Postulated vasoactive neuropeptide immunopathology affecting the blood–brain/blood–spinal barrier in certain neuropsychiatric fatigue-related conditions: A role for phosphodiesterase inhibitors in treatment?

Donald R Staines[1,2]
Ekua W Brenu[2]
Sonya Marshall-Gradisnik[2]

[1]Queensland Health, Gold Coast Population Health Unit, Southport, Gold Coast, Queensland, Australia; [2]Faculty of Health Science and Medicine, Population Health and Neuroimmunology Unit, Bond University, Robina, Queensland, Australia

Abstract: Neuropsychiatric symptoms occur in a number of neurological fatigue-related conditions including multiple sclerosis (MS), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and chronic fatigue syndrome (CFS). These conditions have been attributed variably to neuroinflammatory and neurodegenerative processes. While autoimmune pathology, at least in part, has long been suspected in these conditions proof has been elusive. Autoimmune pathomechanisms affecting the blood–brain barrier (BBB) or blood–spinal barrier (BSB) may predispose the BBB/BSB to 'leakiness' and be a precursor to additional autoimmune events resulting in neuroinflammatory or neurodegenerative processes. The aim of the paper is to postulate immunopathology of the cerebrospinal perivascular compartment involving certain vasoactive neuropeptides, specifically pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP), in the etiology of certain neuropsychiatric fatigue-related conditions such as MS, ALS, PD, and CFS. Vasoactive neuropeptides (VNs) such as PACAP and VIP have critical roles as neurotransmitters, vasodilators including perfusion and hypoxia regulators, and immune and nociception modulators. PACAP and VIP are widely distributed in the central nervous system (CNS) and have key roles in CNS blood vessels including maintaining functional integrity of the BBB and BSB. Autoimmunity affecting these VNs would likely have a detrimental effect on BBB and BSB functioning arguably predisposing to further pathological processes. Virchow–Robin spaces (VRS) are perivascular compartments surrounding small vessels within the CNS which contribute to the BBB and BSB integrity and contain PACAP and VIP receptors. Autoimmunity of these receptors would likely affect BBB and VRS function and therefore may contribute to the etiology of these conditions by affecting CNS and immunological homeostasis, including promoting neuropsychological symptomatology. PACAP and VIP, as potent activators of adenylate cyclase (AC), have a key role in cyclic adenosine monophosphate (cAMP) production affecting regulatory T cell (Treg) and other immune functions. Phosphodiesterase enzymes (PDEs) catalyze cAMP and PDE inhibitors (PDEIs) maintain cAMP levels and have proven and well known therapeutic benefit in animal models such as experimental allergic encephalomyelitis (EAE). Therefore PDEIs may have a role in therapy for certain neuropsychiatric fatigue-related conditions.

Keywords: vasoactive neuropeptides, multiple sclerosis, Parkinson's disease, chronic fatigue syndrome, phosphodiesterase inhibitors, cyclic AMP, adenylate cyclase, Virchow–Robin spaces

Correspondence: Donald R Staines
Gold Coast Population Health Unit,
10–12 Young Street, Southport 4215,
Queensland, Australia
Tel +61 7 5509 7222
Fax +61 7 5561 1851
Email don_staines@health.qld.gov.au

Introduction

Neuropsychiatric symptoms occur in a number of neurological fatigue-related conditions including multiple sclerosis (MS),[1] Parkinson's disease (PD),[2] amyotrophic lateral

sclerosis (ALS)[3] and chronic fatigue syndrome (CFS).[4] While autoimmune pathology, at least in part, has long been suspected in these conditions proof has been elusive. The present paper asserts a provocative hypothesis that autoimmune pathomechanisms affecting the blood–brain barrier (BBB) or blood–spinal barrier (BSB) may predispose the BBB/BSB to 'leakiness' and be a precursor to additional autoimmune events resulting in neuroinflammatory or neurodegenerative processes, compounded possibly on a genetically susceptible background or exposure to environmental factors.

The paper examines the potential role for vasoactive neuropeptides (VNs) such as pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP) in the possible autoimmune etiology of these disorders through effects on the blood–brain/spinal barriers (BBB/BSB) whereby neuropsychiatric symptomatology may occur. PACAP and VIP, as potent activators of adenylate cyclase (AC), have a key role in cyclic adenosine monophosphate (cAMP) production affecting BBB/BSB function along with regulatory T cell (Treg) and other immune functions.

Phosphodiesterase enzymes (PDEs) catalyse cAMP and PDE inhibitors (PDEIs) maintain cAMP levels and have proven and well known therapeutic benefit in animal models such as experimental allergic encephalomyelitis (EAE). Therefore PDEIs may have a role in therapy for certain neuropsychiatric fatigue-related conditions.

Functions of PACAP and VIP

PACAP and VIP are widely distributed in the central (CNS) and peripheral (PNS) including autonomic (ANS) nervous systems and peripheral tissues including heart, lung, pancreas, adrenal gland, gonads, and gastrointestinal tract as well as immune cells and lymphatic system.[5,103] PACAP and VIP have critical roles as neurotransmitters, vasodilators including perfusion and hypoxia regulators, and immune and nociception modulators. They have key roles in blood vessels in the CNS[84] and VIP is associated with maintaining functional integrity of the BBB.[83] PACAP and VIP influence regulatory T cell (Treg)[29] and other immune functions. Their role as anti-inflammatory modulators is of particular interest[89] considering the implications for loss of immune and inflammatory regulation should they fail, for example through autoimmunity of their receptors. PACAP and VIP are potent activators of AC and thus have a key role in cAMP production.

Virchow–Robin spaces (VRS) are perivascular compartments surrounding small vessels within the CNS which contribute to BBB and BSB integrity and modulate immune responses.[90] VRS contain microglia and these cells are known to be influenced by PACAP and VIP in immunoregulation.[8] Autoimmunity of these VNs or their receptors would be expected to affect BBB/BSB and VRS function and therefore may contribute to the etiology of neuropsychiatric-related neurodegenerative and other conditions by affecting CNS and immunological homeostasis.

Autoimmunity as an etiology in MS, ALS, PD, and CFS remains controversial. While autoimmunity is reasonably well established in MS,[88] an autoimmune etiology in PD,[85] which may involve the BBB,[86] and ALS[87] is only just emerging. Autoimmunity affecting PACAP and VIP has been postulated as a possible contributing factor in MS and ALS,[79] PD[80] and CFS.[81]

Role of PACAP and VIP in the blood–brain/blood–spinal barrier

The multiple functions of PACAP and VIP impact on maintaining BBB/BSB function. Moreover PACAP and VIP have specific and complex roles in the CNS including high level neurological functioning such as memory and learning.[6] PACAP and VIP have a well described neuroprotective role[7] as well as being inflammatory mediators in microglial activation,[8] therefore their failure may be linked to neuroinflammatory and other disease processes in the BBB/BSB and the CNS.

PACAP and VIP exert potent effects in metabolism as they have a vital role in cAMP production and regulation through AC activation. Immunological dysregulation of vital biochemical and/or epigenetic mechanisms affecting PACAP and VIP resulting in down-regulation of cAMP are possible pathways by which disease entities become manifest. Their role along with other neurotrophic factors,[9] in maintaining cAMP levels[10] are important for maintaining the integrity of the BBB and the BSB.

Conditions such as MS, PD, and ALS are increasingly being investigated for possible disruption of the BBB or the BSB.[11–13] Zlokovic[107] asserts BBB breakdown due to the disruption of the tight junctions may initiate and or contribute to a "vicious circle" of disease process resulting in progression synaptic and neuronal dysfunction and loss in disorders including PD, ALS, and MS. The BBB and the BSB are the main barriers between the brain and spinal cord parenchyma and the intravascular compartment and are therefore important in keeping immune hydrophilic macromolecules and neuroactive hormones from interfering with brain and spinal neurological processes. Generally the CNS parenchyma does not express MHCII molecules consequently the CNS has long been regarded as an immune privileged site. However certain cells such as pericytes and microglia within the BBB and BSB

are able to express MHCII molecules on activation and initiate T helper cell (Th4) immunological responses.[66] PACAP and VIP suppress the expression of MHCII molecules[102] and this likely has an important role in maintaining the immune-protected status of the CNS through maintaining the integrity of the BBB/BSB. Disruption of BBB and BSB are well known in certain pathological states, for example, injury and inflammation and this disruption may involve VRS.

VRS contain interstitial fluid and, while some functional contact with sub-arachnoid spaces may occur for solute exchange, there is doubt whether they contain CSF.[14] VRS have important connections with lymphatic drainage of the head and neck[15] as well as having intricate pial relations and providing a surface for activity of neuropeptides, hormones and cytokines. Pial cells may have a role in protecting the brain from exogenous catecholamines[16] and VRS may have a complex role in leukocyte recruitment across, and maintenance of the BBB.[17] They are a likely site for antigen presentation and antigen presenting cell (APC) engagement and MHCII recognition. Perivascular microglia in the VRS undertake APC recognition in the CNS.[18] Hence it is possible CNS inflammation may be initiated within the CNS and not in the periphery alone. As PACAP and VIP and their receptors are located throughout the CNS parenchyma and vasculature they not only serve a role in immunomodulation they could also serve as antigens which might be subject to autoimmunity. Whether VN- or VN receptor-activated autoimmune Th cells arise from the periphery and undertake autoimmune processes in CNS and other sites is unknown and is an important question for further research.

PACAP and VIP are known to have neuroprotective effects through hypoxia protection on passage through the BBB[19] via a transport mechanism which enables the intact peptides to enter the parenchymal space of the brain.[20] Additionally PACAP and VIP have protective effects on neurons and glial cells.[21] These VNs therefore may have a significant role in blood BBB/BSB function and likely assist in immune regulation of VRS in the brain and spinal cord. The present paper asserts that, in view of the many vital roles of PACAP and VIP in CNS neuroregulatory and immunological function including BBB function, autoimmunity to these VNs or their receptors will have significant effects on homeostasis possibly resulting in neuropsychiatric symptoms.

PACAP and VIP in neuroimmunological dysregulation

PACAP and VIP exert antiinflammatory activities and loss of their function, for example through autoimmune compromise, could become manifest as unmodulated activation of immune responses. Hence effects of VN autoimmunity may extend beyond the BBB and BSB and a complex multisystem explanation for certain neuropsychiatric fatigue-related conditions is possible.

PACAP and VIP exert influence over inflammatory control mechanisms including influencing Th1 to Th2 shift and suppression of proinflammatory activity through the PKA/cAMP pathway.[23] For example tumor necrosis factor alpha (TNF-α) has a reciprocal modulating relationship with cAMP in vascular dysfunction involving endothelial cells[24] and this may prove to be an analogous mechanism in VRS. Implications for the BBB/BSB functioning are important as the VRS has been identified as a location for immunoreactive lymphocytes in penetration of neuronal parenchyma.[25] Also VIP has been identified in connection with neuronal function and VRS, which suggests that VIP may have a regulatory function associated with vasodilatation.[26] We assert that important immunoregulation occurs in perivascular spaces and VRS and that this may involve PACAP/VIP regulation. Many regulatory and antiinflammatory functions of PACAP and VIP are dependent on the Th2-directed cytokines[93] eg, interleukin-10 (IL-10) and IL-4 and these could be compromised in PACAP/VIP failure. Moreover 'leakiness' in the BBB and BSB may initiate, or encourage development of relapses in, neurological conditions such as MS.[27]

Regulatory T cells (Tregs) function to control autoreactive T cells in the periphery[28] and possibly within the brain parenchyma. Treg function is substantially influenced by these VNs[29] and they may also have influence over Th17 direction.[30] Loss of Treg function in VRS will therefore have significant implications for inflammatory control. Moreover Th17 development occurs under IL-6 and transforming growth factor beta (TGF-β) influence[31] and this may be a key switching point from a protective Treg phenotype to an autoimmune Th17 phenotype. Certainly Th1 and Th17 ratios are important in brain and spinal inflammation regulation.[32] Further, autoreactive Th cells within the CNS are likely to be critical determinants of disease development.

Interestingly a number of seemingly unrelated disorders may be implicated in postulated PACAP/VIP autoimmune pathology. For example, Crohn's disease (CD) and MS are inflammatory disorders with known Th1-directed cytokines and loss of Foxp3 Treg function.[33,34] In one study in MS patients Treg numbers were unchanged but their function may have been diminished.[92] As VRS have important roles in controlling macrophage and perivascular infiltrates in MS,[35] VRS immunological function in relation to VNs becomes

of considerable interest. Complement-fixing myelinolytic antigens have been identified in the VRS in early MS,[36] which indicates the possible involvement of VRS in immune activity. However autoimmunity directed at VN guanine nucleotide protein-coupled receptors (GPCRs) is currently unproven and loss-of-function autoimmunity to GPCRs generally is not well documented,[22] although parallels exist with other conditions eg, Sjogren's syndrome which has T cell and/or B cell antibody targeting of acetylcholine GPCRs.[37]

The role of PACAP and VIP in linking the innate and acquired immune systems[38] suggests there would be significant effects on immunological and neurological homeostasis if they are compromised and include their complex influences on inflammatory regulation involving microglia.[105] As noted above, macrophages and microglia in VRS express MHC class II molecules, albeit in low levels constitutively, and interact with lymphocytes from the blood in initiating and promoting immune responses to foreign antigens in the brain. However not all inflammatory events within the CNS lead to MS.[104] Nevertheless, sites deficient in the BBB include the subarachnoid space and pial surface, and circumventricular organs may be more prone to macromolecule penetration of CSF and this may have implications regarding autoimmune dysfunction within the CNS.[39] Collections of macrophages may occur in VRS following trauma in a pro-inflammatory context[40] and pathological dilatation of VRS may occur from a variety of causes including ischemia.[41] These pathological features rely on PACAP/VIP protection to ameliorate their effects.

The distribution of VRS in anatomically specific locations implies that compromise of VRS will affect the functions of those anatomical locations. For example VRS located in the nucleus tractus solitarius in the dorsal medulla oblongata suggests that their compromise may have a role in impairing viscerosensory and autonomic functions.[42] Capillary diversity within the subfornical organ (SFO) and area postrema (AP) may function as low-resistance pathways for the rapid dispersion of blood-borne hormones inside their organ boundaries and this may have a role in regulation of blood pressure and body fluids.[43] Hence these functions linked to VRS microanatomy may be particularly susceptible to PACAP/VIP compromise.

Vascular compromise within the CNS, possibly as a result of PACAP/VIP compromise, may give rise to features consistent with certain forms of dementia. Fronto-temporal dementia (FTD) is a neurodegenerative disease in which a vascular component is suggested and immunoreactivity of Bax, a proapoptotic protein regulated in part by PACAP/VIP in astrocytes, suggests a role for autoimmunity in the pathology of FTD.[44] Astrogliosis in FTD corresponds with SPECT hypoperfusion, suggesting that astrocyte disruption may be related to disturbances of cerebral perfusion in FTD.[45] Cognitive dysfunction is associated with reduced cerebral blood flow in different types of dementia.[46] Moreover VRS dilatation associated with microvessel abnormality may contribute to the diagnosis of vascular dementias.[47] Changes in social behavior occur in cerebrovascular comprise and may result from an FTD-like syndrome.[48] Similarly, reduction in cortical blood flow has been identified in CFS patients;[49,50] however these findings were not replicated in a study of twins with CFS.[51] FTD however is recognized in ALS.[91]

Other mechanisms such as water channel function are influenced by PACAP/VIP. The astroglial water channel aquaporin (AQP4) is essential for the maintenance of BBB integrity.[52] Antibodies to AQP4 have a highly specific role in neuromyelitis optica (NMO) and characteristically bind to cerebral microvessels, pia mater and VRSs.[53] Secretin is important for other aquaporin expression via vasopressin, secretin receptor-null mice for example have reduced renal expression of AQP2 and AQP4.[54] Thus VIP impairment may influence water channel function. Additionally an association with VRS has been identified supporting the view that cortical nerve cells release VIP in the perivascular space during periods of activity and thus contribute to local vasodilatation associated with neuronal function. There is an important relationship whereby ATP over-expression has a down-regulatory impact on AQP4 expression.[55] Adenylate cyclase compromise could have an impact on ATP levels by failure to convert to cAMP, arguably maintaining elevated levels of ATP with adverse consequences for AQP4 function.

Treatment in PACAP/VIP postulated autoimmune neuropsychiatric fatigue-related disorders

Receptors for PACAP and VIP exhibit a number of sub-types which are located in specific anatomical locations. Autoimmunity may affect these different sub-types in different anatomical regions. Thus it may be possible that VN receptor subtypes specific for the substantia nigra (SN) may be involved in, for example, PD. PACAP activates receptors of PAC1R, VPAC1R, and VPAC2R families, and PACAP has been identified widely in the brain including the SN as well as in other organs and blood cells.[56] Moreover, PACAP has been shown to be effective in treatment in rat models of PD.[57] Both PACAP and VIP have neuroprotective effects in PD models by inhibiting the production of inflammatory mediators.[58] PACAP specifically protects

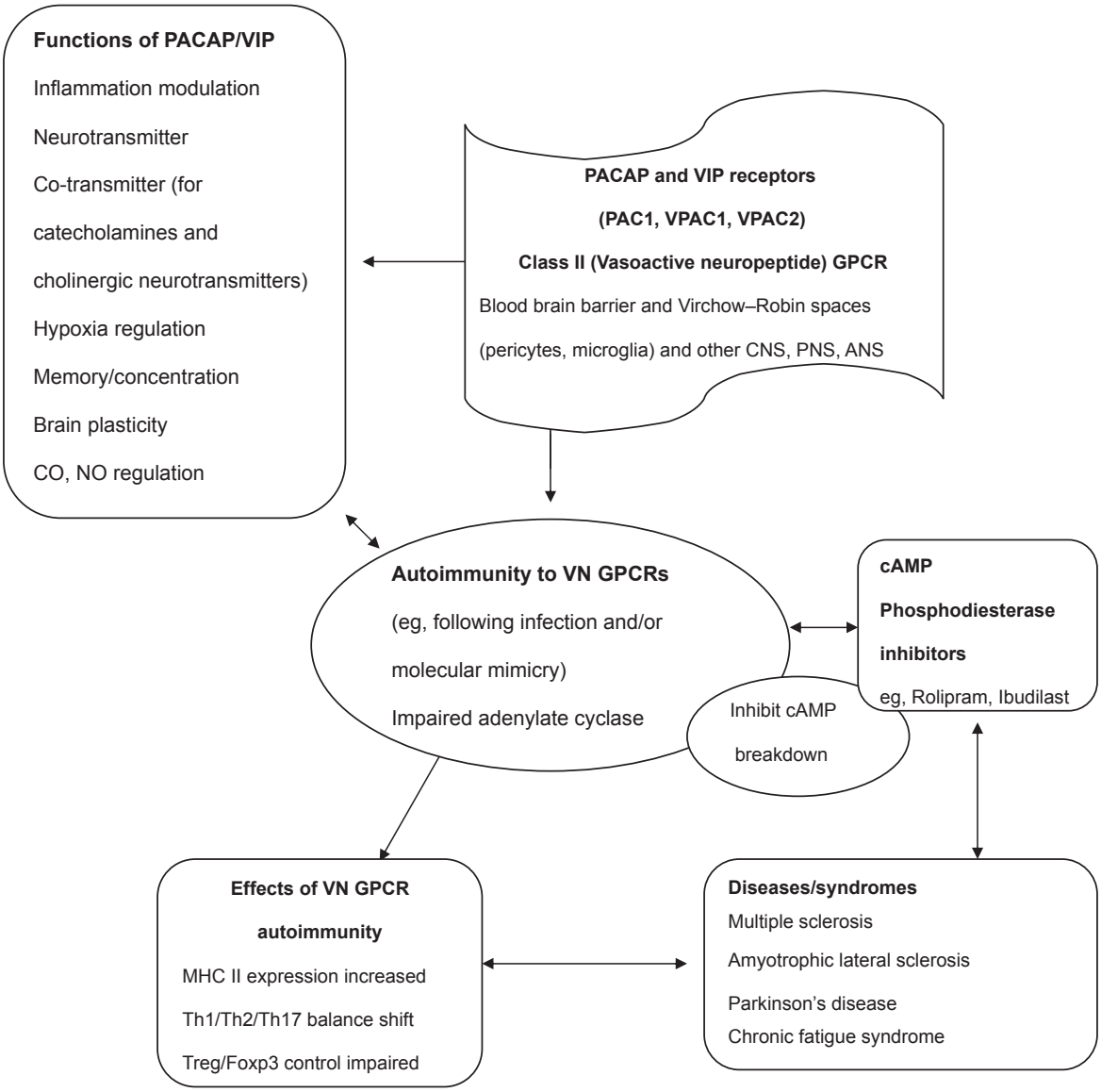

Figure 1 Postulated effect of PACAP/VIP autoimmunity on blood–brain barrier and CNS predisposing to neuroinflammation and/or neurodegeneration including potential treatment with phosphodiesterase inhibitors.

Abbreviations: ANS, autonomic nervous system; CO, carbon monoxide; CNS, central nervous system; GPCR, G protein-coupled receptors; NO, nitric oxide; PACAP, pituitary adenylate cyclase-activating polypeptide; VIP, vasoactive intestinal peptide; VN, vasoactive neuropeptides.

against the neurotoxicity induced by rotenone[59] as well as protecting against oxidative stress-induced apoptosis.[60] In a murine model for PD, Delgado and Ganea[61] note that the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) activates microglia and promotes dopaminergic neuronal loss. These pathologies are significantly decreased by VIP thus suggesting that VIP may be useful in treatment of neuropathological conditions such as PD. Conversely these findings might suggest that a defect in specific PACAP/VIP function could act adversely on SN cells and, perhaps in concert with other environmental factors, contribute to a clinical presentation consistent with PD.

PACAP/VIP are critical for neuronal survival. In a series of ALS patients, CSF levels of VIP were found to be significantly lower compared with controls.[62] VIP has demonstrated potent effects on neurite outgrowth in spinal cord cultures suggesting its use in treatment of ALS.[63] Interestingly Sun and colleagues[64] noted impaired VIP receptor (VPAC2) production in activated T cells in MS patients, suggesting transcription irregularity at promoter regions of the VPAC2 gene. These findings may be extremely important as more widespread impairment of VIP, if proven in patients with MS, may explain the manifestations associated with this condition. Also, in another paradigm, oligodendrocyte

apoptosis is suggested as the earliest change leading to MS. Tissue injury may be amplified over months or years as a result of oligodendrocyte cell death associated with myelin phagocytosis initiating local macrophage scavenger activity.[65] This paradigm is consistent with PACAP/VIP failure.

There are significant treatment implications from this VN autoimmune hypothesis. As noted above, PACAP and VIP exert potent effects in metabolism as they have a vital role in cAMP production and regulation through AC activation. PDEs metabolise cAMP as a means of feedback regulation of cAMP levels. PACAP/VIP compromise could result in impaired AC activation and hence impaired cAMP production. Thus phosphodiesterase inhibitors (PDEIs), novel therapeutic substances used to promote cAMP levels[106] may have a role in treatment of VN autoimmune conditions. PDEIs have been identified as a mechanism to modulate neuronal activity in psychiatric and neurodegenerative disorders.[94]

Drugs such as rolipram, a phosphodiesterase type 4 inhibitor, activate cAMP-response element binding proteins (CREB) signalling as well as enhancing cAMP levels through impeding cAMP catabolism.[67] Imipramine also appears to have a key role in cAMP metabolism and therefore may have a role in combination drug therapy[68,69] as this pathway may share features with activity of PACAP and VIP as cAMP enhancers.

Rolipram was developed as an antidepression drug but has been found to have antiinflammatory and immunoregulatory activities.[70,71] Mediation of rolipram's antiinflammatory effects is suggested through modulation of antigen presenting functions of dendritic cells and lowering of MHC II expression. An IL-10-dependent mechanism is suggested in vitro[72] but an IL-10-independent mechanism is suggested in vivo.[77] Mediation of autoimmune protection in islet cells is associated with suppression of inducible nitric oxide synthase (iNOS) mRNA[73] and suppression of macrophage activation along with NO suppression is noted in mouse peritoneal macrophages.[74] Perhaps contrarily, NO generation might be a contributing factor to the therapeutic benefit in EAE achieved by Rolipram in rats.[78] However there is evidence that rolipram stabilises the endothelial junctions of the BBB/BSB to reduce permeability to inflammatory cells and reduce EAE severity.[96] The efficacy of PDEIs in MS is being considered and clinical trials are indicated.[97] Models of PD[98–100] and ALS[101] also suggest a role for PDEIs in treatment. Theoretically some of the pathology of these diseases may be reversible with PDEIs. Experimental models in EAE, for example, show promising results.[95]

Rolipram has a protective effect in experimental autoimmune neuritis associated with downregulation of interferon-gamma (IFN-γ) and inflammatory chemokines as well as upregulation of IL-4 in the PNS.[75] Unfortunately side-effects such as nausea, vomiting, and headache are reported suggesting the need for less side effect-inducing analogues in therapy[76] and continuous administration may be necessary to sustain its therapeutic effect.[77] However PDEIs have proven to be of benefit in vitro and clinical trials in humans, particularly using more recent generation drugs, may be considered provided side effects are not a barrier.[82]

Conclusion

Certain neurological fatigue-related conditions such as MS, ALS, PD, and CFS often present with fatigue and other neuropsychiatric symptoms including memory and concentration loss, emotional lability, and confusion. Whether these conditions reflect related neuroinflammatory and neurodegenerative molecular pathology is speculative. However multisystem involvement including cerebrospinal effects of these conditions could be explained in part through PACAP and VIP compromise. In particular, cerebrovascular and spinovascular effects at the ultramicroscopic level involving BBB/BSB may contribute to these disorders. Compromise of PACAP and VIP or their receptors may have a role and should be the subject of further research. Evidence for compromise of these VN receptors at different levels including VN receptor mRNA, protein transcription, cellular migration and trafficking, cell membrane localization and possible antibody or T cell targeting may provide an explanation for some of the etiology of these disorders. Alternatively, antagonist short-form variants of VNs themselves may exist through faulty protein manufacture and produce a similar clinical picture.

If PACAP and VIP do indeed fail, through autoimmunity of their receptors, MHCII molecules usually expressed at low levels in BBB, eg, in microglia/pericytes, and normally kept in check by PACAP and VIP, may be at risk of becoming overexpressed. This may be the initiating event for BBB/BSB breakdown and subsequent neuroinflammation and/or neurodegeneration in MS, PD, and ALS. Why CFS does not proceed to result in further demonstrable neurodegenerative pathology is not clear under this model although confirmatory studies have not been done to date. The possibility that PACAP/VIP failure also leads to Treg impairment, possibly through impaired Foxp3 expression, is also yet to be investigated. Treg impairment has been described in MS patients suggesting possible PACAP/VIP failure.

PDEs metabolize cAMP and serve to regulate cAMP levels. PACAP/VIP compromise will result in impaired AC activation and hence impaired cAMP production. Noting the cAMP-protecting effects of PDEIs there is evidence to suggest PDEIs may have a role in treatment for certain neuropsychiatric fatigue-related conditions.

Disclosure

The authors report no conflicts of interest in this work.

References

1. Ghaffar O, Feinstein A. The neuropsychiatry of multiple sclerosis: a review of recent developments. *Curr Opin Psychiatry.* 2007;20:278–85.
2. Galvin JE. Cognitive change in Parkinson disease. *Alzheimer Dis Assoc Disord*. 2006;20:302–10.
3. Phukan J, Pender NP, Hardiman O. Cognitive impairment in amyotrophic lateral sclerosis. *Lancet Neurol*. 2007;6:994–1003.
4. Michiels V, Cluydts R. Neuropsychological functioning in chronic fatigue syndrome: a review. *Acta Psychiatr Scand*. 2001;103:84–93.
5. Vaudry D, Gonzalez BJ, Basille M, et al. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol Rev*. 2000;52:269–324.
6. Zhou CJ, Shioda S, Yada T, et al. PACAP and its receptors exert pleiotropic effects in the nervous system by activating multiple signalling pathways. *Curr Protein Pept Sci*. 2002;3:423–39.
7. Brenneman DE. Neuroprotection: a comparative view of vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide. *Peptides*. 2007;28:1720–6.
8. Delgado M, Leceta J, Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit the production of inflammatory mediators by activated microglia. *J Leukoc Biol*. 2003;73:155–64.
9. Igarashi Y, Utsumi H, Chiba H, et al. Glial cell line-derived neurotrophic factor induces barrier function of endothelial cell forming the blood-brain barrier. *Biochem Biophys Res Commun*. 1999;261:108–12.
10. Baranczyk-Kuzma A, Audus KL, Guillot FL, et al. Effects of selected vasoactive substances on adenylate cyclase activity in brain, isolated brain microvessels and primary cultures of brain microvessel endothelial cells. *Neurochem Res*. 1992;17:209–14.
11. Correale J, Villa A. The blood-brain-barrier in multiple sclerosis: functional roles and therapeutic targeting. *Autoimmunity*. 2007;40:148–60.
12. Rite I, Machado A, Cano J, et al. Blood-brain barrier disruption induces in vivo degeneration of nigral dopaminergic neurons. *J Neurochem*. 2007;101:1567–82.
13. Garbuzova-Davis S, Haller E, Saporta S, et al. Ultrastructure of blood-brain barrier and blood-spinal cord barrier in SOD1 mice modelling ALS. *Brain Res*. 2007;1157:126–37.
14. Ozturk MH, Aydinogoz U. Comparison of MR signal intensities of cerebral perivascular (Virchow–Robin) and subarachnoid spaces. *Comput Assist Tomogr*. 2002;26:902–4.
15. Esiri MM, Gay D. Immunological and neuropathological significance of the Virchow–Robin space. *J Neurol Sci*. 1990;100(1–2):3–8.
16. Zhang ET, Inman CB, Weller RO. Interrelationships of the pia mater and the perivascular (Virchow–Robin) spaces in the human cerebrum. *J Anat*. 1990;170:111–23.
17. Bechmann I, Galea I, Perry VH. What is the blood-brain barrier (not)? *Trends Immunol*. 2007;28:5–11.
18. Becher B, Bechmann I, Greter M. Antigen presentation in autoimmunity and CNS inflammation: how T lymphocytes recognise the brain. *J Mol Med*. 2006;84:532–43.
19. Takeda N, Murozono M, Watanabe S, et al. Neuroprotective effects of novel derivatives of vasoactive intestinal peptide and pituitary adenylate cyclase-activating peptide in two brain ischemic models on mice. *Masui*. 2005;54:240–8.
20. Banks WA, Uchida D, Arimura A, et al. Transport of pituitary adenylate cyclase-activating polypeptide across the blood-brain barrier and the prevention of ischemia-induced death of hippocampal neurons. *Ann N Y Acad Sci*. 1996;805:270–7.
21. Masmoudi-Kouki O, Gandolfo P, Castel H, et al. Role of PACAP and VIP in astroglial functions. *Peptides*. 2007;28:1753–60.
22. Vauquelin G, von Mentzer B. 2007. *G-protein coupled receptors.* Molecular Pharmacology. New Jersey: Wiley.
23. Delgado M, Abad C, Martinez C, et al. PACAP in immunity and inflammation. *Ann N Y Acad Sci*. 2003;992:141–57.
24. Koga S, Morris S, Ogawa S, et al. TNF modulates endothelial properties by decreasing cAMP. *Am J Physiol*. 1995;268:C1104–13.
25. Tomimoto H, Akiguchi I, Akiyama H, et al. T-cell infiltration and expression of MHC class II antigen by macrophages and microglia in a heterogeneous group in leukoencephalopathy. *Am J Pathol*. 1993;143:579–86.
26. Zhang ET, Mikkelsen JD, Fahrenkrug J, et al. Prepro-vasoactive intestinal polypeptide-derived peptide sequences in cerebral blood vessels of rats: on the functional anatomy of metabolic autoregulation. *J Cereb Blood Flow Metab*. 1991;11:932–8.
27. Odoardi F, Kawakami N, Klinkert WE, et al. Blood-borne soluble protein antigen intensifies T cell activation in autoimmune CNS lesions and exacerbates clinical disease. *Proc Natl Acad Sci U S A*. 2007;104:18625–30.
28. Romagnani S. Regulation of the T cell response. *Clin Exp Allergy*. 2006;36:1357–66.
29. Delgado M, Chorny A, Gonzalez-Rey E, et al. Vasoactive intestinal peptide generates CD4+CD25+ regulatory T cells in vivo. *J Leukoc Biol*. 2005;78:1327–38.
30. Leceta J, Gomariz RP, Martinez C, et al. Vasoactive intestinal peptide regulates Th17 function in autoimmune inflammation. *Neuroimmunomodulation*. 2007;14(3–4):134–8.
31. Oukka M. Interplay between pathogenic Th17 and regulatory T cells. *Ann Rheum Dis*. 2007;66:S87–90.
32. Stromnes IM, Cerretti LM, Liggitt D, et al. Differential regulation of central nervous system autoimmunity by T(H)1 and T(H)17 cells. *Nat Med*. 2008;14:337–42.
33. Ricciardelli I, Lindey KJ, Londie M, et al. Anti tumour necrosis-alpha therapy increases the number of FOXP3 (+) regulatory T cells in children affected by Crohn's disease. *Immunology*. 2008;125:178–83.
34. Vandenbark AA, Culbertson NE, Bartholomew RM, et al. Therapeutic vaccination with trivalent T cell receptor (TCR) peptide vaccine restores deficient FoxP3 expression and TCR recognition in subjects with multiple sclerosis. *Immunology*. 2008;123:66–78.
35. van Horssen J, Bo L, Vos CM, et al. Basement membrane proteins in multiple sclerosis-associated inflammatory cuffs: potential role influx and transport of leukocytes. *J Neuropath Exp Neurol*. 2005;64:722–9.
36. Gay FW. Early cellular events in multiple sclerosis. Intimations of an extrinsic myelinolytic antigen. *Clin Neurol Neurosurg*. 2006;108:234–40.
37. Gordon TP, Bolstad AI, Rischumeller M, et al. Autoantibodies in primary Sjogren's syndrome: new insights into mechanisms of autoantibody diversification and disease pathogenesis. *Autoimmunity*. 2001;34:123–32.
38. Delgado M, Ganea D. Inhibition of IFN-gamma-induced janus kinase-1-STAT1 activation in macrophages by vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *J Immunol*. 2000;165:3051–7.
39. Broadwell RD, Sofroniew MV. Serum proteins bypass the blood-brain fluid barriers for extracellular entry to the central nervous system. *Exp Neurol*. 1993;120:245–63.
40. Mueller CA, Schluesener HJ, Conard S, et al. Spinal cord injury induces lesional expression of the proinflammatory and antiangiogenic cytokine *EMAP II. J Neurotrauma*. 2003;20:1007–15.

41. Shiratori K, Mrowka M, Toussaint A, et al. Extreme unilateral widening of Virchow–Robin spaces: case report. *Neuroradiology*. 2002;44:990–2.
42. Gross PM, Wall KM, Pang JJ, et al. Microvascular specialization promoting rapid interstitial solute dispersion in nucleus tractus solitarius. *Am J Physiol*. 1990;259:R1131–8.
43. Gross PM. Morphology and physiology of capillary systems in subregions of the subfornical organ and area postrema. *Can J Physiol Pharmacol*. 1991;69:1010–25.
44. Nichol KE, Kim R, Cotman CW. Bcl-2 family protein behaviour in frontotemporal dementia implies vascular involvement. *Neurology*. 2001;56(11 Suppl 4):S35–40.
45. Martin JA, Craft DK, Su JH, et al. Astrocytes degenerate in frontotemporal dementia: possible relation to hypoperfusion. *Neurobiol Aging*. 2001;22:195–207.
46. Osawa A, Maeshima S, Shimamoto Y, et al. Relationship between cognitive function and regional cerebral blood flow in different types of dementia. *Disabil Rehabil*. 2004;26:739–45.
47. Patankar YF, Mitra D, Varma A, et al. Dilatation of the Virchow–Robin space is a sensitive indicator of cerebral microvascular disease: study in elderly patients with dementia. *AJNR Am J Neuroradiol*. 2005;26:1512–20.
48. Nakano S, Asada T, Yamashita F, et al. Relationship between antisocial behaviour and regional cerebral blood flow in frontotemporal dementia. *Neuroimage*. 2006;32:301–6.
49. Ichise M, Salit IE, Abbey SE, et al. Assessment of regional cerebral perfusion by 99Tcm-HMPAO SPECT in chronic fatigue syndrome. *Nuel Med Commun*. 1992;13:767–72.
50. Yoshiuchi K, Farakas J, Natelson BH. Patients with chronic fatigue syndrome have reduced absolute cortical blood flow. *Clin Physiol Funct Imaging*. 2006;26:83–6.
51. Lewis DH, Mayberg HS, Fischer ME, et al. Monozygotic twins discordant for chronic fatigue syndrome: regional cerebral blood flow SPECT. *Radiology*. 2001;219:766–73.
52. Zhou J, Kong H, Hua X, et al. Altered blood-brain barrier integrity in adult aquaporin-4 knockout mice. *Neuroreport*. 2008;19:1–5.
53. Jarius S, Paul F, Franciotta D, et al. Mechanisms of disease: aquaporin-4 antibodies in neuromyelitis optica. *Nat Clin Pract Neurol*. 2008;4:202–14.
54. Chu JY, Chung SC Lam AK, et al. Phenotypes developed in secretin receptor-null mice indicated a role for secretion in regulating renal water reabsorption. *Mol Cell Biol*. 2007;27:2499–511.
55. Lee M, Lee SJ, Choi HJ, et al. Regulation of AQP4 protein expression in rat brain astrocytes: role of P2X7 receptor activation. *Brain Res*. 2008;1195:1–11.
56. Kausz M, Murai Z, Arimura A, et al. Distribution of pituitary adenylate cyclase activating polypeptide (PACAP) immunoreactive elements in the brain stem of rats studied by immunohistochemistry. *Neurobiology (Bp)*. 1999;7:19–31.
57. Reglodi D, Tamas A, Lubics A, et al. Morphological and functional effects of PACAP in 6-hydroxydopamine-induced lesion of the substantia nigra in rats. *Regul Pept*. 2004;123(1–3):85–94.
58. Dejda A, Sokolowska P, Nowak JZ. Neuroprotective potential of three neuropeptides PACAP, VIP and PHI. *Pharmacol Rep*. 2005;57:307–20.
59. Wang G, Qi C, Fan GH, et al. PACAP protects neuronal differentiated PC12 cells against the neurotoxicity induced by a mitochondrial complex 1 inhibitor, rotenone. *FEBS Lett*. 2005;579:4005–11.
60. Vaudry D, Pamantung TF, Basille M, et al. PACAP protects cerebellar granule neurons against oxidative stress-induced apoptosis. *Eur J Neurosci*. 2002;15:1451–60.
61. Delgado M, Ganea D. Neuroprotective effect of vasoactive intestinal peptide (VIP) in a mouse model of Parkinson's disease by blocking microglial activation. *FASEB J*. 2003;17:944–6.
62. Werdelin L, Gjerris A, Boysen G, et al. Neuropeptides and neural cell adhesion molecule (NCAM) in CSF from patients with ALS. *Acta Neurol Scand*. 1989;79:177–81.
63. Iwasaki Y, Ikeda K, Ichikawa Y, et al. Vasoactive intestinal peptide influences neurite outgrowth in cultured rat spinal cord neurons. *Neurol Res*. 2001;23:851–4.
64. Sun W, Hong J, Zang YC, et al. Altered expression of vasoactive intestinal peptide receptors in T lymphocytes and aberrant Th1 immunity in multiple sclerosis. *Int Immunol*. 2006;18:1691–700.
65. Barnett MH, Sutton I. The pathology of multiple sclerosis: a paradigm shift. *Curr Opin Neurol*. 2006;19:242–7.
66. Balabanov R, Beaumont T, Dore-Duffy P. The role of central nervous system microvascular pericytes in activation of antigen-primed splenic T-lymphocytes. *J Neurosci Res*. 1999;55:578–87.
67. Conti AC, Blendy JA. Regulation of antidepressant activity by cAMP response element binding proteins. *Mol Neurobiol*. 2004;30:143–55.
68. Itoh T, Tokukura M, Abe K. Effects of rolipram, a phosphodiesterase 4 inhibitor, in combination with imipramine on depressive behaviour, CRE-binding activity and BDNF level in learned helplessness rats. *Eur J Pharmacol*. 2004;498(1–3):135–42.
69. Knuuttila JE, Toronen P, Castren E. Effects of antidepressant drug imipramine on gene expression in rat prefrontal cortex. *Neurochem Res*. 2004;29:1235–44.
70. Sommer N, Loschmann PA, Northoff GH, et al. The antidepressant rolipram suppresses cytokine production and prevents autoimmune encephalomyelitis. *Nat Med*. 1995;1:244–8.
71. Castro A, Jerez MJ, Gil C, et al. Cyclic nucleotide phosphodiesterases and their role in immunomodulatory responses: advances in the development of specific phosphodiesterase inhibitors. *Med Res Rev*. 2005;25:229–44.
72. Kambayashi T, Wallin RP, Ljunggren HG. cAMP-elevating agents suppress dendritic function. *J Leukoc Biol*. 2001;70:903–10.
73. Beshay E, Prud'homme GJ. Inhibitors of phosphodiesterase isoforms III or IV suppress islet-cell nitric oxide production. *Lab Invest*. 2001;81:1109–17.
74. Beshay E, Croze F, Prud'homme GJ. The phosphodiesterase inhibitors pentoxifylline and rolipram suppress macrophage activation and nitric oxide production in vitro and in vivo. *Clin Immunol*. 2001;98:272–9.
75. Abbas N, Zou LP, Pelidou SH, et al. Protective effect of Rolipram in experimental autoimmune neuritis: protection is associated with down-regulation of IFN-gamma and inflammatory chemokines as well as up-regulation of IL-4 in peripheral nervous system. *Autoimmunity*. 2000;32:93–9.
76. Dal Piaz V, Giovannoni MP. Phosphodiesterase 4 inhibitors, structurally unrelated to rolipram, as promising agents for the treatment of asthma and other pathologies. *Eur J Med Chem*. 2000;35:463–80.
77. Xu H, Strassmann G, Chan CC, et al. Protective effect of the type IV phosphodiesterase inhibitor rolipram in EAU: protection is independent of IL-10- inducing activity. *Invest Ophthalmol Vis Sci*. 1999;40:942–50.
78. Martinez I, Puerta C, Redondo C, et al. Type IV phosphodiesterase inhibition in experimental allergic encephalomyelitis of Lewis rats: sequential gene expression analysis of cytokines, adhesion molecules and the inducible nitric oxide synthase. *J Neurol Sci*. 1999;164:13–23.
79. Staines DR. Are multiple sclerosis and amyotrophic lateral sclerosis autoimmune disorders of endogenous vasoactive neuropeptides? *Med Hypotheses*. 2008;70:413–8.
80. Staines DR. Is Parkinson's disease an autoimmune disorder of endogenous vasoactive neuropeptides? *Med Hypotheses*. 2007;69:1208–11.
81. Staines DR. Postulated vasoactive neuropeptide autoimmunity in fatigue-related conditions: a brief review and hypothesis. *Clin Devel Immunol*. 2006;13:25–39.
82. Staines DR. Phosphodiesterase inhibitors may be indicated in the treatment of postulated vasoactive neuropeptide autoimmune fatigue-related disorders. *Med Hypotheses*. 2006;66:203–4
83. Benagiano V, Virgintino D, Maiorano E, et al. VIP-like immunoreactivity within neurons and perivascular neuronal processes of the human cerebral cortex. *Eur J Histochem*. 1996;40:53–6.
84. Henning RJ, Sawmiller DR. Vasoactive intestinal peptide: cardiovascular effects. *Cardiovasc Res*. 2001;49:27–37.
85. Monahan AJ, Warren M, Carvey PM. Neuroinflammation and peripheral immune infiltration in Parkinson's disease: an autoimmune hypothesis. *Cell Transplant*. 2008;17:363–72.

86. Desai BS, Monahan AJ, Carvey PM, et al. Blood-brain barrier pathology in Alzheimer's and Parkinson's disease: implications for drug therapy. *Cell Transplant*. 2007;16:285–99.
87. Niebroj-Dobosz I, Dziewulska D, Janik P. Auto-antibodies against proteins of spinal cord cells in cerebrospinal fluid of patients with amyotrophic lateral sclerosis (ALS). *Folia Neuropathol*. 2006;44:191–6.
88. Pender MP, Greer JM. Immunology of multiple sclerosis. *Curr Allergy Asthma Rep*. 2007;7:285–92.
89. Delgado M, Ganea D. Anti-inflammatory neuropeptides: A new class of endogenous immunoregulatory agents. *Brain Behav Immun*. Epub 2008 Jun 14.
90. Wuerfel J, Haertle M, Waiczies H, et al. Perivascular spaces – MRI marker of inflammatory activity in the brain? *Brain*. 2008;131:2332–40.
91. Strong MJ. The syndromes of frontotemporal dysfunction in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler*. Epub 2008 Aug 27.
92. Costantino CM, Baecher-Allan C, Hafler DA. Multiple sclerosis and regulatory T cells. *J Clin Immunol.* Epub 2008 Sep 2.
93. Gonzalez-Rey E, Varela N, Chorny A, et al. Therapeutical approaches of vasoactive intestinal peptide as a pleiotropic immunomodulator. *Curr Pharm Des*. 2007;13:1113–39.
94. Menniti FS, Faraci WS, Schmidt CJ. Phosphodiesterases in the CNS: targets for drug development. *Nat Rev Drug Discov*. 2006;5:660–70.
95. Paintlia AS, Paintlia MK, Singh I, et al. Combination therapy of lovastatin and rolipram provides neuroprotection and promotes neurorepair in inflammatory demyelination model of multiple sclerosis. *Glia*. Epub 2008 Aug 20.
96. Folcik VA, Smith T, O'Bryant S, et al. Treatment with BBB022A or rolipram stabilizes the blood-brain barrier in experimental autoimmune encephalomyelitis: an additional mechanism for the therapeutic effect of type IV phosphodiesterase inhibitors. *J Neuroimmunol*. 1999;97(1–2):119–28.
97. Dinter H. Phosphodiesterase type 4 inhibitors: potential in the treatment of multiple sclerosis? *BioDrugs*. 2000;13:87–94.
98. Weishaar RE, Cain MH, Bristol JA. A new generation of phosphodiesterase inhibitors: multiple molecular forms of phosphodiesterase and the potential for drug selectivity. *J Med Chem*. 1985;28:537–45.
99. Giorgi M, D'Angelo V, Esposito Z, et al. Lowered cAMP and cGMP signalling in the brain during levodopa-induced dyskinesias in hemiparkinsonian rats: new aspects in the pathogenetic mechanisms. *Eur J Neurosci*. 2008;28:941–50.
100. Yang L, Calingasan NY, Lorenzo BJ, et al. Attenuation of MPTP neurotoxicity by rolipram, a specific inhibitor of phosphodiesterase IV. *Exp Neurol*. 2008;211:311–4.
101. Tomimatsu N, Arakawa Y. Survival-promoting activity of pituitary adenylate cyclase-activating polypeptide in the presence of phosphodiesterase inhibitors on rat motoneurons in culture: cAMP-protein kinase A-mediated survival. *J Neurochem*. Epub 2008 Sep 18.
102. Lu J, Zheng MH, Yan J, et al. Effects of vasoactive intestinal peptide on phenotypic and functional maturation of dendritic cells. *Int Immunopharmacol*. 2008;8:1449–54.
103. Delgado M, Pozo D, Ganea D. The significance of vasoactive intestinal peptide in immunomodulation. *Pharmacol Rev*. 2004;56:249–90.
104. Carson MJ. Microglia as liaisons between the immune and central nervous systems: functional implications for multiple sclerosis. *Glia*. 2002;40:218–31.
105. Pocock JM, Kettenmann H. Neurotransmitter receptors on microglia. *Trends Neurosci*. 2007;30:527–35.
106. Bender AT, Beavo JA. Cyclic nucleotide phosphodieskrases: molecular regulation to clinical use. *Pharmacol Rev*. 2006;58:488–520.
107. Zlokovic BV. The blood–brain barrier in health and chronic neurodegenerative disorders. *Neuron*. 2008;57:178–201.