This is to certify that the

dissertation entitled

Behavioral, Cellular and Metabolic Components of Drug Tolerance to Pentobarbital-Induced Hypothermia and Ataxia

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Pharmacology and Toxicology/Neuroscience

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Date November 15, 1990

O-12771

BEHAVIORAL, CELLULAR AND METABOLIC COMPONENTS OF DRUG TOLERANCE TO PENTOBARBITAL-INDUCED HYPOTHERMIA AND ATAXIA

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pharmacology and Toxicology/Neuroscience

1990

ABSTRACT

BEHAVIORAL, CELLULAR AND METABOLIC COMPONENTS OF DRUG TOLERANCE TO PENTOBARBITAL-INDUCED HYPOTHERMIA AND ATAXIA

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The development of behavioral tolerance to pentobarbital-induced hypothermia and ataxia, as separate from cellular and metabolic tolerances, was established. Pentobarbital was administered to 4 groups of rats, 2 groups of which received intermittent (INT) i.p. treatment. One of these (INT/EXP; intermittently-treated, drug-deficit experienced animals) experienced the drug effects by testing for rotarod performance (RR, ataxia measure) and body temperature (BT, hypothermia measure) after drug injection. The other (INT/NonEXP; intermittently-treated animals that were protected from experience) was tested for RR & BT before receiving drug and then prevented from drug-induced hypothermia by maintenance of body temperature and ataxia experience by a towel-wrap restraint after drug administration. Two groups received chronic treatment (i.p. and in ground chow), one with drug-effect experience (CHR/EXP) and one prevented from experiencing drug effects (CHR/NonEXP). Results of a Postchronic Test, with all groups experiencing the drug effects, was compared to Prechronic Test effects to assess the degree of tolerance. The INT/EXP group demonstrated behavioral tolerance for drug-induced

hypothermia, but not for drug-induced ataxia. The INT/NonEXP group actually showed increased sensitivity to pentobarbital-induced ataxia. Prominent tolerance was noted in both chronic groups for hypothermia and ataxia, without any difference between them. After Postchronic Testing, chronic treatment was discontinued for 9 days (withdrawal) and then, 10 days of extinction training (vehicle-behavioral testing) was conducted. The two intermittent groups demonstrated no change in the hypothermia or ataxia during the Postwithdrawal and Postextinction Drug Tests. However, in CHR/EXP rats tolerance to both hypothermia and ataxia was decreased at the Postwithdrawal Test, with a greater loss at the Postextinction Test. CHR/NonEXP animals showed a prominent loss of tolerance at the Postwithdrawal Test only. Brain concentrations of pentobarbital in identically-treated rats (up to the Postchronic Period) yielded evidence of metabolic tolerance in the two chronic treatment groups. Evidence for cellular tolerance was also produced in these two groups when the brain concentrations were correlated with the hypothermia. Behavioral tolerance to drug effects was expressed after both chronic and intermittent pentobarbital treatment and existed separate from cellular and metabolic tolerance.

To my loving husband, Allan, for his support and encouragement, without whom this dissertation, and all it stands for, would not have been possible.

Also, to Lars, whose life taught me the importance of understanding pharmacological intervention, how it can increase the quality of life and how it can shorten life.

ACKNOWLEDGEMENTS

I would like to thank Cynthia Clingan, Neelu Shukla, Staci Haarer and Clare Casey for their technical and managerial assistance required for this study. I would also like to acknowledge the many student workers (army of undergrads, as it were) who made it possible to conduct this study without automation, some of whom are Suzanne Rado, Cecilia Ho, Rhonda Norton, Jacquie Stoudt, Scott Giraud, Leslie Tilletson, Traci Kimbrough, Robyn Turner, MaryJo Charron, Hussein Huraibi, and Debbie Burkholder. If I have forgotten anyone, please forgive me. I would also like to thank Marlee Langham and Rob Durham for their help with computer programs and their patience with me using their equipment.

Most of all, I would like to thank Dr. Richard Rech for his patience, guidance, and inspiration. Dr. Rech has been more than a mentor, he is a friend.

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LIST OF ABBREVIATIONS AND NOMENCLATURE

Treatment Groups:

INT/EXP - intermittent drug exposure (i.p. injection every 4th day) during the Chronic Treatment Period / drug deficit experience (ataxia-rotarod testing and hypothermia-body temperature measurements)

INT/NonEXP - intermittent drug exposure (i.p. injection every 4th day) during the Chronic Treatment Period / protection from drug deficit experience by prevention of ambulation with a towel wrap and body temperature maintenance with a heat lamp

CHR/EXP - chronic drug exposure (daily i.p. injection plus drug in ground chow) during the Chronic Treatment Period / drug deficit experience (ataxia-rotarod testing and hypothermia-body temperature measurements)

CHR/NonEXP - chronic drug exposure (daily i.p. injection plus drug in ground chow) during the Chronic Treatment Period / protection from drug deficit experience by prevention of ambulation with a towel wrap and body temperature maintenance with a heat lamp

Behavioral manipulations:

RR - rotarod testing

BT - body temperature measurement

RR & BT - rotarod testing (every 15 minutes) and body temperature measurements (every 10 minutes) for two hours

BTW - protection from drug deficit experience by prevention of ambulation with a towel wrap and body temperature maintenance with a heat lamp

Prechronic - measurements obtained from INT/EXP and CHR/EXP during the Pre-chronic Dose-response Test Period

Postchronic - measurements obtained for all groups to a Dose-Response Test after the Chronic Treatment Period

Postwithdrawal - measurements obtained for all groups to a single test dose after a 10-day withdrawal period

Postextinction - measurements obtained for all groups to a single test dose after 10 days of extinction training

i.p. - intraperitoneal

rpm - revolutions per minute

i.d. - inner diameter

ANOVA - analysis of variance

ANCOVA - analysis of covariance

CS - conditioned stimulus, classical 'Pavlovian conditioning' - e.g. bell

UCS - unconditioned stimulus, classical 'Pavlovian conditioning' - e.g. food

INTRODUCTION

Adaptation to changes in environment is inherent to life. Adaptations may be manifested structurally, physiologically, biochemically, and/or behaviorally. Drug administration is an example of environmental change. Adaptation to drug effects can produce drug tolerance and dependence in some situations, while it can produce drug sensitization in other situations (LeBlanc and Cappell, 1977).

Drug tolerance is a phenomenon in which repeated administration of a drug is accompanied by a decrease in a drug effect, requiring an increased amount of drug to produce the same effect (Kalant et al., 1971; Krasnegor, 1978; LeBlanc and Cappell, 1977; Schuster, 1978). A parallel shift to the right in the dose-response curve demonstrates drug tolerance (Goudie and Griffiths, 1986; Schuster, 1978).

A given drug generally produces multiple responses with multiple mechanisms for these responses. Drug tolerance likewise has multiple mechanisms, resulting in various levels of tolerance for each drug effect. Drug tolerance has been split into two major mechanistic categories: dispositional and functional (Goldstein, 1979; Goudie and Griffiths, 1986;

Kalant et al., 1971; Krasnegor, 1978; LeBlanc and Cappell, 1977; Schuster, 1978).

Dispositional or pharmacokinetic tolerance occurs in chronically-treated subjects when a decreased drug concentration reaches the site of drug action as compared to control subjects. This may be produced by changes in drug absorption, distribution, metabolism, and/or excretion (Krasnegor, 1978; Schuster, 1978). Examples of mechanisms for dispositional tolerance which more rapidly decrease the plasma concentration of the drug include induction of drug metabolizing enzymes, increased production of non-target tissue binding proteins, or increased elimination at the kidney.

Functional tolerance is a decrease in the functional effect of the drug without a decrease in drug concentration at the site of action. The traditional concept of functional tolerance is that of cellular tolerance. Cellular or pharmacodynamic tolerance occurs when there is a decreased response of the target cells to the same concentration of the drug at the site of drug action that initially produced greater deficits. Possible mechanisms may include changes in drug or neurotransmitter receptor numbers, changes in the production or release of second messengers, or changes in levels of endogenous chemicals which act at the drug receptor or for which availability to their receptors is affected by the drug.

It has been demonstrated that some types of functional drug tolerance are affected by manipulations that also alter associative learning/conditioning. Examples of these manipulations are environmental conditioning, task practice, reinforcement loss, stimulus strength, etc., all involving stimulus and/or response expectancies. It has been suggested by some researchers that there is a separate tolerance component, behavioral tolerance, which is due to a 'more specific development of learned behavioral changes that allow the organism to accommodate to the drugged condition' (Tang and Falk, 1978).

It is, presumably, behavioral tolerance that can be altered by stimulus and response expectancies rather than the classical cellular tolerance defined as a target-cell physiological change altered by the presence of the drug. Behavioral tolerance involves a mechanism similar to behavioral conditioning or learning. Development of this type of tolerance requires an association between the experience of the drug effect and specific behaviors which are affected. Behavioral tolerance is thought to be an adaptation to the alterations in behavioral stimuli or response patterns caused by the drug effects. Classical cellular and dispositional tolerances are adaptations to the drug and/or its proximate physiological effects. These adaptations can be expressed in terms of decreased neuronal sensitivity without requiring drug-deficit experience for cellular tolerance or reduced

delivery of drug to the site of action for dispositional tolerance.

Adaptations may be useful and/or harmful. Drug tolerance complicates many therapeutic applications (Schuster, 1978). Tolerance may develop to both wanted and unwanted drug effects. An understanding of what behavioral contributions are made to the tolerance of certain drug effects may be useful in therapeutic drug management by allowing an approach toward enhancing or diminishing tolerance development to some drug effects.

Many drugs (such as opiates, barbiturates, alcohol) which have actions on the central nervous system and produce drug tolerance upon repeated administration also produce physical dependence and an accompanying withdrawal syndrome when chronic drug treatment ceases. Although these two phenomena do not always exist together, there is some correlation between physical dependence and functional tolerance with many drugs. The overt physiological changes which produce cellular tolerance remain in effect for a relatively short time after discontinuation of drug administration before re-adaptation occurs. Most of the symptoms of withdrawal syndromes appear to be rebound effects relating to compensations developed during chronic drug treatment. Often, these symptoms are alleviated by administration of the chronically-administered drug or another drug in the same class. Thus, the physiological changes which also produce drug tolerance may be the manifestations which produce withdrawal symptoms upon

sudden discontinuation of drug administration (Dews, 1978; Schuster, 1978).

Drug dependence also may have a behavioral conditioning component which is related to behavioral tolerance (Balster, 1985; O'Brien et al., 1986; Siegel, 1983). This relationship of drug tolerance and drug dependence appears to play a part in drug addiction. Cappell and LeBlanc (1981) construe drug tolerance and drug dependence as independent variables to which drug consumption is a dependent variable. Psychological factors are important in initiating and maintaining the repeated drug administration in human drug abuse leading to drug tolerance and drug dependence, but drug tolerance and drug dependence are also important in perpetuating and increasing drug consumption. Therefore, an understanding of the factors involved in drug tolerance, which also may be involved in drug dependence, is important for understanding and combatting drug abuse (Krasnegor, 1978; Schuster, 1978).

Although dispositional tolerance and cellular tolerance are accepted as two separate physiological adaptations, the relationship of behavioral tolerance to these other types of tolerance has not been established. Previous studies of behavioral tolerance have looked at what type of behavioral manipulations can produce and affect this type of tolerance, but none have attempted to demonstrate the separation of behavioral and cellular tolerance. This study was designed to determine if some types of behavioral tolerance are 1) developed due to repetitions of drug experience with the

behaviors in question without the necessity of chronic administration; and 2) separable from cellular tolerance, which theoretically would not require repeated experiences of drug exposure during performance of the behaviors. Many components of drug tolerance may be involved in the total tolerance to a single drug effect. These components may include dispositional, functional-cellular and functional-behavioral types of tolerance. The goals of this study were to determine what components exist within the total drug tolerance for the motor ataxic and hypothermic effects of sodium pentobarbital. More specifically, is there a separable component that fits the definition of behavioral tolerance and what contribution does this behavioral tolerance make to the total tolerance observed after both intermittent and chronic pentobarbital administration?

Specific Aims:

1) To develop tolerance to the hypothermia and ataxia effects of pentobarbital after both chronic and intermittent drug treatment.
2) To attribute the tolerance developed to these pentobarbital-induced deficits within each experimental group either to learned adaptations based on experience and/or to cellular/metabolic tolerance due to chronic exposure to the drug.
3) To determine the presence of cellular and metabolic types of tolerance in the chronic treatment groups.

4) **To determine whether the cellular and/or metabolic types of tolerance are attributable to chronic drug treatment without requiring specific drug experience, and whether behavioral tolerance is attributable to drug-deficit experience without requiring development of cellular tolerance.**

RATIONALE

Simple behaviors were studied for ease of testing sufficiently large numbers of subjects without long training periods, allowing for the time-course of drug effects to be determined, and for more widespread significance to normally observed drug effects. Both a physiological drug effect (hypothermia) and a behavioral drug effect (ataxia) were chosen. Both of these deficits are controlled by the central nervous system. The hypothermia was measured by recording the rectal temperature and the ataxia measured by rotarod performance.

The purpose of this study was to separate behavioral, cellular, and metabolic tolerance to an extent that the existence and autonomy of each could be examined. Separation of the components was attempted by the design of the study that incorporates exclusionary requisites. Four groups of animals with the following types of tolerance development were produced: (INT/EXP) behavioral tolerance only; (CHR/EXP) behavioral and cellular/metabolic tolerance; (INT/NonEXP) no tolerance; (CHR/NonEXP) cellular/metabolic tolerance only. The existence of behavioral tolerance was determined by comparing the tolerance developed in INT/EXP and INT/NonEXP

for intermittent treatment and in CHR/EXP and CHR/NonEXP for chronic treatment. The existence of cellular/metabolic combined tolerance was determined by comparing INT/EXP to CHR/EXP and INT/NonEXP to CHR/NonEXP. Separation of the cellular and metabolic components was produced through analysis of brain concentration time courses and their associated behaviors. In this manner the existence of metabolic tolerance was determined by comparing the brain concentration time courses of INT/EXP and INT/NonEXP to those of CHR/EXP and CHR/NonEXP. The existence of cellular tolerance was determined by comparing the behavioral deficits produced by the corresponding brain concentrations of INT/EXP to those of CHR/EXP and of INT/NonEXP to those of CHR/NonEXP.

Both cellular and metabolic tolerance development require a continuous exposure to the drug for an extended period. A dosing frequency greater than every other day is required to produce either cellular or metabolic tolerance for pentobarbital (Belknap et al., 1977; Cappell et al., 1981; Okamoto et al., 1986). Cellular/metabolic tolerances were eliminated in the present study in two groups of rats by an intermittent dosing schedule of one dose every four days.

Behavioral tolerance was minimized in two groups of rats by attenuating the experience to the drug effects to be measured. Minimizing the experience to the motor ataxia was accomplished by immobilizing the animals in a towel wrap. Minimizing the experience to the hypothermia was attained by maintaining the body temperature at the predrug control

temperature with the aid of a heat lamp similar to the procedure used by Alkana et al. (1983).

The chronic exposure groups were maintained on pentobarbital by adding the drug to their ground chow. This level of drug is not enough to produce overt effects even though it is adequate (along with the daily injected drug) for chronic maintenance.

The first part of this study was designed to determine the contribution and autonomy of behavioral tolerance to the total tolerance. Since it was not possible to separate out the metabolic tolerance from the cellular tolerance with the design used in this part of the study, these two components are referred to together as cellular/metabolic tolerance. The design included four groups of rats: INT/EXP with experience to drug effects, intermittent drug exposure and development of behavioral tolerance only; CHR/EXP with experience to the drug effects, chronic drug exposure, and development of both behavioral and cellular/metabolic tolerances; INT/NonEXP with no experience to the drug effects, intermittent drug exposure and no tolerance development; and CHR/NonEXP with no experience to the drug effects, chronic drug exposure and development of cellular/metabolic tolerance only. Thus, a comparison between INT/EXP and INT/NonEXP should determine if behavioral tolerance has been developed and whether it is separable from cellular/metabolic tolerance. A comparison between CHR/EXP and CHR/NonEXP should determine the relative contribution of behavioral tolerance to the total tolerance

development after chronic administration in drug-experienced rats.

The experimental design involved a training period, an acclimation period, a preliminary or prechronic dose-response determination, a tolerance development period, a postchronic dose-response determination, a withdrawal period, a postwithdrawal single dose-effect test, an extinction period, a postextinction single dose-effect test, a retention period, and a retention single dose-effect test (see Table 1).

The training period allowed training for the rotarod performance as well as introduction to the towel wrap and rectal temperature procedure. The animals were then habituated to the two-hour towel wrap, repeated rectal temperature measurements and rotarod performance tests during the first six days of the study to allow subjects to adjust to the stress of this schedule. All groups received the same non-drug experiences to eliminate any differences due to maintenance and testing treatments.

The preliminary dose-response determination was made in duplicate from the animals in INT/EXP and CHR/EXP during the second six days. The animals in INT/NonEXP and CHR/NonEXP received the same drug exposure at this time but experience was minimized by the towel wrap and temperature maintenance with a heat lamp. Three doses of pentobarbital were administered in a random order and then repeated. Matched pairs of subjects were assigned (INT/EXP to INT/NonEXP and CHR/EXP to CHR/NonEXP) to receive the same dosing order.

During the tolerance development period the animals in CHR/EXP and CHR/NonEXP received daily i.p. pentobarbital injections and pentobarbital in their ground chow for initiation and maintenance of the cellular/metabolic tolerance. The animals in INT/EXP and INT/NonEXP received i.p. saline for three days, then an i.p. dose of pentobarbital on the fourth day and ground chow without pentobarbital. During the three saline days for INT/EXP and INT/NonEXP all animals in all groups were wrapped in the towel during the treatment time.

Rotarod performance was tested for test-dose effects over the tolerance development period in INT/EXP and CHR/EXP (experienced), but was monitored only after vehicle during this period in INT/NonEXP and CHR/NonEXP (nonexperienced). On the fourth day the animals in INT/EXP and CHR/EXP were tested after pentobarbital administration and the animals in INT/NonEXP and CHR/NonEXP were tested after saline administration. In order to preserve the equivalent items of treatment of all animals, the animals in INT/EXP and CHR/EXP received saline and then were towel wrapped, and the animals in INT/NonEXP and CHR/NonEXP received pentobarbital and then were towel wrapped on the fourth day also.

This four-day cycle was continued for 36 days, allowing for nine spaced days of the pentobarbital-testing experience for the animals in INT/EXP and CHR/EXP, as well as 36 continuous days of chronic pentobarbital exposure for the animals in CHR/EXP and CHR/NonEXP. The tolerance development

was maximized during these 36 days by the accelerating chronic dosing method of Okamoto and Boisse (1981) for both the chow exposure for the animals in CHR/EXP and CHR/NonEXP and for the i.p. pentobarbital administration for all groups. The matched pairs from INT/EXP and INT/NonEXP as well as CHR/EXP and CHR/NonEXP were assigned for equivalent dosing increases for the i.p. pentobarbital administration.

The postchronic dose-response determinations, testing for drug effects on both body temperature and rotarod performance, were made for all groups to measure tolerance development in each group. Three doses were administered in a random order to the matched pairs. The doses in the intermittent treatment groups (INT/EXP and INT/NonEXP) were the same as the prechronic determination (20, 28 and 40 mg/kg). Due to the large amount of cellular/metabolic tolerance produced in the chronic treatment groups (CHR/EXP and CHR/NonEXP) the postchronic test doses were shifted upward in dose (28, 40, and 80 mg/kg). This was done in an attempt to produce effective doses comparable to those in the intermittent treatment animals in order to achieve maximum sensitivity for distinguishing behavioral tolerance in these groups.

The dose of pentobarbital that produced a just-significant, short-lived effect (15-45 minutes in length) was chosen for each animal from the postchronic dose determination data. This dose was used subsequently as the test dose for the postwithdrawal and postextinction tests (see below).

The pentobarbital administration, both by injection and in the ground chow, was then discontinued and the animals were monitored for withdrawal signs. A ten-day withdrawal period was allotted for cellular tolerance and metabolic tolerance to dissipate completely (Okamoto et al., 1976). All animals in all groups were then tested with a single test dose of pentobarbital (indicated above) to determine the amount of total tolerance that was lost after withdrawal. Any remaining tolerance at this test is presumed to derive from a behavioral tolerance mechanism.

Removal of the behavioral component of tolerance was calculated to be achieved by application of ten days of extinction trials, since behavioral tolerance is similar in characteristics to conditioning/learning phenomena. Loss of any behavioral tolerance was then determined by postextinction testing with the single pentobarbital test dose.

The determination of cellular and metabolic tolerance components was achieved in the second part of the study. The training period, acclimation period, prechronic dose-response determination, and tolerance development period were repeated in new rats assigned to the same groups. A time-course for brain concentrations was determined by sacrificing the animals at various times after drug injection on day 49, immediately after a final ataxia and hypothermia measurement was obtained. The brain pentobarbital concentrations of the intermittent animals were compared with the chronic animals to determine the relative contribution of the metabolic tolerance component

to the total tolerance observed in the chronic groups. An analysis of covariance was used to statistically control the brain levels, therefore effectively eliminating the metabolic tolerance. This allowed the existence of cellular tolerance to be ascertained by comparing between experienced INT/EXP and CHR/EXP, as well as between nonexperienced INT/NonEXP and CHR/NonEXP. Thus, an accounting of the significance of metabolic and/or cellular tolerance components induced by chronic drug treatment was accomplished.

METHODS

Subjects - Food - Housing

Male Sprague-Dawley rats (Harlan) weighing 175-200 grams at the start of the study were maintained on a regular light-dark cycle (dark between 7 p.m. and 7 a.m.) in temperature- and humidity-controlled animal quarters. Animals were housed individually with water available ad libitum. Food, as Lab Blox®, was available ad libitum, except during the tolerance development period (days 13-48), when ground chow with or without pentobarbital was available ad libitum only from 5 p.m. to 7 a.m. If the body weight of a subject receiving pentobarbital dropped below 80% of the control diet animals' body weights, a supplement of Very Vanilla Sego® was made available between 3 p.m. and 5 p.m.

Training

After allowing the animals to accommodate to the facilities, they were trained for 3-5 days on the rotarod (RR). This involves the subjects' learning to walk on an elevated rotating cylinder (18 cm width, 10.5 cm diameter, and 9 rpm), the training criterion being maintenance on the rotarod for at least 180 seconds for three consecutive trials

(Rech et al., 1966; Akera et al., 1973; Commissaris and Rech, 1983).

During these training days the animals were also introduced and adapted to the towel wrap and rectal temperature probe. The towel wrap (BTW) involves securing the animal snuggly, but not tightly, in a hand towel with Acco® clips to render the animal immobile. Body temperature (BT) was determined using a telethermometer (Yellow Springs Model 46 TUC) with a plastic coated probe which was inserted into the rectum to a distance of 4 cm for 40 seconds (Commissaris et al., 1982).

Drug Dosing

Solutions of sodium pentobarbital in 0.9% saline were administered i.p. at a volume of one ml/kg. Test doses, varied by group (see below), were 0 (vehicle), 20, 28, 40, and 80 mg/kg. During the chronic administration period (days 13-48) the drug dosing was gradually increased (Okamoto and Boisse, 1981), starting at 30 mg/kg, in 3 mg/kg increments, as tolerance was displayed in INT/EXP and CHR/EXP on the RR and BT testing days. Sodium pentobarbital was also administered in ground chow placed in weighted cups at an initial dose of two mg/g during the chronic administration days 12-48. Gradual increases in the dose of pentobarbital in the ground chow were made as the subjects consumed 80% or more of the amount consumed by control diet rats.

Treatment and Testing Schedule

Subjects were randomly divided into four groups of 12 rats each (N=48); INT/EXP, INT/NonEXP, CHR/EXP, and CHR/NonEXP. INT/EXP and CHR/EXP experienced the motor deficits and the hypothermia produced after pentobarbital administration while performing on the rotarod and without body temperature maintenance (RR & BT). INT/NonEXP and CHR/NonEXP did not experience the motor deficits or hypothermia after pentobarbital administration because they were rendered immobile by the towel wrap while their body temperature was maintained within control range through the application of heat lamps (BTW). INT/EXP and INT/NonEXP received intermittent administration (every fourth day) of pentobarbital, while CHR/EXP and CHR/NonEXP received chronic administration of pentobarbital, during the tolerance development period of the study. The entire seven periods of sequential treatment lasted 72 days.

The subjects were manipulated as follows (see also Table 1):

Days 1-6 (Training period): Rats in INT/EXP and CHR/EXP received vehicle at 10 a.m., then were wrapped in the body towel and had their body temperature maintained within the control range for two hours of monitoring the body temperature every ten minutes (body towel wrap or "BTW"). Rats in INT/NonEXP and CHR/NonEXP received vehicle at 8 a.m., then were tested on the rotarod every 15

Table 1: Schedule of Treatment Periods and Pentobarbital Exposure for All Four Experimental Groups

RAT GROUP	Period Schedule - Days						
	1-6: IP Vehicle	7-12: Prechronic IP Drug Test	13-48: Chronic Drug or Vehicle Treatment	49-51: Postchronic Drug Tolerance Test	52-61: Withdrawal Period	62-71: Extinction Training	72: Post-extinction Test
1 (INT. EXP.)#	Towel-Wrap, Heat Lamp, BT* monitored for 2 hrs	Measure RR & BT Effects for 2 hrs	IP Vehicle & Towel-Wrap Daily; Test IP Drug Every 4th Day for RR & BT	Test 3 IP Drug Doses for RR & BT	Stop Chronic Vehicle; Test IP Drug ǂ for RR & BT on Day 61	Daily Vehicle then Test RR & BT	Test IP Drug ǂ for RR & BT
2 (CHR. EXP.)#	Same as Group 1	Same as Group 1	Drug in Diet; IP Drug & Towel-Wrap, 3 Days; IP Vehicle & Towel-Wrap Every 4th Day, Then Test IP Drug for RR & BT	Same as Group 1	Stop Chronic Drug; Test IP Drug ǂ for RR & BT on Day 61	Same as Group 1	Same as Group 1
3 (INT. NONEXP.)#	Measure RR & BT★ Effects for 2 hrs	Towel-Wrap, Heat Lamp, BT monitored for 2 hrs	IP Vehicle & Towel-Wrap, 3 Days; IP Vehicle for RR & BT every 4th Day, Then IP Drug & Towel-Wrap & BT monitored for 2 hrs	Same as Group 1	Same as Group 1	Same as Group 1	Same as Group 1
4 (CHR. NONEXP.)#	Same as Group 3	Same as Group 3	Drug in Diet; IP Drug & Towel-Wrap Daily; IP Vehicle for RR & BT Every 4th Day	Same as Group 1	Same as Group 2	Same as Group 1	Same as Group 1

INT. - intermittent drug treatment, EXP. - repeated experience with drug effect on RR performance, CHR. - chronic drug treatment, NONEXP. - drug test doses administered after conducting the RR test.

* BT - Body temperature.

★ RR - Rotarod performance.

ǂ Drug dose adjusted for each animal to a dose causing significant RR decrement for at least 30 min but no longer than 60 min at the Postchronic Drug Test.

minutes and had their body temperatures monitored every ten minutes for two hours (rotarod and body temperature or RR & BT).

Days 7-12 (Pre-chronic dose-response test period): Rats in INT/EXP and CHR/EXP received twice each of three doses of pentobarbital (20, 28, and 40 mg/kg i.p.) randomly assigned, one dose/day, at 8 a.m. and then were tested for RR & BT. Rats in INT/NonEXP and CHR/NonEXP received twice each of the three doses of pentobarbital also but at 10 a.m. followed by BTW.

Days 13-51 (Chronic period and Postchronic dose-response test period): Rats in INT/EXP and INT/NonEXP received control ground chow from 5 p.m. to 7 a.m., while rats in CHR/EXP and CHR/NonEXP received ground chow containing pentobarbital (2 mg/g initially) over the same period.

Days 13-15, 17-19, 21-23, 25-27, 29-31, 33-35, 37-39, 41-43, and 45-47 (chronic treatment days): Rats in INT/EXP and INT/NonEXP received i.p vehicle at 8 a.m. followed by BTW. Rats in CHR/EXP and CHR/NonEXP received i.p pentobarbital (starting at 30 mg/kg) at 10 a.m. followed by BTW.

Days 16, 20, 24, 28, 32, 36, 40, 44, and 48 (chronic test days): Rats in INT/EXP and CHR/EXP received i.p vehicle at 8 a.m. followed by BTW, then i.p. pentobarbital at 10 a.m. followed by testing of RR

& BT. Rats in INT/NonEXP and CHR/NonEXP received i.p. vehicle at 8 a.m. followed by testing of RR & BT, then i.p. pentobarbital at 10 a.m. followed by BTW.

Days 49-51 (Postchronic dose-response test period): Rats in all groups received three doses of pentobarbital (20, 28, and 40 mg/kg for INT/EXP and INT/NonEXP; 28, 40, and 80 mg/kg for CHR/EXP and CHR/NonEXP) randomly assigned to one dose/day, following which RR & BT were tested. From the measurements obtained from this dose-response test period, a test dose was chosen for each rat which produced only a short-lived behavioral deficit. This calculated dose was used as the test dose for the Postwithdrawal test and Postextinction test.

Days 52-60 (Withdrawal period): Rats in all groups were maintained in their home cages without drug treatment and monitored for withdrawal signs (such as hyperexcitability, tremor, spasticity, hyperthermia, loss of body weight, convulsions).

Day 61 (Postwithdrawal test day): Rats in all groups received the above-calculated i.p. pentobarbital test dose followed by RR & BT testing.

Days 52-71 (Extinction period): Rats in all groups received i.p. vehicle followed by RR & BT testing.

Day 72 (Postextinction test day): Rats in all

groups received the i.p. pentobarbital test dose followed by RR & BT testing.

Day 82 (Retention test day): Rats in all groups received the i.p. pentobarbital test dose followed by RR & BT testing.

Analysis of Brain and Serum Pentobarbital Concentrations

The above treatment and testing schedules were repeated in other rats to day 48. Each group contained 20-21 rats. Four rats from each group were started at one time with three to four days in between starting times for each set. On day 49 from the start of the treatment schedules, all groups received 40 mg/kg of pentobarbital and then were tested for RR & BT. Rats were sacrificed by decapitation in groups of at least four (one from each group of INT/EXP, INT/NonEXP, CHR/EXP, and CHR/NonEXP) each at 15, 30, 60, and 120 minutes immediately after the last BT recording and RR test (within five minutes). The trunk blood and the brain were collected from each subject and frozen at -90 °C until time was available for extraction of the pentobarbital and gas chromatographic analysis of the sample extracts.

Extraction of the pentobarbital from the brain was modified from the methods of Belknap et al., 1977, and Commissaris et al., 1982. The brain samples were thawed, weighed, and homogenized in two volumes of 0.1 N HCl. A 500 µl sample of the brain homogenate was then added to a 50 ml silanized centrifuge tube containing one ml distilled water

and 2.5 μg amobarbital (in 100 μl methanol) as an internal standard for the gas chromatographic analysis. Then 2 ml of 0.4 N HCl was added and the sample was sonicated. Chloroform (15 ml) was added and the sample was vortexed for 30 seconds then centrifuged. The bottom (chloroform) layer was removed with a transfer pipet and filtered through anhydrous sodium sulfate into a second 50 ml silanized centrifuge tube. Next 5 ml of 1.0 N NaOH were added then the sample was vortexed and centrifuged. The chloroform layer was then removed with a transfer pipet and discarded. HCl (5 ml of 1.0 N) was added and the sample was vortexed. Next 10 ml of chloroform were added and the sample was vortexed and centrifuged. The bottom (chloroform) layer was filtered through anhydrous sodium sulfate into a 15 ml conical tube. The sample was then dried with a gentle stream of nitrogen. Finally, 25 μl of TMAH (trimethylaniliniumhydroxide in methanol; MethElute®, Pierce Biochemicals) were added prior to injection of the sample onto the gas chromatographic column, 4 mm id packed with SP2250® (Supelco) in a Varian Aerograph 2400 gas chromatograph with an HP 3392A integrater. The carrier gas was nitrogen with a flow rate of 60 ml/min. The injection port temperature was 250 °C and the column temperature was 180 °C. Detection was by flame ionization at 250 °C with a mixed dry air and hydrogen flame. Pentobarbital determination was by peak area ratio method with the 2.5 μg amount of amobarbital as an internal standard. A pentobarbital recovery of 95% or greater after the extraction was achieved for the tissue

samples. A pentobarbital standard curve was generated that was linear between 0.5 and 120 μg/sample.

Statistical Analysis

Factorial and repeated measures analyses of variance (ANOVAs) were used to analyze all of the body temperature data and the rotarod duration of ataxia data. If the variances for the groups being compared were nonhomogeneous, the non-parametric Kruskal-Wallis or Mann-Whitney U tests were performed. For the rotarod time course and peak effect data, nonparametric Kruskal-Wallis and Mann-Whitney U tests were employed. Regression analysis was performed on the postchronic log dose-response data. Repeated measures ANOVA was used for the brain concentration time course data. Analysis of covariance (ANCOVA) was employed for the analysis of the brain concentrations related to their corresponding behavioral deficits.

RESULTS

For the number of subjects in each group, at each test, see Table 4, Appendix A. For the final pentobarbital doses reached from the maximally accelerated dosing during the chronic treatment period by the rats of INT/EXP, INT/NonEXP, CHR/EXP and CHR/NonEXP see Table 5, Appendix A.

Hypothermia Tolerance

All hypothermia data were analyzed with ANOVA and Tukey's test for multiple comparisons. For data in which the variances between groups being compared were nonhomogeneous, a non-parametric (Kruskal Wallis or Mann-Whitney U) test was used to confirm ANOVA results. For F values and probability levels see Appendix B.

Comparing the time course of the hypothermic effect of pentobarbital after the tolerance development period in INT/EXP and INT/NonEXP to that of the initial dose-response determination reveals tolerance development after intermittent treatment (See Figures 1, 2 and 3). INT/EXP (Figure 1) demonstrated significant tolerance at all time points except the 90-minute time point for the low dose (20 mg/kg). INT/NonEXP (Figure 1) demonstrated significant tolerance only

at 10-40 minutes for the low dose (20 mg/kg). For the middle dose (28 mg/kg), significant tolerance was observed at 10-40 minutes and at 120 minutes for both INT/EXP and INT/NonEXP, while INT/EXP also demonstrated tolerance at 80-110 minutes (see Figure 2). At the high dose (40 mg/kg) INT/EXP displayed significant tolerance from 20-110 minutes, while INT/NonEXP demonstrated significant tolerance from 10-90 minutes (see Figure 3).

As demonstrated by the prechronic bars in Figures 7, 8 and 9, peak hypothermia, duration of hypothermia and total hypothermia (approximation of area under the time course curve) were reliable dose-dependent measurements of the pentobarbital hypothermia effect. In accordance, these measurements were also examined for tolerance development.

INT/EXP (Figure 7) also demonstrated a significant tolerance with respect to the peak hypothermia produced by pentobarbital after the low and high doses. INT/NonEXP (Figure 7) displayed a significant tolerance with respect to the peak hypothermia only at the high dose. There was a significant decrease in the duration of the pentobarbital-induced hypothermia after the low and middle doses for INT/EXP (Figure 8) as compared to the prechronic controls. There was no change in the duration of the pentobarbital hypothermia for INT/NonEXP (Figure 8) at any of the three doses.

This demonstrates there was a trend for increased tolerance in INT/EXP over that observed in INT/NonEXP, which was best demonstrated by the low-dose data. This trend was

further demonstrated by the total hypothermia determination (area under the curve approximation, Figure 9), which was an addition of all hypothermia readings produced for each time point from 10-120 minutes. Again, significant tolerance to total hypothermia was demonstrated for INT/EXP (Figure 9) for all three doses. INT/NonEXP (Figure 9) demonstrated significant tolerance to total hypothermia only at the highest dose.

No significant difference in the postchronic time course for the hypothermic effect was observed between INT/EXP and INT/NonEXP for the low (Figure 1) and middle (Figure 2) doses, although there was a trend for INT/EXP to demonstrate less hypothermia. This trend was reversed for the high dose (Figure 3), and achieved significance at the 20-minute time point where INT/NonEXP produced less hypothermia than INT/EXP. There was no significant difference between INT/EXP and INT/NonEXP for peak hypothermia (Figure 7), overall duration of hypothermia (Figure 8) and total hypothermia (Figure 9) at all three doses postchronically.

Comparing the dose-response time courses of the CHR animals to those produced at the prechronic dose-response determination demonstrates significant cellular/metabolic tolerance (see Figures 4 through 6). The CHR animals received larger amounts of pentobarbital (28, 40, and 80 mg/kg), in essence a shifted dose-response curve due to the large amount of cellular/metabolic tolerance in these animals. There was significant tolerance from 20 to 120 minutes in both CHR/EXP

and CHR/NonEXP produced for the 28 mg/kg dose (Figure 4). The 40 mg/kg dose (Figure 5) produced similar results with significant tolerance at 20-120 minutes for both CHR/EXP and CHR/NonEXP. Although there was no 80 mg/kg dose for the prechronic measures, a significant difference would surely have been produced compared to CHR/EXP and CHR/NonEXP body temperature measures, since the 80 mg/kg dose in naive rats is within the lethal range of the pentobarbital dose-response curve (Barnes and Eltherington, 1973). The peak hypothermia (Figure 7), overall duration of hypothermia (Figure 8) and total hypothermia (Figure 9) for CHR/EXP and CHR/NonEXP demonstrated similar results as seen with the time courses. Both CHR/EXP and CHR/NonEXP were significantly tolerant for both measures at the 28 and 40 mg/kg doses, while the 80 mg/kg measurements for CHR/EXP and CHR/NonEXP were similar to the prechronic 40 mg/kg dose hypothermia levels.

Behavioral tolerance was not apparent in the CHR animals at the postchronic dose-response determination (see Figures 4 through 6). CHR/EXP was not significantly different from CHR/NonEXP at any time point of the time courses. There was also no significant difference between EXP and NonEXP in the CHR animals for peak hypothermia (Figure 7), duration of hypothermia (Figure 8) and total hypothermia (Figure 9) for all three dose comparisons.

The postchronic test period tolerance observed for all four groups can be compared in Figure 10 for the 28 mg/kg dose and Figure 11 for the 40 mg/kg dose. These time courses

demonstrate the large amount of cellular/metabolic tolerance produced in the CHR groups as compared to the INT groups and the prechronic testing.

Further characterization of the tolerance types involved in the total tolerance seen for each group was attempted by observing the loss of tolerance after a ten-day withdrawal period and after a ten-day extinction training period. A test dose was chosen for each subject based on their rotarod performance during the Postchronic Testing (see Table 6, Appendix A for individual doses). Comparisons were made within each group of a postwithdrawal test and a postextinction test to the postchronic test. Additionally, to determine if a significant amount of tolerance was lost due to extinction trials, the postextinction test was compared to the postwithdrawal test.

INT/EXP demonstrated no tolerance loss after withdrawal and extinction training for peak, duration, total and time course of hypothermia (see Figures 12, 13, 14 and 15). This would indicate that a conditioning-type of adaptation was not present or that the extinction trials employed were not sufficient to extinguish the conditioning-like behavioral tolerance. The INT/NonEXP group showed no significant change in the pentobarbital-induced hypothermia for peak, duration, total or time course comparing postchronic, postwithdrawal and postextinction tests, as would be expected (see Figures 12, 13, 14 and 16).

Although the CHR groups showed no differences to indicate there was behavioral tolerance development in the EXP group, a comparison of the postchronic, postwithdrawal and postextinction data for extent of hypothermia indicates that a conditioning-like component was indeed present. For the animals in CHR/EXP, a small trend (non-significant) for loss of tolerance was observed for the peak hypothermia (Figure 12), overall duration of hypothermia (Figure 13) and total hypothermia (Figure 14) at the postwithdrawal test, although a significant loss of tolerance for all three measures was seen for CHR/NonEXP after withdrawal.

A significant loss of tolerance was observed at the postextinction test for CHR/EXP regarding all three measures, while there was no significant additional loss in CHR/NonEXP after extinction trials. The overall duration of hypothermia (Figure 13) and total hypothermia (Figure 14) for CHR/EXP showed significant tolerance loss between the postwithdrawal and postextinction trials, which further supports a conditioning-like component for this tolerance. The time courses for CHR/EXP and CHR/NonEXP (Figures 17 and 18) also demonstrate that CHR/EXP had lost significant tolerance after withdrawal and additionally after extinction trials, while the tolerance observed in CHR/NonEXP was significantly lost after withdrawal with just a slight trend (non-significant) for additional loss after extinction trials.

Ataxia Tolerance

Data on peak and duration aspects of pentobarbital ataxia were analyzed with ANOVA and Tukey's test for multiple comparisons. For data in which the variances between groups being compared were nonhomogeneous, a non-parametric (Kruskal Wallis or Mann-Whitney U) test was used to confirm ANOVA results. For time-course data, the non-parametric Kruskal Wallis and Mann-Whitney U tests were performed. For F values (where appropriate) and probability levels see Appendix II.

Examination of the prechronic dose-response rotarod data demonstrated a fast developing (probably behavioral) tolerance by comparing the first three days to the last three days of the six day period (see Figure 45). This decreased drug-induced ataxia became significant at the highest dose (40 mg/kg). Recall that the second three days of this period repeated the random drug dosing order of the first three days, thus a change in dosing patterns does not complicate these results. Due to this rapid tolerance development the prechronic ataxia measures will only include the first three days of the prechronic dose-response determination data.

Figures 19 through 21 depict the postchronic time courses for the motor ataxia in the INT animals, compared with prechronic ataxia measures. INT/EXP demonstrates no tolerance over all three doses as compared to prechronic testing. However, a significantly increased ataxia was produced in INT/NonEXP as compared to prechronic testing for the two lower doses (20 mg/kg and 28 mg/kg). This increased ataxia for

INT/NonEXP was also significant as compared to INT/EXP scores at the middle dose (28 mg/kg) time points from 105-120 minutes.

The peak ataxia, duration of ataxia, and total ataxia (approximation of the area under the time course curve) were demonstrated as reliable dose-dependent measures of pentobarbital ataxia by the prechronic data representations in Figures 25, 26 and 27.

There was a significantly increased overall duration of ataxia and a significantly decreased overall performance (total ataxia) in INT/NonEXP as compared to the prechronic testing and as compared to INT/EXP postchronic testing for the low and middle doses (see Figure 26 and 27). Thus, in terms of duration of ataxia and total ataxia, INT/NonEXP actually demonstrated an enhanced effect on postchronic testing, relative to the prechronic test. No similar enhancement of these ataxia measures was observed for INT/EXP. No significant differences were observed between the prechronic testing and postchronic testing of INT/EXP and INT/NonEXP for the peak ataxia (Figure 25), although trends similar to the duration of ataxia and total ataxia effects were noted.

The CHR animals manifested significant tolerance development when compared to the 28 and 40 mg/kg prechronic ataxia measures. This ataxia tolerance was observed with the time courses (Figures 22 and 23), as well as the duration of ataxia (Figure 26) and total ataxia (Figure 27).

Although there was no significant difference between CHR/EXP and CHR/NonEXP at any dose postchronically, the CHR animals demonstrated a slight, non-significant trend for behavioral tolerance to pentobarbital-induced ataxia for the 28 and 40 mg/kg dose time courses (see Figures 22 and 23). There was also a trend (non-significant) at these doses for CHR/EXP to display more tolerance than CHR/NonEXP with respect to peak ataxia (Figure 25), duration of ataxia (Figure 26) and total ataxia (Figure 27), which was reversed at the 80 mg/kg dose.

The cellular/metabolic tolerance to ataxia for the CHR animals was depicted in Figures 28 and 29. These time courses clearly demonstrate the degree of tolerance in CHR/EXP and CHR/NonEXP for the 28 and 40 mg/kg doses as compared to the lack of tolerance in INT/EXP and INT/NonEXP (actually, enhanced effect in INT/NonEXP).

The postwithdrawal and postextinction tests for INT/EXP and INT/NonEXP failed to demonstrate any loss of tolerance to the pentobarbital ataxia (see Figures 30-33).

Although there was only a slight trend for behavioral tolerance demonstrated postchronically for CHR/EXP, the postwithdrawal and postextinction tests provided affirmation of that behavioral component (see Figures 30, 31 and 34). After withdrawal CHR/EXP demonstrated some tolerance loss, as would be expected for the cellular/metabolic component, but a significant amount of additional tolerance was lost after extinction trials (see Figures 30, 31 and 34). CHR/NonEXP

demonstrated significant tolerance loss after withdrawal, but little additional loss after extinction trials (see Figures 30, 31 and 35), indicating that only cellular/metabolic tolerance was predominant at the postchronic test.

The regression coefficients (Table 2) from the log dose-response graphs (Figures 36 through 41) were compared. There were no significant differences between any regression coefficients in any of the six graphs. There was, however, a slight trend for CHR/EXP, CHR/NonEXP, and INT/NonEXP to produce a decrease in slope as compared to INT/EXP for all four graphs and as compared to the Prechronic data in the peak effect graphs (Figures 36 and 38). A trend for a very similar, also non-significant, change in slope was observed in the duration of hypothermia (Figure 37), hypothermia peak (Figure 36), total hypothermia (Figure 38), ataxia peak (Figure 39) and
total ataxia (Figure 41) for the NonEXP groups. The common bond for these trends was that all three groups (CHR/EXP, INT/NonEXP, and CHR/NonEXP) had experience with the drug in the towel wrap. INT/NonEXP and CHR/NonEXP, which displayed very similar slopes in five graphs, had only experienced the drug within the towel wrap. CHR/EXP had slopes which were less effected and more similar to INT/EXP, perhaps due to some experience with the drug outside the towel wrap.

TABLE 2

Pentobarbital Postchronic Log Dose-Response Regression Coefficients
Hypothermia Duration and Peak,
Ataxia Duration and Peak

Groups	Hypothermia		Ataxia	
	Peak	Duration	Peak	Duration
Prechronic	-5.85	176.8	-55.7	166.6
INT/EXP	-5.48	254.1	-74.2	199.4
CHR/EXP	-4.97	201.1	-46.0	133.6
INT/NonEXP	-3.33	134.8	-16.1	176.9
CHR/NonEXP	-3.69	145.3	-16.2	111.1

Pentobarbital Brain Concentration and Behavior Comparisons

The group means for hypothermia, ataxia, and pentobarbital brain concentrations are shown in Table 3.

The pharmacokinetic tolerance component was determined by comparing the intermittently-treated animals to the chronically-treated rats after a 40 mg/kg test dose (see Figure 42). INT/EXP had a significantly greater pentobarbital brain concentration compared to CHR/EXP at all time points ($F=14.068$, $p<0.002$). Likewise, INT/NonEXP had a significantly greater brain pentobarbital concentration than CHR/NonEXP at all time points ($F=14.238$, $p<0.002$). There was obviously a large amount of pharmacokinetic tolerance present in the CHR animals. The pharmacokinetic tolerance observed in the CHR groups is due to increased metabolism and can not be attributed to a change in blood:brain distribution (Okamoto and Boisse, 1975).

A determination of the presence of cellular tolerance requires use of a statistical treatment (analysis of covariance) to statistically control brain pentobarbital concentrations, essentially removing the difference in these concentrations between the CHR and INT animals. By using analysis of covariance, a comparison of the pentobarbital-induced hypothermia produced in INT/EXP and CHR/EXP ($F=4.618$, $p<0.05$), as well as that produced in INT/NonEXP and CHR/NonEXP ($F=7.571$, $p<0.01$) demonstrates the existence of significant cellular tolerance for hypothermia as a result of chronic

TABLE 3

Pentobarbital (40 mg/kg) Hypothermia, Ataxia and Brain Concentration Group Means ± SEM
Duplication of the Postchronic Testing Procedure

Group	n	Body Temperature (°C)	Motor Performance (seconds)	[Brain] (μg/g)
		15minute		
INT/EXP	4	-0.85 ± .16	0 ± 0	40.1 ± 4.7
INT/NonEXP	5	-1.26 ± .08	0 ± 0	40.7 ± 1.4
CHR/EXP	5	-0.38 ± .30	15 ± 14	27.7 ± 2.2
CHR/NonEXP	5	-0.42 ± .29	19 ± 18	25.0 ± 3.0
		30minute		
INT/EXP	6	-1.05 ± .16	3 ± 3	34.8 ± 5.7
INT/NonEXP	5	-2.00 ± .22	0 ± 0	40.9 ± 4.1
CHR/EXP	6	-0.13 ± .45	70 ± 15	19.0 ± 2.3
CHR/NonEXP	6	-0.62 ± .44	53 ± 18	22.8 ± 3.2
		60minute		
INT/EXP	4	-1.43 ± .42	23 ± 23	27.4 ± 2.4
INT/NonEXP	6	-2.07 ± .30	0 ± 0	26.7 ± 3.2
CHR/EXP	5	-0.06 ± .13	90 ± 0	14.7 ± 2.2
CHR/NonEXP	5	0.10 ± .17	90 ± 0	9.9 ± 0.3
		120minute		
INT/EXP	4	-0.50 ± .64	69 ± 21	16.0 ± 3.9
INT/NonEXP	5	-0.44 ± .37	90 ± 0	14.5 ± 3.1
CHR/EXP	4	0.83 ± .15	90 ± 0	7.7 ± 1.6
CHR/NonEXP	4	0.18 ± .20	90 ± 0	8.8 ± 1.9

pentobarbital treatment (see Figure 43). Perhaps a simpler representation of the cellular tolerance difference between INT and CHR groups is displayed in Figure 44. This figure depicts the regression lines produced from the mean hypothermia produced at the mean brain concentration of each time point for each group. Unfortunately, there is no non-parametric equivalent to analysis of covariance for similar treatment of the ataxia data.

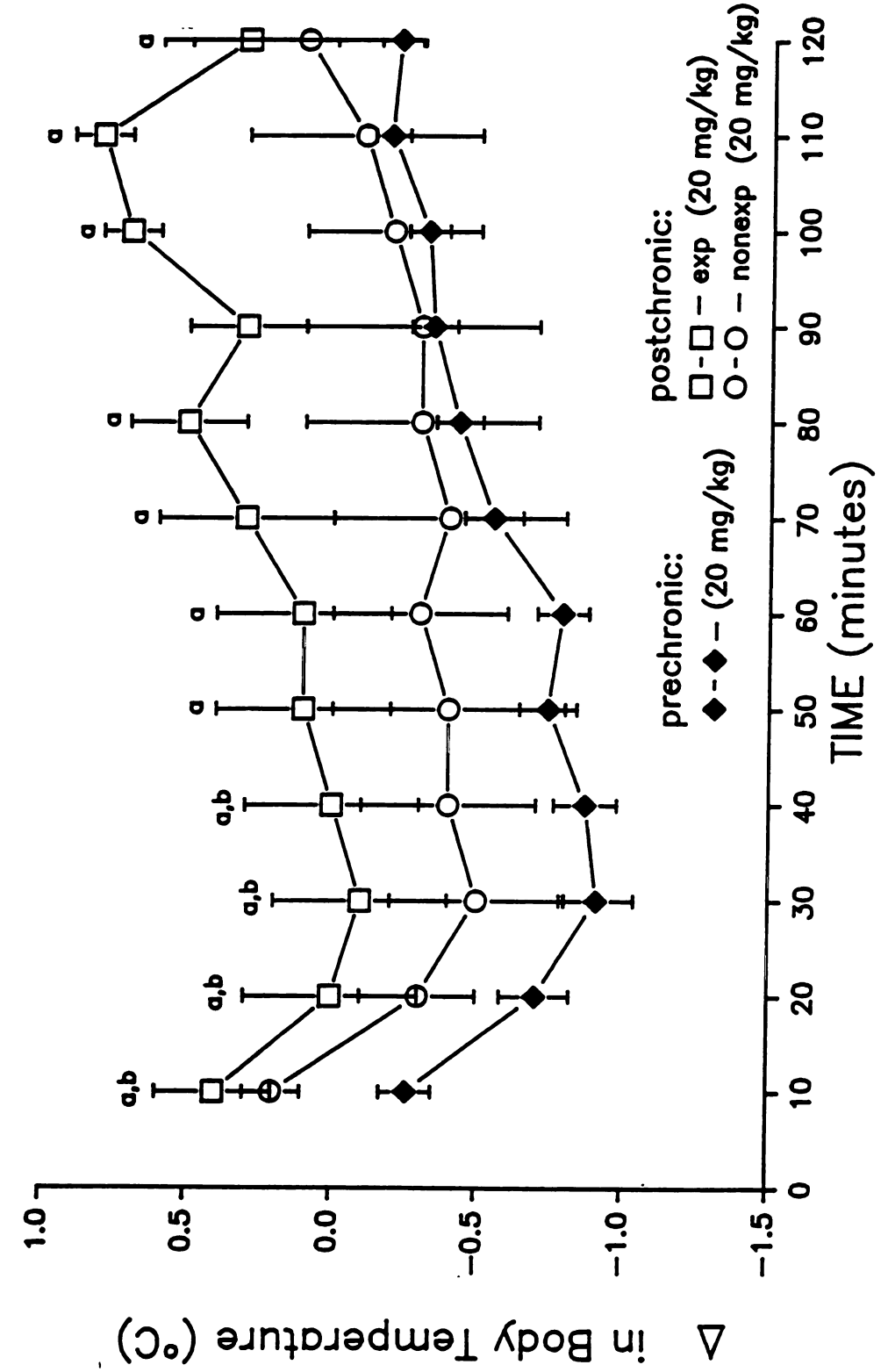

FIGURE 1: Hypothermia tolerance development to a 20 mg/kg pentobarbital test dose after intermittent drug treatment. Time course of change in body temperature ± s.e.m. a-INT/EXP sig. diff. from prechronic, b-INT/NonEXP sig. diff. from prechronic, ($p<0.05$).

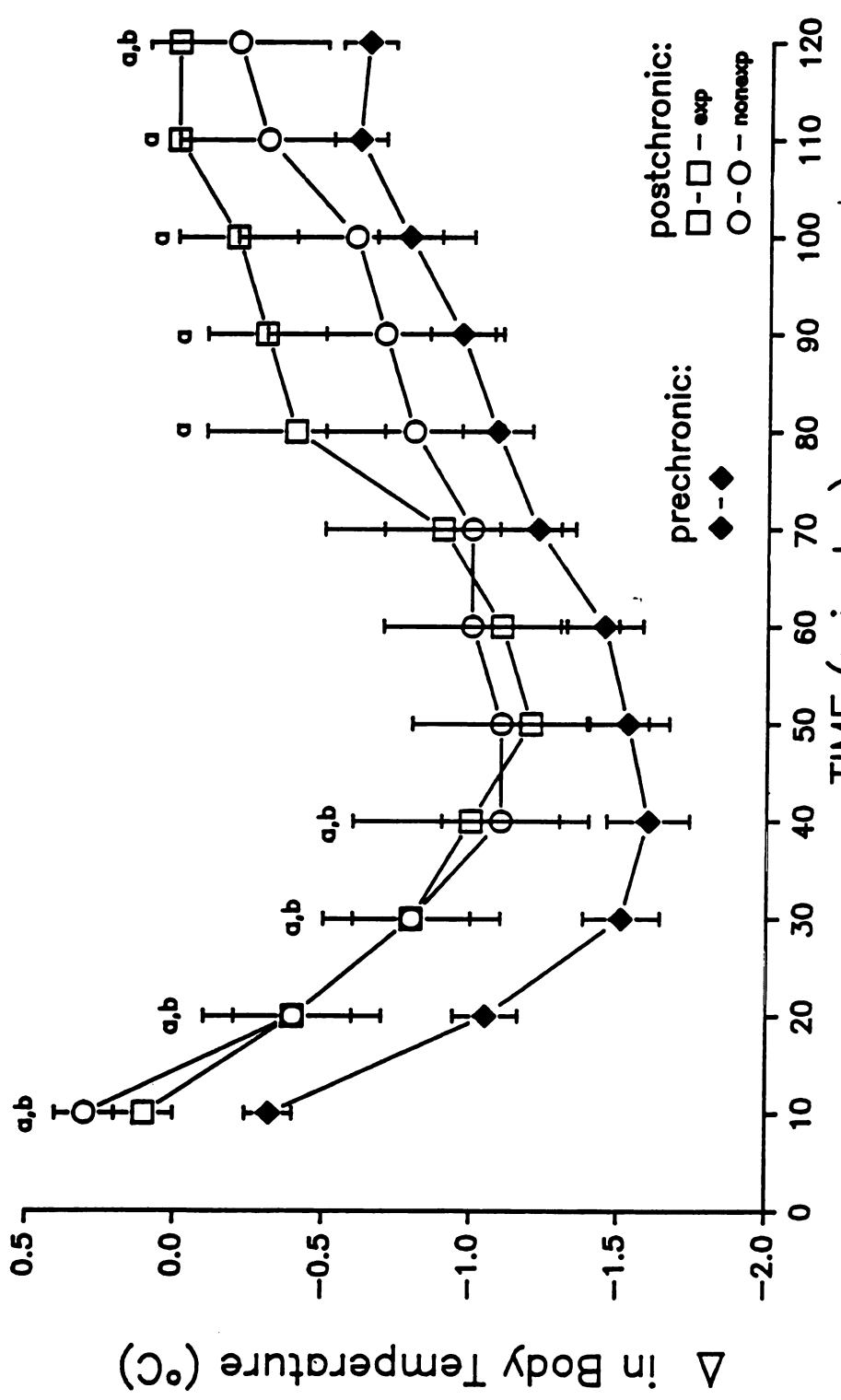

FIGURE 2: Hypothermia tolerance development to a 28 mg/kg pentobarbital test dose after intermittent drug treatment. Time course of change in body temperature ± s.e.m. a-INT/EXP sig. diff. from prechronic, b-INT/NonEXP sig. diff. from prechronic, ($p<0.05$).

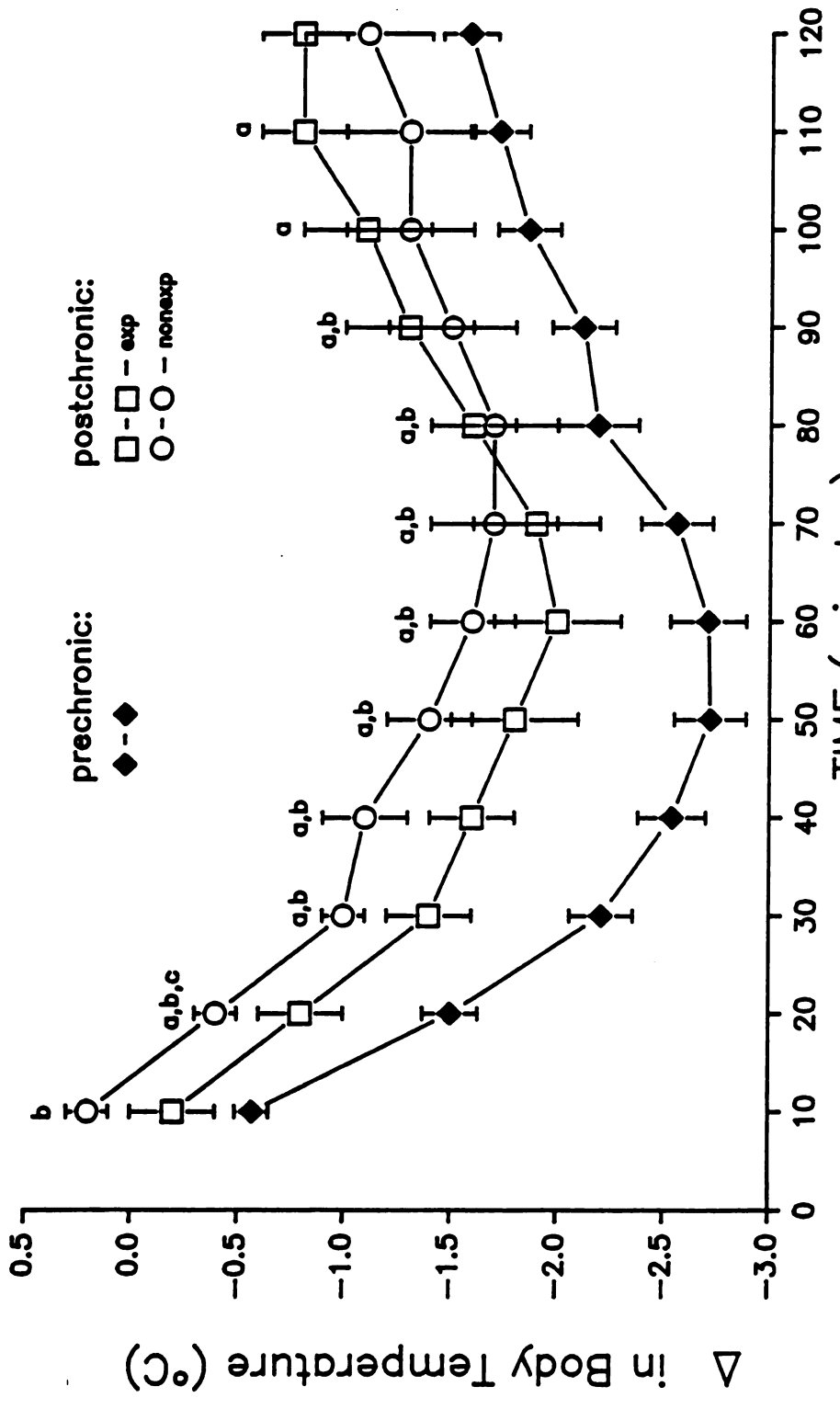

FIGURE 3: Hypothermia tolerance development to a 40 mg/kg pentobarbital test dose after intermittent drug treatment. Time course of change in body temperature ± s.e.m. a-INT/EXP sig. diff. from prechronic,, b-INT/NonEXP sig. diff. from prechronic, c-EXP sig. diff. from NonEXP, ($p<0.05$).

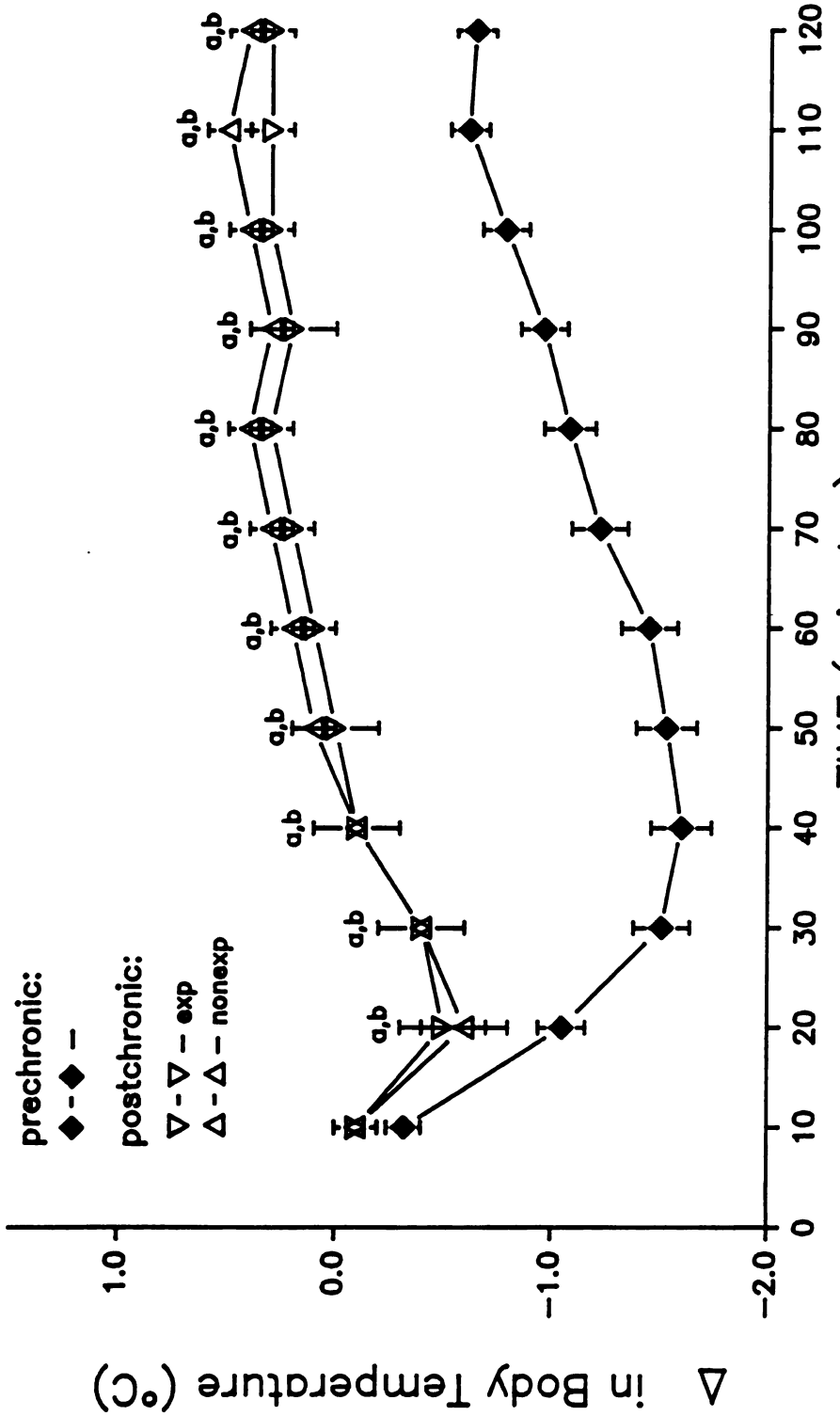

FIGURE 4: Hypothermia tolerance development to a 28 mg/kg pentobarbital test dose after chronic drug treatment. Time course of change in body temperature ± s.e.m. a-CHR/EXP sig. diff. from prechronic, b-CHR/NonEXP sig. diff. from prechronic, ($p<0.05$).

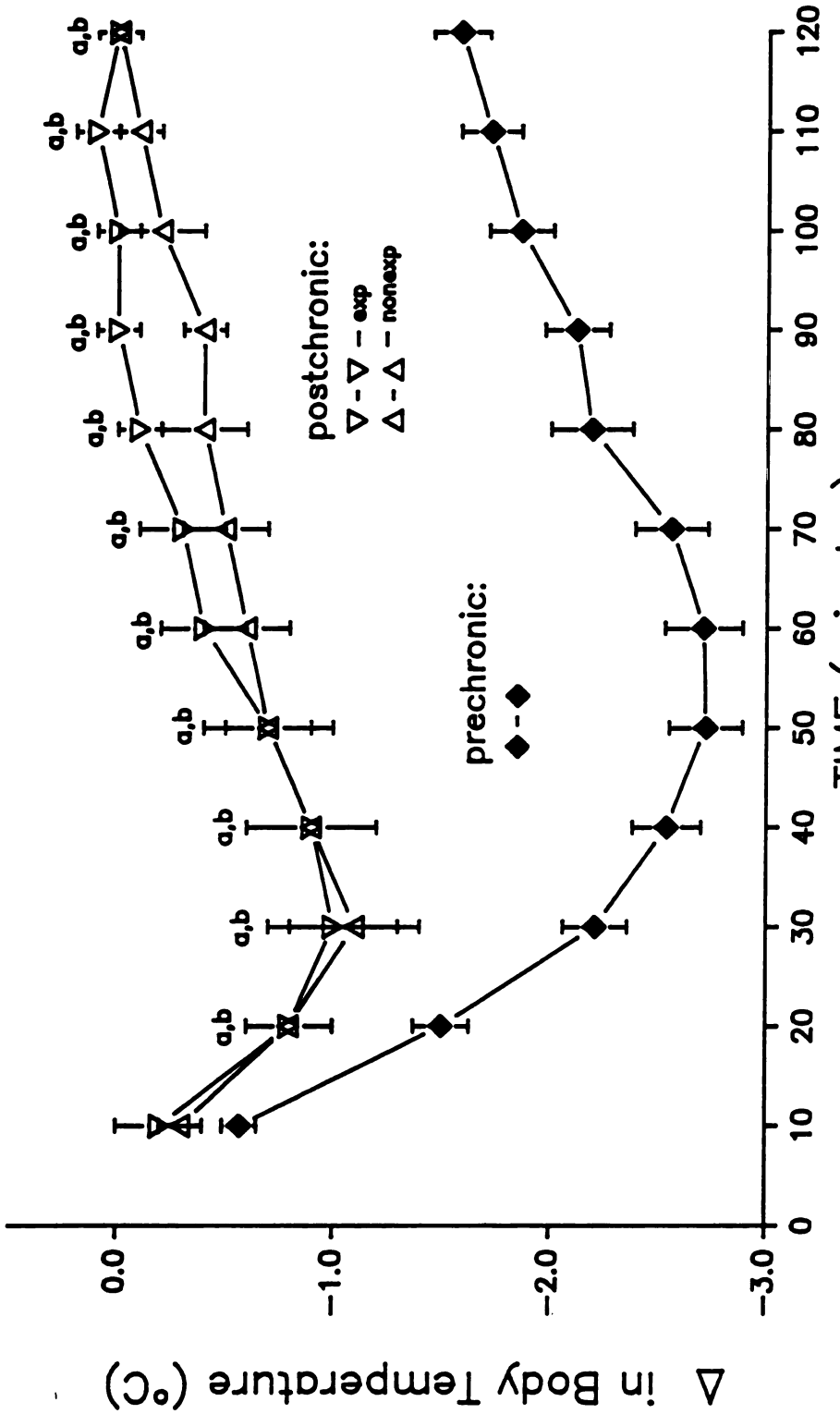

FIGURE 5: Hypothermia tolerance development to a 40 mg/kg pentobarbital test dose after chronic drug treatment. Time course of change in body temperature ± s.e.m. a-CHR/EXP sig. diff. from prechronic, b-CHR/NonEXP sig. diff. from prechronic, ($p<0.05$).

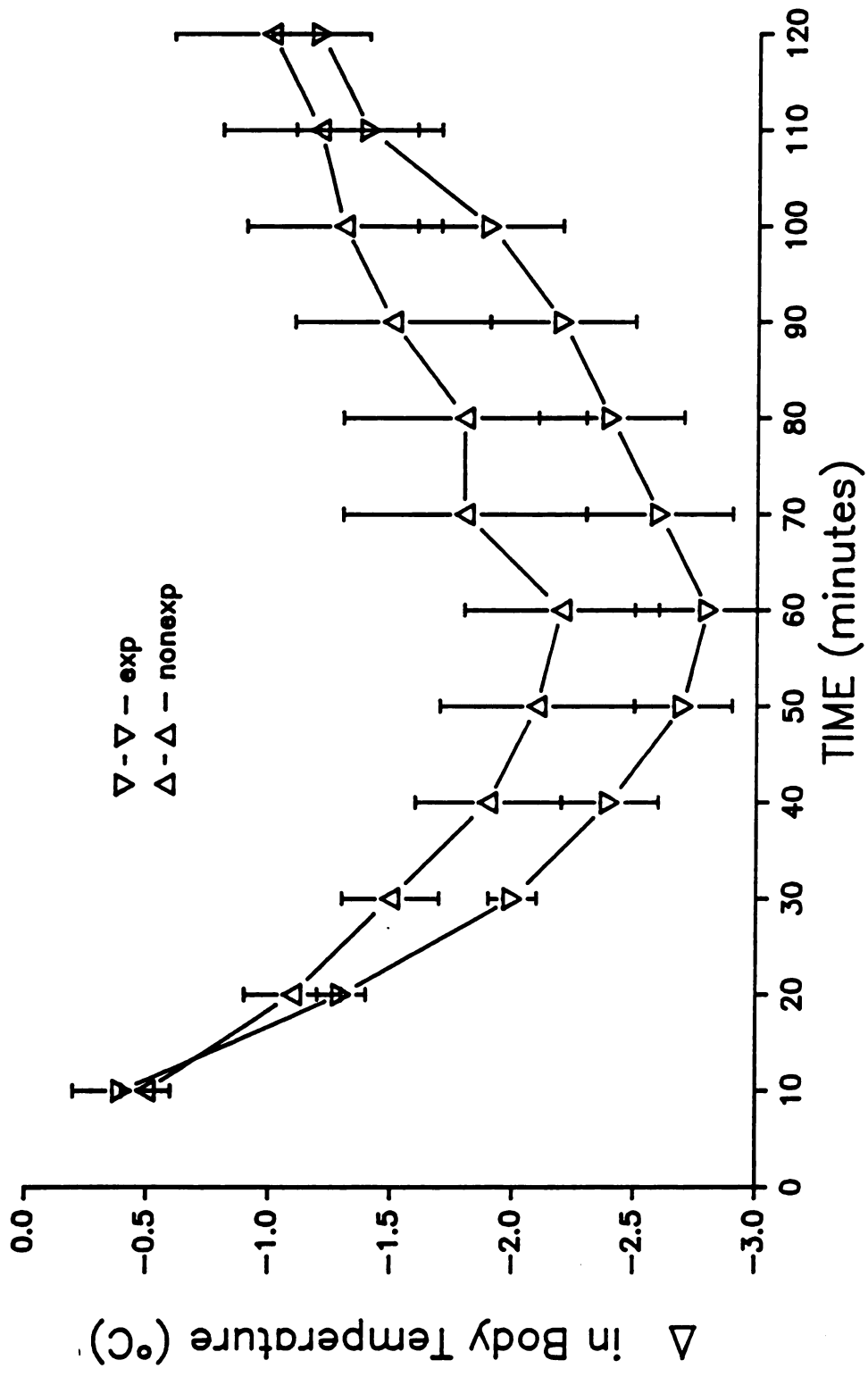

FIGURE 6: Hypothermia tolerance development to an 80 mg/kg pentobarbital test dose after chronic drug treatment. Time course of change in body temperature ± s.e.m.

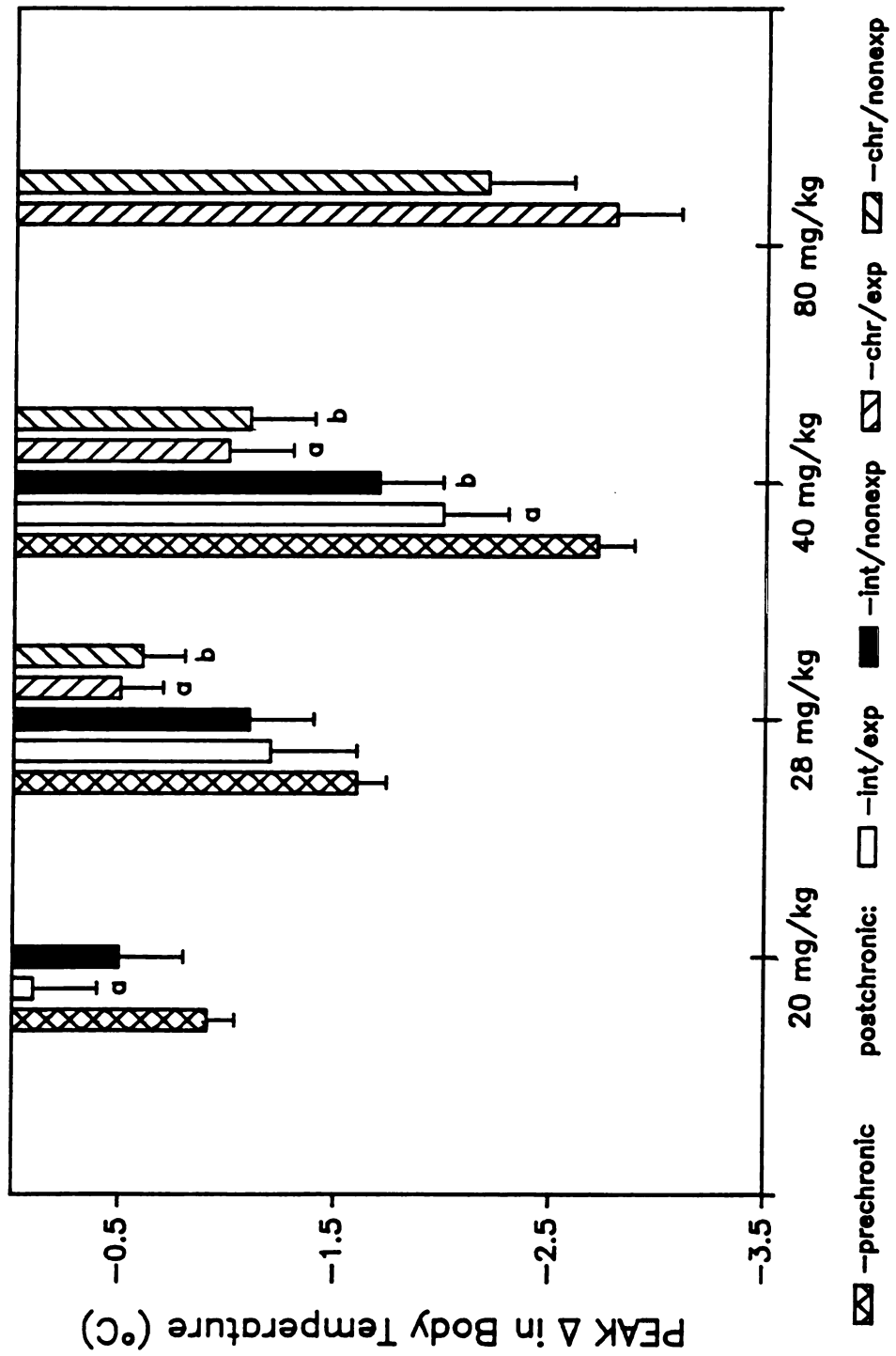

FIGURE 7: Pentobarbital tolerance development to peak hypothermia ± s.e.m. after intermittent or chronic drug treatment. a-EXP sig. diff. from prechronic, b-NonEXP sig. diff. from prechronic, ($p<0.05$).

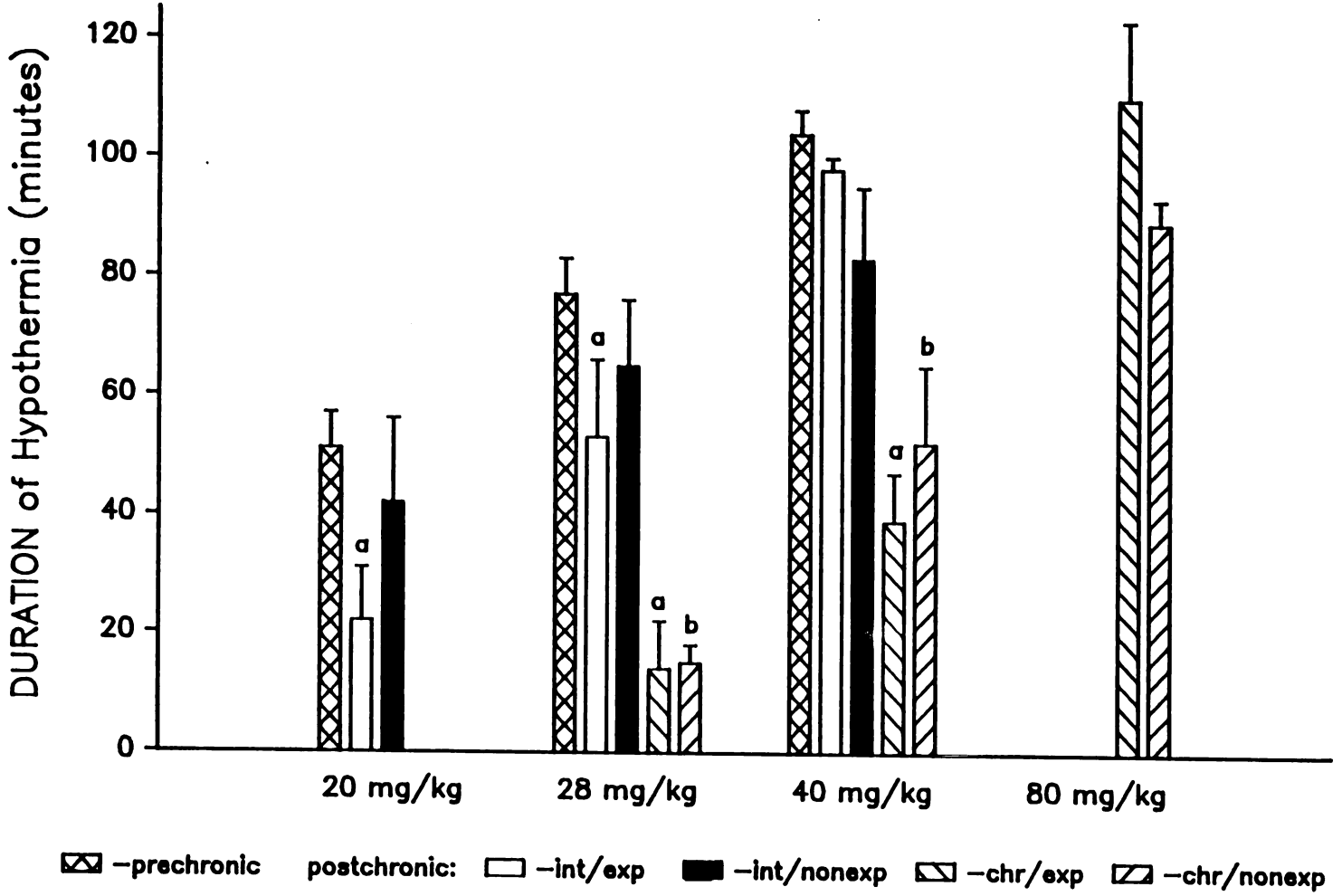

FIGURE 8: Pentobarbital tolerance development to duration of hypothermia ± s.e.m. after intermittent or chronic drug treatment. a-EXP sig. diff. from prechronic, b-NonEXP sig. diff. from prechronic, ($p<0.05$).

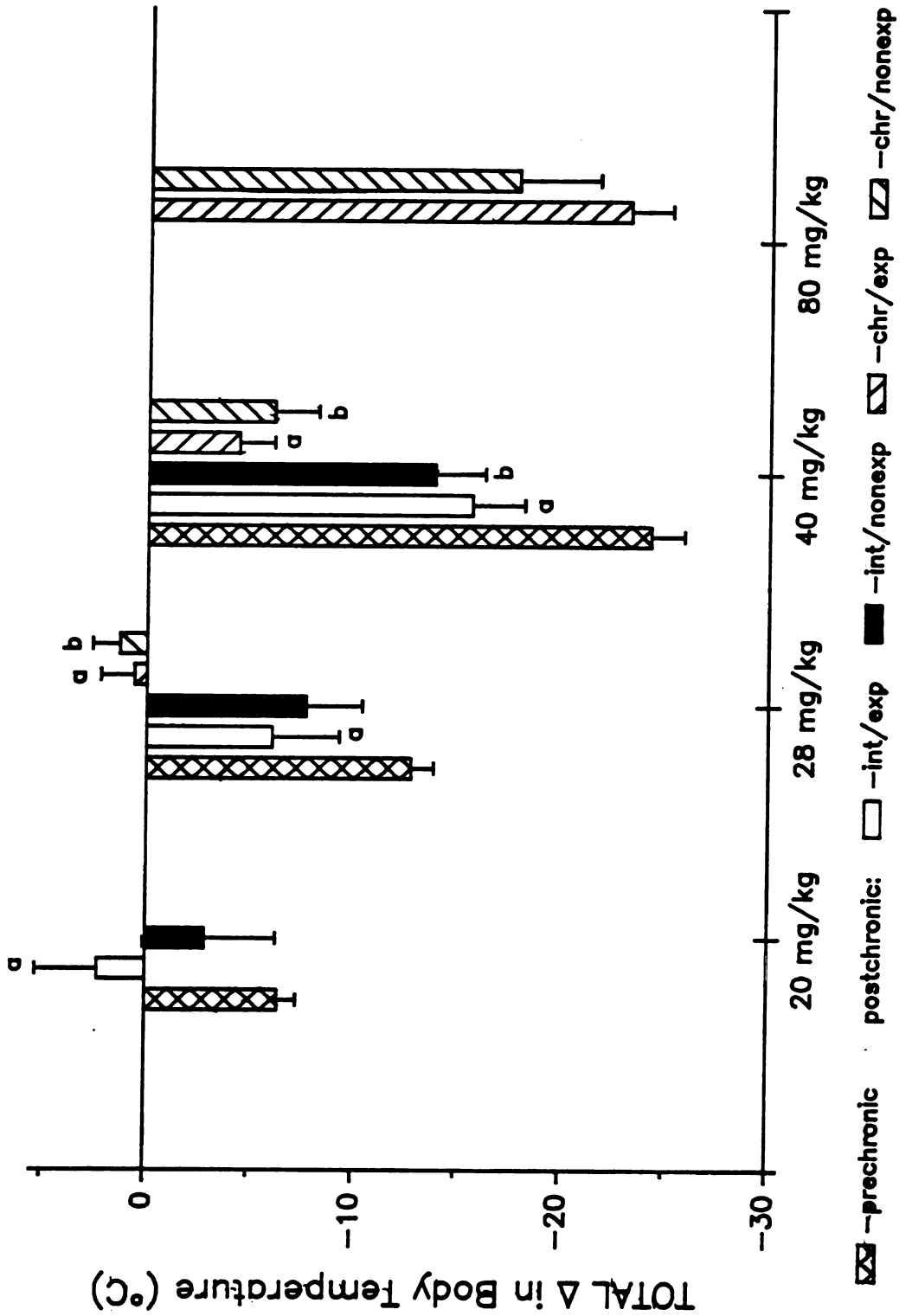

FIGURE 9: Pentobarbital tolerance development to total hypothermia (see text for details) ± s.e.m. after intermittent or chronic drug treatment. a-EXP sig. diff. from prechronic, b-NonEXP sig. diff. from prechronic, ($p<0.05$).

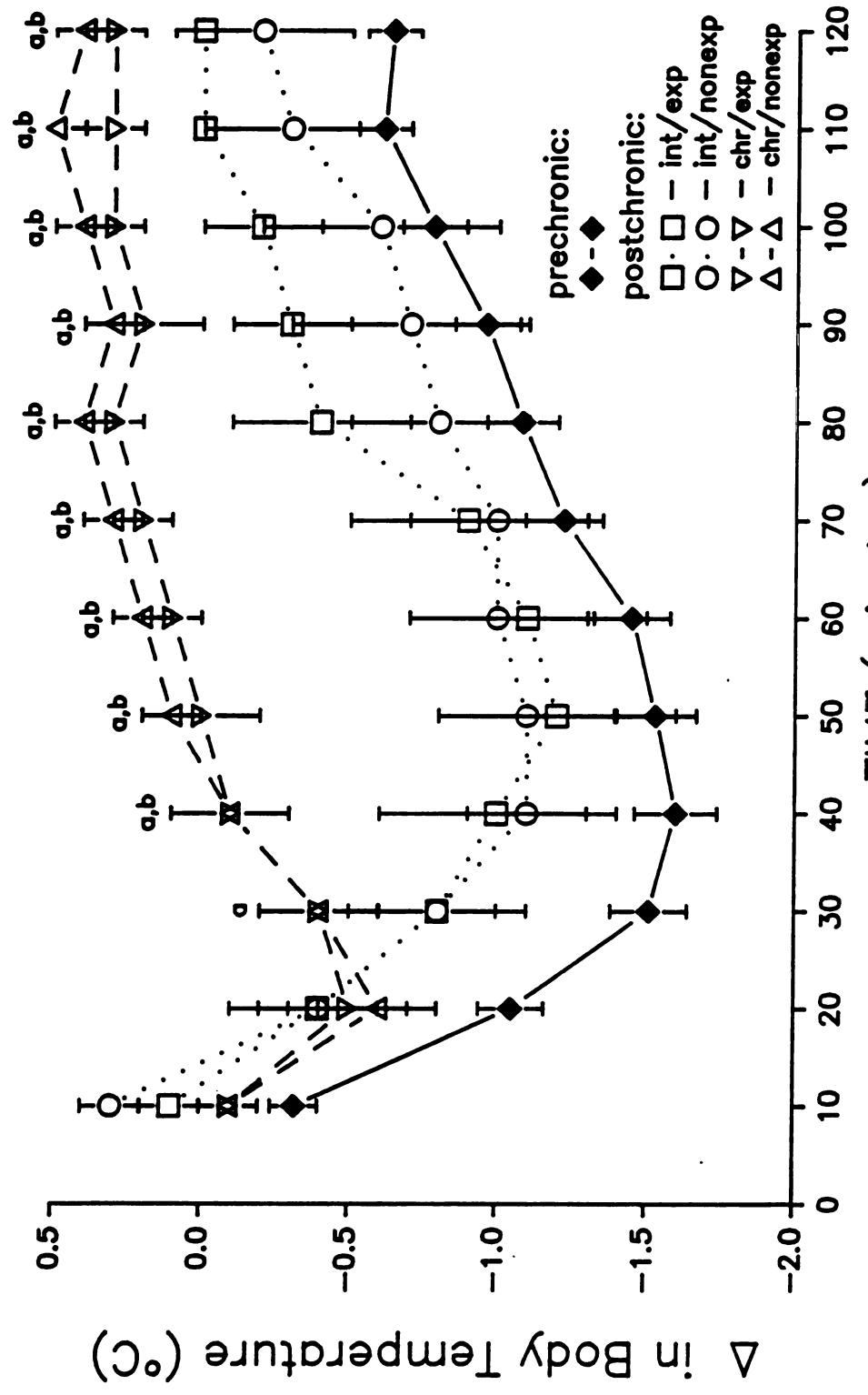

FIGURE 10: Hypothermia time course for a 28 mg/kg pentobarbital test dose. Comparison of intermittent vs. chronic drug treatment. Change in body temperature ± s.e.m. a-CHR/EXP sig. diff. from INT/EXP, b-CHR/NonEXP sig. diff. from INT/NonEXP, ($p<0.05$).

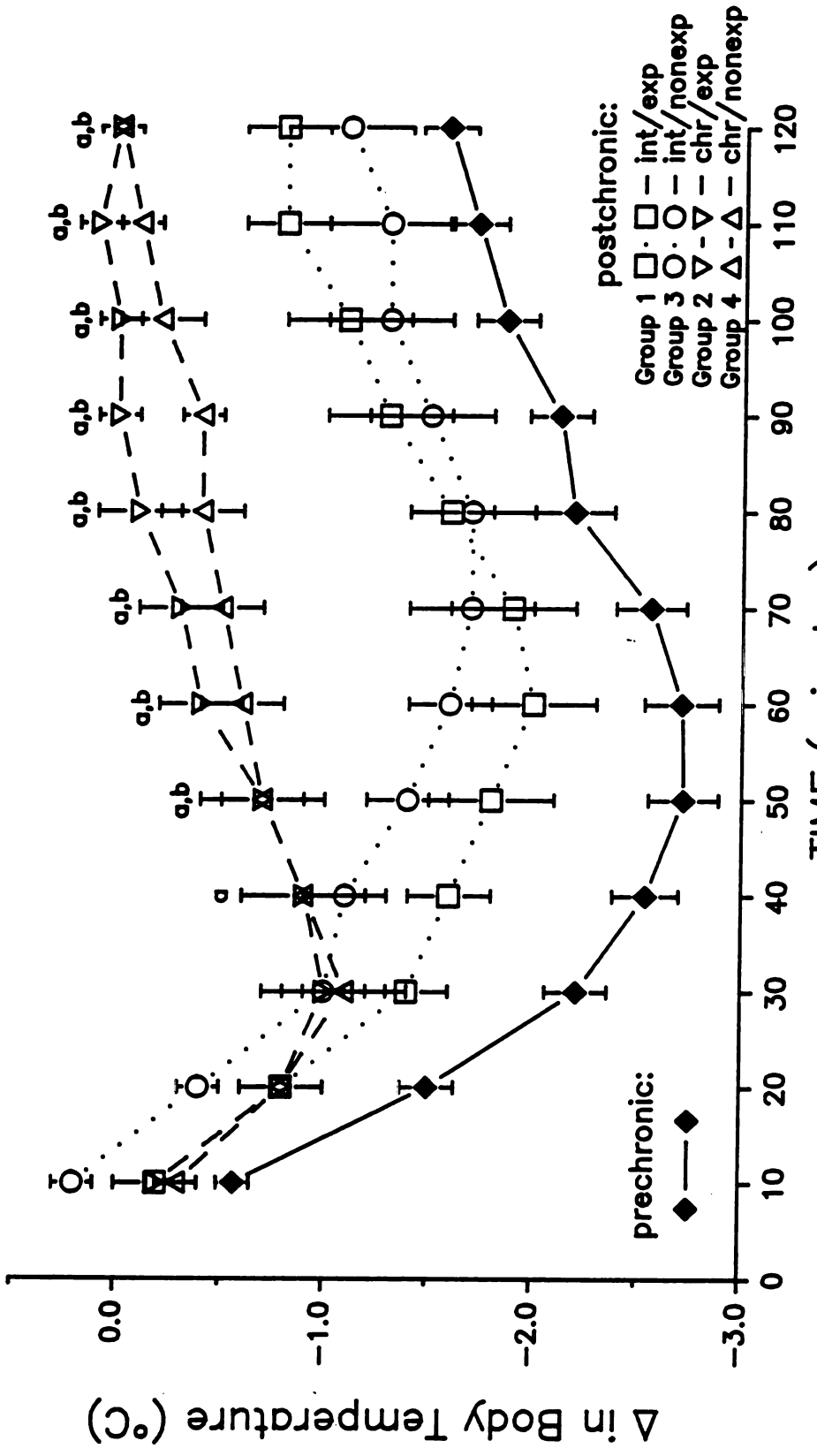

FIGURE 11: Hypothermia time course for a 40 mg/kg pentobarbital test dose. Comparison of intermittent vs. chronic drug treatment. Change in body temperature ± s.e.m. a-CHR/EXP sig. diff. from INT/EXP, b-CHR/NonEXP sig. diff. from INT/NonEXP, ($p<0.05$).

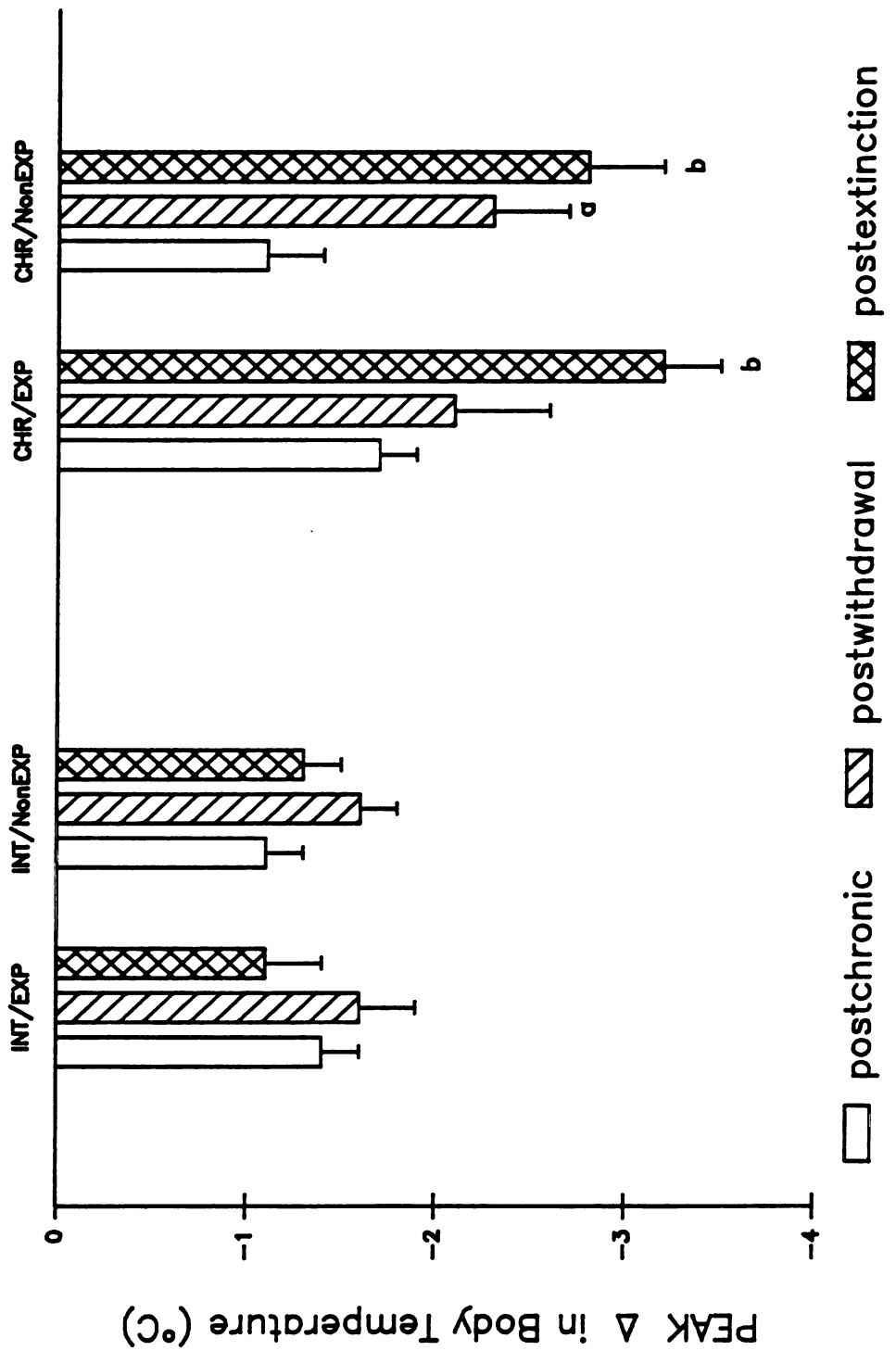

FIGURE 12: Loss of pentobarbital tolerance to peak hypothermia ± s.e.m. after a withdrawal period and extinction trials. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, ($p<0.05$).

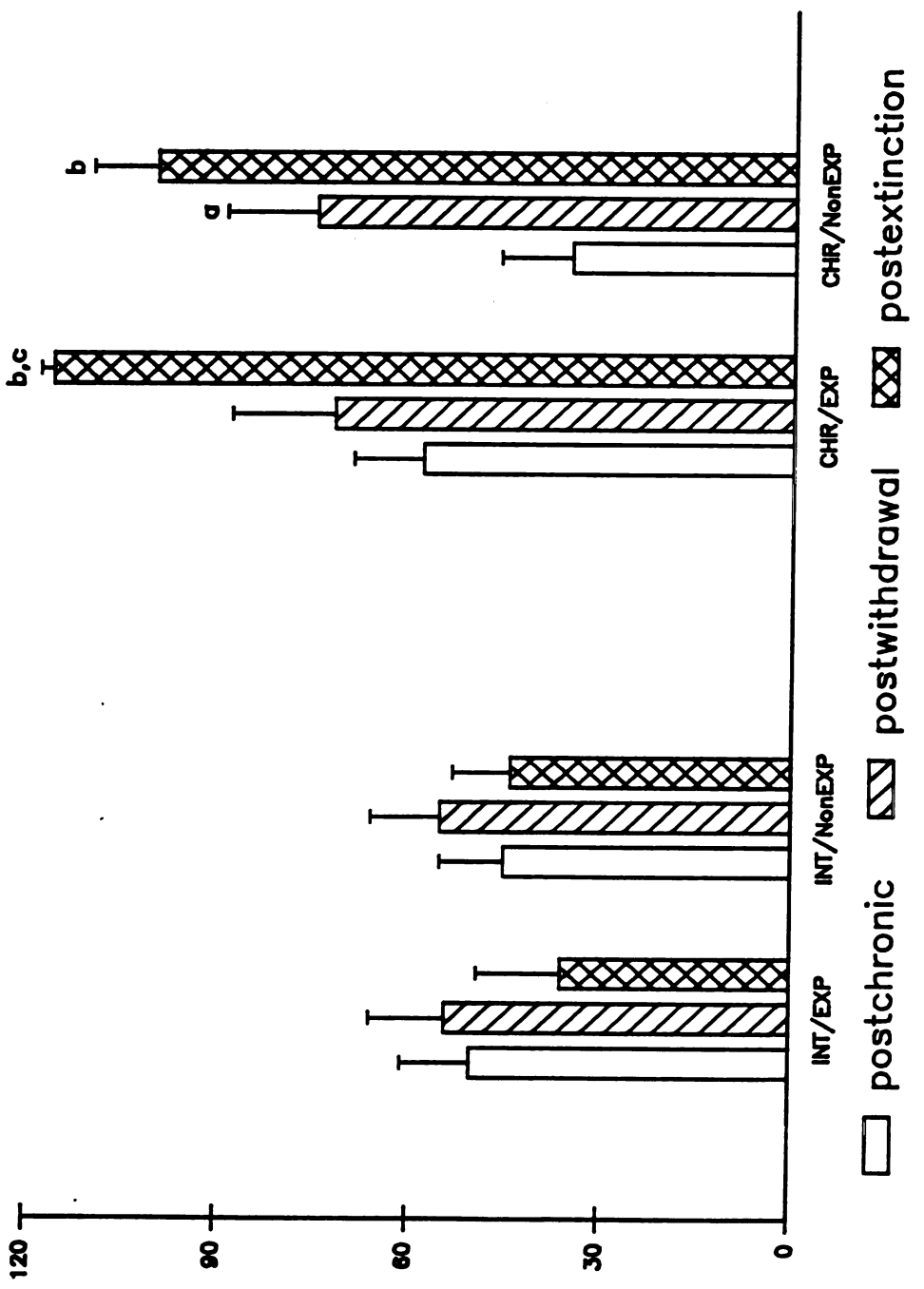

FIGURE 13: Loss of pentobarbital tolerance to duration of hypothermia ± s.e.m. after a withdrawal period and extinction trials. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, c-postextinction sig. diff. from postwithdrawal, ($p<0.05$).

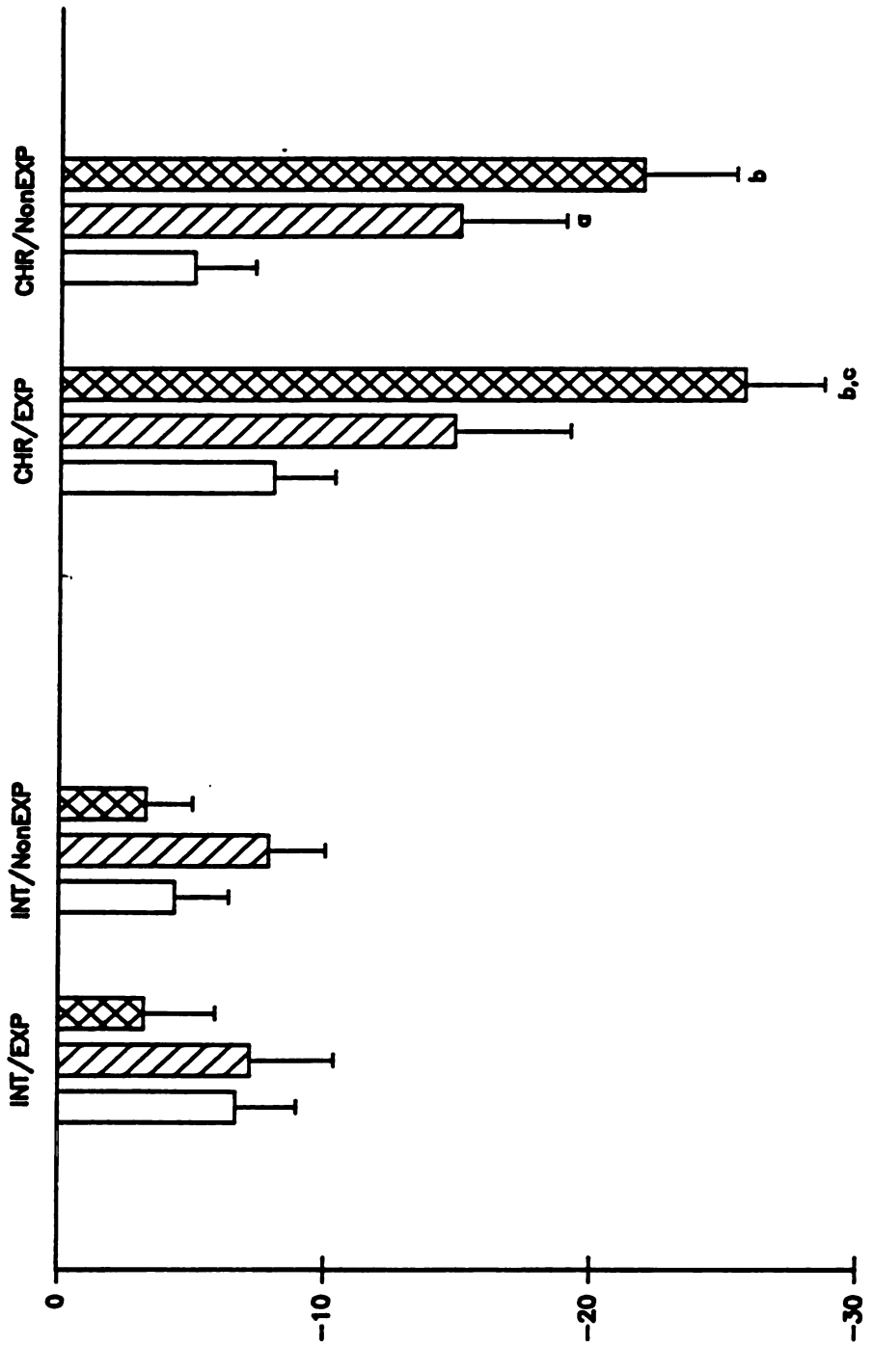

FIGURE 14: Loss of pentobarbital tolerance to total hypothermia ± s.e.m. after a withdrawal period and extinction trials. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, c-postextinction sig. diff. from postwithdrawal, ($p<0.05$).

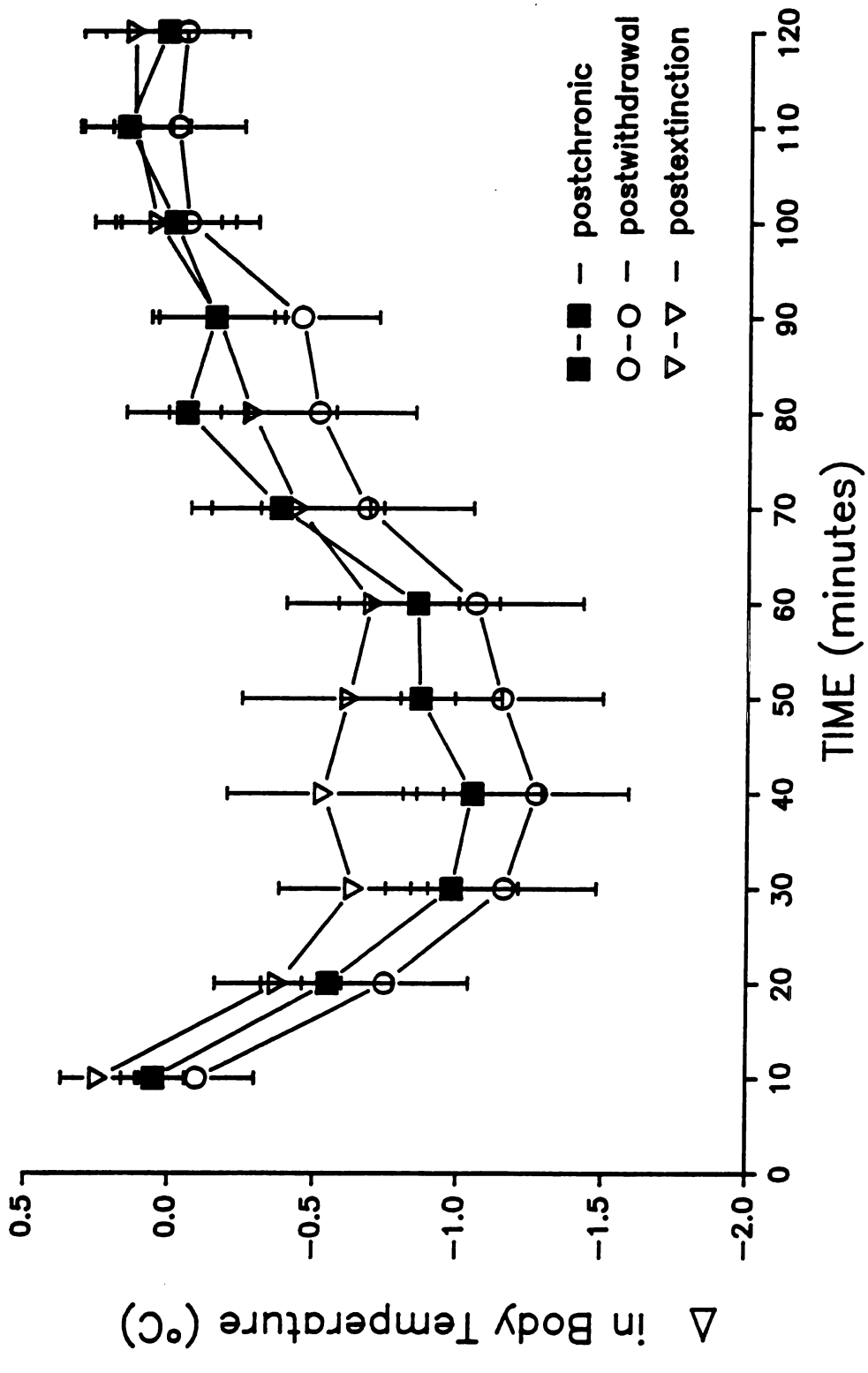

FIGURE 15: INT/EXP loss of pentobarbital tolerance to hypothermia after a withdrawal period and extinction trials. Time course of change in body temperature ± s.e.m.

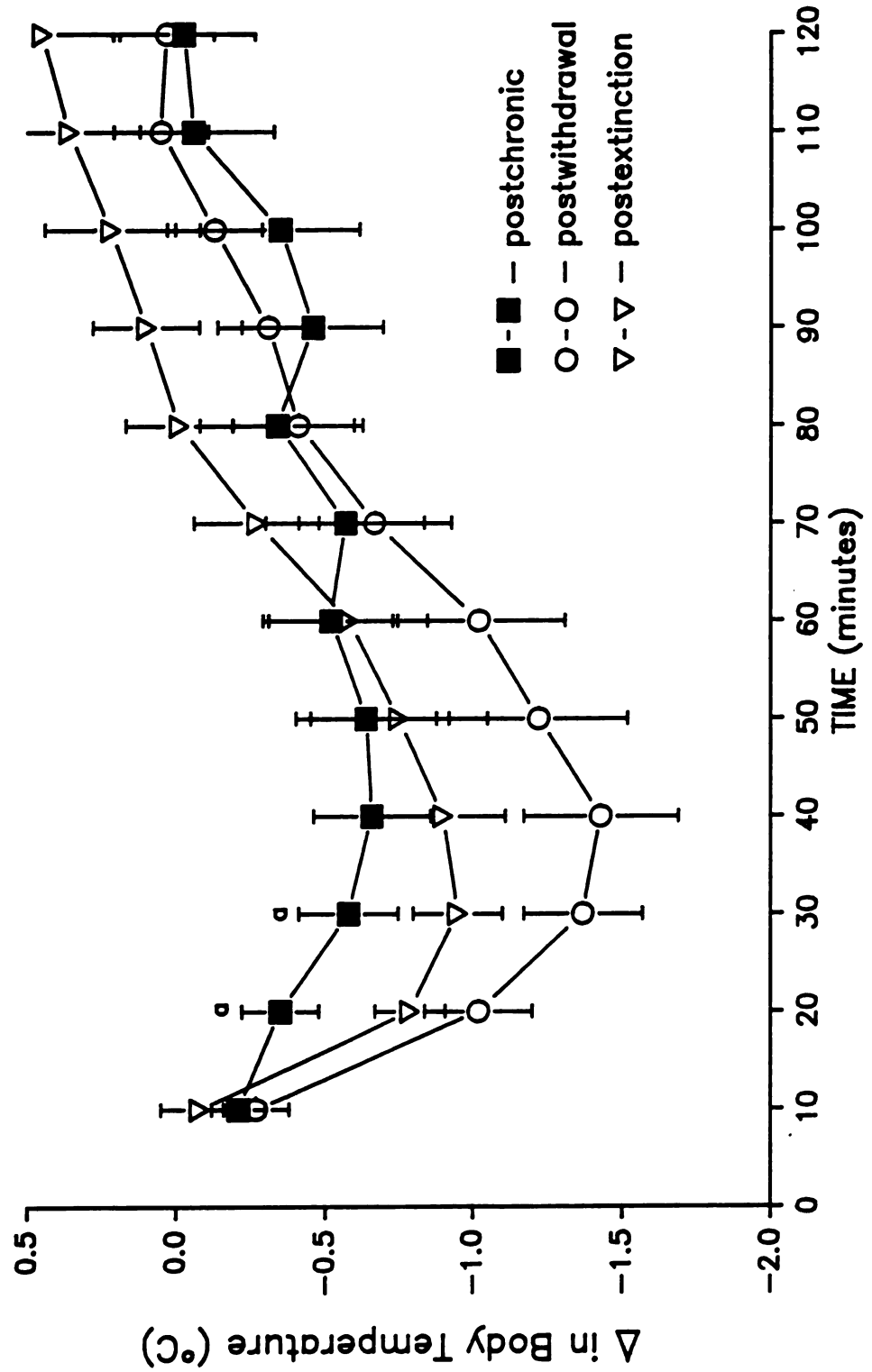

FIGURE 16: INT/NonEXP loss of pentobarbital tolerance to hypothermia after a withdrawal period and extinction trials. Time course of change in body temperature ± s.e.m. a-postwithdrawal sig. diff. from postchronic, ($p<0.05$).

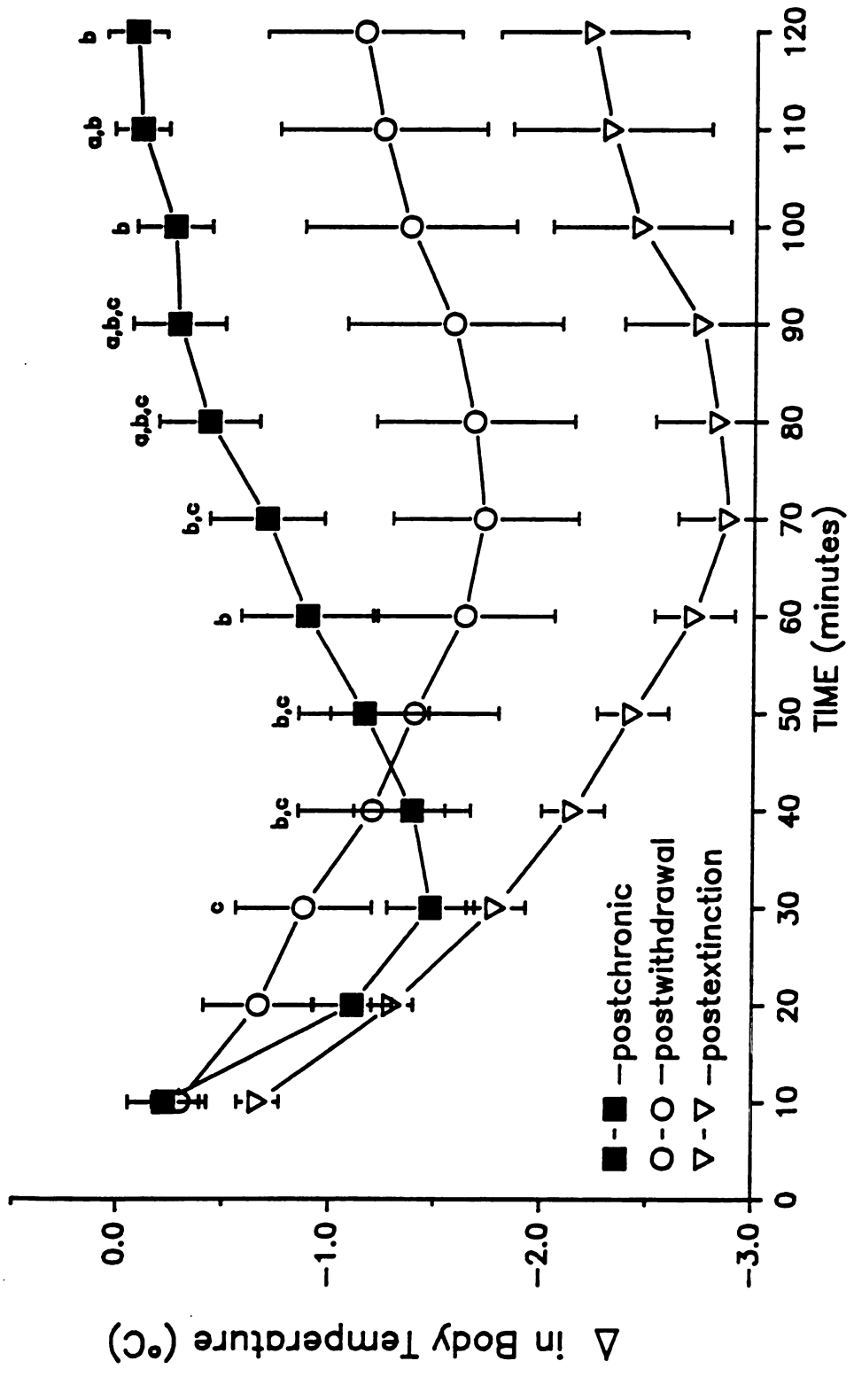

FIGURE 17: CHR/EXP loss of pentobarbital tolerance to hypothermia after a withdrawal period and extinction trials. Time course of change in body temperature ± s.e.m. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, c-postextinction sig. diff. from postwithdrawal, ($p<0.05$).

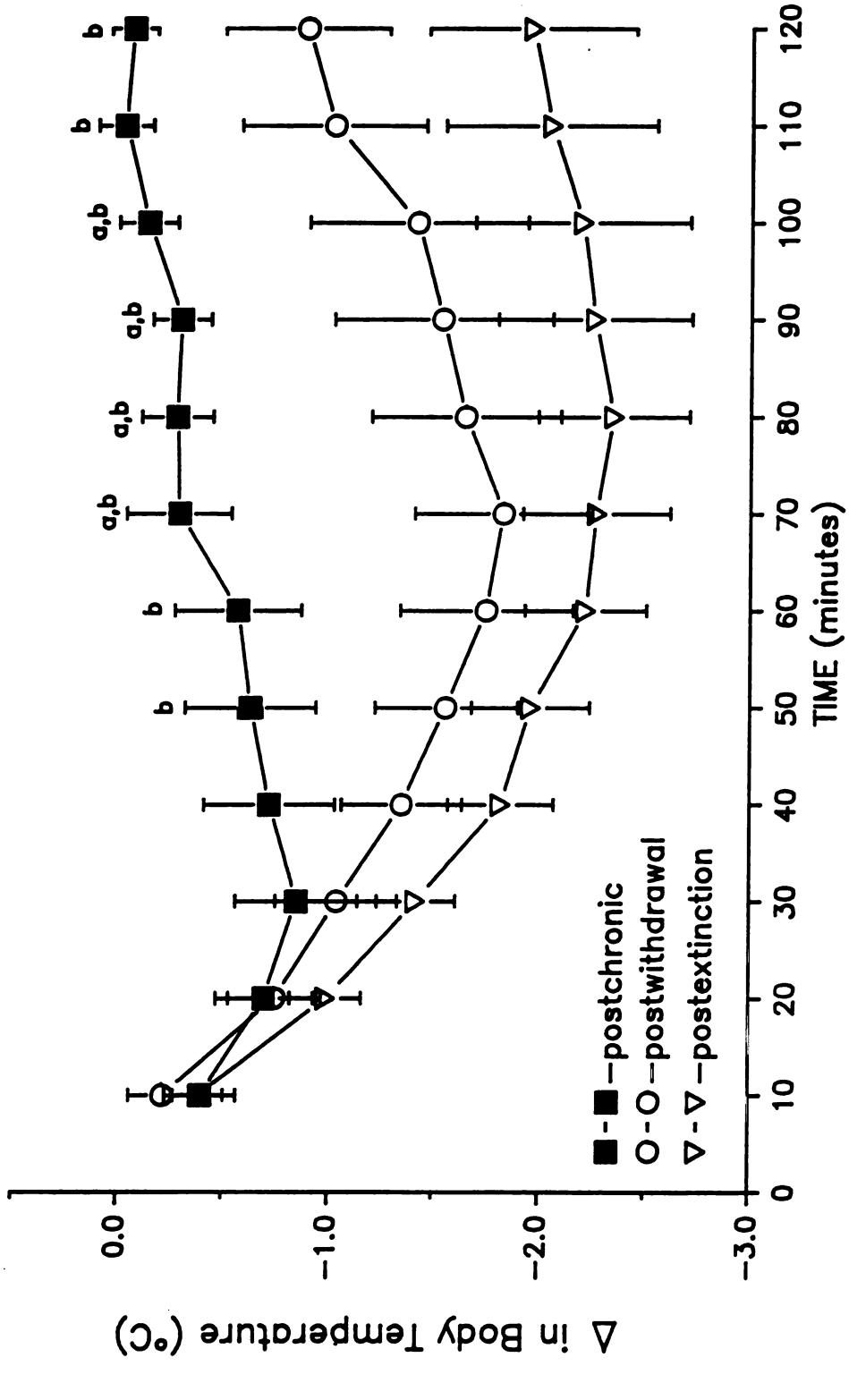

FIGURE 18: CHR/NonEXP loss of pentobarbital tolerance to hypothermia after a withdrawal period and extinction trials. Time course of change in body temperature ± s.e.m. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, ($p<0.05$).

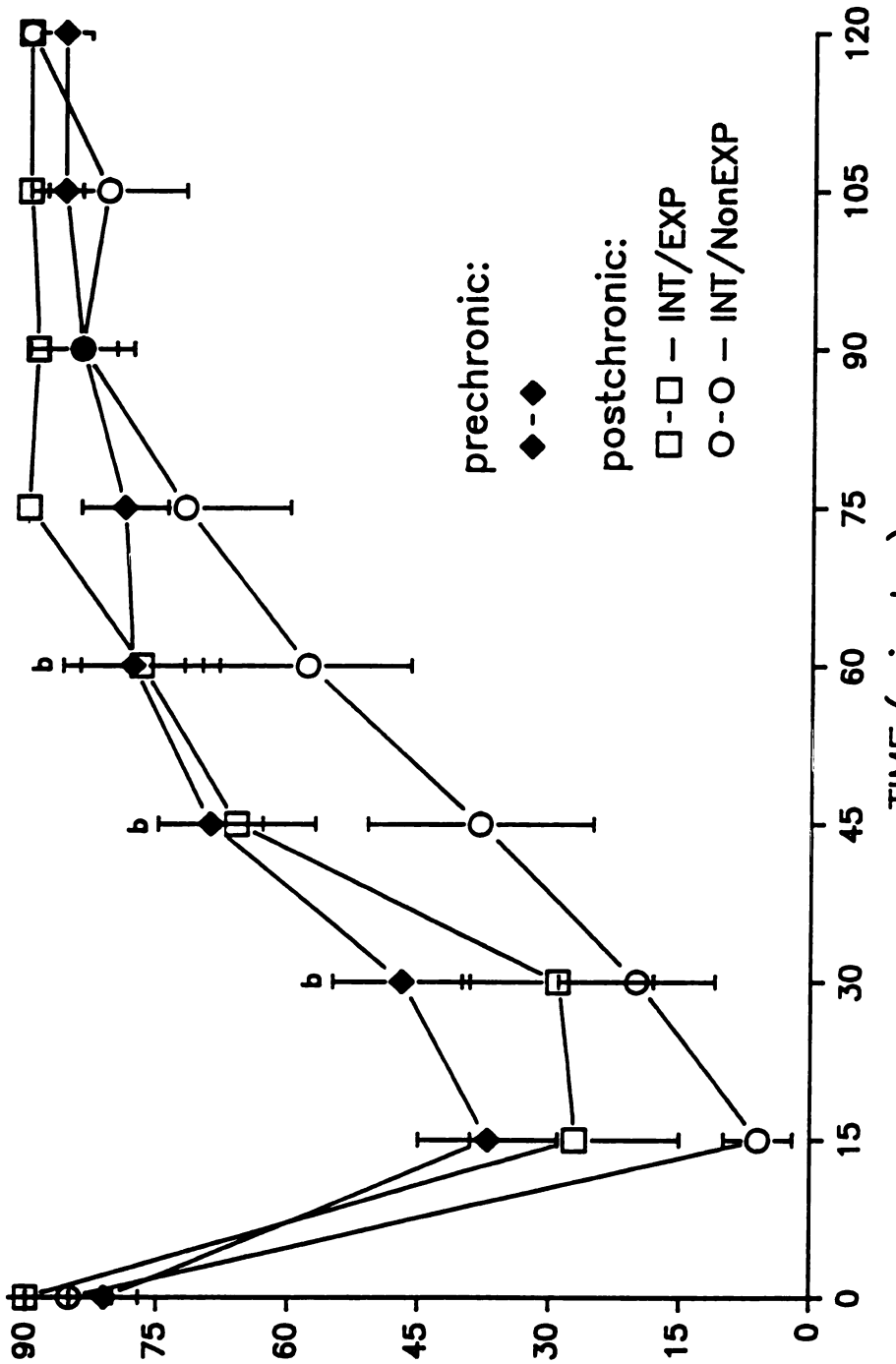

FIGURE 19: Ataxia tolerance development to a 20 mg/kg pentobarbital test dose after intermittent drug treatment. Time course of rotarod performance ± s.e.m. b-INT/NonEXP sig. diff. from prechronic, ($p<0.05$).

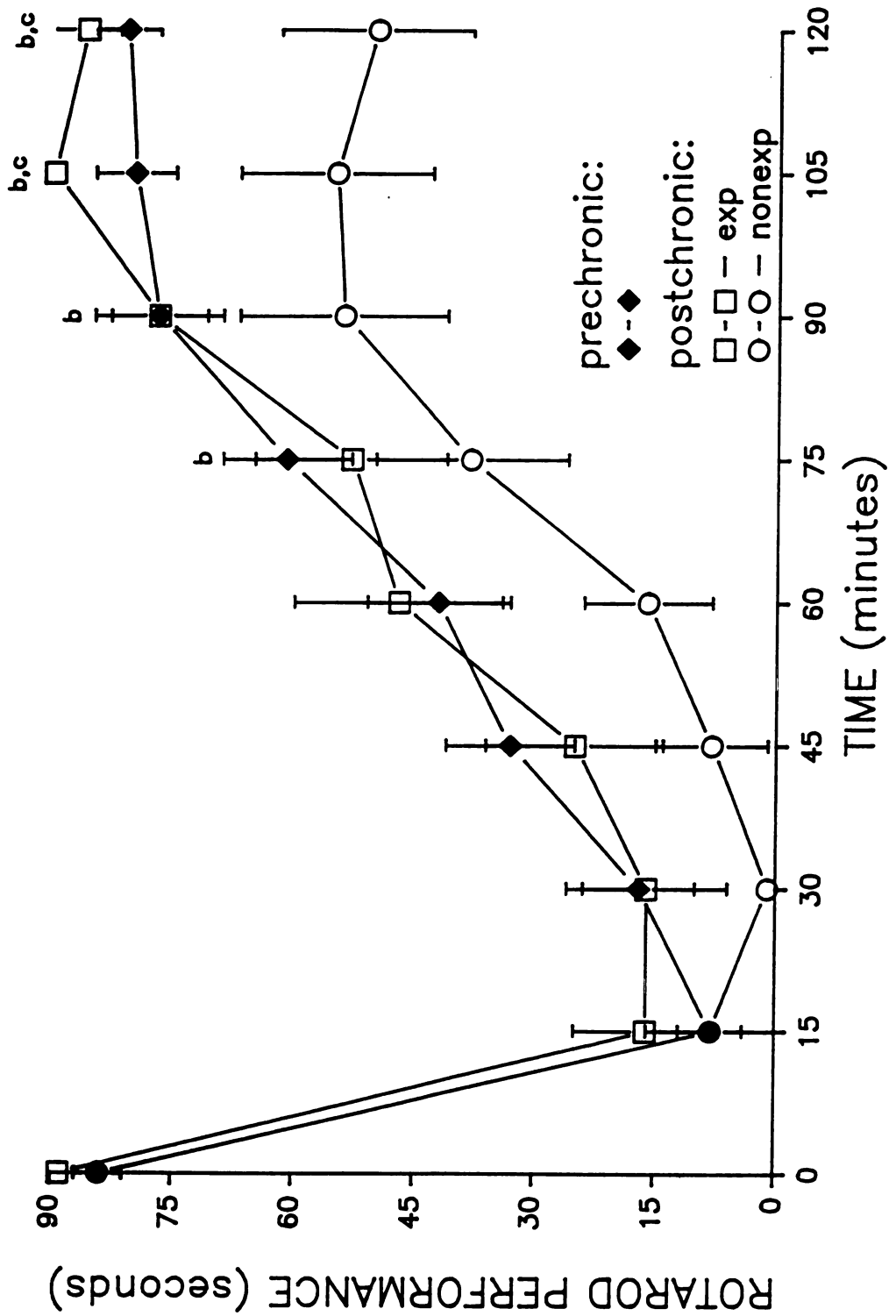

FIGURE 20: Ataxia tolerance development to a 28 mg/kg pentobarbital test dose after intermittent drug treatment. Time course of rotarod performance ± s.e.m. b-INT/NonEXP sig. diff. from prechronic, c-EXP sig. diff. from NonEXP, ($p<0.05$).

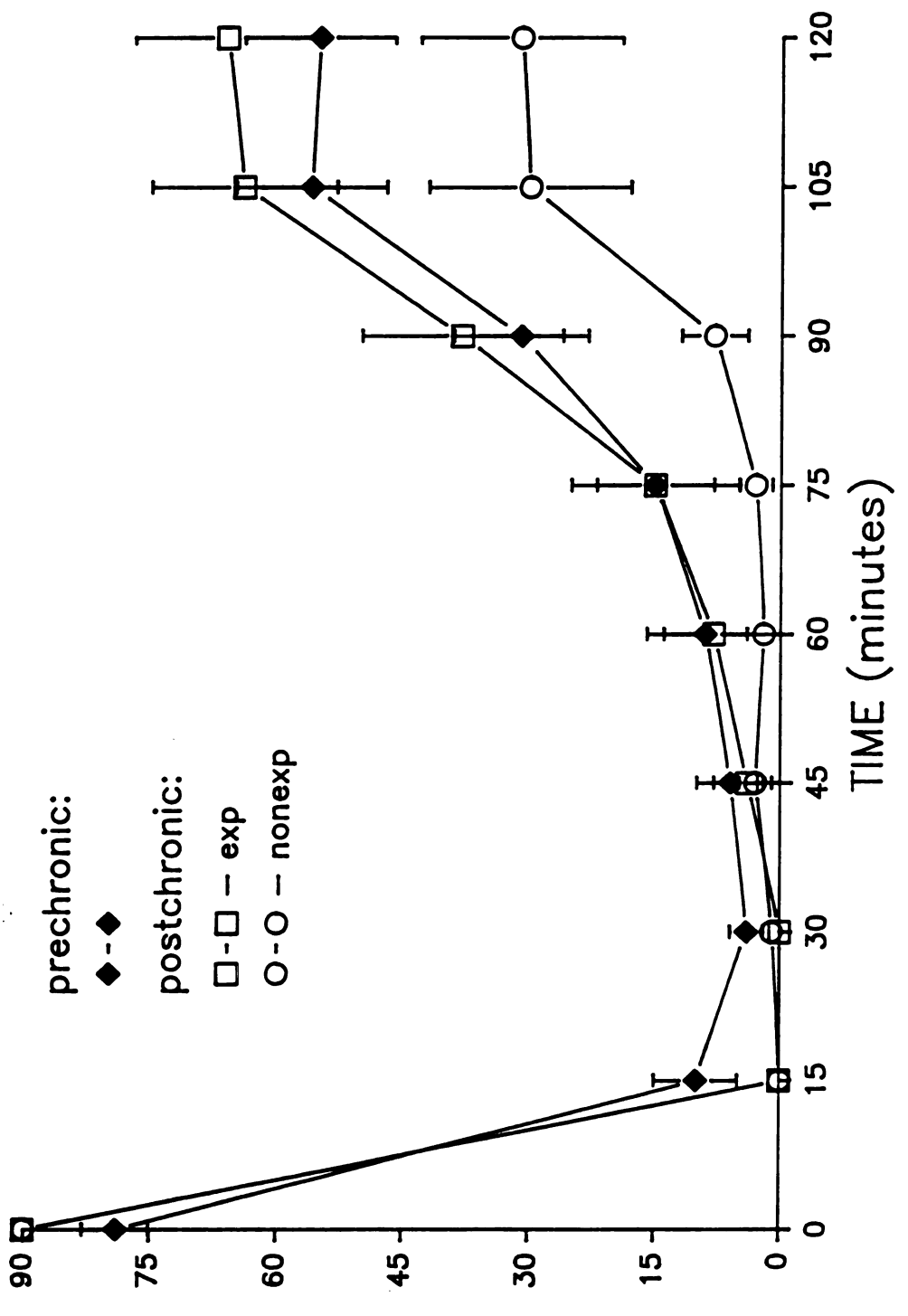

FIGURE 21: Ataxia tolerance development to a 40 mg/kg pentobarbital test dose after intermittent drug treatment. Time course of rotarod performance ± s.e.m.

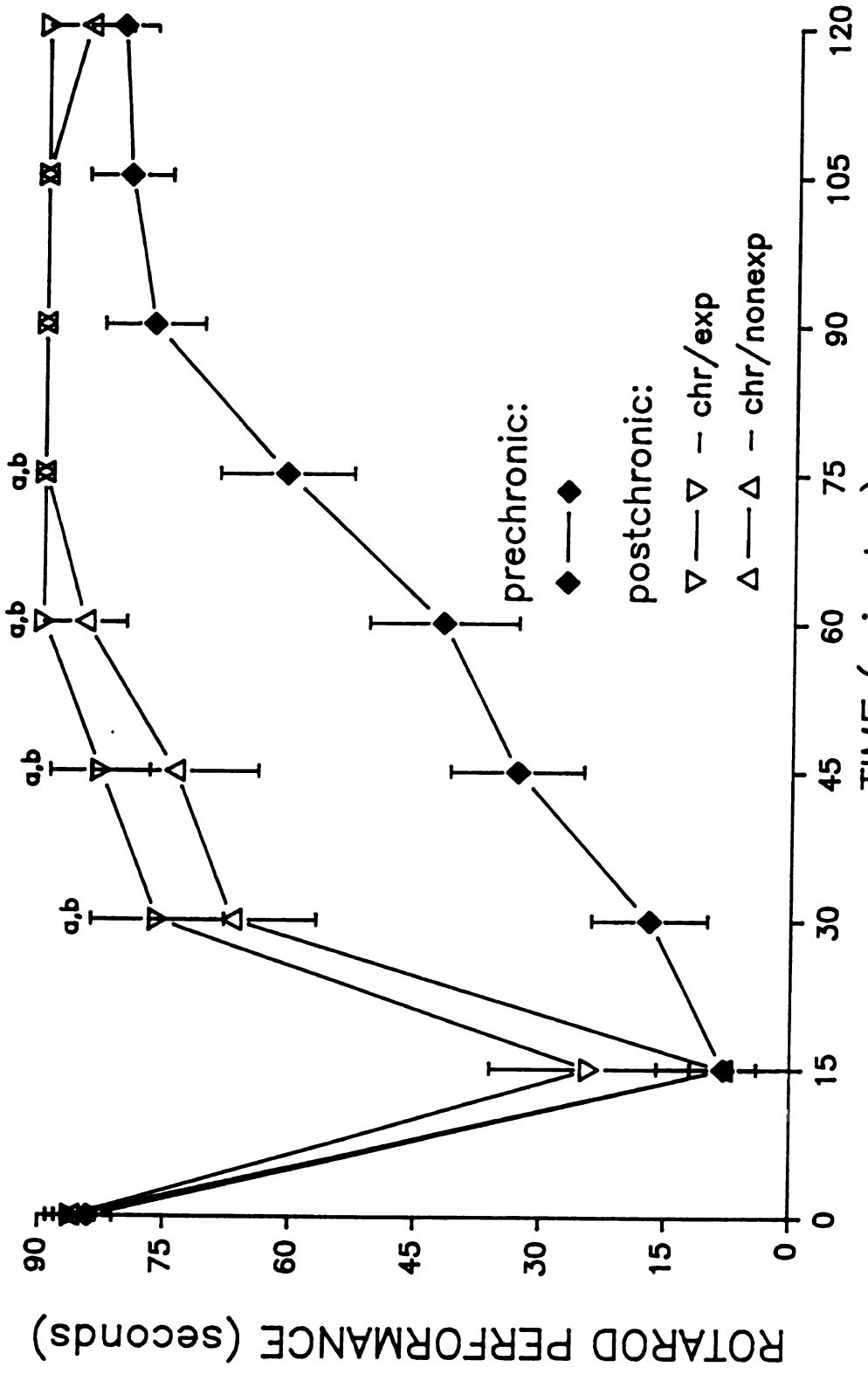

FIGURE 22: Ataxia tolerance development to a 28 mg/kg pentobarbital test dose after chronic drug treatment. Time course of rotarod performance ± s.e.m. a-CHR/EXP sig. diff. from prechronic, b-CHR/NonEXP sig. diff. from prechronic, ($p<0.05$).

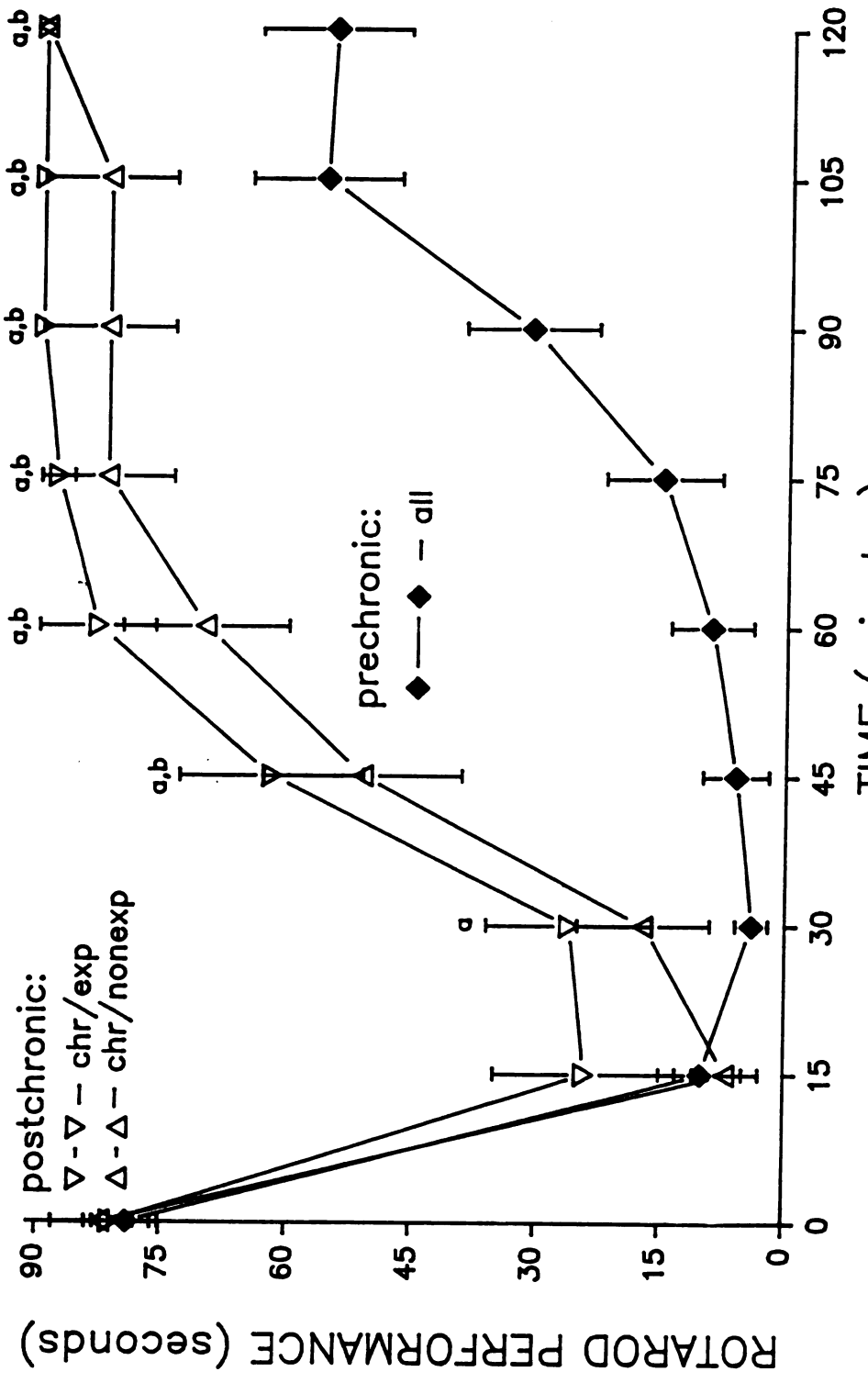

FIGURE 23: Ataxia tolerance development to a 40 mg/kg pentobarbital test dose after chronic drug treatment. Time course of rotarod performance ± s.e.m. a-CHR/EXP sig. diff. from prechronic, b-CHR/NonEXP sig. diff. from prechronic, ($p<0.05$).

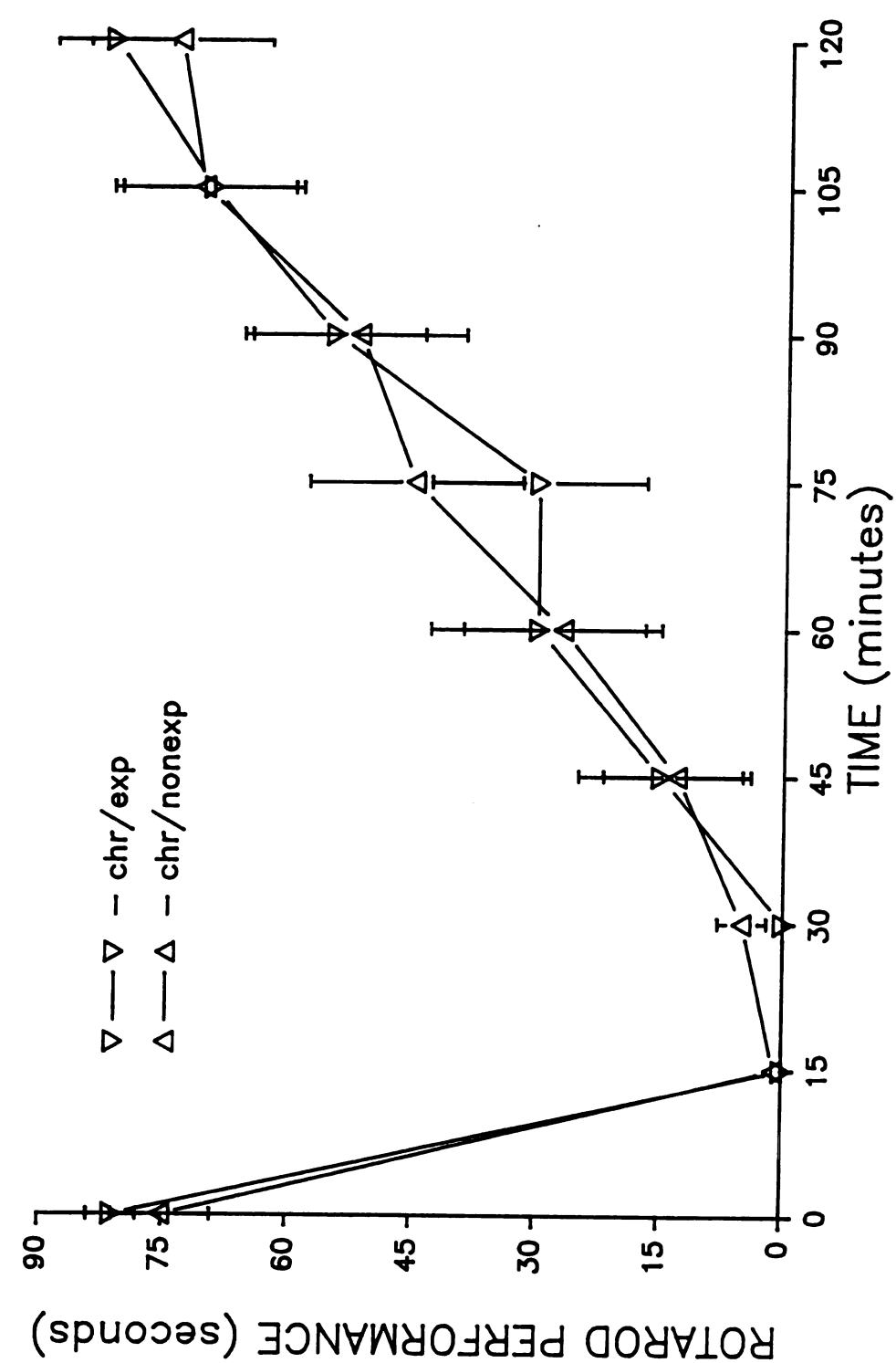

FIGURE 24: Ataxia tolerance development to an 80 mg/kg pentobarbital test dose after chronic drug treatment. Time course of rotarod performance ± s.e.m. ($p<0.05$).

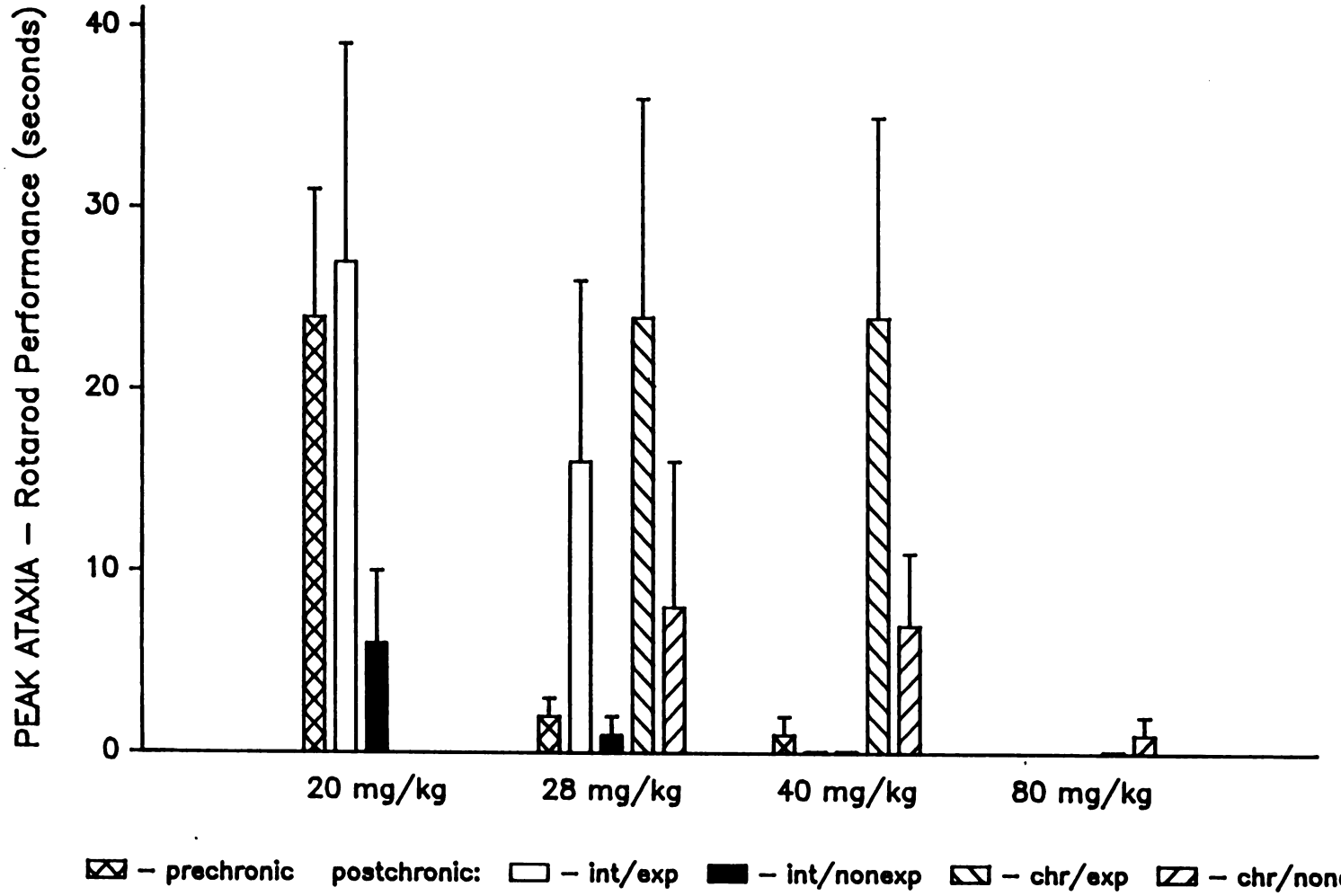

FIGURE 25: Pentobarbital tolerance development to peak ataxia ± s.e.m. after intermittent or chronic drug treatment.

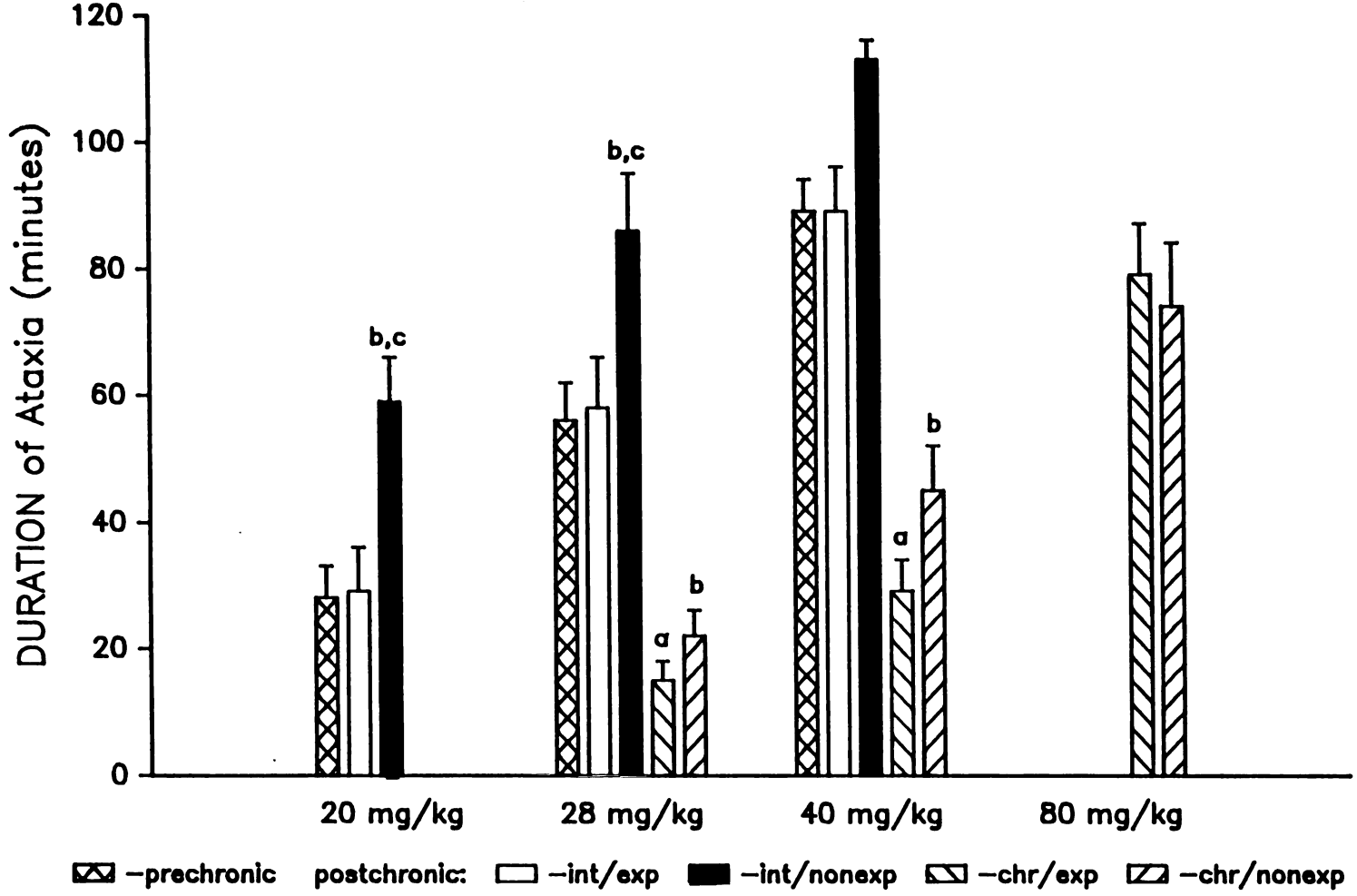

FIGURE 26: Pentobarbital tolerance development to duration of ataxia ± s.e.m. after intermittent or chronic drug treatment. a-EXP sig. diff. from prechronic, b-NonEXP sig. diff. from prechronic, c-EXP sig. diff. from NonEXP, ($p<0.05$).

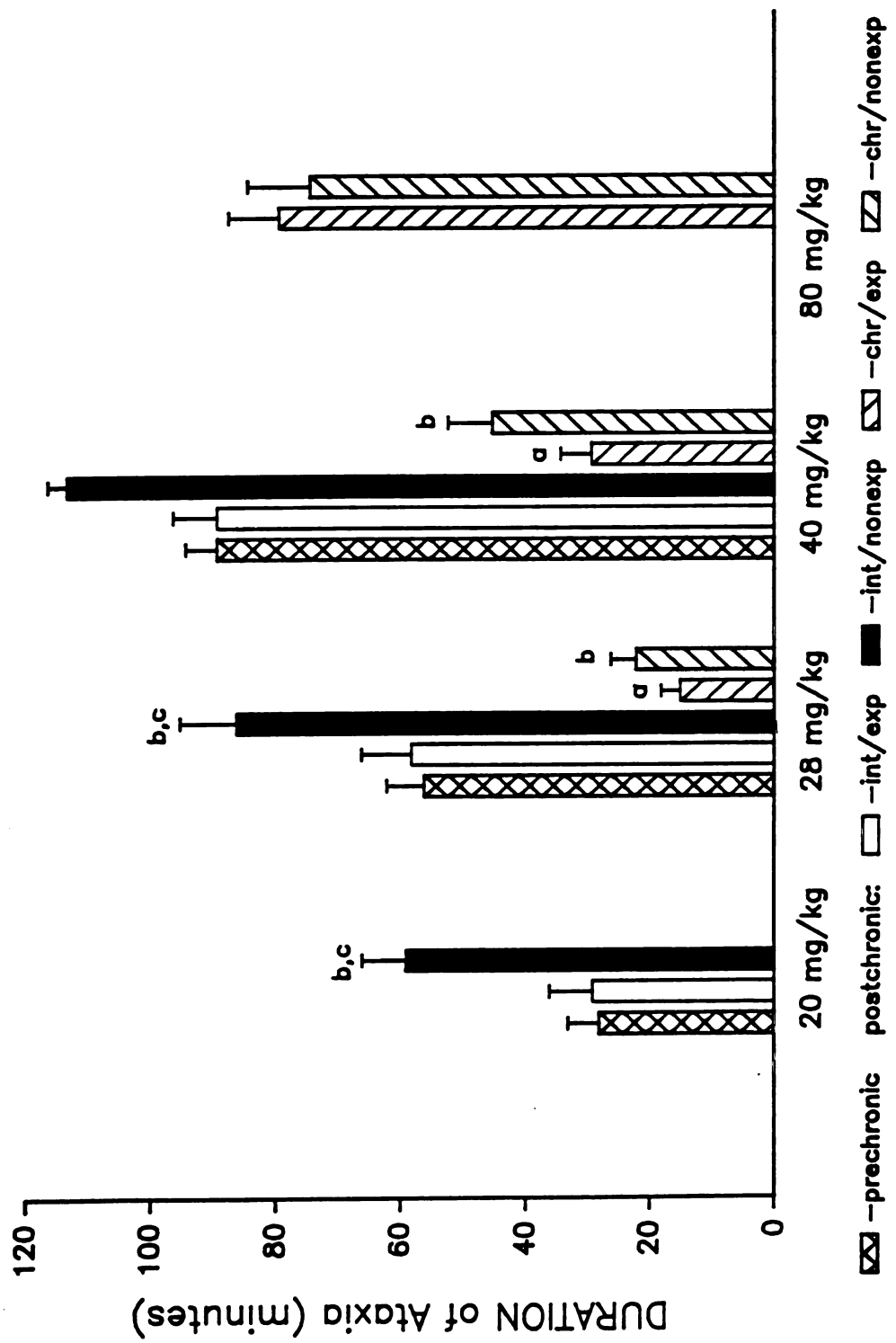

FIGURE 26: Pentobarbital tolerance development to duration of ataxia ± s.e.m. after intermittent or chronic drug treatment. a-EXP sig. diff. from prechronic, b-NonEXP sig. diff. from prechronic, c-EXP sig. diff. from NonEXP, ($p<0.05$).

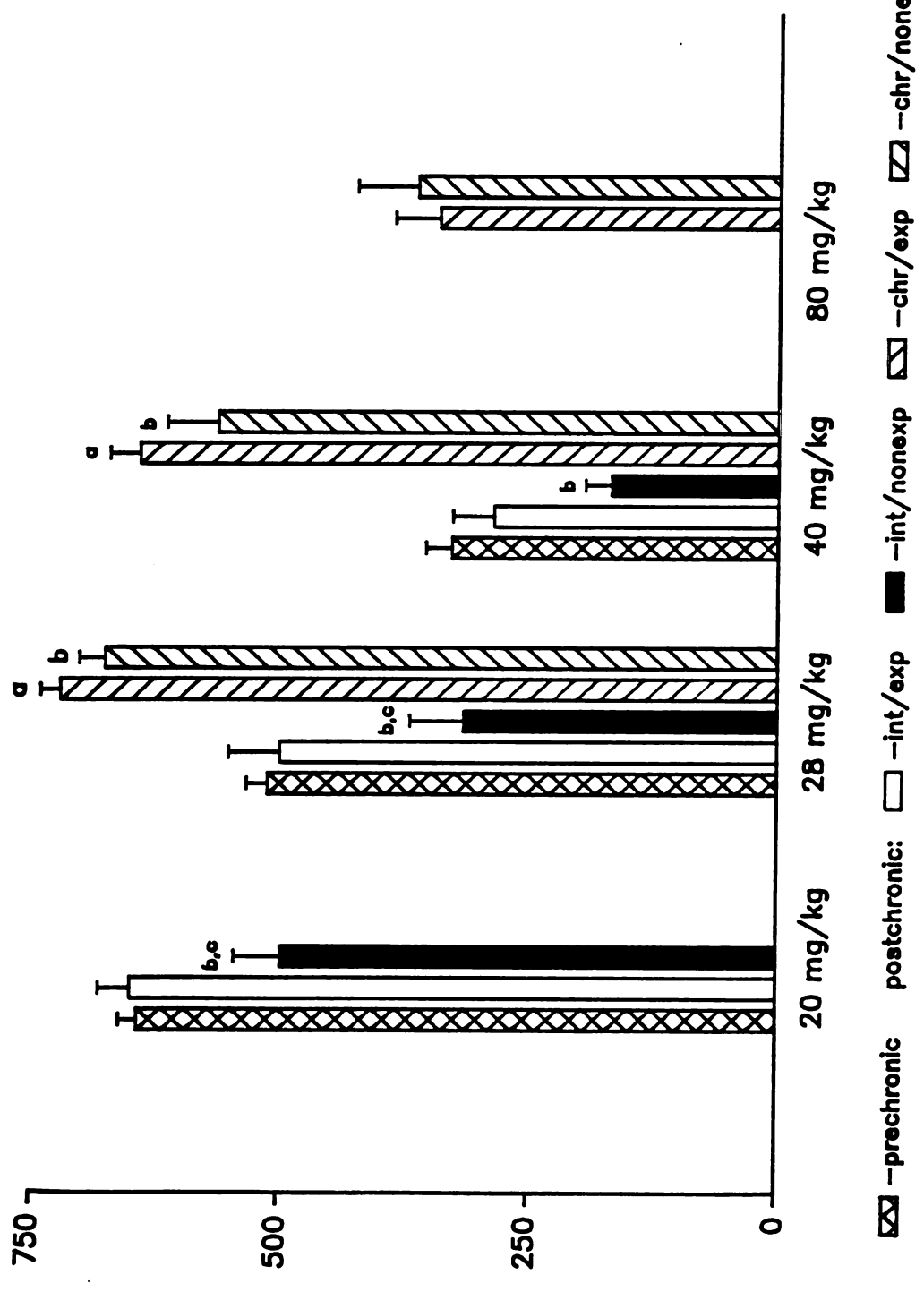

FIGURE 27: Pentobarbital tolerance development to total ataxia (see text for details) ± s.e.m. after intermittent or chronic drug treatment. a-EXP sig. diff. from prechronic, b-NonEXP sig. diff. from prechronic, c-EXP sig. diff. from NonEXP, ($p<0.05$).

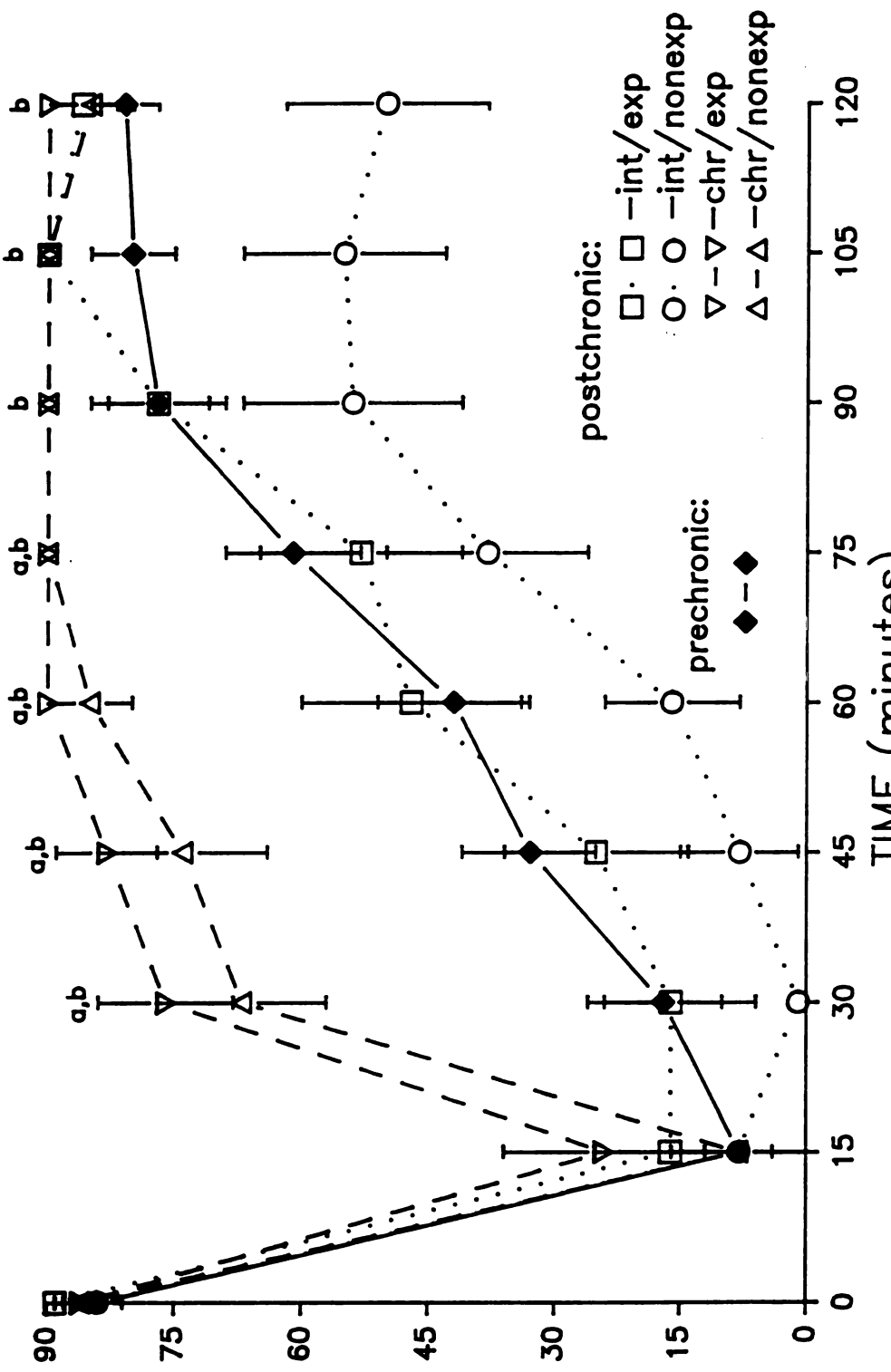

FIGURE 28: Ataxia time course for a 28 mg/kg pentobarbital test dose. Comparison of intermittent vs. chronic drug treatment. Rotarod performance ± s.e.m. a-CHR/EXP sig. diff. from INT/EXP, b-CHR/NonEXP sig. diff. from INT/NonEXP, ($p<0.05$).

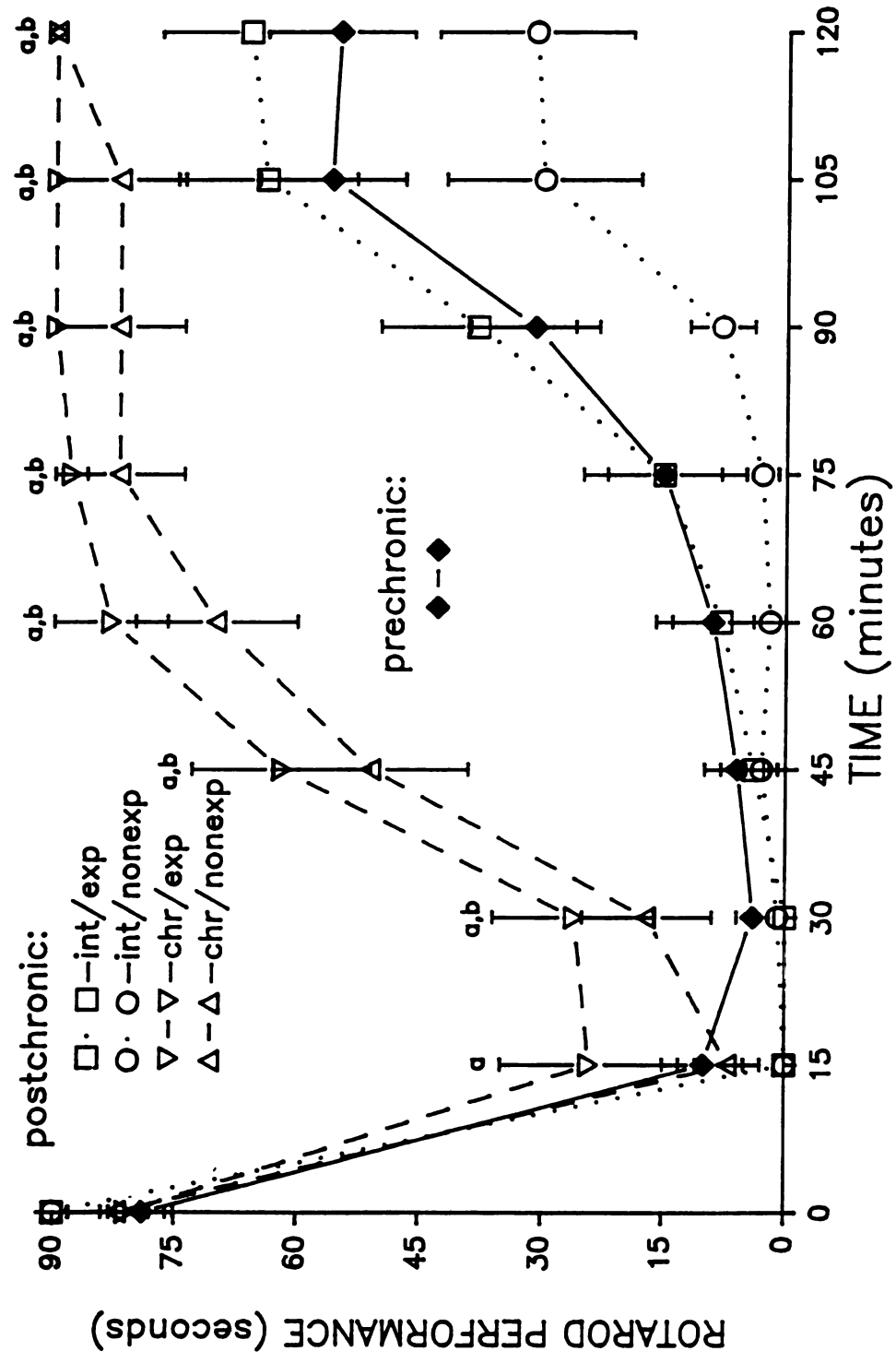

FIGURE 29: Ataxia time course for a 40 mg/kg pentobarbital test dose. Comparison of intermittent vs. chronic drug treatment. Rotarod performance ± s.e.m. a-CHR/EXP sig. diff. from INT/EXP, b-CHR/NonEXP sig. diff. from INT/NonEXP, ($p<0.05$).

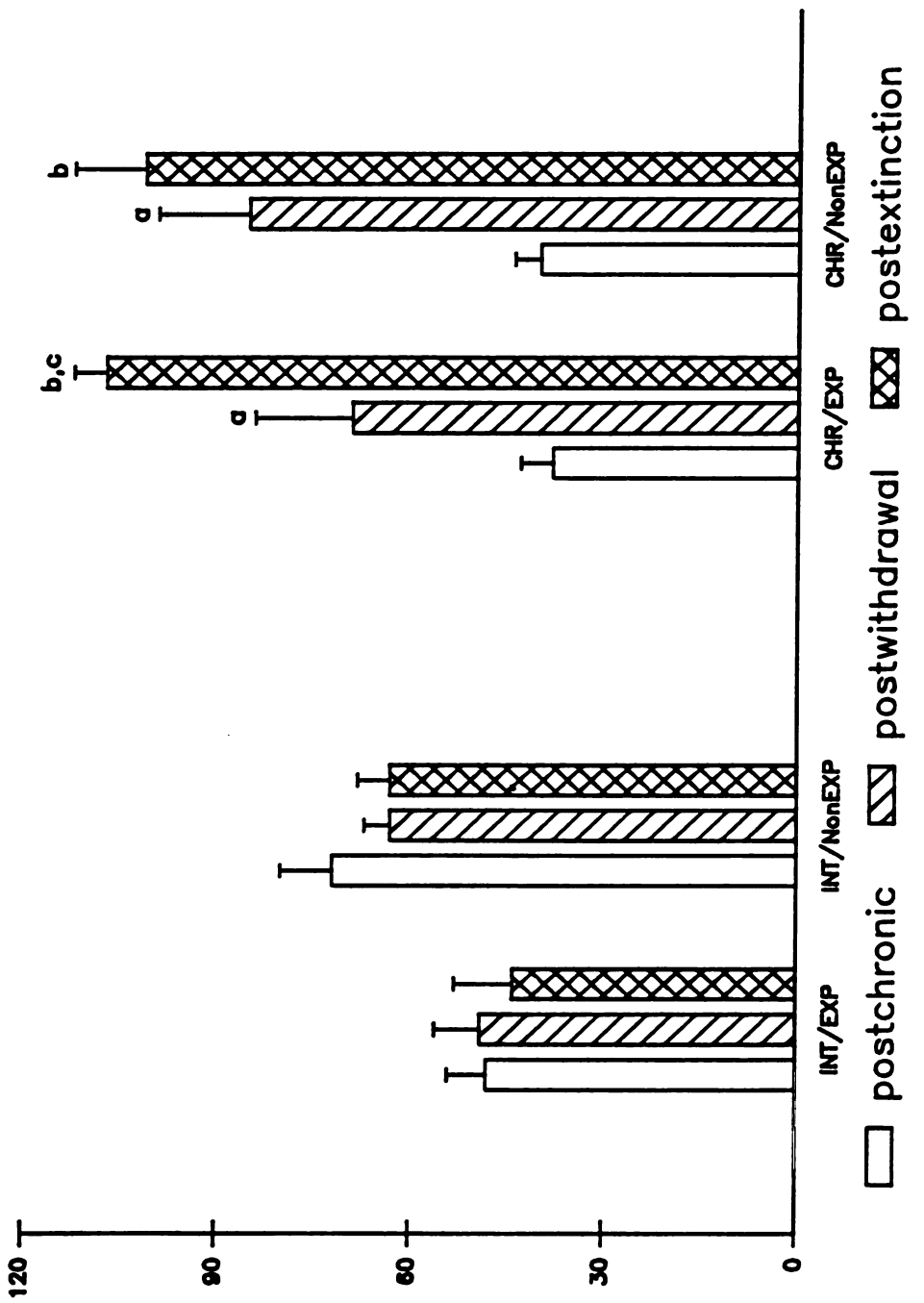

FIGURE 30: Loss of pentobarbital tolerance to duration of ataxia ± s.e.m. after a withdrawal period and extinction trials. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, c-postextinction sig. diff. from postwithdrawal, ($p<0.05$).

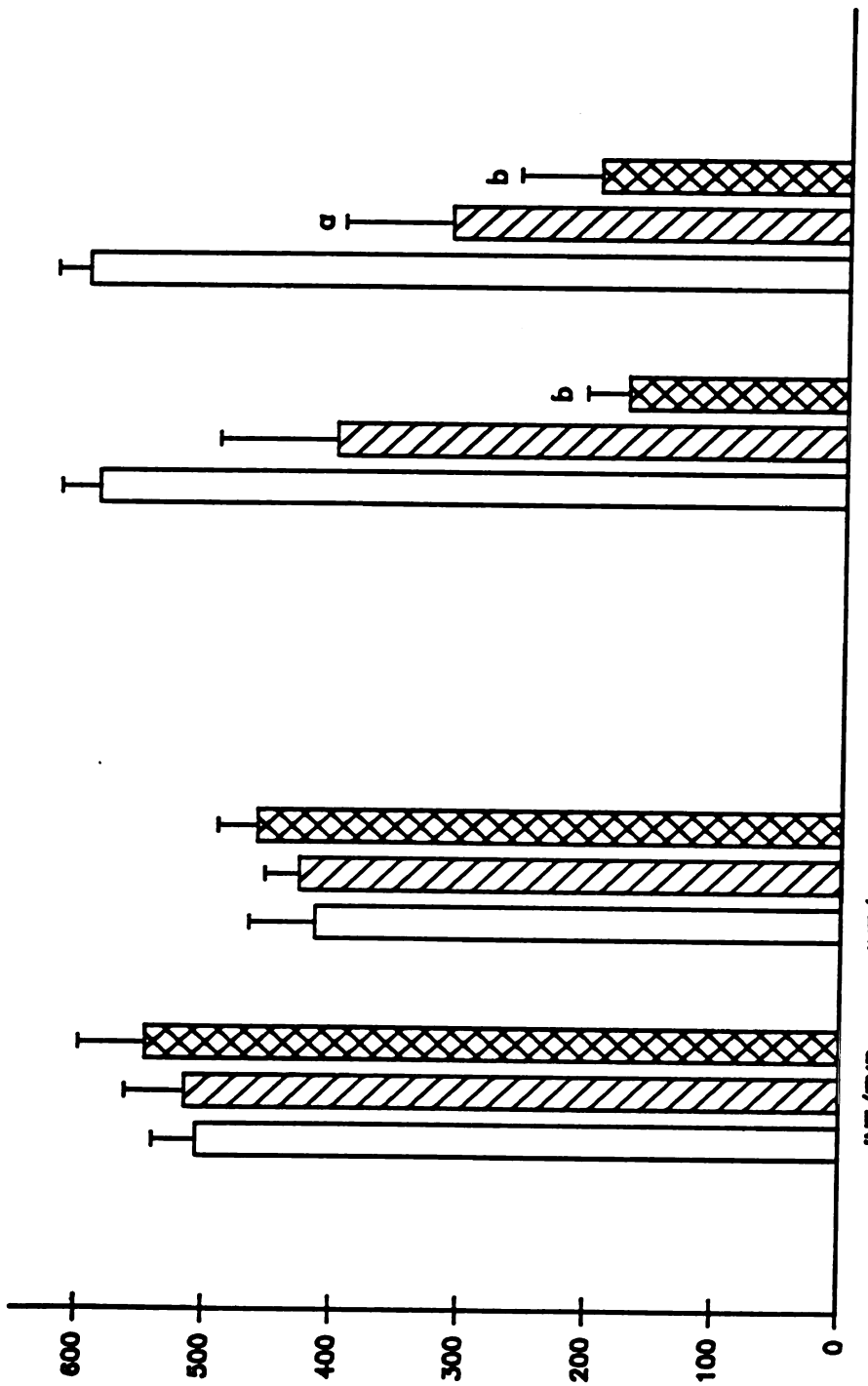

FIGURE 31: Loss of pentobarbital tolerance to total ataxia ± s.e.m. after a withdrawal period and extinction trials. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, ($p<0.05$).

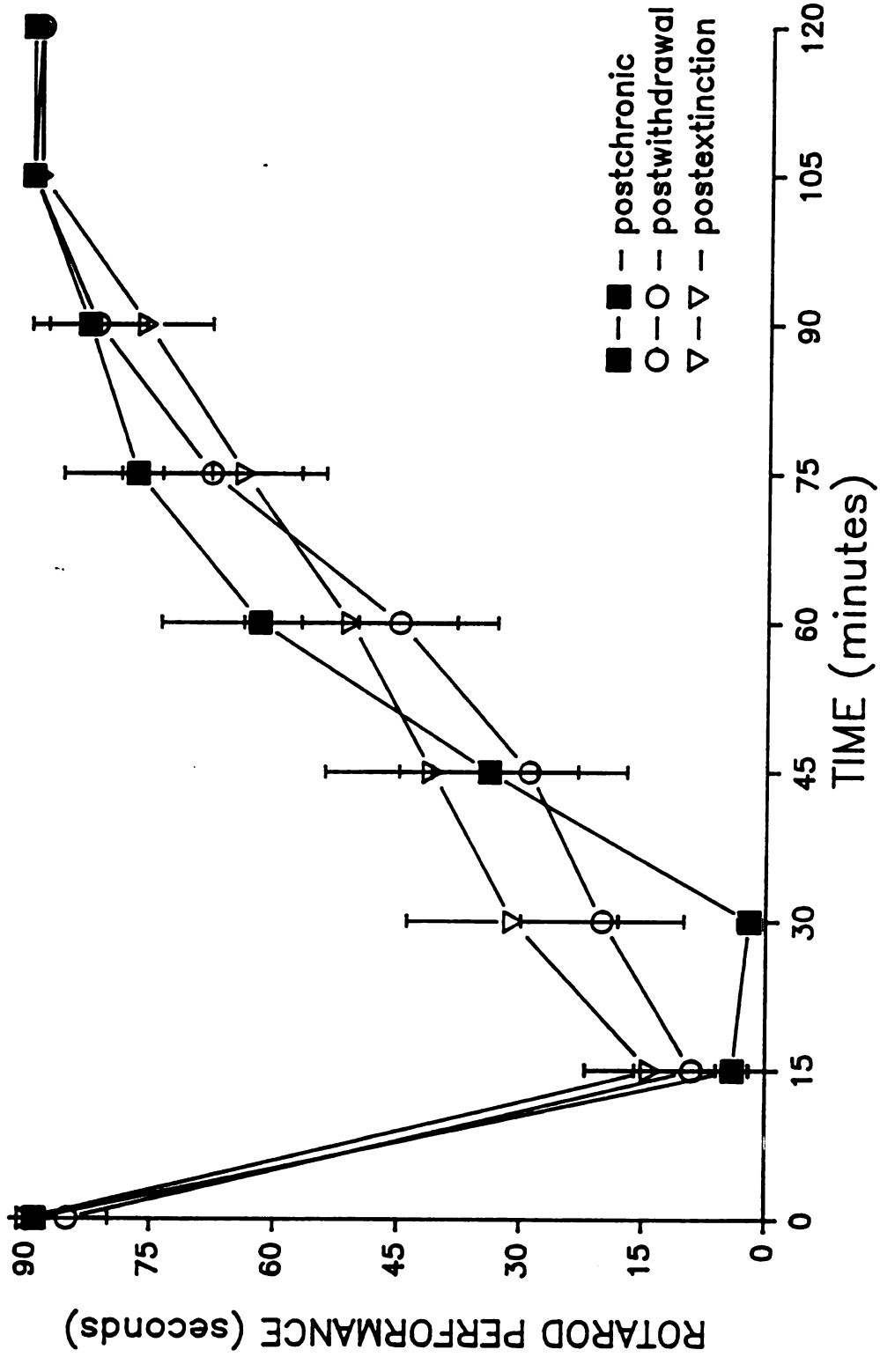

FIGURE 32: INT/EXP loss of pentobarbital tolerance to ataxia after a withdrawal period and extinction trials. Time course of rotarod performance ± s.e.m.

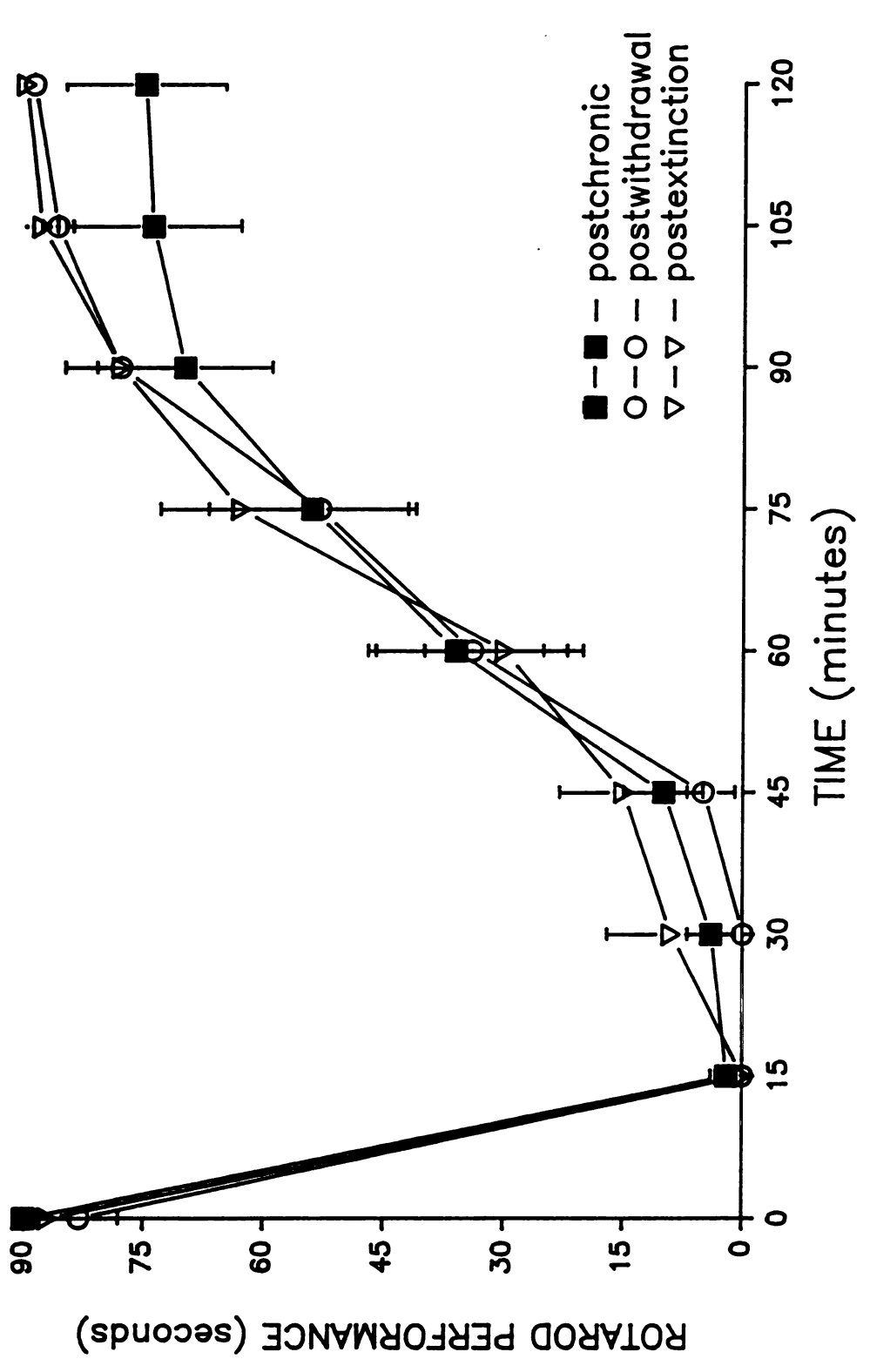

FIGURE 33: INT/NonEXP loss of pentobarbital tolerance to ataxia after a withdrawal period and extinction trials. Time course of rotarod performance ± s.e.m.

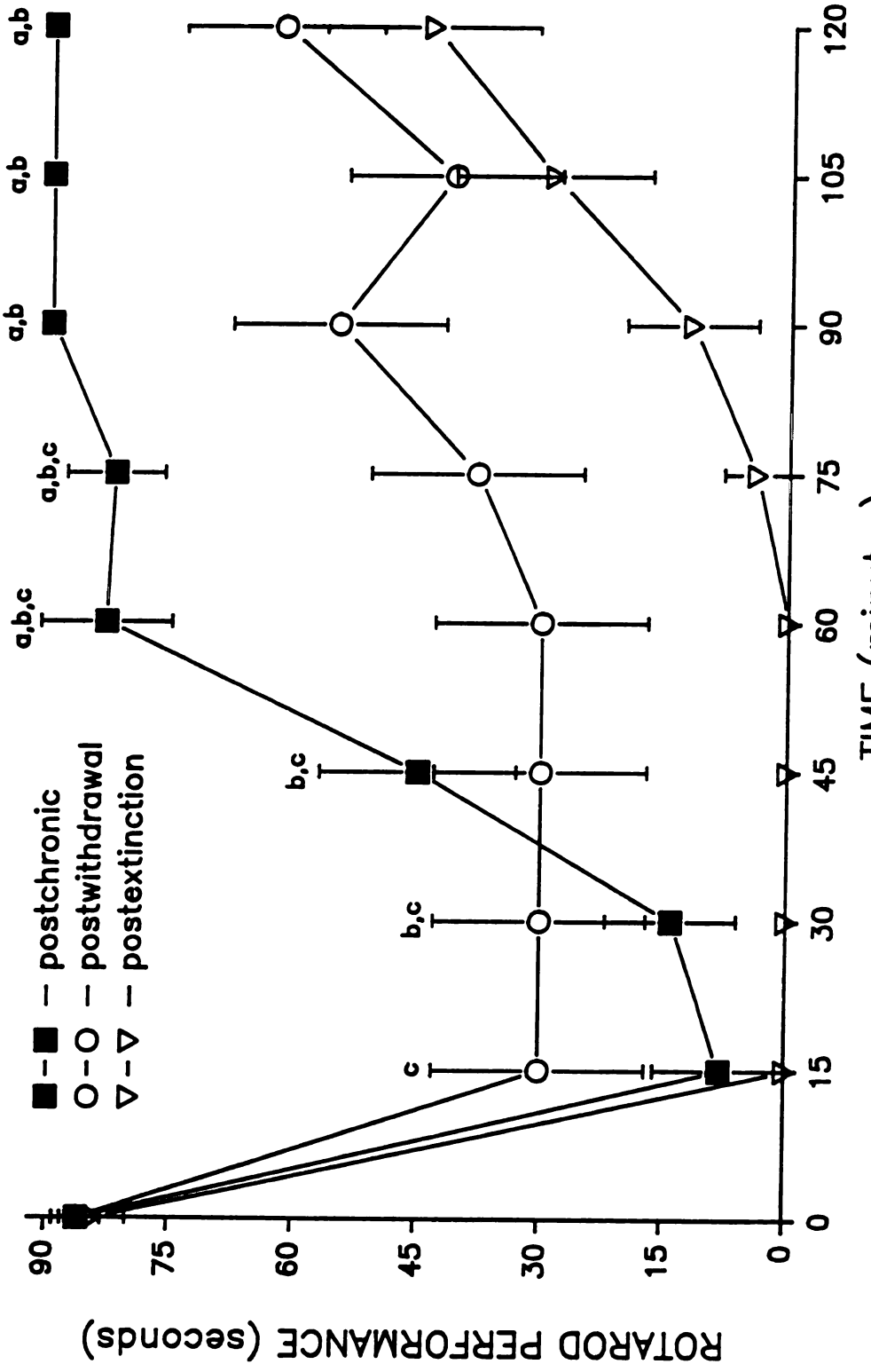

FIGURE 34: CHR/EXP loss of pentobarbital tolerance to ataxia after a withdrawal period and extinction trials. Time course of rotarod performance ± s.e.m. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, c-postextinction sig. diff. from postwithdrawal, ($p<0.05$).

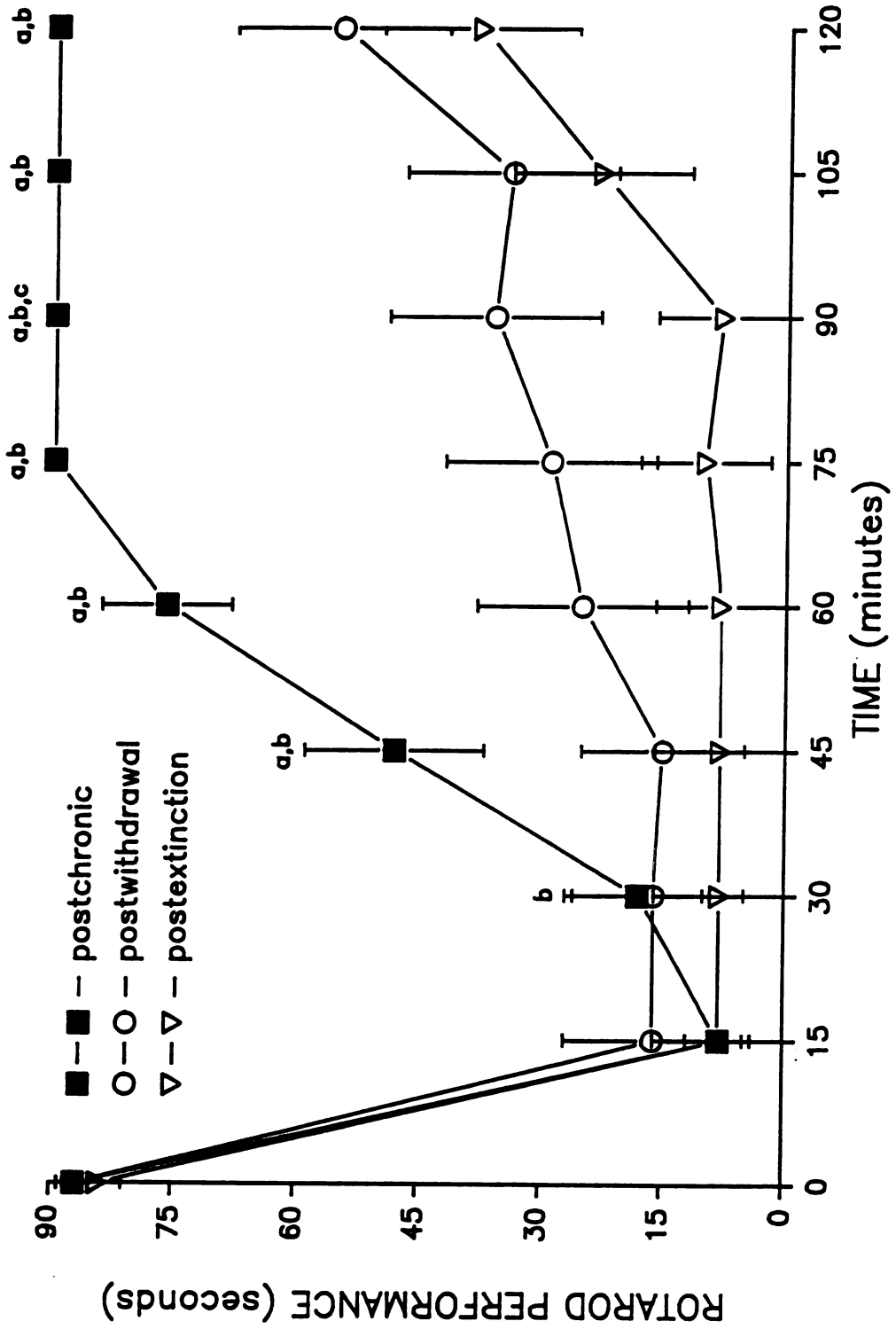

FIGURE 35: CHR/NonEXP loss of pentobarbital tolerance to ataxia after a withdrawal period and extinction trials. Time course of rotarod performance ± s.e.m. a-postwithdrawal sig. diff. from postchronic, b-postextinction sig. diff. from postchronic, c-postextinction sig. diff. from postwithdrawal, ($p<0.05$).

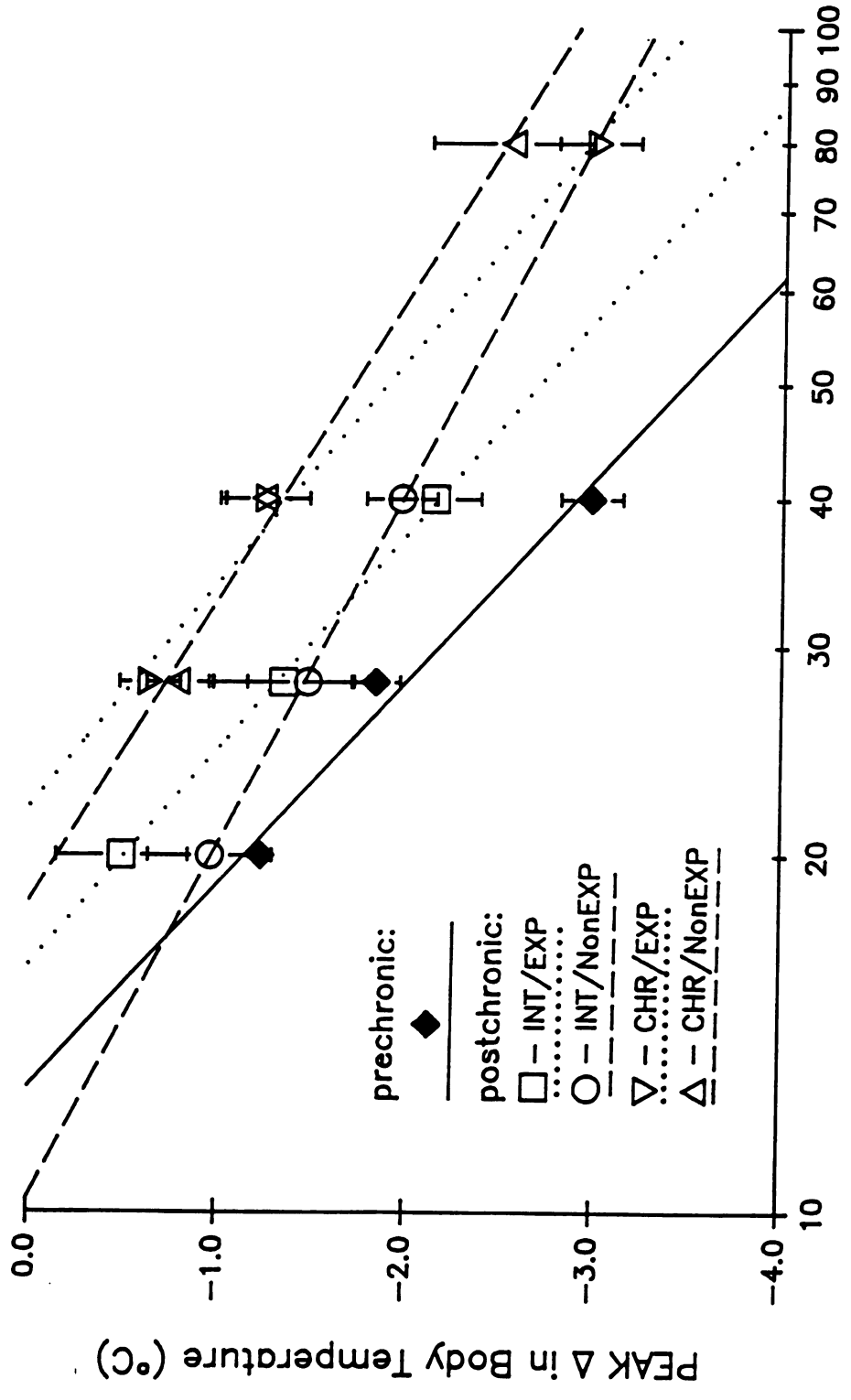

FIGURE 36: Pentobarbital log dose-response regression analysis for peak hypothermia ± s.e.m. No significant change in regression line slope for any treatment group.

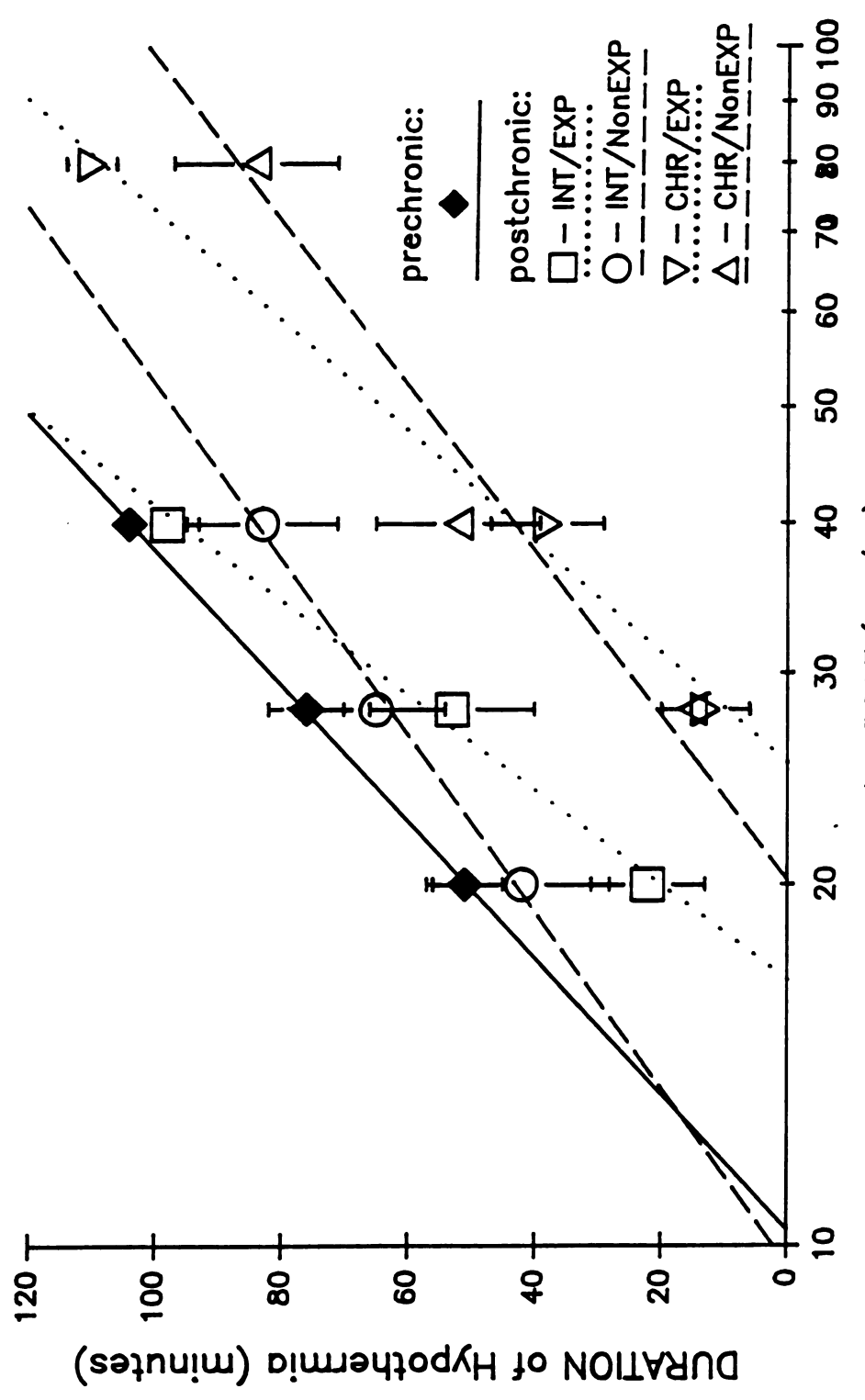

FIGURE 37: Pentobarbital log dose-response regression analysis for duration of hypothermia ± s.e.m. No significant change in regression line slope for any treatment group.

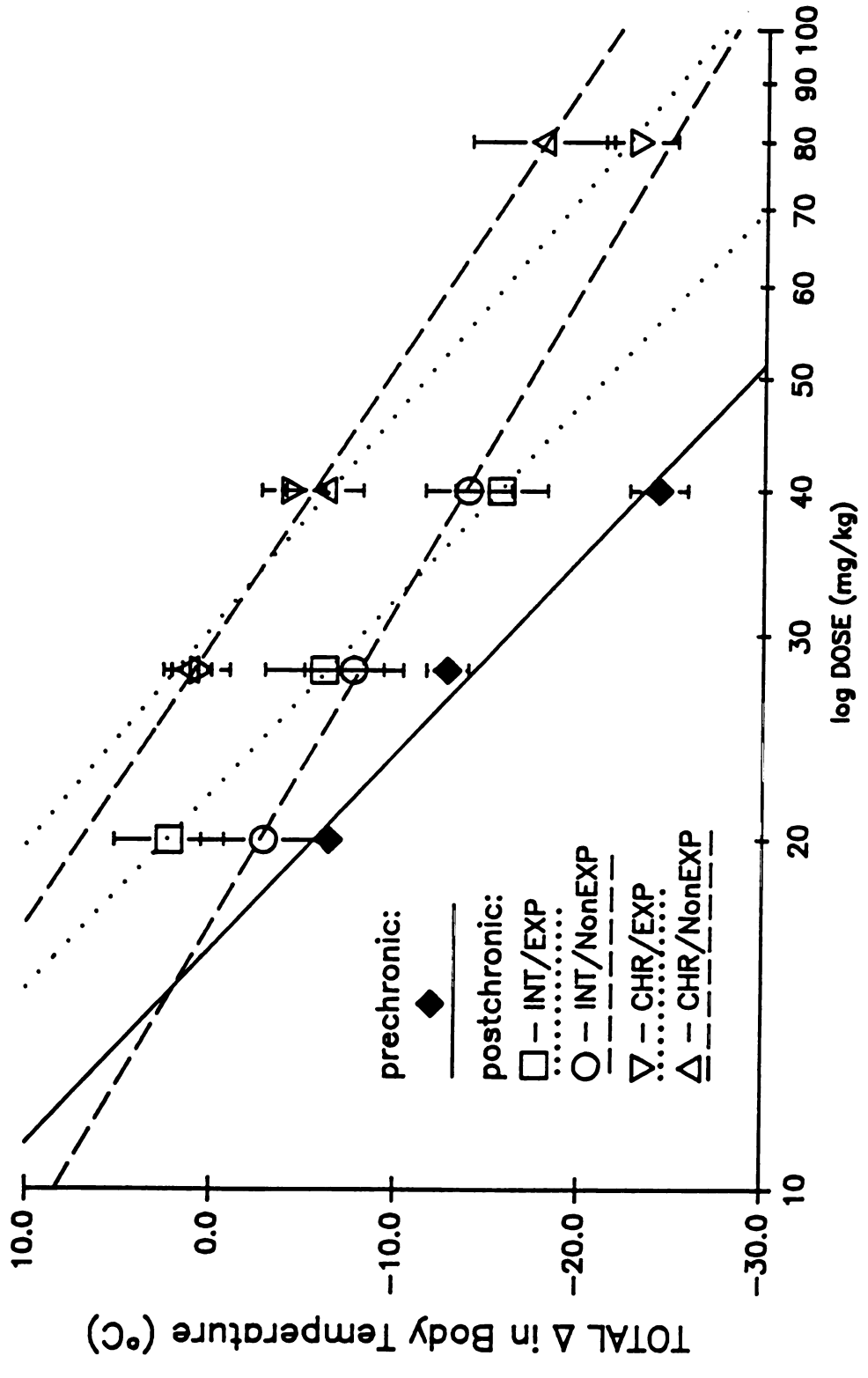

FIGURE 38: Pentobarbital log dose-response regression analysis for total hypothermia ± s.e.m. No significant change in regression line slope for any treatment group.

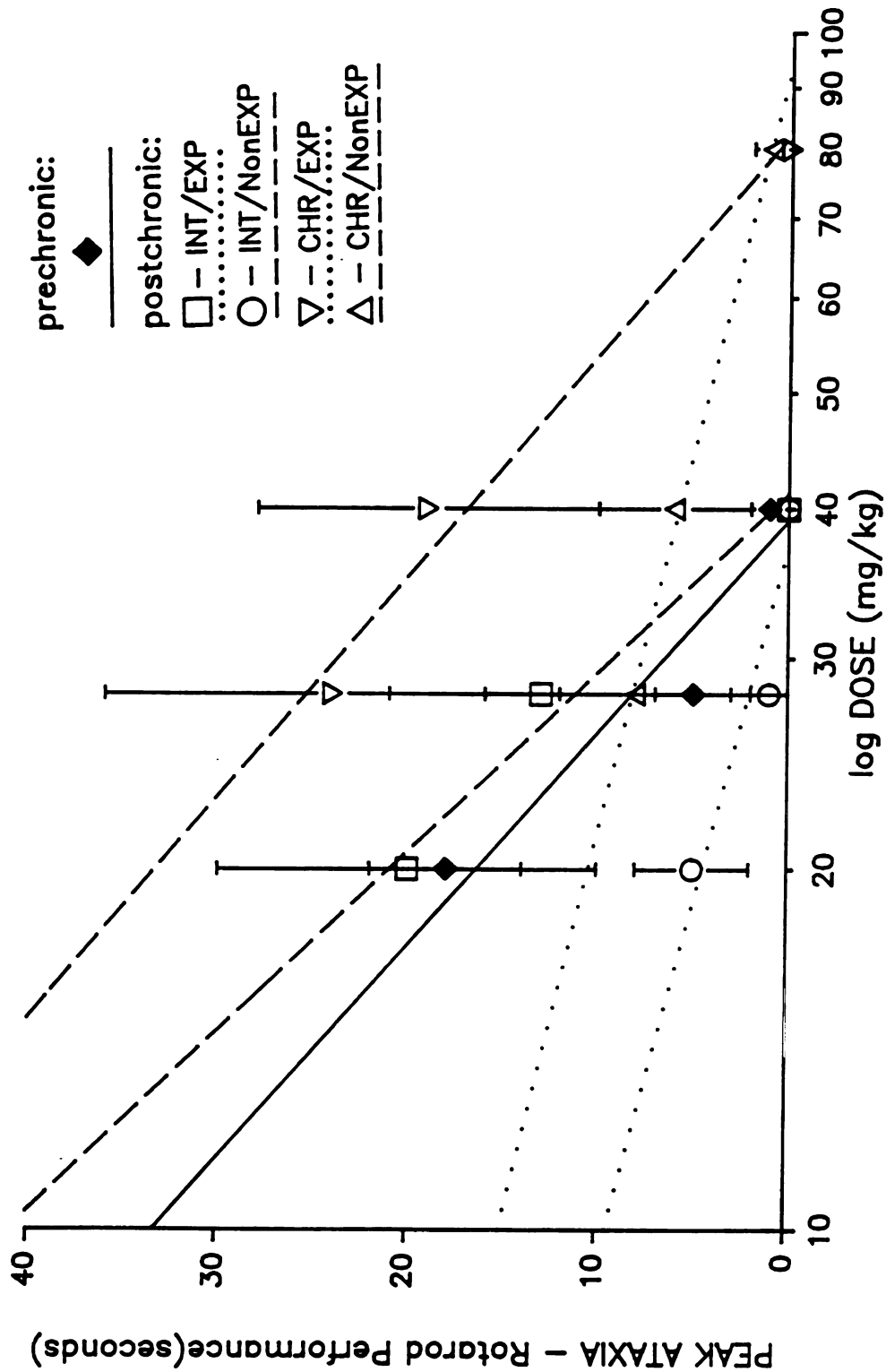

FIGURE 39: Pentobarbital log dose-response regression analysis for peak ataxia ± s.e.m. No significant change in regression line slope for any treatment group.

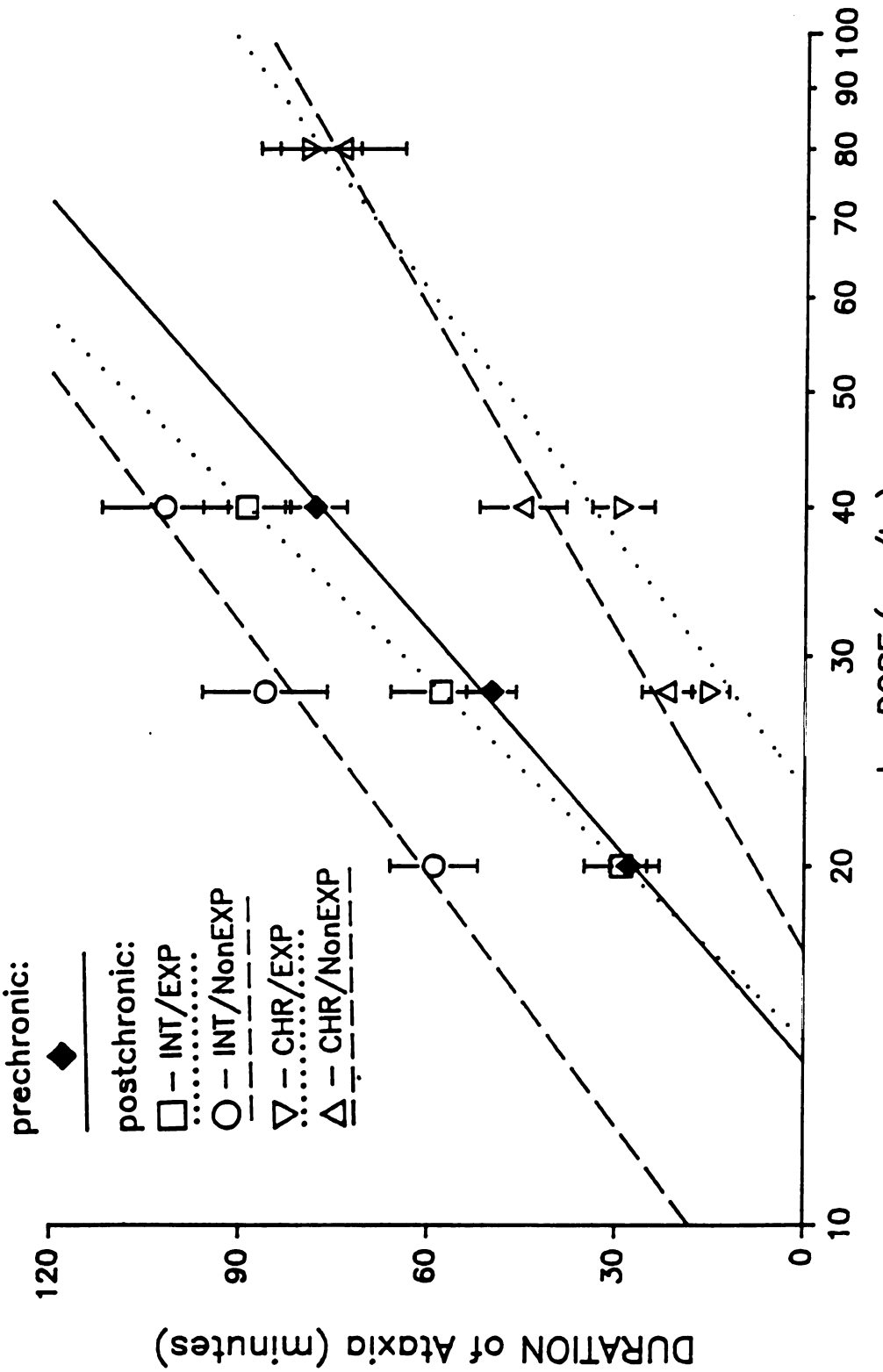

FIGURE 40: Pentobarbital log dose-response regression analysis for duration of ataxia ± s.e.m. No significant change in regression line slope for any treatment group.

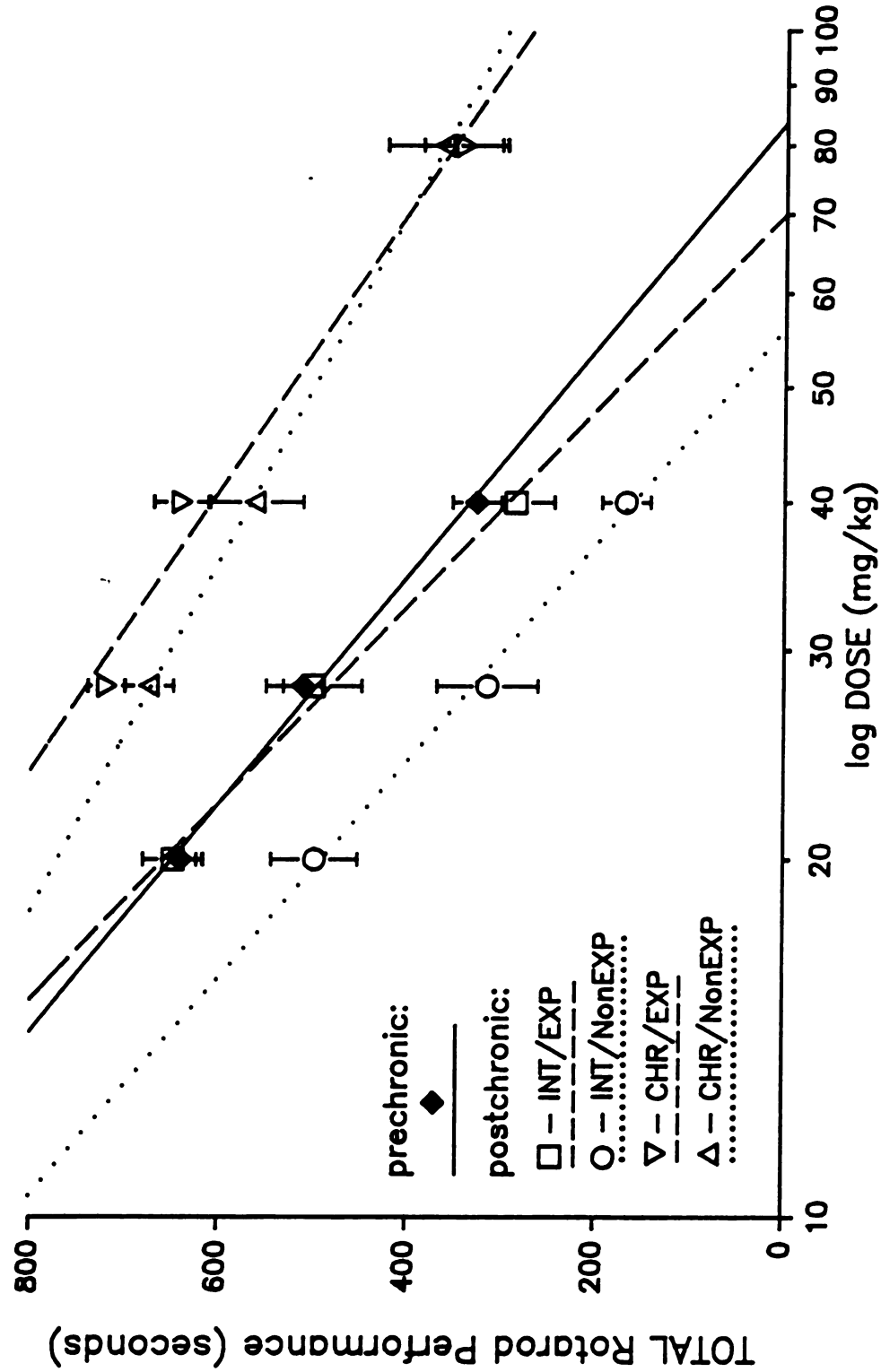

FIGURE 41: Pentobarbital log dose-response regression analysis for total ataxia ± s.e.m. No significant change in regression line slope for any treatment group.

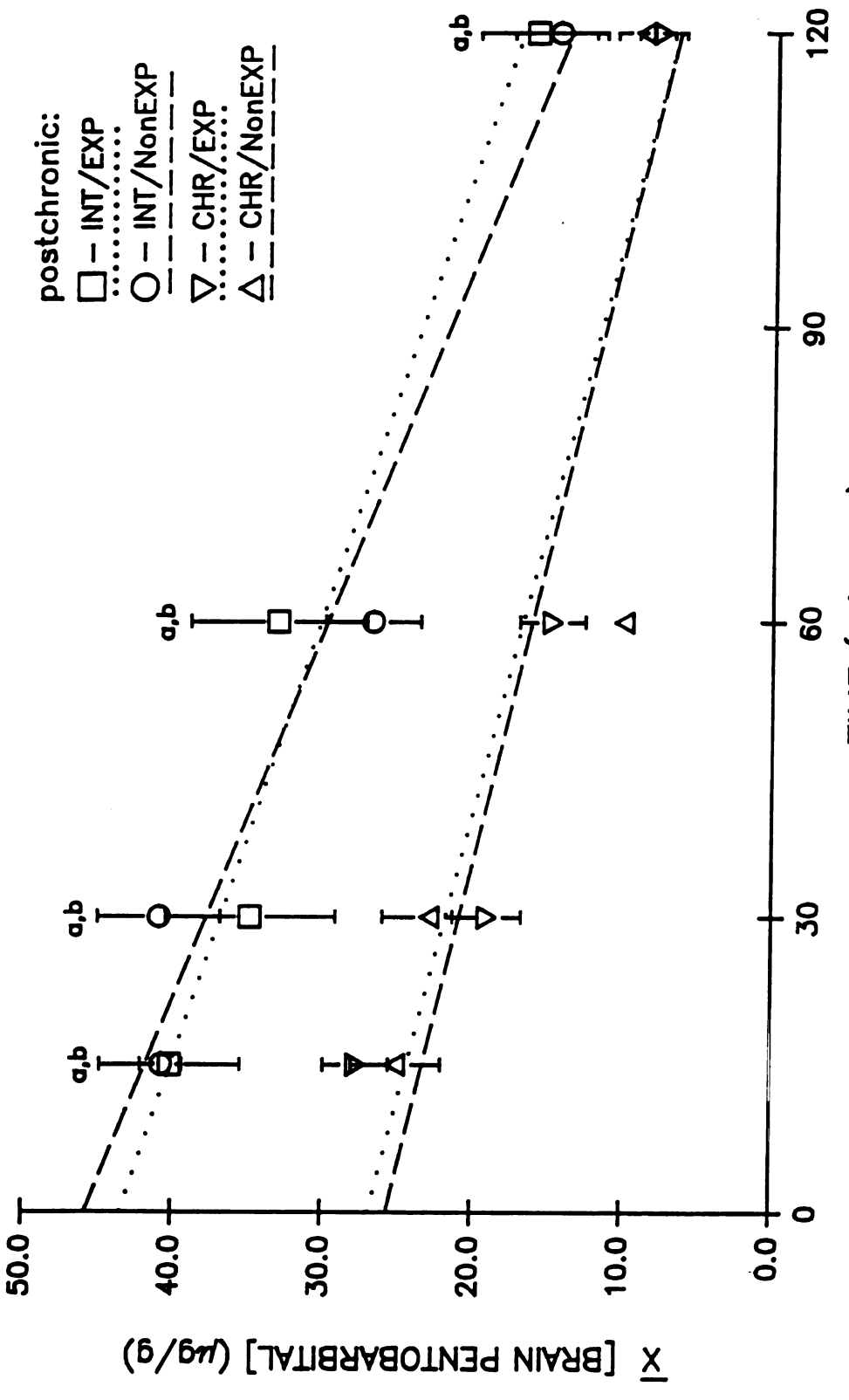

FIGURE 42: Pentobarbital brain concentrations ± s.e.m. as a function of time after injection. Comparison of intermittent vs. chronic drug treatment. a-CHR/EXP sig. diff. from INT/EXP, b-CHR/NonEXP sig. diff. from INT/NonEXP, ($p<0.05$).

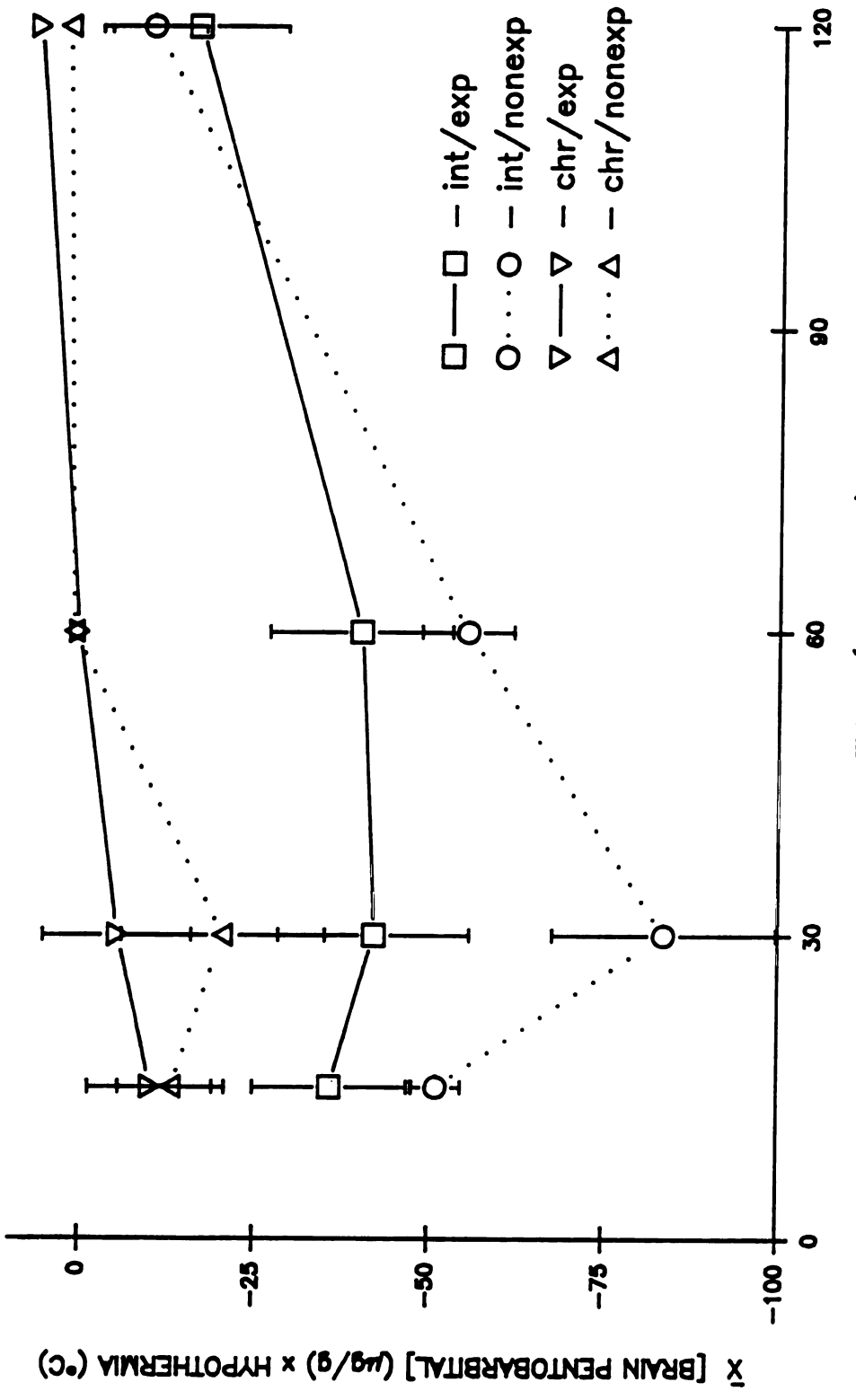

FIGURE 43: Representation of ANCOVA, mean of the pentobarbital brain concentration multiplied by the hypothermia ± s.e.m. Comparison of INT vs. CHR: for EXP sig. at $p<0.05$ and for NonEXP sig. at $p<0.01$).

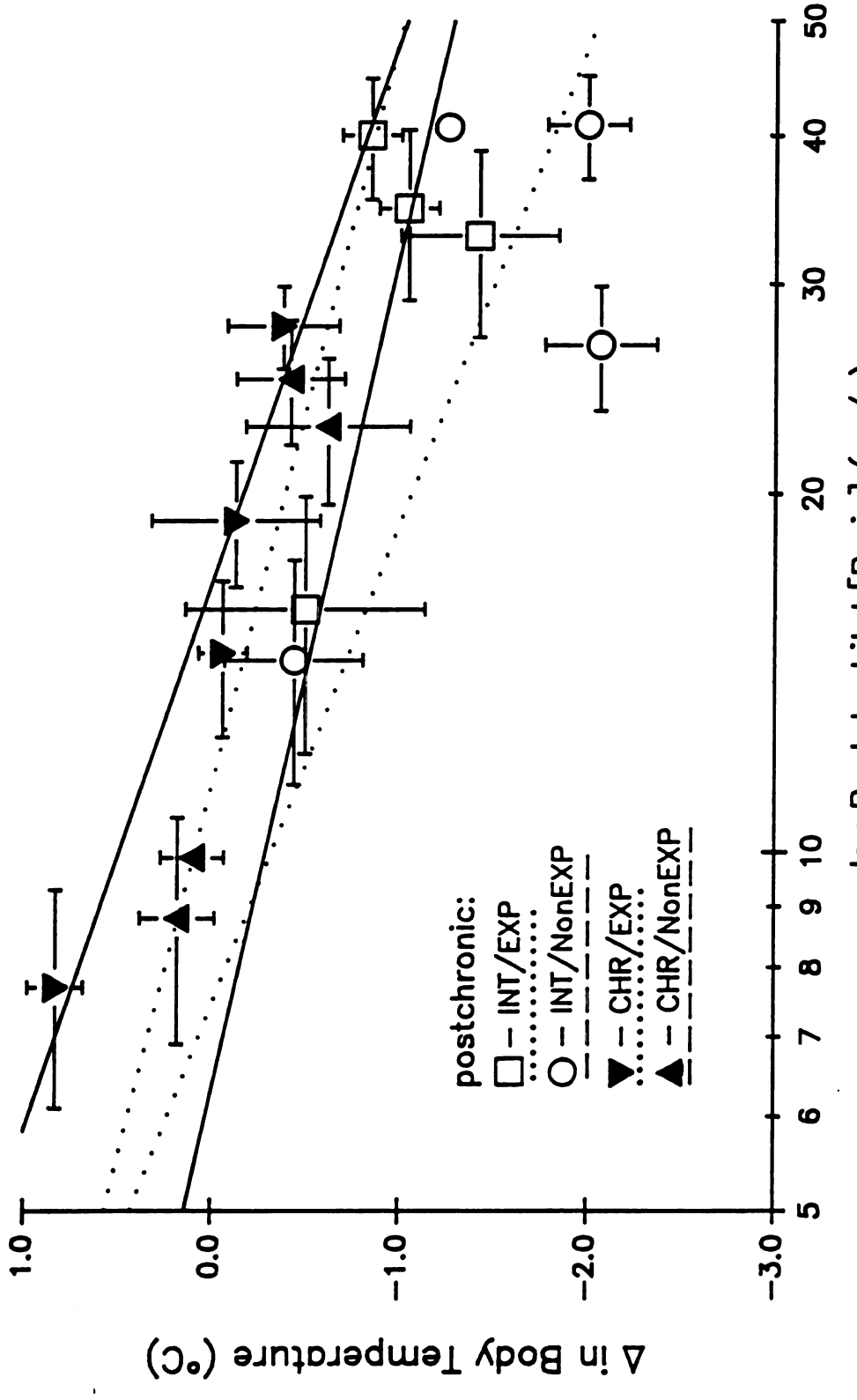

FIGURE 44: Regression analysis of mean hypothermia ± s.e.m. as a function of mean pentobarbital brain concentration ± s.e.m. Comparison of EXP vs. NonEXP.

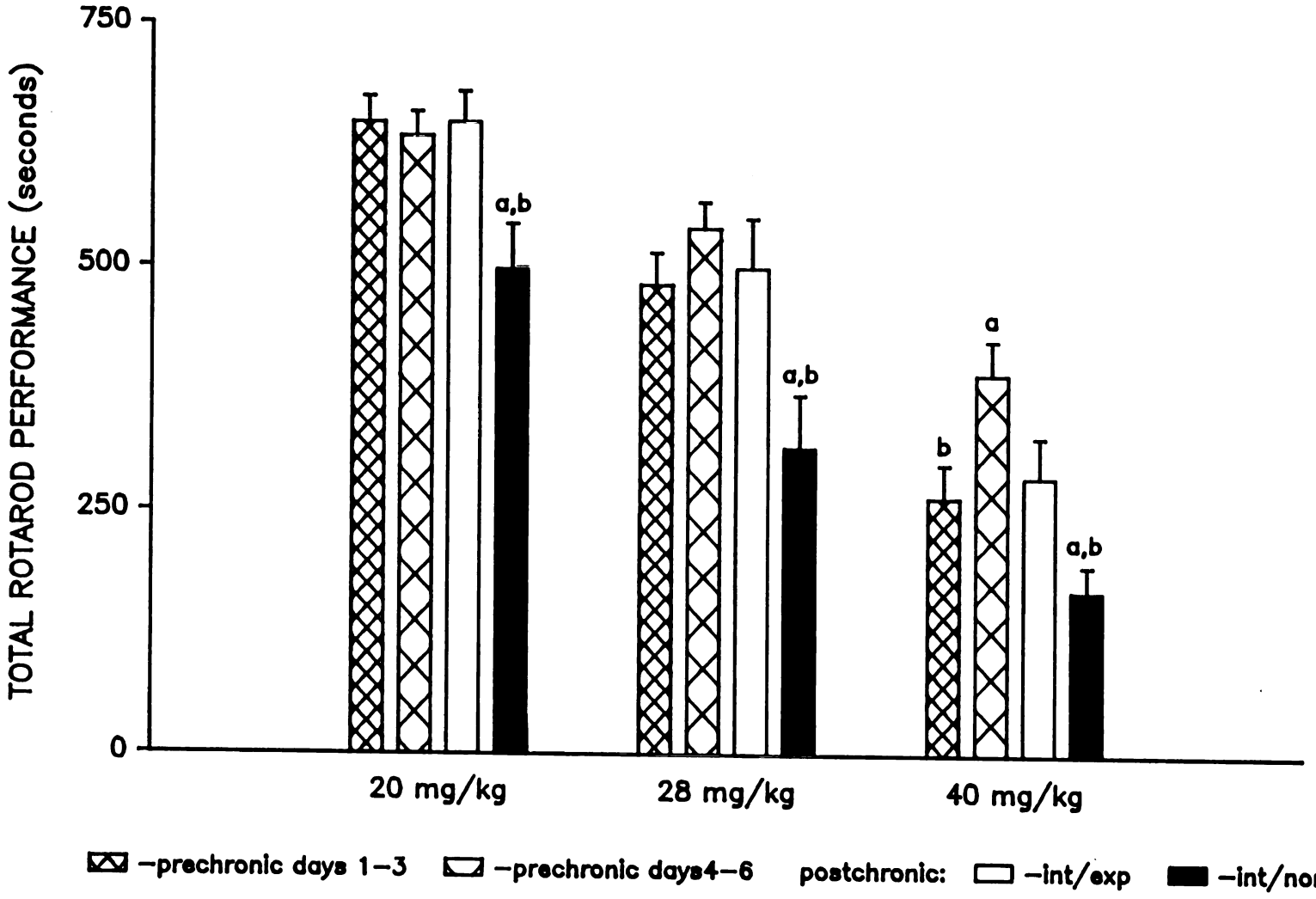

FIGURE 45: Tolerance development to total ataxia ± s.e.m. during prechronic days 1-3, prechronic days 4-6, and after intermittent drug treatment with or without drug-effect experience. a-sig. diff. from prechronic days 1-3, b- sig. diff. from prechronic days 4-6, ($p<0.05$).

DISCUSSION

The postchronic body temperature data from the INT animals indicated that behavioral tolerance, separable from metabolic and cellular tolerances, developed. INT/EXP demonstrated significant tolerance, as compared to the prechronic measures, that INT/NonEXP lacked (see Figures 1 through 3 and 7 through 9). However, there was no difference between the EXP and NonEXP groups.

The slight tolerance development to the hypothermic effect demonstrated by the NonEXP animals may have been due to an inability to totally eliminate drug-induced hypothermia using the BTW procedure or to some generalization of various tolerance phenomena. Due to the large number of animals being maintained, at times the body temperatures may have exceeded the control range for a short time, providing some experience in the NonEXP group to the loss of temperature control. Even though subjects were monitored for up to 20 minutes after turning off the heat lamp (sometimes up to three hours), some animals may also have experienced some prolonged hypothermia that was undetected after being returned to their home cages. An automated temperature control system for each animal could have eliminated this problem.

The lack of dissipation of tolerance to hypothermia after withdrawal in the INT animals was expected since there should have been negligible cellular or metabolic tolerance produced with this dosing schedule. Tolerance loss after extinction trials was expected for the INT/EXP animals but was not observed.

It is possible that the extinction trials were unsuccessful in the INT/EXP animals due to their previous behavioral experiences in the absence of the drug effect. The drug-dosing cues (i.p. administration) were partially reinforced since these animals received saline injections during the chronic drug treatment period. Siegel (1983) demonstrated that partial reinforcement inhibits the development of an association or extinguishes the association of the CS (conditioned stimulus, cues for drug administration) with the UCS (unconditioned stimulus, drug effect). Since the extinction trials in the current study utilized i.p. administration of saline as the conditioned stimulus, the previous partial reinforcement of this stimulus may have caused a failure for the extinction trials to disassociate the drug administration from the drug effect. The stimulus cues for drug effect (or other influence) which developed to become the CS in these animals may have been weakened or may have been something other than the drug administration.

Other possible conditioned stimuli are the testing procedure, environment, altered response pattern and low-level drug effects (Schuster et al., 1966; Demellweek and Goudie,

1983). INT/EXP animals had experienced the testing procedure without pentobarbital during the training period. They had also never experienced the drug outside of the testing procedure. The animals were all in the same room (environment) for all treatments, there was no change in environment to allow cues for conditioning drug effects. The design of this study may have forced these INT/EXP subjects to utilize low level drug cues as the only consistent CS for the drug effect. Evidence that low level drug cues were involved as the CS can be observed from the postchronic dose-response effects on all the animals. For all measures the greater tolerance observed in the experienced subjects as compared to the non-experienced subjects was diminished with increasing dose, and was often reversed at the highest dose. As drug dosing increases the low-level drug cues were altered or missing, which may have resulted in a loss of the conditioned response (i.e., a loss of behavioral tolerance). An introduction of different cues for drug testing days and the chronic treatment (towel wrap) days such as different environments involving changes in visual, auditory, and/or olfactory stimuli may have allowed for extinguishable cues for drug effect.

It is possible that this conditioned behavior was difficult to extinguish using extinction trials that did not employ the drug experience outside of the testing procedure, particularly when the subjects had previous nondrugged testing experience. These hypotheses gain support by the observance

of successful extinction trials in CHR/EXP for both hypothermic and ataxic tolerance. This group had drug experience outside the testing procedure (in the towel wrap) and did not have partial reinforcement of drug administration cues during the chronic drug treatment period.

A difference between the EXP and NonEXP groups of the INT animals was also demonstrated in the amount of pentobarbital-induced ataxia produced. There was an increased sensitivity to the ataxic effect of pentobarbital in the NonEXP group after the chronic treatment period. Furthermore, no apparent tolerance development was measured for the EXP group, as compared to the prechronic dose-response measurements (see Figs 19 through 21 and 25 through 27).

Behavioral tolerance to rotarod-measured ataxia has been demonstrated previously in this laboratory (Commissaris and Rech, 1981). A comparison of the methods employed for the current study and that of Commissaris and Rech (1981) reveals some differences. These differences are in the sex of the subjects, the dose of pentobarbital, and the other behavioral experiences of the subjects (mainly the towel wrap). It is possible that the male subjects and the larger doses of pentobarbital used in the current study may not be as optimum for behavioral tolerance development to ataxia as the previous study's female subjects and the 15 mg/kg dose of pentobarbital. In the current study the higher doses of pentobarbital did not allow for much intermediate drug effect practice. The animals generally went from a rotarod

performance score of 0 seconds to that of 90 seconds within one or two trials. The device used in this study was a single speed rotarod. Ataxia changes could be measured more sensitively with the use of a variable speed rotarod or an inclined plane. Additional senitivity would also be achieve by the use of parametric statistics applicable to data obtained from a variable speed rotarod or an inclined plane.

The apparent lack of behavioral tolerance to ataxia in INT/EXP of the current study may also be a reflection of a non-contiguous drug treatment effect relating to the behavioral experience other than the rotarod training (the towel wrap) that was offset by drug-effect experience (i.e., behavioral tolerance). A comparison of the first three days to the last three days of the initial (prechronic) dose-response determination indicates there was a trend for less ataxia in the last three days as compared to the first three days (see Figure 45) at the middle dose, which becomes significant at the high dose. This was probably due to a rapidly developing behavioral tolerance during this prechronic testing. (Due to these results the prechronic scores reported were only of the first three days of the prechronic dose-response determination for the ataxia. The hypothermia data did not demonstrate a significant difference between the first three days and the last three days of the initial dose-response determination probably due to a more slowly developing behavioral tolerance, and thus all six days of measurements were reported.) At the postchronic test of the

40 mg/kg dose in the INT/EXP animals, the level of ataxia was no longer significantly less than the score for the prechronic days 1-3 determination. Therefore, it appears that INT/EXP actually lost tolerance over the chronic treatment period.

This discrepancy may relate to a conditioned behavior similar to learned helplessness (Maier et al., 1983) produced by the animals' repeated non-drug experiences of inability to ambulate in the towel wrap. It has been demonstrated by Carder (1978) that behavioral experiences that do not involve exposure to the drug or the specific drug-effect testing procedure can alter the drug effect in the specific test. This learned helplessness may explain the increased sensitivity to the drug effect in INT/NonEXP as well as the apparent lack of tolerance in INT/EXP. Although a slight trend (non-significant) for an increase in sensitivity was observed at the 40 mg/kg dose in INT/EXP postchronically as compared to the last three days (4-6) of the prechronic testing, there was no difference as compared to the first three days of prechronic testing (Figure 45). It is likely that the increased sensitivity also expected to occur in INT/EXP by a learned helplessness type of effect was offset by the behavioral tolerance produced in these subjects. Thus, the behavioral tolerance in INT/EXP may be masked by a non-drug behavioral experience that was not anticipated in the design of this study that enhanced the drug effect to impair rotarod performance. Furthermore, a learned helplessness-like effect may have been extinguished during the extinction

training period, resulting then in a decreased drug effect. A trend for this type of decreased impairment was observed for both the pentobarbital-induced hypothermia and ataxia (see Figures 12 through 15, 30, 31 and 32) for INT/EXP.

Restraint stress may also play a role in the unexpected results observed in this study. Restraint stress alters hormone levels (Rupe et al., 1963), drug metabolism (Rupe et al., 1963; Stitzel and Furner, 1967), the regulation of many neurotransmitters (Hendley et al., 1977) and the drug responses (Chung and Brown, 1976; Martin and Papp, 1979). Any one of these alterations could affect tolerance development in these animals. Since the amount of restraint stress is equivalent in the groups being compared, these factors do not play a role in the differences between EXP and NonEXP animals.

Chronic pentobarbital treatment produced profound cellular/metabolic tolerance, as expressed by decreased hypothermia and ataxia. Behavioral tolerance for ataxia was only suggested in CHR/EXP at the postchronic tests by a slight trend (non-significant) for increased tolerance to ataxia due to EXP in the low and middle dose measures (Figures 22, 23, 25, 26 and 27). However, a conditioned component to the tolerance in the CHR/EXP animals became apparent at the postwithdrawal and postextinction testing phases for both the tolerances to hypothermia and ataxia (Figures 30, 31 and 34). Withdrawal either failed to or only partially reversed tolerance to hypothermia and ataxia in CHR/EXP, suggesting that a masked behavioral tolerance was uncovered by withdrawal

which then maintained significant tolerance at the postwithdrawal test. This unmasked behavioral tolerance was then diminished, as would be expected with a conditioned-tolerance component, by extinction trials, as evidenced by the significant tolerance loss at the postextinction test in the CHR/EXP animals. This conditioned component was absent in the CHR/NonEXP animals, which displayed significant tolerance loss only after withdrawal and not from extinction training (Figures 30, 31 and 35).

The slight trend for additional loss of tolerance after extinction trials observed in CHR/NonEXP could be explained if all of the metabolic tolerance was not lost after the ten day withdrawal period. However, previous studies have indicated that metabolic tolerance to barbiturates dissipates within 6-10 days (Aston, 1965; Stevenson and Turnbull, 1968). Since it has also been established that cellular tolerance dissipates within 7-10 days (Okamoto et al, 1976), the significant tolerance remaining within the CHR/EXP animals at the postwithdrawal test, that is lost after extinction trials, must be attributed to a conditioned behavioral tolerance component.

The appearance of a large conditioned component of tolerance in CHR/EXP revealed by the postwithdrawal and postextinction tests introduces a discrepancy with regard to the postchronic test results. The postchronic tests failed to reveal a major behavioral tolerance component when comparing CHR/EXP tolerance to that of CHR/NonEXP. It is

possible that a behavioral component was present but was overshadowed or masked by the prominent cellular/metabolic tolerance developed by the chronic drug treatment. The total tolerance produced after chronic dosing may have a ceiling/floor effect imposed by this experimental design. Even though an accelerated dosing regimen was used during the chronic treatment period, the acceleration may not have been enough to produce maximal functional tolerance development.

Okamoto et al (1978) demonstrated that chronic dosing failed to produce significant functional tolerance when the dose did not challenge the function being tested (anesthesia/consciousness). Almost all of the animals in both CHR groups achieved the criteria for increasing the dose on every test day. Perhaps if the dosing increase was greater for these animals and thus more challenging to the functional tolerance development, a larger amount of total functional tolerance could have been achieved and a difference between the CHR/EXP and CHR/NonÊXP groups would have been observed at the postchronic testing.

Alternatively, if CHR/EXP had received initial training to develop behavioral tolerance first and then been treated with chronic drug, perhaps an initial behavioral tolerance may then have augmented a cellular/metabolic tolerance. Another possible explanation for a functional tolerance ceiling is that development of behavioral tolerance produces less of a demand for cellular tolerance development.

The approach for demonstrating behavioral tolerance after chronic drug treatment in this study is unconventional. The conventional approach is an additive one, illustrating behavioral tolerance by demonstrating a significant increase in tolerance for animals with task-practice after drug administration as compared to animals receiving drug after task-practice following the chronic treatment period. The unconventional approach exhibited in the current study is a subtractive approach. After subtracting the cellular and metabolic components of drug tolerance, the behavioral tolerance is uncovered and its conditioning nature is confirmed by subtracting it with extinction trials.

Changes in the peak effect would traditionally be attributed to cellular tolerance, whereas changes in duration of effect would be attributed to metabolic tolerance. However, these distinctions are far from absolute. Behavioral tolerance can cause changes in both the peak effect and duration of effect (Commissaris and Rech, 1981; San-Marina et al., 1989). The decrease in duration may be due to an increase in acute behavioral tolerance which may be strengthened by conditioning (Tabakoff et al, 1986). The decrease in peak effect may be due to conditioning from the drug exposure combined with behavioral testing experience. Small decreases in peak effect and duration of effect were observed for the INT/EXP animals with regard to hypothermia. The behavioral experience of the towel wrap combined with drug exposure in INT/NonEXP appeared to produce an increase in both

the peak ataxia and duration of ataxia after the pentobarbital test doses. The potential mechanism(s) for this increased sensitivity remain unresolved, though a development of "learned helplessness" was postulated above. It would be possible to test whether the BTW actually produces a "learned helplessness" on its own. Naive animals would be exposed to five weeks of daily BTW, then a dose-effect test performed for pentobarbital ataxia and compared to animals maintained in home cages without BTW experience.

Thus, behavioral experiences during intermittent barbiturate administration may have an influence on the amount of behavioral deficit produced by the drug. This did not appear to be the case with chronic administration in this model. Although there were large decreases in the peak effects and duration of effects after chronic administration there was no appreciable difference between the CHR/EXP and CHR/NonEXP animals for the total tolerance level to hypothermia. These effects were undoubtedly largely due to cellular and metabolic tolerance. There was a slight trend for behavioral tolerance development to ataxia in the lower test doses for the peak effect, duration and total effect (see Figures 25, 26 and 27). It is not possible to determine from these data whether this was due to (a) an increase in tolerance in CHR/EXP due to drug exposure combined with RR or (b) to an enhanced deficit in CHR/NonEXP due to drug exposure combined with BTW. Therefore, analysis of the postchronic log dose-response curves was performed. Even though there was a

lack of significant differences in the regression analyses, there was a consistent trend through all six graphs. This trend suggests that exposure of the towel wrap in combination with the pentobarbital produces an increase in drug effect. From this analysis it appears that the towel-wrap behavioral experience combined with drug exposure did influence the peak ataxia and may have also influenced the hypothermia peak effect, duration of effect and total effect to some extent (Figures 36, 37, 38, and 39). This suggests that the learned helplessness type of behavior was increased by the combination of the drug and the towel wrap (both being incapacitating and leading to a "learned helplessness") and may not have been limited to the ataxia.

Additional evidence for pharmacokinetic tolerance was demonstrated by the brain concentration time courses. The CHR animals have lower pentobarbital brain concentrations at all time points as compared to the INT animals. Since there was such a large difference between the CHR and INT brain levels, even at 15 minutes, the brain/serum ratios were determined at 15 minutes and were found to show no significant difference between any of the groups. This indicates that there was no change in the ability of pentobarbital to cross the blood-brain barrier. The pharmacokinetic tolerance in the CHR animals was most likely due to changes in metabolism as indicated by Okamoto et al. (1975) and Commissaris et al. (1982).

Additionally, cellular tolerance was demonstrated by an analysis of covariance of the brain pentobarbital concentrations and their corresponding hypothermic deficits. The depiction of this analysis, Figure 43, reveals not only a difference in amount of effect between the chronic and intermittent treatment groups, but also a change in shape between the experienced and non-experienced groups. Whether this change in shape was due to the drug-effect experience or to the drug-towel wrap experience is not discernible without naive animal data for comparison.

The cellular tolerance expressed to the ataxia could not be determined due to the non-parametric nature of the data. Again, the solution to this problem would be to employ a variable speed rotarod or an inclined plane to produce data that can be analyzed with parametric statistical tests (Analysis of Covariance).

SUMMARY AND CONCLUSIONS

The aims of this study were achieved as follows:

1) Significant tolerance to the pentobarbital-induced hypothermia was observed after both chronic and intermittent treatment. Significant tolerance to the pentobarbital-induced ataxia was observed after chronic treatment but not after intermittent treatment.

2) INT/EXP animals expressed a tolerance to pentobarbital-induced hypothermia that was attributed to learned adaptation based on drug exposure combined with behavioral testing experience.

INT/NonEXP animals expressed an enhanced sensitivity to pentobarbital-induced ataxia that was attributed to learned adaptation (learned helplessness; Maier et al., 1983) based on drug exposure combined with the towel wrap experience.

Development of behavioral tolerance to pentobarbital-induced ataxia may have been masked in the INT/EXP by a non-drug related 'learned helplessness' type of effect (Carder, 1978).

CHR/EXP animals expressed a tolerance to pentobarbital-induced hypothermia and ataxia which was attributed to

components of learned adaptation, unmasked after Postwithdrawal and Postextinction testing, and cellular/metabolic tolerance.

CHR/NonEXP animals expressed tolerance to pentobarbital-induced hypothermia and ataxia which was attributable to cellular/metabolic tolerance.

3) Analysis of brain pentobarbital concentrations determined that pharmacokinetic tolerance was present after chronic pentobarbital treatment. Analysis of pentobarbital-induced hypothermia as correlated with the brain pentobarbital concentrations (by analysis of covariance) determined that cellular tolerance was also present after chronic pentobarbital treatment.

4) Cellular and metabolic tolerances can occur without requiring experience to specific drug deficits.

Behavioral tolerance (or sensitivity) occurs as a consequence of behavioral experiences, without requiring chronic drug treatment or the development of cellular tolerance.

The following conclusions can be drawn with respect to pentobarbital-induced hypothermia and ataxia:

1) Behavioral experiences (whether drug-related or not) can alter drug effects by learned adaptations.

2) These learned adaptations are responsible for the drug-tolerance or drug-sensitivity observed after intermittent

drug treatment without requiring the production of cellular or metabolic drug tolerance.

3) These learned adaptations are also a component of the total drug tolerance observed after chronic drug treatment which includes the presence of cellular and metabolic tolerances. Behavioral tolerance was uncovered in the chronic treatment group after a withdrawal period and confirmed after extinction trials, using a subtractive approach.

4) Behavioral tolerance is separable from both cellular and metabolic types of tolerance.

5) Cellular or metabolic tolerance development does not require specific experience to drug effects.

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APPENDICES

APPENDIX A

TABLE 4

NUMBER OF SUBJECTS FOR EACH TEST (N) AND GROUP (n)

TEST	N	INT/EXP n	INT/NonEXP n	CHR/EXP n	CHR/NonEXP n
PreCHR	50	12	--	13	--
PostCHR	46	12	11	12	11
PostCHR vs. PostWITH vs. PostEXT	46	12	11	12	11

APPENDIX A (continued)

TABLE 5

PENTOBARBITAL CHRONIC TREATMENT PERIOD DOSING PAIRS

INT/EXP Determining Animal	INT/NonEXP Dependent Animal	Dose Achieved (mg/kg)	CHR/EXP Determining Animal	CHR/NonEXP Dependent Animal	Dose Achieved (mg/kg)
76	138	36	79	139	44
78	150	40	80	140	46
82	94	36	89	141	46
84	128	36	95	146	46
85	81	38	96	75	46
92	137	32	97	107	46
93	118	36	102	112	46
101	77	38	105	135	46
111	90	38	114	120	46
115	106	36	117	86	46
125	104	30	121	103	46
126	100	38	122	88	46
127	109	42	123	91	46
129	98	36	124	142	46
130	119	38	131	110	46
136	83	34	132	116	46
145	139	36	133	87	46
154	144	36	134	108	46
143	---	32	149	152	46
---	147	42	153	151	46
---	148	38			
		$\bar{X}$ = 36.5			$\bar{X}$ = 45.9

APPENDIX A (continued)

TABLE 6

PENTOBARBITAL TEST DOSES
for Postchronic vs. Postwithdrawal vs. Postextinction Tests

INT/EXP		INT/NonEXP		CHR/EXP		CHR/NonEXP	
rat	dose	rat	dose	rat	dose	rat	dose
26	28	31	28	37	40	27	40
28	28	39	20	40	80	29	80
33	20	41	20	42	40	30	28
34	40	47	20	48	40	32	28
36	28	49	20	53	40	35	40
38	20	51	20	54	80	44	40
43	20	52	20	56	40	45	40
55	28	62	20	59	80	50	80
66	20	63	20	60	40	58	80
67	20	65	28	58	40	61	40
70	20	71	28	59	28	72	40
74	28			73	80		
X = 25.0±1.8		**22.2±1.1**		**52.3±6.0**		**48.7±6.2**	

APPENDIX B

TABLE 7

PENTOBARBITAL HYPOTHERMIA
Prechronic and Postchronic
F-values and Probability Levels

GROUPS		20 mg/kg Dur	20 mg/kg Pkht	20 mg/kg Total	20 mg/kg TCI	28 mg/kg Dur	28 mg/kg Pkht	28 mg/kg Total	28 mg/kg TCI	40 mg/kg Dur	40 mg/kg Pkht	40 mg/kg Total	40 mg/kg TCI	80 mg/kg Dur	80 mg/kg Pkht	80 mg/kg Total	80 mg/kg TCI
INT/EXP vs.	F	1.53	0.94	1.29	0.31	0.51	0.07	0.14	0.91	1.48	0.35	0.27	**2.75**	----	----	----	----
INT/NonEXP	p<	.231	.345	.271	.983	.483	.800	.710	.537	.238	.560	.611	**.002**	----	----	----	----
CHR/EXP vs.	F	----	----	----	----	0.02	0.41	0.12	0.38	0.79	0.00	0.40	0.39	4.14	0.96	1.61	1.19
CHR/NonEXP	p<	----	----	----	----	.886	.531	.730	.962	.386	.967	.534	.959	.055	.339	.218	.293
PreChr. vs.	F	**5.06**	**7.10**	**12.8**	0.99	3.10	2.21	**5.42**	0.99	0.51	**4.91**	**5.77**	0.80	----	----	----	----
INT/EXP	p<	**.028**	**.010**	**.002**	.453	.084	.143	**.023**	.458	.477	**.031**	**.019**	.638	----	----	----	----
PreChr. vs.	F	0.38	0.93	1.87	1.11	0.80	1.30	3.41	1.69	**5.30**	**7.54**	**8.56**	**3.79**	----	----	----	----
INT/NonEXP	p<	.542	.339	.177	.351	.373	.259	.070	.072	**.025**	**.008**	**.005**	**.001**	----	----	----	----
PreChr. vs.	F	----	----	----	----	**22.5**	**18.3**	**29.2**	**11.7**	**63.3**	**23.0**	**31.0**	**12.7**	----	----	----	----
CHR/EXP	p<	----	----	----	----	**.001**	**.001**	**.001**	**.001**	**.001**	**.001**	**.001**	**.001**	----	----	----	----
PreChr. vs.	F	----	----	----	----	**25.8**	**13.1**	**35.5**	**12.8**	**29.9**	**21.5**	**26.8**	**10.0**	----	----	----	----
CHR/NonEXP	p<	----	----	----	----	**.001**	**.002**	**.001**	**.001**	**.001**	**.001**	**.001**	**.001**	----	----	----	----

Dur - ANOVA for duration of hypothermia
Pkht - ANOVA for peak hypothermia for each rat
Total - ANOVA for Total hypothermia for each rat
TCI - Repeated-Measures ANOVA of the time course for time course * group interaction

APPENDIX B (continued)

TABLE 8

PENTOBARBITAL HYPOTHERMIA
Postchronic vs. Postwithdrawal vs. Postextinction Tests
F-values and Probability Levels

	INT/EXP		CHR/EXP		INT/NonEXP		CHR/NonEXP	
	F	p<	F	p<	F	p<	F	p<
Dur	1.20	.325	**8.65**	**.001**	0.65	.590	**8.51**	**.001**
Pkht	0.97	.420	**6.26**	**.003**	2.03	.131	**5.42**	**.005**
Total	1.10	.350	**10.95**	**.002**	1.95	.169	**7.06**	**.006**
minutes								
10	**3.01**	**.044**	1.90	.149	**5.12**	**.007**	1.83	.163
20	1.55	.219	2.52	.075	**4.76**	**.009**	2.49	.079
30	1.00	.405	**3.81**	**.019**	**4.27**	**.013**	2.52	.077
40	2.08	.121	**3.39**	**.030**	2.65	.067	**4.08**	**.015**
50	1.07	.376	**5.42**	**.005**	1.23	.315	**4.55**	**.015**
60	0.70	.558	**8.62**	**.001**	2.27	.101	**6.46**	**.003**
70	0.50	.683	**12.20**	**.001**	1.69	.190	**9.27**	**.001**
80	0.87	.468	**14.93**	**.001**	2.05	.128	**9.53**	**.001**
90	1.29	.293	**14.06**	**.001**	1.69	.191	**7.71**	**.001**
100	2.06	.125	**14.08**	**.001**	1.94	.144	**8.28**	**.001**
110	1.55	.221	**12.24**	**.001**	1.11	.362	**8.88**	**.001**
120	2.02	.130	**12.78**	**.001**	2.34	.094	**8.84**	**.001**

APPENDIX B (continued)

TABLE 9

PENTOBARBITAL ATAXIA
Prechronic vs. Postchronic and EXP vs. NonEXP
Probability Levels for Duration, Peak and Total

GROUPS	20 mg/kg			28 mg/kg			40 mg/kg			80 mg/kg		
	Dur. p<	Peak p<	Total p<	Dur. p<	Peak p<	Total p<	Dur. p<	Peak p<	Total p<	Dur. p<	Peak p<	Total p<
INT/EXP vs. INT/NonEXP	.005	.203	.022	.032	.211	.035	.262	.307	.060	----	----	----
CHR/EXP vs. CHR/NonEXP	----	----	----	.208	.301	.246	.073	.235	.310	.682	.307	.829
Prechr. vs. INT/EXP	.803	.392	.845	.730	.984	.612	.907	.149	.449	----	----	----
Prechr. vs. INT/NonEXP	.004	.112	.005	.015	.198	.003	.091	.587	.011	----	----	----
Prechr. vs. CHR/EXP	----	----	----	.001	.507	.001	.001	.067	.001	----	----	----
Prechr. vs. CHR/NonEXP	----	----	----	.001	.214	.004	.001	.332	.001	----	----	----

APPENDIX B (continued)

TABLE 10

PENTOBARBITAL ATAXIA
Prechronic vs. Postchronic Time Course
Mann-Whitney U Test Probability Levels

TIME	INT/EXP			INT/NonEXP			CHR/EXP		CHR/NonEXP	
	20 mg/kg	28 mg/kg	40 mg/kg	20 mg/kg	28 mg/kg	40 mg/kg	28 mg/kg	40 mg/kg	28 mg/kg	40 mg/kg
0	.070	.312	.090	.389	.508	**.039**	.704	.103	.823	.720
15	.321	.904	.070	.061	.206	.243	.598	.462	.206	.964
30	.209	.763	.101	**.016**	.142	.383	**.001**	**.011**	**.001**	.078
45	.654	.591	.530	**.006**	.059	.874	**.001**	**.001**	**.003**	**.001**
60	.940	.917	.319	**.011**	.087	.604	**.001**	**.001**	**.005**	**.001**
75	.149	.602	.256	.261	**.040**	.235	**.014**	**.001**	**.018**	**.001**
90	.973	.822	.544	.910	**.040**	.140	.101	**.001**	.116	**.002**
105	.217	.149	.573	.947	**.045**	.129	.149	**.010**	.166	.083
120	.217	.530	.549	.259	**.011**	.259	.149	**.011**	.587	**.017**

APPENDIX B (continued)

TABLE 11

PENTOBARBITAL ATAXIA
Postchronic EXP vs NonEXP Time Course
Mann-Whitney U Test Probability Levels

TIME	INT/EXP vs. INT/NonEXP			CHR/EXP vs. CHR/NonEXP		
	20 mg/kg	28 mg/kg	40 mg/kg	28 mg/kg	40 mg/kg	80 mg/kg
0	.317	.900	.338	.926	.228	.696
15	.328	.376	.296	.184	.539	.296
30	.312	.305	.296	.366	.625	.131
45	.070	.293	.563	.495	.465	.284
60	.113	.130	.563	.296	.121	.442
75	.068	.180	.926	1.000	.856	.224
90	.442	.115	.078	1.000	.277	.788
105	.317	**.011**	.055	1.000	.277	.677
120	1.000	**.013**	.066	.296	1.000	.852

APPENDIX B (continued)

TABLE 12

PENTOBARBITAL POSTCHRONIC LOG DOSE-RESPONSE REGRESSION LINE PARALLELISM
T-test Values for
Hypothermia Duration and Peak,
Ataxia Duration and Peak

Groups	Hypothermia		Ataxia	
	Peak	Duration	Peak	Duration
Prechr. vs. INT/EXP	0.239	1.426	0.689	0.810
Prechr. vs. CHR/EXP	0.861	0.659	0.405	1.141
Prechr. vs. INT/NonEXP	1.641	0.734	1.339	0.240
Prechr. vs. CHR/NonEXP	1.954	0.786	1.775	1.812
INT/EXP vs. CHR/EXP	0.341	1.151	0.650	1.907
INT/NonEXP vs. CHR/NonEXP	0.220	0.165	0.005	1.616
INT/EXP vs. INT/NonEXP	1.062	1.682	1.505	0.488
CHR/EXP vs. CHR/NonEXP	1.186	1.463	1.009	0.812