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Chrna7 genotype is linked with alpha7 nicotinic receptor expression but not alpha7 RNA levels

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Abstract,¹

Studies using the radio-labeled nicotinic receptor antagonist [¹²⁵I]- α -bungarotoxin, which binds to $\alpha 7$ subunit containing nicotinic receptors, have demonstrated that mouse strains vary considerably in the number of $\alpha 7$ -containing nicotinic receptors in brain. In addition, brain region specific differences in α -bungarotoxin binding between the mouse strains C3H/Ibg and DBA/2 have been linked to polymorphisms in *Chrna7*, the gene that encodes the $\alpha 7$ subunit. In the studies described here, we evaluated whether the relationship between *Chrna7* genotype and individual differences in α -bungarotoxin binding levels in adult brain might be due to an effect of *Chrna7* genotype on $\alpha 7$ RNA levels. Quantitative autoradiography of coronal brain slices from F2 mice derived from the parental strains C3H/Ibg and DBA/2 demonstrate that *Chrna7* genotype is not linked to $\alpha 7$ RNA levels. In contrast, quantitative autoradiography confirmed the linkage of *Chrna7* genotype with α -bungarotoxin binding levels in hippocampus, striatum, and more precisely defined areas within these brain regions where *Chrna7* genotype is associated with the level of α -bungarotoxin binding. The fact that *Chrna7* genotype is linked to individual differences in α -bungarotoxin binding, but not $\alpha 7$ RNA levels, suggests that the observed linkage between *Chrna7* genotype and α -bungarotoxin levels may be due to genetic influences on the post-transcriptional regulation of $\alpha 7$ nicotinic receptor expression.

Keywords

nicotinic receptor; genetics; mice; gene expression; post-transcriptional

Introduction, 2

Neuronal nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated cation channels that are expressed throughout the central and peripheral nervous systems. To date, eleven neuronal nAChR subunits ($\alpha 2$ – $\alpha 7$, $\alpha 9$, $\alpha 10$, and $\beta 2$ – $\beta 4$) have been identified in mammals (Gotti et al. 2006). One of the most abundant nAChR subunits in brain is the $\alpha 7$ subunit. This subunit has been shown to form homomeric nAChRs that bind the snake toxin α -bungarotoxin

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(α -BTX) with high affinity (Couturier et al. 1990;Seguela et al. 1993;Chen and Patrick 1997;Orr-Urtreger et al. 1997;Drisdel and Green 2000).

Previous studies in rodents have demonstrated that there is a significant relationship between levels of α -BTX binding in hippocampus and several behavioral measures which include sensitivity to nicotine-induced seizures, auditory gating, and pre-pulse inhibition of the startle reflex (Miner et al. 1985;Miner and Collins 1989;Stevens et al. 1996;Stevens et al. 2001a). Animals with lower levels of α -BTX binding in hippocampus are less sensitive to nicotine-induced seizures and exhibit reduced auditory gating and pre-pulse inhibition of the startle reflex relative to animals with higher levels of α -BTX binding. In addition, a recent report has demonstrated that significant reductions in $\alpha 7$ nAChRs in mouse brain lead to substantial alterations in hippocampal circuit function (Adams et al. 2008). Another recent study also has shown that elevating the expression of $\alpha 7$ in the hippocampus improves learning and increases the phosphorylation of tau (Ren et al. 2007). In humans, altered expression of $\alpha 7$ nAChRs is associated with neuropsychiatric conditions including schizophrenia (Freedman et al. 1995;Guan et al. 1999) and Alzheimer disease (Wevers et al. 1999;Hellstrom-Lindahl et al. 1999;Yu et al. 2005). Since individual differences in levels of α -BTX correlate with many behavioral, physiological, and pathological measures, it is important to understand the molecular mechanisms that regulate the expression of the $\alpha 7$ subunit.

Previous studies have reported that the mouse strains C3H/Ibg and DBA/2 possess different alleles of the gene *Chrna7*, which encodes the nAChR $\alpha 7$ subunit. The strain-specific alleles of *Chrna7* have been linked to individual differences in the expression of $\alpha 7$ nAChRs in adult brain (Stitzel et al. 1996;Stitzel et al. 1998), nicotine-induced seizure sensitivity (Stitzel et al. 1998), and auditory gating (Stevens et al. 2001b). Furthermore, the C3H and DBA/2 alleles of *Chrna7* were found to be linked to adult differences in the neuroanatomical distribution of $\alpha 7$ nAChRs (Adams et al. 2001) as well as the temporal and spatial development of $\alpha 7$ nAChRs during embryonic development (Adams et al. 2006).

The molecular basis for the *Chrna7*-linked mouse strain-specific differences in $\alpha 7$ expression is not known. However, a recent study reported that the C3H *Chrna7* promoter drives higher levels of gene expression *in vitro* than does the DBA/2 *Chrna7* promoter (Mexal et al. 2007). This finding is consistent with the observation that the C3H allele of *Chrna7* is linked to higher expression levels of $\alpha 7$ nAChRs in hippocampus and colliculli of C3H xDBA/2 F2 intercross animals (Stitzel et al. 1996;Stitzel et al. 1998) and suggests that the strain differences in $\alpha 7$ expression may be due, in part, to differences in the transcription of $\alpha 7$ mRNA. Moreover, studies have shown that polymorphisms in the promoter for the human gene for $\alpha 7$, *CHRNA7*, are associated with deficits in auditory gating, a trait associated with schizophrenia and reduced expression of $\alpha 7$ nAChRs (Leonard et al. 2002).

In this report, $\alpha 7$ RNA levels and α -BTX binding levels were measured by quantitative autoradiography in discrete brain nuclei of F2 mice derived from a cross between C3H/Ibg and DBA/2 mice in order to evaluate whether the linkage between the genetic variants of *Chrna7* and the expression of $\alpha 7$ nAChRs in adult mice may be due, in part, to an effect of *Chrna7* genotype on $\alpha 7$ RNA expression.

Results, 3

Comparison of α -BTX binding levels by *Chrna7* genotype in discrete brain nuclei

Chrna7 genotype has been reproducibly found to associate with levels of α -BTX binding in crude homogenates from the hippocampus, colliculli and striatum in F2 mice from a C3H x DBA/2 cross (Stitzel et al. 1996;Stitzel et al. 1998;Adams et al. 2001). To more precisely define brain nuclei within these regions where *Chrna7* genotype is linked to α -BTX binding levels,

α -BTX binding was evaluated by quantitative autoradiography in coronal brain slices of C3H x DBA/2 F2 intercross mice that had been genotyped for *Chrna7* (Figure 1). In the hippocampus, *Chrna7* genotype was linked to α -BTX binding levels in all three areas of CA1 evaluated in both the anterior and posterior aspects of this brain region (Table 1). *Chrna7* genotype also was linked to α -BTX binding in the CA3 areas assessed, but only in the anterior area of the hippocampus. Consistent with the results from crude homogenates, the C3H allele of *Chrna7* was associated with higher levels of α -BTX binding. In contrast, *Chrna7* was not linked to α -BTX binding in the dentate gyrus in either the anterior or posterior areas of the hippocampus. In the striatum, *Chrna7* genotype was significantly linked to α -BTX levels in the caudate nucleus but not in the nucleus accumbens shell. Also in agreement with previous findings (Stitzel et al. 1996; Stitzel et al. 1998), the DBA/2 allele of *Chrna7* was linked to higher levels of α -BTX binding in this brain region. Previous studies have reported no linkage of *Chrna7* genotype with α -BTX binding in crude homogenates prepared from cortex and midbrain/thalamus. Similarly, *Chrna7* genotype was not linked to α -BTX binding levels in any cortical or thalamic nuclei in this study. There also was no significant linkage of *Chrna7* genotype with α -BTX binding in any of the nuclei of the colliculli. This finding is not consistent with previous results which indicated that the C3H allele of *Chrna7* is linked to higher α -BTX binding in this brain region. Lastly, a significant association between *Chrna7* genotype and levels of α -BTX binding was observed in the nigro-striatal bundle. This region was not previously shown to exhibit a *Chrna7*- α -BTX association.

Comparison of $\alpha 7$ RNA levels by *Chrna7* genotype in discrete brain nuclei

In order to determine whether *Chrna7* genotype also is linked to $\alpha 7$ RNA levels, quantitative autoradiography of $\alpha 7$ RNA levels was performed in coronal brain sections from the same F2 intercross mice (Figure 2). In all, 27 brain nuclei were evaluated (Table 2). A significant effect of $\alpha 7$ genotype on $\alpha 7$ RNA levels was observed in only one of the nuclei quantitated (anterior hippocampal hilus). These data indicate that the observed linkage of *Chrna7* genotype with the expression of $\alpha 7$ nAChRs is not due to an effect of *Chrna7* genotype on $\alpha 7$ RNA levels.

Comparison of $\alpha 7$ RNA levels in three brain regions from C3H and DBA/2 mice

To further address the relationship between the levels of $\alpha 7$ RNA and α -BTX binding, $\alpha 7$ RNA levels were measured by quantitative RT-PCR in hippocampus, striatum and cortex from C3H and DBA/2 mice. Previous studies have demonstrated that relative to DBA/2 mice, C3H mice possess higher levels of α -BTX binding in the hippocampus, lower levels in the striatum and the same levels in cortex (Miner et al. 1986; Miner and Collins 1989; Stitzel et al. 1996; Stitzel et al. 1998). A preliminary report also indicated that there may be C3H/DBA/2 $\alpha 7$ RNA level differences in the hippocampus (Stevens et al. 1996). However, as shown in Figure 3, there were no significant differences in $\alpha 7$ RNA levels for any of these brain regions between C3H and DBA/2 mice. These data are consistent with the autoradiographic data and indicate that the variation in the expression of $\alpha 7$ nAChRs in adult brain between these two strains is not likely the result of differences in $\alpha 7$ RNA levels.

Discussion, 4

Previous studies have shown that strain-specific variants of the nAChR subunit gene *Chrna7* are linked with levels of α -BTX binding in a brain region-specific manner (Stitzel et al. 1996; Stitzel et al. 1998). The results presented in this report confirm these findings as well as define more precisely the brain nuclei where *Chrna7* genotype is linked with levels of α -BTX binding. In hippocampus, *Chrna7* genotype correlated with α -BTX binding in many but not all nuclei examined. In accordance with previous results with whole hippocampal dissections, F2 animals homozygous for the C3H/Ibg variant of *Chrna7* had higher levels of α -BTX binding than F2 animals homozygous for the DBA/2 variant of *Chrna7*. The association between

Chrna7 genotype and receptor levels was also observed in the caudate putamen, the predominant brain nucleus that makes up the striatum in gross dissections. Once again, these data are in agreement with what has been reported in whole striatum (Stitzel et al. 1996; Stitzel et al. 1998); mice with the DBA/2 variant of *Chrna7* have more α -BTX binding in the striatum than those animals homozygous for the C3H/1bg variant of *Chrna7*. The DBA/2 allele of *Chrna7* was also found to be linked with higher levels of α -BTX binding in the nigrostriatal bundle, the neuronal pathway between the substantia nigra and the striatum. The linkage of *Chrna7* genotype with α -BTX in this brain region has not been reported previously and would not have been detected in crudely dissected tissue. Lastly, in agreement with previous reports, *Chrna7* genotype was not linked to α -BTX binding levels in any cortical or thalamic region examined.

In contrast to previous findings, no significant relationship was found between *Chrna7* genotype and α -BTX binding levels in nuclei from either superior or inferior colliculi. The lack of a significant relationship between genotype and receptor levels in the nuclei of the colliculi might relate to the fact that the effect of *Chrna7* genotype on α -TX binding levels was smallest for colliculi among the whole dissected regions. Thus, a significant relationship between *Chrna7* genotype and α -BTX binding levels in colliculi might not always be detected due to the small margin for error. Support for this possibility comes from the observation that there was an observed trend that C3H/1bg *Chrna7* homozygotes had higher, although statistically insignificant, levels of α -BTX binding in all four of the nuclei of the colliculi that were quantitated relative to their DBA/2J *Chrna7* homozygous counterparts.

The observation that *Chrna7* genotype significantly correlates with α -BTX binding levels in many, but not all, hippocampal nuclei suggests that there are different genotypic effects on α -BTX binding levels not only across brain regions, but also among the specific nuclei of a single brain region. In other words, different combinations of genes seem to be involved in regulating the levels of α -BTX binding both across and within regions.

The results reported here also indicate that the association between *Chrna7* genotype and α -BTX binding is not due to an effect of *Chrna7* genotype on $\alpha 7$ RNA levels. Only one of 27 brain nuclei exhibited a relationship between *Chrna7* genotype and $\alpha 7$ RNA levels. The finding that *Chrna7* genotype is not linked to $\alpha 7$ RNA levels contrasts with a recent study that reported that polymorphisms in the *Chrna7* promoter between C3H and DBA/2 influence gene expression in an *in vitro* reporter gene assay (Mexal et al. 2007). One interpretation of these apparently disparate findings is that the *in vitro* experiments do not accurately represent the *in vivo* effect of the polymorphisms. Although this possibility cannot be ruled out, there are other plausible explanations. For example, the *in vitro* reporter gene experiments indicated that the DBA/2 promoter region only reduced gene expression by 22% relative to the C3H *Chrna7* promoter region. Such a small difference in expression may be difficult to measure accurately via autoradiography or quantitative RT-PCR of whole cortex, hippocampus, or striatum. In fact, $\alpha 7$ RNA levels in every region of the hippocampus that was examined (except the anterior hilus) exhibited a trend of being lower ($15.4\% \pm 2.9$ lower on average) in animals homozygous for the DBA/2 allele of *Chrna7*. Therefore, a small effect of *Chrna7* genotype on $\alpha 7$ RNA levels should not be entirely excluded. Another plausible explanation for the disparity between the reporter gene assay and *in vivo* RNA levels is that the effect of the promoter polymorphism is exerted developmentally but not in adult tissue. Consistent with this possibility, previous studies have demonstrated that the spatial and temporal expression of α -BTX binding sites in the hippocampus during embryonic development differs between C3H and DBA/2 mice and that these differences are linked to the alleles of *Chrna7* (Adams 2003; Adams et al. 2006).

The observation that *Chrna7* genotype significantly influences $\alpha 7$ nAChR expression in adult brain with no, or at best, little effect on $\alpha 7$ RNA levels suggests that the influence of *Chrna7*

genotype on α -BTX binding levels in adult brain may be through a post-transcriptional mechanism. A post-transcriptional mechanism in which polymorphisms in the DBA/2 allele of *Chrna7* lead to reduced $\alpha 7$ receptor production per amount of $\alpha 7$ RNA would also explain the finding by Marks et al. (1996) that another mouse strain, ST/bJ, has, on average, significantly more α -BTX binding per amount of $\alpha 7$ RNA than do DBA/2 animals. Although the mechanism by which the variant alleles of *Chrna7* might affect the expression of $\alpha 7$ post-transcriptionally is not known, one possibility would be through strain-specific alterations in the sequence of the 3' UTR of *Chrna7*. The 3' UTR is known to be involved in several processes including mRNA stability, polyA tail addition and translational efficiency (Mazumder et al. 2003; Shyu et al. 2008; Bolognani and Perrone-Bizzozero 2008). Preliminary data also indicate that there are, in fact, multiple polymorphisms in the 3' UTR between the C3H and DBA/2 alleles of *Chrna7* (J. Stitzel, unpublished data). Experiments to assess whether the 3' UTR polymorphisms affect gene expression in vitro are in progress. Should these studies provide evidence that polymorphisms in the 3' UTR affect gene expression, they not only would provide a plausible mechanism by which the variant alleles of *Chrna7* affect the expression of the nAChR $\alpha 7$ subunit in adult brain but also would establish a role of the *Chrna7* 3' UTR in regulating the expression of the $\alpha 7$ subunit.

Finally, it remains possible that the linkage between *Chrna7* genotype and α -BTX binding is not due to a polymorphism or polymorphisms in *Chrna7*, but rather in a gene that is linked to *Chrna7*. Based upon data from congenic mouse strains in which we have exchanged the region of mouse chromosome 7 between C3H and DBA/2 mice that contains *Chrna7* (Adams 2003; Adams et al. 2006), we can narrow the chromosomal segment responsible for the linkage between *Chrna7* genotype and α -BTX binding levels to an approximately 10 cM region in the central region of mouse chromosome 7. This region contains about 300 genes. Studies to identify which of these genes are polymorphic between C3H and DBA/2 and thus warrant further investigation are currently underway. Regardless, the data reported here confirm that there is a gene or gene on mouse chromosome 7, possibly *Chrna7*, that significantly influence the expression of $\alpha 7$ nicotinic receptors in a manner that appears to be independent of $\alpha 7$ RNA levels.

Materials and methods, 5

Animals

C3H/Ibg and DBA/2J mice were maintained in a specific pathogen free colony at the Institute for Behavioral Genetics (IBG). F2 mice were derived from the F1 hybrids obtained by mating the parental C3H/Ibg and DBA/2J strains. Offspring of the matings were weaned at 25 days of age and housed with 1–4 same sex siblings. Female mice were used for all experiments. Mice were maintained on a 12 hour light/12 hour dark cycle (lights on 0700 h to 1900 h) and had free access to food (Teklad 22/5 rodent diet, Harlan, Madison, WI) and water.

Genotyping

Chrna7 genotype of each mouse was determined using Southern blot hybridization exactly as described previously (Stitzel et al. 1996; Stitzel et al. 1998).

Preparation of brain slices

Each mouse was killed by cervical dislocation and its brain was removed, quick-frozen in isopentane (-35°C) and stored at -70°C until sectioning. Brains were sectioned using a cryostatic microtome (IEC, Needham, MA) set at -14°C . Coronal sections (14 μm thick) were thaw mounted on glass microscope slides that had been treated with gelatin/chromium alum and poly-L-lysine. After sectioning, slides were stored at -70°C until use.

[¹²⁵I]-α-Bungarotoxin binding

The binding of [¹²⁵I]-α-BTX to tissue sections was achieved as described previously (Pauly et al. 1989). Sections were initially incubated for 30 minutes at 22°C in Tris/KRH buffer containing no toxin. The sections were subsequently transferred to buffer containing 1 nM [¹²⁵I]-α-BTX (GE Healthcare, Piscataway, NJ) and 0.01% bovine serum albumin and incubated for 4 hours at 22°C. Non-specific binding was determined in the presence of 1 mM L-nicotine. Following incubation, the sections were washed four times in ice-cold buffer (20 minutes per wash), once in 0.1x buffer for 20 seconds and once in distilled water for 20 seconds. The sections were then dried, desiccated, and stored overnight before apposition to film (β-Max Hyperfilm, GE Healthcare). Slides were placed in cassettes so that an approximately equal number of animals from each α7 genotype were included per cassette. Sample size was from 8–11 per *Chrna7* genotype. α-BTX tissue standards were also included in each film cassette. These standards were generated by mixing different amounts of [¹²⁵I]-α-BTX with tissue homogenates. Exact concentrations of α-BTX per mg tissue were determined in weighed aliquots. The tissue homogenates were subsequently frozen and sectioned into 14 μm slices and applied to slides. Films were developed after both a 1 week and 2 week exposure.

In situ hybridization of α7 RNA

On the day of hybridization, slides were incubated for 15 minutes in 4% paraformaldehyde in PBS (phosphate-buffered saline; 137 mM NaCl, 2.5 mM KCl, 16 mM Na₂HPO₄, 4 mM NaH₂PO₄, pH 7.4) to fix the tissue. The slides were subsequently washed for 5 minutes in PBS and air dried. The tissue sections were then acetylated by incubation in 15 mM acetic anhydride/0.1 M triethanolamine (pH 8) for 10 minutes. Slides were then rinsed for 2 minutes in 2x SSC and dehydrated through a graded ethanol series of 50%, 70%, 95%, 100%, and 100%. The slides were then dried and stored under vacuum until hybridization was initiated.

Radio-labeled ([³⁵S]-α-UTP, (PerkinElmer, Waltham, MA)) anti-sense cRNA probes were generated from a plasmid that contains the 5 prime (5') 558 bp of the mouse α7 cDNA as described elsewhere (Saragoza et al. 2003). The radio-labeled α7 cRNA (1 x 10⁷ cpm/ml) was added to a hybridization solution that consisted of 50% formamide, 10% dextran sulphate, 300 mM NaCl, 10 mM Tris (pH 8), 1 mM EDTA, 0.5x Denhardt's solution, 10 mM dithiothreitol (DTT), and 0.5 mg/ml tRNA. Hybridization was initiated by applying 100 μl of the hybridization solution to a 24 mm x 60 mm coverslip and subsequently placing the cover slips over the section-containing slides. The edges of the coverslips were sealed with DPX mountant (BDH Laboratory Supplies, England) and the samples were hybridized overnight at 58°C.

Following hybridization, the slides were washed at room temperature for 15 minutes in 4x SSC after removal of the DPX. The coverslips were then removed and the slides were washed four more times in 4x SSC (5 minutes each at room temperature) and transferred to a solution consisting of 500 mM NaCl, 10 mM Tris (pH 8), 1 mM EDTA, and 20 μg/ml of RNaseA and incubated for 30 minutes at 37°C. Sections were then washed at increasing stringencies to a final wash of 0.1x SSC at 60°C for 30 minutes. All washes contained 1 mM DTT to prevent oxidation of the probes. The slides were subsequently dehydrated by a graded ethanol series, air dried, and exposed to film (β-max Hyperfilm, GE Healthcare). A 3 day and 7 day exposure was performed for all sections. Approximately equal numbers of slides from animals of each α7 genotype were included in each film cassette. Sample sizes were 8–11 per *Chrna7* genotype (adjacent slides to those used for α-BTX autoradiography). ³⁵S standards generated by dot-blotting various dilutions of the radio-labeled α7 probe onto a nylon membrane were also included in each film cassette.

Quantitation of films

Films were quantitated using NIH Image version 1.61. Standard curves were established using the radioactive standards to ensure that exposures for both $\alpha 7$ hybridization and α -BTX binding were within the linear response range of the film. The long exposures for both $\alpha 7$ hybridization and α -BTX binding were used for quantification because preliminary analyses indicated that signals from all regions to be evaluated fell within the linear portion of the standard curve of the long exposures. Between three and six measurements were taken from each brain nucleus per animal and the measurements were averaged to provide a more reliable estimate of binding or hybridization for the given brain nucleus. For α -BTX binding, 32 brain nuclei were quantitated while 26 nuclei were quantitated for $\alpha 7$ hybridization. Gray level measurements provided by NIH Image were converted to fmol/mg (α -BTX binding) or fmol ($\alpha 7$ hybridization) by means of linear regression analysis using the curves generated by the [125 I]- α -BTX and [35 S]- $\alpha 7$ cRNA standards. Again, all regression analyses were conducted within the linear portion of the standard curves. Non-specific α -BTX binding, as measured by the presence of 1 mM nicotine in the binding assay, and non-specific RNA hybridization, as determined by hybridization with sense strand $\alpha 7$, did not differ from film background. Because the standard curves were constructed to take into account film background, no additional measures were necessary to eliminate non-specific binding. Experimenter was blind to *Chrna7* genotype during quantitative analysis.

Quantitative RT-PCR (QRT-PCR)

Single strand cDNA was synthesized from total RNA (2 μ g) using random hexamer primers and the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). To limit amplification from genomic DNA, primer sets for each gene were designed to cross intron/exon boundaries using the OligoToolkit program (<http://oligos.qiagen.com/oligos/toolkit.php>). Primer sequences: *Chrna7* Forward: 5'-GCAGATCATGGATGTGGATG-3', *Chrna7* Reverse: 5'-CAAGACGTTGGTGTGGAATG-3', *Gapdh* Forward: 5'-AACTTTGGCATTGTGGAAGG-3', *Gapdh* Reverse: 5'-CACATTGGGGGTAGGAACAC-3'. BLAST searches were done to confirm primer specificity. PCR amplification of cDNA was performed using 500 nM of primers and the Quantitect SYBR Green PCR Kit (Qiagen, Valencia, CA). PCR cycling conditions were 50 °C for 2 min, 95 °C for 15 min, and 40 cycles of 94 °C for 15 sec, 58 °C for 30 sec, and 72 °C for 30 sec. Formation of PCR products was monitored using an ABI Prism 7000 detection system (Applied Biosystems, Foster City, CA). Standard curves were generated by measuring relative mRNA transcript levels obtained with specific primer sets from pooled cDNA samples (one sample from each experimental group) at 10-fold serial dilutions (95 ng, 9.5 ng, and 0.95 ng). A similar standard curve was performed for the housekeeping gene GAPDH. All standard curves and sample assays were done in triplicate. Linear regression was performed by the ABI Prism 7000 SDS v1.1 software to extrapolate mRNA amounts in nanograms from the standard curve. The correlation coefficient was always >0.95. For each subject, values for the gene of interest were normalized to the GAPDH mRNA levels, which were not significantly different ($p > 0.05$) between mouse strains across brain regions (data not shown). For each experiment, samples without the addition of reverse transcriptase enzyme or the addition of cDNA were included to assess amplification from genomic DNA and nonspecific product formation, respectively. A melt curve analysis was also conducted to examine the uniformity of product formation, primer-dimer formation, and amplification of nonspecific products. Student's t-test was used to assess significant differences in $\alpha 7$ RNA levels between C3H and DBA/2 mice.

Statistics

A One-Way ANOVA of genotype versus either α -BTX binding or $\alpha 7$ RNA levels was used to evaluate the relationship between $\alpha 7$ genotype and either α -BTX binding levels or $\alpha 7$ RNA levels. Post hoc analysis was by means of Duncan's Multiple Range Test. The Student's t-test was used to compare α -BTX binding levels per $\alpha 7$ RNA level between animals homozygous for the strain-specific variants of the $\alpha 7$ subunit gene.

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Abbreviations

UTR	untranslated region
α-BTX	α -bungarotoxin
nAChR	nicotinic acetylcholine receptor

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Figure 1. [¹²⁵I]-α-bungarotoxin binding to coronal brain sections from C3H x DBA/2 F2 intercross mice. Representative sections indicating the quantitated regions are shown. Abbreviations: FR, frontal; CI, cingulate; PA, parietal; SO, stratum oriens; SR, stratum radiatum; SLM, stratum lacunosum moleculare; PO, polymorphic region. N = 8 per Chrna7 genotype

Figure 2.

In situ hybridization of an $\alpha 7$ anti-sense [^{35}S]-labeled cRNA probe to coronal brain sections from C3H x DBA/2 F2 intercross mice. Representative sections indicating the quantitated regions are shown. Abbreviations: FR, frontal; CI, cingulated, PA, parietal; PY, pyramidal. N = 8 per *Chrna7* genotype.

Figure 3.

Quantitate RT-PCR of $\alpha 7$ RNA levels in three brain regions of C3H and DBA/2 mice. Quantitative RT-PCR was performed on total RNA isolated from cortex, hippocampus and striatum to determine $\alpha 7$ RNA levels. Results indicated that there are no differences in $\alpha 7$ RNA levels between C3H and DBA/2 mice in any of these regions. Data shown are mean $\pm$ SEM. N = 6 per sample.

Table 1
Influence of Chrna7 Genotype on α -BTX Binding Levels

REGION	Chrna7 GENOTYPE			F ratio	F prob. *
	HH	HD	DD		
Neocortex					
cingulate	1.26 $\pm$.23	0.94 $\pm$.03	1.06 $\pm$.06	1.61	0.22
frontal	1.43 $\pm$.24	1.13 $\pm$.09	1.3 $\pm$.10	1.05	0.36
parietal	1.22 $\pm$.17	1.03 $\pm$.04	1.09 $\pm$.09	0.86	0.43
cortex (layers 1–4)	0.89 $\pm$.04	0.92 $\pm$.02	0.92 $\pm$.02	0.39	0.68
cortex (layers 5–6)	1.02 $\pm$.06	1.08 $\pm$.06	1.15 $\pm$.08		
Basal ganglia					
anterior caudate nucleus	1.52 $\pm$.21	2.01 $\pm$.20	2.38 $\pm$.21	4.07	0.03
nucleus accumbens, core	0.82 $\pm$.05	0.82 $\pm$.03	0.90 $\pm$.06	1.13	0.34
nucleus accumbens, shell	0.72 $\pm$.03	0.75 $\pm$.03	0.80 $\pm$.13	0.37	0.7
Septum	1.94 $\pm$.44	1.11 $\pm$.08	1.34 $\pm$.17	2.64	0.09
Epithalamus					
medial habenula	2.06 $\pm$.18	2.43 $\pm$.31	2.63 $\pm$.23	1.36	0.27
zona incerta	1.22 $\pm$.08	1.51 $\pm$.37	1.25 $\pm$.10	0.45	0.64
nigrostriatal bundle	6.06 $\pm$.42	7.44 $\pm$.52	9.94 $\pm$.94	7.41	0.0026
Thalamus					
ventromedial thal. nucleus	3.78 $\pm$.43	4.05 $\pm$.24	4.11 $\pm$.24	0.34	0.72
dorsal tegmental nucleus	22.2 $\pm$ 2.2	23.6 $\pm$ 2.1	20.1 $\pm$ 1.5	0.99	0.38
lateral geniculate	4.68 $\pm$.33	5.21 $\pm$.38	5.65 $\pm$.22	2.44	0.11
Rhinencephalon, Anterior					
CA1 sps anterior	2.64 $\pm$.19	1.91 $\pm$.19	1.29 $\pm$.10	18.48	0.00003
CA1 sr anterior	2.35 $\pm$.18	1.65 $\pm$.17	1.15 $\pm$.08	17.7	0.00003
CA1 slm anterior	4.62 $\pm$.28	3.49 $\pm$.25	2.94 $\pm$.18	13.81	0.0001
CA3 slu anterior	4.89 $\pm$.32	3.63 $\pm$.17	3.16 $\pm$.19	14.57	0.00003
CA3 so anterior	4.91 $\pm$.33	3.64 $\pm$.15	3.01 $\pm$.20	17.02	0.00003
DG po anterior	5.26 $\pm$.36	4.56 $\pm$.51	4.55 $\pm$.24	2.04	0.15
Rhinencephalon, Posterior					
CA1 sps posterior	4.00 $\pm$.45	2.29 $\pm$.24	2.23 $\pm$.34	7.66	0.002
CA1 sr posterior	4.00 $\pm$.46	2.31 $\pm$.23	2.47 $\pm$.45	4.87	0.015
CA1 slm posterior	6.53 $\pm$.69	4.52 $\pm$.33	4.83 $\pm$.26	5.89	0.007
CA3 slu posterior	6.65 $\pm$.66	5.01 $\pm$.45	5.80 $\pm$.32	2.87	0.07
CA3 so posterior	7.56 $\pm$.82	5.88 $\pm$.51	7.24 $\pm$.65	1.72	0.2
DG po posterior	7.55 $\pm$ 1.06	5.42 $\pm$.37	6.37 $\pm$.32	2.83	0.08
Mesencephalon					
superior colliculus	7.59 $\pm$.76	7.93 $\pm$.65	7.03 $\pm$.25	0.73	0.49
sup. coll., superficial grey	2.97 $\pm$.12	2.63 $\pm$.12	2.63 $\pm$.15	0.84	0.44
inferior colliculus, cortex	1.99 $\pm$.24	2.37 $\pm$.37	1.70 $\pm$.21	1.14	0.34
inferior colliculus, core	9.20 $\pm$.24	9.3 $\pm$.50	8.10 $\pm$.50	1.01	0.38

REGION	Chrna7 GENOTYPE			F ratio	F prob. *
	HH	HD	DD		
red nucleus	11.60±1.1	12.9±1.3	10.1±1.1	1.13	0.34

Data represented as mean ± SEM;

* Data analyzed by means of One-way ANOVA

Table 2Influence of *Chrna7* Genotype on $\alpha 7$ RNA levels

REGION	Chrna7 GENOTYPE			F ratio	F prob. *
	HH	HD	DD		
Neocortex					
cingulate cortex, area 1	.093 ± .009	.092 ± .008	.096 ± .007	0.08	0.93
cingulate cortex, area 2	.100 ± .01	.095 ± .011	.104 ± .009	0.21	0.81
frontal cortex area 1	.100 ± .01	.093 ± .008	.096 ± .007	0.15	0.86
frontal cortex area 2	.105 ± .012	.106 ± .01	.116 ± .01	0.33	0.72
parietal cortex, area 1	.102 ± .011	.096 ± .008	.098 ± .007	0.11	0.9
parietal cortex, area 2	.122 ± .013	.132 ± .019	.126 ± .014	0.1	0.91
Basal Ganglia					
caudate putamen	.101 ± .011	.098 ± .011	.103 ± .009	0.07	0.93
nucleus accumbens	.091 ± .009	.087 ± .008	.092 ± .007	0.1	0.91
nucleus accumbens, shell	.086 ± .006	.087 ± .007	.087 ± .006	0.01	1
Septum	.099 ± .012	.082 ± .008	.108 ± .013	1.07	0.36
Rhinencephalon, Anterior					
pyramidal cell layer, CA1	0.205 ± .03	0.204 ± .036	.176 ± .029	0.26	0.77
pyramidal cell layer, CA2	0.358 ± .048	.310 ± .059	.245 ± .047	1.18	0.32
pyramidal cell layer CA3	0.270 ± .041	.269 ± .049	.231 ± .034	0.26	0.77
dentate gyrus, granule cell	0.248 ± .037	.225 ± .039	.198 ± .031	0.48	0.62
hippocampal hilus	0.047 ± .008	.060 ± .003	.070 ± .005	4.02	0.03
Rhinencephalon, Posterior					
pyramidal cell layer, CA1	0.286 ± .048	.268 ± .050	.235 ± .044	0.31	0.74
pyramidal cell layer, CA2	0.326 ± .054	.328 ± .059	.277 ± .053	0.26	0.77
pyramidal cell layer CA3	0.377 ± .056	.425 ± .077	.374 ± .061	0.19	0.83
dentate gyrus, granule cell	0.280 ± .042	.263 ± .053	.262 ± .047	0.05	0.95
hippocampal hilus	0.073 ± .023	.072 ± .014	.060 ± .009	0.23	0.8
Amygdala	.233 ± .032	.350 ± .064	.338 ± .05	1.38	0.27
Mesencephalon					
superior Colliculi	.136 ± .018	.133 ± .021	.129 ± .016	0.04	0.96
sup Collic., superficial grey	.096 ± .012	.081 ± .009	.100 ± .014	0.65	0.53
inferior Colliculi, cortex	.120 ± .013	.132 ± .015	.120 ± .017	0.2	0.82
inferior Colliculi, core	.191 ± .026	.235 ± .032	.195 ± .029	0.69	0.51
red nucleus	.236 ± .025	.213 ± .033	.222 ± .034	0.12	0.89
dorsal thalamic nucleus	.093 ± .004	.088 ± .011	.106 ± .040	0.77	0.48

Data represented as mean ± SEM;

* Data analyzed by means of One-way ANOVA