Modulation of the Mouse Prefrontal Cortex Activation by Neuronal Nicotinic Receptors during Novelty Exploration but not by Exploration of a Familiar Environment

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Organization of locomotor behavior is altered in mice knockout for the β2 subunit of the nicotinic receptor—β2−/− mice—during novelty exploration. We investigated the neuronal basis of this alteration by measuring activation of the immediate early gene c-fos in the brains of wild-type (WT) and β2−/− mice after exploration of a novel or a familiar environment. Results show 1) no constitutive difference between WT and β2−/− mice in c-fos gene expression in any brain region, 2) novelty exploration triggered activation of the hippocampus and the reward circuit while exploration of a familiar environment increased activation in the amygdala, and 3) in β2−/− mice, exploration of novelty, but not familiarity, induced an increase in activation in the prefrontal prefrontal cortex (PFC) compared with WT mice. c-Fos immunoreactivity after different stages of learning in a maze increased similarly in the prelimbic area of both WT and β2−/− mice, while their performance differed. In WT mice, exploration of a novel environment triggered an increase in c-Fos expression in the reward circuit and the hippocampus, while in β2−/− mice, the amygdala and the motor cortex were additionally activated. We also highlight the role of nicotinic receptors during activation of the PFC, specifically during free exploration of a novel environment.

Keywords: c-Fos, nAChRs, novelty, prefrontal cortex, reward circuit

Introduction

The β2 subunit—containing nicotinic cholinergic receptors—β2*nicotinic cholinergic receptors (nAChR)—are transmembranal allosteric proteins, widely expressed throughout the mammalian brain (for review, Changeux and Edelstein 2005). Previous investigations have shown that mice bearing the null mutation for the β2 subunit of the nicotinic cholinergic receptor—β2−/− mice (Picciotto et al. 1995)—have increased locomotor activity and decreased exploratory behavior when confronted with a novel environment (Granon et al. 2003; Granon and Changeux 2006; Besson et al. 2007). Locomotor sequences were disorganized during novelty exploration (Maubourguet et al. 2008), but not when mice were habituated to an environment (Wiklund et al. 2008). The implication of the ventral tegmental area—VTA—and the specific contribution of the cholinergic system in these behaviors have been demonstrated by rescue experiments using lentiviruses (Maskos et al. 2005; Avale et al. 2008). Since the potential contributions of other related brain regions remain elusive, here we explored other neuronal correlates of these behavioral deficits using 3 distinct exploratory behavioral situations: 1) the first involved free exploration in a novel environment; 2) the second involved repeated exploration of the same environment, thus providing a control for processes associated with familiarity versus novelty; 3) the third was designed to compare more constrained exploration with free exploration.

In previous experiments (Granon et al. 2003), β2−/− mice showed better spatial learning than wild-type (WT) mice during the early learning stages. After a few days of learning, however, performance of both strains were similar. We assessed the neuronal bases of the behavioral performance in WT and β2−/− mice using quantitative expression of the immediate early gene c-fos, which reflects enhanced neuronal physiological activity (Bisler et al. 2002; Staiger et al. 2002; Majdan and Shatz 2006; Lim et al. 2009). The quantification of c-Fos immunoreactive nuclei provides evidence of the regional patterns of brain areas that are simultaneously activated in specific cognitive/behavioral tasks.

Our results support the view that β2*nAChRs modulate the prefrontal cortex (PFC) activity, specifically in flexible choice making.

Materials and Methods

Animals

Thirty-eight β2−/− mice and 38 C57Bl/6J—WT—mice were used in these experiments. They were 4 months old males arriving from the rearing facilities (Charles Rivers Laboratory, France) 2-3 weeks before the experiment. Extensive genomic analysis has been carried out to determine the percentage of C57Bl6/J genetic background in the β2−/− line that originally derives from a 129/Sv embryonic state cell line. Since then, the line has been backcrossed more than 20 generations with the WT C57Bl6/J line, which is above the 10 backcrosses recommended by the Banbury conference (Banbury Conference on Genetic Background in Mice 1997). Using more than 400 genomic markers, the β2−/− line was confirmed to be at more than 99.99% C57Bl6/J.

The animals were treated according to the ethical standards defined by the Institut Pasteur and Centre National de la Recherche Scientifique for animal health and care in strict compliance with the EEC recommendations (no.86/609).

Two weeks before being tested in any behavioral test, mice were placed in individual cages in a ventilated and temperature- and humidity-controlled room with a 12/12 light/dark cycle (light at 8:00 AM).
For the open field experiment (open field novelty exploration WT n = 10, β2−/− n = 10; familiar open field exploration WT n = 10, β2−/− n = 9, and no open field WT n = 10, β2−/− n = 10), they received food pellets and water ad libitum. For the maze learning experiment (WT n = 8, β2−/− n = 9), they were food deprived for 10 days before the beginning of any learning procedure. Once the weight of the mice reached 85% of their free feeding weight, habituation of the learning procedure started.

Open Field Exploratory Behavior
As described previously (Granon et al. 2003; Maskos et al. 2005; Besson et al. 2007), the open field consists of an opaque white plastic circular tank 1 m in diameter and 40 cm in height, located in an isolated room with large and distinct distal cues permanently fixed to the walls. The light was set at 100 Lux at the center of the open field. Each mouse was transferred from its individual cage in a transport box, taken to the experimental room, and from there to the center of the open field for a unique session of 30 min (open field novelty condition). For the familiar open field exploration, mice were treated as for the novelty condition twice a day for 5 consecutive days. For the control condition (no open field), the animals were taken from their home cage, placed in the same transport box, transported to the experimental room, and then taken back immediately to their home cage. At the end of each session, the mouse was returned to its own cage and perfused 90 min later. The open field was cleaned between each animal.

Maze Learning
The apparatus has been described in detail elsewhere (Granon et al. 2003). Briefly, it consists of 4 arms forming a cross. One arm contained a food cup containing sucrose pellets at the end. The opposite arm had a similar food cup filled identically but it was covered with a grid, making the food unavailable. Two longer arms were used as starting points. The goal of the task was to reach the food cup where the food is available from 1 of the 2 pseudorandomly starting arms. The animals were placed in the maze for 3 trials per day, with an intertrial interval of 2 min during which the animals were returned to their home cage.

Behavioral Measures and Conditions
Open Field Exploratory Behavior
During the open field sessions, a video camera connected to a computer equipped with a video tracking system (View-point, Lyon, France), located above the open field, automatically recorded the locomotor activity (total distance in centimeters) of each animal in absence of any observer.

Maze Learning
After stabilization of their weight to 85% of their free feeding weight, mice were familiarized to the maze for 2 days for 15 min with food scattered in every arm—maze visit condition—before the learning protocol started. At that stage, all animals ate in the maze during the 2 first min. Learning consisted of 3 trials per day where animals were gently placed in 1 of the 2 start arms. Performance was measured by latency to reach the food (a maximum of 2 min was allowed). The animals from each genotype were randomly and blindly assigned to the 5 learning phases: no exploration (animals were food deprived and did not visit the maze), maze visit day 2 (animals visited and ate in the maze but did not learn any specific location as food was scattered all over the maze), learning day 5 (animals learn the food cup location for 5 consecutive days), learning day 10 (animals learn the food cup location for 10 consecutive days), and learning day 15 (animals learn the food cup location for 15 consecutive days).

Immunocytochemistry
At the end of each experimental condition, the animals were returned to their home cage for 90 min to allow for the synthesis and transport of c-Fos proteins to the nuclei of activated neurons. This interval (90 min) was selected as it is within the period of peak production (between 90 and 120 min) for c-Fos protein after a specific, initiating event (Bisler et al. 2002). The animals were then sacrificed with pentobarbital and perfused transcardially with 150 mL of phosphate-buffered saline (PBS), followed by 50 mL 4% paraformaldehyde depolymerized in saline phosphate buffer. The brains were extracted and postfixed overnight in the cold room. Sections (70 μm) were cut throughout the entire brain using a vibratome (Leica). Residual free aldehydes in the tissue were neutralized in PBS containing 50 mM NH4Cl, 1 mM lysine, and 1 mM glycine. Endogenous peroxidases were neutralized in PBS containing 3% H2O2 for 20 min. Brain sections were treated for 30 min in a solution of PBS containing 0.1% Triton to permeabilize the brain cells and 1% bovine serum albumin (BSA) and 5% normal goat serum (NGS) to saturate nonspecific binding sites. The c-Fos proteins were immunolabeled in rotating vials at 4 °C for 38 h. We used a 1:8000 dilution of the purified polyclonal rabbit immunoglobulin G (IgG) anti-human c-Fos (AB-5) human (Calbiochem #PC38) in PBS with 1% BSA, 1% NGS, 0.05% NaN3 (sodium azide, Sigma S-2002), and 0.1% Triton. After 3 rinses (10 min each) in PBS, the first antibody was tagged successively with purified and biotinylated goat anti-rabbit IgGs (Vector BA-1000) diluted 1:600 in PBS containing 1% BSA and 1% NGS for 2 h and then with an avidin-peroxidase complex (Vectastain Elite PK 6100) for 30 min at room temperature. After several rinses in Tris-buffered saline, peroxidase was revealed using H2O2 (33%) and diaminobenzidine (Sigma D-5905) as the chromogen. The peroxidase reaction was stopped after 3 min. Sections were mounted on SuperFrost glass slides dehydrated in ethanol and coverslipped with Eukitt.

Regions of Interest
We quantified c-fos expression in 6 distinct brain regions: prelimbic cortex (Prl), primary motor cortex (M1), cornu ammonis field 1 of the hippocampus (CA1), basolateral nucleus of the amygdala (BLA), nucleus accumbens (NuAcc), and the VTA. These regions are known to be necessary for either spatial cognition, such as the hippocampus (Poucet and Save 2005; Renaudineau et al. 2009) and the Prl (de Saint Blanquat et al. 2010), or are part of a network which showed a different role in β2−/− and WT mice (Maskos et al. 2005; Ballesteros-Yáñez et al. 2010). We systematically sampled the median section of the whole rostrocaudal extent of each of these brain structures (from bregma: Prl +1.98 mm, M1 +0.62 mm, CA1 −3.28 mm, BLA −0.94 mm, NuAcc +1.34 mm, VTA −3.40 mm; Paxinos and Franklin 2004).

Image Acquisition and Quantification of c-Fos-Positive Nuclei
Sections were viewed under a Nikon Eclipse 1000 light microscope, and images were acquired using a digital camera (Nikon DXM 1200). A π×10 Plan Apo objective gave both good resolution of c-Fos immunoreactive (c-Fos+) nuclei and identification of the cytoarchitectonic fields. The focus was set on the upper face of each section before digitization. The final magnification, calibrated with a micrometer object slide (Zeiss), scaled 100 μm in the tissue to 80 pixels in the images. Digitized images were then quantified on a Sun station using an in-house developed multisolution algorithm (Olivio-Marin 2002). The contour of each region of interest (ROI) was drawn on the monitor screen for each digital image. The software automatically calculated the surface of the ROI, converted the number of c-Fos-positive nuclei into gray spots, and computed their density per square micrometer. The immunocytochemical background was eliminated from the quantification process by a denoising algorithm using a threshold value that is image and level dependent and can be computed automatically from the data (Olivio-Marin 2002). This program makes it possible to select and count cells automatically without experimenter bias (counts were conducted without knowledge of the group assignments). In order to derive accurate, absolute cell counts, it would be necessary to use stereological methods (Coggshall and Lekan 1996), but the goal of the present study, as it is the case in other work (e.g., Albasser et al. 2010), was to compare relative numbers of activated cells between groups. In each coronal section, we sampled both ipsi- and contralateral regions separately and then averaged the density of the spots for each structure per animal. The results were expressed as SPOTS Per square millimeter of cerebral tissue.
Statistical Analysis
For open field experiments, the behavioral and c-Fos quantification data were analyzed with analysis of variance (StatView) with genotype and conditions (when applicable) as between-subject main factors or with Student’s t-test.

Statistical correlations (Z score correlation, StatView) were performed concerning the number of c-Fos neurons between the different brain areas for each experimental condition of the open field experiment and between the number of c-Fos neurons and the distance covered in the open field to control for a putative locomotor effect.

For the maze learning experiment, due to a limited number of animals in each learning stage (n = 3 in each group per stage), we conducted nonparametric Mann-Whitney test to assess the genotype effect.

Results

Open Field Novelty Exploratory Behavior
For the open field exploration experiment, statistical analysis (t-test) conducted on distance covered during novelty exploration showed a significant genotype effect (t_{df} = 4.01, P = 0.0008). These results, in agreement with previous studies (Granon et al. 2003; Besson et al. 2007; Avale et al. 2008; Maubourguet et al. 2008), validate the use of these mice for the present study. The results showed that β2/− mice have an hyperactive phenotype, covering more distance (Fig. 1A) compared with WT mice when exposed to a novel environment.

β2/− and WT Comparison in Basal Condition—No Exploration
In baseline condition (no exploration), regional c-fos expression was similar in β2/− and WT mice in all ROIs (Fig. 1B,C). Statistical analysis showed no difference between the 2 genotypes for Prl (F < 1, not significant [NS]), NuAcc (F < 1, NS), VTA (F_{1,18} = 2.4, P = 0.1, NS), CA1 hippocampus (F < 1, NS), basolateral amygdala (F_{1,18} = 1.5, P = 0.24, NS), and motor cortex M1 (F_{1,18} = 1.2, P = 0.3, NS).

Novelty Exploration in WT Mice
When WT mice were exposed to a novel open field for 30 min, they showed a significant increase in density of c-Fos neurons compared with basal levels (no exploration groups, Fig. 1B) in the PFC (F_{1,18} = 5.8, P = 0.03), the VTA (F_{1,18} = 7.1, P = 0.02), the NuAcc (F_{1,18} = 21.2, P = 0.0002), and the hippocampus (F_{1,18} = 10.5, P = 0.005). No increase in c-fos levels was observed in the amygdala (F_{1,18} = 1.07, P = 0.31) and the motor cortex (F_{1,18} = 2.3, P = 0.15).

Novelty Exploration in β2/− Mice
In β2/− mice, as in WT, there is a significant increase in c-fos expression triggered by novelty exploration in the PFC (F_{1,18} = 9.8, P = 0.006), the VTA (F_{1,18} = 6.6, P = 0.02), the NuAcc (F_{1,18} = 19.9, P = 0.0003), and the hippocampus (F_{1,18} = 40.2, P < 0.0001) (Fig. 1C).

In addition to what is observed in WT mice, we also found an increase in c-fos expression in the amygdala (F_{1,18} = 7.3, P = 0.01) and the motor cortex (F_{1,18} = 13.9, P = 0.002) in β2/− mice following exploration of the novel environment.

These results suggest that exploration of a novel environment is sufficient to induce a significantly greater activity than the baseline condition in the amygdala and the motor cortex in β2/− mice but not in WT.

We performed correlation analyses between the number of c-Fos+ neurons in different brain areas. In WT mice, there were...
no correlation between the number of c-Fos* neurons in any brain region or between the number of c-Fos* neurons and the distance covered during exploration of the novel open field (data not shown). By contrast, in β2−/− mice, the number of c-Fos* neurons in the prelimbic area of the PFC was correlated with that of the NuAcc, CA1, amygdala, and motor cortex M1 (Table 1). The distance covered by the β2−/− mice was correlated with the number of c-Fos* neurons only in the VTA (Table 1), suggesting that the higher locomotor level of these mice is unlikely to account for increased cFos expression in all brain regions. No significant correlation was observed between locomotor activity and the number of c-Fos* neurons in WT mice for any brain region.

The same significant correlations were found between the Prl and the amygdala and CA1 in animals not exposed to novelty exploration (Table 2), suggesting that novelty exploration, specifically in β2−/− mice, triggers correlated activation between the Prl and the NuAcc and the motor cortex.

**β2−/− and WT Mice Comparison during Exploration of a Novel Environment**

The only region in which expression of c-fos was significantly greater in β2−/− mice as compared with WT was the prelimbic area of the PFC (F_{1,18} = 6.6, P = 0.02) (Figs 1D and 2). WT and β2−/− mice showed similar density of c-Fos* nuclei in the VTA (F < 1, NS), the NuAcc (F_{1,18} = 2.3, P = 0.1, NS), the hippocampus (F_{1,18} = 1.3, P = 0.3, NS), the amygdala (F < 1, NS), and the motor cortex (F_{1,18} = 2.2, P = 0.2, NS).

**Exploration of a Familiar Environment in WT and β2−/− Mice**

We measured c-fos expression after six 30-min sessions of exploration of the same environment in order to compare brain activations associated with novelty exploration and that associated with familiarity to the environment. Behavioral data, illustrated in Figure 3A, showed that both WT and β2−/− mice habituated with repeated exposure (repetition effect: F_{3,35} = 30.4, P < 0.001 and interaction genotype × repetition: F_{3,35} = 2.47, P = 0.034). We found a significant genotype effect only for the first (t = 2.7, degrees of freedom [df] = 17, P = 0.016), second (t = 2.2, df = 17, P = 0.044), and sixth (t = 2.72, df = 17, P = 0.014) exploration sessions.

c-fos expression data, illustrated in Figure 3B, showed a similar increased activation in WT and β2−/− mice that was specific to the amygdala after exploration of a familiar environment compared with exploration of a novel environment (P < 0.0001). The level of c-fos expression did not significantly differ in any other brain area after exploration of a familiar environment compared with the novel environment in both WT and β2−/− mice (all other P values > 0.05).

c-fos expression was similar in WT and β2−/− mice in all brain areas after exploration of a familiar environment (all P values > 0.14), except the NuAcc where it is significantly increased in β2−/− mice (t = 2.65, df = 17, P = 0.017).

**β2−/− and WT Mice Comparison during the Different Phases of Spatial Learning**

We checked whether c-fos expression in the prelimbic area of the PFC would be altered by a novel maze learning procedure. The learning curve (Fig. 4A) showed a difference between β2−/− and WT, as we previously observed (Granon et al. 2003), with β2−/− mice learning faster than WT mice at day 5 (Z = 2.6, P = 0.009) and day 10 (Z = 2.0, P = 0.05) of learning, but at day 15 (Z = 1.3, P = 0.18). We measured c-Fos* nuclei in the prelimbic area of WT and β2−/− mice (3 per behavioral stage and genotype) after different stages of learning (Fig. 4B). We found no difference between WT and β2−/− mice that were food deprived but not tested in maze exploration (Z = 1.5, P = 0.13, NS) or those given 2 days of exploration of the novel maze (Z = 0.2, P = 0.8). Neither was there a genotype effect at different stages of spatial learning: day 5 (Z = 0.6, P = 0.5, NS), day 10 (Z = 0.2, P = 0.8), and day 15 (Z = 0.2, P = 0.8).

Due to the lack of difference between WT and β2−/− mice, we pooled the 2 groups in order to check whether the c-fos expression in the prelimbic area was altered by spatial learning. We compared c-fos expression in the prelimbic area after the different learning stages using Fisher tests (Fig. 4B). Statistical analyses show that c-Fos expression is significantly greater in the prelimbic area of mice exploring the maze as compared with mice only subjected to food deprivation (P < 0.0001) and in mice in the first stage of learning (day 5) compared with mice in the second stage of learning (day 10, P = 0.03), indicating that exploration of the novel maze is associated with an increased activation of the prelimbic area.

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**Table 1**

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Note: Ptxc, prelimbic area of the prefrontal cortex.

***P < 0.0001, **P < 0.01, *P < 0.05.

**Table 2**

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Note: Ptxc, prelimbic area of the prefrontal cortex.

***P < 0.0001, **P < 0.01, *P < 0.05.
Our present results show that in WT mice, exploration of a novel environment triggers an increase in c-fos expression in the VTA, the NuAcc and the prelimbic area of the PFC, and the hippocampus. In β2−/− mice, the amygdala and the motor cortex were additionally activated.

**Discussion**

Our present results show that in WT mice, exploration of a novel environment triggers an increase in c-fos expression in the VTA, the NuAcc and the prelimbic area of the PFC, and the hippocampus. In β2−/− mice, the amygdala and the motor cortex were additionally activated.

**Cerebral Activation during Exploration of a Novel Environment**

The implication of the hippocampus in spatial exploration and learning has been recognized in rodents for decades (review in Poucet and Save 2005), with place cell firing in area CA1 of the hippocampus encoding spatial features needed for the memory...
of a particular location (e.g., Cressant et al. 2002) and early gene expression imaging its activation in different phases of spatial memory (e.g., Jones et al. 2001; Armin et al. 2006; Renaudineau et al. 2009). A similar form of activation has also been shown in humans (Wittman et al. 2007). Field potential and functional magnetic resonance imaging (fMRI) studies in human and nonhuman primates reported hippocampal activation triggered by the presentation of novel items compared with familiar items (review in Ranganath and Rainer 2003).

Our results showed similar activation of the hippocampus after open field exploration in WT and β2−/− mice, in agreement with the fact that β2−/− mice show normal habituation in spatial exploration (Granon et al. 2003; Wiklund et al. 2008) but do not exhibit any spatial learning deficit or hippocampal-like dysfunction until they age (Zoli et al. 1999).

The involvement of the different regions of the reward circuit is more puzzling. Some previous works have suggested that novelty would be rewarding and would constitute the basis for novelty seeking, with novelty acting as an exploration bonus (Krebs et al. 2009), possibly as novelty has a high biological valence (Mesulam 1998), and is a natural potent feature of external stimuli (Tulving et al. 1996). Recent fMRI data in the human brain have implicated the substantia nigra/VTA system (Wittman et al. 2007; Krebs et al. 2009), the NuAcc (Krebs et al. 2009), and the PFC (Daffner et al. 2000; Ranganath and Rainer 2003) in novelty exploration. However, there is little evidence in rodent models that suggests specific brain regions underlying the association between novelty exploration and reward (Blevins and Bardo 1999; Dulawa et al. 1999). Here, we provide new data giving evidence that exploration of a novel environment is sufficient to activate major components of the reward circuit (the VTA, the NuAcc, and the prelimbic area of the PFC). In addition, we recently showed, in a different behavioral context, that exploration of a novel object also activates the same brain areas (Avale et al. 2011). Altogether, the reward circuit seems to be particularly sensitive to novelty.

It is noteworthy that β2−/− mice show similar activation of brain regions to WT mice with 2 notable exceptions: 1) in β2−/− mice there is significant activation in the amygdala and motor cortex following bouts of exploration compared with those not exposed to the open field and 2) activation of the prelimbic region is significantly increased in β2−/− mice compared with WT mice. The elevated c-fos expression observed here in the prelimbic cortical field of the WT mice might be tentatively related to the significantly higher density of spines, bearing mostly excitatory synapses, recently observed in this same field (Ballesteros-Yañéz et al. 2010). In the β2−/− mice, this density of spines is significantly reduced (Ballesteros-Yañéz et al. 2010), while the expression of c-fos is significantly increased, in the same field, as observed in the present study. We hypothesize that this cognitive field would have a higher physiological activity, as a result of the more active inputs from motor cortex and amygdala as observed here, and/or a reduced inhibition.

Figure 4. Effects of a spatial learning in WT and β2−/− mice. (A) Performance of WT and β2−/− mice after 5, 10, and 15 days of learning. (B) Density of c-Fos+ immunoreactive nuclei in WT mice (white bars) and in β2−/− mice (black bars) after different stages of learning. Ordinate axis is the density of c-Fos+ nuclei expressed per square millimeter of brain tissue section. Mean values are given ± standard error of the mean. * Indicates a significant difference between 2 learning stages or between groups (P < 0.05).
dorsolateral PFC in subjects for whom a cognitive task requires higher attentional control (Cazalis et al. 2003).

Another interesting piece of data are that the activation of the prefrontal region is positively correlated with that of the NuAcc, M1, the hippocampus, and the amygdala only in β2-/- mice after novelty exploration (see Table 1). Such pattern was not found in WT mice for any behavioral conditions (data not shown). In β2-/- mice, these correlations were not found after exploration of a familiar environment (data not shown). It is noticeable, however, that the correlations between the prefrontal area and the hippocampus and the amygdala were already significant in the baseline condition (Table 2).

There are established functional relationships between these brain areas for the integration of motivational, memory, and emotional information (O’Donnell and Grace 1995; Larochelle et al. 2000; Sesack et al. 2003). Specifically, data showing c-fos activation indicate functional interactions between the PFC and the NuAcc that would change as a function of the motivational state of the animals (Moscarello et al. 2007). It is interesting that these brain areas were activated in a correlational manner in β2-/- mice exposed to novelty, as these mice have been shown to exhibit altered motivational states (Picciotto et al. 1998; Maskos et al. 2005; Besson et al. 2007; Avale et al. 2011) and increased response to novelty compared with WT mice (Granon et al. 2003).

The absence of β2*nAChRs, which behaviorally triggers increased locomotor response to novelty, may thus alter homeostatic regulation within the emotional and motivational circuits, suggesting that these receptors are important in returning activity in these circuits to a steady state.

Cerebral Activation during Exploration of a Familiar Environment
In contrast to familiar stimuli or context, novelty engages a high level of attentional processing. These attentional processes also rely on prefrontal activation, shown in both humans and rodents (review in Kehagia et al. 2010). Activity in cholinergic and noradrenergic neurons that project to the PFC is sensitive to novelty, indicating that these neuromodulatory systems are crucial for orienting attention to and enhancing memory for novel stimuli (Sara et al. 1995; Yu and Dayan 2005). Studies in human and nonhuman primates have shown that familiarity results in a reduction in the population-level activity (Ranganath and Rainer 2003); in our experiment, however, activation triggered by exploration of a novel context did not decrease when the context became familiar. Regarding the role of attentional mechanisms in novelty processing as well as in contextual memory, activation of the PFC in our experiments may reflect attentional processing. Finally, it is likely that mice maintain a high level of attention during each exploratory session, independent of its level of familiarity, which may not be the case in human subjects during laboratory experiments. Alternatively, it could also reflect the triggering of memory processes by activation of the same brain circuits as the ones involved during the first exposure to the same environment.

β2-/- Mice Showed Significant Activation in the Amygdala and the Motor Cortex as Compared with the Control
It is noticeable that in baseline conditions—no open field or no maze exploration—β2-/- mice showed no difference to WT mice for c-fos expression in any measured brain region. However, lacking β2*nAChRs would provoke the recruitment of the reward mesocorticolimbic circuit and additional brain regions such as the amygdala and motor cortex. The mechanisms of the crucial involvement of the nicotinic receptors in the amygdala-prefrontal circuit, in relation to the reward circuit, should be further investigated. It may help understanding of the neural and molecular bases of the relationship between novelty seeking and reward on one hand and of novelty seeking and emotion on the other. Such relationship may be crucial for understanding dysfunction that gives rise to pathological states during which the processing of emotional or motivational stimuli is altered, for example, in depression, schizophrenia, posttraumatic stress disorder, or addiction (Hains and Arnsten 2009; Koob 2009).

Cerebral Activation during Free Exploration versus Constrained Learning: The Prefrontal Activation Is Significantly Increased in β2-/- Mice as Compared with Control Mice
There was no difference between WT and β2-/- mice in expression of c-fos in the prefrontal region after spatial learning, at any stage, although the performance differed. This suggests that the role of nicotinic receptors in the PFC is specific to the type of novelty processes engaged in open field free exploration. It would be triggered by flexible behaviors engaged by open choice, such as those available in an open space with no explicit goal to be reached, as in the case of the open field experiment. In a maze task driven by food motivation, flexible behaviors are less required and exploration can be viewed as being more constrained. Recent electrophysiological recordings in the prefrontal PFC of rats showed neuronal firing "before" choices were made, suggesting the involvement of the prefrontal area of the PFC in prospective behaviors (de Saint Blanquat et al. 2010). These results matched our own showing the recruitment of the prefrontal area when choices are open. Similarly, we show here the role of the prefrontal area in the beginning of the maze learning task, but this does not require β2*nAChRs. It is also a phase during which attentional processes, known to be dependent on the prefrontal/prelimbic activity (Granon et al. 1996, 2000), are triggered. This dichotomy between the implication of β2*nAChRs during the self-organization of behavior in open field exploration versus their not being required during exploration of a more constrained environment suggests a specific role of the β2*nAChRs in flexible choices.

These results support the view that β2*nAChRs modulate the dialogue between the PFC, which provides a cognitive control of choices, and the reward, emotional and memory systems, which provide cues for optimal motivational ranking.

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