08:42:58 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 06/14/91

Active

Project #: G-33-608 Cost share #: Rev #: 3
Center # : 10/24-6-R6834-2A0 Center shr #: OCA file #:
Work type : RES
Contract#: 5 R01 DA06305-02 Mod #: B R DTD 6/11/91 Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA:
Main project #: PE #: N/A

Project unit: CHEMISTRY Unit code: 02.010.136
Project director(s):
ZALKOW L H CHEMISTRY (404)894-4045

Sponsor/division names: DHHS/PHS/ADAMHA / ALCOHOL, DRUG ABUSE & MENTAL
Sponsor/division codes: 108 / 004

Award period: 901201 to 911130 (performance) 920228 (reports)

Sponsor amount	New this change	Total to date
Contract value	878.37	223,095.30
Funded	878.37	223,095.30
Cost sharing amount		0.00

Does subcontracting plan apply ?: N

Title: IRREVERSIBLE ANTAGONISTS OF COCAINE & OTHER STIMULANTS

PROJECT ADMINISTRATION DATA

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Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N):
Defense priority rating : N/A NIH supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
ISSUED TO CARRYOVER UNEXPENDED FUNDS FROM PRIOR YEAR'S PROJECT - G-33-66

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 02/17/92

Project No. G-33-608 Center No. 10/24-6-R6834-2A0

Project Director ZALKOW L H School/Lab CHEMISTRY

Sponsor DHHS/PHS/ADAMHA/ALCOHOL, DRUG ABUSE & MENTAL

Contract/Grant No. 5 R01 DA06305-02 Contract Entity GTRC

Prime Contract No.

Title IRREVERSIBLE ANTAGONISTS OF COCAINE & OTHER STIMULANTS

Effective Completion Date 911130 (Performance) 920228 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	Y	
Final Report of Inventions and/or Subcontracts	Y	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

CommentsCONTINUED BY G-33-625

Subproject Under Main Project No.

Continues Project No. G-33-667

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other	N
	N

NOTE: Final Patent Questionnaire sent to PDPI.

1. SUMMARY OF PLANS FOR NEXT YEAR OF SUPPORT

No significant changes are planned over those outlined in the original proposal. Specifically we will:

A. Chemistry

1. Complete all synthetic work related to the mazindol series of imidazo-isoindols.
2. Complete all synthetic work related to the methylphenidate series.
3. Complete all synthetic work on the dopamine series.
4. Complete all synthetic work on the "other series" (substituents on single phenyl ring) of GBR compounds.
5. Work on the synthesis of analogs of the GBR series that might be potential "pseudo" irreversible antagonists.
6. Work on the synthesis of potential irreversible antagonists based on new structures as time permits.

B. Pharmacology

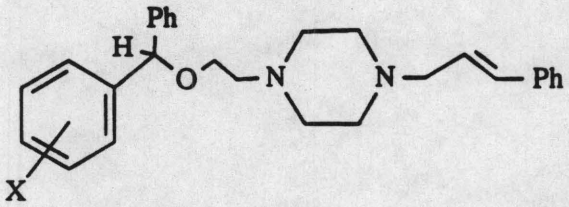
1. Biochemical
   a. Do *in vitro* testing of compounds in the series listed above to determine their effects on [^{3}H]methylphenidate binding and [^{3}H]dopamine transport with respect to potency and irreversibility of binding
   b. Do *ex vivo* testing of compounds in the series listed above which show irreversible activity *in vitro* in order to correlate their behavioral effects with their effects on [^{3}H]methylphenidate binding and [^{3}H]dopamine transport.
2. Behavioral
   a. Test the ability of the compounds identified as irreversible inhibitors of [^{3}H]methylphenidate binding against the low dose stimulant effects of members of the methylphenidate and amphetamine class of stimulant drugs.
   b. Test the ability of the compounds identified as irreversible inhibitors of [^{3}H]methylphenidate binding against the high dose stereotypy effects of members of the methylphenidate and amphetamine class of stimulant drugs.

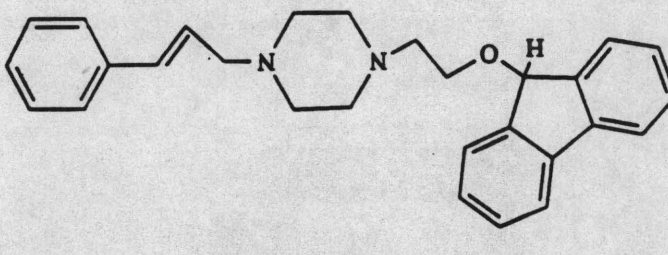
2. DESCRIPTION OF WORK CONDUCTED IN CURRENT YEAR (12/01/90 TO 11/30/91)

A. Chemistry

1. GBR Series

In addition to the work described in the last continuation report, the synthesis and characterization of the 3-maleimido compound **1a**, the fluorenyl compound **2** and the *bis*-alkylenyl piperazine **3** have been accomplished. Also much larger quantities of the 3 and 4-isothiocyanates (**1c-d**) and the 4-maleimido (**1b**) GBR compounds have been synthesized for more extensive biological testing.

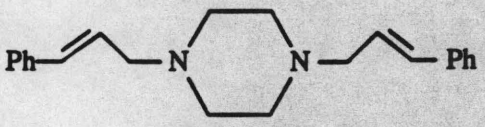




1a X=3-maleimido **1c** X=3-N=C=S

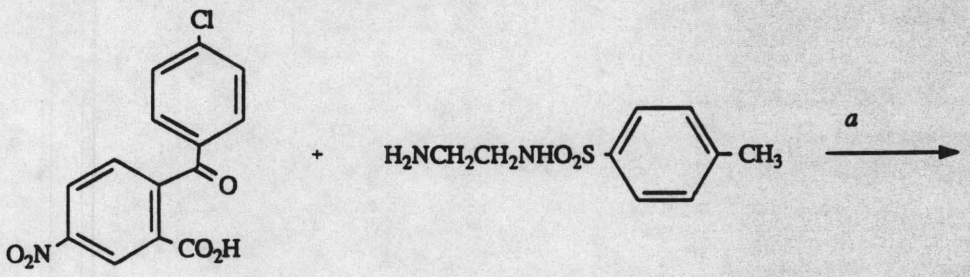
1b X=4-maleimido **1d** X=4-N=C=S

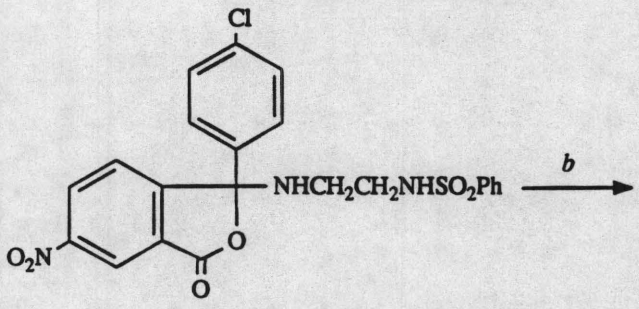
2

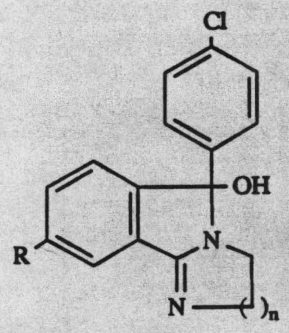


3

2. Mazindol Series







4a R=NO_2 n=1

4b R=NH_2 n=1

4c R=H n=2

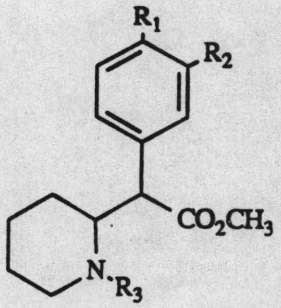
a. Toluene, heat (remove water); *b*. Conc. H_2SO_4, 70 C.

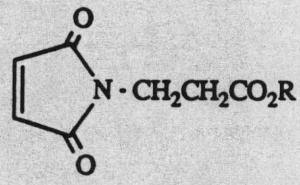
The previous described approach to the synthesis of derivatives of 8-nitro or 8-amino mazindol has been abandoned after it was unequivocally shown that it did not yield the right products. A new methodology, based on work in the patent literature is shown above.

Compound **4a** had been made in 27% yield starting from 4-nitrophthalic anhydride. Catalytic reduction of **4a** to **4b**, without reduction of the carbon-nitrogen double bound, appears to be successful although a pure product has not yet been isolated. We are currently attempting to purify **4b** and convert it to a potential irreversible binding compound. In addition, the synthesis of the ring expanded product **4c** has been accomplished.

3. Methylphenidate Series

We previously described the synthesis of *p*-hydroxy methylphenidate (**5a**). In order to prevent problems with the synthesis of potential irreversible binding compounds, the synthesis of N-methyl methylphenidate was attempted. Reductive amination (with formaldehyde and sodium cyanoborohyride) gave a mixture of products in low yield which could not be separated (may have been an *erythro/threo* mixture). Clark-Eschweiler conditions gave a mixture from which the N-methyl compound (**5b**) and the *bis*-hydroxymethyl compound (**5c**) could be isolated.





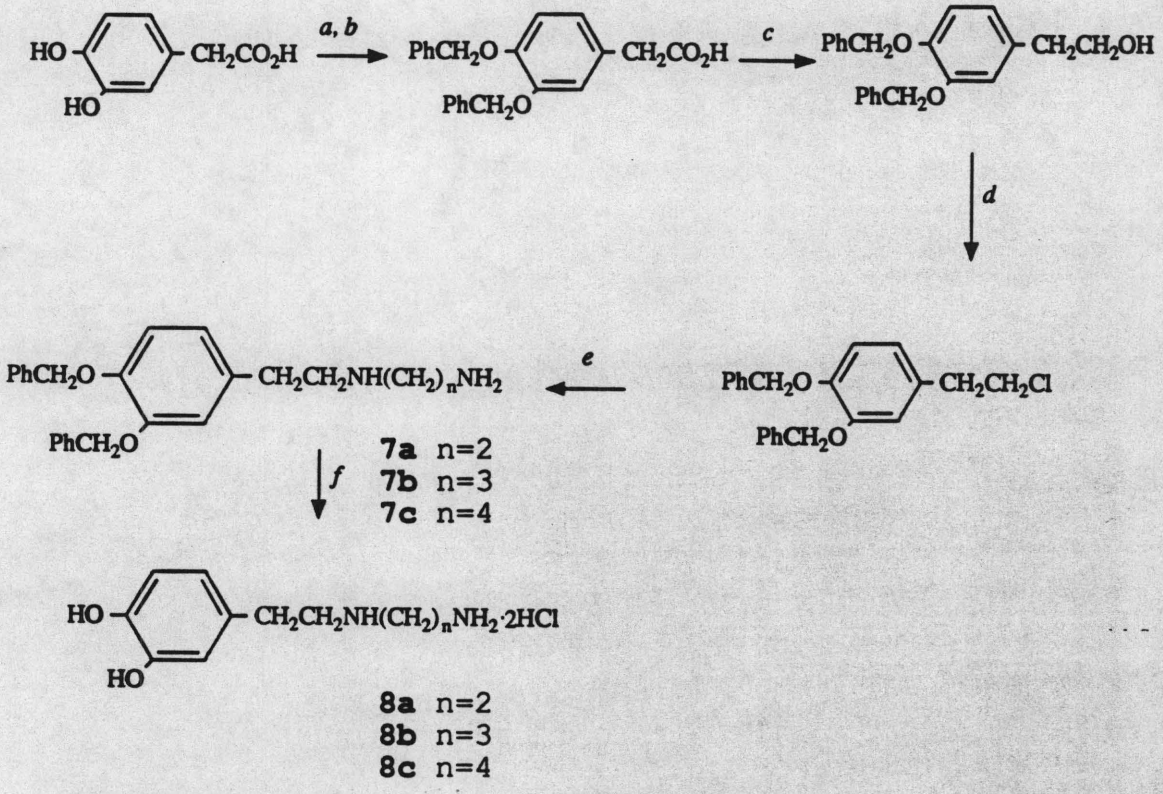
5a R_1=OH, R_2=R_3=H
5b R_1=OH, R_2=H, R_3=CH_3
5c R_1=OCH_2OH, R_2=CH_2OH, R_3=CH_3
5d R_1=OH, R_2=R_3=CHO
5e R_1=**6**, R_2=R_3=H

6a R=Et
6b R=H

The ethyl ester of maleimido ß-alanine ethyl ester (**6a**) has been synthesized, but to date conditions have not been found to hydrolyze it to the free acid **6b**. Our plan is to couple the free acid to the protected compound **5d**, which after deprotection would yield the potential irreversible binding compound **5e**.

4. Dopamine series

The synthetic methodology outlined in the scheme below has been employed to make **8a-c** and the corresponding dibenzyloxy protected intermediates **7a-c**. The synthesis of corresponding maleimido and bromoacetamide derivatives is underway.



a. NaH, $PhCH_2Cl$, DMF; *b*. MeOH/H_2O, NaOH; *c*. BH_3·THF; *d*. $SOCl_2$; *e*. $H_2N(CH_2)_nNH_2$; *f*. Pd/H_2, HCl.

B. Pharmacology

1. *In vitro* studies

a. [^{3}H]methylphenidate binding

The potency of synthesized affinity labels, parent compounds, and synthetic intermediates was assessed by generating inhibition curves for the respective compounds. These curves measured the ability of the test compounds to inhibit binding of [^{3}H]methylphenidate to the stimulant recognition site. The following results were obtained (in most cases, these are averages of three experiments; triplicate determinations at five to seven concentrations were made in each experiment):

1. GBR SERIES

Compound	IC_{50} -nM
GBR-12783	12
1a	970
1b	1677
1c	283
1d	493
2	2000
3	1683

(Other intermediates are described in Table 1, Deutsch *et al.*, attachment #1).

2. MAZINDOL SERIES

Compound	IC_{50}-nM
mazindol	16
4a	2267
4c	5

3. METHYLPHENIDATE SERIES

Compound	IC_{50}-nM
5a	115
5b	1080
5c	443

4. DOPAMINE SERIES

Compound	IC_{50}-nM
7a	3567
7b	1583
7c	1282
7d	12667

The studies initiated last year of the ability of the affinity labels synthesized by us to bind irreversibly at the stimulant receptor on the dopamine transporter were continued. Compound **1c** was shown to bind potently and irreversibly at the site, while the inhibition caused by compound **1a** was found to be only partially irreversible. The 3-amino substituted precursor of these compounds was demonstrated, as expected, to bind only reversibly (see Deutsch *et al*, attachment #1)

Preparatory to characterizing the irreversible inhibition caused by the GBR derivatives in greater depth, the time course for the reaction of **1d** with the [^{3}H]methylphenidate was determined. It was found to be extremely rapid, even at 0 C.

Scatchard analysis of the effect of **1d** on [^{3}H]methylphenidate binding was conducted. Data analysis remains to be completed, but preliminary results suggest that the compound leads to a reduction in B_{max}.

Protection of the site from irreversible inhibition by **1d** using saturating amounts of GBR-12783 was attempted.

Complete protection using this technique was not observed.

Studies were conducted to compare the [^{3}H]methylphenidate binding site to the sites recognized by other radiolabeled psychomotor stimulants. The following information was gained:

--Similar to other stimulant binding, [^{3}H]methylphenidate binding increases in phosphate buffer compared to Tris buffer.

--The stimulant drug amfonelic acid is a competitive inhibitor of the high affinity [^{3}H]methylphenidate binding site.

--When examined over 20 h, [^{3}H]methylphenidate binding is severely reduced. Studies showed it was not due to deterioration of the radioligand, suggesting that either the receptor itself is degrading, or an inhibitory compound is being elaborated. This differs from studies with [^{3}H]GBR and [^{3}H]mazindol compounds, whose binding is relatively constant over 20 h.

b. [^{3}H]Dopamine transport studies

The potency of test compounds in inhibiting [^{3}H]dopamine transport into striatal synaptosomes was assessed. IC_{50}'s shown below are derived from inhibition curves in which inhibition at five to seven different concentrations of test compound was determined. This is an ongoing study; to date, GBR-12783 and its derivatives have been investigated.

Compound	IC_{50} (nM)
GBR-12783	5.5
1b	380
1c	108
1d	92

The time course for inactivation of [^{3}H]dopamine transport by **1b** has been examined. Preliminary results indicate that maximum inhibition occurs within 5-10 min at 0 C.

2. Behavioral studies

The effects of **1c**, **1d**, Fourphit and mazindol derivative **4c** (with an expanded imidazole ring) on the hyperactivity caused by low dose cocaine and amphetamine were examined. Despite solubility problems with some of these compounds which limited the amount of drug which could be delivered by certain routes, some interesting results were obtained. The results are summarized in the table below. Until the remaining statistical analyses of them are completed, they should be considered preliminary. Representative graphs are shown in Figs. 1-4. The test compounds were delivered by intravenous and intraperitoneal injection, as well as microinjection directly into the nucleus accumbens.

Fourphit, which we have demonstrated blocks the hyperactivity caused by cocaine in male rats (see Schweri *et al.*, attachment #2), has similar effects in female rats. In some experimental paradigms, **1c** and **1d** potentiated the effects of low dose cocaine; no conditions were found in which it blocked cocaine's behavioral effects. Compound **1c** was ineffective in preventing the stimulation caused by *d*-amphetamine.

Table 1. Summary of the effects of test compounds on stimulant-induced hyperactivity.

TEST COMPOUND	DOSE (mg/Kg)	ROUTE	CHALLENGED WITH:	AT(HRS)	SEX	RESULTS
FOURPHIT	20	IV	COCAINE	24	F	⇓
1c	2	IV	COCAINE	24	M	⇔
1c	2	IV	COCAINE	0.5	M	⇑
1c	7.6 nmols	intra-accumbens	COCAINE	24	M	⇔
1c	2	IV	D-AMPHET	24	M	⇔
1d	2	IV	COCAINE	24	M	⇔
1d	2	IV	COCAINE	0.5	M	⇔
1d	25	IP	COCAINE	24	M	⇑
1d (NBR RATS)	25	IP	COCAINE	24	M	⇔
4c	1.25	IV	COCAINE	0.25	M	⇔

Cocaine dose was 15 mg/kg, i.p., as the hydrochloride salt. All studies done in Sprague-Dawley rats, except where noted. Rats were challenged with stimulant at the time shown in hours after injection with the test compound. Activity was compared to rats injected with vehicle instead of test compound, before challenge with the stimulant. Results show change in activity with respect to controls. Six to eight rats were in each treatment group.

3. *Ex vivo* studies

[^{3}H]methylphenidate binding studies were conducted on the striatal tissue of the rats used in the behavioral studies summarized above. The rats were sacrificed shortly after the termination of each behavioral experiment, the tissue was removed and frozen until the series was completed, at which time all the samples were analyzed using the same radioreceptor assay. No reduction in [^{3}H]methylphenidate binding was observed as a function of

treatment. Recently, however, further statistical analysis of one experimental series suggested a possible negative correlation between binding and activity of the individual rats treated with **1c**, while rats treated with vehicle in the same experiment showed a possible positive correlation between binding and activity. We intend to analyze this trend further, and examine the data from the other experimental paradigms we have utilized to determine if the same relationship between binding and behavior is observed in treated versus control rat.

3. NO HUMAN SUBJECTS

4. NO CHANGE IN THE USE OF ANIMALS (ASSURANCE #A1575)

5. PUBLICATIONS:

Preprints of the following articles are included:

#1 "Synthesis and Pharmacology of Irreversible Affinity Labels as Potential Cocaine Antagonists. 1. Aryl 1,4-diaklypiperazines Related to GBR-12783"; submitted for publication as an accelerated communication in *Molecular Pharmacology*.

#2 "Fourfit: a Selective Probe for the Stimulant Recognition Site on the Dopamine Transpordt Complex" accepted for publication in *JPET* pending revision.

Copies of abstracts from presentations at:

#3 The American Chemical Society Meeting, April 1991

#4 The Federation of American Societies of Experimental Biology Meeting, April 1991.

#5 Society of Neuroscience Meeting, November 1991.

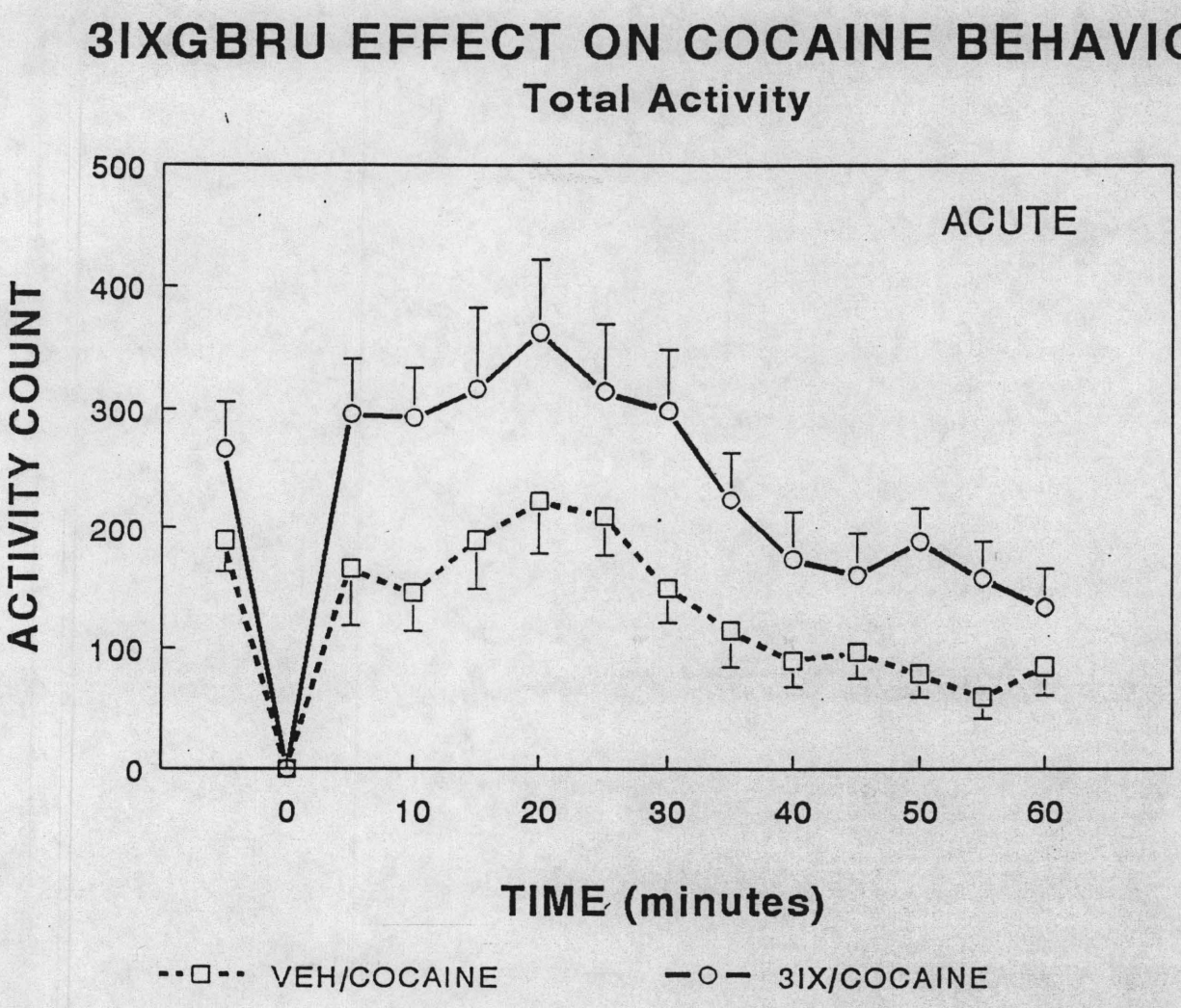


Fig. 1. Effect of 1c (2 mg/kg, i.v.) administered 30 min. prior to treatment of Sprague-Dawley rats with (-)cocaine hydrochloride (15 mg/kg, i.p.). Total activity shown on y-axis includes both ambulatory and non-ambulatory activity counts.

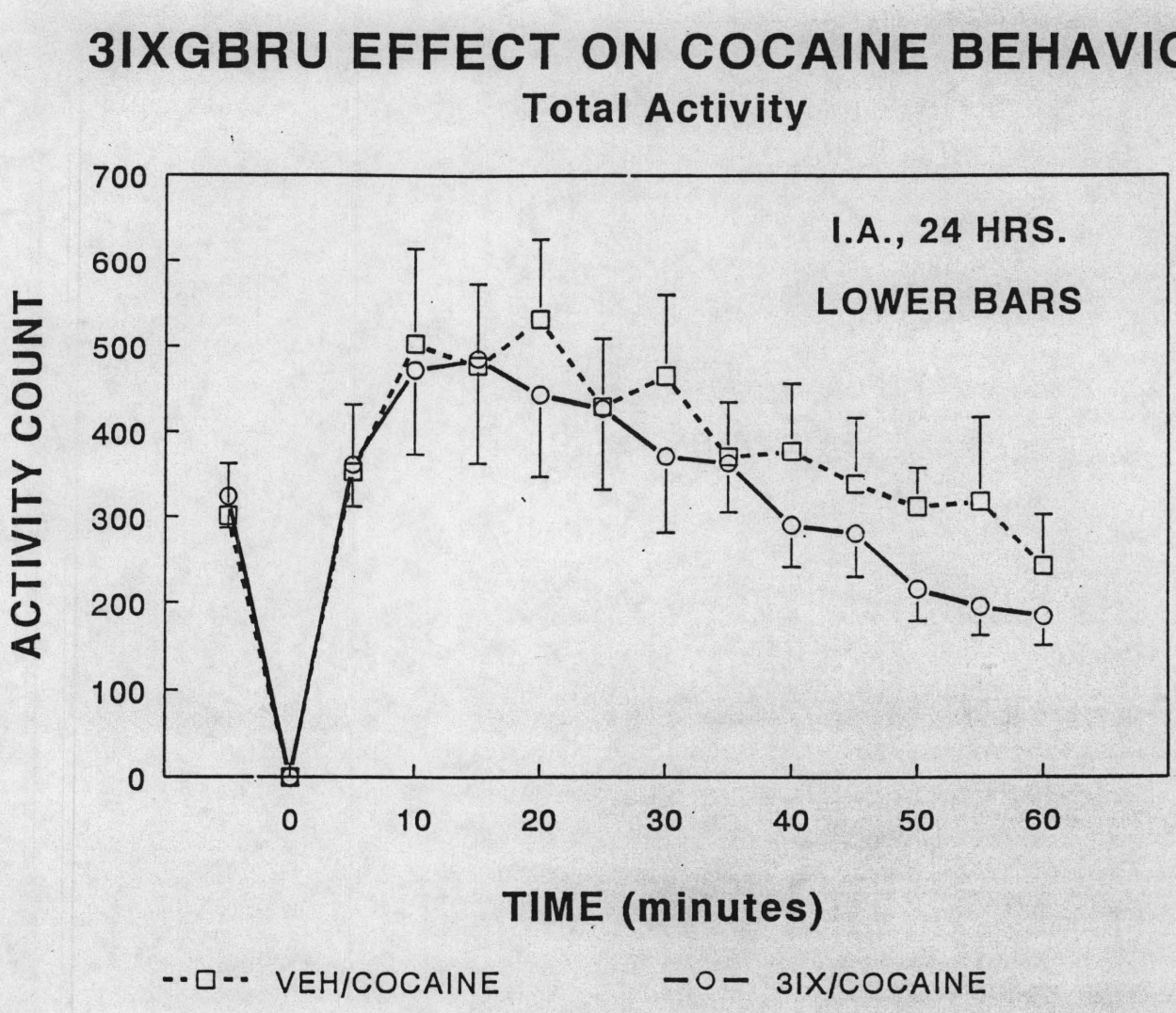


Fig. 2. **Effect of 1c (7.6 nmols) administered by microinjection bilaterally into the nucleus accumbens approximately 24 hrs prior to treatment of Sprague-Dawley rats with (-)cocaine hydrochloride (15 mg/kg, i.p.). Total activity shown on y-axis includes both ambulatory and non-ambulatory activity counts.**

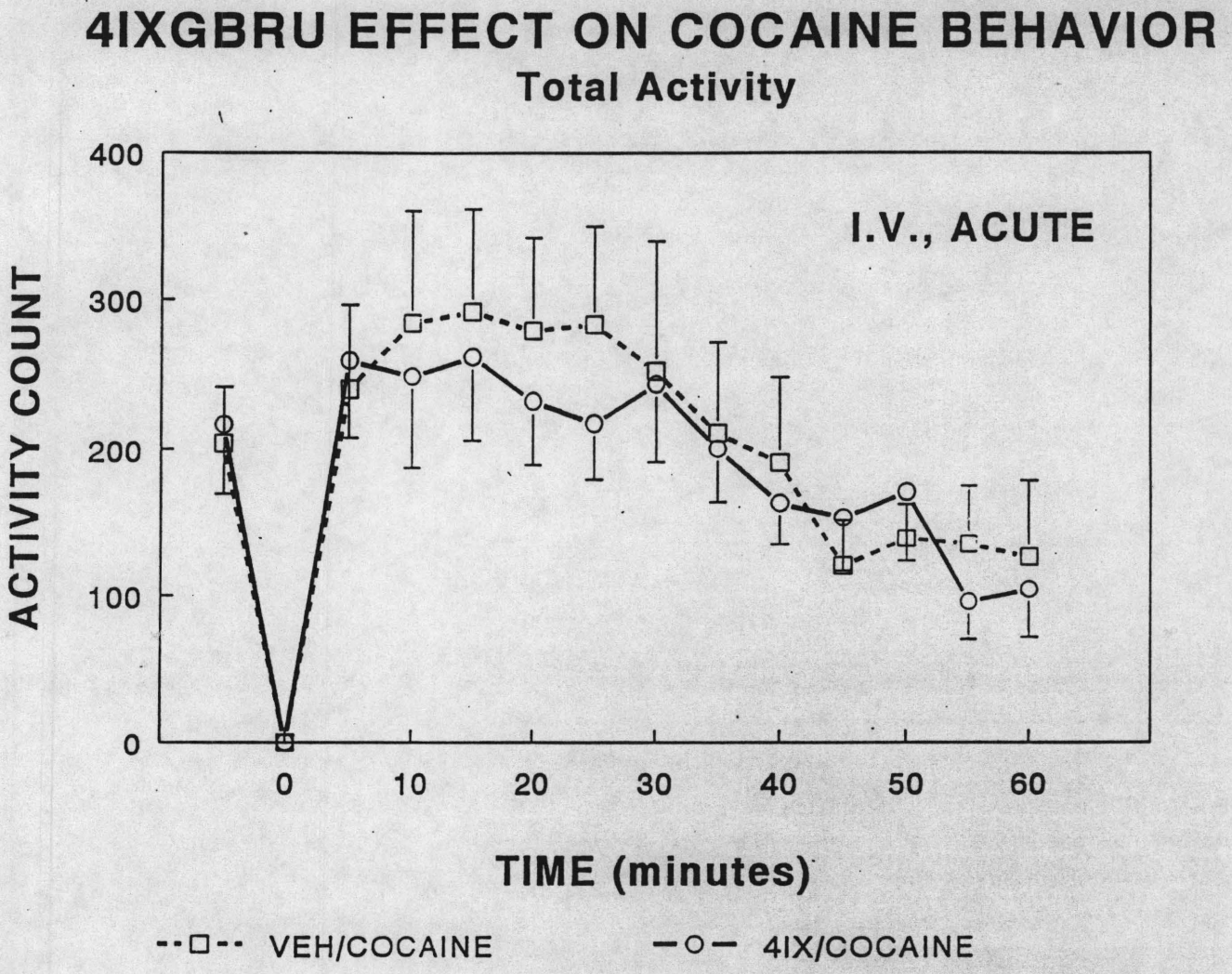


Fig. 3. Effect of **1d** (2 mg/kg, i.v.) administered 30 min. prior to treatment of Sprague-Dawley rats with (-)cocaine hydrochloride (15 mg/kg, i.p.). Total activity shown on y-axis includes both ambulatory and non-ambulatory activity counts.

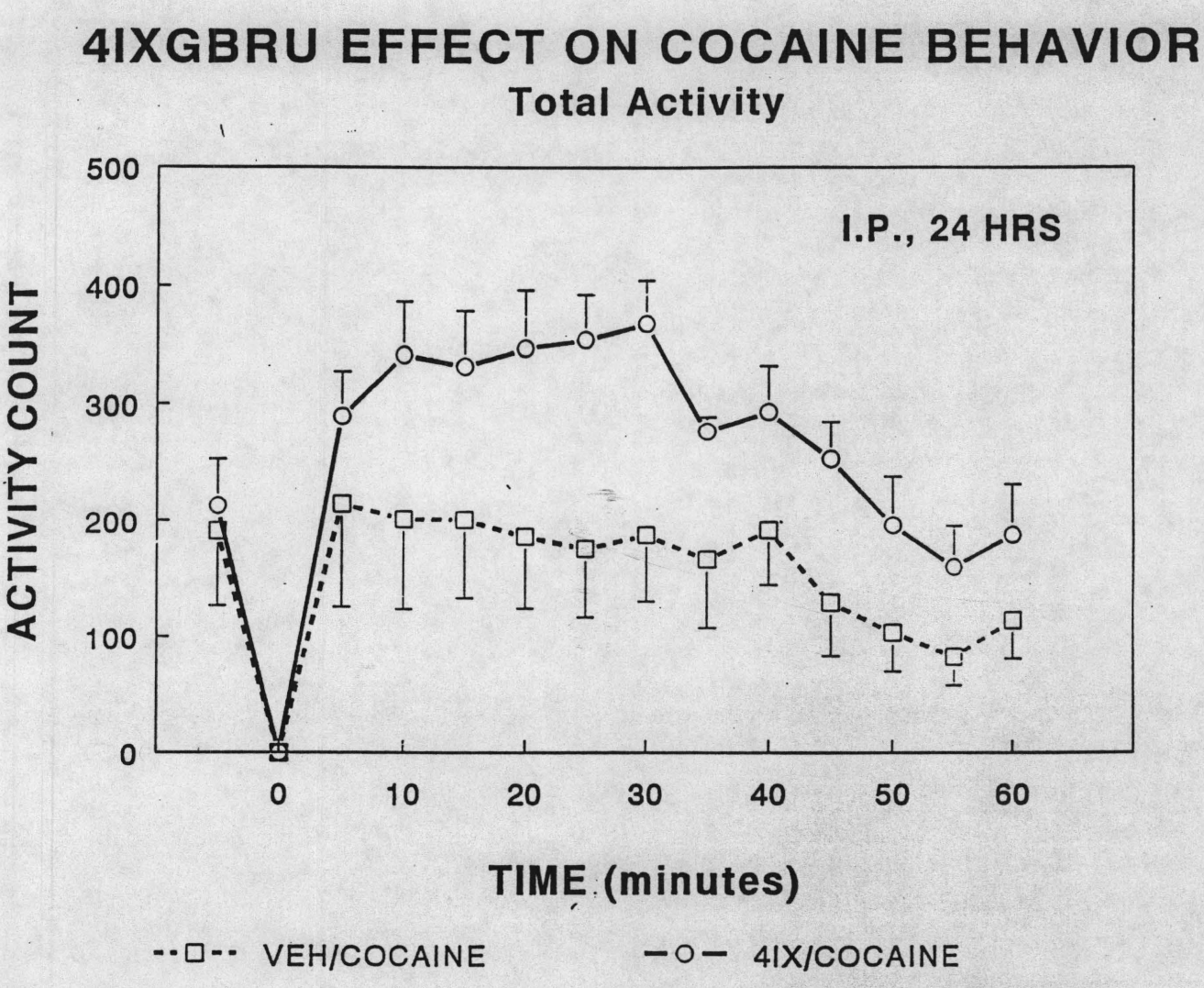


Fig. 4. Effect of **1d** (25 mg/kg, i.p.) administered approximately 24 hrs prior to treatment of Sprague-Dawley rats with (-)cocaine hydrochloride (15 mg/kg, i.p.). Total activity shown on y-axis includes both ambulatory and non-ambulatory activity counts.