BRIEF REPORT

Abnormal Social Behavior in Nicotinic Acetylcholine Receptor $\beta 4$ Subunit–Null Mice

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ABSTRACT

Introduction: Tobacco addiction has a strong social component. Therefore, nicotinic acetylcholine receptors (nAChR) may influence social behavior. Because the $\beta 4$ nicotinic receptor subunit is important for possibly related behaviors, such as anxiety-like behavior and the effects of nicotine, we studied the social behavior of mice null for the $\beta 4$ nAChR subunit.

Methods: To measure social behavior, we used the intruder test for social memory in wild-type and littermate $\beta 4$ null mice. In addition, we used a nonsocial olfactory memory test as a control.

Results: In the intruder test, $\beta 4$ null mice showed social amnesia: Wild-type mice spent less time actively interacting with a younger intruder on Day 2 than on Day 1, but $\beta 4$ null mice interacted for a similar time on both days. In the nonsocial olfactory memory test, control littermates and $\beta 4$ null mice learnt the associations to a similar extent, showing that the amnesic phenotype in the intruder test is specific for social settings.

Conclusions: We conclude that nAChRs that contain the $\beta 4$ subunit are important for social behaviors. As those receptors are necessary to observe several effects of nicotine including withdrawal, it is tempting to speculate that the social component of tobacco use is related to the same neuronal circuits responsible for continuing tobacco use in smokers.

INTRODUCTION

The addictive properties of nicotine are believed to be mediated by neuronal nicotinic acetylcholine receptors (nAChRs) in the central nervous system. These are pentameric ligand-gated cation channels that upon acetylcholine or nicotine binding permeate cations and may subsequently become desensitized (Dani & De Biasi, 2001). Neuronal nAChRs are involved in several processes, including memory (Levin & Simon, 1998), anxiety (File, Cheeta, & Kenny, 2000; Ross et al., 2000), antinociception (Marubio et al., 1999), and autonomic nervous system function (De Biasi, Nigro, & Xu, 2000; Xu, Gelber, et al., 1999; Xu, Orr-Urtreger, et al., 1999). Nicotinic receptors may also be involved in neurological disorders such as nocturnal epilepsy, schizophrenia, Parkinson's disease, and Alzheimer's disease (Dani & De Biasi, 2001; Leonard et al., 2001; Levin & Simon, 1998; Rusted, Newhouse, & Levin, 2000).

The $\beta 4$ subunit gene is found in mouse chromosome 9 (syntenic to human chromosome 15q25) in a gene cluster that also comprises the $\alpha 5$ and the $\alpha 3$ subunits. In the mouse brain, the expression pattern of those nAChR subunits is restricted to few regions and is strongly overlapping. Areas of expression include the medial habenula, the interpeduncular nucleus, the ventral tegmental area ($\alpha 3$ and $\alpha 5$), the olfactory bulb ($\alpha 3$ and

$\beta 4$), and other few areas (Salas, Cook, Bassetto, & De Biasi, 2004; Salas, Pieri, Fung, Dani, & De Biasi, 2003). The interest in the physiological and pathological functions of these subunits has recently increased due to the results of genome-wide association studies (GWAS) in humans. In those studies, single nucleotide polymorphisms in $\alpha 3$, $\alpha 5$, and $\beta 4$ have been repeatedly linked to tobacco use and addiction, susceptibility to lung cancer, and addiction to other drugs of abuse (Amos et al., 2008; Berrettini et al., 2008; Bierut et al., 2008; Chen et al., 2011; Thorgeirsson et al., 2008; Wang et al., 2009).

Our lab showed that $\beta 4$ null mice display a strong phenotype that includes lower anxiety-like behavior (Salas et al., 2003), decreased response to the effects of acute nicotine (Kedmi, Beaudet, & Orr-Urtreger, 2004; Salas, Cook, Bassetto, & De Biasi, 2004), and absent nicotine withdrawal somatic signs (Salas, Pieri, & De Biasi, 2004). Interestingly, during our investigations on the anxiolytic-like effect of this mutation, we found that social isolation was more anxiogenic-like in $\beta 4^{-/-}$ mice than in wild-type littermates, at least as measured by increases in heart rate recorded by telemetry (Salas et al., 2003). Based on that finding, we decided to study the social behavior of these mice in more detail. We performed an experiment that measures social memory: the intruder test, in which an adult “resident” mouse is presented with a juvenile “intruder” mouse and active

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social interaction is measured. We show here that $\beta 4$ null mice are impaired in such test of social behavior, implicating nAChRs containing this subunit in this critical behavior. As a control for nonsocial olfaction and memory, we performed an experiment in which mice learnt to look for chocolate buried under sand mixed with different scents. In this control experiment, no differences were found between wild-type and $\beta 4$ null mice, which suggests that the observed phenotype is social and not a general memory or olfactory problem.

METHODS

Animals

Female and male 2- to 5-month-old wild-type mice and littermates lacking the $\beta 4$ subunit of nAChRs (Xu, Orr-Urtreger, et al., 1999) were studied. Intruder mice were wild-type mice 22–30 days old of the same sex as the resident mouse. Mice were backcrossed into the C57BL/6J background for 6–9 generations. Animals were housed in groups of 2–5 under a 12-hr light/dark cycle, with access to food and water *ad libitum*, with experiments performed during the light phase. The experimenters were blind to the genotypes until data were gathered and analyzed. All procedures were approved by the Baylor College of Medicine Animal Care and Use Committee and followed the guidelines for animal intramural research from the National Institutes of Health.

Intruder Test

The intruder test was performed on adult male and female mice ($N_{+/+} = 18$; $N_{-/-} = 21$) isolated into a clean cage for at least 1 hr. After the acclimation period, a juvenile mouse (intruder, 22–30 days old, wild type) was introduced into the cage, and active interaction was manually recorded for 2 min (Day 1). Interaction was defined as any behavior initiated by the resident mouse directed toward the intruder, such as sniffing or close following. After the 2-min period, the intruder was removed and the resident mouse stayed isolated until the experiment was completed for all the mice in its cage, at which moment all mice from that cage would return to the home cage. The following day (Day 2), the procedure was repeated with the same pairs of mice as on Day 1. On Day 3, the process was repeated again, but this time the intruder mouse was a new juvenile who had no previous contact with the resident.

Nonsocial Olfactory Memory Test

Male and female mice ($N_{+/+} = 11$ [7 male, 4 female], $N_{-/-} = 12$ [4 male, 8 female]) were trained to find small pieces of chocolate buried under sand mixed with a scent (0.1% of garlic, onion, paprika, cinnamon, ginger, pepper, coriander, or nutmeg). On Day 1, mice were presented thrice with small pieces of chocolate in a soda cap with sand (chocolate both on top and buried), which were placed in a regular mouse cage with no bedding. On Days 3 and 4, they were presented with two soda caps, one with and one without buried chocolate. Day 5 was as Days 3 and 4, but two scents (garlic and onion) that were not going to be used again were mixed with the sand. Finally, mice were trained on three pairs of scents: pepper/paprika, ginger/coriander, and cinnamon/nutmeg. Pepper, ginger, and nutmeg always predicted chocolate. This happened 9 times a day (thrice for each pair of scents) for 5 days. The experimenter recorded the first cap that the mouse dug searching for chocolate.

Statistical Analyses

For the intruder test, we performed ANOVA (days, three levels; genotype, two levels) followed by LSD post-hoc tests. For the nonsocial olfactory memory test, we used ANOVA with repeated measures (days, five levels; genotype, two levels). In both cases, we used a value of $p < 0.05$ as statistical significance.

RESULTS

$\beta 4$ Null Mice Show Social Amnesia

As shown in Figure 1, wild-type mice displayed the expected behavior in the intruder test: a high interaction time on Day 1, followed by a marked decrease in interaction on Day 2. On Day 3, the time of interaction increased again to basal levels because of the introduction of a new intruder. In contrast, in $\beta 4$ null mice, despite normal behavior on Days 1 and 3, a decrease in interaction time was not observed on Day 2. On ANOVA, we found effects of day ($F(2,37) = 12.2$, $p < 0.001$), genotype ($F(2,37) = 14.6$, $p < 0.00001$), and genotype $\times$ day interaction ($F(3, 36) = 4.8$, $p < 0.01$). Post-hoc LSD test gave a value of $p < 0.001$ for genotype. No differences were observed among sex. In mice, this phenotype has been termed “social amnesia,” and it is a landmark of mutations that severely affect social behavior (Ferguson et al., 2000).

$\beta 4$ Null Mice Show Normal Nonsocial Olfactory Memory

Wild-type control and $\beta 4$ null mice were studied in an olfactory-dependent memory paradigm (Rondi-Reig, Libbey, Eichenbaum, & Tonegawa, 2001). In this experiment, mice learn the association between a particular scent and the likelihood of finding chocolate buried under the sand mixed with that scent. After a training period, mice are presented with two choices: two soda caps filled with scented sand, of which only one contains buried chocolate. The first scratched cap is recorded as the choice. Mice were presented with choices 9 times a day for 5 days. At Day 1, random choices are expected, and approximately 50% correct choices were recorded for both genotypes. At Day 5, mice have learned the scent–chocolate pairings and both genotypes showed correct choices close to 90%. As shown in Figure 2, both wild-type and $\beta 4$ null mice learnt these associations to a similar extent, suggesting that the $\beta 4$ nAChR subunit null mutation affects neither olfaction nor nonsocial memory. Using ANOVA, we obtained $F(2,21) = 0.78$, $p > 0.1$ for genotype; $F(4, 84) = 22.3$, $p < 0.0001$ for day; and $F(4,84) = 1.95$, $p > 0.1$ for interaction.

DISCUSSION

We show here that mice null for the $\beta 4$ subunit of the nAChR display an abnormal social phenotype that can be hypothesized to be “social amnesia.” In addition, we show that in a nonsocial olfactory memory experiment, these mice perform as wild-type mice, which suggests that the observed phenotype may not reflect a general defect in memory or olfaction.

Figure 1. $\beta 4^{-/-}$ mice show impaired social memory in the intruder test. On Day 1, adult $\beta 4^{-/-}$ mice and wild-type control littermates were single housed for at least 1 hr and exposed to a younger wild-type intruder. Time of active interaction was manually measured for 2 min, after which the intruder was removed. On Day 2, the same procedure was repeated. On Day 3, the same procedure was repeated with a different, unfamiliar intruder. * $p < 0.05$ versus Day 1 in same genotype (ANOVA).

Figure 2. $\beta 4^{-/-}$ mice show normal memory in a nonsocial olfactory memory test. After training to dig under soda caps for small pieces of chocolate, mice were exposed to pairs of soda caps filled with scented sand. In each pair, one scent predicted chocolate. By Day 5, mice had learnt the scents that predict chocolate at about 80%, both in $\beta 4^{+/+}$ and in $\beta 4^{-/-}$ littermates. No differences were observed between genotypes (ANOVA).

The molecular and neural mechanisms of social behavior are far from being well understood. Several mutant mouse studies have shed light on possible genes participating in this important behavior. Among others, oxytocin and its receptor (Ferguson et al., 2000; Lee, Caldwell, Macbeth, Tolu, & Young, 2008), the arginine vasopressin system (Bielsky, Hu, Szegda, Westphal, & Young, 2004), and genes in the serotonergic pathway (Millan et al., 2004) seem to be key players. $\beta 2$ -containing nAChRs in the prefrontal cortex (PFC) have also been shown to be involved in social behaviors (Avale et al., 2011), and our data add nAChRs that contain the $\beta 4$ subunit to the cadre of influencing factors. Our results speak to the complexity of both social behavior and the nicotinic system. In fact, the relationship between the $\beta 4$ subunit and PFC function is indirect: There are no $\beta 4$ receptors in the PFC, and any influence of $\beta 4$ -containing receptors on PFC is probably mediated by other brain areas.

The pattern of expression of $\beta 4$ nAChR subunit mRNA is highly restricted in the mouse brain: Very high levels are found only in the pineal gland, medial habenula, mitral cells of the olfactory bulb, and dorsal motor nucleus of the vagus. Intermediate levels are found in several other areas, such as the interpeduncular nucleus, the superior colliculus, and the locus

coeruleus (Salas et al., 2003). Our data provide clues on possible brain regions where social behaviors may be computed. A region potentially responsible for the $\beta 4$ null phenotype is the olfactory bulb, as this brain area has been shown before to be involved in social behaviors (Sanchez-Andrade, James, & Kendrick, 2005). The medial habenula and interpeduncular nucleus might also be important for these behaviors. As already mentioned, the IPN expresses high levels of $\beta 4$ (Salas et al., 2003) and, interestingly, is monosynaptically connected with the olfactory bulb (Mameli et al., 2003).

The $\beta 4$ subunit gene belongs to a cluster that also contains the $\alpha 3$ and $\alpha 5$ nAChR subunits. These three subunits had not received much scientific attention until recently when a number of GWAS linked them to tobacco use, lung cancer, peripheral disease, and alcohol abuse (Amos et al., 2008; Berrettini et al., 2008; Bierut et al., 2008; Chen et al., 2011; Thorgeirsson et al., 2008; Wang et al., 2009). It is therefore possible that SNPs in these subunits also affect social behavior, which should be studied in humans. In fact, tobacco smoke and addiction in general have a strong social component, and the $\beta 4$ subunit, perhaps together with the $\alpha 5$ and $\alpha 3$ subunits with which it tends to form functional channels, might influence both addiction and social behavior.

In conclusion, we have shown that nAChRs containing the $\beta 4$ subunit are necessary for normal social behavior. Further work is needed to study the implications of this observation both in rodents and humans.

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DECLARATION OF INTERESTS

The authors have no competing interests to declare.

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