

Developmental regulation of the geniculate ganglion by neurotrophins BDNF and NT-4

Ami Patel and Robin F Krimm

Anatomical Sciences and Neurobiology, School of Medicine, University of Louisville

INTRODUCTION

The geniculate ganglion is a sensory ganglion made up of primary sensory neurons that innervate the taste buds of the anterior two thirds of the tongue, the soft palate, and somatosensory cells of the outer ear. During development, brain-derived neurotrophic factor (BDNF) and neurotrophin 4 (NT-4) maximally influence the survival of these geniculate ganglion neurons. Specifically, 50% of the geniculate ganglion neurons die in BDNF null mice (*bdnf*^{-/-}) as well as NT-4 null mice (*nt-4*^{-/-}) (Liu et al., 1995). BDNF/NT-4 double mutant mice lose about 90-95% of geniculate ganglion neurons. The loss of almost all the neurons in the double mutant mice indicates that most of the geniculate ganglion neurons depend on one or more of the two neurotrophins for survival during development. Though a similar endpoint reduction in neurons occurs in mice lacking BDNF or NT4, the developmental processes regulated by BDNF and NT4 are unknown. To determine when during development BDNF and NT-4 regulate geniculate ganglion neuron loss, we quantified geniculate ganglion neuron loss on embryonic days 12.5, 14.5, 16.5 and 18.5 for *bdnf*^{-/-}, *nt-4*^{-/-} and wild-type embryos.

METHODS

Immunohistochemistry and Volume Measurements

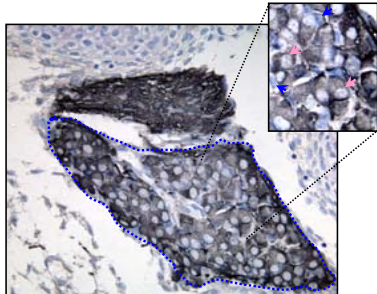


FIGURE 4. Embryonic day 14.5 (E14.5) *bdnf*^{-/-} geniculate ganglion. Horizontal serial sections of the mouse embryo head were used to quantify neurons of the geniculate ganglion. Immunohistochemistry against neuronal class III β tubulin, a neuronal marker, was used to identify and count neuron profiles, counted only in sections where the nucleus was visible. Neurons (pink arrows) were easily distinguished from non-neurons (blue arrows) during quantification. Six sections per geniculate ganglion were counted. The dashed line illustrates the area used for volume calculations. Volume was measured for all the sections in which the geniculate ganglion was located.

Formulas were used to estimate total neuron number

$$\text{Total Profiles} = \frac{\text{Counted profiles}}{\text{Volume of counted}} \times \text{Total Volume}$$

$$\text{Total Neurons} = \text{Total Profiles} \times (5/5+d)$$

BDNF Regulates Geniculate Ganglion Neuron Number Beginning After E12.5 and Continuing Through E18.5 of Development.

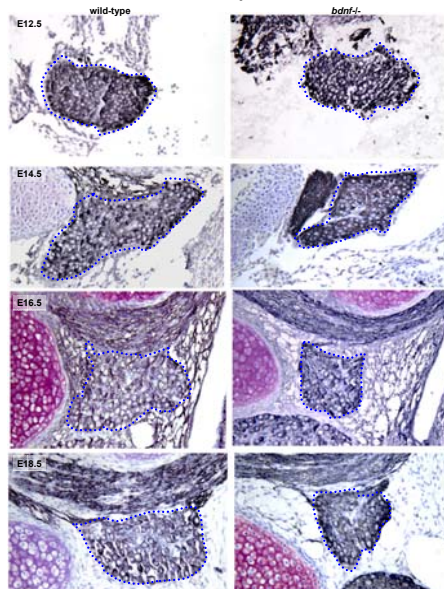
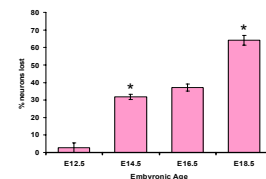


FIGURE 2. Images of the TUJ-1 labeled geniculate ganglion in wild-type (left) and *bdnf*^{-/-} mice (right). At E12.5, the geniculate ganglion appears to be the same size between *bdnf*^{-/-} mice and wild-type mice. However, by E14.5 the geniculate ganglion appeared smaller in *bdnf*^{-/-} mice compared to wild-type mice.

	E12.5		E14.5		E16.5		E18.5	
	wild-type	<i>bdnf</i> ^{-/-}	wild-type	<i>bdnf</i> ^{-/-}	wild-type	<i>bdnf</i> ^{-/-}	wild-type	<i>bdnf</i> ^{-/-}
Mean of Volume	1,577.6 ± 1125 (n=3)	1,474.6 ± 1,123 (n=3)	1,434.6 ± 986 (n=3)	830.2 ± 581* (n=3)	1,455.0 ± 979.6 (n=3)	670.2 ± 619* (n=3)	1,291.2 ± 524 (n=3)	636.2 ± 319* (n=3)
Mean no. of Neurons	946.4 ± 48.53 (n=3)	917 ± 35.3 (n=3)	722 ± 43.1 (n=3)	492 ± 19* (n=3)	567.2 ± 111 (n=3)	348.2 ± 14.9* (n=3)	748.22 ± 144.23 (n=3)	238 ± 19* (n=3)

There was no significant difference in either geniculate ganglion volume or neuron number between wild-type and *bdnf*^{-/-} animals at E12.5. Where as for E14.5, E16.5 and E18.5 embryos, *bdnf*^{-/-} animals showed a significant loss in geniculate ganglion volume and neuron number compared to wild-type animals. (*p<0.05). * = significantly different from wild-type values.

Neuron losses in BDNF-/- mice



BDNF^{-/-} mice lose geniculate ganglion neurons between E12.5 and E14.5 and between E16.5 and E18.5 of development. * = significantly different from previous age.

NT-4 Regulates Geniculate Ganglion Neuron Number Beginning as Early as E12.5 and Ending by E16.5 of Development.

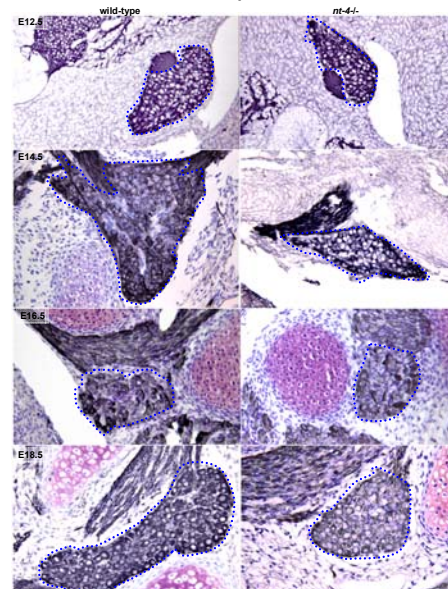
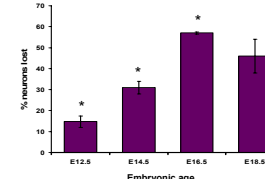


FIGURE 3. Images of the TUJ-1 labeled geniculate ganglion in wild-type (left) and *nt-4*^{-/-} mice (right). As early as E12.5 the geniculate ganglion appeared smaller in *nt-4*^{-/-} mice compared to wild-type mice and the difference continues up to E18.5 of development.

	E12.5		E14.5		E16.5		E18.5	
	wild-type	<i>nt-4</i> ^{-/-}	wild-type	<i>nt-4</i> ^{-/-}	wild-type	<i>nt-4</i> ^{-/-}	wild-type	<i>nt-4</i> ^{-/-}
Mean of Volume	1,577.6 ± 1125 (n=3)	1,464.0 ± 1,015 (n=3)	1,434.6 ± 986 (n=3)	935.2 ± 690* (n=3)	1,455.0 ± 979.6 (n=3)	670.2 ± 619* (n=3)	1,291.2 ± 524 (n=3)	636.2 ± 319* (n=3)
Mean no. of Neurons	946.4 ± 48.53 (n=3)	744.2 ± 74.9* (n=3)	722 ± 43.1 (n=3)	498 ± 17* (n=3)	567.2 ± 111 (n=3)	279.7 ± 47.9* (n=3)	748.22 ± 144.23 (n=3)	481 ± 48.23* (n=3)

There was a significant difference in geniculate ganglion neuron number between wild-type and *nt-4*^{-/-} animals by E12.5 which continued for E14.5, E16.5 and E18.5 embryos (p<0.05). * = significantly different from wild-type values.

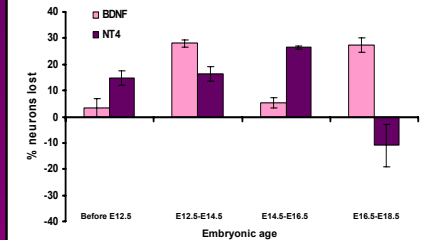
Neuron losses in NT-4-/- mice



Geniculate ganglion neurons are lost as early as E12.5 and losses continue until E16.5 of development. No additional losses occur after E16.5 of development. * = significantly different from previous.

Developing geniculate ganglion neurons are influenced by BDNF and NT-4 at different time points

Percent neurons lost between each embryonic age



BDNF has a significant influence on geniculate ganglion neuron number at two distinctive time points. There is a loss of about 30% of the neurons early in development in *bdnf*^{-/-} mice before the onset of target innervation between E12.5-E14.5. The absence of BDNF also causes major neuron loss of approximately another 30% of geniculate ganglion neurons between E16.5-E18.5. On the other hand, absence of NT-4 lead to loss of geniculate ganglion neurons by E12.5, which is earlier than losses in *bdnf*^{-/-} mice. Neuron loss ends in *nt-4*^{-/-} mice by E16.5, which is earlier than for *bdnf*^{-/-} mice.

CONCLUSIONS

- 1) BDNF regulates number of geniculate ganglion neurons at two distinctive ages, the first is just before or at the onset of target innervation and the second is well after target innervation.
- 2) NT-4 regulates neuron number by E12.5 but stops influencing geniculate ganglion development by E16.5.
- 3) NT-4 regulates neuron number in the geniculate ganglion earlier in development than BDNF.

REFERENCES

Liu, X., Ermfors, P., Wu, H., and Jaenisch, R. (1995). Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. *Nature* 375, 238-41.

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