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Stress-Induced Tau Phosphorylation: Functional Neuroplasticity or Neuronal Vulnerability?

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Abstract

Abnormally phosphorylated tau protein is a key component of the pathology seen in neurodegenerative tauopathies, such as Alzheimer's disease (AD). Despite its association with disease, tau phosphorylation (tau-P) also plays an important role in neuroplasticity, such as dendritic/synaptic remodeling seen in the hippocampus in response to environmental challenges, such as stress. To define the boundaries between neuroplasticity and neuropathology, studies have attempted to characterize the paradigms, stimuli, and signaling intermediates involved in stress-induced tau-P. Supporting an involvement of stress in AD are data demonstrating alterations in stress pathways and peptides in the AD brain and epidemiological data implicating stress exposure as a risk factor for AD. In this review, the question of whether stress-induced tau-P can be used as a model for examining the relationship between functional neuroplasticity and neuronal vulnerability will be discussed.

Keywords

Alzheimer's disease; corticotropin-releasing; hippocampus; hypothalamus; neurofibrillary tangles; phosphorylation; stress; tau

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder affecting approximately 40% of the population over 80 years of age [1] and is defined neuropathologically by the presence of amyloid- β ($A\beta$) plaques and neurofibrillary tangles (NFT). NFTs are intracellular inclusions composed of aggregated, hyperphosphorylated forms of the microtubule-associated protein, tau. Hyperphosphorylated tau has reduced ability to bind and stabilize microtubule networks and can self-aggregate into paired helical filaments (PHFs), which comprise NFTs. The prevalence of NFTs in the brain is correlated with cognitive decline and neuronal loss in AD, suggesting that pathological changes in tau may be involved in neurodegeneration.

With less than 1% of AD cases having identified genetic loci, significant research has focused on environmental factors that may be involved in sporadic AD. Of the numerous environmental influences that are under investigation, propensity to experience "psychological distress" in humans has been demonstrated to confer a three-fold increase in risk of AD [2,3]. These epidemiological studies provided important groundwork for rodent studies attempting to define causal links between stress exposure and AD neuropathological endpoints. Many of these rodent studies have focused on the ability of acute physiological or psychological stress episodes to induce tau-P in the hippocampus [4–11].

In terms of disease relevance, acute stress-induced tau-P appears to be a rapid and transient phenomenon, suggesting an involvement in stress adaptation and neuroplasticity. Given the propensity of phosphorylated tau to dissociate from microtubules, the transient nature of tau-P induced by acute stress can be seen as an attempt to promote modulation of networks to form new memories or strengthen existing synapses.

POST-TRANSLATIONAL CHANGES IN TAU AND NEURONAL VULNERABILITY

Tau is a soluble phospho-protein that stabilizes axonal microtubules to maintain cellular morphology and facilitate neurite outgrowth and differentiation [12–14]. Six isoforms of tau exist in brain and are produced by alternative mRNA splicing of a single gene on chromosome 17. Isoforms of tau differ by the inclusion of inserts in the N-terminal domain and tandem repeats within the C-terminal microtubule-binding domain. Recent studies demonstrate that tau mediates Golgi membrane and dynein binding to microtubules to facilitate neuronal integrity [15,16]. Tau binding/stabilization of axonal microtubules is also crucial for trafficking of intracellular organelles and vesicles [17]. One of the primary events that regulate tau-microtubule interactions is phosphorylation of tau, which alters tau binding and function [12, 13].

Pathological alterations in tau occur in several neurodegenerative disorders, termed tauopathies, and include AD, frontotemporal dementia, Pick's disease, supranuclear palsy, and corticobasal degeneration. While the tau pathology in many of these diseases is of sporadic origin, rare mutations in the tau gene have also been identified and can lead to neurodegenerative tauopathies [18].

In AD, tau becomes hyperphosphorylated and aggregates, forming PHFs within the somatodendritic compartment [19–21]. PHF bundles accumulate as insoluble NFTs in the entorhinal cortex and CA1 subfield of the hippocampus before appearing in cortical areas. The incidence of NFTs is positively correlated with cognitive deficit and neuronal loss in AD, which has positioned pathological changes in tau as critical in neurodegeneration. Although the specific links between tau alterations and neurotoxicity are controversial, tau phosphorylation (tau-P) is considered one of the earliest cytoskeletal changes in AD and a critical step in the formation of NFTs. Whereas tau is normally phosphorylated at 2–3 moles/mole of protein, tau from the AD brain is hyperphosphorylated at a 7–10 molar ratio, and at more than 25 distinct sites [22].

With the development of a variety of specific antibodies, studies have proposed a sequence of tau alterations, which include conformation, truncation and phosphorylation changes that lead to NFT. A folded conformational change in the N and C-termini on tau is thought to be the earliest pathological event and can be detected using conformational-specific tau antibodies (e.g., MC1) [23]. Caspase-cleavage of tau at the C-terminus has also been demonstrated to be an early pathologic change in tau and can be detected with neopeptide antibodies to truncated tau [24–28]. Other antibodies are directed to immature NFTs by recognizing tau-P at several N-terminal serines and threonine, which are thought to be the first residues to be hyperphosphorylated [29]. This is in contrast to antibodies directed toward phosphorylation sites in the C-terminus of tau, which recognize hyperphosphorylated tau found within more mature, late-stage NFTs [30].

STRESS SIGNALING PATHWAYS AND ALZHEIMER'S DISEASE

Corticotropin-releasing factor (CRF) is best known as the hypothalamic neuropeptide that activates the stress response (reviewed in [31]). CRF exerts its biological effects by binding to

two G protein-coupled receptors (CRFRs) that activate adenylate cyclase. CRFR1 is expressed in the anterior pituitary and can account for the neuroendocrine actions of the peptide. CRFR1 is also expressed widely in brain, including AD-relevant regions as isocortex, hippocampus, and amygdala [32]. CRFR2 is also expressed in brain, but has a distinct and more limited distribution than CRFR1 [32].

Reduced cortical CRF and increased hypothalamic immunoreactivity is a prominent neurochemical change in AD, which occurs early in disease progression, and is focused in areas vulnerable to AD neuropathology (for review [33]). In addition, marked increases in CRF binding have been described in specific cortical regions of the AD brain, suggesting upregulation of CRFRs in areas vulnerable to AD pathology [34]. It is thought that some of these changes may result from sequestration of CRF by a specific binding protein (CRF-BP) [35]. In concordance with this hypothesis, competitive antagonists to CRF-BP enhance performance on learning and memory tasks [35]. Although studies have documented alteration of the CRF system, clinical correlations and isolation of molecular intermediates are still needed to identify the precise consequences of these changes during AD.

Another crucial variable in the stress cascade are glucocorticoids (GC), dominant stress hormones that are released as a result of CRF signaling and that mediate physiological changes during stress. The idea that excessive amounts of GC could be causal in endangering hippocampal neurons was first suggested in the 1970s, with the demonstration that the magnitude of GC hypersecretion could predict the extent of neuronal loss in the hippocampus in aged rats (reviewed in [36,37]). These studies provided the foundation for subsequent studies demonstrating direct links between GC exposure and neurotoxicity (reviewed in [38]). In several studies where GC concentrations have been raised into the stress range for extended periods, significant hippocampal neuron loss has been observed, particularly in the pyramidal cells of CA3 [39]. In concordance with these rodent studies, increased circulating levels of GC have been reported with increasing age, are a prominent feature of AD, and may play a prominent role in AD pathology [36,37,40,41]. Although studies on changes in the CRF system and the ability of excess GC to endanger hippocampal neurons have provided important groundwork for identifying the role of stress in AD, the specific molecular intermediates connecting stress to development of neuropathology have remained elusive.

Another important link between the stress axis and AD came with the discovery that exposure to physiological [4–10] and psychological stress could induce tau-P in rodents [11]. Because the circuitry and patterns of neuronal activity that mediate responses to physiological and psychological stressors differ [44–47], the mechanisms underlying tau phosphorylation induced by different stress models have not yet been deciphered thoroughly. For example, both physiological and psychological stress can induce tau-P independently of glucocorticoid secretion [4,11], although most stressors have not been tested. Other mechanisms underlying stress-induced tau-P have been suggested to involve modulation of temperature sensitive tau phosphatases [8,9], which is particularly relevant to strong physiological stressors such as starvation, cold-water stress, and heat shock. In addition, this mechanism has been used to explain tau-P seen during hibernation [48] or exposure to anesthesia [49,50]. Recent studies that have found that dexamethasone treatment and/or stress exposure can increase $A\beta$ production and tau accumulation in transgenic mouse models of AD [42,43].

Although the data thus far appears to provide a convincing case for a temperature-dependent mechanism for some stressors, more mild stressors appear to have distinct underlying mechanism that may not be directly tied to the kinase-phosphatase balance. In the case of psychological stress, such as restraint, genetic and pharmacologic studies of CRFRs have demonstrated that CRFR1 is required for stress-induced tau-P and that CRFRs regulate tau kinases and stress-induced tau-P in an opposing manner [11]. The potential AD relevance of

stress-induced tau-P was also investigated, with studies investigating the role of repeated (chronic) emotional stress on tau-P and tau solubility. When a repeated stress paradigm was administered, stress-induced tau-P was not seen to be transient and resulted in sustained increases in tau-P even 24 h after the final stress exposure [11]. Furthermore, as a result of repeated stress, portions of phosphorylated tau were also sequestered to detergent-soluble fractions (cf acute stress), which is where the bulk of PHFs in the AD brain are found [51, 52]. Whether or not the development of detergent-soluble tau species as a result of stress exposure can lead to toxicity and contribute to pathology is currently under investigation.

CONCLUSIONS

Because known genetic links comprise a low percentage of AD cases, identifying potential environmental contributors in AD is critical. Although age is considered the primary risk factor for AD, numerous studies have also implicated chronic stress in this regard, with changes in stress steroids and CRF system intermediates being altered early in AD progression. These studies, taken together with the recently discovered relationship between the CRF system in stress-induced tau-P, provide a more complete picture of the potential mechanisms linking stress and AD endpoints.

From the relevant literature, there seems little question that stress can be an exacerbating influence in combination with genetic and other factors (e.g., aging) but the limits of this have not been explored. Although it remains to be determined whether prolonged stress exposure can lead to bona-fide tau pathology, recent data demonstrating tau-P and solubility changes with repeated stress exposure certainly begs the question of whether an animal can be stressed into senility. Given that the balance between stress-induced tau-P and development of insoluble tau aggregates may be quite delicate, using stress-induced tau-P as a model to examine these dynamics may prove useful for further determining the boundaries between of the relationship between stress, AD tau pathology and neuroplasticity.

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REFERENCES

1. Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM. Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement* 2007;3:186–191. [PubMed: 19595937]
2. Wilson RS, Evans DA, Bienias JL, Mendes de Leon CF, Schneider JA, Bennett DA. Proneness to psychological distress is associated with risk of Alzheimer's disease. *Neurology* 2003;61:1479–1485. [PubMed: 14663028]
3. Wilson RS, Bennett DA, Mendes de Leon CF, Bienias JL, Morris MC, Evans DA. Distress proneness and cognitive decline in a population of older persons. *Psychoneuroendocrinology* 2005;30:11–17. [PubMed: 15358438]
4. Korneyev A, Binder L, Bernardis J. Rapid reversible phosphorylation of rat brain tau proteins in response to cold water stress. *Neurosci Lett* 1995;191:19–22. [PubMed: 7659281]
5. Papasozomenos SC. Heat shock induces rapid dephosphorylation of tau in both female and male rats followed by hyperphosphorylation only in female rats: implications for Alzheimer's disease. *J Neurochem* 1996;66:1140–1149. [PubMed: 8769877]
6. Korneyev AY. Stress-induced tau phosphorylation in mouse strains with different brain Erk 1 + 2 immunoreactivity. *Neurochem Res* 1998;23:1539–1543. [PubMed: 9821159]

7. Yanagisawa M, Planel E, Ishiguro K, Fujita SC. Starvation induces tau hyperphosphorylation in mouse brain: implications for Alzheimer's disease. *FEBS Lett* 1999;461:329–333. [PubMed: 10567721]
8. Planel E, Yasutake K, Fujita SC, Ishiguro K. Inhibition of protein phosphatase 2A overrides tau protein kinase I/glycogen synthase kinase 3 beta and cyclin-dependent kinase 5 inhibition and results in tau hyperphosphorylation in the hippocampus of starved mouse. *J Biol Chem* 2001;276:34298–34306. [PubMed: 11441005]
9. Planel E, Miyasaka T, Launey T, Chui DH, Tanemura K, Sato S, Murayama O, Ishiguro K, Tatebayashi Y, Takashima A. Alterations in glucose metabolism induce hypothermia leading to tau hyperphosphorylation through differential inhibition of kinase and phosphatase activities: implications for Alzheimer's disease. *J Neurosci* 2004;24:2401–2411. [PubMed: 15014115]
10. Feng Q, Cheng B, Yang R, Sun FY, Zhu CQ. Dynamic changes of phosphorylated tau in mouse hippocampus after cold water stress. *Neurosci Lett* 2005;388:13–16. [PubMed: 16005567]
11. Rissman RA, Lee KF, Vale W, Sawchenko PE. Corticotropin-releasing factor receptors differentially regulate stress-induced tau phosphorylation. *J Neurosci* 2007;27:6552–6562. [PubMed: 17567816]
12. Mandell JW, Banker G. A spatial gradient of tau protein phosphorylation in nascent axons. *J Neurosci* 1996;16:5727–5740. [PubMed: 8795628]
13. Li Y, Black MM. Microtubule assembly and turnover in growing axons. *J Neurosci* 1996;16:531–544. [PubMed: 8551337]
14. Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J Cell Biol* 2002;126:1051–1063. [PubMed: 11901170]
15. Farah CA, Perreault S, Liagzoghli D, Desjardins M, Anton A, Lauzon M, Paiement J, Leclerc N. Tau interacts with golgi membranes and mediates their association with microtubules. *Cell Motil Cytoskeleton* 2006;73:710–724. [PubMed: 16960886]
16. Magnani E, Fan J, Gasparini L, Golding M, Williams M, Schiavo G, Goedert M, Amos LA, Spillantini MG. Interaction of tau protein with the dynactin complex. *EMBO J* 2007;26:4546–4554. [PubMed: 17932487]
17. Ebner A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E. Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J Cell Biol* 1998;143:777–94. [PubMed: 9813097]
18. Spillantini MG, Murrell JR, Goedert M, Farlow M, Klug A, Ghetti B. Mutations in the tau gene (MAPT) in FTDP-17: the family with Multiple System Tauopathy with Presenile Dementia (MSTD). *J Alzheimers Dis* 2006;9(3 Suppl):373–380. [PubMed: 16914875]
19. Binder LI, Frankfurter A, Rebhun LI. The distribution of tau in the mammalian central nervous system. *J Cell Biol* 1985;1:1371–1378. [PubMed: 3930508]
20. Wischik CM, Novak M, Edwards PC, Klug A, Tichelaar W, Crowther RA. Structural characterization of the core of the paired helical filament of Alzheimer disease. *Proc Natl Acad Sci U S A* 1988;13:4884–4888. [PubMed: 2455299]
21. Braak E, Braak H, Mandelkow EM. A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. *Acta Neuropathol* 1994;87:554–567. [PubMed: 7522386]
22. Kopke E, Tung YC, Shaikh S, Alonso AC, Iqbal K, Grundke-Iqbal I. Microtubule-associated protein tau. Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. *J Biol Chem* 1993;268:24374–24384. [PubMed: 8226987]
23. Weaver CL, Espinoza M, Kress Y, Davies P. Conformational change as one of the earliest alterations of tau in Alzheimer's disease. *Neurobiol Aging* 2000;21:719–727. [PubMed: 11016541]
24. Ding H, Matthews TA, Johnson GV. Site-specific phosphorylation and caspase cleavage differentially impact microtubule interactions and tau aggregation. *J Biol Chem* 2006;281:19107–14. [PubMed: 16687396]
25. Gambin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, Garcia-Sierra F, LaPointe N, Miller R, Berry RW, Binder LI, Cryns VL. Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci U S A* 2003;100:10032–7. [PubMed: 12888622]

26. Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, LaFerla FM, Rohn TT, Cotman CW. Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology. *J Clin Invest* 2004;114:121–130. [PubMed: 15232619]
27. Guo H, Albrecht S, Bourdeau M, Petzke T, Bergeron C, LeBlanc AC. Active caspase-6 and caspase-6-cleaved tau in neuropil threads, neuritic plaques, and neurofibrillary tangles of Alzheimer's disease. *Am J Pathol* 2004;165:523–531. [PubMed: 15277226]
28. Guillozet-Bongaarts AL, Garcia-Sierra F, Reynolds MR, Horowitz PM, Fu Y, Wang T, Cahill ME, Bigio EH, Berry RW, Binder LI. Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease. *Neurobiol Aging* 2005;26:1015–1022. [PubMed: 15748781]
29. Biernat J, Mandelkow EM, Schroter C, Lichtenberg-Kraag B, Steiner B, Berling B, Meyer H, Mercken M, Vandermeeren A, Goedert M, et al. The switch of tau protein to an Alzheimer-like state includes the phosphorylation of two serine-proline motifs upstream of the microtubule binding region. *EMBO J* 1992;11:1593–1597. [PubMed: 1563356]
30. Greenberg SG, Davies P, Schein JD, Binder LI. Hydrofluoric acid-treated tau PHF proteins display the same bio-chemical properties as normal tau. *J Biol Chem* 1992;267:564–569. [PubMed: 1370450]
31. Sawchenko, PE.; Yuan, ZF.; Laplante, F.; Rissman, RA.; Bittencourt, JC. Corticotropin-releasing hormone: Integration of adaptive responses to stress.. In: Squire, LR., editor. *Encyclopedia of Neuroscience*. Elsevier Ltd; 2009. p. 239-245.
32. Van Pett K, Viau V, Bittencourt JC, Chan RKW, Li H-Y, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE. Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. *J Comp Neurol* 2000;428:191–212. [PubMed: 11064361]
33. Horgan J, Miguel-Hidalgo JJ, Thrasher M, Bissette G. Longitudinal brain corticotropin releasing factor and somatostatin in a transgenic mouse (TG2576) model of Alzheimer's disease. *J Alzheimers Dis* 2007;12:115–127. [PubMed: 17917156]
34. De Souza EB, Whitehouse PJ, Kuhar MJ, Price DL, Vale WW. Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease. *Nature* 1986;319:593–595. [PubMed: 3003585]
35. Behan DP, Heinrichs SC, Troncoso JC, Liu XJ, Kawas CH, Ling N, De Souza EB. Displacement of corticotropin releasing factor from its binding protein as a possible treatment for Alzheimer's disease. *Nature* 1995;378:284–287. [PubMed: 7477348]
36. Sapolsky RM, Krey LC, McEwen BS. Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J Neurosci* 1985;5:1222–1227. [PubMed: 3998818]
37. Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocr Rev* 1986;7:284–301. [PubMed: 3527687]
38. Sapolsky RM. Glucocorticoids, stress, and their adverse neurological effects: relevance to aging. *Exp Gerontol* 1999;34:721–32. [PubMed: 10579633]
39. Watanabe Y, Gould E, McEwen BS. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Res* 1988;588:341–345. [PubMed: 1393587]
40. Davis KL, Davis BM, Greenwald BS, Mohs RC, Mathé AA, Johns CA, Horvath TB. Cortisol and Alzheimer's disease, I: Basal studies. *Am J Psychiatry* 1986;143:300–305. [PubMed: 3953862]
41. Masugi F, Ogihara T, Sakaguchi K, Otsuka A, Tsuchiya Y, Morimoto S, Kumahara Y, Saeki S, Nishide M. High plasma levels of cortisol in patients with senile dementia of the Alzheimer's type. *Methods Find Exp Clin Pharmacol* 1989;11:707–710. [PubMed: 2560104]
42. Green KN, Billings LM, Roozendaal B, McGaugh JL, LaFerla FM. Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci* 2006;26:9047–9056. [PubMed: 16943563]
43. Kang JE, Cirrito JR, Dong H, Csernansky JG, Holtzman DM. Acute stress increases interstitial fluid amyloid-beta via corticotropin-releasing factor and neuronal activity. *Proc Natl Acad Sci USA* 2007;104:10673–10678. [PubMed: 17551018]
44. Sawchenko PE, Brown ER, Chan RK, Ericsson A, Li HY, Roland BL, Kovacs KJ. The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. *Prog Brain Res* 1996;107:201–222. [PubMed: 8782521]
45. Watts AG. The impact of physiological stimuli on the expression of corticotropin-releasing hormone (CRH) and other neuropeptide genes. *Front Neuroendocrinol* 1996;17:281–326. [PubMed: 8812298]

46. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* 1997;20:78–84. [PubMed: 9023876]
47. Dayes CV, Buller KM, Crane JW, Xu Y, Day TA. Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. *Eur J Neurosci* 2001;14:1143–1152. [PubMed: 11683906]
48. Arendt T, Stieler J, Strijkstra AM, Hut RA, Rudiger J, Van der Zee EA, Harkany T, Holzer M, Hartig W. Reversible paired helical filament-like phosphorylation of tau is an adaptive process associated with neuronal plasticity in hibernating animals. *J Neurosci* 2003;23:6972–6981. [PubMed: 12904458]
49. Planel E, Richter KE, Nolan CE, Finley JE, Liu L, Wen Y, Krishnamurthy P, Herman M, Wang L, Schachter JB, Nelson RB, Lau LF, Duff KE. Anesthesia leads to tau hyper-phosphorylation through inhibition of phosphatase activity by hypothermia. *J Neurosci* 2007;27:3090–3097. [PubMed: 17376970]
50. Run X, Liang Z, Zhang L, Iqbal K, Grundke-Iqbal I, Gong CX. Anesthesia induces phosphorylation of tau. *J Alzheimers Dis* 2009;16:619–626. [PubMed: 19276556]
51. Iqbal K, Zaidi T, Thompson CH, Merz PA, Wisniewski HM. Alzheimer paired helical filaments: bulk isolation, solubility, and protein composition. *Acta Neuropathol* 1984;62:167–77. [PubMed: 6538056]
52. Rubenstein R, Kascsak RJ, Merz PA, Wisniewski HM, Carp RI, Iqbal K. Paired helical filaments associated with Alzheimer disease are readily soluble structures. *Brain Res* 1986;372:80–88. [PubMed: 2939924]