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When a trophic process turns toxic: Alzheimer's disease as an aberrant recapitulation of a developmental mechanism

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ABSTRACT

Here we review the idea that Alzheimer's disease (AD) results from aberrant activation of a normal developmental mechanism. This process operates in primarily vulnerable, subcortical nuclei with a distinguishing embryological provenance: the basal rather than the alar plate. All cells are dependent for growth on calcium influx yet these neurons retain a sensitivity to trophic factors into maturity. However, as the brain matures this action becomes detrimental such that the trophic process could turn toxic if triggered in adult brain, in retaliation to an initial insult. The signalling molecule driving this trophic-toxic mechanism is a 14mer peptide (T14) that acts on the alpha-7 receptor to enhance calcium entry, inducing excitotoxicity and proliferation of the receptor, perpetuating a feedforward cycle of neurodegeneration including production of beta-amyloid and p-tau. The T14 system has been previously unrecognised as a basic biological process, yet its pharmaceutical manipulation could have valuable clinical applications.

1. Introduction

The idea discussed here takes as its starting point two general shortcomings of conventional approaches to Alzheimer's Disease (AD). First, the rationale based on a malfunction of the generic cell, for example, APOE, Aβ expression, tau phosphorylation, or inflammation: whilst these events might form part of the later-stage clinical picture, they could be dysfunctions occurring in *any* neuron irrespective of its location, morphology, or neurochemical signature. By contrast we know that, early on, AD degeneration starts in a region-selective manner, where only certain cell groups are primarily vulnerable (Braak and Del Tredici, 2012). Second, pathological cellular features might form part of the eventual toxic cascade in AD, but their mere presence does *not* actually prompt any explanation why the neurodegenerative process has, in the first place, began.

Neurodegeneration does not automatically follow after brain injury nor stroke, and this should surely prompt the question: why not? Moreover, the literature (Theofilas et al., 2015; Attems et al., 2012; Woolf, 1996; Rossor, 1981) reveals a large number of reports identifying the primarily vulnerable cells in AD, i.e., those that degenerate first, as the interconnecting subcortical cell groups extending from the basal forebrain to Raphe nuclei, ventral tegmental area, substantia nigra, locus coeruleus and motor neurons. These vulnerable nuclei can be definitively differentiated from all other brain cells at a very early stage in life, by their embryological provenance: the basal rather than the alar plate (Woolf, 1996) (Fig. 1A).

This fundamental distinction, is apparent as early as 4 weeks gestation and surely suggests that the cells primarily prone to degeneration could well have very different basic properties from other brain areas. Such differentiating properties might in turn provide an important clue regarding the selective vulnerability of these key cells, and hence offer an eventual insight into the cause of AD. A key difference is that the basal plate derived cells retain their developmental potential into maturity, with an increased sensitivity to trophic agents not seen in the alar-plate derived populations (Woolf, 1996). This persistent capacity for growth, based on a normal developmental system controlled by feedback in the early stages of life, could provide a clue as to why, in adult life, such cells will now succumb to a continuing process of gradual cell loss.

The ultimate trigger for cell growth is the influx of calcium (Dickie et al., 1996) (Fig. 1B): however, in functional excess intracellular calcium can lead to excitotoxicity. Various factors determine whether calcium influx is ultimately beneficial or detrimental. Not only does amount, in turn related to duration of influx (Day and Greenfield, 2002, 2004) tip the balance from trophic to toxic but, less obviously, age can be crucial (Eimerl and Schramm, 1994).

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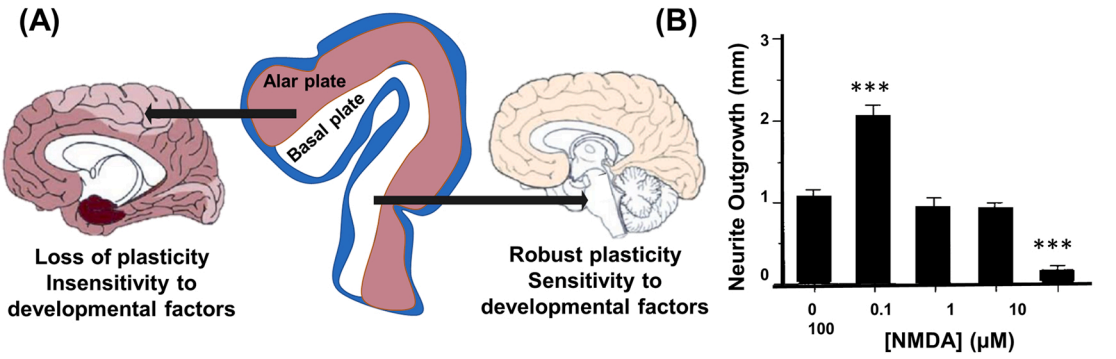

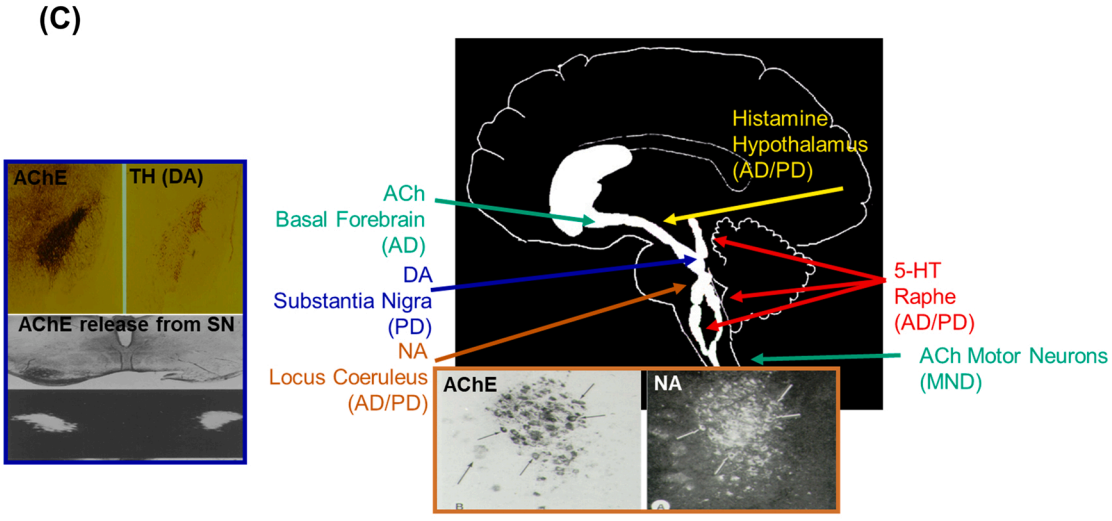

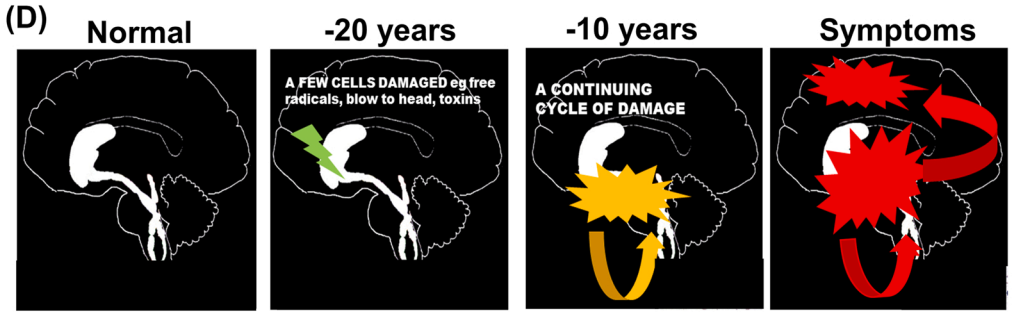

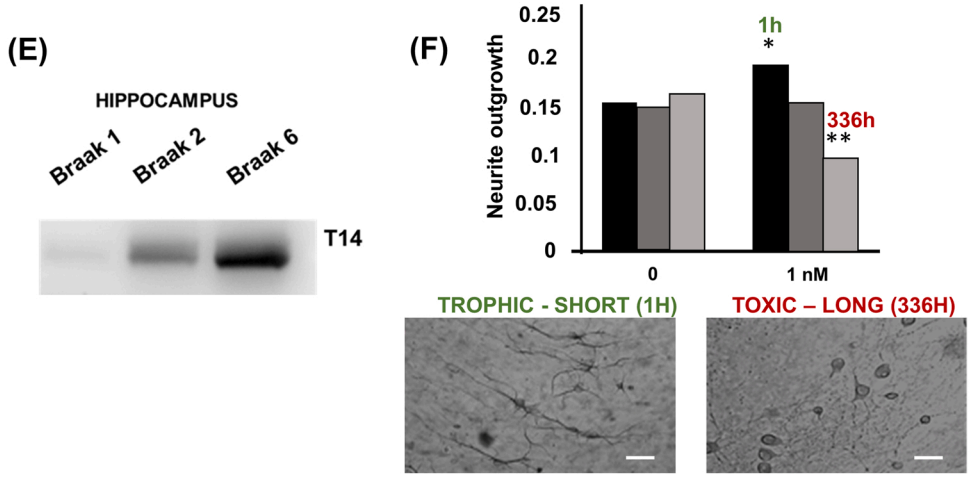

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Fig. 1. (A) Basic features differentiating basal from alar plate derived cells. Aspects relating to retention of developmental mechanism are shown in blue. (B) Neurite growth induced by NMDA via calcium influx in organotypic culture of rat dopamine neurons (Dickie et al., 1996). (C) Schematic of the areas comprising the iso-dendritic core and its association with neurodegenerative diseases independent of neurotransmitter, with staining of noradrenaline and acetylcholinesterase co-expressing in locus coeruleus (Albanese and Butcher, 1980) and Distribution and release of acetylcholinesterase in the substantia nigra (Llinas and Greenfield, 1987). (D) Schematic of the process of neurodegeneration involving T14 during the evolution of the neurodegeneration and appearance of the AD symptoms. (E) Expression of T14 in hippocampus of different Braak stages detected by Western Blot (Greenfield et al., 2022). (F) Effect of AChE-peptide on neurite outgrowth from MAP2-immunopositive neurones. Organotypic hippocampal cultures were exposed to the peptide variants for 1, 24 and 336 h. Below, MAP2 immunochemistry images of representative hippocampal organotypic cultures. Cultures treated with AChE-peptide (10 μM) showed a marked decrease in neurite outgrowth as compared to control cultures. Scale bar= 50 μm.

Accordingly, if any of the basal plate derived cell groups are damaged for whatever reason, the outcome will be different from that of an equivalent insult to their alar plate derived counterparts. Unlike in the rest of the brain, this damage will trigger in retaliation compensatory cell growth selectively retained by these particular cells into adulthood. However, the problem is that now the brain is fully mature the amount of calcium influx that would normally have enabled cell growth during development, will be excitotoxic in the context of the older brain: hence more cells will die and the cycle of cell loss will be perpetuated and amplified. The erstwhile *feedback* system has now become one of *feed-forward* toxicity that we recognise as neurodegeneration. As the damage becomes more extensive it will spread beyond the initial cell group to the adjacent interconnecting nuclei enhancing the ensuing malfunction, and accounting for the frequent observation of a co-pathology of AD with Parkinson's disease (Theofilas et al., 2015) (Fig. 1C, D). Moreover, such redundancy would account more generally for the lengthy time window that has always bedevilled the treatment of AD, from the onset of cell loss to the appearance of cognitive impairment. Nonetheless dysfunctions associated with the brainstem-midbrain nuclei might be apparent earlier, ie problems arising from deficiencies in NA and/or 5-HT, in turn linked to depression, as indeed reported (Botto et al., 2022). It would only be when the damage progressing within the basal plate nuclei was sufficiently extensive to affect the target areas of hippocampus and cortex, linked to more sophisticated functions of memory and language, that a diagnosis of AD would be possible. The next step is to identify the pivotal signalling molecule driving this inappropriately activated developmental mechanism in these vulnerable cells.

2. The pivotal signalling molecule in the vulnerable cells

If the cells prone to AD degeneration are characterised by an inappropriately activated signalling system for cell growth, it might be expected that they operate by means of a common transmitter system: but this is not the case. A range of diverse amines characterise and operate in the respective nuclei. However, irrespective of diverse transmitter systems, all of these cells do express a common molecule: the very familiar enzyme acetylcholinesterase (AChE) (Fig. 1C).

In addition to its traditional role in cholinergic transmission, it has been acknowledged for some 50 years that AChE is present in a widespread range of neurons and non-neuronal cells, albeit in the absence of its normal substrate ACh and the synthesising enzyme choline acetyltransferase (ChAT) (Albanese and Butcher, 1980). Although not a requirement for its familiar catalytic role, AChE is released into CSF (Appleyard et al., 1987; Greenfield et al., 1986), detectable locally from brain areas linked to neurodegeneration such as the substantia nigra, (Dally et al., 1996; Dally and Greenfield, 1994; Taylor et al., 1989) where its secretion can be visualised in real-time (Llinas and Greenfield, 1987) and monitored on-line (Taylor et al., 1989) in the freely moving animal (Taylor et al., 1990) (Fig. 1C). As a signalling molecule independent of cholinergic transmission, AChE has electrophysiological (Webb and Greenfield, 1992; Webb et al., 1996) biochemical/pharmacological (Dajas et al., 1993), and behavioural (Weston and Greenfield, 1985) actions which in all the studies cited cannot be replicated by another enzyme (butyrycholinesterase) which will nonetheless also hydrolyse ACh.

Could this non-classical, non-enzymatic function of AChE, featuring as it does in all the basal plate derived cells, have any effect as a trophic agent? AChE can indeed promote cell growth (Greenfield, 1996): it has a non-cholinergic, trophic action in organotypic cell cultures of rat midbrain (Day and Greenfield, 2002), via activation of calcium influx (Whyte and Greenfield, 2003). However, in higher doses, or for a protracted period of administration, the trophic action of AChE can turn toxic.

Since (i) AChE is present in all the cell populations primarily vulnerable in neurodegeneration, irrespective of the diverse transmitter systems and (ii) calcium influx can switch from trophic to toxic effects dependent on age, then (iii) neurodegeneration could be a brain region-selective, aberrant form of development, with 'non-cholinergic' AChE as the key signalling molecule. However, it is unlikely that a protein of such size could be a signalling molecule in its entirety, but much more feasible that only a smaller part, independent of its enzymatic site, would be necessary for the effects seen both in vitro and in vivo.

Two separate labs have each reported the prevalence in embryonic and early postnatal development of a monomeric form of AChE (G1) (Arendt et al., 1992; Garcia-Ayllon et al., 2010). In AD brain and blood, this single catalytic subunit, the monomer G1, is far more dominant and recapitulates the profile found mainly in developing brain. Not only do these studies suggest that neurodegeneration could somehow be a reactivated form of a developmental process, but they also indicate that the oligomerization of AChE could be critical. The G4 form of AChE is dependent on disulphide bonds which include a 14mer peptide at the C terminus, with homology to Aβ (Greenfield and Vaux, 2002). The potentially bioactive active part of the AChE molecule has thus been identified as this peptide, 'T14′. In the normal mature brain, the vulnerable basal plate derived cells retain a distinct (Woolf, 1996) developmental growth mechanism in which the T14 molecule has the appropriate excitotoxic actions (Day and Greenfield, 2003) to be a central driver. If the basal plate derived cells are damaged, for example by free radicals, ischaemia or a blow to the head, the onset of neurodegeneration would be triggered by inappropriate mobilisation of the erstwhile trophic T14 system in the subcortical cell groups. The increasing cell loss triggers further mobilisation of the T14 process: within the basal plate derived cells continuing degeneration spread within subcortical regions without any cognitive impairment becoming apparent for some 10–20 years, until reaching hippocampus and cortex. As more cells attempt to mobilise the T14 system as compensation for cell death, it causes even more damage that eventually spreads to the hippocampus and cortex, when the characteristic memory loss and confusion become apparent (Fig. 1D). Changes in T14 are even detectable at the asymptomatic stages of AD (Braak stage I and II).

Once the peptide has been cleaved, various reports suggest the target for T14 is an allosteric site on the alpha-7 receptor (Greenfield et al., 2004) (Fig. 2A), which has a calcium permeability, and thus potential for mediating trophic-toxic effects, even greater than NMDA (Seguela et al., 1993). However, the most cogent reason for suspecting that this receptor might be the target for T14, is that it co-expresses effectively in lockstep with the appearance of AChE in the developing brain (Broide et al., 1996), even in areas where there is no cholinergic transmission. In fact, the alpha7 receptor, though classified as part of the nicotinic receptor family, can play a role in non-cholinergic neurons: choline, available from the diet, can act as an alternative primary ligand and hence confer complete independence from ACh systems (Alkondon

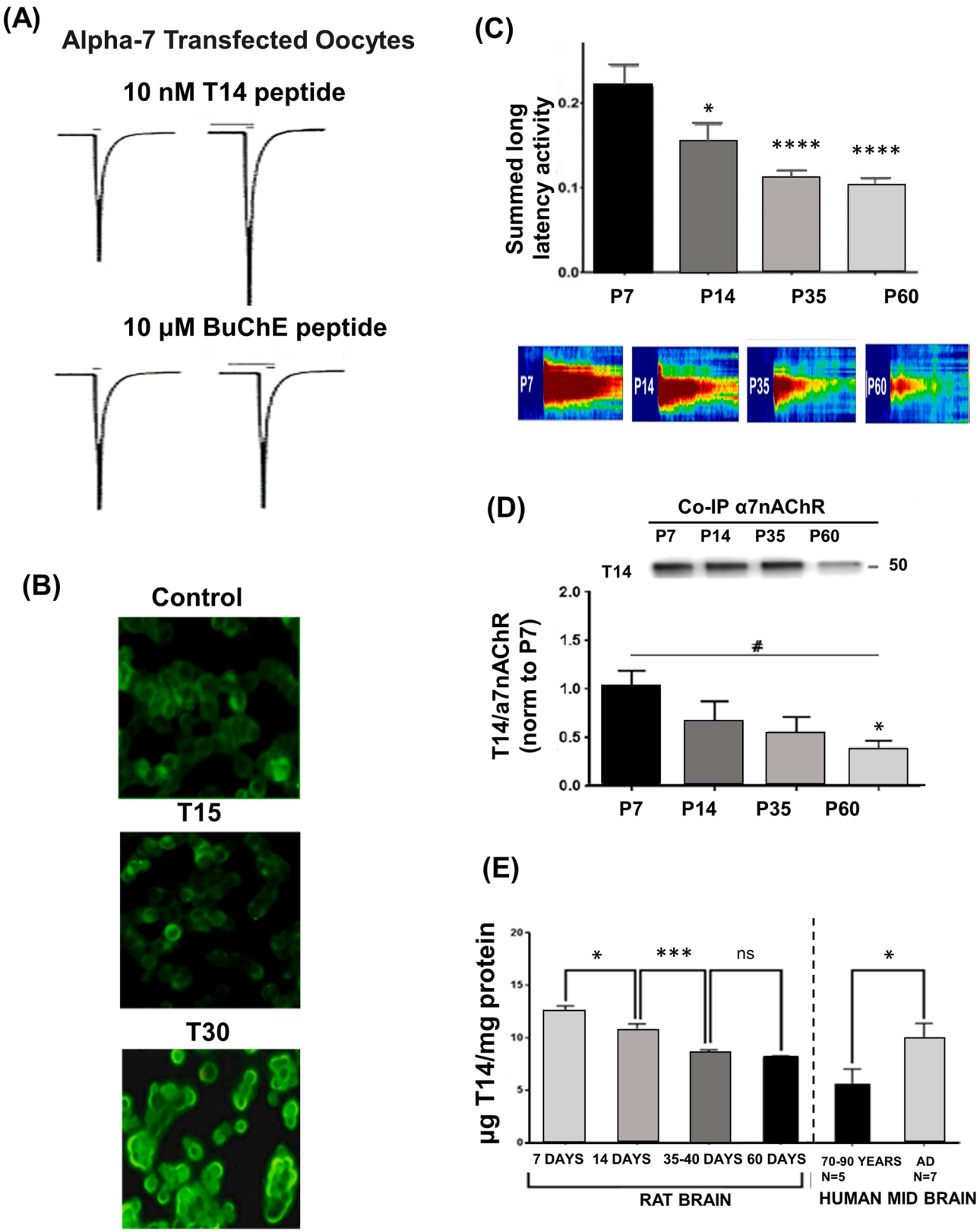

Fig. 2. (A) Potentiation of AChE currents by T14 using frog oocytes expressing the alpha7 nicotinic receptor (Greenfield et al., 2004). (B) Overexpression of the alpha7 nicotinic receptor (green) after T14 treatment for 24 h in GH4-ha7 cells (Bond et al., 2009). Levels of T14 in 4 different rat ages as detected in (C) ex vivo brain slices using optical imaging (Ferrati et al., 2018), (D) co-immunoprecipitation with its target using western blot (Brai et al., 2018) and (E) using ELISA in brain homogenate of rat and human midbrain (Garcia-Rates et al., 2016).

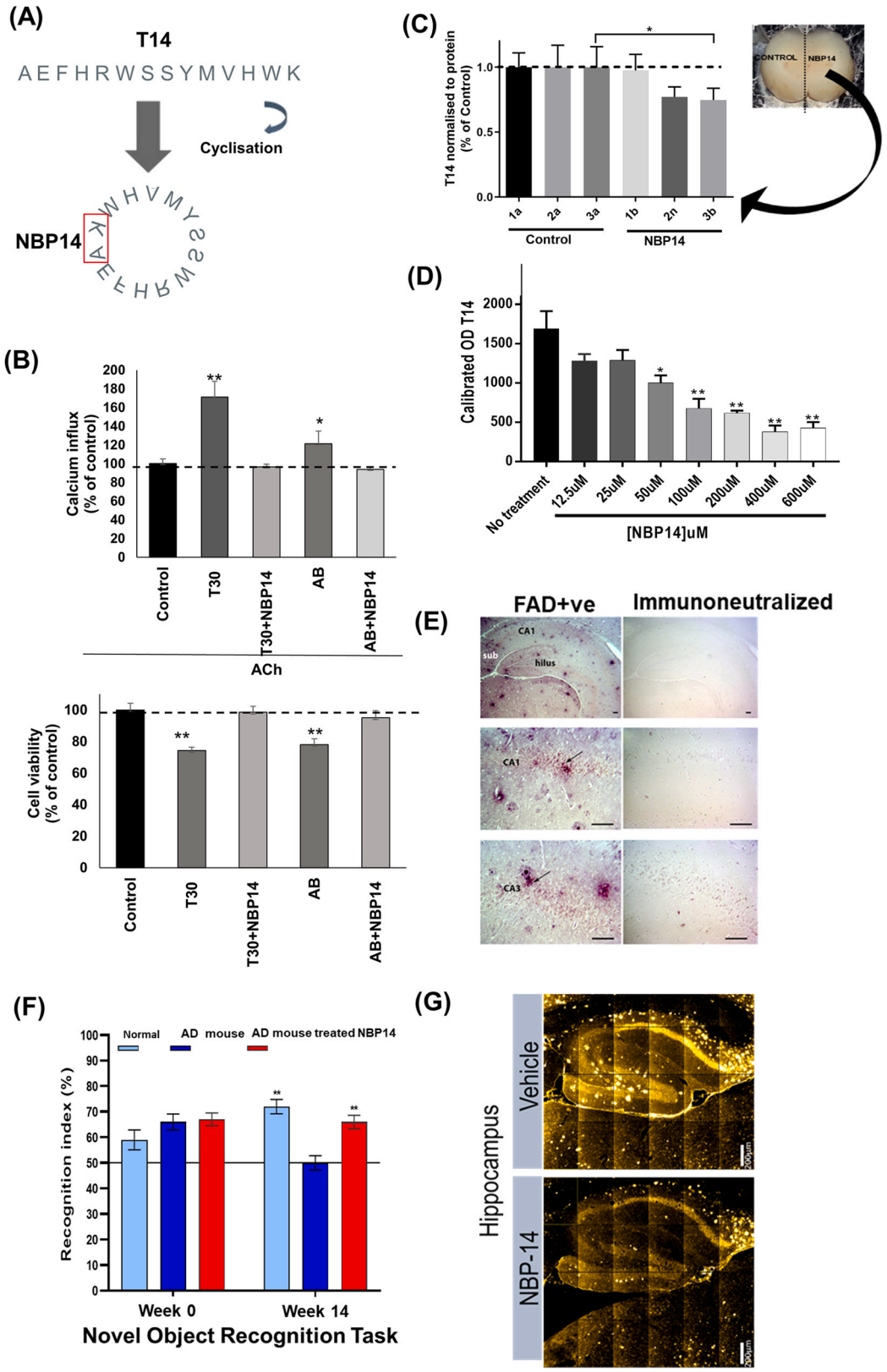

Fig. 3. Characterisation of NBP14. (A) Cyclisation of the T14 peptide becomes NBP14. (B) Effect of NBP14 against T14 and amyloid beta in PC12 cells using Calcium and cell viability read out (Garcia-Rates et al., 2016). (C) Effect of NBP14 in endogenous T14 detected by western blot in ex vivo rat brain slices (Brai et al., 2018). (D) Displacement of T14 from the alpha7 nicotinic receptor by NBP14 in human AD tissue (Greenfield et al., 2022, In Press). (E) Staining of T14 in the brain of 5XFAD AD model; immunoneutralization confirms the T14 staining (Greenfield et al., 2022, In Press). (F) Effect of intranasal NBP14 (after 6 and 14 weeks) in cognition (NOR) in 5XFAD animals. (G) Reduction of amyloid beta expression in 5XFAD hippocampus after 14 weeks (Greenfield et al., 2022, In Press).

et al., 1997).

The action of T14 on calcium influx was first observed in *ex vivo* slices of guinea pig brain using electrophysiology recording (Bon and Greenfield, 2003). Subsequently toxicity was observed in vitro and *ex vivo*, more specifically in PC12 tissue cultures where it triggered the increase in both Aβ and tau, and was more potent than amyloid in enhancing excessive calcium influx, leading to a drop in cell viability accompanied by a compensatory release of AChE (Garcia-Rates et al., 2016). The action of T14 at this receptor is modulatory, i.e. *enhances* calcium influx via an allosteric site (Greenfield et al., 2004), leading to further upregulation of its target (Bond et al., 2009) and thus perpetuating its potential toxicity (Fig. 2B).

Using in vitro and *ex vivo* preparations, the levels of T14 have been shown to be high in development and subsequently reduce with age, and associated with neuronal activity as demonstrated in *ex vivo* brain slices and VSDI (Ferrati et al., 2018; Garcia-Rates et al., 2016; Badin et al., 2016; Brai et al., 2018) (Fig. 2C, D).

The significance of the peptide in neurodegeneration has now been demonstrated by its increase in the CSF of Alzheimer patients to levels corresponding to those seen in early (rat) brain development (Garcia--Rates et al., 2016) whilst immunoreactivity for T14 appears with increasing intensity in the midbrain (part of the basal plate derived complex) (Garcia-Rates et al., 2016) and in the hippocampus in correspondence with later Braak staging in AD brain tissue (Greenfield et al., 2022 In press) (Fig. 2E).

3. New developments in the treatment of Alzheimer's disease

'NBP14' is a new genre of drug for combatting AD, a cyclised form of the toxic 14mer peptide itself (Fig. 3A). It has proved in both in vitro (Garcia-Rates et al., 2016*)* and *ex vivo* studies (Ferrati et al., 2018; Brai et al., 2018; Badin et al., 2016), to be non-toxic when applied alone, but protective against a range of linear peptides: Aβ, T14, and T30, the more stable peptide containing the active component T14 (Bond et al., 2009). The drug appears to have no biochemical effects other than antagonism of its linear counterpart on: calcium influx, cell viability, and AChE release in PC12 cells, where it also blocks the toxic effects of amyloid (Garcia-Rates et al., 2016) (Fig. 3 B). In addition, within the CNS, NBP14 blocks the action of T14 on large scale neuronal activity, as monitored with optical imaging in living brain slices (Badin et al., 2016). In a subsequent *ex vivo* preparation, it was shown that NBP14 competes with T14 for its binding site (Brai et al., 2018) (Fig. 3C). Studies in human brain using AlphaLISA techniques show the displacement of T14 by NBP14 show a clear dose-response (Fig. 3D) Greenfield et al., 2022. The IC50 of 61.4 μM (advance AD Braak III-VI) is comparable order of magnitude to that of the IC50 of 1.1 μM found in membranes of PC12 cells (Garcia-Rates et al., 2016).

Further validation of the bioactivity of the T14 system and the efficacy of its blockade with the cyclised variant has been demonstrated by investigating the chronic in vivo effects of NBP14 in a transgenic mouse model following long-term administration in vivo. In this study, memory capacity of the 5XFAD mice, expressing T14 (Fig. 3E), and treated with intranasal NBP14, only after 14 weeks (Fig. 3F), was comparable to wild type mice with a decrease in Aβ at 6 weeks after the treatment had started (Oakley et al., 2006) (Fig. 3G).

4. Conclusion

Over the past two decades, empirical evidence has mounted for a link between the basal plate derived nuclei, their persistent sensitivity to trophic factors, and their vulnerability to neurodegeneration. It is now possible, finally, to start putting these various pieces of the jigsaw together with reference to the novel T14 signalling system: in so doing we can appreciate a more dynamic picture of the neurodegenerative process and thereby gain insights for, at last, laying to rest the spectre of AD that haunts us all and developing a treatment to arrest this devastating disease.

Declaration of interest statement

Sara Garcia-Ratés, is an employee of Neuro-Bio Ltd. Susan Greenfield is the founder and CEO of Neuro-Bio Ltd and holds shares in the Company. She is the inventor in all Neuro-Bio patents.

References

Albanese, A., Butcher, L.L., 1980. Acetylcholinesterase and catecholamine distribution in the locus ceruleus of the rat. Brain Res. Bull. 5, 127–134.

Alkondon, M., Pereira, E.F., Cortes, W.S., Maelicke, A., Albuquerque, E.X., 1997. Choline is a selective agonist of alpha7 nicotinic acetylcholine receptors in the rat brain neurons. Eur. J. Neurosci. 9, 2734–2742.

Appleyard, M.E., Green, A.R., Greenfield, S.A., 1987. Acetylcholinesterase activity rises in rat cerebrospinal fluid post-ictally; effect of a substantia nigra lesion on this rise and on seizure threshold. Br. J. Pharmacol. 91, 149–154.

Arendt, T., Bruckner, M.K., Lange, M., Bigl, V., 1992. Changes in acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease resemble embryonic development–a study of molecular forms. Neurochem. Int. 21, 381–396.

Attems, J., Thal, D.R., Jellinger, K.A., 2012. The relationship between subcortical tau pathology and Alzheimer's disease. Biochem. Soc. Trans. 40, 711–715.

Badin, A.S., Morrill, P., Devonshire, I.M., Greenfield, S.A., 2016. (II) Physiological profiling of an endogenous peptide in the basal forebrain: age-related bioactivity and blockade with a novel modulator. Neuropharmacology 105, 47–60.

Bon, C.L., Greenfield, S.A., 2003. Bioactivity of a peptide derived from acetylcholinesterase: electrophysiological characterization in guinea-pig hippocampus. Eur. J. Neurosci. 17, 1991–1995.

Bond, C.E., Zimmermann, M., Greenfield, S.A., 2009. Upregulation of alpha7 nicotinic receptors by acetylcholinesterase c-terminal peptides. PLoS One 4, e4846.

Botto, R., Callai, N., Cermelli, A., Causarano, L., Rainero, I., 2022. Anxiety and depression in Alzheimer's disease: a systematic review of pathogenetic mechanisms and relation to cognitive decline. Neurol. Sci.

Braak, H., Del Tredici, K., 2012. Where, when, and in what form does sporadic Alzheimer's disease begin? Curr. Opin. Neurol. 25, 708–714.

Brai, E., Simon, F., Cogoni, A., Greenfield, S.A., 2018. Modulatory effects of a novel cyclized peptide in reducing the expression of markers linked to Alzheimer's Disease. Front. Neurosci. 12, 362.

Broide, R.S., Robertson, R.T., Leslie, F.M., 1996. Regulation of alpha7 nicotinic acetylcholine receptors in the developing rat somatosensory cortex by thalamocortical afferents. J. Neurosci. 16, 2956–2971.

Dajas, F., Silveira, R., Costa, G., Castello, M.E., Jerusalinsky, D., Medina, J., et al., 1993. Differential cholinergic and non-cholinergic actions of acetylcholinesterase in the substantia nigra revealed by fasciculin-induced inhibition. Brain Res. 616, 1–5.

Dally, J.J., Greenfield, S.A., 1994. The release of acetylcholinesterase in vivo is regulated by dopaminergic systems in the guinea-pig substantia nigra. Neurochem. Int. 25, 339–344.

Dally, J.J., Schaefer, M., Greenfield, S.A., 1996. The spontaneous release of acetylcholinesterase in rat substantia nigra is altered by local changes in extracellular levels of dopamine. Neurochem. Int. 29, 629–635.

Day, T., Greenfield, S.A., 2002. A non-cholinergic, trophic action of acetylcholinesterase on hippocampal neurones in vitro: molecular mechanisms. Neuroscience 111, 649–656.

Day, T., Greenfield, S.A., 2003. A peptide derived from acetylcholinesterase induces neuronal cell death: characterisation of possible mechanisms. Exp. Brain Res. 153, 334–342.

Day, T., Greenfield, S.A., 2004. Bioactivity of a peptide derived from acetylcholinesterase in hippocampal organotypic cultures. Exp. Brain Res. 155, 500–508.

Dickie, B.G., Holmes, C., Greenfield, S.A., 1996. Neurotoxic and neurotrophic effects of chronic N-methyl-D-aspartate exposure upon mesencephalic dopaminergic neurons in organotypic culture. Neuroscience 72, 731–741.

Eimerl, S., Schramm, M., 1994. The quantity of calcium that appears to induce neuronal death. J. Neurochem. 62, 1223–1226.

Ferrati, G., Brai, E., Stuart, S., Marino, C., Greenfield, S.A., 2018. A multidisciplinary approach reveals an age-dependent expression of a novel bioactive peptide, already involved in neurodegeneration, in the postnatal rat forebrain. Brain Sci. 8.

Garcia-Ayllon, M.S., Riba-Llena, I., Serra-Basante, C., Alom, J., Boopathy, R., Saez-Valero, J., 2010. Altered levels of acetylcholinesterase in Alzheimer plasma. PLoS One 5, e8701.

Garcia-Rates, S., Morrill, P., Tu, H., Pottiez, G., Badin, A.S., Tormo-Garcia, C., et al., 2016. (I) Pharmacological profiling of a novel modulator of the alpha7 nicotinic receptor: Blockade of a toxic acetylcholinesterase-derived peptide increased in Alzheimer brains. Neuropharmacology 105, 487–499.

Greenfield, S., 1996. Non-classical actions of cholinesterases: role in cellular differentiation, tumorigenesis and Alzheimer's disease. Neurochem. Int. 28, 485–490.

Greenfield, S., Vaux, D.J., 2002. Parkinson's disease, Alzheimer's disease and motor neurone disease: identifying a common mechanism. Neuroscience 113, 485–492.

Greenfield, S.A., Appleyard, M.E., Bloomfield, M.R., 1986. 6-Hydroxydopamine-induced turning behaviour in the rat: the significance of acetylcholinesterase in cerebrospinal fluid. Behav. Brain Res. 21, 47–54.

Greenfield, S.A., Day, T., Mann, E.O., Bermudez, I., 2004. A novel peptide modulates alpha7 nicotinic receptor responses: implications for a possible trophic-toxic mechanism within the brain. J. Neurochem. 90, 325–331.
Greenfield, S.A., Cole, G.M., Coen, C.W., Frautschy, S., Singh, R.P., Mekkittikul, M., et al., 2022. A novel process driving Alzheimer's disease validated in a mouse model: therapeutic potential. Alzheimers Dement 8, e12274.
Llinas, R.R., Greenfield, S.A., 1987. On-line visualization of dendritic release of acetylcholinesterase from mammalian substantia nigra neurons. Proc. Natl. Acad. Sci. USA 84, 3047–3050.
Oakley, H., Cole, S.L., Logan, S., Maus, E., Shao, P., Craft, J., et al., 2006. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. J. Neurosci. 26, 10129–10140.
Rossor, M.N., 1981. Parkinson's disease and Alzheimer's disease as disorders of the isodendritic core. Br. Med J. (Clin. Res. Ed.) 283, 1588–1590.
Seguela, P., Wadiche, J., Dineley-Miller, K., Dani, J.A., Patrick, J.W., 1993. Molecular cloning, functional properties, and distribution of rat brain alpha 7: a nicotinic cation channel highly permeable to calcium. J. Neurosci. 13, 596–604.
Taylor, S.J., Haggblad, J., Greenfield, S.A., 1989. Measurement of cholinesterase activity released from the brain "on-line" and in vivo. Neurochem. Int. 15, 199–205.
Taylor, S.J., Jones, S.A., Haggblad, J., Greenfield, S.A., 1990. "On-line" measurement of acetylcholinesterase release from the substantia nigra of the freely-moving guinea-pig. Neuroscience 37, 71–76.
Theofilas, P., Dunlop, S., Heinsen, H., Grinberg, L.T., 2015. Turning on the light within: subcortical nuclei of the isodentritic core and their role in Alzheimer's Disease Pathogenesis. J. Alzheimers Dis. 46, 17–34.
Webb, C.P., Greenfield, S.A., 1992. Non-cholinergic effects of acetylcholinesterase in the substantia nigra: a possible role for an ATP-sensitive potassium channel. Exp. Brain Res. 89, 49–58.
Webb, C.P., Nedergaard, S., Giles, K., Greenfield, S.A., 1996. Involvement of the NMDA receptor in a non-cholinergic action of acetylcholinesterase in guinea-pig substantia nigra pars compacta neurons. Eur. J. Neurosci. 8, 837–841.
Weston, J., Greenfield, S.A., 1985. Application of acetylcholinesterase to the substantia nigra induces stereotypy in rats. Behav. Brain Res. 18, 71–74.
Whyte, K.A., Greenfield, S.A., 2003. Effects of acetylcholinesterase and butyrylcholinesterase on cell survival, neurite outgrowth, and voltage-dependent calcium currents of embryonic ventral mesencephalic neurons. Exp. Neurol. 184, 496–509.
Woolf, N.J., 1996. Global and serial neurons form A hierarchically arranged interface proposed to underlie memory and cognition. Neuroscience 74, 625–651.