weniger als 0.8 µCi/ml ^{125}J-T_4 inkubiert wurden (VK3.8%). Eine Beziehung der Ergebnisse zu Filtrationsdauer oder -druck findet sich nicht (Tabelle I). Durch Verwendung heparinisierter Vakutainer-Röhren lässt sich der gelegentlich auftretende Konzentrations-Polarisationseffekt und geringere Filtrationsleistung umgehen. Unsere an klinisch gesunden Versuchspersonen gefundenen Werte (Tabelle II) stehen in guter Übereinstimmung mit jenen anderer Autoren[4].

Tabelle I. Freies Thyroxin (PFT_4) im Serum in Abhängigkeit von der Dauer der Ultrafiltration

Patient	1.0	1.5	2.0	2.5	3.0	3.5	4.0
K.,U.	0.018[a]	0.019[a]	0.019[a]	0.020[a]			
N.,P.				0.0325[b]	0.0345[b]	0.0310[b]	0.0305[b]

(Stunden, [a]) oder N_2-Druck (atü, [b]). Mittelwerte aus je 3–4 Einzelbestimmungen.

Tabelle II. Gesamtes, prozentual freies und absolut freies Thyroxin im Serum von Gesunden (P) und Patienten unter T_4/T_3-Substitution nach Strumektomie (S)

	T_4 (µg/100 ml)	PFT_4	AFT_4 (ng/100 ml)
P (18)	7.06	0.030	2.17
	1.63	0.010	0.79
S (12)	8.95	0.022	2.0
	1.91	0.001	0.80

Mittelwerte ± 1 SD. ( ): Zahl der Fälle.

Während die Vorteile der PFT_4-Bestimmung mittels Gleichgewichtsdialyse gegen pH-stabile Pufferlösungen in der geringen Menge benötigten Probenmaterials zu suchen sind, lassen sich mittels Ultrafiltration mühelos 30–40 Proben/Tag gewinnen und am folgenden aufarbeiten.

Summary. A rapid determination of free thyroxine in human serum using ^{125}J-thyroxine and pressure ultrafiltration is presented. Assaying per cent free thyroxine (PFT_4) more than 30 specimen/day can easily be established. The mean value of healthy controls is 0.030 ± 0.010 (1 SD). Interference of results with height of pressure applied and duration of ultrafiltration resp. could not be detected. Both precision and sensitivity of this method allow further research and routine clinical employment.

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A Method and its Pharmacological Application to Determine the Duration of Arousal Pattern Based on Amplitude Changes of EEG Signals in Rats

After the first attempt at clinical use of potentials generated by the brain, it was very soon recognized that there exists a specific low voltage and high frequency EEG pattern now known as desynchronisation[1], activation pattern[2] or arousal reaction[3]. The arousal reaction is used as parameter for studying the mode of action of many centrally acting drugs. In this respect, the following aspects of the arousal phenomenon can be measured: arousal threshold, amplitude and frequency of EEG signals during arousal, and duration of the arousal action. The first 3 parameters using the existing methods can be expressed in an exact and objective way. However, with the duration of arousal many authors[4–6] still analyse this parameter exclusively by the visual inspection of the EEG records. This method is subjective, and difficulty is often encountered in determining the end of arousal, since transition of arousal and control pre-arousal state is not sharp. Moreover, the oscillation of the amplitude during the arousal period may very often reach the pre-arousal amplitude value, confusing the observer as to whether arousal is really terminated or not.

Our preliminary analysis of the frequency and amplitude of the activation pattern indicated that the amplitude of EEG signals is a better parameter for quantification of the duration of the arousal than the frequency spectrum. This led us to the use of the integrative method[7] for amplitude analysis of the electroencephalogram in pre-arousal and arousal period, with the aim of developing a simple and objective way for determination of the duration of arousal.

Materials and methods. Adult albino male rats (200–250 g) of the Wistar strain (TNO animals farm Delft) were used. Electrodes for the EEG recording were essentially similar to those described earlier[8] and were slightly modified (H. van Riezen, personal communication). Implantation was carried out under Hypnorm® (10 mg Fluanison and 0,2 mg Fentanyl in 1 ml) anaestheticum in doses of 1 ml/kg i.p. Following surgery the wound of the rats was treated with penicillin powder. Before the experiment the rats were allowed at least a week for recovery during which period they were caged individually.

The EEG was recorded while the animals were in a sound proof cabin with dimmed light. The animals were left overnight in the cabin before the experiment started. During the experimental session the rats had freedom of movement, twisting of the wires being prevented by the use of a swivel.

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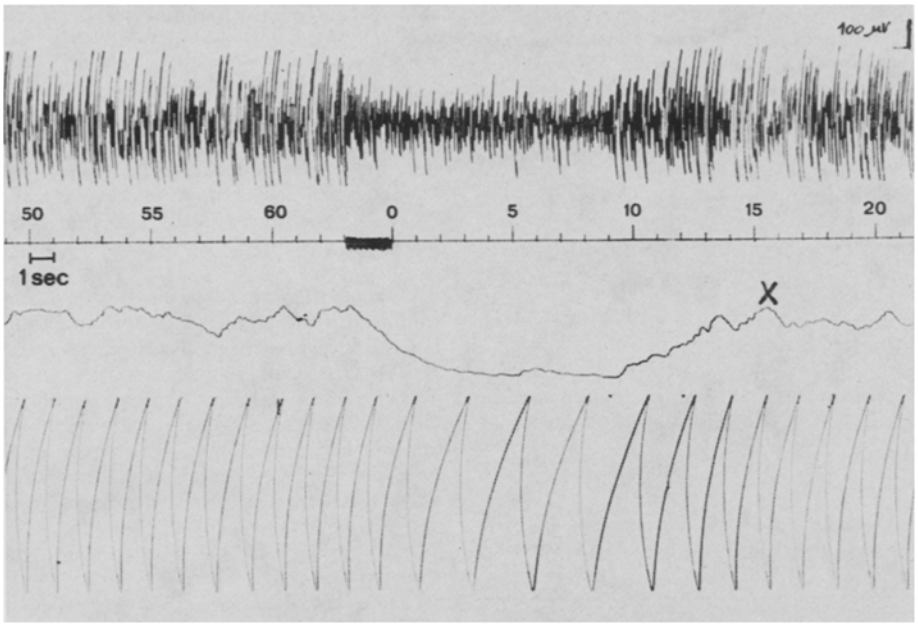

Electrocorticogram of the rat. The upper record is a frontal left-frontal right lead. Below this is a time marker with a 2 sec acoustic stimulation (200 Hz, 20 dB) indicated by the solid line. The middle record is the averaged amplitude of the recorded EEG signals. The lower record is taken from the integrator. The averaged line indicates that in the pre-arousal period there are no marked oscillations of the amplitude in last 10 sec. In this period the number of integrated resets is 7. Since in the last 30 sec before the acoustic stimulation is applied, the amplitude value oscillated in the same range, the mean value of the resets in 3 subsequent periods of 10 sec was also 7. After the sound stimulation, the amplitude of the EEG-signals decreased, averaged line declines and the number of integrated resets decresead also. However, after a relatively short period of time, the amplitude begins to increase and in the period between 10 and 20 sec after the acoustic stimulation, the number of the integrated resets became equal to the control value. This indicated that the arousal was terminated. However, the precise point of the end of arousal within this 10 sec is determined by the averaged line, using arbitrarily the highest point (indicated by X) which does not exceed the highest point of the prearousal averaged line. The exact time for the duration of arousal for recording on the Figure is 17.5 sec, as counted from the onset of the acoustic stimulus.

The electrocorticogram was monitored on a Grass model 78, the EEG signals being amplified and averaged through a Grass 7P3 and electronically integrated with a Grass 7P10 integrator. The integrator was calibrated in a way that 20 resets in a 1 min period corresponded to 150 µV. By knowing the number of integrator resets for a given period of time, the average integrated voltage of an EEG recording in the coresponding time can be calculated. Amplification of the EEG signals was kept constant during the experiment.

Results. a) Determination of the duration of arousal. The first recording session normally began 2–3 h after connection of the implanted electrodes. The arousal was induced by acoustic stimulation (200 Hz, 20 dB for 2 sec) using a sound generator (Exact Electronic, model 126). EEG recording lasted at least 1 min before acoustic stimulation was applied. A longer recording session was normally not necessary, but was considered if there were pronounced variations of amplitude in the pre-arousal period. Recording of the EEG after acoustical stimulation was continued until the termination of arousal. However, this recording was limited to 1 min, if the arousal duration was less than 60 sec. During the recording of the EEG in the pre-arousal period the number of integrative resets every 10 sec was noted. If an arousal response after sound stimulation was pronounced, then the amplitude of the EEG signals was decreased and the number of integrative resets also decreased in comparison with the number of resets in the pre-arousal period. The control amplitude value of the pre-arousal period was given by the mean value of the resets counted every 10 sec in 3 subsequent time periods during the last 30 sec before sound stimulation was applied. After induction of the arousal we considered that it is terminated when the control pre-arousal amplitude was regained. This could be demonstrated by counting the integrated resets in one of the 10 sec period in the post-arousal state. If the number of integrative resets in one of the 10 sec periods of the post-arousal recording became equal to the number of resets taken as the control value in the pre-arousal period, then it was considered that arousal was terminated. The exact point of the end of arousal within a 10 sec period was established by means of an averaged line. We have chosen arbitrarily the highest point of an averaged line which does not exceed the highest point of the averaged pre-arousal signals as the point of arousal termination (Figure).

b) The application of the above method for study of the effect of drugs on the relationship between the duration of arousal and amplitude value of EEG.

Animals treated with depressory drugs in doses which increase the amplitude value of EEG signals, show, in most cases a decrease of duration of arousal. This should be considered as the occurence of the well-known type of 'association' of the 2 phenomena (i.e. increase of amplitude and decrease of arousal duration). However, using our method we observed that these 2 phenomena are not necessarily associated. When rats were treated with 5 mg/kg of the recently described GABA-like substance 1-hydroxy-3-aminopyrrolidone-2 (HA-966)[9], there was

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an increase of the voltage and decrease of the duration of arousal in the 1st h after injection. However, in the next $^1/_2$-h the duration of arousal was normalized but the value of amplitude was still significantly higher comparing with the control value. Clearly this suggests the existence of the time differences in the recovery of the structures which are involved in the synchronisation and arousal after the administration of a CNS depressant drug.

Discussion. In spite of the fact that averaged signals of linear characteristics give better visual presentation of the amplitude variation in arousal than normal EEG records, we considered them to be insufficient criteria for the determination of the end of arousal. This is due to the fact that oscillating averaged line can, for short periods of time, be equal or greater than the pre-arousal averaged amplitude value, thus giving a false impression that arousal has been terminated. Evidence that arousal has indeed not terminated came from the integrated amplitude value, which in a period of 10 sec did not reach the control pre-arousal value. For this reason we suggest the simultaneous use of both parameters – integrated and averaged signals – as a more accurate way to determine the end of arousal.

The use of the integrated EEG-signals together with an averaged amplitude line gives not only objective criteria for determination of the end of arousal, but also the possibility to compare the changes in the duration of the arousal and amplitude values during the pre-arousal and arousal period. Using this method in experiments with the rats treated with the GABA-like substance HA-966, we have shown that the increase of the amplitude in the pre-arousal period was not followed by changes in the duration of arousal reaction in the recovery time. This indicates that certain drugs may have a 'dissociative' effect, showing that synchronisation in the pre-arousal period and duration of the arousal might be two independent phenomena not necessarily correlated. It is of interest to note that the substance HA-966 has earlier been described to induce dissociation between EEG-pattern and behaviour[8]. In this respect it would be of importance to conduct similar studies with other 'dissociation' inducing drugs.

In addition we should like to point out that the method described for the determination of the duration of arousal with the parallel registration of amplitude can be applied to any other animal with any type of stimulus for the induction of activation pattern and is particularly suitable for laboratories without computer facilities.

In our experiments we intended to exclude the small spontaneous variation of the amplitude, thus we chose the relatively small number of resets (20) to correspond for 150 μV in 1 min period time. However, the possibility still exists of using a higher number of resets (60–80 etc). If this variation is applied than the arbitrary end of arousal should be considered when the post-arousal amplitude of EEG signals reaches the 90% of control pre-arousal voltage value. The exact point of the end of arousal within the 10 sec time interval already selected is determined by the averaged line as described before.

Résumé. On décrit une méthode établie pour déterminer la durée de la désynchronisation corticale en utilisant les changements d'amplitude de signaux électro-corticographiques. Un intégrateur Grass a été utilisé pour enregistrer d'amplitude moyenne et pour intégrer les signaux EEG. La phase de désynchronisation est considérée comme terminée quand l'amplitude des signaux, avant l'excitation, réapparaît et reste à ce niveau pendant 10 sec. Le point culminant de la ligne de moyenne pendant la période de 10 sec (qui n'exède pas le point culminant de la ligne de moyenne en la phase précédant l'excitation) est choisie comme indiquant la fin de la phase de désynchronisation. L'usage de la méthode démontra que les effets d'un nouveau congénère du GABA, l'HA-966 sur la synchronisation de l'EEG et sur le raccourcissement de la réaction d'éveil peuvent exister indépendamment l'un de l'autre.

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Improved Method for Separation and Identification of Serum Transferrins: Thin Layer Acrylamide-Gel Electrophoresis with Acrinol Pretreatment

For the separation and identification of serum transferrins, starchgel electrophoresis[1] has been widely employed in combination with the labelling of the protein with radioactive iron (Fe^{59}) followed by autoradiography[2]. It is still desirable, however, to develop more rapid, accurate and safe method for these purposes. A combination of acrinol pretreatment and thin layer acrylamide-gel electrophoresis seems to fulfill the above requirements, as demonstrated in the present study. Using this method one can identify transferrin bands on the gel clearly and rapidly without any use of the hazardous radioactive isotope and subsequent autoradiography.

For the acrinol pretreatment, a preparative method[3] of transferrin using acrinol and ethanol was modified to the smaller scale. As a routine procedure, 100 μl murine serum, mainly *Rattus rattus* in our case, was mixed with 1 μl of 0.6 m*M* $FeCl_3$ solution and diluted 4 times with 300 μl of 5 m*M* *Tris* buffer (pH 8.8). To that mixture was added 400 μl of 0.6% acrinol solution prepared just before use. After 30 min, coagulated proteins were removed by centrifugation at 3,000 rpm for 10 min at 0 °C. An equal volume of 95% cold (–10 °C) ethanol was added to the supernatant. After standing the mixture in an ice bath for 30 min, the protein fraction was spun down at 3,000 rpm for 10 min at 0 °C, after which it was dissolved in 100 μl of 5 m*M* *Tris* buffer and subjected to the thin layer acrylamide-gel electrophoresis.

The procedures for the thin layer acrylamide-gel electrophoresis essentially followed a previous report[4]. Acrylamide was dissolved at the final concentration of 3.8%, methylenbisacrylamide (BIS) 0.2%, tetramethylethylendiamine (TEMED) 0.3% and ammonium persulfate 0.036% in *Tris* citrate buffer (pH 7.4) containing

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