Pharmacology Biochemistry & Behavior, Vol. 24, pp. 1577–1582, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Effects of Pimozide on Positive and Negative Incentive Contrast With Rewarding Brain Stimulation

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Received 7 October 1985

PHILLIPS, A. G. AND F. G. LEPIANE. *Effects of pimozide on positive and negative incentive contrast with rewarding brain stimulation.* PHARMACOL BIOCHEM BEHAV **24**(6) 1577–1582, 1986.—Positive and negative contrast effects with brain-stimulation reward were inferred from significant differences in rate/intensity curves obtained by ascending, random and descending orders of current presentation. The neuroleptic drug pimozide caused a dose-related attenuation of both positive and negative contrast. A dose of 0.1 mg/kg blocked positive contrast obtained by comparing ascending and random rate/intensity curves. Negative contrast and threshold current intensities for brain-stimulation reward at sites in the ventral tegmental area were unaffected by the low dose (0.1 mg/kg) of pimozide. Higher doses of pimozide (0.25, 0.4 mg/kg) blocked both positive and negative contrast effects, and caused a significant elevation in threshold current intensities under the random order condition. Two doses (0.25, 0.4 mg/kg) of pimozide were associated with performance deficits as the response rates at the maximum current intensities were attenuated significantly with each order of current presentation. These demonstrations of both positive and negative contrast are consistent with the theoretical position linking brain-stimulation reward to incentive motivation. Furthermore the effects of the dopamine receptor antagonist pimozide are consistent with a role for dopamine in brain-stimulation reward and also raise the possibility of dopaminergic involvement in incentive contrast phenomena.

Positive contrast Negative contrast Brain-stimulation reward Dopamine Pimozide Rats

BOTH positive and negative contrast effects have been observed following an increase or decrease in the current intensities used to maintain self-stimulation behavior in the lateral hypothalamus [11]. In view of the sparse evidence for positive contrast from studies using natural rewards (cf. [6]), further experiments were conducted to confirm and extend the analysis of positive contrast effects with rewarding brain-stimulation. Positive incentive contrast accompanied successive increases in current intensity, a procedure used routinely to determine rate/intensity functions for self-stimulation behavior [12]. This effect was confirmed by Koob [9] in a comprehensive analysis that utilized ascending, descending and random series to present a range of current intensities. Although a comparison of the entire rate/intensity curves in the latter study failed to confirm a negative contrast effect with systematic decrements in current intensities, robust negative contrast effects have been reported with animals running for brain-stimulation reward in a shuttle box [1].

Contrast effects may serve as a sensitive index of changes in the incentive value of rewarding stimuli and as such may be a useful procedure for detecting pharmacological manipulation of brain-stimulation reward. Alterations of reward value would not necessarily produce symmetrical changes in both positive and negative contrast. Nevertheless, enhancement of contrast effect should be observed following pharmacological increases in reward value at specific current intensities. Conversely, a reduction in the relative change between adjacent current intensities should lead to decrements in positive and negative contrast at the most sensitive current intensities.

Neuroleptic drugs including pimozide have been used extensively to study the role of dopamine (DA) in brain-stimulation reward [4]. Although pimozide at high doses may interfere with operant behavior [3], there is now general agreement that it has selective effects on brain-stimulation reward at moderate doses [7, 14, 16]. The present experiment examined the possible consequences of such pharmacological attenuation of rewarding brain-stimulation by pimozide on incentive contrast effects obtained with systematic changes in the intensity of electrical brain-stimulation. In addition, changes in operant responding at optimal current intensities were used to monitor drug-induced motor deficits.

METHOD

Subjects

Twelve male Wistar rats weighing 280–320 g at the time of surgery were housed individually in stainless steel cages located in a climatically controlled colony room with a 12 hr light/dark cycle. Food and water were available ad lib.

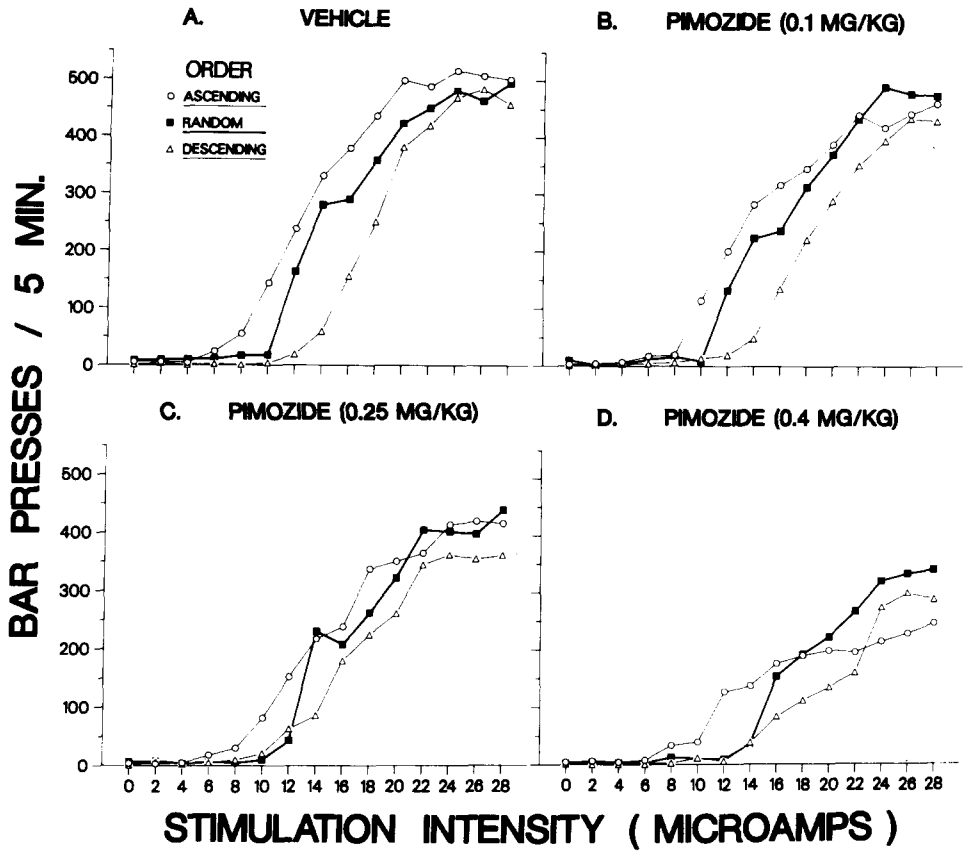

FIG. 1. The effects of pimozide (0.1–0.4 mg/kg) on rate-intensity curves for rewarding brain-stimulation at sites in the ventral tegmentum, obtained with ascending, random or descending orders of current presentation.

TABLE 1

THRESHOLD CURRENT INTENSITIES (μA) FOR SELF-STIMULATION IN THE VTA AS A FUNCTION OF THE ORDER OF CURRENT PRESENTATION AND PRETREATMENT WITH PIMOZIDE (0.0–0.4 mg/kg)

	Dose (mg/kg)			
Order	Vehicle	0.1	0.25	0.4
Ascending	12.50 ± 1.18	13.00 ± 1.45	14.33 ± 1.55	17.17 ± 21.0
Random	14.00 ± 0.92	15.33 ± 1.31	15.83 ± 1.14	16.50 ± 1.28
Descending	16.17 ± 0.76	16.50 ± 1.05	15.50 ± 1.16	20.33 ± 1.61

Data presented as mean ± s.e.m.

Surgery and Histology

Each animal was anaesthetized with sodium pentobarbital (50 mg/kg), placed into a stereotaxic apparatus, and a small diameter (0.005 in., Plastic Products Co.) nichrome bipolar electrode was implanted chronically. The uninsulated electrode tips were aimed at DA containing cell bodies in the ventral tegmental area (VTA). The stereotaxic coordinates with the mouthbar located 4.2 mm below the interaural line were : anterior from stereotaxic zero = +2.3 mm; lateral =0.8 mm; dorsal=2.1 mm. At the completion of the experiment all subjects were sacrificed, their brains removed rapidly and stored in 10% buffered Formalin. For histological confirmation of electrode placements, each brain was frozen, sectioned at 30 μ and the sections containing electrode tracts were mounted and stained with cresyl violet.

Procedure

Testing for self-stimulation was conducted in 5 Plexiglas

TABLE 2

SELF-STIMULATION RATES (BAR-PRESSES/5 MIN) AT MAXIMUM CURRENT INTENSITIES AS A FUNCTION OF THE ORDER OF CURRENT PRESENTATION AND PRETREATMENT WITH PIMOZIDE (0.0–0.4 mg/kg)

	Dose (mg/kg)							
	Vehicle		0.1		0.25		0.4	
Order Current (μA)	26	28	26	28	26	28	26	28
Ascending	508 ± 29	501 ± 26	446 ± 39	464 ± 39	418 ± 51	415 ± 46	229 ± 61	247 ± 61
Random	464 ± 29	495 ± 25	181 ± 39	479 ± 44	397 ± 51	438 ± 36	334 ± 44	341 ± 44
Descending	484 ± 29	458 ± 25	438 ± 44	433 ± 42	354 ± 56	359 ± 55	299 ± 61	288 ± 54

Data are presented as means ± s.e.m.

chambers (46×30×24 cm). Depression of a small bar 2.5 cm wide, activated a constant current stimulator which could deliver various intensities (0–200 μA) of 60 Hz sine wave at a fixed duration (0.2 sec) through a flexible cable, to the chronic stimulating electrodes. The lever presses were recorded on Sodeco counters. Following the establishment of stable self-stimulation behavior, each subject was tested with a range of stimulation currents from 0–28 μA.

These current intensities were presented in one of three orders; ascending (0–28 μA), descending (28–0 μA), or random (selected from a table of random numbers). Each current was available for a 5-min period and up to 10 priming stimuli were administered at the start of each period, if no responding occurred in the first 10 sec. Contrast effects were defined as significant differences between rate/intensity curves obtained under the ascending versus random (positive contrast) and descending versus random (negative contrast) orders of presentation.

Following confirmation that both positive and negative contrast effects could be obtained with ascending and descending orders of presentation respectively, the sequence of testing was repeated with three doses of pimozide (0.1, 0.25, 0.4 mg/kg). The drug was dissolved in a warm solution containing tartaric acid (6:1 part pimozide). Injections were given intraperitoneally, 3 hours before testing. The exact sequence of current intensities under the random condition was varied for each drug dose. In addition to studying the influence of pimozide on contrast effects with brain-stimulation reward, a comparison of the dose effects across the four random orders of stimulus presentation permitted a further analysis of neuroleptic effects on both the rewarding (threshold current intensities) and performance (bar-press rates at maximum current intensities) correlates of VTA self-stimulation behavior.

RESULTS

Positive and Negative Contrast Effects

As may be seen in Fig. 1A, the ascending order of current presentation was accompanied by bar-pressing rates that were higher than those observed with random shifts in current intensity. The opposite effect was obtained with a descending order of presentation. A two-way ANOVA of bar-pressing rates under each order condition across the range of current intensities from 10–24 μA, revealed a significant main effect for order of current presentation, $F(2,22)=17.76$, $p<0.01$. Post-hoc analyses with Duncan's Multiple Range test indicated significant differences ($p<0.05$) between the ascending and random orders as well as between the random and descending conditions. These data confirmed the presence of positive and negative contrast effects respectively with rewarding brain-stimulation of the VTA.

Effects of Pimozide on Positive and Negative Contrast

The effects of pimozide (0.1, 0.25, 0.4 mg/kg) on both positive and negative contrast effects with VTA stimulation are shown in Fig. 1B, C, D. A separate ANOVA was computed for each dose condition and post-hoc comparisons between the order of stimulus presentation were made whenever a significant main effect for order was obtained. A significant order effect was observed with the lowest dose (0.1 mg/kg) of pimozide, $F(2,22)=15.31$, $p<0.01$, and post-hoc tests revealed a significant difference between the random and descending orders of presentation, but not with the random and ascending orders. Therefore these data indicate a significant attenuation of the positive contrast effect and no effect of this dose on negative contrast (see Fig. 1B).

A similar statistical analysis of the data obtained after treatment with the second dose of pimozide (0.25 mg/kg) again identified a significant order effect, $F(2,22)=4.88$, $p<0.02$, but in this instance only the ascending and descending orders were significantly different from each other. In the absence of significant differences between rate/intensity curves for both the ascending and random comparison and random versus descending orders, pimozide at a dose of 0.25 mg/kg appeared to have blocked both positive and negative contrast effects (Fig. 1C).

The final analysis compared the rate/intensity curves obtained following treatment with the high dose of pimozide (0.4 mg/kg), and there was no significant main effect for the order in which current intensities were presented, $F(2,22)=1.98$, $p>0.05$. Again, both positive and negative contrast effects appeared to have been blocked by pimozide (Fig. 1D).

Effects of Pimozide on Reward Threshold and Bar-Pressing Rate at Maximum Current Intensities

Threshold current intensities were defined as the lowest intensity required to maintain a rate of 50 or more bar presses in a 5 min period. Individual current thresholds were computed for each subject under each of the four drug conditions for each order of current presentation. The mean scores for each condition are given in Table 1. A statistical analysis of these data using a two-way ANOVA identified a significant main effect for order of presentation,

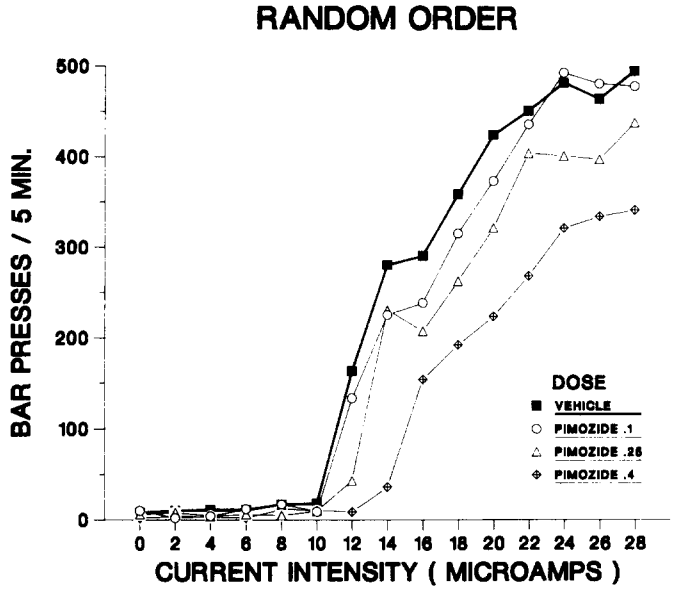

FIG. 2. The effects of pimozide (0.1–0.4 mg/kg) on rate-intensity curves for rewarding brain-stimulation at sites in the ventral tegmentum obtained with random orders of current presentation.

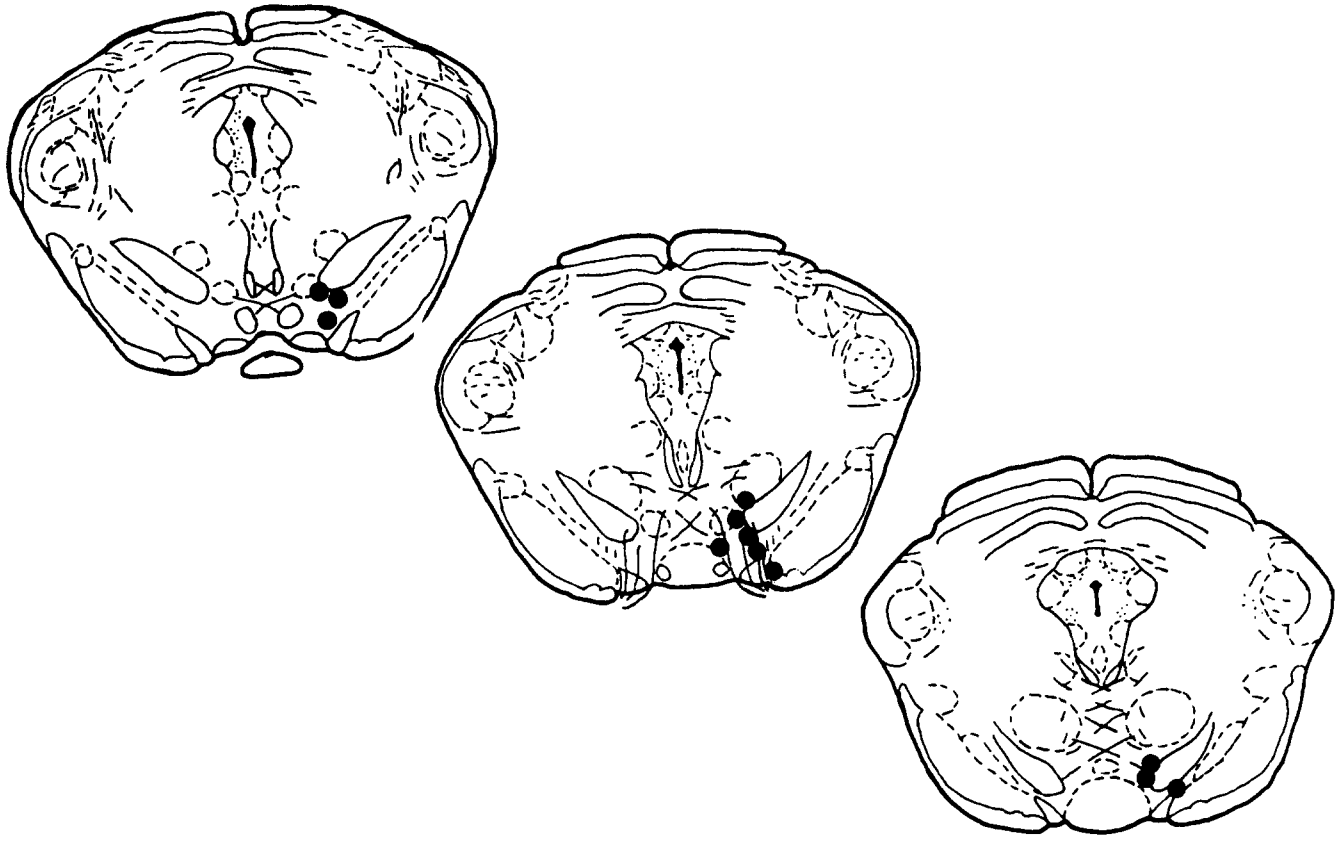

FIG. 3. Location of the tips of bipolar stimulating electrodes in the ventral tegmentum on coronal representations of the mesencephalon redrawn from König and Klippel [8], plates 46b, 48b, 49b.

$F(2,22)=9.95$, $p<0.01$, and drug dose, $F(3,33)=14.77$, $p<0.01$. Post-hoc analyses indicated a significant difference between the descending and random orders but not with ascending versus random conditions Similar post-hoc tests of the dose effect with the random order of current intensities identified a significant difference between the 0.25 and 0.40 mg/kg doses of pimozide as compared with the vehicle condition. Together, these analyses indicate that current thresholds for rewarding brain-stimulation were significantly elevated with a descending order of presentation, as compared to the random and ascending conditions. Furthermore, a 0.4 mg/kg dose of pimozide was required to produce a significant increase in threshold values when the ascending and descending current orders of presentation were employed.

As an index of possible performance effects accompanying the various doses of pimozide, bar-pressing rates at 26 and 28 μA were compared under each order of current presentation (see Table 2). Under the random sequence, a significant main effect was observed for dose, $F(3,33)=12.71$, $p<0.01$. Similar effects were obtained for both the ascending, $F(3,33)=28.06$, $p<0.01$, and descending, $F(3,33)=5.19$, $p<0.01$, conditions. Post-hoc tests identified significant differences between the high dose of pimozide (0.40 mg/kg) and the vehicle as well as the two lower doses of pimozide for the random condition. Under this condition, the maximum response rates observed with a 0.25 mg/kg dose of pimozide also differed significantly from the vehicle condition, whereas the lowest dose (0.10 mg/kg) did not. Similar differences between maximum response rates obtained after vehicle injections and the three doses of pimozide were confirmed by post-hoc analyses of the data from the ascending and descending orders of current presentation.

Effects of Pimozide on Random Rates/Intensity Curves

A comparison of rate/intensity curves under the random condition obtained following vehicle and drug conditions allowed for a further analysis of neuroleptic effects self-stimulation of the VTA, in a manner unconfounded by positive and negative contrast effects (see Fig. 2). A two-way ANOVA yielded significant main effects of drug dose, $F(3,33)=34.8$, $p<0.01$, and current intensity, $F(7,77)=35.8$, $p<0.01$, there was also a significant dose $\times$ current interaction. Significant differences were observed between the vehicle condition and the two higher doses of pimozide (0.4, 0.25 mg/kg). Post-hoc comparisons between bar-pressing rates at intensities ranging from 12–24 μA showed significant differences at all current intensities for 0.4 mg/kg. A significant reduction in bar-pressing rates was observed across most of the intensity range with a dose of 0.25 mg/kg; the two exceptions being at 14 and 22 μA. No significant differences were observed between vehicle treatment and 0.1 mg/kg pimozide.

Histology

The location of the tips of the stimulating electrodes in the brains of each of the 12 rats employed in this study are shown in Fig. 3. The majority of electrode placements (N=10) were located in the ventral tegmentum, between the interpeduncular nucleus and the medial lemniscus or substantia nigra. One electrode terminated on the ventral edge of the red nucleus and another was localized to the medial aspect of the substantia nigra pars reticulata.

DISCUSSION

This experiments confirms the induction of both positive and negative contrast effects with increments and decrements in current intensities used to elicit brain-stimulation reward. The robust positive and negative contrast effects seen here with VTA electrode placements are consistent with the theory linking brain-stimulation reward to incentive motivation [10,15]. In spite of the difficulties associated with the induction of positive contrast in runway experiments by augmenting the number of food pellets [6], the repeated observations of positive contrast or elation effects with electrical brain-stimulation ([9, 11, 12], present results) would appear to validate this phenomenon.

As in an earlier study by Koob [9], the present effects were obtained in conjunction with the generation of ascending and descending rate/intensity curves; a procedure commonly used to study the pharmacology of brain-stimulation reward. As such, these data may be used to endorse Koob's concern regarding the interpretation of the relationship between a given intensity of electrical stimulation and reward value presumably associated with the direct activation of a neural substrate of brain-stimulation reward. In the presence of robust positive and negative contrast effects, any pharmacological alteration of self-stimulation may reflect either direct effects on the value of the rewarding stimulus, the incentive effects associated with shifts between specific current intensities, or a combination of these two factors; assuming of course no drug-induced performance deficits. The possible confounding effects of incentive contrast in drug studies of self-stimulation could be overcome to a degree by using a random order of current intensities to generate rate/intensity functions. Recently we have established that comparable rate/intensity functions are generated from both within session (present study) and between session random manipulation of current intensities (Phillips and Druhan, unpublished observation).

An analysis of the effects of pimozide on the rate/intensity curves obtained with the random presentation sequences revealed a significant shift to the right after injections of pimozide at doses of 0.25 and 0.4 mg/kg. Threshold current intensities required to maintain bar-pressing rates of 50 or more responses per 5 min interval also were increased significantly with the two higher doses of pimozide. It should be noted however, that maximum bar-pressing rates were attenuated significantly with these doses of pimozide (0.25, 0.4 mg/kg). The presence of such performance deficits can confound the interpretation of drug effects on brain-stimulation reward but the significant effect of these doses of pimozide on both rate/intensity curves and threshold current intensities demonstrates that pimozide can block the rewarding effects of brain-stimulation at electrode placements in the VTA. A similar pattern of results was reported recently with electrode placements in the lateral hypothalamus, used in conjuction with the reward summation function paradigm [14]. Together, these data support the role of dopaminergic neurons in both reward and motor processes [13].

In view of the evidence provided here and elsewhere for a direct attenuation of the value of brain-stimulation reward by neuroleptics [3, 7, 14, 16], this would seem to be the most probable means by which pimozide attenuated incentive contrast effects in the present study. Although attenuation of brain-stimulation reward may be the most parsimonious explanation of pimozide's effects on incentive contrast, other factors also may play a role. The selective effect of pimozide

at a dose of 0.1 mg/kg on positive contrast in the absence of any corresponding effects of brain-stimulation reward, as measured by changes in threshold currents or rate/intensity curves raises the possibility of dopaminergic mediation of incentive contrast. Dopaminergic pathways have been related to incentive motivational processes [4,10] and as such could mediate some aspect of the abrupt enhancement or depression of motor activity associated with shifts in reward value [2]. Obviously this proposition cannot be pursued effectively with brain-stimulation reward obtained mainly from direct activation of dopaminergic neurons in the VTA. Future studies should employ shifts in the concentration of sapid solutions such as sucrose or perhaps saccharin and evidence should be sought for selective effects of neuroleptics on incentive contrast independent from changes in responding for the primary reward per se.

The question remains as to whether different mechanisms underlie the induction of positive and negative contrast. The relative ease with which negative contrast can be obtained in a wide variety of circumstances may be indicative of important differences between these two phenomena. Studies of the duration of these effects following shifts in intensity of brain-stimulation reward indicate that elation effects may be less persistent than negative contrast [11]. In the present study, positive contrast appears to be more sensitive to the effect of pimozide than negative contrast, although this may simply reflect relative differences in the magnitude of these two effects. However, long-term treatment with the antidepressant drug desipramine has a selective facilitatory effect on the ascending rate/intensity measure of VTA self-stimulation which in turn may be related to positive contrast effects associated with this procedure [5].

Additional pharmacological studies of positive and negative contrast are clearly warranted as they may reveal important information regarding the neural bases of these phenomena.

ACKNOWLEDGEMENTS

This research was supported by Program Grant 23 from the Medical Research Council of Canada. We thank J. Druhan for valuable discussion.

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