Amounts of ACTH released by various levels of heptapeptide ($\varkappa$ µg) versus the amounts of ACTH released by 4 µg of CRF 91

Date	amounts of heptapeptide $\varkappa$ µg	ACTH released by $\varkappa$ µg / ACTH released 4 µg CRF 91	Limits of confidence	F	λ
29. 2. 1960	4.0	2.02	1.65–2.58	274.0	0.039
7. 3. 1960	2.0	1.32	0.85–2.23	49.3	0.096
4. 10. 1960	1.0	1.43	0.73–3.17	24.0	0.140
8. 3. 1960	0.5	1.43	1.10–1.93	137.7	0.057
9. 3. 1960	0.1	1.04	0.66–1.78	47.6	0.098
6. 10. 1961	0.1	1.16	0.74–1.87	54.0	0.092
15. 12. 1959	0	0.66	0.51–0.82	205.0	0.047
12. 10. 1961	0	0.51	0.33–0.73	105.9	0.066

and 0.5 µg; the dose of 4 µg of heptapeptide doubles the release of ACTH obtained with 4 µg of CRF 91. By lowering progressively the doses of heptapeptide one reaches the point where 0.1 µg of this material is still able to release an amount of ACTH equal to the amount released by 4 µg of CRF 91. When the hypophyses receive no stimulation they release only one half of the amount of ACTH released by 4 µg of CRF 91 and one fourth of that released by 4 µg of the heptapeptide.

In this series of experiments we were thus able to demonstrate that the heptapeptide H-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-OH was capable of releasing corticotropin *in vitro*. No definite conclusion can be drawn at present concerning the *in vivo* activity of this peptide and its possible physiological role.

Résumé. Au cours d'une série d'expériences décrites en détail nous avons observé le phénomène suivant: l'heptapeptide H-Met-Glu(NH_2)-His-Phe-Arg-Try-Gly-OH est susceptible d'augmenter *in vitro*, de façon statistiquement significative, la libération de corticotropine par des antéhypophyses de rat isolées.

M. Privat de Garilhe and C. Gros, with the technical collaboration of Yveline Lozac'h and Bernadette Garnuchot

Laboratoire de Chimie Biologique de la Faculté des Sciences, Paris (France), le 3 novembre 1961.

Cumulative Record of Motor Activity. (A New Procedure and its Use for the Study of Central Stimulant Drugs)

The motor activity of small animals is measured in a variety of ways. The rotations of a squirrel-wheel or a revolving cage[1], or the interruptions of light beams[2] are counted with impulse counters over standard periods of time[3]. The movements of a jiggle-box are measured on a sooted drum[4] or recorded electrically[5]. The movements of a platform of an animal container are converted into electrical currents with a piezo electric crystal[6] and registered on a kymographion or electronic recorder. The various methods have recently been critically reviewed[7].

Counts over standard periods of time are easy to use for measuring the potency of drugs on motor activity, but have the disadvantage that information on how motor activity proceeds in time is lost. Continuous measurements of animal induced movements do indeed give a time course relationship but it is often difficult to obtain statistical data from such recordings.

In operant behaviour research[8], extensive use has been made of cumulative recorders (Gerbrands recorders), to which square pulses of 24 V and 35–40 msec duration are fed, resulting in excursions of a pen in steps of 0.25 mm. If rotations of wheels, interruption of lightbeams or movements of animal-cages can be converted into pulses of standard duration, a cumulative record of motor activity can be obtained on a cumulative recorder. A cumulative record has the advantage that a complete time-response curve is obtained, while motor activity can easily be determined from such a curve. A procedure for integration pulses over standard periods of time can as well be used. With such a procedure practically the same information can be obtained as with the cumulative procedure provided that integration is carried out over short periods of time (30 sec or less).

The lightbeam method is in use in our laboratory and we therefore developed pulse-shapers for converting an interruption of a beam into a pulse of 40 msec duration. A diagram of the transistorized pulse-shaper is given in Figure 1.

The animal cage consists of a box (dimensions 36 cm l; 24 cm d, and 20 cm h) situated in a ventilated soundproof box and illuminated with a house light of constant intensity. The dimensions of the cage are not critical but are chosen for studying groups of two mice, or one or two rats. For larger animals or larger groups the cage should be extended. Three lightbeams were placed 6 cm apart over the depth of the cage and opposite to them 3 CdS photoelectric cells. Each photoelectric cell is connected with the input of a pulse-shaper. The output terminals of the three pulse-shapers are connected with the input terminal of the stepping device of a Gerbrands recorder. If less than 100 pulses/min are recorded, there is no

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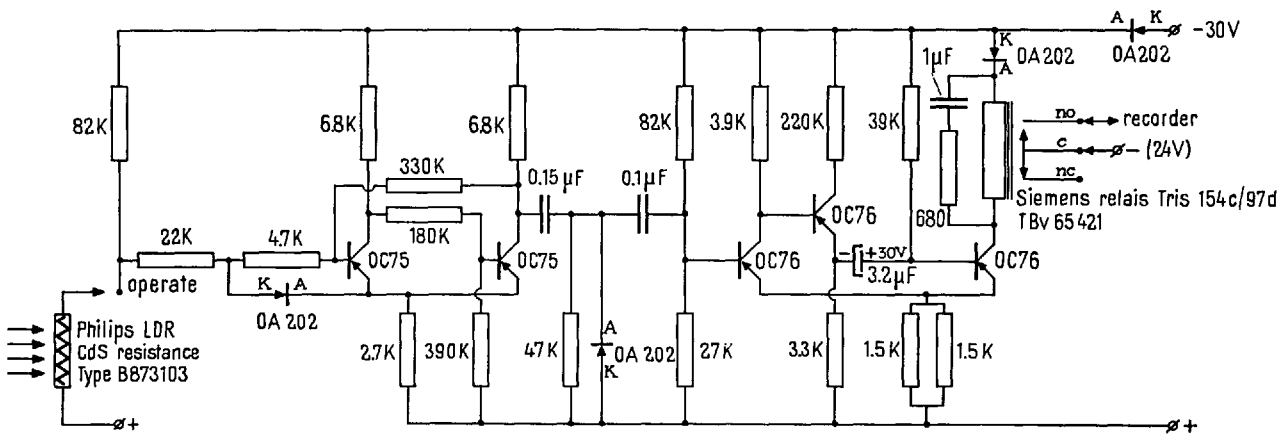

Fig. 1. Diagram of transistorized pulse-shaper which is used for the conversion of irruptions of a lightbeam into square pulses of 40 msec duration. The resistance of the CdS photoelectric cell[9] is about 10 kΩ during illumination. A relais is kept in operation and is released over a period of 40 msec when the resistance of the photoelectric cell is increased to more than 50 kΩ as result of interruption of the lightbeam. The C-stud of the relais is connected with the ground of a 24 V dC power supply. The NO-stud is connected with the stepping device of the recorder so that when a pulse comes in the stepper is momentary grounded.

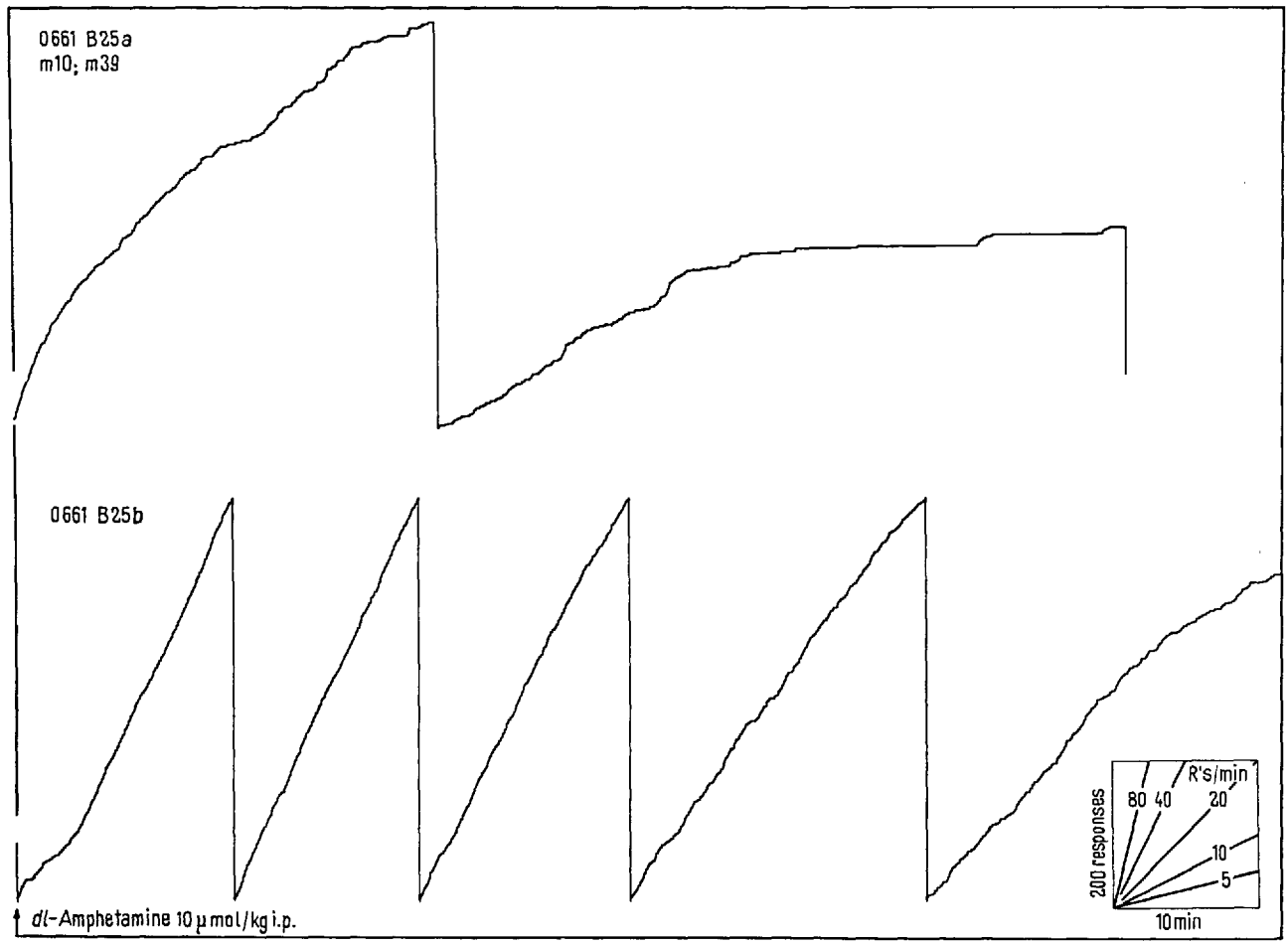

Fig. 2. (a) Cumulative record of the spontaneous motor activity of two mice (m 10 and m 39). Activity is high immediately after placing the animals in the cage, but gradually subsides (exploratory behaviour). The pen of the recorder returns to its starting position after 500 pulses have come in (500 pulses is 12.5 cm). (b) When exploratory behaviour has subsided, which is mostly the case after about 1 h, the animals are injected intraperitoneally with a dose of *dl*-amphetamine. After a short latency time (about 2 min), spontaneous motor activity increases rapidly to a maximum whereafter it levels off gradually. Motor activity can be determined from the slope of the curve at any time after the injection. The maximum value, which is usually obtained 10–15 min after injection, is used for the calculation of the dose-response curve.

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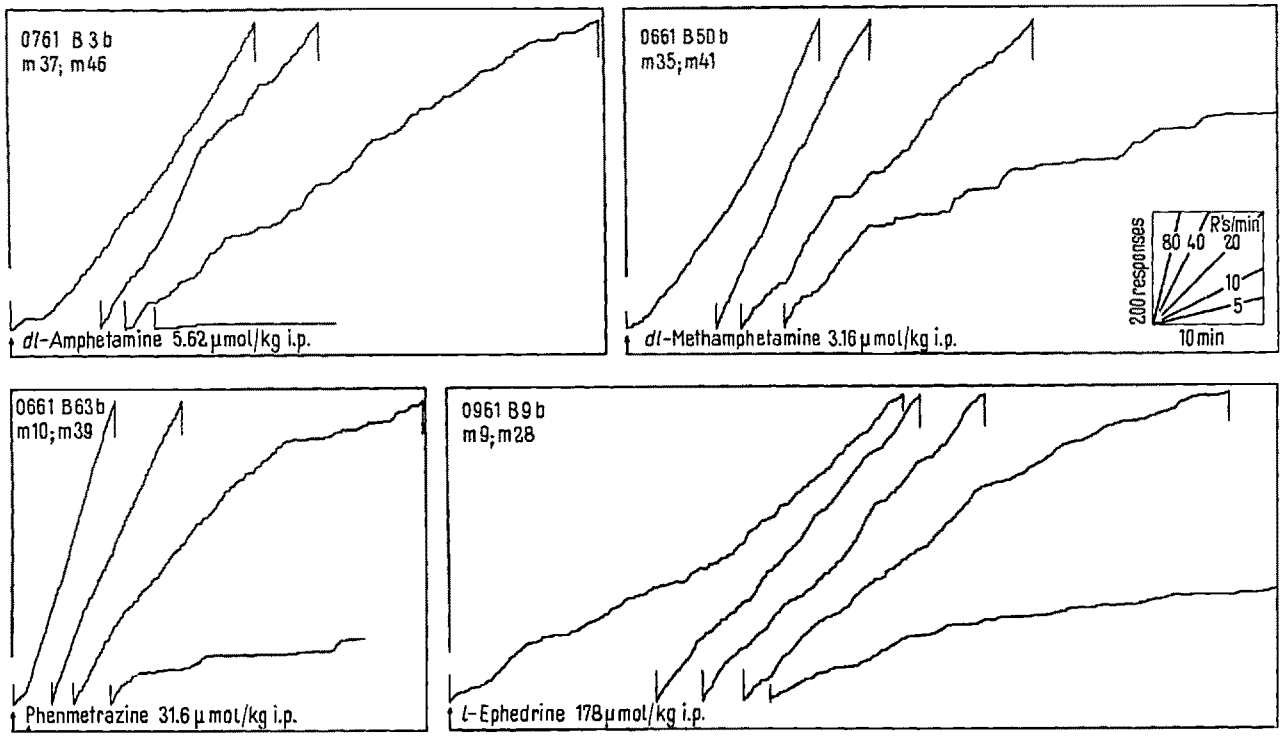


Fig. 3. Cumulative records of motor-activity in groups of two mice as induced by various amphetamine-like drugs. The original curves are shifted along the time-axis for easier reproduction. (a) *dl*-amphetamine, (b) *dl*-methamphetamine, (c) phenmetrazine and (d) (−)-ephedrine. Notice a difference in the initial slope as compared with the duration of action of the various compounds.

significant overlapping. Otherwise one lightbeam can be turned off.

A cumulative record of the spontaneous motor activity of two mice simultaneously is presented in Figure 2a. Activity is high immediately after placing the animals in the box and subsides when they have explored their new environment. This type of behaviour, known as exploratory behaviour, can be suitably followed on such a cumulative record. The use of the lightbeam method is irrevelant to this procedure. Other methods can be used if the correct pulse-shapers can be built.

The lightbeam method is useful for studying the action of amphetamines on motor activity. The effect of *dl*-amphetamine in two mice injected after they have achieved a steady activity of less than 5 pulses/min is given in Figure 2b. From this typical cumulative record, the activity can be read as the slope of the time-response curve 10 to 15 min after administration of the drug. For determining the slope, a straight part of the record of at least 5 cm is used in the steepest part of the activity-time record. For practical reasons, a perspex linescale with lines of various slopes has been made with the aid of which the motor activity as the number of pulses/min can immediately be read from the record.

If amphetamine is injected intraperitoneally, the latency-time is about 2–5 min while shortly thereafter maximal activity is obtained. In Figure 3 similar experiments are presented for various drugs in which the curves have been shifted along the time-axis for easier reproduction. From these curves it may be noted that amphetamine and methylamphetamine have a long duration of action in doses in which they produce a moderate activity. Phenmetrazine, however, causes a provoked increase in motor activity although it is of short duration. Therefore phenmetrazine is here found to be more potent than is usually stated in the literature on the subject. Our findings may give an explanation of why addiction to phenmetrazine is easily developed although this compound is a relatively 'weak' central stimulant. In contrast to phenmetrazine, the onset of action of ephedrine is very slow (Figure 3d).

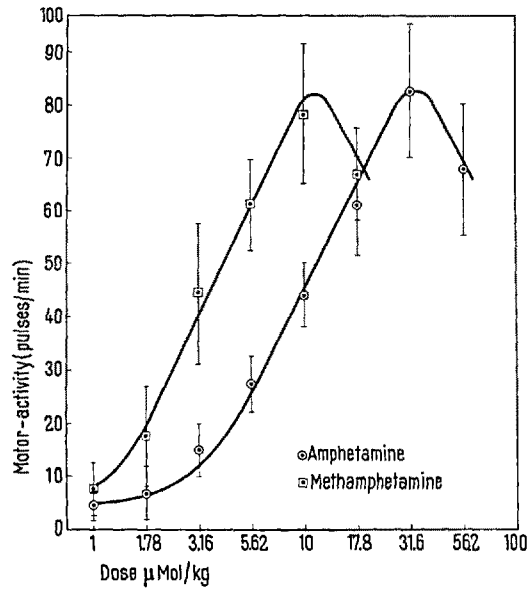
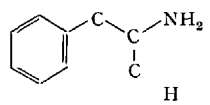
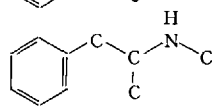
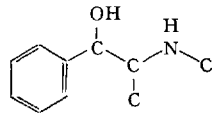
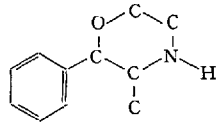


Fig. 4. Log dose-response curves for *dl*-amphetamine and *dl*-methamphetamine obtained by plotting for each dose the motor activity as calculated from experiments such as given in Figure 2 and 3. Each point is the average of 4 or more estimations with the standard deviation. The groups of two R Q mice were chosen at random out of a population of sixty. Notice that the curves are parallel and that an auto-inhibition occurs.

Potency of some amphetamine-like drugs. ED_{50} is determined from the log dose-response curves as the dose for which the motor activity is 45 pulses/min

Drug	Formula	ED_{50} μMol/kg	ED_{50} mg HCl salt/kg
dl-Amphetamine		9.7	1.7
dl-Methamphetamine		3.6	0.67
(−)-Ephedrine		170	34
Phenmetrazine		30	6.8

For 4 or 6 geometric increasing doses of each drug, cumulative time-response records were made and the activity determined by measuring the slope. The activity in responses/min were plotted *versus* the dose, using a logarithmic dose-scale (see Figure 4). Parallel lines were obtained over a considerable dose range. With higher doses, an auto-inhibition occurs. From the dose-response curves thus obtained (Figure 4), the dosage producing 50% effect was calculated. This dose is used as a measure for the central stimulating action. The potency of a few amphetamine-like drugs determined from dose-response curves are given in the Table.

In a number of pairs of mice, two latency times were observed for amphetamine. After 30–60 min there is again an increase in activity with moderate to high doses. This might be an indication that amphetamine could be methylated into the more potent methylamphetamine.

Time-response curves and dose-response curves of drugs and of combinations of drugs with their specific antagonists form the basis of a proper understanding of the mechanism of action of drugs in general.

The shape of drug-induced cumulative records of locomotor-activity and the dose-response curves may therefore provide valuable information on the mechanism of action and the classification of central stimulant drugs.

Zusammenfassung. Eine Methode zur kumulativen Registrierung spontaner motorischer Aktivität kleiner Tiere wird beschrieben. Die Wirksamkeitsbestimmung aus dem kumulativen Rekord und die analytische Bedeutung der Methode für die Wirkungsweise zentralstimulierender Substanzen wird diskutiert.

J. M. VAN ROSSUM,
with the assistance of W. F. H. VAN AMEROM, J. L. DAAMEN, J. A. TH. M. HURKMANS, G. J. J. MEGENS, and K. A. PETERS

Department of Pharmacology and Technical Center, R. C. University, Medical School, Nijmegen (The Netherlands), September 21, 1961.

Effects of Selective Intracranial Section and Stimulation of Vago-Accessory Roots. III. Course and Distribution of the Cardio-Inhibitory Fibers of the Bulbar Root of the Accessory Nerve

It has been previously reported that, in the dog, marked cardio-inhibitory effects can be obtained from, and mediated by, the bulbar root of the accessory nerve[1]. This root, fusing into a common trunk with the vagus nerve at level of the upper part of the nodose ganglion[2], seems therefore to represent an additional channel through which the pre-ganglionic cardio-inhibitory axons leave the oblongata. The arrangement of these 'accessory' fibers into the cardiac branches of the vagal trunk and the pattern of distribution to the intrinsic structures of the heart have now been investigated.

Intracranial section of either vagal or bulbar accessory root has been made unilaterally, in dogs, under aseptic conditions, and the efferent axons then allowed to degenerate. 20–30 days after, the animals were anaesthetized with chloralose and both vagi were cut at the neck. The peripheral stump of the vagal trunk ipsilateral to the chronic radicotomy (thus containing either vagal or bulbar accessory efferent fibers only) and its cardiac branches were gently dissected free and stimulated at different levels, the effects being recorded by conventional EKGraphy. Samples of vagal trunk and cardiac branches were then taken and processed according to the Marchi's method for degenerating myelinated fibers and the Cajal's method for axons.

EKGraphic records were also taken in acute preparations, subjected to selective intracranial stimulation of vago-accessory roots.

The results can be summarized as follows: (a) Cardio-inhibitory responses, including cardiac (or ventricular) arrest, can be obtained by stimulation of the vagal trunk, after degeneration of either vagal or bulbar accessory fibers, though more readily in the latter case. The same applies, generally, to the main cardiac branches (recurrent cardiac, cranio- and caudovagal nerves, on the right side; ventromedial and dorsal cervical cardiac nerves, left innominate and recurrent nerves, on the left side[3,4]) of the vagal trunk, with the exception of the ventromedial cervical cardiac and, possibly, left innominate nerves, which appear to be, after chronic vagal radicotomy, almost completely without effects. (b) After chronic section of the bulbar accessory root, no degenerated myelinated fibers have been traced into the cardiac branches of the vagal trunk (as after chronic vagal radicotomy). A small, compact group of degenerated myelinated fibers, present in the vagal trunk just cranially to the exit of the cranialmost branch (ventromedial cervical cardiac nerve, on the left; recurrent cardiac nerve, on the right side), enters the recurrent nerve. (c) Electrical stimulation of the bulbar root of the acces-

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