P.1.h.028 Knockdown of Bcl-xL in the rat hippocampus increased immobility in the forced swim test

Shishkina G.T., Lanshakov D.A., Bannova A.V., Agarina N.P., Dygalo N.N.

The Federal Research Center Institute of Cytology and Genetics of Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

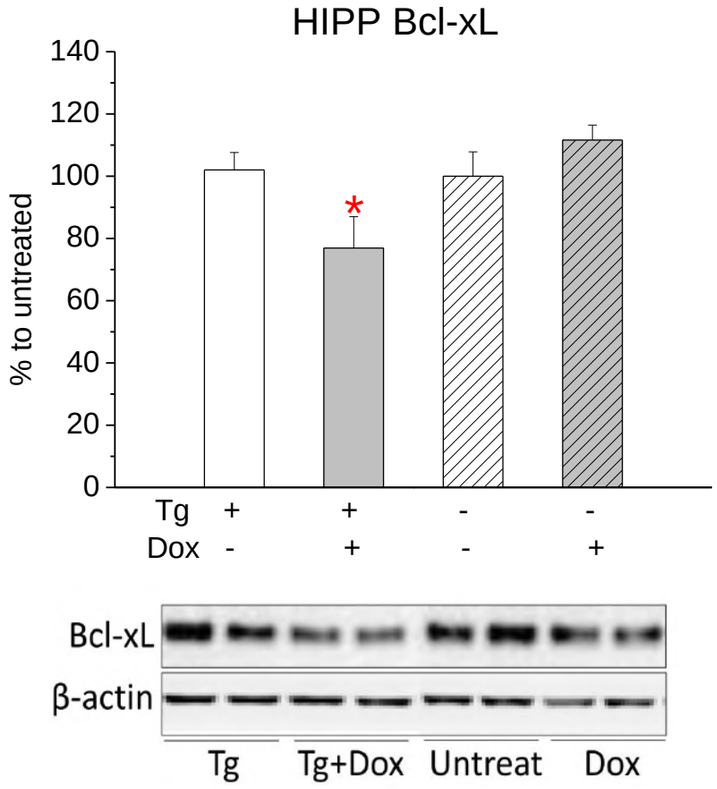
Introduction

The duration of immobility, a behavioral measure of depression in rodents in the forced swim test (FST) (Porsolt et al., 1978), was shown to be negatively correlated with FST-induced increase in the hippocampal Bcl-xL expression (Shishkina et al., 2010). We hypothesized that the attenuation of Bcl-xL expression during period of FST exposure would increase immobility in the test session. To further evaluate the relation between hippocampal Bcl-xL and immobility, in the present study, a doxycycline (Dox)-controllable Bcl-xL gene knockdown was applied.

Methods

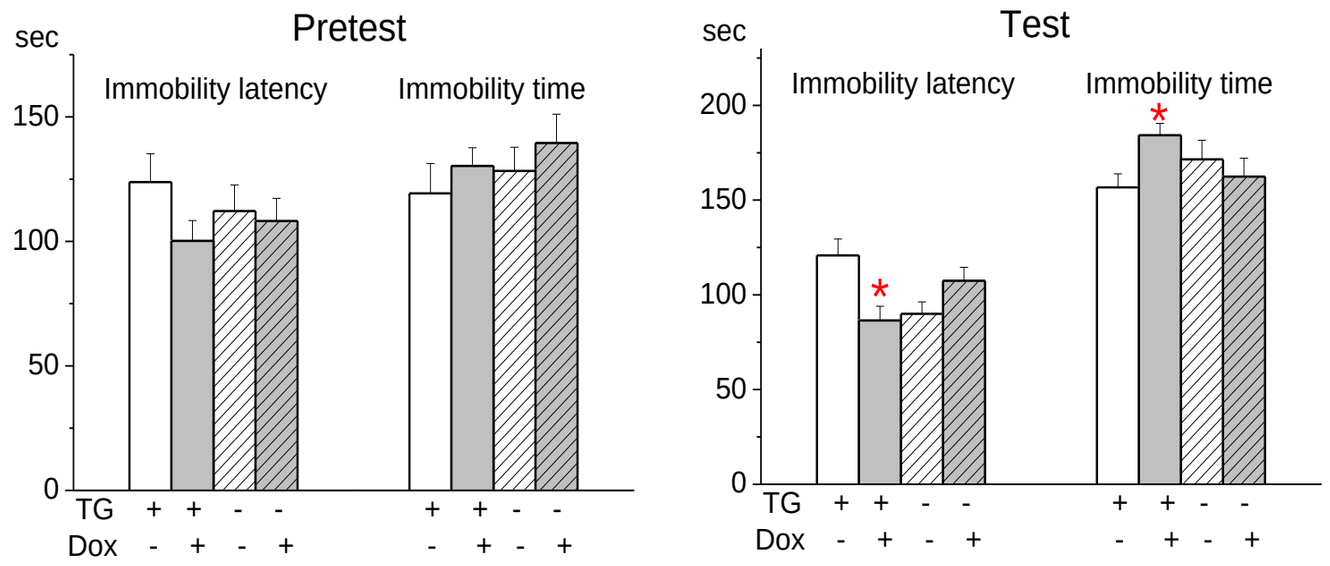
Lentiviral vector with transgene cassette (Tg), containing Dox-dependent (tet-on) shRNA targeting Bcl-xL, was stereotaxically injected bilaterally into the hippocampus in 5-μL volume at the following coordinates, as calculated from bregma: AP -3.0; ML ±1.5; DV -2.7 (Paxinos,Watson, 1998). Three weeks after viral vector infusion, half of the animals from intact and Tg groups began to receive Dox in the drinking water (2 mg/ml). Starting from the seventh day of Dox consumption, a two-day FST was performed. Thirty min after the second swim session, Bcl-xL and BDNF protein levels were determined in the hippocampus and frontal cortex by immunoblotting. The behavioral and neurochemical data were analyzed by two-way ANOVA with Tg and Dox as factors, followed by LSD post hoc test.

1 Analyses of the efficacy of Bcl-xL knock-down



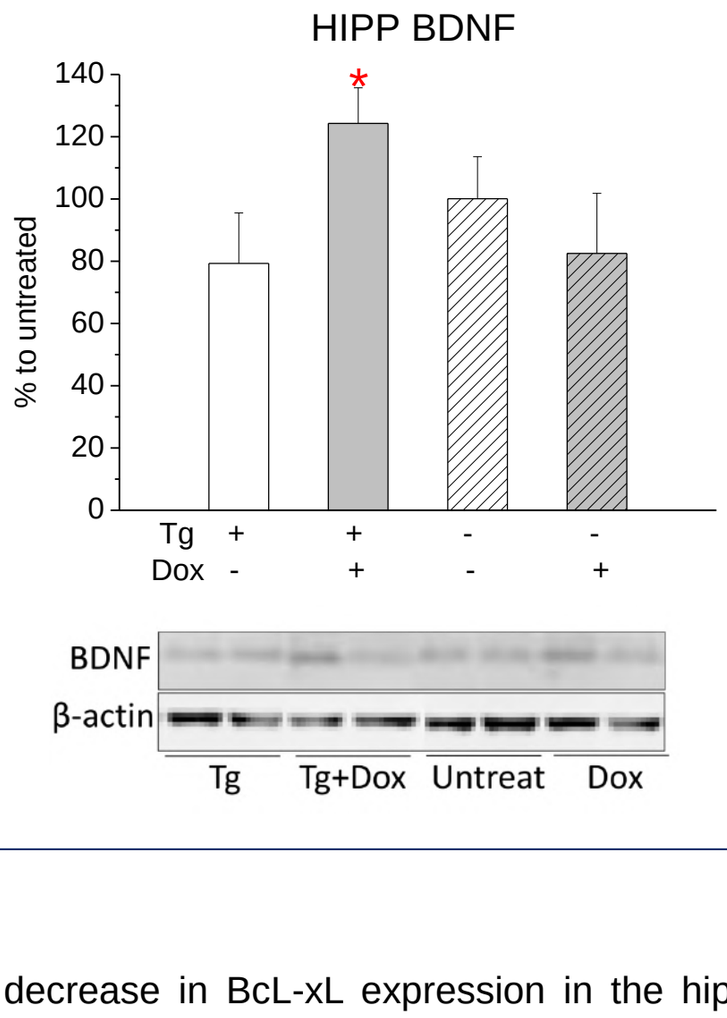
The total Bcl-xL protein levels in the hippocampus of Tg+Dox animals were significantly reduced compared to Tg group ($p<0.05$; effect of Tg x Dox interaction: $F(1, 26)=5.719$, $p=0.0243$).

2 Behaviors in the forced swim test



No effects of Tg or Dox, or interactions between factors on immobility latency and immobility time were observed during 5 min of the pretest session. In the test session, the knockdown of Bcl-xL expression was accompanied by the decrease in the latency time to the first immobility ($p<0.01$ vs. Tg; effect of Tg x Dox interaction: $F(1, 43)=12.074$, $p=0.0012$) and the increase in the total time of immobility ($p<0.05$ vs. Tg; effect of Tg x Dox interaction: $F(1, 43)=4.752$, $p=0.0347$) in the 5-min test session. Thus, Bcl-xL knockdown during the period of FST produced a pro-depressant-like effect in the test.

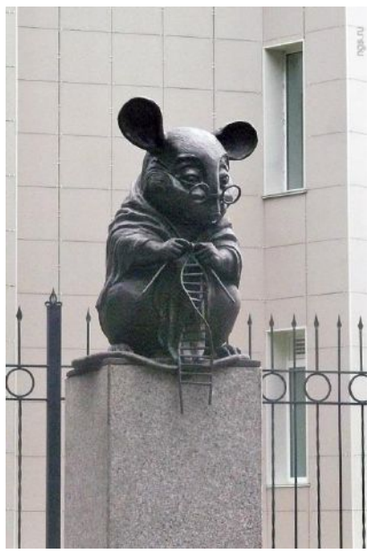
3 BDNF protein (% to those in untreated animals in the hippocampus



Together with the decreased Bcl-xL expression in the hippocampus of Tg+Dox animals, we observed a significant increase in BDNF protein levels in this structure ($p<0.01$ vs. Tg; effect of Tg x Dox interaction: $F(1, 27)=4.383$, $p=0.0458$), effect that may be adaptive in conditions of decreased Bcl-xL levels. However, although it was demonstrated that BDNF can increase Bcl-xL expression in the hippocampus (Chao et al., 2011), the increase in BDNF levels in conditions of decreased Bcl-xL expression was not shown.

Conclusion

The results of the present study indicate that the decrease in BcL-xL expression in the hippocampus during the FST period was accompanied by the pro-depressant effects in the test, providing an additional support for the involvement of hippocampal anti-apoptotic protein in behavioral responses to forced swim stress (Dygalo et al., 2012). In addition, the knockdown of Bcl-xL expression was also accompanied by the increase in BDNF expression, but it is not clear whether there is causal relationship between these two events.



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The authors have declared that no competing interests exist.