

## The subcellular localization of administered N-acetylneuraminic acid in the brains of well-fed and protein restricted rats

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1. This study investigated the subcellular localization of injected N-acetylneuraminic acid (NeuNAc) in brain. Forty pregnant rats were distributed into four groups. Two groups were given a 200 g casein/kg diet and the other two groups a 100 g casein/kg diet throughout gestation. One group from each of the low- and high-protein groups were given their respective diets for the first 11 d of lactation. On day 12 of lactation, 2.5  $\mu\text{Ci}$  [ $^{14}\text{C}$ ]NeuNAc/kg body-weight were injected intraperitoneally into their pups. After 1 h the pups were killed, their brains removed and subjected to subcellular fractionation. On day 16 of lactation the other two groups were similarly treated.

2. In all groups of animals 80% of the [ $^{14}\text{C}$ ]NeuNAc incorporated into the brains was found in the synaptosomal fraction and the remainder distributed among the other subcellular fractions in proportion to their total NeuNAc content.

3. These results suggest that NeuNAc exerts its effects on behaviour via the synaptic membrane.

Among the structural and biochemical changes induced by early malnutrition in the brain are a reduction in the extent of dendritic arborization (Salas *et al.* 1974) and a decreased brain content of ganglioside and glycoprotein N-acetylneuraminic acid (NeuNAc; Merat & Dickerson, 1974; Morgan and Naismith, unpublished results).

Our recent work has shown that early stimulation reduces the abnormalities in behaviour caused by malnutrition as well as resulting in an elevated brain ganglioside and glycoprotein NeuNAc content (Morgan & Winick, 1980*a*). We have also demonstrated that [ $^{14}\text{C}$ ]NeuNAc administered intraperitoneally during the first 30 d of life becomes incorporated into the brain gangliosides and glycoproteins of well-fed and undernourished rat pups (Morgan & Winick, 1980*b*). When malnourished pups were given daily 1 mg NeuNAc/50 g body-weight there was a permanent reduction in the expected behavioural abnormalities secondary to malnutrition as well as an increase in brain NeuNAc content.

All cell membranes contain NeuNAc which contributes to the negative charge of the membrane. Neurotransmitters are positively charged and if NeuNAc were concentrated in the synaptic membrane its negative charge might facilitate neurotransmission by facilitating the binding of transmitter molecules to the membrane. We therefore investigated the hypothesis that NeuNAc administered intraperitoneally to rat pups becomes incorporated into the synaptosomal membrane and so affects neurotransmission.

### MATERIALS AND METHODS

Forty littermate pairs of pregnant rats of the Sprague–Dawley strain were selected on the basis of body-weight and caged in pairs. On the third day of gestation they were randomly distributed into two groups and housed individually. One group was fed *ad lib.* a diet containing 200 g casein/kg. The other group was given a diet with 100 g casein/kg (Morgan & Winick, 1980*a*). These diets were fed to their respective groups throughout the experimental period.

On the third day post partum the litters were reduced to eight pups. [ $^{14}\text{C}$ ]NeuNAc (2.5  $\mu\text{Ci}$ /kg body-weight) was injected intraperitoneally into the pups from each of ten litters of protein restricted and ten litters of well-fed dams on day 12 post partum. After

Table 1. *Brain weights (g) of well-fed and malnourished rat pups*  
(Mean values with their standard errors)

Age (d)...	12		16	
	Mean	SE	Mean	SE
Well-fed	0.95	0.02	1.35	0.04
Undernourished	0.77	0.03*	1.18	0.03*

\* Values for malnourished pups were statistically significantly different from those for control pups ( $P < 0.001$ ; Student's *t* test (Bruning & Kintz, 1968)).

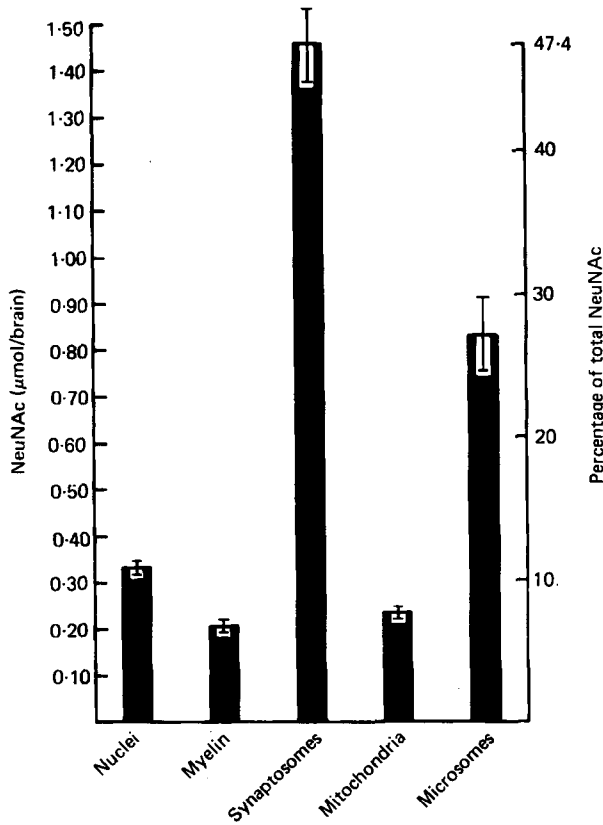


Fig. 1. The N-acetylneuraminic acid (NeuNAC) content of the subcellular fractions of the brains of 12-d-old well-fed rat pups. Values are means with their standard errors represented by vertical bars for ten determinations.

1 h the rats were killed and their brains removed. Similarly, at day 16 post partum, ten litters of protein restricted and ten litters of well-fed pups were injected with NeuNAC and their brains removed.

The brains of whole litters were pooled and homogenized (medium-tissue 10:1, v/w) in 0.32 M-sucrose adjusted to pH 7.0 (with sodium hydroxide). The homogenates were subjected to subcellular fractionation using the method reported by Wolfe (1961) to give nuclear, myelin-rich, synaptosomal, mitochondrial and microsomal fractions. These

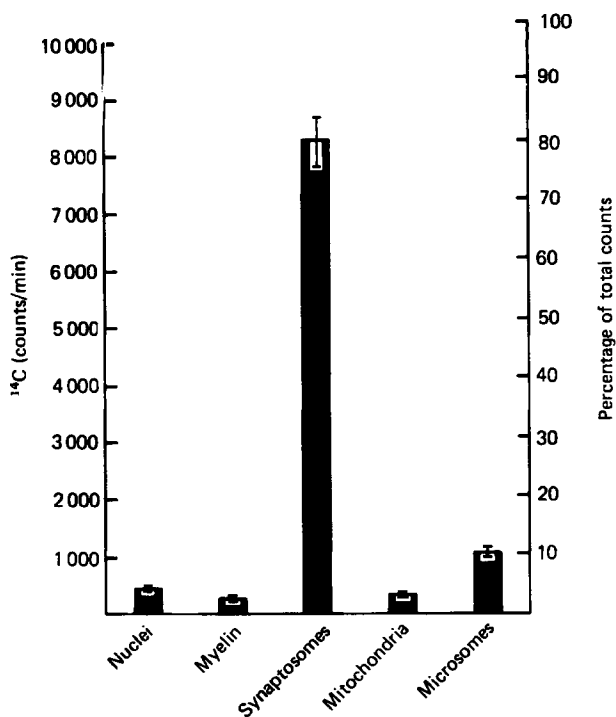


Fig. 2. Incorporation of [4-<sup>14</sup>C]N-acetylneuraminic acid (NeuNAc) into the subcellular fractions of the brains of 12-d-old well-fed rat pups. Values are means with their standard errors represented by vertical bars for ten determinations.

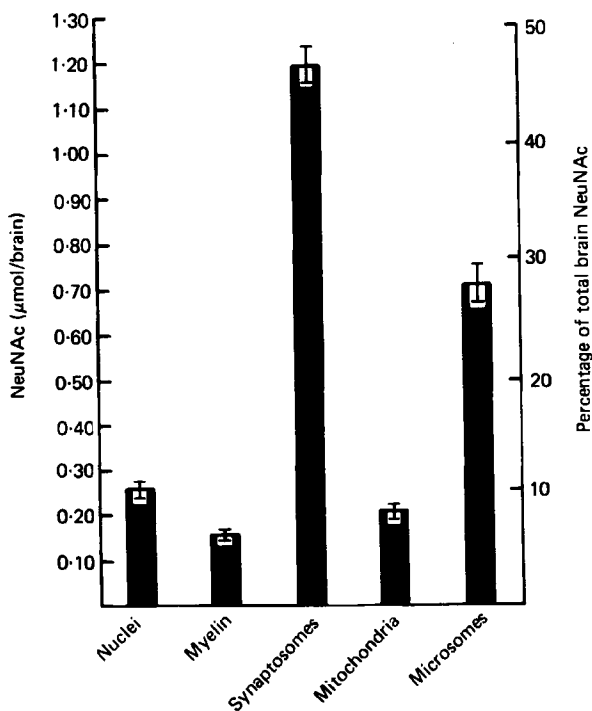


Fig. 3. The N-acetylneuraminic acid (NeuNAc) content of the subcellular fractions of the brains of 12-d-old protein restricted rat pups. Values are means with their standard errors represented by vertical bars for ten determinations.

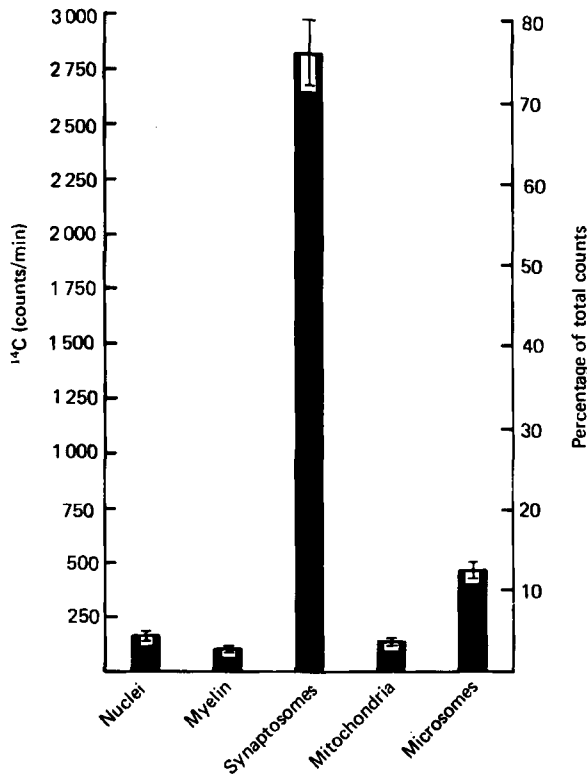


Fig. 4. Incorporation of [4-<sup>14</sup>C]N-acetylneuraminic acid (NeuNAc) fractions of the brains of 12-d-old protein restricted rat pups. Values are means with their standard errors represented by vertical bars for ten determinations.

extracts were divided into two equal portions. One portion was dissolved in liquid solubilizer and counted for incorporation of radioactively labelled NeuNAc. The other portion was hydrolyzed with 0.1 M-sulphuric acid for 2 h at 80°, centrifuged and the supernatant fraction assayed for total NeuNAc content by the thiobarbituric acid assay (Warren, 1959).

#### RESULTS

Table 1 shows that at both time-points measured, the protein restricted rats had brains that were significantly smaller than their well-fed counterparts.

Fig. 1 shows the NeuNAc content of the brain subcellular fractions in the well-fed rats at day 12. From Fig. 1 it can be seen that the percentage distribution of brain NeuNAc was: (%) 10.8 nuclei, 6.6 myelin, 47.4 synaptosomes, 7.8 mitochondria, 27.4 microsomes.

Fig. 2 shows the incorporation of radioactive NeuNAc into the subcellular brain fractions of the same well-fed rats. Here we see that 80% of the radioactive NeuNAc was incorporated into the synaptosomal fraction. The amount incorporated into the other fractions was in proportion to their NeuNAc content.

Fig. 3 shows the NeuNAc content of the subcellular brain fractions of the protein restricted rats. Although every fraction contained less total NeuNAc than in the well-fed group the distribution of NeuNAc between the fractions was the same in the two groups. Most of the NeuNAc was found in the synaptosomal fraction, i.e. 46.7%. The nuclei contained (%) 10.1, myelin 6.1, mitochondria 8.0 and microsomes 27.7.

Fig. 4 shows the subcellular incorporation of [4-<sup>14</sup>C]NeuNAc. Here once again the

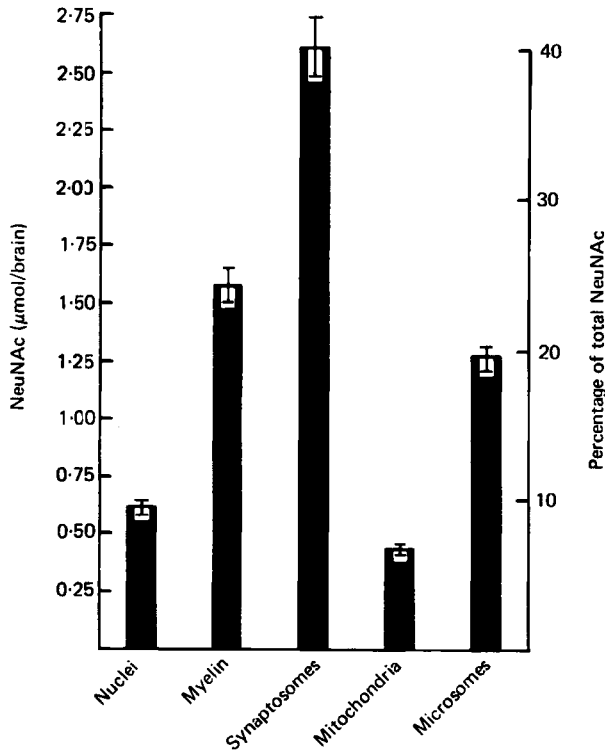


Fig. 5. The N-acetylneuraminic acid (NeuNAc) content of the subcellular fractions of the brains of 16-d-old well-fed rat pups. Values are means with their standard errors represented by vertical bars for ten determinations.

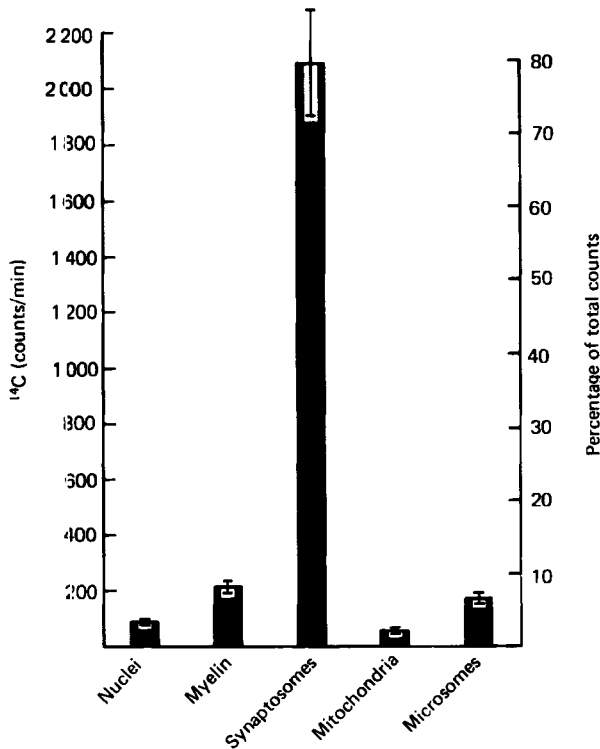


Fig. 6. Incorporation of [4-<sup>14</sup>C]N-acetylneuraminic acid (NeuNAc) into the subcellular fractions of the brains of 16-d-old well-fed rat pups. Values are means with their standard errors represented by vertical bars for ten determinations.

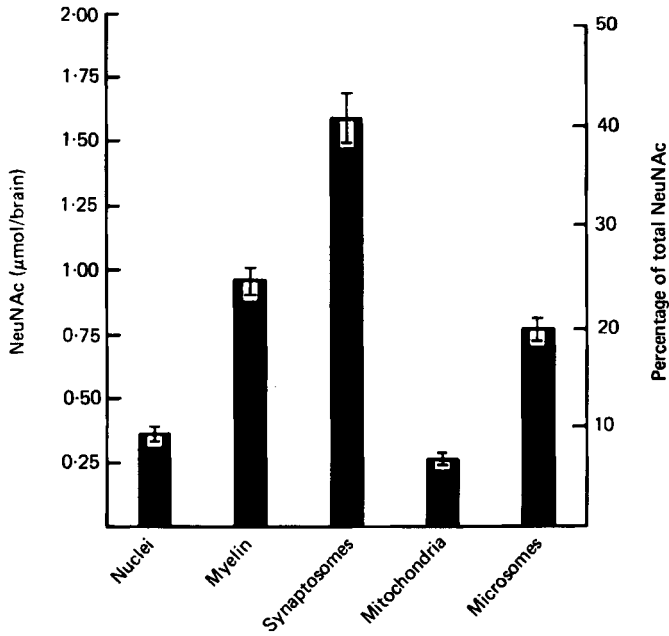


Fig. 7. The NeuNAc content of the subcellular fractions of the brains of 16-d-old protein restricted rat pups. Values are means with their standard errors represented by vertical bars for ten determinations.

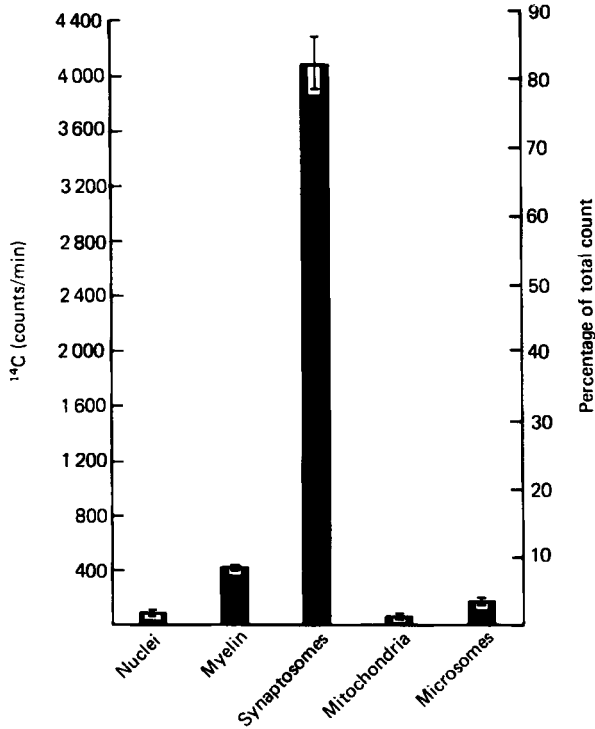


Fig. 8. The NeuNAc content of the subcellular fractions of the brains of 16-d-old protein restricted rat pups. Values are means with their standard errors represented by vertical bars for ten determinations.

results mimic those of the well-fed rats. Again less [4-<sup>14</sup>C]NeuNAc was incorporated into each fraction but the relative levels of incorporation into the various fractions was the same. Of the labelled NeuNAc 76% was incorporated into the synaptosomal fraction and the remainder was divided up between the other fractions in proportion to their NeuNAc content.

Figs. 5 and 6 show the values for the 16-d-old well-fed rats. In Fig. 5 it is once again apparent that over 40% of the brain NeuNAc was contained in the synaptosomal fraction. In Fig. 6 we see that approximately 80% of the injected [4-<sup>14</sup>C]NeuNAc became incorporated into the synaptosomal fraction.

Figs. 7 and 8 show the values for the 16-d-old protein restricted rat pups. Fig. 7 reiterates what was observed in the 12-d-old protein restricted animals namely that the synaptosomal fraction contained more NeuNAc than any other fraction. Fig. 8 illustrates the values for incorporation of radioactive NeuNAc into the brains of the same animals. As with the other groups of animals between 70 and 80% of the total NeuNAc incorporated found its way to the synaptosomal fraction. The other fractions took up NeuNAc in proportion to their total NeuNAc content.

#### DISCUSSION

These results confirm our earlier findings that the brains of protein deprived rats at days 12 and 16 post partum contain less NeuNAc than their well-fed controls. We have also once again shown that 12-d-old pups incorporate less [4-<sup>14</sup>C]NeuNAc into their brains when given the isotope intraperitoneally whereas 16-d-old pups incorporate much more (Morgan & Winick, 1980b).

Several workers have studied the subcellular localization of ganglioside NeuNAc in brain. Many like Lapetina *et al.* (1967), Wolfe (1961) and Brunngraber *et al.* (1967) have shown that the major portion is located in the synaptosomes. Others like Ledeen (1978) maintain that NeuNAc is equally distributed among all neural cell membranes. We have provided evidence to support the former view. Both in the well-fed and protein restricted groups the synaptosomal fraction contained by far the major portion of the brain NeuNAc. Further, at days 12 and 16 post partum, which are times of rapid proliferation of neuronal processes, the synaptosomes clearly incorporate NeuNAc more readily than any other brain fraction.

Previous results from our laboratory have clearly shown that intraperitoneal injections of NeuNAc during the first 3 weeks of life give rise to an increased brain ganglioside and glycoprotein NeuNAc content. Moreover the increased NeuNAc content is associated with an amelioration of the behavioural abnormalities shown by undernourished rats which persists into adulthood (Morgan & Winick, 1980b).

Neural cell membranes contain twenty times as much NeuNAc as any other type of cell membrane in the body and it clearly has an important role to play (Ledeen, 1978). The results reported here support the view that NeuNAc exerts its effects on behaviour at the synapse. The negative charge associated with it may facilitate binding of neurotransmitters to the synaptosomal membrane as suggested by Weseman *et al.* (1971). On the other hand it could alter the containing membrane of the synaptic vesicles in some way and so affect its ability to take up substrates for neurotransmitter synthesis (Weseman, 1969). Further experiments are planned to clarify which mechanism is operative.

#### REFERENCES

- Bruning, J. L. & Kintz, B. L. (1968). *Computational Handbook of Statistics*, pp. 12-15. Glenview, Illinois: Scott Foresman and Co.
- Brunngraber, E. G., Dekirmenjian, M. & Brown, R. D. (1967). *Biochem. J.* **103**, 73.
- Lapetina, E. G., Soto, E. F. & DeRobertis, E. (1967). *Biochim. Biophys. Acta* **135**, 33.
- Ledeen, R. W. (1978). *J. Supramol. Struct.* **8**, 1.

- Merat, A. & Dickerson, J. W. T. (1974). *Biol. Neonate* **25**, 158.  
Morgan, B. L. G. & Winick, M. (1980*a*). *J. Nutr.* **110**, 425.  
Morgan, B. L. G. & Winick, M. (1980*b*). *J. Nutr.* **110**, 416.  
Salas, M., Diaz, S. & Nieto, A. (1974). *Brain Res.* **73**, 139.  
Warren, L. (1959). *J. biol. Chem.* **234**, 1971.  
Weseman, W. (1969). *FEBS Lett.* **3**, 80.  
Weseman, W., Henkel, R. & Marx, R. (1971). *Biochem. Pharm.* **20**, 1961.  
Wolfe, L. S. (1961). *Biochem. J.* **79**, 348.