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ABSTRACT

Learning processes have been investigated for regularities of the changes in the strength of a reflex that result from manipulations of several parameters of a stimulus. Most of this work has been carried out in the tradition of associative theory. One consequence of this emphasis was that for a long time several phenomena that can be characterized properly as learning were ignored. This group includes habituation, sensitization, pattern recognition and acquisition of motor skills. However, now it is common to find references in the literature to behavioral changes that are not the result of some associative process. Among the nonassociative phenomena, habituation has received the most attention in the contemporaneous study of learning. Habituation is defined as a decrement in the response that results from its repeated elicitation. If we include as learning all those behavioral changes that are a product of experience, habituation would be one of the most elementary forms of learning. Additionally, habituation is observed in a large variety of species and organisms like PC-12 cells. Since they are a model of the neuronal system, the explanation of the behavioral changes that take place during habituation can become one of the building blocks of a more general theory of behavioral plasticity. This work describes the principal characteristics of the process of habituation that permit a definition of habituation as a basic process of learning and illustrates some methodological problems faced in the study of its parameters. A mathematical model that explains behavioral changes observed in PC-12 cells in situations of repetitive stimulation is proposed. (Contains 26 references, 11 figures, and 5 tables.) (Author/NB)

Mathematical Model of the Habituation Process as a Learning Basic Phenomena in PC-12 Cells

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Mathematical model of the habituation process as a learning basic phenomena in PC- 12 cells

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Abstract

Learning processes have been investigated for regularities of the changes in the strenght of a reflex that result from manipulations of several parameters of a stimulus. Most of this work has been carried out in the tradition of associative theory. One consequence of this emphasis was that, for a long time, several phenomena, that can be characterized properly as learning, were ignored. This group includes habituation, sensitization, patterns recognition and acquisition of motor skills. However, nowadays is common to find in the literature references to behavioral changes that are not result of some associative process. Among the nonassociative phenomena, habituation has received the larger attention in the contemporaneus study of learning. Habituation is defined as a decrement in the response that results from its repeated elicitation. If we include as learning all those behavioral changes that are a product of experience then habituation would be one of the most elementary forms of learning. Additionally, since habituation is observed in a large variety of species and organisms, like PC 12 cells. Since they are a model of the neuronal system, the explanation of the behavioral changes that take place during habituation can become one of the building blocks of a more general theory of behavioral plasticity. In this work we set up the principal characteristics of the process of habituation that permit a definition of habituation as a basic process of learning, we illustrate some methodological problems faced in the study of its parameters, we propose a mathematical model that explains behavioral changes observed in PC 12 cells in situations of repetitive stimulation.

Introduction

The most important means by which the environment alters behavior in humans is learning. Learning is the process of acquiring knowledge about the world. The study of learning has taught us about the logical capabilities of the brain and has proven a powerful approach to evaluating mental processing (Lieberman,1993). In the study of learning we can ask several related questions : What types of environmental relantionships are learned most easily ? What conditions optimize learning ? How many different forms of learning are there ? What are the stages of memory formation ?

Learning can occur in the absence of over behavior but its occurrence can only be inferred from changes in behavior (Gould,1986). Behavioral change, as well as other that can not be detected simply by observation of the organism´s behavior, all reflect alterations in the brain produced by learning. Thus, although purely behavioral studies have defined many important principles of learning, many of the fundamental questions about learning require direct examination of the brain (Bolles & Beecher, 1988).

Learning can be assessed by providing the subject with repeated learning experiences and observing progressive changes in performance.

Psychologists study learning (Thompson & Robinson, 1979) by exposing animals to information about the world, usually specific types of controlled sensory experience. Two major procedures have emerged from such studies, and these procedures give rise to two major classes of learning : nonassociative and associative. Nonassociative learning results when the animal is exposed once or repeatedly to a single type of stimulus. This procedure provides an opportunity for the animal to learn about the properties of the stimulus. In associative learning the organism learns about the relationship of one stimulus to another (classical conditioning) or about the relationship of a stimulus to the organim´s behavior (operant conditioning).

Two forms of nonassociative learning are very common in everyday life : habituation and sensitization (Dworkin & Dworkin, 1990). *Habituation* is a decrease in a behavioral response to a repeated, nonnoxious stimulus. An example of habituation is the failure of a person to show a startle response to a loud noise that has been repeatedly present. Sensitization (or pseudoconditioning) is an increased response to a wide variety of stimuli following an intense or noxious stimulus. For example, a sensitized animal responds more vigorously to a mild tactile stimulus after it has received a painful pinch. Moreover, a sensitizing stimulus can over-ride the effects of habituation.

Pheochromocytomas are tumors composed of cells which have morphological and functional characteristics comparable to those of normal catecholamine-storing chromaffin cells, or

pheochromocytes (Peppers & Holz, 1986). The term is derived from the Greek *phaios* (dusky), and alludes to the dark reddish brown color, or chromaffin reaction, which is produced when normal adrenal medulla or other catecholamine-storing tissue is immersed in solutions of dichromate-containing fixatives or other oxidizing agents. It is synonomous with the more awkward and less widely used term "chromaffinoma."

The PC12 cell line is a clone of pheochromocytoma cells which has become extensively employed as a tissue culture model for studies of neuronal development and function (Desale, Sociola & Delogu, 1996). This line was established in continuous monolayer culture in 1975 from a transplantable adrenal medullary tumor which arose in an irradiated rat.

Cultured PC12 cells closely resemble chromaffin cells in that they contain numerous dense-core chromaffin-type granules and synthesize and store large amounts of catecholamines. The principal catecholamines contained in the cells are dopamine and norepinephrine(Greene & Rein, 1977). The enzyme required to synthesize epinephrine is absent. Exposure of the cells to depolarizing level of potassium ion or to nicotinic cholinergic agonists elicits a Ca^{++} -dependent release of the stored dopamine and norepinephrine. PC12 cells also synthesize and secrete acetylchorline and at least two neuropeptides, neuropeptide Y and neurotensin (Huang, Tsay & Kao, 1996) . Both of these neuropeptides are also present in norepinephrine-containing chromaffin cells in the normal rat adrenal medulla.

An important feature of PC12 cells is that they bear specific receptors for, and exhibit specific responses to, nerve growth factor (NGF), a protein which profoundly influences the growth and development of certain cells such as sympathetic and sensory neurons. Exposure of PC12 cells to physiological levels (10^{-10}M) of NGF causes them to be converted to a phenotype similar to that of sympathetic neurons. For example, after several days of teatment with NGF, PC12 cells cease proliferation, extend long neurites, become capable of generating action potentials, produce synaptic-like vesicles, acquire greater numbers of nicotinic and muscarinic acetylcholine receptors, and undergo increases in the levels of several neuronal marker proteins (Inoue & Kenimer, 1988 ; Inoue, Nakasawa, Fujimori & Takanaka, 1989). The effects of NGF on PC12 cultures are reversible (Banett & Georgiou, 1996). Removal of the factor causes the cells to lose their neurites and to revert to the proliferating chromaffin-cell like state.

Several unique characteristics of the PC12 line have led to its widespread use as an experimental model :

1. The cells are highly differentiated and can be used as a model for either the chromaffin cell or the sympathetic neuron, as well as to study the means whereby NGF causes a transition between these two phenotypic states.

2. PC12 cells respond to NGF but, unlike sympathetic neurons, do not require the factor for their survival in serum-containing medium. This allows direct comparison of non-NGF-treated PC12 cultures with those that have been exposed to the factor for various times.

3. It is possible to monitor the initial events that occur when PC12 cells are first exposed to NGF. This is not presently feasible with normal neurons, because normal neurons are exposed to NGF in vivo.

4. Since PC12 cells proliferate, mutant subclones which are deficient in specific properties may be produced and selected. These in turn can be of use in evaluating the functional roles of particular gene products and for genetic dissection of the separate elements involved in regulation of complex neuronal properties such as neurite outgrowth.

In addition to these unique characteristics, the PC12 line offers general advantages common to cell culture systems.

Large numbers of homogeneous, replicate cultures of PC12 cells can easily be grown for experimentation and biochemical analysis. Furthermore, both the cellular and biochemical environments in which the cells are maintained can be definably manipulated for experimental purposes (Kamoto, Tanoka & Yagsawa, 1996).

Current studies employing PC12 cells are varied. For instance, one use includes analysis of the molecular mechanisms by which the synthesis and release of neurotransmitters and neuropeptides are regulated (Dobashi, Bhattacharjce, Tojoshina & Akiyama, 1996). Another major direction has been to discover the mechanism of action of NGF. This has ranged from studies of NGF receptor properties to characterization of the molecules involved in the outgrowth and regeneration of neurites (Wu & Bradshow, 1996). PC12 cells have also served as a source of material useful as an adjunct to other studies. For example, messenger RNA isolated from the cells has been employed to clone the gene for tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines (Toran-Allerand, 1996).

As a first step towards the understanding of the biochemical processes in the cells PC-12, we propose a descriptive mathematical model which fits well the experimental data on the simulation of PC-12 cells by K^+, ACh, and ATP.

Namely, the hypothesis of this paper consist that PC-12 cells can demostrate the habituation as a learning basic phenomena due that they change their conduct like result of the experience taking in mind the learning definition and according to "Habituation means to become insensitive to stimulant that does not have a special mean or consequence to habituation conduct" (Thompson & Spencer, 1996). "Habits are a bond to stimulus-response that automatically acquire and gradually at times, through out different possibilities of the effort of stimulus - response" (Kandel, 1989). "Habituation refers to a decreasing conduct response that occurs when a stimulus, initially new, is presented repeatedly" (Barry, 1990). "Habituation is proportionally inverse to the stimulus intensity. Stimulus very intensive produce an insignificant habituation" (Ben-Shakhar, 1994).

Methods

The time course of [3H] norepinephrine release from PC-12 cells was determined as described Mc Fadden and Koshland (1990), except that measurements were made at 25ºC.

The experimental observations that we processed contain four sets of data.

1) Data on a continuous stimulation of PC-12 cells by the solvent of K^+ (69 μM NaCl /56 μM KCl). The percent of liberation of [3H] norepinephrine per minute was measured every 5 minutes. There were performed 5 similar experiments with no time breaks during each experiment. The obtained sets of data are given in the Table 1 in the columns A through E.

2) Data on the stimulation of PC-12 cells by the solvent of K^+ (69 μM NaCl / 56 μM KCl). The percent of liberation of [3H] norepinephrine per minute was measured every 5 minutes. During the experiment there were 2 time breaks, each 20 minutes long. There were performed 5 similar experiments. The obtained sets of data are given in the Table 2 in the columns A through E.

3) Data on the stimulation of PC-12 cells by the solvent of ACh (1 μM). The percent of liberation of [3H] norepinephrine per minute was measured every 5 minutes. During the experiment there was a time break for 60 minutes. There were performed 5 similar experiments. The obtained sets of data are given in the Table 3 in the columns A through E.

4) Data on the stimulation of PC-12 cells by the solvent of ATP (300 μM). The percent of liberation of [3H] norepinephrine per minute was measured every 5 minutes. During the

experiment there was a time break for 25 minutes. There were performed 5 similar experiments. The obtained sets of data are given in the Table 4 in the columns A through E.

We have processed the experimental data presented in the Tables 1 through 4, and calculated the mean values of measured quantities together with the standard deviations. The results of calculations are presented in the Tables 1 through 4 respectively. As one can see, the standard deviations of experimental data from the mean values are small enough, and the mean values represent the experimental data with good accuracy. Full squares on the Figures 1 through 4 depict the mean values of the rate of liberation of [3H] norepinephrine versus time in each of the four experiments.

Results

The results of experimental observations (Figures 2-4) demonstrate the adapting effect of the cells which is expressed in the decay of the maximum of the response of the cells subjected to the square-wave stimulus. It follows from the experimental data that the adaptation effect depends qualitively on the type of stimulant (K^+, ACh, or ATP), on "waiting time", i.e. the period of time between the succeeding simulations and perhaps, on the initial concentration of a stimulant. Indeed, in the experiment N2 where the cells were stimulated by K^+ (69 μM NaCl / 56 μM KCl), there is a strong decay in the maximums of the rate of liberation of [3H] norepinephrine after 20 minutes breaks. This effect is much less expressed, though visible when PC-12 cells were stimulated by the solvent of ACh (1 μM), with time break 60 minutes (experiment 3), and is almost absent in the case of the experiment 4 where the cells were stimulated by the solvent of ATP (300 μM). In the latter case the cells "forget" the stimulus of the first part of the experiment, so that the second application of the stimulus, 25 minutes later, almost reproduces the first part.

Given below is a descriptive mathematical model which we use to analyze the experimental data given in the Tables 1 through 4 and presented on the Figures 1 through 4.

Mathematical Model

One possible mathematical model for the description of the adapting effect of the cells subjected to the periodic square-wave stimulation was proposed by Segel, Goldbeter, Devreotes, & Knox (1986) and developed by Li & Goldbeter (1989). The main idea of these papers was to introduce a concentration of two types of biochemical reactions with two different time scales, and to make use of the "activity" as a weighted linear combination of

concentrations. From mathematical point of view, the problem reduces to the resolution of a linear system of ordinary differential equations with periodic piecewize-constant coefficients. It is important to note that in every interval of time where the coefficients of the system are continuous, the solution of the aforementioned linear system of ordinary differential equations consists of linear combination of exponents (in general, with complex arguments), polynomials, and the products of the two.

In our model, we start with the fit curves that describe the experimental data given in Tables 1 through 4. Presented on Figures 1 through 4 are the points (black full squares) which depict the rate of liberation of norepinephrine with respect to time (that is, percent of liberation of [3H] norepinephrine per minute). We have found that the functional form of the curves that describe all sets of data of four experiments presented in the Tables 1-4 is

$$\frac{dS(t)}{dt} = \frac{1}{\sqrt{a + b(t - t_0)}} \qquad (1)$$

where $dS(t)/dt$ is the rate of liberation of norepinefrina, and a, b and t_0 are coefficients that depend on the type of experiment. The values of the coefficients are given in Table 5. The curves (1) for all of the experiments are presented on the Figures 1 through 4 as solid lines. Note that the equation (1) determines the function $S(t)$ up to an arbitrary constant.

As one can see, the fit curves are not exponential, but rather the inverse square roots. To obtain the differential equation of the model, we integrate equation (1) with respect to t. We get

$$S(t) = S_0 - \frac{2}{b}\sqrt{a} + \frac{2}{b}\sqrt{a + b(t - t_0)}\ , \qquad (2)$$

where S_0 is the value of $S(t)$ at $t = t_0$. Thus, excluding time t from equation (1) with the help of equation (2), we obtain

$$\frac{dS(t)}{dt} = \frac{k}{S(t) - S_0 + k\sqrt{a}}, \qquad (3)$$

$$k = \frac{2}{b}\ . \qquad (4)$$

Let us introduce the function

$$\Sigma(t) = S(t) - S_0 + k\sqrt{a}\,. \qquad (5)$$

This function determines $S(t)$ up to a constant. Subsituting the function $\Sigma(t)$ given by equation (5) into equation (3), we get the following nonlinear differential equation

$$\frac{d\Sigma(t)}{dt} = \frac{k}{\Sigma(t)}\,. \qquad (6)$$

The equation (6) with the coefficient k given by the expression (4) and presented in the Table 5 describes all the observed experimental data and is a mathematical descripition of the fit curves (1). Hence, we obtain a mathematical model which provides (on the time scale of observations) the fit curves for the set of experimental data represented in the Tables 1 - 4.

The adequate mathematical frame for the description of adaptation effect of the cells subjected to a repeated stimulus is a differential equation (or a system of such equations) with time-dependent, piecewize-continuous coefficients. According to this approach, we consider the coefficient k in equation (3) to be time-dependent and piecewize-constant. The functional form of $k = k(t)$ depends on the type of experiment (i.e. on the stimulation of the cells by K^+, ACh, or ATP), and on "waiting times" T_W during which there is no stimulation of the cells. It is important to note that according to experimental data the equations (3) and (6) are applicable only on that intervals of time where the measurements of $dS(t)/dt$ are made. We have no observed experimental data out of the mentioned time intervals. This is why the functions $k = k(t)$ for each of four experiments are also determined only on that intervals. Graphical representation of $k(t)$ is given on Figures 5-8. Provided that the initial values of $S(t)$ are given, we can calculate the dynamics of the response of PC-12 cells to a continuous or repeated stimulating.

Discussion

The differential equation (5) of our model is a nonlinear differential equation of the first order. The solutions of this equation give us curves whose derivatives fit well the experimental data presented on Figures 1 - 4. The model itself depends on two parameters: a and b (or a and k).

The values of these parameters, according to the available experimental data, are listed in the Table 5. As it was mentioned above, the adapting effect of PC-12 cells depend on the type of stimulant (K^+, ACh, or ATP) and on the "waiting time" T_W during which no stimulation of the cells is made. In general, it may also depend on the number of breaks during the experiment. Therefore, the coefficients of our model depend, at least, on the type of stimulant and on the "waiting time" T_W. At the time being, we have no sufficient experimental data to provide analytical dependencies of the parameters of the model on T_W. Indeed, experiments on stimulation of PC-12 cells by ACh and ATP were conducted only for the single value of "waiting time" T_W (equal to 60 min in the case of ACh and to 25 min in case of ATP), with no continuous stimulation and with no other values of T_W. The same note concerns two experiments with K^+, where there were no other measurements of $dS(t)/dt$ except for $T_W = 0$ and $T_W = 20$ min. We can define, there is habituation when $d^2S/dt^2 \rightarrow 0$ in all experiments, Figures 9 through 12. Also we have no quantitative dependencies of parameters on the type of stimulant. That is why the proposed model, which describes the observed experimental data with good accuracy, is only a first step toward the investigation and quantitative description of complex biochemical processes which follow the periodic stimulation of the cells and lead to the adaptation effect of the cells. Further studies of the phenomenon should provide the representative set of experimental data with various values of "waiting times" for each type of the stimulant as well as various values of initial concentration of the stimulants. This will give the possibility to take all the mentioned factors into account, to proceed in the working out the adequate advanced theory, and to obtain a more deep insight into the nature of the adaptation mechanism of the cells in connection with the process of learning.

Conclusions

Habituation is the process by which an organism learns to repress its response to a repetitively presented stimulus (Kandel, Schwratz & jessel, 1991). Habituative learning has been described in a wide variety of organisms, including molluses, insects, and mammals (Scholz, 1987). Thompson and Spencer (1966) compiled a list of characteristics found in behavioral studies of habituative learning that is now widely used as a definition of habituation. Typically, the response to a stimulus decreases exponentially with repetition of the stimulus, asymptotically approaching a habituated level. The response recovers spontaneously if the stimulus is withheld

for a sufficient period of time ; the rate of recovery may depend on the amount of previous stimulation.

Thus, for a time after repetitive stimulation, the organism remembers not to respond to that stimulus ; this memory is by definition short-term (Wagner, 1979). After habituation and subsequent recovery of the response, the response to a second set of repetitive stimuli habituates more rapidly. This indicates a memory of habituation that persists even after the response is recovered ; this memory is generally long-term.

PC12 cells, rat pheochromocytoma cells that differentiate upon treatment with nerve growth factor to resemble sympathetic neurons, have been used as a model system for the study of habituation. These cells secrete norepinephrine (NE) in response to K^+, acetylcholine (ACh) or ATP.

When cells are repetitively stimulated with ACh, the amount neurotransmitter released in response to each successive stimulus is decreased, meeting many of the criteria for habituation. ATP is a neurotransmitter or neuromodulator that is secreted from both adrenergic and cholinergic nerve terminals after stimulation, and possibly from other nerve types as well, and is, therefore, expected to have a widespread distribution in both the central and peripheral nervous systems. In PC12 cells, ATP acts by opening cell-surface channels, allowing calcium influx, and depolarizing the cell. To our knowledge, habituation of the response to ATP has not previously been demonstrated..

The observation of habituation of neurotransmitter release in PC12 cells suggested that habituative learning in its most fundamental form can result from molecular interactions occurring within single cells. We wondered whether clonal cells essentially free of synaptic connections and lacking input from other cell types would also exhibit characteristics of short- and long-term memory after habituation.

To test this, PC12 cells were repetitively stimulated with either ACh or ATP, and , after various rest intervals, the response to stimulation and the rate of habituation to a second series of stimuli were measured. A model based on the simplest possible general mechanism for habituation is described. Equations based on this model could be used to analyze the kinetics of habituation and recovery of neurotransmitter release and fit these data well.

- The descriptive mathematical model proposed is an adequate standard for the description of a phenomena of habituation in PC12 cells, subjected to repeatedly stimulus which functions could be applied for the study of other neurotransmitters released by neuronal cells.
- The decreasing capacity response with repeatedly stimulus is clearly observed when the PC12 cells are successively stimulated by K^+ , ACh and ATP solutions.

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Table 1

Time, min	A	B	C	D	E
8	3.25	3.26	3.25	3.27	3.24
13	2.30	2.29	2.31	2.30	2.32
18	2.10	2.10	2.11	2.12	2.10
23	1.75	1.74	1.75	1.76	1.77
28	1.80	1.80	1.79	1.80	1.80
33	1.50	1.51	1.50	1.49	1.50
38	1.55	1.55	1.54	1.53	1.54
43	1.25	1.26	1.24	1.24	1.25
48	1.20	1.20	1.19	1.19	1.20
53	1.10	1.11	1.10	1.10	1.10
58	1.05	1.06	1.07	1.05	1.05
63	1.05	1.05	1.05	1.04	1.04
68	1.00	1.00	1.00	1.00	1.01
73	0.98	0.98	0.97	0.98	0.98
78	0.98	0.97	0.95	0.96	0.97

Experiment 1. Data on a continuous stimulation of PC-12 cells by a K^+ (69 μM NaCl /56 μM KCl) solution. The percent of liberation of [3H] norepinephrine per minute was measured every 5 minutes. Five more the similar experiments were performed with no time breaks.

Table 2

Time, min	A	B	C	D	E
10	2.48	2.49	2.47	2.48	2.48
15	1.42	1.40	1.43	1.42	1.40
20	1.40	1.39	1.40	1.38	1.38
25	1.00	1.01	1.00	1.10	1.00
30	1.00	1.00	0.99	1.00	1.00
50	1.40	1.41	1.42	1.41	1.40
55	0.90	0.90	0.90	1.00	0.93
60	0.75	0.75	0.76	0.78	0.78
65	0.70	0.71	0.73	0.75	0.75
70	0.73	0.71	0.70	0.70	0.73
90	0.98	0.97	0.98	0.98	0.98
95	0.85	0.85	0.85	0.86	0.85
100	0.75	0.76	0.77	0.77	0.75
105	0.77	0.76	0.75	0.77	0.73
110	0.75	0.75	0.73	0.75	0.70

Experiment 2. Data on the stimulation of PC-12 cells by a K^+ (69 μM NaCl / 56 μM KCl) solution. The percent of liberation of [3H] norepinephrine per minute was measured every 5 minutes. During the experiment there were 2 time breaks, each 20 minutes long. There were performed 5 similar experiments.

Table 3

Time, min	A	B	C	D	E
15	1.20	1.40	1.20	1.30	1.20
30	0.90	1.00	0.91	1.10	0.95
35	0.50	0.60	0.50	0.60	0.51
40	0.40	0.50	0.40	0.50	0.40
45	0.30	0.35	0.30	0.40	0.31
50	0.22	0.22	0.20	0.30	0.22
110	1.00	1.10	1.10	1.00	1.10
115	0.40	0.50	0.50	0.40	0.41
120	0.30	0.40	0.40	0.31	0.29
130	0.21	0.30	0.30	0.25	0.21
135	0.20	0.20	0.20	0.20	0.19

Experiment 3. Data on the stimulation of PC-12 cells by a ACh (1 μM) solution. The percent of liberation of [3H] norepinephrine per minute was measured every 5 minutes. During the experiment there was a time break for 60 minutes. *There were performed 5 similar experiments.*

Table 4

Time, min	A	B	C	D	E
28	3.30	3.10	3.00	3.30	3.20
33	2.20	2.10	2.20	2.15	2.00
38	1.80	1.75	1.81	1.70	1.82
43	1.50	1.49	1.53	1.51	1.51
48	1.30	1.29	1.31	1.32	1.30
53	1.20	1.18	1.25	1.22	1.21
78	3.20	3.10	3.20	3.20	3.10
83	1.60	1.58	1.62	1.61	1.60
88	1.10	1.15	1.12	1.13	1.10
93	1.10	1.10	1.09	1.08	1.07
98	1.00	1.00	1.00	1.00	1.00
103	1.00	1.00	0.98	0.98	1.00

Experiment 4. Data on the stimulation of PC-12 cells by a ATP (300 μM) solution. The percent of liberation of [3H] norepinephrine per minute was measured every 5 minutes. During the experiment there was a time break for 25 minutes. There were performed 5 similar experiments.

Table 5

Coefficients in the Equation (1).

Experiment	a	b	k	t_0, min
Experiment 1, $8 \le t \le 78$	0.094	0.014	143.9	8
Experiment 2, $10 \le t \le 30$	0.162	0.047	42.55	10
Experiment 2, $50 \le t \le 70$	0.504	0.093	21.48	50
Experiment 2, $90 \le t \le 110$	1.045	0.049	40.16	90
Experiment 3, $25 \le t \le 50$	0.694	0.799	2.50	25
Experiment 3, $110 \le t \le 135$	1.000	0.960	2.08	110
Experiment 4, $28 \le t \le 53$	0.099	0.023	85.84	28
Experiment 4, $78 \le t \le 103$	0.100	0.045	44.42	78

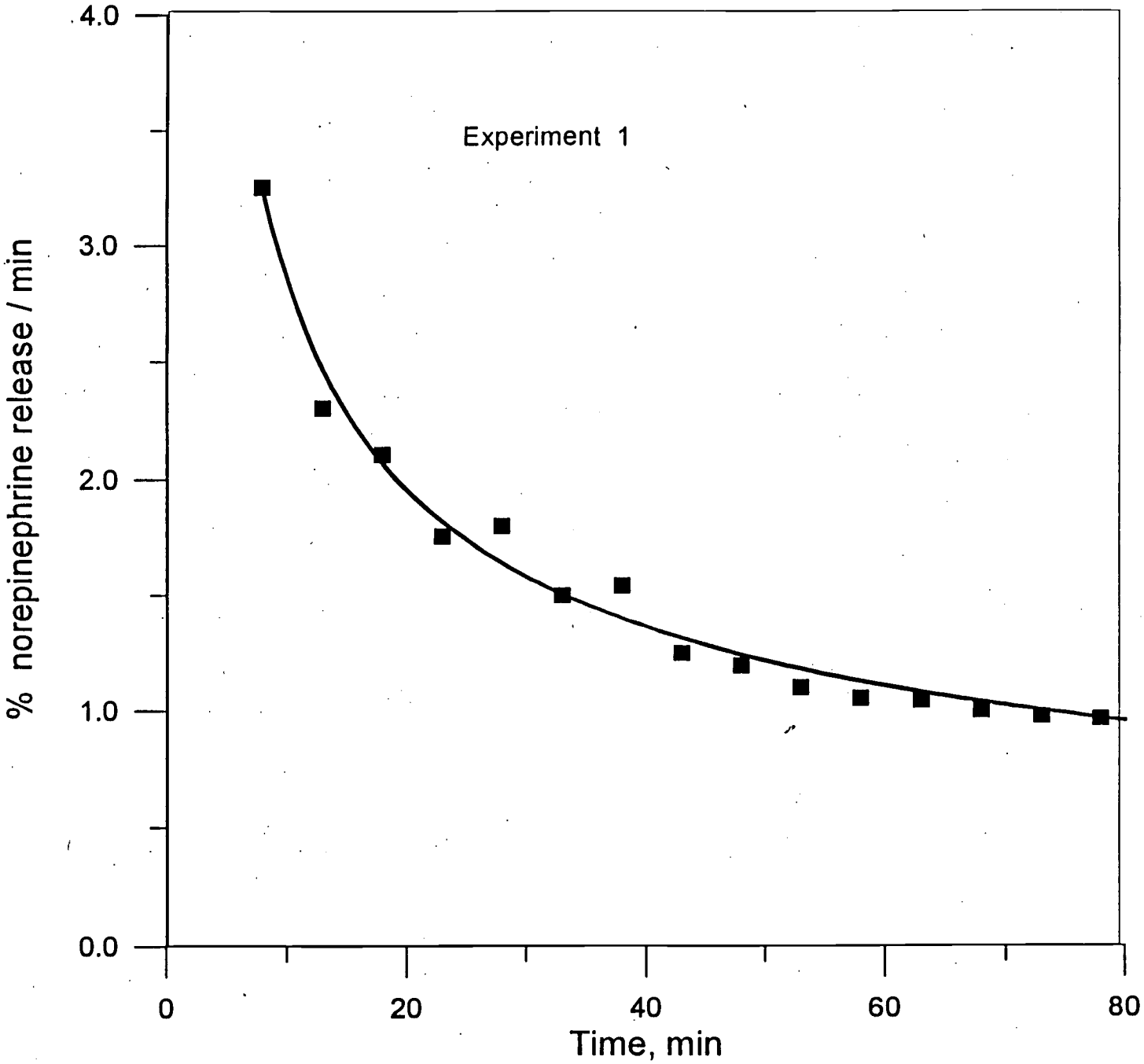

Figure 1. The points (black full squares) which depict the rate of liberation of norepinephine with respect to time (that is, percent of liberation of [3H] norepinephine per minute) for the experiment 1.

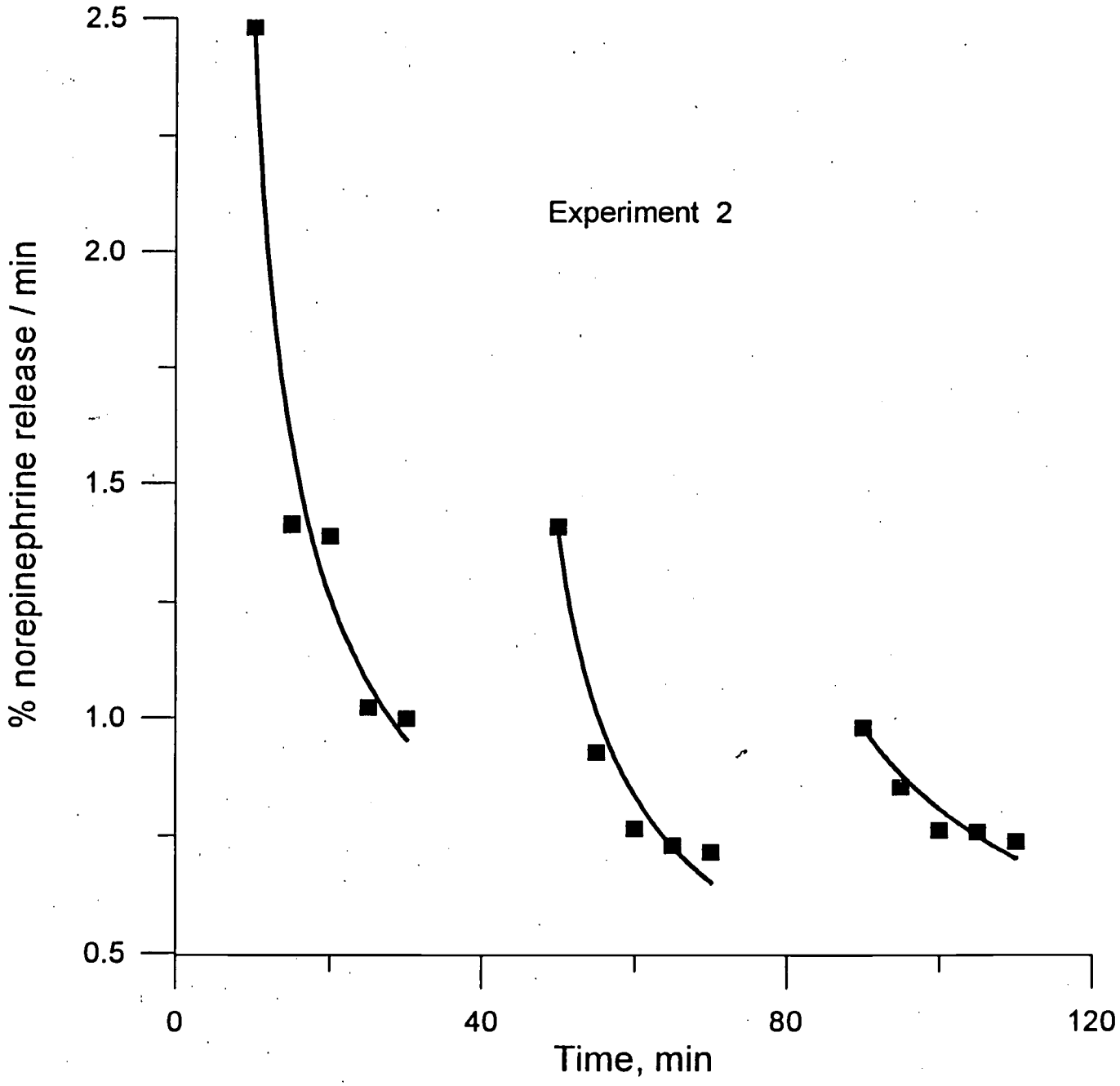

Figure 2. The point which depict the rate of liberation of norepinephine with respect to time for the experiment 2

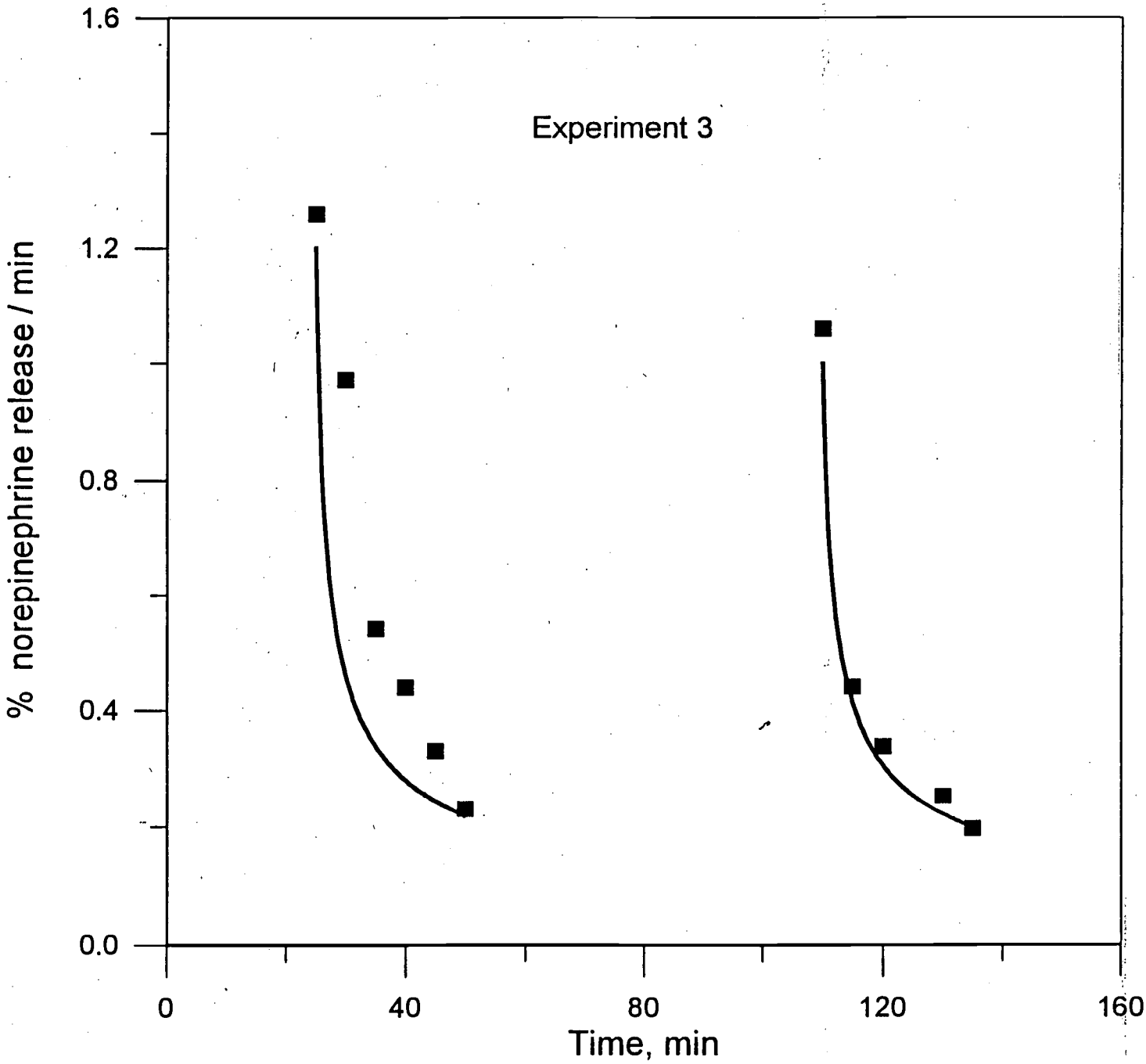

Figure 3. The points which depict the rate of liberation of norepinephine with respect to time for the experiment 3.

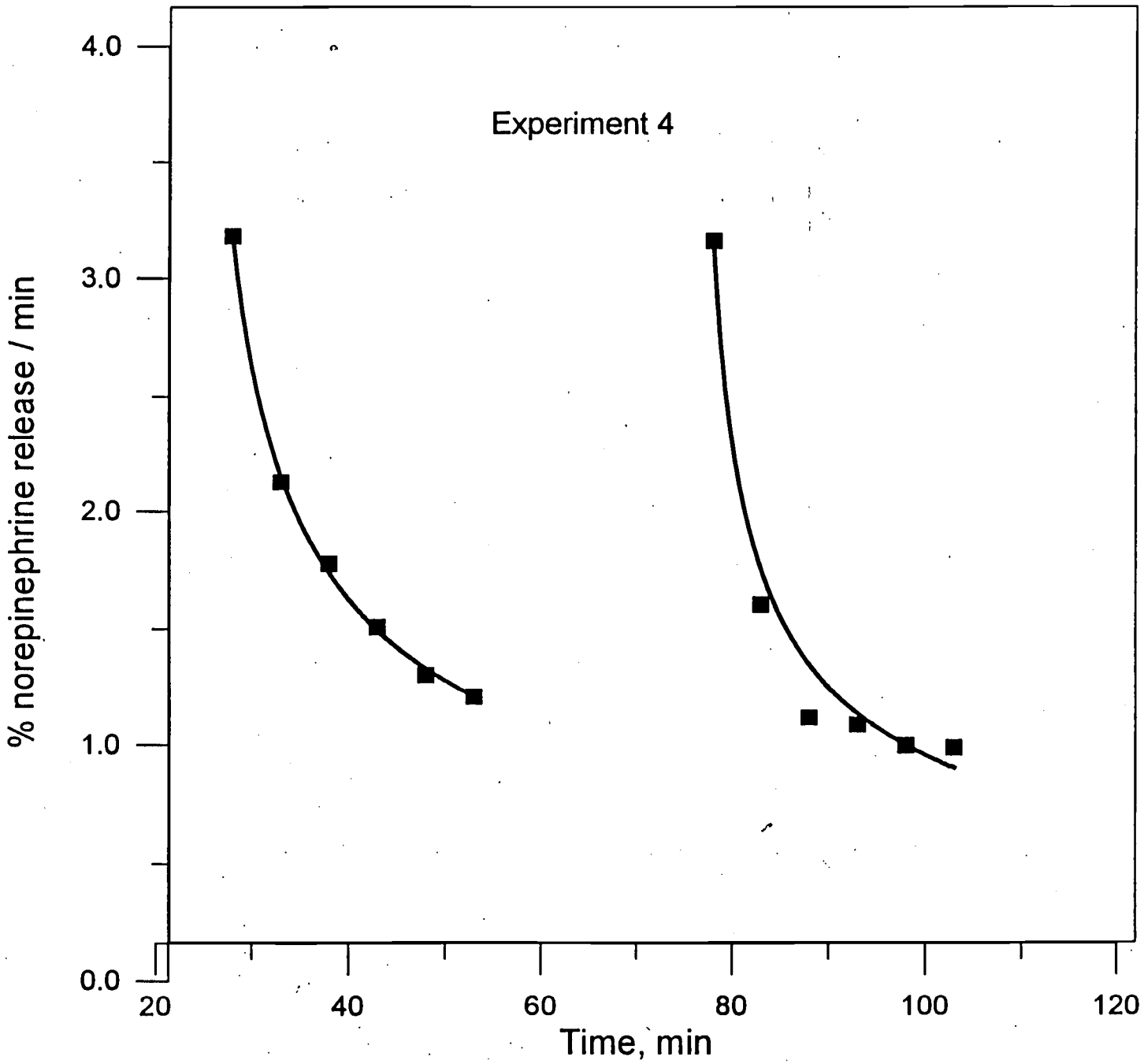

Figure 4. The points which depict the rate of liberation of norepinephine with respect to time for the experiment 4.

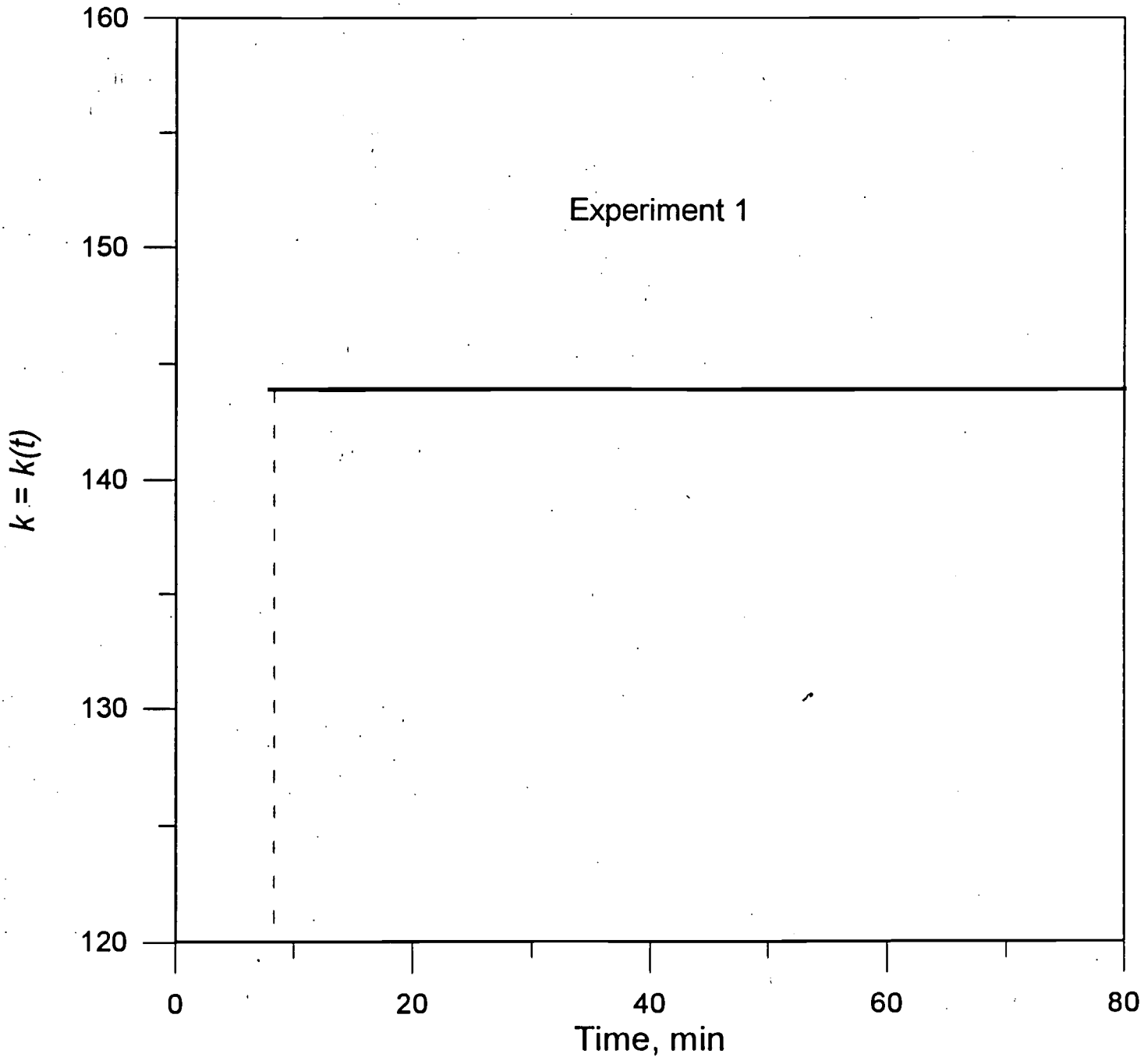

Figure 5. Graphical representation of k(t) for the experiment 1.

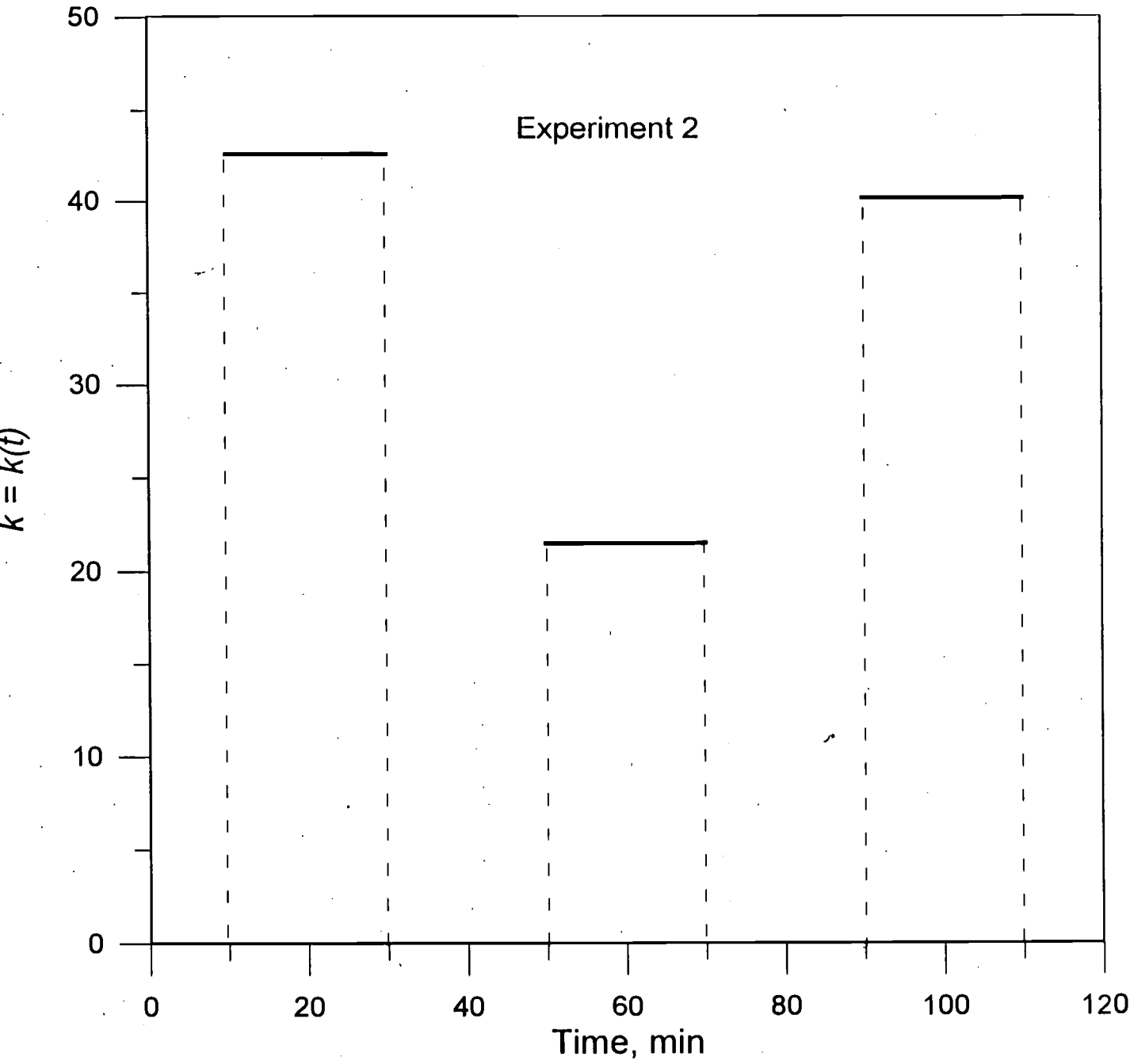

Figure 6. Graphical representation of k(t) for the experiment 2.

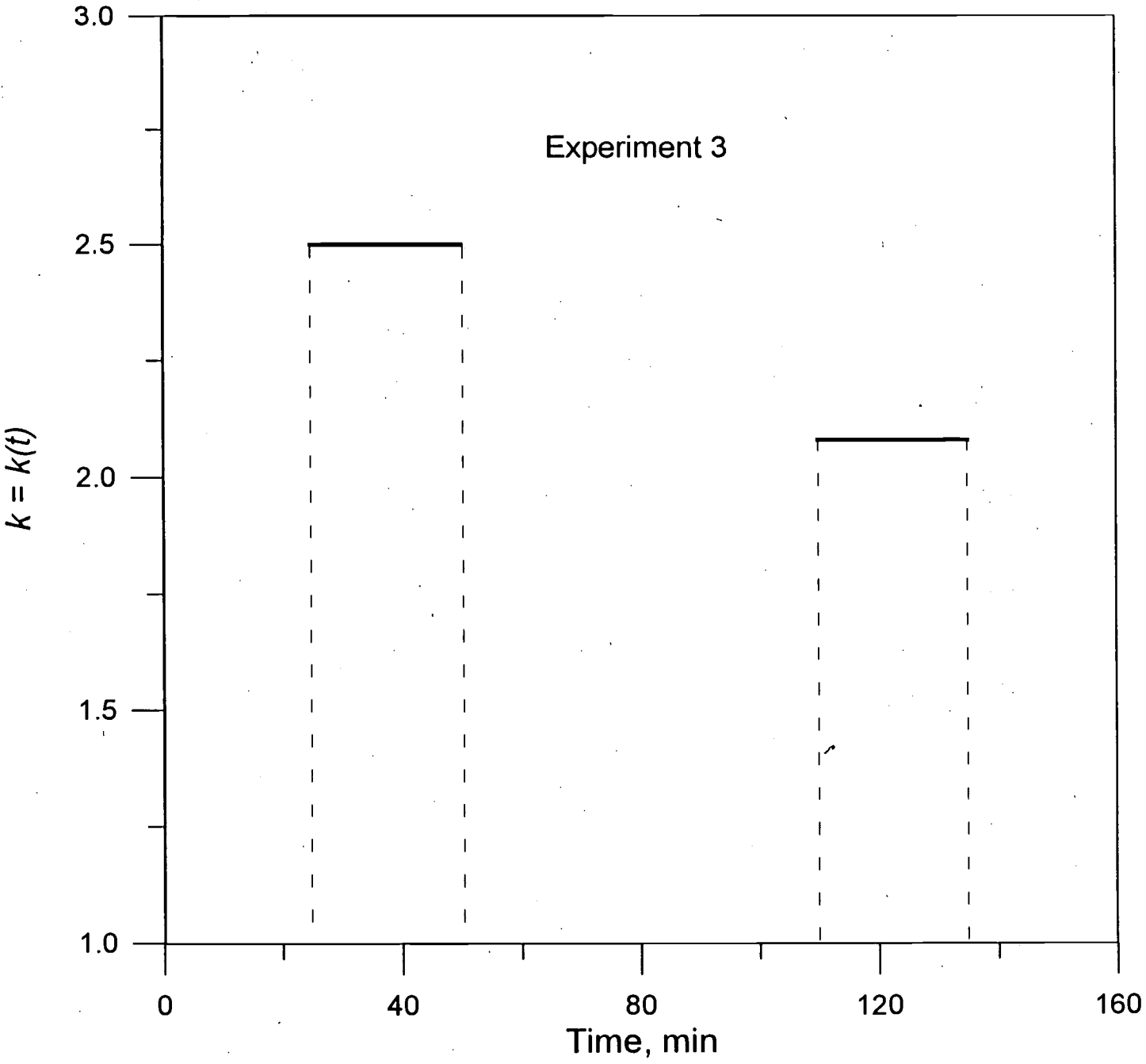

Figure 7. Graphical representation of k(t) for the experiment 3.

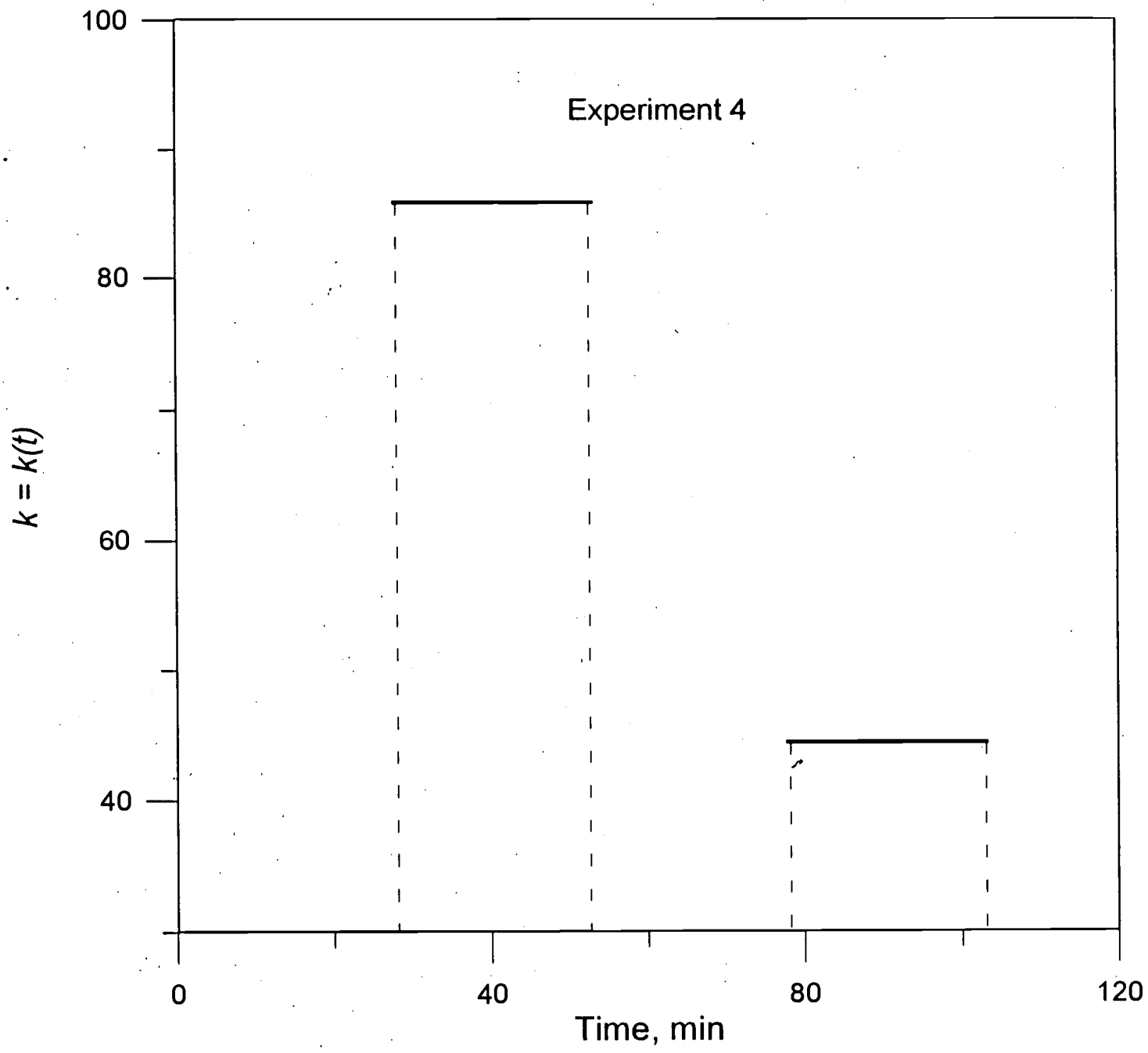

Figure 8. Graphical representation of k(t) for the experiment 4.

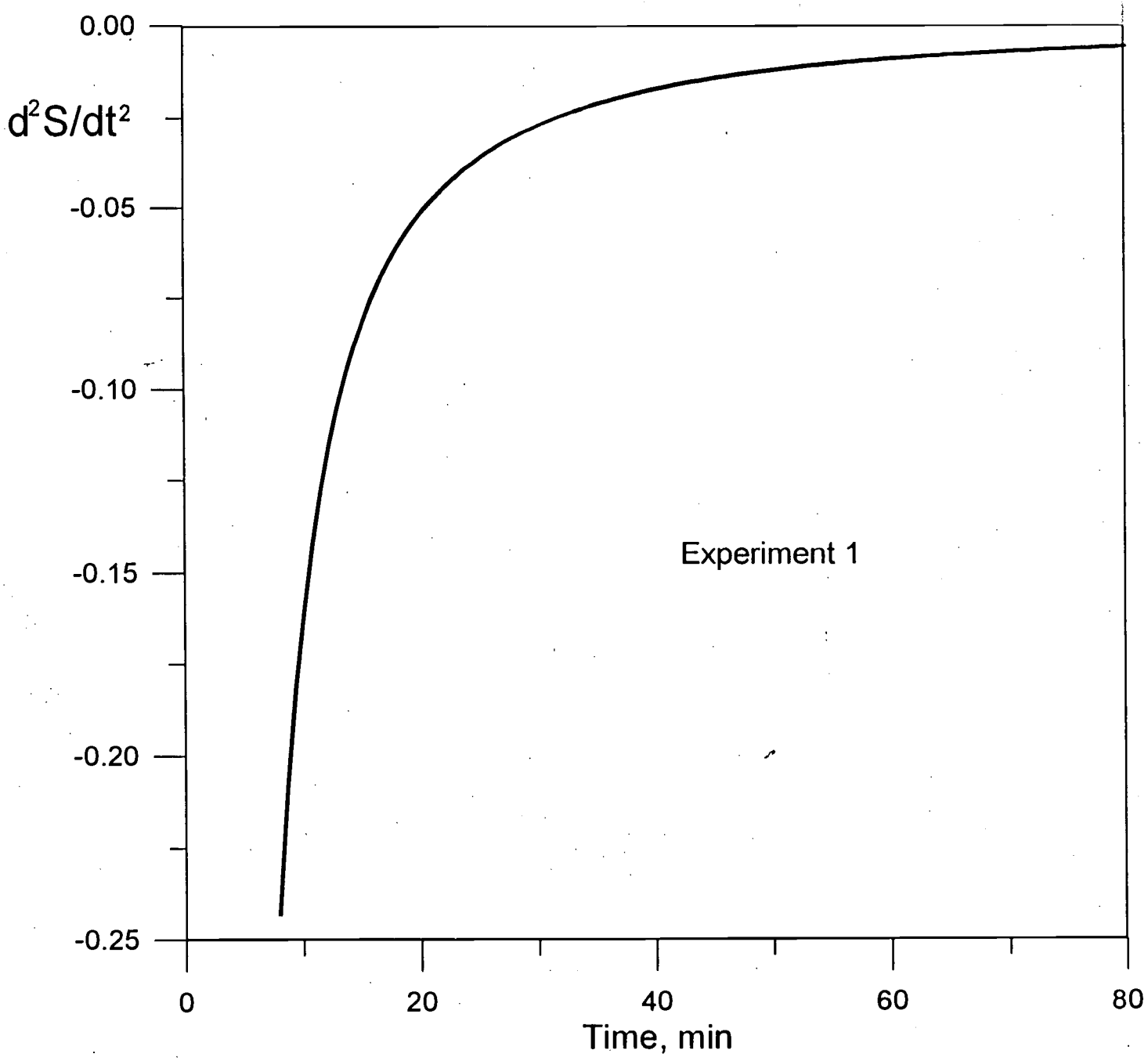

Figure 9. Graphical representation of d^2S/dt^2 respect to time for the experiment 1. We can define, there is habituation when $d^2S/dt^2 \rightarrow 0$.

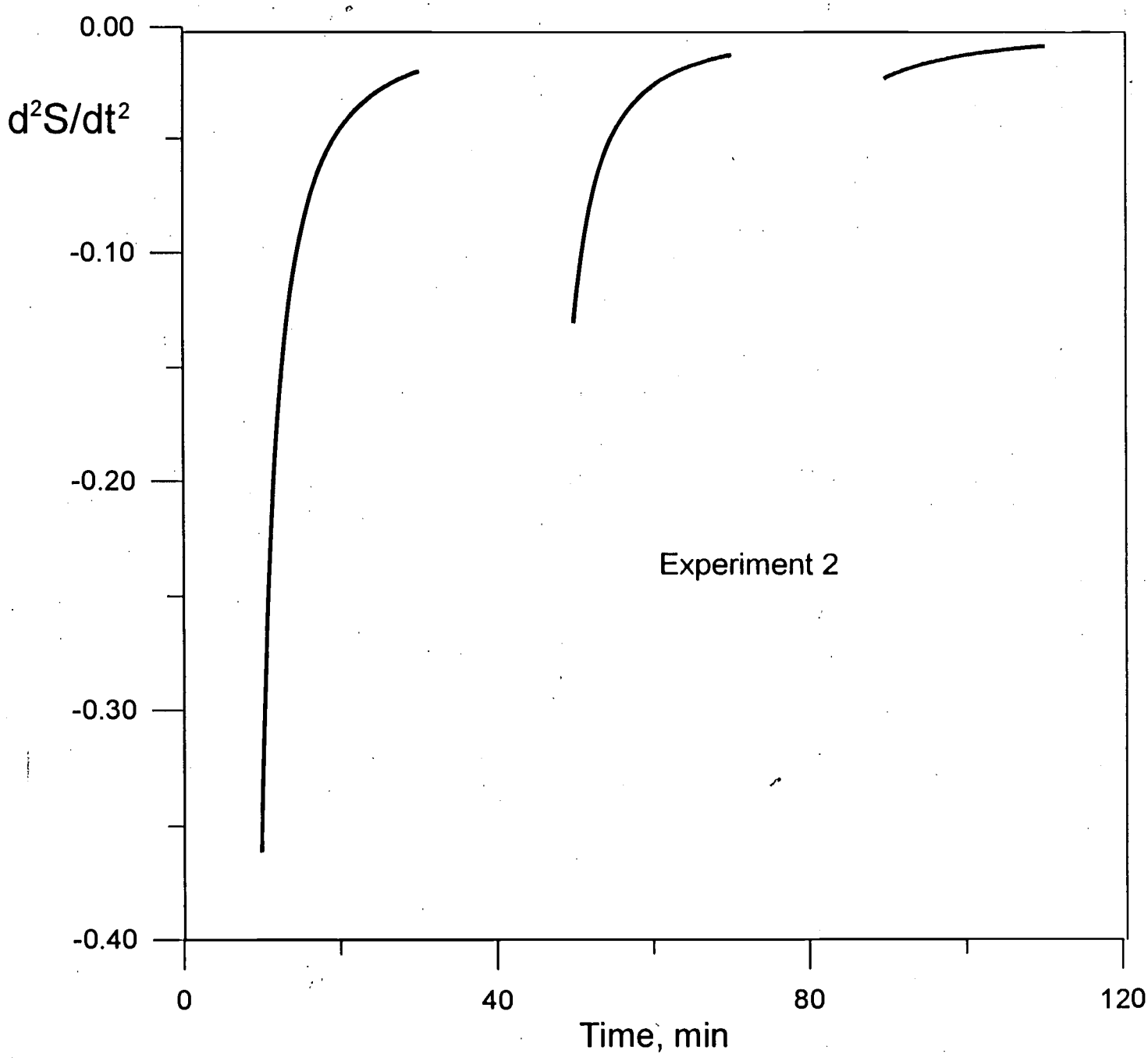

Figure 10. Graphical representation of d^2S/dt^2 respect to time for experiment 2.

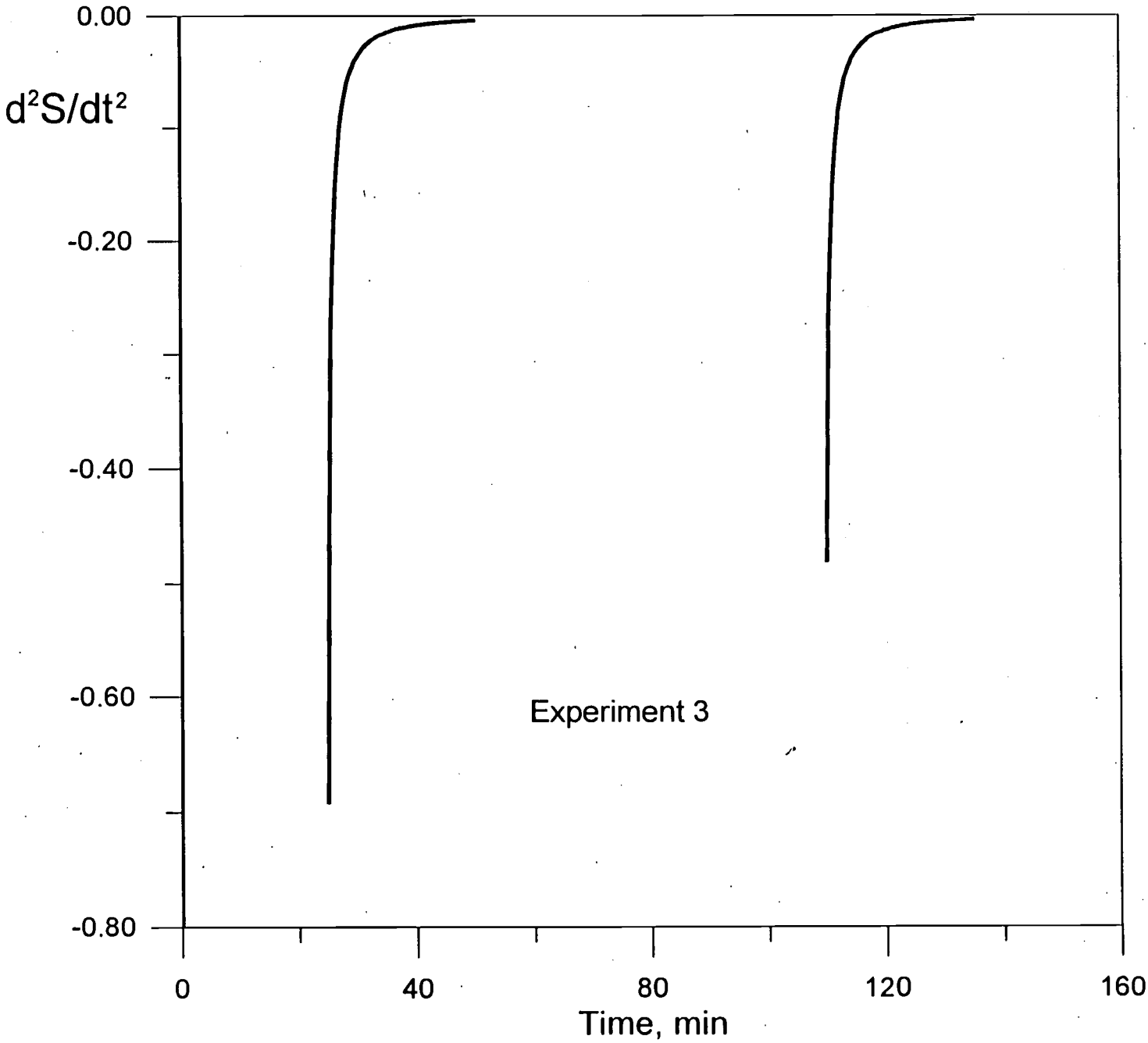

Figure 11. Graphical representation of d^2S/dt^2 respect to time for experiment 3.

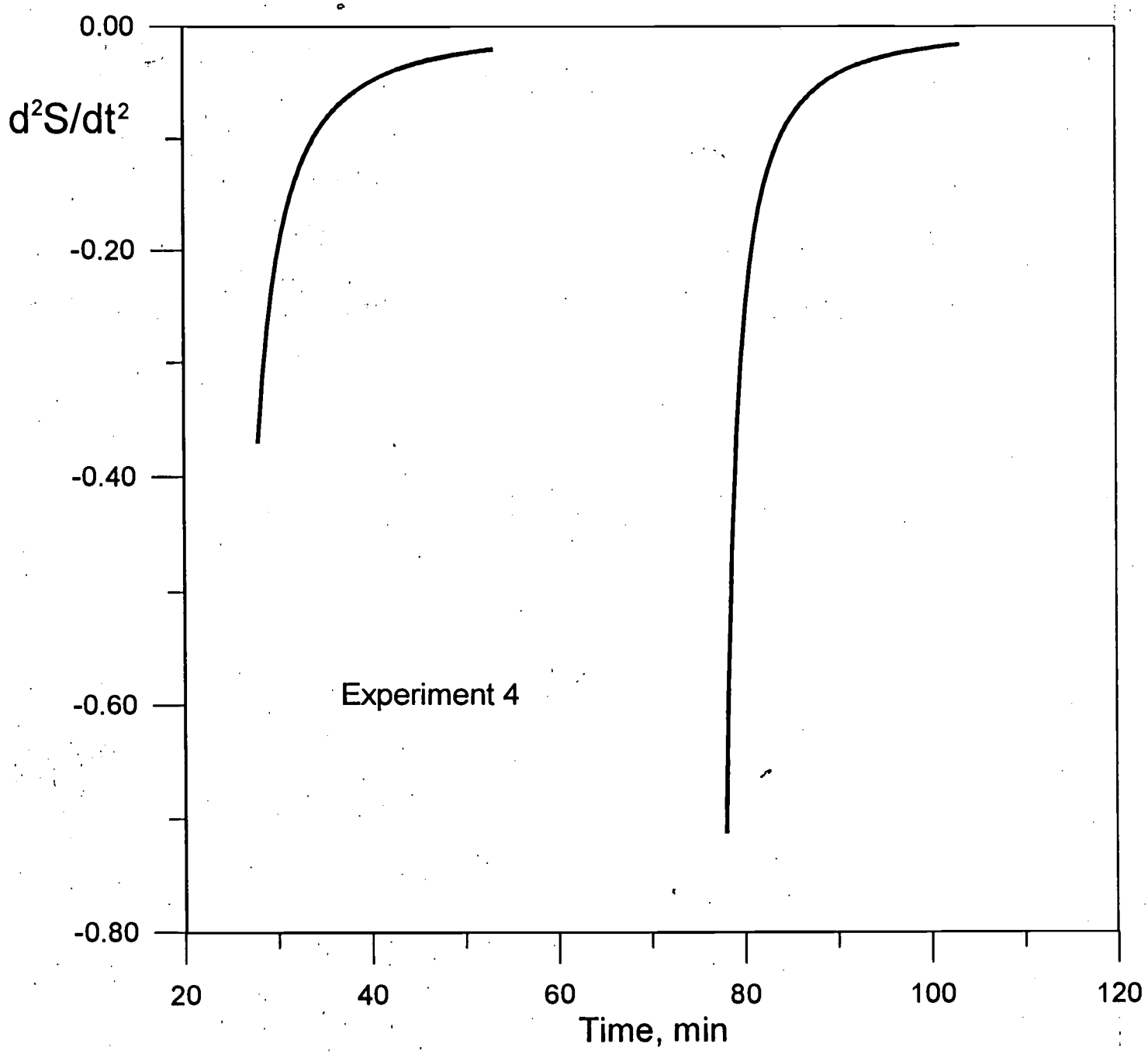

Figure 12. Graphical representation of d^2S/dt^2 respect to time for experiment 4.

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