The vasopressin-memory hypothesis: a citation network analysis of a debate

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Abstract

The 1970's saw growing interest in the vasopressin-memory hypothesis, proposed by David de Wied and his collaborators in Utrecht. This rose to a peak in the 1980's that saw a flurry of papers published from diverse sources critical of the experimental foundations of this idea. In subsequent years, interest in this hypothesis declined markedly as shortcomings were recognised. Here we study this debate using citation network analysis to identify the influential papers in this debate and the citation links between them. The issues raised have contemporary relevance to current controversy about the interpretation of studies using intranasal oxytocin.

Introduction

When Starling first introduced the term 'hormone' to refer to substances produced at one site that were conveyed by the blood to act at distant targets, he recognised that these substances might exert many different actions at different targets, but he conceived that a unity of purpose embraced them all. *"In the multiplicity and diversity of the physiological effects produced by these various chemical messengers one is apt to lose sight of the fact that we are here investigating one of the fundamental means for the integration of the functions of the body"* [1]. In the 1950's, the concept that hormone secretion from the pituitary gland is

governed by the hypothalamus became established through Geoffrey Harris' notion of hypothalamic releasing factors [2]. It then became natural to think that hormones released from the pituitary might also act on the brain to induce behavioral responses that were congruent with their peripheral actions.

David de Wied in Utrecht pioneered such studies, initially by considering behavioral effects of ACTH and α-MSH, but soon by concentrating on vasopressin, and he coined the term "neuropeptide" to describe its central actions. His studies pursued the idea that vasopressin is specifically involved in certain types of memory. How such effects might be congruent with the peripheral actions of vasopressin was never clearly articulated, but in the 1970's his studies and those of his close collaborators attracted considerable interest, particularly when reports began to appear that intranasal application of vasopressin might have beneficial effects on memory in man. That interest diminished when it appeared that the apparent effects of vasopressin on memory might be accounted for by effects on arousal and attention, and when it became clear that the blood-brain barrier efficiently obstructs the entry of vasopressin into the brain.

However, De Wied's work stimulated interest in the central release of vasopressin, leading to the recognition of an extensive network of vasopressin fibres within the brain [3], and recognition that vasopressin is released centrally from these fibres [4]. Here, we focus on the relationship between the central (brain) actions of vasopressin and its peripheral actions. Our motivation comes from the current interest in the behavioural effects of vasopressin and the related peptide oxytocin, and in using systemic (intranasal) administration therapeutically for its presumed direct effects on the brain. We begin by analysing the rise and fall of the belief that vasopressin, secreted into the circulation from the pituitary gland or when administered systemically, acts not only at its well established targets of the kidney and vasculature, but also on the brain to selectively enhance memory functions.

The extensive literature relating to de Wied's work has been synthesised in a work of exceptional scholarship by McEwen [5]. Here we approached this controversy in a different way, by studying the evolution of the controversy through *citation network analysis,* compartmenting the network of interacting papers according to whether papers were supportive or critical of the vasopressin-memory hypothesis in the manner of a claim-specific network (e.g. [6]).

Network analysis of the vasopressin-memory debate.

De Wied developed the *vasopressin-memory hypothesis* in conjunction with a number of collaborators, mainly based in Utrecht. Tracking the citations to the papers they published on vasopressin reveals a progressive growth in interest from its origins in 1965 to 1980. This was followed by a surge in interest between 1981 and 1987, followed by a monotonic decline, although de Wied and his collaborators continued to publish on this hypothesis at an undiminished rate throughout the 1990's (Figure 1). The surge reflected the publication of a number of studies that together amounted to a comprehensive rebuttal of the theory, at least in its original form.

To understand the structure of the network we generated a catalogue of papers that were involved in the debate, and classified them by whether they were supportive of the hypothesis or critical of it. This method has recently been used to study the structural dynamics of a number of scientific controversies [6-8]. To catalogue papers involved in the debate, we began with de Wied's most highly cited paper. This, published in *Nature* in 1971, presented an early and core element of the evidence [9]. We retained the 370 papers that cited this paper from the Web of Science (WoS) core collection.

We then retrieved all bibliometric data held on these papers via the WoS 'Full Record and Cited References' download function. From these, we constructed an edge-list by parsing data into a 'Source' column (all identified publications) and a 'Target' column (all identified references). This describes a network as a set of vertices (papers) connected by 'edges' (citation links). We constructed a vertex attribute list that included information on year of publication, all authors, journal of publication, title of paper, type of paper (review, article etc.), total number of citations, and WoS accession number. We then classified each paper according to whether the results or arguments were supportive, critical, or 'passive' toward the vasopressin-memory hypothesis.

In Gephi, we imported the vertex attribute list (contained in a .csv file) into the workstation, then imported the edge-list (in a .csv file). From this, a graph [G] was constructed of the vertex set [V] and edge set [E]:

$$G = (V, E)$$

This produced a network with 13,653 vertices and 23,240 edges. This is many times larger than the original set of papers because it includes all of their references. After a degree centrality analysis, we identified another 61 publications that had been cited at-least 15 times by the 370 citing papers that had not been retrieved from the original search. These were retained because their high citation score meant that they were likely to be relevant to the debate that unfolded. Their bibliographic data and cited references were added to the

network.

Restricting this network to *only* papers fully retrieved gave a network of 436 vertices and 4,620 edges. The vertices included 109 papers by de Wied or by his close collaborators. Another 71 papers were critical of key aspects of the hypothesis, while 109 papers were structurally supportive by providing corroborating evidence or extended arguments in favour. Another 146 papers were "passively supportive"; these referred to the hypothesis in a positive or neutral manner but did not provide or aim to provide any experimental test of it. We checked our assignation of papers against McEwen's reading of their content where we could [5], and conferred amongst ourselves in other cases, collating key quotes.

The first critical paper [10] in this network was published in 1975 and the vast majority were published between 1981 and 1985. Accordingly, we restricted our analysis to the period between 1971 and 1991, a period that 94% of all critical publications and 77% of all publications retrieved. We removed all papers not directly relevant to the assessment of the hypothesis (passive papers), leaving a network of 250 vertices and 3,010 edges (67 critical papers; 83 supportive papers written by scientists who were not collaborators of De Wied; and 100 papers authored by De Wied and his collaborators).

We visualised this network using Gephi [11]and the 'ForceAtlas 2' algorithm [12], a force-directed layout for directed networks that pulls vertices together if they share an edge and pushes vertices away from one another if they do not (Figure 2). The network has a non-random structure; vertices are clustered according to their classification showing that papers of a particular classification tend to cite others of the same classification (Table). The paucity of isolates (vertices unconnected to the largest connected structure) and the density of edges demonstrates extensive interaction between different classifications of paper. The network conforms to the typical structure of citation networks in that the in-degree distribution is highly skewed with a long-tailed distribution typical of a scale-free network. De Wied's 1971 paper is, obviously, the most highly cited paper in this network with 199 citations. Sixty-one papers received no citations from other papers of this network, and 20% of papers were responsible for 67% of all citations.

Until 1980, almost all papers citing de Wied were either actively or passively supportive. Between 1971 and 1980, we identified just ten critical papers in this network, and these were cited just 47 times within the network in this period. However after 1981 came a storm of papers critical of the hypothesis, including 57 that appear in the network. In this period, 634 of the citations within the network were to the 67 critical papers: 312 of these came from *other* critical papers, but 128 were from de Wied and his collaborators (Table).

Thus de Wied and his collaborators engaged with their critics, and the critics engaged with the responses from de Wied. It is, in this sense, a model of what we tend to think science ought to be like – an open and fruitful debate.

Between 1981 and 1991, we see a dramatic change in the structure of communication. In terms of the number of new publications, critics, supporters, and De Wied's collaboration group had very similar outputs. About 25% of citations were to critical papers, about 21% to supportive papers, and 54% to De Wied's papers. However, in this period, 1,672 citations were to papers published between 1971 and 1980, of which 198 were to critical papers, 1,155 to De Wied's papers, and 319 to supportive papers. Excluding the citations to these earlier publications demonstrates a major shift in the debate. Of the papers published between 1981 and 1991, De Wied's 52 publications gathered 233 citations, the 57 critical papers gathered 436 citations, and the supportive papers gathered 225 citations. Thus, there was not only an increase in the rate of publication of critical papers but also an increase in their influence.

By 1991, it seems that, whatever the merits of the arguments, de Wied had lost the debate. Insofar as his hypothesis survived at all, it survived only in a form modified beyond recognition from its early form, yet still entwined in innumerable qualifications and uncertainties. Hope that it would yield any beneficial therapy for cognitive dysfunctions had all but evaporated, and by 1991, the critics had all but left the field.

Before discussing that debate, we briefly summarise what is currently known of the vasopressin systems of the brain. We then discuss the experimental evidence that led de Wied to propose the vasopressin-memory hypothesis, and then the evidence that challenged this hypothesis, ending by reflecting on what lessons might be learned for a current, related controversy concerning intranasal oxytocin.

The vasopressin systems of the brain

The oxytocin/vasopressin signalling system is thought to have evolved from the ancestral nonapeptide vasotocin by gene duplication. Both peptides are present in virtually all vertebrates, including mammals, birds, reptiles, amphibians and fish, and oxytocin- and vasopressin-like peptides have also been identified in molluscs, annelids, nematodes and arthropods. Members of this peptide family share high sequence similarity, and they appear to be involved in many similar functions in different species across the animal kingdom [13].

By our current understanding, the vasopressin and oxytocin systems of the brain comprise multiple compartmented systems that subserve a huge diversity of functions [14]. Oxytocin and vasopressin are produced by magnocellular neurons that project to the posterior

pituitary from where they secrete their products into the systemic circulation. In rats, both oxytocin neurons and vasopressin neurons are osmoresponsive: vasopressin and oxytocin are secreted to regulate antidiuresis and (in rodents) natriuresis respectively. Both hormones have a wide variety of other peripheral actions; oxytocin is essential for milk-ejection in lactation and for controlling the progress of parturition [15], and vasopressin has pressor effects on the peripheral vasculature [16], but both have a diversity of other actions in the periphery.

The magnocellular neurons are conspicuously aggregated in the supraoptic and paraventricular nuclei of the hypothalamus, but many are dispersed between these aggregations; some are clustered in small "accessory nuclei". It is now clear that some magnocellular oxytocin neurons give rise to axon collaterals that innervate many forebrain regions [17]. In addition, a relatively small population of "parvocellular" oxytocin neurons in the paraventricular nucleus do not project to the pituitary but to the caudal brainstem (where they regulate gastric reflexes [18]), to the spinal cord (where they regulate pain pathways, thermogenesis, penile erection and ejaculation [13]), and to the larger population of magnocellular oxytocin neurons [13].

The paraventricular nucleus also contains several populations of centrally projecting vasopressin neurons with apparently diverse functions: some of these are involved in regulating ACTH secretion[19-21], some in the behavioral responses to stressors [22, 23], some in the regulation of thermoregulation and fever [24], some in the control of blood pressure [25]. Many other neurons in different brain regions also produce vasopressin: a large population in the suprachiasmatic nucleus regulates circadian rhythms and the sleep-wake cycle [26], and this nucleus is also innervated by vasopressin-containing retinal ganglion cells that are intrinsically photosensitive [27]. Other vasopressin populations in the olfactory bulb and the accessory olfactory nucleus [28], and the piriform cortex [29] are involved in processing olfactory information and appear to be involved in social recognition [30]. Yet others are present in the septum [31]. The vasopressin and oxytocin pathways of the brain are also sexually dimorphic [32], and both systems [33] are involved in the regulation of a variety of reproductive and social behaviors [34-36]. Vasopressin and oxytocin are not only released from axons [37], but also from neuronal dendrites [38]. Their actions involve autocrine actions on the cells of origin, paracrine effects on neighbouring cells, effects on neurons in multiple target areas mediated by axonal release, and effects on neurons mediated by "neurohormonal" release within the brain [34, 39].

However, in 1965, when de Wied reported the findings that first implicated vasopressin in memory processes [40], the exuberant central vasopressin system was unknown. Then, it was believed that vasopressin was secreted only from the posterior pituitary gland,

from the nerve terminals of hypothalamic neuroendocrine neurons. It had long been recognised that vasopressin was involved in body homeostasis by its regulation of fluid and electrolyte balance and by its involvement in stress, so it did not seem unreasonable to ask whether vasopressin, by feedback actions on the brain, might also have a central role in the behavioral response to stress.

The vasopressin-memory hypothesis: experimental foundations

1.Behavioral experiments

De Wied's first experiments were on rats trained to avoid an electric shock [40]. Rats were placed in a 'shuttle-box' with two compartments separated by a 2-inch high barrier. A *conditioned stimulus* (the sound of a buzzer or illumination of a lamp) was produced for 5 s, after which an electric shock (the *unconditioned stimulus*) was delivered through the mesh floor, and the rat could avoid this shock by crossing the barrier to the other side of the box within the 5-s window. Rats were given ten trials on each of 14 days, and "good learners" were selected for extinction testing. For this, ten trials were given on each of 9-14 days without the unconditioned stimulus, and *retention* was measured as the number of avoidances per session.

De Wied [40] reported that removing the posterior and intermediate lobes of the pituitary did not affect the speed of learning, but reduced the retention of the response. He then showed that systemic administration of either 1 IU (~2 μg) pitressin – a pituitary extract rich in vasopressin – or doses of 1-10 μg of purified lysine vasopressin (LVP) could correct the deficit when injected subcutaneously during the extinction test period. In *Nature* the following year [41], de Wied and Bohus reported that subcutaneous injections of pitressin enhanced retention of the conditioned avoidance whether given either during training or during extinction testing. From this, they proposed that vasopressin was involved in the maintenance, but not in the acquisition, of learned avoidance behaviour, and in associated experiments they excluded the involvement of ACTH in these effects. It should be noted that the doses involved in these studies are very high – the total vasopressin content of the rat pituitary is about 1 μg [42].

In 1971, de Wied used another conditioned avoidance test, in which rats learned to avoid a shock by jumping onto a pole in the middle of the test cage. Rats were trained over three days with ten trials each day [9]. The following day was the first extinction session, in which rats were again given ten trials but no shocks. Rats that showed at least eight

avoidances were then given a subcutaneous injection of peptide at various times after the last trial, and were tested in further extinction sessions. Subcutaneous injections of 1 µg lysine vasopressin (LVP) enhanced the retention of learned avoidance behaviors when given 2 h after the last trial, but not when given after 6 h, suggesting an effect on memory *consolidation*. No effects were seen with oxytocin, or with angiotensin, insulin or growth hormone (given to control for effects of vasopressin on blood pressure and carbohydrate metabolism).

In 1972 de Wied introduced a *passive avoidance* test [43]. Rats were placed in a cage with two areas - a 'shock-box' and a 'runway' - separated by a door. The shock box was dark whereas the runway was brightly lit, so a rat with a normal preference for dark, enclosed spaces would move from the runway to the shock box as soon as the door was opened. Rats became acclimatised to the shock-box for one day. On the next day, they were placed on the runway and allowed to enter the shock-box for 10 s. They were then replaced on the runway, and the door was reopened allowing the rat to re-enter the shock-box. On the third run, an electric shock was delivered through the floor in the shock-box. Retention of the learned fear was tested 24 and 48 h later by measuring the time taken for the rat to enter the shock-box from the runway. A longer latency to re-enter the box was interpreted as reflecting memory of the association between entry into the box and the shock.

2. Studies in Brattleboro rats

The Brattleboro rat is a variant of the Long-Evans rat which lacks endogenous vasopressin. Bohus et al. (1975) compared learning of all three major learning paradigms (shuttle box, pole jumping, and passive avoidance) between Wistar rats, and Brattleboro rats homozygous and heterozygous for diabetes insipidus [44]. The Brattleboro rats had quicker extinction of learned behaviours than Wistar rats, and this apparent memory deficit could be corrected by giving a subcutaneous injection of vasopressin immediately after the learning trial.

3. Translational implications

In 1974, de Wied reported that subcutaneous injection of 10 µg vasopressin improved retention of the passive avoidance response when given either 1 h before the acquisition trial *or 1 h before the first retention trial in rats with CO_2-induced amnesia of the learned response*.[45] This suggested that clinically useful memory treatments might be developed using vasopressin.

4. Central actions

In 1976, de Wied's group published studies of the effects of vasopressin administered into the brain by intracerebroventricular (i.c.v.) injection [46]. Like systemic administration, i.c.v. administration of vasopressin or of fragments of the peptide improved retention of pole jumping, even at very low doses (1 ng), implying that vasopressin or centrally-produced metabolites of vasopressin had been acting centrally in his previous experiments. Because the covalent ring of vasopressin was effective in reducing extinction, de Wied suggested this was the behaviourally active part of the compound. The same year, the group used anti-serum to vasopressin (enough to neutralise 2.5 ng of the peptide) to inactivate the neuropeptide in the brain [47]. This, given i.c.v., improved retention of the passive avoidance response if given up to 2 h before or 3 h after the acquisition trial.

5. Summary

While de Wied and his collaborators published many other papers and reviews on vasopressin and memory, these nine papers were the most highly cited of his research papers before 1983, and they comprise the core experimental foundations of the hypothesis. From these, we can recognise that the hypothesis combined four claims:

1) The effects of systemically applied vasopressin on the performance of rats in certain behavioral tasks reflects specific effects on both the *consolidation* and *retrieval* of memories.

2) Systemically applied vasopressin acts *centrally* to exert its effects on memory.

3) *Endogenous* vasopressin is implicated in these memory processes.

4) Systemically applied vasopressin can reverse induced memory deficits and might therefore be of therapeutic value in man.

Each of these claims became the subject of determined assault by a diverse body of critics, an assault that came to a head in the 1980's. We consider the fate of each in turn.

The critical assault

1. The interpretation of behavioral studies.

While the outcomes of the behavioral tests used by de Wied and collaborators were interpreted as evidence of enhanced consolidation and retrieval of memory, others suggested that the outcomes were consequences of peripheral actions of vasopressin, reflecting effects on attention, arousal, or emotionality. Particular attention was given to the fact that systemic injections of large doses of vasopressin have prolonged cardiovascular effects, and it was noted that these were likely in themselves to be aversive. Since the behavioral tasks

concerned were mainly aversively motivated, it was suggested that the post-task administration of vasopressin, by inducing additional fear as a result of its pressor actions, enhanced the memory of the association between footshock and the conditioned stimulus. In 1981, Koob and colleagues confirmed that subcutaneous doses of vasopressin that prolonged extinction of active avoidance behavior also had pressor effects, and they showed that a vasopressin antagonist given systemically would block both effects [48].

In response, in 1984, de Wied et al. reported that *central* administration of vasopressin antagonists could block the behavioral effect of systemically applied vasopressin without affecting the pressor response [49]. The antagonists also blocked the passive avoidance response to i.c.v. injections of low doses of either vasopressin or a behaviorally active fragment of vasopressin that lacked pressor activity (and hence apparently did not act via the classical vasopressin receptors).

In response to this, in 1985, Koob and colleagues confirmed that central administration of a vasopressin antagonist could block the effects of peripherally applied vasopressin – but, in their hands, only at doses sufficient to block the pressor response [50, 51]. Thus it seemed that two distinct actions of vasopressin might be involved in the behavioural response: a peripheral, pressor action of vasopressin, and possibly also a central action of a metabolite that did not act through the classical vasopressin receptors.

Others asked whether vasopressin could enhance memory in *positively*-reinforced tasks. It appeared that vasopressin had effects in some but not all such tasks. For example, Sara et al. [52] reported that vasopressin facilitated learning in an appetitively motivated task but had no effect on retrieval. Hostetter et al. found no effect of vasopressin in a food reward task but did find an effect on fear-motivated behavior [53]; they subsequently reported an inability to reproduce de Wied's findings on passive avoidance behavior [54]. In 1983, Ettenberg et al. used a simple one-trial appetitive situation [55, 56]. Post-training administration of vasopressin reduced the latency of thirsty rats to contact a drinking tube in an environment with which they had experienced only one previous exposure. This was consistent with the notion that vasopressin facilitates memory consolidation and/or retrieval in this positively reinforced task. However, they went on to show that (i) vasopressin alone could act as an effective unconditioned stimulus in conditioned taste and place aversion tests, indicating that systemically applied vasopressin was aversive ; (ii) lithium, a nausea-inducing aversive agent could mimic the apparently memory-enhancing effects of vasopressin; and (iii) vasopressin affected locomotor activity of rats consistent with an effect on arousal. They concluded that,

in this paradigm at least, the apparent effects of systemically applied vasopressin on memory could be attributed to its aversive pressor effects.

As mentioned. de Wied had reported that low doses of vasopressin given i.c.v. did not evoke pressor effects but had similar effects on memory as systemic applications [46]. However, Sahgal et al. [57, 58] saw differences between the effects of central and systemic vasopressin. They reported that, in the shuttle-box task, i.c.v. vasopressin lengthened the latency to re-enter the box in some rats but shortened it in others. This, they argued, was consistent with an effect on *arousal*. Cognitive performance is impaired by either very low or very high levels of arousal, so the apparent bimodal effects of vasopressin might reflect enhancing effects of arousal on rats with a low level of arousal, but deleterious effects in rats with an already optimal level of arousal. They thus proposed that the effects of systemically applied vasopressin involved reinforcement mechanisms as well as effects on arousal, whereas those of centrally applied vasopressin reflected an effect on arousal alone.

2. *Endogenous vasopressin?*

The main evidence for a role for endogenous vasopressin came from the original finding of a behavioral deficit after posterior lobectomy, which presumably eliminated peripheral but not central vasopressin; and from studies of Brattleboro rats. Many subsequent studies were conducted with Brattleboro rats, but many of these failed to find *any* memory defects [10, 59, 60], and some reported that Brattleboro rats performed *better* than the relevant strain control rats [61-63]. Apparently, different laboratory strains of Brattleboro rats had different behavioral features – presumably reflecting the consequences of laboratory in-breeding producing additional phenotypic features that had nothing to do with vasopressin.

In 1983, Danguir [64] described what seems an obvious explanation of cognitive deficits in homozygous Brattleboro rats. These rats drink about their own body weight in water each day and excrete it in copious amounts of dilute urine; because they have to spend such a disproportionate amount of time drinking and urinating, their sleep is grossly disturbed. The sleep pattern could be restored by continuous intravenous infusions of either vasopressin (0.48 μg per day) or of water. Danguir's paper was published in *Nature,* but had been cited only 14 times by others in the ten years after it was published, and never by de Wied and his collaborators and supporters, or by any of the major critics of Brattleboro rat studies. The paper was inconvenient for both the supporters of de Wied, in proposing an explanation of the cognitive defects of Brattleboro rats that did not implicate a central action of vasopressin, and for those critics who failed to find any relevant cognitive deficit in those rats.

As a postscript to de Wied's work on Brattleboro rats, in 2006, he was posthumously an author of a study of neuropsychological functioning in human subjects with familial neurohypophysial diabetes insipidus and unaffected family members [65]. Of 63 quantified neuropsychological parameters, few were significantly different between affected and unaffected subjects. The authors concluded that, in this human disease, the central vasopressin systems may be less affected than the magnocellular system. To date there appear to have been no published post mortem studies of the brains of affected individuals.

The results of posterior lobectomy in rats [40] had seemed to suggest that deficits in memory followed the loss of systemic vasopressin – but there was scepticism about whether enough endogenous vasopressin would enter the brain after systemic secretion to induce any central effects. De Wied's collaborators in Utrecht set about answering this by measuring CSF levels of vasopressin (and of oxytocin) after peripheral injections of 5 µg of either peptide. In 1983 they reported the results [66]: the last sentence of their abstract states "*The present results demonstrate that neurohypophysial hormones do cross the blood-brain barrier in amounts obviously sufficient to induce central actions*." This exuberant claim however was preceded by a precise statement of the actual extent of passage: "*These data reveal that approximately 0.002% of the peripherally applied amount of AVP or OXT reached the central nervous system at 10 min after injection*."

This outcome was supported by a 1986 study which measured vasopressin in tissue samples of various brain regions, including the hippocampus, after subcutaneous injection of 5 µg/kg of vasopressin. When the brains of anesthetised rats were perfused to be free of blood, there was no detectable increase in vasopressin content of any area of the brain except in the median eminence, where there is no effective blood-brain barrier [67].

The Mens et al. paper [66], now a classic for its elegant and careful quantification of the efficacy of the blood-brain barrier for oxytocin and vasopressin, was never cited by de Wied or his immediate collaborators in Utrecht. It is, using a term now commonly used in Scientometrics, a "sleeping beauty"[68]. Most papers in the scientific literature are cited most often in the first few years after publication and at declining levels thereafter, but some are "awakened" after a period of relative quiescence. Cited an average of 8 times per year in the 8 years after publication, Mens et al. "slept" for the next 19 years, with an average citation rate of 3/year, and then "re-awakened" between 2013 and 2019, with an average of 15 citations/year, reaching 218 citations in its life to date.

3. Vasopressin and memory in man

The translational potential of vasopressin for enhancing memory was tested in diverse small studies using intranasal application of large doses of vasopressin or of desglycinamide-arginine-vasopressin (DGAVP), a metabolite of vasopressin that in some tests appeared more potent than vasopressin itself. Two short reports published in 1978 in the *Lancet* attracted considerable attention. In one, (cited 226 times to date) twenty-three inpatients with minor pulmonary or gastroenterological diseases were given vasopressin or placebo by intranasal spray: the twelve patients given vasopressin performed better in memory tests, and in many other tests, including tests of attention and concentration [69]. In the other [70] (cited 175 times), four patients with amnesia were reported to have improved after vasopressin. Other small supportive studies followed [71, 72]; as summarised by one of de Wied's collaborators in 1983 [73], these indicated that vasopressin–like peptides do have behavioral effects in humans based on the fact that "most studies do find something, be it a clinical impression of improvement or objective test results." But many studies, had disappointing outcomes. For example, Beckwith et al. [74], Gais et al. [75], Bruins et al.[76] and Snel et al. [77] found no effect on memory consolidation or retrieval; they found some effects on learning, but these seemed to be associated with effects of vasopressin on arousal and attention. Studies in patients with memory defects were also generally disappointing: Jenkins et al.[78, 79] and Koch-Henriksen et al. [80] concluded that DGAVP was of little practical value in conditions where memory deficiency is the predominant disorder. Others reported no benefits of vasopressin for subjects with memory impairment induced by ethanol [81, 82] or ECT treatment [83] or of 92 µg DGAVP in patients with Alzheimer's [84, 85]. Thus, from studies with intranasal vasopressin, it appeared that what effects *were* observed were not associated with consolidation or retrieval of memories as expected from the de Wied hypothesis, were likely to be associated with non-specific effects on arousal or attention, and were of little if any therapeutic value.

Perhaps the "killer" experiment for this line of investigation was conducted by Ang and Jenkins in dogs [86]. They administered radiolabelled vasopressin intranasally or intravenously and studied the resultant appearance of label in the CSF and plasma. By either route of application, concentrations of label in the plasma exceeded CSF levels by about 100 fold. They then checked how much of the label corresponded to intact vasopressin by HPLC, and found no evidence that *any* intact peptide penetrated into the CSF by either route. They also found no passage of radiolabelled DGAVP into CSF after intravenous administration. In 1987, Riekinnen et al. [87] asked whether DGAVP penetrated the brain following intranasal administration. In an open single dose study administered 2 mg of DGAVP intranasally to 42

patients (a dose 20 fold higher than used in earlier memory tests): CSF concentrations after this enormous intranasal dose peaked at about 60 pg/ml.

4. The fate of the 'vasopressin-memory' hypothesis

By the end of the 1980's, the vasopressin-memory hypothesis was in disarray. The studies on Brattleboro rats seemed to imply that complete absence of vasopressin had no clear consequences for cognitive function. Peripherally applied vasopressin penetrated the brain in at most tiny amounts, and the behavioral effects could be attributed to effects of the resulting increase in blood pressure on either fear or arousal. Centrally applied vasopressin might also exert its apparent effects on memory via changes in arousal. However given the growing evidence of diverse central sites of action of vasopressin, diverse central sources and diverse physiological roles, the possibility that apparently specific effects on memory processes reflected incidental actions in the brain seemed impossible to exclude. De Wied persevered with the hypothesis, arguing that it was not vasopressin itself that acted, but a putative metabolite [88] that was active through a novel (still unidentified) receptor at some undetermined site in the brain. Prospects of an effective therapy for memory deficits in man had dissolved.

One 'spin-off' from this work persisted, that vasopressin was involved in "social memory" through its central actions, an idea pursued in the early years by Dantzer and his colleagues [89, 90]. This particular notion has now been subsumed by a broader hypothesis, that vasopressin is involved in affiliative behaviour, involving multiple component effects at different brain sites [14, 91-95].

Reflections

Here we have followed the central strand of De Wied's hypothesis, but a second strand involved the peptide oxytocin: this, it was claimed had effects on memory processes that were opposite to those of vasopressin – that oxytocin was a neuropeptide of "forgetting". While vasopressin administered i.c.v. immediately after learning improved passive avoidance retention, oxytocin *reduced* retention in a dose-dependent manner [96, 97]. It is currently accepted that oxytocin, like vasopressin, affects memory processes by actions in the hippocampus, but it is also recognised that these effects are a facet of its broader involvement in social behavior [95].

However, in the last twenty years there has been an explosion of attention given to the possibility that intranasal administration of oxytocin might have therapeutic value for its purported effects on social behavior. This literature has been criticised for statistical and methodological considerations that led Walum et al. [98] to conclude that "there is a high probability that most of the published intranasal oxytocin findings do not represent true effects". Where there *are* true effects, it is far from clear that these reflect central actions of oxytocin, rather than incidental consequences of peripheral actions of the type that confounded studies of vasopressin on memory. Oxytocin receptors are present on many peripheral tissues, including the heart, gut, kidney, pituitary and reproductive organs [99].

Very little of the large amounts of oxytocin delivered in intranasal studies enters the brain. In 2018, Lee et al. [100] studied the passage of oxytocin into CSF after intranasal or intravenous injection of 80 IU oxytocin (160 µg) to rhesus monkeys (body weight 6-11 kg), a dose at least ten fold higher than the total body content. Six monkeys were used in this study, and deuterated oxytocin was administered to distinguish it from endogenous oxytocin. Blood and CSF samples were taken at various times after administration. Following i.v. injection, plasma concentrations rose in all monkeys within 10 min to between 25 and 75 ng/ml, declining rapidly thereafter. Repeated CSF measurements were reported for five of the same monkeys, all of which showed some increase at some time point. At 60 min, the average CSF concentration was increased by 100 pg/ml after i.v. administration. This is broadly consistent with the findings of Mens et al. [66] in rats. In that study, subcutaneous injections of 2 µg of peptide increased plasma concentrations to 28 ng/ml vasopressin and 39 ng/ml oxytocin within 5 min, accompanied by prolonged increases in CSF concentrations of about 25 pg/ml for vasopressin and about 70 pg/ml for oxytocin - about 1000 fold lower than the maximal concentrations achieved in plasma.

A major factor in undermining the vasopressin-memory hypothesis was the evidence that many of the effects of systemically applied vasopressin that had been interpreted as central actions on memory could be adequately explained as the consequences of peripheral effects of vasopressin on blood pressure. However, while studies with intranasal oxytocin in humans uniformly use massive doses of oxytocin that raise plasma levels to unphysiologically high levels, and despite evidence that very little of these large doses enters the brain, few authors have attempted to control for peripheral effects of intranasal oxytocin. One exception is a recent study by Quintana et al. [101] who found a significant effect of intranasal administration of 8 and 24 IU oxytocin on right (but not left) amygdala activity and

pupil diameter – but a similar effect with a much lower dose (1 IU) given intravenously to mimic the changes in peripheral levels produced by intranasal administration. Clearly, more such studies controlling for peripheral effects are needed.

We based this analysis primarily on analysis of citations. We do not hold that citations are a good reflection of study quality, but they are a reflection of influence. Critics of an apparently established theory are generally placed at a considerable structural disadvantage by citation and publication practices. Journals are thought to be generally unwilling to publish negative studies, and negative studies are cited less often than positive studies, leading to publication bias that reflects what has been called a "file-drawer" effect [102].

We examined this by searching on PubMed for human studies of vasopressin and memory, leading to the identification of 44 studies of intranasal vasopressin or vasopressin analogs published between 1978 and 1992. Of these, we identified 18 as supportive of some effect on memory that were interpreted by the authors as consistent with de Wied's hypothesis, and 26 studies interpreted as inconsistent. Thus in this period there is no evidence of publication bias. However the supportive studies have been cited (WoS) on average 76 times (SEM 17), while the negative studies have been cited on average just 24 times (SEM 4). It is hard to see that this reflects a difference in publication date or Journal of publication: in 1978, two of the first positive studies appeared; these have been cited 226 times [69] and 175 times [70] respectively. These were swiftly followed by two negative studies also published in *Lancet* cited 57 times[82] and 42 times[79] respectively. It is hard to recognise these differences as a reflection of study quality: one of the positive studies in *Lancet*[70] was an open uncontrolled study of just four patients. Nor do they obviously reflect differences in the notability of the authors: in 1983, Laczi et al. reported positive effects of intranasal vasopressin in patients with diabetes insipidus[103], and in the same year and in the same journal the same authors reported no effect in patients with alcohol-induced memory impairments[104]. The supportive study has been cited 50 times, the negative study just 9 times.

While the strength of science is often touted as being in its self-correcting nature, there is an obvious circularity in this. To claim that science is self correcting is to assume that current notions are correct while those that have been superseded are wrong. But there are powerful structural forces in science that can lead to the dogmatic persistence of some motions, while other structural forces lead to the systematic neglect of inconvenient evidence. In this case, without needing to take any position on the merits of the vasopressin-memory hypothesis, we can through citation network analysis address the neutral question of what it

was that enabled the critics of this hypothesis to "win" against these structural odds in the debate in the 1980's.

What is apparent is that the vasopressin-memory hypothesis rested on several independent lines of experimental evidence. An attack on any one alone could never be decisive; failed replications are seldom damning because no replication is ever exact; there are *always* methodological differences, and *always* flexibility of interpretation. In the vasopressin-memory debate, the decisive assault came because each pillar on which the hypothesis was built was subjected to determined assault by multiple independent critics. These critical strands were drawn together by the key critics to make an apparently comprehensive and devastating case against the hypothesis.

It is not possible to say however that the hypothesis is dead; the controversy is now largely forgotten, and while the hypothesis as originally proposed is no longer pursued to any serious extent, reviews of vasopressin still commonly cite effects on memory as though they are an accepted part of the canon. There is little comfort for those who hold that science is self-correcting in this.

At the same time, there is no doubt that de Wied's canon spurred intense and ultimately highly productive interest in the role in the brain of neuropeptides – a term that he coined. It is now universally accepted that a vast diversity of neuropeptides, including vasopressin, are released from many different populations of neurons to modulate many physiological functions and to regulate associated behaviors, and also that many peptides of peripheral origin act on the brain. However, while it seems reasonable to expect that the central and peripheral actions of *peripherally*-produced peptides will be congruent, there is no reason to think that in general this will be true of peptides produced at multiple sites in both the brain and the body, and we must be particularly cautious when the doses that we apply are greatly in excess of physiological levels.

The studies of de Wied and his collaborators that involved peripheral administration of vasopressin used doses that can be readily recognised as unphysiologically high: their standard dose of 1 μg is, in a rat, equivalent to the total pituitary content of vasopressin[42]. Similarly high and often much higher doses of systemically applied oxytocin have been widely used in behavioral studies in rodents and man[99]. Even given the very low rate of penetrance of the blood-brain barrier, it is impossible to be certain that such doses do not have central effects. But one general moral that can be derived from the de Wied canon is that

peripheral effects of peptides at high doses can have complex and unphysiological consequences that can subvert the interpretation of behavioral tests.

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Competing interests

None

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Figure Legends

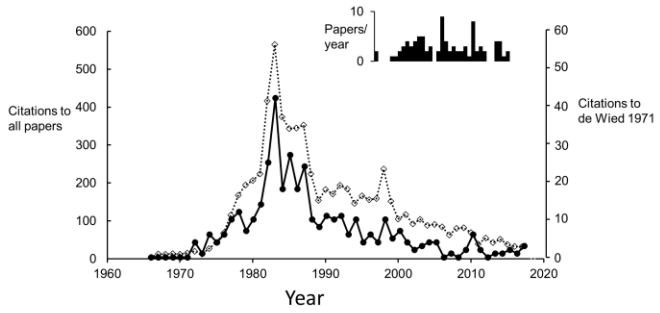

Figure 1.
From the *Web of Science*, we identified 82 papers published with de Wied as author that addressed the vasopressin-memory hypothesis. The dotted line shows the total citations per year to these papers (left hand axis). Citations to his most highly cited paper are shown by the black line (right hand axis – note the difference in scale). The distribution of these papers by year from the first (in 1996) to the last (in 2000) is shown in the inset. The graphs show the steady growth in citations up to 1982, with a surge in interest that lasted until 1988, followed by a steady decline thereafter. Citations to the most highly cited paper show the same pattern as to all papers, and this paper was selected as the seed for citation network analysis.

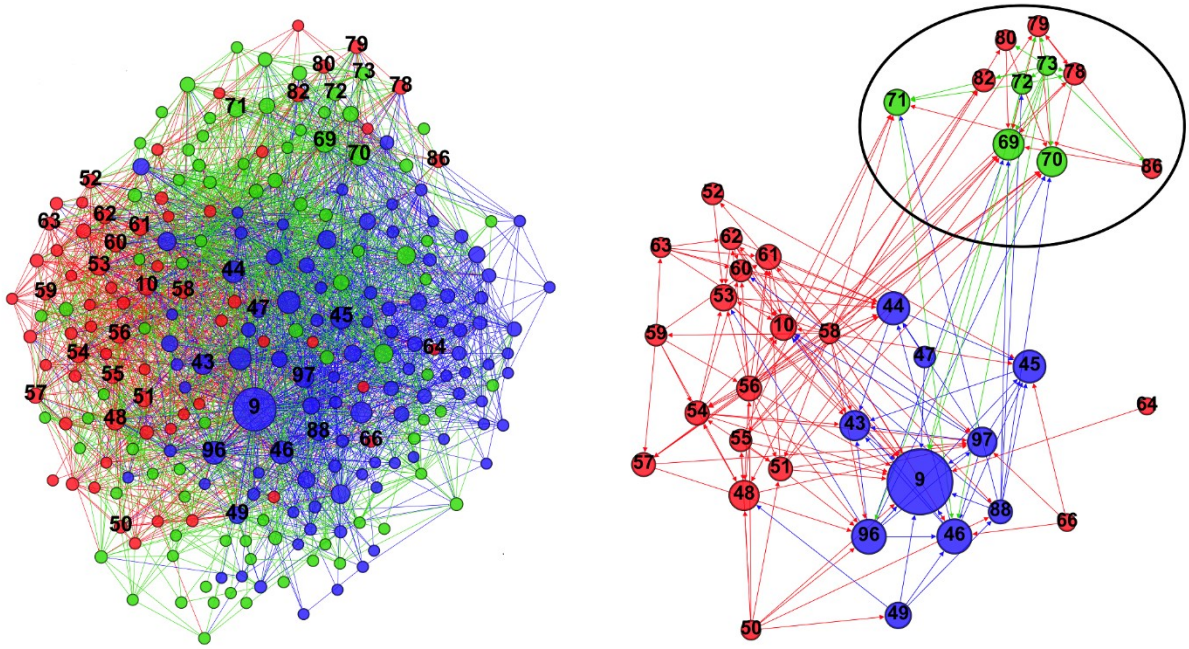

Figure 2.
Left. Citation network of 250 papers published between 1971 and 1991 showing citation links between papers authored by de Wied and his collaborators (blue), his critics (red) and his supporters (green). The numbers associated with each vertex refer to the list of references. Layout: ForceAtlas 2 algorithm. Vertices are sized by their in-degree and bound within a scale of 20 to 80 in Gephi's node size range. Edges are coded by the color of the source. The critical papers tend to cluster together because of the high density of citations between them, as do the papers from de Wied and collaborators.

Right. Papers within the network shown in Figure 2 that have been cited in the text. The numbers associated with each vertex refer to the list of references; the layout and sizing is as on the left. The area enclosed by an oval encompasses the human studies. Note the extensive reciprocal interactions between the critical network (red) and the papers from de Wied and his collaborators (blue).

Table:
250 primary research papers were identified as being centrally involved in the debate, and were classified as (i) *Collaborators*: 100 papers authored by de Wied or his close collaborators; (ii) *Supporters*: 83 papers not authored by de Wied or his collaborators but materially supportive of the vasopressin-memory hypothesis as proposed by de Wied; (iii) *Critics:* 67 papers materially critical of the vasopressin-memory hypothesis. The table displays how each of these groups cites papers of the three classifications.