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# Neuronal cell adhesion molecule deletion induces a cognitive and behavioral phenotype reflective of impulsivity

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**Cell adhesion molecules, such as neuronal cell adhesion molecule (Nr-CAM), mediate cell–cell interactions in both the developing and mature nervous system. Neuronal cell adhesion molecule is believed to play a critical role in cell adhesion and migration, axonal growth, guidance, target recognition and synapse formation. Here, wild-type, heterozygous and Nr-CAM null mice were assessed on a battery of five learning tasks (Lashley maze, odor discrimination, passive avoidance, spatial water maze and fear conditioning) previously developed to characterize the general learning abilities of laboratory mice. Additionally, all animals were tested on 10 measures of sensory/motor function, emotionality and stress reactivity. We report that the Nr-CAM deletion had no impact on four of the learning tasks (fear conditioning, spatial water maze, Lashley maze and odor discrimination). However, Nr-CAM null mice exhibited impaired performance on a task that required animals to suppress movement (passive avoidance). Although Nr-CAM mutants expressed normal levels of general activity and body weights, they did exhibit an increased propensity to enter stressful areas of novel environments (the center of an open field and the lighted side of a dark/light box), exhibited higher sensitivity to pain (hot plate) and were more sensitive to the aversive effects of foot shock (shock-induced freezing). This behavioral phenotype suggests that Nr-CAM does not play a central role in the regulation of general cognitive abilities but may have a critical function in regulating impulsivity and possibly an animal's susceptibility to drug abuse and addiction.**

Keywords: Addiction, cell adhesion molecules, cognition, emotionality, general cognitive ability, impulsivity, learning, transgenic mice

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Cell adhesion molecules (CAMs) mediate cell–cell interactions in both the developing and mature nervous system. Cell adhesion molecules of the immunoglobulin superfamily mediate several aspects of nervous system development, including cell adhesion and migration, axonal growth, fasciculation and guidance, as well as target recognition, synapse formation and plasticity (Grumet 1997; Sakurai *et al.* 2001).

The elucidation of neuronal cell adhesion molecule (Nr-CAM) expression in mammals has indicated that Nr-CAM may have multiple functions at different locations during nervous system development. Neuronal cell adhesion molecule is expressed in a number of cortical regions, including the hippocampus, olfactory bulb and the corpus callosum (Lustig *et al.* 2001). In the hippocampus, Nr-CAM is expressed in the major cell layers, including the pyramidal cell layer and the granule cell layer of the dentate gyrus, as well as in the molecular layer (Backer *et al.* 2002).

Neuronal cell adhesion molecule is ubiquitously expressed in the hippocampus across the life span of mice (Sandi *et al.* 2005). Given that neural cell adhesion molecules have important functions related to synaptic plasticity (Knafo *et al.* 2005), and the hippocampus' and limbic brain regions' pervasive roles in learning and memory processes, it was of interest to characterize the role of Nr-CAM in regulating cognitive abilities. To that end, here we assess the impact of Nr-CAM on a range of learning tasks (representing multiple cognitive domains), and subsequently, on behaviors indicative of sensory/motor abilities, emotionality and exploration.

In addition to its potential role in learning, recent reports have implicated Nr-CAM in regulating the susceptibility to drug abuse and addiction (Ishiguro *et al.* 2006), a characteristic that is similar to other Nr-CAMs (Kahn *et al.* 2005). Specifically, it has been observed that mice with reduced levels of Nr-CAM expression manifest less drug-conditioned place preference, in concordance with the human data that support reduced addiction vulnerability in individuals with similar haplotypes (Hall *et al.* 2004; Lin *et al.* 2005). However, it is difficult to interpret this later data given the paucity of information regarding the role of Nr-CAM in learning *absent* motivational states supported by drugs of abuse. Nonetheless, the suggestion that Nr-CAM contributes to addiction vulnerability and learned drug-related behaviors provided the impetus to assess the role of this adhesion molecule in a wider range of cognitive processes and to more thoroughly characterize the behavioral effects of Nr-CAM deletion in mice.

We have developed a test battery with which to assess the 'general' learning abilities of laboratory mice (Kolata *et al.* 2005, 2007; Matzel *et al.* 2003, 2006). Here, a similar test battery was used to assess the impact of a brain-wide



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deletion of the gene for the Nr-CAM. Mice were assessed on five learning tasks (Lashley maze, odor discrimination, passive avoidance, spatial water maze and fear conditioning) and 10 measures of sensory/motor function, emotionality and stress reactivity. These sensory motor tests included measures of pain sensitivity, co-ordination/strength, exploration of novel environments, light/dark preferences, emotionality (e.g. defecation evoked by novel environments or aversive stimulation) and measures of general activity (e.g. running wheel performance).

## Methods

### *Subjects*

The Nr-CAM Knockout mice were established using homologous recombination to delete the second exon containing the ATG translation initiation codon, as has been described previously (Sakurai *et al.* 2001). Outbred mice (mixture of 129SvEvS6/Tac and Swiss Webster (CFW)) were maintained by brother-sister mating. The animals used for experimentation were Nr-CAM Knockout ($n = 21$) heterozygous ($n = 14$) and wildtype ($n = 12$) as detected by PCR analysis in previous studies (Sakurai *et al.* 2001). Litter mates including heterozygotes and homozygotes were used for the different groups. Only male animals were used in this study. All animals were 3–4 months old at the start of behavioral testing, an age that corresponds to young adulthood. This experiment was conducted in three balanced replications, with approximately one week intervening between the start of each replication.

Homozygotes and heterozygotes were detected by polymerase chain reaction analysis and, in previous studies (total >600 mice), have been found to be indistinguishable in terms of overall body size, activity and growth rate (and similar results are reported here).

Animals were individually housed in clear boxes lined with wood shavings in a humidity- and temperature-controlled vivarium adjacent to a suite of testing rooms. A 12-h light/dark cycle was maintained, and all training and testing took place between 1000 and 1700 h. To mitigate any differential stress responses to handling at the start of behavioral testing, each mouse was removed from its home cage and held by an experimenter for 90 seconds/day for the week preceding behavioral testing.

### *General behavioral training and testing methods*

The battery of tests and associated analysis regimen used here provides a method with which to compare the impact of a manipulation (e.g. a gene deletion) on general and domain-specific learning abilities, as well as a means with which to assess the impact of variations in sensory/motor abilities on cognitive performance. The learning tasks that are included in the battery each make different demands on sensory, motor and motivational systems, and performance on individual tasks do not obviously influence performance on other tasks in the battery. On all cognitive tasks, performance was assessed during the acquisition phase of learning, and as such, measures of performance were sensitive to differences in learning *rates* and do not reflect constraints (e.g. 'ceiling effects') that may influence asymptotic performance. With the exception of fear conditioning, learning was assessed at very short ($\leq$20 min) retention intervals, providing an index of learning that was minimally impacted by variations in long-term retention. During fear conditioning (in which a noise conditioned stimulus (CS) is paired with foot shock), mice typically develop fear of the context. Furthermore, mice are reluctant to drink water (our dependent measure) for varying periods of time after exposure to shock. So as to obtain a more pure index of CS fear (independent of context fear), assessment of fear conditioning was conducted in an associatively 'neutral' context (i.e. one in which shock had not previously been administered) after a relatively long retention interval (24 h).

Consistent with previously reported procedures (Matzel *et al.* 2003, 2006), here mice were first assessed for activity and exploratory behaviors in an open field, followed by training and testing in five learning tasks, and finally, on a series of additional tests of activity, exploration and sensory/motor performance. Two of the learning tasks (Lashley maze and odor discrimination) required food deprivation, for which *ad libitum* food was removed from the animals' home cages at the end of the light cycle approximately 40 h prior to the start of training (≈16 h prior to their acclimation to the relevant apparatus). During the deprivation period, animals were provided with food in their home cages for 60 min/day during the last 2 h of the light cycle. This deprivation schedule was deemed 'mild' (animals rarely lose more than 6% of their free-feeding body weight during this period) but is sufficient to maintain stable food-motivated performance on these tasks. In the one task that required water deprivation (fear conditioning), the same schedule was followed, except that free access to water was limited to 90 min/day. To familiarize the animals with novel foods used as reinforcers (in the Lashley maze and odor discrimination tasks), on the day prior to apparatus acclimation, all animals were provided (in their home cages) with three of the food pellets utilized as reinforcers in the upcoming test.

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 multiple training/test trials were employed. As noted above, all tests were conducted in three balanced replications, and all animals were trained and tested under nominally identical conditions.

Animals were tested on cognitive and noncognitive tasks in the following order: (1) open field, (2) Lashley maze, (3) passive avoidance, (4) water maze, (5) odor discrimination, (6) fear conditioning, (7) balance beam, (8) rod suspension, (9) pain sensitivity, (10) balance platform, (11) shock-induced freezing and (12) light/dark preference. Body weights were obtained weekly and after a 24-h period of deprivation (just prior to the start of Lashley maze training). At the completion of all other tests, a subgroup of knockout ($n = 8$) and wildtype ($n = 7$) mice (drawn from two of the three replications that constituted all other tests) were assessed for acoustic startle responses. Each of the above tasks required 1–3 days for completion, and one 'rest' day intervened between each successive task, with the exception of the last cognitive task (fear conditioning) and the first noncognitive task (balance beam), between which 1 week intervened. Accordingly, the entire test regimen (administered in three balanced replications) was completed in 40 days. Different experimenters ($n = 4$) trained or tested animals in different tasks, and no experimenter was aware of an animal's genotype or performance on other tasks. Each task in this battery is described in detail below.

### *Tests of learning*

#### *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 mice to locate the goal box decreases, as does their errors (i.e. wrong turns or retracing).

The maze was constructed of black Plexiglass. A 2 cm wide × 0.1 cm deep white cup was located in the rear portion of the goal box and half of a 45-mg BioServe (rodent grain; Frenchtown, NJ, USA) pellet served as reinforcers. Illumination was 80 lux at the floor of the maze. The maze was isolated behind a shield of white Plexiglass to mitigate against extramaze landmark cues.

Food-deprived animals were acclimated and trained on two successive days. 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 was confined for 6 min in the last (goal) alley, where three food pellets were present in the food cup. 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 trial. The apparatus was cleaned during

each ITI, and the sequence was repeated for five trials. Both the latency and errors (i.e. wrong turns or path retracing) to enter the goal box were recorded on each trial. For purposes of scoring 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 to better represent animals' performance.

### *One-trial passive avoidance*

In order not to duplicate stimuli (i.e. shock) used to train fear conditioning (below), we utilized aversive auditory and visual stimuli to motivate avoidance. Upon stepping off the platform, animals were exposed to a compound of a bright light and a loud oscillating tone (i.e. a '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 (<5 lux) 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 comprised 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 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 a bright (550 lux) white light and an oscillating siren (a 4/second transition from 600 to 12 Hz tone, 60 dBc above the 50 dBc background; Radio Shack model 2730057) was initiated.

Animals were placed on the platform behind the exit blocked by the Plexiglass 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. Upon contact with the floor, the door to the platform was lowered and the aversive stimulus (light and noise) was presented for 4 second, at which time the platform door was opened to allow animals to return to the platform, where subjects remained for 1 min. At the end of this interval, animals were returned to their home cages for a 1-h retention period. Subsequently, mice were returned to the safe platform for 2 min, then the door was opened and the latency of the animal to exit the platform and step onto the grid floor was recorded, completing training and testing. The ratio of post-training to pre-training step-down latencies were calculated for each animal and served to index learning.

### *Spatial water maze*

For this task, animals are introduced into a round pool of opaque water from which they can escape onto a hidden (i.e. submerged) platform. We employ a protocol in which mice exhibit significant reductions in their latency to locate the escape platform across six training trials. 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 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 light emitting diodes (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). In total, these cues provided the only illumination of the maze, totaling 16 lux at the water surface. A video camera was mounted 180 cm above the center of the water surface.

On the day prior to training, each animal was confined to the escape platform for 300 seconds. Training was conducted on the subsequent day. On training day, animals were started from a unique location on each of six trials. (The pool was conceptually divided into four equal 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 seconds. Each animal was left on the platform for a total of 30 seconds, 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 lux) box lined with wood shavings. On each trial, a 90-second limit on swimming was imposed, at which time any animal that had not located the escape platform was placed by the experimenter onto the platform, where it remained for 30 seconds. Animals were observed from a remote (outside of the pool's enclosure) video monitor, and animals' performance was recorded on videotape for subsequent analysis.

### *Odor discrimination and choice*

A black Plexiglass of 60 cm square field with 30 cm high walls was located in a dimly lit (40 lux) testing room with a high ventilation rate (3 min volume exchange). Three 4 × 4 × 2 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.)

Rodents rapidly learn to use odors to guide appetitively reinforced behaviors. In a procedure based on one designed by Sara *et al.* (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 was 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 is 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).

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. Only in 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 seconds and then returned to its home cage to begin a 6-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 were changed on each trial. On each trial, the latency to retrieve the food and errors were recorded. An error was recorded any time when 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.

### *Associative fear conditioning*

In fear conditioning, animals are exposed to a stimulus (i.e. a CS; white noise) that terminates in the onset of a mild foot shock (i.e. an unconditioned stimulus; US). These noise–shock (CS–US) pairings come to elicit conditioned fear responses when animals are subsequently presented with the noise. 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, training and testing were conducted in separate distinct contexts.

Two distinct experimental chambers (i.e. contexts; 32 × 28 × 28 cm, l × w × 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 lux), had clear Plexiglass 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), 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/seconds 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 recorded 8 licks/seconds, a rate that approximates continuous licking.

In the training chamber, a 0.6-mA constant-current scrambled foot shock (US) could be delivered through the grid floor. In both the training and test chambers, a 40 dBc 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 seconds 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-second noise terminated with the onset of a 500-milliseconds foot shock. With our present parameters, we have observed that asymptotic performance (as evident in group means) is reached with four to six 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 reacclimated 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-noise interval and in the presence of the noise was recorded, with a 600-second 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 noise CS served 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.

## ***Tests of unlearned behaviors and fitness***

With the exception of open-field testing (which was conducted *prior* to tests of learning), animals began testing in a series of noncognitive tasks 1 week after the completion of cognitive testing.

### *Open-field exploration and activity*

A square field (46 × 46 cm) with 13 cm high walls of white Plexiglass was utilized. The apparatus was located in a brightly lighted room (400 lux) with a background noise of 65 dBc. The field was conceptually divided into a grid comprised 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. Three measures were recorded: total movements (in both walled and unwalled quadrants, percentage of movements in the unwalled quadrants and bolli deposited throughout the 4 min test.

Animals were placed in the center of the field. After 20 seconds 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. It should be noted that a 4-min test was explicitly chosen (based on pilot work) because only minimal changes in behavior (e.g. that which accompanies habituation) were observed over this interval. Thus, we presume that open-field performance was most sensitive to *unlearned* behavioral tendencies.

### *Balance beam*

Animals were placed on a 40 × 0.7 × 2 cm (l × w × h) beam suspended 30 cm above the ground. In a 4-min test, mice exhibit wide variability in the amount of movement along its length.

### *Rod suspension*

Animals are hung from their front paws from a 0.7-cm rod [coated in black shrink tubing (Archer; Fort Worth, TX, USA)] suspended 20 cm above ground. The rod was 3 cm in length and terminated on black Plexiglass walls (which prevented animals from climbing onto the rod). Latency to drop from the rod (an index of grip strength) was recorded.

### *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 served as the index of pain sensitivity.

### *Screen hanging*

Animals are placed in the underside of a wire mesh screen tilted 45° 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.

### *Balance platform*

All four paws of animals are placed on a 3-cm round platform (60 lux illuminations) 30 cm above the ground. Latency to fall off the platform was recorded.

### *Shock-induced freezing*

Freezing after the offset on an unsignaled shock is often interpreted as a measure of fear, which is to some degree a reflection of pain sensitivity. Animals were acclimated for 20 min to a 25-cm square chamber (60 lux 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-milliseconds constant-current scrambled foot shock 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 occurred between 10 and 10.5 min. During and for a brief time (500 milliseconds) 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 both rear paws of the animals to move 2 cm) served as the dependent variable.

### *Light/dark preference*

A 10 × 36-cm chamber divided across its length in two equal halves was utilized. One half was white and brightly lit (100 lux), and the

other half was black and dim (5 lux). The two halves were divided by a center wall with a 3-cm square opening that joined the black and white sides. Animals were placed in the black side of the chamber and allowed to explore for 4 min. The latency to first enter into the white chamber, percent of total time in the white chamber and number of crossings between the black and white chambers were recorded.

*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 dBc). A 200 milliseconds, 60 dBc 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 was computed during a 500-milliseconds window beginning at the onset of the noise, and the two responses were averaged for each animal.

*Body weight and food consumption under mild deprivation*
Body weights during periods of free feeding were compared at the start and completion of behavioral testing. In addition, the percent change in body weight was recorded after 18 h of deprivation (prior to testing in the Lashley maze).

## Results

For clarity, the presentation of results will be grouped according to the nature of the test. The order of discussion of these tests does not in all cases follow the actual order in which the tests were administered (as described above).

### *Learning tasks*

*Spatial water maze*
Performance in the water maze is dependent on the stability of extramaze cues, and is said to indicate the animals' representation of its environment as a 'cognitive map' (Morris 1981). Acquisition and performance in the spatial version of the Morris water maze is dependent on an intact hippocampus (Hooge & De Deyn 2001; Pearce *et al.* 1998). Across the six trials of training, analysis of variance (ANOVA) showed that all animals improved their performance ($F_{5,220} = 29.38$, $P < 0.0001$) (Fig. 1a), i.e. a reduced latency to locate the hidden platform was observed across trials. However, no group effect ($F_{2,44} = 0.89$, ns) or a group × trial interaction ($F_{10,220} = 1.25$, ns) was observed, indicating similar rates of acquisition across the three groups.

*Odor discrimination and choice*
Rodents rapidly learn to use odors to guide appetitively reinforced behaviors, as indicated here by a reduction in the errors committed in locating a food reward when that food was signaled by an odor cue (Fig. 1b). Analysis of variance showed a significant decrease in errors across trials ($F_{3,129} = 204$, $P < 0.0001$). However, no difference was observed between groups ($F_{2,43} = 1.41$, ns), and no group × trial interaction was observed ($F_{6,129} = 0.88$, ns), indicating that all groups learned at a similar rate.

*Lashley maze*
This task is strongly dependent on egocentric navigation, a strategy that differentiates performance on this task from that in the spatial water maze (see above). Over trials, the latency of all groups to locate the goal box decreased, as did their errors ($F_{4,168} = 8.47$, $P < 0.001$) (Fig. 1c). However, the groups did not differ ($F_{2,43} = 2.36$, ns), and no group × trial interaction was observed ($F_{8,168} = 0.82$, ns), indicating that all groups learned to navigate the maze at similar rates.

*Fear conditioning*
One transgenic animal became ill and was removed from this and subsequent analyses. For illustration, lick rates before

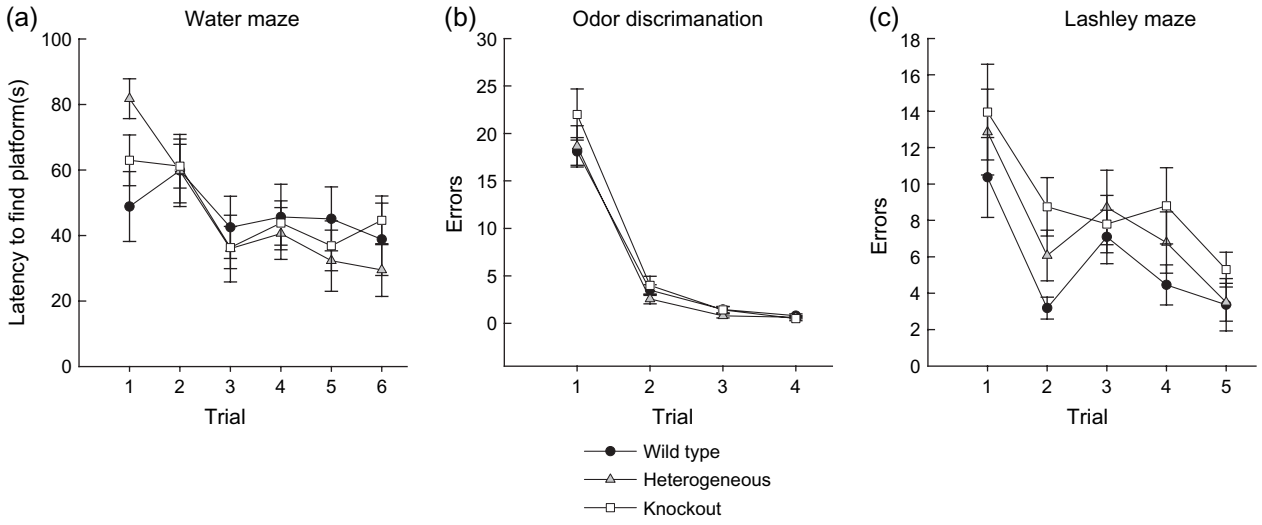


**Figure 1: Assessment of learning with multiple test trials.** (a) The average latency to find the hidden platform across eight training trials in the water maze for Nr-CAM knockouts, heterogeneous and wild-type mice. (b) The average number of errors to locate food associated with a target odor across four training trials. (c) The average number of errors to locate food in the Lashley maze. No group differences were detected in any of these tasks. Error bars represent SEM.

and during the white noise CS presentation were converted to ratios (latency to complete 25 licks during the period immediately preceding the CS/latency to complete 25 licks during the CS), where values lower than 1.0 indicate suppression of responding during the white noise CS (Fig. 2a). An ANOVA was conducted on the pre-CS and CS lick latencies. All groups exhibited a significant increase in the latency to complete 25 licks during the CS ($F_{1,43} = 182$, $P < 0.001$), but no difference was observed between groups ($F_{2,43} = 0.26$, ns) and no interaction was observed ($F_{2,43} = 0.51$, ns), indicating that similar levels of suppression across the three groups.

*Passive avoidance*

After stepping from a platform, animals were exposed to a compound stimulus of bright light and a loud siren. After this exposure, mice exhibit an increased latency to step from the platform. Step latencies prior to exposure to the aversive stimulation and after exposure to the aversive stimulation were converted to ratios (step latency prior to exposure/step latencies after exposure), where values lower than 1.0 indicate suppression of responding (Fig. 2b). An ANOVA was conducted on the pre- and post-training step latencies. A significant difference between pre- and post-training latencies was observed ($F_{1,43} = 37.3$, $P < 0.001$), as was a difference between groups ($F_{2,43} = 3.30$, $P < 0.05$). Lastly, an interaction between the groups and the pre- and post-training latencies were observed ($F_{2,43} = 6.39$, $P < 0.01$), indicative of the lack of post-training suppression in Nr-CAM knockout mice.

### *Unlearned behaviors and physical characteristics*

*Body weights*

The non-deprived body weights of the three groups of animals did not differ at either the start or completion of behavioral testing ($Fs_{2,43} \leq 1.06$, ns). Heterogeneous, wild-type and knockout groups did not differ in response to a period of food deprivation, shedding ≈5–8% of body weight ($Fs_{2,43} \leq 1.26$, ns).

*Pain sensitivity*

One Nr-CAM knockout mouse was not included in this and subsequent tests because of illness. Knockout animals reacted quicker to a painful stimulus than wild-type mice ($F_{2,42} = 5.26$, $P < 0.05$) (Fig. 3b). Differences in pain reactivity could affect learning tasks that entail painful reinforcement, such as associative fear conditioning. However, as mentioned above, acquisition of fear responses during associative fear conditioning did not differ between the three groups.

*Shock-induced freezing*

Neuronal cell adhesion molecule knockouts suppressed movement significantly longer after delivery of an unsignaled foot shock ($F_{2,42} = 9.16$; $P < 0.001$) (Fig. 3a). Consistent with the above test of pain sensitivity, this result suggests that the knockout mice are more sensitive to the effects of painful stimulation.

*Strength and co-ordination*

Knockouts and heterozygous mice showed impairments in grip strength, measured by latency to fall from an apparatus in two sensory/motor tasks. Knockout and heterozygous mice were quicker to fall when suspended from the front paws from an elevated rod ($F_{2,42} = 5.25$, $P < 0.05$) (Fig. 4c) and an elevated screen ($F_{2,42} = 3.38$, $P < 0.05$). These deficits appear to be unrelated to balance, as all groups fell with a similar latency from the balance platform ($F_{2,42} = 1.16$, ns) (Fig. 4d).

*Open field*

Animals were assessed in the open field *prior* to tests of learning. The three groups of mice did not differ in their total

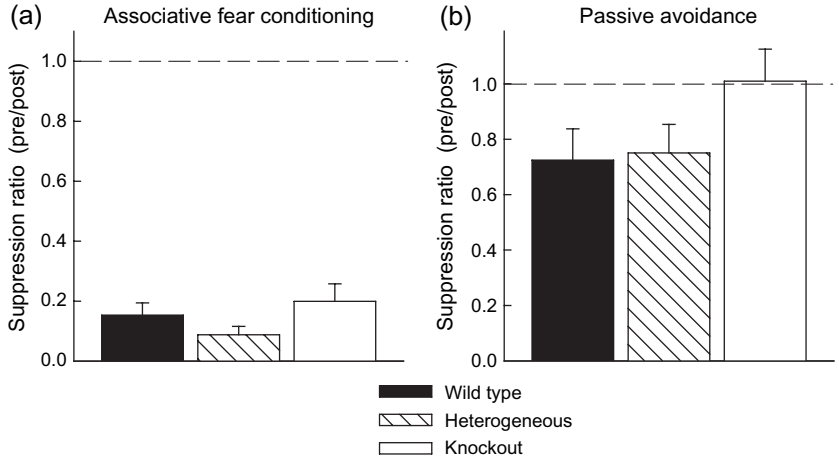


**Figure 2: Assessment of learning with single test trials.** (a) Animals were exposed to a white noise paired with foot shock. Fear of the white noise was subsequently assessed by white noise-induced suppression of licking (for water). Conditioned fear is expressed as a ratio of the latency to make 25 licks before (pre) and after (post) CS presentation, where values lower than 1.0 are indicative of suppression. No differences were detected between groups. (b) Upon stepping down from a safe platform, animals were exposed to bright light and a loud siren. Shown are step-down latencies as a ratio of before (pre) and after (post) exposure to this aversive stimulus in this passive avoidance task Nr-CAM knockout mice exhibited a lack of suppression relative to each of the other groups. Error bars represent SEM.

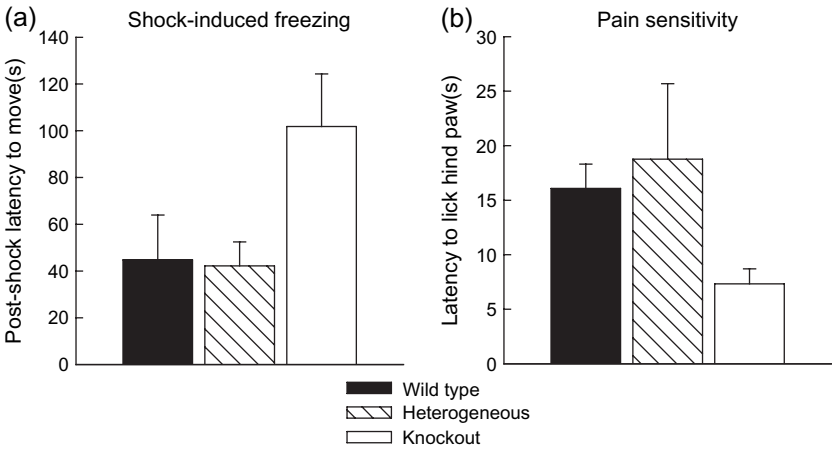


**Figure 3: Tests of fear and pain sensitivity.** (a) Group mean latencies to reinitiate movement after foot shock are depicted. Nr-CAM knockout mice exhibited significantly more freezing than either heterogeneous or wild-type mice. (b) Group average latencies to lick a hind paw after being placed on a hot plate. Nr-CAM mice licked a hind paw significantly faster than either of the other groups. Error bars represent SEM.

movements (grid crossings) made in the open field, indicating similar levels of non-specific activity across groups ($F_{1,43} = 1.61$, ns). A difference between groups was observed in the percentage of activity in the unwalled areas of the open field ($F_{2,43} = 8.75$, $P < 0.001$), where Nr-CAM mutant animals exhibited more activity in the unwalled areas relative to both wild-type and heterozygous mice ($F$s $\leq 9.64$, $P$s $< 0.001$) (Fig. 5a). Activity in the open quadrants of an open field is often interpreted as an index of an individual mouse's inclination for novelty seeking or exploration. Alternatively, animals more prone to novelty seeking may be less susceptible to consequences of stress, so behavior in the unwalled areas of an open field may also reflect variations in stress reactivity. However, no differences were observed between groups in bolli deposited in the novel open field ($F_{2,43} = 0.85$, ns). Because bolli deposits in a novel environment

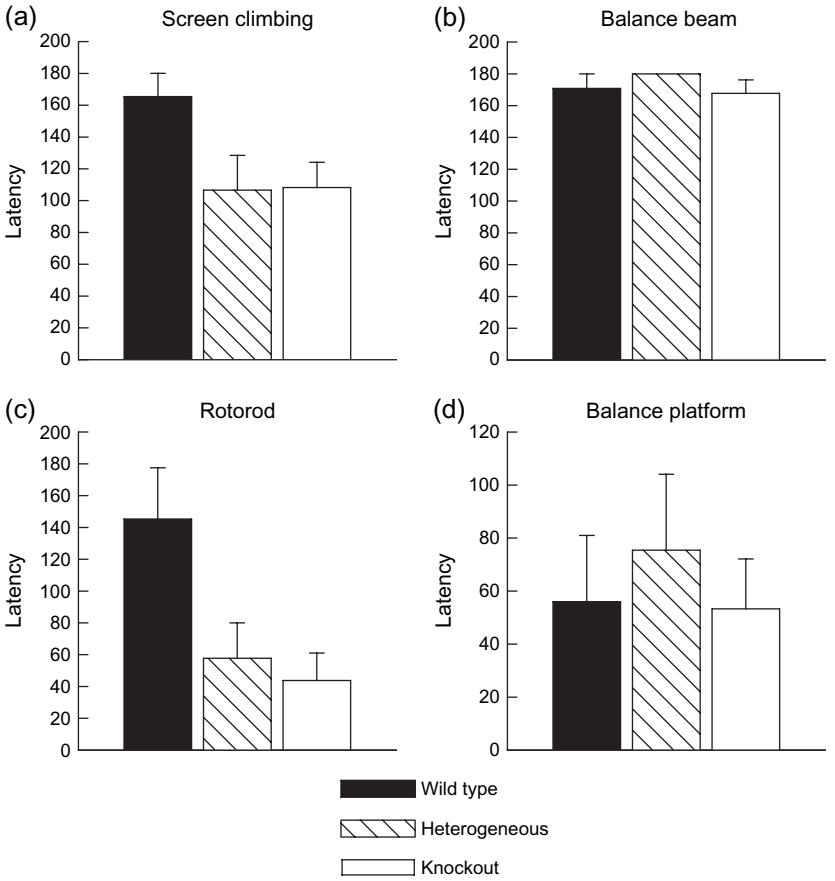


**Figure 4: Tests of motor performance.** (a) Animals were placed on the bottom of an elevated screen set 45°to horizontal. Nr-CAM knockout and heterogeneous animals fell from the screen with significantly shorter latencies than did wild-type animals. (b) Animals were placed on a narrow elevated beam. No differences between groups were found in the latency to fall from the beam. (c) Group averages to fall off the apparatus in the rotorod test are shown (d) Group averages to fall off the apparatus in the balance platform task are shown. Error bars represent SEM.

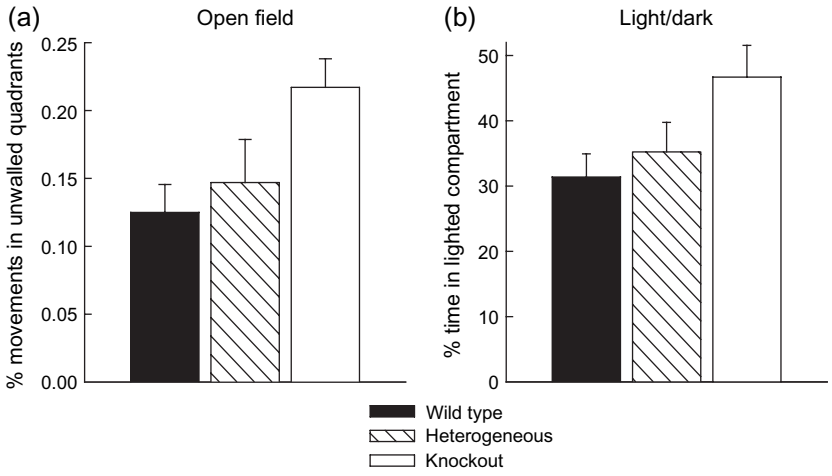


**Figure 5: Tests of activity and exploration.** (a) Group mean percent of time spent in the unwalled quadrants of a novel open field. Nr-CAM mutant mice spent significantly more time in these unwalled areas. (b) Group mean percent time in the lighted half of a dark/light preference box. Nr-CAM mutant mice spent significantly more time in the lighted half of the box. Error bars represent SEM.

are often interpreted to reflect stress reactivity, this result renders less viable any interpretation of the differences in exploratory patterns exhibited by the three groups to differences in stress reactivity.

*Dark/light preference*

Conceptually similar to the open-field test (which was administered as the first test in the behavioral battery), the light/dark preference test was administered at the completion of all behavioral testing. When given a choice between dark and lighted compartments, mice typically spend more time in the dimly lit chamber. As with the open field, this behavior is often interpreted as an index of exploration or novelty seeking. The three groups did not differ in the total crossings between the light and dark compartments ($F_{2,42} = 0.61$, ns) or in the latency to first enter the lighted side of the box ($F_{2,42} = 1.79$, ns) (although Nr-CAM-deficient mice exhibited a tendency to enter the lighted side more *slowly* than either the heterozygous or wild-type animals). However, the percent of time spent in the lighted portion of the box (Fig. 5b) differed between groups ($F_{2,42} = 3.31$, $P < 0.05$), and Nr-CAM mutant animals were found to spend a higher percentage of time in the lighted compartment relative to both heterozygous and wild-type animals ($F$s $\geq 6.02$, $P < 0.05$).

*Auditory startle responding*

Auditory startle responses were obtained from a group of Nr-CAM mutant ($n = 8$) and wild-type ($n = 7$) animals drawn from two of the three replicates that comprised all other tests in this battery. (Apparatus and scheduling difficulties prohibited testing all animals in this task.) Nr-CAM mutant animals exhibited nominally higher startle amplitudes than did wild-type controls (means $= 0.48 + 0.07$ and $0.37 + 0.08$, respectively), but this difference was not significant, $t(13) = 1.08$.

## Discussion

Genetic and molecular biological studies have recently linked the Nr-CAM (in both mouse and human populations) with susceptibility to and consequences of addiction (Ishiguro *et al.* 2006; Minana *et al.* 2000), aberrant drug-seeking behavior and dysfunctional brain-based drug reward systems (Hitzemann *et al.* 2003). In support of these contentions, it has been observed that mice with reduced levels of Nr-CAM expression manifest less drug-conditioned place preference (Hall *et al.* 2004; Lin *et al.* 2005). However, it was previously unknown whether these deficits in drug conditioning reflect a specific influence of Nr-CAM on drug-motivated behaviors, or whether perturbations of Nr-CAM expression impact learning in a more general manner. Neuronal cell adhesion molecule's expression in numerous cortical regions, especially the hippocampus, coupled with other Nr-CAM's involvement in synaptic plasticity (Panicker *et al.* 2003; Welzl & Stork 2003) suggest that this CAM might be involved more broadly in the acquisition of learned responses. Here, the performance of Nr-CAM knockout mice was compared with wild-type and heterozygous mice on a series of tests of learned and unlearned behaviors. It was determined that brain-wide deletion of Nr-CAM did not affect performance in the majority of our cognitive and sensory/motor tasks. However, behavioral differences in certain specific tasks provide support for the hypothesis that Nr-CAM may play a role in behaviors symptomatic of addiction vulnerability.

Wild-type, heterogeneous and knockout mice did not differ significantly from one another during acquisition in a spatial navigation test (Morris water maze), which depends heavily on hippocampal processes (Bohbot *et al.* 1996; Deacon *et al.* 2002). This is revealing as the hippocampus is one of the cortical regions where Nr-CAM is abundantly expressed, and other neuronal CAMs have been implicated in the acquisition of spatial/hippocampal tasks (Cambon *et al.* 2003; Sandi *et al.* 2005). The results here suggest that Nr-CAM's function in the hippocampus is unrelated to performance in tasks that require animals to form a 'cognitive map' such as the water maze, and thus distinguishes Nr-CAM from other CAM molecules.

Wild-type, heterogeneous and knockout mice did not differ in their acquisition of learned responses in an egocentric navigation task (Lashley III maze), an odor discrimination task or an associative fear-conditioning task. Because the discrimination

task used here is dependent on olfactory abilities, the lack of performance differences related to the expression of Nr-CAM suggests that deletion of this Nr-CAM does not impair olfaction, at least within the limited parameters employed here (Schellinck *et al.* 2004). Similarly, the lack of impairment in the water maze (which depends on the integration of visual cues) and in fear conditioning (in which animals respond to a noise CS) suggests that at least within the limited range of these tests, visual and auditory abilities were unaffected by Nr-CAM deletion.

Suppression of ongoing behavior in response to a signal (i.e. CS) that predicts impending foot shock is often described as a 'conditioned emotional response' (i.e. conditioned 'fear'; Fanselow & Kim 1994; LeDoux 1997). The lack of an effect of Nr-CAM deletion on fear conditioning is consistent with the absence of group differences in defecation in novel environments, a common measure of unlearned emotionality. In combination, these results suggest that Nr-CAM deletion does not overtly impact emotionality.

Data discussed thus far suggest that brain-wide deletion of Nr-CAM spares forms of learning that are dependent on two components of the limbic system, i.e. the hippocampus (upon which performance in the water maze and odor discrimination tasks rely) and the amygdala [upon which fear conditioning is dependent (Blair *et al.* 2001; Davis 1992; Phillips & LeDoux 1992)], while the lack of an impairment in odor discrimination consequent to Nr-CAM deletion suggests that fornix function (which is necessary for odor discrimination; Fagan *et al.* 1985) is spared. Finally, the absence of any impact of Nr-CAM deletion on performance in the Lashley maze is further evidence of normal amygdala and hippocampus function (Dickson & Vanderwolf 1990).

Passive avoidance was the one learning task in the present series of tests where Nr-CAM knockout animals showed impaired performance. Specifically, a brain-wide deletion of Nr-CAM significantly reduced performance of an avoidance response that requires animals to learn to inhibit an overt behavior. Nr-CAM knockout mice did not show an increase in step-down latencies after the step was paired with the onset of aversive stimulation (bright light and siren). This result suggests that Nr-CAM-deficient mice could either not learn the relationship between their behavior and the presentation of the aversive stimulus or that they could not effectively suppress the target behavior. Based on the results of other tests of learning described here, the latter possibility is more parsimonious with available evidence. Furthermore, impaired performance in passive avoidance is not likely attributable to impaired auditory abilities (as necessary to detect the aversive siren used in this task) as mutant animals exhibited a normal (or slightly elevated) auditory startle response and performed normally in a fear-conditioning task in which the danger signal (CS) was white noise.

Mice are highly exploratory (Crawley *et al.* 1997) and in the passive avoidance task must learn to inhibit this tendency to avoid contact with the aversive event. In at least two measures of exploration (entries into the walled quadrants of the open field and time spent in the lighted side of a dark/light box), Nr-CAM-deficient mice exhibited more exploration, a result that may account for this performance deficit in passive avoidance.

A propensity for drug self-administration has previously been associated with aberrant passive avoidance learning (Seth *et al.* 2002), and lack of inhibitory control is a hallmark of addiction (Dawe & Loxton 2004). Passive avoidance responses are routinely employed in drug studies to explore the genetic basis of drug vulnerability differences among mice (Bignami 1987; Crawley 2000), and impaired passive avoidance responding has been associated with drug usage (Barrionuevo *et al.* 2000), prenatal exposure to drugs of abuse (Petkov *et al.* 1991) and susceptibility to drug usage (Hishida 1996; Sakurai *et al.* 2001). Notably, impaired passive avoidance responses, like those expressed in Nr-CAM knockout mice, have been observed in rodents with other Nr-CAM deficiencies (Baydas *et al.* 2005; Cambon *et al.* 2003; Foley *et al.* 2000). Data from our other cognitive tasks imply that Nr-CAM knockouts are not impaired (or facilitated) across a wide range of learning domains. However, the impairment in this one task, in combination with the propensity for more exploration in Nr-CAM knockout mice, suggests a specific inability to withhold behavioral responding, not an impairment of general or domain-specific cognitive abilities.

Assessment of sensory/motor performance also produced results suggesting that the lack of Nr-CAM creates a genotype useful in explorations of drug abuse and addiction. In two tests of pain sensitivity and responsiveness, Nr-CAM knockout mice displayed heightened nociception (a decrease in paw lick latencies on a hot plate and prolonged freezing after an unsignaled foot shock) relative to wild-type animals. Increased sensitivity to pain has been implicated in the susceptibility to drug abuse and addiction (Lehofer *et al.* 1997).

In the open field, Nr-CAM knockout mice spent significantly more time in the unwalled quadrants of the apparatus without an increase in total activity. Exploration of the center quadrants of the open field is often interpreted as indicative of novelty seeking (Stansfield *et al.* 2004), and novelty seeking is one of the hallmarks of abuse and addiction in human populations and rodent models of abuse (for reviews, see Laviola *et al.* 2000; Spear 2000). Neuronal cell adhesion molecule knockout's open-field behavior could be viewed as increased novelty seeking indicative of increased vulnerability to drug abuse type behaviors, as similar open-field behavior has been in other mouse models of addiction (Bowirrat & Oscar-Berman 2005; Gingras & Cools 1997; Stanfield & Trice 1988).

In total, the present results suggest that deletion of Nr-CAM does not promote general deficits in learning. However, Nr-CAM knockout mice performed deficiently in a task that required the suppression of behavior (i.e. passive avoidance). This latter result suggests that these animals may be abnormally impulsive, a result consistent with their propensity to explore stressful areas of a novel open field. In combination with the heightened pain sensitivity exhibited by these animals, these results suggest that Nr-CAM may play a critical function in establishing an animal's susceptibility to drug abuse and addiction, a speculation supported by recent molecular and genetic work (Ishiguro *et al.* 2006).

## References

Backer, S., Sakurai, T., Grumet, M., Sotelo, C. & Bloch-Gallego, E. (2002) Nr-CAM and TAG-1 are expressed in distinct populations of

developing precerebellar and cerebellar neurons. *Neuroscience* **113**, 743–748.
Barrionuevo, M., Aguirre, N., Del Rio, J.D. & Lasheras, B. (2000) Serotonergic deficits and impaired passive-avoidance learning in rats by MDEA: a comparison with MDMA. *Pharmacol Biochem Behav* **65**, 233–240.
Baydas, G., Ozveren, F., Tuzcu, M. & Yasar, A. (2005) Effects of thinner exposure on the expression pattern of neural cell adhesion molecules, level of lipid peroxidation in the brain and cognitive function in rats. *Eur J Pharmacol* **512**, 181–187.
Bignami, G. (1987) Avoidance methods in neurobehavioural toxicity assessments. *Zentralbl Bakteriol Mikrobiol Hyg [B]* **185**, 36–47.
Blair, H., Schafe, G.E., Bauer, E.P., Rodrigues, S.M. & LeDoux, J.E. (2001) Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn Mem* **8**, 229–242.
Bohbot, V., Otahal, P., Lui, Z., Nadel, L. & Bures, J. (1996) Electroconvulsive shock and lidocaine reveal rapid consolidation of spatial working memory in the water maze. *Proc Nat Acad Sci U S A* **93**, 4016–4019.
Bowirrat, A. & Oscar-Berman, M. (2005) Relationship between dopaminergic neurotransmission, alcoholism, and reward deficiency syndrome. *Am J Med Genet B Neuropsychiatr Genet* **132**, 29–37.
Cambon, K., Venero, C., Berezin, V., Bock, E. & Sandi, C. (2003) Post-training administration of a synthetic peptide ligand of the neural cell adhesion molecule, C3d, attenuates long-term expression of contextual fear conditioning. *Neuroscience* **122**, 183–191.
Crawley, J.N. (2000) *What's Wrong with My Mouse*. Wiley-Liss, New York.
Crawley, J.N., Belknap, J.K., Collins, A., Crabbe, J.C., Frankel, W., Henderson, N., Hitzemann, R.J., Maxson, S.C., Miner, L.L., Silva, A.J., Wehner, J.M., Wynshaw-Boris, A. & Paylor, R. (1997) Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology (Berl)* **132**, 107.
Davis, M. (1992) The role of the amygdala in fear and anxiety. *Ann Rev Neurosci* **15**, 353–375.
Dawe, S. & Loxton, N.J. (2004) The role of impulsivity in the development of substance use and eating disorders. *Neurosci Biobehav Rev* **28**, 343–351.
Deacon, R.M., Bannerman, D.M., Kirby, B.P., Croucher, A. & Rawlins, J.N. (2002) Effects of cytotoxic hippocampal lesions in mice on a cognitive test battery. *Behav Brain Res* **133**, 57–68.
Dickson, C.T. & Vanderwolf, C.H. (1990) Animal models of human amnesia and dementia: hippocampal and amygdala ablation compared with serotonergic and cholinergic blockade in the rat. *Behav Brain Res* **41**, 215–227.
Fagan, A., Eichenbaum, H. & Cohen, N. (1985) Normal learning set and facilitation of reversal learning in rats with combined fornix-amygdala lesions: implications for preserved learning abilities in amnesia. *Ann N Y Acad Sci* **444**, 510–512.
Fanselow, M.S. & Kim, J.J. (1994) Acquisition of contextual pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behav Neurosci* **108**, 210–212.
Foley, A.G., Hartz, B.P., Gallagher, H.C., Rønn, L.C., Berezin, V., Bock, E. & Regan, C.M. (2000) A synthetic peptide ligand of neural cell adhesion molecule (NCAM) IgI domain prevents NCAM internalization and disrupts passive avoidance learning. *J Neurochem* **74**, 2607–2613.
Gingras, M.A. & Cools, A.R. (1997) Different behavioral effects of daily or intermittent dexamphetamine administration in Nijmegen high and low responders. *Psychopharmacology (Berl)* **132**, 188–194.
Grumet, M. (1997) Nr-CAM: a cell adhesion molecule with ligand and receptor functions. *Cell Tissue Res* **290**, 423–428.
Hall, F.S., Sora, I., Drgonova, J., Li, X.F., Goeb, M. & Uhl, G.R. (2004) Molecular mechanisms underlying the rewarding effects of cocaine. *Ann NY Acad Sci* **1025**, 47–56.
Hishida, S. (1996) Application of operant conditioning techniques to forensic toxicology: experimental studies on alcohol and abusable drugs. *Nihon Hoigaku Zasshi* **50**, 292–319.
Hitzemann, R., Hitzemann, B., Rivera, S., Gatley, J., Thanos, P., Shou, L.L. & Williams, R.W. (2003) Dopamine D2 receptor binding, Drd2 expression and the number of dopamine neurons in the BXD recombinant inbred series: genetic relationships to alcohol and other drug associated phenotypes. *Alcohol Clin Exp Res* **27**, 1–11.
Hooge, R. & De Deyn, P. (2001) Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev* **36**, 60–90.
Ishiguro, H., Liu, Q.R., Gong, J.P., Hall, F.S., Ujike, H., Morales, M., Sakurai, T., Grumet, M. & Uhl, G.R. (2006) NrCAM in addiction vulnerability: positional cloning, drug-regulation, haplotype-specific-expression and altered drug reward in knockout mice. *Neuropsychopharmacology*.
Kahn, L., Alonso, G., Normand, E. & Manzoni, O.J. (2005) Repeated morphine treatment alters polysialylated neural cell adhesion molecule, glutamate decarboxylase-67 expression and cell proliferation in the adult rat hippocampus. *Eur J Neurosci* **21**, 493–500.
Knafo, S., Barkai, F., Herrero, A.I., Libersat, F., Sandi, C. & Venero, C. (2005) Olfactory learning-related NCAM expression is state, time, and location specific and is correlated with individual learning capabilities. *Hippocampus* **15**, 316–325.
Kolata, S., Light, K., Townsend, D., Hale, G., Grossman, H. & Matzel, L.D. (2005) Variations in working memory capacity predict individual differences in general learning abilities among genetically diverse mice. *Neurobiol Learn Mem* **84**, 242–246.
Kolata, S., Light, K., Hale, G. & Matzel, L.D. (2007) Selective attention is the primary determinant of the relationship of working memory to general cognitive ability in outbred mice. *Learn Mem* **14**, 22–28.
Laviola, G., Adriani, W., Terranova, M.L. & Gerra, G. (2000) [Psychobiologic risk factors and vulnerability to psychostimulants in adolescents and animal models]. *Ann Ist Super Sanita* **36**, 47–62.
LeDoux, J.E. (1997) The amygdala: contributions to fear and stress. *Semin Neurosci* **6**, 231–237.
Lehofer, M., Liebmann, P.M., Moser, M., Legl, T., Pernhaupt, G., Schauenstein, K. & Zapotoczky, H.G. (1997) Decreased nociceptive sensitivity: a biological risk marker for opiate dependence? *Addiction* **92**, 163–166.
Lin, Z., Walther, D., Yu, X.Y., Li, S., Drgon, T. & Uhl, G.R. (2005) SLC18A2 promoter haplotypes and identification of a novel protective factor against alcoholism. *Hum Mol Genet* **14**, 1393–1404.
Lustig, M., Erskine, L., Mason, C.A., Grumet, M. & Sakurai, T. (2001) Nr-CAM expression in the developing mouse nervous system: ventral midline structures, specific fiber tracts, and neuropilar regions. *J Comp Neurol* **434**, 13–28.
Matzel, L.D., Han, Y.R., Grossman, H., Karnik, M.S., Patel, D., Scott, N. & Specht, S.M. (2003) Individual differences in the expression of a ''General'' learning ability in mice. *J Neurosci* **23**, 6423–6433.
Matzel, L.D., Townsend, D.A., Grossman, H., Han, Y.R., Hale, G., Zapulla, M., Light, K. & Kolata, S. (2006) Exploration in outbred mice covaries with general learning abilities irrespective of stress reactivity, emotionality, and physical attributes. *Neurobiol Learn Mem* **86**, 228–240.
Minana, R., Climent, E., Barettino, D., Segui, J.M., Renau-Piqueras, J. & Guerri, C. (2000) Alcohol exposure alters the expression pattern of neural cell adhesion molecules during brain development. *J Neurochem* **75**, 954–964.
Morris, R.G.M. (1981) Spatial localization does not require the presence of local cues. *Learn Mot* **12**, 239–260.
Panicker, A.K., Buhusi, M., Thelen, K. & Maness, P.F. (2003) Cellular signalling mechanisms of neural cell adhesion molecules. *Front Biosci* **8**, d900–d911.
Pearce, J.M., Roberts, A.D.L. & Good, M. (1998) Hippocampal lesions disrupt navigation based on cognitive maps but not heading vectors. *Nature* **396**, 75–77.
Petkov, V.D., Konstantinova, E.R., Petkov, V.V. & Vaglenova, J.V. (1991) Learning and memory in rats exposed pre- and postnatally to alcohol. An attempt at pharmacological control. *Methods Find Exp Clin Pharmacol* **13**, 43–50.
Phillips, R.G. & LeDoux, J.E. (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci* **106**, 274–285.
Sakurai, T., Lustig, M., Babiarz, J., Furley, A.J., Tait, S., Brophy, P.J., Brown, S.A., Brown, L.Y., Mason, C.A. & Grumet, M. (2001) Overlapping functions of the cell adhesion molecules Nr-CAM and L1 in cerebellar granule cell development. *J Cell Biol* **154**, 1259–1274.
Sandi, C., Woodson, J.C., Haynes, V.F., Park, C.R., Touyarot, K., Lopez-Fernandez, M.A., Venero, C. & Diamond, D.M. (2005) Acute

stress-induced impairment of spatial memory is associated with decreased expression of neural cell adhesion molecule in the hippocampus and prefrontal cortex. *Biol Psychiatry* **57**, 856–864.
Sara, S.J., Roullet, P. & Przybyslawski, J. (2001) Retrieval and reconsolidation: toward a neurobiology of remembering. *Learn Mem* **6**, 88–96.
Schellinck, H.M., Arnold, A. & Rafuse, V.F. (2004) Neural cell adhesion molecule (NCAM) null mice do not show a deficit in odour discrimination learning. *Behav Brain Res* **152**, 327–334.
Seth, P., Cheeta, S., Tucci, S. & File, S.E. (2002) Nicotinic – serotonergic interactions in brain and behaviour. *Pharmacol Biochem Behav* **71**, 795–805.
Spear, L.P. (2000) The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev* **24**, 417–463.
Stanfield, B.B. & Trice, J.E. (1988) Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections. *Exp Brain Res* **72**, 399–406.
Stansfield, K.H., Philpot, R.M. & Kirstein, C.L. (2004) An animal model of sensation seeking: the adolescent rat. *Ann N Y Acad Sci* **1021**, 453–458.
Welzl, H. & Stork, O. (2003) Cell adhesion molecules: key players in memory consolidation? *News Physiol Sci* **18**, 147–150.

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