α7 Nicotinic Receptor Mediation of CNS Inflammatory Response Examined by Magnetic Resonance Imaging and Bioluminescence Imaging

G. H. Turner1, J. Hao1, A. R. Simard1, J. Wu2, P. Whiteaker4, R. J. Lukas3, and F-D. Shi2
1Neuroimaging Research, Barrow Neurological Institute, Phoenix, AZ, United States, 2Neurology, Barrow Neurological Institute, Phoenix, AZ, United States, 3School of Medicine, Nankai University, Tianjin, China, People’s Republic of, 4Neurobiology, Barrow Neurological Institute, Phoenix, AZ, United States

Introduction
Nicotinic acetylcholine receptors (nAChRs) are members of a diverse family of ligand-gated ion channels that serve as targets for acetylcholine and nicotine [1]. They play critical roles throughout the brain and body by mediating cholinergic excitatory neurotransmission, modulating the release of neurotransmitters, and having longer-term effects on gene expression and cellular interactions [1]. In addition to their function in neuromuscular junctions and in neurons, studies have shown that many immune cell types express nAChR subunits and that binding of nicotine or acetylcholine to α7-nAChR leads to a suppression of inflammation [2-5]. Nicotine administration has been shown to attenuate inflammation in an experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis [6]. Although the involvement of α7-nAChR in CNS autoimmune disease has been suggested, the extent to which α7-nAChRs mediate the effect of nicotine on clinical and pathological hallmarks of EAE has not been explored. This study used a combination of in vivo MRI and bioluminescence imaging to examine the effect of nicotine on EAE in α7-nAChR knockout mice.

Methods
C57BL/6 wild-type (α7+/+) and nAChR α7 knockout mice (α7−/−) were injected with 200 µg of MOG35-55 peptide containing 500 µg of non-viable, desiccated Mycobacterium tuberculosis. On the day of and 2 days after immunization, the mice were inoculated with 200 ng of pertussis toxin intraperitoneally. Mice received 100 mg/ml nicotine (Nic) or PBS (n=6-8/group) for 28 days upon EAE induction through continuous infusion from osmotic minipumps (Durect Corp, Cupertino, CA). The resulting nicotine plasma levels (~40 ng/ml) were consistent with those in human smokers. Mice were monitored daily for symptoms and scored on the following scale with 0.5 increments: 0, no symptoms; 1, flaccid tail; 2, hindlimb weakness; 3, complete hindlimb paralysis; 4, complete hindlimb paralysis with forelimb weakness; 5, moribund or deceased.

In vivo MRI was performed on a 7 Tesla small-animal scanner (Bruker BioSpin, Billerica, MA). Coronal fat-suppressed T2-weighted images were acquired over the entire brain of each animal (RARE; TE1=14.5 ms, TE2=65.5 ms, TR=4500 ms, FOV=2.8 cm, eight averages, 40 coronal slices, scan time 28 minutes). For imaging of ROS generation in brain, bioluminescence images in live mice were captured with a 1 min acquisition time using a cooled IVIS imaging system (Xenogen IVIS-200, Alameda, CA) after injection of 27 mg/kg DHE (Molecular Probes, Eugene, OR).

Results
Differences in clinical scores are significant (p<0.05) at day 10 post-immunization between the α7+/+ group and the other groups, but not across both PBS groups and the α7−/− group (Figure 1, A). Visualization and quantification of brain inflammation by in vivo bioluminescence imaging at day 14 post-immunization and PBS/nicotine treatment reveals significant ROS differences between α7+/+ Nic and both PBS groups. There is only a moderate, non-significant, difference between the α7−/− Nic and PBS groups (Figure 1, B). T2 weighted periventricular images, obtained 14 days after immunization plus Nic/PBS treatment, are shown in Figure 1C. Arrows indicate focal lesions located around the lateral ventricles and increased signal intensity. Nicotine exposure diminished EAE-induced brain lesion volume in α7+/+ mice (p<0.01) but had only a partial effect in α7−/− (p=0.051).

Conclusion
The principal findings in this study are that although α7-nAChR deficiency prevents nicotine from protecting against clinical manifestations in EAE mice, many parameters relating to inflammatory and autoimmune response affected by nicotine exposure are only partially attenuated in α7−/− mice. These results indicate that cholinergic modulation of inflammation involves not only α7-nAChR alone but also likely involves several nAChR subtypes.

References