AMERICAN ASSOCIATION OF ORTHODONTISTS FOUNDATION

Report for 1996 award

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TITLE OF PROJECT ANALYSIS OF IMMEDIATE EARLY GENE EXPRESSION DURING ORTHODONTIC MOVEMENTS IN RATS
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The purpose of the study was to examine if the immediate early genes (IEGs) Egr-1, c-*jun* and c-*fos* are induced *in vivo* when an orthodontic force is applied to the maxillary molars of Sprague-Dawley rats.

Using a well characterized model for tooth movement (1) we examined the expression of *c-fos* during OTM using semi-quantative reverse transcription- polymerase chain reaction (RT-PCR). Briefly, seventy young male (40-50 day old) Sprague-Dawley rats were divided into seven groups corresponding to times 0, 3, 6, 12, 24 hours and 7 and 14 days, with five rats each in sham and OTM groups. From previous studies these time points best represent the biochemical changes being studied. OTM was achieved by using a NiTI coil to apply 40 grams of force between the maxillary first molar and incisor. One hemimaxilla was used for total RNA isolation. The RNA was extracted from the roots and surrounding alveolar bone using standards protocols. RT-PCR was used to follow induction of c-fos mRNA.
As shown in Figure 1, *c-fos* was expressed in both treated and sham groups and at all time points. *c-fos* expression was observed to be cyclic in nature (Figure 2). Within 3 hours of applying a 40-gram force, there was a 1.7 fold induction in *c-fos* mRNA expression when compared to its respective control ($p < 0.05$). The next significant induction of 1.9 fold was detected at 24 hours post force application. A final 1.5 fold induction was seen at 7 days post appliance activation. *c-fos* mRNA induction was not detected at the following time points 6 hours, 12 hours and 14 days.

1. King GJ, Keeling SD, McCoy EA, Ward TH. Measuring dental drift and orthodontic tooth movement in response to various initial forces in adult rats. Am J Orthod Dentofacial Orthop 1991;99**:**456-465.

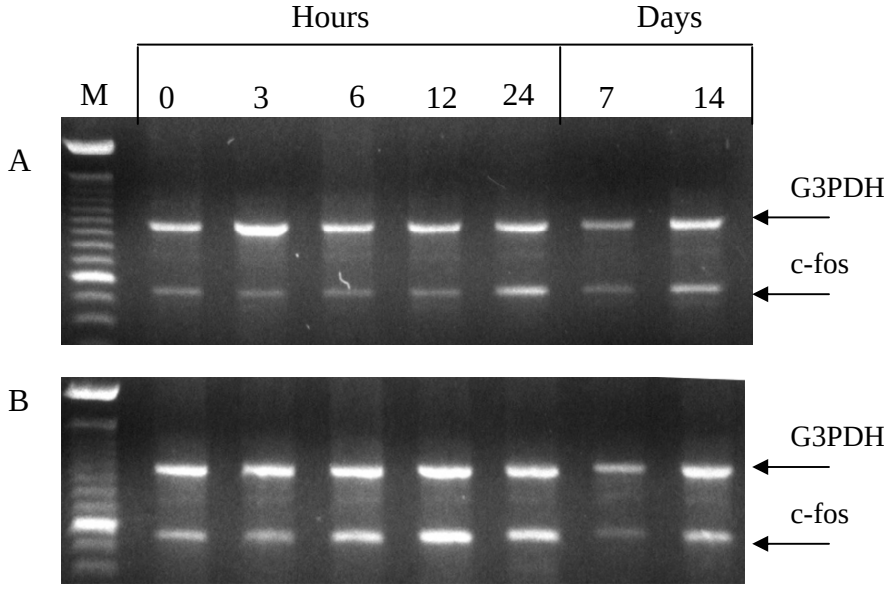

Figure 1. Representative gel of RT-PCR product for *c-fos* mRNA expression in alveolar bone (day 0) and at various time points following OTM (A) or sham treated (B). Glyceraldehyde-3 phosphate dehydrogenase (G3PDH) was the housekeeping gene. An anti-sense 21-base pair (5'-TCCTACTACCATTCCCCAGCC-3') probe complementary to mRNA encoding a specific region in the c-fos gene and its sense probe were synthesized and. used to amplify cDNA of c-fos. Equal amounts of RNA were reverse transcribed (RT) using the protocol in the Perkin Elmer GeneAmp RT-PCR Kit. The 20 ul RT reaction then underwent PCR using 1.25 U of Taq polymerase and primers for the genes in a 50 ul volume. G3PDH expression was utilized to serve as a control for variations between PCR reactions and allow the calculation of a normalized value for *c-fos* gene expression. Equal volumes of PCR products were electrophoresed in a 2.0% agarose gel, stained with ethidium bromide, visualized with an UV transilluminator, and scanned.

Figure 2. Relative mRNA expression of *c-fos* as determined by densitometry. Densitometric analysis of gel pictures utilizing a Hewlett-Packard desktop scanner with NIH Image (v1.57) software provided a determination of the intensity of the bands. For each sample at each time point the ratio of the integrated optical densities (IOD) of the *c-fos* band to G3PDH band was calculated. In the sham operated rats, at each time point, the ratio of *c-fos*/G3PDH was set to 1 and the expression of the *c-fos*/G3PDH in treated rats was expressed as a ratio of this value. Mean and standard deviation of the *c-fos*/G3PDH ratio for each group and at each time point was calculated. For comparison of two groups at the same time interval, the unpaired Student's *t*-test was used. p 0<05 was considered significant.