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Intracerebral Implantation of Carbachol in the Rat: its Effect on Water Intake and Body Temperature

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HULST, S. G. Th. *Intracerebral implantation of carbachol in the rat: its effect on water intake and body temperature.* PHYSIOL. BEHAV. 8 (5) 865–872, 1972.—Intracerebral carbachol produces a fall in body temperature as well as drinking in the rat when implanted in various subcortical structures, related to the emotion-motivation limbic circuit. These effects are due to a central cholinergic stimulation since they can be prevented by the systemic administration of the centrally active anticholinergic substance atropine sulphate and to a lesser degree by methylatropine nitrate. By withholding water during the first hr following carbachol implantation it could be shown that the hypothermic response is independent from water intake. When carbachol as well as atropine sulphate are implanted in two localisations, which both induce hypothermia as well as drinking following carbachol stimulation, atropine sulphate nearly always blocked drinking, but practically only when atropine sulphate was applied caudally to carbachol did it block hypothermia. The results suggest a drinking and hypothermic circuit within the limbic system, anatomically linked but functionally different and independent.

Water intake Body temperature Carbachol Atropine Intracerebral implantation

INTRACEREBRALLY implanted carbachol elicits vigorous drinking in the water satiated rat [16, 17]. Grossman [16] and Fisher and Coury [13] found that cholinergic stimulation within perifornical region, as well as the limbic system, the hypothalamus and the midbrain induced drinking. It was postulated that the emotion-motivation limbic circuit, as proposed by Papez [29, 30] is intimately involved in the mediation of drinking. Further evidence for such a circuit has been presented [12, 26, 27, 32, 33]. In the rat hypothermia has been found following cholinergic stimulation of the anterior hypothalamus, lateral hypothalamus, preoptic region, nucleus lateralis septi and the area between the thalamic nuclei and the nucleus ruber [20, 22, 23, 25]. In a previous study [20] it was suggested that cholinergically evoked drinking and hypothermia follow roughly parallel pathways in the limbic system and the diencephalon. As the fibre systems for these two phenomena need not necessarily be the same, further experiments were conducted to evaluate the previous results; these studies are reported here.

MATERIALS AND METHODS

Male white rats of an inbred Wistar strain weighing 180–190 g were used. Crystalline substances were implanted into various subcortical structures as follows. A stainless steel plate, equipped with 12 holes, was used. Tubes, with a diameter of 0.8 mm, of the same material, protruding 2.50 mm from the lower side of the plate, were attached to the holes in the plate. This plate was fixed to the rat skull with dental cement (for details see [20]). Through the tubes needles of different length, containing crystalline substances at the tip, could be directed into the brain. The operated animals were allowed to recover from the operation and get used to drinking from a calibrated pipet for one week, while placed in individual cages. Rats in which the plate was not fixed properly, or which showed aggressive behavior, not regaining pre-operative body weight in one week or abnormal body temperature were excluded from the experiments.

On the day of the experiment an 18 gauge needle, with a small amount of crystalline carbachol or atropine sulphate (approximately 5 μg) at the tip, was placed into the brain of the water satiated conscious rat. The needles were cut at different lengths (6–11 mm) so that the crystalline substances could be placed at different depths into the brain. Four implantations, including one needle without any substances at the tip, were performed at each site. The effects on water intake and body temperature were regarded to be reliable when the differences between the effects of the three drug implantations were not greater than the changes found by the insertion of an empty needle (i.e. water intake less than 2.3 ml and change in body temperature less than 0.3°C).

Each experiment lasted 2½ hr. Every 30 min following implantation a probe of a Yellow-Spring Telethermometer was inserted 5 cm deep into the rectum of the rat and

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temperature was read. An additional measurement was performed 15 min following implantation. At the same time water intake was measured and the total amount of water consumed during the test period was calculated. Not more than two localisations in one rat were investigated and, by alternating implantations, it was ensured that both sites retained their reactivity to carbachol. As both localisations were reached via different tubes, no succeedingly deeper structures were explored in one rat. Implantations took place with an interval of at least three days. Thereafter animals were sacrificed. The places which had been reached by the needle were stained with Evans Blue. Brains were sectioned to examine the sites of these places using De Groot's atlas for the rat brain as a reference [15]. Only those localisations were taken into account which could be clearly defined on macroscopical and microscopical examination. A total of 201 different sites of implantation were investigated.

RESULTS

1. *Implantation of Carbachol in Subcortical Structures*

The insertion of an empty needle never resulted in changes in body temperature exceeding 0.3°C nor in drinking of more than 2.3 ml water during the test period. Effects were defined as follows for purposes of analysis: carbachol was considered to have an effect on body temperature when temperature rose or fell more than 1.0°C, and to have induced drinking when water intake exceeded 5.0 ml during the test period. From a total of 201 different sites of implantation 93 resulted in hypothermia as well as drinking, 19 only in hypothermia, 50 only in drinking, 9 showed a hyperthermia as well as drinking, 8 only hyperthermia and 22 implantations were without effect on body temperature or water intake.

Table 1 summarizes the effects of carbachol implantation

TABLE 1

MEAN AND MAXIMAL CHANGES IN BODY TEMPERATURE AND WATER INTAKE FOLLOWING CARBACHOL IMPLANTATION IN 201 DIFFERENT SITES IN SUBCORTICAL STRUCTURES. RESPONSES ARE DIVIDED INTO SIX CATEGORIES.

Structure		Hypothermia + Drinking	Hypothermia	Drinking	Hyperthermia + Drinking	Hyperthermia	No effect
Ventricle (lateral)	a.	−1.9(4) (−2.6)	−1.7(2) (−2.5)		+1.4(1)		(3)
	b.	9.7(4) (17.5)		10.8(2) (16.0)	16.0(1)		
Nucleus lateralis septi	a.	−2.1(18) (−4.2)	−1.7(2) (−1.8)				(1)
	b.	11.9(18) (20.0)		8.0(3) (10.0)			
Nucleus caudatus	a.	−1.7(7) (−2.7)	−1.1(1)		+1.0(1)	+1.5(1)	(1)
	b.	9.7(7) (16.5)		11.0(7) (16.5)	6.5(1)		
Diagonal band	a.	−2.2(7) (−3.0)					
	b.	12.0(7) (18.3)					
Area preoptica	a.	−1.1(3) (−1.3)	−1.9(1)				
	b.	13.0(3) (16.3)		14.9(4) (22.0)			
Fornix	a.	−2.0(4) (−2.8)					
	b.	11.8(4) (17.2)		10.6(3) (14.8)			
Area anterior hypothalami	a.	−1.8(2) (−2.6)					
	b.	11.5(2) (13.0)					
Anteroventral hippocampus	a.	−2.3(4) (−2.8)	−1.4(2) (−1.4)				
	b.	14.3(4) (21.7)					
Dorsocaudal hippocampus	a.				+2.0(1)		(3)
	b.			6.0(1)	6.2(1)		
Medio-antero-ventral thalamus	a.	−1.5(4) (−2.2)	−1.8(2) (−2.2)				(1)
	b.	12.9(4) (18.0)		13.6(2) (18.0)			

TABLE 1—continued

Structure		Hypothermia + Drinking	Hypothermia	Drinking	Hyperthermia + Drinking	Hyperthermia	No Effect
Medio-antero-dorsal thalamus	a.	−2.2(9) (−4.6)	−1.2(1)		+1.1(2) (+1.2)		
	b.	13.6 (9) (21.5)		12.8 (3) (17.5)	10.4 (2) (11.3)		
Medio-postero-ventral thalamus	a.		−1.9(1)			+1.4(1)	
	b.			7.0 (2) (7.0)			
Medio-postero-dorsal thalamus	a.		−1.6(3) (−2.1)				
	b.			7.7(3) (9.5)			
Latero-antero-ventral thalamus	a.		−1.2(1)			+1.9(1)	(2)
	b.			6.8(2) (8.4)			
Latero-antero-dorsal thalamus	a.	−1.5(18) (−3.0)					(2)
	b.	11.9(18) (21.0)		10.5(8) (15.0)			
Latero-postero-ventral thalamus	a.	−1.2(1)	−2.1(1)			+1.8(2) (+2.0)	(1)
	b.	8.7(1)		10.2(3) (12.6)			
Latero-postero-dorsal thalamus	a.	−1.5(1)					(5)
	b.	9.7(1)		9.2(3) (11.5)			
Medial forebrain bundle and lateral hypothalamus	a.	−2.2(8) (−2.7)	−2.8(1)		+1.5(2) (+1.5)	+1.5(1)	
	b.	9.7(8) (15.7)		15.1(1)	10.3(2) (12.0)		
Gyrus cinguli	a.	−2.2(2) (−2.6)	−2.6(1)			+1.0(1)	(1)
	b.	10.8(2) (16.5)		9.8(1)			
Zona incerta	a.	−2.5(1)			+1.5(2) (+2.0)	+2.6(1)	(2)
	b.	11.0		6.9 (2) (8.3)	7.0(2) (8.0)		

a = Mean change in body temperature. Between brackets number of localisations and maximal response.
b = Mean water intake. Between brackets number of localisations and maximal response.

on body temperature and water intake according to the structures stimulated. For purpose of analysis the thalamus was considered to be restricted to an area bounded by planes A 5.8 and 1.4, V + 1.5 and − 2.0, and L. 0.0 and − 2.0. The thalamus was further arbitrarily subdivided in eight areas, using planes A 4.2, L. 1.0 and V 0.0. In the areas where the combination of hypothermia and drinking is most frequently found—nucleus lateralis septi, fornix, diagonal band of Broca, anteroventral hippocampus, medio-antero-dorsal and latero-antero-dorsal thalamus and the area of the medial forebrain bundle and the lateral hypothalamus—the hypothermia is mostly greater than 2.0°C. An exception is the latero-antero-dorsal thalamus. In the latero-antero-ventral, medio-postero-ventral and medio-postero-dorsal thalamus implantation of carbachol induced either drinking or hypothermia, while implantation in the nucleus caudatus, area preoptica and medio-antero-ventral thalamus resulted in either hypothermia or drinking or both. In a small number of implantations a hyperthermia was found which was generally constricted to an area caudally from the anterior hypothalamus, ventrally from the thalamus and the posterior hypothalamus.

2. *Influence of Pretreatment with Atropine*

In order to learn whether these changes result from a central cholinergic stimulation, the influence of pretreatment with atropine sulphate and methylatropine nitrate in doses of 4 mg/kg body weight i.p. was studied in 20 rats (40 localisations) in which carbachol was implanted widely spread through the subcortical structures. These doses were found to influence neither water intake nor body temperature following

i.p. administration. These anticholinergic compounds were injected i.p., dissolved in 0.25 ml 0.9% NaCl, 1 hr before the implantation of carbachol. In 10 operated rats the effect of i.p. administration of atropine sulphate, methylatropine nitrate as well as 0.9% NaCl was studied. The mean maximal changes in body temperature after atropine sulphate administration (+ 0.2 ± 0.3°C) and after methylatropine nitrate (− 0.3 ± 0.2°C), were not significantly different from the temperature changes after 0.9% NaCl (+ 0.1 ± 0.3°C). Neither atropine sulphate, methylatropine nitrate nor 0.9% NaCl caused any water intake during the test period.

Table 2 summarizes separately the effect of pretreatment with atropine sulphate and methylatropine nitrate on both parameters, subdivided in several categories according to

TABLE 2

EFFECT OF PRETREATMENT WITH ATROPINE SULPHATE AND METHYLATROPINE NITRATE ON CHANGES IN BODY TEMPERATURE AND WATER INTAKE FOLLOWING CARBACHOL IMPLANTATION IN SUBCORTICAL STRUCTURES

1. Fall in body temperature ≥ 2.0°C

Pretreatment	Number	Fall in body temperature (°C)	p†
0.25 ml 0.9% NaCl i.p.	13	−2.45±0.32*	$p<0.01$
0.25 ml Atropine sulphate (4 mg/kg) i.p.	13	−0.16±1.10	$p<0.01$
0.25 ml Methylatropine nitrate i.p.	13	−0.54±0.67	$p<0.01$

2. Fall in body temperature 1.0–2.0°C

Pretreatment	Number	Fall in body temperature (°C)	p
0.25 ml 0.9% NaCl i.p. —	16	−1.44±0.30	$p<0.01$
0.25 ml Atropine sulphate (4 mg/kg) i.p.	16	+0.23±0.90	$p>0.05$
0.25 ml Methylatropine nitrate (4 mg/kg) i.p.	16	−1.16±0.69	$p<0.01$

3. Change in body temperature <1.0°C

Pretreatment	Number	Change in body temperature (°C)	p
0.25 ml 0.9% NaCl i.p.	6	−0.63±0.33	$p<0.05$
0.25 ml Atropine sulphate (4 mg/kg) i.p.	6	+0.28±0.70	$p>0.05$
0.25 ml Methylatropine nitrate (4 mg/kg) i.p.	6	−0.32±0.73	$p>0.05$

4. Rise in body temperature ≥1.0°C

Pretreatment	Number	Rise in body temperature (°C)	p
0.25 ml 0.9% NaCl i.p.	5	+1.48±0.27	$p<0.01$
0.25 ml Atropine sulphate (4 mg/kg) i.p.	5	+0.74±0.30	$p>0.05$
0.25 ml Methylatropine nitrate (4 mg/kg) i.p.	5	+1.60±0.47	$p<0.01$

TABLE 2—continued

5. Water intake $\geqslant 10$ ml

Pretreatment	Number	Water intake (ml)		p
0.25 ml 0.9% NaCl i.p.	21	13.81±2.90	$p<0.01$	$p<0.01$
0.25 ml Atropine sulphate (4 mg/kg) i.p.	21	4.30±3.40		
0.25 ml Methyl-atropine nitrate (4 mg/kg) i.p.	21	9.00±4.00	$p<0.05$	

6. Water intake <1.0 ml

Pretreatment	Number	Water intake (ml)		p
0.25 ml 0.9% NaCl i.p.	19	5.38±3.40	$p<0.01$	$p>0.05$
0.25 ml Atropine sulphate (4 mg/kg) i.p.	19	2.05±3.00		
0.25 ml Methyl-atropine nitrate (4 mg/kg) i.p.	19	4.04±3.00	$p<0.05$	

*mean±standard deviation
†Student *t*-test

magnitude of response. Only the centrally active compound, atropine sulphate, is constantly capable of blocking both effects of carbachol. Both variables were also influenced by methylatropine nitrate pretreatment and its blocking effect was significant when carbachol induced a fall in body temperature greater than 2°C and more than 10 ml water intake. This blocking effect of methylatropine nitrate, however, was less than that of atropine sulphate. This could either implicate a peripheral cholinergic participation in both mechanisms or metabolic breakdown of methylatropine nitrate to a compound which can pass the blood brain barrier.

3. *Relation Between Changes in Body Temperature and Water Intake*

Although the previous results suggest that both phenomena are structurally linked but probably functionally independent, it was felt of interest to study the effect of carbachol implantation on body temperature with and without available water. In 24 rats water was withheld during the first hr following carbachol implantation in localisations widely spread through the subcortical structures while temperature was measured. After one hr rats were allowed to drink and during the remaining 150 min of the test period water intake as well as body temperature were recorded. Temperature changes in the first hr following implantation were compared when water was not withheld with those when water was withheld. As was expected, carbachol implantation induced changes in body temperature or water intake or both. Since the scope of this series of experiments was to investigate whether withholding of water did have any effect on changes in body temperature presumably induced by carbachol, it was decided to take all temperature changes into consideration, even those less than 1°C. Figure 1 shows the relation between the two temperature changes. This figure shows that changes in body temperature in the first hr following implantation of carbachol are independent of the amount of water consumed. Using a Spearman rank correlation test a correlation coefficient $r_s = 0.9380$ was found which is significant ($p < 0.01$) and a regression line ($y = 1.16x + 0.14$) could be drawn.

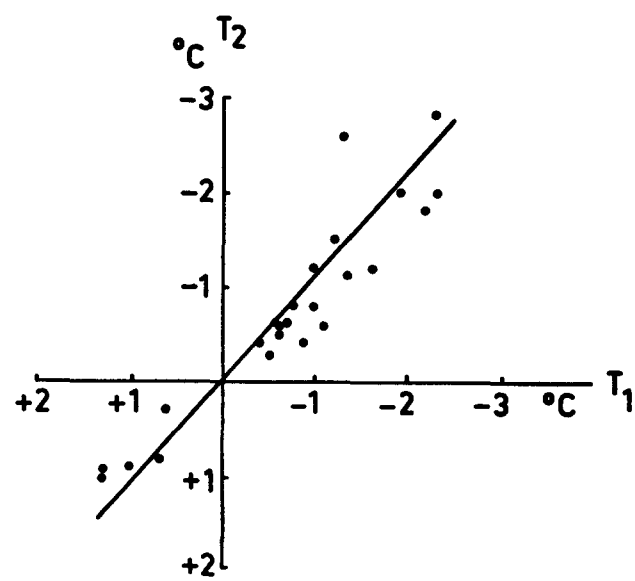

FIG. 1. Relation between temperature changes in the first hr following carbachol implantation with (T_2) and without (T_1) water withdrawal.

4. *Influence of Intracerebral Implantation of Atropine Sulphate on Carbachol Induced Drinking and Hypothermia*

To examine further support for the hypothesis that the subcortical structures contain two separate cholinergic circuits for drinking and hypothermia, carbachol as well as atropine sulphate was implanted in two different localisations in both of which carbachol induced hypothermia of at least 1.0°C and drinking of at least 10.0 ml. After having found two such sites in one rat a second experiment was performed in which in one site carbachol and in the other atropine sulphate was implanted and the effect of these two implantations on body temperature and water intake were compared with the results obtained from the two separate carbachol implantations. The site of atropine sulphate and carbachol implantation were then reversed and again water intake and body temperature recorded. In two subsequent experiments the reactivity to carbachol implantation in the two localisations was ascertained separately. When the effects of the two latter implantations were comparable with the former, the results of the combined carbachol and atropine sulphate implantations were regarded to be reliable. Intracerebrally applied atropine sulphate was considered to have blocked the carbachol induced hypothermia and drinking when during the combined implantations the fall in body temperature was less than 0.3°C and water intake less than 2.3 ml. Reliable results were obtained in 28 rats.

The localisations of the stimulated areas are summarized in Fig. 2 as superimposed on a drawing representing section L.1.1. from De Groot [15] The two localisations stimulated in one rat are connected by a line. These experiments showed that centrally applied atropine sulphate is able to block the effect of carbachol on water intake and body temperature. In general, atropine sulphate blocked carbachol induced drinking in both combined implantations (bidirectional blockade), but carbachol induced hypothermia only in one of the two combinations (monodirectional blockade). This mono-directional blockade occurred mostly when atropine sulphate was applied caudally to carbachol (monodirectional posterior-anterior blockade), and scarcely when it was applied rostrally to carbachol (monodirectional anterior-posterior blockade). In five combined implantations atropine sulphate did not block the hypothermia in either localisation and in four it did not block carbachol induced drinking. Table 3 shows the frequency distribution of the different types of blockade. The number of observations does not permit further analysis of the different kinds of blockade.

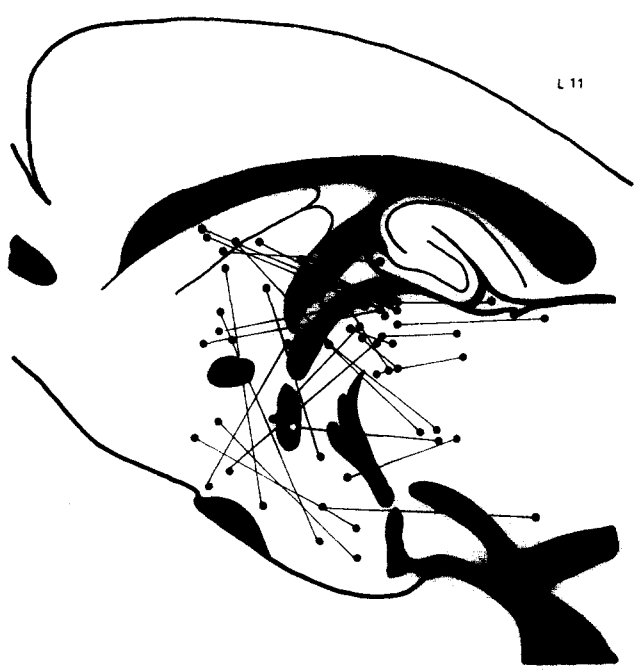

FIG. 2. Reconstruction of 28 pairs of combined implantations of carbachol and atropine sulphate superimposed on a drawing representing section L 1.1 from De Groot. The localisations stimulated in one rat are connected by a drawn line.

TABLE 3.

FREQUENCY DISTRIBUTION OF THE DIFFERENT INFLUENCES OF INTRACEREBRAL APPLICATION OF ATROPINE SULPHATE ON HYPOTHERMIA AND WATER INTAKE FOLLOWING CARBACHOL STIMULATION OF SUBCORTICAL STRUCTURES

Blockade	Hypothermia		Water Intake	
Monodirectional		20		3
posterior-anterior	15		1	
anterior-posterior	5		2	
Bidirectional		3		21
No blockade		5		4
Total	20	28	3	28

DISCUSSION

The present data confirm results of a number of authors who proposed that there exists within various subcortical structures, related to the limbic circuit, a cholinergic drinking circuit [12, 13, 16, 17, 24, 26, 27, 32, 33]. The present data also suggest a circuit which, following carbachol stimulation, induces hypothermia. From the results in the first experiment it can be seen that this circuit is anatomically closely linked to the drinking circuit, since over half of effective implantations resulted in hypothermia as well as drinking (N = 93). There seem to be more implantation sites in which drinking is induced by carbachol (N = 152 {93 + 50 + 9}) than hypothermia (N = 112 {93 + 19}). That the combination of carbachol induced drinking and hypothermia is probably only due to anatomical factors is suggested by comparing the results from stimulated sites in the areas where the combination of hypothermia and drinking is most frequent with those found in regions where they are evoked separately and where either hypothermia or drinking or both occur (Table 1). This is further supported by the results from the third series of experiments, in which it was shown that the withholding of water during the first hour after carbachol implantation does not influence the magnitude of the hypothermic response.

The results from the last series of experiments not only support the circuit hypothesis, but also suggest a functional difference in both circuits. The fact that atropine sulphate administration to the drinking circuit nearly always inhibits carbachol induced drinking suggests that there exists hardly an alternative pathway within this circuit. In the hypothermia circuit, however, there seem to exist alternative pathways since the hypothermic effect of carbachol stimulation is only to be blocked in one of the two localisations. This mono-

directional blockade suggests a one-way circuit for carbachol induced hypothermia. These suggestions derived from the present data are only valid when one assumes that the effects of this kind of stimulations are comparable to the stimuli as they occur physiologically. Whether this is a thirst circuit [12] or a circuit which after cholinergic stimulation induces drinking even without physiological need, is still unsettled. Evidence thus far reported in the literature is in favour of a facilitating mechanism. Water deprived rats will work as hard for water as water satiated rats after carbachol stimulation [21]. Carbachol stimulation in water deprived rats induces drinking of amounts far exceeding their physiological needs [18], while water deprived animals will drink just enough to equal their needs [8]. Lesions in the lateral hypothalamus induce a reversible adipsia. Application of carbachol in the area preoptica of these rats does not induce drinking whether they are still adipsic or not [36]. Water deprivation as well as the injection of a hypertonic saline solution induce ADH secretion as well as drinking and cholinergic stimulation has been shown to augment ADH secretion [4, 11, 34, 35], but no effect of ADH injections on water intake has been found [1, 10] and carbachol induced drinking is hardly ever accompanied by an antidiuresis [19]. Carbachol induced drinking seems, according to the above mentioned data from the literature, not to depend on intra- and extracellular dehydration which in itself are strong stimuli to consume water [2, 14].

The present data suggest also a separate cholinergic innervated hypothermic circuit. There are, however, suggestions in the literature that hypothermia and drinking are functionally closely related. Local warming of the preoptic area induces drinking in the goat [3]. Injections of hypertonic saline solutions induce drinking as well as hypothermia in the rat [28] while deprivation alone also induces hypothermia [7].

Although several theories are suggested for thermoregulation due to effects of neurotransmittors, the fact that in the rat no neurotransmittor has been found to induce only hyperthermia makes it difficult to propose such a theory for this animal. In the rat nearly all neurotransmittors studied cause hypothermia, but only carbachol has been shown to induce hypothermia following implantation in many subcortical structures as well as hyperthermia in a circumscribed area caudally from the anterior hypothalamus, ventrally from the thalamus and the posterior hypothalamus. This probably means that besides a system composed of a center for heat production (posterior hypothalamus) and a center for heat loss (anterior hypothalamus) there is a second system involved, which is widely spread through the central nervous system and is situated within the limbic circuit. The reaction of an organism to its environment is composed of at least two components. Firstly a direct reaction via the sensorial specific projection system and secondly via a non-specific relatively diffusely located system in the formatio reticularis and its functionally connected structures, i.e. the limbic circuit [31]. As has been suggested for carbachol induced drinking there is also evidence for behavioral influences on body temperature [5, 6, 9]. Cholinergic stimulation of parts of the limbic circuit is known to influence the behavioral status of the animal. As the regulation of body temperature may not only be directed towards maintaining body temperature at a constant level but also towards preventing major changes due to the behavior of the rat, it is suggested that a cholinergic temperature circuit within the limbic circuit is involved in this part of temperature regulation.

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