadings From the Principal-Components Analysis (n = 26) for Performance on the Six Learning Tasks as Well as the Three Behavioral Assays of Exploration

Variable	Factor 1
Lashley III maze: Errors	0.33
Odor discrimination: Errors	0.70
Fear conditioning: Post- and pre-CS lick latencies	0.49
Spatial plus-maze: Errors	0.38
Water maze: Latencies	0.25
Reinforced alternation: Errors	0.37
Open field: % open activity	0.19
Elevated plus-maze: % open entries	−0.66
Dark–light test: % open time	−0.59
Eigenvalue	2.04
Percentage of variance	0.22

Note. In measures of exploration (i.e., open field, elevated plus-maze, and dark–light test), more activity is reflected in higher values. In the Lashley III maze, odor discrimination, water maze, reinforced alternation, and spatial plus-maze tasks, better performance (faster acquisition) is reflected in lower values (e.g., fewer errors, shorter latencies). In the raw form, better performance in the fear conditioning task is expressed as higher values. To simplify interpretation of the variable loadings, we converted fear conditioning scores 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). Because 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 for the large part of exploratory tasks is positively correlated with subjects' actual learning performance (e.g., more exploration is associated with better learning), although open-field exploration is negatively correlated with subjects' actual learning performance. CS = conditioned stimulus.

their overall level of locomotor activity was not impacted by drug treatment. Likewise, CDP-treated mice did not display any change in closed-arm or total-arm entries in the elevated plus-maze, $t(25) = 0.16$, *ns*, and $t(25) = 0.60$, *ns*, respectively. On the basis of factor-analytic approaches, these measures have each been shown to belong to a dimension of general locomotor activity (Cruz et al., 1994; Ramos et al., 1997; Rodgers & Johnson, 1995). Thus, all measures obtained here that reflect a component of locomotor activity indicate that there was no significant effect on the level of general locomotion resulting from administration of CDP.

Measures of Motor Strength–Coordination

CDP treatment had no effect on subjects' balance, as evidenced by their performance on the balance beam, in which CDP-treated mice exhibited a similar amount of movement prior to falling as did nontreated mice, $t(25) = 0.65$, *ns*. Likewise, no significant difference between groups was observed in their latencies to fall from the beam, $t(25) = 1.77$, *ns*. Mice treated with CDP moved a greater distance per second than did nontreated mice when climbing a suspended screen, $t(25) = -2.59$, $p < .05$ (data not shown), although their latency to fall from the screen was not significantly affected, $t(25) = 1.54$, *ns*. In contrast, mice treated with CDP did exhibit significantly shorter latencies to fall from a suspended rod, $t(25) = 2.28$, $p < .05$ (data not shown). It is notable that tests in which these abilities were assessed operate under the premise that mice are reluctant to fall and thus assume that the latency to fall reflects a deficit in grip strength or balance. However, a reduction in anxiety or fear reactivity (as would be induced by CDP) may suppress a subject's reluctance to fall, and, as such, CDP-treated mice may exhibit a shorter latency to fall in part simply because of decreased motivation. It is not possible from the present data to

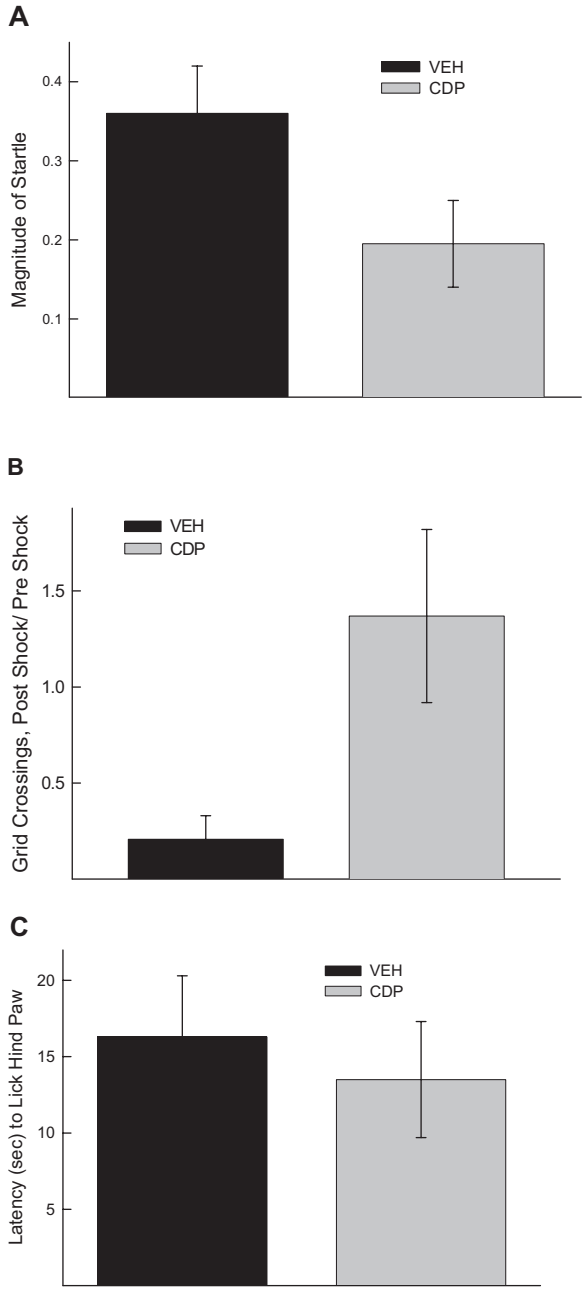

Figure 5. Chlorodiazepoxide- (CDP) treated mice exhibited a significant decrease in their startle magnitude (A) and less suppression of movement immediately following a shock (B). However, CDP-treated mice exhibited no reduction in pain sensitivity, as indexed by their latency to lick a hind paw when placed on a hot surface (C). Error bars indicate standard error of the mean. VEH = vehicle.

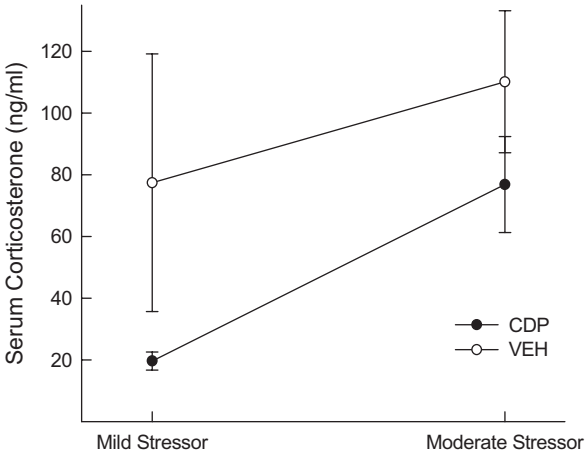

Figure 6. Mice treated with chlorodiazepoxide (CDP) exhibited lower serum corticosterone levels after stressors of both mild and moderate intensity (confinement on an elevated platform in a bright, noisy room). A significant difference between groups was found only at the lower stress level. Error bars indicate standard error of the mean. VEH = vehicle.

determine the degree to which these deficits reflect motor or motivational impairments. Overall, the modest and variable effects of CDP on motor performance suggest that CDP administration did not unilaterally compromise measures of motor coordination and strength in a manner that is likely to principally account for impairments in cognitive behaviors that are movement dependent.

Discussion

To assess the role of stress reactivity and anxiety in regulating general learning ability and its relationship to exploratory behavior, we used a pharmacological manipulation that has been shown to modulate exploratory behaviors. Indeed, mice treated with CDP exhibited a decrease in the latency to move after unexpected shock, as well as a corresponding increase in movement following shock, which was not attributable to reduced pain sensitivity. Similarly, they displayed a diminished startle response in reaction to an acoustic startle-inducing stimulus. This pattern was mirrored by physiological indexes of stress reactivity; CDP-treated mice displayed a reduced corticosterone response to environmental stress. Consistent with prior research (De Boer, Slangen, & Van der Gugten, 1991; File & Cooper, 1985; Pellow, Chopin, File, & Briley, 1985), differences in these measures demonstrate that CDP-treated mice were less reactive to stress-provoking and anxiety-provoking stimuli.

The reduction in stress reactivity induced by CDP produced an interesting pattern of behaviors interpreted to reflect exploration. Although CDP-treated mice exhibited a diminished propensity to explore the inner quadrants of a novel open field, on all other measures, the exploratory pattern of CDP mice was in the direction of heightened exploratory behavior on measures of exploration that are sensitive to stress. This increase is especially meaningful in that there was no corresponding change in locomotor activity, as assessed by the amount of total activity in the open field and the number of entries into closed arms or total arm entries in the elevated plus-maze, indicating an increase in exploratory activity rather than an increase in nonspecific general activity. Of the behavioral tests measuring exploration, exploratory behavior assessed in the open field is said to be more ambiguous (Archer, 1973; File, 1985; Walsh & Cummins, 1976) as well as more sensitive to changes in direction of behavioral effects as a function of minor variations in pharmacological parameters, such as acuteness versus chronicity of injection (Crawley, 1985) or drug dosage (File, 1985). In this task, CDP-treated mice tended to perseverate in ambulation along the walls of the field, in a pattern consistent with fixed motor responses observed during select learning tasks (see discussion of reinforced alternation and spatial plus-maze, below). Consequently, the pattern of results obtained on the aggregate of exploratory measures in the present study lends itself to interpretation as largely enhanced exploration for the purpose of drawing conclusions about relationships between stress reactivity, exploratory behaviors, and general learning abilities. This effect is in line with extensive research that has shown that various manipulations or traits associated with reduced anxiety or stress reactivity are characterized by an increase in exploratory behavior (Crawley, 1985; File, 1985; Ramos et al., 1997).

In contrast with the enhancing effects on exploration, reductions in anxiety and stress reactivity did not produce a parallel enhancement in learning ability, as would be expected if stress reactivity was a codeterminant of both variables or if increased exploration caused improved learning (e.g., by promoting a mouse's interaction with its environment). Mice treated with CDP exhibited learning performance that was comparable with that of mice injected with saline in most of the learning tasks. In reinforced alternation and spatial plus-maze navigation, CDP-treated mice were somewhat more impaired than were nontreated mice (although, at least in the case of the spatial plus-maze, this impairment was likely the result of stereotypic behavior and not necessarily a cognitive deficit per se). Overall, these results are generally consistent with other studies on the effects of CDP on learning performance, in which it has consistently been observed that CDP has little or no consistent impact on short-term memory (File, 1985; Thiebot, 1985). Converging with this, learning performance across tasks for CDP-treated mice demonstrated an equivalent degree of loading on a factor indicative of general learning ability as did performance of saline-treated mice, demonstrating a comparable expression of general learning abilities in mice with attenuated stress responsivity. Thus, it appears that the stress response in these mice did not impact the expression of general learning abilities (although domain-specific deficits cannot be ruled out). Crucially, in direct contradiction of predictions derived from a stress–anxiety account of the relation between general learning ability and exploratory activity, CDP did not produce an enhancement of learning performance in any of the tasks, as would be the case if reduced stress reactivity and superior general learning abilities were causally related.

It is important to note that the present combination of CDP effects on stress responsivity, exploration, and learning occurred in the absence of significant impacts on pain sensitivity and ataxic effects on motor coordination–strength. Pain sensitivity was comparable in CDP-treated and nontreated mice and therefore is not responsible for the reduced tendency of these animals to react to unexpected shock. Although mice administered CDP showed a tendency to fall more quickly from a rod suspended above the ground, they were not significantly impaired in their ability to hang

from and climb a suspended screen or balance on an elevated beam. Thus, measures of muscle strength–coordination yielded a general pattern of small effects that are not likely to have significantly impacted learning performance or exploration. In addition, total activity in the open field and in the elevated plus-maze was unchanged, suggesting that general locomotor activity (Cruz et al., 1994; Rodgers & Johnson, 1995) was not impacted by any sedative influence of CDP. Further, the pattern of effects characteristic of anxiolytic action in the dark–light test observed here is particularly compelling evidence that CDP was inducing anxiolytic rather than sedative actions because of the clear dissociation between doses that produce anxiolytic versus sedative effects in this test (Crawley, 1985). Finally, the pattern of anxiolytic effects in the dark–light test and on tests of stress reactivity (corticosterone elevations) were obtained near the completion of all testing, providing compelling evidence that CDP was producing anxiolytic effects throughout the duration of our test battery.

The pattern of effects of CDP on learning is particularly illuminating in light of our original aim: Down-regulation of stress reactivity yielded an effect only on selective learning tasks while having no influence on general cognitive abilities. Consequently, as previously elucidated, it appears that the pattern of individual differences in aggregate learning performance attributed to general learning ability is not regulated by differences in stress reactivity, that is, lower endogenous stress reactivity does not likely promote a corresponding increase in general learning abilities. This finding, taken by itself as well as in concert with the effects of stress reduction on exploration, provides evidence that variations in stress reactivity do not underlie the relationship between general learning abilities and exploratory propensity. Specifically, an increase in exploratory behavior driven by attenuation of stress responsivity was not accompanied by a corresponding change in general learning abilities in the characteristic pattern of the relationship previously observed between them (Matzel et al., 2003, 2006). This conclusion is supported by the finding that behavioral and physical measures indicative of stress reactivity and anxiety do not load on the common factor underlying general learning abilities and exploratory behaviors (Matzel et al., 2006). Similarly, prior attempts failed to relate cognitive performance to relationships that exist between individual differences in exploratory tendencies and physiological stress sensitivity in young animals (Vallee et al., 1997; but see Dellu et al., 1996; Touyarot, Venero, & Sandi, 2004, for contradictory results in aged animals). These results suggest that the relationship between these variables may be based on a secondary process that is not specific to general cognitive ability. Thus, converging evidence suggests that the relationship between exploratory proclivity and general learning ability is not mediated by individual differences in stress reactivity. Consequently, it appears that a facet of exploratory behavior other than stress inhibition is responsible for this relationship.

The above conclusion is consistent with previous research on the separate components of the process described as exploration, which appears to implicate novelty seeking more strongly than stress reactivity in cognitive functions. Research has demonstrated that exposure to novel stimuli evokes both a novelty-seeking drive and anxiety, which combine to regulate the ensuing pattern of exploration behavior (Denenberg, 1969; File, 1985). These two functions, novelty seeking and anxiety, have been shown to represent independent dimensions (Berton, Ramos, Chaouloff, & Mormede, 1997; Montgomery, 1955; Whimbey & Denenberg, 1967) dissociated in their regulation of exploration (Denenberg, 1969) and differentially sensitive to environmental manipulations (Zimmerman, Stauffacher, Langhans, & Wurbel, 2001). For instance, manipulations impinging on anxiety and stress reactivity induce marked changes only in exploratory tests explicitly designed to assess measures of anxiety (Vallee et al., 1997) while producing inconsistent effects on measures of more pure exploration that are less dependent on anxiety (Fernandez-Teruel, Escorihuela, Castallano, Gonzalez, & Tobena, 1997). In contrast, manipulations designed to act on novelty seeking or exploration yield marked changes in more anxiety-independent exploration behaviors (Fernandez-Teruel et al., 1992; Van Waas & Soffie, 1996) but inconsistent effects on measures of emotionality assessed via exploration (Fernandez-Teruel et al., 1997). Moreover, it appears that manipulations impinging on anxiety act primarily on the neural systems for fear and stress reactivity (Meaney et al., 1996), only secondarily affecting learning and memory (e.g., through attenuation of age-related deficits; Meaney, Aitken, Bhatnagar, Van Berkel, & Sapolsky, 1987). Conversely, manipulations designed to act on novelty seeking induce changes in neural structures and systems directly underlying learning and memory (Greenough, 1975; Kempermann, Kuhn, & Gage, 1997), alluding to a more cognitive-specific action of novelty seeking. On the basis of these findings, the latter appears to be a more likely candidate for a mediator of a cognitive link to exploration. In concert with these findings, results of the present study mitigate against a role for stress reactivity in this relationship, thereby suggesting that mediation of this relationship is alternatively executed via novelty seeking. These conclusions coincide with unpublished research in our laboratory (Grossman et al., 2007) demonstrating that exploratory tasks designed explicitly to minimize a stress component and assess a more pure component of novelty seeking yield greater correlations with general learning ability. Collectively, these studies provide evidence that novelty seeking (rather than stress reactivity) is the underlying component of exploratory behavior that is responsible for its relationship with general learning abilities.

The set of effects on learning tasks that was found here illustrates the implications of such a technique for research addressing the impact of stress on cognitive abilities. Whereas a number of studies describe effects of stress on particular learning or cognitive tasks, there is a paucity of research on the effects of stress on general learning abilities that extend beyond a specific domain of learning or task demands. Our results indicate that there are no unilateral or unitary effects of attenuation of the stress response on aggregate learning performance, consistent with previous findings of inconsistent effects of stress on various learning abilities (Shors, 2004). Furthermore, stress attenuation–manipulation of stress does not appreciably alter expression of general learning ability. Therefore, although there is much evidence demonstrating effects on learning tasks produced by alterations in stress or anxiety, general learning ability appears to be largely impervious to manipulations that reduce stress responsivity. The fact that the effect of stress on cognition seems to be largely dependent on specific domains, and subject to unique properties of different tasks, suggests that it is mediated through a number of distinct mechanisms, in support of implications of prior research (Lupien & Lepage, 2001; Lupien & McEwen, 1997). As indicated above, these results demonstrate that effects of stress on cognition do not appear to operate through a

central process via general cognition. This finding confirms, and in turn accounts for, the collection of conflicting findings regarding the nature of the relationship between stress and cognition–learning. Thus, despite the wealth of evidence for effects of alterations in stress or anxiety on learning tasks, general learning ability does not appear to necessarily reflect stress responsivity.

In conclusion, converging evidence appears to indicate that stress reactivity is not a determinant of individual differences in general learning abilities. Instead, the effects of stress reactivity on learning and cognition appear to be largely dependent on individual systems and/or performance demands. Parallel to this, individual differences in stress reactivity cannot account for the relationship between exploratory propensity and general learning ability, suggesting the possibility that it is instead novelty seeking that regulates this association.

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