# 3 Results

## 3.1 Method validation

### 3.1.1 HPLC

- #### 3.1.1.1 Separation of iodothyronines by HPLC

Figure 5 shows the HPLC chromatogram for $T_4$, $rT_3$, $T_3$, 3,3'-$T_2$ and 3,5-$T_2$ obtained by using a gradient of acetonitrile and water (containing 1% acetic acid), with a linear increase from 36% acetonitrile at time zero to 38% after 25 min. Using this method, the [$^{125}$I]-$T_4$, [$^{125}$I]-$rT_3$, [$^{125}$I]-$T_3$, [$^{125}$I]-3,3'-$T_2$, and 3-Br-[5-$^{125}$I]-$T_1$ tracers, and the endogenous amounts of the above-mentioned iodothyronines of the tissue probes, were eluted with retention times of 22-24 min, 18-20 min, 14-15 min, 9-10 min and 5-6 min, respectively (fig 5).

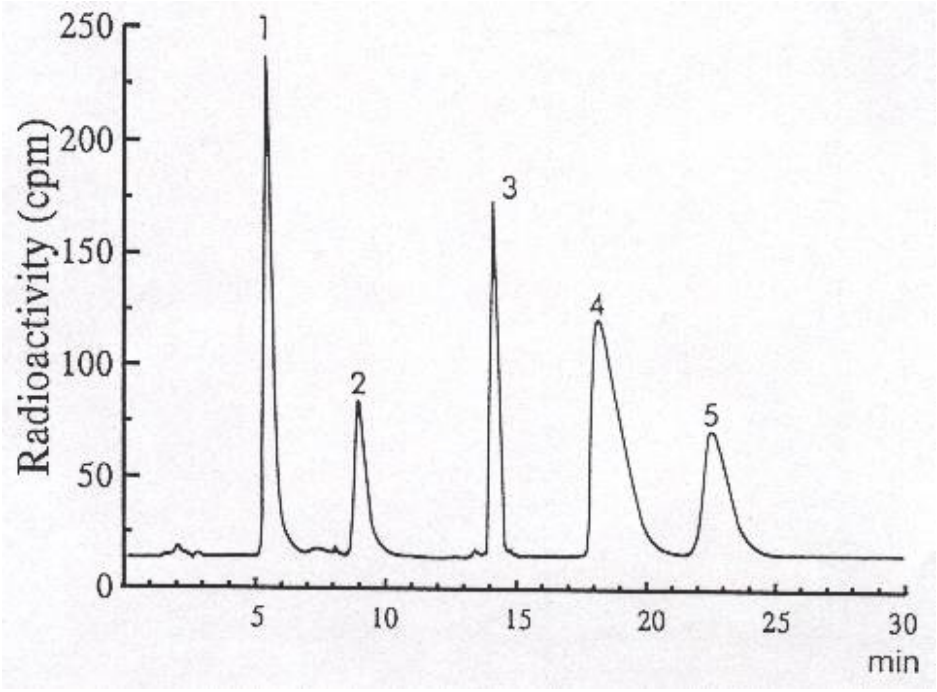


**Fig 5**. HPLC chromatogram resulting from the separation of the 3-Br-[5-$^{125}$I]-$T_1$ (**1**), [$^{125}$I]-3,3'-$T_2$ (**2**), [$^{125}$I]-$T_3$ (**3**), [$^{125}$I]-$rT_3$ (**4**), [$^{125}$I]-$T_4$ (**5**).

The entire process took 35 min for each probe. Five minutes at 2 ml/min flow rate were required to wash the column with 100% acetonitrile to eliminate contaminants contained in the tissue samples. Five additional minutes at 2 ml/min flow rate were needed to re-equilibrate the column with the solution of acetonitrile and 1% acetic acid-water (36:64) before the next probe was injected.

- **3.1.1.2 Recovery of iodothyronines after extraction and HPLC**

After extraction and HPLC separation and purification, recovery rates for labeled iodothyronines ranged from 45% to 50% for $T_4$, 60% to 70% for $rT_3$, 60% to 75% for $T_3$, 60% to 70% for 3,3'-$T_2$, and 65% to 80% for 3,5-$T_2$. Approximately 15% to 20% were lost in the filters and another 5% to 10% during HPLC.

### 3.1.2 RIA for 3,5-$T_2$

- **3.1.2.1 RIA sensitivity for 3,5-$T_2$**

Figure 6 represents the standard curve showing the displacement of the 3-Br-5-[$^{125}$I]-$T_1$ tracer and the specific antibody to 3,5-$T_2$, effected by increasing concentrations of non-radioactive 3,5-$T_2$. The lowest standard concentration of 0.48 fmol/tube induced a 20% inhibition of 3-Br-5-[$^{125}$I]-$T_1$ binding. This sensitivity threshold allowed the detection of 1.0 fmol/g and 0.8 pmol/l of 3,5-$T_2$ in tissue and in serum, respectively.

- **3.1.2.2 Cross-reactivity of 3,5-$T_2$ antibody with iodothyronines**

Table 1 shows the abilities of various iodothyronines and iodotyrosines, tested in four or five different concentrations, to displace the binding of 3,5-$T_2$ to its specific antibody. Cross-reaction of the 3,5-$T_2$ antibody with almost all

compounds tested was minimal. In particular, Diac and 3-$T_1$ cross-reacted with the 3,5-$T_2$ antibody to the extent of 0.13% and 0.65%, respectively. Particular attention was paid to the cross-reaction with $T_3$. Following the addition of several supraphysiological concentrations of non-radioactive $T_3$ to the serum of four healthy controls, the serum was extracted and subsequently processed for 3,5-$T_2$ measurement. Applying this procedure, $T_3$ cross-reacted with the 3,5-$T_2$ antibody to the extent of 0.06%.

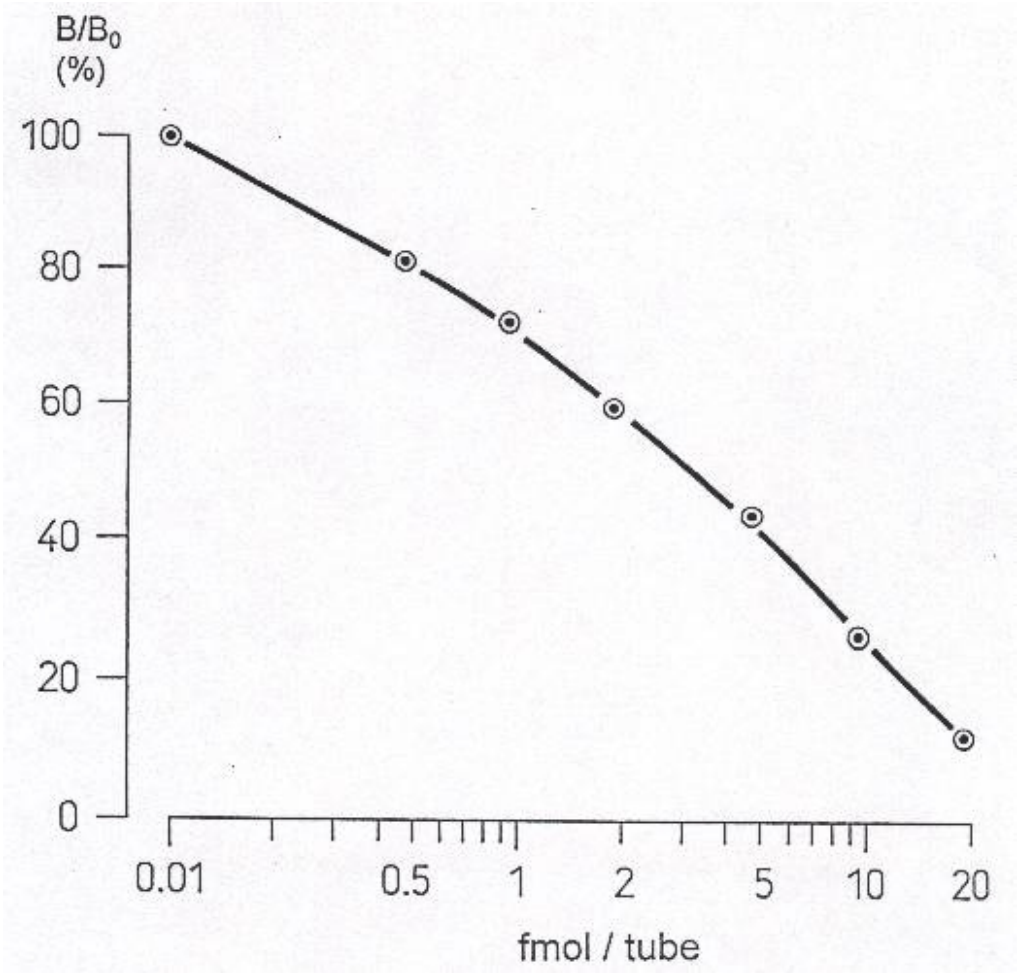


**Fig 6**. Standard curve for 3,5-$T_2$. Increasing concentrations of non-radioactive 3,5-$T_2$ were added to displace the binding of the 3-Br-[5-$^{125}$I]-$T_1$ tracer and its specific antibody.

**Table 1**. Relative cross-reactivities of several iodothyronines with 3,5-$T_2$ antibody.

| Compounds | Relative Reactivity (%) |
|---|---|
| 3,5-$T_2$ | 100 |
| 3,5-$br_2$-$T_0$ | < 0,14 |
| $T_3$ | 0,06 |
| $T_4$ | 0,001 |
| $rT_3$ | 0,001 |
| Tetrac | < 0,0001 |
| Triac | 0,0006 |
| Diac | 0,13 |
| 3',5'-$T_2$ | 0,001 |
| 3,3'-$T_2$ | 0,005 |
| 3-$T_1$ | 0,65 |
| 3'-$T_1$ | 0,0005 |
| DIT | < 0,0001 |
| MIT | < 0,0001 |

Tetrac, tetraiodothyroacetic acid; Triac, triiodothyroacetic acid; Diac, diiodothyroacetic acid; DIT, diiodotyrosine; MIT, monoiodotyrosine.

- **3.1.2.3 Cross-reactivity of 3,5-$T_2$ antibody with drugs**

Table 2 shows the effects of several drugs used for the treatment of intensive care patients on the binding of 3-Br-5-[$^{125}$I]-$T_1$ to its specific antibody. Four of the 12 drugs tested showed no detectable effects at all. The highest cross-reactivity was measured for imipinem, vancomycine, and norepinephrine (1.3 x $10^{-7}$; 1.2 x $10^{-7}$, and 7.4 x $10^{-7}$, respectively). The cross-reactivity of all other drugs was between one and three orders of magnitude lower.

**Table 2**. Cross-reactivity of various drugs with antiserum to 3,5-$T_2$.

| Drugs | Cross-reactivity (by wt) |
|---|---|
| Imipinem | $1{,}3 \times 10^{-7}$ |
| Cefotaxime | $1 \times 10^{-10}$ |
| Vancomycine | $1{,}2 \times 10^{-7}$ |
| Dopamine | $3{,}7 \times 10^{-8}$ |
| Norepinephrine | $7{,}4 \times 10^{-7}$ |
| Dobutamine | $1 \times 10^{-10}$ |
| Fentanyl | $1 \times 10^{-10}$ |
| Furosemide | $1{,}8 \times 10^{-8}$ |
| Propofol | $1{,}2 \times 10^{-8}$ |
| Norcuron | $1 \times 10^{-10}$ |
| Heparin | *a* |

[a] Heparin (0.25-100 IU/assay tube) did not affect 3-Br,5-[$^{125}$I]-$T_1$ binding to antiserum.

- **3.1.2.4 Recovery of "cold" 3,5-$T_2$**

After the addition of 0 pmol/l, 2.5 pmol/l, 5 pmol/l, and 10 pmol/l of non-labeled 3,5-$T_2$ to pooled serum obtained from euthyroid patients, the endogenous concentrations of 3,5-$T_2$ were determined and subtracted, and the mean (± SD) recoveries of each added amount of 3,5-$T_2$ were calculated. These were 98.5 ± 7.3%, 96 ± 9.6%, and 101 ± 8.1%, respectively.

- **3.1.2.5 Inter- and intra-assay coefficients of variation**

Inter- and intra-assay coefficients of variation (CVs) for tissue samples were determined in four tests. The intra-assay CVs ranged from 6.6% to 7.8%, the inter-assay CVs from 7.7% to 8.2%. The measurements were performed using

two different samples, both of which caused approximately 50% inhibition of 3,5-$T_2$ antibody binding (table 3).

**Table 3**. Variability of the 3,5-$T_2$ measurements in brain tissue.

| sample | tissue conc. (fmol/g) mean ± SEM | No. of assay | CV (%) |
|---|---|---|---|
| **Intra-assay CV** | | | |
| **1** | **29 ± 2.9** | **4** | **6.6** |
| **2** | **42 ± 3.1** | **4** | **7.8** |
| **Inter-assay CV** | | | |
| **1** | **19 ± 2.1** | **8** | **7.7** |
| **2** | **23 ± 1.3** | **8** | **8.2** |

CV, Coefficient of variation.

### 3.1.3 RIA for 3,3'-$T_2$

- #### 3.1.3.1 RIA sensitivity for 3,3'-$T_2$

Figure 7 represents the standard curve showing the displacement of the 3,3'-$T_2$ tracer from the specific antibody to 3,3'-$T_2$, effected by increasing concentrations of non-radioactive 3,3'-$T_2$. The lowest standard concentration of 0.48 fmol/tube induced a 10% inhibition of 3,3'-[$^{125}$I]-$T_2$ binding. This

sensitivity threshold allowed the detection of 1.8 fmol/g and 1.5 pmol/l of 3,3'-$T_2$ in tissue and in serum, respectively.

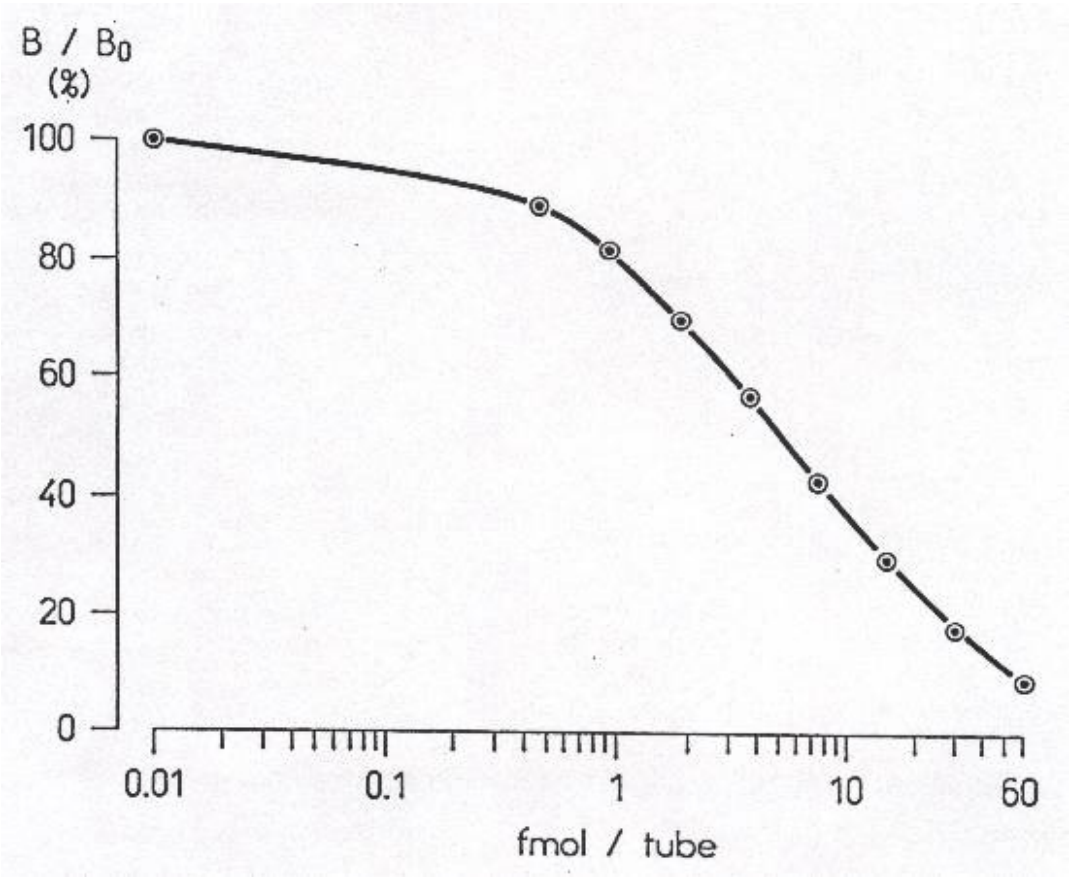


**Fig 7**. Standard curve for 3,3'-$T_2$. Increasing concentrations of non-radioactive 3,3'-$T_2$ were added to displace the binding of the 3,3'-[$^{125}$I]-$T_2$ tracer and its specific antibody.

- **3.1.3.2 Cross-reactivity of 3,3'-$T_2$ antibody with iodothyronines**

Table 4 shows the proportions of various iodothyronines and iodotyrosines, tested in four or five different concentrations, that bound to the specific antibody of 3,3'-$T_2$. Cross-reaction of the 3,3'-$T_2$ antibody with the majority of the compounds tested was minimal. In particular, only 0.04% of Triac reacted with the 3,3'-$T_2$ antibody.

**Table 4**. Relative cross-reactivities of several iodothyronines with 3,3'-$T_2$ antibody.

| Compounds | Relative Reactivity (%) |
|---|---|
| 3,3'-$T_2$ | 100 |
| 3,5-$T_2$ | 0,00006 |
| $T_3$ | < 0,001 |
| $T_4$ | < 0,00003 |
| $rT_3$ | 0,007 |
| Tetrac | < 0,0001 |
| Triac | < 0,04 |
| Diac | 0,00001 |
| 3',5'-$T_2$ | < 0,00003 |
| 3-$T_1$ | 0,0001 |
| 3'-$T_1$ | < 0,001 |
| DIT | < 0,00001 |
| MIT | 0,004 |

Tetrac, tetraiodothyroacetic acid; Triac, triiodothyroacetic acid; Diac, diiodothyroacetic acid; DIT, diiodotyrosine; MIT, monoiodotyrosine

- **3.1.3.3 Cross-reactivity of 3,3'-$T_2$ antibody with drugs**

Table 5 shows the effects of several drugs used for the treatment of intensive care patients on the binding of 3,3'-[$^{125}$I]-$T_2$ to its specific antibody. Two of the 12 drugs tested showed no detectable effects at all. The highest cross-reactivity was measured for fentanyl ($2 \times 10^{-5}$). The cross-reactivity of all other drugs was between one and five orders of magnitude lower.

**Table 5**. Cross-reactivity of various drugs with antiserum to 3,3'-$T_2$.

| Drugs | Cross-reactivity (by wt) |
|---|---|
| Imipinem | $5{,}6 \times 10^{-6}$ |
| Cefotaxime | $1 \times 10^{-6}$ |
| Vancomycine | $9 \times 10^{-8}$ |
| Dopamine | $1 \times 10^{-7}$ |
| Norepinephrine | $8 \times 10^{-7}$ |
| Dobutamine | $9 \times 10^{-6}$ |
| Fentanyl | $2 \times 10^{-5}$ |
| Furosemide | $1 \times 10^{-10}$ |
| Propofol | $1 \times 10^{-8}$ |
| Norcuron | $1 \times 10^{-10}$ |
| Heparin | *a* |

[a] Heparin (0.25-100 IU/assay tube) did not affect 3,3'-[$^{125}$I]-$T_2$ binding to antiserum.

- **3.1.3.4 Recovery of "cold" 3,3'-$T_2$**

After the addition of 0 pmol/l, 4.7 pmol/l, 20 pmol/l, and 78 pmol/l of non-labeled 3,3'-$T_2$ to pooled serum obtained from euthyroid patients, the endogenous concentrations of 3,3'-$T_2$ were determined and subtracted, and the mean (± SD) recoveries of each added amount of 3,3'-$T_2$ were calculated. These were 97.9 ± 6.6%, 95 ± 8.2% and 98.7 ± 7.3%, respectively.

- **3.1.3.5 Inter- and intra-assay coefficients of variation**

The inter- and intra-assay CVs for tissue samples were determined in five tests. The intra-assay CVs ranged from 6.8% to 7.2% and the inter-assay CVs from 7.4% to 8.6%. The measurements were performed using two different

samples, both of which caused approximately 50% inhibition of 3,3'-$T_2$ antibody binding (table 6).

**Table 6**. Variability of the 3,3'-$T_2$ measurements in brain tissue.

| sample | tissue conc. (fmol/g) mean ± SEM | No. of assay | CV (%) |
|---|---|---|---|
| **Intra-assay CV** | | | |
| **1** | **69 ± 3.9** | **4** | **6.8** |
| **2** | **74 ± 3.3** | **4** | **7.2** |
| **Inter-assay CV** | | | |
| **1** | **56 ± 4.1** | **8** | **7.4** |
| **2** | **43 ± 4.5** | **8** | **8.6** |

CV, Coefficient of variation.

## 3.2 Clinical Studies

### 3.2.1 Serum concentrations of 3,5-$T_2$

- #### 3.2.1.1 Healthy controls

The mean concentration of 3,5-$T_2$ for the group of healthy controls (n = 62) was 16.2 ± 6.4 pmol/l. Linear regression analysis conducted to investigate the effects of age and sex on the variable "hormone" revealed no significant effect.

Therefore, in all further calculations the serum levels of 3,5-$T_2$ of all healthy controls were compared with those of the respective group of the patient sample. The normal range of 3,5-$T_2$ was defined as the mean ± 2 standard deviations (fig 8).

- **3.2.1.2 Patients with thyroid disorders**

Figure 8 shows the results for the serum concentrations of 3,5-$T_2$ in patients with thyroidal disorders. The serum levels of 3,5-$T_2$ were significantly enhanced in patients with hyperthyroidism ($p$ = 0.009), whereas hypothyroid patients showed decreased concentrations of 3,5-$T_2$. Four of the 9 patients with hyperthyroidism had serum 3,5-$T_2$ concentrations above the normal range, and 7 of the 8 hypothyroid patients had serum 3,5-$T_2$ concentrations that were not measurable.

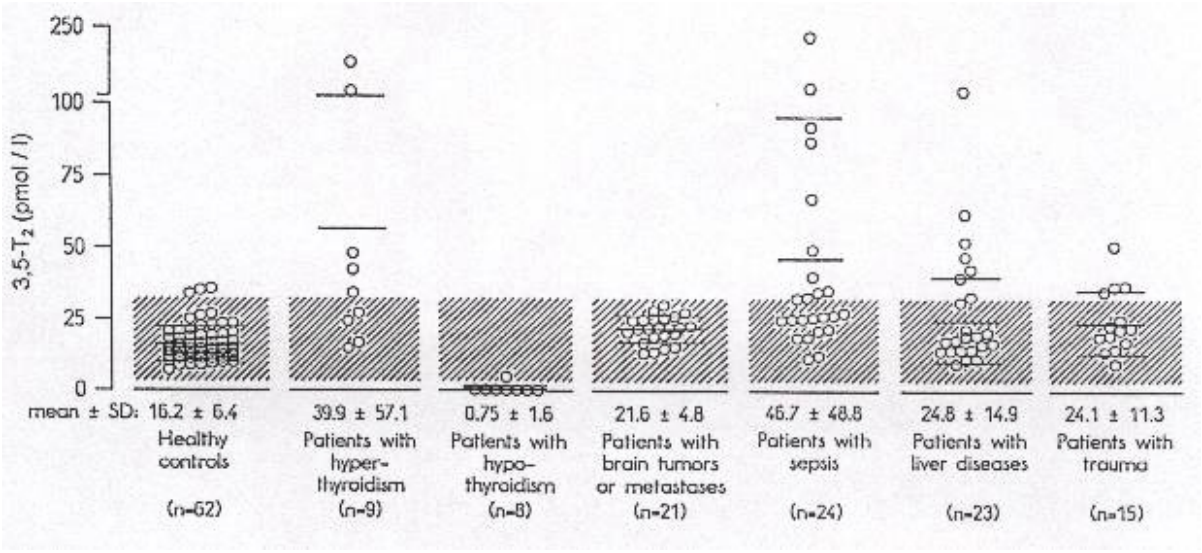


**Fig 8**. Serum concentrations of 3,5-$T_2$ in healthy controls and in patients with thyroidal and NTI. Values are the mean ± SD. The *hatched area* indicates the normal range (3.4-29 pmol/l).

- **3.2.1.3 Patients with different somatic, nonthyroidal diseases**

Figure 8 shows the full results for serum concentrations of 3,5-$T_2$ in the controls and in the patient groups with different somatic, nonthyroidal disorders. Serum levels of 3,5-$T_2$ were elevated in the patients with sepsis ($p$ = 0.004), liver diseases ($p$ = 0.004), head injury ($p$ = 0.015), and brain tumors and metastases ($p$ = 0.003). Eight of the 24 patients with sepsis, 6 of the 23 patients with liver diseases, and 4 of the 15 patients with head injury had serum 3,5-$T_2$ concentrations above the normal range.

- **3.2.1.4 Acute stress**

Table 7 shows that an acute stress such as delivering a lecture at a clinical conference did not significantly alter serum concentrations of 3,5-$T_2$.

| | $T_4$ (nmol/l) | $T_3$ (nmol/l) | 3,3'-$T_2$ (pmol/l) | 3,5-$T_2$ (pmol/l) | TSH (mU/l) | Cortisol (nmol/l) |
|---|---|---|---|---|---|---|
| Control day (3 p.m.) | 92.8±23.4 | 1.72±0.32 | 81.0±30.4 | 29.9±11.3 | 1.04±0.4 | 340±61 |
| Lecture (3 p.m.) | 100.8±25 | 1.88±0.37 | 75.4±28.7 | 25.5±8.4 | 1.37±0.6 | 552±265 |
| *P* (Wilcoxon) | 0.01 | 0.05 | NS | NS | NS | <0.01 |
| Control day (5 p.m.) | 95.1±24.3 | 1.74±0.30 | 73.9±33.4 | 30.6±9.9 | 1.01±0.4 | 274±85 |
| Lecture (5 p.m.) | 101±29.3 | 1.88±0.37 | 74.7±20.7 | 21.8±9.9 | 1.04±0.4 | 551±172 |
| *P* (Wilcoxon) | NS | 0.01 | NS | NS | NS | 0.001 |

NS: not significant

**Table 7**. Thyroid hormone and cortisol serum concentrations in 10 physicians before (3 p.m.) and after (5 p.m.) delivering a lecture and on a control day. Values are the mean ± SD.

- **3.2.1.5 Sleep deprivation**

As shown in table 8, a whole night's sleep deprivation did not significantly affect the serum concentration of 3,5-$T_2$.

| | $T_4$ (nmol/l) | $T_3$ (nmol/l) | 3,3'-$T_2$ (pmol/l) | 3,5-$T_2$ (pmol/l) | TSH (mU/l) |
|---|---|---|---|---|---|
| During sleep | 78.3±7.1 | 1.48±0.24 | 83.3±47.3 | 37.3±15.1 | 1.01±0.6 |
| During sleep deprivation | 91.8±7.1 | 1.97±0.33 | 80.8±49.7 | 37.8±24.3 | 1.82±0.8 |
| *P* (Wilcoxon) | <0.001 | <0.001 | NS | NS | <0.001 |

NS: not significant

**Table 8**. Mean thyroid hormone concentrations of six healthy volunteers measured at 20-min intervals between midnight and 6 a.m. during nights of sleep and nights of sleep deprivation, respectively. Values are the mean ± SD.

### 3.2.2 Serum concentrations of 3,3'-$T_2$:

- **3.2.2.1 Healthy controls**

The mean serum concentration of 3,3'-$T_2$ for the group of healthy controls (n = 62) was 46.6 ± 20.0 pmol/l. Linear regression analysis conducted to investigate the effects of age and gender on the variable 'hormone' revealed a significant age effect (t = -3.661, $p$ = 0.006). Thus the 3,3'-$T_2$ concentrations declined significantly with increasing age. No effect of sex was noted (t = 0.715, $p$ = 0.48). The four groups of patients with different somatic, NTI differed considerably with respect to age (see Methods). We therefore established a specific age- and sex-matched control group for each patient group. The normal range of 3,3'-$T_2$ was defined as the mean ± 2 standard deviations measured in the respective control group.

- **3.2.2.2 Patients with thyroid disorders**

Figure 9 shows the results for serum concentrations of 3,3'-$T_2$ in the controls and in the patients with thyroid disorders. The serum levels of 3,3'-$T_2$ were significantly enhanced in patients with hyperthyroidism ($p = 0.01$) and subnormal in those with hypothyroidism ($p = 0.001$). Seven of the 9 patients with hyperthyroidism had serum 3,3‘-$T_2$ concentrations above the normal range, whereas 11 of the 12 hypothyroid patients had serum 3,3‘-$T_2$ concentrations below the normal range, one of whom had 3,3‘-$T_2$ serum concentrations that were not measurable.

- **3.2.2.3 Patients with different somatic, nonthyroidal diseases**

In the group of patients with nonthyroidal diseases, the serum levels of 3,3'-$T_2$ were lower in patients with brain injury ($p = 0.006$), normal in patients with sepsis ($p = 0.06$), and elevated in patients with liver diseases ($p = 0.04$) and in patients with brain tumors and metastases ($p = 0.01$) compared with controls. Although the serum levels of 3,3'-$T_2$ in the patients with brain injury were significantly reduced, the values of all but two patients were still within the normal range. In contrast, 9 of the 24 patients with sepsis had 3,3'-$T_2$ concentrations above the normal range, although their mean levels of 3,3'-$T_2$ did not differ significantly from those measured in the corresponding control group. Six of the 22 patients with liver diseases and 12 of the 23 patients with brain tumors and metastases had elevated serum levels of 3,3'-$T_2$ (fig 9).

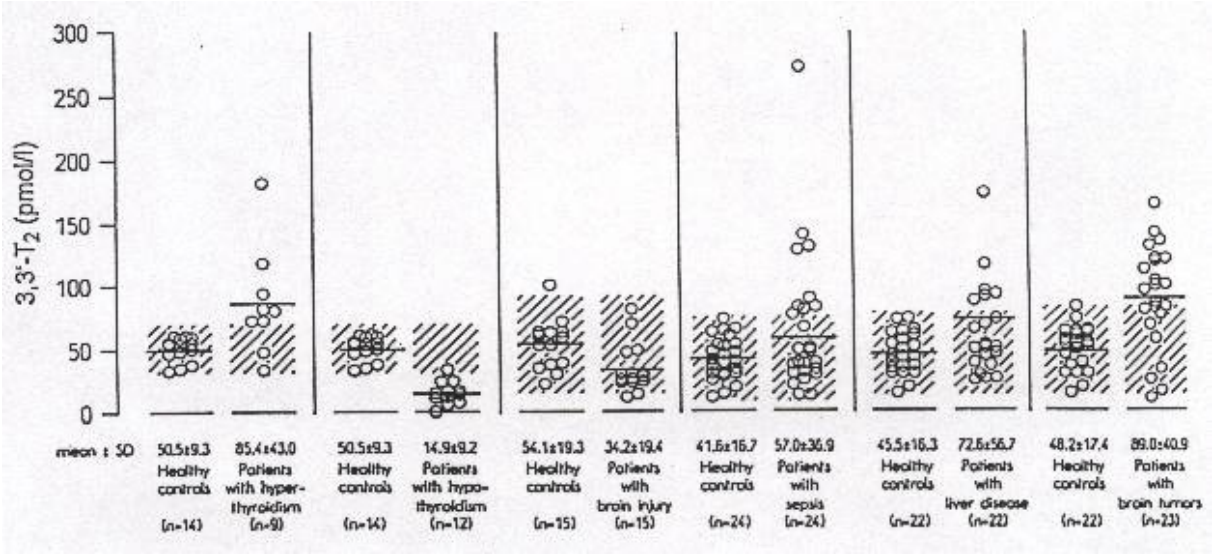


**Fig 9**. Serum concentrations of 3,3'-$T_2$ in healthy subjects and in patients with thyroidal and NTI. Values are the mean ± SD. The *hatched areas* indicate the normal range. The hormone measurements in the samples of each patient group were performed together with those in the samples of the respective age- and sex-matched control subjects.

- **3.2.2.4 Acute stress**

Table 7 shows that an acute stress such as delivering a lecture at a scientific conference had no significant effect on serum concentrations of 3,3'-$T_2$.

- **3.2.2.5 Sleep deprivation**

As reported in table 8, the serum concentrations of 3,3'-$T_2$ in a group of 6 volunteers subjected to a whole night's sleep deprivation remained unaffected.

| | $T_4$ (nmol/l) | $fT_4$ (pmol/l) | $T_3$ (nmol/l) | $rT_3$ (nmol/l) | TSH (mU/l) |
|---|---|---|---|---|---|
| Hyperthyroidism (n = 9) | 178 ± 32** | 58.7 ± 29.5** | n.m. | n.m. | < 0.01** |
| Hypothyroidism (n = 8) | 44 ± 26** | 3.8 ± 3.3** | n.m. | n.m. | 37 ± 34** |
| Sepsis (n = 24) | 83 ± 30 | 15.1 ± 6.7 | 0.74 ± 0.37** | 2.13 ± 1.13** | 0.91 ± 1.12 |
| Liver diseases (n = 22) | 54 ± 5** | n.m. | 0.51 ± 0.18** | 1.73 ± 1.46** | 1.50 ± 1.40 |
| Head trauma (n = 15) | 90 ± 21 | 14.2 ± 4.7 | 1.35 ± 0.91* | 1.00 ± 0.65* | 1.40 ± 2.13 |
| Brain tumors (n = 21) | 6.7 ± 9.8** | n.m. | 0.18 ± 0.22** | 2.14 ± 1.03** | 0.02 ± 0.02** |
| Healthy controls (n=62) | 94 ± 15 | 12.5 ± 2.0 | 2.01 ± 0.32 | 0.54 ± 0.13 | 1.21 ± 0.60 |
| Normal range | 64 – 124 | 8.5 - 16.5 | 1.37 – 2.65 | 0.28 - 0.80 | 0.10 - 2.41 |

* $p < 0.05$
** $p < 0.01$
n.m. not measured

**Table 9.** Serum concentrations (means ± SD) of different iodothyronines and thyrotropin (TSH) in patients with thyroid disorders and different NTI.

### 3.2.3 Serum concentrations of other iodothyronines and thyrotropin (TSH)

The $T_4$, $fT_4$, $T_3$, $rT_3$, and TSH concentrations in the patient groups with thyroidal disorders, in those with different somatic, nonthyroidal diseases, and in the healthy control group are listed in Table 9. Hyperthyroid patients had increased serum concentrations of both $T_4$ and $fT_4$, whereas serum concentrations of these hormones were decreased in hypothyroid patients. All four patient groups with NTI had significantly reduced serum levels of $T_3$. The lowest serum levels of $T_3$ were seen in patients with brain tumors and metastases, some of whose $T_3$ and $T_4$ concentrations were not measurable. $RT_3$ serum concentrations were significantly increased in the four groups of patients with NTI.

The results of the stress experiments are presented in Tables 7 and 8. Statistical analysis of the stress experiments was performed using the Wilcoxon rank test as described above (2.7). Table 7 shows that delivering a lecture induced significant increases in serum levels of $T_4$ (at 3 p.m.) and $T_3$ (at both 3 and 5 p.m.). TSH levels were significantly higher before the lecture, at 3 p.m., than after it, at 5 p.m. ($p$ = 0.01). No such difference occurred on the control day. Concentrations of cortisol were also measured in order to better evaluate the severity of the stress effects. These levels were found to be significantly elevated at both measuring times (Table 7). As can be seen in Table 8, sleep deprivation induced a significant increase in serum concentrations of $T_4$, $T_3$, and TSH.

### 3.2.4 Tissue levels of 3,5-$T_2$

- #### 3.2.4.1 Human brain areas of healthy donors

  Tissue concentrations of 3,5-$T_2$ in different parts of the brain and in the pituitary glands obtained from 5 human donors at autopsy as well as in the temporal and occipital cortices obtained during neurosurgery are shown in figure 10 B. 3,5-$T_2$ was detectable in all tissue samples: The concentrations ranged from 70 to 130 fmol/g and were markedly similar in the different regions of the brain and in the pituitary glands. In particular, the mean 3,5-$T_2$ tissue concentrations ranged from 70 ± 15 fmol/g in the cortical frontal lobe samples to 135 ± 23 fmol/g in the pons. Intermediate values were obtained in the striatum and in the cerebellum (108 ± 28 fmol/g and 88 ± 45 fmol/g, respectively). The mean 3,5-$T_2$ concentrations in the pituitary glands were 128 ± 47 fmol/g. Higher concentrations of 3,5-$T_2$ were determined in the cortical samples excised intra-operatively.

  The tissue $T_3$/3,5-$T_2$ molar ratios (fig 10 C) ranged from 23:1 in the cerebellum to 17:1 in the cortical frontal lobe, with an intermediate value in the pons (19:1). The $T_3$/3,5-$T_2$ molar ratios in the cortical samples obtained intra-

operatively from donors were found to be slightly decreased when compared with the cortical samples obtained at autopsy (13:1).

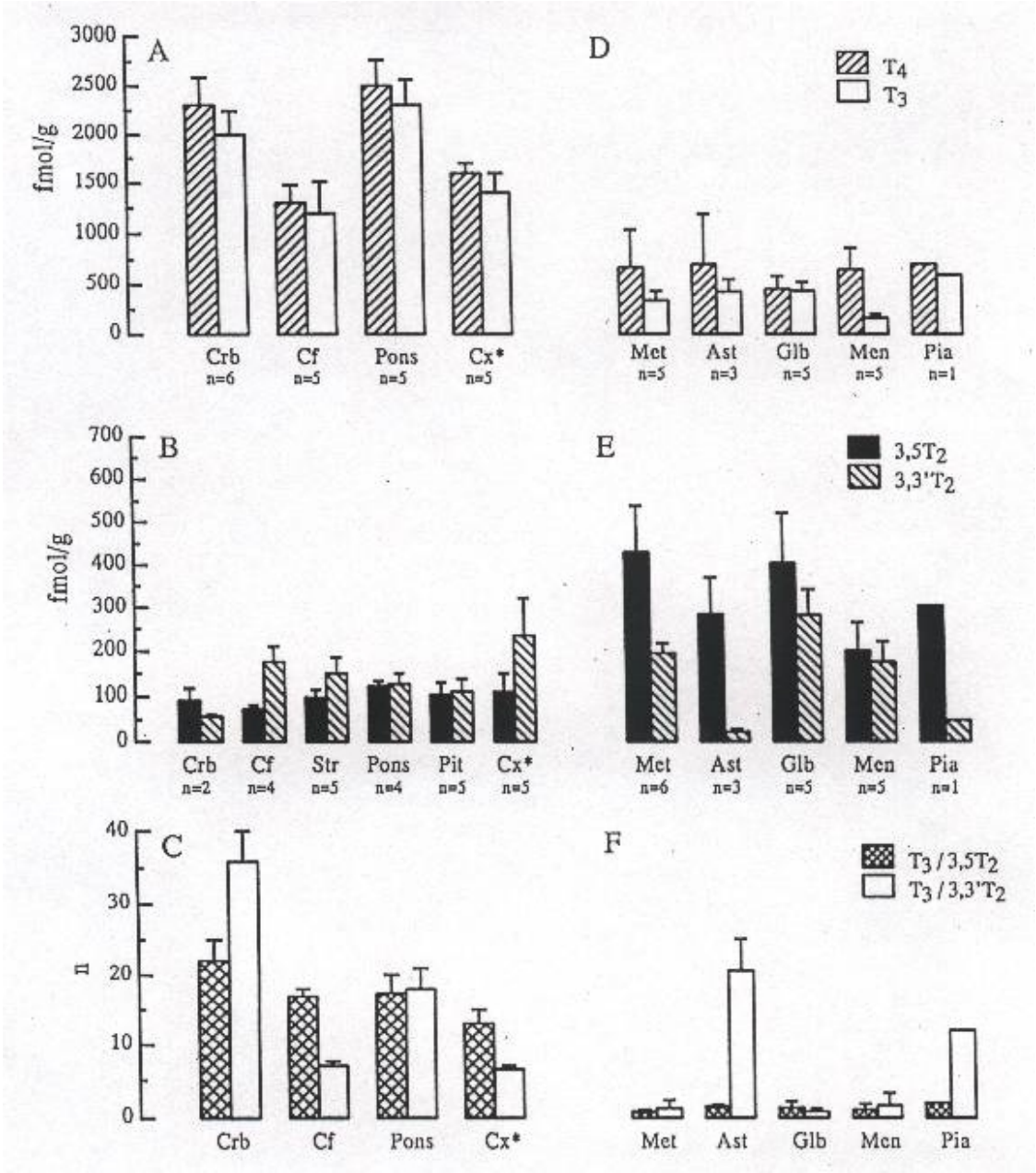


**Fig 10**. $T_4$, $T_3$ (A), 3,5-$T_2$ and 3,3'-$T_2$ (B) concentrations and molar ratios of $T_3$/3,5-$T_2$ and $T_3$/3,3'-$T_2$ (C) in samples from different areas of the adult human brain and pituitary obtained either at autopsy or intra-operatively (*). $T_4$, $T_3$ (D), 3,5-$T_2$ and 3,3'-$T_2$ (E) concentrations and molar ratios of $T_3$/3,5-$T_2$ and $T_3$/3,3'-$T_2$ (F) in tissue sample from different forms of human brain tumors, metastases and a pituitary adenoma. Abbreviations: Crb=Cerebellum; Cf=Cortex frontalis; Str=Striatum; Pit=Pituitary; Cx=Cortex cerebralis; Met=Metastases; Ast=Astrocytomas; Glb=Glioblastomas; Men=Meningiomas; Pia=Pituitary adenoma.

- **3.2.4.2 Human brain tumors and metastases**

The concentrations of 3,5-$T_2$ in the various brain tumors and metastases are shown in figure 10 E. The mean 3,5-$T_2$ concentrations over all 21 samples was 336 ± 106 fmol/g. The highest mean concentration was found in the metastases (427 ± 110 fmol/g), followed by the glioblastomas (403 ± 118 fmol/g) and the astrocytomas (283 ± 86 fmol/g). The lowest values were measured in the meningiomas (197 ± 66 fmol). The level of 3,5-$T_2$ in the pituitary adenoma was 304 fmol/g. Thus, these values were considerably higher than the 3,5-$T_2$ concentrations measured in the different non-tumoral regions of the human brain (fig 10 B and E).

The tissue $T_3$/3,5-$T_2$ molar ratios were found to be markedly constant over all brain tumor and metastases samples analyzed. They ranged from 0.9:1 in the metastases to 1.5:1 in the astrocytomas (fig 10 F).

### 3.2.5 Tissue levels of 3,3‘-$T_2$

- **3.2.5.1 Human brain areas of healthy donors**

The 3,3'-$T_2$ concentrations in tissue from different parts of the brain obtained from 5 donors at autopsy and in samples obtained from the temporal and occipital cortex during neurosurgery are shown in figure 10 B. 3,3'-$T_2$ was detectable in all tissue samples, the concentrations ranging from 50 to 300 fmol/g. In detail, mean tissue 3,3'-$T_2$ concentrations ranged from 178 ± 44 fmol/g in the cortical frontal lobe samples to 58 ± 10 fmol/g in the cerebellum and reached intermediate values in the striatum and pons (152 ± 40 fmol/g and 128 ± 27 fmol/g, respectively). Mean tissue concentrations of 3,3'-$T_2$ in the pituitary glands were 118 ± 18 fmol/g. In the cortical lobe samples obtained intra-operatively, concentrations of 3,3'-$T_2$ were about 30% higher than those

measured in the frontal cortical lobe samples obtained at autopsy (240 ± 80 fmol/g).

Tissue $T_3$/3,3'-$T_2$ molar ratios ranged from 38:1 in the cerebellum to 7:1 in the cortical frontal lobe. Intermediate values were found in the pons (19:1). The mean $T_3$/3,3'-$T_2$ molar ratio in the cortices obtained intra-operatively was as low as 5:1 (fig 10 C).

- **3.2.5.2 Human brain tumors and metastases**

The concentrations of 3,3'-$T_2$ in different brain tumors are shown in figure 10 E. They ranged from 15 to 350 fmol/g. The highest concentrations were found in the glioblastomas (281 ± 61 fmol/g), followed by the brain metastases (224 ± 32 fmol/g) and the meningiomas (202 ± 45 fmol/g). The lowest values were determined in the astrocytomas (20 ± 6 fmol/g). The tissue level of 3,3'-$T_2$ in the pituitary adenoma was 48 fmol/g.

Tissue $T_3$/3,3'-$T_2$ molar ratios differed somewhat according to the brain tumor analyzed. They ranged between 1.2:1 in the glioblastomas to 20:1 in the astrocytomas (fig 10 F).

### 3.2.6 Tissue levels of other iodothyronines in human brain areas of healthy donors

The concentrations of $T_4$ and $T_3$ measured in tissue samples from different areas of the human brain are shown in figure 10 A. Thyroid hormones were demonstrable in both the tissues obtained intra-operatively and those excised post-mortem. In the autopsy tissues, mean $T_4$ concentrations ranged from 1320 ± 179 fmol/g in the cortical frontal lobe samples to 2530 ± 268 fmol/g in the pons, with intermediate values in the cerebellum (2310 ± 285 fmol/g). Mean $T_3$ concentrations ranged from 2308 ± 265 fmol/g in the pons to 1220 ± 313 fmol/g in the frontal lobe, with intermediate values of 2023 ± 245 fmol/g in the cerebellum.

Slightly higher values of $T_4$ (1612 ± 110 fmol/g) and $T_3$ (1416 ± 208 fmol/g) were measured in the cortical samples obtained intra-operatively (fig 10 A).

Tissue $T_3/T_4$ molar ratios were relatively constant in all brain regions investigated (mean: 0.77 ± 0.1; n=21; range: 0.6-0.9). Slightly higher values of $T_4$ and $T_3$ were measured in the cortical samples obtained intra-operatively.

The results of the experiments conducted to evaluate the effects of post-mortem delay on iodothyronine concentrations are given in fig 11. This figure shows that storing the brain samples at 4 °C for 24 h led to a fall in tissue hormone concentrations of between 11% and 27% ($T_4$: -11%; $T_3$: -16%; 3,3'-$T_2$: -27%; 3,5-$T_2$: -15%). The respective decreases after a storage period of 96 h at 4 °C before freezing were 43% for $T_4$, 28% for $T_3$, 50% for 3,3'-$T_2$, and 29% for 3,5-$T_2$. These data imply that the 3,5-$T_2$ and 3,3'-$T_2$ levels measured in the different regions of the adult human brain at autopsy and presented in figure 10 B are probably 30% to 50% too low. This is consistent with the fact that the concentrations of 3,5-$T_2$ and 3,3'-$T_2$ measured in the tissue samples obtained intra-operatively (Cx* in figure 10 B) were indeed approximately 30% higher than those determined in tissue obtained post-mortem.

### 3.2.7 Tissue levels of other iodothyronines in human brain tumors and metastases

Mean tissue concentrations of $T_4$ in the brain tumors and metastases are shown in figure 10 D. $T_4$ mean tissue concentrations were 624 ± 97 fmol/g over the 21 samples analyzed. They ranged from 698 ± 492 fmol/g in the astrocytomas to 453 ± 133 fmol/g in the glioblastomas. Intermediate values were detected in the meningiomas (648 ± 215 fmol/g) followed by the metastases (671 ± 164 fmol/g). The $T_4$ tissue level in the pituitary adenoma was 706 fmol/g.

The mean tissue concentration of $T_3$ in all 21 brain tumors and metastases was 345 ± 44 fmol/g. The highest mean $T_3$ tissue concentrations were found in the glioblastomas (440 ± 84 fmol/g), followed by the astrocytomas (427 ± 126 fmol/g) and

the metastases (337 ± 93 fmol/g). Finally, the meningiomas had the lowest $T_3$ concentrations (160 ± 37 fmol/g). The tissue level of $T_3$ in the pituitary adenoma was 599 fmol/g (fig 10 E).

Tissue molar ratios of $T_4$ to $T_3$ ranged from 1.1:1 in the glioblastomas to 4.6:1 in the meningiomas. Intermediate values were found in the astrocytomas (2.3:1), pituitary adenoma (1.2:1), and metastases (3:1).

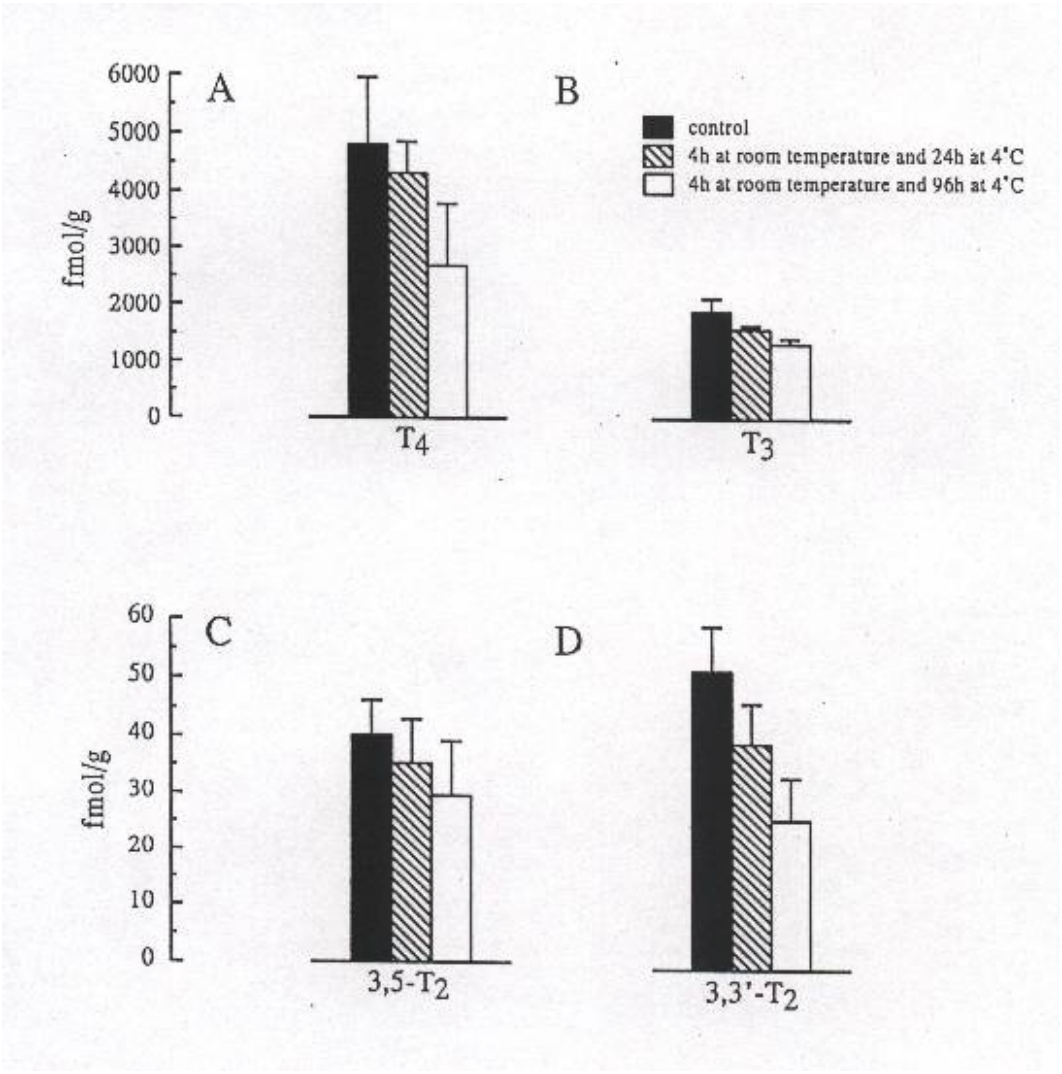


**Fig 11**.Tissue concentrations of $T_4$ (A), $T_3$ (B), 3,5-$T_2$ (C), and 3,3'-$T_2$ (D) obtained from the striata of rats (n = 4) at different intervals post-mortem.

## 3.3 Animal studies

### 3.3.1 Serum concentrations of 3,5-$T_2$ and 3,3'-$T_2$ in control animals

The serum concentrations of 3,5-$T_2$ and 3,3'-$T_2$ measured in control animals (n = 8) were 11.4 ± 0.8 pmol/l and 15.7 ± 5.7 pmol/l for 3,5-$T_2$ and 3,3'-$T_2$, respectively.

### 3.3.2 Tissue levels of 3,5-$T_2$

- #### 3.3.2.1 Homogenates of various brain areas of the rat

  Concentrations of 3,5-$T_2$ were investigated in twelve different areas of the brain, in the pituitary glands, and in the liver of the rat. Figure 12 A shows that 3,5-$T_2$ was measurable in 6 of the 12 brain areas and in the liver, but was not measurable in the pituitary glands. The 3,5-$T_2$ brain concentrations ranged from 16.6 ± 1.9 fmol/g in the cerebellum to 45.8 ± 1.5 fmol/g in the amygdala. Intermediate values were obtained in the parieto-occipital cortex (22.5 ± 3.0 fmol/g), followed by the midbrain (22.6 ± 3.9 fmol/g), the medulla (31.5 ± 7.5 fmol/g), and the striatum (32.1 ± 3.6 fmol/g). The highest concentrations of 3,5-$T_2$ were measured in the liver (91.4 ± 13.9 fmol/g).

  The concentration of 3,5-$T_2$ expressed as fmol/mg protein ranged from 0.17 ± 0.02 fmol/mg protein in the cerebellum to 0.39 ± 0.01 fmol/mg protein in the amygdala. In the liver the concentrations of 3,5-$T_2$ were 0.48 ± 0.25 fmol/mg protein.

  $T_3$/3,5-$T_2$ tissue molar ratios were measured in the different regions of the brain and in the liver of the rat. They ranged from 14:1 to 99:1 in the amygdala and midbrain, respectively. In the liver, concentrations of $T_3$ were 28-fold higher than those of 3,5-$T_2$.

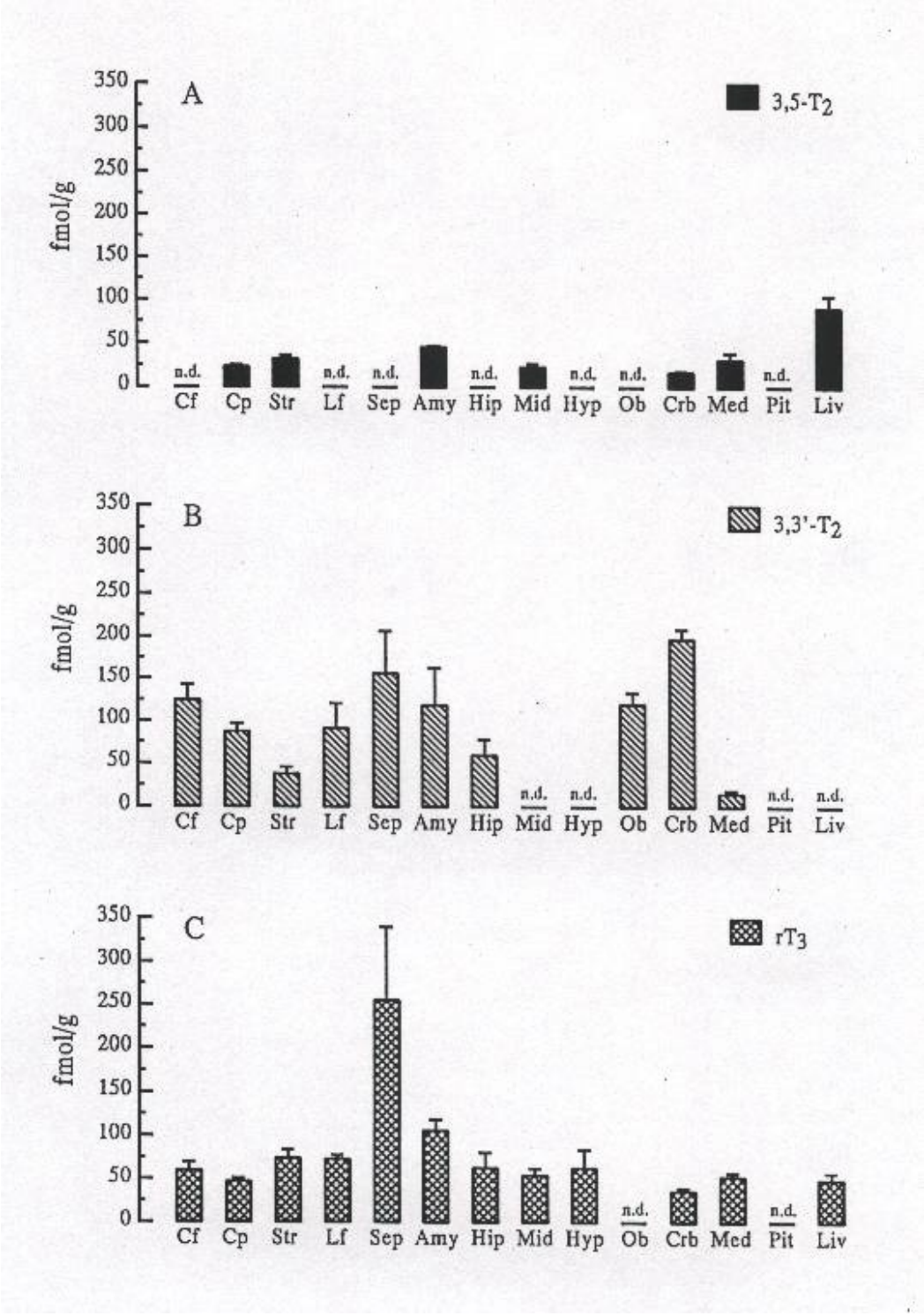


**Fig 12**. Regional distribution of 3,5-$T_2$ (A), 3,3'-$T_2$ (B), and $rT_3$ (C) in different areas of the adult rat brain (n = 8). Abbreviations: frontal cortex (Cf); parieto-occipital cortex (Cp); striatum (Str); limbic forebrain (Lf); septum (Sep); amygdala (Amy); hippocampus (Hip); midbrain (Mid); hypothalamus (Hyp); olfactory bulb (Ob); cerebellum (Crb); medulla (Med); pituitary (Pit); liver (Liv); not detectable (n.d.).

- **3.3.2.2 Subcellular fractions of brain areas of the rat**

Concentrations of 3,5-$T_2$ were investigated in the subcellular fractions of the amygdala and the parieto-occipital cortex (fig 14 C and D). A preliminary study showed that 3,5-$T_2$ was detectable neither in the amygdala nor in the parieto-occipital cortex in a pool of two brain regions for one hormone determination (fig 13 A).

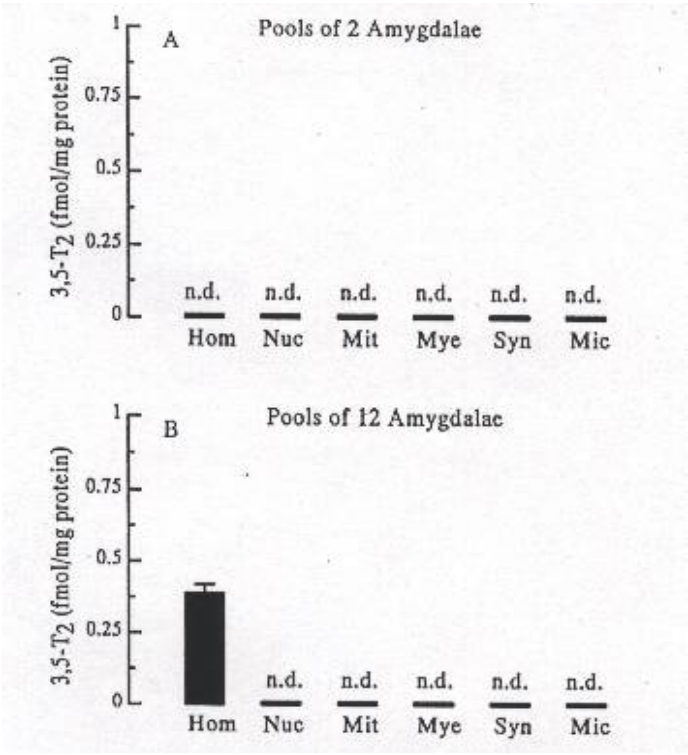


**Fig 13**. 3,5-$T_2$ distribution in homogenate and subcellular fractions in pools of 2 (A) and 12 (B) amygdalae of euthyroid male rats (n = 8 and 48, respectively). Note that in the homogenate, 3,5-$T_2$ levels were determined in aliquots of only 1/10 of the original homogenate used for subcellular fractionation (see 2.3.2.2). Abbreviations: homogenate (Hom); nuclei (Nuc); mitochondriae (Mit); myelin (Mye); synaptosomes (Syn); microsomes (Mic); non detectable (n.d.).

Therefore, in a second study, 12 amygdalae were pooled to clearly define whether 3,5-$T_2$ was present or not in the subcellular fractions under investigation. Figure 13 B shows that in the pool of 12 amygdalae the concentrations of 3,5-$T_2$ were again not detectable in any of the subcellular fractions investigated.

### 3.3.3 Tissue levels of 3,3'-$T_2$

- #### 3.3.3.1 Homogenates of various brain areas of the rat

Figure 12 B shows the mean concentrations of 3,3'-$T_2$ in various areas of the rat brain. Concentrations of 3,3'-$T_2$ were investigated in 12 regions of the brain, in the liver, and in the pituitary glands of the rat. While 3,3'-$T_2$ was not measurable in the liver or in the pituitary glands, in 10 of the 12 brain areas its concentrations ranged from 15.2 ± 3.8 fmol/g (medulla) to 197.3 ± 12.4 fmol/g (cerebellum). Intermediate values were measured in the striatum (37.3 ± 8.1 fmol/g), in the hippocampus (59.1 ± 18.2 fmol/g), in the parieto-occipital cortex (86.8 ± 9.7 fmol/g), in the limbic forebrain (90.2 ± 30 fmol/g), in the amygdala (118 ± 45), in the olfactory bulbs (119.6 ± 13.8 fmol/g), in the frontal cortex (124.3 ± 17.8 fmol/g), and in the septum (155.9 ± 51 fmol/g).
The concentrations of 3,3'-$T_2$ expressed as fmol/mg protein. They ranged from 2.1 ± 0.01 fmol/mg protein to 0.18 ± 0.01 fmol/mg protein in the cerebellum and in the medulla, respectively.

- #### 3.3.3.2 Subcellular fractions of brain areas of the rat

Concentrations of 3,3'-$T_2$ were investigated in the subcellular fractions of the parieto-occipital cortex and the amygdala of the rat. Figure 14 E and F shows that in both the parieto-occipital cortex and the amygdala, 3,3'-$T_2$ was measurable only in the synaptosomes. Synaptospmal 3,3'-$T_2$ concentrations ranged from 1.7 ± 0.3 fmol/mg protein to 0.48 ± 0.02 fmol/mg protein in the parieto-occipital cortex and in the amygdala, respectively.

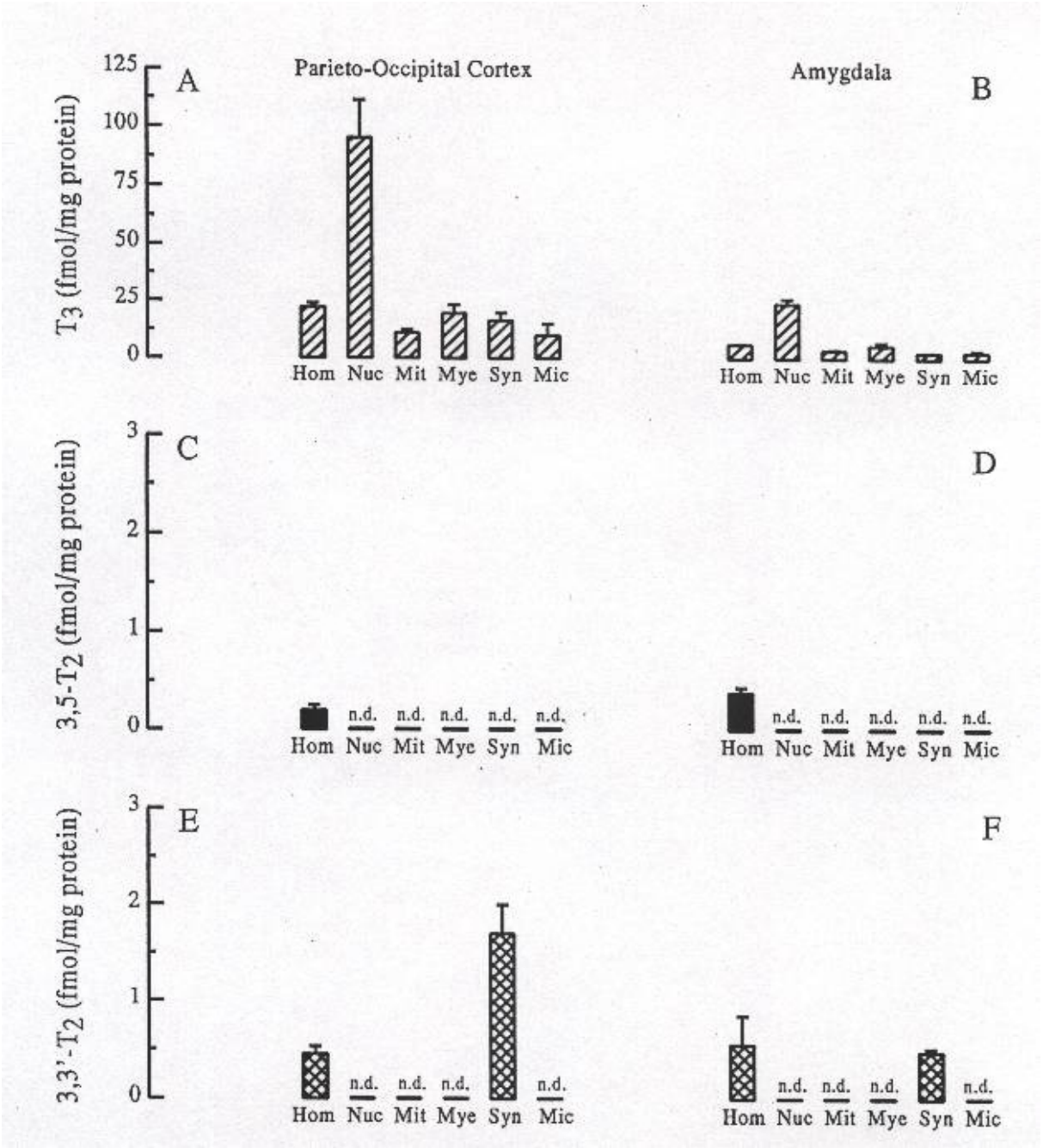


**Fig 14**. $T_3$ (A; B), 3,5-$T_2$ (C; D), and 3,3'-$T_2$ (E; F) distribution in homogenate and subcellular fractions of parieto-occipital cortex (A; C; E) and amygdala (B; D; F) of euthyroid male rats (n = 8 - 48 according to hormone determination and area of the brain under investigation). Note that in the homogenate, iodothyronine levels were determined in aliquots of only 1/10 of the original homogenate used for subcellular fractionation. For the determination of 3,3'-$T_2$ two areas have been pooled for one hormone determination. For the determination of 3,5-$T_2$ in C and D, 4 and 12 areas were pooled, respectively, for one hormone determination. Abbreviations: homogenate (Hom); nuclei (Nuc); mitochondria (Mit); myelin (Mye); synaptosomes (Syn); microsomes (Mic); not detectable (n.d.).

### 3.3.4 Tissue levels of other iodothyronines

- #### 3.3.4.1 Homogenates of various brain areas of the rat

The concentrations of $T_4$, $rT_3$, and $T_3$ were measured in 12 regions of the brain, in the liver, and in the pituitary glands of the rat.

$T_4$ was measurable in all 12 regions of the brain, in the liver, and in the pituitary glands. The olfactory bulb exhibited the highest brain $T_4$ concentrations (6255 ± 958 fmol/g), followed by the medulla (5423 ± 807 fmol/g), the septum (5253 ± 1875 fmol/g), the striatum (4895 ± 899 fmol/g), the hypothalamus (4629 ± 631 fmol/g), the limbic forebrain (3695 ± 419 fmol/g), the cerebellum (3380 ± 218 fmol/g), the midbrain (3270 ± 295 fmol/g), the hippocampus (3090 ± 321 fmol/g), the amygdala (1720 ± 202 fmol/g), the parieto-occipital cortex (1130 ± 172 fmol/g), and the frontal cortex (1051 ± 82 fmol/g). The concentrations of $T_4$ in the pituitary glands and in the liver were 27.7 ± 7.5 pmol/g and 71.0 ± 9.4 pmol/g, respectively (fig 15 A).

Concentrations of $rT_3$ were detectable in all regions of the brain examined with the exception of the olfactory bulb. $RT_3$ was detectable in the liver but not in the pituitary glands. In the eleven areas of the brain where $rT_3$ was measurable, its concentrations varied between 255 ± 91 fmol/g and 36.2 ± 3.1 fmol/g in the septum and in the cerebellum, respectively. Intermediate values were obtained in the following brain regions: amygdala (105.7 ± 12.6 fmol/g), striatum (74.3 ± 9.9 fmol/g), limbic forebrain (73.2 ± 4.4 fmol/g), hypothalamus (63.3 ± 21.4 fmol/g), hippocampus (62.8 ± 18.1 fmol/g), frontal cortex (59.7 ± 9.9 fmol/g), midbrain (54.5 ± 8.7 fmol/g), medulla (52.4 ± 5.1 fmol/g), and parieto-occipital cortex (46.7 ± 4.0 fmol/g). The concentrations of $rT_3$ in the liver were 49.6 ± 7.4 fmol/g (fig 12 C).

Concentrations of $T_3$ were measurable in all brain areas investigated as well as in the pituitary glands and in the liver. They ranged from 2821 ± 150 fmol/g

in the midbrain to 792 ± 60 fmol/g in the amygdala. Intermediate values were determined in the following brain regions: hypothalamus (2540 ± 66 fmol/g), frontal cortex (2085 ± 370 fmol/g), limbic forebrain (1810 ± 190 fmol/g), striatum (1986 ± 101 fmol/g), septum (1802 ± 180 fmol/g), hippocampus (1278 ± 93 fmol/g), olfactory bulbs (1265 ± 75 fmol/g), medulla (1172 ± 69 fmol/g), parieto-occiptal cortex (1130 ± 247 fmol/g), and cerebellum (1052 ± 76 fmol/g). The concentrations of $T_3$ in the pituitary glands and in the liver were 3980 ± 95 fmol/g and 2106 ± 170 fmol/g, respectively (fig 15 B).

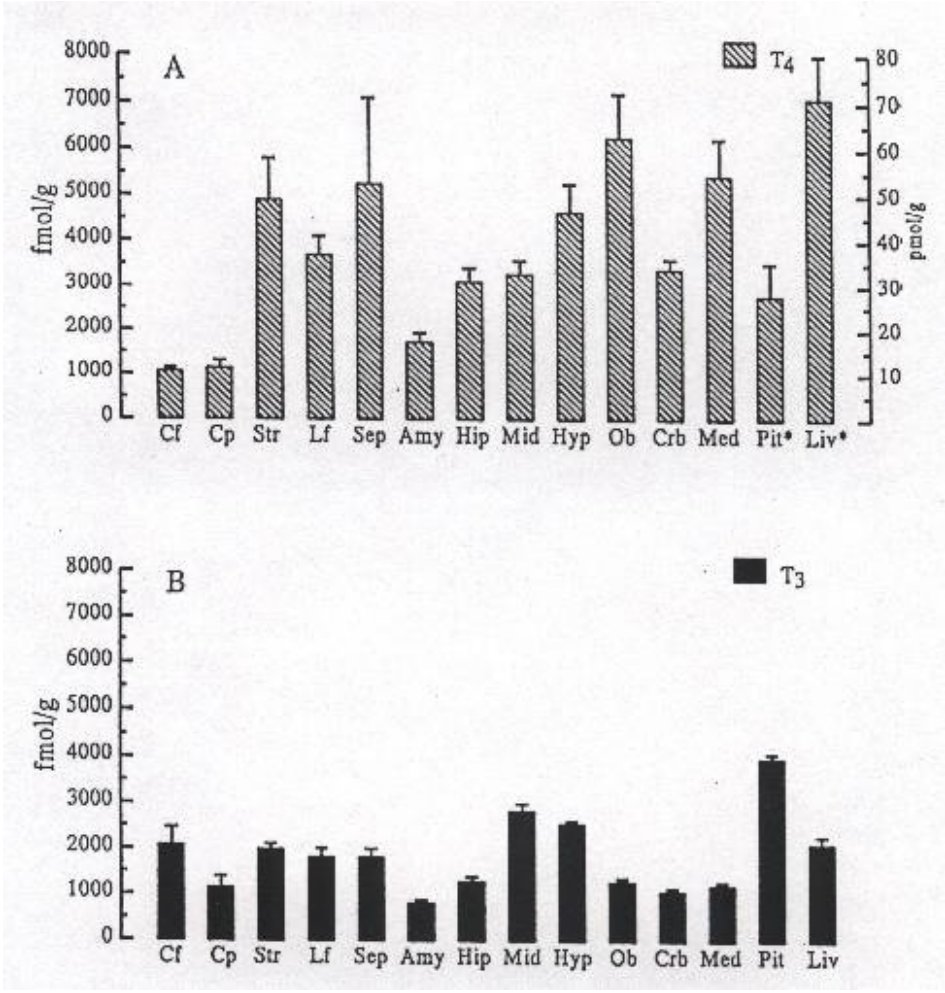


**Fig 15**. Regional distribution of $T_4$ (A) and $T_3$ (B) in different areas of the adult rat (n=8) brain. * The values for the concentrations of $T_4$ in pituitary and liver are given in the right Y axis. Abbreviations: frontal cortex (Cf); parieto-occipital cortex (Cp); striatum (Str); limbic forebrain (Lf); septum (Sep); amygdala (Amy); hippocampus (Hip); midbrain (Mid); hypothalamus (Hyp); olfactory bulb (Ob); cerebellum (Crb); medulla (Med); pituitary (Pit); liver (Liv).

The tissue $T_4/T_3$ molar ratios showed that $T_4$ was always more concentrated than $T_3$ in the various regions of the brain investigated, the only exception being the frontal and parieto-occipital cortex, where concentrations of $T_3$ were found to be two-fold higher and equal to those of $T_4$, respectively. In particular, $T_4$ appeared to be between 1.7 and 5 times more concentrated than $T_3$ in the midbrain and in the olfactory bulb, respectively. Figure 16 shows the comparison between the concentrations of $T_4$, $T_3$, $rT_3$, 3,3'-$T_2$, and 3,5-$T_2$ in the parieto-occipital cortex and amygdala, respectively.

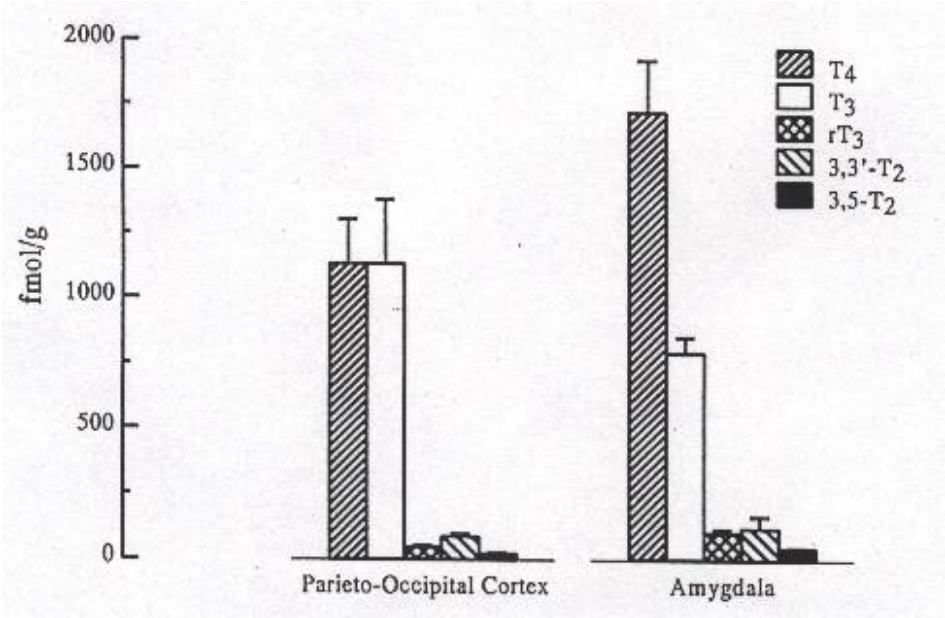


**Fig 16**. Comparison of the concentrations of $T_4$, $T_3$, $rT_3$, 3,3'-$T_2$, and 3,5-$T_2$ in the parieto-occipital cortex and in the amygdala of euthyroid male rats (n = 8).

- **3.3.4.2 Subcellular fractions of brain areas of the rat**

Figure 14 A shows the concentrations of $T_3$ in several subcellular fractions of the parieto-occipital cortex and amygdala in the rat. $T_3$ was detected in all subcellular fractions both in the parieto-occipital cortex and in the amygdala. In the parieto-occipital cortex, nuclei exhibited the highest $T_3$ concentrations (95

± 16.5 fmol/mg protein), followed by myelin (19.5 ± 3.8 fmol/mg protein), synaptosomes (16.5 ± 3.3 fmol/mg protein), mitochondria (11 ± 1.5 fmol/mg protein), and microsomes (10 ± 5.1 fmol/mg protein).

In the amygdala, nuclei also displayed the highest $T_3$ concentrations (24 ± 1,9 fmol/mg protein), followed by myelin (5.8 ± 1.2 fmol/mg protein), mitochondria (3.5 ± 0.54 fmol/mg protein), microsomes (2.9 ± 1 fmol/mg protein), and synaptosomes (2.6 ± 0.2 fmol/mg protein).

Investigation of $rT_3$ concentrations in the subcellular fractions of the amygdala (i.e., one of the regions of the brain where $rT_3$ was most concentrated) and the parieto-occipital cortex (the largest area of the brain examined), respectively, showed that $rT_3$ was not detectable in any of the fractions of the amygdala or the parieto-occipital cortex. Pools of 12 amygdalae for one hormone determination showed again that $rT_3$ was not detectable in any of the subcellular fractions investigated.

### 3.3.5 Effects of antidepressant drugs on brain subcellular concentrations of 3,5-$T_2$ and 3,3'-$T_2$

The effects of subchronic administration of desipramine on subcellular concentrations of 3,5-$T_2$ and 3,3'-$T_2$ were investigated in the parieto-occipital cortex and in the amygdala.

Desipramine administered in doses of 5 mg/kg, 20 mg/kg, and 40 mg/kg surprisingly caused an increase in 3,5-$T_2$ concentrations in the mitochondria and myelin of the amygdala (fig 17 A). The tissue concentrations of 3,5-$T_2$ were measurable in the mitochondria already after the dose of 5 mg/kg, whereas a more pronounced effect was detected at the doses of 20 mg/kg and 40 mg/kg of desipramine. In the myelin of the amygdala, the 5 mg/kg dose of desipramine was also effective to increase concentrations of 3,5-$T_2$. However, the doses of 20 mg/kg and 40 mg/kg induced a more marked increase in levels of 3,5-$T_2$. No effects of desipramine on 3,5-$T_2$ levels were detected in the synaptosomal and microsomal fractions (fig 17 A).

Concentrations of 3,5-$T_2$ in the parieto-occipital cortex were not affected by treatment with desipramine (fig 17 B).

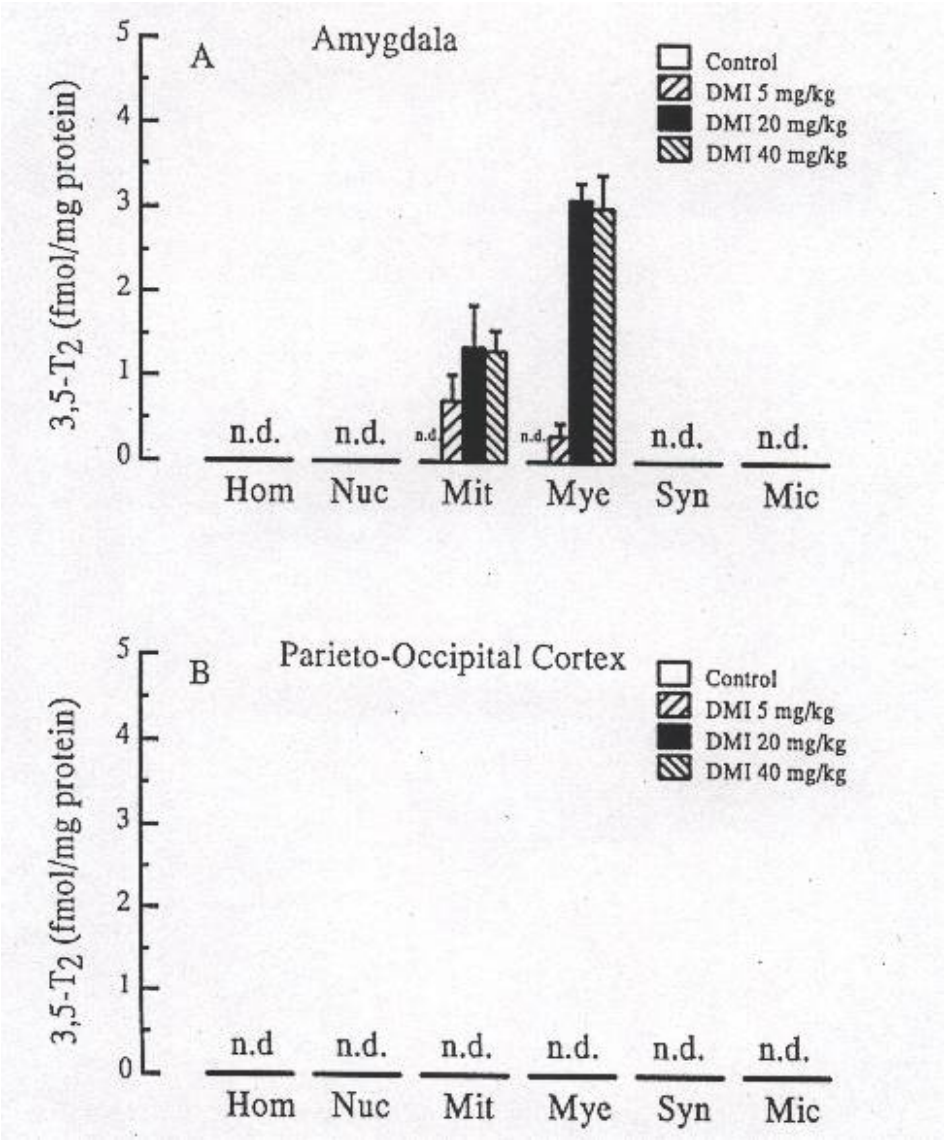


**Fig 17**. Concentrations of 3,5-$T_2$ in subcellular fractions of the amygdala (A) and parieto-occipital cortex (B) after administration of desipramine for 14 days (5, 20, and 40 mg/kg/day, decapitation between 11 a.m. and 1 p.m.) to 10 male euthyroid rats. Note that for the determination of 3,5-$T_2$ in A and B, 2 areas have been pooled for one hormone determination. For the determination of 3,5-$T_2$ in the homogenate, aliquots of only 1/10 of the original homogenate used for subcellular fractionation were used. Abbreviations: homogenate (Hom); nuclei (Nuc); mitochondria (Mit); myelin (Mye); synaptosomes (Syn); microsomes (Mic); not detectable (n.d.).

The effects of a 14-day treatment with desipramine on subcellular concentrations of 3,3'-$T_2$ in the amygdala and in the parieto-occipital cortex were further investigated. As shown in figure 18 A and B, no significant effects were detected either in the amygdala or in the parieto-occipital cortex.

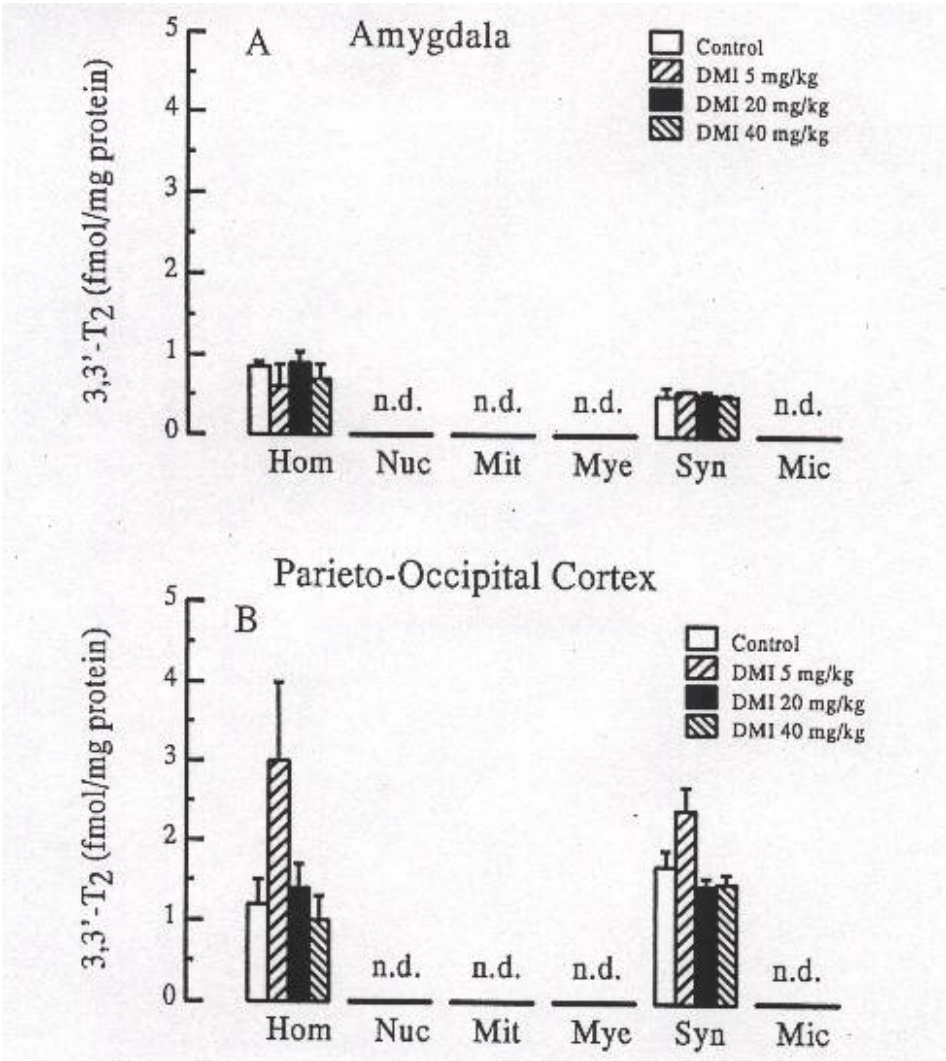


**Fig 18**. Concentrations of 3,3'-$T_2$ in subcellular fractions of the amygdala (A) and parieto-occipital cortex (B) after administration of desipramine for 14 days (5, 20, and 40 mg/kg/day, decapitation between 11 a.m. and 1 p.m.) to 10 euthyroid male rats. Note that for the determination of 3,3'-$T_2$ in A and B, 2 areas have been pooled for one hormone determination. For the determination of 3,3'-$T_2$ in the homogenate, aliquots of only 1/10 of the original homogenate used for subcellular fractionation were used. Abbreviations: homogenate (Hom); nuclei (Nuc); mitochondria (Mit); myelin (Mye); synaptosomes (Syn); microsomes (Mic); not detectable (n.d.).

### 3.3.6 Effects of circadian variation on concentrations of 3,5-$T_2$ and other iodothyronines

The investigation of circadian effects on brain concentrations of $T_4$ and $T_3$ in the cerebellum are part of a previous study published by Campos-Barros et al. (1997). $T_4$, $T_3$, and 3,5-$T_2$ levels in the liver and in the midbrain, as well as 3,5-$T_2$ concentrations in the cerebellum, have not been previously reported.

As reported by Campos-Barros et al. (1997) there were significant circadian variations in TSH, $T_4$, and $T_3$ serum concentrations.

5'DII activity as well as tissue $T_4$ and $T_3$ concentrations in the cerebellum also displayed significant circadian variations (fig 21 A and B).

In this study we determined that tissue $T_4$ and $T_3$ concentrations displayed significant daily variations in the liver ($F = 4.8$, $p < 0.01$ and $F = 4.3$, $p < 0.02$, respectively; fig 19 A and B) and in the midbrain ($F = 3.8$, $p < 0.05$ and $F = 9.6$, $p < 0.001$, respectively; fig 20 A and B). The tissue $T_4$ and $T_3$ concentrations exhibited significant ($0.005 < p > 0.01$) circadian rhythms with amplitudes ranging from 32% to 21% of the daily mean value (fig 19, 20 and 21).

Tissue concentrations of 3,5-$T_2$ showed significant daily variations in the liver ($F = 4.9$, $p < 0.002$; fig 19 C), in the midbrain ($F = 3.03$, $p < 0.02$; fig 20 C), and in the cerebellum ($F = 3.2$, $p < 0.01$; fig 21 C). The tissue 3,5-$T_2$ concentrations exhibited significant ($0.03 < p < 0.01$) circadian variations with amplitudes ranging from 28% to 22% of the daily mean value. Similar to $T_3$ levels, 3,5-$T_2$ concentrations reached a maximal value in the cerebellum at the beginning of the light phase, between 8 a.m. and 1 p.m., in the midbrain between 11 a.m. and 3 p.m., and in the liver between 11 a.m. and 4 p.m. Figure 22 shows that in the cerebellum, the circadian variations of $T_4$, $T_3$, and 3,5-$T_2$ are parallel.

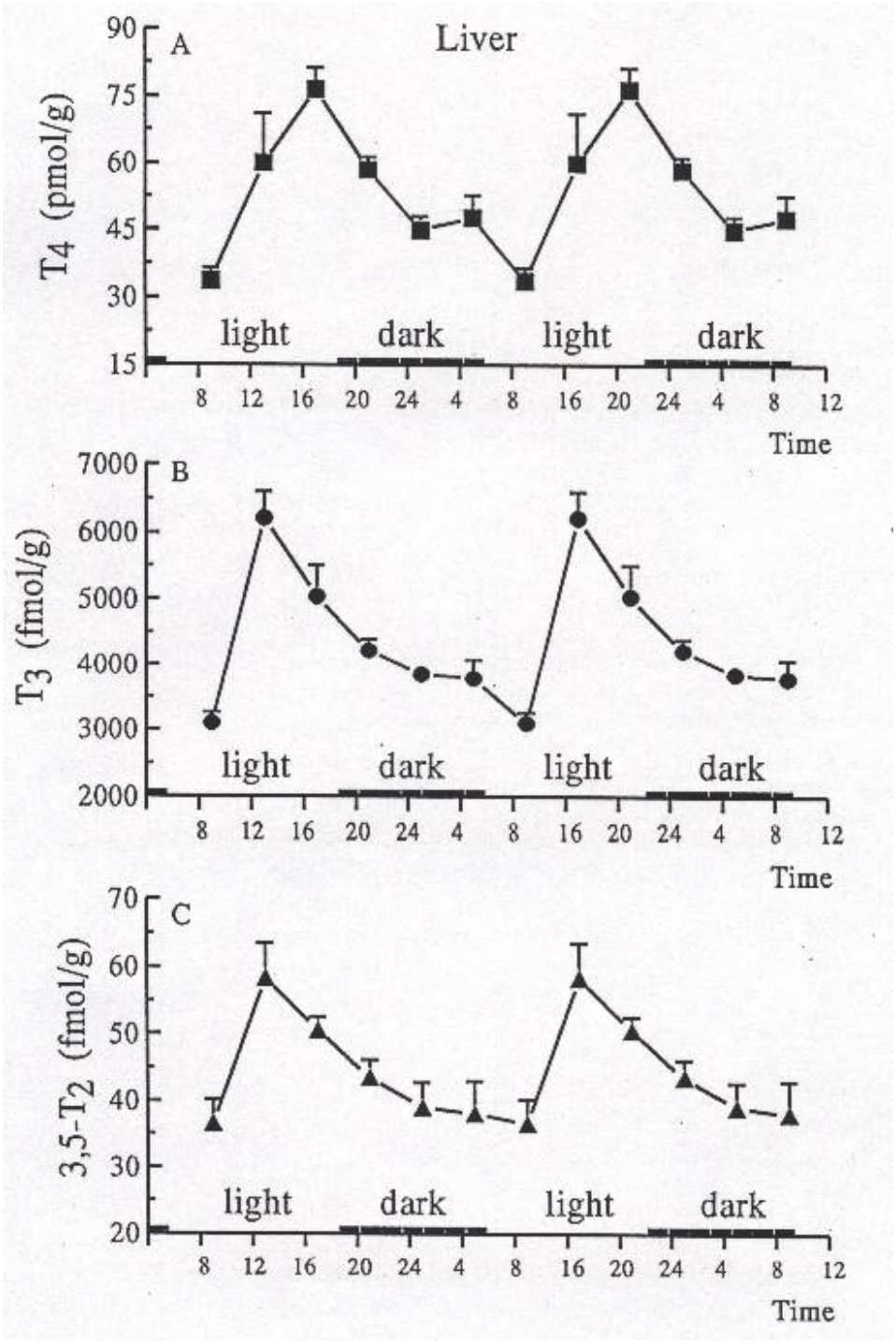


**Fig 19**. Twenty-four hour rhythms of tissue $T_4$ (A), $T_3$ (B), 3,5-$T_2$ (C) concentrations in the liver of euthyroid male rats (n = 6) entrained to a regular 12:12 h light-dark photoperiod. Horizontal bold segments of the X axis indicate the dark phase of the photoperiod.

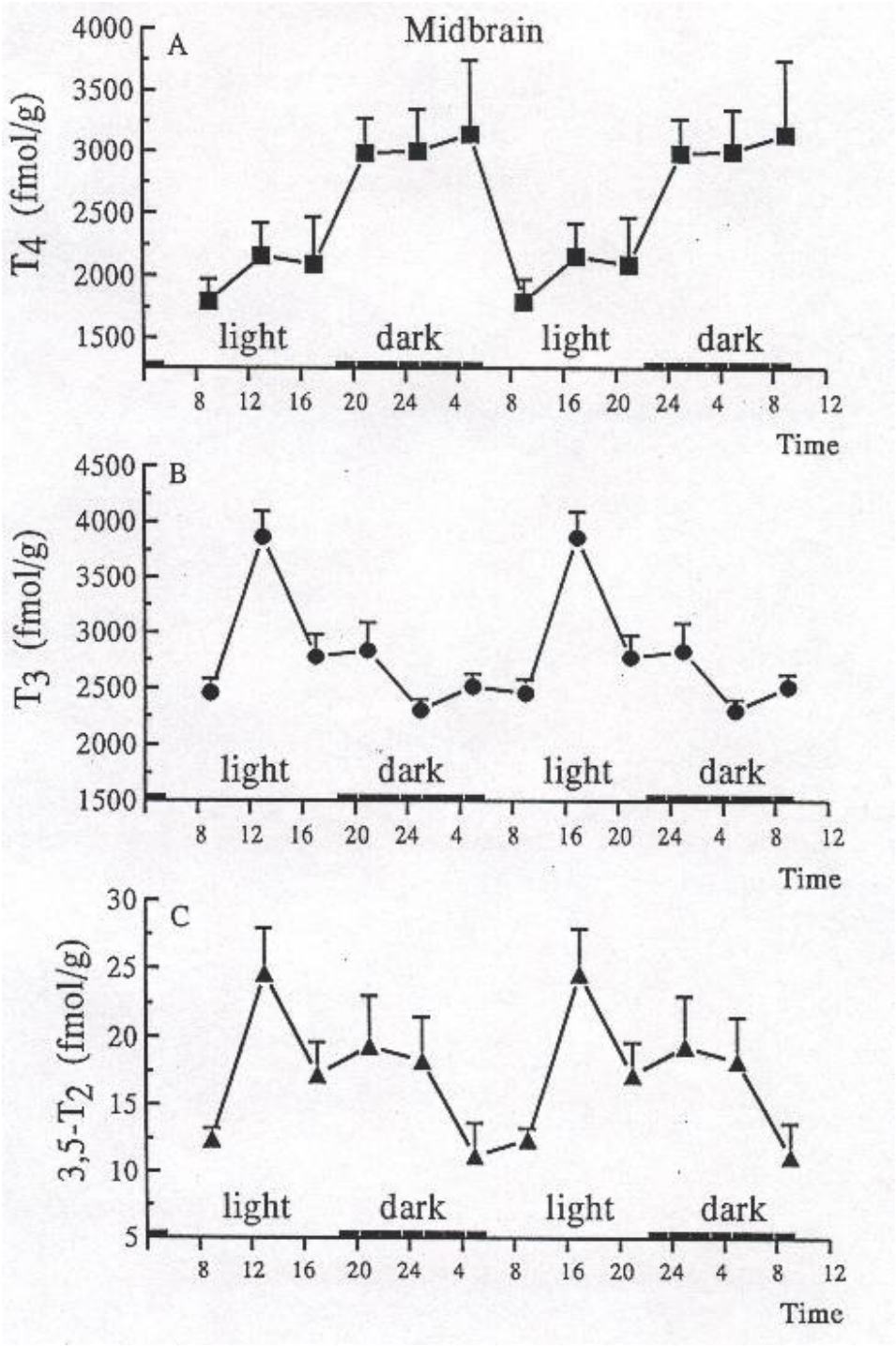


**Fig 20**. Twenty-four hour rhythms of tissue $T_4$ (A), $T_3$ (B), and 3,5-$T_2$ (C) concentrations in the midbrain of euthyroid male rats (n = 6) entrained to a regular 12:12 h light-dark photoperiod. Horizontal bold segments of the X axis indicate the dark phase of the photoperiod.

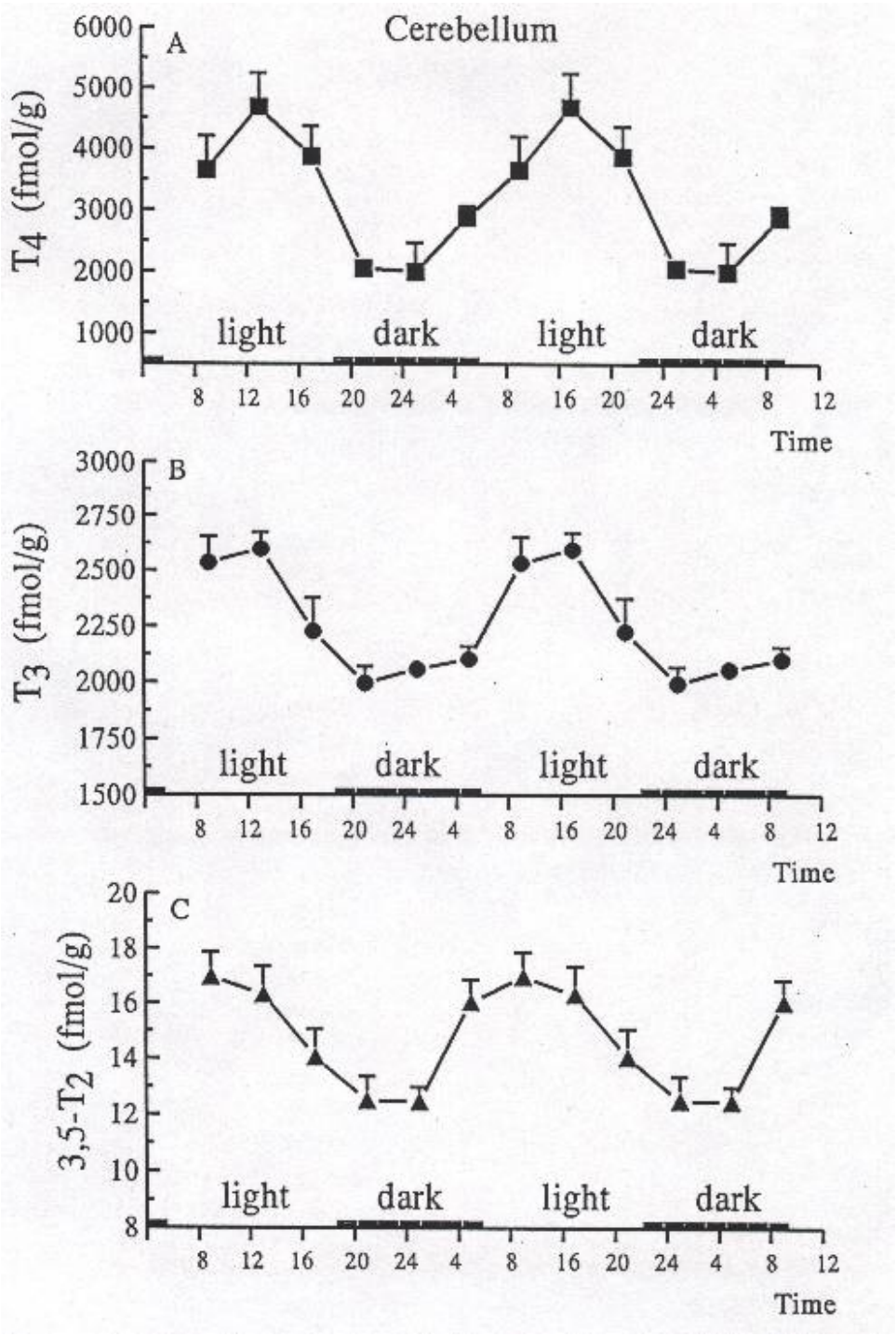


**Fig 21**. Twenty-four hour rhythms of tissue $T_4$ (A), $T_3$ (B), and 3,5-$T_2$ (C) concentrations in the cerebellum of euthyroid male rats (n = 6) entrained to a regular 12:12 h light-dark photoperiod. Horizontal bold segments of the X axis indicate the dark phase of the photoperiod.

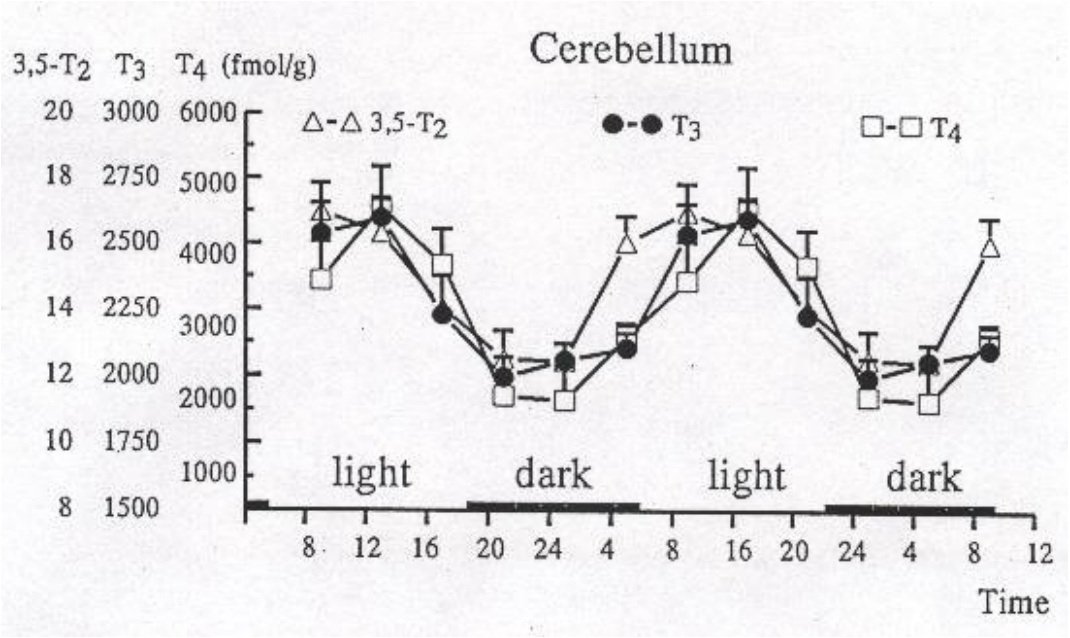


**Fig 22**. Simultaneous plot of the circadian rhythms of tissue $T_4$, $T_3$ and 3,5-$T_2$ concentrations in the cerebellum.