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The neuropharmacology of behavior related to cued discrimination tasks studied following temperature stress was the focus of this work. Prostaglandin E2 (PGE2) microinjected into the medial anterior hypothalamus/preoptic area (MAHPOA) heat gain sites of rats that were at a normal core temperature resulted in an improvement in task performance by a decrease in error rate. The PGE2 response changes that define changes in motivation, that were elicited by changes in core temperature. This differential response was defined as a change in an additional factor. Further studies would be required to determine the exact nature of this response and its robustness.

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Performance Modification Following Alteration of Molecular
 Mechanisms of Thermoregulation

C. Wayne Simpson, PI. and Garth E. Resch, Co-PI.

I. PHARMACOLOGY OF NEUROTRANSMITTERS FOR USE IN CUED DISCRIMINATION

A. Five Neurotransmitter-Elicited Responses: Unanesthetized Rat

1. Agonist-elicited Heat Gain and Heat Loss Responses

Five agonists were tested in PGE2-sensitive heat gain sites. Examples of typical heat gain and heat loss responses are illustrated in the figures (Figs. 1-5). Four elicited a 0.5 C or greater rise in Tc (PGE2, Ach, Nic, and 5-HT) and one elicited a fall in Tc (NE). The site response to the four heat gain agonists in the rat data in this report occur at anatomically comparable sites and elicit comparable amplitudes of rise in Tc as reported by us in previous hamster experiments.

2. Effective Doses of Agonists in Unanesthetized Unrestrained Rats

To determine minimum effective doses to use in the cued discrimination task dose response tables were completed for PGE2 (Table 1). Effective doses were determined for 5-HT, Ach, and NE (Table 2); dose response work is continuing. Microinjections of these ligands were made into MAHPOA heat gain sites in awake unrestrained rats. Tc was measured for 20 min. before and 30 min. following microinjection. In individual rats a 0.5 C change in Tc was used as the criterion for a positive response. Statistical criteria were used for the determination of significant threshold values in groups of rats. PGE2 elicited a significant rise in Tc at doses between 10[-12]gm and 10[-30]gm but not at 10[-33]gm or 10[-36]gm. Ach elicited increased Tc from 10[-12]gm. 5-HT elicited increased Tc from 10[-12]gm. NE elicited significant decreases in Tc at doses of 10[-5]gm. These data show that the PGE2-sensitive MAHPOA heat gain site responds, in fact, to both heat gain and a heat loss agonist. The responses are also shown to occur at much lower doses than previously reported.

B. Effect of Neurotransmitter on Heat Gain: Anesthetized vs Unanesthetized

1. PGE2-elicited Change in Tc of Awake vs Anesthetized Rats

The amplitude of rise in Tc in unanesthetized rats (Table 3) was statistically the same as the response in ketamine anesthetized rats. The data show no significant difference between the change in Tc following injection of 10[-12]gm of PGE2 into the MAHPOA of awake rats at 1.15 C and anesthetized rats at 0.89 C. These data confirm and validate the use of ketamine anesthetized rats for screening of heat gain agonists.

2. PGE2-elicited rise in Tc vs Starting Tc

The Tc response amplitude following 10[-12]gm of PGE2 was measured in four starting Tc ranges in awake rats (Table 4) and in eight starting Tc ranges in anesthetized rats (Table 5). The only significant difference was a reduced amplitude that occurred in the awake rat in the 39.0 to 39.4 C starting Tc range all lower ranges. No significant differences occurred in the ketamine anesthetized rats. Three conclusion can be drawn from these data. First, that whether the rat is awake or anesthetized in the euthermic starting Tc range, the PGE2-elicited rise in Tc is the same. Second, that higher starting Tc ranges in awake rats only effects the rats Tc response to PGE2 when the animal nears 40 C. Third, that lower Tc ranges, seen in anesthetized rats, show no Tc response amplitude differences from euthermic to the lowest starting Tc range (34.0 to 34.4 C). These data agree with those of Malkinson, Veale, and Cooper (1988) who also showed no change in Tc responses at different hypothermic starting Tc values and a suppressed response at Tc values near 40 C. That work differed from that in this report in that Malkinson et al. used urethane anesthesia instead of ketamine (plus 10% acepromazine).

These data confirm year one annual report data demonstrating neurochemical site discrimination. In addition the new data demonstrate the multiple agonist site in unanesthetized rats and agree with previous data in the hamster.

II. BEHAVIOR

A. Training Modifications

Training of rats to the cue discrimination task was done for the primary protocol and for three modified protocols. In all 241 training sessions was conducted on 23 rats between June of 1988 and June of 1989.

A single training session consisted of four parts of 10 or more trials each. The four parts were initial baseline, drug treatment, saline vehicle treatment, and final baseline. Initial baselines varied from 10 to 50, one minute trials. Each trial consisted of approximately 50 seconds of cue presentation and 10 seconds of an inter-trial interval. During each trial the rat was scored for barpresses during correct, incorrect, and inappropriate intervals of each trial. The scores were expressed as number, rate, and percent.

1. Increasing Cue Complexity (Addition of Inappropriate Cue)

One suggested modification of basic protocols specified in the grant objectives was the increase in task complexity to assess cue discrimination capability and to assess the effects of drugs on

Codes
/or

A-1

discrimination between the cues. The increase in task complexity consisted of the presentation of a visual cue (horizontal light bar on a dark background) instead of the correct cue for 10 % of the cue presentation time between ITI's.

The comparison of incorrect and inappropriate scores following the MAHPOA injection of PGE2 resulted in a 27% decrease for incorrect and a 56% decrease for inappropriate responding (Table 6). The decrease error rate in task performance was reflected in the significant decrease of both the incorrect ($P < .05$) and inappropriate ($P < .03$) scores following PGE2 compared to saline vehicle injections. Performance improved by the addition of the inappropriate cue due to a decrease in error rate. The PGE2 effects were elicited at 10[-12]gm discussed in section I.A. above. The PGE2-elicited effects occurred only in sites that also elicited as rise in Tc, demonstrating site specificity for the response.

2. Baseline Trial Number and Control of Tc.

Control of a motivation stimulus, Tc, was done by testing cued performance a two temperature intervals, ie. <35 C and >38.0 C (Table 7). Total response score increased, when evaluated as either number or as rate, in both the correct and incorrect cue conditions when the Tc value was experimentally decreased by increasing the number of baseline trials. The data show a proportional increase in correct and incorrect scores with lowered Tc. Therefore, a change of Tc away from normal elicits an increase in motivation as indicated by a proportional increase in the behavioral responding scores.

3. Alternating Correct and Inappropriate Cue Presentation

An attempt was made to accelerate improved discrimination of the correct cue. The behavior protocol was modified to permit comparison of the barpress response scores between the correct and inappropriate cue. Each cue was presented on the screen for approximately 50% of the trials during a session. Performance scores decreased and the protocol modification was rejected.

B. Central Effects of PGE2 on Cued Discrimination Performance

1. Euthermic

The effects of PGE2 on cue discrimination scores improved compared to saline vehicle controls in rats with Tc values of 38.0 to 39.8 C (Table 6). The scores improved due to a decrease in error rate rather than an increase in correct responses. The Tc values of the rats were determined by the rat in a protocol with 10 trials per session each for drug and saline treatments and no more than 20 trials for the initial baseline. The decrease in error rate was true for scores during the ITI and for the inappropriate cue.

The effect was brain site specific as indicated by the observation that improved performance after PGE2 occurred only when the PGE2 injection was made into an MAHPOA site that resulted in an increase in Tc value. Control sites in which no Tc rise occurred following PGE2 injection showed no performance improvement.

2. Hypothermic vs Euthermic

The improvement in task performance following PGE2 injection occurred in the euthermic group of rats but did not occur in hypothermic rats (Table 7). Two groups of rats at different Tc values were compared for a reduction of the incorrect score following PGE2 injection into the MAHPOA. The rats with Tc values >37 C showed the improved performance as described in the preceding section; however, the rats with Tc values <35 C showed no response compared with saline vehicle controls. Rats at Tc <35 C did show increased motivation due to the lowered Tc value, ie. a proportional increase in correct and incorrect scores, but no improvement in performance following PGE2, ie. as shown by a differential decrease in error rate compared to correct scores or compared to saline vehicle controls.

3. Protocol Variations with PGE2

Several variations in injection schedules and inter-session times were done. Since none showed an improved discriminating capability compared to the protocol adopted and used for the observations in preceding sections, none of these modifications were adopted.

C. Control of Experimental Parameters

1. Sham Controls on Central Drug Injection in Cued Discrimination

A group of six rats were run in the experimental protocol exactly as drug injected animals with the exception that after the injector was inserted into the guide tube, no drug or saline injection was given. The data constitutes a control on all handling and procedures in the experiment except drug or saline vehicle effects. The data show an overall significant F-test and follow-up pairwise comparisons showed that the drug and saline treatment sessions were not significantly different from each other.

2. Ambient Temperature (Ta) Elicited Motivation

An ambient temperature of -4 C was a sufficient stimulus to motivate a shaved rat to barpress for heat. The data throughout all of the experiments demonstrate this point. It is well established in the literature that animals do not press at normal room temperatures and do at Ta's below 0 C. This was verified on a few rats at temperatures of 0 and 2 C and barpress rates were close to zero.

3. Colonic Temperature (Tc) Elicited Motivation

An issue that was raised in the reviews of the renewal proposal was the definition of motivation and appropriate control data demonstrating the same. Terminology is only useful when it is defined operationally in terms of data. Cue discrimination experiments were conducted at Tc values >37 C and <35 C (Table 7). The barpress performance scores show a non-differential increase in all parameters of the experiment at the lower Tc value compared to normal. These data demonstrate a change in performance referred to as a change in motivation in agreement with other reports of barpressing in the cold for heat reinforcement.

4. Brain Site Controls for PGE2-elicited Behavioral Response

The data show that changes in behavioral response following PGE2 injection into an MAHPOA brain site occurred only when the injection was made into a site that elicited a rise in Tc, ie. a heat gain control site (Table 6, col. 3 vs 1 & 2). PGE2 injections into tissue surrounding a heat gain site elicits no rise in Tc and no changes in behavior. Heat gain sites were differentiated neurochemically and stereotaxically in the first annual report. Behavioral changes have now been elicited from these same sites. These data indicate that behavioral and autonomic controls operate out of the same discrete MAHPOA injection sites. These data also constitute a data basis for exploring neuron pathways from the MAHPOA to sites elsewhere in the brain that are important for consolidating a behavioral response.

5. Control on Drug Effects

Just as PGE2 injection into a non-heat gain site elicited no response, so to a saline vehicle injection into an active heat gain response elicits no significant rise in Tc or change in behavior. The PGE2 was dissolved in saline so saline represents a control for any possible effects of the vehicle. Injections of PGE2 and saline were given on the same day in different sessions of the same experiment. PGE2 responsive sites were typically verified a day before and after the day of a behavioral experiment.

Four other characteristics of the drug-elicited response are an increase in Tc, a decrease in ITI behavioral responding, a limited duration of response to bolus injection, and blockade with specific antagonists. The characteristic response to the drug, and not vehicle, is a decrease error rate, no change in correct score and a rise in Tc. The limited duration of response is demonstrated in the two sessions that follow the drug treatment session, ie. the barpress rates return to baseline. Finally a specific antagonist, SC19220, inhibits the PGE2-elicited rise in temperature. The antagonist effect on behavior has not yet been tested and should be in future work.

III. Preliminary Observations Related To Second Grant Proposal Objectives

A. Pharmacology

1. Effect of Peptides in PGE2-sensitive MAHPOA Heat Gain Sites

Four peptides were screened in the PGE2-sensitive MAHPOA heat gain sites in preliminary experiments in a few animals. AVP elicited a hypothermia (mean change in Tc of -0.8 C at 10⁻⁶gm), ie. an effect opposite to the PGE2 effect. Alpha-MSH (0.49 C at 10⁻¹²gm), NPY (0.6 C at 10⁻⁶gm), and beta-endorphin (0.6 C at 10⁻⁶gm) elicited a rise in Tc. AVP and beta-endorphin elicited responses at fairly high doses; however, alpha-MSH only required doses comparable to those of the neurotransmitter, near 10⁻¹²gm, to elicit a rise in Tc. These data show that alpha-MSH, which is an endogenously occurring substance, is active in the brain site of interest, that modulates autonomic and behavioral responses.

B. Behavior

1. Central Effects of Alpha-MSH on Cued Performance

Alpha-MSH injected into the PGE2-sensitive MAHPOA heat gain site of a few animals elicited effects similar to those of PGE2. The response differed in a most interesting way. PGE2 improved discrimination performance 1) between correct and inappropriate cues and 2) between correct cue and no cue. Alpha-MSH improved discrimination performance more substantially than PGE2 but only between correct cue and no cue presentations, ie. there was no apparent effect on performance response to inappropriate cues. These data suggest the two drugs may be useful to differentiate components in a behavioral control pathway. Alpha-MSH has been implicated in human learning studies. Alpha-MSH data from our preliminary animal studies agrees with human studies which show improved performance on sequentially learned symbols.

An additional comparison between alpha-MSH and PGE2 showed a difference in potency effecting the cue discrimination behavior. The alpha-MSH elicited rise in Tc does not need to be as large to elicit a more substantial behavioral change when compared to the effects of PGE2. Specifically, a 0.1 C rise in Tc was still associated with a behavioral response, whereas a 0.5 C or greater change in Tc was necessary for PGE2. Therefore alpha-MSH appears to be more important for the behavioral component than for the autonomic component of the elicited response.

2. Interaction With Cold Stress Factors (Glucocorticoid with PGE2)

Plasma glucocorticoids rise in response to many kinds of stress and consequently has been referred to as a stress indicator, that modulates the stress response. Consistent with the general observation, glucocorticoid also rises to cold stress. Data in the literature and confirming preliminary data from this laboratory show that during cold exposure glucocorticoid effects the responding heat gain system. That is, adrenalectomized rats are not cold tolerant (ie. maintain Tc in -4 C ambient) but intact animals are. A brain site and mechanism of the glucocorticoid effect on cold tolerance has not previously been demonstrated, only in peripheral tissues. Brain sites of action and mechanism of action were suggested by preliminary data from this laboratory.

Corticosterone replacement into the MAHPOA site restores cold tolerance in adrenalectomized rats. Tc measured at 30 min intervals during exposure to Ta = -4 C shows a 0 to 0.6 C rise in Tc maintained for 2 to 3 hrs. by intact controls vs a Tc drop of 4.3 to 6.6 C at 60 to 90 minutes of cold exposure for adrenalectomized rats. MAHPOA corticosterone replacement resulted in normal Tc values vs intact controls at 60 to 90 min of cold exposure.

The same MAHPOA sites controls behavioral and autonomic responses to neurotransmitter and peptide microinjection. The preliminary data suggest that one way in which the Glucocorticoid may effect heat gain is by control of PGE2 effectiveness. PGE2-elicited Tc responses of 0.62 C within 30 min in intact controls decreased to 0.2 C after adrenalectomy and returned to values of 0.68 C after MAHPOA

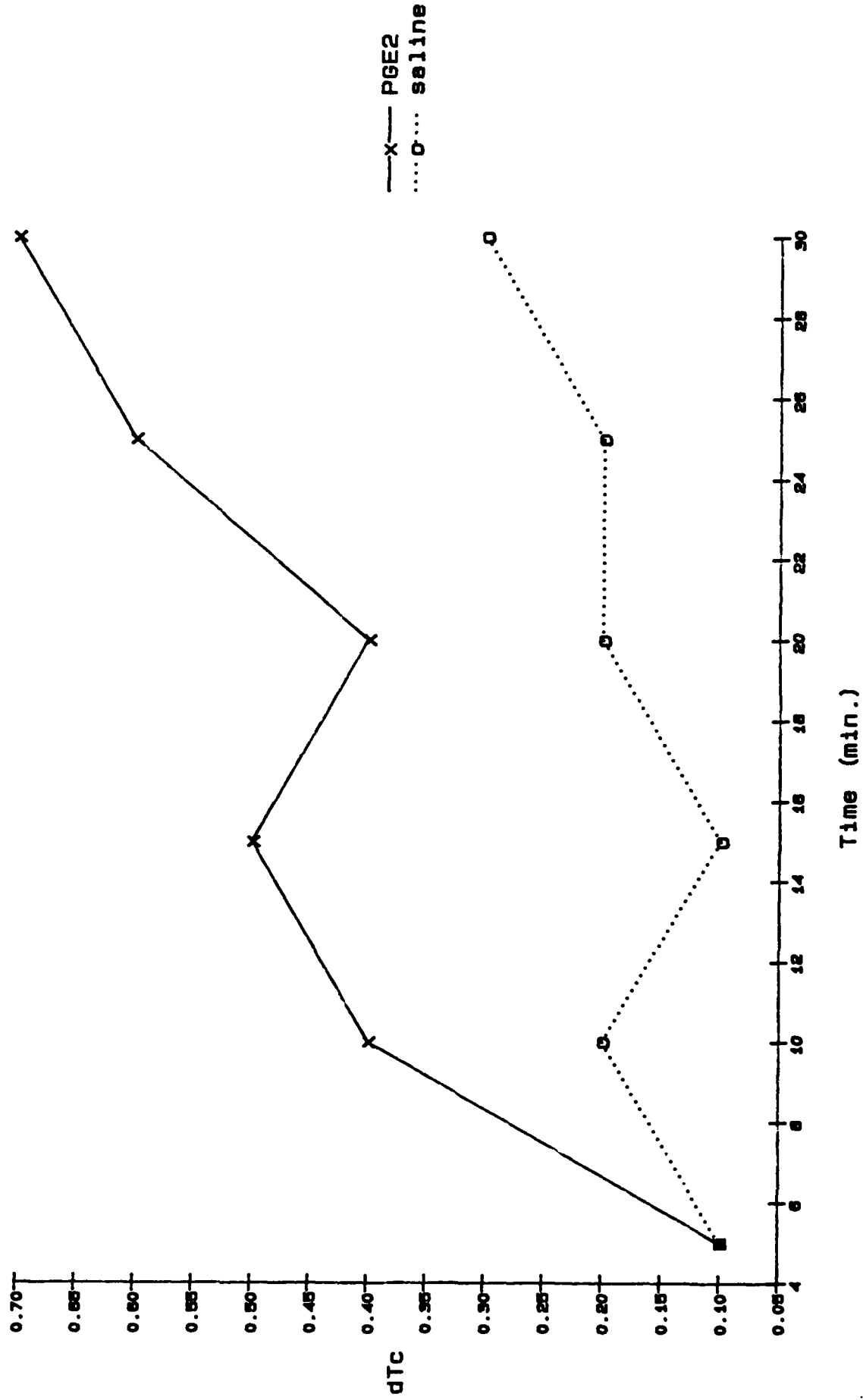
replacement of corticosterone. The data indicate effects of PGE2 are altered by corticosterone injected at the same brain site. Therefore, sites identified as by this laboratory afford the opportunity to study molecular interactions that influence autonomic and/or behavioral responses.

PGE2EG

Figure 1

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CHANGE IN Tc AFTER PGE2 INJECTION INTO RAT MAHPOA

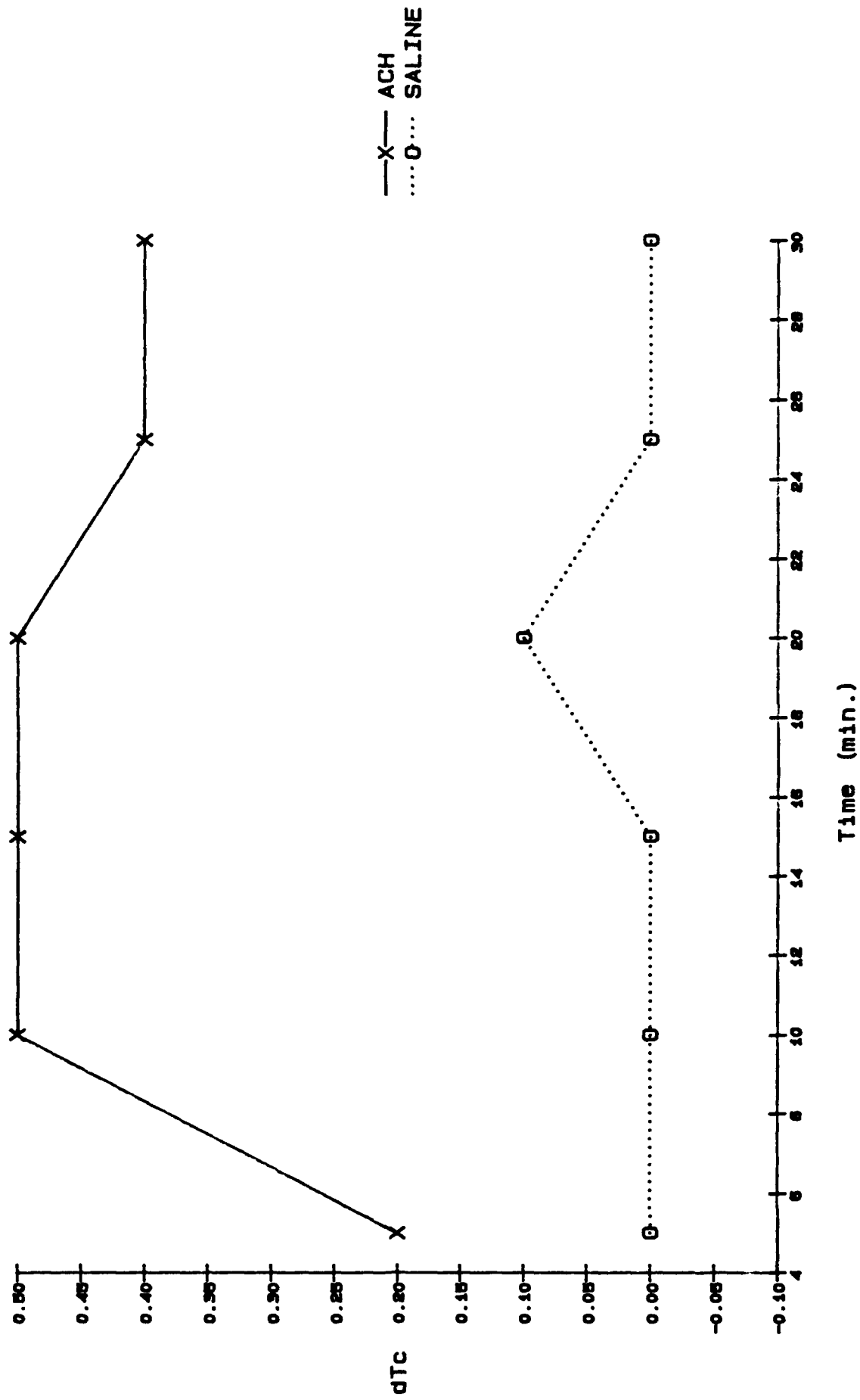


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Figure 2

CHANGE IN Tc AFTER ACH INJECTION INTO RAT MAHPOA



—X— ACH
.....O... SALINE

Figure 3

CHANGE IN Tc AFTER NICOTINE INJECTION INTO RAT MAHPOA

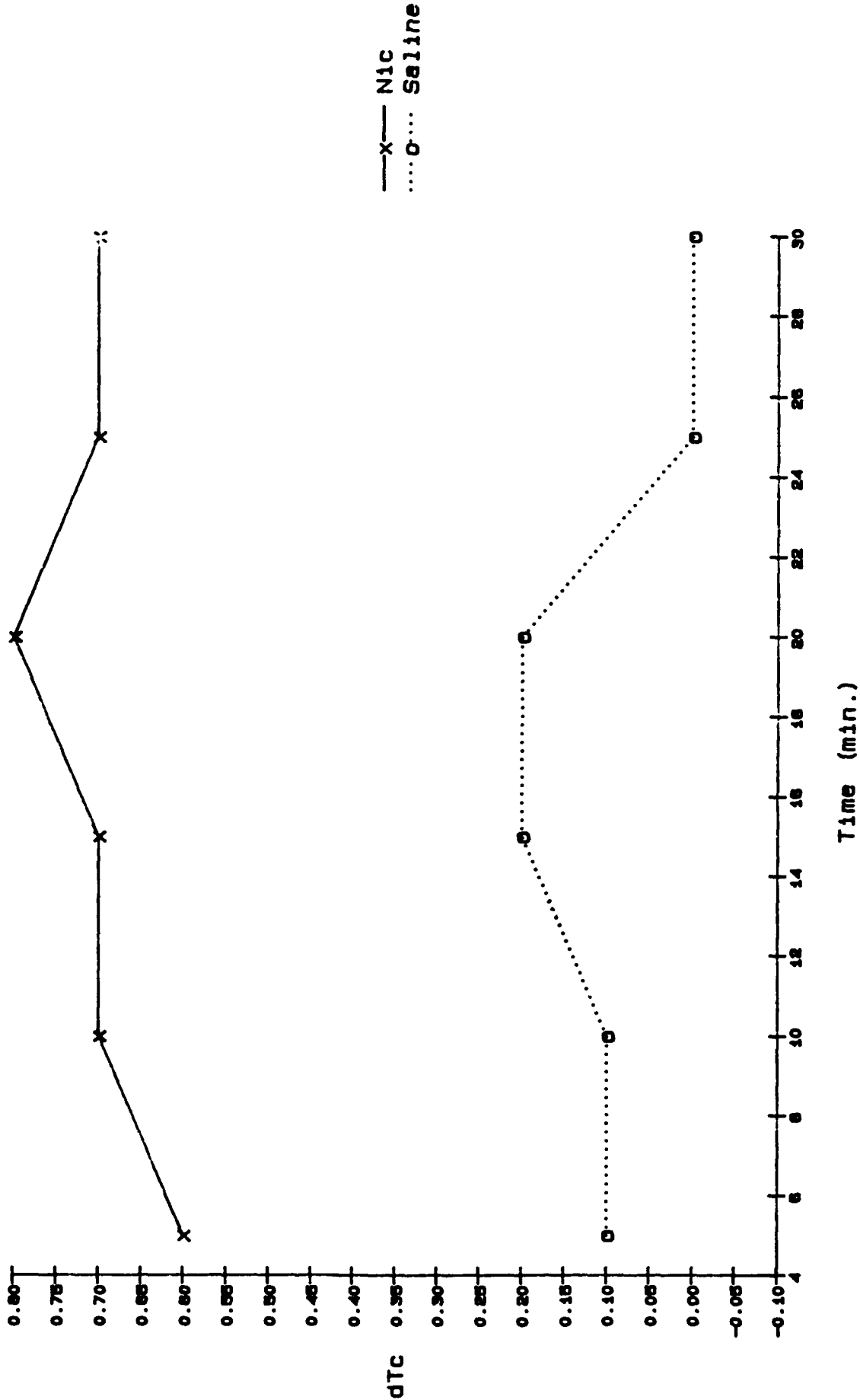
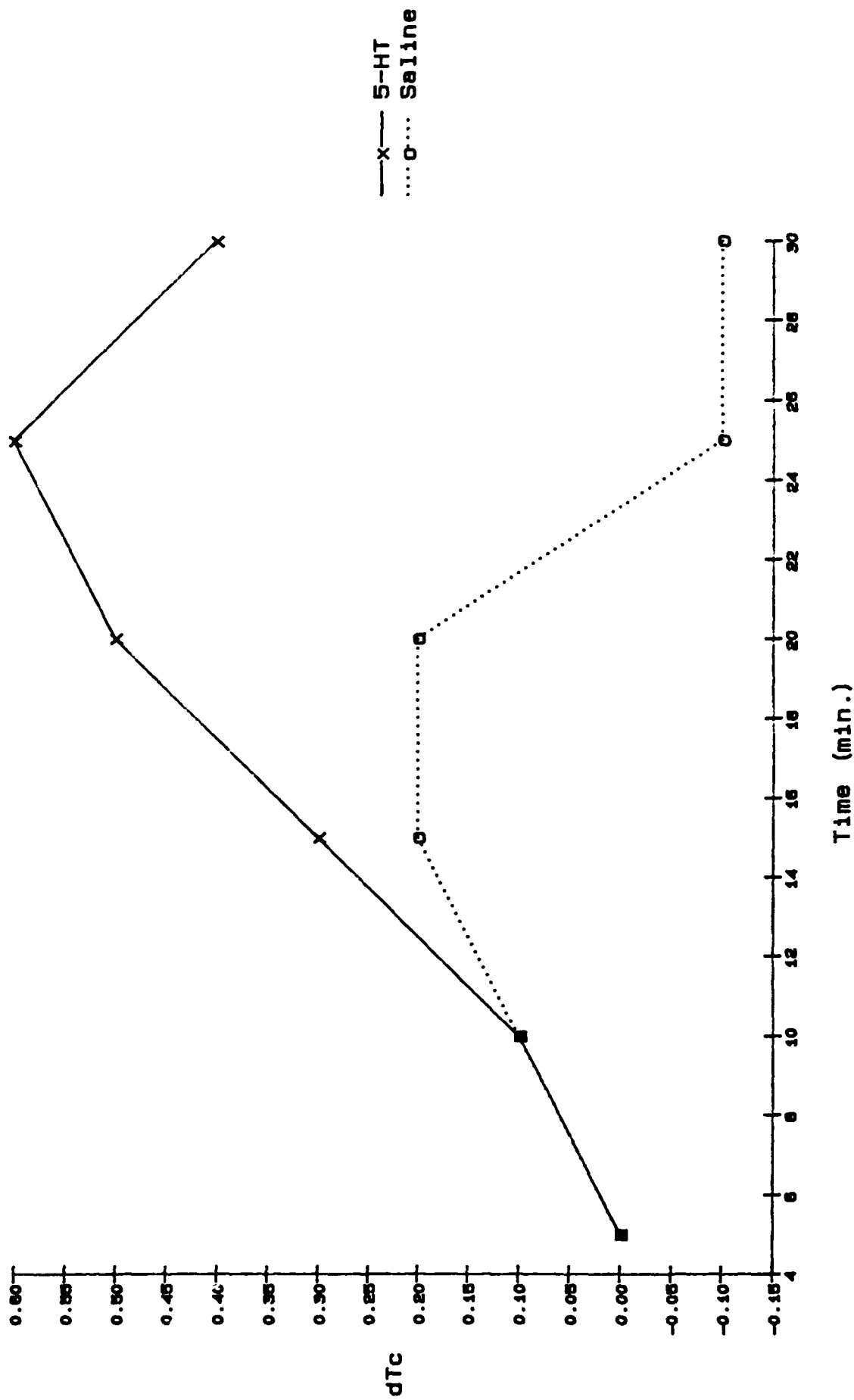


Figure 4

EG5HT

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CHANGE IN Tc AFTER 5-HT INJECTION INTO RAT MAHPOA



NEEG

Figure 5

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CHANGE IN Tc AFTER NOREPINEPHRINE
INJECTION INTO RAT MAHPOA

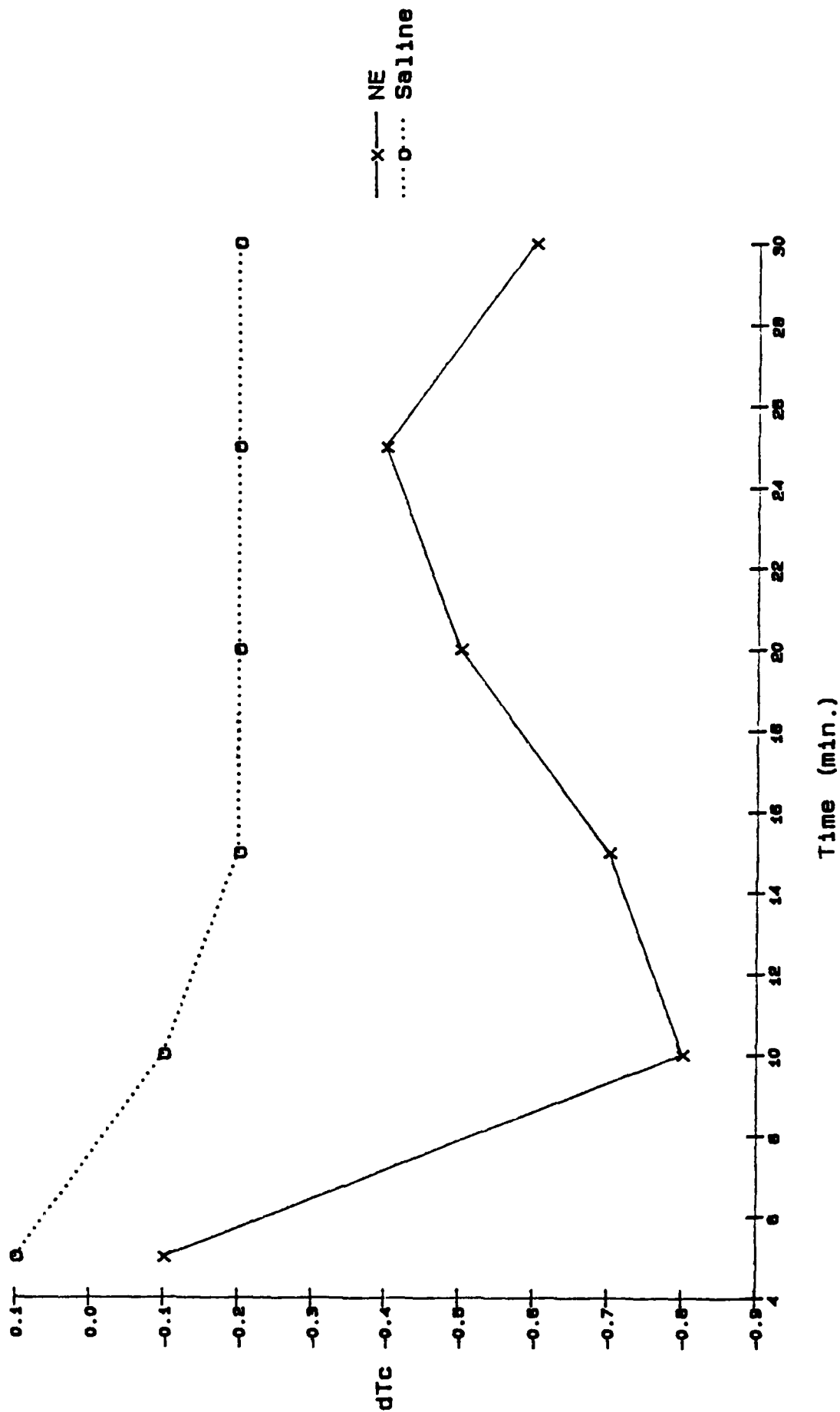


Table 1

PGEAWKDR 5R x 7C

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PGE2 DOSE RESPONSE IN UNANESTHETIZED RATS

0	1 10 ^[-12]	2 10 ^[-18]	3 10 ^[-21]	4 10 ^[-24]	5 10 ^[-30]	6 10 ^[-33]

1						
2 Mean	0.72	0.62	0.68	0.60	0.41	0.17
3 SD	0.31	0.22	0.28	0.24	0.22	0.41
4 N	22	5	8	5	11	9
5 P	0.00	0.01	0.01	0.14	0.02	0.48

0	7 10 ^[-36]					

1						
2 Mean	0.00					
3 SD	0.55					
4 N	4					
5 P	0.72					

Notes:

1. Data represent the mean maximum change in colonic temperature within 30min. after 1ul injections of PGE2 into previously identified MAHPOA heat gain sites.
2. Grams of PGE2 injected in 1ul is given at the head of each column.
3. Statistical significance was determined using the independent t-test comparing each dose against their saline vehicle controls.

Table 2

A5NAWKDR 5R x 3C

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EFFECTIVE DOSES OF ACH, 5-HT, AND NE
IN UNANESTHETIZED RATS

0	1 ACH 10[-12]	2 5-HT 10[-12]	3 NE 10[-5]
1			
2 Mean	0.60	0.58	-0.51
3 SD	0.15	0.21	0.70
4 N	11	16	10
5 P	0.0001	0.0001	0.008

Notes:

1. Data represent the mean maximum rise in colonic temperature within 30min. after 1ul injections of each agonist at the dose in grams into previously identified PGE2-sensitive MAHPOA heat gain sites.
2. Statistical significance was determined using the independent t-test comparing each dose against their saline vehicle control.
3. The minus sign in column 3 indicates a fall in mean colonic temperature after NE injection.

Table 3

CHANGE IN T_c AT THE SAME STARTING T_c IN
 AWAKE VS ANESTHETIZED RATS
 FOLLOWING 1 pg PGE₂*

	Awake	Anesthetized
	1.0	1.2
	1.4	1.4
	1.6	0.5
	0.6	0.5
		0.7
		1.0
		0.9
Mean	1.15	0.89
SD	0.44	0.34
N	4	7

* Values represent the increase in colonic temperature (T_c) following the injection of 1 pg PGE₂ in 1 ul into the medial preoptic anterior hypothalamus; P = 0.30; anesthesia = ketamine HCl (50 mg/kg, IP); starting colonic temperature (T_c) values for both group; was between 37.4 and 37.9 C.

Table 4

CHANGE IN T_c VS STARTING T_c IN
 AWAKE UNRESTRAINED RATS
 FOLLOWING 1 pg PGE₂*

	I 37.4-37.9	II 38.0-38.4	III 38.5-38.9	IV 39.0-39.4
	1.0	0.7	1.1	0.5
	1.4	1.7	0.6	0.8
	1.6	1.2	1.2	0.7
	0.6	0.7	1.1	0.7
		0.8	0.7	0.5
		0.5	1.0	0.7
		0.8	0.5	0.5
		0.7		0.6
		0.8		1.0
		0.5		0.7
		1.2		0.6
				0.5
				0.5
				0.8
				0.7
				0.7
				0.9
				0.9
				0.5
				0.5
				0.5
Mean	1.15	0.87	0.89	0.66*
SD	0.44	0.36	0.28	0.16
N	4	11	7	21

* Values represent the increase in colonic temperature (T_c) following the injection of 1 pg PGE₂ in 1 ul into the medial preoptic anterior hypothalamus; P < 0.02 for group IV vs groups I through III; ns between all other groups.

Table 5

CHANGE IN Tc VS STARTING Tc IN
ANESTHETIZED RATS
FOLLOWING 1 pg PGE2*

	I	II	III	IV	V	VI	VII	VIII
	34.0 to 34.4	34.5 to 34.9	35.0 to 35.4	35.5 to 35.9	36.0 to 36.4	36.5 to 36.9	37.0 to 37.4	37.5 to 37.9
	0.9	1.8	1.2	1.0	0.8	0.9	0.9	1.2
	0.6	0.5	1.9	0.8	0.8	0.5	0.8	1.4
	0.8	0.9	0.7	0.6	0.8	0.5	0.8	0.5
	0.6	0.6	1.0	1.8	0.9	1.3	0.7	0.5
	0.6		0.7	0.5	0.8	1.2	0.8	0.7
	0.6			0.9	0.8	1.8	1.6	1.0
	0.9			0.7	0.5	0.5	0.5	0.9
				0.6	0.6	0.5	0.7	
				1.2	1.3	0.8	0.8	
				0.9	1.0	0.6	1.1	
					0.8		0.7	
					1.2		0.6	
					1.0		0.6	
					0.5		0.8	
					0.5			
Mean	0.71	0.95	1.1	0.9	0.82	0.86	0.81	0.89
SD	0.15	0.59	0.50	0.38	0.24	0.44	0.27	0.34
N	7	4	5	10	15	10	14	7

* values represent the increase in colonic temperature (Tc) following the injection of 1 pg PGE2 in 1 ul into the medial preoptic anterior hypothalamus; ns between all groups.

Table 6

Cued Discrimination Barpress Performance:
PGE2 vs Saline in Rats at Tc 38.0 to 39.9

Parameter	PGE2 > 0.5 C 10 trials N=10		PGE2 > 0.5 C last 5 trials N=10		PGE2 < 0.5 C 10 trials N=6	
	PGE2	Saline	PGE2	Saline	PGE2	Saline
Number Correct per Trial	14.8 7.6 P< .25	13.6 9.6	17.3 7.1 P< .73	16.7 9.4	15.8 7.4 P< .43	14.8 8.6
Number Incorrect per Trial	2.1 2.5 P< .05	2.8 3.6	2.7 2.5 P< .03	3.8 3.5	2.4 2.0 P< .87	2.4 2.7
Percent Correct	86.8 19.1 P= .06	81.1 21.7	87.1 11.4 P< .03	80.0 18.6	87.0 10.2 P< .86	87.3 14.2
Percent Incorrect	13.1 19.1 P= .06	18.8 21.7	12.8 11.4 P=.06	18.6 17.0	13.1 10.2 P< .86	12.7 14.2
Rate Cor/min.	18.0 9.3 P<.25	16.2 11.4	20.4 9.0 P<.73	19.8 11.4	18.0 8.4 P<.45	16.2 10.2
Rate Incr/min.	12.6 15.6 P<.05	17.4 21.6	16.2 15.0 P<.02	22.8 20.4	13.2 12.0 P=1.0	13.2 16.8
Number Inapprop. per Trial	8.0 11.3 P<.03	18.7 11.3				
Change in Tc	0.60 0.10 P<.0001	0.12 0.13			0.20 0.22 P<.69	0.15 0.14

Table 6 Footnotes

NOTE: The data support the interpretation that a PGE2 enhanced an attentional component which reduced error rate, based on the following five observations:

a. Differences ($P < .05$) were found in the number of incorrect barpresses during the ITI, between the PGE2 drug treatment and its saline vehicle control (row 2 col 1) for animals that showed a rise in Tc response (row 8 col 1). The same was not true (row 2 col 3) ($P < .87$) for animals that showed no Tc response (row 8 col 3) to PGE2 (ie. sham site injection).

b. No differences ($P < .25$) were found in the number of correct barpresses during presentation of a correct cue, between the PGE2 drug treatment and its saline vehicle control (row 1 col 1) for animals that showed a rise in Tc response (row 8 col 1) to PGE2. The same was true (row 1 col 3) ($P < .43$) for animals that showed no Tc response (row 8 col 3) to PGE2 (ie. sham site injection).

c. A pattern similar to that shown in note "a." and "b." above was observed for strike (barpress) rate data (rows 5 and 6). Strike rate to PGE2 treatment was significantly lower ($P < .05$) than saline during the ITI (for the rate of incorrect strikes) (row 6 col 1). Strike rate to PGE2 treatment was not significantly different ($P < .25$) from saline during the correct cued presentation (row 5 col 1). The reduction of incorrect strike rate following PGE2 occurred only for animals that previously showed a rise in Tc to PGE2, and was not observed for animals microinjected with PGE2 in a non-responding site (ie. sham site injection).

d. Well trained animals presented with an inappropriate cue (ie. different from the correct cue and not reinforced) showed a statistically significant ($P < .03$) decrease in number of strikes during PGE2 treatment compared with the saline vehicle control.

e. Comparison between parameters showed a lower score for number of incorrect and inappropriate vs correct.

Table 7

Motivation vs Attention***
 Comparison of PGE2 in a Rat at Tc of 35 C vs 38 C*

parameter	Tc < 35 C	Tc > 38 C**
number of correct strikes	23.0 11.2	14.9 7.7
	P<0.0001	
number of incorrect strikes	4.0 3.1	2.1 2.6
	P<0.0001	
total number of strikes per trial	28	17
percent correct	85% 15.4	87% 19.2
	P=0.23	
correct per min.	27.8 13.6	17.9 9.3
	P<.0001	
incorrect per min.	24.0 18.5	13.2 16.6
	P<.0001	

* PGE2 treatment period ran for 10 trials in the behavior box. Values represent mean (+-SD) number of bar press strikes per trial.

** Note that Tc value given is after PGE2 elicited at least a 0.5 C rise in Tc

*** Interpretive Notes on Motivation vs Attention

- a. Increase number of total responses in a trial indicate an increase in motivation in the animal starting trials at Tc values of 35 C vs 38 C.
- b. Increase number of responses occurred in both the correct and incorrect parameters when the animal started trials at Tc values of 35 C vs 38 C. Therefore no differential response, ie. between the correct and incorrect parameters, was observed indicating no change in an attentional mechanism occurred.
- c. The conclusions in notes "a and b" were consistent with the % correct values in that the proportion of correct and incorrect were not different for trials starting at the different Tc values.

SUMMARY: Completion of Year Two Objectives

A. NEUROPHARMACOLOGY IN UNANESTHETIZED RATS

Agonist-elicited response to five ligands, PGE2, ACH, Nic, 5-HT, and NE were demonstrated in the previously defined MAHPOA PGE2-sensitive heat gain sites unanesthetized unrestrained rats (I.A.1).

A complete dose response was done for PGE2 in unanesthetized rats (I.A.2), and the minimum effective dose for three other agonists, ACH, 5-HT, and NE, were completed.

Heat gain responses were elicited unanesthetized rats and did not differ from those previously demonstrated in ketamine anesthetized rats (I.B.1).

The PGE2-elicited rise in Tc was unaffected by the starting Tc value except at values near 40 C (I.B.2).

These data fulfilled the neuropharmacologic objectives for the second grant year.

B. BEHAVIOR TRAINING

1. Training in Cued Discrimination

At the end of the first year of funding, basic training protocols were developed but only a few animals were trained to a level for experimentation. At the end of the second year of funding, 23 rats were fully trained and 241 individual behavioral experiments were completed (see II.A.).

2. Temperature Challenge To Cued Discrimination

Original protocols called for stepwise reduction of ambient temperature below -4 C as a means of achieving a decrement in performance. The first few trials indicated that a better protocol to accomplish this objective was to lower the animal's Tc by varying the number of trials in the initial baseline (see II.A.2.).

3. Increasing Task Complexity

Task complexity was increased by adding an inappropriate cue which was not reinforced following a barpress. The difficulty of the task for the rat was sufficient that no additional increases in the difficulty of performing the task for reinforcement was done. Response to inappropriate cue is described in section II.A.1. of this report.

4. Modifications of Cue Presentation Formats

Variations of cue presentation were done to maximize training efficiency. Duration of cue presentation vs ITI were varied from a 50:10 ratio to a 30:30 ratio within a 60 sec trial. It was found that the 50:10 ratio was most successful since animals were best able to maintain Tc. A second variation of cue presentation involved varying the ratio of correct to inappropriate cue presentation. Ratios of 90:10 to 50:50 were done. A ratio of 90:10 resulted in the most

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successful protocol to achieve criterion performance (II.A.3.).

C. BEHAVIORAL EFFECTS OF CENTRAL PGE2

1. Euthermic

PGE2 microinjection into the PGE2-sensitive MAHPOA heat gain sites of rats that were at a normal Tc value resulted in a improvement in task performance by a decrease in error rate (II.B.1).

2. Hypothermic vs Euthermic

The PGE2-elicited behavioral response was compared in rats at normal and hypothermic Tc values (II.B.2). Rats with Tc values below 35 C showed increased motivation to barpress for heat reinforcement when compared to rats at Tc values above 37 C. Hypothermic rats did not show the differential improvement in performance following PGE2 injection shown by euthermic animals.

D. CONTROL OF EXPERIMENTAL PARAMETERS IN BEHAVIORAL EXPERIMENTS

Controls were done for 1) sham injection procedure (II.C.1), 2) ambient temperature effects (II.C.2), 3) colonic temperature effects (II.C.3), 4) brain site of injection (II.C.4), and 5) control of drug specific effects (II.C.5). These controls made possible the discrimination of motivational effects from PGE2-elicited performance improvement by decrease in incorrect scores, which were defined as attentional effects.

E. CONCLUSIONS

A review of the original grant objectives show that all of the objectives were met for the two years of funding. If a third year of the original proposal had been funded, the electrophysiology and push-pull perfusion of sites that support behavioral and autonomic responses would have been included in the study. Aspects of those objectives have been included in a renewal proposal.

The data from studies accomplished in the two years of funding and data from preliminary experiments suggested extensions and modifications of experiments that can be used 1) to test the hypotheses of drug effects on attentional and motivational factors controlling task performance; 2) extend the study to account for molecular modulation of the brain site by stress factors; and 3) extend the study to include effects of neuropeptides which preliminary data show may influence behavioral and/or autonomic responses.

The principle finding from the original proposal links task performance behaviors with drug effects in specific brain sites. The data demonstrate a PGE2-elicited improvement in performance by a differential decrease in incorrect vs correct scores. The PGE2 response differed from the proportional response changes that define changes in motivation, that were elicited by changes in Tc. The differential response was defined as a change in an attentional

factor. The experiments were originally designed to discover drug (eg. PGE2) effects on behavior. The exact nature of the results was not predicted nor anticipated from the literature. Therefore, the drug effects on behavior in this report, while consistent with previous literature, represents a new finding, which may be potentially applicable to Air Force interests.

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