

Y-Maze Behavior After an Analog of ACTH 4–9, Evidence for an Attentional Alteration

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Abstract. Adult, male, Swiss-Webster mice were injected with either control solution or various doses of a long-acting analog of ACTH 4–9. The drug produced significant alterations in performance in an equiangular Y-maze. In comparison with control performance, both 25 and 50 µg/kg of drug produced a significant increase in repeated two-arm entries. This response pattern is consistent with the hypothesis that the drug produced a focusing of attention.

Key words: ACTH 4–9 – Attention – Y-maze performance

Fragments of adrenocorticotrophic hormone (ACTH) without intrinsic corticotrophic activity may manifest neurotropic activity. These neurotropic actions may include effects upon learning and extinction, attention (see Van Riezen et al. 1977; Walker and Sandman 1979 for recent summaries), grooming, and analgesia (Gispén et al. 1975, 1976). Heretofore the attentional theory of neuropeptide activity has been supported by physiological measures of arousal (e.g., Sandman et al. 1977) and studies involving various forms of dimensional shifts in learning tasks (e.g., Sandman et al. 1973). In the latter, enhanced reversal learning and impaired extradimensional shifting have been taken as evidence for peptide facilitation of attention.

A less complex, but potentially useful attentional measure is initial performance in a Y-maze. Mice exposed to an equiangular three-arm maze typically show various combinations of response perseveration and alternation. Three-arm entries represent response alternation and are consistent with an influence of motor bias or stereotypy (e.g., Kokkinidis and Anisman 1977, 1978). Repeated two-arm entries on the

other hand demand complex successive reversals of motor responses and may reflect non-motor factors such as an attentional bias or sensory perseveration (op. cit.). Drugs affecting attentional processes typically increase the proportion of two-arm entries at the expense of response perseveration (Kokkinidis and Anisman 1977, 1978). The present design utilized Y-maze performance to examine whether the neuropeptide ORG 2766, an analog of ACTH 4–9, would influence attention in a design in which learning was probably not directly involved. Our findings are consistent with the presence of an attentional effect for the compound.

Materials and Methods

Adult, male, Swiss-Webster mice ($N = 42$; 25–30 g each, Charles River Farms) were group housed six per cage with food (Teklad 4.0% fat rodent diet S-0836) and tap water continuously available, and 12 h day-night cycles (lights on = 7 a.m. – 7 p.m.).

All testing was carried out from 3 p.m. – 6 p.m. Mice were individually removed from housing and injected IP 1 ml/0.1 kg with one of three doses (25, 50, 100 µg/kg) of ORG 2766 [H-Met (O₂)-Glu-His-Phe-D-Lys-Phe-OH] in 0.9% sodium chloride vehicle. Testing began 15 min later. The subject was removed from a 28.5 × 17.5 × 12.5 Plexiglas holding cage and placed in an equiangular three-arm maze for a single 15-min observation period. Dimensions of individual maze arms were 17.5 × 6.5 × 7.5 cm. The maze was washed thoroughly with tap water between tests. Air circulation equipment in continuous operation provided masking noise of 40 dB.

Performance was assessed as proportion of alternation responses per total responses. This measure has been described in detail by Kokkinidis and Anisman (1977, 1978). Briefly, alternation responses (consecutive three-arm entries) were divided by total arm entries (alternations plus perseveration responses) to yield a net score between 0.0 and 1.0. Statistical analysis was by univariate analysis of variance with Sheffé limits for post-hoc comparisons (Dixon and Massey 1969).

Results

Data from vehicle-injected mice indicated a predominant pattern of response bias (i.e., three-arm entries),

Table 1. Effects of Org 2766 on Y-maze performance in the mouse. Analysis of variance based upon Dixon and Massey (1969)

ORG 2766 μg/kg	N	Proportion of alternations in 15 min ($\bar{x} \pm \text{SEM}$)
Control	19	0.67 \pm 0.01
25	9	0.62 \pm 0.03 ^a
50	9	0.60 \pm 0.02 ^a
100	5	0.73 \pm 0.04

^a Differs from control performance $P < 0.05$ (Scheffe procedure); F ratios for 25 and 50 μg/kg vs. control are 7.2, 4.6, $P < 0.01$, 0.001 all other groups are equivalent

and this was reduced significantly by low and intermediate dose of peptide (Table 1).

It might be questioned if these results reflected an underlying non-specific behavioral activation or depression. Since the data are ratios and therefore independent of absolute activity this seems unlikely. However, to test further whether these results reflected some change in activity level, five correlations of total arm entries by proportion of alternations were calculated (one for each cell, one for all subjects regardless of treatment). Regression coefficients of total activity upon proportional alternations for control, the three doses in increasing order, and total subjects were 0.20, 0.18, 0.11, -0.04 , and 0.19. Fisher transformations to Z scores indicated Z ratios of 0.8, 0.5, 0.3, 0.1 and 1.3 respectively. In all cases these results are below levels reliably different from chance.

Discussion

It should be noted that the dose response curve was not monotonic. Bitonic responses have also been noted for other peptides (Frederickson et al. 1978; Krivoy et al. 1979). The findings may reflect indirect "neuromodulatory" process similar to those described by Krivoy et al. (1979). As previously noted the predominance of three-arm entries may also reflect peptide induced motor bias. Finally, the findings might indicate an attentional disorganization, being even higher than the control mean, although not reliably so. Such processes have been described by Meldman (1970), who notes that attention may be ordered upon a continuum in which the highest extreme involves disorganized behaviors. Once an attentional optimum is exceeded performance may be predicted to decline.

Whether or not this last explanation in fact accounts for the present results, overall, the intermediate dose effects are consistent with the experimental hypothesis, and may be taken to support and extend prior studies on neuropeptides and attention. Future studies might outline further localized central injections (Kokkinidis and Anisman 1977) to further test the attentional nature of the Y-maze effect.

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References

- Dixon WJ, Massey FJ (1969) Introduction to statistical analysis, 3rd edn. McGraw Hill, New York
- Frederickson RCA, Burgis V., Harrell CE, Edwards JD (1978) Dual actions of substance P on nociception: possible role of endogenous opioids. *Science* 199, 1359–1362
- Gispén WH, Buitelaar J, Wiegant VM, Terenius L, DeWied D (1976) Interaction between ACTH fragments, brain opiate receptors, and morphine induced analgesia. *Eur J Pharmacol* 39:393–398
- Gispén WJ, Wiegant VM, Greven HM, DeWied D (1975) The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH, structure activity studies. *Life Sci* 17:645–652
- Kokkinidis L, Anisman H (1977) Perseveration and rotational behavior elicited by *d*-amphetamine in a Y-maze exploratory task: differential effects of intraperitoneal and unilateral intraventricular injection. *Psychopharmacology* 52:123–128
- Kokkinidis L, Anisman L (1978) Abatement of stimulus perseveration following repeated *d*-amphetamine treatment: absence of behaviorally augmented tolerance. *Pharmacol Biochem Behav* 8:557–563
- Krivoy WA, Couch JR, Henry JL, Stewart JM (1979) Synaptic modulation by substance P. *Fed Proc* 38:2344–2347
- Meldman MJ (1970) Diseases of attention and perception. Pergamon, New York
- Sandman CA, Alexander WD, Kastin AJ (1973) Neuroendocrine influences on visual discrimination and reversal learning in albino and hooded rats. *Physiol Behav* 11:613–617
- Sandman CA, George J, McCanne TR, Nolan JD, Kaswan J, Kastin AJ (1977) MSH/ACTH 4–10 influences behavioral and physiological measures of attention. *J Clin Endocrinol Metab* 44:884–891
- VanRiezen HH, Rigter H, Dewied D (1977) Possible significance of ACTH fragments for human mental performance. *Behav Biol* 20:311–324
- Walker B, Sandman CA (1979) Influence of an analog of the neuropeptide ACTH 4–9 on mentally retarded adults. *Am J Ment Defic* 83:346–352

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