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DSIP-Induced Changes of the Daily Concentrations of Brain Neurotransmitters and Plasma Proteins in Rats

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GRAF, M., J. B. BAUMANN, J. GIRARD, H. J. TOBLER AND G. A. SCHOENENBERGER. *DSIP-induced changes of the daily concentrations of brain neurotransmitters and plasma proteins in rats.* PHARMAC. BIOCHEM. BEHAV. **17**(3) 511–517, 1982.—The influence of delta sleep-inducing peptide (DSIP) on the brain neurotransmitters 5-HT, dopamine and norepinephrine and plasma proteins/corticosterone concentrations for four time points within the 24 hr following IV injection of 30 nmol/kg was investigated in rats. DSIP administered in the morning or in the evening respectively induced changes in nearly all measured parameters. Different effects were observed for different times of administration. The most marked changes were found in the level of serotonin during daytime. In view of the multivariate results obtained by measuring several parameters at multiple time points, a method was developed to describe the time-dependent changes. By means of "circadian rhythm statistics" based on a statistical likelihood analysis we found that multiple and different changes within the factor's daily variation are induced by one injection of DSIP. A multidimensional scaling of the results provides further insights into the correlations of the DSIP-induced effects on plasma and brain factors which are therefore tentatively termed "programming functions." These apparently involve not just sleep induction but also act on multiple parameters within the 24 hr rest-activity period.

Delta sleep-inducing peptide Time-dependence Daily rhythm Circadian statistics Rat
Neurotransmitters Plasma proteins

ISOLATION, characterization and synthesis of the delta sleep-inducing peptide (DSIP) have previously been reported [22,23]. A short review of its possible multiple functions recently appeared [11]. We reported the capacity of DSIP to shift the circadian locomotor activity in time for rats after repeated daily IV injections prior to the onset of increased locomotor activity (arousal/waking). In addition, phosphorylation of the serine in position 7 produced a quicker and more pronounced change of the circadian locomotor rhythms within a constant light-dark period=Zeitgeber [1] than the original compound. This effect was achieved by the dose of 100 pmol, i.e. 300 times lower than with the original peptide [6]. In normal and more drastically in insomniac humans DSIP qualitatively and quantitatively enhanced EEG and behavioral sleep during the night following administration, i.e., in analogy to the circadian sleep rhythmicity. It simultaneously influenced basic psychophysiological parameters in the sense of a circadian shift [5,21].

In the last decade it has been established that the catecholamines norepinephrine (NE) and dopamine (DA) as well as serotonin (5-HT) influence the sleep-EEG patterns in different mammals and humans [10,15]. Furthermore, circadian variations of plasma factors like proteins or hormones are well known [7, 19, 25, 27] and might influence time and dosage of drug administration in general [2,20]. For these reasons we attempted to investigate the influence of DSIP on these humoral parameters with regard to acute or delayed effects within their diurnal rhythms.

METHOD

All reagents were analytical grade and used without further purification. Serotonin (5-HT), norepinephrine (NE) and dopamine (DA) were purchased from Fluka, Switzerland. Synthetic DSIP was obtained from Bachem Fine Biochemicals, Switzerland.

Male inbred rats (KBS/K60, 200–250 g) from our own colony were adapted in groups of 5/cage to constant room

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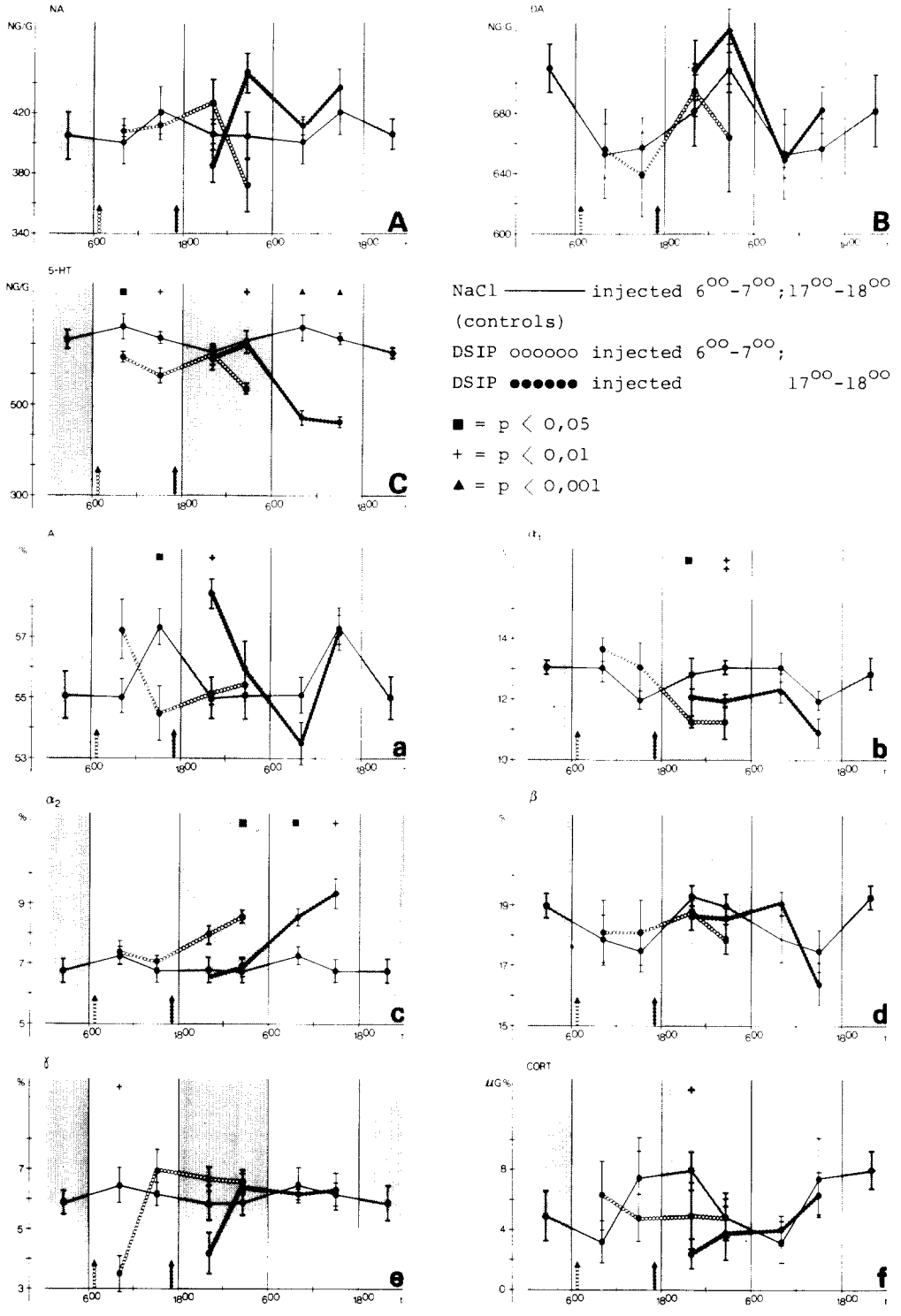

FIG. 1. Capital letters = brain factors; Small letters = plasma factors:

A (NA)	Noradrenaline	f (CORT)	Corticosterone
B (DA)	Dopamine	dashed area	darkness
C (5-HT)	Serotonin	t	daytime
a (A)	Albumin	NG/G	content in ng/g wet weight brain tissue
b (α_1)	α_1-Globulin		
c (α_2)	α_2-Globulin	%	percentage of total protein
d (β)	β-Globulin		
e (γ)	γ-Globulin		

All values are indicated as the mean ± s.e. Control: n = 10, DSIP: n = 5.

conditions for 21 days. A regular light ("Truelite" source; 0600–1800) and dark (1800–0600) cycle was maintained. The animals had free access to standard food and water ad lib. Groups of 4×5 test animals and 4×5 control rats were randomly formed, and food was retrieved 12 hr prior to the experiments. The groups were divided into evening animals (=E) and morning animals (=M). The M rats were injected from 0600–0630, i.e., at the beginning of the light (sleeping) period with either 30 nmol of DSIP/kg or 0.4 ml of saline=controls. The E animals of the test and control groups were injected from 1700–1730, i.e., just before the beginning of the dark (arousal/activity) period. Ten animals (5 test and 5 control rats) of the M (morning injection) and the E (evening injection) groups were sacrificed simultaneously 4, 9, 16 and 21 hr after the respective injections by decapitation in a separate room. This experimental design permitted measurements over time with respect to the injections on one hand, and within 24 hr following administration on the other.

The pituitaries, pineal glands and the whole brains (minus olfactory bulbs) were removed, immediately transferred to dry ice and kept frozen at −30°C. Norepinephrine, dopamine and serotonin were determined by the method of Shellenberger and Gordon [24]. The blood was collected from the decapitated trunk into ice-cooled tubes containing EDTA to prevent coagulation. The blood was centrifuged at 4°C for 10 min at 3000 × g and the plasma frozen at −30°C. All determinations for any group of animals killed together were carried out simultaneously. Plasma protein electrophoresis was routinely performed in the clinical laboratories and quantified by scanning in a Densicomb apparatus (Clifford, USA). Corticosterone was estimated by radioimmunoassay [4].

RESULTS

In a first approach the values measured were evaluated with a *t*-test whereby DSIP animals were compared to NaCl injected controls. Fig. 1 shows that not only the values of the variables measured but also their time courses appear to be changed under DSIP treatment.

Depending on the time of injection DSIP seems to interfere with regulatory processes. These interferences manifest themselves in alterations of the levels measured and their time courses. Beside the neurotransmitters NE, DA and 5-HT, plasma proteins and plasma corticosterone levels and their time courses are changed by DSIP.

DSIP apparently influences multiple biochemical parameters known to reflect circadian rhythmicity on one hand and to be involved in the mechanism of sleep regulation on the other. As only 4 time points were measured and as the standard deviations were rather large, only very general information about the 24 hr rhythmicity of the observed values can be gained. For this purpose the following nonparametric procedure for qualitative analysis was developed:

Given 4 time points and 3 levels Low, Middle, High for the variables measured, $3^4=81$ different circadian rhythms can be defined (e.g., LLHH, HLML, etc.). The analysis proceeds as follows: For each treatment group (DSIP-M, DSIP-E, Controls) each of the 81 possible rhythms was formulated as a hypothesis and tested as follows: The measurements for the High and Middle periods for the particular rhythm tested were pooled into group 1 and the measurements for the Middle and Low periods into group 2, and the groups 1 and 2 were tested for a difference in central trends with a Mann-Whitney U-test. Rhythms with all equal levels (HHHH, LLLL, MMMM) were not considered. The confidence level ($1-\alpha$) was used as a sort of likelihood probability p_i, i=1–81 for the individual rhythms.

The likelihood probabilities p_i were normalized to a sum of 1.0 for all "possible" rhythms. Then for each time t an estimate of the level L_t (t=1–4) was calculated by multiplying for each rhythm i its likelihood probability with the interval value v_{it} (1=Low, 2=Middle, 3=High) and summing up all products for each time interval:

$$L_t = \sum_{i=1}^{81} p_i \cdot v_{it}$$

The values L_t for the 4 time intervals and the 3 treatment groups are depicted in Fig. 2. The U-test designed for the comparison of two groups was selected instead of a Kruskal Wallis test for 3 groups in order to avoid a possible overinterpretation of the results. The sampling statistics of the new procedure were not further investigated, the results obtained therefore have now to be interpreted as mere descriptive procedure for illustrating a qualitative pattern of rhythmicity in cases where due to a restricted number of time points and restricted information on error distribution, parametric tests and cosinor type analysis cannot be carried out seriously. However, the representation of results in Fig. 2 which directly reflects the significance of the variations is in good agreement with the representation in Fig. 1. Therefore, we assume that this method in our case is appropriate for reflecting a rough qualitative description of circadian variations. Figure 2 suggests the following influences of morning (M) and evening (E) injections of DSIP upon the daily variations of these parameters: an acceleration of the rhythmic changes of dopamine, 5-HT and corticosterone occurring in both cases (M,E) of DSIP treatment whereas the variations of noradrenaline being slowed down (M) or accelerated (E).

Furthermore, there appeared to be some hidden common structures in the observed values. This can be elucidated by an analysis of the correlation structure of the parameters investigated. The correlation structure of the nine observables measured can be discussed on the basis of the Spearman rank correlation matrix (Table 1) instead of interpreting pairwise correlation of the observables measured. It is possible to investigate the question whether the variabilities of the values observed reflect independent phenomena, or whether they can be formally interpreted as results of a restricted number of latent processes. For this purpose the Spearman rank correlation matrix (Table 1) for the 9 variables was submitted to a multidimensional scaling procedure [9]. The rank correlation coefficients were taken as "similarities" of the observables. Multidimensional scaling represents observables as points in a geometric space such that similarities are displayed as distances, i.e., short distances represent strong similarities (positive correlations) and long distances strong dissimilarities (negative correlations). The lower the necessary dimensionality of the representation space, the less is the number of independent latent variables to which the similarity structure investigated can formally be referred. Visual inspection of multidimensional scaling results allows to see clusterings of similar variables and separation of dissimilar variables and can induce the formulation of hypotheses. Starting from $n-1=8$ dimensions and reducing the dimensionality in our case a representation of the correlation structure is possible with negligible inaccuracy to a 4-"dimensional" space and with satisfactory accuracy to a 3-"dimensional" space as shown in Fig. 3A and 3B. In Fig. 3A the multidimensional scaling results in 4

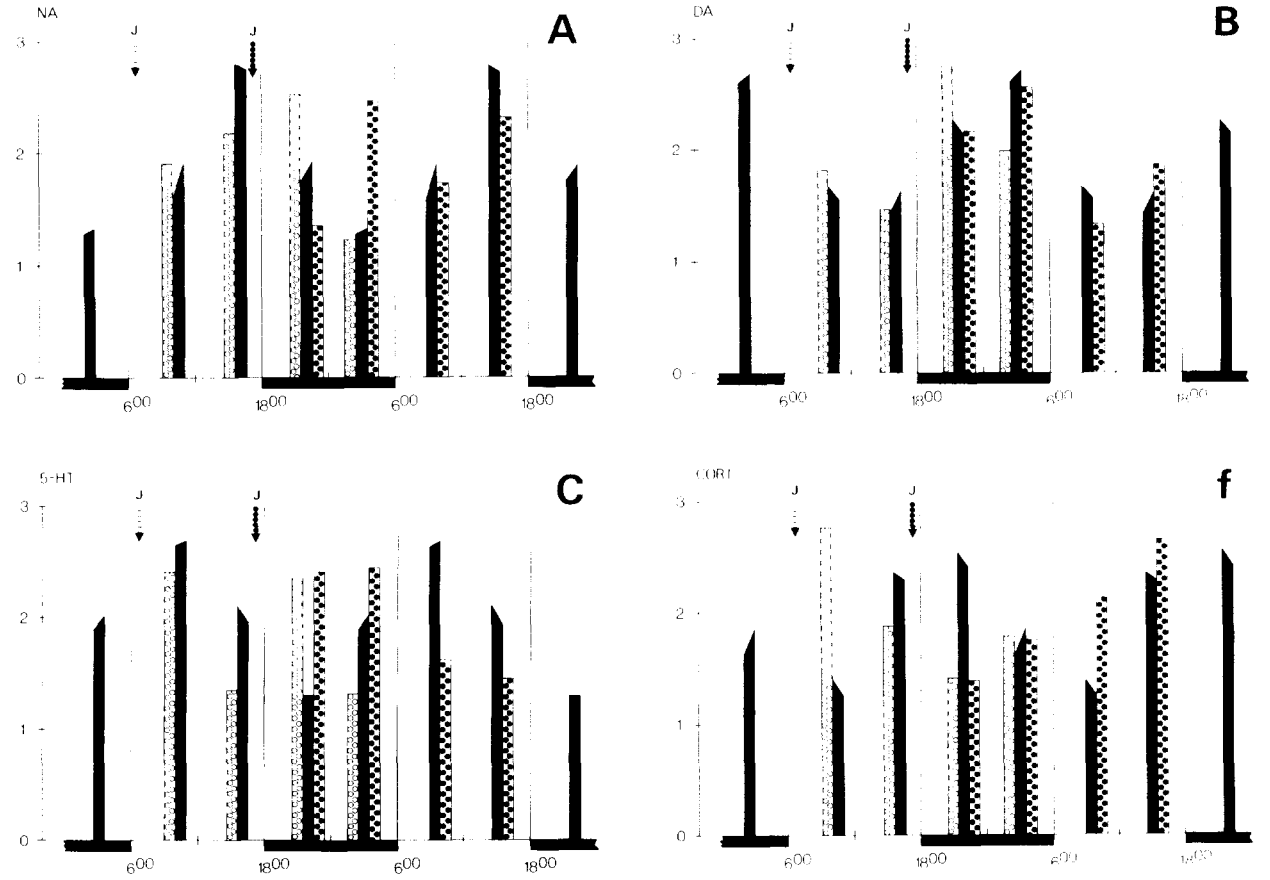

FIG. 2. Letters same as for Fig. 1. Dark horizontal bars=darkness; Ordinate=arbitrary units (see text); Dark vertical columns=controls. Left upper edge of the dark vertical columns=Control value (n=5) determined at the same time as the rats after the morning DSIP injection (n=5) (open circled columns). Right upper edge of the dark vertical columns=Control value (n=5) determined at the same time as the rats after the evening DSIP injection (n=5) (black circled columns). J=Injection (NaCl or DSIP).

dimensions are depicted. The 3 dimensions shown represent the first 3 dimensions in a solution space of 4 dimensions. The close distances between NA, 5-HT and surprisingly serum α_1-globulin suggest an interdependent reaction of these parameters after DSIP administration. DA more distant might be discussed as positively correlated to this group. Plasma corticosterone is positively correlated with γ-globulin variations whereas albumin, α_2- and β-globulin do not. However, elimination of the fourth dimension and representation of the multidimensional scaling results in 3 dimensions (Fig. 3B) still give the same qualitative configuration of the observables as shown in Fig. 3A. A positive correlation between albumin and α_2-globulin might be possible. This result indicates that the number of latent processes that manifest themselves through the nine variables investigated seems to be lower than 9. Probably about 3–4 different independent latent processes seem to cause the DSIP-induced variation of the 9 variables.

DISCUSSION

The frequent sampling paradigm employed in the present study does not allow for accurate characterization of the rhythmicity of the parameters being examined. However, this pilot study was designed for a first exploration of a possible influence of DSIP upon multiple biochemical parameters over 24 hr. As saline controls were carried out simultaneously the results of the 4 time points after a morning and evening injection are comparable and informative for this purpose. Furthermore, a comparison of the correlation between the parameters measured in connection to DSIP administration was possible.

We are also aware of the fact that some non-responses in behavioral studies with DSIP are observed. Some reasons which might explain this finding are listed below. However, in our experiments we did not focus on all or more effects but rather a quantitative shift of mean levels. In addition, where a weak statistical difference is indicated one of the five rats might be attributed to be a non-responder, in the higher probability all 5 were responders whereas the other groups with no t-statistical difference merged.

The primary interest in DSIP was its sleep-inducing property. Therefore, some aspects of the parameters measured in relation with the sleep-waking cycle are briefly discussed. Rhythmic daily variations of serum proteins and hormones are well known [7, 19, 25, 27]. In contrast, whether serum proteins or hormones exhibiting a circadian pattern reflect rather than influence the sleep-waking cycle is poorly understood. We found that the morning (M) and the evening (E) administration of DSIP influenced all serum protein concen-

TABLE 1

SPEARMAN RANK CORRELATION MATRIX OF THE NINE VARIABLES INVESTIGATED

		1 Dopa	2 Nora	3 Sero	4 Cort	5 Albm	6 Alf1	7 Alf2	8 Beta	9 Gamm
I	Dopa	1.000								
II	Nora	.381	1.000							
III	Sero	.179	.152	1.000						
IV	Cort	−.042	−.025	−.110	1.000					
V	Albm	−.014	.156	.138	.048	1.000				
VI	Alf1	−.031	−.189	.136	−.100	−.131	1.000			
VII	Alf2	−.131	.116	−.173	−.078	−.057	−.013	1.000		
VIII	Beta	.127	−.020	−.181	−.063	−.584	−.078	−.334	1.000	
IX	Gamm	.014	−.003	.049	.129	−.303	−.524	−.094	.022	1.000

tration patterns in a similar manner (Fig. 1a to 1e). However, significant differences to the control values were found. At present, it is unclear how these changes were induced and the poor correlation between these parameters (Fig. 3A and 3B) additionally confuses the understanding. Several explanations are possible, but further speculations concerning these results would be presumptuous, because little is known about the endogenous regulation of the protein concentrations in serum.

Biochemical research dealing with induction and regulation of sleep has primarily focused on the role of the classical neurotransmitters, 5-hydroxytryptamine, norepinephrine and dopamine. Several close correlations with the quality and quantity of sleep have been established [10,15].

5-HT is regarded as the transmitter responsible for slow-wave sleep (SWS). It seems to parallel and reflect the vigilance states and to be correlated with the locomotor activity [3,17]. However, a priming role or 5-HT for paradoxal sleep (PS) has also been shown.

Norepinephrine is thought to have a positive priming function for REM-sleep at concentrations below arousal level [10]. Reductions of the NE-content of neurons produced hypersomnia [10,15]. Daily rhythms of the NE-content in hypothalamus, striatum and pons were found to be correlated with the vigilance state [14,25].

Dopamine controls the motoric behavioral response of the arousal phase. Metabolism of both catecholamines seems to correlate positively with the appearance and persistence of PS [12,26]. The chronorhythms of DA (ultradian, partially correlated to GH and corticosterone) [18,25] and NE (circadian) [14, 18, 25] in the brain are consistent with rhythmic changes of catecholamine receptors in this organ [16,28]. Attempts to correlate one amine to one effect led to integrating hypotheses. It was suggested that the sleep-waking cycle is governed by two neuronal systems which exhibit antagonistic effects. The first is responsible for waking and arousal and is influenced by the second system which is responsible for sleep by deactivation of inhibition of the waking mechanisms [15]. These systems use excitatory and inhibitory transmitters and postulate several kinds of modulators all acting together as an overall complex mechanism. However, other findings postulate unknown degradation and synthesis controls within the transmitter systems in addition to the known modulation mechanisms [8,17]. The complex underlying mechanisms of sleep cannot be explained

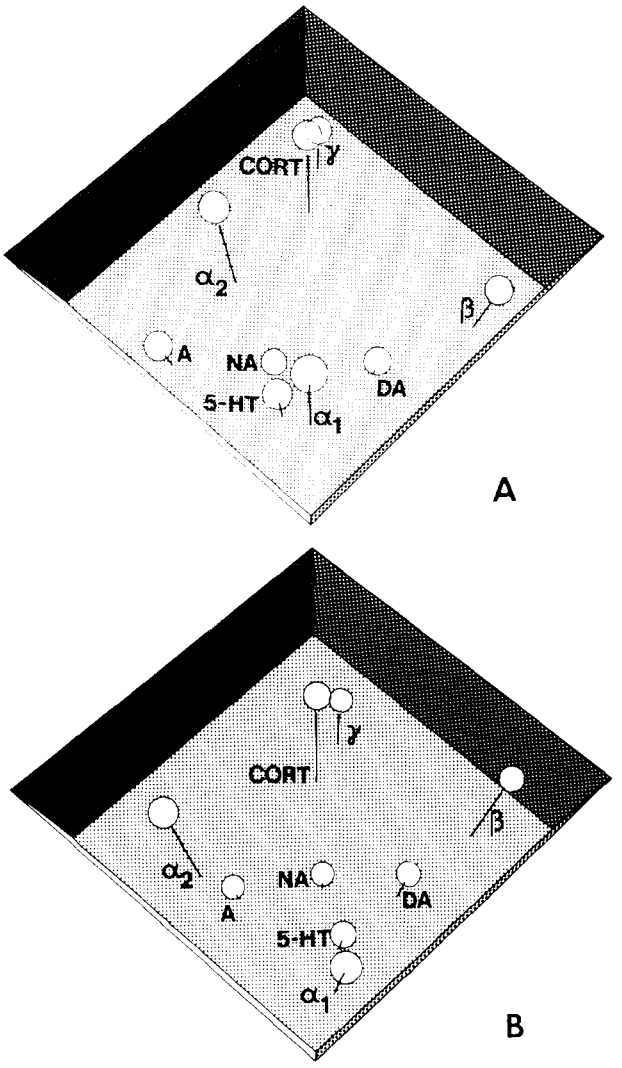

FIG. 3. A. Representation of multidimensional scaling results in 4 dimensions. The 3 dimensions shown represent the first 3 dimensions in a solution space of 4 dimensions. Fig. B. Representation of multidimensional scaling results in 3 dimensions. Elimination of the 4th dimension still gives the same qualitative configuration of the observables. Letters from Fig. 1.

by simple correlation or comparison of single substance effects or systems. In order to approach an analysis of multiple interdependent regulation systems we investigated the influence of DSIP upon the transmitter concentrations in the brain with time using two different time points for administration. The "acute" changes 4 and 9 hr after injection were compared with the "late" effects after 16 and 21 hr respectively. The results are seen within the normal daily changes and can be explained only by interactions of DSIP with the regulation of these parameters. It was not possible to describe the variations by simple comparative statistics. We had to develop a new curve statistic by which we were able to suggest with accurate probability a possible interfering function of DSIP on the circadian organisation of several parameters. As DSIP seems likely to interfere with regulation processes that are also triggered by the light/dark cycle and only two injection times were investigated, at present the interaction scheme with these supposed but yet unknown processes cannot be further characterized.

Although the Spearman rank correlation matrix given in Table 1 seems not to offer a clear interdependence of the variables, submitting the values to a multidimensional scaling procedure and reducing the "dimensionality" of the multiple variables measured led to the least common number of the involved regulating processes. In our study (with 9 variables) not more than three or four latent processes, i.e., common structural functions seem to exist. Therefore, we may conclude that with respect to the rhythmic organisation of multiple factors DSIP apparently exerts an interesting kind of activity tentatively termed "programming" function. The sleep-enhancing capacity previously used for monitoring its isolation and testing the synthetic compound in different species may be understood in this context as a subset embedded in the overall circadian rhythms. Furthermore, the results strongly suggest that in experimental work with DSIP attention must be paid to the following points: time of injection, the time of measuring the parameters within 24 hr and the time elapse from the time of injection, the parameter investigated and the DSIP concentrations vs the duration of the injection. Finally, it seems possible that similar phenomena occur with other CNS-active peptides. This would explain the multidimensional effect obtained upon intracerebral or peripheral injections of a variety of those peptides by measuring multiple compounds and/or reactions. However, the different effects of DSIP discussed have to be confirmed and extended.

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