

GENE AND PROTEIN EXPRESSION OF THE NGF AND ITS COGNATE RECEPTORS IN DIFFERENT TESTICLE MORPHOTYPES OF A GREY SQUIRREL POPULATION (*SCIURUS CAROLINENSIS*) LIVING IN CENTRAL ITALY



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Introduction

Grey squirrel (*Sciurus carolinensis*) (Fig.1) is an invasive alien species representing a serious threat to Eurasian red squirrel (*Sciurus vulgaris*) (Fig.2) conservation. In line with one of the tasks of the EU Biodiversity 2020 Strategy, as part of the LIFE Project BIO/IT/000204 U-SAVEREDS, this work aimed at safeguarding the native red squirrel in Umbria, central Italy, limiting the diffusion of the non-native species. Various studies reported that nerve growth factor (NGF) and its receptors (NTRK1, NGFR) play a critical role in the regulation of mammalian reproduction [1,2], this is the first investigation on the NGF system expression in the grey squirrel testicles.



Figure 1. Eastern gray squirrel (*Sciurus carolinensis*)



Figure 2. Eurasian red squirrel (*Sciurus vulgaris*)

Materials & Methods

Eighteen squirrels were captured in the Umbria Region, using the Tomahawk live traps (mod. 202.5, Tomahawk Live Trap Co., WI, USA), during the 2016–2018 management plan. The animals underwent external or internal orchiectomies.

Within few minutes, for each animal, one testicle intended for the investigation of the gene and protein expression were washed in a RNase-free phosphate-buffered saline solution and then frozen at -80 °C [1], the other testicle for the histological procedures to assess the morphotype. The testicles were classified into 3 different morphotypes [3]: active spermatogenesis, pubertal, immature animals. Real Time PCR: Testicle NGF, NTRK1 and NGFR mRNA were evaluated by Real Time PCR, using BACT as housekeeping (Table 1).

Western blot analysis: testicle NGF, NTRK1 and NGFR protein were highlighted using three different antibodies: mouse anti-NGF (sc-365944), mouse anti-NTRK1 (sc-803998) and mouse anti-NGFR (sc-271708) (Fig. 3,4).

Results

We found that only NGF significantly increased in pubertal with respect to both active spermatogenesis and immature morphotypes. Western Blot revealed the presence of bands matching the expected size for NGF and its receptors. Significant differences among group means were calculated by ANOVA followed by Tukey post hoc test.

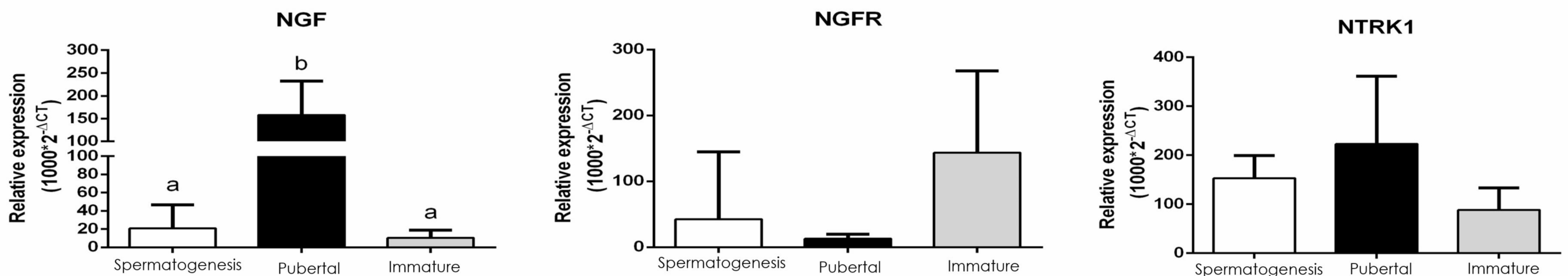


Figure 3. Histograms of nerve growth factor (NGF) nerve growth factor receptor (NGFR) and neurotrophic tyrosine kinase receptor 1 (NTRK1) transcription values (medians and 95% confidence intervals) in testicles of grey squirrels in spermatogenesis, pubertal and immature phase. Data are shown as ΔCt (relative to β -actin) values.

Conclusion

These data suggest that in the grey squirrel population here investigated the NGF system is involved in the testicle development and function. The increase in NGF expression in pubescent animals testifies a possible involvement of this molecule during puberty. This highlights NGF system contribution to the reproductive success of this species by a mechanism not yet understood.

References

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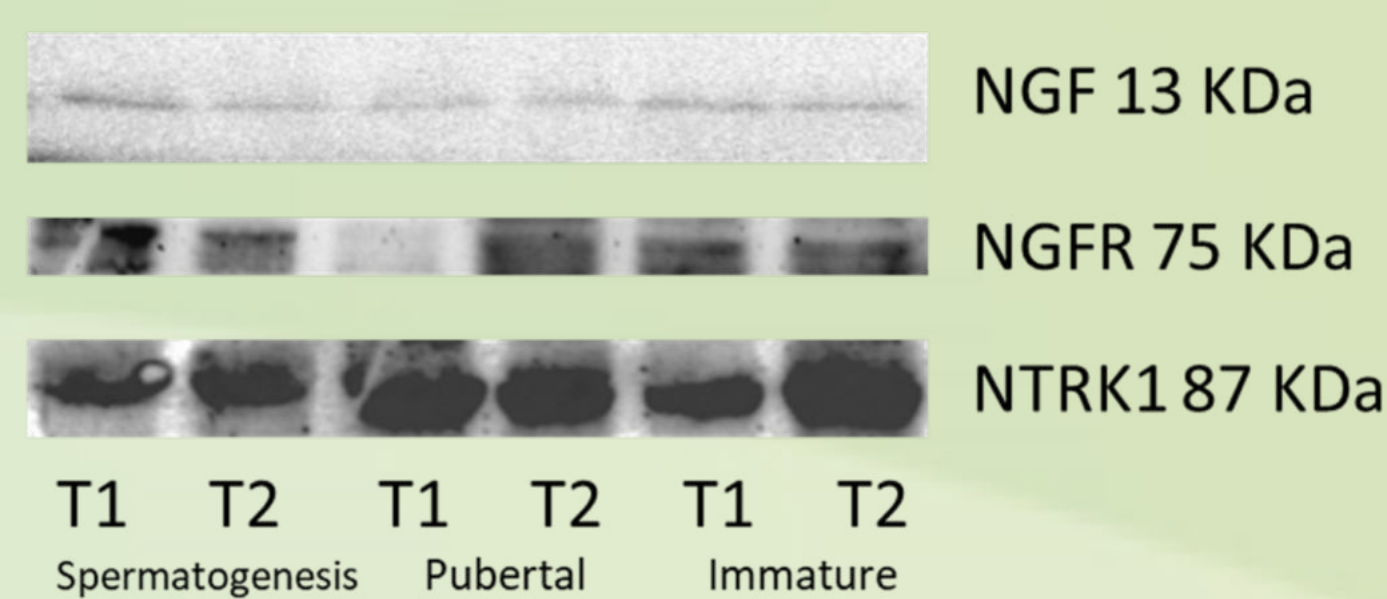


Figure 4. Immunoblot showing NGF, NGFR, NTRK1 proteins in testicle lysate in two different squirrels belonging to spermatogenesis, pubertal and immature phase. Total protein extracts were separated by SDS-PAGE and analyzed by immunoblotting with the specific antibodies.

Gene		Primers	bp
NGF	F	TCCACCCACCCAGTCTC	178
	R	GCTCGGCACTTGGTCTCA	
NTR1	F	TCCGACCAATGCTGCCATCC	261
	R	AGGCGTGTCTCGGTTCTCG	
NGFR	F	GGAGGACACGAGTCTGAGC	295
	R	CAGTGGAGAGTCTGCAAAG	
BACT	F	TGTGATGGACTCCGGAGAC	186
	R	TGATGTCACGCACGATTCC	

Table 1. Primers for NGF, NTRK1, NGFR and BACT (used as internal standard) for Real Time-PCR quantification. NGF: nerve growth factor; NTRK1: neurotrophic tyrosine kinase receptor 1; NGFR: nerve growth factor receptor; BACT: β -Actin; bp: base pair.

