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The role of NMDA receptor-dependent activity of noradrenergic neurons in attention, impulsivity and exploratory behaviors

P. E. Cieślak[†], N. Llamosas[‡], T. Kos[§], L. Ugedo[‡], K. Jastrzębska[†], M. Torrecilla[‡] and J. Rodriguez Parkitna[†,*]

[†]Laboratory of Transgenic Models, Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland, [‡]Department of Pharmacology, Faculty of Medicine and Dentistry, University of the Basque Country, UPV/EHU, Leioa, Spain, and [§]Department of Behavioral Neuroscience & Drug Development, Institute of Pharmacology Polish Academy of Sciences, Krakow, Poland
**Corresponding author: J. Rodriguez Parkitna, Laboratory of Transgenic Models, Department of Molecular Neuropharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, 31-343 Krakow, Poland.*
E-mail: Jan.Rodriguez@if-pan.krakow.pl

Activity of the brain's noradrenergic (NA) neurons plays a major role in cognitive processes, including the ability to adapt behavior to changing environmental circumstances. Here, we used the *NR1*^{DbhCre} transgenic mouse strain to test how NMDA receptor-dependent activity of NA neurons influenced performance in tasks requiring sustained attention, attentional shifting and a trade-off between exploration and exploitation. We found that the loss of NMDA receptors caused irregularity in activity of NA cells in the locus coeruleus and increased the number of neurons with spontaneous burst firing. On a behavioral level, this was associated with increased impulsivity in the go/no-go task and facilitated attention shifts in the attentional set-shifting task. Mutation effects were also observed in the two-armed bandit task, in which mutant mice were generally more likely to employ an exploitative rather than exploratory decision-making strategy. At the same time, the mutation had no appreciable effects on locomotor activity or anxiety-like behavior in the open field. Taken together, these data show that NMDA receptor-dependent activity of brain's NA neurons influences behavioral flexibility.

Keywords: Attention, behavioral flexibility, locus coeruleus, NMDA receptors, noradrenergic neurons

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Behavioral states and state-dependent sensory information processing are regulated by the noradrenergic (NA) system (Berridge & Waterhouse 2003). More than half of NA nerve terminals in the central nervous system derive from neurons projecting from the locus coeruleus (LC) of the pons. The LC activity is involved in regulation of arousal, stress response, attention and learning (Aston-Jones & Cohen 2005; Berridge 2008; Sara & Bouret 2012). The role of the LC in response to stress or danger could be viewed as part of a general involvement in behavioral flexibility, the ability to adapt behavior to dynamic changes in the environment. Thus, NA signaling could serve as a facilitator of change in the cognitive state in response to the perceived environmental contingencies, suppressing old behaviors and allowing acquisition of new ones. It should be noted that while the LC is the main source of NA in the forebrain, the NA neurons in the medulla provide additional inputs targeting mainly the ventral forebrain, notably including the nucleus accumbens (NAc) (Delfs *et al.* 1998; Ricardo & Koh 1978). The activity of these projections probably complements the role of LC in stress response, and was shown to also affect emotional processing and reinforcement learning (Kerfoot *et al.* 2008; Rinaman 2011).

The activity of the LC correlates with arousal and was observed to follow one of two distinct patterns, phasic or tonic (Aston-Jones & Cohen 2005; Berridge & Waterhouse 2003). The phasic mode, trains of action potentials interspersed with periods of lower activity, was associated with good performance in tasks requiring vigilance. Conversely, high tonic activity, increased but regular frequency of discharges, was associated with distractibility and poor performance in tasks requiring focused attention. These observations led to formulation of the adaptive gain theory, which links activity of LC neurons with inverted-U relationship between the level of arousal and behavioral performance (Aston-Jones & Cohen 2005; Aston-Jones *et al.* 1999; Usher *et al.* 1999). The model predicts that an intermediate level of LC firing corresponds to focused attention and optimal performance, while high firing rate may produce a state of scanning attentiveness, which could facilitate behavioral flexibility. It was also reported that activation of high-affinity receptors (i.e. postsynaptic $\alpha 2$) at moderate NA neuron activity levels could promote network connectivity and enhance working memory, while further increase in NA release would lead to activation of low-affinity receptors (i.e. $\alpha 1$) thus shifting control of behavior toward the amygdala and striatum (Arnsten 2011; Berridge *et al.* 2012).

Independently, it was hypothesized that activation of LC neurons and subsequent release of NA in targeted brain areas facilitates neural network reorganization (a 'network reset') that permits attentional shifting and rapid behavioral adaptation to changing environmental circumstances

(Bouret & Sara 2004, 2005). This notion is supported by observation that pharmacological activation of NA signaling facilitates attentional shifting in rats performing a maze-based decision-making task (Lapiz & Morilak 2006), while NA deafferentation of the rat medial frontal cortex impairs the ability to shift attention (Devauges & Sara 1990). In line with this observations, Yu and Dayan (2005) proposed a model in which NA signals unpredicted task changes (i.e. unexpected changes in the cue-target relationship), enabling learning in noisy and changeable environments and thus facilitating adaptation to unexpected events.

Activity of LC neurons is controlled by the sum of intrinsically active conductances (Alreja & Aghajanian 1991; Williams *et al.* 1984) and a complex network of synaptic inputs, mainly excitatory (Aston-Jones *et al.* 1991; Samuels & Szabadi 2008; Williams *et al.* 1984). The glutamatergic inputs modulate the LC activity by acting on N-methyl-D-aspartate (NMDA) and non-NMDA receptors (Aston-Jones *et al.* 1991; Jodo & Aston-Jones 1997; Sara & Hervé-Minvielle 1995; Williams *et al.* 1991). We have previously shown that selective ablation of NR1 gene in cells expressing dopamine β-hydroxylase (Dbh) induces the loss of functional NMDA receptors in NA neurons of the LC (Rodriguez Parkitna *et al.* 2012). At the behavioral level, disruption of NMDA receptor-dependent glutamatergic input to NA cells was associated with attenuated development of specific morphine-induced psychomotor sensitization and withdrawal, but has no apparent effects on locomotor activity and anxiety levels or spatial memory performance. Here, we use the *NR1*^{DbhCre} strain to examine the role of NMDA receptor-dependent signaling in NA neurons on the performance of tasks dependent on attention, exploration and behavioral flexibility as well as its contribution to the spontaneous activity of the LC.

Materials and methods

Animals

Generation and genotyping of the *NR1*^{DbhCre} strain was described previously (Rodriguez Parkitna *et al.* 2012). The strain was bred to be congenic with the C57BL/6N. Animals were housed in a conventional facility in Plexiglas cages (Type II L, 2–5 animals per cage) on a 12 h light/dark cycle, on aspen laboratory bedding (MIDI LTE E-002, Abedd), without additional environmental enrichment and with an ambient temperature of 22 ± 2°C. Unless indicated otherwise, mice had *ad libitum* access to water and chow (RM1 A (P), Special Diets Services). All experiments were conducted in accordance with the European Union guidelines for the care and use of laboratory animals (2010/63/EU) and the Spanish law for the care and use of laboratory animals (RD 53/2013). Experimental protocols were reviewed and approved by the local Bioethics Committee (Krakow, Poland; permit number 1000/2012 issued on November 26, 2012) and the local Committee for Animal Experimentation at the University of the Basque Country (Leioa, Spain; permit number 224M/2012 issued on March 19, 2012). Mutant mice had the genotype Cre Tg/0; NR1 flox/flox and control animals were 0/0; flox/flox or 0/0; flox/wt. All behavioral experiments were performed on male mice during the light phase, by an experimenter blinded to the genotype. Animals of both sexes were used in the electrophysiology measurements. Four cohorts of mice were used in the experiments. First was tested for open-field activity and hole board exploration (19 male mice, mean age 11.21 ± 0.52 weeks; weight before test 25.96 ± 0.30 g). The second cohort was tested in the go/no-go and two-armed bandit (TAB) tasks (21 male mice, mean age 14.33 ± 0.94 weeks; weight before restriction 26.82 ± 0.70 g and 23.45 ± 0.58 g before first session). The third cohort was used in the attentional set-shifting task (ASST) (26 male mice, mean age 17.08 ± 0.86 weeks; weight before restriction 28.92 ± 0.69 g and 23.55 ± 0.64 g before first test). The fourth cohort (40 mice) included both male and female mice were used in electrophysiology experiments.

Open field

A cohort of animals (9 controls + 10 mutants) was tested in a square box (40 × 40 × 40 cm^3), which was illuminated in the central part. Light intensity was approximately 120 lux in the center area (20 × 20 cm^2) and approximately 50 lux outside the center area. Each session lasted 30 min. The total distance traveled, as well as the number of entries and time spent in the center area, were measured using Any-Maze video tracking software (version 4.99m, Stoelting Co., Wood Dale, IL, USA).

Hole board exploration

The experiment was performed on the same cohort of mice as the open-field test. Mice explored the open-field apparatus containing hole board (40 × 40 cm^2) with 16 holes (5 cm apart, 3 cm diameter, 4 cm depth) in a grid pattern for 10 min. There were additional spatial cues (colored cards in different geometric shapes) located on the walls of the apparatus to facilitate orientation. Spontaneous exploration (head dipping) was recorded with a video camera over two consecutive days. Recordings were further analyzed to determine the frequency of head dipping and the number of holes visited. Head dipping was defined as head placement into one of the holes to a minimum depth at which the ears were level with the floor of the apparatus.

Instrumental conditioning

Animals were allowed to acclimatize to the laboratory for 1 week and were then food deprived over another week to reduce their weight to 80–85%. Experiments were performed in operant chambers (ENV-307W-CT, Med Associates Inc., Fairfax, VT, USA) enclosed in cubicles that were equipped with a fan to provide ventilation and to mask extraneous noise. Each chamber was fitted with two nose-poke ports equipped with a photo-beam and yellow light-emitting diode, with one port located on each side of a central food receptacle. The food dispenser was loaded with 20 mg standard food pellets (#F0071, 20 mg, Dustless Precision Pellet, BioServ, Flemington, NJ, USA). A house light and a 65 dB, 2.9 kHz tone generator were located in the top of the wall opposite the operant wall. Mice were trained to nose-poke into the active port (cue-light on) for a food reward. The other port was inactive (cue-light off). The port assignments were counterbalanced. Animals were trained on a fixed-ratio 1 schedule of reinforcement (FR1) until they reached the criterion of 30 reinforced responses within 1 session. Sessions ended when an animal collected 40 food pellets or after 40 min.

Go/no-go discrimination

Experiments were performed on the cohort of mice (11 controls + 10 mutants) that earlier underwent instrumental conditioning. The procedure was based on the method described by McDonald *et al.* (1998), with modifications. Briefly, mice were first trained to respond to a target stimulus (cue-light in the nose-poke port) presented for 30 seconds during the first phase and then for 10 seconds during the second phase of training. Each trial started with the house light switched on and a pre-cue period ranging from 9 to 24 seconds, after which the target stimulus was presented. Responses during the final 3 seconds of the pre-cue period caused the trial to reset, and the trial started from the beginning. The house light was turned off 3 seconds after reward delivery, and a 10-second inter-trial interval (ITI) followed. The completion criteria for both phases were 40 completed trials and fewer than 5 misses over 2 consecutive sessions. The simple reaction time (SRT) was measured for five consecutive days, during which the target stimulus was presented for 5 seconds. In the go/no-go discrimination task, animals had to discriminate between two signal

types: 'go' signals (5 seconds, identical to target stimulus in SRT procedure) and 'no-go' signals, which consisted of both cue-lights and a continuous 65 dB 2.9 kHz tone presented for 5 seconds. The 'go' signal was always presented in the same port (left or right, counterbalanced between animals). Refraining from responding during 'no-go' signal presentation was scored as a 'correct rejection' and was rewarded with a food pellet. Conversely, a nose-poke during 'no-go' into any port resulted in immediate trial termination and transition into the ITI, without reward delivery ('false alarm'). Animals were tested for 10 consecutive sessions (each comprising 40 trials), during which 'go' and 'no-go' signals were presented randomly. The discriminability index (d') and response bias (β) were calculated as described by (Stanislaw & Todorov 1999):

$$d' = z(\text{Hits}) - z(\text{False alarms}) \quad (1)$$

$$\beta = e^{\left(\frac{(z(\text{False alarms}))^2 - (z(\text{Hits}))^2}{2}\right)^2} \quad (2)$$

where, z indicates the z-score (standard score).

Attentional set-shifting task

The procedure was based on the method developed by Birrell and Brown (2000). To strengthen the formation of an attentional set and to avoid the effects of satiation, we used two-session procedure with an additional intra-dimensional shift phase (IDS2), as described previously by Kos *et al.* (2011). A cohort of mice (14 controls + 12 mutants) was food restricted to reduce their weight to approximately 85%. Animals were tested in a three-compartment apparatus made of black acrylic with a wire grid floor. The starting compartment was connected via guillotine doors with two identical testing compartments. A bowl with water was placed in the starting compartment, and two digging bowls were located in the testing compartments. First, during training days, animals were habituated to the testing chamber for 5 min. Then, a food reward was placed in the bowls (#F05301, 20 mg, chocolate flavor, Dustless Precision Pellets, BioServ), which were filled with digging medium (wood shavings), requiring animals to dig in the medium to reach the food pellet. The training days were then followed by two testing days. During the test, the digging bowls were marked with olfactory and tactile cues. The first testing day (session 1) started with the simple discrimination (SD) phase with only one stimulus dimension (digging medium). Next, in the compound discrimination (CD) phase, an additional but irrelevant stimulus dimension (odor) was introduced. In the CD reversal (CDR) phase, reward contingencies within the relevant dimension (medium) were reversed. In the IDS phase, new exemplars of media and odors were used, and the digging medium remained the relevant dimension. In the intra-dimensional shift reversal (IDSR) phase, as in the CDR phase, the reward contingencies within the relevant dimension (medium) were reversed. The second testing day (session 2) started with IDS2 and IDS2R phases, which were equivalent to IDS and IDSR, but different media and odors were used. Next, in the extra-dimensional shift (EDS) phase, shifts in attentional sets from one stimulus dimension (digging medium) to another stimulus dimension (odor) were required. In the final extra-dimensional shift reversal (EDSR) phase, reward contingencies within new relevant dimension (odor) were reversed. The completion criterion was 8 correct (rewarded) choices out of 10 consecutive trials. Non-rewarded choices were scored as errors.

TAB task

The task follows in part the procedure described by (Kim *et al.* 2009) and is equivalent to a probabilistic reversal-learning task. A cohort of animals previously tested in the go/no-go paradigm was used in this experiment. Animals were allowed *ad libitum* access to food for 3 weeks. After that period, mice were food restricted again and training started. During training, mice had to respond to a target stimulus (cue-light) presented pseudo-randomly in one of the two nose-poke ports. There were 60 stimulus presentations per session, with 10 seconds inter-trial interval (ITI) between them. A correct response to a target stimulus within 10 seconds of its presentation resulted in the delivery of a 20 mg food pellet with 0.8 probability. During target stimulus presentation, responses to the inactive port had no consequence. A lack of a response was scored as an omission. Animals underwent training sessions until they reached a criterion of fewer than five omissions over two consecutive days. In the TAB task, both nose-poke ports were active (cue-light on) and animals had to distinguish between 'correct' and 'incorrect' choice options with different reward probabilities, 0.8 vs. 0.2, respectively. Trials were organized in blocks, and the probability of reward delivery associated with each port was constant within a block of trials but was reversed across blocks. During each session, there were three probability reversals (block changes). The total number of trials in a single session was 150, and the length of blocks varied from 30 to 45 trials per block. Animals had to decide which port to choose within 10 seconds from the start of the trial, and after this period, a 10-second ITI was initiated. The lack of a response was scored as an omission. The 'win-stay', 'win-shift', 'lose-stay' and 'lose-shift' ratios were calculated as fractions of the sum of all choices excluding omissions and the first choice during the session.

Electrophysiological procedures

Single-unit extracellular recordings of mouse LC neurons were performed on 2 cohorts of naïve animals (male and female, 18 mutants and 22 controls) as previously described by Torrecilla *et al.* (2013). The rationale to include mice of both sexes was to increase the size of the sample. Mice were anesthetized with chloral hydrate (400 mg/kg, intraperitoneal). The mouse was placed in the stereotaxic frame with the skull positioned horizontally. A burr hole was drilled in the skull and the recording electrode was placed 1.5 mm posterior to lambda and 0.2–1.2 mm from the midline and lowered into the LC, which was usually encountered at a depth between 2.7 and 4.0 mm from the brain surface. Then, a catheter (Terumo Surflo®) was inserted in the peritoneum for additional administrations of anesthetic. In order to maintain a full anesthetic state (no reaction to a tail or paw pinch), supplementary doses were given as needed. The body temperature was maintained at approximately 37°C for the entire experiment using a heating pad.

The recording electrode was filled with 2% solution of pontamine sky blue in 0.5% sodium acetate and broken back to a tip diameter of 1–2 μm. The electrode was lowered into the brain using a hydraulic microdrive (David Kopf® Instruments, Tujunga, CA, USA, model 640). The LC neurons were identified using the following standard criteria: spontaneous activity displaying a regular rhythm and firing rate between 0.5 and 5 Hz, characteristic spikes with long-lasting (>2 milliseconds), positive–negative waveform action potentials and a biphasic excitation-inhibition response to pressure applied to the contralateral hind paw (paw pinch), as was previously described in mice (Gobbi et al. 2007; Torrecilla *et al.* 2013). All neurons recorded from each mouse were analyzed according to the criteria defined above and using the computer software Spike2 (version 6).

The extracellular signal from the electrode was preamplified with a headstage, amplified with a high-input impedance amplifier and monitored with an oscilloscope and audio monitor. Two Spike2 scripts were used to analyze the spontaneous burst activity (burst.s2s) and the coefficient of variation (meaninx.s2s) of the neurons. The basal firing period (3 min) was used to determine whether a neuron showed spontaneous burst firing (bursty neurons) or not (non bursty neurons), and the regularity of the firing by measuring the coefficient of variation. A cell with highly irregular activity, exhibiting pauses of 10 seconds or longer was excluded from the analysis of the coefficient of variation. Spontaneous burst firing of LC neurons was defined as a train of at least two spikes in which the first inter-spike interval was shorter than or equal to 80 milliseconds and a termination interval greater than or equal to 160 milliseconds (Torrecilla *et al.* 2013). If the firing pattern of a neuron fit the burst criteria (bursty neurons), we measured the burst set frequency, spikes that fired in burst and intraburst frequency.

Statistical analysis

Data analyses were performed using GraphPad Prism 7 software package (GraphPad Software, La Jolla, CA, USA) and the R statistical

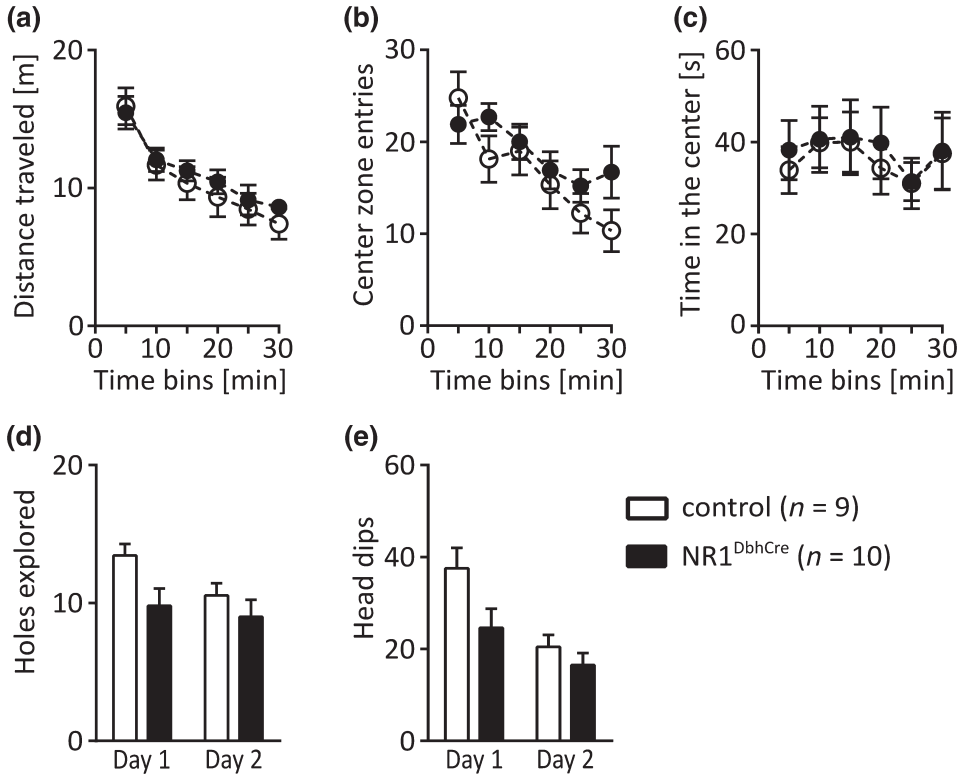

Figure 1: Exploratory behavior in the open field. (a) The first stage of the experiment was free exploration of the open field illuminated in the center. The graph shows the distance traveled during the 30-min session in 5-min bins. (b) Number of entries into the center zone. (c) Time spent in the illuminated center zone of the open field. (d) and (e) In the second phase of the experiment, a hole board was placed in the open field and animals could explore freely during two sessions on consecutive days. (d) Shows the number of holes explored and (e) the number of head dips into the holes. Error bars represent SEM.

language. Electrophysiological data sets were tested for normality using the Kolmogorov-Smirnov one sample normality test. When any data set failed the normality test, the two-tailed Mann–Whitney test was used. Otherwise, statistical differences were determined by unpaired two-tailed *t*-test. Fisher's exact test was used to compare the proportion of cells that displayed spontaneous burst firing. A difference of $P < 0.05$ was considered significant. Behavioral data were analyzed using ANOVA followed by Bonferroni *post hoc* analysis or Student's *t*-test in cases where only two groups were compared. Behavioral data was not tested for normality and no results were excluded as outliers.

Results

Open-field exploration

The *NR1*^{DbhCre} strain was generated by crossing DbhCre mice with a strain carrying a floxed variant of the NR1 (*Grin1*) gene, as described previously (Rodriguez Parkitna *et al.* 2012). Deletion is restricted to cells expressing the dopamine β-hydroxylase and in the LC it causes complete loss of NMDA receptor-dependent currents. Despite the mutation, *NR1*^{DbhCre} mice show no overt phenotype. To test the effects of the mutation on exploratory behavior, we performed a series of experiments in an open field. First, animals were introduced to an empty open field with illumination in the center area. There was no difference in the distance traveled between control and *NR1*^{DbhCre} animals [Fig. 1a, *time* ($F_{5,85}$) = 44.39, $P < 0.0001$, *genotype* ($F_{1,17}$) = 0.24, $P = 0.6329$, *time* × *genotype* ($F_{5,85}$) = 0.56, $P = 0.7296$]. Mice generally avoided exploration of the illuminated area, but genotype had no effect on the number of center zone entries [Fig. 1b, *time* ($F_{5,85}$) = 9.81, $P < 0.0001$, *genotype* ($F_{1,17}$) = 1.02, $P = 0.3271$, *time* × *genotype* ($F_{5,85}$) = 1.61, $P = 0.1671$] and the time spent in the center zone [Fig. 1c, *time* ($F_{5,85}$) = 0.58, $P < 0.7139$, *genotype* ($F_{1,17}$) = 0.18, $P = 0.6785$, *time* × *genotype* ($F_{5,85}$) = 0.06, $P = 0.9973$]. Accordingly, no genotype effect on movement speed was observed (Fig. S1, Supporting Information). These results are similar to previously reported activity of *NR1*^{DbhCre} mice in a dimly lit open field (Rodriguez Parkitna *et al.* 2012). Finally, in the second phase of the experiment, a hole board was inserted into the open field. There was a trend toward decreased exploration of the board by *NR1*^{DbhCre} mice, which was observable in the number of holes explored [Fig. 1d, *day* ($F_{1,17}$) = 5.02, $P = 0.0388$, *genotype* ($F_{1,17}$) = 3.93, $P = 0.0637$, *day* × *genotype* ($F_{1,17}$) = 1.61, $P = 0.2218$] and total head dips into the holes [Fig. 1e, *day* ($F_{1,17}$) = 19.73, $P = 0.0004$, *genotype* ($F_{1,17}$) = 4.13, $P = 0.0580$, *day* × *genotype* ($F_{1,17}$) = 2.52, $P = 0.1308$]. In summary, the mutation had no effect on

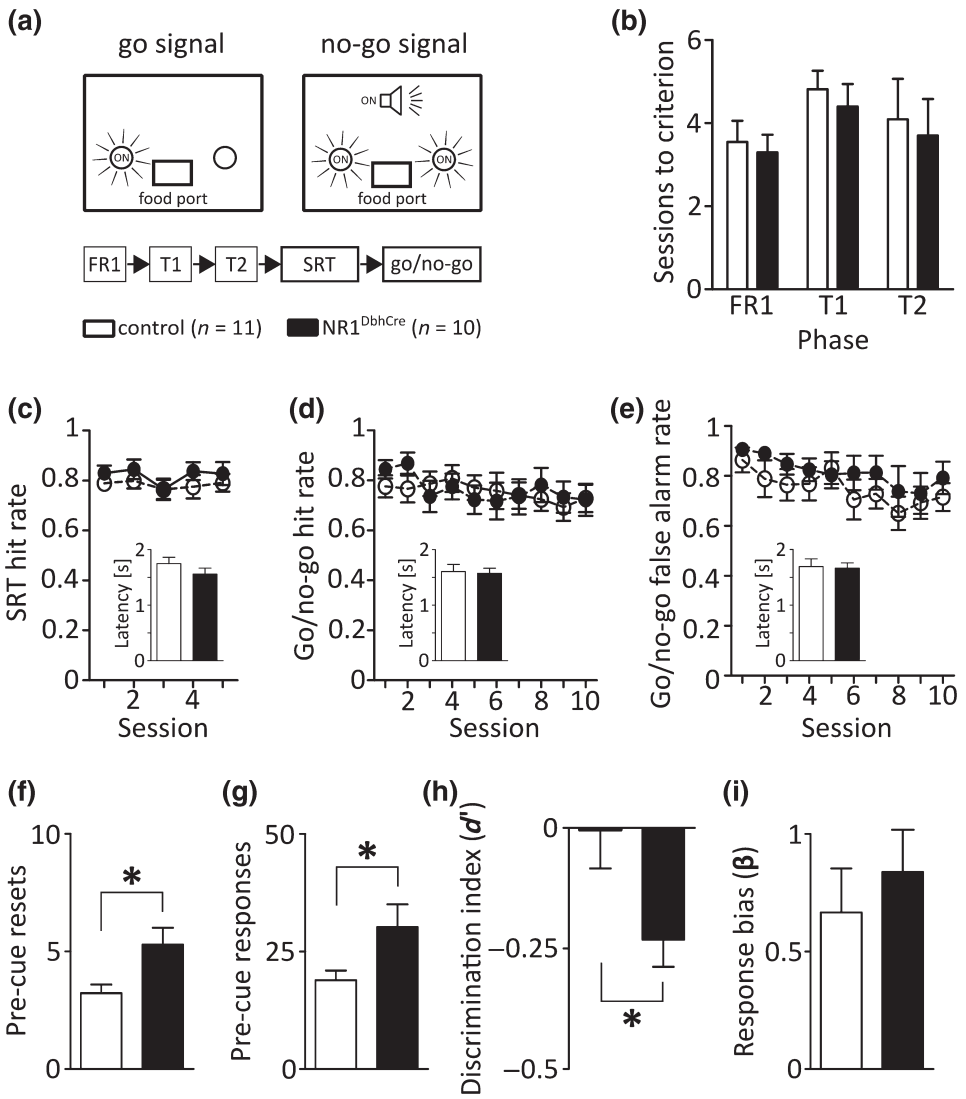

Figure 2: Sustained attention and response inhibition in the go/no-go discrimination task. (a) Schematic representation of the cues corresponding to go and no-go signals. (b) The graph shows the mean number of sessions required to reach criterion during training phases; FR1, T1, training phase 1; T2, training phase 2. (c) Fraction of correct responses (hit rate) in the simple reaction time task. The inset shows the mean response latencies. (d) and (e) Correct responses to presentation of go signals (hit rate) and incorrect responses to no-go signals (false alarm rate), during the signal discrimination phase. The insets show corresponding latencies. (f) Mean number of pre-cue resets during the signal discrimination phase (instrumental responses during the last 3 seconds of the pre-cue period). (g) Mean total number of responses during the pre-cue period. (h) Mean discriminability indices (d') calculated based on the performance during the entire test. (i) Mean response biases (β). Data shown in panels (f)–(i) are collapsed across sessions, session by session analysis is shown in Fig. S2. Error bars represent SEM. Statistically significant differences (t-test) $P < 0.05$ are marked with '*'.

locomotor activity or anxiety-like behavior. In the hole board test, both control and mutant mice showed a decrease in exploratory activity on the second day.

Sustained attention and response inhibition in the go/no-go task

We assessed how the mutation affected sustained attention and response inhibition by measuring the SRT and performance in the go/no-go discrimination task (Fig. 2a). There were no significant differences between genotypes in the number of sessions completed to meet the criteria in the training phases (Fig. 2b). Both control and *NR1*^{DbhCre} animals showed similar hit rates and response latencies in the SRT [Fig. 2c, *session* ($F_{4,76}$) = 1.58, $P = 0.1877$, *genotype* ($F_{1,19}$) = 0.76, $P = 0.3941$, *session* × *genotype* ($F_{4,76}$) = 0.42, $P = 0.7930$]. In the go/no-go paradigm, both control and *NR1*^{DbhCre} mice retained a high rate of hits [Fig. 2d,

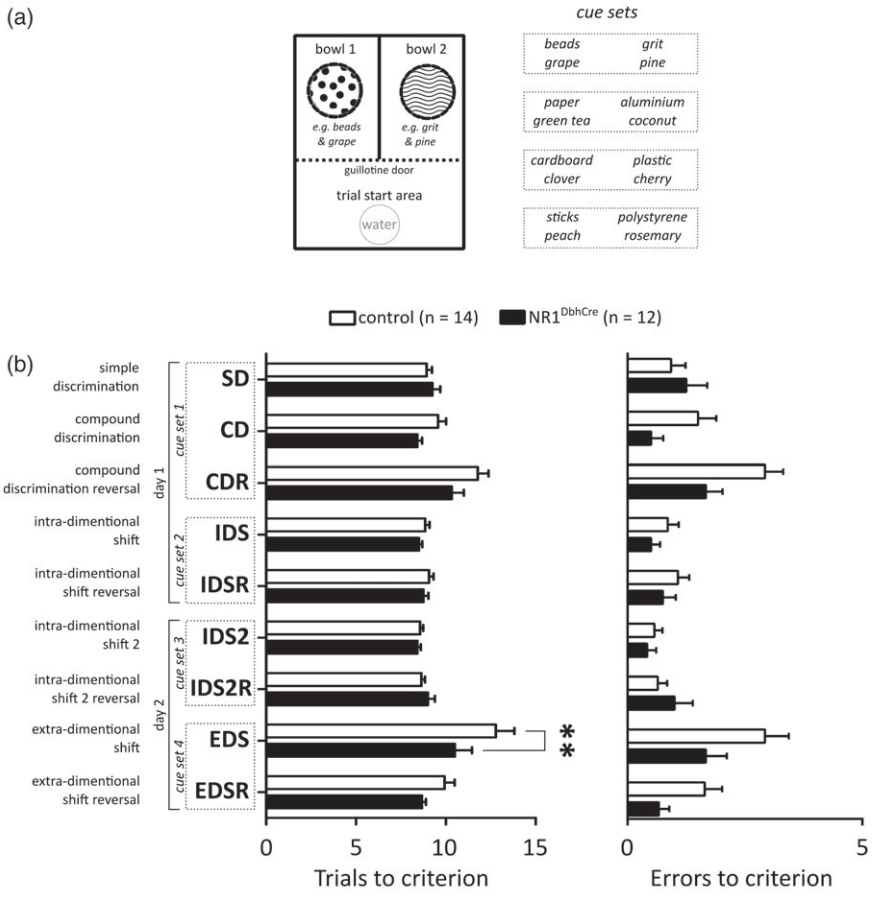

Figure 3: Performance in the attentional set-shifting task. (a) Schematic representation of the apparatus (left) and list of the odors and digging media combinations used in the study (right). (b) Number of trials completed in subsequent phases of the task (left) and number of errors (right), before the criterion of 8 correct choices out of 10 consecutive trials was reached. The phases of the experiment are listed on the left, along with the sequence of cue set changes. The order of cue presentations was counterbalanced. Error bars represent SEM. Statistically significant difference (Bonferroni) $P < 0.01$ is indicated with '**'.

session ($F_{9,171}$) = 2.86, $P = 0.0036$, *genotype* ($F_{1,19}$) = 0.02, $P = 0.9037$, *session* × *genotype* ($F_{9,171}$) = 1.68, $P = 0.0976$], but they showed a limited ability to refrain from responding to no-go signals, as evidenced by the high false alarm rate [Fig. 2e, *session* ($F_{9,171}$) = 4.97, $P < 0.0001$, *genotype* ($F_{1,19}$) = 0.73, $P = 0.4027$ and *session* × *genotype* ($F_{9,171}$), $P = 0.8105$]. However, mutant mice had a significantly higher number of pre-cue period resets [Fig. 2f, $t_{19} = 2.597$, $P = 0.0177$] and responses [Fig. 2g, $t_{19} = 2.229$, $P = 0.0381$]. Moreover, *NR1*^{DbhCre} mice had a decreased ability to discriminate between go and no-go signals (the d' parameter, Fig. 2h, $t_{19} = 2.280$, $P = 0.0343$). There was no significant effect of the genotype on response bias (the β parameter, Fig. 2i, $t_{19} = 0.6764$, $P = 0.5070$). Thus, the mutation increased impulsivity (pre-cue period resets) and possibly reduced the ability to discriminate between reward-predicting signals.

Attentional set-shifting

To measure cognitive flexibility, we used the ASST. In this test, animals were presented with two dimensions of cues, tactile and olfactory, that guided them to a hidden food pellet (Fig. 3a). Mice first learned to use the tactile cues (digging media) to find the reward (Fig. 3b, SD, CD). The mice were then tested to determine whether they learned to use a new set of tactile cues (IDS, IDS2) and the reversals of significance between the tactile cues (CDR, IDSR, IDS2R). Although olfactory cues were present during all phases of the task except SD, they switched positions independently of the location of the reward. The extended training was intended to form an attentional set directed at the tactile cues. Attentional set formation was confirmed in case of both control and mutant animals (Fig. S3). There was a significant general genotype effect on number of trials to criterion [Fig. 3b, *test phase* ($F_{8,192}$) = 10.18, $P < 0.0001$, *genotype* ($F_{1,24}$) = 11.36, $P = 0.0025$, *test phase* × *genotype* ($F_{8,192}$) = 1.62, $P = 0.1223$] and errors to criterion [Fig. 3b, *test phase* ($F_{8,192}$) = 7.79, $P < 0.0001$, *genotype* ($F_{1,24}$) = 14.01, $P = 0.0010$, *test phase* × *genotype* ($F_{8,192}$) = 1.81, $P = 0.0773$]. Moreover, in the EDS phase, where olfactory cues predicted the location of the reward and tactile cues were no longer relevant, the *NR1*^{DbhCre} mice required significantly fewer attempts than controls to reach the criterion. Taken together, these data indicate that the mutation enhanced behavioral flexibility by facilitating attentional set-shifting.

Exploration-exploitation trade-off in the TAB task

To examine how the mutation affected the balance of the trade-off between exploration and exploitation, we tested the mice in a TAB task. In the first phase of the procedure, animals were trained to respond to a target stimulus

presented pseudo-randomly in one of the two available nose-poke ports. There were no effects of genotype on the number of sessions required to achieve the criterion in the training phase (Fig. 4a, $t_{15} = 0.8831$, $P = 0.3911$). Then, animals were tested in the TAB task for the ability to distinguish between correct and incorrect choice options with different reward probabilities, 0.8 vs. 0.2, respectively. During each session, the probability of reward delivery was reversed between blocks of trials (Fig. 4b). All animals improved their performance during the experiment, and there was a significant increase in the number of rewards obtained [Fig. 4c, *session* ($F_{14,210} = 6.91$, $P < 0.0001$, *genotype* ($F_{1,15} = 0.49$, $P = 0.4965$, *session* × *genotype* ($F_{14,210} = 0.74$, $P = 0.7356$]. The proportion of omitted trials remained constant [Fig. 4d, *session* ($F_{14,210} = 1.52$, $P = 0.1075$, *genotype* ($F_{1,15} = 0.32$, $P = 0.5825$, *session* × *genotype* ($F_{14,210} = 0.42$, $P = 0.9683$]. The behavioral strategies of mice were evaluated by assessing the probability of selecting the same operant that was previously selected when the previous response was rewarded ['win-stay', Fig. 4e, *session* ($F_{14,210} = 13.43$, $P < 0.0001$, *genotype* ($F_{1,15} = 3.68$, $P = 0.0744$, *session* × *genotype* ($F_{14,210} = 1.32$, $P = 0.1971$] or performing a response on the other operant when the previous choice was not rewarded ['lose-shift', Fig. 4f, *session* ($F_{14,210} = 0.86$, $P = 0.6057$, *genotype* ($F_{1,15} = 0.66$, $P = 0.4276$, *session* × *genotype* ($F_{14,210} = 1.09$, $P = 0.3715$]. Cumulative analysis of choices made during the experiment showed a significant increase in the probability of win-stay responses in *NR1*^{DbhCre} mice [Fig. 4g, *strategy type* ($F_{3,60} = 84.00$, $P < 0.0001$; *genotype* ($F_{1,60} = 0.00$, $P = 1.0000$; *strategy type* × *genotype* ($F_{3,60} = 4.68$, $P = 0.0053$]. These results suggest that the mutation shifted decision-making strategies toward exploitation over exploration.

Electrophysiological properties of LC neurons in anesthetized NR1^{DbhCre} *mice*

Finally, we examined whether the loss of NMDA receptors affected the spontaneous activity of LC neurons in anesthetized animals. All LC neurons recorded from control and *NR1*^{DbhCre} mice displayed a typical biphasic excitation-inhibition response to a pinch of the contralateral paw (Fig. 5a). While the trend toward increased mean firing rate of LC neurons recorded in *NR1*^{DbhCre} mice did not reach significance (Fig. 5b, $P = 0.063$), we found increased irregularity of firing in the mutant group compared with controls (Fig. 5c, $P = 0.0067$). LC neurons from both control and *NR1*^{DbhCre} mice displayed spontaneous burst firing; however, the mutation had a significant effect on the proportion of neurons displaying burst activity, increasing the number of bursty neurons in this group (Fig. 5d, control: 36 of 77 recorded cells; *NR1*^{DbhCre}: 40 of 60 recorded cells, $P = 0.0098$).

Analysis of firing patterns in bursty neurons showed that inactivation of NMDA receptors did not affect the firing rate (Fig. 5e left, $P = 0.6323$) but significantly decreased the regularity of the pattern. The coefficient of variation was greater in the mutant group compared with that recorded in the control group (Fig. 5e right, $P = 0.0378$). Nevertheless, properties of the bursts themselves: the distribution of the inter-spike intervals, burst set frequency, the proportion of spikes that fired in burst and the intraburst frequency were not affected by the mutation (Fig. 5f–j). Taken together, these results indicate that the mutation increased the number of LC neurons with spontaneous burst activity and decreased their firing regularity.

Discussion

We found that the inactivation of NMDA receptors in the NA neurons alters animal performance in tasks depending on attention and behavioral flexibility. We had previously reported that *NR1*^{DbhCre} mice showed normal locomotor activity and no increase in anxiety-like behaviors (Rodriguez Parkitna *et al.* 2012). In line with this, here we observed no effects of the mutation on distance traveled or anxiety-like behavior in the open field, in contrast to the phenotypes observed in case of tonic optogenetic stimulation of LC neurons (Carter *et al.* 2010; McCall *et al.* 2015). The only potential indication toward a difference in behavior in a novel environment was a trend toward decreased exploration of the hole board maze. Taken together these results suggest that the *NR1*^{DbhCre} phenotype was not related to altered anxiety levels.

To some extent surprisingly, mutant mice showed a normal ability to learn to detect and respond to the stimulus presented during training in the go/no-go experiment. No deficits in the ability to maintain focused attention during the task were observed, as evidenced by relatively few misses. However, in the final part of the experiment when the no-go signal was introduced, effects of the mutation became apparent. First, we observed increased responsiveness during the pre-cue period and pre-cue resets performed by mutant animals, which is an indicator of impulsive behavior, a deficit in waiting impulsivity. This, however, was not associated with a difference in response latencies or a significantly increased number of false alarms during the no-go trials and thus normal response inhibition. Independently of the genotype, the false alarm rate was generally high (~75%) and showed only a relatively small reduction over the duration of experiment. Accordingly, the discrimination between signals was poor, the d' values are close to 0. This limits, to an extent, interpretation of the results. Poor discrimination between signals in a similarly constructed go/no-go task was previously reported to result from deficits in learning of instrumental omission contingency or because of generalization of the approach behavior elicited by the go signal (Gubner *et al.* 2010). This interpretation appears plausible, because the *NR1*^{DbhCre} mice are congenic with the C57BL/6 strain, which had been observed to show poor discrimination. Taken, together the results of the go/no-go experiment indicate that the mutation resulted in increased waiting impulsivity and had no appreciable effects on sustained attention, although methodological limitations urge caution with the latter observation and preclude a conclusion with regard to signal discrimination.

Cumulative analysis of choices in the TAB task showed that mutant mice were more likely to select the win-stay strategy. A possible explanation for the behavior would be that

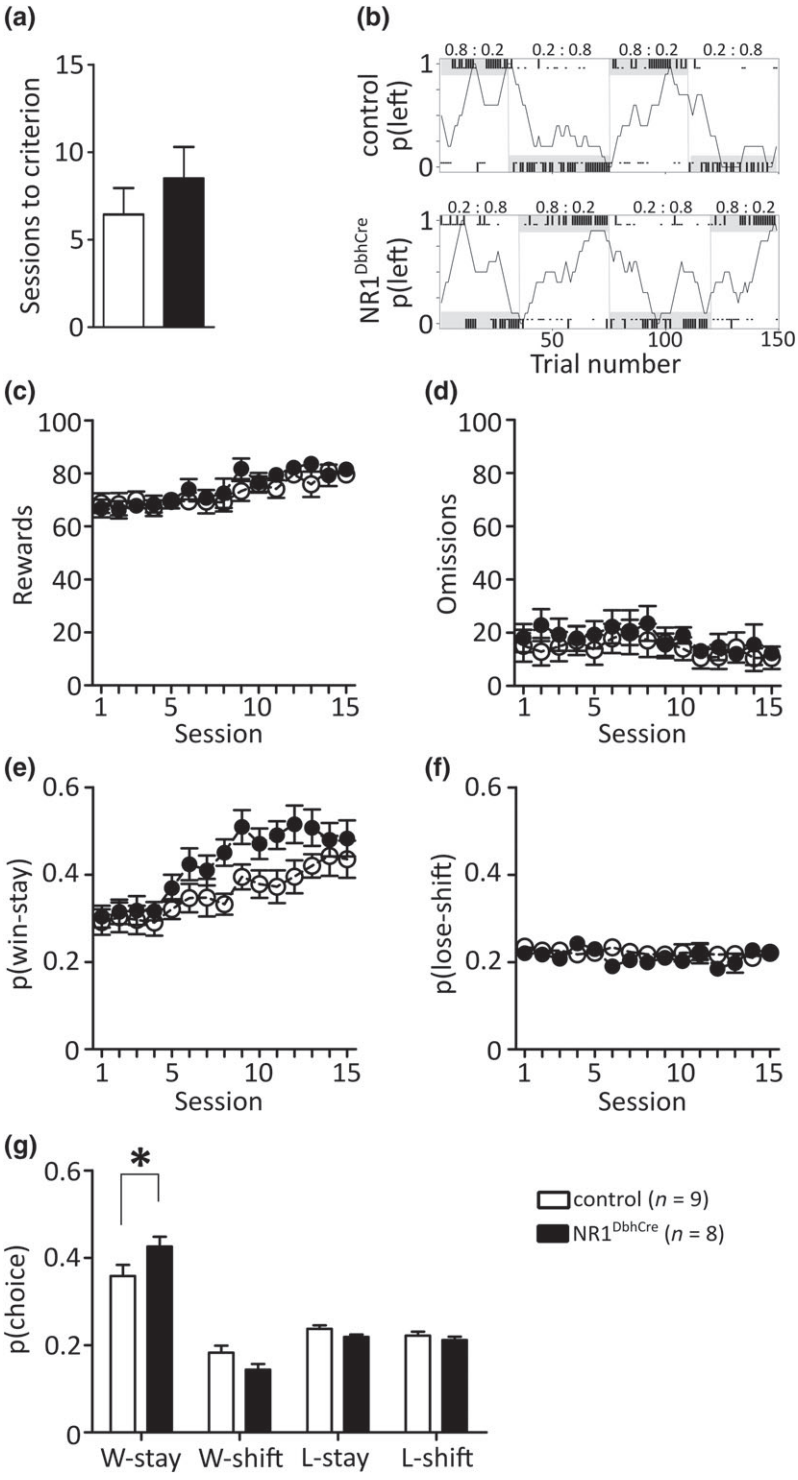

Figure 4: Behavioral strategy in the two-armed bandit task. (a) Mean number of sessions required to reach the performance criterion during the training phase with constant outcome probability ($P = 0.8$). (b) Sample TAB sessions. The solid line shows the probability of choosing the left side in a moving average during the 11th session for representative *NR1*^{DbhCre} and control mice. Dotted vertical lines denote block transitions. Numbers indicate reward probabilities in each block. Shaded gray bars indicate the correct side associated with a 0.8 probability of food pellet delivery; top and bottom correspond to left and right, respectively. The ticks and dots indicate rewarded and unrewarded choices, respectively. No tick is shown if no choice was made (omission). (c) and (d) The graphs show the mean number of rewards earned and omitted trials during each session. (e) Exploitation, defined as the probability of selecting the same choice option as before or after a rewarded trial (win-stay). (f) Probability of selecting an alternative choice option after a non-rewarded trial (lose-shift). (g) Probabilities of different strategies in the TAB task based on the data from the whole experiment. Error bars represent SEM. Statistical significance (Bonferroni) $P < 0.05$ is marked with a '*'.

since mutation facilitates attentional shifting in the ASST, it also facilitates adaptation to changes in reward contingency in the TAB task, and as a result facilitates exploitation of the rewarded choices. *NR1*^{DbhCre} mice required significantly fewer trials to reach the criterion in the EDS phase of the ASST, resembling the effects of treatment with the NMDA receptor NR2B-subtype specific antagonist Ro 25-6981 (Kos *et al.* 2011). This result is similar to the effects of pharmacological facilitation of NA signaling on attentional set-shifting (Devauges & Sara 1990; Snyder *et al.* 2012) and opposite to

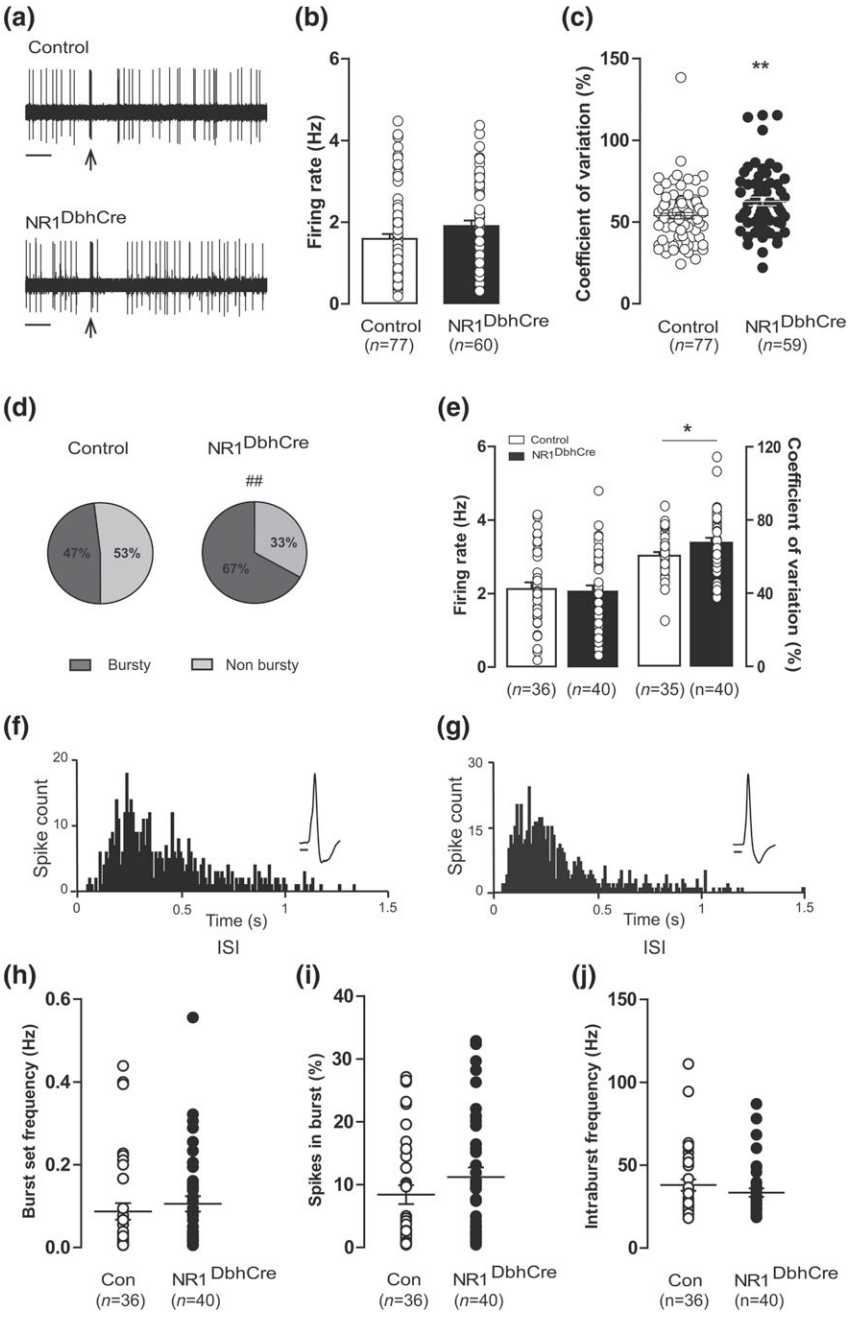

Figure 5: *In vivo* electrophysiological properties of locus coeruleus neurons. (a) Extracellular signals of action potentials of LC neurons and the biphasic excitation-inhibition response (pinch of the contralateral paw) in control and *NR1^{DbhCre}* mice. Arrows indicate paw pinch. Scale bars, 2 seconds. (b) and (c) The graphs show the firing rate (b) and coefficient of variation (c) of LC neurons in both groups. (d) Relative amount of LC neurons with spontaneous burst firing (bursty neurons) in control and *NR1^{DbhCre}* mice. (e) The graph shows the firing rate and coefficient of variation of bursty neurons from control and *NR1^{DbhCre}* mice. (f) and (g) Inter-spike interval histograms (10 milliseconds bins) and waveforms of bursty neurons recorded from control (f) and *NR1^{DbhCre}* mice (g). Scale bars, 2 milliseconds. (h), (i) and (j) The graphs show the burst set frequency (h), spikes in burst (i) and intraburst frequency (j) of bursty neurons from control and *NR1^{DbhCre}* mice. Bars represent mean and error bars SEM. Values from a single neuron are shown by circles. Statistical significance $P < 0.05$ (*t*-test) is marked with a '*', $P < 0.01$ (*t*-test) is marked with '**' and $P < 0.01$ (Fisher's exact test) is marked with '##'.

optogenetic inhibition of LC firing or lesions of the dorsal NA bundle (Janitzky *et al.* 2015; Tait *et al.* 2007). The result is also similar to the observed effects of elevated NA neuron activity leading to activation of alfa1 adrenergic receptors in the prefrontal cortex (Berridge *et al.* 2012; Lapiz & Morilak 2006). An additional possibility to consider is that the altered activity of NA neurons outside of the LC, particularly in the A1 and A2 areas, contributed to the increased sensitivity to positive reinforcement. These neurons have projections into the NAc, which is a part of the brain's reward system and is involved in reinforcement learning and decision-making (Delfs *et al.* 1998). Finally, it should be also noted that enhanced performance in the ASST could also be influenced by an enhancement of olfactory learning in *NR1^{DbhCre}* mice, which speculatively might had facilitated the shift from tactile to olfactory cues.

We show that loss of NMDA receptors in the LC increased the proportion of neurons that display burst activity and reduced the regularity of the firing pattern of these cells. This could be in agreement with the upregulation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated synaptic activity in the LC of the

NR1^{DbhCre} mice, earlier described by our group (Rodriguez Parkitna *et al.* 2012). Accordingly, pharmacological blockade of the glutamatergic input to the LC reduces both spontaneous burst activity and firing rate irregularity (Tung *et al.* 1989). Therefore, a compensatory increment in the AMPA/kainate receptor-mediated synaptic input to the LC might underlie the greater proportion of LC neurons with burst pattern and their increased irregularity observed in the *NR1*^{DbhCre} mice. Furthermore, although the pattern of firing of the LC in anesthetized mice likely differs from activity in awake animals, it should be noted that an increase in bursting would lead to higher noradrenaline release at target areas (Berridge & Abercrombie 1999; Devoto *et al.* 2005; Florin-Lechner *et al.* 1996). Hypothetically, if increased number of bursty neurons in the LC of *NR1*^{DbhCre} mice caused greater noradrenaline release in forebrain areas, this could facilitate attentional shifting and promote reorganization or a 'reset' of local neuronal networks, accelerating adaptation to a change in the environment (Bouret & Sara 2004, 2005).

In conclusion, our results show that a selective loss of NMDA receptors resulted in enhanced behavioral flexibility, similarly to that which was observed after activation of NA receptors in the prefrontal cortex (Berridge *et al.* 2012; Lapiz & Morilak 2006). Accordingly, we have observed in anesthetized mutant mice increased occurrence of bursty neurons in the LC, which speculatively could indicate higher levels of NA neuron activity during behavioral tests. Thus, the NMDA receptor-dependent signaling is necessary to adjust NA neuron activity for optimal performance, regulating the balance between exploration and exploitation as well as limiting impulsivity.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Figure S1: Movement speed during the open-field test. (a) Mean movement speed [*time* ($F_{5,85}$) = 43.98, $P < 0.0001$, *genotype* ($F_{1,17}$) = 0.22, $P = 0.6417$, *time* × *genotype* ($F_{5,85}$) = 0.54, $P = 0.7468$]. (b) Maximum speed [*time* ($F_{5,85}$) = 2.19, $P < 0.0632$, *genotype* ($F_{1,17}$) = 1.74, $P = 0.2051$, *time* × *genotype* ($F_{5,85}$) = 1.12, $P = 0.3544$].

Figure S2: Session by session go/no-go analysis. (a) Mean number of pre-cue resets [*session* ($F_{9,171}$) = 0.22, $P = 0.9912$, *genotype* ($F_{1,19}$) = 6.75, $P = 0.00177$, *session* × *genotype* ($F_{9,171}$) = 0.62, $P = 0.7829$]. (b) Mean total number of responses during the pre-cue period [*session* ($F_{9,171}$) = 0.46, $P = 0.8990$, *genotype* ($F_{1,19}$) = 4.97, $P = 0.0381$, *session* × *genotype* ($F_{9,171}$) = 0.48, $P = 0.8846$]. (c) Mean discriminability indices (d') [*session* ($F_{9,171}$) = 0.84, $P = 0.5806$, *genotype* ($F_{1,19}$) = 6.85, $P = 0.0170$, *session* × *genotype* ($F_{9,171}$) = 1.26, $P = 0.2618$]. (d) Mean response biases (β) [*session* ($F_{9,171}$) = 0.65, $P = 0.7566$, *genotype* ($F_{1,19}$) = 6.01, $P = 0.0241$, *session* × *genotype* ($F_{9,171}$) = 1.14, $P = 0.3365$].

Figure S3: Attentional set formation. (a) Comparison of the number of trials to criterion in extra-dimensional shift (EDS) phase and preceding intra-dimensional shift (IDS2) phase in groups of control ($t_{26} = 4.022$, $P = 0.0004$) and mutant ($t_{22} = 2.133$, $P = 0.0443$) animals. (b) Attentional 'shift cost' – a measure of successful set formation, calculated as a ratio of trials needed to reach criterion in the EDS as compared to the IDS2 phase (Control: $t_{13} = 3.916$, $P = 0.0018$; Mutant: $t_{11} = 2.117$, $P = 0.0579$; one sample *t*-test). Statistically significant differences $P < 0.05$ are marked with '*', $P < 0.01$ with '**' and $P < 0.001$ with '***'.