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GEORGE KARPATI
Professor, Department of Neurology/Neurosurgery, McGill University, Montreal, Quebec, Canada H3A 2B4.

Letter to the Editor

We welcome comments from our readers. Short communications stand the best chance of publication. The Editor reserves the right to take extracts from the longer ones.

The importance of vasopressin in memory

'Die Wahrheit ist das Kind der Zeit, nicht die Autorität'

BERTOLD BRECHT, *Leben des Galilei*

SIR:

In their article entitled 'What is the importance of vasopressin in memory processes?' (*TINS*, June 1983)[14], Don M. Gash and Garth J. Thomas critically evaluated the validity of our thesis that vasopressin influences memory processes by direct action on the brain. They argue that, in Hegelian fashion, work from other laboratories has suggested the antithesis; namely, that memory processes are unaffected by vasopressin.

Our thesis that vasopressin does influence memory processes was based on the following observations:

(a) *Vasopressin has a long-term effect on active avoidance behavior.*

A single injection of vasopressin or a related fragment administered before acquisition[17], after completion of training[10], or during extinction[9] increases resistance to the extinction of pole-jumping avoidance behavior for several days.

(b) *The influence of vasopressin and related peptides is time dependent.*

To be active, vasopressin must be administered within several hours before or after a particular learning session. This has been demonstrated in active as well as passive avoidance behavior[3,9]. Studies on passive avoidance behavior have shown that vasopressin affects consolidation as well as retrieval processes. Again the effect is long-term.

(c) *Vasopressin and related peptides prevent or reverse retrograde amnesia for a passive avoidance response in rats[26] or a maze-learning test in mice[32].*

In Gash and Thomas's interpretation of the data there is still the possibility of a role for vasopressin in behavior. In their article they raised the following points:

(1) *The use of the Brattleboro rat homozygous for diabetes insipidus (the HO-DI rat) is of doubtful validity.*

The Utrecht group found that these rats showed impaired passive avoidance behavior which could be restored by administration of vasopressin or a non-endocrine fragment DGAVP[4,11]. In HO-DI rats active avoidance learning in a multiple-trial test procedure (shuttle-box and pole-jumping avoidance behavior) was normal but extinction was facilitated[4]. These findings have not been confirmed. Gash and Thomas (see Brito *et al.*[6]) did however, find a defective reference memory in HO-DI rats but surprisingly did not mention it. Bailey and Weiss[1] also observed a deficiency in passive avoidance behavior of male and female HO-DI rats as compared to heterozygous controls, but both groups of HO-DI rats performed better than Long-Evans animals. Celestian *et al.*[8] also found that HO-DI rats performed better during extinction of shuttle-box avoidance behavior than did control rats, but Celestian *et al.* had discarded 70% of their HO-DI rats as non-learners. Four different investigators working in the Rudolf Magnus Institute at different times have observed the impaired performance in passive avoidance behavior[4,5,11,20]. Differences may arise from the selection of control rats. The Brattleboro rats we used were originally given to us by Professor J. Sloper and Dr J. Lee (Charing Cross Hospital Medical School, London) in 1969. In 1974 the Central Breeding Laboratories in Zeist, The Netherlands, took over the breeding of test and control strains and developed a homozygous 'normal' variant which is equivalent to the Brattleboro rat except for the genetic lesion and is thus a true control. These animals became available in 1975/1976 and since then we have used only these animals.

(2) *There have been problems in replicating the effect of vasopressin in normal rats but other groups, using essentially the same technique, have replicated and confirmed our findings[2,19,22,28].*

Gash and Thomas[14] cite Hostetter *et al.*'s studies[16] as using the same experimental design as ours. Unfortunately this is not true. Rigter[25] has critically evaluated the passive avoidance procedure which we use and has shown that one cannot omit any of the steps we take to demonstrate the effect of vasopressin. Hostetter *et al.*[16] did precisely this and omitted the first habituation trial.

Gash and Thomas[14] point out that we may bias our results by using a selection procedure in the pole-jumping avoidance test. Indeed, we select only those rats which make seven or more avoidances out of ten at the third (not the first) acquisition session. This criticism may apply, but in general we do not discard more than 10% of our rats.

It is not difficult to explain the controversies reported in the literature over the influence of neuropeptides on behavior. One of the reasons is that test procedures are not always reproduced precisely or described as carefully as is necessary.

(3) *Sahgal* et al.[27] *have also found effects of vasopressin on passive avoidance behavior but post-trial intracerebroventricular administration of vasopressin produced a bimodal effect that included facilitated as well as attenuated passive avoidance behavior.*

Hagan *et al.*[15] found the same on extinction of pole-jumping avoidance behavior and this bimodal effect appeared to

be time dependent. Sahgal *et al.*[27] argued that these effects indicate influences of vasopressin on other than memory processes; they infer an influence on arousal. From this view vasopressin would improve the behavior at low levels of arousal and attenuate it at high levels of arousal. This may well be a possibility, since passive avoidance behavior is associated with emotion and arousal; but how could it explain the long-term effect of a single dose of vasopressin on active and passive avoidance behavior? According to McGaugh[23] a long-term effect such as this indicates an influence on memory processes.

(4) *Le Moal* et al.[22] *have pointed out that one interpretation of our data is that vasopressin influences avoidance behavior through its visceral effects, perhaps by increasing blood pressure.* Such a conclusion was derived from studies in which a vasopressin antagonist administered systemically prevented vasopressin (also administered systemically) from increasing blood pressure, and also abolished its behavioral effects. The authors, however, failed to perform a similar experiment using much lower doses of the agonist and antagonist administered intracerebroventricularly. In addition, microinjection of picogram amounts of vasopressin into places remote from those brain areas involving projections of vasopressin neurons to parasympathetic and orthosympathetic divisions of the autonomic nervous system[29] facilitates passive avoidance behavior[18]. Moreover, centrally but not peripherally administered vasopressin antiserum impairs passive avoidance behavior[30]. The intracerebroventricular administration of vasopressin in relatively high quantities may increase peripheral blood pressure as Pittman *et al.*[24] have shown. Yet Versteeg *et al.*[31] have shown that intracerebroventricular administration of vasopressin, oxytocin and their fragments reduces the pressor response of rats to stimulation of the mesencephalic reticular formation, suggesting that these peptides prevent rather than stimulate centrally (emotionally) induced increases in blood pressure. One may further question whether the amounts of vasopressin released during learning and retention of passive avoidance behavior[20] are sufficient to induce the same visceral effects as the pharmacological amounts of vasopressin administered peripherally and needed to demonstrate its influence on memory processes. It is thus questionable whether the studies by Ettenberg *et al.*[13] on the aversive effects of systemically administered vasopressin, as cited by Gash and Thomas, are relevant to the problem.

A final point I wish to make is that the behavioral (memory) effects of vasopressin are dissociated from the classical endocrine effect of this nonapeptide. This we discovered in 1971[21] when DGLVP was isolated from hog pituitary material. We found that this peptide was almost completely devoid of effects on blood pressure and water retention but that it had retained its behavioral effects[12]. We have recently found that vasopressin is converted by brain membrane enzymes to several fragments which are 1 000 times as active as the parent molecule in facilitating passive avoidance behavior[7]. We found that one of these fragments, [pGlu^4]AVP-(4-8), is active at sub-picogram doses when administered intracerebroventricularly, that it has high affinity for putative vasopressin receptors in the brain, and that its effect is blocked by the vasopressin antagonist ($d(CH_2)_5Tyr(Me)$-AVP).

I regard vasopressin and oxytocin as

prototypes which have helped us to demonstrate the important memory-modulating effects of the neurohypophyseal hormones. The time has now come for investigators to work with the newly found highly potent and selective neuropeptides which the brain appears to generate from the neurohypophyseal hormones.

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D. DE WIED

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

Reply from Don M. Gash and Garth J. Thomas

SIR:

Does vasopressin have significant effects on memory? More specifically, as DeWied has proposed, does vasopressin facilitate memory consolidation and retrieval? This thesis is of considerable biological importance and has, correctly, focused attention upon the role of peptides in behavior. The issue we addressed in *TINS* (June, 1983)[1] was whether the results from numerous studies testing this thesis could be most logically interpreted as demonstrating that vasopressin directly modulates memory processes. We concluded that, while data could be selected to support a direct role for vasopressin, considerable evidence suggests that other explanations are more likely. One concept which does unify much of the work conducted to date is that vasopressin has direct visceral (autonomic) effects which may indirectly influence other behaviors including higher cognitive functions.

DeWied has presented arguments supporting his thesis in the accompanying letter. We cannot address those points derived from his studies which have not been independently confirmed[2–4]. Also, several of the experiments cited are not relevant to his thesis. For example, studies on injections of vasopressin[5] or vasopressin antiserum into the brain[6] will not clearly distinguish between the direct and indirect effects of vasopressin on behavior. The important issue is that studies by other groups generally have either failed to replicate key experiments or have obtained data which can be interpreted as resulting from the visceral effects of vasopressin. The following amplifies our concerns and addresses the key arguments raised by DeWied.

Vasopressin-deficit rats do not have clearly defined memory deficits, and often show longer retention latencies (i.e. a better 'memory') of shock avoidance behavior than normal animals.

A number of studies (see Table I) have focused on the behavior of Brattleboro rats, homozygous for the diabetes insipidus trait (HO-DI rats). These animals originated as a mutant of the Long-Evans strain and totally lack the ability to synthesize vasopressin[7]. Investigations conducted independently of the Rudolf Magnus Institute would suggest that vasopressin-deficient rats do not have significant memory deficits as measured by shock avoidance tests. Systematic differences in procedures

TABLE I. Investigations of memory deficits in the vasopressin-deficient Brattleboro rat

	Refs
A. Memory deficits found	
1. DeWied, Bohus, and van Wimersma Greidanus (1975)	27
2. Bohus, van Wimersma Greidanus and DeWied (1975)	28
3. Laczi, Fekete and DeWied (1983)	13
B. Mixed effects of vasopressin on memory found	
1. Bailey and Weiss (1979)	29
2. Brito, Thomas, Gingold and Gash (1981)	8
C. No memory deficits found	
1. Celestian, Carey and Miller (1975)	30
2. Miller, Barranda, Dean and Brush (1976)	31
3. Bailey and Weiss (1978)	32
4. Brito, Thomas, Gash and Kitchen (1982)	9
5. Carey and Miller (1982)	33
6. Williams, Carey and Miller (1983)	34