INTRODUCTION

Epigenetic mechanisms involve self-perpetuating changes in chromatin structure and function that have been shown to regulate neuronal differentiation, neurodegeneration, circadian rhythms, seizure, memory, drug addiction, and stress response. Chromatin contains regions highly condensed (heterochromatin), and regions less condensed (euchromatin). The different regions reflect distinct functional states: euchromatin is actively transcribed, whereas heterochromatin is usually transcriptionally inactive. Post-translational modifications of histone tail residues, such as acetylation or methylation, can remodel the structure of chromatin activating or repressing gene expression. Several lines of evidence suggest an important role for BDNF in the pathogenesis of anxiety and mood disorders. Moreover, a human polymorphism in the BDNF gene (Val66Met) that causes a Met5Val substitution in codon 66 of proBDNF has been associated with major susceptibility to cognitive deficits, neuropsychiatric and neurodegenerative diseases, eating and metabolic disorders. The BDNF Val66Met transgenic mouse is the only existing animal model that recapitulates the phenotypic hallmarks of the human polymorphism. Indeed, both human and mice BDNF<sub>Val</sub> alleles carry show reduced hippocampal volume and cognitive deficits [1,2]. Moreover, the human and the mouse BDNF Val66Met-carrier show alterations in the extinction of fear conditioning, a type of learning involved in phobia and post-traumatic stress disorder. The aim of this work was to study the impact of the BDNF Val66Met polymorphism on the epigenetic mechanisms that regulate expression of genes involved in synaptic plasticity, such as the transcriptional factors CREB and c-Fos, the neurotrophic factor BDNF and the NMDA receptor subunits. To analyse epigenetic modifications at the gene promoters, we employed the chromatin immunoprecipitation (ChIP) technique followed by amplification of immunoprecipitated DNA fragments by quantitative q-PCR (qChIP) and specific primers. Chromatin was immunoprecipitated with specific antibodies that recognize post-translational modifications in the H3 histone protein [anti-acetyl histone H3 (Lys9,14), marker of active gene transcription].

MATERIALS AND METHODS

Animals. BDNF Val66Met founder were kindly donated from Prof. Lee, Cornell University, New York. Three months old male BDNF<sub>Val</sub> and BDNF<sub>Met</sub> mice were sacrificed and hippocampi were dissected.

Chromatin Immunoprecipitation (ChIP). Chromatin was extracted in formaldehyde 1% to crosslink DNA to histone proteins, and then homogenized in Homogenization Buffer (PBS IX + 1% SDS). Chromatin was fragmented with 13 cycles of sonication (20” ON/40” OFF) and diluted ten-fold with Dilution Buffer (IX 1X Magenta ChIP, Upstate). Immunoprecipitation was carried out overnight at 4 C by using anti-acetyl histone H3 (Lys9,14) or anti-trimethyl histone H3 (Lys27) antibodies (both from Millipore) or IgG. Not immunoprecipitated chromatin (Input) was used to normalize qChIP data. Immunoprecipitated DNA was purified by spin columns (Magna ChIP, Millipore).

qChIP. Levels of acetylation in Lys9,14 and trimethylation in Lys27 at gene promoter was determined by quantitative real-time PCR. qPCR was performed on ABI7900HT thermocycler by gene promoter specific primers and SYBR green. 10 min at 95° C, 15 sec at 55° C and 1 min at 60° C for 40 cycles.

Real-time PCR. Total RNA was extracted from the hippocampus (RNeasy Mini kit, Qiagen) and single-stranded genomic DNA fragments of about 500 bp. It is possible to note the enrichment of DNA fragments with a length between 300 and 600 bp. Sonicated chromatin sample, lane 3: DNA Ladder 100 bp. It is possible to note the enrichment of DNA fragments with a length between 300 and 600 bp.