Accumulation of Highly Stable ΔFosB-Isoforms and Its Targets inside the Reward System of Chronic Drug Abusers - A Source of Dependence-Memory and High Relapse Rate?

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Factor kappa B; PBS: Phosphate Buffered Saline; PMI: Post Mortem Interval; SD: Standard Deviation

Parameter	Opioid Group (n=15)	Control Group (n=15)
Age (years): Mean/S		

Figure 2: Significant (Wilcoxon-Mann-Whitney U, two-sided test) positive immunoreactions of (A) ΔFosB (Pr> |Z| =<0.0001), (B) Ser27- ΔFosB (Pr> |Z| =<0.0001), (C) Cdk5 (Pr> |Z| =0.0089), (D) CREB (Pr> |Z| =0.0825), (E) BDNF (Pr> |Z| =0.0003), (F) NFκB (Pr> |Z| =0.0240), (G) JunD (Pr> |Z| =<0.0001) proteins in the HPC of chronic drug addicts in comparison to non-drug users. Description of Box-Whisker-Plot: diamond indicates sample mean, box indicates the range from 25 and 75 percentiles, whiskers are bounded at 1.5 times the interquartile range and dots represent values outside the range of whiskers.

Figure 3: Significant (Wilcoxon-Mann-Whitney U, two-sided test) positive immunoreactions of (A) ΔFosB (Pr> |Z| =<0.0001), (B) Ser27- ΔFosB (Pr> |Z| =<0.0001), (C) Cdk5 (Pr> |Z| =0.0006), (D) CREB (Pr> |Z| =0.0095), (E) BDNF (Pr> |Z| =0.0092), (F) NFκB (Pr> |Z| =0.0077), (G) JunD (Pr> |Z| =0.0016) proteins in the NAc of chronic drug addicts in comparison to non-drug users. Description of Box-Whisker-Plot: diamond indicates sample mean, box indicates the range from 25 and 75 percentiles, whiskers are bounded at 1.5 times the interquartile range and dots represent values outside the range of whiskers.

Figure 4: Positive immunoreaction: brown nuclear staining patterns of ΔFosB of the medium spiny neurons (MSN) of NAc of a long-term drug abuser (DAB; 3,3´-diaminobenzidine), 200 X magnification; scale bar represents 100 µm.

Figure 5: Another positive immunoreaction: brown nuclear staining patterns of ΔFosB of the medium spiny neurons (MSN) of NAc of a long-term drug abuser (DAB; 3,3´-diaminobenzidine), 200 X magnification; scale bar represents 100 µm.

Furthermore, the expression of ΔFosB in post-mortem NAc tissue samples was proven by means of immunoblotting of chronic opioid addicts and compared this with the tissue of control individuals: Stable ΔFosB isoforms with a size of 35 kD and 37 kD were detected in the NAc of subjects with chronic drug abuse (n=15), even after a PMI of 8.47 ± 2.61 days (Table 1), with both the polyclonal and the m MTa¾ ‘

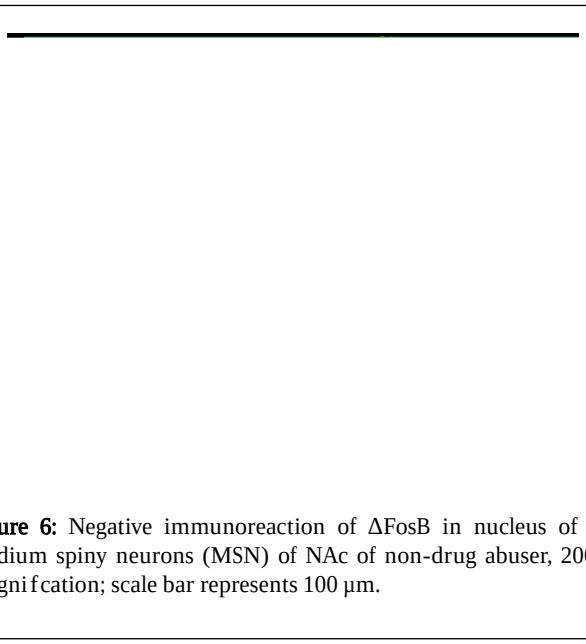

Figure 6: Negative immunoreaction of ΔFosB in nucleus of the medium spiny neurons (MSN) of NAc of non-drug abuser, 200 X magnification; scale bar represents 100 µm.

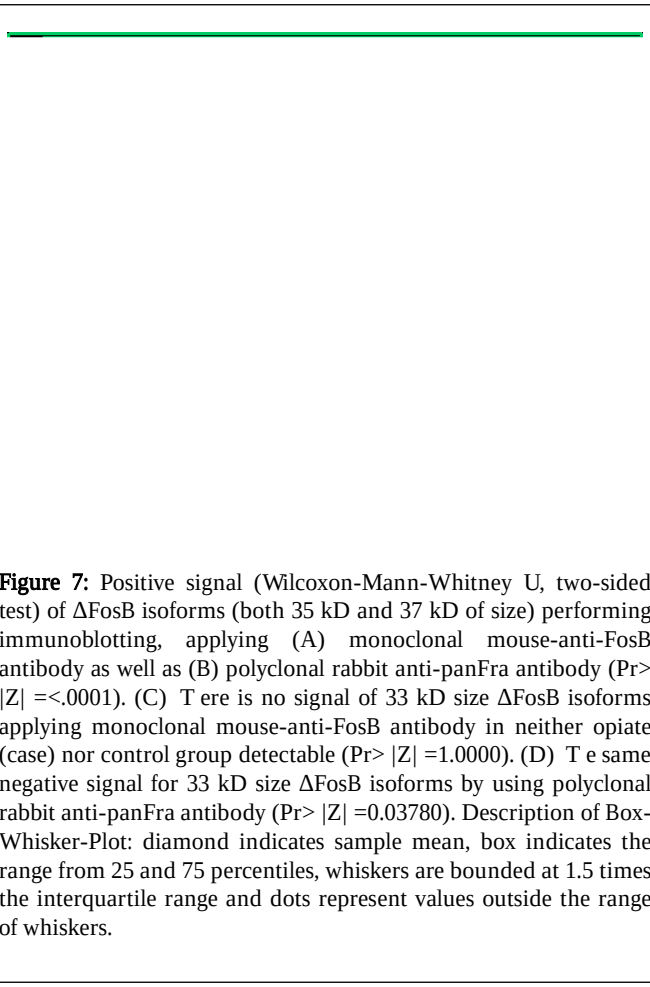

Figure 7: Positive signal (Wilcoxon-Mann-Whitney U, two-sided test) of ΔFosB isoforms (both 35 kD and 37 kD of size) performing immunoblotting, applying (A) monoclonal mouse-anti-FosB antibody as well as (B) polyclonal rabbit anti-panFra antibody (Pr> |Z| =<.0001). (C) There is no signal of 33 kD size ΔFosB isoforms applying monoclonal mouse-anti-FosB antibody in neither opiate (case) nor control group detectable (Pr> |Z| =1.0000). (D) The same negative signal for 33 kD size ΔFosB isoforms by using polyclonal rabbit anti-panFra antibody (Pr> |Z| =0.03780). Description of Box-Whisker-Plot: diamond indicates sample mean, box indicates the range from 25 and 75 percentiles, whiskers are bounded at 1.5 times the interquartile range and dots represent values outside the range of whiskers.

Discussion

The underlying biological process of drug addiction, namely the impact of repeated drug intake on the human brain, implies specific adaptations of individual neurons. This, in turn, alters the mode of operation of affected neurons, which again alters the way in which specific neural circuits' function, within which these neurons usually operate. In combination, these effects lead to a specific behavioral complex: dependence, tolerance, sensitization, and craving. This defines the status of addition, as already published by Koob and Le Moal [1] and Wise [2].

As repeatedly confirmed by previous studies such as Lynch [21] and Robison et al. [22], the accumulation of stable 35-37 kD ΔFosB isoforms in the NAc as a consequence of chronic exposure to different stimuli, including drugs of abuse such as cocaine or morphine, appears to be responsible for sustained behavioral changes, inducing addiction or "drug-sickness", as well as sustained neuroplasticity. Besides NAc, recent findings of Eagle et al. underscore the important role of IEGs and particularly ΔFosB inside HPC regarding learning and memory formation [23].

Although we assumed that ΔFosB might be a comparatively stable transcription factor, with an approximate half-life of 10 h in PC12 cells, as revealed by Ulery et al. [12], our recent results have considerably exceeded our expectations, especially with regard immunoblotting results.

Based on a study Chen et al. where the authors describe the consistently regulation of cyclin-dependent kinase 5 (Cdk5) by ΔFosB in the HPC of mice, we were able to demonstrate an increased immunoreactivity of Cdk5 in both NAc and HPC of chronic drug abusers [24]. As a result, the evidence of the fact that Cdk5 is one of the downstream target genes for ΔFosB could be proven by our findings. Toward CREB as a constitutivedcɹM com ᵐa ˢ v]osB we▯

Alibhai et al. [33]. However, recent fndings by Teyssier et al. [34] clearly show that the ΔFosB pathway in brain tissue of depressed patients was chronically activated at the mRNA level, suggesting that a