

Astrocyte reactivity is associated with decreased levels of N-Acetyl-Aspartate in the absence of neurodegeneration in the rat brain

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Target audience

This work should be of interest to all persons using magnetic resonance spectroscopy to study neurodegeneration *in vivo*.

Purpose

N-Acetyl-Aspartate (NAA) is one of the most abundant metabolite in the brain and constitutes the major peak detected by magnetic resonance spectroscopy (MRS) *in vivo* (Moffet *et al.*, 2007). NAA function in the brain is still unclear but this neuronal metabolite has received considerable interest as a biomarker for neurodegeneration. Indeed, NAA is produced in neurons and a decrease in its concentration is classically interpreted as neuronal degeneration or at least dysfunction (Moffet *et al.*, 2007). However, it is now recognized that neurons are not the only players in the game. Astrocyte reactivity occurs in response to many pathological brain situations (Escartin and Bonvento, 2008), including neuronal degeneration. Reactive astrocytes display morphological and functional changes that could result in changes in the concentration of some brain metabolites more specifically localized in astrocytes such as myo-inositol. In addition, astrocytes reactivity could trigger indirect effects on neurons and their metabolism.

In this study, we aimed at dissecting the MRS signature associated with astrocyte reactivity *per se*. We took advantage of a model of selective astrocyte activation using overexpression of the cytokine ciliary neurotrophic factor (CNTF, Escartin *et al.*, 2006). CNTF leads to a selective activation of astrocytes in absence of detectable effects on neurons. Using this model, we show that, contrary to the classical interpretation, a decrease in the NAA to creatine ratio can occur in absence of neurodegeneration.

Methods

Animal model: Experiments were performed using a rat model (10 animals) of astrocyte activation induced by stereotactic injection of lentiviral vectors encoding for CNTF (lenti-CNTF) into the right striatum (Escartin *et al.*, 2006). The contralateral striatum was injected by a control lentiviral vector that encodes for beta-galactosidase (lenti-LacZ).

NMR Experiments: MRS data were acquired on a 7 T Agilent magnet in two voxels positioned in each striatum (Fig. 1). A LASER (Localization by Adiabatic Selective Refocusing) sequence with echo time TE=40 ms was used. Repetition time was 2 s. Chemical shift localization error was less than ±5% over the 2-4 ppm range. Using LCModel, concentrations could be measured with good precision (Cramér-Rao lower bounds <5%) for total N-acetyl-aspartate (NAA+NAAG), myo-inositol (Ins), total choline (Cho), glutamate and taurine relative to total creatine (tCr). All concentrations were calculated relative to tCr, as it is usually performed, in particular in a clinical context.

Results

Lenti-CNTF injection promotes a sustained, extensive and selective activation of astrocytes, as evidenced by overexpression of GFAP and vimentin and cellular hypertrophy. Importantly, neurons display normal morphological and molecular features (Fig. 2 and Escartin *et al.*, 2006). Indeed, previous studies have reported that CNTF overexpression does not alter the electrophysiological properties of neurons (Beurrier *et al.*, 2010) and even more, promote significant neuroprotective effects against various insults (Mittoux *et al.*, 2002, Beurrier *et al.*, 2010).

Examples of spectra are given in Fig. 3, with visually striking differences. Statistical analysis (N=10) confirmed significant changes (significance threshold: $p < 0.05$, paired *t*-test) in metabolite concentration in this model: in the lenti-CNTF injected striatum, levels of the astrocyte metabolites Ins and Cho were increased (+38% and +19%, respectively) whereas levels of the neuronal metabolite NAA and glutamate were decreased (-17% and -12%, respectively) (Fig. 4). Although concentrations were calculated relative to tCr, it is interesting to note that the absolute tCr signal (arbitrary units) was identical in both groups. Note that for each single animal, the decrease of tCho and Ins and the increase of NAA could be detected. In contrast, an experiment was performed on a non-injected rat where no difference could be seen between both hemispheres.

Discussion

The increase of Ins and Cho levels in reactive astrocytes does not come as a surprise, since they are both preferentially compartmentalized in astrocytes. In contrast, the decrease in NAA (and Glu) is unexpected given that neurons are not dysfunctional with CNTF. To understand how astrocyte reactivity can influence NAA levels, we are currently characterizing the molecular changes induced by astrocyte reactivity using quantitative PCR, biochemistry, histology and high performance liquid chromatography.

Conclusion

Our results suggest that reactive astrocytes alone, in the absence of neuronal degeneration, result in decreased levels of NAA and Glu as observed by MRS. Although the mechanisms by which activated astrocytes regulate neuronal metabolism remain to be elucidated, our work challenges the MRS dogma of decreased neuronal metabolites, in particular NAA, associated with neuronal degeneration.

References

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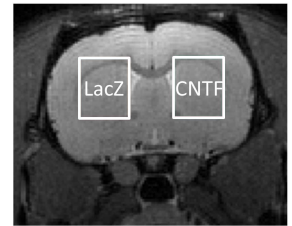


Fig.1: Position of the 40.8 μL voxels in the rat striatum.

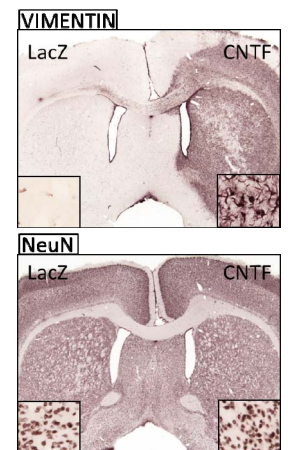


Fig.2: lenti-CNTF injection leads to astrocyte activation (upper image) with no detectable effect on neurons (bottom image).

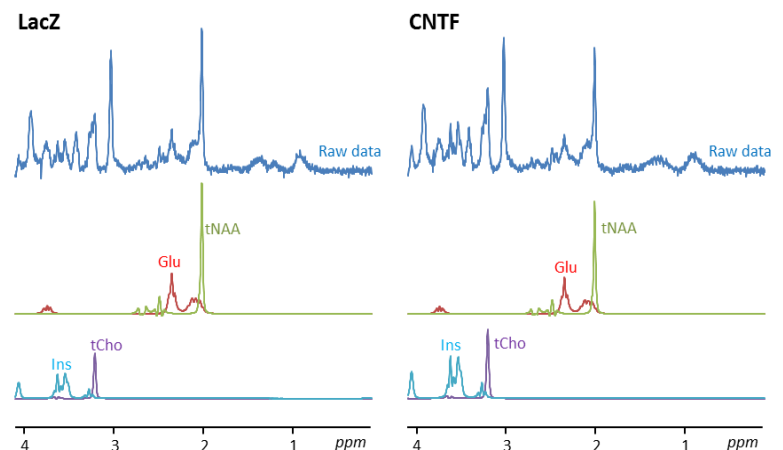


Fig.3: Examples of spectra (no filtering applied) obtained in control and CNTF striatum. The decrease of NAA and the increase of tCho and Ins are visually striking.

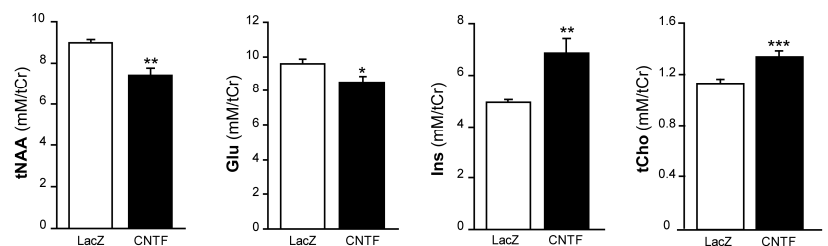


Fig.4: Astrocyte activation by CNTF alters the concentration of metabolites measured by MRS on 10 rats, with neuronal NAA and Glu decreasing, and astrocytic Ins and tCho increasing (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).