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HEALTHY AGING RESEARCH

Supporting evidence for using Perispinal Etanercept to inhibit TNFα when treating neuropathologies including dementia, chronic stroke, neuropathic pain or traumatic brain injury: Role of TNF in the regulation of normal brain activity (Part I)

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Abstract

This first of three parts of the review examines the evidence for the involvement of the pro-inflammatory cytokine, Tumour Necrosis Factor-alpha (TNFα) in regulating normal brain activity. The second part examines changes in TNFα implicated in several neuropathologies. Part III reviews the clinical evidence based on Part I and II for use of anti-TNF therapy to target and for treating these health problems, including chronic stroke, dementias, neuropathic pain or traumatic brain injury. All of these can become chronic illnesses and are of major incidence with a grossly unmet need to improve their treatment. The intent of the three part review is to present the overwhelming scientific and medical basis why research studies and trials to evaluate the use of the perispinally administered anti-TNFα drug, Etanercept, are justified to allow it to become a front-line standard therapy.

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Introduction

TNF, although a protein principally characterized for its role as a signalling factor in the immune system, has other roles. Unfortunately, much unjustified criticism has been targeted at the use of TNF medical treatment for neurological disorders, limited by the premise that TNF's only role is in causing inflammation, indirectly affecting the neuropathology. This disposition ignores the major role of TNF as a glial-derived regulator of neural transmission. Whilst it is correct that TNF is a key mediator of the inflammatory response, nevertheless, this viewpoint is grossly oversimplified and there is now a wealth of information that TNF has more immediate and sinister roles as a significant regulator of the global levels of neuronal activity, that often goes awry in neurological disease processes. To quote from a 2008 article [1], "Amgen (the pharmaceutical company that developed the product Etanercept) seems to think the reported rapid clinical response after treatment (using the Perspinal Etancercept approach) - which neutralises TNFα – does not make sense. But this assumes that TNFα acts only in its "traditional role" of causing inflammation, and takes no account of its recently realised function as a neurotransmitter"[1]. Subsequently, TNFα has become well established as a direct regulator of neuronal synaptic activity. It is in

this context, as detailed below, that targeting TNF in the brain holds major significance, not only for treating the dementias, but also its great benefits in reducing long term pain during rehabilitation from traumatic brain injury (TBI) or chronic stroke.

Characteristics of a prolonged state of Long Term Depression (LTD) in neuronal activity

Firstly, aspects of the global regulation of brain activity will be explained. Long-term depression (LTD) is a neural condition where an activity-dependent loss of function in the nerve connections or synapses, lasting hours or longer can take place, usually after prolonged, repeated nerve stimuli. The most common neurotransmitter involved in LTD is L-glutamate, the major stimulating signal (or excitatory neurotransmitter) in the brain. Glutamate acts on 2 major classes of nerve cell surface receptors, the ionotropic (ligand gated ion channels for K^+, Na^+ or Ca^{2+}) or metabotropic types (reviewed in [2, 3]). The ionotropic (i) glutamate receptors (iGluR) mediate most excitatory neurotransmission in the brain and include the subclasses N-methyl-D-asparate receptors (NMDARs), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs), and kainate receptors (KARs). The other class are the metabotropic (m) glutamate receptors (mGluRs), are seven transmembrane domain G-protein coupled receptors with no inherent ion channel activity. There are 8 different mGluRs that signal indirectly to regulate nerve cell biochemistry.

LTD can either result from overactivity, due to strong synaptic stimulation or from underactivity caused by the persistent lack of, or weak synaptic stimulation. The AMPARs are the most abundant fast acting nerve receptors in the brain and include four different types, iGluR1-4, each capable of binding a glutamate molecule. The AMPARs exist as tetramers, usually as a homodimer of GluR2 with another homodimer of either GluR1, GluR3 or GluR4. The net outcome of LTD at the molecular level is the phosphorylation of the AMPA glutamate receptors causing their down-regulation and removal (by internalization) from the surfaces of the nerve synapses, thereby reducing levels of neuronal excitation.

Long-term potentiation (LTP), the opposing process to LTD, is the long-lasting increase in synaptic strength. Thus, LTP or LTD involve rapid adjustments in the strength of signalling and firing of the neuronal synapses in response to changes in nerve activity, mainly manifested by the regulation of AMPAR trafficking (moving onto to increase or off the outer nerve cell membrane to decrease activity) and levels of expression on the surfaces of the synapses at the nerve endings. “Synaptic plasticity” is a term which refers to global changes in the strength of the adhesive connections bridging between the neurons (via their synapses) and also varies with their firing rate, depending upon changes in neurotransmitter levels released into the synaptic clefts at the nerve endings and in numbers of receptors located around the synapses. All of these factors determine the overall responses of the nerve cells to neurotransmitter signalling and receptor binding.

A good example of such effects occurs during periods of acute stress (such as from psychological shock like Post Traumatic Stress Disorder or PTSD) which causes inhibition of LTP whilst enabling the induction of LTD in the dorsal hippocampus [4, 5].

TNFα and the regulation of synaptic plasticity and neuronal function

Homeostatic “synaptic plasticity” is a feedback response to compensate for functional disturbances in the nervous system. Typically, synaptic activity becomes strengthened when neuronal firing is chronically suppressed or weakened to provide a compensatory mechanism attempting to overcome the repressed levels of neuronal firing, thereby helping to maintain function at a steady state level. At both the whole cell and entire network levels, artificially manipulating nerve activity can lead to the global up- or downscaling of the transmission efficacy of the nerve synapses. The regulation of the interconnected synaptic networks in the brain with respect to synaptic plasticity is a core component of the processes involved in learning and memory.

“Excitotoxicity” is a pathological process in which nerve cells become damaged and killed after their exaggerated and continuous stimulation by the neurotransmitter, glutamate, which acts by promoting

the nerve-associated microglial cell release of TNFα [6]. By its actions, TNFα modifies nerve receptor trafficking and acts as a glial cell/astrocyte-released mediator of homeostatic synaptic scaling [[7]; reviewed in [8]]. Glial cells, sometimes called neuroglial or glia are not nerve cells and are not directly involved in nerve signalling, but rather support the nerve signalling and synapse forming abilities of neurons. Astrocytes are glial cells with a star-shape and hence, the derivation of their name. Thus, TNFα, predominantly produced by glial cells, directly regulates the surface expression of the calcium-permeable glutamate receptor levels on nerve endings, which greatly increases neuronal cell vulnerability to excitotoxicity [reviewed in [9];[10-12]]. Upon severe neural trauma, excitotoxicity is the major cause of nerve cell death. In this fashion, TNFα has now been established as an integral and key regulator of synaptic transmission, as well as global nerve survival and function.

Defining the function of TNFα as a central regulator of neuronal synaptic plasticity

Synaptic plasticity, underlying the basis for learning and memory, is also linked to aberrant forms of learning such as drug addiction and neuropathic pain and not just the activity-dependent refinements in the connectivity of the brain as it forms during our development. Synaptic plasticity results, in part, from changes in the number of the AMPA-type glutamate receptors at the excitatory synapses and TNFα has been shown to directly regulate brain neuron AMPA receptor trafficking, causing their dramatic exocytosis to rapidly increase their surface expression.

As a glial cell-released factor, TNFα regulates the surface expression of the iGluR2-lacking types of AMPAR (the iGluR2 subunit inhibits calcium (Ca^{2+}) channel activation by these receptors), thereby modulating the threshold for channels conducting Ca^{2+} required for synaptic plasticity and neuronal excitotoxicity. The iGluR2 deficient subclasses of glutamate receptors have been implicated in a number of disease states. Hence, for example, the up-regulation of the regulatory AMPA receptor GluR2 (iGluA2) subunits occurring during subcortical ischemic-induced vascular dementia, is repressed in Alzheimer's disease [13].

Neurodegenerative disease and post injury environments are characterized by abnormally high levels of TNF that have been found responsible for the neuronal cytotoxicity and dysfunction [14-18] and TNF can directly induce neuronal cell death [19-22]. Another mechanism of TNF-induced neuronal death which occurs during LTD is via the TNF-induced surface expression of the AMPARs [9, 11]. The precise regulation of AMPAR numbers on the postsynaptic plasma membrane has been shown in many studies to be an essential controller of synaptic activity [23-26] and the TNF mediated dysregulation of the iGluR trafficking causes nerve excitotoxic vulnerability (reviewed in [10]),[27]. TNF can induce the rapid increase in levels of steady state iGluR1 and iGluR2 containing AMPAR via cell surface accumulation, within 15 minutes after application to hippocampal neuron cultures [12, 28-30] through a phosphoinositide 3 kinase (PI3K) signalling-dependent mechanism [9].

It has been known for many years that long term administration of TNF, over the low 1-100nM range, results in LTP in hippocampal slices [31]. TNF induced activity can be observed in cortical neurons [27] as well as in the intact spinal cord [32], suggesting that this represents a common *in vivo* response of neurons to elevated TNF during post-injury inflammation across the entire CNS. However, higher TNF levels in the CNS are likely to contribute to AMPAR-dependent neuronal cell death [33, 34]. The specific blockade of Ca^{2+}-permeable AMPARs (CP-AMPARs) by selective inhibitors prevents this TNF induced excitotoxicity in the intact spinal cord [30, 35].

Continued high frequency stimulation inducing LTD was also shown to modulate the metabotropic glutamate receptors in the dentate gyrus, mainly mediated by activation of mGluR5, although a partial involvement of the metabolic mGluR1 was found, and TNFR1 was directly implicated as an intermediary factor inducing the switch from LTP to LTD [36, 37]. Hence, TNF can also regulate LTP to LTD switching via mGluR changes as well.

To summarise at this point, these data are consistent with the hypothesis that TNF is capable of inducing

the rapid changes in GluR activity, either via mGluRs or a rise in CP-AMPARs in a dose-responsive manner, which contributes to excitotoxic vulnerability, and hence to the ensuing loss of brain function (Fig. 1).

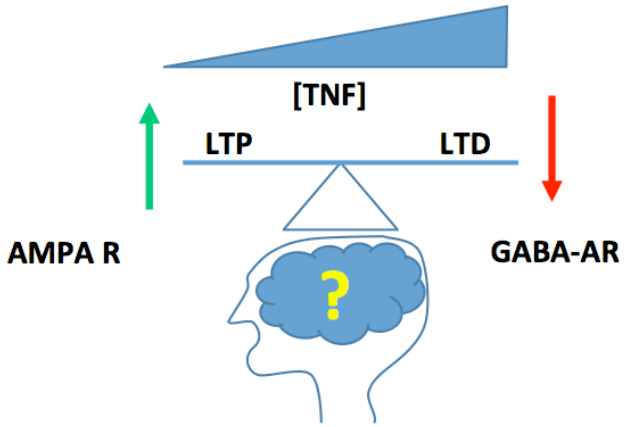

Figure 1. Global brain function is dependent on the overall balance between opposing activities of different neural receptor types and TNF levels

AMPA iGluR receptor levels promote excitatory neurotransmission whereas the GABAR receptors are inhibitory. Increased TNF levels as shown in the blue triangle will shift the balance towards Long Term Depression (LTD) or global repression.

TNFα also regulates neuronal inhibition by affecting the endocytosis of the GABA-A receptor, the principle mediator of "fast" inhibition in the brain

As outlined above, glutamate receptor (GluR) trafficking induced by TNFα underlies the basic regulation of homeostatic synaptic plasticity and scaling of neuronal activity. Homeostatic synaptic plasticity entails the uniform adjustments in the strength of all synapses on a given nerve cell in response to prolonged changes in the cell's electrical activity, and is critical for maintaining the stability of the neuronal circuits in the brain. During prolonged periods of low activity, the excitatory synapses on neurons strengthen due to insertion of additional AMPA receptors whilst the opposite acting, inhibitory synapses weaken simultaneously, due to removal of Gamma Amino Butyric Acid-type A receptors (GABA-ARs). The latter are a major source of fast inhibitory synaptic transmission in the CNS and hence, the balance together with the opposing activity of AMPARs and levels of TNF play crucial roles in regulating the overall activity levels of the neuronal networks in the brain (Fig. 1).

TNFα, produced by the glial cells, is also a critical mediator of these changes, such that should TNF levels become increased, as part of the damage response following brain/spinal cord injury or other nervous system disorders, it causes nerve cell/brain regional tissue death [9, 32-33, 38-41]. The GABA-AR levels at the neuronal synapses show a U-shaped response to TNF and are down-regulated by low levels of TNF (0.01-0.1 mM), but then become significantly increased by higher TNF levels (0.1-1 mM), whereas the AMPAR receptor levels show a direct linear response, increasing with TNF levels over the same concentration range [40].

Much like the action of the amnesia-inducing drugs, such as gamma hydroxybutyrate (GHB) or Rohypnol, which both act by rapidly enhancing the levels of GABA-R activity, TNF over-production by glial cells will cause similar states of LTD in neuronal activity within the brain, associated with accumulative nerve and brain tissue damage. Therefore, inhibiting TNFα signaling, either pharmacologically or genetically will modulate the changes in synaptic strength induced by the increased TNF-mediated chronic activity blockade and excitotoxicity. The implication from these observations is that the TNFα is of glial origin and that glia detect the levels of neuronal activity to feedback and regulate homeostatic signals via TNFα production and release (see review [42]).

It should be noted at this point that the changes in neuronal circuit responses occur very rapidly within 15-60 minutes and can be completely inhibited by the presence of soluble TNFR1 receptor [7, 9, 12, 29, 42]. These observations are consistent with the effects of the anti-TNFα targeted Perispinal Etanercept (PSE) therapy, which are also rapid with responses detected in treated patients occurring over a similar fast time course [43-49] (see Part III).

Defining the function of TNFα in chronic brain diseases and traumatic brain injury

Neuroinflammation is a hallmark of almost every neurological disorder, from Traumatic Brain Injury (TBI) such as acute head injury and stroke to most neurodegenerative diseases, including the dementias, and it is a major contributor to the disruption of neuronal function and cell death.

How does inflammation, driven by the release of pro-inflammatory cytokines, damage brain tissue?

The evidence described above documents that in the normal course of regulating homeostatic plasticity, TNFα is an intrinsic factor involved in neuronal function. It also implies that during disease states, or as a result of brain tissue damage, infiltration of immune cells into the neuronal tissue with their associated increase in local or even more remote systemic production of TNFα, will exacerbate the situation to cause brain dysfunction by disrupting normal neuronal regulation. In this regard, TNFα is rather unique amongst cytokines and has been critically linked to a variety of neuronal insults, such as stroke and head/spinal trauma, and to the neurodegenerative diseases, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and the disorders of depression, migraine and neuropathic pain [18, 48, 50-52]. Hence, understanding exactly how TNFα contributes to these disorders is essential. Perispinal Etanercept therapy, by targeting TNF to mitigate its ensuing damage represents a new and important advance in treating such diseases. It is also a very important development in neurobiological understanding of brain function. These are topics described in more detail in Part II and III.

To summarise Part I, TNFα is endogenously released by glia in a nerve activity-dependent manner, but also released at much higher levels by activated astrocytes, microglia, and infiltrating immune cells during neuro-inflammatory insults. TNFα plays important functions in the regulation of normal neuronal processes, but this function can become chronically dysregulated during inflammatory disorders, contributing to associated neuronal damage.

References

1. Clark IA. Alzheimer's Puzzlement. New Sci. 2008;199(2672):25.
2. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate receptor ion channels: structure, regulation, and function. Pharmacological reviews. 2010;62(3):405-96.
3. Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annual review of pharmacology and toxicology. 2010;50:295-322.
4. Wong TP, Howland JG, Robillard JM, Ge Y, Yu W, Titterness AK, et al. Hippocampal long-term depression mediates acute stress-induced spatial memory retrieval impairment. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(27):11471-6.
5. Xu L, Anwyl R, Rowan MJ. Behavioural stress facilitates the induction of long-term depression in the hippocampus. Nature. 1997;387(6632):497-500.
6. Figiel I, Dzwonek K. TNFalpha and TNF receptor 1 expression in the mixed neuronal-glial cultures of hippocampal dentate gyrus exposed to glutamate or trimethyltin. Brain research. 2007;1131(1):17-28.
7. Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF-alpha. Nature. 2006;440(7087):1054-9.
8. Turrigiano GG. More than a sidekick: glia and homeostatic synaptic plasticity. Trends in molecular medicine. 2006;12(10):458-60.
9. Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25(12):3219-28.
10. Pickering M, Cumiskey D, O'Connor JJ. Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. Experimental physiology. 2005;90(5):663-70.
11. Ogoshi F, Yin HZ, Kuppumbatti Y, Song B, Amindari S, Weiss JH. Tumor necrosis-factor-alpha (TNF-alpha) induces rapid insertion of Ca2+-permeable alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)/kainate (Ca-A/K) channels in a subset of hippocampal pyramidal neurons. Experimental neurology. 2005;193(2):384-93.
12. Leonoudakis D, Zhao P, Beattie EC. Rapid tumor necrosis factor alpha-induced exocytosis of glutamate receptor 2-lacking AMPA receptors to extrasynaptic plasma membrane potentiates excitotoxicity. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008;28(9):2119-30.
13. Mohamed NE, Zhao Y, Lee JH, Tan MG, Esiri MM, Wilcock GK, et al. Upregulation of AMPA receptor GluR2 (GluA2) subunits in subcortical ischemic

vascular dementia is repressed in the presence of Alzheimer's disease. Neurochemistry international. 2011.

14. Shohami E, Ginis I, Hallenbeck JM. Dual role of tumor necrosis factor alpha in brain injury. Cytokine & growth factor reviews. 1999;10(2):119-30.
15. Perry RT, Collins JS, Wiener H, Acton R, Go RC. The role of TNF and its receptors in Alzheimer's disease. Neurobiology of aging. 2001;22(6):873-83.
16. Szelenyi J. Cytokines and the central nervous system. Brain research bulletin. 2001;54(4):329-38.
17. Sedger LM, McDermott MF. TNF and TNF-receptors: From mediators of cell death and inflammation to therapeutic giants - past, present and future. Cytokine & growth factor reviews. 2014;25(4):453-72.
18. Tuttolomondo A, Pecoraro R, Pinto A. Studies of selective TNF inhibitors in the treatment of brain injury from stroke and trauma: a review of the evidence to date. Drug design, development and therapy. 2014;8:2221-38.
19. Zhao X, Bausano B, Pike BR, Newcomb-Fernandez JK, Wang KK, Shohami E, et al. TNF-alpha stimulates caspase-3 activation and apoptotic cell death in primary septo-hippocampal cultures. Journal of neuroscience research. 2001;64(2):121-31.
20. Fontaine V, Mohand-Said S, Hanoteau N, Fuchs C, Pfizenmaier K, Eisel U. Neurodegenerative and neuroprotective effects of tumor Necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2002;22(7):RC216.
21. Yang D, Zhai Y, Zhang M. LIGHT, a new member of the TNF superfamily. Journal of biological regulators and homeostatic agents. 2002;16(3):206-10.
22. Zou JY, Crews FT. TNF alpha potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF kappa B inhibition. Brain research. 2005;1034(1-2):11-24.
23. Carroll RC, Beattie EC, von Zastrow M, Malenka RC. Role of AMPA receptor endocytosis in synaptic plasticity. Nature reviews Neuroscience. 2001;2(5):315-24.
24. Malinow R, Malenka RC. AMPA receptor trafficking and synaptic plasticity. Annual review of neuroscience. 2002;25:103-26.
25. Song I, Huganir RL. Regulation of AMPA receptors during synaptic plasticity. Trends in neurosciences. 2002;25(11):578-88.
26. Bredt DS, Nicoll RA. AMPA receptor trafficking at excitatory synapses. Neuron. 2003;40(2):361-79.
27. Leonoudakis D, Braithwaite SP, Beattie MS, Beattie EC. TNFalpha-induced AMPA-receptor trafficking in CNS neurons; relevance to excitotoxicity? Neuron glia biology. 2004;1(3):263-73.
28. Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, et al. Control of synaptic strength by glial TNFalpha. Science. 2002;295(5563):2282-5.
29. Rainey-Smith SR, Andersson DA, Williams RJ, Rattray M. Tumour necrosis factor alpha induces rapid reduction in AMPA receptor-mediated calcium entry in motor neurones by increasing cell surface expression of the GluR2 subunit: relevance to neurodegeneration. Journal of neurochemistry. 2010;113(3):692-703.
30. Yin HZ, Hsu CI, Yu S, Rao SD, Sorkin LS, Weiss JH. TNF-alpha triggers rapid membrane insertion of Ca(2+) permeable AMPA receptors into adult motor neurons and enhances their susceptibility to slow excitotoxic injury. Experimental neurology. 2012;238(2):93-102.
31. Tancredi V, D'Arcangelo G, Grassi F, Tarroni P, Palmieri G, Santoni A, et al. Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. Neuroscience letters. 1992;146(2):176-8.
32. Ferguson AR, Christensen RN, Gensel JC, Miller BA, Sun F, Beattie EC, et al. Cell death after spinal cord injury is exacerbated by rapid TNF alpha-induced trafficking of GluR2-lacking AMPARs to the plasma membrane. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008;28(44):11391-400.
33. Hermann GE, Rogers RC, Bresnahan JC, Beattie MS. Tumor necrosis factor-alpha induces cFOS and strongly potentiates glutamate-mediated cell death in the rat spinal cord. Neurobiology of disease. 2001;8(4):590-9.
34. Noh KM, Yokota H, Mashiko T, Castillo PE, Zukin RS, Bennett MV. Blockade of calcium-permeable AMPA receptors protects hippocampal neurons against global ischemia-induced death. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(34):12230-5.
35. Corona JC, Tapia R. Ca2+-permeable AMPA receptors and intracellular Ca2+ determine motoneuron vulnerability in rat spinal cord in vivo. Neuropharmacology. 2007;52(5):1219-28.
36. Wang Q, Chang L, Rowan MJ, Anwyl R. Developmental dependence, the role of the kinases p38 MAPK and PKC, and the involvement of tumor necrosis factor-R1 in the induction of mGlu-5 LTD in the dentate gyrus. Neuroscience. 2007;144(1):110-8.
37. Cumiskey D, Butler MP, Moynagh PN, O'Connor JJ. Evidence for a role for the group I metabotropic glutamate receptor in the inhibitory effect of tumor necrosis factor-alpha on long-term potentiation. Brain research. 2007;1136(1):13-9.
38. Hermann GE, Holmes GM, Rogers RC. TNF(alpha) modulation of visceral and spinal sensory processing. Current pharmaceutical design. 2005;11(11):1391-409.
39. Beattie MS, Hermann GE, Rogers RC, Bresnahan JC. Cell death in models of spinal cord injury. Progress in brain research. 2002;137:37-47.

40. Stuck ED, Christensen RN, Huie JR, Tovar CA, Miller BA, Nout YS, et al. Tumor necrosis factor alpha mediates GABA(A) receptor trafficking to the plasma membrane of spinal cord neurons in vivo. Neural plasticity. 2012;2012:261345.
41. Huie JR, Baumbauer KM, Lee KH, Bresnahan JC, Beattie MS, Ferguson AR, et al. Glial tumor necrosis factor alpha (TNFalpha) generates metaplastic inhibition of spinal learning. PloS one. 2012;7(6):e39751.
42. Stellwagen D. The contribution of TNFalpha to synaptic plasticity and nervous system function. Advances in experimental medicine and biology. 2011;691:541-57.
43. Tobinick E. Rapid improvement of chronic stroke deficits after perispinal etanercept: three consecutive cases. CNS drugs. 2011;25(2):145-55.
44. Tobinick EL, Chen K, Chen X. Rapid intracerebroventricular delivery of Cu-DOTA-etanercept after peripheral administration demonstrated by PET imaging. BMC research notes. 2009;2:28.
45. Tobinick E. Perispinal etanercept produces rapid improvement in primary progressive aphasia: identification of a novel, rapidly reversible TNF-mediated pathophysiologic mechanism. Medscape journal of medicine. 2008;10(6):135.
46. Tobinick EL, Gross H. Rapid improvement in verbal fluency and aphasia following perispinal etanercept in Alzheimer's disease. BMC neurology. 2008;8:27.
47. Tobinick EL, Gross H. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. Journal of neuroinflammation. 2008;5:2.
48. Tobinick E. Deciphering the physiology underlying the rapid clinical effects of perispinal etanercept in Alzheimer's disease. Current Alzheimer research. 2012;9(1):99-109.
49. Tobinick E, Rodriguez-Romanacce H, Levine A, Ignatowski TA, Spengler RN. Immediate neurological recovery following perispinal etanercept years after brain injury. Clinical drug investigation. 2014;34(5):361-6.
50. Ignatowski TA, Spengler RN, Dhandapani KM, Folkersma H, Butterworth RF, Tobinick E. Perispinal etanercept for post-stroke neurological and cognitive dysfunction: scientific rationale and current evidence. CNS drugs. 2014;28(8):679-97.
51. Tobinick E. Perispinal etanercept for neuroinflammatory disorders. Drug discovery today. 2009;14(3-4):168-77.
52. Stoeck K, Schmitz M, Ebert E, Schmidt C, Zerr I. Immune responses in rapidly progressive dementia: a comparative study of neuroinflammatory markers in Creutzfeldt-Jakob disease, Alzheimer inverted question marks disease and multiple sclerosis. Journal of neuroinflammation. 2014;11(1):170.