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Original investigations

Hallucinogenic and stimulatory amphetamine derivatives: fingerprinting DOM, DOI, DOB, MDMA, and MBDB by spectral analysis of brain field potentials in the freely moving rat (Tele-Stereo-EEG)

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Abstract. Telemetric recordings of field potentials from frontal cortex, hippocampus, striatum and reticular formation of freely moving rats were analysed before and after injection of the enantiomeric hallucinogenic amphetamine derivatives *R*-DOB [(−)-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane], *R*-DOM [(−)-1-(2,5-dimethoxy-4-methylphenyl)-2-amino-propane] and *R*-DOI [(−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] as well as the nonhallucinogenic amphetamine derivatives *S*-MBDB [(+)-N-methyl-1-(1,3-benzodioxol-5-yl)butanamine] and *S*-MDMA [(+)-3,4-methylenedioxymethamphetamine] and *S*-(+)-amphetamine. The frequency analysis of the field potentials revealed a clearcut difference between them. The spectral patterns emerging after injection of the non-hallucinogens were characterized by a general decrease of power, the changes in the alpha$_2$ and delta band being the most prominent, whereas only after the application of the hallucinogenic compounds was a contrasting increase of power observed in the alpha$_1$ frequency band, especially in the striatum. As increases in alpha$_1$ power have been correlated in the same pharmacological model to serotonergic control mechanisms, the results are in line with the hypothesis that 5-HT$_2$ receptors, predominantly occurring in the striatum, might be involved in the hallucinogenic action of drugs.

Key words: Hallucinogenics – Amphetamine – Rat – Tele-Stereo-EEG

In order to study structure-function relationships of pharmacological compounds in animals, very sensitive methods of monitoring the physiological effects of the particular drugs are necessary. In addition, their actions have to be measured with the aid of a variety of parameters in order to be able to differentiate very closely related drugs from each other. The recently described method of Tele-Stereo-EEG as developed in our laboratory has been used to characterize a number of compounds known to act on particular transmitter receptors in dopaminergic as well as serotonergic systems (Dimpfel et al. 1987 and submitted). Particular changes in the spectral patterns computed from the telemetrically recorded brain field potentials could be related to specific neurotransmitter control mechanisms in this model. This method proved to be very sensitive; the power within 24 frequency ranges reliably reflected the dose and time dependence of drug action. Therefore, this model appeared suitable to compare hallucinogenic and nonhallucinogenic drugs.

As the electrophysiological fingerprint of the action of amphetamine is already known (Dimpfel et al. 1986a, 1987), it seemed particularly interesting to compare the hallucinogenic amphetamine derivaties DOM, DOI and DOB to the nonhallucinogenic amphetamine derivatives MDMA and MBDB (Oberlender and Nichols 1988). As all three hallucinogenic compounds have high affinity for the 5HT$_2$ receptor, whereas MBDB and MDMA do not, it was hoped to learn something about the functional consequences of different types of receptor interactions in terms of changes in the bioelectric activity of the brain of awake, undisturbed animals, and also to relate them to the hallucinogenic or nonhallucinogenic properties of these compounds.

Material and methods

The procedure for telemetric recording of field potentials from freely behaving rats and for computing the power density spectra has been published in detail by Dimpfel et al. (1986b). In brief, inbred male adult Fisher-344 rats were kept under a reversed day-night cycle and implanted with four stainless steel electrodes, mounted on a base plate, into frontal cortex, hippocampus, striatum, and reticular formation. The plate carried a microplug for a four-channel radiotransmitter. During the experimental sessions, which began 2 weeks after surgery, the transmitted field potentials were analysed in real time using fast Fourier transformations. The resulting power density spectra were segmented into six frequency bands, each representing the integrated power over a certain frequency range (for definition of frequency ranges see legend to Fig. 1). The data from three pre-drug periods of 15 min each were compared to those from continuously monitored 15-min post-drug periods.

The drugs were injected intraperitoneally in a volume of 1 ml/kg in the following doses:

S-(+)-amphetamine 0.2 and 0.4 mg/kg);
S-(+)-MBDB [(+)-N-methyl-l-(1,3-benzodioxol-5-yl)butanamine] 1.6 and 2.4 mg/kg;
S-(+)-MDMA [(+)−3,4-methylenedioxy-methamphetamine] 0.4, 0.8, 1.6, and 2.4 mg/kg;
R-(−)DOB [(−)-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane] 0.1 mg/kg;

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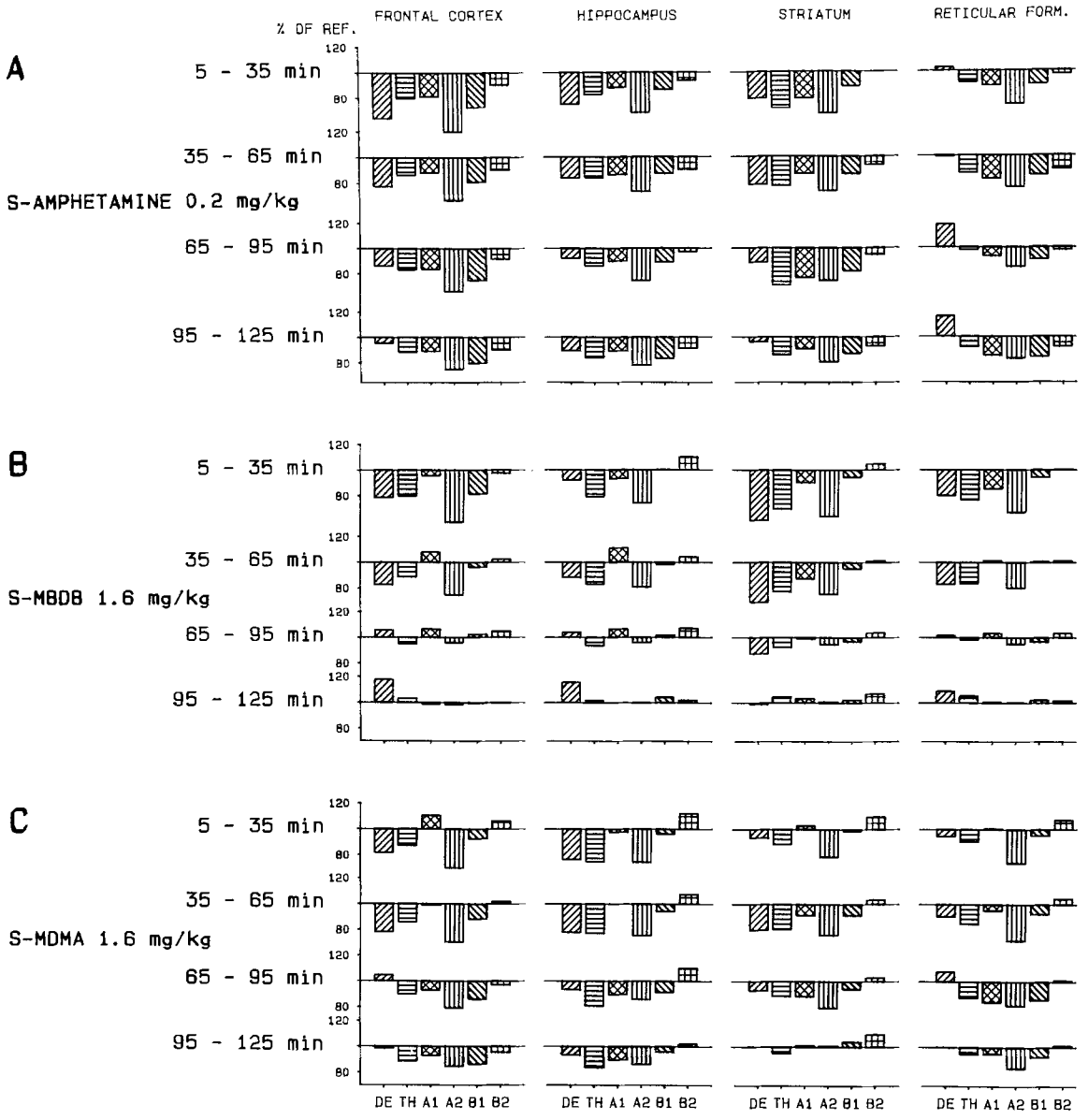

Fig. 1 A–C. Changes in frequency patterns in the first 2 h after IP injection of 0.2 mg/kg *S*-amphetamine (**A**), 1.6 mg/kg *S*-MBDB (**B**) and 1.6 mg/kg *S*-MDMA (**C**). The delta band (*DE*) was defined from 1.25 to 4.50 Hz, theta (*TH*) from 4.75 to 6.75 Hz, $alpha_1$ (*A1*) from 7.00 to 9.50 Hz, $alpha_2$ (*A2*) from 9.75 to 12.50 Hz, $beta_1$ (*B1*) from 12.75 to 18.50 Hz, and $beta_2$ (*B2*) from 18.75 to 35.00 Hz. The ordinates give the power in per cent of the reference value, which is the mean power of the 45-min predrug period in each experiment

R-(−)DOM [(−)-1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane] 0.2 mg/kg;
R-(−)DOI [(−)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] 0.1 mg/kg.

The reactions to the different drugs and dosages were analysed with a total of 32 animals, which went into experiments repeatedly with a minimum drug-free interval of 4 days between successive measurements (in general, 1 week). The data presented here are averages over five to eight single experiments (see Table 1).

To test for significant differences between drug action and the effect of placebo injection, a *F*-approximation to the T^2-trace test criterion was calculated on the basis of the logarithms of the data obtained from the six frequency bands in the power spectra (Ahrens and Läuter 1974). This multivariate statistic was computed separately for the four brain areas (cf., Dimpfel et al. 1986b). In the univariate case, e.g., to analyse data of an individual frequency band from one brain area, the univariate test is equivalent to the test with the *t* criterion.

Results

The intraperitoneal administration of 0.4 mg/kg (+)-amphetamine resulted in clearcut changes in the frequency spectrum, consisting of decreases of power, mainly in the delta and $alpha_2$ band but also, though less prominent, in the theta and $beta_1$ band (Fig. 2A). The power content in the $alpha_1$ and $beta_2$ bands were practically unchanged. With the exception of $alpha_1$ power decreases, essentially the same pattern could also be observed after 0.2 mg/kg

Table 1. Multivariate approximate *F*-statistics for the comparison of the respective drug with placebo on the basis of data of the frequency bands (=six variates) in the power spectra from the four brain areas

	Dose (mg/kg)	*n*	Frontal cortex	Hippocampus	Striatum	Reticular formation
S-Amphetamine	0.2	6	1.29	0.97	1.33	0.91
	0.4	6	4.47	4.08	4.55	4.07
S-MBDB	1.6	6	0.70	0.57	1.90	1.44
	2.4	5	5.84	3.63	4.73	6.10
S-MDMA	0.4	8	0.80	1.22	0.45	1.37
	0.8	5	0.15	0.44	0.26	0.67
	1.6	8	0.64	0.79	1.00	0.97
	2.4	5	18.38	7.81	11.51	5.94
R-DOB	0.1	5	2.86	1.45	1.77	3.45
R-DOM	0.2	7	5.90	4.11	7.35	3.45
R-DOI	0.1	5	2.70	0.56	2.78	1.64

F-values were calculated for data from 5 to 65 min after IP injection. Variance/covariance was estimated on the basis of 98 groups from a part of our data base with a total of 602 experiments carried out under identical conditons. $F(6,499) > 2.13$ corresponds to $P < 0.05$, $F(6,499) > 2.86$ corresponds to $P < 0.01$. *n*: number of experiments

(+)-amphetamine (Fig. 1 A), but the changes did not reach statistical significance (Table 1) and the pattern faded during the recording time, whereas is was rather stable over the 2 h after 0.4 mg/kg.

In terms of spectral patterns, nearly the same changes could be observed after the injection of 1.6 and 2.4 mg/kg MBDB (Fig. 1 B, Fig. 2 B) or MDMA (Fig. 1 C, Fig. 2 C). With respect to the overall efficacy as judged by the statistical *F*-values computed for the data from the six frequency bands from the four brain areas as an index for the dissimilarity from placebo (Table 1), 0.4 mg/kg amphetamine and 2.4 mg MBDB seemed to be rather equipotent; the equivalent dose of MDMA lies between 1.6 and 2.4 mg/kg. When comparing the effects of MBDB with the the pattern observed after injection of 0.4 mg/kg amphetamine, a minor dissimilarity became apparent in that the decrease in theta power in the frontal cortex developed only gradually within the first hour after injection. The 2.4 mg/kg dose of MDMA even resulted in a clear increase in theta power in the frontal cortex for 45 min after injection.

A somewhat similar pattern of power decreases could be observed after the administration of DOB (Fig. 3 A). However, in addition, and in contrast to the effect of the drugs described above, a clear power increase could be observed in the alpha$_1$ range, particularly within the striatum, and later on also in the frontal cortex and hippocampus. The changes were visible to nearly the same extent for 2 h after the injection. The changes in the reticular formation were indiscernible from those after the injection of 0.4 mg/kg (+)-amphetamine during the recording session.

Virtually the same pattern of changes as observed after DOB could be seen after giving 0.2 mg/kg DOM (Fig. 3 B) to the rats. However, there were some subtle differences. Whereas in the case of DOM the power decreases remained throughout the recording period, these decreases tended to cease during the action of DOB. On the other hand, the increase in alpha$_1$ power became much more prominent with time, exceeding by far that observed after DOB. With regard to the reticular formation, again no clear difference could be observed in comparison to (+)-amphetamine or DOB.

The effects of DOI (Fig. 3 C) were rather similar to those reported for DOM. Its action started with an increase of alpha$_1$ power in the striatum, and later on power increases within this frequency band became visible in the frontal cortex and the hippocampus, too. These changes were stronger than those noted after injection of DOB but less pronounced than the effects of DOM. In contrast to the results with the latter two, a late increase in the alpha$_1$ power could be observed in the reticular formation. Similar to DOM, but different from DOB, the power decrease in other frequency bands faded with time.

Taken together, there was a clearcut difference between amphetamine, MDMA and MBDB on the one hand and DOM, DOB or DOI on the other. This difference consisted in a prominent increase of alpha$_1$ power, particularly within the striatum. As can be inferred from Table 2, this striatal alpha$_1$ increase proved to be statistically significant in most comparisons to placebo or to the non-hallucinogenic amphetamine derivatives. In the frontal cortex the equivalent power change was less pronounced, and it was just detectable within the hippocampus. Particularly remarkable is the fact that this increase was seen first in the striatum and did not develop within the other brain areas before at least about 20–30 min had passed after the injection.

Discussion

Hallucinogens and CNS stimulants are two entirely different classes of drugs which, however, are not so easily discriminated in an animal model. Up to now, one of the most powerful methods has been use of the drug discrimination paradigm. This technique has allowed differentiation between hallucinogenic and nonhallucinogenic amphetamines derivatives (Young and Glennon 1986; Oberlender and Nichols 1988). Though this method is based on observation of the highest integrative level of CNS activity, namely behavior, one would like to know more about the mechanisms leading to this discrimination in terms of functional parameters measured at a lower level of integration. Recording the field potentials within several brain areas in the freely moving rat provides such a means, because it reflects the

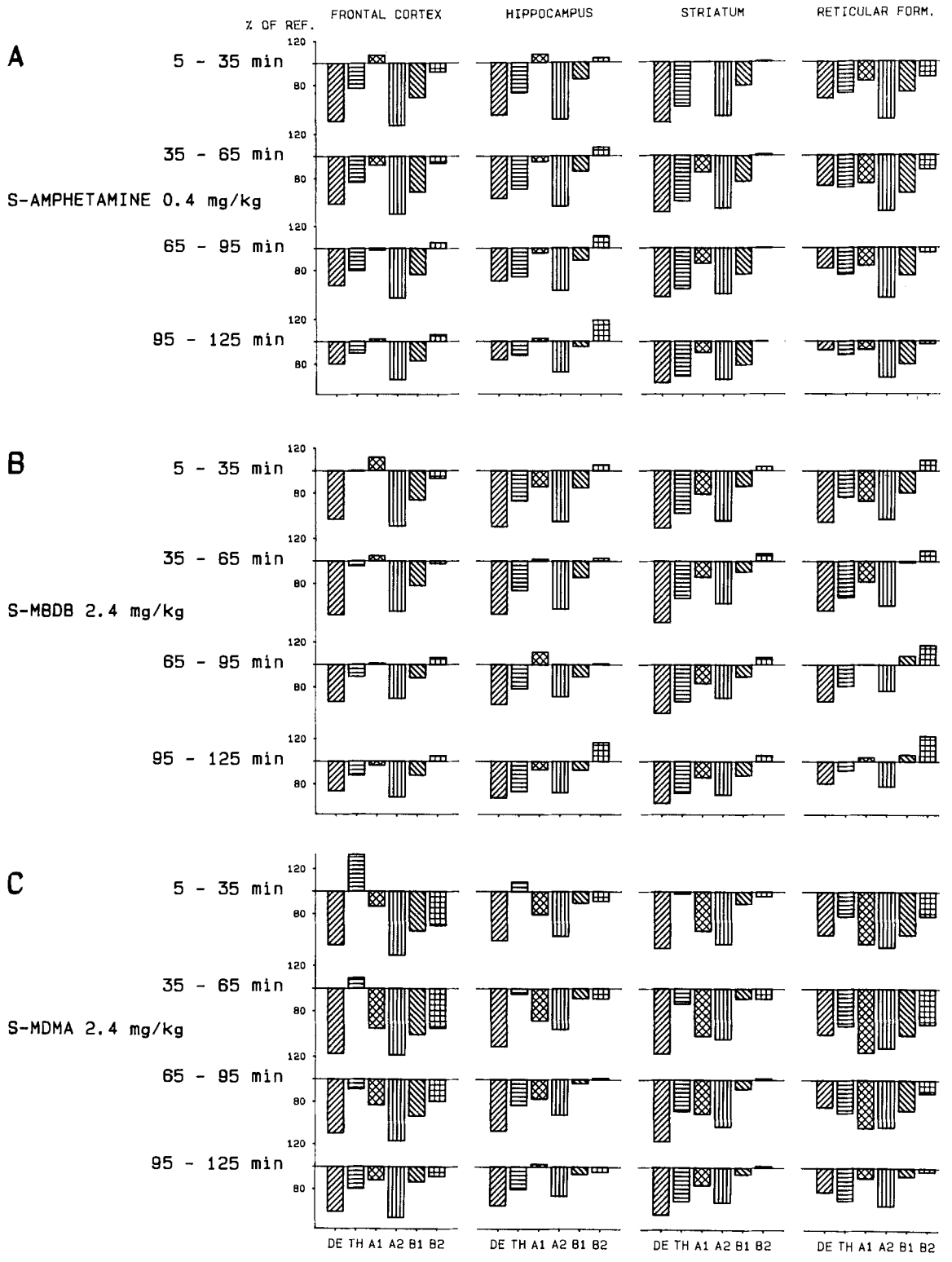

Fig. 2A–C. Changes in frequency patterns in the first 2 h after IP injection of 0.4 mg/kg *S*-amphetamine (**A**), 2.4 mg/kg *S*-MBDB (**B**) and 2.4 mg/kg *S*-MDMA (**C**). For definition of the axes see legend to Fig. 1

Fig. 3A–C. Changes in frequency patterns in the first 2 h after IP injection of 0.1 mg/kg *R*-DOB (**A**), 0.2 mg/kg *R*-DOM (**B**) and 0.1 mg/kg *R*-DOI (**C**). For definition of the axes see legend to Fig. 1

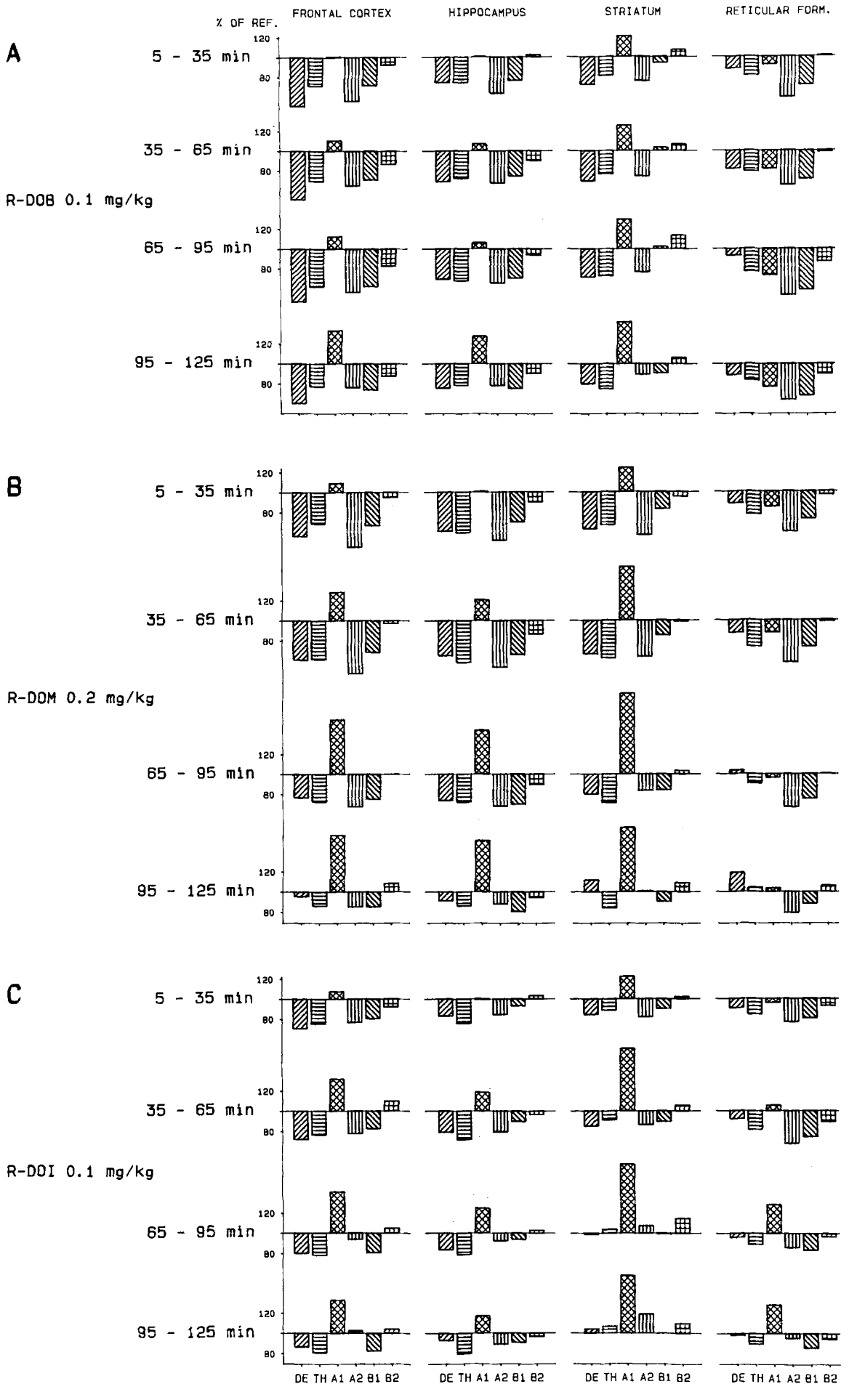
A
B
C
% OF REF.
FRONTAL CORTEX
HIPPOCAMPUS
STRIATUM
RETICULAR FORM.
5 - 35 min
35 - 65 min
65 - 95 min
95 - 125 min
R-DOB 0.1 mg/kg
R-DOM 0.2 mg/kg
R-DOI 0.1 mg/kg
120
80
DE TH A1 A2 B1 B2

Table 2. Univariate approximate *F*-statistics for the data of the alpha$_1$ frequency band from the striatum under different experimental conditions. Comparison of *R*-DOB (0.1 mg/kg), *R*-DOM (0.2 mg/kg), and *R*-DOI (0.1 mg/kg) with placebo, *S*-amphetamine, *S*-MBDB, and *S*-MDMA

		R-DOB	*R*-DOM	*R*-DOI
5–65 min after injection				
Placebo		2.37	6.17	5.73
S-amphetamine	0.2 mg/kg	3.46	6.96	6.69
	0.4 mg/kg	1.39	3.60	3.64
S-MBDB	1.6 mg/kg	2.03	4.69	4.64
	2.4 mg/kg	2.58	5.38	5.31
S-MDMA	1.6 mg/kg	1.02	3.13	3.17
	2.4 mg/kg	9.57	15.40	14.36
65–125 min after injection				
Placebo		3.03	13.06	10.89
S-amphetamine	0.2 mg/kg	3.60	11.71	10.41
	0.4 mg/kg	2.15	8.71	7.81
S-MBDB	1.6 mg/kg	0.71	5.17	4.72
	2.4 mg/kg	2.36	8.70	7.89
S-MDMA	1.6 mg/kg	1.82	8.66	7.62
	2.4 mg/kg	4.56	12.96	11.64

F-values were calculated for data from 5 to 65 and for 65 to 125 min min after IP injection. Variance/covariance was estimated on the basis of 98 groups from a part of our data base with a total of 602 experiments carried out under identical conditions. $F(1,504) > 2.74$ corresponds to $P < 0.10$, $F(1,504) > 3.86$ corresponds to $P < 0.05$

activities of neurotransmitter balance, i.e. the net efficacy of their mutual interactions.

The comparison of various hallucinogenic and nonhallucinogenic amphetamine derivatives using Tele-Stereo-EEG shows a clearcut difference between them, namely that all hallucinogenic compounds increase the power within the alpha$_1$ frequency range, particularly in the striatum, whereas amphetamine, MDMA or MBDB do not. As there is no other obvious difference between them with respect to the other frequency bands, there is a good chance that this feature is not coincidential. The question now arises as to the meaning of this alpha$_1$ increase. In previous studies we were able to correlate increases of power in the alpha$_1$ frequency band with interactions involving serotonin receptors of the 5-HT$_{1A}$ type or with serotonin uptake inhibition (Dimpfel et al. submitted). In both cases alpha$_1$ increases were observed, but those after injection of the 5-HT$_{1A}$ receptor agonists were confined to the hippocampus and, to a lesser degree, to the frontal cortex. Serotonin uptake inhibitors effected the same changes of spectral power, but also produced similar changes in the field potentials from striatum. Thus, it can safely be assumed that the changes produced by DOM, DOB and DOI also reflect changes in serotonergic control of local electrical activity, possibly resulting from direct receptor interaction.

This would be in line with reports showing that indolealkylamines as well as phenylalkylamines bind to the 5-HT$_2$ receptors (Lyon et al. 1988). The distribution of these receptors is somewhat different from that reported for the 5-HT$_{1A}$ receptors in as much as the hippocampus has a very low density of 5-HT$_2$ receptors, whereas in the striatum there are hardly any 5-HT$_{1A}$ receptors (Pazos et al. 1985; Pazos and Palacios 1985). On the other hand the frontal cortex contains both types. This receptor distribution is reflected by the changes in alpha$_1$ power after the administration of the different receptor specific drugs. This leads us to the conclusion that the local brain electrical activity governed by drug specific interactions on the receptor level can be characterized by spectral analysis of the field potentials recorded from those particular brain areas.

The action of amphetamine was restricted mainly to power decreases in the delta and particularly alpha$_2$ frequency band. The same spectral pattern of changes, including those involving the theta and beta$_1$ band, was observed after the injection of a dopamine D$_1$-receptor specific agonist imitating the action of dopamine (Dimpfel et al. 1987). As amphetamine is known for its ability to release dopamine, this pattern can be correlated to dopaminergic control of local brain electric activity. This also means that MDMA and MBDB may work by affecting dopaminergic control mechanisms. There is fairly strong evidence for this with MDMA, derived from several other studies. For example, Yamamoto and Spanos (1988) reported on the acute effects of MDMA on dopamine release in the awake-behaving rat. Their results suggest that this dopamine release was region, time and dose dependent. Johnson et al. (1986) have shown that MDMA induces the release of dopamine from prelabeled slices of rat caudate, with the *S*-(+)-isomer of MDMA being more potent than the (−). Steele et al. (1987) have also shown that MDMA inhibits the reuptake of dopamine into rat striatal synaptosomes, again with a stereoselectivity for the (+)-isomer. Finally, in rats trained to discriminate saline from (±)-MDMA, (+)-amphetamine produces full substitution (Oberlender and Nichols 1988).

The situation is not quite as clear in the case of MBDB. The studies by Johnson et al. (1986) and Steele et al. (1987) demonstrate that this compound seems to have a negligible effect on in vitro dopaminergic function. Furthermore, MBDB does not substitute in (+)-amphetamine-trained rats, nor does amphetamine substitute in rats trained to discriminate saline from (+)-MBDB (Nichols and Oberlender 1989). These results contrast with those for MDMA, and suggest that while MDMA has a component of action that may be "amphetamine-like", there is much less support for that in the mechanism of action of MBDB. Even so, our present findings suggest similarities in the action of amphetamine, MDMA, and MBDB. It has to be kept in mind, however, that the comparison made above is based on data from experiments with single doses, which were chosen according to previous experience from behavioural studies, but which may not be sufficiently equivalent. For a detailed analysis of differences in the action of amphetamine, MDMA and MBDB the complete dose-activity relationships will have to be investigated.

Taken together, our results provide evidence for the hypothesis that dopaminergic mechanisms might be responsible for the stimulatory properties of amphetamines, whereas serotonergic mechanisms, particularly those involving the 5-HT$_2$ receptor, might be responsible for the hallucinogenic properties of certain substituted amphetamine derivatives.

Brain 5-HT$_2$ receptors have been implicated in the mechanism of action of both phenylisopropylamine, and tryptamine hallucinogens such as LSD (see, for example, Glennon et al. 1986; Titeler et al. 1988). After injecting 0.05 mg/kg LSD, during the first hour we could observe

a pattern of changes very similar to those recorded after DOM, including an increase in alpha$_1$ power in the striatum (Dimpfel et al. 1988). Therefore we are quite sure that the common changes of the spectral content of the recorded field potentials are not coincidental, but reflect certain properties of the drugs which might be directly or indirectly related to their hallucinogenic potential. Analysis of field potentials recorded from several brain areas in the awake-behaving rat might bridge the gap between receptor research and the study of behavior and enable us to learn more about drug actions at an intermediate integrative level of the brain.

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References

Ahrens H, Läuter J (1974) Mehrdimensionale Varianzanalyse. Akademieverlag, Berlin

Dimpfel W, Spüler M, Nickel B, Tibes U (1986a) 'Fingerprints' of central stimulatory drug effects by means of quantitative radioencephalography in the rat (Tele-Stereo-EEG). Neuropsychobiology 15:101–108

Dimpfel W, Spüler M, Nickel B (1986b) Radioelectroencephalography (Tele-Stereo-EEG) in the rat as a pharmacological model to differentiate the central action of flupirtine from that of opiates, diazepam and phenobarbital. Neuropsychobiology 16:163–168

Dimpfel W, Spüler M, Koch R, Schatton W (1987) Radioelectroencephalographic comparison of memantine with receptor-specific drugs acting on dopaminergic transmission in freely moving rats. Neuropsychobiology 18:212–218

Dimpfel W, Spüler M, Borbe HO (1988) Monitoring of the effects of antidepressant drugs in the freely moving rat by radioelectroencephalography (Tele-Stereo-EEG). Neuropsychobiology 19:116–120

Glennon RA, Titeler M, Young R (1986) Structure-activity relationships and mechanism of action of hallucinogenic agents based on drug discrimination and radioligand binding studies. Psychopharmacol Bull 22:953–958

Johnson MP, Hoffmann AJ, Nichols DE (1986) Effects of the enantiomers of MDA, MDMA and related analogues on [^{3}H]serotonin and [^{3}H]dopamine release from superfused rat brain slices. Eur J Pharmacol 132:269–276

Lyon RA, Titeler M, Seggel MR, Glennon RA (1988) Indolealkylamine analogs share 5-HT$_2$ binding characteristics with phenylalkylamine hallucinogens. Eur J Pharmacol 145:292–297

Nichols DE, Oberlender RA (1989) Structure-activity relationships of MDMA-like substances. In: National Institute on Drug Abuse. Res Monogr Ser (in press)

Oberlender R, Nichols DE (1988) Drug discrimination studies with MDMA and amphetamine. Psychopharmacology 95:71–76

Pazos A, Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1-receptors. Brain Res 346:205–230

Pazos A, Cortés R, Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. II. Serotonin-2-receptors. Brain Res 346:231–249

Steele TD, Nichols DE, Yim GKW (1987) Stereochemical effects, of 3,4-methylenedioxymethamphetamine (MDMA) and related amphetamine derivatives on inhibition of uptake of [^{3}H]-monoamines into synaptosomes from different regions of the rat brain. Biochem Pharmacol 36:2297–2303

Titeler M, Lyon RA, Glennon RA (1988) Radioligand binding evidence implicates the brain 5-HT$_2$-receptor as a site of action for LSD and phenylisopropylamine hallucinogens. Psychopharmacology 94:213–216

Yamamoto BK, Spanos LJ (1988) The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. Eur J Pharmacol 148:195–204

Young R, Glennon RA (1986) Discriminative stimulus properties of amphetamine and structurally related phenalkyamines. Med Res Res 6:99–130

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