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Anxiolytic-Like Action of Melatonin on Acquisition but not Performance of DRL

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NEVILLE, K. AND N. McNAUGHTON. *Anxiolytic-like action of melatonin on acquisition but not performance of DRL.* PHARMACOL BIOCHEM BEHAV 24(6) 1497–1502, 1986.—The behavioural effects of melatonin have been attributed to a general reduction in motor activity; interference with memory fixation; a decrease in emotionality; or an anxiolytic action. The present experiments compared the effects of melatonin with an anxiolytic benzodiazepine, chlordiazepoxide (Librium), on a schedule of differential reinforcement of low rates of response (DRL) increasing 'burst' responding and premature responding. No doses of melatonin tested (0.03–8.1 mg/kg, IP) affected performance of well-learned DRL. Both low (0.03 mg/kg) and high (1.0 mg/kg) doses of melatonin impaired acquisition of DRL in a similar manner to chlordiazepoxide (5.0 mg/kg) and to much the same extent. Chlordiazepoxide had its usual effects on both acquisition *and* performance of DRL. These results show that melatonin shares a subset of the effects of chlordiazepoxide. The nature of the effects favours an 'anxiolytic' hypothesis of melatonin action rather than the other hypotheses so far proposed.

Melatonin Chlordiazepoxide DRL Anxiety Benzodiazepines Nonreward Behavioural inhibition

IN reviewing the behavioural effects of administration of melatonin Datta and King [5] cover a number of hypotheses. The simplest, and the one with least support, is that melatonin generally reduces motor activity. A second hypothesis is that melatonin acts to interfere with 'memory fixation,' that is to say interfering with retention as opposed to acquisition of learned responses. A third hypothesis is that melatonin might decrease emotionality or some other feature of motivational systems. Related to this is Romijn's [19] suggestion that the pineal is a tranquillising organ.

The behavioural evidence in relation to these three hypotheses is somewhat sparse. However, a number of data suggest that the last of them in particular is worth testing. Firstly, there is the report of Marangos *et al.* [15] that melatonin inhibits diazepam binding, suggesting that melatonin interacts directly or indirectly, with the benzodiazepine receptor. Secondly, there is the observation that melatonin causes increased GABA levels [1]. If this represents an increase in GABAergic activity it would parallel a proposed common action of a variety of anxiolytic drugs [8,17]. Thirdly, melatonin can both induce sleep and potentiate barbiturate-induced sleep time (see [5]). Fourthly, like a variety of anxiolytic drugs, melatonin can act as an anticonvulsant—in this case its action is not obtained through an effect on the benzodiazepine receptor itself [10]. Whether its anticonvulsant action is at some other site in the GABA-benzodiazepine receptor complex (e.g., that affected by barbiturates) has not yet been determined. Fifthly, both 'central' and 'peripheral' type of benzodiazepine receptor have been identified in the pineal [13,18].

The present experiments, therefore, set out to investigate the possiblity that, among other potential actions, melatonin would share some of the behavioural properties of the anxiolytic drugs. Melatonin can inhibit the pituitary-adrenal response to stressors (see [5]). So it was decided to use a task which did not involve shock in order to minimise the involvement of the pituitary-adrenal system.

One task which anxiolytics have been consistently reported to impair is that of differential reinforcement of low rates of responding (DRL) [3,9]. This impairment in DRL has been reported to have a specific form which allows it to be differentiated from the changes in DRL produced by non-anxiolytic drugs [2,21]: both an increase in premature responding close to the correct time and a specific increase in bursts of responses.

In our first experiment, therefore, the effects of a number of doses of melatonin were assessed for their capacity to change responding on a DRL 15 second schedule. For comparison the effects of 5 mg/kg chlordiazepoxide were also tested. It was expected that both melatonin and chlordiazepoxide would have the following effects: (1) Shift the distribution of inter-response (IRTs) to shorter times reducing the number of reinforcements received by the animals; (2) increase the number of very short IRTs (i.e., less than or equal to 2 seconds). These responses are referred to below as bursts.

In this experiment testing was carried out during the first two hours of darkness of the rats' light/dark cycle. During this time melatonin levels are as low as during the light part of the cycle. During the third hour of darkness they rise rapidly and then remain high until commencement of the light phase [7, 12, 16].

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The data from Experiment 1 showed that melatonin injections do not reproduce the effects of anxiolytic drugs on performance of DRL. CDP was shown to be effective both before and after administration of melatonin and melatonin was ineffective whether given in small and increasing doses or given in a single extremely large dose (8.1 mg/kg) to animals which had not previously received melatonin.

However, experiments completed shortly after Experiment 1 [14] showed that CDP interfered with a successive discrimination task in two separate ways. Firstly, when the task was well learned CDP interfered with it through a form of state dependency; secondly, during acquisition of the task CDP interfered in a way which was not state dependent. In Experiment 1, the ABA design of drug administration makes it impossible to rule out state-dependency as the mechanism through which CDP is having its effects. Experiment 2 was carried out, therefore, to test whether melatonin would reproduce the effects of CDP if given during acquisition of DRL.

The testing of melatonin on acquisition of DRL required a between groups design for drug administration. For this reason larger numbers of subjects had to be run and hence a smaller number of doses had to be tested than in Experiment 1. The groups chosen were: a vehicle injected control, a group receiving 0.03 mg/kg melatonin, a group receiving 1.0 mg/kg melatonin and a group receiving 5 mg/kg CDP. The CDP was used for comparison with the effects of melatonin and to ensure the sensitivity of the experimental measures to anxiolytics. The high dose of melatonin was used for comparison with other behavioural experiments in the literature. The low dose was used in the hope of producing a more physiological effect.

Previous behavioural and pharmacological work has frequently used doses in the 100–1000 μg range. Gibbs and Vriend [6] found that intravenous injection of 2 μg of melatonin raised plasma levels of melatonin by 80 pg/ml one hour after injection. Normal diurnal variation in plasma melatonin is between a low of 20 pg/ml and a high of 40 pg/ml [11]. Previous workers must, therefore, have produced increases in, and absolute levels of, melatonin that are well outside the physiological range. The dose of 0.03 mg/kg (approximately 10 μg/rat) used in Experiment 2 would bring the change in level of melatonin during the experiment closer to the physiological range. This dose is higher than that used by Gibbs and Vriend to allow for the change in route of injection, to IP, and in the hope that melatonin levels would stay high throughout the experimental session. No assay was available to check for actual plasma levels of melatonin.

Because of the large number of animals involved Experiment 2 was run throughout the light part of the rats' light/dark cycle.

METHOD

Subjects

Male rats, approximately 3 months old at the beginning of the experiment, were placed on 23 hour food deprivation for 10 days prior to experimentation. Their body weights ranged from 250 to 350 grams at the beginning of the experiment and subjects were fed daily for one hour of free feeding for the duration of this experiment. Subjects were housed in groups of 5 rats per cage in a room with regulated heating (21±2°C) on a 12:12 hour light/dark cycle. The dark period commenced at 6.30 p.m. Water was available to subjects ad lib in their home cages. In Experiment 1 ten Wistar rats were used: They had previously been tested for spontaneous alternation in a T-maze to accustom them to being handled, but were otherwise experimentally naive. In Experiment 2, 32 naive Sprague-Dawley rats were used. Feeding occurred at 8.30 p.m. in Experiment 1 and 5.00 p.m. in Experiment 2.

Apparatus

Four automated Skinner boxes (Campden Instruments) each of internal dimensions 24.5 cm by 22.5 cm high by 23 cm deep, with a grid floor, were used to train and test all subjects. Each box contained a food hopper, one fixed lever and one retractable lever, three small separate lights each of 2.8 watts located on the same side of the box as the hopper and levers and a main light of 2.8 watts located in the centre of the roof. Only the retractable lever, the main light and the food hopper were used in this experiment. An Acorn Atom microcomputer programmed in ONLIBASIC operated the Skinner boxes and recorded and printed out the data from each box. The Skinner boxes and computer were located in a separate room to the home cages. Noyes food pellets, each weighing 45 mg, were used as food reinforcement.

Procedure

After 10 days of 23 hour food deprivation, training in the Skinner boxes commenced. To begin with, all rats were trained to receive food from the food hopper at the sound of the automated food deliverer advancing (to deliver a pellet) using a non-contingent variable interval schedule of 30 seconds between pellets. Each session continued for 15 minutes and each subject was given two sessions per day for three days. The lever was retracted during these two sessions. As there were four boxes, rats were removed from their home cages and run in squads of four in the same order every day. All subjects were then trained to bar press by placing them on a continuous reinforcement schedule (CRF), contingent on pressing the retractable lever. Each CRF session lasted for 30 minutes and subjects were given one session per day (Days 1–3). A print-out giving the number of bar presses made by each rat was obtained at the end of each day. At the end of the CRF training, the eight rats with the highest bar pressing rates on the last day were chosen for DRL training.

For three days (Days 4–6), the eight rats were trained on a DRL 5 seconds schedule and were then advanced to a schedule of DRL 10 seconds for the next four days (Days 7–10). A schedule of DRL 15 seconds was then employed and maintained for the remainder of the experiment. All DRL sessions lasted one hour and each subject was run in the same Skinner box at the same time once every day. Training took place between 6.30–8.30 p.m. in Experiment 1 and 8.00 a.m. and 5.00 p.m. in Experiment 2.

Injections (Experiment 1)

The injection procedure was as follows. Melatonin (Sigma Chemical Co.) was dissolved in propylene glycol and then mixed with 0.9% saline solution. The dosage of melatonin administered varied from 0.033 mg/kg to 8.1 mg/kg and the ratio of propylene glycol (PG) to saline solution increased as the dosage increased in order to dissolve the greater weight of drug used. The ratio of PG to saline solution used with each dosage is given in Table 1.

On melatonin treatment days, subjects were either injected 30 minutes before the session with melatonin in the

TABLE 1

AMOUNTS OF PROPYLENE GLYCOL AND SALINE USED AS VEHICLE FOR VARYING DOSES OF MELATONIN

Melatonin (mg/kg)	PG:Saline Solution
0.033; 0.01; 0.3	1:14
0.9; 2.7	1:4
8.1	1:1

TABLE 2

SUMMARY OF INJECTION SCHEDULES

	Day									
	24	25	26	27	28	29	30	31	32	33
A	S	C5.0	S	S	M0.03	V	M0.1	V	M0.3	V
B	S	S	S	S	M0.03	V	M0.1	V	M0.3	V
C	S	C5.0	S	S	V	V	V	V	V	V
D	S	S	S	S	V	V	V	V	V	V
	Day									
	34	35	36	37	38	39	40	41	42	43
A	M0.9	V	M2.7	V	M8.1	V	V	C5.0	S	S
B	M0.9	V	M2.7	V	M8.1	V	V	C5.0	S	S
C	V	V	V	V	V	V	M8.1	S	C5.0	S
D	V	V	V	V	V	V	M8.1	S	C5.0	S

Each schedule (A,B,C,D) was delivered to 2 rats. M indicates melatonin, C indicates chlordiazepoxide HCl, V indicates propylene glycol/saline vehicle (see Table 1), S indicates saline. Numbers are doses in mg/kg.

vehicle or with the vehicle only using a volume of 1.0 ml/kg. Chlordiazepoxide hydrochloride (Librium, Roche) in powder form was dissolved in saline solution made to a concentration of 5 mg/ml to give a dosage of 5 mg/kg of CDP as 1.0 ml/kg and injected 10 minutes before the session. Control injections for CDP treatment consisted of saline solution only. Each injection was carried out using a 1 cc Plastipak disposable syringe with a detachable Monoject disposable hypodermic needle (25 ga/5/8A).

The schedule of drug injections is given in Table 2. From day 21 of the experiment (day 11 of DRL 15) to day 27 the 8 rats were divided into two groups of 4. On day 25 one of the two groups was injected with 5 mg/kg CDP, in all other cases the rats were injected with saline. From day 28 and for the rest of the experiment two new groups were formed with two rats from the previous CDP group and two rats from the previous saline group in each. One of these groups (Melatonin) received alternating days on which either melatonin or vehicle was injected. The second (Vehicle) received vehicle up until day 40.

From day 40 the rats remained grouped ("Melatonin" vs. "Vehicle") as before and received melatonin or CDP as indicated in Table 2.

Injections (Experiment 2)

All drugs were made up in a 50:50 mixture of propylene glycol and saline. This mixture was also given to the vehicle control group. All injections were given in a volume of 1 ml/kg. Four separate groups of 8 rats each received respectively: vehicle only, 0.033 mg/kg melatonin; 1.0 mg/kg melatonin; 5 mg/kg CDP. Injections were started on the last day of CRF training and continued throughout acquisition of DRL 5, DRL 10, and DRL 15 seconds.

Data Analysis

The IRT for each response during a session was recorded for each subject classified in one second bins, with all responses greater than 30 seconds pooled in the 30th bin. At the end of a session the cumulated IRT distribution for each rat was printed.

The number of responses in each bin was logarithmically transformed to normalise the data [22] and submitted to analysis of variance. Three factors were tested, together with their factorial interactions: group (e.g., CDP vs. saline); days; and bins (i.e., IRT). Changes in shape of the IRT curve were assessed through extraction of linear, quadratic, cubic and quartic orthogonal polynomial components [22].

It should be noted that in Experiment 1 the group factor represents sampling bias in assignment of rats to group initially, while group × days detects effects of the drugs. In Experiment 2 the group factor represents the overall effects of the drugs, while the groups × days interaction detects

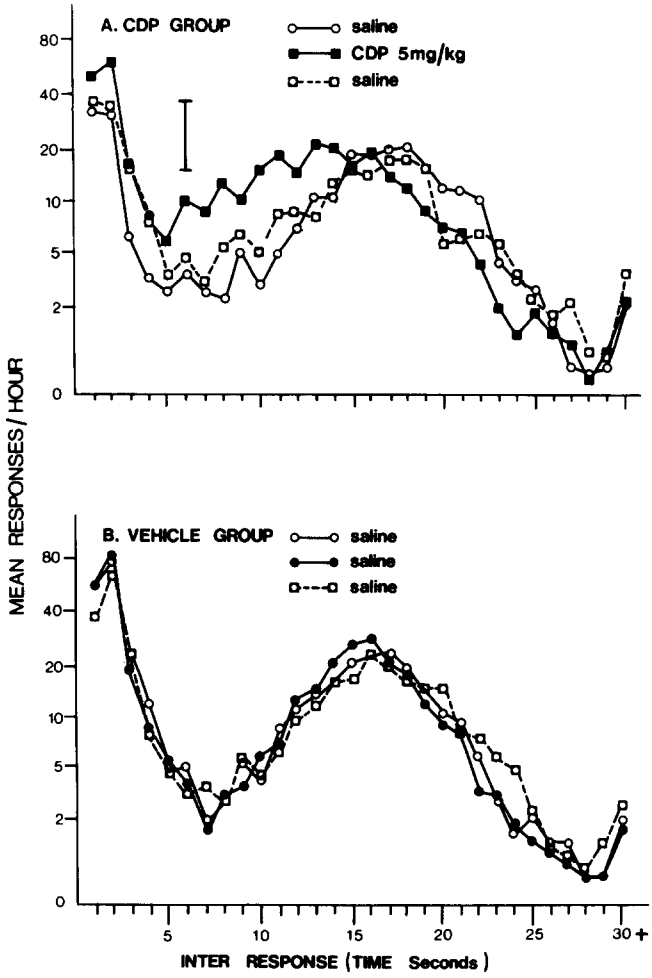

FIG. 1. Effects of administration of chlordiazepoxide (5 mg/kg IP) on inter-response time distribution under DRL 15. A: Mean IRT curves for four rats receiving saline, CDP, saline on successive days. B: Mean IRT curves for four rats tested together with those shown in A, but receiving saline on all three days. The response scale is the result of logarithmic transformation, the vertical bar represents 2 standard errors.

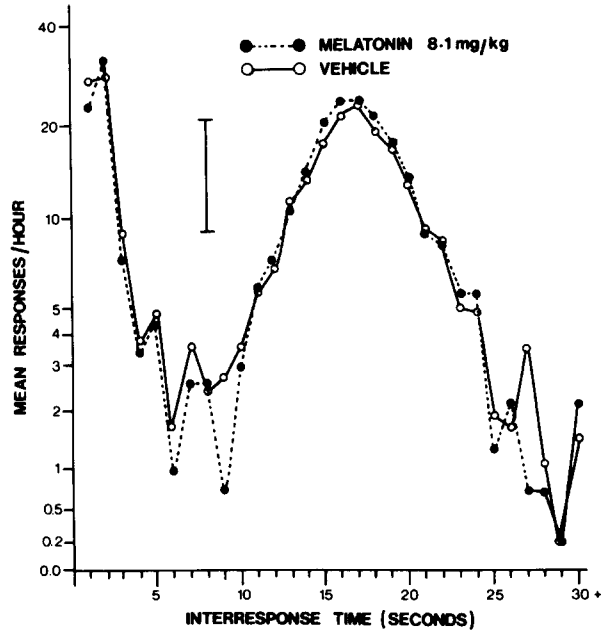

FIG. 2. Lack of effects on well-learned DRL of administration of 8.1 mg/kg melatonin. The largest F ratio for melatonin in the analysis of variance of this data was 1.03. Graphical details as for Fig. 1.

differential actions of the drugs on rate of acquisition. DRL 5, DRL 10, DRL 15 were each analysed separately.

The data for some rats for some days were lost due to printer failure and these were entered as missing values.

RESULTS

Experiment 1

Baseline. Data for days 21, 22 and 24 were analysed to determine whether acquisition of DRL 15 had stabilised. Day 23 was excluded as two rats failed to receive reward because of equipment problems. There was no significant variation across days.

CDP trials. Days 24, 25 and 26 represent an ABA design for the effects of CDP (see Table 1). As can be seen from Fig. 1A CDP injected on day 25 produced both a shift of the main body of the IRT curve to the left and an increase in bursts of the form previously described in the literature [21]. No similar changes on day 25 were observed in the rats receiving vehicle (Fig. 1B). These effects of the drug were demonstrated statistically by significant linear and cubic changes in the IRT curve (group × days × bins: linear, $F(2,522)=6.87$, $p<0.005$; cubic, $F(2,522)=4.79$, $p<0.025$).

Days 41, 42 and 43 involve a similar design (see Table 1). In both groups of rats administration of CDP produced similar effects to those found on day 25. Again the effects were highly significant (group × days × bins: linear, $F(2,522)=34.94$, $p<0.001$; cubic, $F(2,522)=9.13$, $p<0.001$).

Melatonin trials. Days 29–39 were analysed together. There was no sign of an effect of melatonin at any of the doses given (from 0.033 to 8.1 mg/kg; group × days × bins: all $F<1.0$).

Since the melatonin injections on days 29–39 had been given in increasing dosage it was possible that the lack of observed changes was due to the fact that the melatonin group had habituated to the drug. On day 40 therefore the largest dose of melatonin (8.1 mg/kg) was given to the "vehicle" group, which had previously received no melatonin. The "melatonin" group received vehicle on day 40. Analysis of days 39 and 40 together showed no evidence of an effect of melatonin (largest $F=1.03$). The data obtained are shown in Fig. 2.

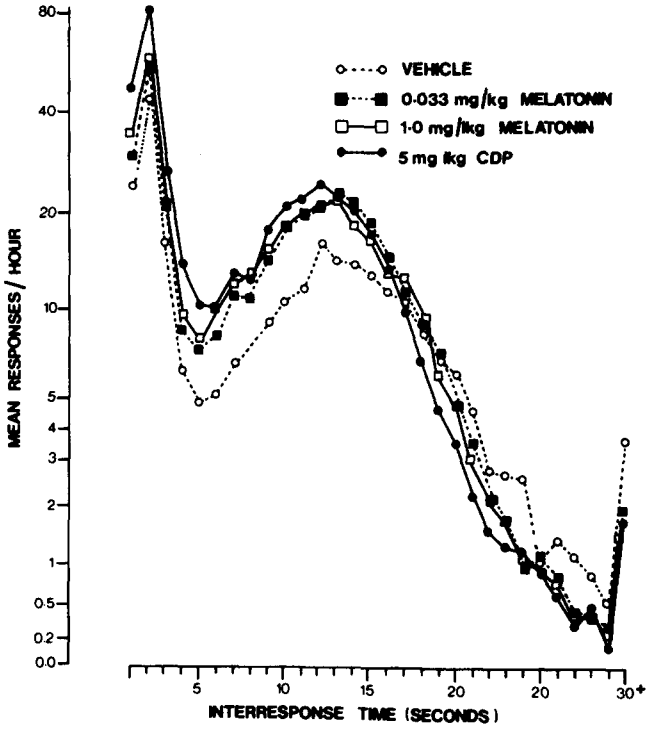

FIG. 3. Comparison of the effects of melatonin (0.033 and 1.0 mg/kg) and chlordiazepoxide HCl (5 mg/kg) on acquisition of DRL. The data shown are for essentially stabilised responding in the second of two four-day blocks of DRL 15 seconds. Similar results were obtained for DRL 5, DRL 10 and the first block of DRL 15. Graphical details as for Fig. 1.

Experiment 2

During acquisition of DRL there was progressive impairment of performance by all drug conditions. Analysing the first four days of DRL 15 as a block this impairment was demonstrated by significant linear and cubic components of the bins × group interaction (linear, $F(3,2755)=21.1$, $p<0.0001$; cubic, $F(3,2755)=11.2$, $p<0.0001$). But there was considerable change in the IRT functions across the four days between the different groups (group × days × bins: Linear, $F(9,2755)=7.4$; $p<0.0001$; quadratic, $F(9,2755)=4.4$, $p<0.0005$).

Within the second four day block of DRL 15 performance was more stabilised across days (group × days × bins; Linear, $F(9,3016)=2.4$, $p<0.05$; quadratic, $F=1.14$; Note: the difference in *df* between this and the previous 4 days is due to missing values). The form of the observed changes was essentially the same in the two four day blocks so the latter, being the more stable, is considered further below.

The data for the second 4 day block of DRL 15 acquisition are presented in Fig. 3. As can be seen from this, the general form of deviation of the drug IRT curves from the vehicle IRT curve is an increase in bursting (IRTs<2 sec), an increase in premature responses (3–14 sec) and a decrease in slow, rewarded, responses. (Most noticeable from 23 sec IRT onwards.) The shape of the IRT curve differs significantly between groups (group × bins: Linear, $F(3,3016)=59.6$, $p<0.0001$; quadratic, $F(3,3016)=6.9$, $p<0.0005$; cubic, $F(3,3016)=7.2$, $p<0.0005$).

The difference in linear trend effectively represents a combination of increased burst responding and decreased slow responses. Post hoc testing of the slope coefficients for the four groups with Student-Newman-Keuls showed highly significant differences ($p<0.001$) between all pairs of groups except the comparison of high with low dose melatonin ($q=2.4$, $P=2$, $n=3016$, $p<0.10$). Thus CDP produced a significantly greater slope than either dose of melatonin and they in turn produced significantly greater slopes than vehicle.

The difference in cubic trend effectively represents the increase in premature as opposed to burst responses. As with the linear coefficient, the cubic coefficient for CDP was further from that of the control group than either of the melatonin coefficients. However, in this case the difference between CDP and melatonin was not significant ($q=1.79$, $P=3$, $n=3016$, $p<0.20$), while all three drug treatments differed significantly from vehicle ($p<0.005$).

Overall we may conclude: that melatonin has essentially similar effects to CDP on acquisition of DRL; that the effects of CDP are somewhat greater than melatonin, particularly in relation to burst responding as opposed to premature responding; and that while 1.0 mg/kg melatonin may have slightly more effect than 0.03 mg/kg the difference is negligible and does not, in this case, achieve acceptable levels of significance.

DISCUSSION

The present experiments show that melatonin given at a dose of 0.03 mg/kg IP during acquisition of DRL has similar effects to CDP. Acute administration of melatonin, even at a dose as high as 8.1 mg/kg, after DRL is well learned had no effects. Melatonin thus shares some but not all of the effects of CDP and probably other anxiolytics.

Even given during acquisition of DRL, melatonin did not have quite as large an effect as CDP. Since increasing the dose of melatonin from 0.03 to 1.0 mg/kg produced little change it seems that even here melatonin lacked some component of CDP action. Further study with a benzodiazepine receptor blocker would determine whether in this case melatonin is in fact interacting with the benzodiazepine receptor—or whether CDP is, perhaps, interacting with the benzodiazepine receptors in the pineal [13,18].

With the low dose injection, it was hoped that plasma levels of melatonin during behavioural testing would have been close to the diurnal physiological range (see the introduction). However, the similarity in effects of the two doses of melatonin suggests that even the low dose has effectively saturated the receptor systems involved. It may be premature, therefore, to related the observed effects to the normal physiological action of melatonin.

In other experiments [14], *chronic* administration of CDP interfered with acquisition but not stable performance of successive discrimination. If this pattern of effects of CDP were repeated with DRL we could conclude from the present experiments that exogenous (and possibly endogenous) melatonin shares an 'anxiolytic' property of CDP but does not produce 'state dependence' similar to that produced by CDP.

The form of change in IRT distribution produced by melatonin was characteristic of anxiolytics as opposed to other classes of drug [2,21]. However, these previous studies investigated acute administration on a stable baseline. It is premature, therefore, to conclude that melatonin has an action in this test typical of all anxiolytics, although its effects did closely resemble those of CDP. On the other hand, the

present results exclude at least two previous hypotheses: (1) that melatonin generally reduces motor activity—in this case responding increased; (2) that melatonin interferes with "memory fixation"—the obtained IRT curves strongly suggest that the melatonin-treated rats can remember the nature of the task, but fail to inhibit responding for *long* enough to be efficient.

Further work will be needed to determine whether melatonin is "tranquillising" [19] or is changing emotionality or some other aspect of motivation or responses to stressors [4–5]. However, the specific form of the changes in the IRT curve would favour a role for melatonin as an endogenous anxiolytic.

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REFERENCES

1. Anton-Tay, F. Pineal brain relationships. In: *The Pineal Gland*, edited by G. E. W. Wolstenholme and J. Knight. (eds.) Ciba Foundation Symposium on the Pineal Gland. London: Churchill Livingstone, 1971, pp. 213–227.
2. Canon, J. G. and A. S. Lippa. Use of DRL in differentiating anxiolytic and neuroleptic properties of CNS drugs. *Pharmacol Biochem Behav* **6**: 591–593, 1977.
3. Dantzer, R. Behavioural effects of benzodiazepines: a review. *Biobehav Rev* **1**: 71–86, 1977.
4. Datta, P. C., F. K. Hoehler and C. A. Sandman. Effects of melatonin on startle reflex in rat. *Peptides* **2**: Suppl 1, 155–160, 1981.
5. Datta, P. C. and M. C. King. Melatonin: effects on brain and behaviour. *Neurosci Biobehav Rev* **4**: 451–458, 1980.
6. Gibbs, F. P. and J. Vriend. The half-life of melatonin elimination from rat plasma. *Endocrinology* **109**: 1796–1798, 1981.
7. Goldman, B., V. Hall, C. Hollister, S. Reppert, P. Roychoudhury, S. Yello and L. Tamarkin. Diurnal changes in pineal melatonin content in four rodent species: relationships to photoperiodism. *Biol Reprod* **24**: 778–783, 1981.
8. Graeff, F. G. Minor tranquillisers and brain defence systems. *Braz J Med Biol Res* **14**: 239–265, 1981.
9. Gray, J. A. Drug effect on fear and frustration. In: *Handbook of Psychopharmacology*, vol 8, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York: Plenum Press, 1977, pp. 433–529.
10. Green, R. A., D. J. Nutt and P. J. Cowen. Using Ro 15-1788 to investigate the benzodiazepine receptor in vivo: studies on the anticonvulsant and sedative effect of melatonin and the convulsant effect of the benzodiazepine Ro 05-3663. *Psychopharmacology (Berlin)* **78**: 293–295, 1982.
11. Grota, L. J., V. Snieckus, O. de Silva, H. W. Tsui, W. R. Holloway, A. J. Lewy and G. M. Brown. Radioimmunoassay of melatonin in rat serum. *Prog Neuropsychopharmacol* **5**: 523–526, 1981.
12. Illnerova, H., J. Vanecek and K. Hoffman. Regulation of the pineal melatonin concentration in the rat (Rattus Norvegicus) and in the Djungarian hamster (Phodopus Sungorus) *Comp Biochem Physiol* **74**: 155–159, 1983.
13. Lowenstein, P. R., R. Rosenstein, E. Caputti and D. P. Cardinati. Benzodiazepine binding sites in human pineal gland. *Eur J Pharmacol* **106**: 399–403, 1985.
14. McNaughton, N. Chlordiazepoxide and successive discrimination: Different effects on acquisition and performance. *Pharmacol Biochem Behav* **23**: 487–494, 1985.
15. Marangos, P. J., J. Patel, F. Hirata, D. Sondheim, S. M. Paul, P. Skolnick and F. K. Goodwin. Inhibition of Diazepam binding by tryptophan derivatives including melatonin and its brain metabolite N-acetyl S-methoxy kynurenamine. *Life Sci* **29**: 259–267, 1981.
16. Pang, S. F., P. L. Tang, H. S. Yu and M. K. Yip. The level of N-acetylserotonin and melatonin in the brain of male rats: diurnal variations and effects of pinealectomy. *J Exp Zool* **219**: 271–276, 1982.
17. Paul, S., P. Marangos and P. Skolnick. The benzodiazepine-GABA-chloride-ionophore receptor complex: common site of minor tranquillisers action. *Biol Psychiatry* **16**: 213–229, 1981.
18. Quiron, R. High density of [3H] RO5-4864 'peripheral' benzodiazepine binding sites in the pineal gland. *Eur J Pharmacol* **102**: 559–560, 1984.
19. Romijn, H. J. Mini review: the pineal, a tranquillizing organ? *Life Sci* **23**: 2257–2274, 1978.
20. Sampson, P. H. Behaviour and pineal functioning. In: *Frontiers of Pineal Physiology*, chapter 8, edited by M. D. Altschule. Cambridge: MIT Press, 1975, pp. 204–222.
21. Sanger, D. J., M. Key and D. E. Blackman. Differential effects of chlordiazepoxide and d-amphetamine on responding maintained by a DRL schedule of reinforcement. *Psychopharmacologia* **38**: 159–171, 1974.
22. Snedecor, G. W. and W. G. Cochran. *Statistical Methods*, 6th Edition. Ames, IA: Iowa State University Press, 1967.
23. Zar, J. H. *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice-Hall Inc., 1974.