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The role of NR4A transcription factors in memory formation

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Abstract

In various physiological contexts, *Nr4a* genes are transcribed in response to external stimuli as part of an immediate early response that initiates a cascade of gene expression ultimately leading to distinct physiological outcomes in each of these contexts. The signaling pathway that initiates *Nr4a* gene expression in most of these contexts consists of elevated intracellular cAMP activating PKA, which in turn leads to phosphorylation of CREB and new gene synthesis. This cAMP-PKA-CREB pathway is a central molecular pathway in the formation of a long-term memory. Indeed, learning induces *Nr4a* family gene expression, and long-term memory formation requires at least two waves of transcription after learning, suggesting that NR4A nuclear receptors may contribute to the second of these waves of gene expression. In this article, we review insights gained in other physiological contexts regarding *Nr4a* function and regulation and highlight how these lessons can be applied to the study of memory formation.

Keywords

Memory; Consolidation; Hippocampus; Nuclear Receptor; Transcription

Introduction

The NR4A family of transcription factors consists of three members, NR4A1 (NGFI-B/Nur77/TR3), NR4A2 (NURR1/HZF-3/RNR1), and NR4A3 (NOR1/MINOR/TEC), each of which was identified roughly 20 years ago in studies examining activity-dependent processes in neurons or during neuronal differentiation [46,69,76]. Yet, the last twenty years of research has demonstrated that these transcription factors contribute to a broad array of processes including energy metabolism, atherosclerogenesis, T-cell receptor (TCR)-mediated apoptosis, inflammatory response, hypothalamic-pituitary axis (HPA) regulation and reproductive processes [24,55,66,89,109,111,119]. The unifying principle that emerges from these studies is that NR4A transcription factors are the first-wave transcriptional response to environmental cues that cause diverse, but critical, changes in cellular physiology. A common set of signaling pathways, including PKA and MAPK activity, converge to induce the expression of *Nr4a* genes after an environmental stimulus. The transcriptional activity and genetic targets of NR4A family members are further refined by

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post-translational modifications and protein-protein interactions that specify the second-wave transcriptional response to the stimulus. The formation of a long-term memory requires PKA, MAPK and two waves of transcription in the hours following learning [28], suggesting that, in analogy to the role of NR4A elsewhere, NR4A transcription factors may act to shape the second of these memory promoting transcriptional waves. Only recently have the insights learned about NR4A family function from other systems been applied to examine how these transcription factors might contribute to the activity-dependent processes that underlie memory formation.

Signaling cascades important for memory storage regulate Nr4a gene expression

The three genes encoding NR4A transcription factors, *Nr4a1*, *Nr4a2*, and *Nr4a3*, are immediate early genes that can be induced by a variety of stimuli, including activation of G protein-coupled receptors, tyrosine receptor kinases and direct activation of intracellular protein kinase pathways. The specific intracellular signaling cascades responsible for *Nr4a* gene induction depends on the stimulus and cell type involved, and in many of these contexts all three *Nr4a* genes are induced in parallel. For instance, the expression of all three *Nr4a* genes increases after lipopolysaccharide treatment of macrophages, a process that appears to be mediated by the NFκ-B signaling pathway [80]. In fibroblasts stimulated with growth factors, increased *Nr4a* family gene expression requires mitogen- and stress-activated kinases (MSKs) and is blocked by a dominant negative form of the transcription factor cAMP-response element binding protein (CREB) [19]. Consistent with the regulation of *Nr4a* family members by CREB, increased expression of *Nr4a* family genes was observed in the neuron-like PC12 cell line expressing a constitutively active CREB derivative (VP16-CREB). This increase in expression was mimicked by increasing cAMP with forskolin and activation of protein kinase A (PKA), which leads to phosphorylation of CREB and recruitment of the coactivator CREB-binding protein (CBP) [44]. The effect of forskolin on *Nr4a* gene expression in PC12 cells was blocked by overexpression of a dominant negative form of CREB [23]. Additionally, the induction of *Nr4a* gene expression in skeletal muscle by β-adrenergic receptor activation is mediated by elevated cAMP production, PKA activation, MAPK signaling, and CREB-mediated transcription [79]. PKA and CaMKII contribute to *Nr4a* gene expression in corticotroph-derived cells, but MAPK activity is not necessary for *Nr4a* gene expression in these cells [42]. In the neuroblastoma cell line N2A, treatment with MEK inhibitors impairs the induction of *Nr4a2* gene expression by forskolin, whereas MEK inhibitor treatment enhances the induction of *Nr4a2* by forskolin in the glioblastoma line C6 [49]. In both of these cell lines, PKA activity is required for *Nr4a2* expression [49]. Thus, the contribution of MAPK and NFκB signaling to the induction of *Nr4a* family gene expression depends on the cell type and signal examined, but the involvement of the cAMP-PKA-CREB pathway is a common feature (Figure 1). The signals that induce *Nr4a* family gene expression differ slightly among different cellular contexts, but the molecular pathways that drive *Nr4a* gene expression in these various contexts (NFκB, MAPK, CaMKII, PKA, and CREB) coincide strikingly well with molecular mechanisms thought to underlie long-term memory formation [28], suggesting the possibility that NR4A transcription factors may also contribute to the cascade of gene expression that leads to memory storage (Figure 1).

NR4A proteins are ligand-independent nuclear receptors

The three NR4A transcription factors compose a single subfamily within the nuclear receptor superfamily [9,62]. Nuclear receptors have a common structure consisting of a weakly conserved amino-terminal A/B region containing the activation function (AF)-1 transactivation domain, a highly conserved DNA-binding domain (DBD), and a highly

conserved carboxy-terminal ligand-binding domain (LBD) (Figure 2A). Classically, nuclear receptor activation is accomplished by the binding of a lipophilic ligand in a hydrophobic pocket within the LBD. Ligand binding causes a conformational shift that regulates the recruitment of coactivators and corepressors to the AF-2 transactivation region within the LBD [27]. In contrast, NR4A activity is independent of the LBD [77,103], relying instead on the AF-1 domain [15,57,58,102,103]. Because they have no known ligand, NR4A proteins are described as orphan nuclear receptors. The presence of this unusual mechanism of action is supported by the fact that the hydrophobic coactivator-binding cleft observed in the LBD of other NRs is replaced in the NR4A subfamily with a hydrophilic surface that does not support classical coactivator recruitment to AF-2, whereas the AF-1 domain interacts directly with the coactivators SRC-2, p300 and PCAF [103]. Additionally, the LBD of NR4A proteins adopts a fold resembling the ligand bound state of other nuclear receptors, and bulky side-chain hydrophobic residues are tightly packed in place of the ligand-binding pocket [101]. Thus, a major difference between NR4A and classical nuclear receptors is ligand-independent regulation, and activity of the NR4A family is regulated at the level of gene expression and protein stability [66]. Given the apparent role of NR4A proteins as a multi-purpose response system to environmental stimulation in various cellular contexts, it is perhaps fitting that these proteins do not require a specific ligand for activation.

NR4A proteins regulate target gene expression as monomers, homodimers, and heterodimers

The C-terminal LBD of NR4A proteins have lost ligand-binding and transactivation functions, but this region retains at least one major role: protein-protein dimerization (Figure 2B). Dimerization is a feature of many members of the nuclear receptor family, where the DNA binding sites for dimers, called hormone response elements (HRE), typically consist of two direct repeats of the canonical half-site AGGTCA separated by differing length nucleotide spacers for different nuclear receptor partner combinations [60, 86, 115]. Initial studies using a yeast genetic selection approach, identified the binding site of NR4A *monomers* as the NGFI-B response element (NBRE) consisting of the core nuclear receptor binding site preceded by two additional adenosines (AAAGGTCA)[106]. Two highly conserved zinc finger domains (>90%) within the NR4A DBD mediate the sequence-specific interaction with the core AGGTCA, but a separate domain, the A-box within the C-terminal extension (CTE), is responsible for interaction with the two extended A-T base pairs [107]. Each of the three NR4A family members is capable of activating transcription by binding the NBRE sequence in reporter systems [17, 78, 117]. Activation from certain NBRE variants has been reported to be impaired by co-expression of combinations of NR4A members [72], suggesting that NR4A family members may have opposing actions at monomer-regulated target genes. Later work identified a variant of the Nur response element (NurRE) as a functional binding site for NR4A1 homodimers in the promoter of POMC [88]. The NurRE sequence resembles the NBRE site identified by Wilson and colleagues [106] but is instead composed of an everted repeat of the octanucleotide AAAGGTCA separated by 6 base pairs [58] (Figure 2B). Interestingly, different NR4A family members combine to form heterodimers that synergistically enhance NurRE-mediated gene expression to a greater extent than homodimers alone [58]. An additional level of complexity arises from dimerization of NR4A1 and NR4A2, but not NR4A3, with the alpha and gamma isoforms of RXR [1, 85, 92], a retinoid receptor that acts as a partner to a variety of nuclear receptor [61, 115]. NR4A-RXR heterodimers bind to DR5 sequences composed of direct repeats of the core nuclear receptor half-site AGGTCA followed by the NBRE site separated by a 5-bp spacer between the core half-sites (Figure 2B). In addition to binding DR5 sequences through RXR and NR4A DBDs, these dimers may be recruited to NBRE sequence independently of the RXR DBD [25]. As a partner to several nuclear receptors,

RXR is often a “silent” partner, because RXR ligand-binding capacity can be suppressed by these interactions [25, 43]. In contrast, NR4A-RXR heterodimers are responsive to the RXR ligand, 9-*cis* retinoic acid [85, 117]. Strikingly, this ligand dependence is bestowed on NR4A-mediated activation of NBRE-regulated sequences even in the absence of the DBD of RXR [25]. Thus, the ability of NR4A nuclear receptors to dimerize greatly increases the potential range of targets and means of NR4A regulation (Figure 2B). Monomeric activation of NBRE can be achieved by individual NR4A members, or in a retinoic acid-sensitive manner by heterodimers with RXR at NBRE or DR5 sequences. Further, NurRE-regulated genes can be activated to varying levels depending on whether they are bound by homodimers or heterodimers composed of NR4A factors [58]. This richness of target sequence selection may be one factor that allows the NR4A family of transcription factors to be a general mechanism that engages distinct responses to stimuli in differing cellular contexts.

The transcriptional activity of NR4A proteins is regulated by post-translational modification

As discussed above, signaling cascades that are important for memory storage regulate expression of the genes encoding NR4A nuclear receptors. These signaling cascades also regulate the activity of the newly synthesized NR4A proteins by means of post-translational modifications. Shortly after the discovery of NR4A1, this protein was determined to be heavily phosphorylated by cellular stimulation [22], and the extent of this phosphorylation depends on the stimulus [29,38]. For instance, the MAPK pathway member RSK is capable of phosphorylating NR4A1 in the C-terminal extension of the DBD (Figure 2A)[108], an event that has been associated with reduced DNA binding capacity [32,53], impaired transactivation ability [38,53], and exclusion of NR4A1 from the nucleus [65]. Although this phosphorylation event appears to be mediated by MAPK members RSK and MSK in certain cell types [20,108], AKT may carry out this role in other contexts [65,82]. Thus, in certain contexts the MAPK pathway limits the ability of newly synthesized NR4A to drive gene expression by leading to nuclear export (Figure 1). In contrast, MAPK activity is required for full induction of NR4A reporters by cAMP in corticotrophs [42]. This regulation was not reflected in *Nr4a* transcript levels, but instead appears to be mediated by MAPK-dependent phosphorylation enhancing the activity of NR4A transcription factors [42]. Another pathway known to be important for memory formation, cAMP-PKA signaling, is capable of rapidly reversing inhibitory phosphorylation in the DBD of NR4A, enhancing the DNA-binding and transcriptional activity achieved by NR4A dimers but not monomers [57]. Perhaps this interplay between MAPK- and PKA-mediated post-translational modifications allows the activity of stimulus-induced NR4A family members to be fine tuned for particular responses. A recent study found that NR4A1 is acetylated in the LBD by the histone acetyltransferase p300 [37]. This work goes on to suggest that this acetylation event blocks the degradation of NR4A1 protein leading to enhanced expression of NR4A1 target genes [37]. Thus, in addition to regulation of *Nr4a* gene expression, signaling molecules involved in long-term memory formation such as PKA and MAPK [28] also regulate the subcellular localization and activity of newly synthesized NR4A proteins.

NR4A family members are expressed in distinct but overlapping regions of the brain

The genes encoding NR4A nuclear receptors were each cloned from neurons or neuronal precursors and are expressed throughout the brain with both overlapping and distinct regions of expression [94,112,118]. Indeed, anatomical profiling of nuclear receptor expression patterns shows that all three of the *Nr4a* genes fall into a cluster that is expressed

predominantly in the central nervous system (Figure 3) [9]. The most striking example of distinct expression profiles is that of *Nr4a2*, the only *Nr4a* family member expressed at high levels in the substantia nigra pars compacta and ventral tegmental area [94,112,118]. In contrast, expression of *Nr4a1*, *Nr4a2*, and *Nr4a3* overlap in areas CA1 and CA3 of the hippocampus [112], although the relative abundance in these areas differs for the three genes. Because the relative levels of NR4A family members can impact NR4A-mediated transcriptional activity [58,72], these differences in expression may lead to very different target gene activation profiles in different brain regions.

NR4A2 contributes to the development of midbrain dopaminergic circuitry

The bulk of research on the role of NR4A nuclear receptors in the central nervous system has focused on the role of NR4A2 in the dopaminergic system [87]. Based on high expression of NR4A2 in tyrosine hydroxylase-expressing (TH⁺) dopaminergic neurons found in the ventral tegmental area (VTA) and substantia nigra pars compacta (SN), the role of this transcription factor in the development of dopaminergic was examined using knockout mouse lines [14,95,116]. Homozygous null mice (*Nr4a2*^{-/-}) die shortly after birth, and prenatal development of the dopaminergic system is strongly disrupted by this mutation. Specifically, markers of dopaminergic cell fate fail to be expressed in ventral dopaminergic precursors, followed by the death of these precursors around birth [14,95,116]. As would be expected, this loss of dopaminergic neurons leads to a substantial reduction in dopamine levels in the striatum of newborn *Nr4a2*^{-/-} pups, and a reduction in dopamine was also observed in heterozygous null (*Nr4a2*^{+/-}) pups [14,95,116]. Recently, a conditional knockout system (see [28] for review) was used to show that *Nr4a2* plays a cell-autonomous role in the development of dopaminergic neurons and is required for the maintenance, in addition to the induction, of this cell lineage [36]. Work on the dopaminergic system provides the most complete characterization of how an NR4A transcription factor functions in the brain, but the breadth of NR4A functions in other tissues suggests that this function is unlikely to be the only role of NR4A2 within the brain.

NR4A2 function may contribute to the etiology of schizophrenia

The role of NR4A2 in the development and maintenance of dopaminergic neurons in the VTA and SN has received considerable attention because of the involvement of the dopaminergic system in neuropsychiatric disorders, such as addiction [41], depression [73], Parkinson's Disease [52], and schizophrenia [40]. Intriguingly, missense mutations that reduce the activity of NR4A2 have been identified in schizophrenic patients but not controls [10,16]. These mutations represent a small fraction of the patients analyzed [10,16], and some studies have failed to find an association between *Nr4a* genes and schizophrenia [13], but given the complex etiology of schizophrenia it is perhaps not surprising that any single mutation would be found in only a small portion of patients [40]. Further support for a role of NR4A function in schizophrenia is provided by the observation that both *Nr4a1* and *Nr4a2* expression is reduced in postmortem cortical tissue from schizophrenic patients [113]. Interpretation of this observation should be tempered by the fact that treatment with antipsychotic drugs can alter the expression of *Nr4a* genes [7,45,56,105]. Acute treatment with antipsychotic drugs typically increases *Nr4a* gene expression, but in some brain regions chronic treatment may reduce expression [7]. Increased *Nr4a* expression in the striatum and nucleus accumbens appears to be maintained without sensitization during chronic antipsychotic treatment [7,105], suggesting that these changes could contribute to the efficacy of neuroleptic drugs.

Further support for a relationship between NR4A function and schizophrenia has come from studies of *Nr4a2* heterozygous null (*Nr4a2*^{+/-}) mice. This *Nr4a2* mutant mouse model has

no apparent reduction in midbrain TH⁺ dopaminergic neurons at 2–3 months of age [47], but displays an age-dependent decrease in TH⁺ neurons and striatal dopamine levels between 10 and 15 months of age [35]. At birth, dopamine levels in the brains of *Nr4a2*^{+/-} mice are reduced significantly (40–50%) [14,95,116]. At 5 months of age, dopamine levels have normalized to wild-type levels in the striatum, but reduced dopamine levels are still observed in some regions, such as the prefrontal cortex and hippocampus of male mice [90]. *Nr4a2*^{+/-} mice have been proposed as a mouse model of schizophrenia because they display behaviors that are thought to be endophenotypes of schizophrenia and are responsive to antipsychotic drugs. For instance, these mice display hyperactivity in novel environments and an enhanced response to the psychotomimetic drug PCP. The hyperactivity phenotype is blocked by haloperidol treatment [90]. Additionally, *Nr4a2*^{+/-} mice have deficits in long-term memory in the passive avoidance task [90], which is consistent with the observation that schizophrenic patients often present with cognitive deficits [74]. These studies suggest that *Nr4a* family function may be involved in specific endophenotypes of schizophrenia, but given the constitutive nature of the mutation it is difficult to determine whether these phenotypes are a consequence of independent functions of NR4A2 in multiple brain regions or due to alterations in the dopaminergic system. Further work is required to determine the contribution of NR4A proteins in non-dopaminergic neurons to endophenotypes of schizophrenia.

NR4A family members have been implicated in hippocampus-dependent memory

Despite the identification of NR4A family members as activity-induced transcription factors in neurons or neuronal precursors nearly 20 years ago, the role of NR4A transcription factors in activity-dependent neuronal plasticity and memory formation has only recently begun being investigated. The first studies to show activity-dependent regulation of *Nr4a* family genes in the intact brain, rather than cell lines, examined seizure-induced gene expression in the hippocampus, a brain region that is required for spatial and contextual memory formation [2,3,64,67]. The genes encoding both NR4A1 and NR4A2 are robustly induced by seizure activity [26,83,104,114]. Further, the seizure-induced expression of *Nr4a1* and *Nr4a2* in the hippocampus is attenuated in CREB mutant mice [50], demonstrating that these genes are regulated in an activity-dependent and CREB-dependent manner in the hippocampus.

As might be predicted from the similarity between the signaling cascades that mediate memory storage [28] and those that induce *Nr4a* genes in various cellular contexts [66], *Nr4a* family gene expression increases within the hippocampus after learning of hippocampus-dependent tasks [59,70,84,100]. Learning of a spatial discrimination task leads to increased expression of *Nr4a2* mRNA in the CA1 and CA3 subregions of the dorsal hippocampus [84]. Similarly, training in contextual fear conditioning, a hippocampus-dependent task that involves learning an association between a specific training context and an aversive foot-shock [63], increases expression of *Nr4a1* in area CA1 of the dorsal hippocampus [59,100], an effect that is blocked in mice with a defect in CaMKII signaling that impairs efficient memory formation [33,99]. The overlap of mechanisms between *Nr4a* gene activation and the mechanisms of memory formation, the observation that *Nr4a* family gene expression increases in the hippocampus under conditions that generate a memory, and the importance of *Nr4a* family members in a variety of context for generating changes in cellular physiology in response to environmental signals together suggest the possibility that NR4A transcription factors may also contribute to memory formation. Several recent studies provide further support for this hypothesis. Injection of antisense oligodeoxynucleotides targeting *Nr4a2* into the hippocampus impairs long-term memory and reversal learning in an appetitive spatial learning task [18]. Consistent with this finding, mice lacking one copy of

the *Nr4a2* gene have impaired long-term memory for the hippocampus-dependent passive avoidance task [90]. Also, memory enhancement by intrahippocampal injection of HDAC inhibitors is accompanied by increased expression of both *Nr4a1* and *Nr4a2* [97]. These studies begin to address the role of NR4A transcription factors in memory formation, but considerations brought to light by previous studies of NR4A function in other cellular and anatomical contexts should be used to guide work on the role of NR4A nuclear receptors in memory formation.

NR4A transcription factors regulate genes implicated in memory formation

In addition to the role of signaling mechanisms that activate *Nr4a* gene expression in the formation of memories, several of the target genes transcribed in response to NR4A proteins have been implicated in memory formation (Figure 1). For instance, NR4A2 has been shown to regulate expression of the gene encoding BDNF [98], a neurotrophic factor that contributes to the formation of hippocampus-dependent memories [6,30,48,54,71]. Expression of several components of the NF κ -B signaling cascade increase upon overexpression of NR4A proteins [81], including the NF κ -B family member c-Rel that is required for hippocampus-dependent memory formation [5,51]. Other intriguing NR4A target genes include two receptor protein tyrosine phosphatases (RPTPs) [34,81], a class of molecule implicated in excitatory synapse formation [21,110], and the protein kinase C target MARCKS that can modulate memory formation [11,68,96]. The established connection between molecules upstream and downstream of NR4A transcription factors in memory storage provides a strong impetus for further investigation of the role of this family in memory formation.

Future directions to examine the role of NR4A function in memory formation

NR4A family members play differing roles in distinct cell types. Because of the intrinsic heterogeneity of brain tissue, this issue needs to be considered in the study of NR4A proteins in memory formation. Manipulations of NR4A function in a particular brain region may impact distinct biological processes in different cell types within a single brain region. For instance, lipopolysaccharide (LPS) injection into the substantia nigra activates microglia leading to an inflammatory response that destroys tyrosine hydroxylase-expressing (TH⁺) dopaminergic neurons [31,93]. Treatment with LPS is accompanied by increased expression of NR4A2 in microglia and astrocytes, and the knockdown of NR4A2 in these cells speeds neuronal loss. Thus, it appears that NR4A2 functions in a neuroprotective role by reducing the NF κ B-driven astrocytic inflammatory response through a novel interaction with a transcriptional repression complex [93]. In the same brain region, NR4A2 plays a complementary role within midbrain dopaminergic neurons to maintain the TH⁺ neuronal cell fate in the substantia nigra, presumably through the role of NR4A2 in activating transcription of target genes important for defining the identity of these neurons [36]. The co-occurrence of these two distinct roles in different cell types within the same brain region highlights the importance using cell-type specific approaches to investigate the contribution of these pleiotropic genes to memory formation.

A related concern in studying the role of NR4A proteins in memory formation is that NR4A proteins may impact memory formation through distinct mechanisms in different brain regions. This concern is clearly illustrated by the role of NR4A2 in the development and maintenance of the dopaminergic system [36,116] and the role of dopamine signaling in memory formation [8,75]. Intrahippocampal infusion of D1/D5 receptor antagonists impairs long-term memory [8,75,91], and intrahippocampal infusion of D1 agonists has even been found to enhance long-term memory [91]. For these reasons, it will be important to

specifically manipulate NR4A function in memory-related brain structures, such as the hippocampus, without impairing function in midbrain dopaminergic areas.

NR4A family transcription factors contribute to gene expression individually, as monomers and as homodimers, and as heterodimers with NR4A family members and with RXR. As discussed previously, heterodimers can synergistically enhance expression at NurRE sites [58] or have opposing action at certain NBRE variants [72]. Thus, reducing expression of a single NR4A family member could have an effect on target genes controlled by multiple NR4A proteins or theoretically produce a paradoxical enhancement in expression of certain NR4A targets. In contrast to the unique requirement for individual family members potentially produced by the synergistic or opposing interaction of NR4A family members, NR4A family members appear to be somewhat interchangeable in certain cellular contexts. For instance, considerable overlap was observed in the genes regulated by overexpression of the three NR4A family members in a macrophage-derived cell line [81]. In HeLa cells, both NR4A1 and NR4A2 act in a similar manner to promote cell survival and reduce anchorage dependence [39]. Also, the elimination of self-reactive T cells by TCR-mediated apoptosis appears to be mediated by both NR4A1 and NR4A3, the two family members expressed in this cell type [17,55,109,111]. Mice expressing a dominant negative form of NR4A1 that inhibits all NR4A family members [17] have deficits in TCR-mediated T-cell selection [12,120], but *Nr4a1* homozygous null mutant mice have normal T-cell selection, apparently due to redundant functions of NR4A1 and NR4A3 [17]. Thus, an approach that simultaneously impairs the function of all three NR4A family members was required to reveal the role of this signaling pathway in TCR-mediated apoptosis. A similar approach was required to provide compelling genetic evidence that PKA signaling plays a role in long-term memory formation for similar reasons [3,4], and may be important for understanding the role of NR4A transcription factors in learning and memory.

Conclusions

Signaling mechanisms that are critical for memory formation regulate the genes encoding the NR4A family transcription factors, and these genes have increased expression after learning in brain structures required for memory formation. Functional analysis of the role of this family in memory has begun for one family member, NR4A2, but more work is required to establish the importance of these transcription factors in the cascade of gene expression that mediates memory storage. From a clinical perspective, a possible contribution of these nuclear receptors to cognitive function is particularly exciting because they represent potential targets for pharmacological agents. The connection of this gene family with schizophrenia, a disorder associated with impaired cognitive performance, suggests that modulation of NR4A function may be a useful avenue for improving cognitive function in patients with neuropsychiatric disorders.

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Figure 1.

The cAMP-PKA-CREB pathway, a signaling cascade that is critical for memory formation, induces expression of the genes encoding NR4A nuclear receptors. Newly translated NR4A proteins translocate into the nucleus to drive transcription of NR4A target genes. MAPK signaling is capable of activating NR4A-mediated transcription or reducing this activity by nuclear export of these proteins, depending on the cellular context. Nuclear export is opposed by PKA signaling. Several NR4A target genes, including BDNF, c-REL, and receptor protein tyrosine phosphatases (RPTPs), have been previously implicated in memory formation.

Figure 2.

A. NR4A primary protein structure consists of an amino (NH₂) -terminal A/B transactivation domain (TAD) that includes the activator function (AF)-1 region implicated in coactivator recruitment. The DNA-binding domain (DBD) and C-terminal extension (CTE) both contribute to target sequence specificity and contain nuclear localization signals (NLS) that regulate the nuclear import of NR4A proteins. The carboxy (COOH) -terminal ligand-binding domain (LBD) contains the AF-2 domain and three putative nuclear export sequences. Phosphorylation sites (pSer) on NR4A proteins regulate nuclear-cytoplasmic shuttling and DNA-binding capacity. **B.** NR4A proteins activate transcription of target genes as monomers at NBRE sites or as dimers as NurRE sites. Additionally, heterodimers formed between NR4A1 or NR4A2 and RXR (in orange) confer retinoid-sensitive (9-*cis* RA) activation to genes targeted by NBRE or DR5 sites.

Figure 3.

Hierarchical clustering of nuclear receptors based on the anatomical distribution of nuclear receptor gene expression places each of the three *Nr4a* genes (*Nr4a1*/NGFI-B, *Nr4a2*/NURR1, *Nr4a3*/NOR-1) in a cluster of nuclear receptors (indicated by IC in the dendrogram) that “are expressed either predominantly or most abundantly in the CNS” (modified from Bookout *et al.* 2006).