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Excitatory Amino Acid Receptors in Normal and Abnormal Vestibular Function

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Contents

Abstract

Although excitatory amino acid (EAA) receptors have been investigated extensively in the limbic system and neocortex, less is known of the function of EAA receptors in the brainstem. A number of biochemical and electrophysiological studies suggest that the synapse between the ipsilateral vestibular (VIIIth) nerve and the brainstem vestibular nucleus (VN) is mediated by an EAA acting predominantly on kainate or α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. In addition, there is electrophysiological evidence that input from the contralateral vestibular nerve via the contralateral VN is partially mediated by

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N-methyl-D-aspartate (NMDA) receptors. Input to the VN from the spinal cord may also be partially mediated by NMDA receptors. All of the electrophysiological studies conducted so far have used in vitro preparations, and it is possible that denervation of the VN during the preparation of an explant or slice causes changes in EAA receptor function. Nonetheless, these results suggest that EAA receptors may be important in many different parts of the vestibular reflex pathways. Studies of the peripheral vestibular system have also shown that EAAs are involved in transmission between the receptor hair cells and the vestibular nerve fibers. A number of recent studies in the area of vestibular plasticity have reported that antagonists for the NMDA receptor subtype disrupt the behavioral recovery that occurs following unilateral deafferentation of the vestibular nerve fibers (vestibular compensation). It has been suggested that vestibular compensation may be owing to an upregulation or increased affinity of NMDA receptors in the VN ipsilateral to the peripheral deafferentation; however; at present, there is no clear evidence to support this hypothesis.

Index Entries: Excitatory amino acid receptors; *N*-methyl-D-aspartate; lesion-induced plasticity; vestibular system; vestibular compensation.

Introduction

Excitatory amino acid (EAA) receptors have been demonstrated to mediate excitatory synaptic transmission in many parts of the central nervous system (CNS). Although these receptors have been studied most extensively in the limbic system and neocortex (*see* Collingridge and Lester, 1989 for a review), in recent years there has been an increase in EAA research in the hindbrain, particularly the cerebellum (e.g., Kano and Kato, 1987; Burgoyne et al., 1988; Smith, 1989; Garthwaite and Beaumont, 1989; Billard and Pumain, 1989; Larson-Prior et al., 1990; Wood et al., 1990; Schramm et al., 1990; Monaghan and Beaton, 1991; Sorimachi et al., 1991) and in the spinal cord (e.g., Grillner et al., 1981; MacDermott et al., 1986; Polc, 1987; Davies et al., 1988; Forsythe and Westbrook, 1988; Ohta and Grillner, 1989; Alford and Williams, 1989; Gerber and Randic, 1989a,b; Hornfeldt and Larson, 1989; Stein and Schild, 1989; Cazalets et al., 1990; Murase et al., 1990; Jansen et al., 1990; Long et al., 1990; Raigorodsky and Urca, 1990; Turski et al., 1990; White et al., 1990; Ziskind-Conhaim, 1990; Sundaram and Sapru, 1991). By contrast, relatively little research has been conducted on EAA receptors in the brainstem: Two main areas of brainstem EAA research have been the nucleus tractus solitarii (e.g., Kubo and Kihara, 1988; Shirasaki et al., 1990; Tell and Jean, 1990; Kessler et al., 1990; Nakagawa et al., 1990) and the vestibular nuclei (*see* Table 1) (*see also* Dye et al., 1989). Although the density of the *N*-methyl-D-aspartate (NMDA) EAA receptor subtype is much lower in the brainstem than in cortical areas (Monaghan and Cotman, 1985), studies of the nucleus tractus solitarii and vestibular nucleus suggest that NMDA receptors may nonetheless make an important contribution to synaptic function in these brainstem areas.

The vestibular nucleus (VN) is a sensory-motor nucleus that is ideal for studying the contribution of EAA receptors to synaptic function and behavior. Many VN neurons (e.g., type I neurons in the medial VN; *see* Fig. 1) receive monosynaptic input from the vestibular portion of the VIIIth nerve (i.e., the vestibular nerve), and project monosynaptically to motoneurons involved in short-latency vestibulo-ocular and vestibulospinal reflexes (*see* Wilson and Melvill Jones, 1979 for a review). These reflex pathways are similar across a wide range of mammalian and submammalian species, and can be used to study the evolution of a behavioral response, from the stimulation of the sensory receptors to muscle contraction. Studies of the effects of EAA-modulating drugs on vestibular reflex behavior can therefore be used to complement physiological and pharmacological studies of the VN.

Table 1
Evidence for EAA Receptors in the Brainstem Vestibular Nucleus*

Type of study	Spec.	Preparation	Authors
Biochemical, vest. n	Cat	In vitro	Raymond et al. (1984)
Biochemical, VN	Rat	In vitro	Kaneko et al. (1989)
Biochemical	Sq. m.	In vitro	Henley and Igarashi (1991)
Binding, MVN	Rat	In vitro	Monaghan and Cotman (1985)
Binding, VN	Rat	In vitro	Touati et al. (1989)
Binding, VN	Rat	In vitro	Raymond et al. (1989)
Electrophysiol., MVN	Rat	In vitro, slice	Gallagher at al. (1985)
Electrophysiol., MVN	Rat	In vitro, slice	Lewis et al. (1987)
Electrophysiol., VN	Frog	In vitro, explant	Knopfel (1987)
Electrophysiol., VN	Frog	In vitro, explant	Cochran et al. (1987)
Electrophysiol., VN	Frog	In vitro, explant	Knopfel and Dieringer (1988)
Electrophysiol., MVN	Rat	In vitro, slice	Lewis et al. (1989)
Electrophysiol., MVN	Guin.	In vitro, slice	Smith et al. (1990)
Electrophysiol., MVN	Rat	In vitro, slice	Doi et al. (1990)
Electrophysiol., MVN	Guin.	In vitro, slice	Smith and Darlington (1992)
Electrophysiol., MVN	Guin.	In vitro, slice	Serafin et al. (1991a)
2-Deoxyglucose, VN	Rat	In vivo	Nehls et al. (1990)
Behavioral	Guin.	In vivo	Smith and Darlington (1988)
Behavioral	Guin.	In vivo	Darlington and Smith (1989)
Behavioral	Guin.	In vivo	Sansom et al. (1990)
Behavioral	Guin.	In vivo	de Waele et al. (1990)
Behavioral	Frog	In vivo	Luneburg and Flohr (1990)
Behavioral	–	In vivo	Pettorossi et al. (1990)
Behavioral	Guin.	In vivo	Sansom et al. (in preparation)

*Summary of evidence relating to the function of excitatory amino acid receptors in the vestibular nucleus complex—vest. n: vestibular nerve; VN: vestibular nucleus complex; MVN: medial vestibular nucleus; electrophysiol.: electrophysiological study; spec.: species; sq. m: squirrel monkey; guin.: guinea pig.

In addition to its advantages in studying the contribution of EAA receptors to normal synaptic function, the VN is also useful for studying EAA-related neuronal plasticity. Numerous studies have documented the adaptive capabilities of the VN under such conditions as altered visual feedback during the vestibulo-ocular reflex (*see* Lisberger, 1988 for a review) and deafferentation of the vestibular nerve fibers (*see* Smith and Curthoys, 1989 for a review). However, at present, few studies of EAA receptors in the vestibular reflex pathways have been conducted compared to other areas of the nervous system, and therefore, the contribution of these receptors to normal vestibular function and vestibular plasticity is poorly understood.

EAA Receptors and Normal Vestibular Function

Receptor Hair Cell/Vestibular Nerve Synapse

Although the identity of the neurotransmitter(s) that mediate(s) transmission between the vestibular receptor hair cells in the labyrinth and the vestibular nerve fibers remains uncertain, there is some evidence to support the hypothesis that an EAA is involved: Glutamate (Glu) has been found to cause an increase in vestibular nerve fiber activity in the isolated frog labyrinth, and this is blocked by the application of Glu receptor antagonists (Annoni et al., 1984; Valli et

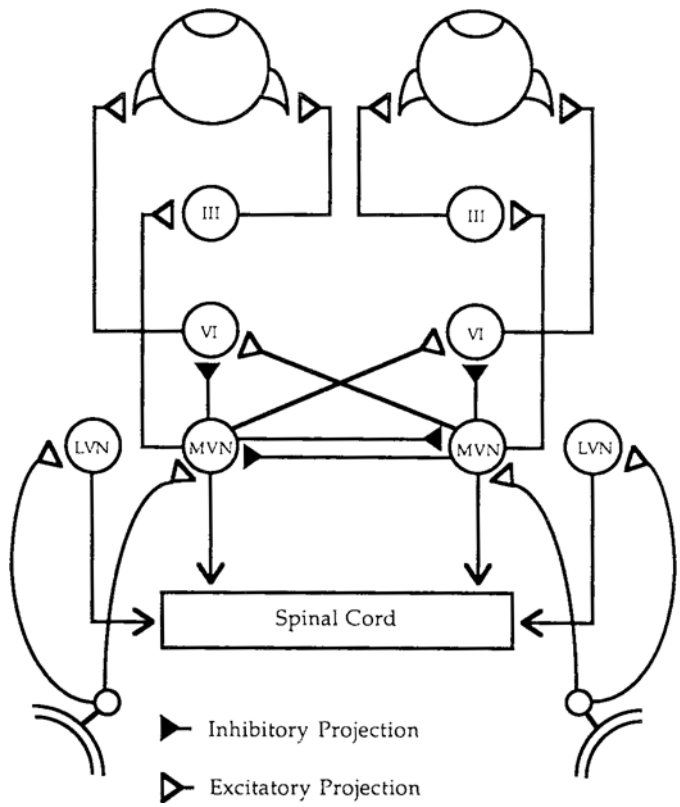

Fig. 1. Simplified schematic diagram of some of the vestibular reflex pathways. The semicircular canals in the diagram represent the entire vestibular labyrinth, from which the vestibular nerve carries vestibular input to the ipsilateral brainstem vestibular nucleus complex. MVN: medial vestibular nucleus; LVN: lateral vestibular nucleus. The other subnuclei of the vestibular nucleus complex have been omitted for simplicity. The vestibulo-ocular reflex pathways shown are those concerning the horizontal vestibulo-ocular reflex (*see* Shimazu, 1983 for a review). VI: abducens nucleus (nucleus of VIth cranial nerve); III: oculomotor nucleus (nucleus of the IIIrd cranial nerve). The inhibitory and excitatory nature of the projections from the MVN and LVN to the spinal cord are not indicated because of their complexity. Note that the brainstem commissural projections between the bilateral MVN are functionally inhibitory, i.e., some of these commissural projections are from inhibitory neurons, and others are from excitatory neurons that synapse with inhibitory interneurons in the MVN to which they project.

al., 1985); application of low concentrations of Glu or kainate to the cat labyrinth in vivo also causes depolarization of vestibular nerve fibers (Dechesne et al., 1984), although in this study, it was not clear whether this was a specific EAA receptor effect. Using embryonic and newborn mouse vestibular epithelia, Raymond and Desmadryl (1985) found that the development of the neurotoxic effects of EAAs parallels the development of EAA binding sites on the corresponding fibers of the vestibular nerve. Recent in vitro studies of the isolated frog labyrinth using intracellular recording suggest that endogenous EAAs may act both on the presynaptic hair cells as well as the postsynaptic vestibular nerve fibers; since quisqualic acid and kainic acid were more potent than NMDA, the authors concluded that both the presynaptic and postsynaptic EAA receptors may be mainly of the nonNMDA type (Prigioni et al., 1990). Biochemical studies have also identified a kainate binding protein on the dendrites of vestibular nerve neurons (Dechesne et al., 1991).

Vestibular Nerve/Vestibular Nucleus Synapse

Biochemical Studies

One of the first indications that EAA receptors might be important in transmission between the vestibular nerve and the VN was the demonstration by Dememes et al. (1984) of retrograde transport of [^{3}H]-D-aspartate in the vestibular nerve fibers. Following this result, Raymond et al. (1984) reported that the presynaptic terminals of vestibular nerve fibers took up Glu and that Glu uptake was significantly decreased after vestibular nerve section. These results led to the suggestion that the vestibular nerve used Glu or aspartate (Asp) as a neurotransmitter (Dememes et al., 1984; Raymond et al., 1984,1988). This hypothesis ran counter to the previously popular hypothesis that the transmitter used by the vestibular nerve is acetylcholine. Recent direct tests using electrical stimulation of the vestibular nerve and intracellular recording from identified second-order VN neurons suggest that, at least in the medial VN (MVN), acetylcholine does not function as a transmitter at the synapse between first- and second- order vestibular neurons (Cochran et al., 1987; Knopfel, 1987; Lewis et al., 1989); whether acetylcholine mediates vestibular nerve input to the lateral VN (LVN) or to other parts of the VN complex remains to be determined (Ito et al., 1981; Matsuoka et al., 1985; Ujihara et al., 1988).

Binding studies have shown that there is a high density of Glu receptors in the VN, especially in the MVN (Touati et al., 1989; Raymond et al., 1989), which is mainly involved in the control of eye movement (*see* Wilson and Melvill Jones, 1979 for a review). The MVN has a higher density of NMDA binding sites than the other VN subnuclei (Monaghan and Cotman, 1985). The functional consequences of the differential distribution of NMDA binding sites across the various subnuclei of the VN is unknown.

Electrophysiological Studies

The results from electrophysiological studies are generally consistent with the pharmacological and biochemical evidence that an EAA is likely to be a transmitter used by the vestibular nerve. However, it should be cautioned that, so far, all of the electrophysiological evidence comes from in vitro preparations (*see* Table 1).

The first electrophysiological evidence that the transmitter mediating vestibular nerve input to the MVN is an EAA was reported by Gallagher et al. (1985) using rat brainstem slices. These authors found that synaptic responses evoked in second-order MVN neurons by electrical stimulation of the root of the VIIIth nerve could be blocked by superfusion with the low-potency EAA receptor antagonist α-aminoadipate, without change in the cell's resting membrane potential or membrane resistance. Further studies from this laboratory have confirmed that MVN neurons are depolarized by a range of EAAs, including NMDA, kainate, quisqualate, and homocysteate, and that excitatory post-synaptic potentials (EPSPs) evoked in single neurons by electrical stimulation of the root of the VIIIth nerve can be blocked or depressed by application of the nonselective EAA antagonist kynurenic acid or non NMDA EAA antagonists, such as DL-α-aminoadipic acid or DL-2-amino-4-phosphonobutyric acid (Lewis et al., 1987,1989). Lewis et al. (1989) reported that the selective NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (D-APV) did not depress, but facilitated, MVN EPSPs evoked by stimulation of the VIIIth nerve; on the other hand, NMDA depolarized second-order neurons, and this effect could be blocked by D-APV. VIIIth nerve-evoked EPSPs also remained unchanged in Mg^{2+}- free artificial cerebrospinal fluid (ACSF). Taken together, these results suggest that, although rat MVN neurons have NMDA receptors, the synapse with the vestibular portion of the VIIIth nerve (the vestibular nerve) is mediated by non NMDA EAA receptors. The authors speculated that the facilitation of the VIIIth nerve-evoked EPSPs in MVN neurons by application of an NMDA antagonist may be the result of the existence of NMDA receptors on presynaptic vestibular nerve terminals that serve to inhibit the release of the EAA transmitter (Lewis et al., 1989).

Much of what is currently known about EAA receptors in the VN comes from studies of the isolated frog medulla by Knopfel (1987) and Cochran et al. (1987). Knopfel (1987) found that VN field potentials evoked by electrical stimulation of the ipsilateral VIIIth nerve were not reduced by D-APV in either normal (1 m*M*) or low (10–50 μ*M*) Mg^{2+} concentrations, but that a late component of field potentials evoked by stimulation of the contralateral VIIIth nerve was reduced by D-APV in low, but not in normal Mg^{2+} concentrations. Recording intracellularly from single VN neurons, Knopfel found that, in 1 m*M* Mg^{2+}, D-APV reduced the slow-rising EPSPs evoked by stimulation of the contralateral VIIIth nerve (cEPSPs), but not the fast-rising EPSPs resulting from stimulation of the ipsilateral VIIIth nerve (iEPSPs). In low Mg^{2+}, both iEPSPs and cEPSPs showed an increase in amplitude and area; although D-APV strongly depressed cEPSPs, it also reduced a late component of iEPSPs. NMDA was found to increase evoked EPSPs, and this effect was abolished in the presence of D-APV. NMDA (but not quisqualate or kainate) was also found to cause an increase in the input resistance of VN neurons, resulting in membrane potential shifts that could be abolished by D-APV. Knopfel attributed the latter effect to the negative slope conductance of the NMDA receptor-mediated ion channel. From his studies of frog medullary explants, Knopfel concluded that VN NMDA receptors do not make a major contribution to synaptic transmission between the ipsilateral vestibular nerve and second-order VN neurons, but that input from the contralateral vestibular nerve, via the contralateral VN, is partially NMDA receptor-mediated *(see* Fig. 2).

Cochran et al. (1987), using Mg^{2+}-free solutions, reported that field potentials evoked by electrical stimulation of the ipsilateral VIIIth nerve could be blocked by the nonselective EAA antagonist kynurenic acid, but not by selective NMDA antagonists, such as D-APV, also suggesting that, although the vestibular nerve input to the ipsilateral VN uses an EAA, it acts on mainly non-NMDA receptors. Intracellular recordings supported this conclusion: iEPSPs could be blocked by low concentrations of kynurenic acid, but even at high concentrations, D-APV reduced only a long latency component of iEPSPs. By contrast, cEPSPs could be abolished by low concentrations of kynurenic acid or D-APV. Stimulation of the spinal cord resulted in EPSPs that could be blocked by low concentrations of kynurenic acid or D-APV. Depolarizations induced by NMDA could be blocked by kynurenic acid.

Consistent with the results of Knopfel (1987), the results of Cochran et al., (1987) suggest that fast-rising monosynaptic EPSPs evoked from the ipsilateral vestibular nerve are mediated by mainly nonNMDA EAA receptors (i.e., kainate/α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid [AMPA] receptors) rather than NMDA receptors, whereas slow-rising EPSPs evoked from the contralateral VN or the spinal cord are partially mediated by NMDA receptors.

Recently, Doi et al. (1990) have used extracellular recording in rat brainstem slices to examine the effects of EAA antagonists on the changes in firing rate of single MVN neurons evoked by electrical stimulation of the ipsilateral VIIIth nerve root or the brainstem commissural fibers, which cross the midline from the contralateral MVN. For the majority of MVN neurons, the response to stimulation of the ipsilateral VIIIth nerve was suppressed by kynurenic acid or the kainate/AMPA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), whereas in only a small number of cases was it suppressed by D,L-APV. By contrast, for the majority of neurons, the response to commissural stimulation was suppressed by kynurenic acid, CNQX, or D,L-APV. Kynurenic acid, CNQX, and D,L-APV were found to decrease the resting activity of many neurons; however, there was no obvious correlation between the effect of these EAA antagonists on resting activity and their effect on the response to electrical stimulation of the ipsilateral VIIIth nerve or the vestibular commissures.

Smith et al. (1990) have also reported that the resting activity of many MVN neurons in guinea pig brainstem slices is strongly depressed by NMDA antagonists, such as 3-([+]-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP)

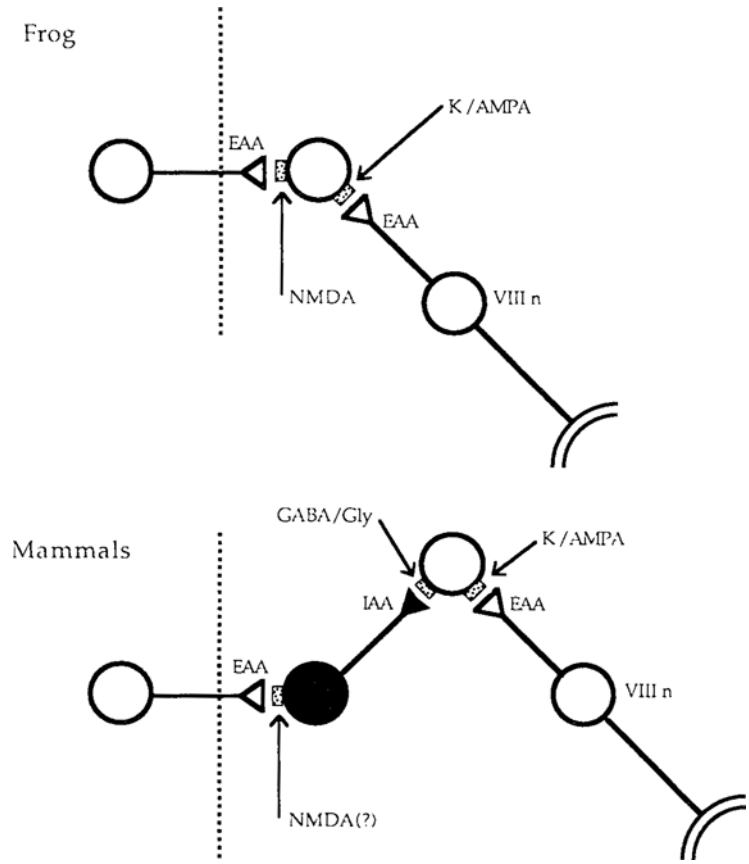

Fig. 2. Simplified schematic diagram showing the labyrinthine and brainstem commissural projections to the VN in frog and mammals, and the neurotransmitter receptors that are likely to mediate these inputs. The semicircular canals represent the entire vestibular labyrinth. VIII n: VIIIth cranial nerve (the vestibulocochlear nerve). The hatched line represents the midline of the brainstem, and the excitatory projection across it represents the excitatory commissural projections characteristic of some MVN type I neurons. In frog, these commissural projections are predominantly excitatory in function, resulting in the excitation of contralateral MVN type I neurons. In mammals, however, the excitatory commissural projections synapse on inhibitory interneurons (type II neurons, shown in black), which in turn inhibit ipsilateral type I neurons (*see* Shimazu and Precht, 1966 and Ozawa et al., 1974; *see also* Shimazu, 1983 for a review); therefore, the commissural projections in mammals are said to be functionally inhibitory. The majority of evidence from frog and mammals suggests that at least one transmitter used by the vestibular nerve is an excitatory amino acid (EAA), acting on mainly kainate/AMPA receptors (K/AMPA). Evidence from the frog suggests that brainstem commissural input to the VN is partially mediated by NMDA receptors. Evidence from mammals suggests that the transmitter mediating commissural inhibition between the MVN is an inhibitory amino acid (IAA), either GABA and/or glycine (Gly), and it is likely that one of these is the transmitter used by type II neurons (Precht et al., 1973). The transmitter mediating excitatory input to type II neurons is unknown, but it is possible that it is an EAA acting on NMDA receptors (*see* Doi et al., 1990).

and ([+]-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,1 0-imine maleate) (MK801), at concentrations as low as 1 μ*M* (*see* Fig. 3). In recent studies by Serafin et al. (1991a), MVN neurons in guinea pig brainstem slices were found to be depolarized by NMDA, and this effect could be blocked by the NMDA antagonist D (–)-2-amino-5-phosphonopentanoic acid (D-AP5); NMDA also induced a decrease in membrane resistance and an increase in spontaneous activ-

ity. Serafin et al. found that, in some MVN neurons, inducing a 10–30 mV hyperpolarization while simultaneously applying NMDA caused a prolonged oscillation of the membrane potential. These oscillations continued in the presence of tetrodotoxin, but could be blocked by D-AP5 or the Ca^{2+} channel blocker cobalt.

Taken together, the electrophysiological studies that have been conducted in frog medullary explants and brainstem slices from rat and guinea pig confirm that VN neurons, particularly MVN neurons, have functional EAA receptors that participate in mediating major excitatory inputs from the ipsilateral labyrinth, the contralateral VN, and the spinal cord. Evidence from both frog (Knopfel, 1987; Cochran et al., 1987) and mammals (Lewis et al., 1989; Doi et al., 1990) suggests that EAA input from the ipsilateral vestibular nerve is mediated predominantly by kainate/AMPA receptors, giving rise to fast EPSPs, whereas input from the contralateral VN and possibly the spinal cord is partially mediated by NMDA receptors, giving rise to slow EPSPs. As in other CNS areas, the function of the NMDA receptors appears to be voltage-dependent, with high Mg^{2+} concentrations decreasing the conductance of the NMDA receptor-associated ion channel (Knopfel, 1987). In this respect, it is interesting that in rat and guinea pig brainstem slices, NMDA antagonists reduce not only stimulation-evoked EPSPs, but also the resting activity of many MVN neurons (Smith et al., 1990; Doi et al., 1990), suggesting that the level of depolarization provided by endogenous transmitter release and perhaps the activity of voltage-dependent Ca^{2+} channels (Serafin et al., 1990,1991b,c) is sufficient to maintain the open state of the NMDA ion channel. This may not occur in frog VN neurons, because VN neurons in that species generate little resting activity even in vivo (Ozawa et al., 1974). A particularly interesting finding is that NMDA can induce changes in membrane resistance and depolarization shifts that can result in oscillatory behavior (Knopfel, 1987; Serafin et al., 1991a). Whether this phenomenon may relate to plastic processes

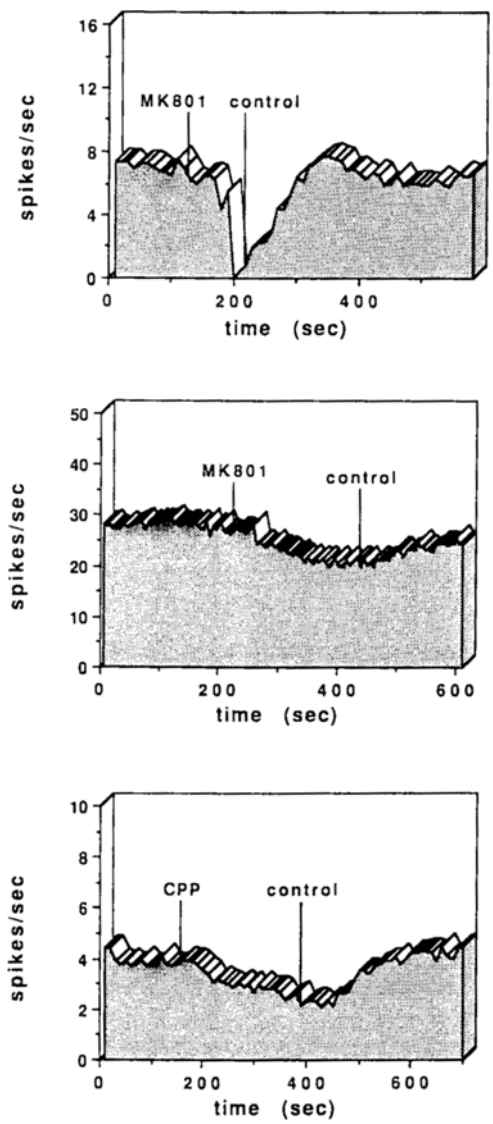

Fig. 3. Examples of the effects of the selective NMDA antagonists MK801 and CPP on the resting activity of single medial vestibular nucleus (MVN) neurons in guinea pig brainstem slices. Data points represent successive bins of averaged resting activity for one neuron. Top: effect of 1 μ*M* MK801 on a neuron from a labyrinthine-intact animal; bin width = 10 s. Middle: effect of 1 μ*M* MK801 on a neuron from a compensated animal at 6–8 wk postunilateral labyrinthectomy; bin width = 5 s. Bottom: effect of 10 n*M* CPP on a neuron from a labyrinthine-intact animal; bin width = 10 s. "MK801" or "CPP" indicates the onset of the artificial cerebrospinal fluid (ACSF) solution containing the drug. "Control" indicates the offset of the drug solution and the onset of the control ACSF. Modified from Smith et al. (1990) and Smith and Darlington (1991).

induced by denervation is an interesting question. Finally, it should be remembered that, since all of these electrophysiological results have been obtained in vitro, it cannot be assumed that EAA receptors will necessarily operate in the same way in vivo: It is possible that the denervation involved in preparing the in vitro explant or slice alters NMDA receptor function.

Behavioral Studies

Given the above electrophysiological results, it might be predicted that injection of EAA agonists or antagonists into the VN of a behaving animal would have profound effects on the function of vestibular reflexes. However, since the NMDA ion channel has a negative slope conductance by virtue of the voltage-dependent blockade by Mg^{2+}, the activity of NMDA receptor ion channels in vivo will depend on, among other factors, the concentration of Mg^{2+} in the extracellular fluid. Some of the in vitro electrophysiological studies of the VN (e.g., Cochran et al., 1987) have been conducted using low (i.e., 10–50 μ*M*) Mg^{2+} concentrations to potentiate the function of the NMDA ion channel, and therefore, it is possible that the NMDA receptor complex functions differently in vivo than would be predicted on the basis of in vitro results. Unfortunately, to date there have been few in vivo studies of the contribution of VN NMDA receptors to the generation of vestibular reflex behavior.

de Waele et al. (1990) implanted cannulae unilaterally into the VN of labyrinthine-intact guinea pigs and delivered EAA antagonists by osmotic minipump. Any imbalance in neural activity between the two VN would be reflected in an imbalance in ocular motor (i.e., eye deviation or spontaneous ocular nystagmus) and/or postural (i.e., head and body deviation in the roll and/or yaw planes) reflexes. Chronic infusion of CNQX (2 or 10 m*M*) did not cause any imbalance in ocular motor or postural reflexes (the latter were assessed by X-ray photography). By contrast, chronic infusion of D,L-APV (10 or 20 m*M*) resulted in an ocular motor and postural syndrome similar to that produced by a deafferentation of the ipsilateral vestibular nerve (i.e., a labyrinthine syndrome): The ipsilateral eye was deviated downward and backward; the contralateral eye was deviated forward and upward; a spontaneous ocular nystagmus occurred with an ipsilateral slow phase; the cervical column was rotated in the roll plane toward the perfused side; the head was also rotated in the yaw plane toward the perfused side; there was a spiroid rotation of the thoracolumbar column, with the lumbar and last thoracic vertebrae directed toward the contralateral side and first thoracic vertebrae directed ipsilaterally; the ipsilateral forelimb was flexed, and the contralateral forelimb and hindlimb extended. Perfusion with saline produced no ocular motor or postural symptoms. Histological analysis indicated that the cannulae were localized to the superior border of the VN complex and that the effects of the D,L-APV were not owing to neurotoxicity.

Sansom et al. (in preparation) have also implanted cannulae unilaterally into the VN of labyrinthine-intact guinea pigs, and have examined the effects of injections of CPP on ocular motor and postural behavior. Preliminary results indicate that injections of 10 n*M* or 10 m*M* CPP *do not* produce the labyrinthine syndrome reported for D,L-APV in de Waele et al.'s (1990) study. In addition, injections of 10 n*M* or 1 μ*M* NMDA also produced no obvious effects on ocular motor or postural balance. Histological analysis indicated that the cannulae were localized to the MVN or the ventral border of the MVN and LVN.

The explanation for the discrepancy between the results of de Waele et al. (1990) using D,L-APV and Sansom et al. (in preparation) using CPP is unclear. It is possible that differences in cannula placement within the VN complex or the concentration of the NMDA antagonist that actually reached the VN NMDA receptors accounts for the discrepant results. It is also possible that D,L-APV has additional actions on nonNMDA EAA receptors that CPP does not (*see* Collingridge and Lester, 1989 for a review).

Since the available electrophysiological evidence is based entirely on in vitro preparations,

the study of the behavioral effects of EAA receptor-modulating drugs is critical to understanding the normal function of EAA receptors in the vestibular system. More research in this area is urgently needed.

Based on the available electrophysiological data, predicting the effects of EAA antagonists on the various vestibular pathways is not a straightforward matter. Given the evidence that kainate/AMPA receptors mediate input to VN neurons from the vestibular nerve (Cochran et al., 1987; Lewis et al., 1989; Doi et al., 1990), it might be predicted that unilateral administration of a kainate/AMPA antagonist into the VN would result in a labyrinthine syndrome directed to the ipsilateral side. However, the results of de Waele et al. (1990) do not support this prediction: No such effect was produced by administration of CNQX. Predicting the effects of NMDA antag-onists is more complicated. Electrophysiological evidence from the frog suggests that excitatory input from the contralateral VN is partially mediated by NMDA receptors (Knopfel, 1987; Cochran et al., 1987), and therefore, it might be predicted that unilateral adminstration of an NMDA antagonist into the VN in that species would result in an ipsilaterally directed labyrinthine syndrome. However, brainstem commissural interaction between the VN in mammalian species is largely inhibitory in function: Type I neurons in the MVN on one side send axons across the midline and excite type II neurons in the opposite MVN, which in turn inhibit type I neurons on the same side (*see* Shimazu, 1983 for a review; *see* Fig. 2). Consequently, if NMDA receptors mediate the excitatory synapses between type I neurons in one MVN and contralateral type II neurons, unilateral administration of an NMDA antagonist might be expected to produce a labyrinthine syndrome *contralateral* to the side of the perfusion, as a result of reduced excitation of type II neurons on the perfused side and consequent disinhibition of type I neurons on the same side. However, de Waele et al. (1990) reported a labyrinthine syndrome directed to the *ipsilateral* side, and Sansom et al. (in preparation) reported no effect. It is possible that NMDA receptors mediate other excitatory inputs to type I neurons (e.g., from the spinal cord), or that they participate indirectly in the integration of input from the vestibular nerve, by amplifying the responses of kainate/AMPA receptors. Finally, it should be recognized that abducens nucleus, which participates in the control of horizontal eye movement through motoneurons that innervate the ipsilateral lateral rectus muscle and interneuron projections to the oculomotor nucleus innervating the contralateral medial rectus (*see* Shimazu, 1983 for a review), also has neurons with NMDA receptors (Durand et al., 1987; Ouardouz and Durand, 1991). Since the abducens nucleus is close to the MVN, the presence or absence of ocular motor effects from cannula injections of EAA antagonists may be partially the result of effects on abducens NMDA receptors. The contribution that NMDA receptors make to the function of abducens neurons is unclear at present; therefore, it is difficult to predict exactly what ocular motor effects the action of an NMDA antagonist within one abducens nucleus may produce (Durand et al., 1987; Ouardouz and Durand, 1991).

EAA Receptors and Vestibular Function Following Unilateral Deafferentation of the Vestibular Nerve Fibers

Very little research has been done on the function of EAA receptors under abnormal conditions, such as following deafferentation of one VIIIth nerve (unilateral labyrinthectomy, UL). However, it must be remembered that all of the previously reviewed in vitro electrophysiological evidence relates to a condition that is functionally equivalent to a bilateral labyrinthectomy i.e., a bilateral VIIIth nerve neurectomy. It is difficult, therefore, to know whether the latter evi-

dence is most relevant to the normal or the partially deafferented condition of the VN.

Following UL, a syndrome of ocular motor and postural disorders occurs that is a result of the imbalance in excitatory inputs to the two VN complexes (*see* Smith and Curthoys, 1989 for a review). In mammalian species, this imbalance is amplified by the mutually inhibitory interaction between the bilateral VN via the brainstem commissures (*see* Fig. 2). Over time, many of the ocular motor and postural symptoms disappear in a process of behavioral recovery known as *vestibular compensation* (*see* Smith and Curthoys, 1989; de Waele et al., 1989; Flohr et al., 1989; Smith and Darlington, 1991, for reviews). In many species, compensation of symptoms, such as spontaneous ocular nystagmus (SN) and static postural imbalances (e.g., roll head tilt and yaw head tilt), occurs within 2–3 d following the UL (*see* Fig. 4). Since vestibular compensation is not the result of a regeneration of the peripheral vestibular receptor cells or a functional recovery in the vestibular nerve (Sirkin et al., 1984; Smith and Curthoys, 1988; Cass and Goshgarian, 1991), it is presumed to be owing to CNS plasticity. Consistent with this hypothesis, electrophysiological and biochemical studies have shown that a partial regeneration of resting activity occurs within the VN ipsilateral to the UL, with a time-course that correlates with vestibular compensation (*see* Smith and Curthoys, 1989 for a review). The precise mechanisms of vestibular compensation are poorly understood. However, it has been speculated that EAA receptors may contribute to the adaptive VN neuronal changes that are responsible for the behavioral recovery, for example, through upregulation (Cochran et al., 1987; Knopfel and Dieringer, 1988; Smith and Darlington, 1988; Darlington and Smith, 1989; de Waele et al., 1990; Luneburg and Flohr, 1990; Pettorossi et al., 1990).

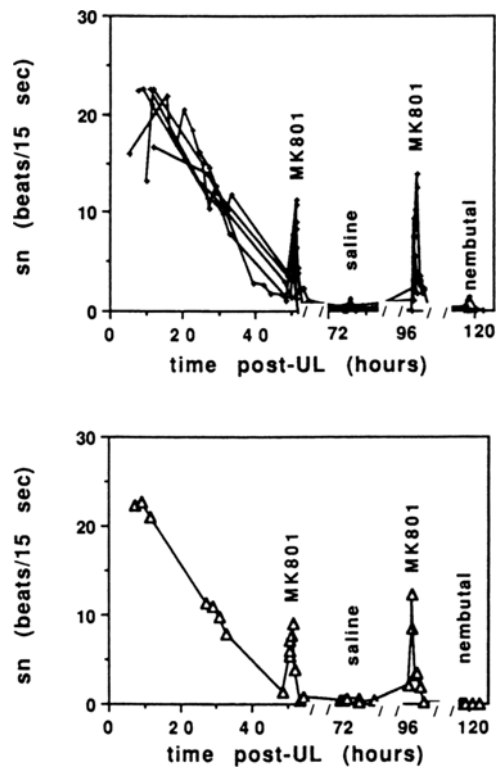

Fig. 4. The compensation of spontaneous ocular nystagmus (sn) in the guinea pig and the effect of single injections of MK801 (0.5 mg/kg, ip, dissolved in saline) on the maintenance of sn compensation. Sn was measured as frequency of quick phases (beats)/15 s interval. Time postUL indicates time following unilateral surgical labyrinthectomy. Top: mean sn for five animals. Bottom: mean sn for one animal, to show more clearly the pattern of decompensation of sn. In both diagrams, "MK801" refers to a single 0.5 mg/kg ip injection; "saline" refers to an equivalent volume single ip injection of saline; and "nembutal" refers to a single ip injection (0.1–0.5 mg/kg) of Nembutal, used to show that another drug with sedative effects does not produce similar decompensation. Modified from Smith and Darlington (1988) and Darlington and Smith (1989).

Biochemical Studies

Raymond et al. (1989) have examined levels of Glu receptor binding in the rat VN between 2 wk and 1 yr postUL. Contrary to the hypothesis that an upregulation of EAA receptors might account for the recovery of resting activity in the VN ipsilateral to the UL, Raymond et al. found no evidence for an increase in the number or affinity of VN Glu receptors during vestibular compensation. However, it is possible that changes occurred in specific subtypes of EAA receptors that were not discernible from examining overall Glu binding levels. It is also possible that changes in EAA receptors occurred earlier than 2 wk postUL and

served a transient function in vestibular compensation, similar to the role of NMDA receptors in the induction of long-term potentiation in the hippocampus (*see* Collingridge and Bliss, 1987 for a review).

Recently, Henley and Igarashi (1991) have used amino acid assays of the VN complex to investigate changes in the levels of amino acids in the VN during vestibular compensation in squirrel monkeys. Immediately following UL, a reduction in Glu in the ipsilateral VN, relative to labyrinthine-intact controls, might be expected because of the loss of Glu input from the deafferented vestibular nerve. However, by 10 mo postUL, normal levels of Glu were found in the ipsilateral VN, suggesting an enhancement of Glu input to the ipsilateral VN. Henley and Igarashi (1991) suggest that the increased Glu input may originate from the spinal cord (Cochran et al., 1987).

Electrophysiological Studies

The possibility that changes in EAA receptors might account for vestibular compensation was first investigated by Cochran et al. (1987) and Knopfel and Dieringer (1988). Using medullary explants from frogs that had received a UL at least 2 mo previously, Cochran et al. found that VN EPSPs evoked by electrical stimulation of the contralateral VIIIth nerve (cEPSPs) could be blocked by kynurenic acid or D-APV, similar to the effect on cEPSPs in explants from labyrinthine-intact animals. Knopfel and Dieringer (1988), using a similar preparation from frogs that had been labyrinthectomized 6–9 wk previously, investigated whether NMDA receptors contributed to the enhancement of brainstem commissural input from the contralateral VN, which Dieringer and Precht (1977) had previously shown to be correlated with vestibular compensation in frog. Knopfel and Dieringer (1988) found that, in explants from compensated frogs, VN field potentials evoked by stimulation of the contralateral VIIIth nerve were about twice the amplitude of those in explants from labyrinthine-intact frogs. cEPSPs in explants from compensated frogs had a faster rise time than cEPSPs in control explants, but were, on average, less sensitive to D-APV (although those cEPSPs from the two groups that had similar rise times had similar sensitivities to D-APV). These results suggested that NMDA receptors make a lesser contribution to commissural inputs to the ipsilateral VN in compensated frogs than in labyrinthine-intact frogs. Whether other EAA receptor subtypes contribute to the enhancement of commissural inputs that occurs in compensated frogs remains to be tested.

At present, no systematic electrophysiological studies of VN EAA receptors during vestibular compensation have been carried out in a mammalian species. Smith and Darlington (1992) have confirmed that MVN neurons in brainstem slices from compensated guinea pigs show decreases in firing rate in response to CPP and MK801 (*see* Fig. 3), but from extracellular recordings, there was no evidence to suggest that the response of these neurons to NMDA antagonists was substantially different from MVN neurons in slices from labyrinthine-intact animals.

Behavioral Studies

To date, the majority of investigations into the possible contribution of EAA receptors to vestibular compensation have been behavioral, and all of these have focused on the NMDA receptor because of its involvement in other forms of CNS plasticity. Smith and Darlington (1988) investigated the effects of single ip injections of MK801 (0.5 mg/kg) and CPP (1 mg/kg) on spontaneous nystagmus (SN), and postural symptoms in compensated guinea pigs between 2–7 d postUL. Both NMDA antagonists caused a return of SN (i.e., "decompensation") after it had compensated, but there was comparatively little effect on postural symptoms (*see* Fig. 4). Similar ip injections of MK801 and CPP during the first 24 h postUL were found to disrupt the development of compensation (Darlington and Smith, 1989). Surprisingly, the decompensatory effect of the NMDA antagonists could not be obtained after approx 2 wk postUL, even with doses of CPP 10 times those that had produced decompensation at earlier times (Darlington and Smith, 1989). In further studies, CPP injections into the IVth ventricle, via

a cannula close to the MVN, were found to disrupt the development and maintenance of postural and ocular motor compensation during the first 3 d postUL (Sansom et al., 1990, in preparation; *see* Fig. 5). Pettorossi et al. (1990) have found that injections of APV into the IIIrd ventricle, at concentrations as low as 130 μM, also disrupt the development of vestibular compensation.

de Waele et al. (1990), also using guinea pigs, have shown that unilateral injections of D,L-APV directly into the VN ipsilateral to the UL produce a return of ocular motor and postural symptoms in compensated animals during the first week postUL. These authors used X-ray analysis of the spinal vertebrae to show that the imbalance in vestibulo-spinal reflexes caused by the injection was identical to that seen in the uncompensated stage immediately following the UL. However, de Waele et al. (1990) observed a similar labyrinthine syndrome, directed toward the side of the injection, in labyrinthine-intact guinea pigs. It is not clear, therefore, whether VN NMDA receptors function any differently during vestibular compensation, or whether they continue to contribute to VN resting activity in their normal way. In the case of systemic or intraventricular injections where the drug is distributed bilaterally to both VN complexes, NMDA antagonists might cause decompensation, because the VN ipsilateral to the UL has less excitatory input relative to the VN on the contralateral side and, therefore, relies more on excitatory input mediated by NMDA receptors.

Recently, Luneburg and Flohr (1990) have reported that systemic administration (0.5 or 2 mg/kg) of MK801 disrupted the development and maintenance of vestibular compensation of roll head tilt in frogs. Single injections (2 mg/kg) were sufficient to produce decompensation in a partially compensated frog. However, no decompensation was obtained after approx 6 wk postUL.

These results suggest that NMDA antagonists disrupt compensation in guinea pigs and frogs, but that there may exist a species-dependent critical period following UL during which the decompensatory effect can be obtained (Darlington and Smith, 1989; Luneburg and Flohr, 1990). It is possible that, as in long-term potentiation *(see* Collingridge and Bliss, 1987 for a review), NMDA receptors contribute to the induction, but not the maintenance, of the plasticity responsible for vestibular compensation. It is interesting to note that both of the electrophysiological studies of VN NMDA receptors that have been conducted in compensated frogs were conducted at least 6 wk following UL (Cochran et al., 1987; Knopfel and Dieringer, 1988); therefore, it is conceivable that modifications of VN NMDA receptor function occurred earlier in the compensation process. However, since much of the behavioral evidence is based on systemic administration of NMDA antagonists, the possibility must also be recognized that NMDA antagonists could disrupt compensation by acting on parts of the CNS other than the VN.

Further studies will be needed to determine whether NMDA and other EAA receptors contribute to vestibular compensation through alterations in their function. At present, most of the studies of NMDA receptors in relation to vestibular compensation have preceded the systematic investigation of EAA receptors in normal vestibular function. Therefore, one problem has been a lack of information about the labyrinthine-intact state that is necessary to determine whether NMDA receptor function has changed as a result of UL. The consequence of this inadequacy has been that many studies have shown that NMDA antagonists disrupt compensation, but it is not clear whether this result signifies a change in the operation of the NMDA receptor, or whether the NMDA receptor is simply one of many neurotransmitter receptors on whose normal function the ipsilateral VN is reliant in order to maintain its resting activity in the absence of input from the ipsilateral vestibular nerve.

Conclusions

The study of EAA receptors in the vestibular system is at a very early stage. The available biochemical and electrophysiological evidence suggests that EAA receptors are likely to be

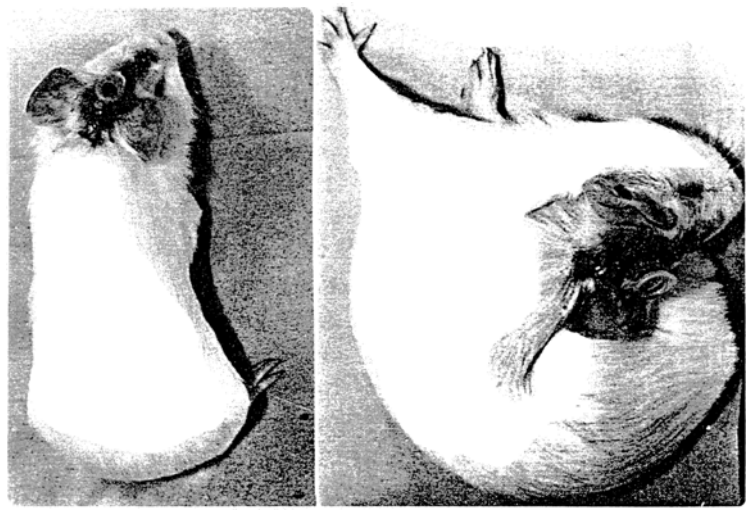

Fig. 5. Effect of a single cannula injection of CPP (40 m*M*, dissolved in 5 uL of artificial cerebrospinal fluid [ACSF], pH approx 7.4) into the IVth ventricle on the posture of a compensated (2–3 d postunilateral labyrinthectomy, UL) guinea pig. Left: compensated animal before injection; note the absence of head and body deviation about the yaw (vertical) axis. Right: compensated animal following CPP injection; note the distinct yaw deviation toward the side of the UL (the right side), similar to that observed in the uncompensated stage. Control injections of ACSF alone did not produce this postural effect. From Sansom et al. (1990).

important at a number of levels in the vestibular reflex pathways, including the synapse between the receptor cells that transduce head movement stimuli and first-order neurons in the vestibular nerve, and the synapse between vestibular nerve fibers and central VN neurons. There is now considerable evidence from several species to support the view that the vestibular nerve/VN neuron synapse is mediated by an EAA acting predominantly on nonNMDA receptors (i.e., kainate/AMPA receptors). It is possible that NMDA receptors mediate polysynaptic input from the vestibular nerve (Cochran et al., 1987; Knopfel, 1987) and that they contribute to monosynaptic input in some way that is not currently recognized (e.g., NMDA receptors on presynaptic vestibular nerve fibers; Lewis et al., 1989). EAA receptors, particularly the NMDA subtype, are likely to be involved in brainstem commissural interaction between the bilateral VN (Knopfel, 1987; Cochran et al., 1987; Doi et al., 1990), although exactly which VN neurons (e.g., MVN type I or type II neurons) use EAA receptors in this interaction remains unclear. In addition to the possibility that EAA receptors change as a result of the denervation involved in the preparation of explants and slices, a further disadvantage of in vitro studies is the difficulty in identifying the responsive neuronal types. For example, both type I and type II MVN neurons, which are usually defined by their response to head movement, may receive input from different components of the ipsilateral vestibular nerve and brainstem commissural fibers (Shimazu and Precht, 1965,1966), and therefore, it may not be possible to distinguish these cell types using gross electrical stimulation in vitro. Future behavioral and in vivo electrophysiological studies will reveal the extent to which results from in vitro VN preparations accurately represent the contribution of EAA receptors to normal vestibular function; these studies should also indicate which cell types have EAA receptors and how EAA receptors contribute to processing information about head movement.

A better understanding of the function of VN EAA receptors under abnormal circumstances, such as the deafferentation of one VIIIth nerve, will depend on defining the normal modes of operation of the VN EAA receptors in the labyrinthine-intact state. At present, there is considerable evidence that antagonists for the NMDA receptor disrupt vestibular compensation, but it is not clear whether this effect is owing to a direct disruption of the adaptive changes within the VN ipsilateral to the UL, and whether it entails some change in the number, affinity, or efficacy of the receptor, compared to the labyrinthine-intact state. However, given the finding in two species that there is a critical period for the decompensatory effect of NMDA antagonists (Darlington and Smith, 1989; Luneburg and Flohr, 1990), it is conceivable that the NMDA receptor is involved in the induction of vestibular compensation processes, and that a change in its function may trigger secondary changes, such as phosphorylation and protein synthesis, via second messenger pathways.

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