What is the physiological role of the neurotransmitter transporter 4 (NTT4) in the central nervous system?

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The neurotransmitter transporter 4 (NTT4) is a member of the solute carrier family 6 (SLC6) or the neurotransmitter transporter family of membrane transporters. Members of this family have a significant physiological relevance and have been implicated in several disorders and conditions such as anxiety, epilepsy, attention deficit hyperactivity disorder (ADHD), cocaine addiction, obesity, and Hartnup disorder (Brõer, 2006).

NTT4 (SLC6A17) is distributed widely across the central nervous system in the pre-synaptic axon terminals of most glutamatergic and a small sub-section of GABAergic neurons (el Mestikawy *et al.*, 1997). It belongs to the branch of "orphan" transporters, which was initially called so because the function of the members of this branch could not be identified. However, since the identification of SLC6A15, SLC6A19 and SLC6A20 as amino acid transporters, it is alleged that the orphan transporters might in fact all be transporters of amino acids (Brõer, 2006). Although NTT4 is a member of this amino acid transporter branch, its function is still unknown. The aim of this study was to identify substrate(s) transported by NTT4, and thus hypothesize its function in the central nervous system.

*Xenopus laevis** oocytes were chosen as an appropriate expression system to study the function of the transporter. Confocal microscopy on oocytes expressing eGFP- NTT4 suggested that the transporter was expressed on the oocyte surface membrane. This was further confirmed by the surface biotinylation of oocytes expressing NTT4.

Radioactive uptake experiments initially suggested that the uptake of radiolabeled leucine, proline, glycine and glutamine was significantly higher in oocytes expressing NTT4 as compared to non-injected oocytes. To confirm that this uptake was due to NTT4, a conserved arginine residue (R85) was mutated to serine in an attempt to make the transporter non-functional. However, uptake of labelled leucine, proline, glycine and glutamine was still observed in oocytes expressing NTT4-R85S.

To confirm that the R85S mutation made the transporter non-functional, the same mutation was created in SLC6A15 (B0AT2), the closest homolog of NTT4. Radioactive uptake of proline showed that the R85S mutation reduced transport activity to background levels. To further analyse if NTT4 needed to be modified to be functional, two putative phosphorylation sites were identified. These two residues were mutated and the uptake of leucine, proline, glycine and glutamine was measured. However, there was no significant difference in the uptake of these amino acids in oocytes expressing the wild type and phosphorylation mutants of NTT4.

Although the function of NTT4 is still unknown, further studies using electrophysiology and potential protein interactions of the transporter could elucidate its role in the central nervous system.

Brõer S. (2006) *Neurochemistry International*, **48:** 559-67.
el Mestikawy S, Wehrle R, Masson J, Lombard MC, Hamon M & Sotelo C. (1997) *Neuroscience*, **77:** 319-33

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* Procedures to remove oocytes were approved by the animal ethics committee of the ANU.