Role of NCAM in Emotion and Learning

Lisa Conboy, Reto Bisaz, Kamila Markram, and Carmen Sandi

Introduction

In this review, we will discuss the role of the neural cell adhesion molecule [1] in emotion and learning. Classically, the behavioral role attributed to NCAM has been in learning, memory, and neural plasticity [2, 3]. However, increasing evidence presented over the past few years is also unraveling a role of NCAM on emotional behavior. After a brief introduction about the NCAM molecule, we will start reviewing its role in emotion, particularly in unlearned emotional responses, such as anxiety and aggression. In the second part of this review, we will address the role of NCAM in learning and memory processes to finally propose a role for this molecule at the interface between emotion and learning.

General Features of NCAM in the Central Nervous System: Molecular Structure and Function

NCAM is a member of the immunoglobulin superfamily of cell adhesion molecules. It is characterized by the presence of immunoglobulin homology domains (Ig-domains) in its extracellular part. NCAM is encoded by a single gene on chromosome 9 in mice [4] and 11 in humans [5] and undergoes differential splicing of the messenger RNA [6, 7]. The three main splice variants of NCAM are named according to their approximate molecular weights: NCAM180, NCAM140, and NCAM120. Within the central nervous system, NCAM180 appears to be the isoform enriched at postsynaptic sites, while NCAM140 is expressed both in neurons (pre and postsynaptically) and glia, and NCAM120 predominantly in glia [8, 9].

L. Conboy (✉)
Laboratory of Behavioral Genetics, Brain and Mind Institute, Swiss Federal Institute of Technology (EPFL), 1015, Lausanne, Switzerland
e-mail: lisa_conboy@hotmail.com

V. Berezin (ed.), *Structure and Function of the Neural Cell Adhesion Molecule NCAM*, *Advances in Experimental Medicine and Biology* 663, DOI 10.1007/978-1-4419-1170-4_18,

Post-translational attachment of long chains of the polysaccharide polysialic acid (PSA) to NCAM (PSA-NCAM) allows NCAM an additional mechanism to control synaptic functioning. In the adult brain, PSA-NCAM is mainly present in regions capable of undergoing some kind of structural plasticity [10], such as the hypothalamo-neurohypophyseal system [11], the olfactory bulb [12], the piriform and entorhinal cortices [13], the amygdala [14] hippocampus [13], and prefrontal cortex [14]. PSA is proposed to decrease NCAM-mediated membrane–membrane adhesion in vitro [15, 16], presumably due to its very large hydrated volume or negative charge or both [9, 17, 18].

NCAM is highly expressed at synaptic junctions. Neuronal activity regulates the functioning of synapses with a potential to either enhance or depress synaptic strength. During development, selective expression of cell adhesion molecules is proposed to regulate embryogenesis by dictating patterns of cell differentiation followed either by stabilization or selective elimination of synapses, as a mechanism of finetuning cellular connections [19]. Moreover, during development, neurite outgrowth is associated with the cell being in a de-adhesive state. When the neurite reaches and innervates the correct area, adhesiveness is increased so that the cell becomes locked in position. This modification in adhesion is controlled by homophilic cell–cell binding via cell adhesion molecules such as NCAM [20]. In addition to development, learning, and memory, LTP, aging, stress, and neuro-regeneration are all events that can also stimulate synaptic reorganization. Cumulative evidence indicates a key role for NCAM in the neural remodeling accompanying all these events.

NCAM and Emotion

Altered emotional behaviors have mainly been studied in adult male mice expressing a null mutation in the NCAM gene. Initial work demonstrated that NCAM-KO mice show enhanced anxiety in an emergence test, as indicated by their increased latencies to leave from a small box and explore an open environment [21]. An increased anxiety-like behavior of NCAM-deficient mice was also found in the light/dark avoidance test [22]. This effect was then shown to be gender and genetic background-independent and could be influenced by application of a low dose of the 5-HT_{1A} serotonin receptor agonists, either buspirone or 8-hydroxy-2-(di-n-propylamino)-tertraline (8-OH-DPAT), which at the same dose showed no anxiolytic effect in wild-type controls [22]. This suggested a functional alteration of the serotonergic system in these mice likely to be involved in their altered anxiety-like responses. Although the authors found little evidence for the existence of regional differences in serotonin receptor expression, they did find a slight reduction in the level of the 5-HT_{1A} receptor expression in the hippocampus and the amygdala of NCAM-KO mice.

In support of an altered serotonergic system is also the finding that NCAM-KO mice show enhanced aggression toward an unfamiliar intruder male, which was

accompanied by a greater post-intruder hormonal stress response [23]. Analysis of c-fos mRNA levels to monitor neuronal activation after the intruder stress revealed greater neuronal activity when compared with wild type controls in limbic areas, including the amygdala, a brain region known to be involved in modulating emotional responses.

Exploratory behavior is a basic adaptive behavioral response in rodents. When an animal is presented with a new environment, it is normally motivated to explore. Perturbations in this response are indicative of alterations in emotionality. NCAM-KO mice were found to show enhanced exploratory activity in response to challenging and novel environments, such as the light/dark avoidance test [22] and the elevated plus maze [24], which has been proposed to be related to their enhanced amygdala activation and/or the greater stress response (see above). On its turn, this enhanced exploratory behavior of NCAM-deficient mice could also explain the contradictory anxiolytic behavior, which was reported in the elevated plus maze [24].

In order to further investigate the role of specific NCAM isoforms in emotional behaviors, the effect of manipulating the levels of the NCAM180 isoform was analyzed in the presence or absence of endogenous NCAM in mice. While transgenic overexpression of NCAM180 was without apparent behavioral and morphological effects, its expression in NCAM-deficient mice rescued many of the effects induced by NCAM ablation; i.e., it counteracted the following effects observed in NCAM-KO: (1) the enhanced intermale aggression displayed in the intruder test [25]; (2) the increased anxiety-like behavior in the light/dark avoidance test; and (3) partially, the hyperactivity displayed in the elevated plus maze [24]. Transgenic induction of NCAM180 in NCAM-KO mice also prevented the hypersensitivity of NCAM-KO mice to the anxiolytic effects of buspirone, which suggests the involvement of NCAM180 on the development and/or maintenance of the functionality of the serotonergic system, which might represent an important link between NCAM and the regulation of emotional behaviors. Together, these studies indicate that deletion of the NCAM gene is associated with abnormal emotional responding and that the NCAM180 isoform plays a pivotal role in emotional behavior.

Research to elucidate the role of NCAM in learning and memory has been greatly aided by the development of mimetic peptides with the ability to bind and modulate the activity of NCAM. In addition to the above described findings in genetically mutant mice, there is upcoming evidence from pharmacological studies that further supports a role for NCAM in emotional behaviors. Peptides which interact either with NCAM homophilic binding (P2 peptide; a 12-amino-acid peptide derived from the second immunoglobulin-like (Ig) module of NCAM and able to interact with cis-homophilic) have been shown to alter emotional behaviors. For example, administration of the P2 peptide intracerebroventricularly was shown to reduce anxiety, associated with performance in a learning task (i.e. the T-maze), and exploratory activity in an open field test [26]. Another peptide, C3d, binds to the NCAM IgI module and is able to trigger intracellular signaling cascades in vitro, which are similar to those activated by homophilic NCAM binding [27]. Intracerebroventricular injection of this peptide had no acute effect on exploratory

behavior in an open field test, but dramatically reduced exploratory activity when C3d injected rats were re-exposed to the same arena 3-h and 24-h post-injection. This effect was not a consequence of sensorimotor impairments as peptide-treated rats performed without any significant differences on a rotarod and when explorative motility was accessed in an activity cage [28].

Emotional behavior is to a large extent associated with the development of psychiatric disorders. A few recent studies have shown some evidence to implicate NCAM in mood disorders. Thus, an increase in the CSF levels of the soluble 100–120kDa NCAM fragments has been found in patients with bipolar mood disorder type I and recurrent unipolar major depression [29, 30]. This increased presence of 120-kDa NCAM fragment in the CSF was postulated to derive form enhanced proteolytic cleavage of most of the extracellular region of transmembrane NCAM isoforms [31]. The complex nature of the extracellular region of the NCAM molecule has been also analyzed by creating transgenic mice that overexpress a soluble extracellular fragment of NCAM from the neuron-specific enolase promoter which leads to expression of this transgene in late neuronal differentiation (maximally in the neocortex and hippocampus) in adult mice [32]. Although these mice have normal sensory and motor functions, as compared to wild-type controls they display enhanced basal activity in the open field and in response to amphetamine and MK-801. However, and despite presenting a decreased number of synaptic terminals of subpopulations of GABAergic interneurons in the amygdala, these mice showed no alterations in anxiety-levels when tested in the zero maze [32].

Further evidence for the importance of NCAM and its polysialylated form PSA-NCAM in depression comes also from animal studies, which show that prolonged exposure of rodents to aversive stimuli (i.e. chronic stress protocols are widely used to model depression-like symptoms in rodents) lead to a reduction of NCAM mRNA and protein levels mainly in the hippocampus and the prefrontal cortex [33–43]. In contrast, PSA-NCAM expression was upregulated in the hippocampus, but downregulated in the amygdala of rats, exposed to 3 weeks of chronic stress [44]. Interestingly, recent studies in NCAM-KO (either constitutive or conditional, in which the cre-recombinase is regulated under the control of the αCaMKII promoter) mice have shown that these mice show enhanced depressive-like symptoms such as higher immobility time in the tail suspension test (Bisaz et al. unpublished observations) and a lower preference for sucrose solution [45]. In addition, chronic antidepressant treatments of rats and mice, with either the selective serotonin reuptake inhibitor fluoxetine or the tricyclic antidepressant imipramine, have shown to increase PSA-NCAM expression in the hippocampus and the prefrontal cortex [14, 46, 47]. These findings strongly support the hypothesis of a critical link between stress-related mood disorders and altered NCAM and PSA-NCAM expression [45].

In conclusion, the revised work indicates an important association between NCAM and emotion, particularly in domains such as anxiety, intermale aggression and depression. (See Table 1 for a comprehensive summary.) We will now review the literature that indicates a link between NCAM and learning and memory processes.

Table 1 Role of NCAM and PSA-NCAM in emotion

Model	Effect	Location	References
Anxiety			
NCAM$^{-/-}$ mice	Enhanced anxiety in an open field emergence test		[21]
NCAM$^{-/-}$ mice	Enhanced anxiety in Light/Dark avoidance test		[22, 24]
	Reduced anxiety in Elevated plus maze due to increased locomotion		[22, 24]
NCAM$^{-/-\ 180+}$ mice	Normal anxiety in Light/dark avoidance test and Elevated plus maze		[24]
ST8SiaIV/PST$^{-/-}$ mice	Reduced Anxiety in the Elevate Plus maze		[81]
Startle response and prepulse inhibition			
ST8SiaII/STX$^{-/-}$ mice	No effect in Startle response and Prepulse inhibition		[84]
NCAM$^{-/-}$ mice	No effect in Prepulse inhibition and facilitation		[109]
NCAM$^{-/-}$ mice	Reduced sensitivity to acoustic, but enhanced sensitivity to tactile stimuli		[59]
	Reduced footshock sensitization in startle response		[59]
Cleavage of PSA-NCAM by endoN	No effect in Startle response, 72-h post-enzyme injection	Bilateral in dorsal HC	[95]
Aggression			
NCAM$^{-/-}$ mice	Enhanced intermale aggression in an intruder aggression test		[23, 24]
NCAM$^{-/-\ 180+}$ mice	Normal intermale aggression in an intruder aggression test		[24]
Exploration and locomotion			
NCAM$^{-/-}$ mice	No effect on exploration and locomotion in open field test		[21]
NCAM$^{-/-}$ mice	Enhanced exploratory behavior in Light/dark avoidance test and Elevated plus maze		[22, 24]
C3d injected rats	No effect in exploratory behavior in the open field, 15-min post-injection Reduced exploration when retested 24 h later	Ventricle	[28]
C3d injected rats	No effect in mobility in the activity cage, 24-h post-injection	Ventricle	[28]
C3d injected rats	No effect in exploratory behavior in the open field test, 48-h post-injection	Ventricle	[67]
FGL injected rats	No effect in exploratory behavior in open field test, 24-h post-injection	Ventricle	[70]

(continued)

Table 1 (continued)

Model	Effect	Location	References
ST8SiaII/STX$^{-/-}$ mice	Enhanced exploratory behavior in the open field and enhanced rearing		[84]
FGL injected rats	No effect on anxiety and locomotion in the open field test, when injected for two consecutive days before test	Ventricle	[70]
P2 injected rats	Reduced exploratory behavior in the open field test, 1-h post-injection	Ventricle	[26]
Cleavage of PSA-NCAM by endoN	No effect in exploratory behavior in the open field test, 48-h post-enzyme injection	Bilateral in dorsal HC	[95]
ST8SiaIV/PST$^{-/-}$ mice	No effect in exploratory behavior in the open field test		[81]
Depression			
Soluble NCAM in CSF of patients with mood disorders	Enhanced levels of soluble NCAM120 fragment in CSF of patients with bipolar and major depression		[29]
NCAM$^{-/-}$ mice	Reduced immobility in the Forced swim test due to enhanced swimming activity		[24]
NCAM$^{-/-180+}$ mice	Enhancement of immobility (but below wt) and reduction of swimming activity (but above wt) in Forced swim test		[24]
NCAM$^{-/-}$ mice	Enhanced immobility in the tail suspension test and reduced sucrose preference in the sucrose preference test		[44]
FGL injected NCAM$^{-/-}$ mice	Reduction of depressive-like behaviors (in tail suspension test and sucrose preference test) by FGL		[44]
Aged conditional NCAM$^{-/-}$ mice	Enhanced depressive-like behavior in the tail suspension test		Bisaz et al. unpublished data
Stress			
NCAM$^{-/-}$ mice	Enhanced CORT levels after intruder (social) stress		[23]
CORT administration	Increase total NCAM expression after acute CORT administration	Forebrain chicken	[97]
	Increased total NCAM expression 8-h and 24-h post- injection	Frontal cortex	[40]
	Reduced total NCAM expression after 21-day chronic treatment	Frontal cortex, hypothalamus	[40]
	Reduced PSA-NCAM levels after 21-day chronic treatment	Piriform cortex, dentate gyrus	[32, 41]

Acute stress	Reduced NCAM180 protein levels immediately after stress	Hippocampus	[99]
	Reduction in all NCAM isoforms immediately after stress	Prefrontal cortex	[99]
	No effect in NCAM isoform expression immediately after stress	Amygdala, cerebellum	[99]
Chronic stress	Reduced totalNCAM protein expression after 21-day chronic restrain stress	Hippocampus	[37]
	Increased PSA-NCAM expression after 21-day chronic restrain stress	Hippocampus	[37]
	Increased PSA-NCAM after 21-day chronic restrain stress	Dentate gyrus	[38]
	No effect on PSA-NCAM after 42-day chronic restrain stress	Dentate gyrus	[38]
	Reduced total NCAM mRNA after 21-day chronic restrain stress	Hippocampus	[33]
	No effect on total NCAM mRNA after 21-day chronic restrain stress	Prefrontal cortex, frontal cortex, striatum and thalamus	[33]
	Reduced NCAM140 after 21-day chronic restrain stress	Hippocampus	[34]
	Reduced NCAM140 in high reactive rats after 21 days of chronic psychosocial stress	Hippocampus	[35]
	Reduced PSA-NCAM after 21-day chronic restrain stress	Central medial and medial amygdala	[43]
	Reduced totalNCAM mRNA after 21-day chronic restrain stress	Hippocampus	[39]
	Reduced NCAM180 in early aged rats after 28-day unpredictable chronic stress during adulthood	Hippocampus	[36]
	No effect in PSA-NCAM levels in early aged rats after 28 days unpredictable chronic stress during adulthood	Hippocampus	[36]

NCAM in Learning: Functional Studies

Learning can be defined as a process by which new information is acquired, whereas memory is the process by which this information is retained. The process of transferring learned information into memory is known as consolidation and is intimately linked to the functioning of the hippocampus [48, 49]. Cognitive tests for rodents have been developed to capitalize on normal behavioral responses often using fear, hunger or innate curiosity to motivate and strengthen learning. For example, in the watermaze, animals are forced to learn a novel spatial map in order to escape from water, whereas in avoidance and fear conditioning paradigms electrical shocks are used to initiate associative memory formation. In particular, a key role and requirement of NCAM function during learning has been particularly demonstrated using hippocampus-dependent tasks, including avoidance conditioning [50] and spatial learning [51]. In addition, the role of NCAM in "emotional learning" is more typically studied with fear conditioning paradigms. Classical contextual and auditory fear conditioning are often utilized to this end. These tasks involve the induction of learned fear (generally manifested as a freezing response) to an initially neutral stimulus (respective, either a new context or a tone) that is associated with a naturally aversive stimulus (normally a footshock). Recall of the fear response can be tested by re-exposing the animal to either the context or tone and evaluating its conditioned fear (freezing) responses. Acquisition and consolidation of either fear conditioning modality relies on the basolateral complex of the amygdala [52–58], while the hippocampus is also required in the case of contextual fear conditioning [59].

The role of NCAM in learning has been investigated by a variety of approaches, including the examination of particular behaviors in genetically mutant (transgenic or knockout) mice, after application of "blocking" NCAM-related antibodies or peptides that affect (either mimicking or impairing) NCAM functioning. Constitutive NCAM-KO are perturbed in the Morris water maze when compared to wild-type controls [21]. As mentioned in the introduction, NCAM expression is critical during post-natal development and, therefore, one of the problems with the constitutive NCAM-KO mice is that their altered learning and memory might be related to the elimination of NCAM during such period (and its consequent developmental effects), but unrelated to the absence of NCAM in the adult brain. To overcome this problem, a conditional knockout mouse was generated where expression of NCAM was controlled by a Cre-recombinase system using a forebrain specific promoter. In this conditional knockout mouse, NCAM expression is reduced starting at around P22 onwards and, therefore, its deficit occurs after the major neurodevelopmental events have already taken place [60]. This conditional KO of NCAM also leads to a spatial memory deficit in adulthood, demonstrating that post-natal NCAM expression is required for learning and memory. Evidence that NCAM mediated processes contribute to fear memories also stem from work performed in constitutive NCAM-KO mice. In these mice, both auditory and contextual fear memories are impaired [24], suggesting that NCAM-mediated consolidation processes might also be implicated in brain regions other than the

hippocampus, because auditory fear conditioning relies on the amygdala, but does not implicate the hippocampus.

The role of NCAM in non-associative learning was also tested by examining habituation (a decrease in behavioral responding to a stimulus) and sensitization (increased responding to an aversive stimulus) processes in NCAM-KO mice. While acoustic and tactile responses were altered in NCAM-KO mice (as evaluated by their startle responses), their ability to habituate to these stimuli was the same as wild type mice, demonstrating habituation learning is intact. In contrast, NCAM-KO mice exhibited impaired footshock sensitisation learning when compared with the wild type controls [61]. Footshock sensitization is a form of contextual conditioning, during which the context becomes a fear inducing stimulus leading to an increase in startle response to a tone [62].

In addition to the work reviewed above in genetically mutant mice, the critical role played by cell adhesion molecules in learning-related synaptic plasticity has been further demonstrated using blocking antibodies and peptides that bind to NCAM. Through these pharmacological approaches, the temporal implication of NCAM molecules during memory consolidation could also be explored. For example, studies where antibodies against NCAM were infused by intracerebroventricular injection in rats trained in the passive avoidance task found NCAM antibodies to induce memory impairment only when they where administered between 6 and 8 h post-training, but they were ineffective if given at the time of training or at any other time point up to 10 h following training [63]. In agreement with these observations, NCAM-specific antibodies were also found to impair passive avoidance learning in chicks when administered to a brain region critical for that learning (the intermediate medial hyperstriatum ventrale) at around 6–8 h post-training time [1, 64, 65]. The requirement of NCAM in long-term memory formation was similarly demonstrated through the use of oligonucleotides directed to NCAM [66].

In vitro evidence demonstrated that the C3d peptide could disrupt NCAM mediated cell adhesion and modulate neuritogenesis and synaptogenesis. When given to rats following training in the passive avoidance task, C3d prevented memory consolidation but only when the peptide was administered within a restricted time window either 20 min before training or at 6–8 h post-training [67]. Moreover, the C3d peptide also impaired both acquisition and recall of the Morris water maze [68] and the consolidation of contextual fear memories when administered 5.5 post-training [69].

After homophilic binding, NCAM promotes neurite outgrowth through mechanisms involving its interaction with the fibroblast growth factor receptor (FGFR1) [25, 70, 71]. The region of NCAM that binds FGFR1 is found in the second FnIII module of NCAM. A 15-aa peptide mimicking this region, termed the FGL peptide, has been shown to bind to and activate FGFR1 and to stimulate neurite outgrowth [25]. In vitro, the FGL peptide promotes synaptogenesis, synaptic outgrowth and pre-synaptic functioning [25, 72], as opposed to the C3d peptide that it similarly induces synaptogenesis and synaptic outgrowth, but impairs presynaptic functioning [27]. In vivo, FGL effects also contrast to those induced by C3d peptide (for details, see above). FGL strongly enhances spatial memory as shown in experiments, in which it was administered in rats immediately after the first and second day of

spatial training in the water maze [72]. This peptide was ineffective if given 2 days prior to training indicating that it does not perturb learning of new information. Moreover, post-training FGL treatment also improved performance in a subsequently given reversal learning challenge, suggesting that FGL is beneficial in promoting behavioral flexibility [72].

The FGL peptide has also significantly enhanced our understanding of the role of NCAM in the prototypic emotional learning task, fear conditioning. Intracereboventricular infusion of FGL just after fear conditioning improved contextual memory performance when tested 24 h, and 7 and 28 days later. However, auditory fear memories were only enhanced when tested 28 days later, but not earlier, suggesting different consolidation mechanisms for conditioned fear to tones, which might become apparent only after longer time periods. FGL did not affect emotional responses per se, having no affect on open field behavior when administered for 2 days prior to testing. Therefore, similarly to its effect in spatial learning, FGL was shown to also enhance emotional learning.

NCAM in Learning: Correlative Studies

Functional studies reviewed above suggest a role for NCAM in memory formation. However, they do not allow one to address whether the functional consequences derived from interference with NCAM (expression or function) are due to a primary effect on already existing molecules or, instead, on learning-induced NCAM regulation. This is a critical issue as the former possibility might imply a non-specific effect of treatments on normal circuit functioning, whereas the latter would highlight NCAM as a key player in memory-associated circuit remodeling [68]. We will review here those studies that have addressed a potential regulation of NCAM expression by learning experiences.

The 6–8 h post-training period was highlighted by interventive (antibody injection) studies as critical for the involvement of NCAM in memory consolidation, Interestingly, in chicks, NCAM was found to be enriched in synaptic active zones in a memory-relevant region (the lobus parolfactorius) 5–6 h after a one-trial passive avoidance learning experience [73]. In rats, spatial training in the water maze was found to induce an increase in synaptosomal, but not total, expression level of hippocampal NCAM140 24 h post-training [68]. In the zebrafish, using an active avoidance paradigm (fish learn to cross a hurdle to avoid mild electric shocks when presented with a conditioned light signal) NCAM mRNA levels were increased in the optic tectum (a region important for avoidance learning) 3 h following avoidance conditioning, indicating that some learning-dependent changes in NCAM expression are transcriptionally mediated [74].

In addition to this temporal increase in NCAM expression observed several hours after training, there is also evidence that NCAM expression is decreased for a restricted period on the first phases of memory consolidation. Work in simple invertebrate organisms such as the sea snail *Aplysia* has allowed the study

of neurobiological mechanisms linked to different types of learning, notably habituation and sensitisation [75]. Sensitisation in *Aplysia* is induced by presenting a noxious stimulus to the tail of the snail whereupon it withdraws its siphon and gill. If this is followed by stimulation of the siphon, it will withdraw both the siphon and gill in a more sensitized manner [76]. The neuronal circuit involved in long-term sensitisation has been identified [77]. Elements of this circuit (a siphon sensory neuron synapsing onto a gill motor neuron) can be isolated and cultured in vitro and synaptic facilitation can be induced artificially by presenting a puff of 5-TH directly to the cultured sensory neuron [78]. Interestingly, *Aplysia* expresses a homologue of mammalian NCAM known as apCAM. Induction of long-term sensitisation in cultured sensory neurons of *Aplysia* is accompanied by the growth of new synaptic connections and requires downregulation of apCAM [79].

Remarkably, in rodents NCAM is also selectively degraded 2–6 h post-training and this is necessary for passive avoidance memory consolidation [67]. Moreover, in this study the authors demonstrated that the C3d peptide, which impairs memory formation, seemed to prevent the temporal reduction in NCAM that occurs 2–6 h following learning in the passive avoidance paradigm indicating, as had been demonstrated in *Aplysia*, that a temporal reduction in NCAM expression is required for effective learning and memory.

PSA-NCAM in Learning

Polysialylation of NCAM is a potent modulator of NCAM functioning that significantly impacts on the role of NCAM in learning. Substantial evidence indicates that this posttranslational modification mechanism plays a key role in activity-dependent synaptic plasticity [2, 80] and memory formation. Most of the original work on this topic focused in the hippocampus. The requirement of PSA-NCAM for spatial learning has been indicated by different approaches. Removal of PSA from NCAM by endo-neuraminidase NE (endo-N), an enzyme which specifically cleaves α-2, 8-linked PSA, impedes the acquisition and retention of spatial memory [81, 82]. Mice expressing a null mutation in the polysialyltransferase (PST) gene, an enzyme critical for the postnatal polysialylation of NCAM, are impaired in spatial learning [83]. Conversely, a synthetic PSA-mimetic peptide administered in the mouse hippocampal CA3 region 5 h after massed training in the water maze was shown to significantly improve recall up to 4 weeks after training [84].

The role of PSA-NCAM in fear conditioning has received much attention in the past few years. Mice deficient in the PST gene were shown to display a very mild deficit in contextual fear conditioning. By contrast, auditory fear conditioning was normal in these PST-KO mice [83]. Strikingly, contextual fear conditioning was also impaired by application of PSA or PSA-NCAM 6 h, but not 2 h, following training [85]. Adult mice lacking the prenatally important St8SiaII/STX polysialyltransferase exhibit impaired memories (but not acquisition) in fear learning-related paradigms, auditory and contextual fear conditioning [86], suggesting an involvement

of PSA-NCAM in memory processes related to fear conditioning. However, since PSA-NCAM expression levels in the amygdala of adult St8SiaII/STX knockout mice are normal, the possibility exist that their alterations in emotional learning are due to developmentally caused alterations in amygdala function.

Given the differential disruption of auditory fear conditioning in PST as compared to SXT-KO mice it initially seemed, at least in adulthood, PSA-NCAM may be more important in regulating hippocampal learning when compared with amygdala-dependent learning. To resolve this issue, our group examined the role of PSA-NCAM in the amygdala during fear learning and found that auditory fear conditioning under conditions that employed a high intensity shock (1 mA) enhances the amygdaloid expression of PSA-NCAM 12 h post-training [87]. However, fear learning appeared not to require the induction of PSA-NCAM since endo-N cleavage failed to prevent either fear learning or its consolidation. However, removal of PSA-NCAM in the amygdala enhanced memory extinction, suggesting that PSA-NCAM modulations during emotional learning may be important in determining the intensity of the memory trace.

On the other hand, there is extensive evidence showing that PSA-NCAM levels in the hippocampus are modified by learning experiences. Notably, selective enhancement of PSA-NCAM-positive cells in the rat hippocampal dentate gyrus (in particular in a population of cells located at the dentate infragranular zone) has been found following initiation of learning in numerous behavioral tasks. For example, a temporal modification of PSA-NCAM levels is known to occur 10–12 h after passive avoidance [88–92], water maze [93, 94], olfactory learning [95, 96] and contextual fear conditioning [97, 98]. This up-regulation in PSA-NCAM can be sustained, being in some cases also evident at 24 h post-training [68, 94]. Moreover, repetitive training in the water maze induces PSA-NCAM upregulation following each training session while animals are still improving their performance levels [93] suggesting that enhanced expression of PSA-NCAM in the hippocampus is a molecular signature of plasticity-related to hippocampal learning. Despite PSA-NCAM being a marker for immature neurons, the spatial learning-dependent increase in PSA-NCAM does not result from increased neurogenesis or progenitor cells survival [95], indicating some other function for this selective modification in PSA-NCAM. Together, these studies suggest that selected enhancement of hippocampal PSA-NCAM can facilitate memory formation, whereas the role of amygdaloid PSA-NCAM in memory function deserves further studies.

NCAM and PSA-NCAM: Sensitive Indices of "Emotional Learning"

In this review about the role of NCAM and PSA-NCAM in learning, we have detailed many instances were both NCAM and PSA-NCAM are important for "emotional learning" particularly in the context of auditory and contextual

fear conditioning. We should also note that in our view the concept of "emotional" learning goes beyond fear learning tasks, since in our view virtually all animal learning models involve an emotional motivation too (for example, escaping from water stress in the water maze task). Work from our lab has found that NCAM [99] and PSA-NCAM [98] expression in the hippocampus are regulated by emotional learning depending on the intensity of the emotional experience. Contextual fear conditioning was found to induce time- and shock-intensity dependent alterations in the expression of hippocampal NCAM and PSA-NCAM. The intensity of the training experience can be modulated by altering the shock received by the animal, applying 0.2, 0.4 or 1 mA, which corresponds to low, medium and high intensity shocks, respectively. Previously, we showed that the intensity of the shock has a positive correlation with both the extent and duration of conditioned fear and post-training corticosterone levels [100]. Training rats at a moderate intensity (0.4 mA) led to a significant enhancement of hippocampal PSA-NCAM 12 h post-training [98], similarly to changes found after passive avoidance conditioning and spatial learning [68, 88]. By contrast, 24 h hours post-training only animals trained at 1 mA showed a significant enhancement of NCAM expression and, interestingly, this group also exhibited the greatest retention of the task and highest post-training corticosterone induction.

We have recently examined the regional specificity of contextual fear conditioning on hippocampal PSA-NCAM expression. We found [97] differential expression of hippocampal PSA-NCAM in the ventral and dorsal hippocampus that corresponds to a different functional involvement of these discrete regions in learning tasks [51]. Context exposure alone led to a significant increase in PSA-NCAM in the ventral and dorsal hippocampal dentate gyrus 24 h post-training [97]. However, following training in the contextual fear conditioning task (i.e., when context was paired with a shock), PSA-NCAM expression was only enhanced in the dorsal hippocampus. Moreover, infusion on Endo-N to the dorsal, but not the ventral, hippocampus impaired retention of the contextual memory [97]. More recently, we have demonstrated that prevention of a very rapid reduction in PSA-NCAM in the ventral hippocampus of rats exposed to the radial arm water maze is linked to a facilitation of memory retrieval (Conboy et al. unpublished observations).

Exposing rats during 30 min to a traumatic experience (i.e., predator stress, more specifically cat exposure) immediately following massed training in the radial arm water maze can impair recall of the platform location and induce a correlative reduction in hippocampal NCAM180 expression [101]. In contrast, the novel anti-depressant treatment agomelatine can prevent this stress-induced deficit in memory retrieval. Our recent findings show that in parallel to this behavioral effects, agomelatine facilitates the synaptic insertion of NCAM within 30 min of cessation of the learning task (Conboy et al. unpublished observations).

For a more comprehensive list of the role of NCAM and PSA-NCAM in learning and memory see Table 2.

Table 2 NCAM and PSA-NCAM in learning and memory

Model	Effect	Location	References
Passive avoidance			
NCAM expression	Increased expression in synaptic active zones 5–6-h post-training	Lobus parolfactorius (chick striatum)	[73]
NCAM expression	Decreased due to enhanced NCAM180 internalization 3–4-h post-training. Could be prevented by C3 infusion	Dentate gyrus	[67]
Anti-NCAM infusion	Impaired when infused 5–6-h and tested 48-h post-training	Ventricle	[63]
Polyclonal anti-NCAM (1 and 2) infusion	Impaired when injected 6–8-h post-training and tested 24-h post-training No effect at any other time point	Intermediate medial hyperstriatum ventrale (IMHV) of chick	[64]
Polyclonal anti-NCAM R1 and R2 infusion	Impaired when injected 5.5-h post-training and tested 24-h post-training	IMHV (chick)	[65]
Polyclonal anti-NCAM R1 infusion	Impaired when injected 5.5-h post-training and tested 48 h later No effect when tested 24 h later	Ventricle	[1]
Anti-NCAM	Impaired the facilitatory effect of CORT on learning	Forebrain (chick)	[99]
C3 infusion	Impaired when infused during training and 6–8 post-training and tested 48 h later, but not 24-h post-training. Prevents NCAM180 internalization 3–4 post-training in dentate gyrus	Ventricle	[67]
C3 infusion	Impaired when infused pre-training and tested 48 post-training	Ventricle	[28]
NCAM antisense oligonucleotides infusion	Impaired when injected twice: both 12 h before training and immediately afterwards and tested 3-h and 24-h post-training	IMHV (chick)	[66]
PSA-NCAM expression	Increase 12-h and 24-h post-training	Hippocampus	[102]
PSA-NCAM expression	Increase 12–24-h post-training, can be abolished with amnesia inducing agents	Hippocampus	[110]
PSA-NCAM expression	Increase 10–12 h post-training	Dentate gyrus	[88]
PSA-NCAM expression	Increase 10–12 h post-training	Dentate gyrus (granular cells)	[93]

PSA-NCAM expression	Increase 10–12 h, persisting up to 24–48-h post-training	Layer II of the enthorinal, perirhinal and piriform cortex	[90]
PSA-NCAM expression	Increase 12-h post-training at subtriangular septal zone in GABAergic interneurons	Septal nuclei and septohippocampal pathway	[91]
St8SiaII/STX$^{-/-}$ mice	Impaired		[86]
Active avoidance			
NCAM expression	Increased 3-h post-training	Optic tectum (zebrafish)	[74]
Auditory fear conditioning			
Constitutive NCAM$^{-/-}$ mice	Impaired tone memory		[85]
Constitutive NCAM$^{-/-}$ mice infused with Fc	Impaired when injected pre-training (4 h) and tested 24 h and 72 h later	Dorsal hippocampus	[85]
NCAM$^{-/-180-}$ mice	Impaired tone memory		[24]
NCAM$^{-/-180+}$ mice	Impaired tone memory		[24]
FGL infusion	No effect 24-h and 7-day post-training, but: Enhanced 28-day post-training	Ventricle (post-training)	[72]
NCAM-Fc infusion (C57Bl/6J mice)	No effect when injected pre-training (4 h) or post-training (2 or 6 h) and tested 24 h and 72 h later	Dorsal hippocampus	[85]
NCAM-Fc infusion (Constitutive NCAM$^{-/-}$ mice)	No effect when injected pre-training (4 h) and tested 24 h and 72 h later	Dorsal hippocampus	[85]
PSA-NCAM expression	No effect	Dentate gyrus	[97]
Cleavage of PSA-NCAM by endoN	No effect	Dorsal dentate gyrus	[97]
PSA-NCAM expression	Increase in BLA and CE after 1 mA, not 0.4 mA, 24-h post-training	Amygdala	[87]
Cleavage of PSA-NCAM by endoN	No effect	Amygdala	[87]
pr 2 infusion	No effect 24-h and 28-day post-training	Amygdala	[87]
PSA-NCAM-Fc infusion (C57Bl/6J mice)	No effect when injected pre-training (4 h) or post-training (2 or 6 h) and tested 24 h and 72 h later	Dorsal hippocampus	[85]

(continued)

Table 2 (continued)

Model	Effect	Location	References
PSA infusion (C57Bl/6J mice)	No effect when injected pre-training (4 h) or post-training (2 or 6 h) and tested 24 h and 72 h later	Dorsal hippocampus	[85]
PSA-NCAM-Fc infusion (Constitutive NCAM$^{-/-}$ mice)	Enhanced when injected pre-training (4 h) and tested 24 h and 72 h later (when compared to NCAM$^{-/-}$ injected with Fc)	Dorsal hippocampus	[85]
St8SiaII/STX$^{-/-}$ mice	Impaired		[86]
St8Sia-IV/PST$^{-/-}$ mice	No effect		[83]
St8Sia-IV/PST$^{-/-}$ mice	No effect		[85]
Auditory fear extinction			
Cleavage of PSA-NCAM by endoN	Enhanced extinction memory 24-h post-training	Amygdala	[87]
Contextual fear conditioning			
NCAM expression	Decrease 12 h post-training after 0.2, 0.4 and 1 mA Increase 24-h post-training after 1 mA	Hippocampus	[98]
C3 infusion	Impaired when injected 5.5-h post-training and tested 2–3 and 7 days later. (0.4 mA) No effect when injected 2 days pre-training	Ventricle	[69]
FGL infusion	Enhanced contextual fear 24-h, 7-day and 28-day post-training	Ventricle (post-training)	[72]
NCAM-Fc infusion (C57Bl/6J mice)	No effect when injected pre-training (4 h) or post-training (2 or 6 h) and tested 24 h and 72 h later	Dorsal hippocampus	[85]
Constitutive NCAM$^{-/-}$	Impaired context memory		[85]
NCAM$^{-/-180-}$ mice	Impaired context memory		[24]
NCAM$^{-/-180+}$ mice	Impaired context memory		[24]
Constitutive NCAM$^{-/-}$ mice infused with Fc	Impaired when injected pre-training (4 h) and tested 24 h and 72 h later (when compared to NCAM$^{+/+}$)	Dorsal hippocampus	[85]
NCAM-Fc infusion (Constitutive NCAM$^{-/-}$ mice)	Impaired when injected pre-training (4 h) and tested 24 h and 72 h later (when compared to NCAM$^{-/-}$ injected with Fc)	Dorsal hippocampus	[85]

PSA-NCAM expression	No effect 12 and 24 h post-training after 0.2 and 0.4 mA Decrease 12 and 24 h post-training after 1 mA	Hippocampus	[98]
PSA-NCAM expression	Increase 12-h post-training after moderate shock intensities (0.4 mA) Decrease 12-h post-training after traumatic shock intensity (1 mA)	Dentate gyrus	[109]
PSA-NCAM expression	Increase 24 h post-training in dorsal, but not ventral hippocampus (both after 0.4 and 1 mA shock)	Dentate gyrus	[97]
Cleavage of PSA-NCAM by endoN	Impaired	Dorsal hippocampus	[97]
PSA-NCAM-Fc infusion (C57Bl/6J mice)	Impaired when injected pre-training (4 h) or 6 h post-training and tested 24 h and 72 h later No effect when injected 2-h post-training and tested 24 h and 72 h later	Dorsal hippocampus	[85]
PSA infusion (C57Bl/6J mice)	Impaired when injected pre-training (4 h) and tested 24 h and 72 h later No effect when injected 2-h or 6-h post-training and tested 24 h and 72 h later	Dorsal hippocampus	[85]
PSA-NCAM-Fc infusion (Constitutive $NCAM^{-/-}$ mice)	Enhanced when injected pre-training (4 h) and tested 24 h and 72 h later (when compared to $NCAM^{-/-}$ injected with Fc)	Dorsal hippocampus	[85]
PSA-NCAM expression	No effect at any time point (30 min, 24 h) and intensity (0.4, 1 mA)	Amygdala	[87]
Cleavage of PSA-NCAM by endoN	No effect	Amygdala	[87]
St8Sia-II/$STX^{-/-}$ mice	Impaired		[86]
St8Sia-IV/$PST^{-/-}$ mice	Impaired (mildly)		[85]
Spatial learning			
NCAM expression	No effect immediately post-training	Hippocampus, thalamus, striatum, PFC, frontal cortex	[111]
NCAM expression	Increase 24-h post-training	Hippocampus	[112]
C3 infusion (pre-training infusions)	No effect when averaged over all days Impaired on second trial on day one	Ventricle	[28]

(continued)

Table 2 (continued)

Model	Effect	Location	References
C3d infusion	Impaired in learning Impaired in probe test	Ventricle	[112]
FGL infusion after 1st and 2nd training session	Enhanced on 2nd training session in first trial Enhanced 24-h, 7-day and 14-day post-training No effect in probe trials Enhanced reversal learning	Ventricle	[72]
Conditional hippocampus NCAM$^{-/-}$ mice	Impaired (day 3)	Hippocampus	[60]
Constitutive NCAM$^{-/-}$ mice	Impaired during acquisition Impaired in probe trial		[21]
NCAM$^{-/-180-}$ mice	Impaired acquisition Impaired probe trial memory Increased floating		[24]
NCAM$^{-/-180+}$ mice	No effect		[24]
Polyclonal anti-NCAM	Impaired	Ventricle	[113]
PSA-NCAM expression	Increase 10–12-h post-training	Dentate gyrus (granular cells)	[93]
PSA-NCAM expression	Increase 12-h post-training at subtriangular septal zone in GABAergic interneurons	Septal nuclei and septohippocampal pathway	[91]
PSA-NCAM expression	Increase 10–12-h post-training	Enthorinal cortex	[92]
PSA-NCAM expression	Increase 12-h post-training	Dentate gyrus	[109]
PSA-NCAM expression	Increase 12-h post-training Negative correlation between PSA-NCAM expression and performance in WM	Dentate gyrus	[114]
PSA-NCAM expression	Increase 12-h post-training, no effect on neurogenesis	Dentate gyrus	[95]
PSA-NCAM expression	Increase 24-h post-training in synaptosomes	Hippocampus	[112]
Cleavage of PSA-NCAM by endoN	Impaired on learning day 2, impaired in probe test	Dorsal hippocampus	[81]
Cleavage of PSA-NCAM by endoN	Impaired learning	Ventricle	[112]
pr2 infusion	Enhanced learning	Hippocampus	[115]

St8Sia-II/STX$^{-/-}$ mice	No effect		[86]
St8Sia-IV/PST$^{-/-}$ mice	Impaired learning, impaired reversal learning		[83]
Water maze followed by passive avoidance			
PSA-NCAM expression	Increase 10–12-h post-training	Dentate gyrus (granular cells)	[93]
PSA-NCAM expression	Increase 12-h post-training	Dentate gyrus	[94]
Olfactory discriminative learning and memory			
NCAM expression	No effect at any time point of measurement (3rd, 5th day of learning, 3-day post-training) Positive correlation between learning performance and NCAM expression	HippocampusPiriform cortex	[116]
Constitutive NCAM$^{-/-}$ mice	Impaired odor discrimination No effect short-term memory No effect odor detection thresholds		[117] [112]
Polyclonal anti-NCAM	Impaired when injected 5.5-h post-training and tested 48 h later	Ventricle	[113]
PSA-NCAM expression	Increase 12-h post-training	Dentate gyrus	[96]
PSA-NCAM expression	Increase 24-h post-training in the hippocampus, but not piriform cortex	Hippocampus Piriform cortex	[116]

Studies were conducted on rats or mice unless specified otherwise
Compounds infused: C3 or C3d, synthetic peptide ligand of NCAM with an affinity for the IgI domain and the capability of inhibiting NCAM-mediated neurite outgrowth in vitro
FGL, synthetic 15 amino acid peptide corresponding to the binding site of NCAM for the fibroblast growth factor receptor 1 (FGFR1)
pr2, synthetic peptide mimicking PSA
NCAM-Fc or PSA-NCAM-Fc, recombinantly produced proteins in which the extracellular portion of NCAM is fused with a human Fc fragment of IgGs. Fc alone has no effect when infused into mouse brains

Mechanisms Related to NCAM Actions on Learning

Humans retain the ability to form new memories, in the absence of dementia, throughout their whole life, which indicates that the implicated brain structures must retain the potential to continuously restructure their synapses. Adult learning and memory has been proposed to imply, to a certain extent, a replay of neurodevelopmental events and as such utilize the same plasticity-related molecules, including NCAM [102, 103]. Numerous studies have shown modifications of hippocampal synaptic morphology as a result of learning and memory [104–107]. As the selective expression of cell adhesion molecules during neurodevelopment is important in determining synaptic structuring [19], analogous cell adhesion molecule modulations presumably regulate synaptic restructuring during memory formation.

At the conceptual level, the reviewed evidence led us to propose the following role for NCAM during memory consolidation. During the early consolidation period, 2–6 h following training NCAM expression seems to be downregulated [67]. A transient increase in spine number has been found in the hippocampus in the early hours after a training experience [104–106]. In *Aplysia*, it has been shown that the growth of new synaptic connections requires endocytosis and degradation of the NCAM homologue apCAM [79]. It is therefore conceivable that the temporal reduction in NCAM found in rodents may similarly enable synaptic loosening to facilitate synaptic growth. During the later periods of consolidation, robust learning and memory has been associated with an enhancement of synaptosomal NCAM expression [68, 98]. In chicks, avoidance training induces the localisation of NCAM in the synaptic active zone of the lobus parolfactorius 5–6 h after a one-trial passive avoidance learning experience [73], indicating that NCAM may localize to newly formed synapses. Moreover, contextual fear conditioning leading to a strong memory [100] correlates with an enhancement of synaptically localized NCAM [98]. Correlative work in vitro has suggested that increased concentrations of NCAM can selectively increase synapse formation. For example, transfection of NCAM deficient neurons with any of the three NCAM molecules leads to the formation of synapses preferentially between NCAM-NCAM containing neurons [108].

Learning and memory also require temporal modulations in PSA-NCAM. Learning induced synaptic modifications occurring in the hippocampus are transient [106] indicating that a period of synaptic pruning or selection also contributes to memory consolidation. As PSA attachment to NCAM reduces NCAM mediated cell adhesion, activity dependent upregulation in PSA-NCAM during the later memory consolidation period (12–24 h) [68, 88] may enable synaptic loosening which facilitates selection and pruning of hippocampal connections.

In conclusion, we have presented evidence that NCAM and PSA-NCAM regulate both emotion and learning and memory processes, and we have presented a model suggesting that the functioning of these molecules might be related to the modulation of learning induced by emotional aspects. This evidence supports the use of recently developed NCAM-related compounds, such as the FGL peptide, for the treatment of devastating neurological disorders of cognitive dysfunction like Alzheimer's disease.

References

1. Alexinsky T, Przybyslawski J, Mileusnic R et al (1997) Antibody to day-old chick brain glycoprotein produces amnesia in adult rats. Neurobiol Learn Mem 67:14–20
2. Gascon E, Vutskits L, Kiss JZ (2007) Polysialic acid-neural cell adhesion molecule in brain plasticity: from synapses to integration of new neurons. Brain Res Rev 56:101–118
3. Welzl H, Stork O (2003) Cell adhesion molecules: key players in memory consolidation? News Physiol Sci 18:147–150
4. D'Eustachio P, Owens GC, Edelman GM et al (1985) Chromosomal location of the gene encoding the neural cell adhesion molecule (N-CAM) in the mouse. Proc Natl Acad Sci USA 82:7631–7635
5. Nguyen C, Mattei MG, Mattei JF et al (1986) Localization of the human NCAM gene to band q23 of chromosome 11: the third gene coding for a cell interaction molecule mapped to the distal portion of the long arm of chromosome 11. J Cell Biol 102:711–715
6. Owens GC, Edelman GM, Cunningham BA (1987) Organization of the neural cell adhesion molecule (N-CAM) gene: alternative exon usage as the basis for different membrane-associated domains. Proc Natl Acad Sci U S A 84:294–298
7. Santoni MJ, Barthels D, Barbas JA et al (1987) Analysis of cDNA clones that code for the transmembrane forms of the mouse neural cell adhesion molecule (NCAM) and are generated by alternative RNA splicing. Nucleic Acids Res 15:8621–8641
8. Pollerberg GE, Burridge K, Krebs KE et al (1987) The 180-kD component of the neural cell adhesion molecule N-CAM is involved in a cell-cell contacts and cytoskeleton-membrane interactions. Cell Tissue Res 250:227–236
9. Rutishauser U, Jessell TM (1988) Cell adhesion molecules in vertebrate neural development. Physiol Rev 68:819–857
10. Bonfanti L, Olive S, Poulain DA et al (1992) Mapping of the distribution of polysialylated neural cell adhesion molecule throughout the central nervous system of the adult rat: an immunohistochemical study. Neuroscience 49:419–436
11. Theodosis DT, Bonfanti L, Olive S et al (1994) Adhesion molecules and structural plasticity of the adult hypothalamo-neurohypophysial system. Psychoneuroendocrinology 19:455–462
12. Miragall F, Kadmon G, Husmann M et al (1988) Expression of cell adhesion molecules in the olfactory system of the adult mouse: presence of the embryonic form of N-CAM. Dev Biol 129:516–531
13. Seki T, Arai Y (1991) Expression of highly polysialylated NCAM in the neocortex and piriform cortex of the developing and the adult rat. Anat Embryol (Berl) 184:395–401
14. Varea E, Nacher J, Blasco-Ibanez JM et al (2005) PSA-NCAM expression in the rat medial prefrontal cortex. Neuroscience 136:435–443
15. Cunningham BA, Hoffman S, Rutishauser U et al (1983) Molecular topography of the neural cell adhesion molecule N-CAM: surface orientation and location of sialic acid-rich and binding regions. Proc Natl Acad Sci USA 80:3116–3120
16. Sadoul R, Hirn M, Deagostini-Bazin H et al (1983) Adult and embryonic mouse neural cell adhesion molecules have different binding properties. Nature 304:347–349
17. Hoffman S, Edelman GM (1984) The mechanism of binding of neural cell adhesion molecules. Adv Exp Med Biol 181:147–160
18. Rutishauser U, Acheson A, Hall AK et al (1988) The neural cell adhesion molecule (NCAM) as a regulator of cell-cell interactions. Science 240:53–57
19. Edelman GM (1984) Modulation of cell adhesion during induction, histogenesis, and perinatal development of the nervous system. Annu Rev Neurosci 7:339–377
20. Edelman GM, Crossin KL (1991) Cell adhesion molecules: implications for a molecular histology. Annu Rev Biochem 60:155–190
21. Cremer H, Lange R, Christoph A et al (1994) Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. Nature 367:455–459
22. Stork O, Welzl H, Wotjak CT et al (1999) Anxiety and increased 5-HT1A receptor response in NCAM null mutant mice. J Neurobiol 40:343–355

23. Stork O, Welzl H, Cremer H et al (1997) Increased intermale aggression and neuroendocrine response in mice deficient for the neural cell adhesion molecule (NCAM). Eur J Neurosci 9:1117–1125
24. Stork O, Welzl H, Wolfer D et al (2000) Recovery of emotional behavior in neural cell adhesion molecule (NCAM) null mutant mice through transgenic expression of NCAM180. Eur J Neurosci 12:3291–3306
25. Neiiendam JL, Kohler LB, Christensen C et al (2004) An NCAM-derived FGF-receptor agonist, the FGL-peptide, induces neurite outgrowth and neuronal survival in primary rat neurons. J Neurochem 91:920–935
26. Rizhova L, Klementiev B, Cambon K et al (2007) Effects of P2, a peptide derived from a homophilic binding site in the neural cell adhesion molecule on learning and memory in rats. Neuroscience 149:931–942
27. Kiryushko D, Kofoed T, Skladchikova G et al (2003) A synthetic peptide ligand of neural cell adhesion molecule (NCAM), C3d, promotes neuritogenesis and synaptogenesis and modulates presynaptic function in primary cultures of rat hippocampal neurons. J Biol Chem 278: 12325–12334
28. Hartz BP, Sohoel A, Berezin V et al (2003) A synthetic peptide ligand of NCAM affects exploratory behavior and memory in rodents. Pharmacol Biochem Behav 75:861–867
29. Poltorak M, Frye MA, Wright R et al (1996) Increased neural cell adhesion molecule in the CSF of patients with mood disorder. J Neurochem 66:1532–1538
30. Vawter MP (2000) Dysregulation of the neural cell adhesion molecule and neuropsychiatric disorders. Eur J Pharmacol 405(1–3):385–395
31. Vawter MP, Usen N, Thatcher L et al (2001) Characterization of human cleaved N-CAM and association with schizophrenia. Exp Neurol 172:29–46
32. Pillai-Nair N, Panicker AK, Rodriguiz RM et al (2005) Neural cell adhesion molecule-secreting transgenic mice display abnormalities in GABAergic interneurons and alterations in behavior. J Neurosci 25:4659–4671
33. Nacher J, Pham K, Gil-Fernandez V et al (2004) Chronic restraint stress and chronic corticosterone treatment modulate differentially the expression of molecules related to structural plasticity in the adult rat piriform cortex. Neuroscience 126:503–509
34. Venero C, Tilling T, Hermans-Borgmeyer I et al (2002) Chronic stress induces opposite changes in the mRNA expression of the cell adhesion molecules NCAM and L1. Neuroscience 115:1211–1219
35. Touyarot K, Sandi C (2002) Chronic restraint stress induces an isoform-specific regulation on the neural cell adhesion molecule in the hippocampus. Neural Plast 9:147–159
36. Touyarot K, Venero C, Sandi C (2004) Spatial learning impairment induced by chronic stress is related to individual differences in novelty reactivity: search for neurobiological correlates. Psychoneuroendocrinology 29:290–305
37. Sandi C, Touyarot K (2006) Mid-life stress and cognitive deficits during early aging in rats: individual differences and hippocampal correlates. Neurobiol Aging 27:128–140
38. Sandi C, Merino JJ, Cordero MI et al (2001) Effects of chronic stress on contextual fear conditioning and the hippocampal expression of the neural cell adhesion molecule, its polysialylation, and L1. Neuroscience 102:329–339
39. Pham K, Nacher J, Hof PR et al (2003) Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. Eur J Neurosci 17:879–886
40. Alfonso J, Frick LR, Silberman DM et al (2006) Regulation of hippocampal gene expression is conserved in two species subjected to different stressors and antidepressant treatments. Biol Psychiatry 59:244–251
41. Sandi C, Loscertales M (1999) Opposite effects on NCAM expression in the rat frontal cortex induced by acute vs. chronic corticosterone treatments. Brain Res 828:127–134
42. Nacher J, Gomez-Climent MA, McEwen B (2004) Chronic non-invasive glucocorticoid administration decreases polysialylated neural cell adhesion molecule expression in the adult rat dentate gyrus. Neurosci Lett 370:40–44

43. Sandi C (2004) Stress, cognitive impairment and cell adhesion molecules. Nat Rev Neurosci 5:917–930
44. Cordero MI, Rodriguez JJ, Davies HA et al (2005) Chronic restraint stress down-regulates amygdaloid expression of polysialylated neural cell adhesion molecule. Neuroscience 133:903–910
45. Zharkovsky A, Aonurm A, Soon K, Zharkovsky M (2006) Depression-like phenotype in NCAM-deficient mice. Int J Dev Neuroscience 24:495–603
46. Encinas JM, Vaahtokari A, Enikolopov G (2006) Fluoxetine targets early progenitor cells in the adult brain. Proc Natl Acad Sci USA 103:8233–8238
47. Sairanen M, O'Leary OF, Knuuttila JE et al (2007) Chronic antidepressant treatment selectively increases expression of plasticity-related proteins in the hippocampus and medial prefrontal cortex of the rat. Neuroscience 144:368–374
48. Alvarez P, Squire LR (1994) Memory consolidation and the medial temporal lobe: a simple network model. Proc Natl Acad Sci USA 91:7041–7045
49. Squire LR, Zola SM (1996) Structure and function of declarative and nondeclarative memory systems. Proc Natl Acad Sci USA 93:13515–13522
50. Blozovski D, Harris P (1986) Passive avoidance deficits following lesions of the poster-oventral hippocampo-subiculo-entorhinal area in the developing rat. J Physiol (Paris) 81:374–378
51. Moser E, Moser MB, Andersen P (1993) Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. J Neurosci 13:3916–3925
52. Liang KC, McGaugh JL, Martinez JL Jr et al (1982) Post-training amygdaloid lesions impair retention of an inhibitory avoidance response. Behav Brain Res 4:237–249
53. Parent MB, McGaugh JL (1994) Posttraining infusion of lidocaine into the amygdala basolateral complex impairs retention of inhibitory avoidance training. Brain Res 661:97–103
54. Goosens KA, Maren S (2001) Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. Learn Mem 8:148–155
55. Wilensky AE, Schafe GE, LeDoux JE (1999) Functional inactivation of the amygdala before but not after auditory fear conditioning prevents memory formation. J Neurosci 19:RC48
56. Chang SD, Chen DY, Liang KC (2008) Infusion of lidocaine into the dorsal hippocampus before or after the shock training phase impaired conditioned freezing in a two-phase training task of contextual fear conditioning. Neurobiol Learn Mem 89:95–105
57. Parent MB, West M, McGaugh JL (1994) Memory of rats with amygdala lesions induced 30 days after footshock-motivated escape training reflects degree of original training. Behav Neurosci 108:1080–1087
58. Maren S, Yap SA, Goosens KA (2001) The amygdala is essential for the development of neuronal plasticity in the medial geniculate nucleus during auditory fear conditioning in rats. J Neurosci 21:RC135
59. Phillips RG, LeDoux JE (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274–285
60. Bukalo O, Fentrop N, Lee AY et al (2004) Conditional ablation of the neural cell adhesion molecule reduces precision of spatial learning, long-term potentiation, and depression in the CA1 subfield of mouse hippocampus. J Neurosci 24:1565–1577
61. Plappert CF, Schachner M, Pilz PK (2006) Neural cell adhesion molecule (NCAM) null mice show impaired sensitization of the startle response. Genes Brain Behav 5:46–52
62. Richardson R, Elsayed H (1998) Shock sensitization of startle in rats: the role of contextual conditioning. Behav Neurosci 112:1136–1141
63. Doyle E, Nolan PM, Bell R et al (1992) Intraventricular infusions of anti-neural cell adhesion molecules in a discrete posttraining period impair consolidation of a passive avoidance response in the rat. J Neurochem 59:1570–1573
64. Scholey AB, Rose SP, Zamani MR et al (1993) A role for the neural cell adhesion molecule in a late, consolidating phase of glycoprotein synthesis six hours following passive avoidance training of the young chick. Neuroscience 55:499–509

65. Mileusnic R, Rose SP, Lancashire C et al (1995) Characterisation of antibodies specific for chick brain neural cell adhesion molecules which cause amnesia for a passive avoidance task. J Neurochem 64:2598–2606
66. Mileusnic R, Lancashire C, Rose SP (1999) Sequence-specific impairment of memory formation by NCAM antisense oligonucleotides. Learn Mem 6:120–127
67. Foley AG, Hartz BP, Gallagher HC et al (2000) A synthetic peptide ligand of neural cell adhesion molecule (NCAM) IgI domain prevents NCAM internalization and disrupts passive avoidance learning. J Neurochem 74:2607–2613
68. Venero C, Herrero AI, Touyarot K et al (2006) Hippocampal up-regulation of NCAM expression and polysialylation plays a key role on spatial memory. Eur J NeuroSci 23:1585–1595
69. Cambon K, Venero C, Berezin V et al (2003) Post-training administration of a synthetic peptide ligand of the neural cell adhesion molecule, C3d, attenuates long-term expression of contextual fear conditioning. Neuroscience 122:183–191
70. Saffell JL, Walsh FS, Doherty P (1994) Expression of NCAM containing VASE in neurons can account for a developmental loss in their neurite outgrowth response to NCAM in a cellular substratum. J Cell Biol 125:427–436
71. Niethammer P, Delling M, Sytnyk V et al (2002) Cosignaling of NCAM via lipid rafts and the FGF receptor is required for neuritogenesis. J Cell Biol 157:521–532
72. Cambon K, Hansen SM, Venero C et al (2004) A synthetic neural cell adhesion molecule mimetic peptide promotes synaptogenesis, enhances presynaptic function, and facilitates memory consolidation. J Neurosci 24:4197–4204
73. Skibo GG, Davies HA, Rusakov DA et al (1998) Increased immunogold labelling of neural cell adhesion molecule isoforms in synaptic active zones of the chick striatum 5–6 hours after one-trial passive avoidance training. Neuroscience 82:1–5
74. Pradel G, Schmidt R, Schachner M (2000) Involvement of L1.1 in memory consolidation after active avoidance conditioning in zebrafish. J Neurobiol 43:389–403
75. Kandel ER (2001) The molecular biology of memory storage: a dialog between genes and synapses. Biosci Rep 21:565–611
76. Pinsker HM, Hening WA, Carew TJ et al (1973) Long-term sensitization of a defensive withdrawal reflex in Aplysia. Science 182:1039–1042
77. Hawkins RD, Castellucci VF, Kandel ER (1981) Interneurons involved in mediation and modulation of gill-withdrawal reflex in Aplysia. I. Identification and characterization. J Neurophysiol 45:304–314
78. Glanzman DL, Kandel ER, Schacher S (1989) Identified target motor neuron regulates neurite outgrowth and synapse formation of aplysia sensory neurons in vitro. Neuron 3:441–450
79. Bailey CH, Chen M, Keller F et al (1992) Serotonin-mediated endocytosis of apCAM: an early step of learning-related synaptic growth in Aplysia. Science 256:645–649
80. Kiss JZ, Muller D (2001) Contribution of the neural cell adhesion molecule to neuronal and synaptic plasticity. Rev Neurosci 12:297–310
81. Becker CG, Artola A, Gerardy-Schahn R et al (1996) The polysialic acid modification of the neural cell adhesion molecule is involved in spatial learning and hippocampal long-term potentiation. J Neurosci Res 45:143–152
82. Muller D, Wang C, Skibo G et al (1996) PSA-NCAM is required for activity-induced synaptic plasticity. Neuron 17:413–422
83. Markram K, Gerardy-Schahn R, Sandi C (2007) Selective learning and memory impairments in mice deficient for polysialylated NCAM in adulthood. Neuroscience 144:788–796
84. Florian C, Foltz J, Norreel JC et al (2006) Post-training intrahippocampal injection of synthetic poly-alpha-2, 8-sialic acid-neural cell adhesion molecule mimetic peptide improves spatial long-term performance in mice. Learn Mem 13:335–341
85. Senkov O, Sun M, Weinhold B et al (2006) Polysialylated neural cell adhesion molecule is involved in induction of long-term potentiation and memory acquisition and consolidation in a fear-conditioning paradigm. J Neurosci 26:10888–10898

86. Angata K, Long JM, Bukalo O et al (2004) Sialyltransferase ST8Sia-II assembles a subset of polysialic acid that directs hippocampal axonal targeting and promotes fear behavior. J Biol Chem 279:32603–32613
87. Markram K, Lopez Fernandez MA, Abrous DN et al (2007) Amygdala upregulation of NCAM polysialylation induced by auditory fear conditioning is not required for memory formation, but plays a role in fear extinction. Neurobiol Learn Mem 87:573–582
88. Fox GB, O'Connell AW, Murphy KJ et al (1995) Memory consolidation induces a transient and time-dependent increase in the frequency of neural cell adhesion molecule polysialylated cells in the adult rat hippocampus. J Neurochem 65:2796–2799
89. Doyle E, Regan CM, Shiotani T (1993) Nefiracetam (DM-9384) preserves hippocampal neural cell adhesion molecule-mediated memory consolidation processes during scopolamine disruption of passive avoidance training in the rat. J Neurochem 61:266–272
90. Fox GB, Fichera G, Barry T et al (2000) Consolidation of passive avoidance learning is associated with transient increases of polysialylated neurons in layer II of the rat medial temporal cortex. J Neurobiol 45:135–141
91. Foley AG, Ronn LC, Murphy KJ et al (2003) Distribution of polysialylated neural cell adhesion molecule in rat septal nuclei and septohippocampal pathway: transient increase of polysialylated interneurons in the subtriangular septal zone during memory consolidation. J Neurosci Res 74:807–817
92. O'Connell AW, Fox GB, Barry T et al (1997) Spatial learning activates neural cell adhesion molecule polysialylation in a corticohippocampal pathway within the medial temporal lobe. J Neurochem 68:2538–2546
93. Murphy KJ, O'Connell AW, Regan CM (1996) Repetitive and transient increases in hippocampal neural cell adhesion molecule polysialylation state following multitrial spatial training. J Neurochem 67:1268–1274
94. Murphy KJ, Regan CM (1999) Sequential training in separate paradigms impairs second task consolidation and learning-associated modulations of hippocampal NCAM polysialylation. Neurobiol Learn Mem 72:28–38
95. Van der Borght K, Wallinga AE, Luiten PG et al (2005) Morris water maze learning in two rat strains increases the expression of the polysialylated form of the neural cell adhesion molecule in the dentate gyrus but has no effect on hippocampal neurogenesis. Behav Neurosci 119:926–932
96. Foley AG, Hedigan K, Roullet P et al (2003) Consolidation of memory for odour-reward association requires transient polysialylation of the neural cell adhesion molecule in the rat hippocampal dentate gyrus. J Neurosci Res 74:570–576
97. Lopez-Fernandez MA, Montaron MF, Varea E et al (2007) Upregulation of polysialylated neural cell adhesion molecule in the dorsal hippocampus after contextual fear conditioning is involved in long-term memory formation. J Neurosci 27:4552–4561
98. Merino JJ, Cordero MI, Sandi C (2000) Regulation of hippocampal cell adhesion molecules NCAM and L1 by contextual fear conditioning is dependent upon time and stressor intensity. Eur J NeuroSci 12:3283–3290
99. Sandi C, Rose SP, Mileusnic R et al (1995) Corticosterone facilitates long-term memory formation via enhanced glycoprotein synthesis. Neuroscience 69:1087–1093
100. Cordero MI, Merino JJ, Sandi C (1998) Correlational relationship between shock intensity and corticosterone secretion on the establishment and subsequent expression of contextual fear conditioning. Behav Neurosci 112:885–891
101. Sandi C, Woodson JC, Haynes VF et al (2005) Acute stress-induced impairment of spatial memory is associated with decreased expression of neural cell adhesion molecule in the hippocampus and prefrontal cortex. Biol Psychiatry 57:856–864
102. Doyle E, Nolan PM, Bell R et al (1992) Hippocampal NCAM180 transiently increases sialylation during the acquisition and consolidation of a passive avoidance response in the adult rat. J Neurosci Res 31:513–523
103. Rauschecker JP (1995) Developmental plasticity and memory. Behav Brain Res 66:7–12

104. O'Malley A, O'Connell C, Regan CM (1998) Ultrastructural analysis reveals avoidance conditioning to induce a transient increase in hippocampal dentate spine density in the 6 hour post-training period of consolidation. Neuroscience 87:607–613
105. O'Malley A, O'Connell C, Murphy KJ et al (2000) Transient spine density increases in the mid-molecular layer of hippocampal dentate gyrus accompany consolidation of a spatial learning task in the rodent. Neuroscience 99:229–232
106. Eyre MD, Richter-Levin G, Avital A et al (2003) Morphological changes in hippocampal dentate gyrus synapses following spatial learning in rats are transient. Eur J NeuroSci 17:1973–1980
107. Stewart MG, Davies HA, Sandi C et al (2005) Stress suppresses and learning induces plasticity in CA3 of rat hippocampus: a three-dimensional ultrastructural study of thorny excrescences and their postsynaptic densities. Neuroscience 131:43–54
108. Dityatev A, Dityateva G, Sytnyk V et al (2004) Polysialylated neural cell adhesion molecule promotes remodeling and formation of hippocampal synapses. J Neurosci 24:9372–9382
109. Sandi C, Merino JJ, Cordero MI et al (2003) Modulation of hippocampal NCAM polysialylation and spatial memory consolidation by fear conditioning. Biol Psychiatry 54:599–607
110. Doyle E, Regan CM (1993) Cholinergic and dopaminergic agents which inhibit a passive avoidance response attenuate the paradigm-specific increases in NCAM sialylation state. J Neural Transm Gen Sect 92:33–49
111. Venero C, Tilling T, Hermans-Borgmeyer I et al (2004) Water maze learning and forebrain mRNA expression of the neural cell adhesion molecule L1. J Neurosci Res 75:172–181
112. Arami S, Jucker M, Schachner M et al (1996) The effect of continuous intraventricular infusion of L1 and NCAM antibodies on spatial learning in rats. Behav Brain Res 81:81–87
113. Sandi C, Cordero MI, Merino JJ et al (2004) Neurobiological and endocrine correlates of individual differences in spatial learning ability. Learn Mem 11:244–252
114. Knafo S, Barkai E, Herrero AI et al (2005) Olfactory learning-related NCAM expression is state, time, and location specific and is correlated with individual learning capabilities. Hippocampus 15:316–325
115. Gheusi G, Cremer H, McLean H et al (2000) Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. Proc Natl Acad Sci USA 97:1823–1828
116. Roullet P, Mileusnic R, Rose SP et al (1997) Neural cell adhesion molecules play a role in rat memory formation in appetitive as well as aversive tasks. NeuroReport 8:1907–1911
117. Roullet P, Mileusnic R, Rose SP, Sara SJ et al (1997) Neural cell adhesion molecules play a role in rat memory formation in appetitive as well as aversive tasks. NeuroReport 8:1907–1911
118. Conboy L, Tanrikut C, Zoladz PR, Campbell AM, Park CR, Gabriel C, Mocaer E, Sandi C, Diamond DM (2009) The antidepressant agomelatine blocks the adverse effects of stress on memory and enables spatial learning to rapidly increase neural cell adhesion molecule (NCAM) expression in the hippocampus of rats. Int J Neuropsychopharmacol 12(3):329–41