CHAPTER XI

BROMOCRIPTINE RETARDS TAIL REGENERATION IN 12L : 12D BUT NOT 24L : OD EXPOSED LIZARDS: EVIDENCE FOR PHOTOPERIODIC CONTROL OF PROLACTIN RELEASE MACHANISMS IN LIZARDS.

It is now well established, from studies with pharmacological agents, that dopamine (DA) has an inhibitory role in prolactin (PRL) release. Studies with inhibitors of catecholamine synthesis have shown that a catecholamine is involved in the inhibitory control of PRL release (Clemens, 1976). Several lines of evidence suggest that DA is the main inhibitor of pituitary PRL secretion and that it exerts its effects directly at the level of the lactotroph (for details and references, see Fernandez-Ruiz et al., 1987). Secretion of PRL from the anterior pituitary gland is primarily under negative control by the hypothalamus (Meites and Clemens, 1972) and DA appears to be the hypothalamic inhibitory factor (Macleod, 1976). There is evidence to show that dopaminergic agonists inhibit PRL secretion in intact animals (Takahara et al., 1974). In addition, the inhibitory activity found in hypothalamic extracts has been shown to have properties of catecholamines (Shaar and Clemens, 1974).

Ergot alkaloids have been shown to block PRL secretion (Caron et al., 1978). Bromocriptine (2-bromo-α-ergocryptine), a dopamine receptor agonist, has been shown to reduce PRL levels to normal in patients with both functional hyperpro-

lactinemia as well as those with pituitary tumors (Thorner et al., 1980a). Further, there is now increasing clinical evidence that the drug may lead to a reduction in tumor size and PRL- and growth hormone secreting adenomas in upto 40% of cases (Thorner et al., 1980b). Several other investigations on the inhibitory role of bromocriptine in pituitary PRL release have focused mainly on mammalian species (Melmed, 1981; Archer et al., 1982; Vrontakis et al., 1987). To our knowledge, no investigation has attempted to elucidate the mechanism of PRL release in lacertilians (Ndukuba and Ramachandran, 1989b; Chapter 10 Ramachandran and Ndukuba, 1989b; Chapter 8).

Recent experimental evidence from our laboratory suggests that parachlorophenylalanine (p-CPA), a specific depleter of brain serotonin (Koe and Weisman, 1966; Walker, 1983), retarded tail regeneration in lizards exposed to continuous light (24L : OD), indicating that the stimulatory serotonergic mechanism may be operating under this regime (Ramachandran and Ndukuba, 1989b; Chapter 8). Further evidence exists that pimozide, a potent dopamine receptor antagonist, stimulated the regeneration process in lizards exposed to continuous darkness (OL : 24D), suggesting that the inhibitory dopaminergic mechanism may be functioning under this condition (Ndukuba and Ramachandran, 1989b; Chapter 10). Earlier, we have reported that exogenous ovine-PRL enhanced tail regeneration in lizards maintained under OL : 24D, implicating PRL as a growth

promoter in regenerating lizards (Ndukuba and Ramachandran, (1989a, Chapter 7). However, bromocriptine did not affect the regeneration process in animals exposed to either 24L : 0D or 0L : 24D schedule (Ramachandran and Ndukuba, 1989e, Chapter 9), compelling the authors to test for the possible presence of the dopaminergic system in the intermediate photoperiod of 12L: 12D. The experimental approach in the present study was to inject bromocriptine to lizards obeying the alternating daily light-dark(LD) rhythm since the drug did not influence regenerating lacetilians exposed to constant photoperiods (Ramachandran and Ndukuba, 1989e, Chapter 9).

The data showing that bromocriptine retarded tail regeneration in lizards exposed to 12L : 12D, but not 24L : 0D, providing et al neuropharmacological evidence for the operation of the dopaminergic mechanism under 12L : 12D photoregime, whence it had earlier been hypothesized that both serotonergic and dopaminergic regulatory systems of PRL release may be functioning on par, are presented in this study.

MATERIALS AND METHODS

A total of 80 lizards was used for this investigation and they were balanced for size and sex in order to eliminate any error in the final statistical analysis due to size and sex differences. They were then divided into eight groups of 10 lizards each. Four groups were exposed to the intermediate photoregime of 12L: 12D and the other four to

continuous light-24L : OD. Food and water were provided ad libitum throughout the entire period of experimentation.

Groups 1 and 2. Bromocriptine treated (1mg kg^{-1})

Two groups of 10 lizards each received once daily intraperitoneal (ip) injection of 1mg kg^{-1} bromocriptine, 5 days prior to and 30 days after tail autotomy and were exposed to 12L : 12D and 24L : OD, respectively.

Groups 3 and 4. Bromocriptine treated (2mg kg^{-1})

Two groups of 10 animals each received once daily ip injection of 2mg kg^{-1} bromocriptine, 5 days prior to tail autotomy and 30 days thereafter and were exposed to 12L:12D and 24L:OD, respectively.

Groups 5 and 6. saline treated (0.6%)

Two groups of 10 lizards each served as one control and received once daily ip injection of 0.6% saline, 5 days prior to and 30 days after tail autotomy and were exposed to 12L : 12D and 24L : OD, respectively.

Groups 7 and 8. Control lizards (no saline treatment)

Two groups of 10 lizards each, which served as a second control, were not given any injection. These groups were included in the experiment as a double-check to make sure that

saline injection had no effect on the control data. They were also exposed to 12L : 12D and 24L : 0D respectively.

Preparation of bromocriptine (1mg/ and 2mg/kg^{-1}).

Bromocriptine (2-bromo- α -ergocryptine), as proctinal by Biddle Sawyer Pvt. Ltd., Bombay, India was prepared and stored at 4°C in a refrigerator for the daily injection. The drug was weighed and dissolved in few drops of ethanol. Warmed (40°C) saline (0.6%) was then added to give the required concentration. For the high dose of 2mg/kg^{-1}, twice the weight of bromocriptine used for the low dose (1mg) preparation was utilized. 0.1ml of the prepared solutions was injected giving an approximate dose of 10 µg/animal/day and 20 µg/animal/day, respectively.

Experimental design

Tail autotomy was performed by pinching off the tail at the third segment from the vent. Each group of 10 animals was housed in one cage and four cages were exposed to the 12L : 12D and the other four to 24L : 0D schedules. The detailed description of the light conditions and the dimensions of the experimental cage have been well documented in chapters 2 and 3. This investigation was conducted during the summer month of May and the average daily cage temperature at the level of the animals was 30°C.

Statistical analysis

The length of new growth (regenerate) was measured twice, first with a pair of compass and then with a piece of thread, and the measurements were scored against a ruler graduated in mm. The use of a pair of compass and a piece of thread for taking measurements was designed to cross-check one with the other in order to ensure accuracy, and not for calculating the average of the two. This is an improvement in our earlier method of directly measuring with a ruler graduated in mm, although the improved method has confirmed the accuracy of the old one. The measurements were later used for morphometric calculations. The data on the length of tail regenerated and total percentage replacement were subjected to Student's t test and to Duncan's multiple range test for statistical significance (Duncan, 1955). Values which were different at the $P < 0.01$ and $P < 0.05$ levels were considered to be statistically significant.

RESULTS

The results are shown clearly in figures 1-3. As there was no statistically significant difference between normal (nonsaline) and saline-injected controls, we have opted, for graphical purposes, to represent only the saline-injected controls.

Growth rate, length of tail-regenerated and total percentage replacement

The average length of tail regenerated by the 30th day

TABLE 1. APPROXIMATE NUMBER OF DAYS TAKEN TO REACH THE VARIOUS ARBITRARY STAGES OF TAIL REGENERATION IN BROMOCRIPTINE TREATED AND CONTROL LIZARDS H.FLAVIVIRIDIS EXPOSED TO 24L : OD AND 12L : 12D PHOTOPERIODIC SCHEDULES.

EXPERIMENTAL ANIMALS AND PHOTOREGIMES	WOUND HEALING	BLASTEMA	EARLY DIFFEREN-TIATION	MID-DIFFEREN-TIATION	LATE DIFFEREN-TIATION	GROWTH
24L : OD						
BROMOCRIPTINE ($1mg/kg^{-1}$)	1	3-5	5-7	8	14	30
BROMOCRIPTINE ($2mg/kg^{-1}$)	1	3-5	5-7	8	14	30
SALINE CONTROL (0.6%)	1	3-5	5-7	8	14	30
12L : 12D						
BROMOCRIPTINE ($1mg/kg^{-1}$)	3	5-7	10-12	16	23	30
BROMOCRIPTINE ($2mg/kg^{-1}$)	6	9-11	14-16	20	23	30
SALINE CONTROL	3	5-7	10-12	16	23	30

24L : OD - 24 HOURS OF LIGHT AND 0 HOURS OF DARKNESS (CONTINUOUS LIGHT)

12L : 12D- 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS

* - DAYS AFTER TAIL AUTOTOMY

TABLE 2. LENGTH OF TAIL REGENERATED AND TOTAL PERCENTAGE REPLACEMENT IN BROMOCRIPTINE TREATED AND CONTROL H. FLAVIVIRIDIS EXPOSED TO 24L : OD AND 12L : 12D PHOTOREGIMES DURING THE SUMMER MONTH OF MAY.

EXPERIMENTAL ANIMALS AND PHOTOREGIMES	DAY 10	DAY 15	Day 20	DAY 25	DAY 30	TOTAL % TAIL REPLACEMENT
24L : OD						
BROMOCRIPTINE TREATED $2mg/kg^{-1}$	16.5± 0.56	20.7± 1.25	23.5± 1.48	27.8± 1.50	32.5±1.63*	58.0%
SALINE INJECTED (CONTROL)	17.0± 0.61	20.0± 1.18	22.8± 1.56	27.2± 1.58	32.3±1.68	57.8%
12L : 12D						
BROMOCRIPTINE TREATED $1mg/kg^{-1}$	3.7± 0.78	9.1± 1.22	17.3± 1.69	22.2± 1.77	25.2±1.93	48.4%
BROMOCRIPTINE TREATED $2mg/1kg^{-1}$	--	2.8± 1.53	6.0± 1.67	10.2± 1.93	12.8±2.22	24.6%
SALINE INJECTED (CONTROL)	3.8± 0.74	9.2± 1.07	17.4± 1.62	22.1± 1.86	25.2±1.85	48.4%
NON SALINE (CONTROL)	3.8± 0.74	9.1± 1.13	17.8± 1.53	22.6± 1.74	25.4±1.90	48.8%

24L : OD - 24 HOURS LIGHT AND 0 HOURS DARKNESS (CONTINUOUS LIGHT)
OL : 24D- 0 HOURS LIGHT AND 24 HOURS DARKNESS (CONTINUOUS DARKNESS)
$1mg/kg^{-1}$ - LOW DOSE
$2mg/kg^{-1}$ - HIGH DOSE
* - TOTAL LENGTH OF TAIL REGENERATED IN MM BY THE 30th DAY.

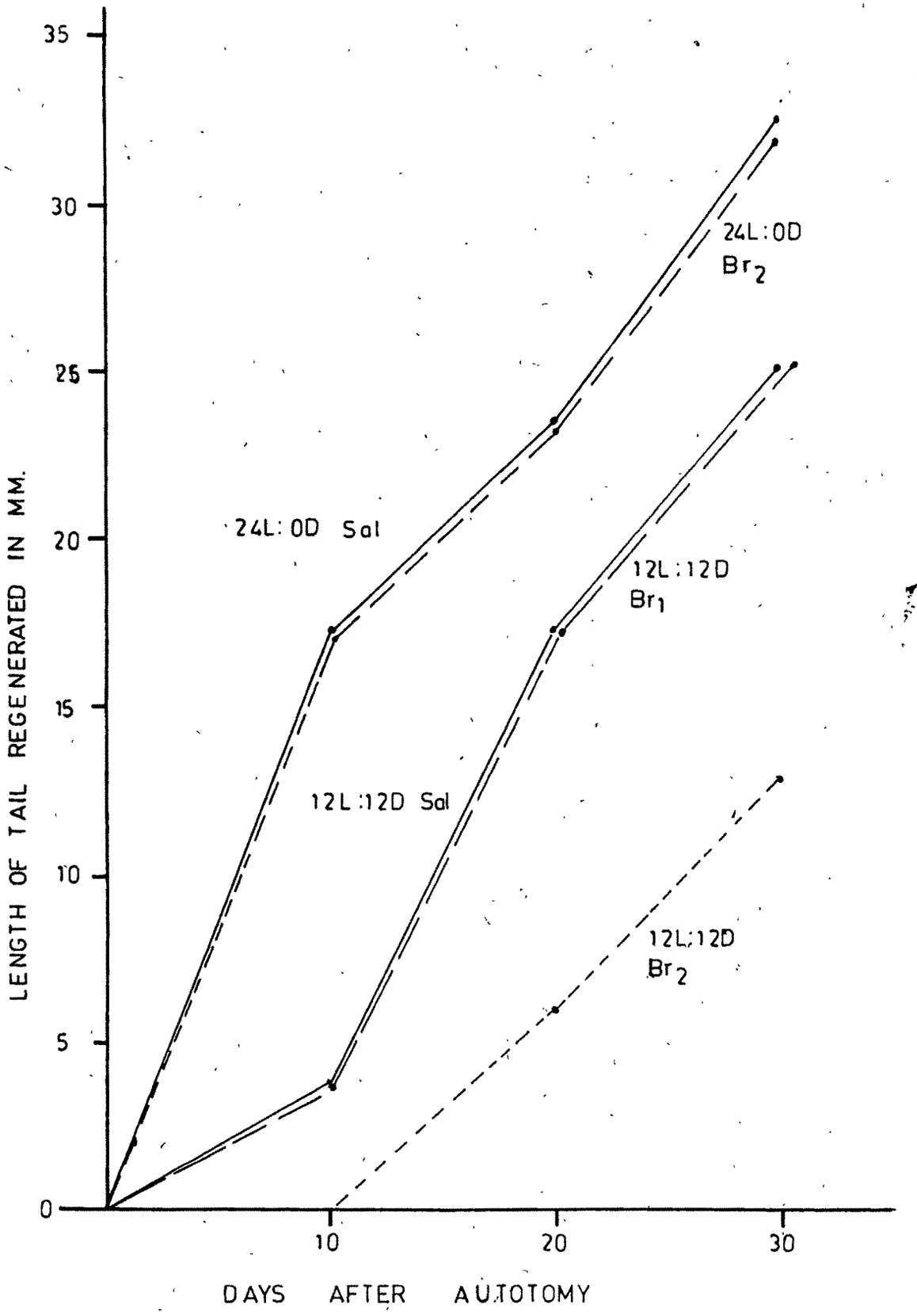

FIG 1 LENGTH OF TAIL REGENERATED DURING 30 DAYS AFTER AUTOTOMY. 24L:0D – CONTINUOUS LIGHT, 12L:12D – 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS. Br_1 – 1mg.kg-1 BROMOCRIPTINE (LOW DOSE), Br_2 – 2mg.kg-1 BROMOCRIPTINE (HIGH DOSE), SAL – SALINE INJECTED CONTROLS.

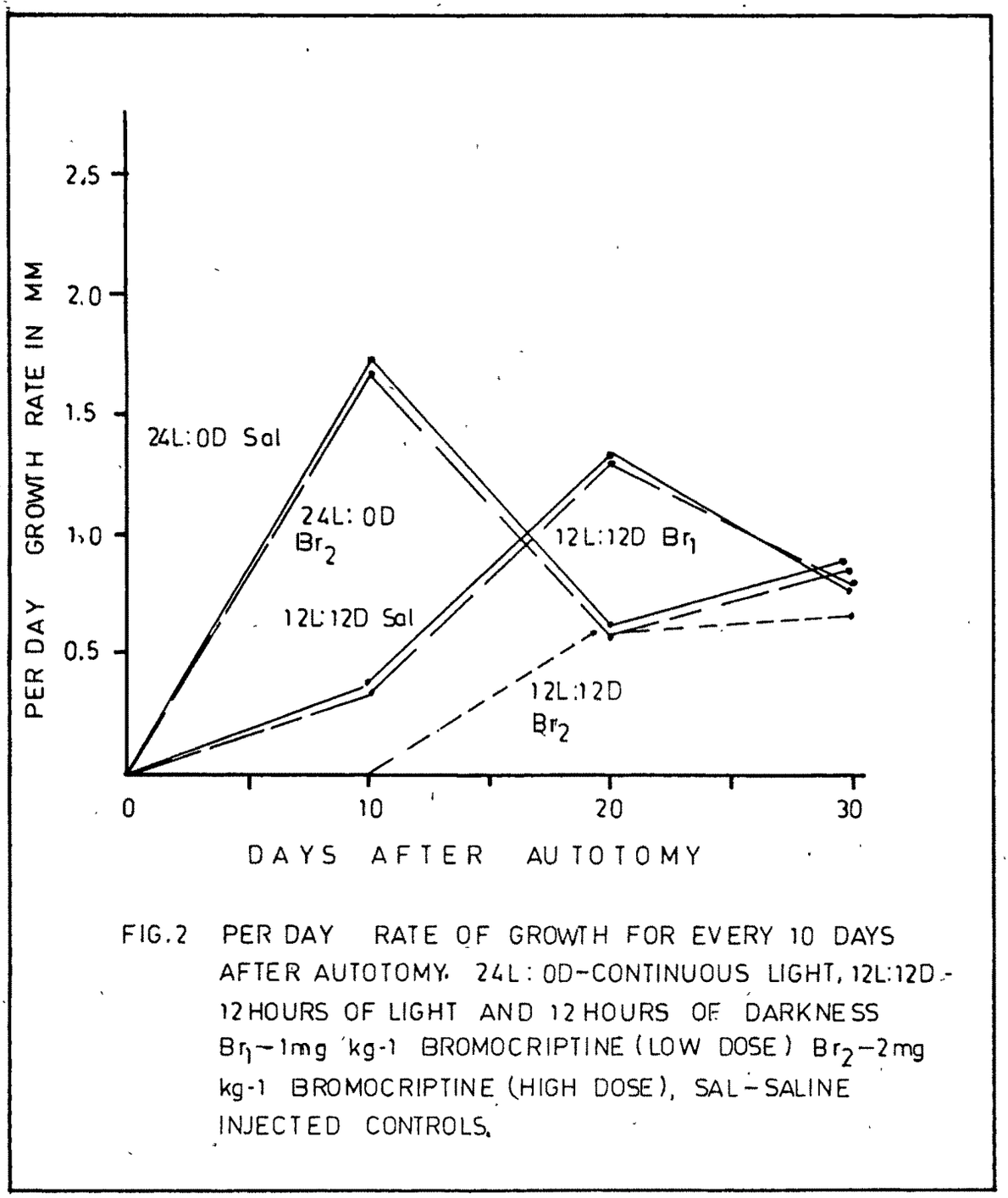

FIG.2 PER DAY RATE OF GROWTH FOR EVERY 10 DAYS AFTER AUTOTOMY. 24L:0D-CONTINUOUS LIGHT, 12L:12D-12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS Br_1-1mg kg-1 BROMOCRIPTINE (LOW DOSE) Br_2-2mg kg-1 BROMOCRIPTINE (HIGH DOSE), SAL-SALINE INJECTED CONTROLS.

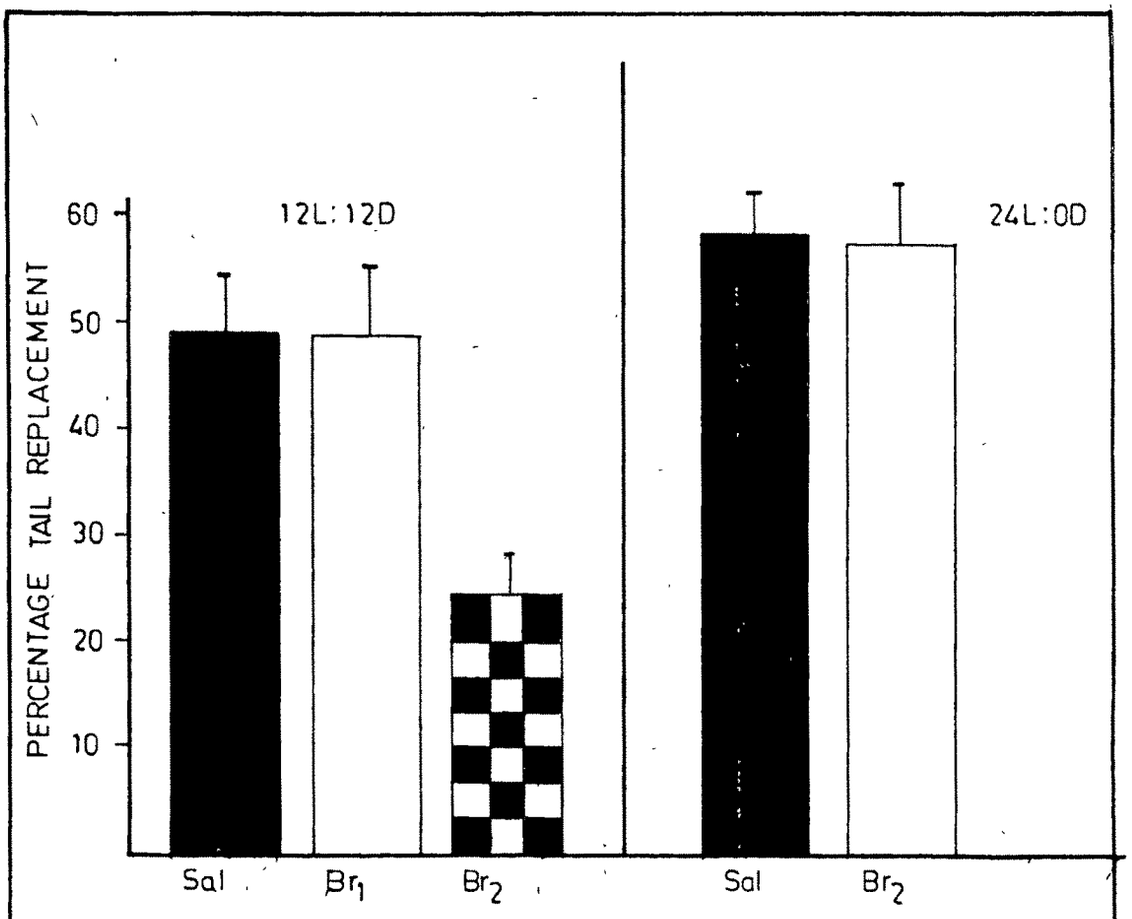

FIG. 3 PERCENTAGE OF TAIL REPLACED AT THE END OF 30 DAYS AFTER AUTOTOMY (± SD SHOWN BY VERTICAL BARS). 24L:0D-CONTINUOUS LIGHT, 12L:12D – 12 HOURS OF LIGHT AND 12 HOURS OF DARKNESS. SAL – SALINE CONTROL. Br_1 – 1 mg kg-1 BROMOCRIPTINE (LOW DOSE), Br_2 – 2 mg kg-1 BROMOCRIPTINE (HIGH DOSE)