SYNAPTIC PLASTICITY AND TRANSSYNAPTIC SIGNALING

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(Eds.)

Synaptic Plasticity and Transsynaptic Signaling

With 119 Figures

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PREFACE

This volume serves two purposes; addressing what we believe is the beginning of a paradigm shift in our understanding of plasticity of synaptic function, and honoring the scientific contributions of John Michael Sarvey. These purposes are inextricably intertwined, because John Sarvey made a number of seminal contributions to our understanding of activity-dependent synaptic plasticity.

John began his training with Dr. Edson Albuquerque, studying cholinergic receptors mediating skeletal muscle contraction. His postdoctoral work in collaboration with Manfred Klee and Ulrich Misgeld led to some of the earliest demonstrations of the utility of brain slices in the study of synaptic function. One of their experiences illustrates the excitement, and controversy, of that era, and how their persistence moved our field forward. Sir John Eccles visited the laboratory and, after witnessing some intracellular recordings from pyramidal neurons first hand, he pronounced the slice useless because there was no evidence of intact synaptic inhibition. When Sir John returned a year later, Sarvey and Misgeld gleefully showed him recordings of robust i.p.s.p.s and, from then on, Eccles was one of the most active proponents of the new *in vitro* slice.

Almost as soon as he established his own laboratory, John Sarvey began making important novel contributions to the field of synaptic plasticity. He was among the first to demonstrate the importance of postsynaptic action potentials, neuromodulatory receptors that raise intracellular [cyclic AMP], and ongoing protein synthesis, for the induction of long-term potentiation (LTP) of synaptic strength. This was the beginning of a career of methodical, thorough and reliable investigations into the mechanisms underlying LTP in the hippocampus. Among these were demonstrations of heterosynaptic LTP in field CA3, and norepinephrine-induced long-lasting potentiation and depression of perforant path synaptic transmission. John, and the many people who have worked in his laboratory, have played key roles in identifying neuromodulators influencing LTP, and the mechanisms of their actions,

including roles for opiate, noradrenergic, muscarinic, GABAergic and metabotropic glutamate receptors.

During his career, John Sarvey also contributed significantly to our knowledge of hippocampal and neocortical synaptic physiology relevant to cognition, learning, and epilepsy. Early studies of the properties of GABAergic responses of visual cortical neurons improved the basis for our understanding of processing in visual cortical circuits. His investigations of how epileptiform activity is produced by direct actions of cholinesterase inhibitors, and by lowering extracellular Ca^{2+} and Mg^{2+} concentrations, have supplied important information. Perhaps most impressive is the understood reliability and integrity of his work, transcending the number of papers published. Moreover, the help, advice and support he always gave freely to colleagues, both junior and senior, stands as one of his greatest contributions, of which he would be the proudest.

The most recent work from Dr. Sarvey's lab is a perfect illustration of the creativity, rigor, tenacity and integrity he has exhibited throughout his career. This work addressed an area of great interest for decades; the functional roles of synaptic release and transsynaptic diffusion of Zn^{2+} ions. Past interpretations of the roles of Zn^{2+} have included stabilization of vesicular opioid peptides, synaptogenesis, and heterosynaptic regulation of NMDA receptors. In this context, John entered the field, joining with a superb team of collaborators and, as usual, brought his creativity, technical and intellectual talents, and careful rigor to the question. This resulted in compelling evidence that Zn^{2+} is rapidly translocated from presynaptic terminals into postsynaptic pyramidal neurons, and that this translocation can be a necessary event in the induction of LTP at mossy fiber-CA3 synapses. Furthermore, work of John and colleagues has also shown that periods of ischemia are associated with Zn^{2+} release that may be a key factor in the extent of long-term brain damage following stroke and other types of traumatic injury. The thorough nature of their work has clarified developmental and other factors that may help explain divergent findings, and will continue to fuel interest in understanding the role of Zinc in transsynaptic signaling and synaptic plasticity.

We hope that two things will be evident as you peruse the chapters in this volume. The first is that neuroscience is in a time of numerous

exciting advances in our understanding of the complexity and subtlety of electrochemical signaling across the synapse. The second is that the interests and work of John Sarvey have significantly influenced the research of most of these contributors, some of the finest scientists in our field. The range of John's influence is one of his unique contributions; this volume includes chapters that consider the importance of neuromodulatory transmitters such as norepinephrine, histamine and acetylcholine in regulating long-term activity-dependent synaptic plasticity, and how these modulators regulate memory formation. Classical LTP, and much newer models of synaptic plasticity such as long-term depression (LTD), spike-timing plasticity and metaplasticity of the threshold for induction of LTP, are all considered. There are chapters that address the functional roles of new/unusual messengers such as hydrogen peroxide, nitric oxide, brain-derived neurotrophic factor, zinc and endocannabinoids in inducing or regulating various forms of activity-dependent plasticity. This volume also contains chapters addressing the basic properties of GABAergic synaptic transmission and their impact on vesicular release, plasticity and epileptiform discharge.

While the neurochemical steps in the induction of long-term plasticity are one continuing research focus, the types of changes that express long-term plasticity in its different phases are of equal importance, and are addressed as well. The many mechanisms by which various types of glutamate and neuromodulatory receptors regulate NMDA receptor activity, leading to calcium influx and release that activates Ca^{2+}-calmodulin dependent protein kinase and Ca^{2+}-activated adenylyl cyclases, and the roles of CREB and rapid nuclear responses in LTP are all addressed. No volume addressing recent advances in our understanding of memory-related plasticity would be complete without considering how rapid, activity and kinase-dependent changes are consolidated into persistent physical alterations in neural circuits, so we have chapters on topics such as synapse preservation and elimination, the transsynaptic bridging molecules integrins, and alterations in dendritic spine morphology. A final chapter addresses the physiological functions of beta amyloid as they might relate to Alzheimer's disease.

It is, of course, impossible for any such volume to be anywhere near exhaustive. The overarching theme of this volume is the vast richness of the bidirectional transsynaptic communication that occurs over time scales both rapid and slow. In this sense, we mean bidirectional both in the sense of messengers moving both pre to postsynaptic and post to presynaptic, and the strength of synapses moving both up and down. We are just beginning to uncover these mechanisms, and it is this knowledge that will crucially inform and constrain our understanding of learning and memory, and higher cognitive function.

John Sarvey had strong personal and scientific connections to many of the authors in this volume. One of his greatest gifts was his ability to stimulate and encourage a love for science in the people who worked with him and with whom he communicated. Many contributors supplied reminiscences of John that emphasized both his impressive intellectual talents and his gentle ability to help, teach and encourage. Some remembered him as the calmest, most composed person in the room, a levelheaded thinker, suggesting he must have had a superabundance of some natural benzodiazepine receptor agonist. Others thought his unfailing enthusiasm must be a sign of high serotonin levels. In discussing the often great frustration associated with allowing graduate students to write drafts of their first papers, Johns' response was that, if we wrote them ourselves, we would have only papers to show for our life's work, while this way, we produce scientists *and* papers.

John Sarvey always relished a challenge much more than the credit associated with overcoming it. One friend remembered his arriving at the Grand Canyon after the Phoenix Society for Neuroscience meeting, sans overnight camping pass, intent upon hiking to the Colorado River. His friends, all outfitted with passes, foolishly attempted to dissuade him from doing the hike. Of course, he left before dawn, flew down the Bright Angel trail to reach the river, turning back up the Kaibab trail for the arduous return trip before lunch. By the time those friends returned from their more leisurely hike the evening of the next day, John had completed the 48km round-trip, slept on a colleagues hotel room floor, and slipped quietly away. Another reminisced about scuba diving together at the Winter Conference on Neural Plasticity in St. Lucia. Having been warned

about the strong currents flowing just outside the bay, they nevertheless found themselves perilously close to being swept into them. After swimming as hard as they could for 10-15 minutes, the nervous friend asked John if they were, in fact, getting closer to their entry point. He drolly replied "We had better, or it's next stop - the Grenadines!"

The stories go on and on, but they all come back to John. He was a quiet, caring, very good man who happened to also be a superb scientist. He lived a life to be proud of, touched us all deeply in so many ways, and we are the richer for having known him.

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CONTENTS

THE THREE FACES OF NOREPINEPHRINE: PLASTICITY AT THE PERFORANT PATH-DENTATE GYRUS SYNAPSE

Carolyn W. Harley, Susan G. Walling, and Robert A.M. Brown*

1. INTRODUCTION

John Sarvey and his collaborators made major contributions to our understanding of the noradrenergic contribution to plasticity in the dentate gyrus of the hippocampus. The present chapter considers, first, the conclusions reached using *in vitro* models, and is based primarily on the work of John Sarvey and his collaborators, and, then, describes *in vivo* data and the interrelationships between the two approaches. These considerations lead us to the proposal that norepinephrine (NE) mediates three distinct forms of plasticity: immediate increases in cell excitability (i.e., likelihood of cell firing to a given synaptic input), immediate increases in synaptic strength and delayed increases in synaptic strength. All of these changes could contribute to the enduring functional alteration of information flow in the dentate gyrus and, thus, could act as memory mechanisms.

A decade after the first report of tetanic stimulation-induced homosynaptic (i.e., presumed glutamate-mediated modulation at glutamate synapses) long-term potentiation (LTP) of the perforant path evoked potential in the dentate gyrus *in vivo* (Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973), we observed that iontophoretic application of NE in the dentate gyrus could induce a potentiation of the perforant path evoked population spike that greatly outlasted the 1- 5 min iontophoresis period (Neuman and Harley, 1983). In ~40% of the experiments population spike potentiation lasted more than 30 min, an accepted criterion for LTP at that time. Potentiation in one rat was monitored for 11 hours. These observations were christened NE-induced long-lasting potentiation to distinguish the phenomenon from the earlier homosynaptic LTP. The heterosynaptic (i.e., NE-activation modulating a glutamate-mediated response) modulation of glutamate synapses by NE in the perforant path of the dentate gyrus did not require high frequency electrical input, and suggested that the co-activation of

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glutamatergic and noradrenergic signals could alter brain information processing for extended periods. The effect appeared to be primarily an alteration of EPSP to spike coupling (E-S potentiation), since the EPSP was not consistently increased. This implied an increase in cell excitability.

2. NEP AND NELLP IN THE FIRST SET OF SARVEY *IN VITRO* STUDIES

In 1985, Stanton and Sarvey carried out a pioneering series of *in vitro* studies to better characterize NE effects in dentate gyrus and to examine their relationship to LTP. In their first report they applied NE to the slice for 30 min and produced two forms of NE-induced potentiation of population spike amplitude (Stanton and Sarvey, 1985a). These two forms were distinguished by their dependence on protein synthesis. The first had the acronym NEP (NE-induced potentiation) and was seen even in the presence of a protein synthesis inhibitor (emetine) that completely blocked LTP in area CA1. NEP occurred when NE was present in the bath and disappeared during the 30 min of NE wash out. The second, NELLP, for NE-induced long-lasting potentiation, occurred only in the absence of the protein synthesis inhibitor and could last throughout a 5-hour recording period. Their standard for NELLP in this paper and subsequent papers, however, was potentiation 30 min after washout. They estimated the NE concentration for the half-maximal response of both forms of NE-potentiation with NELLP requiring a somewhat higher concentration than NEP. They then showed that a priming concentration of an adenylate cyclase activator, forskolin, shifted the NE dose-response curve to the left by an order of magnitude for both forms of NE potentiation. This established a key role for adenylate cyclase in both types of NE-potentiation. The nonselective β-adrenergic antagonist, propranolol, and the selective β1-adrenergic antagonist, metoprolol, blocked NEP and NELLP. NE-potentiation was not seen in CA1. Protein synthesis inhibition 15 min after the application of NE did not block NELLP, establishing an early time window for the protein synthesis requirement. Stanton and Sarvey also compared – and + isomers of NE as both had been reported to produce NELLP in an *in vivo* study {Winson, 1985). *In vitro* only the biologically active – NE was effective.

In a second paper (Stanton and Sarvey, 1985b), they followed up an *in vivo* report that perforant path homosynaptic LTP was reduced in rats with 6-hydroxydopamine (6-OHDA) depletion of NE (Bliss et al., 1983). Using the depletion method of the *in vivo* study they took slices from normal and NE-depleted hippocampi and evaluated LTP. Sarvey's group was one of the few who were able to successfully induce LTP (100 Hz train 2 sec at intensity eliciting 40% maximum spike) in the dentate gyrus *in vitro* without using disinhibition (Wigstrom and Gustafsson, 1983b; Wigstrom and Gustafsson, 1983a) or lowered magnesium (Nguyen and Kandel, 1996) to increase cell excitability. With NE-depletion, LTP of perforant path population spike amplitude could not be induced in dentate gyrus. As rats recovered from 6-OHDA-induced NE depletion their NE levels increased in hippocampus, as did the ability to elicit LTP from their brain slices, further supporting the dependence of LTP on NE. β-adrenoceptor antagonists that blocked NELLP also blocked LTP in the dentate gyrus in normal slices. Forskolin, the adenylate cyclase activator, at a priming concentration, could restore LTP in NE-depleted slices. CA1 LTP was not affected by NE manipulations, suggesting separate roles in the two hippocampal subregions. Further, only NE, not 5-HT, depletion prevented perforant path

LTP *in vitro*, in contrast to the earlier *in vivo* study, suggesting a direct action of NE in the dentate gyrus and an indirect mediation of 5-HT depletion effects, possibly via modulation of NE activity.

In the final paper in this set Stanton and Sarvey reported that LTP in the dentate gyrus and NELLP were both associated with increased cAMP in the dentate gyrus (Stanton and Sarvey, 1985c). With LTP, cAMP rose 3-fold in the first min after the tetanus and was at control levels at 30 min. NE-depletion prevented the normal tetanus-induced increase in cAMP. With NE, cAMP levels increased with a 3- to 4-fold peak at 1 min and a sustained elevation even during washout. NE-depletion did not prevent the cAMP response to NE. Whole slices from NE-depleted rats showed reduced basal cAMP suggesting a contribution of NE to basal cAMP. Earlier, Segal et al. had shown that NE and histamine at 100 μM elevated cAMP in dentate gyrus, but glutamate, GABA, serotonin and acetylcholine did not (Segal et al., 1981). The pharmacological profile was that of a β1 receptor, although glial cells also showed an increase in cAMP to NE stimulation that had a β2 profile. Basal levels of cAMP were higher in dentate gyrus than CA3 or CA1.

Taken together, these studies confirmed NE heterosynaptic long-lasting potentiation of the perforant path population spike in the dentate gyrus *in vitro*, showed that it depended on activation of β-adrenergic receptors, the activation of adenylate cyclase and rapid protein synthesis and demonstrated that a predicted increase in cAMP was associated with NE application. Frequency-induced homosynaptic LTP in dentate gyrus also required the presence of NE and β-adrenoceptor activation and was associated with the elevation of cAMP. NE enhanced the somatic calcium current during the LTP-inducing tetanus (Stanton and Heinemann, 1986). These parallels argued for converging mechanisms underlying both NELLP and LTP in dentate gyrus.

3. FURTHER INVESTIGATIONS OF NEP AND NELLP *IN VITRO*

Our laboratory published similar *in vitro* results (Lacaille and Harley, 1985). Using a 10 min NE application both perforant path EPSP slope (measured at the soma) and population spike amplitude increased, although long-lasting effects were more common with the population spike. With NE, 47% of spike amplitude potentiation could be accounted for by the increase in EPSP slope, suggesting an additional increase in EPSP/spike coupling. EPSP slope potentiation and spike potentiation occurred in all slices exposed to the β-adrenoceptor agonist isoproterenol. The β-adrenoeptor antagonist, timolol, blocked NE potentiation. An α-receptor agonist (phenylephrine) and antagonist (phentolamine) produced only weak or partial effects, in contrast to the β-adrenoceptor agents. When perforant path stimulation was omitted during NE application potentiation was still seen. While this appears to argue against a co-activation requirement for glutamatergic and noradrenergic input to obtain NE-potentiation effects, Stanton and Sarvey's results suggest cAMP would have been elevated during NE washout when stimulation was resumed (Stanton and Sarvey, 1985c). Prolonged cAMP elevation may provide the co-activation requirement. In the co-activation tests there were no long-lasting effects of NE, so the co-activation requirement of NELLP was not probed.

Antidromic activation of the population spike was not altered by NE (Lacaille and Harley, 1985) suggesting the effect required synaptically-elicited action potentials.

Antidromic activation also did not result in enhanced granule cell calcium entry in the presence of NE, while the same stimulation given orthodromically was effective (Stanton and Heinemann, 1986).

In 1987, Stanton and Sarvey revisited the phenomena of NELLP, and of NE depletion effects on LTP, in experiments in which the dendritic EPSP, as well as the population spike amplitude, were examined in response to perforant path stimulation *in vitro* (Stanton and Sarvey, 1987). Examination of the dendritic EPSP was prompted by reports that NE depletion *in vivo* affected the population spike, but not the somatic EPSP (Robinson and Racine, 1985) and that NE iontophoresis decreased the dendritic EPSP, while not altering the somatic EPSP (Winson and Dahl, 1985). Stanton and Sarvey found LTP of both the dendritic EPSP, and the population spike amplitude, were prevented by NE depletion. NE application alone initiated long-lasting potentiation of the dendritic EPSP as well as population spike amplitude. The finding of NE-induced dendritic EPSP potentiation complimented the earlier finding of somatic EPSP potentiation (Lacaille and Harley, 1985) and was reinforced by the observation that NE application produced a long-lasting increase of glutamate release in the dentate gyrus (Lynch and Bliss, 1986;. see also other evidence for presynaptic release increases Chen and Roper, 2003; Kohara et al., 2001). This increase also appears to be β-adrenoceptor dependent.

The earlier finding of differing roles of NE in LTP in dentate gyrus and CA1 was also later replicated measuring EPSP slope. The β-adrenoceptor antagonist propranolol reduced both induction and maintenance of EPSP slope LTP in dentate gyrus, but not in CA1 (Swanson-Park et al., 1999) A dopamine antagonist was effective in CA1, but ineffective in dentate gyrus.

Membrane effects of NE on *in vitro* dentate granule cells were reported in 1987. Haas and Rose replicated the spike potentiating effect of NE, and of a β-adrenoceptor agonist, although only the β-adrenoceptor agonist, isoproterenol, but not NE, potentiated the EPSP (Haas and Rose, 1987). Long-lasting effects on either spike amplitude or EPSP were seen in a minority of experiments. Intracellular recording revealed an NE suppression of the pronounced afterhyperpolarization in dentate granule cells (Haas and Rose, 1987). NE modulation of the afterhyperpolarization was related to reduction of a calcium-mediated potassium current. Evidence was also obtained for inhibition of a potassium A current by the β-adrenoceptor agonist in some granule cells (Haas and Rose, 1987).. The afterhyperpolarization suppression could contribute to enhanced cell excitability and appeared enduring in some recordings. Reduction of the A current would also increase cell excitability. Gray and Johnston reported that the voltage dependent L channel currents were enhanced in granule cells by activation of β-adrenoceptors (Gray and Johnston, 1987), which would promote calcium-mediated plasticity effects upon NE application.

In summary, the *in vitro* studies to this point were consistent with the hypothesis that NE induces a heterosynaptic long-lasting potentiation of the perforant path evoked EPSP and population spike in the dentate gyrus, which is mediated by activation of β-adrenoceptors and the elevation of cAMP. The increase in population spike amplitude is not wholly accounted for by EPSP slope increases and thus implicates an increase in the coupling of the EPSP to spike generation. The latter effect is likely related to postsynaptic changes in membrane channels. The differences and similarities to frequency-induced LTP are examined in the next section.

4. NE INTERACTIONS WITH NON-ADRENERGIC RECEPTORS AND PATHWAY SELECTIVITY

Burgard et al. examined the effects of NMDA antagonists on NELLP and LTP. In their study, the medial perforant path was selectively stimulated and both spike amplitude and EPSP effects of NE were examined (Burgard et al., 1989). LTP spike potentiation was compared to NELLP with the same concentrations of NMDA antagonists. LTP was effective somewhat more often than NELLP (18/20 slices versus 19/27 for NELLP) in inducing spike amplitude potentiation. NMDA antagonists blocked LTP and NELLP, with more complete antagonism of NELLP. Dendritic EPSP potentiation by NE occurred in 7/14 slices and was also antagonized by NMDA receptor blockade. These results suggested both LTP and NELLP depended on NMDA receptors.

In the same year, Dahl and Sarvey reported a pathway selective action of NE in the dentate gyrus. NE in the presence of an α-adrenoceptor antagonist induced long-lasting potentiation of both the medial perforant path population spike and the medial perforant path dendritic EPSP slope (Dahl and Sarvey, 1989). Concurrently, the lateral perforant path-evoked population spike and EPSP slope were depressed. The β-adrenoceptor agonist, isoproterenol, mimicked these effects and the β-adrenoceptor antagonist, propranolol, blocked them. While LTP of either pathway in the dentate gyrus is associated with depression of the other, LTP effects are not preferential for the medial perforant path as the β-adrenoceptor activation effects were.

In further examinations of the pharmacology of NELLP and its relationship to LTP, Burgard and Sarvey reported that 1 μM muscarine facilitated LTP induction while 10 μmol depressed the population spike, and dendritic EPSP, but did not alter LTP induction (Burgard and Sarvey, 1990). By contrast (Burgard et al., 1993) the same concentrations of muscarine reduced (1 μmol muscarine) or blocked (10 μmol muscarine) NELLP induced by the β-adrenoceptor agonist isoproterenol. No facilitation of NELLP was seen at the lower dose. The ability of isoproterenol to enhance cAMP in the dentate gyrus was unaffected by muscarine however. M1/M3 muscarine receptor antagonists blocked the effects of muscarine in both studies. The authors concluded that LTP and NELLP differed in response to muscarine receptor activation and, in particular, they suggested a presynaptic action of muscarine: the reduction of glutamate release prevents a co-activation of NMDA receptors *required* to induce NELLP, while the post-synaptic depolarization effect of LTP is enhanced by muscarine's post-synaptic increase of cell excitability.

The $GABA_B$ agonist, baclofen, had a different action in dentate gyrus relative to other regions of the hippocampus, and its net effect was disinhibition, as described in detail by Burgard and Sarvey. They demonstrated that the $GABA_B$ agonist alone induces a long-lasting potentiation of the population spike, but not the EPSP, while at a subthreshold dose a $GABA_B$ agonist synergizes with subthreshold isoproterenol to produce NELLP of both the spike and dendritic EPSP (Burgard and Sarvey, 1991). NELLP does not depend on sustained NE release, as shown in this study by application of a β-adrenoceptor antagonist after its initiation. Burgard and Sarvey suggest the synergistic action of $GABA_B$ receptor activation is a function of postsynaptic disinhibition, evidenced by a decrease in paired pulse inhibition even at doses subthreshold for direct potentiation.

Dahl and Sarvey investigated the role of NMDA receptors in the pathway selective dendritic EPSP responses elicited by isoproterenol. Both long-lasting potentiation and long-lasting depression were blocked by prior application of an NMDA antagonist (Dahl et al., 1990). After washout the LLP of the medial perforant pathway to isoproterenol recovered, however the isoproterenol-induced LLD with lateral perforant pathway stimulation did not reappear. The data suggest there are enduring effects of NMDA receptor antagonism on the lateral, but not the medial, perforant path effects of adrenergic receptor activation. The authors also showed that it was not necessary to electrically stimulate perforant path fibers during the 30 min period of drug application and the 30 min period of washout in order to obtain LLP and LLD. Again the earlier evidence that cAMP levels are elevated even after NE application and washout (Stanton and Sarvey, 1985c) may account for the ability to elicit LLP and LLD without perforant path stimulation. More difficult to explain is the loss of lateral perforant path LLD after a single exposure to an NMDA antagonist.

In a later study Pelletier et al. revisited the pathway specificity of noradrenergic plasticity in the dentate gyrus. They replicated the long-lasting potentiation of medial perforant path EPSP and the long-lasting depression of the lateral perforant path EPSP with 1 μM isoproterenol reported by Dahl and Sarvey (1989) and evaluated the interaction with LTP. LTP potentiated both pathways with larger potentiation at medial perforant path synapses (Pelletier et al., 1994). The EPSP returned to baseline values when LTP was applied to the lateral perforant path. If LTP was induced prior to isoproterenol application there was further potentiation with isoproterenol of the medial perforant path, but no change in the level of potentiation on the lateral perforant path. Again prior NMDA receptor activation apparently prevented LLD effects of isoproterenol. The selective β1-antagonist, metoprolol, was tested against LTP. Metoprolol blocked the effect of medial perforant pathway LTP, but on the lateral perforant path significantly greater LTP occurred in the presence of metoprolol. Bath application of 20 μM (but not 1 μM) metoprolol for 30 min produced a depression of medial perforant path responses and a potentiation of lateral perforant path responses. These effects continued to increase during the 60 min washout of metoprolol. It was not possible to conclude that metoprolol antagonized the LTP effect in the medial perforant path since it had its own depressing effect that may have masked LTP.

Bramham et al. evaluated propranolol and timolol, two nonselective β-adrenoceptor antagonists, against the medial and lateral perforant path dendritic EPSP LTP. Propranolol blocked LTP in both the medial and lateral pathways, but timolol had no effect at the time of LTP evaluation (Bramham et al., 1997). At later time points the timolol-treated slices showed a greater decline in LTP consistent with evidence for β-adrenoceptor involvement in late phase LTP. The failure of timolol to antagonize early LTP led Bramham et al. to speculate that there was a subpopulation of timolol insensitive β2-receptors in dentate gyrus.

Nguyen and Kandel, using reduced extracellular magnesium, investigated early LTP and late (3 hr) LTP of the medial perforant path dendritic EPSP. Both forms were NMDA-dependent. Late, but not the early, LTP required protein synthesis and activation of cAMP-dependent protein kinase (Nguyen and Kandel, 1996). Segal's early investigation of neurotransmitters in the dentate gyrus that elevate cAMP suggest NE is the most likely candidate to promote elevation of cAMP and consequent activation of cAMP-dependent protein kinase. Sarvey's laboratory showed that spike LTP depended

on NE activation of β-adrenoceptors and that tetani in the slice induced elevation of cAMP when NE was intact. Thus, all data available converge on the hypothesis that enduring LTP of EPSP and spike in the medial perforant path requires NE heterosynaptic facilitation of this homosynaptic mechanism.

While NMDA receptors participate in both NELLP and LTP examined *in vitro*, the two forms of potentiation differ in pathway selectivity with NELLP showing selectivity for the medial perforant path only. The response to other pharmacological tests also suggests NELLP and LTP are likely distinct forms of connectivity modulation despite a common dependence on the cAMP cascade.

5. PRESYNAPTIC EFFECTS OF NE *IN VITRO* AND THE QUESTION OF THE PHYSIOLOGICAL ACTIVATION OF β-ADRENOCEPTORS

Sarvey drew attention to the evidence that there were both pre- and post-synaptic effects of β-adrenoceptor activation (Sarvey et al., 1989). Parfitt et al. (1991, 1992) investigated the presynaptic effect, enhanced release of glutamate, by examining phosphorylation of Synapsin I and Synapsin II in the dentate gyrus. Isoproterenol (250 nM) or norepinephrine (10 μmol) induced phosphorylation of Synapsin I and II. Both the calcium/calmodulin protein kinase II phosphorylation site and the PKA phosphorylation site on Synapsin I were phosphorylated following exposure to these adrenoceptor agonists (Parfitt et al., 1992). Previous work had established that phosphorylation of the calcium/calmodulin protein kinase II site activated enhanced transmitter release and hence could account for NE's effects on glutamate release. Phosphorylation of the calcium/calmodulin protein kinase II site by NE application underscores the likely importance of NE's modulation of calcium as well as cAMP in mediating cellular plasticity in dentate gyrus. In aged rats NE did not induce phosphorylation of the synapsins and the basal phosphorylation of these dentate gyrus proteins was higher than that of younger rats (Parfitt et al., 1991). However, slices from aged rats would show increased phosphorylation with NE if a phosphodiesterase inhibitor were added. Such inhibitors have become popular as memory enhancing candidates for both rodent and human aging populations. Parfitt et al. (1992) found that the time course of dentate gyrus EPSP potentiation and of synapsin phosphorylation in dentate gyrus in response to a lower dose of isoproterenol (250 nM) were similar in their hands (less than 30 min) suggesting NE-induced EPSP potentiation may be dependent on phosphorylation of these presynaptic proteins.

Dahl speculated that the 1 μM concentration of isoproterenol normally used to induced NELLP of the EPSP and population spike in dentate gyrus might be an unrealistically high level of β-adrenoceptor activation *in vivo* that would be unlikely to be sustained given the efficiency of NE uptake (Dahl and Li, 1994a). Dahl undertook a series of studies of the effects of bath applied isoproterenol in the 50-100 nM range. He found short-term increases in population spike amplitude during the 15 min application of these low concentrations of isoproterenol, but no long-term increase and no change in the EPSP slope. With a 30 min washout followed by a repeated application of the same low dose of isoproterenol NELLP of the population spike was observed to the second application of isoproterenol. This demonstrated that a repeated, spaced activation of β-adrenoceptors *in vitro* could engage a long-term potentiation of population spike amplitude, but not of EPSP slope (Dahl and Li, 1994a). A non-selective β-adrenoceptor

antagonist and a selective β1-adrenoceptor antagonist blocked these effects, but an NMDA antagonist did not.

Dahl also examined coapplications of isoproterenol and cholecystokinin, which he predicted would have effects similar to that of the $GABA_B$ agonist, baclofen, previously studied (Burgard and Sarvey, 1991). Co-application of cholecystokinin and a low dose of isoproterenol produced long-lasting depression of the population spike while isoproterenol followed by washout and then cholecystokinin produced long-lasting potentiation of the population spike similar to the sequential effect of low dose isoproterenol alone (Dahl and Li, 1994c). The effect occurred even when the isoproterenol concentration was too low (50 nM) to produce any change when applied alone. β-antagonists prevent the potentiation seen with sequential isoproterenol and cholecystokinin applications, but again an NMDA antagonist does not (Dahl and Li, 1994b).

Dahl concluded that there may be two levels of β-adrenergic modulation in the dentate gyrus: one that requires NMDA receptor activation (the higher 1 μM concentration effect) and one that is NMDA receptor-independent (the lower 50-100 nM concentration effect). Only the higher concentration effect modulates EPSP as well as spike amplitude, the lower concentration effect is specific to spike amplitude. Dahl suggested that the effects of lower concentrations may represent the physiological effects of β-adrenoceptor activation (Dahl and Li, 1994a).

6. *IN VIVO* STUDIES OF NE RELEASE

The question Dahl raises "Is the NMDA-receptor independent effect of β-adrenergic modulation the physiological effect of NE at these receptors?" seems to have been answered in the affirmative when we turn our attention to the results of *in vivo* studies.

The source of dentate gyrus NE is the terminals of the locus coeruleus (LC) (Loy et al., 1980) and activation of the LC has been used in a number of studies to characterize the physiological actions of NE release. In one of the first studies to use LC electrical stimulation to examine modulation of the perforant path evoked potential in the dentate gyrus, Dahl and Winson reported potentiation of population spike amplitude and no change in EPSP slope at the cell body level, although they observed a depression of the EPSP recorded at the dendritic level (Dahl and Winson, 1985).

We used glutamatergic activation of LC and found a potentiation of population spike amplitude in all rats with variable durations, ~5-20 min (Harley and Milway, 1986). The population EPSP slope increased briefly (less than 3 min) in about 50% of the experiments, and decreased briefly, or was unchanged, in the remainder. The different time courses suggested EPSP and spike effects were largely uncorrelated. In a later study (Harley and Sara, 1992), EPSP slope increases were more common (>60%), but again were uncorrelated with spike increases. EPSP slope increases occurred on less than 30% of the evoked potentials with increased population spikes.

Most recently, we have used the neuroactive peptide, orexin, to activate the LC in the urethane-anesthetized rat (Walling et al., 2004). Orexin produces an enduring (more than 3 hr) and gradually increasing β-adrenoceptor-dependent potentiation of population spike amplitude without potentiating the EPSP (see Figure 1). The gradual increase in spike

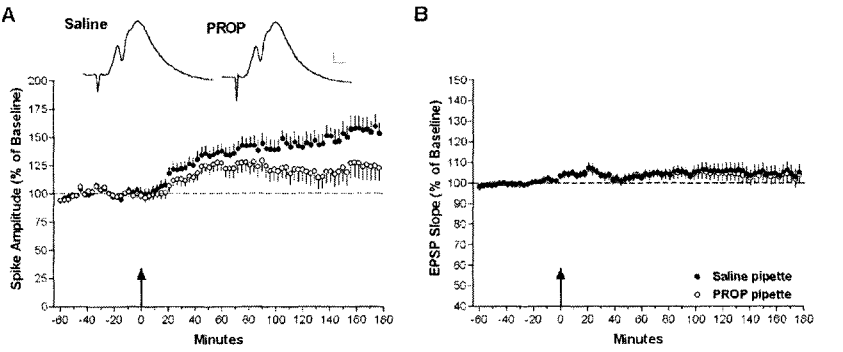

Figure 1. Orexin infused in the locus coeruleus at the arrow produces a significant potentiation of population spike amplitude over the 3 hr recording period (**A**). EPSP slope is not significantly altered (**B**). The β-adrenoceptor antagonist propranolol (PROP) in one of two micropipettes recording the perforant path evoked potential attenuates the spike increase. From Walline et al. (2004) Reprinted with permission from The Journal of Neuroscience.

amplitude is consistent with reports of E-S potentiation recruited by LTP (Jester et al., 1995).

In one study we compared glutamatergic and electrical stimulation of LC (Harley et al., 1989). With electrical stimulation, the potentiating LC effects on population spike amplitude occurred selectively with a preceding ~40 msec interstimulus interval (ISI) as reported previously for LC train stimulation, but repeated LC-perforant path pairings produced 30 min potentiation in about 50% of the experiments. Repeated pairings were associated with enduring population spike increases, but EPSP slope decreased in 60% of the experiments. Long-lasting potentiation occurred more often when potentiation was greater than 140% during the acute stimulation. Ten-Hz stimulation also produced an increase in spike amplitude and no effects on EPSP slope. The β-adrenoceptor antagonist, propranolol, blocked the effects of glutamatergic activation-induced potentiation, as reported previously, but did not alter the potentiating effects of acute LC electrical stimulation-induced potentiation in the same rats. The long-lasting effects of repeated electrical stimulation were not assessed in the presence of a β-adrenoceptor antagonist.

In contrast, Washburn and Moises, using the same stimulation train in the LC or in its efferent dorsal bundle and a 35 msec ISI reported that stimulation giving 50% of the maximal spike amplitude potentiation produced a β-adrenoceptor sensitive potentiation that could also be reduced by clonidine (to activate the terminal NE autoreceptors) or by 6-OHDA depletion (Washburn and Moises, 1989). Sites slightly outside the LC were ineffective in producing spike potentiation. EPSP slope potentiation was not seen (<20% of experiments) at either the somatic or the dendritic level. Repeated pairings were not explored. Thus, LC electrical stimulation may be appropriately selective for NE release under some conditions.

In pharmacological approaches to NE release modulation, Richter-Levin et al. and Sara and Bergis monitored the perforant path evoked potential in the dentate gyrus in anesthetized (Richter-Levin et al., 1991) and awake rats (Sara and Bergis, 1991) receiving the α2 receptor antagonist, idazoxan. Idazoxan increases LC firing and the

release of NE. Population spike amplitude was potentiated in both studies. In anesthetized rats, EPSP slope was depressed at the somatic level. Idazoxan was ineffective in 6-OHDA NE-depleted rats confirming NE's role. In the awake rats no changes were seen in EPSP slope. In awake rats paired pulse inhibition was also tested and found to be enhanced contrary to the expectation that there would be decreased inhibition. Segal et al. using amphetamine, also reported potentiation of population spike amplitude in anesthetized rat without consistent effects on EPSP slope (Segal et al., 1991). Amphetamine-associated spike potentiation was sensitive to both β-adrenoceptor and α1-adrenoceptor blockade.

Electrical stimulation of the paragiganocellularis nucleus that provides the major source of glutamatergic excitation to the LC is another method to activate NE release. This produces potentiation of population spike amplitude with EPSP slope increases in <20% of the experiments (Babstock and Harley, 1992). Peak potentiation occurs in a 35-40 msec window, as reported for direct LC stimulation, and is blocked by the β-adrenoceptor antagonist propranolol.

LC stimulation in awake rats has been examined using glutamatergic activation, and using natural events, to recruit activation of LC neurons. We found that glutamatergic LC stimulation in awake rats (Klukowski and Harley, 1994; Walling and Harley, 2004) produces spike potentiation, which can be transient (less than 20 min) or longer term (+20 min to +3 hr depending on the study). EPSP slope potentiation is rare, again occurring in less than 20% of the experiments. With exploration of novel objects in a hole board or exposure to a novel environment, two manipulations previously shown to activate LC, spike potentiation is seen, but not slope potentiation (Kitchigina, 1997). The spike potentiation effects are transient and are blocked by the β-adrenoceptor antagonist, propranolol.

Taken together, a variety of methods for producing physiological increases in synaptic NE, in both anesthetized and awake rats, produces a modulation of the perforant path evoked potential in the dentate gyrus which resembles that reported by Dahl with low dose isoproterenol activation of β-adrenoceptors. The population spike is potentiated, but EPSP slope is rarely affected.

A second parallel between low dose *in vitro* isoproterenol effects and *in vivo* NE release effects is their response to NMDA antagonism. An NMDA antagonist cannot block lasting or long-term low dose isoproterenol potentiation of spike amplitude {Dahl, 1994}. *In vivo,* using glutamatergic activation of the LC, we showed that intraventricular application of the NMDA antagonist ketamine, sufficient to attenuate LTP, did not attenuate, but enhanced, spike potentiation produced by glutamatergic LC activation (Frizzell and Harley, 1994). The enhancement was ascribed to ketamine's ability to reduce NE reuptake. In these experiments transient EPSP slope and spike amplitude increases were seen with all activations. While transient, the potentiations lasted for many minutes, while LC cellular activation by glutamate produces a burst of activity lasting less than .5 sec (Harley and Sara, 1992). Ketamine significantly increased only spike amplitude and also selectively extended the duration of the spike increase. This supports the notion of an NMDA-independent component of NE potentiation, which increases cell excitability, similar to that seen by Dahl and Li (1994a).

Finally, using microdialysis with concomitant intracerebroventricular (ICV) NE *in vivo* we estimated the synaptic concentration of NE required to produce NELLP *in vivo* (Harley et al., 1996). ICV NE in these studies potentiated spike amplitude with no

consistent EPSP slope change. The estimated synaptic concentration for long-term spike potentiation (more than 2 hr) was 750 nM NE while transient short-term potentiation was seen at ~1/2 that value. While 750 nM NE is 10X the concentration of isoproterenol used by Dahl and Li to produce long-term spike potentiation with spaced repeated applications, NE concentrations used to evoke NELLP *in vitro* are typically at least 10X (10 μM) higher than isoproterenol (1 μM) concentrations. Thus, 750 nM NE is a low concentration in this context, and would be similar to 75 nM of isoproterenol β-adrenoceptor activation.

These calculations support Dahl and Li's hypothesis that physiological release of NE likely produces low concentration β-adrenoceptor activation rather than high concentration β-adrenoceptor activation (Dahl and Li, 1994a). The general findings that population spike increases occur independently of slope increases after NE release *in vivo* (using a variety of release paradigms) and that such physiological release can induce a potentiation that is NMDA receptor independent, also support the hypothesis.

An alternative explanation of the failure to see consistent EPSP slope potentiation *in vivo* could be the problem of combined medial and lateral perforant pathway contributions to the EPSP slope. In all of the *in vivo* studies described, stimulation was sufficient to evoke a medial perforant pathway population spike. The EPSP slope components would likely represent mixed contributions from both pathways. Enhancement of the medial, and depression of the lateral, EPSP components might result, coincidentally, in a low percentage of apparent EPSP increases. Using paragigantocellularis stimulation to activate NE release we found depression of the lateral olfactory tract EPSP, which is mediated by the lateral perforant pathway, in dentate gyrus when preceded by paragigantocellularis stimulation (Babstock and Harley, 1993). The depression was sensitive to β-adrenoceptor blockade by propranolol. The same stimulation potentiated perforant path spike amplitude. Systematic investigations of medial and lateral perforant path components *in vivo* are warranted and have implications for the cognitive role of hippocampal NE.

7. NE IN INTERACTIONS WITH OTHER NEUROTRANSMITTERS

While it may be suggested that, acting on its own, release of NE from LC terminals primarily modulates population spike amplitude *in vivo*, as hypothesized by Dahl in his model in which physiological NE is at a lower level than that typically used in slice experiments, NE in interaction with the release of other neurotransmitters, may have actions more typical of those reported for the 'higher NE concentration' effects. LTP associated glutamate release, in particular, may alter both the level of NE release locally and the intracellular machinery available for influence by NE. NE release in hippocampal slices is increased by activation of NMDA (e.g, Pittaluga and Raiteri, 1992) and AMPA (e.g., Pittaluga et al., 1994) receptors. This glutamate enhancement of NE release is strongest in the dentate gyrus (Andres et al., 1993), is selective for NE over other transmitters (Fink et al., 1989) and is enhanced by memory promoters (e.g,, Desai et al., 1995). Thus, if brain activity promotes the activation of NMDA and AMPA receptors, NE levels should concomitantly be elevated. NE can enhance glutamate release in dentate gyrus also, which may create further positive feedback (Lynch and Bliss, 1986). LTP tetani do increase NE release in dentate gyrus *in vivo* (Bronzino et al., 2001) likely

mediated in part by direct stimulation of NE fibers, but also likely mediated by glutamatergic modulation of local NE release. Elevated levels were sustained for 2 hr following the LTP trains, while spike amplitude was elevated for 24 hr. In a methodological study, levels of NE release varied with the intensity of the LTP manipulation, with stronger manipulations giving rise to more potentiation and higher levels of NE (Bronzino et al., 1999). Thus LTP and NMDA receptor activation in the dentate gyrus are both linked to enhanced NE release.

The ability of NE depletion and β-adrenoceptor blockade to prevent the induction of LTP in dentate gyrus also argues for an key role of NE in LTP plasticity in dentate gyrus, including potentiation of EPSP slope. In the initial NE depletion study *in vivo* Bliss showed that EPSP slope, but not spike potentiation, was impaired (Bliss et al., 1983). We showed that local β-adrenoceptor blockade reduces LTP of EPSP slope, but does not reduce LTP of the population spike *in vivo* (Munro et al., 2001). The reduction of slope potentiation by β-adrenoceptor blockade is largest at later time periods as predicted from brain slice experiments which showed that late phase LTP slope potentiation requires cAMP-dependent protein kinase activation (Nguyen and Kandel, 1996). Thus, physiological circumstances that lead to NMDA receptor activation may always recruit enhanced NE release leading to activation of the 2nd higher concentration NE influence on EPSP slope potentiation described by Sarvey and others.

Decaying LTP of EPSP slope can be restored by electrical stimulation of the LC that elicits exploratory behavior (Ezrokhi et al., 1999). The LC stimulation alone produced a brief EPSP depression followed by mild, but usually nonsignificant potentiation, but when given within minutes or hours of the return to baseline of an LTP-induced EPSP potentiation LC stimulation produced a return to a robust LTP level of EPSP potentiation. Prior LC stimulation, by contrast, did not seem to alter the effectiveness of the LTP train. The authors suggest that either through reduced dephosphorylation, or increased phosphorylation, of the calcium/calmodulin kinase II activation initially set up by LTP NE's β-adrenoceptor recruitment of cAMP restores the activity of the calcium/calmodulin kinase II to return the EPSP to its potentiated level.

The role of β-adrenoceptors in LTP of perforant path spike amplitude *in vivo* varies with the induction protocol. β-adrenoceptors are required for weaker and intermediate induction protocols to generate late phase LTP, but do not appear needed if very strong induction protocols are employed (Straube and Frey, 2003). While EPSP slopes were not measured in these studies, it is likely that they would show similar increases.

In vivo Seidenbecher et al showed that natural reinforcers such as water for water deprived rats or footshock can convert early phase to late phase medial perforant path spike amplitude LTP if given at the same time as, or within 30 min after, the LTP train delivery (Seidenbecher et al., 1997). This effect is blocked by a β-adrenoceptor antagonist. Bergado et al. have similar results with spike amplitude and EPSP slope LTP (Bergado et al., 2001).

Straube et al. (2003) have shown that exposure to a novel environment 15-30 min prior to a weak LTP stimulus converts early phase LTP to late phase LTP (Straube et al., 2003). This conversion is β-adrenoceptor-dependent as well as protein synthesis-dependent. In this study a preference for spike amplitude measures over EPSP measures was ascribed to instability of the latter in recordings in awake rats, however LTP was shown with both measures. The authors note that prior studies of dentate gyrus LTP that only use spike amplitude measures likely also have EPSP potentiation. Isoproterenol

administered ICV, at a dose with no effect on its own, mimicked the ability of novelty to convert weak LTP to late phase LTP and supported the hypothesis that β-adrenoceptors were critical. These data suggest sequential interactions occur with initial β-adrenoceptor priming and subsequent LTP events as well as with concurrent and the reverse sequence effects seen in other studies e.g., Seidenbecher et al., 1997.

Our most recent findings suggest there is a 3rd long-term modulatory effect of synaptically released NE (Walling and Harley, 2004). Glutamatergic activation of LC in awake rats produced the typical potentiation of spike amplitude in the dentate gyrus in the majority of rats tested with no significant, or only a very transient, increase in EPSP slope. See Figure 2. This spike potentiation was maintained over a 3 hr period in some rats, an effect consistently observed previously with orexinergic activation of LC (Figure 1). Thus this study initially found a typical low concentration NE effect on spike, but not slope. Twenty-four hr later the EPSP slope of all rats was significantly potentiated, as was spike amplitude (Figure 2). Input output data suggested the increase in EPSP slope at 24 hr accounts for the spike potentiation. Thus, at 3 hr there is E-S potentiation, but at 24 hr there is synaptic potentiation (Figure 3).

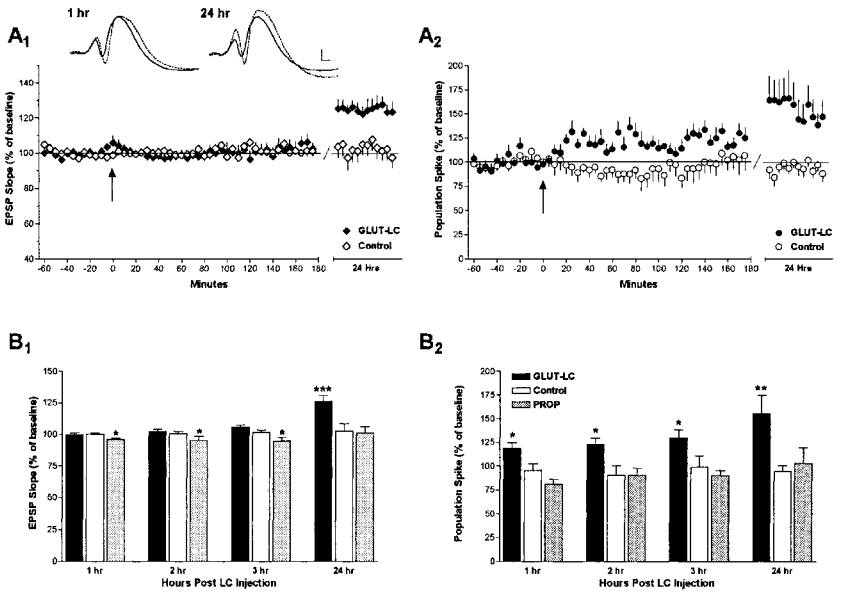

Figure 2. Glutamate infusion in the locus coeruleus at the arrow produces an initial spike potentiation (A_2) with no effect on the EPSP slope (A_1). Twenty-four hours later both EPSP slope and population spike are significantly potentiated. Propranolol prevents the initial and 24 hour effects on EPSP slope (B_1) and spike amplitude (B_2). N=7. From Walling and Harley (2004). Reprinted with permission from The Journal of Neuroscience.

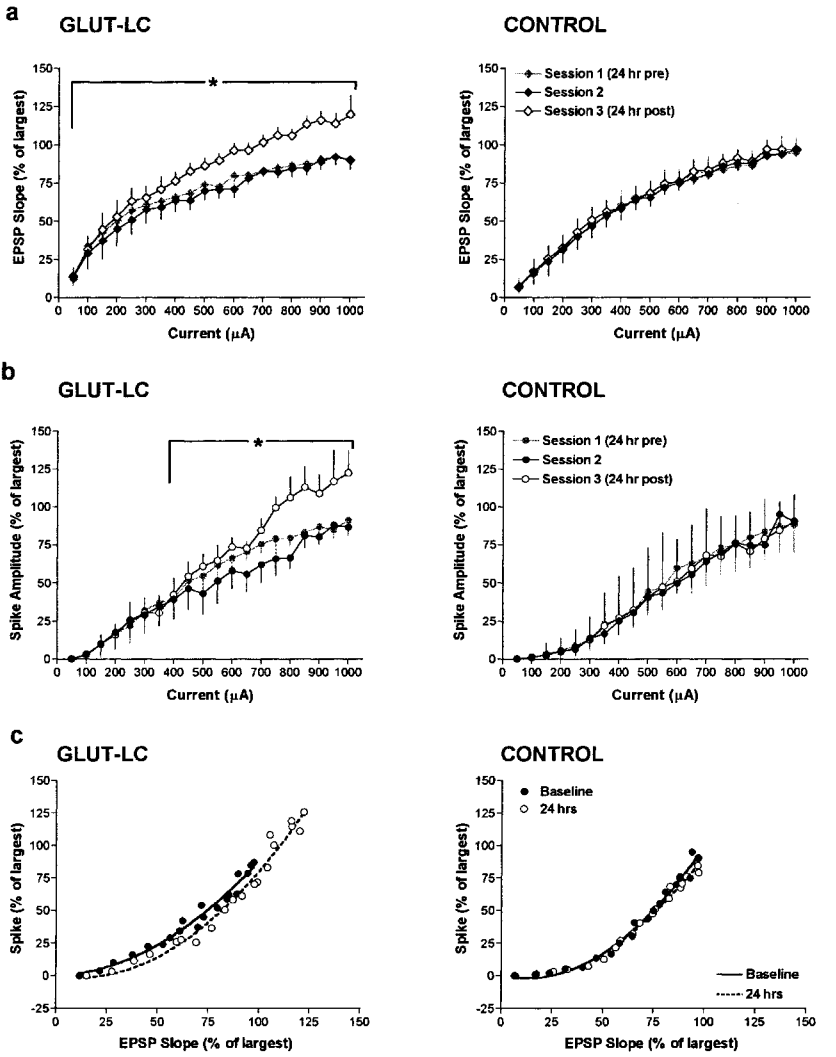

Figure 3. Potentiation of EPSP slope (**a**) and spike amplitude (**b**) at a range of currents prior to (Sessions 1 and 2), and 24 hr following, LC activation by glutamate (N=7). The EPSP-spike relationship is shown in **c**. EPSP slope predicts population spike amplitude both prior to, and 24 hr following, LC-induced potentiation. *indicates significant differences in spike amplitude and EPSP slope 24 hr after LC glutamate infusion relative to baseline. From Walling and Harley (2004). Reprinted with permission from The Journal of Neuroscience.

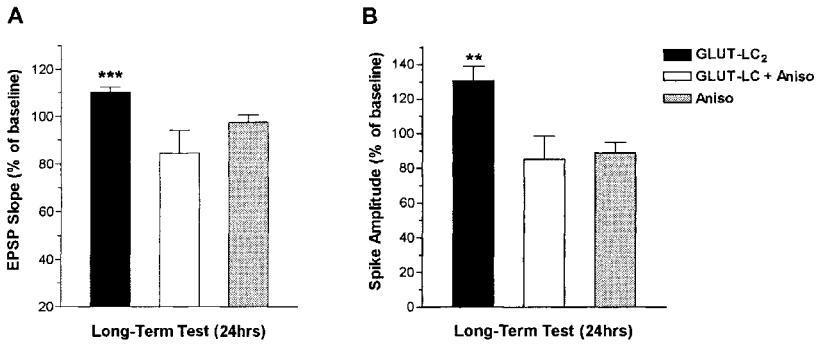

Figure 4. Intracerebroventricular anisomycin (Aniso) given prior to activation of the locus coeruleus (LC) by glutamate (GLUT) prevented the delayed 24 hr potentiation of EPSP slope (**A**) and spike amplitude (**B**) seen in a 2nd group of rats (N=7). Anisomycin alone did not alter recording levels from baseline when taken at the same time point. From Walling and Harley (2004). Reprinted with permission from The Journal of Neuroscience.

The E-S potentiation, characteristic of NE applications in other *in vivo* studies and of the initial effect of LC activation here was no longer seen. The E-S effect had been replaced by a delayed long-term potentiation of EPSP slope and population spike amplitude. The long-term effects were blocked by intraventricular infusion of either a protein synthesis inhibitor (Figure 4) or a β-adrenoceptor blocker (Figure 2). This result parallels a delayed long-term facilitation of synaptic strength described in invertebrates, which is also mediated by cAMP elevation (Emptage and Carew, 1993; Mauelshagen et al., 1998).

8. NE AND PLASTICITY: A SUMMARY AND SUGGESTED MECHANISMS

The present retrospective look at NE effects on the perforant path evoked potential in the dentate gyrus since the early 1980s provides evidence for three distinct effects of noradrenegic modulation on the evoked potential, although only two distinct effects can be described following the initial release of NE.

Low and, possibly physiological, synaptic concentrations of NE produce E-S potentiation. Thus, a given EPSP is associated with a larger spike amplitude than would be predicted by the change in EPSP amplitude alone. This effect can be long-lasting and is typically seen for minutes to hours following a brief period of NE release. Increased NE release associated with LC activation by either glutamatergic or orexinergic activation of LC occurs only in the first microdialysis sample taken at 20 min (Walling et al., 2004).

While E-S potentiation may be related to disinhibition (Staff and Spruston, 2003), our recent measurements of NE suppression of inhibition in dentate gyrus produced by glutamatergic LC activation suggest disinhibitory effects last less than 3 min. The prolonged increase in population spike amplitude is more likely related to changes in intrinsic cell excitability as reported in other systems that exhibit E-S potentiation

(Schrader et al., 2002; Zhang and Linden, 2003; Daoudal and Debanne, 2003; Frick et al., 2004). These changes appear to be post-synaptic changes in voltage-gated channels, particularly potassium channels, that can alter excitability locally in the dendritic arbor. Such channels are known to be modulated by phosphorylation and early evidence for NE modulation of the A type potassium channel was reported by Haas et al. (Haas and Rose, 1987). Recent theoretical models identify the increase in cell excitability as the other half of the Hebb synapse "story", and argue it has an important role in the engram (Schrader et al., 2002; Zhang and Linden, 2003). There is some evidence for an associative component to these changes, although they appear less specific than synaptic changes. The basis of these effects in the dentate gyrus remains to be elucidated.

Higher levels of NE, or low NE levels interacting with other plasticity-promoting neurotransmitters, produce EPSP slope potentiation. In the dentate gyrus, these effects appear intertwined with NMDA-dependent plasticity. NMDA receptor activation enhances NE release and activation of β-adrenoceptor pathways through NE release can enhance NMDA post-synaptic currents (Xie and Lewis, 1997). NE also promotes an increase in evoked presynaptic glutamate release and both the pre- and postsynaptic effects are β-adrenoceptor dependent. NE or β-adrenoceptor agonists also play a role in the conversion of early to late LTP and the recovery of decaying LTP possibly through their enhancement of the L channel calcium current, and promotion of calcium/calmodulin translocation to the nucleus (Mermelstein et al., 2001).

The third effect of NE is the most recently discovered and may be a result of physiological release of NE interacting with glutamatergic input in the dentate gyrus. The conditions for the development of the 24 hr synaptic and spike potentiation effect remain to be described with precision. It is possible that a period of sleep or some other state change must intervene to convert the glutamate-induced burst activation of LC concomitant with the activation of perforant path input into a delayed potentiation of the perforant path connection.

The associative requirements for the E-S potentiation effects characteristic of lower NE levels have not been clarified. However, it is likely that the effects of higher concentrations of NE in promoting synaptic potentiation have an associative component since NMDA antagonists block them. Thus, EPSP slope potentiation by NE appears to require concomitant glutamate release. Examining the associative properties of NE effects in dentate gyrus *in vivo* is complicated by the differential modulation of input from the medial and lateral perforant pathways. The depression observed in the lateral perforant pathway may be related to the disparate effects of opioid and glutamate release in that pathway. The opioids released depress NMDA post-synaptic currents, while NE may enhance the same currents (Xie and Lewis, 1997). LTP stimulation of lateral perforant path fibers releases sufficient opioids for the disinhibitory effects of the opioids to offset their reduction of NMDA currents. If this balance is altered with single pulse stimulation than the competitive reduction of NMDA currents might reduce calcium entry to the level required for LTD. In the delayed EPSP potentiation study (Walling and Harley, 2004), the failure to see EPSP slope potentiation initially could be related to stimulation of a consistent mix of medial and lateral fibers, although this seems unlikely. If it occurred, the later appearance of significant EPSP slope potentiation argues that lateral perforant path depression is transient. *In vitro* low levels of β-receptor activation failed to modulate EPSP slope and a similar lack of modulation seems more likely to account for the *in vivo* observations.

NE is normally released when new events happen in the world (Aston-Jones and Bloom, 1981; Sara and Segal, 1991; Sara et al., 1994; Vankov et al., 1995; Bouret and Sara, 2004). These are clearly moments when the ability to encode new information is most critical. Dahl and Sarvey's early observation still seems pertinent to our present views of NE's role in the dentate gyrus. "Selective persistent NE-induced effects upon neocortical input to the hippocampal formation via the entorhinal cortex may underlie mechanisms of attention, learning and memory (Dahl and Sarvey, 1989)." Our survey of studies on NE's effects in the dentate gyrus suggest NE alters information processing in the dentate gyrus through three β-adrenergic mechanisms: (1) a postsynaptic increase in cell excitability that may be linked to the phosphorylation and closing of voltage-dependent potassium channels in the dendrites of granule cells, (2) an increase in EPSP slope that may be associated with a presynaptic increase in transmitter release through phosphorylation of release proteins like synapsin and/or with an increase in LTP effects through enhancement of NMDA currents, increases in calcium entry via L channels with enhancement of calcium/calmodulin activity, and (3) a delayed potentiation of EPSP slope that resembles that seen in invertebrates, but the mechanism of which has not been identified.

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