Addiction Therapy-2014

Chicago, USA

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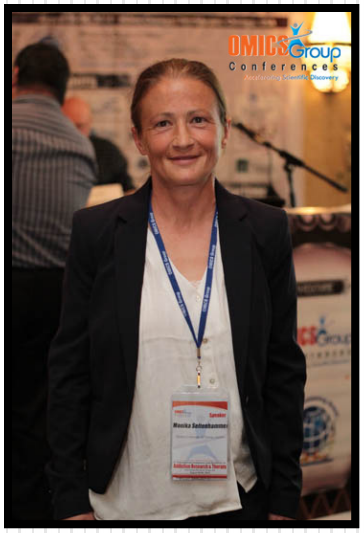

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Detecting Highly Stabilized Cumulative ~35-37 kD Isoforms of ΔFosB in Postmortem Human Brain Tissue Samples of the Nucleus Accumbens (NAc) of Chronic Opioid Abusers

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Overview – Addiction a Global Affair

- Illegal use of drugs still remains a global affair
- Accompanied with rampant health problems (blood borne infections such as HIV, hepatitis B & C), increasing rate of morbidity and mortality (AIDS, liver cirrhosis and liver cancer)
- People of younger ages are concerned particularly: in 2009 ~ 150-270 millions (» 3.3-6.1% of global population aged 15-64 yrs) used illicit substances (World Drug Report of 2011, UNODC)

Overview – Addiction a Global Affair

- 12-21 (midpoint 16.5) million people are chronic opioid (opiates, morphines, heroin, methadone, …) addicts
- Co-Morbidity
- Co-Mortality
- Co-Criminality
- Relapse rate is > 90% (!!!) – again leading to a vicious circle of severe medical, social & other problems

Table 1: Amount of Immediate Drug-Related Deaths (DRDs) according to Cause of death, Austria 2002-2011

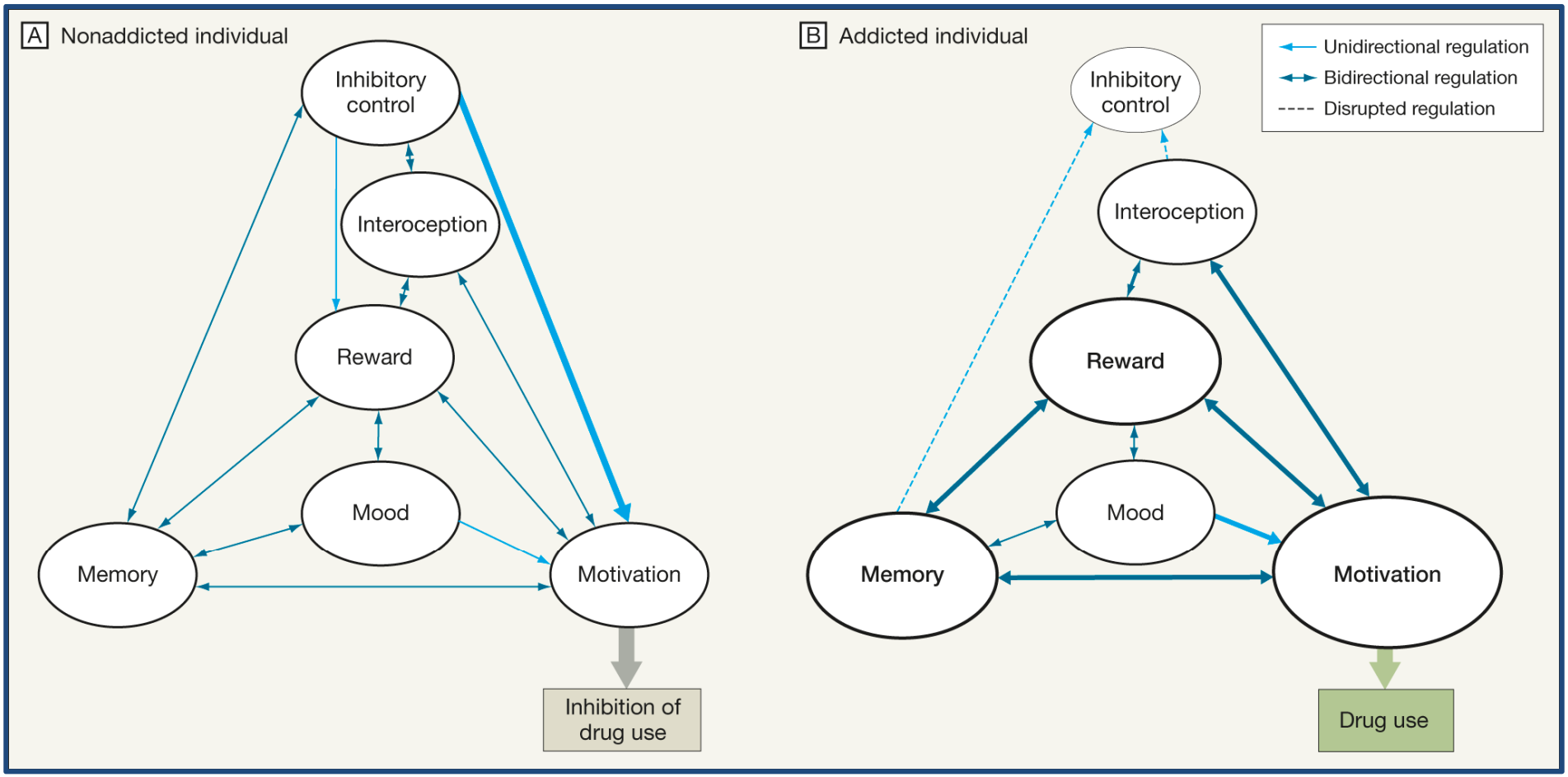
(Annually Drug Report of Austria)

Cause of Death	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Intoxications with Opioids	17	40	38	31	27	9	13	18	13	11
Mixed Intoxications with Opioids	119	115	133	134	137	138	136	153	148	151
Mixed Intoxicatons with Illicit Drugs or NPS* without Opioids	3	8	4	4	5	5	4	1	0	8
Non-verifiable Intoxications	0	0	10	22	28	23	16	15	9	7
Total Amount of directly verified DRDs	139	163	185	191	197	175	169	187	170	177
*: new psychoactive substances										

Stages of Drug Addiction

- 1) First Use of Drugs – Experimentation
  entrance into drug using
- 2) Misuse of Drugs
  risky behaviors, unexplained violence, symptoms of depression and anxiety
- 3) Abuse of Drugs
  craving, preoccupation with the drug, and symptoms of depression, irritability and fatigue if the drug is not used
- 4) Drug Addiction & Dependency
  withdrawal symptoms and compulsive use of the drug despite severe negative consequences to his or her relationships, physical and mental health, personal finances, job security and criminal record

Stages of Drug Addiction – Nonaddicted vs addicted individual



Nestler E.J., 2013

Chronic Drug Abuse / Addiction

- Long-lasting adaptations in specific brain regions (reward system):
  Neuroplasticity, Synaptic plasticity, Structural plasticity, Brain plasticity
- Development of Sensitization
- Development of Tolerance
- Vulnerability to Relapse

Reward System of the Brain

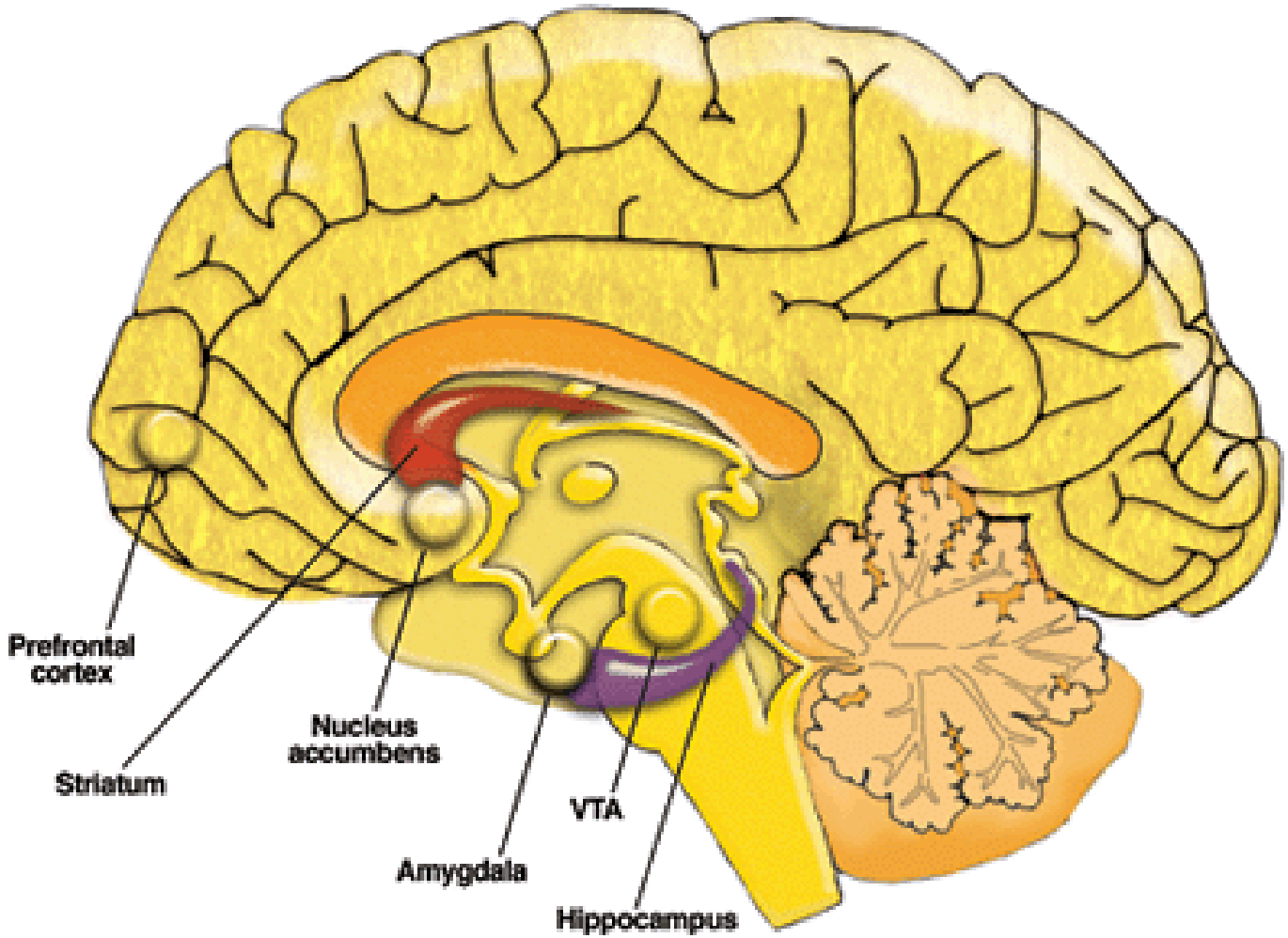
- Mesocorticolimbic and nigrostriatal dopaminergic (DA) pathway regulating behavior, motor activity, reward, and reinforcement in the brain
- Mesolimbic: ventral tegmental area – medial prefrontal cortex – ventral striatum (Nucleus Accumbens or NAc) and amygdala; locomotor stimulant, rewarding & sensitizing properties of drugs of abuse
- Nigrostriatal: substantia nigra – dorsal striatum (caudate putamen or CPU); movement initiation, learning of motor patterns and drug-related habit learning

Induction of ~35-37 kD Isoforms of ΔFosB in the reward system of the brain

- Induction of high levels in region-specific manner following **chronic**, but **not acute**, exposure to variety of psychoactive stimuli:
- Drugs of abuse (opiates, cocaine, amphetamine, nicotne, ethanol, cannabinoids,…)
- Natural rewards (compulsive running, stress, certain lesions)
- Antipsychotic & antidepressant drugs

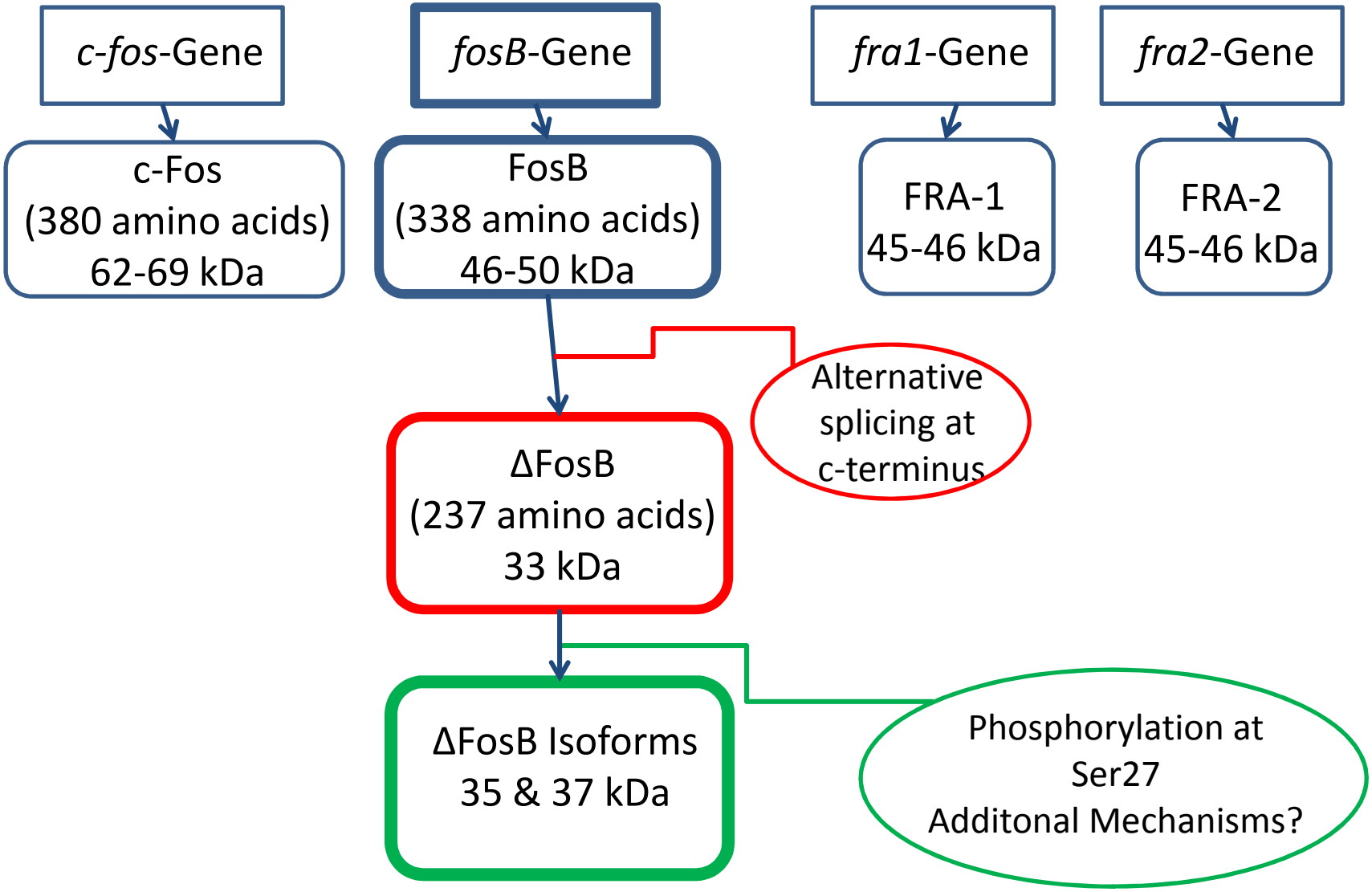
- Complex transcriptional effects which are still not fully understood and therefore remains elucive

Transcription Factor ΔFosB



Kasanetz et al., *Science*, June 24, 2010 DOI: 10.1126/science.1187801

Fos Family Transcription Factors – Immediate Early Genes



Biochemical basis of ΔFosB´s unique stability

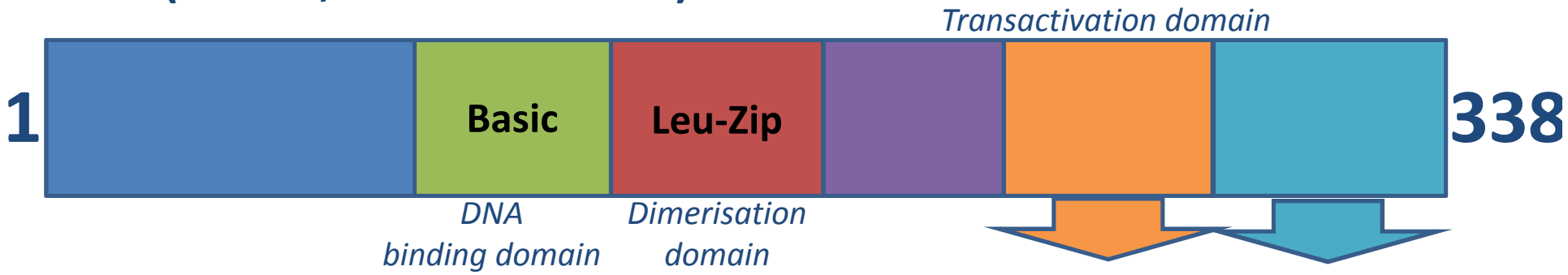
- ΔFosB is generated by alternative splicing and lacks the C-terminal 101 amino acids present in FosB
- Two mechanisms for ΔFosB´s unique stability:

  a) ΔFosB lacks two degron domains present in the C-terminus (one targets for ubiquilation and degradation in the proteasome – the other for degradation by ubiquitin- and proteasome-independent mechanism) → ~33 kD Mr ΔFosB

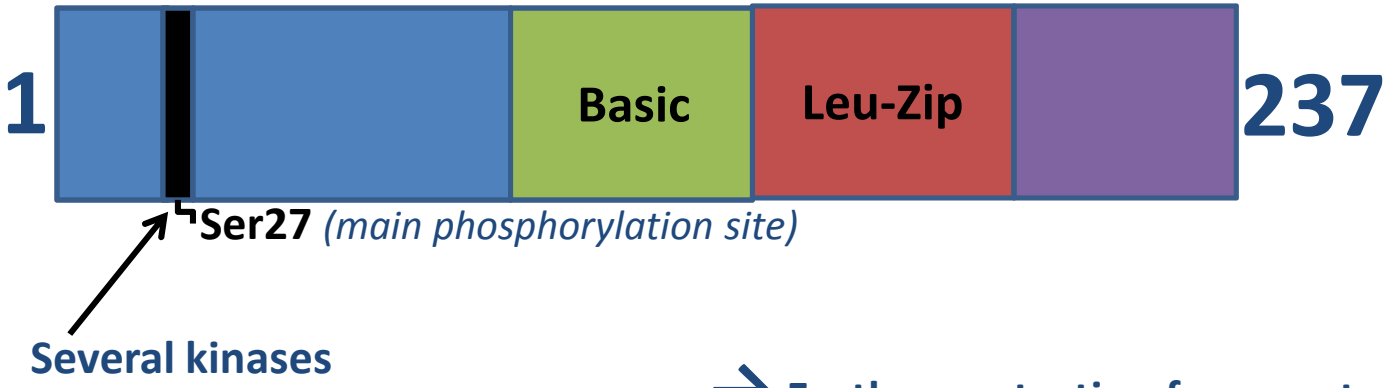
  b) ΔFosB is phosphorylated by several protein kinases at its N-terminus leading to further stabilization → ~35-37 kD Mr ΔFosB

Biochemical basis of ΔFosB´s unique stability

FosB (338 aa, Mr ~ 46-50 kDa)

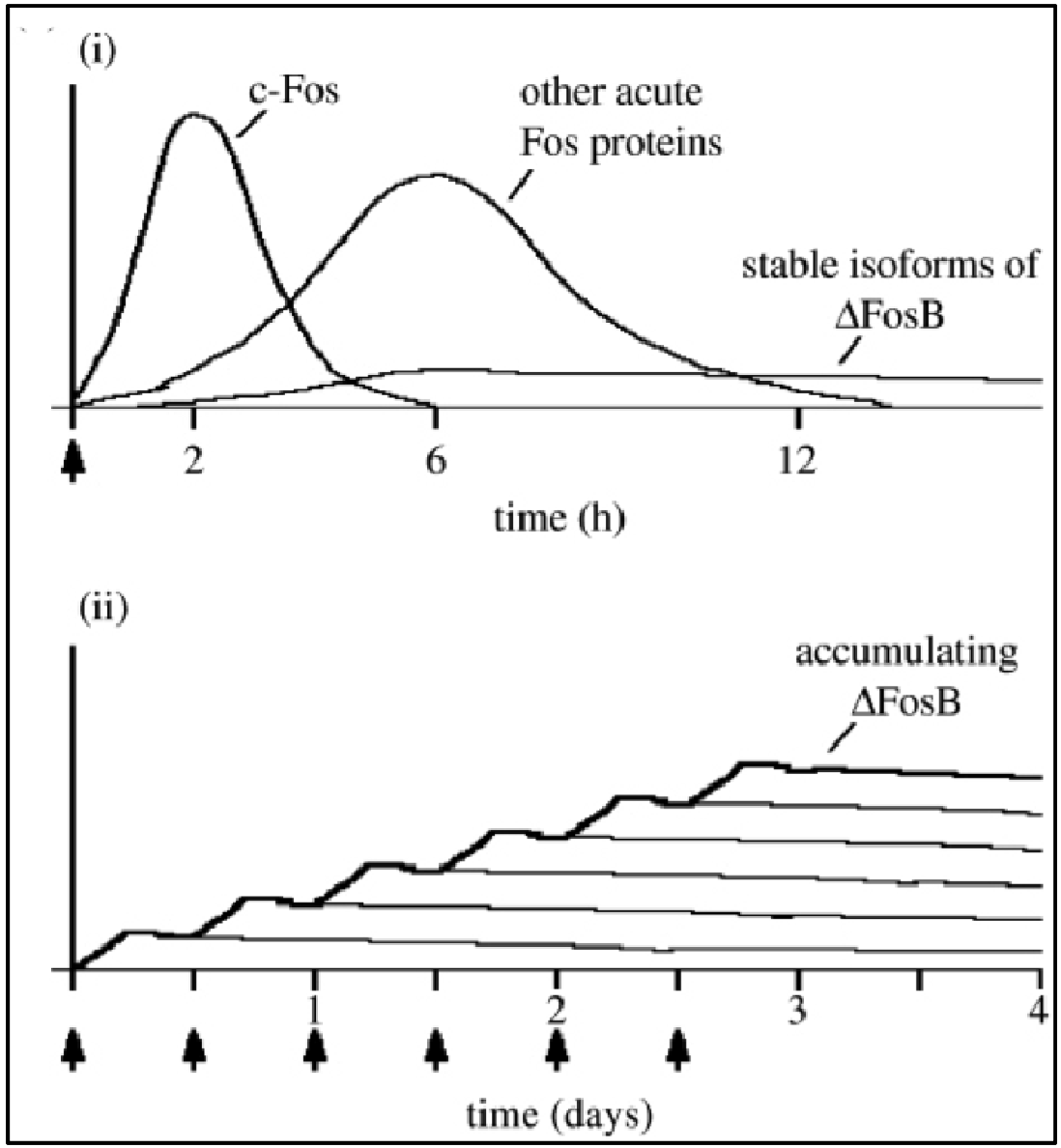


ΔFosB (237 aa, Mr ~ 33 kDa)



→ **Further protection from proteolytic degradation**

Modified according Nestler E.J., 2013



Nestler E.J., 2013

... Almost all studies on FosB and ΔFosB were performed using animal models so far

Table 2: Detection of ΔFosB Protein in Postmortem Human Brain Tissue

Parameter	Opioid Group (n=15)	Control Group (n=15)
Age (years): Mean/Std Deviation	27.0 / ± 7.05	26.87 / ± 6.80
Median	26.0	24.0
Range	18.0	21.0
p=0.96		
PMI (days): Mean/Std Deviation	8.47 / ± 2.61	9.33 / ± 3.87
Median	8.0	11.0
Range	9.0	10.0
p=0.48		
Morphine (ng/g): Mean/Std Deviation	230.5 / ± 92.5	0.0 / ± 0.0
Median	196.5	0.0
Range	590.0	0.0
p≤0.001		
Gender Distribution (F/M)	2 / 13	4 / 11
p=0.65		

Drug Testing

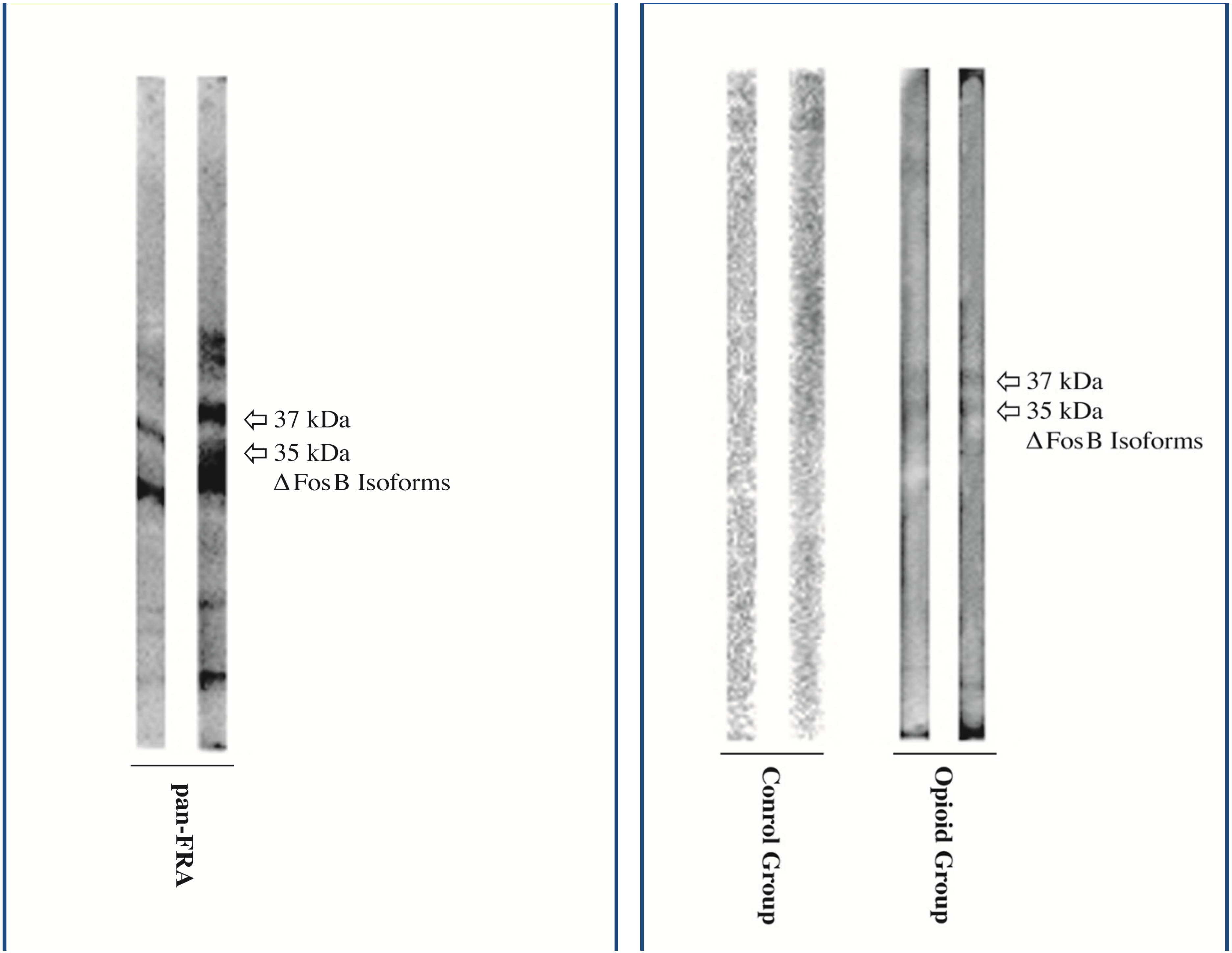
- Determination of morphine concentration in:
  a) Medulla oblongata
  b) Cerebellum
  c) Blood
  d) Urine
  e) Hair
- 6-Monoacetylamine (6-MAM) and other psychoactive substances (e.g. cannabis, benzodiazepine, etc.) performing quantitative toxicological analysis using Gas Chromatography-Mass Spectrometry (GC-MS)

Protein Isolation & Immunoblotting

- Brain tissue samples NAc – flash frozen – storage at -80°C
- Protein extraction according to Korner as described by Hope et al. (1994) with modifications
- Homogenization in lysis-buffer containing Triton®X-114 (removing lipids – phase separation)
- Precipitation of proteins to purify & enrich
- SDS-polyacrylamide gel electrophoresis – Ponceau-S staining

Protein Isolation & Immunoblotting

- Immunoblotting with following antibodies:
  a) monoclonal mouse anti-FosB (SantaCruz, #sc-8013)
  b) monoclonal mouse anti-Delta FosB (Cell Signaling, #9890)
  c) polyclonal rabbit anti-panFRA (SantaCruz, #sc-253)



37 kDa
35 kDa
ΔFosB Isoforms
pan-FRA
37 kDa
35 kDa
ΔFosB Isoforms
Conrol Group
Opioid Group

Summary and Outlook

- Enormous stability of ΔFosB isoforms
- Substantial impact on regulation and expression of numerous key-position genes in the brain e.g. GluA2↑(decreased sensitivity to glutamate, silent synapses), Dynorphin↓(downregulation of *k*-opioid feedback loop), Cdk5↑(expansion of dendritic processes), NF-*k*B↓(expansion of dendritic processes; regulation of cell survival pathways), c-Fos↓(molecular switch from short-lived Fos family proteins induced acutely to ΔFosB induced chronically)

Summary and Outlook

- ΔFosB is stable even after cessation of drug administration or chronic stimulus for several weeks, months or even much longer
- Leading to sustained neuronal plasticity
- Its stability makes it detectable even in postmortem human brain tissue samples with a prolonged PMI of 8.47 / ± 2.61 days
- Represents a key factor concerning vulnerability & relapse

Summary and Outlook

- ΔFosB itself - or any of the numerous genes it regulates - represents potential targets for development of fundamentally novel treatment strategies for drug addiction with particular attention to more personalized therapies when thinking of the high relapse rates.

Aknowledgements

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Thank you for your attention!

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