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Topical Review

Clustering of Neurotransmitter Receptors in the Mammalian Retina

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Introduction

In the mammalian central nervous system (CNS) fast transmission of information between neurons is dependent upon characteristic properties of specialized cell contacts, the synapses. Synaptic contacts show a high degree of morphological specialization which is characterized by a concentration of several types of intracellular, transmembrane and extracellular molecules at the contact site (Craig et al., 1994; Ehlers et al., 1996; Gomperts, 1996; Kirsch & Kröger, 1996; Kirsch et al., 1996; Sheng, 1996; Sheng & Kim, 1996; Sheng & Wyszynski, 1997).

Both the presynaptic and the postsynaptic site (Kirsch & Kröger, 1996; Irie et al., 1997; Nguyen & Südhof, 1997; Song et al., 1999) of the synapse forming neurons develop molecular and morphological specializations. Glial cells also develop cellular specializations at synaptic sites and play an important role in contributing molecules that regulate neurotransmission. Synaptically localized molecules include neurotransmitter receptors, amino acid transporters and neurotransmitter-degrading enzymes as well as structural molecules responsible for neurotransmitter vesicle transport, fusion and recycling and for establishing the cellular contact.

These different families of pre- and postsynaptic molecules are enriched at synaptic sites. They allow the precise alignment of pre- and postsynaptic specializations opposite to each other. To achieve signaling specificity, distinct sets of these molecules are clustered to the correct cellular region and are put in register with each other. For example, presynaptic neurotransmitter accompanied by the necessary exocytotic machinery and corresponding postsynaptic neurotransmitter receptors have to be specifically targeted to distinct types of synapses (Craig et al., 1994; Wallace, 1996; Sheng & Wyszynski, 1997; Kennedy, 1998). To increase the speed and reliability of signal transmission, these synaptic molecules are not only found in the synapses, but they are markedly enriched within synapses. Clustering results from a combination of presynaptic signals, postsynaptic signals and signals located extracellularly in the synaptic cleft (Wallace, 1996; Sheng & Wyszynski, 1997; Kennedy, 1998). In particular, neurotransmitter receptors, the primary mediators of chemical and electrical signal transduction, are actively concentrated at synaptic sites. The distribution of these transmembrane proteins changes during ontogenetic development of the mammalian retina, where receptors are expressed extrasynaptically first in a ubiquitous distribution and later during development are aggregated at synaptic sites (Hartveit et al., 1994; Brandstätter et al., 1996, 1998; Mitchell & Redburn, 1996; Karne et al., 1997; Sassoè-Pognetto & Wässle, 1997; Hu et al., 1998; Koulen, 1999*b*,*c*; Koulen et al., 1996*a*, 1997*b*, 1998*b*,*c*). In addition there is a high specificity for the differential molecular composition of the postsynaptic site (Vardi & Sterling, 1994; Brandstätter et al., 1996, 1997; Koulen et al., 1996*b*; Morigiwa & Vardi, 1999).

This review will focus on how neurotransmitter receptors are distributed and what underlying clustering mechanisms might be involved in their synaptic local-

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ization in one part of the mammalian CNS, the retina, that serves as a model for identical processes in other parts of the CNS.

The Need for Neurotransmitter Receptor Clustering in Retinal Circuitry

The mammalian retina offers several experimental advantages over other parts of the CNS: It is easily accessible and controlled delivery of external stimuli is possible. In addition, its well-described anatomy, physiology and function also enables a detailed study of neuronal function.

In contrast to other regions of the mammalian CNS that contain only specific sets of neurotransmitters with the appropriate receptors and accompanying molecules, the retina contains most known neurotransmission systems (for review *see* Wässle & Boycott, 1991; Brandstätter et al., 1998; Wässle et al., 1998). In the retina the distribution of neurotransmitters, and especially neurotransmitter receptors, is highly specialized to convey specific information only of matching pre- and postsynaptic partners. Furthermore, one single cell type can express different specialized synapses with distinct neurotransmitter, neurotransmitter receptor and neuromodulator composition to accommodate appropriate reactions to different presynaptic partners (Greferath et al., 1995; Koulen et al., 1996*b*; Vardi & Morigiwa, 1997; Fletcher et al., 1998).

All these specializations are needed for optimizing the speed of neurotransmission. The time between the perception of visual stimuli by the retinal photoreceptor cells and the first responses in brain areas responsible for the processing of visual information is approximately 300 msec (Rager & Singer, 1998; Gegenfurtner, 1999). Taking into account the time required for signal transduction (Liebman & Pugh, 1982; Ichikawa, 1985, 1994; Koch & Kaupp, 1985; Zuckerman & Cheasty, 1986; Schmidt & Yguerabide, 1989; Lamb & Pugh, 1992; Hurley, 1994) this speed requires fast synaptic transmission. The characteristic speed of transmission between CNS neurons and interneurons downstream of the photoreceptor cells can only be achieved by molecularly specialized contact sites.

Therefore, both factors, signaling specificity and transmission speed require a highly efficient mechanism to segregate all the necessary components.

Neurotransmitter Receptors of the Mammalian Retina—Clustered at Synapses

The presence of most known neurotransmitter systems in the mammalian retina (Wässle & Boycott, 1991; Pourcho, 1996; Brandstätter et al., 1998; Enz & Cutting, 1998; Feigenspan & Bormann, 1998; Wässle et al., 1998) is very challenging on one hand because a plethora of signaling molecules is present in close proximity to each other. Redundancy in signaling mechanisms as well as in the function of neurotransmission systems has to be considered when analyzing retinal signaling circuits. On the other hand, for the same reason this nervous tissue can help answer a crucial cell-biological question: How do individual neurons target their diversity of neurotransmitter receptors to different synapses to achieve signaling specificity?

Table 1 shows that a large number of neurotransmitter receptors have been localized to synapses of the mammalian retina. In addition, synaptic staining in most studies shows differential staining patterns rather than a ubiquitous distribution. Cell and synapse specific distribution of neurotransmitter receptors is characterized by localization to specific layers or bands. This pattern is caused by a synaptic localization of neurotransmitter receptors combined with the stratification of processes of retinal neurons into functional layers (Wässle & Boycott, 1991; Bisti et al., 1998). An especially well-characterized example is the distribution of the ionotropic GABA receptors (*see* review by Wässle et al., 1998). The separation of retinal synapses into glutamatergic ribbon synapses, which show specific structural specializations and conventional synapses that use neurotransmitters other than glutamate, indicates highly specialized protein distribution mechanisms. The synaptic localization of various neurotransmitter receptors is supported by several studies on the physiology and function of synaptic connections in the mammalian retina (reviewed in Wässle & Boycott, 1991).

Typically, the physiological characterizations of transmission speed and specificity argue for synaptically localized neurotransmitter action (Sabatini & Regehr, 1996). However, this compelling evidence for synaptic localization and associated function of neurotransmitter receptors is supported by only a small number of publications on how this specific clustering and targeting is achieved.

Clustering Molecules—Many Families, One Function

In recent years, many proteins involved in neurotransmitter receptor clustering have been identified in various parts of the central and peripheral nervous system. The protein families that have been characterized by functional assays (loss of synaptic clustering) and localization (colocalization with synaptically localized molecules) are diverse and unrelated to each other. Table 2 provides an overview of these molecules. Typically, specific clustering proteins are associated with a distinct neurotransmitter receptor family. This specificity can

Table 1. Synaptically localized neurotransmitter receptors in the mammalian (if not labeled otherwise, rat) retina

Neurotransmitter receptors	Reference adult	Reference ontogenesis
NMDA receptor	Hartveit et al., 1994	Hartveit et al., 1994
AMPA receptor	Morigiwa & Vardi, 1999 (cat)	
	Qin & Pourcho, 1999 (cat)	
Kainate receptor	Peng et al., 1995	
	Brandstätter et al., 1997	
Dopamine receptor	Veruki & Wässle, 1996	Koulen, 1999*b*
	Veruki, 1997	
Metabotropic glutamate receptor (mGluR)	Nomura et al., 1994	Nomura et al., 1994
	Brandstätter et al., 1996	Brandstätter et al., 1996
	Koulen et al. 1996a	Koulen et al., 1996*a*
	Koulen et al. 1997*b*	Koulen et al., 1997*b*
	Vardi & Morigiwa, 1997	
Glycine receptor	Grünert & Wässle, 1993 (macaque, cat, rabbit, rat)	Pinto et al., 1994 (mouse)
	Greferath et al., 1994*a*	Wässle et al., 1998 (mouse)
	Pinto et al., 1994 (mouse)	
	Sassoè-Pognetto et al., 1994	
	Grunert & Wässle, 1996 (macaque)	
	Koulen et al., 1996*b*	
	Wässle et al., 1998	
	Zucker, 1998 (rabbit)	
$GABA_A$ receptor	Hughes et al., 1991 (cat)	Hu et al., 1998
	Vardi et al., 1992 (cat)	Sassoè-Pognetto & Wässle, 1997
	Grunert & Hughes, 1993 (cat)	Karne et al., 1997 (ferret)
	Greferath et al., 1994*b* (rabbit)	Mitchell & Redburn, 1996
	Vardi & Sterling, 1994 (macaque, man)	
	Greferath et al., 1995	
	Sassoè-Pognetto et al., 1995	
	Koulen et al., 1996*b*	
	Karne et al., 1997 (ferret)	
	Fletcher et al., 1998	
	Zucker & Ehinger, 1998 (rabbit)	
$GABA_B$ receptor	Koulen et al., 1998*c*	Koulen et al., 1998*c*
$GABA_C$ receptor	Enz et al., 1996 (cat, rat, rabbit, macaque)	Koulen et al., 1998*b*
	Koulen et al., 1997*a* (cat)	
	Koulen et al., 1998*b*	
	Fletcher et al., 1998	
Acetylcholine receptor	Hutchins, 1994	

distinguish subclasses or isoforms of receptors, as in the case for the NMDA (N-methyl-D-aspartic acid) receptor and ionotropic GABA (γ-amino-butyric acid) receptors.

Even though the mechanism of action for clustering activity has not been described in complete detail for each clustering protein family, there are several possible mechanisms (Fig. 1). The different mechanisms involve receptor phosphorylation (Gurd, 1985*a,b*; Zuckerman & Cheasty, 1986; Wallace et al., 1991; Kennedy, 1998), linking of the neurotransmitter receptor to the cytoskeleton or other intracellular proteins or establishment of a connection between the neurotransmitter receptor and the extracellular matrix (Table 2, Fig. 1). These mechanisms may differ among receptor types and clustering protein families.

The identification of these molecules in the mammalian retina has just begun. The following paragraphs will summarize the possible series of events that might be involved in neurotransmitter receptor clustering in the mammalian retina. Evidence for the involvement of clustering molecules in synapse formation will also be reviewed.

Possible Sequence of Events in Retina Synaptic Development

Synapse-Associated Proteins (SAPs) — Localization at Ribbon Synapses — Clustering Components of Vertical Information Flow

Vertical information processing — photoperception by photoreceptor cells and transmission of information to

Table 2. Identified molecules that are involved in clustering neurotransmitter receptors at synapses

Neurotransmitter receptor	Family of neurotransmitter receptor clustering molecule	Reference
NMDA receptor	SAP, chapsins, citron	Gurd, 1985*a*,*b* Cho et al., 1992 Woods & Bryant, 1993 Kornau et al., 1995 Brenman et al., 1996 Gomperts, 1996 Kim et al., 1996 Lau et al., 1996 Müller et al., 1996 Sheng, 1996 Sheng & Kim, 1996 Sheng & Wyszynski, 1997 Hata et al., 1998 Rao et al., 1998 Kennedy, 1998 Furuyashiki et al., 1999
NMDA receptor	Yotiao	Lin et al., 1998
NMDA receptor	Alpha-actinin	Wyszynski et al., 1997, 1998*a*
AMPA receptor	GRIP, ABP	Dong et al., 1997 Srivastava et al., 1998 Wyszynski et al., 1998*b*
mGluR	Homer, Vesl	Brakeman et al., 1997 Kato et al., 1997, 1998 Tu et al., 1998 Xiao et al., 1998
Glycine receptor	Gephyrin	Betz et al., 1991 Kirsch & Betz, 1993, 1998 Kirsch et al., 1993 Kuhse et al., 1995 Meyer et al., 1995 Kirsch & Kröger, 1996 Kneussel et al., 1990
Acetylcholine receptor	DG complex, MUSK, agrin, S-laminin, rapsyn/43k	McMahan, 1990 Wallace et al., 1991 Ferns & Hall, 1992 McMahan et al., 1992 Hall & Sanes, 1993 Froehner, 1993 Patthy & Nikolics, 1993 Campanelli et al., 1994 Fallon & Hall, 1994 Sealock & Froehner, 1994 Bowe & Fallon, 1995 Hall, 1995 Wallace, 1996, 1997 Sheng & Wyszynski, 1997 Cartaud et al., 1998 Durbeej et al., 1998 Kassner et al., 1998
$GABA_A$ receptor $GABA_C$ receptor	GABARAP, MAP-1B	Shafit-Zagardo et al., 1998 Hanley et al., 1999 Wang et al., 1999

bipolar cells, ganglion cells and higher CNS areas — uses glutamatergic synapses (for review *see* Brandstätter et al., 1998). Glutamatergic synapses in the retina are specialized morphological structures called ribbon synapses. They differ from conventional CNS synapses that are also present in the retina (*see* next paragraph) in their presynaptic part. Ribbon synapses are characterized by a fanlike structure (appears as a "ribbon" in vertical sec-

Clustering molecules (CM) link neurotransmitter receptors (NTR) of the plasma membrane (PM) to the cytoskeleton (CS)	Clustering molecules (CM) link neurotransmitter receptors (NTR) to non-cytoskeletal intracellular proteins (ICP)	Clustering molecules (CM) link neurotransmitter receptors (NTR) to integral membrane proteins (IMP) that bind to the extracellular matrix (ECM)
Betz et al., 1991	Brenman et al., 1996	McMahan et al., 1992
Froehner, 1993	Kim et al., 1996	Froehner, 1993
Kirsch et al., 1993	Müller et al., 1996	Campanelli et al., 1994
Kirsch and Betz, 1993, 1998	Tu et al., 1996	Fallon and Hall, 1994
Kuhse et al., 1995	Dong et al., 1997	Sealock and Froehner, 1994
Meyer et al., 1995	Wyszynski et al., 1997, 1998 (actinin)	Montanaro et al., 1995
Sheng and Wyszynski, 1997	Lin et al., 1998	Cartaud et al., 1998
Kassner et al., 1998	Srivastava et al., 1998	Durbeej et al., 1998
Lin et al., 1998		
Hanley et al., 1999		
Wang et al., 1999		

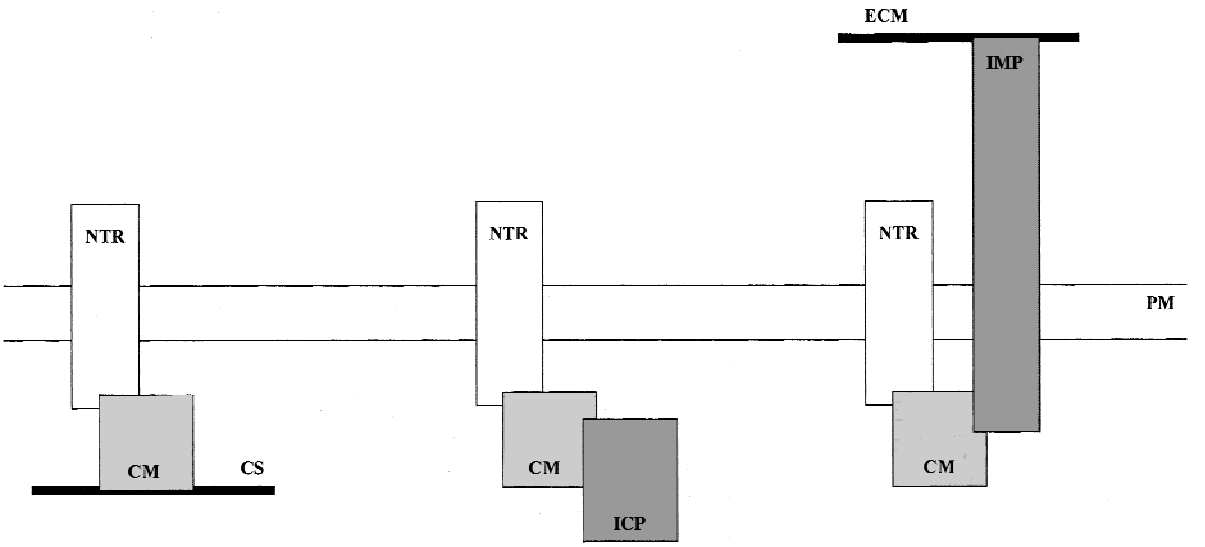

Fig. 1. Interactions of clustering molecules with other proteins.

tions), that is believed to facilitate vesicle fusion and/or function in subcompartmentalizing the presynapse (Rao-Mirotznik, 1995; Vollrath & Spiwoks-Becker, 1996; Brandstätter et al., 1996). These synapses contain a defined number of well characterized postsynaptic elements (Sjöstrand, 1958; Dowling & Boycott, 1966; Rao-Mirotznik, 1995). Ribbon synapses start to develop at the end of the first postnatal week with morphological maturation just prior to eye opening (Weidman & Kuwabara, 1968; Horsburgh & Sefton, 1987).

However, components involved in the development of the function of retinal glutamatergic synapses are expressed and clustered in hot spots in the retina well before the morphological differentiation of ribbon synapses takes place.

Immunocytochemistry data (summarized in Table 3) suggest that clustering molecules of the SAP/chapsin family are expressed at future synaptic sites very early in retina ontogenesis (Koulen, 1999*a*). Typically, the ratio of proteins present at prospective synapses or at synapses compared to extrasynaptic sites increases during development. The same process of segregation into synaptic sites was also observed at later developmental stages for glutamate receptors which are potentially clustered by SAPs (NMDA receptor; Hartveit et al., 1994) but also for other glutamate receptors (Table 3). This sequence of events ((i) synaptic expression of clustering molecules; (ii) expression of neurotransmitter receptors; (iii) final morphological development of the glutamatergic synapse) was also observed for the hippocampus (Rao et al., 1998) and suggests a highly regulated process that is dominated by postsynaptic events. However, an interplay of pre- and postsynaptic differentiation to form functioning synapses has been shown for other synaptic sites (McMahan, 1990; McMahan et al., 1992; Froehner, 1993; Hall, 1995; Wallace, 1996, 1997; Sheng & Wyszynski, 1997; Kennedy, 1998), but remains to be described in the mammalian retina. A wide variety of cotransmitters (Wässle & Boycott, 1991) or molecules involved in neuronal migration and synapse differentiation (Anchan & Reh, 1995; Folli et al., 1996; Cui et al., 1998) could be involved in this process in the mammalian retina.

Table 3. Development of glutamatergic ribbon synapses and expression of glutamate receptors and of clustering proteins at ribbon synapses in the rat retina

Feature	IPL staining at postnatal day	OPL staining at postnatal day	Reference
SAP 90/PSD-95	0	5	Koulen, 1999*a*
Chapsin-110/PSD-93	0	5	Koulen, 1999*a*
SAP 97	2	5	Koulen, 1999*a*
SAP 102	0	5	Koulen, 1999*a*
NMDA receptor	10	–	Hartveit et al., 1994
mGluR 1	1	7	Koulen et al., 1997*b*
mGluR 2	5	–	Koulen et al., 1996*a*
mGluR 4	1	–	Koulen et al., 1996*a*
mGluR 5	3	5	Koulen et al., 1997*b*
mGluR 6	–	7	Nomura et al., 1994
mGluR 7	7	–	Brandstätter et al., 1996
Morphologically defined ribbon synapses	13		Weidmann & Kuwabara, 1968 Horsburgh & Sefton, 1987
Eye opening	15		Tamai & Chader, 1979

Gephyrin — Localization at Conventional Synapses — Clustering Components of Horizontal Information Flow

In addition to the vertical signaling pathway, a larger number of cell types in the mammalian retina are dedicated to transmit neuronal signals laterally (Dowling & Werblin, 1971; Wässle & Boycott, 1991). Amacrine and horizontal cells mediate these horizontal signals that contribute to functions such as lateral inhibition, feedback inhibition, adaptation to different stimulus intensities, and general signal processing of visual information at the retina level. These laterally interacting cells use predominantly GABA and glycine although most other known neurotransmitters are also found in the mammalian retina (Wässle & Boycott, 1991). Consequently, neurotransmitter receptors for these substances are found on all retinal neurons. In contrast to the specialized glutamatergic ribbon synapses these synapses are identical to the majority of synapses in the CNS and are therefore called conventional synapses (Dowling & Boycott, 1966; Dowling & Werblin, 1971).

Conventional synapses are formed earlier during development than ribbon synapses. Like ribbon synapses, synaptic proteins are clustered at future synaptic sites before the morphological features of conventional synapses are fully developed. Table 4 summarizes the expression of proteins found at conventional synapses in the rat retina. Gephyrin, a protein involved in glycine receptor clustering (Kirsch et al., 1993; Meyer et al., 1995; Kirsch & Betz, 1998) and molybdenum metabolism (Stallmeyer et al., 1999) was found to colocalize with glycine receptors and $GABA_A$ receptors in the rat retina (Sassoè-Pognetto et al., 1995). Sassoè-Pognetto and Wässle (1997) have shown that gephyrin is expressed either prior to or at the same time as these neurotransmitter receptors. They have also shown that gephyrin is highly concentrated at future synaptic sites during early ontogenesis.

A large number of other neurotransmitter receptors and subsets of $GABA_A$ receptors (Sassoè-Pognetto et al., 1995) are not clustered by gephyrin. Therefore, more clustering molecules can be expected to be found in the mammalian retina.

MAP-1B Related Clustering Proteins

One of the families of clustering proteins that is involved in targeting neurotransmitter receptors to conventional synapses has been recently identified as microtubule-associated proteins (Hanley et al., 1999; Wang et al., 1999). Like gephyrin, these proteins bind neurotransmitter receptors (Hanley et al., 1999: MAP-1B-$GABA_C$ receptor; Wang et al., 1999: GABARAP-$GABA_A$ receptor) to the microtubles of the cytoskeleton at synapses (Fig. 1). Hanley et al., 1999 also showed that such interactions are highly selective. The interaction is so specific that $GABA_C$ receptors, but not $GABA_A$ receptors, colocalize and interact with MAP-1B.

It appears that the number of clustering molecules parallels and may even exceed the large number of neurotransmitter receptors. Therefore, to identify specifity in synaptic localization and clustering depends critically on the determination of the differential expression and localization of all clustering molecules. The interaction of microtubule-associated proteins and neurotransmitter receptors explains the targeting and clustering of ionotropic GABA receptors and shows that other clustering molecules (*see* Table 2), or not yet identified proteins, may be involved in creating synaptic specificity in the mammalian retina.

Agrin — Is the Clustering Molecule of the Neuromuscular Junction Involved in the Assembly of CNS Synapses?

In addition to clustering molecules and their possible function in the mammalian retina, recent data suggest that another complex of molecules may be involved in the formation of retinal synapses. Synaptically localized proteins of the dystrophin-glycoprotein complex (a complex which interacts with agrin, MUSK, S-laminin and rapsyn; for review *see* McMahan, 1990; McMahan et al.,

Table 4. Development of conventional synapses and expression of key synaptic proteins in rat retina

Feature	IPL staining at postnatal day	OPL staining at postnatal day	Reference
Glycine receptor	1–3	9	Sassoè-Pognetto & Wässle, 1997
$GABA_A$ receptor	1–3	7	Sassoè-Pognetto & Wässle, 1997
	2	8–12	Koulen, 1999*c*
$GABA_B$ receptor	1	1	Koulen et al., 1998*c*
Dopamine D1 receptor	1	8	Koulen, 1999*b*
$GABA_C$ receptor	7	7	Koulen et al., 1998*b*
Gephyrin	1	–	Sassoè-Pognetto & Wässle, 1997
Morphologically defined	3		Sassò-Pognetto & Wässle, 1997
Conventional synapses	11		Weidman & Kuwabara, 1968
			Horsburgh & Sefton, 1987
Eye opening	15		Tamai & Chader, 1979

1992; Froehner, 1993; Hall, 1995; Wallace, 1996, 1997) and was originally characterized to cluster acetylcholine receptors at the neuromuscular junction) appear to be equally important in the formation of CNS synapses (Kirsch & Kröger, 1996, Kröger et al., 1996; Wallace, 1997). Agrin, a protein synthesized by presynaptic neurons and deposited in the synaptic extracellular matrix induces postsynaptic differentiation and neurotransmitter receptor clustering at postsynaptic sites (Nitkin et al., 1987; McMahan, 1990). In the mammalian retina, components of the dystrophin-glycoprotein complex (Montanaro et al., 1995; Koulen et al., 1998*a*; Blank et al., 1999) have been identified and localized to synapses and glial cell specializations. Evidence from mammalian (Biroc et al., 1993) and nonmammalian vertebrate (avian) retina (Ma et al., 1994; Kröger et al., 1996; Mann & Kröger, 1996; Kröger, 1997) indicates that agrin may be involved in the formation of a large number of synapses of the retina. Assuming that agrin and the dystrophin-glycoprotein complex are present at almost all synapses in nonmammalian vertebrate (avian) retina (Koulen et al., 1999) and that this situation is similar in the mammalian retina, the function of this signaling cascade probably mediates not only the clustering of nicotinic acetylcholine receptors but other neurotransmitter receptors as well. Unlike the neuromuscular junction, nicotinic acetylcholine receptors in the retina are limited to a small subset of neurons (Hutchins, 1994).

Function of Neurotransmitters, Neurotransmitter Receptors and Neuronal Activity in the Formation of Synapses

Several lines of evidence indicate that expression of neurotransmitters and neurotransmitter receptors precedes their functions in the adult and the final morphological maturation of synapses (Tables 3 and 4).

Different groups have shown that synaptic activity between neurons is necessary to achieve neuronal and synaptic differentiation in the mammalian retina (Meister et al., 1991; Wong et al., 1991, 1993, 1995; Bodnarenko & Chalupa, 1993; Penn et al., 1994; Bodnarenko et al., 1995). If neurotransmission is blocked, proper development of synaptic contacts does not occur (Wong et al., 1991; Bodnarenko & Chalupa, 1993; Bodnarenko et al., 1995). This finding does not necessarily mean that light stimulus driven neuronal transmission is required for retina synaptic development (Fisher, 1997), but suggests rather that, in fact, most of these processes occur before eye opening (Weidman & Kuwabara, 1968; Horsburgh & Sefton, 1987; Meister et al., 1991; Wong et al., 1991, 1993, 1995; Bodnarenko & Chalupa, 1993; Penn et al., 1994; Bodnarenko et al., 1995).

Conclusions

The need for high velocity and reliability in neuronal signal transmission in the retina created cellular specializations at synaptic sites like in other parts of the mammalian CNS. Neurotransmitter receptors, the receivers and transducers of chemical signaling between neurons, are highly enriched at synaptic sites. Immunocytochemical evidence and studies investigating the interaction between neurotransmitter receptors and intracellular proteins suggest that this targeting is achieved through clustering molecules. These molecules form connections between neurotransmitter receptors and the extracellular matrix and/or the cytoskeleton (*see* Table 2 and Fig. 1). Two questions remain: The exact mechanism of action of clustering molecules and the intercellular signals which determine where future synapses will be established.

The mammalian retina, with its defined function and morphology and its large number of neurotransmitter signaling mechanisms (Wässle & Boycott, 1991), makes it a great model system to determine clustering processes that are important for the understanding of learning and memory, neuronal re- and degeneration and neuronal development. To this end, more localization data of clus-

tering molecules in the mammalian retina and functional assays as described for the spinal cord (Kirsch et al., 1993) are needed. With the dystrophin-glycoprotein complex and agrin, a first approach to the question 'Who clusters the clustering molecules?' is made, but mechanisms that are involved in the consolidation and maintenance of synaptic contacts have to be tested in the mammalian retina to explain the functional data (Meister et al., 1991; Wong et al., 1991, 1993, 1995; Bodnarenko & Chalupa, 1993; Penn et al., 1994; Bodnarenko et al., 1995).

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