

The Role of Cdk5 as a Cell Cycle Suppressor in Post-mitotic Neurons

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Abstract Neurons of the central nervous system (CNS) leave the mitotic cycle when they leave the ventricular zone during embryonic and early postnatal development. Normally, they will never re-enter the cell cycle for the rest of the life of the organism. This rule is now known to be broken in many types of neurodegenerative disease. In these situations, nerve cells at risk for death have greatly elevated expression of cell cycle-related proteins; they have also been found to replicate their DNA. The existence of this pathway to neuronal death through the cell cycle raises the question of how a normal adult neuron suppresses cell division and places high therapeutic value on encouraging the activity of those proteins involved in the process. We have developed several lines of evidence that cyclin-dependent kinase 5 (Cdk5) is one such protein. To function as a cell cycle suppressor, Cdk5 must be located in the nucleus and it must be able to bind its cyclin-like activator, p35. Curiously, however, it does not need to retain kinase activity. Instead, its activity derives from its ability to sequester the E2F1 transcription factor and block its access to the DP1 co-factor, which greatly reduces binding to various cell cycle protein gene promoters thus inhibiting the cycle. Cdk5 stands as an excellent example of proteins whose functions are needed for the regulation of both differentiation and cell division. From this description of dual-specificity proteins, a concept is presented that the processes of division and differentiation are not so much independent as overlapping analog functions that must be balanced both during development and in the adult. The loss of balance would be expected to lead to neurodegeneration in a neuron or cancer in a less highly differentiated cell type.

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1 Introduction

The control of the cell cycle control is a critical capability for all forms of life. From the simplest unicellular organism to the most complex mammal, survival depends on the ability to divide when possible and to stop dividing when conditions require. For a single cell organism such as a yeast cell or a bacterium, the regulation seems fairly straightforward: divide when nutrients are present and stop when they are not. For a neuron in the CNS of an adult human being, the regulatory constraints are more complex. During the early neurogenic phases of development, the progenitors of the various neuronal cell types must divide quickly and consistently. During the final stages of neurogenesis, a pattern of asymmetric divisions is established to allow the generation of the specialized cells that will populate the various brain regions in the adult. As most neurons in the adult CNS complete their last cell division during this period of embryogenesis and early postnatal life, a final challenge faced by the typical adult neuron is how to hold the cell cycle in check for the rest of the life of the organism. The need to do so is clear, as many laboratories have shown that, in different neurodegenerative diseases, the appearance of cell cycle events in mature neurons – re-expression of cell cycle proteins and the replication of DNA – are a near certain harbinger of impending cell death. Because of this tight linkage to disease, we have been engaged in a search for proteins whose functions include cell cycle suppression in mature neurons. Surprisingly, each of these proteins has also been identified as performing a highly specific function in the physiological health of a normal neuron. The enzyme known as cyclin dependent kinase (Cdk5) is one such protein.

2 Cdk5 Plays an Important Role in Neuronal Development

Cdk5 was identified as a member of the Cdk5 family of cell cycle-related kinases by virtue of its ability to bind typical cyclins such as cyclin D (Xiong et al. 1992). It was soon assigned to the status of ‘atypical’ Cdk, as overexpression in cultured cells did not drive the cell cycle. Further, the binding of cyclins such as cyclin D did not enhance kinase activity. The gene for the kinase was subsequently shown to be expressed in the maturing regions of the embryonic nervous system, not in the ventricular zone (Tsai et al. 1993), which further re-enforced the idea that Cdk5 was not a true cell cycle kinase. Rather, reduced Cdk5 activity was found to impede neuronal process growth (Nikolic et al. 1996). Subsequent studies of an engineered null mutation of Cdk5 showed that embryos lacking Cdk5 activity die at the end of embryogenesis, with massive failures of neuronal cell migration and cytological maturation (Gilmore et al. 1998; Ohshima et al. 1996). Cdk5 has two potential cyclin-like activators, p35 and p39. While the genetic inactivation of one of these has cortical lamination defects (Chae et al. 1997), elimination of both genes produces a spectrum of developmental defects that is indistinguishable from that

seen in the Cdk5 knockout embryos (Ko et al. 2001). This finding was yet another piece of evidence identifying Cdk5 as a kinase involved in differentiation and maturation rather than in cell cycle regulation. This differentiation activity of Cdk5, in particular its role in migration and neuronal development, has been largely ascribed to its ability to phosphorylate a variety of cytoskeletal proteins ranging from tau to neurofilament (Hosoi et al. 1995; Veeranna et al. 1995).

3 Cdk5 Serves Important Functions in the Regulation of Synaptic Function

As the roles of Cdk5 in post-mitotic neuronal activity were explored further, several labs reported evidence for its involvement in the phosphorylation of numerous synaptic proteins (Bibb 2003; Cheng and Ip 2003; Fischer et al. 2003; Smith and Tsai 2002; Tan et al. 2003). The list of synaptic proteins whose activity is modified by Cdk5 phosphorylation has grown year by year. The synaptic vesicle phosphoprotein, synapsin I, was identified early on (Matsubara et al. 1996). Later, proteins such as Munc-18, dynamin, amphiphysin and many others were also shown to be synaptic targets. Functional changes in synaptic functions are clearly seen when these phosphorylations are prevented or enhanced. As only one example, Cdk5 phosphorylates TrkB, and blocking Cdk5 activity essentially blocks brain-derived neurotrophic factor (BDNF)-triggered dendritic growth in primary hippocampal neurons (Cheung et al. 2007).

4 Cdk5 is a Cell Cycle Suppressor in Post-mitotic Neurons

Inspired by the appearance of Cdk5 and its cyclin-like activator, p35, immediately after the neuroblasts of the CNS leave the ventricular zone, my laboratory has explored the possible role that Cdk5 might play a role in inhibiting rather than advancing the cell cycle. The first suggestion that this might be true came from a re-examination of the phenotype of *Cdk5*^{-/-} embryonic cortical neurons both in vivo and in vitro (Cicero and Herrup 2005). The migration of most Cdk5-deficient neurons is abnormal, but even those neurons that do achieve normal or near normal positions are defective in their development. This is made most apparent by examining their biochemical maturation. Nestin, a commonly used marker of mitotic neuronal progenitor cells, is robustly expressed in *Cdk5*^{-/-} neurons, even in those that have migrated substantial distances from the ventricular zone. In keeping with this biochemical evidence of an immature state of existence, the levels of Map2 staining, a commonly used marker of mature or maturing dendrites, is virtually absent. This same block in maturation can be found when neurons from mutant and wild type embryos are cultured in vitro. Wild type embryos lose the

immature marker, TuJ1, and increase expression of Map2; *Cdk5*^{-/-} neurons never lose their TuJ1-positive status and fail to show any increase in Map2 staining (Cicero and Herrup 2005). This biochemical evidence for a blocked maturation and persistent precursor status was also seen in the ability of *Cdk5*^{-/-} neurons to regulate their cell cycle. Both in vivo and in vitro, wild type neurons show little or no evidence of cell cycle involvement, whereas *Cdk5*^{-/-} neurons cycle and these cycling neurons can be shown to die (Cicero and Herrup 2005).

5 Cdk5 Inhibits the Cell Cycle in a Kinase-Independent Fashion

The loss of cell cycle control in the Cdk5-deficient neurons suggests that phosphorylation of one or more Cdk5 target substrates is normally required to hold the neuronal cell cycle in check. A potential candidate for just such a substrate was the tumor suppressor gene, retinoblastoma (RB), a recognized target of Cdk5 (Hamdane et al. 2005; Lee et al. 1997). This hypothesis, however, proved incorrect. Culturing of wild type neurons in the presence of roscovitine, a Cdk5 inhibitor, reduced their dendritic complexity but did not release the cell cycle (Cicero and Herrup 2005). Further transfection of a kinase-dead (KD) form of Cdk5 into *Cdk5*^{-/-} neurons was completely effective in restoring full cell cycle control to the mutant neurons (Zhang et al. 2008).

This unexpected set of observations prompted us to examine the behavior of Cdk5 during a typical cell cycle. We verified earlier findings that total Cdk5 levels do not change with cell cycle arrest (by serum withdrawal) or release (by restoration of serum). The subcellular localization of Cdk5, however, changes dramatically. In the arrested cells, Cdk5 is predominantly nuclear; in the cycling cells, Cdk5 is predominantly cytoplasmic (Zhang et al. 2008), suggesting that it was Cdk5 in the nucleus that was the key to neuronal cell cycle suppression. We validated this finding by showing that both wild type Cdk5 and Cdk5 with a nuclear localization signal (NLS) were capable of arresting the incorporation of BrdU in *Cdk5*^{-/-} neurons. By contrast, Cdk5 with a nuclear export signal (NES) was unable to rescue the mutant cell cycle. To prove that nuclear export was the instigating factor, we stimulated wild type neurons to cycle and die with fibrillarized A β ₁₋₄₂ peptide or A β -stimulated microglial conditioned medium (Wu et al. 2000). In the presence of the nuclear export inhibitor, leptomycin-B, the A β peptide could not induce a neuronal cell cycle (Zhang et al. 2008).

These experiments identified the importance of nuclear localization for Cdk5 cell cycle suppression but did not speak directly to the requirements for kinase activity. To approach this problem, we turned first to the neuroblastoma cell line, N2a. When wild type Cdk5 is transfected into log phase N2a cells, the level of BrdU incorporation decreases by five to ten-fold. Nuclear Cdk5 (Cdk5-NLS) is equally

effective, but Cdk-NES was inactive. With Cdk5-NES, cell cycle activity was as high as with control GFP-only transfections (Zhang et al. 2010). We repeated these experiments with a KD form of Cdk5 (Nikolic et al. 1996; Tsai et al. 1994) and validated our earlier findings. KD Cdk5 was as effective as wild type at inhibiting the N2a cell cycle. As with the wild type protein, KD-Cdk5-NLS (nuclear) was a cell cycle suppressor whereas KD-Cdk5-NES (cytoplasmic) was not. This effect was not restricted to neuroblastoma cells. The same set of findings was seen in the ventricular zone of the E14.5 mouse embryo after in utero electroporation of GFP-Cdk5 or its KD isoform (Zhang et al. 2010).

6 Cdk5 Inhibits the Cell Cycle by Sequestering E2F1

These findings leave unanswered the mechanistic question of how nuclear Cdk5 is able to so effectively arrest the cell cycle. We turned to the E2F1 protein for answers. We first used an electrophoretic mobility shift assay (EMSA) to show that, in the presence of Cdk5, occupancy of E2F1 response elements by E2F1 was dramatically reduced (Zhang et al. 2010). We subsequently demonstrated that E2F1 is physically associated with Cdk5 both in situations where the two proteins are overexpressed – in cell lines or neurons – and when co-immunoprecipitations are performed from whole brain.

The association of Cdk5 and E2F1 led us to ask whether there might be any other proteins associated with the complex. We had determined that Cdk5 did not require its kinase activity to block the cycle (Cicero and Herrup 2005; Zhang et al. 2008), but we wished to determine whether Cdk5 required the presence of p35 to form a complex with E2F1. We engineered a p35 binding-deficient Cdk5 isoform (Tarricone et al. 2001) and repeated our N2a and in utero electroporation experiments. To our surprise, the p35 binding-deficient Cdk5 ($\Delta 35$) was ineffective in blocking the cell cycle, suggesting that p35 was a part of the Cdk5-E2F1 complex. We verified this suggestion by co-immunoprecipitation of p35 and E2F1. The critical nature of the presence of p35 for the formation of the complex was shown by the observation that Cdk5- $\Delta 35$ is unable to associate with E2F1 when the two are overexpressed in the presence of p35.

Further insights into the dynamics of the complex were achieved when we examined the behavior of the E2F1 DNA binding co-factor, DP1. In the absence of DP1, the cell cycle stimulatory activity of E2F1 is decreased significantly (Bandara et al. 1993). We showed that the presence of Cdk5 and p35 in the nucleus successfully displaces DP1 from its binding to E2F1, effectively diminishing the efficiency of E2F1 as a promoter binding element. This finding helps to explain the EMSA findings as well as the variety of cell cycle effects reported above. Curiously, when the same combination of proteins was expressed in the cytoplasm, the situation reversed. Now DP1 and E2F1 remained tightly bound whereas Cdk5 was excluded. Clearly there are additional insights left to be had.

7 The Implications of the Central Role of Cdk5 in Neuronal Cell Cycle Suppression

The story of Cdk5 has several important lessons to teach us about the relationships between cancer and neurodegeneration – the subject of this IPSEN meeting. It teaches us that Cdk5 may well be an atypical Cdk, but not because it has *no* role in the cell cycle. It is atypical primarily because its role is to suppress rather than encourage the cell cycle. It is also atypical because it achieves this activity without the use of its kinase activity yet it requires association with its cyclin-like partner, p35. The implication of these characteristics is that the well-known association of the Cdk family of kinases with their activating subunits (the cyclins) and other inhibitory elements (the Cip/Kip and Ink families of Cdk inhibitors) may well have functions beyond changing the extent to which Cdk target substrates become phosphorylated. The associations themselves may prove a significant part of the story of any Cdk.

Finding a role for Cdk5 in cell cycle activity should also remind us of a deeper lesson: cell cycle and cell differentiation are highly inter-related activities and they are often regulated by the same protein. An early example of this was the discovery of a role for the RB tumor suppressor in neuronal differentiation as well as cell cycle control (Lee et al. 1994). Perhaps the most recent example is that of the protein that is mutated in ataxia-telangiectasia, ATM. Long recognized as a cell cycle checkpoint response protein during the DNA damage response, ATM also plays a major role in maintaining the complex economy of neuronal synaptic vesicle functioning (Li et al. 2009). Many other examples of cell cycle proteins functioning in the development and differentiation of neurons have been discovered (reviewed in Frank and Tsai 2009). Cdk5 fits well into this category of dual-specificity proteins.

The discovery of growing numbers of proteins in this class suggests that it may be time to loosen the normally binary view of cell cycle and cell differentiation. A neuron that has left the ventricular zone of the CNS is viewed as permanently post-mitotic. Before it left the ventricular zone, it was dividing; after it left, it was differentiating. A more appropriate description of this event might be to assign both division and differentiation an analog value; this idea is crudely diagrammed in Fig. 1. Beginning with fertilization, the primary activity of the cells of the zygote is division. The mass of the organism must increase. As development proceeds (upwards in the figure), cells move from the totipotency and rapid cell cycle kinetics of the embryonic stem cell through more restricted progenitor cell phases and finally to a stage where terminal differentiation occurs. In most cell types, as this process unfolds, the pressure to divide decreases, as is shown by the lightening and narrowing of the red wedge labeled “Division”. At the same time, the cells are increasingly expressing their differentiation program, indicated by the darkening and the broadening of the green wedge labeled “Differentiation” and the nervous system-specific labels such as “synapse”. If we add the dimension of time specifically to this progression, a single neuron can trace its heritage through this lineage, as shown in Fig. 2.

Fig. 1 The program of development can be seen as two analog processes – division and differentiation – that wax and wane inversely to each other. As development proceeds (from the *bottom* to the *top* of the figure), the two processes change in relative proportion to each other, but neither is ever completely eliminated. ES, embryonic stem

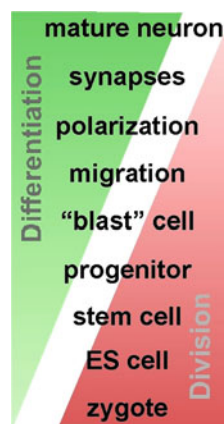
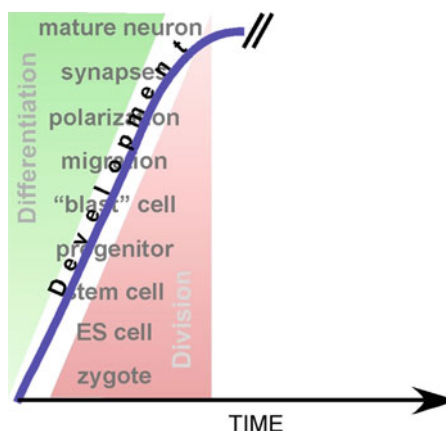


Fig. 2 The trajectory of the lineage of one adult neuron through the progression shown in Fig. 1. Developmental time is shown as the *rightward arrow*



Both cancer and certain forms of neurodegenerative disease can be incorporated into this scheme (Fig. 3). In cancer, certain stem cells or blast-like cells lose their differentiation properties and slip back towards a state where cell division is more aggressive and hence more likely. The description of how this occurs through both genetic and epigenetic means will be left to others in this symposium. For neurodegeneration, the scheme reminds us that the populations of neurons that are at risk for death in diseases such as Alzheimer's, Parkinson's and others display substantial evidence of re-activating the machinery of the cell cycle. In the scheme shown in Fig. 3, this process can be envisioned as a mature neuron slipping downwards; the extent of differentiation decreases while the probability of division increases. At some tipping point, the neuron, while still highly differentiated, triggers a cell cycle, which is ultimately a lethal decision and the result is neurodegenerative disease.

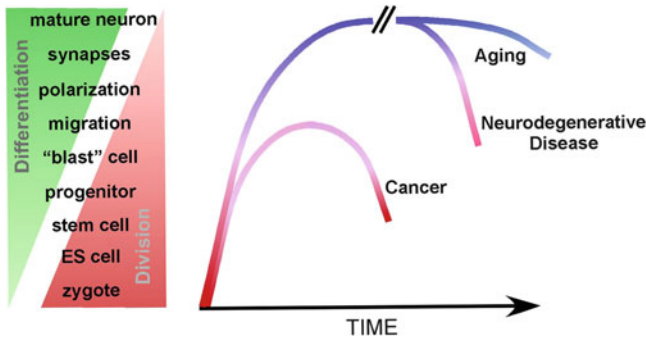


Fig. 3 On this continuum, neurodegenerative disease can be seen as a retreat from the highly differentiated state of the mature neuron, eventually leading to an enhanced susceptibility to cycle re-initiation and cell death. Cancer can be viewed as a similar failure, but usually from a less differentiated blast cell or stem-like state. The trajectory is similar, however: a descent into a state where susceptibility to the re-initiation of the cell cycle is enhanced

This concept of differentiation and division as two competing forces in development is not a new one. What the emerging evidence suggests, however, is that the competition is never ending. A differentiated cell, such as a neuron, must constantly and actively promote differentiation and suppress cell division. It may be speculated that proteins, such as Cdk5, have evolved to efficiently promote both processes. Taking a cue from these dual-specificity proteins, it may be that the most effective strategies for combating both cancer and neurodegeneration are those that involve both the promotion of differentiation and the inhibition of division.

References

- Bandara LR, Buck VM, Zamanian M, Johnston LH, La Thangue NB (1993) Functional synergy between DP-1 and E2F-1 in the cell cycle-regulating transcription factor DRTF1/E2F. *Embo J* 12:4317–4324
- Bibb JA (2003) Role of Cdk5 in neuronal signaling, plasticity, and drug abuse. *Neurosignals* 12: 191–199
- Chae T, Kwon YT, Bronson R, Dikkes P, Li E, Tsai L-H (1997) Mice lacking p35, a neuronal specific activator of Cdk5, display cortical lamination defects, seizures, and adult lethality. *Neuron* 18:29–42
- Cheng K, Ip NY (2003) Cdk5: a new player at synapses. *Neurosignals* 12:180–190
- Cheung ZH, Chin WH, Chen Y, Ng YP, Ip NY (2007) Cdk5 is involved in BDNF-stimulated dendritic growth in hippocampal neurons. *PLoS Biol* 5:e63
- Cicero S, Herrup K (2005) Cyclin-dependent kinase 5 is essential for neuronal cell cycle arrest and differentiation. *J Neurosci* 25:9658–9668
- Fischer A, Sananbenesi F, Spiess J, Radulovic J (2003) Cdk5: a novel role in learning and memory. *Neurosignals* 12:200–208
- Frank CL, Tsai LH (2009) Alternative functions of core cell cycle regulators in neuronal migration, neuronal maturation, and synaptic plasticity. *Neuron* 62:312–326

- Gilmore EC, Ohshima T, Goffinet AM, Kulkarni AB, Herrup K (1998) Cyclin-dependent kinase 5-deficient mice demonstrate novel developmental arrest in cerebral cortex. *J Neurosci* 18: 6370–6377
- Hamdane M, Bretteville A, Sambo AV, Schindowski K, Begard S, Delacourte A, Bertrand P, Buee L (2005) p25/Cdk5-mediated retinoblastoma phosphorylation is an early event in neuronal cell death. *J Cell Sci* 118:1291–1298
- Hosoi T, Uchiyama M, Okumura E, Saito T, Ishiguro K, Uchida T, Okuyama A, Kishimoto T, Hisanaga S (1995) Evidence for cdk5 as a major activity phosphorylating tau protein in porcine brain extract. *J Biochem (Tokyo)* 117:741–749
- Ko J, Humbert S, Bronson RT, Takahashi S, Kulkarni AB, Li E, Tsai LH (2001) p35 and p39 are essential for cyclin-dependent kinase 5 function during neurodevelopment. *J Neurosci* 21: 6758–6771
- Lee EY, Hu N, Yuan SS, Cox LA, Bradley A, Lee WH, Herrup K (1994) Dual roles of the retinoblastoma protein in cell cycle regulation and neuron differentiation. *Genes Dev* 8: 2008–2021
- Lee K, Helbing C, Choi K, Johnston R, Wang J (1997) Neuronal Cdc2-like kinase (Nclk) binds and phosphorylates the retinoblastoma protein. *J Biol Chem* 272:5622–5626
- Li J, Han YR, Plummer MR, Herrup K (2009) Cytoplasmic ATM in neurons modulates synaptic function. *Curr Biol* 19:2091–2096
- Matsubara M, Kusubata M, Ishiguro K, Uchida T, Titani K, Taniguchi H (1996) Site-specific phosphorylation of synapsin I by mitogen-activated protein kinase and Cdk5 and its effects on physiological functions. *J Biol Chem* 271:21108–21113
- Nikolic M, Dudek H, Kwon Y, Ramos Y, Tsai L (1996) The cdk5/p35 kinase is essential for neurite outgrowth during neuronal differentiation. *Genes Dev* 10:816–825
- Ohshima T, Ward JM, Huh CG, Longnecker G, Veeranna Pant HC, Brady RO, Martin LJ, Kulkarni AB (1996) Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. *Proc Natl Acad Sci USA* 93:11173–11178
- Smith DS, Tsai LH (2002) Cdk5 behind the wheel: a role in trafficking and transport? *Trends Cell Biol* 12:28–36
- Tan TC, Valova VA, Malladi CS, Graham ME, Berven LA, Jupp OJ, Hansra G, McClure SJ, Sarcevic B, Boadle RA, Larsen MR, Cousin MA, Robinson PJ (2003) Cdk5 is essential for synaptic vesicle endocytosis. *Nat Cell Biol* 5:701–710
- Tarricone C, Dhavan R, Peng J, Areces LB, Tsai LH, Musacchio A (2001) Structure and regulation of the CDK5-p25(nck5a) complex. *Mol Cell* 8:657–669
- Tsai LH, Takahashi T, Caviness VS Jr, Harlow E (1993) Activity and expression pattern of cyclin-dependent kinase 5 in the embryonic mouse nervous system. *Development* 119:1029–1040
- Tsai LH, Delalle I, Caviness VS Jr, Chae T, Harlow E (1994) p35 is a neuronal-specific regulatory subunit of cyclin-dependent kinase 5. *Nature* 271:419–423
- Veeranna SKT, Link WT, Jaffe H, Wang J, Pant HC (1995) Neuronal cyclin-dependent kinase-5 phosphorylation sites in neurofilament protein (NF-H) are dephosphorylated by protein phosphatase 2A. *J Neurochem* 64:2681–2690
- Wu Q, Combs C, Cannady SB, Geldmacher DS, Herrup K (2000) Beta-amyloid activated microglia induce cell cycling and cell death in cultured cortical neurons. *Neurobiol Aging* 21: 797–806
- Xiong Y, Zhang H, Beach D (1992) D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. *Cell* 71:505–514
- Zhang J, Cicero SA, Wang L, Romito-Digiacomo RR, Yang Y, Herrup K (2008) Nuclear localization of Cdk5 is a key determinant in the postmitotic state of neurons. *Proc Natl Acad Sci USA* 105:8772–8777
- Zhang J, Li H, Yabut OY, Fitzpatrick H, D’Arcangelo G, Herrup K (2010) Cdk5 suppresses the neuronal cell cycle by disrupting the E2F1-DP1 complex. *J Neurosci* 30:5219–5228

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