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# The Effects of Thyrotropin Releasing Hormone on a Visual Discrimination Task in Rats

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ANDREWS, J. S. AND A. SAHGAL. *The effects of thyrotropin releasing hormone on a visual discrimination task in rats.* PHARMACOL BIOCHEM BEHAV **21**(5) 715–719, 1984.—The effects of intracerebroventricular (ICV) administration of thyrotropin releasing hormone (TRH, 1 and 50 μg) were assessed on a two-choice visual discrimination task. The data were analysed using signal detection theory techniques in order to test for changes in cognitive and response factors. No significant changes in performance were observed. In a second experiment, the effects of TRH (100 μg ICV) on performance were compared with amphetamine (AMP, 1 mg/kg, intra-peritoneally, IP) and a metabolite of TRH, histidyl-proline diketopiperazine (DKP, 100 μg ICV). No significant effects on performance as measured by standard indices were observed. However, both TRH and AMP, but not DKP, significantly increased perseverative responding on one lever with respect to saline. In keeping with recent evidence, it is concluded that the traditional non-parametric signal detection parameters of sensitivity and bias are insensitive to certain strategies. Possible mechanisms for the perseveration of responding, and its relationship to stereotypic behaviour, are discussed in the light of the known effects of each compound on dopaminergic systems.

Thyrotropin releasing hormone Histidyl-proline diketopiperazine Amphetamine Visual discrimination Signal detection theory Perseverative responding Rats

THYROTROPIN releasing hormone (TRH) has been identified throughout the brain and has effects which are independent of its classic endocrinological functions. For example, TRH interacts with the mesolimbic dopamine (DA) system to produce increases in locomotor activity [9], and has profound analeptic properties possibly via an interaction with cholinergic mechanisms (see [18]). However, its effects on cognitive behavior are less well documented.

Recently, we have reported that TRH disrupts the acquisition of lever pressing in rats [1], but in contrast to other workers [2, 13, 16], did not observe performance deficits once the task was learned. The fact that amphetamine (AMP) did not have as detrimental an effect as TRH on the acquisition of responding, and the finding that TRH administration was not in itself strongly aversive, suggested that this was not a simple non-specific toxic effect. A possibility arises that TRH might act on motivational or response, rather than sensory, processes; however, many tasks used to describe the effects of TRH are not suitable for testing this hypothesis as the independent performance variables, the sensory and motivational/response parameters, cannot be easily separated.

These problems may be mitigated by the use of discrimination paradigms, and signal detection theory analysis. This approach has been successfully used in evaluating the cognitive effects of several drugs, for example AMP and morphine [12], AMP, chlordiazepoxide and α-flupenthixol [4].

## EXPERIMENT 1: THE EFFECTS OF TRH ON A VISUAL DISCRIMINATION TASK ANALYSED BY SIGNAL DETECTION TECHNIQUES

Signal detection theory considers the ways in which choices are made based on information available. A sensitivity measure, corresponding to sensory factors, or to the efficiency of information processing, is termed d-prime and can be affected by such factors as changes in the intensity of the signal, or efficiency of the neural pathways; the subject has little direct control over this factor and it tends to remain constant. However, this is not true of the second variable: motivational bias, often referred to as beta, which can be altered by a variety of factors, for example, changing the value of the reward, or the introduction of a distracting stimulus.

In the present case, it may be that performance as a whole is unaffected because the sensitivity measure is little changed, but if TRH does have motivational or response effects, then there may be some change in indices related to beta.

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## METHOD

### *Animals and Surgery*

Subjects were 12 male Norwegian Hooded rats (Bantin and Kingman Ltd, Hull, UK), weighing approximately 225 g at the beginning of the experiment. They were individually housed under diurnal conditions (lights on between 0700 and 1900 hr) with water available ad lib, but on a 23 hr food deprivation schedule. All subjects had been used in a previous experiment involving the acute effects of AVP and other drugs on a visual discrimination task (Sahgal, in preparation), and thus had already learned the task.

The rats were implanted, under deep anaesthesia (Nembutal 42 mg/kg), with 23 gauge stainless steel guide cannulae (Plastic Products Inc, VA, USA) aimed at the lateral ventricle; co-ordinates, from bregma: AP −1, LV +1.3, HV −4.5 mm.

### *Apparatus*

Four rodent operant chambers (Campden Instruments Ltd, London, UK) were connected to, and controlled by, Acorn System III microcomputers (Acorn Computers Ltd, Cambridge, UK) running ONLIBASIC software [6]. Each box was fitted with two retractable levers, food-magazine tray with flap, a food pellet dispenser, stimulus and house lights (24 V, 2.9 W). The test room was darkened and white noise (70 dB) present at all times; behavioural testing was carried out between 1300 and 1700 hr.

### *Procedure*

Rats were trained to press a lever for food-reward by means of the autoshaping paradigm described by Sahgal [15]. Following this, they were trained on a two lever visual discrimination task in the following manner: at the beginning of each trial both levers emerged into the box and the stimulus light above one or the other levers illuminated (with equal probability). If the animal pressed the correct lever (designated by the illumination of the light above the lever), a pellet of food was delivered to the magazine tray, the magazine light switched on, levers withdrawn and a short intertrial interval followed during which the house light was also extinguished. If the animal pressed the other lever, or failed to make a response in the allotted time, an additional "time-out" punishment period was added on to the intertrial interval during which the levers were withdrawn, the house light switched off, and no reward given.

Initially, rats were trained with the stimulus light illuminated above the to-be-rewarded lever for 4 sec and the levers made available for 10 sec. The intertrial interval and punishment period for incorrect responding were set at 10 sec. Over a period of 5 weeks these values were gradually changed until the stimulus light was presented for only 0.25 sec, the lever available for 2 sec, and an intertrial interval varying between 15–35 sec introduced; the punishment period was increased to 20 sec. Following several months' testing, performance stabilized around an average of 75% correct responses per session of 50 daily trials.

One week following surgery, rats were retrained on the task until daily performance was similar to that before cannulation. They were then divided into 3 groups of 4 subjects and given saline or one of two doses of TRH (CRB Ltd, Cambridge, UK; 5 or 50 μg in 2 μl saline) in a 3×3 Latin square design in an attempt to minimize possible order effects. At least one day's drug free testing was allowed between test days in order to minimize carry over effects. Two animals did not complete all the treatments and their data were discarded.

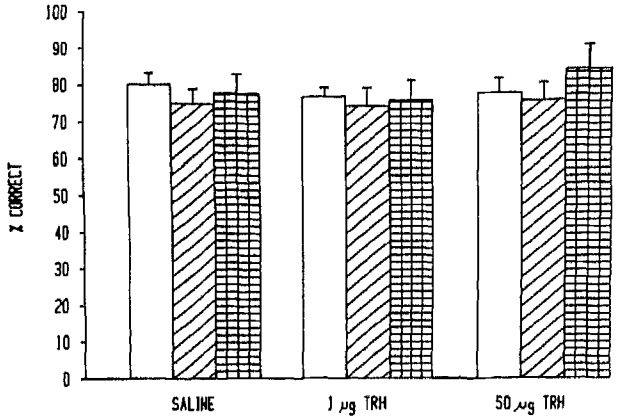


FIG. 1. The effects of 0, 1 or 50 μg of TRH in 2 μl saline ICV, on performance of 10 rats in a visual discrimination task. The % correct scale also represents centiseconds. In each block: unshaded histogram represents % correct score (±SEM); stripes=mean latency to correct response (csec); cross hatch=mean latency to incorrect response (csec). See Experiment 1 text for further details.

### *Data Analysis*

The data were analyzed in terms of the non-parametric signal detection indices SI and RI [7], in which SI is the sensitivity index (equivalent to d-prime), and RI the responsivity index, a response bias measure (equivalent to beta). These are calculated by first computing the probabilities of a hit and a false alarm; the former equals the number of times the animal responded to the left hand lever, given that the left hand stimulus was present, and the latter the number of times it responded to the left hand lever given that the right hand lever was the correct one (note, the right hand lever may be used to calculate values, and this will give identical results). Using appropriate formulae given elsewhere [7], SI and RI may be calculated. Perfect performance yields an SI of 1; RI can range from −1 (completely biased towards one lever), through 0 (no bias) to +1 (complete opposite bias). Thus, efficient behaviour would result in an SI approaching 1, and an RI tending to 0; treatments which impair discrimination ability should reduce SI, while those that produce response perseveration or preference will increase the absolute value of RI (towards one or other lever). For each drug treatment, these indices were compared using a 1-factor repeated measures analysis of variance [17].

## RESULTS AND DISCUSSION

TRH had no effect on overall performance as measured by percent corrects (Fig. 1); this result was also reflected in the SI scores for the three conditions, $F(2,18)=0.18$, ns; Fig. 2, top. Although the RI's tended to be larger on the drug days, this was not significant, $F(2,18)=0.817$, ns; Fig. 2, bottom.

The overall lack of effect of TRH on performance was surprising: 50 μg of TRH was seen to have marked physical effects, producing wet dog shakes (WDS). Besides offering

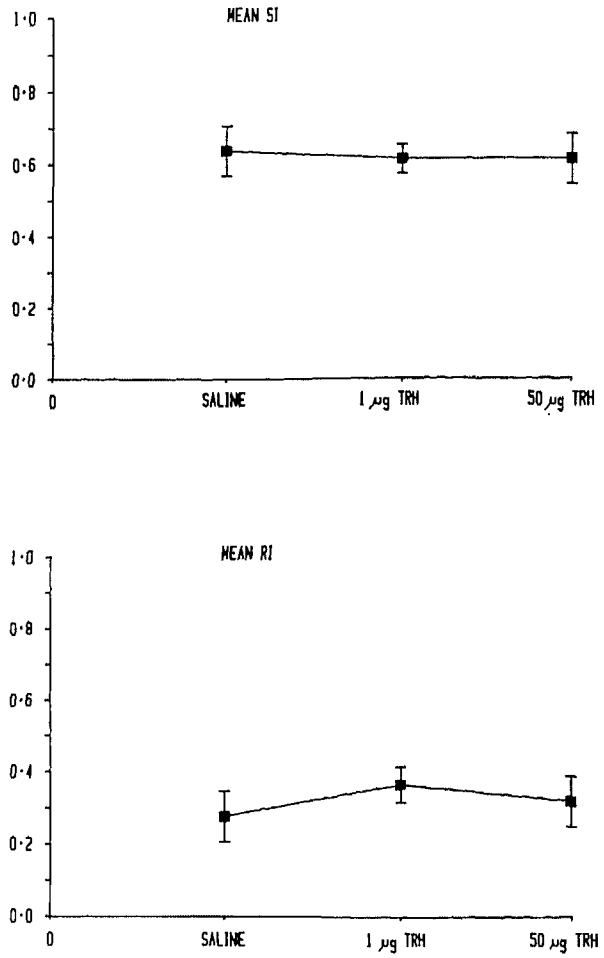


FIG. 2. Top: Mean SI (±SEM) of 10 rats in a visual discrimination paradigm described in Experiment 1. Injections were made ICV in a total volume of 2 μl. Bottom: Mean absolute RI (±SEM) of same rats under the corresponding conditions as above.

little support for cognitive effects, this contradicts several previous studies reporting disruptive effects of TRH on operant behaviour [2, 13, 16]. There are a number of possible explanations for this discrepancy.

The rats in this paradigm may have been overtrained. This is reflected in the stability of their performance, as measured by percent corrects, and they are not perhaps easily disturbed from the task. In this procedure, reinforcement was available after a single response and failure to respond resulted in mild punishment (no reward, no immediate opportunity to gain reward); disruption of performance by TRH has been most noticeable in tasks using high fixed ratio (FR) schedules of reinforcement—usually FR 30 [2,16]. It may be that TRH has a slight negative effect on motivation, but that in this case the immediacy of the reward following a single response offsets some of this disruptive effect on performance. In addition to the changes in RI, rats tended to miss more trials after drug treatment, although this was again non-significant, taken together they might be seen as support for some, albeit mild, disruption of cognitive processes.

However, there is one other possibility for the lack of any clear change in RI: besides the fact that well trained animals may quickly compensate for certain physiological and psychological manipulations, it may be that RI is itself not sensitive enough to changes in patterns of responding.

## EXPERIMENT 2: COMPARISON OF THE EFFECTS OF TRH, DKP AND AMP ON RESPONDING

Several groups have reported no disruption of RI in discrimination tasks following administration of AMP [4,12] except at very high doses. This is surprising: it is well documented that AMP produces stereotypy, and this might be expected to alter response bias, and have apparent effects on discrimination accuracy by altering response topography [14]. However, it appears that AMP-induced perseveration, at doses below those capable of inducing stereotypy, does not preclude the ability to switch from one lever to the other: in fact both response perseveration and switching increase following the administration of AMP [5]. Therefore, although the rat's behaviour alters, bias (RI) appears to be unaffected. Thus, recent work has shown that AMP does increase perseverative responding in multi-choice tasks [4, 5, 12], and, that RI is not sufficiently sensitive to identify the different effects of AMP and morphine on discriminative responding [12].

A similar process may account for the lack of change in RI, following administration of TRH (Experiment 1), and further analysis might show a similarity with AMP on response repetition. Thus, the effects of TRH, a metabolite of TRH, histidyl-proline diketopiperazine (DKP) and AMP, all of which have been reported to exert effects through central DA systems [3, 9, 10], were tested for their effects on SI, RI and response perseveration in rats trained on a visual discrimination task.

### METHOD

*Subjects*

Subjects were 7 Norwegian Hooded rats, with lateral ventricular cannula implants, used in the previous experiment.

*Drugs*

d-Amphetamine sulphate (Sigma Ltd, London, UK) was dissolved in 0.9% saline at a concentration of 1 mg/ml, for subsequent intra-peritoneal injection at 1 mg/kg. This dose was chosen because it has been shown to have no significant effect on SI and RI performance variables, but to cause some increase in response repetition in an auditory discrimination paradigm [12]. TRH (CRB Ltd, Cambridge, UK) was dissolved in saline at 50 μg/μl for ICV injections of 100 μg per rat. These are high doses, which have in this laboratory been observed to cause noticeable behavioural effects, albeit in the case of DKP very short lived.

*Apparatus*

Equipment was the same as in Experiment 1.

*Procedure*

Following the completion of Experiment 1, subjects were given 7 days' testing without drug treatment, in order to prevent carry over effects and consolidate their baseline performance values. Then, using a Latin square design, subjects received ICV injections of saline, TRH and DKP, with a minimum 48 hr between drug treatments. After this proce-

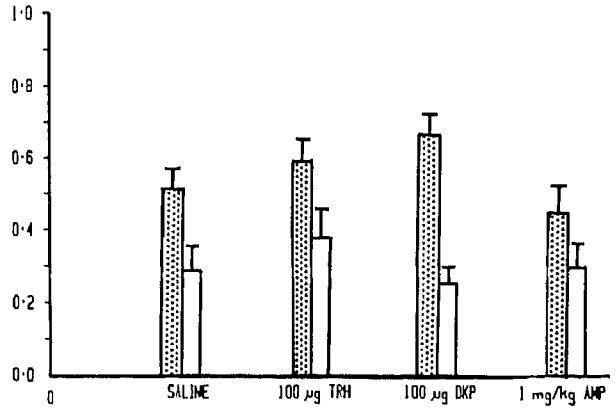


FIG. 3. Stippled bars: Mean SI (±SEM) of 7 rats in the visual discrimination paradigm described in Experiment 2. Saline, TRH and DKP injections were made ICV in 2 μl volume; amphetamine was given IP. Unshaded bars: Mean absolute RI (±SEM).

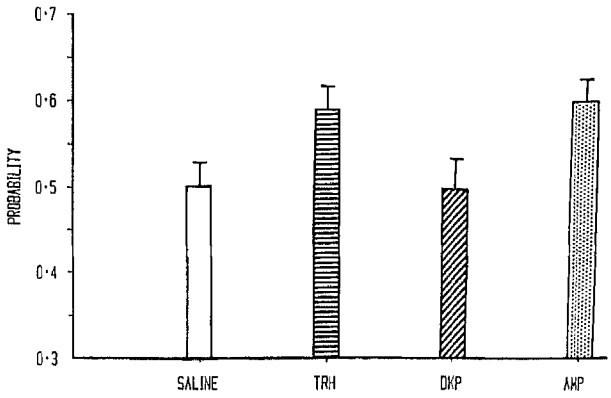


FIG. 4. The effects of 100 μg (in 2 μl saline ICV) of TRH, DKP and 1 mg/kg of amphetamine (IP) on the probability of perseverative responding in Experiment 2. See text for further details.

dure had been completed, all animals were injected with 1 mg/kg of AMP, which was not included in the Latin square design in order that all ICV injections could be completed before possible loss of cannulae. Cannulae placements were verified by the injection of methyl blue dye through the cannulae, followed by inspection of gross sections which showed the distribution of dye throughout the ventricles.

*Data Analysis*

In addition to the established procedures, probability of response repetition was calculated as the number of trials during which the rat responded on the same lever as on the preceding trial, divided by the total number of trials during which the rat responded minus one [12]; the first trial was not counted as it cannot be considered as a change or repetition from the previous, non-existent response.

## RESULTS AND DISCUSSION

TRH and DKP induced small increases, and AMP a small decrease in the average SI with respect to saline (Fig. 3); however, this was not significant, $F(3,18)=2.05$, ns. Interestingly, DKP had by far the largest overall effect on performance as measured by percent corrects and SI. Although administration of TRH caused a slight increase in bias (RI), the RI results were non-significant, $F(3,18)=0.843$, ns; (Fig. 3). However, there was an overall significant difference in the analysis of response perseveration, $F(3,18)=4.46$, $p<0.05$, and this is by far the most interesting result of this study. A posteriori testing using the Newman-Keuls test [17] revealed both TRH and AMP, but not DKP, to be significantly different from saline. This result is illustrated in Fig. 4; AMP markedly increased response repetition with respect to saline, as did 100 μg of TRH, but not DKP. From these results we can conclude that, in keeping with recent studies, RI may not be sensitive enough to detect changes in animals' response patterns.

## GENERAL DISCUSSION

Intracerebroventricular administration of TRH, and its metabolite DKP, had no overall effect on the performance of rats in a visual discrimination task as measured by the signal detection parameters SI and RI. However, TRH affected the pattern of responding, producing response perseveration similar to that observed following peripheral administration of AMP.

Each of the drugs used in this experiment have been reported to affect central dopamine systems; thus the effects of TRH reported here might be interpreted as being mediated by the same mechanism involved in AMP-induced perseveration. However, since stereotypic responses to AMP are not affected by lesioning the mesolimbic DA system [10], this suggests that AMP induced perseveration may be mediated by the nigro-striatal DA system, a system on which several workers have failed to observe significant effects of TRH [9,11]. Given this action of AMP, it is difficult to attribute the effects of the two peptides to the same mechanism. Thus, DKP has been reported to prevent the uptake of DA in the striatum [3], but it has no effect on response perseveration; however, TRH does induce perseverative responding, yet reports as to its effects on striatal DA are at best controversial. If we accept that AMP-induced perseveration is related to stereotypic behaviour, and since TRH has been reported to affect DA in the striatum (e.g., [8]), we might conclude that the dose of TRH employed was high enough to cause such effects, and therefore TRH was acting in an analogous manner to AMP. However, even 100 μg of TRH has, in this laboratory, been repeatedly demonstrated not to cause stereotypic behaviour. It could be argued that this dose is close to the threshold at which stereotypic behaviour becomes obvious, and thus its effects are only apparent under certain conditions.

TRH and AMP could have fundamentally different effects on animals yet produce overtly similar results. AMP may induce a dose-dependent perseveration because it is primarily exerting a motor action [14]: having responded once on one lever the animal is compelled to respond again on the same lever. However, TRH could produce the same effect through a change in response strategy; instead of being compelled to respond in the same way, the rat may change its pattern of responding, repeating its previous response to compensate for, say, a disruption in attention. The advent of

punishment would be expected to prevent the animal from responding completely on one lever, and perhaps momentarily enhance performance in this task (a "win-stay, lose-shift" strategy). This change in strategy to compensate for one type of cognitive deficit may account for the increases in perseveration and changes in bias seen in both experiments.

AMP has been reported to reduce the occurrence of win-stay, lose-shift strategies in rats [5]. If the same result was found with TRH, it would suggest that TRH disrupts performance in a manner analogous to AMP; an opposite result could be due to a disruption of attention, and the adoption of a compensating strategy, whereas no effect might suggest either no cognitive action, or some other role—for example in the maintenance of a learned response. However, in this study, there were no clear effects of TRH, DKP or AMP on a win-stay, lose-shift strategy, but further investigation of this possibility is required.

In conclusion, TRH, although not significantly affecting visual discrimination in a complex operant task, did increase perseverative responding in a manner similar to that found with AMP. However, it is unclear as to whether both drugs exert their effects by the same mechanism. In future research, the rigorous analysis of complex cognitive tasks may reveal differences between superficially similar results obtained using different drugs, differences which may correlate well with the known physiology and pharmacology of these compounds.

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