2001

EPH receptors and memory

Alexander Joseph McNamara
San Jose State University

Follow this and additional works at: https://scholarworks.sjsu.edu/etd_theses

Recommended Citation

McNamara, Alexander Joseph, "EPH receptors and memory" (2001). *Master's Theses*. 2149.
DOI: <https://doi.org/10.31979/etd.wz5s-b778>
https://scholarworks.sjsu.edu/etd_theses/2149

This Thesis is brought to you for free and open access by the Master's Theses and Graduate Research at SJSU ScholarWorks. It has been accepted for inclusion in Master's Theses by an authorized administrator of SJSU ScholarWorks. For more information, please contact scholarworks@sjsu.edu.

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI[®]

EPH RECEPTORS AND MEMORY

A Thesis

Presented to

The Faculty of the Department of Biology

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Alexander Joseph McNamara

May 2001

UMI Number: 1403982

UMI Microform 1403982

Copyright 2001 by Bell & Howell Information and Learning Company.

All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.

Bell & Howell Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

© 2001

Alexander Joseph McNamara

ALL RIGHTS RESERVED

APPROVED FOR THE DEPARTMENT OF BIOLOGY

Dr. Sally Veregge

Dr. Adrian Rodriguez

Dr. Robert Gerla (Genentech Inc.)

APPROVED FOR THE UNIVERSITY

ABSTRACT

EPH RECEPTORS AND MEMORY

By Alexander Joseph McNamara

EphA receptors and their ligands, ephrinA, were previously thought to only play a role in the embryonic development of the central nervous system, however these proteins have been found to be expressed in the adult mouse brain, primarily in the hippocampus. The hippocampus is thought to be the processing region of the brain responsible for certain types of learning and memory. The role of Eph receptors in memory and learning has yet to be explored. This study uses the fear conditioning paradigm and a ketamine induced retrograde amnesia model to examine the role of EphA receptors in memory and learning. The results of this study suggest that activation of the EphA5 receptor through bilateral intrahippocampal infusion of the agonist ligand ephrinA5 (in the form of an immunoadhesin) ameliorates retrograde amnesia produced by anesthesia.

Acknowledgements: It is through the patience and guidance of Robert Gerlai that this paper found its way to completion. Thank you. Thanks are also due to A. Eisert, H. Philips, and V. Hogue. To all of which none of this could have been possible.

Table of Contents

Introduction	Page 1
Material and Methods	Page 14
Statistical Analysis	Page 20
Results	Page 21
Discussion	Page 26
References	Page 33
Figure Legends	Page 41
Figure 1	Page 44
Figure 2	Page 45
Figure 3	Page 46
Figure 4	Page 47
Figure 5	Page 48
Figure 6	Page 49
Figure 7	Page 50
Figure 8	Page 51

INTRODUCTION

EphA receptors, the largest subfamily of receptor tyrosine kinases, have been implicated in the development of the vertebrate central nervous system (CNS) (Friedman and O'Leary 1996). They and their ligands have been shown to act as repellent guidance cues during development for topographical movement of cells and axonal growth cones (Friedman and O'Leary 1996, Gao, Cerretti, Dreyfus, and Zhou 1999, Tessier-Lavigne 1995, and Zhou 1998). The ligands of this receptor subfamily are membrane bound proteins, attached to the membrane by a glycosylphosphatidylinositol (GPI) anchor and mostly found on astrocytes (Meima, Kljavin, Shih, Winslow, and Caras 1997). A recent *in situ* hybridization study has found that a member of this receptor family, EphA5, is expressed in the adult mouse and rat brain, predominantly in the pyramidal and granule cells of the hippocampal formation (Gao, Shinisky, Armanini, Moran, Zheng, and Mendoza-Ramirez 1998, Gerlai, Shinsky, Caras, Gao, Philips, and Winslow 1997, and Gerlai, Shinsky, Shih, Williams, Winer, and Armanini 1999). Other studies using real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR), a sensitive method to quantify mRNA expression, detected mRNA that codes for several EphA receptors and their ligands in the adult mouse hippocampus (Gerlai et al. 1997 and Gerlai et al. 1999). The presence of EphA receptors in the hippocampus, an area of the brain that demonstrates plasticity, is interesting, but not surprising considering that neuronal plasticity must occur during development (Bliss and Collingridge 1993, and Logue, Paylor, and Wehner 1997). The involvement of EphA in neuronal plasticity is not conclusive, but recent *in vitro* (Friedman and O'Leary 1996) and *in vivo* (Gerlai et al.

1997 and Gerlai et al. 1999) studies did find that activation and deactivation of EphA receptors in the hippocampus could improve or impair long term potentiation (LTP). Taken together, the results of localization and electrophysiological studies raise the possibility that EphA receptors are involved in adult neural plasticity and perhaps influence learning and memory. The latter possibility was investigated in a recent study (Gerlai et al. 1999) which showed that inactivation of EphA receptors by intrahippocampal infusion of EphA antagonists impaired the performance of mice in learning paradigms (T-maze spontaneous alteration and context dependent fear conditioning) sensitive to hippocampal dysfunction. Intrahippocampal infusion of an EphA agonist produced opposite effects, that is, performance improved in both tasks. While these results demonstrate that *in vivo* activation or inactivation of EphA receptors leads to correlated changes in learning (Gerlai et al. 1997 and Gerlai et al. 1999), it remains unclear what aspects of learning the receptors may mediate, acquisition or retrieval? By manipulating the receptors before acquisition, performance factors important during acquisition including motivation or sensory perception, were potentially affected, which made it difficult to draw a firm conclusion about whether or not EphA function is associated with memory and learning. The present study addresses these problems and dissociates acquisition from the storage of memory by inducing post-acquisition retrograde amnesia and by investigating whether such amnesia can be ameliorated by post acquisition activation of EphA receptors.

Recent developments in recombinant DNA technology now allow the specific disruption of single genes, which assists in the analysis of molecular mechanisms of

complex behaviors including learning and memory (Tonegawa, Li, Erzurumlu, Jhaveri, Chen, and Goda 1995). Although gene targeting would allow specific disruption of genes encoding EphA receptors or their ligands, it was decided in this study not to use this approach. The results of a gene targeting study may be hindered by several confounding factors, including the flanking of alleles problem, developmental alterations, and compensatory mechanisms (Gerlai 1996 and Gerlai et al. 1998). The flanking of alleles problem refers to the uncontrollable recombinant events that occur when using two different mouse strains to create a transgenic mouse (see figure 1 for more details), as is common practice. It was decided to use a strategy novel in behavioral neuroscience, protein targeting (Gerlai et al. 1998). This technique specifically targets a protein product of a gene rather than the gene itself. Either administration of an antagonist or agonist of the protein of interest can accomplish protein targeting. In the case of this study, an agonist molecule to the EphA receptor, the ligand EphrinA, was made in the form of an immunoadhesin molecule.

To target EphA receptors, a genetically engineered immunoadhesin (figure 2), ephrinA5-IgG, was designed using recombinant DNA technology (Chamow and Ashkenazi 1996). The immunoadhesin mimics the behavior of endogenous ephrinA ligands in that it has the natural receptor-binding domain of the endogenous ligand and it is capable of eliciting dimerization and thus phosphorylation (activation) of its receptors (Chamow and Ashkenazi 1996, Gerlai and Clayton 1999, and Meima et al. 1997). The EphrinA expression level in the adult brain however, is generally very low, suggesting that EphA receptors are not fully activated under baseline physiological conditions

under baseline physiological conditions (Gerlai et al. 1999). EphrinA5-IgG is an EphA agonist that contains the full-length ephrinA5 ligand, including the receptor-binding domain, which primarily recognizes the EphA5 receptor (Chamow and Ashkenazi 1996, Gerlai and Clayton 1999, and Meima et al. 1997). However, ephrinA ligands are promiscuous and can activate several EphA receptors (Orioli and Klein 1997). EphrinA5-IgG primarily recognizes EphA5 but also binds to the receptors to which the endogenous ephrinA5 ligand binds (EphA2-8), which the endogenous ephrinA5 ligand would (Chamow and Ashkenazi et al. 1996). Therefore, this immunoadhesin targets the biologically relevant functional receptor system thereby allowing one to avoid the problem of compensation by sister receptors (figure 1) (Gerlai et al. 1998 and Gerlai and Clayton 1999). Indeed, ephrinA5-IgG has been shown to be capable of eliciting phosphorylation of EphA receptors including EphA5. Furthermore, ephrinA5-IgG can be delivered into the brain at a convenient time during adulthood, so embryonic development remains unaltered.

Memory and Learning

What is memory and learning and how do both work at the cellular level? In recent years, an intense interest in the study of memory and learning has led to fascinating discoveries. Paramount to these findings is a possible mechanism of synaptic plasticity called long term potentiation (LTP). Timothy Bliss and Terje Lomo made the discovery of LTP, in 1973, while they examined neurophysiological activity of the hippocampal neural pathways (Bliss and Lomo 1973). They demonstrated that a short

postsynaptic excitatory potentials. This facilitation of the hippocampal postsynaptic neurons lasted for days, and even weeks in some cases. In the years that followed other studies demonstrated that LTP was associated with a clear alteration in the presynaptic activity (For general review see Milner et al. 1998).

LTP is thought to be a possible model for synaptic modification in the brain. The changes associated with LTP are synapse specific and use-dependent (Bliss and Collingridge 1993). These properties of LTP make it a possible model for induction, maintenance, and recall of memory and learning. The identifying characteristic of LTP is that a sustained increase in the amplitude of the postsynaptic excitatory potential is induced by only short bursts of stimuli to the postsynaptic target. This can be demonstrated when bursts of electrical stimuli are delivered to the Schaffer collateral of the hippocampus. The result is an increase in the amplitude and duration of the excitatory postsynaptic potential in the pyramidal cells of the CA1 region that received the initial stimuli. LTP can also be considered associative in that LTP may be activated by a series of combined weak stimuli from different neural pathways (Bliss and Collingridge 1993). The associative nature of LTP activation can be attributed to the fact that some postsynaptic neurons have N-methyl-D-aspartate receptors (NMDA-R). The NMDA-R must be bound by the neurotransmitter glutamate to remove the Mg^{2+} plug that blocks the receptor-linked channel. However, glutamate alone will not remove the Mg^{2+} plug that prevents Na^{+} and Ca^{2+} from entering the cell via the channel (Gustafsson and Wigstrom 1986). Activation of the NMDA-R channel will only occur if glutamate is bound and the postsynaptic cell is adequately depolarized by a strong cooperative input

from the presynaptic neuron (Bliss and Collingridge 1993). This entire process of LTP induction is important because it allows selectivity of neural pathways and synaptic specific responses to stimuli, even on the same nerve cell. The strengthening of neural networks through the activation of specific postsynaptic synapses by LTP may indeed be the foundation for memory and learning.

The role of NMDA-R channels in synaptic plasticity and LTP has been demonstrated through agents that block the NMDA-R channels, such as D-2-amino-5-phosphonopentanoate (AP5) (Morris et al. 1986). Other synaptic pathways in the hippocampus that exhibit LTP have been shown to be non-NMDA-R dependent. Lack of NMDA-R activity during LTP was determined through electrophysiological recording of the CA3 region after administration of AP5. The excitation of the CA3 region under these circumstances still produced an LTP. The mechanism behind the LTP in the CA3 region is believed to require the actions of a cAMP-dependent protein kinase (PKA). A cascade of molecular events is thought to activate this system of LTP induction (Brandon, Idzerda, and McKnight 1997, Huang, Li, and Kandel 1994, and Villacres, Wong, Chavkin, and Storm 1998).

The role of Ca^{2+} in LTP induction is not completely understood, however Ca^{2+} is thought to activate two second messenger systems in the postsynaptic dendrite. Once Ca^{2+} enters the cell it binds to Ca^{2+} /calmodulin kinase or protein kinase C, each of which remain active through the continued depolarization of the synapse. It is thought that the binding of Ca^{2+} to these protein kinases may create a positive local feedback loop between the presynaptic and postsynaptic membranes (Nicoll, Kauser, and Malenka 1988

between the presynaptic and postsynaptic membranes (Nicoll, Kauser, and Malenka 1988 and Tsein and Malinow 1990). Two strategies are employed for LTP induction, both using second messengers. One acts to increase the resting potential of the postsynaptic membrane and the other increases the release of glutamate from the presynaptic terminal. Increasing the resting potential allows the synapse to reach threshold for depolarization with less stimuli. Also required is an unknown retrograde messenger, thought to be controlled by the aforementioned protein kinase, or at least some other Ca^{2+} dependent mechanism, that act on a second messenger system in the presynaptic terminal to enhance neurotransmitter release (Nicoll et al. 1988 and Tsein and Malinow 1990). Recent evidence suggests that a cAMP-dependent protein kinase interacts with a cAMP responsive element binding protein (CREB) and modifies transcriptional factors (Impey, Mark, Villacres, Poser, Chavkin, and Storm 1996 and Milner et al. 1998). These changes to the pre- and postsynaptic regions may persist for hours or weeks to maintain the LTP. The modification may even alter the structure of the synapse, allowing a more rapid response to mild stimuli in the future. The underlining outcome of these molecular alterations, or rather synaptic plasticity, is the alteration of a matrix of networks that form the foundation of memory and learning.

It has been suggested since 1949 that presynaptic and postsynaptic axons exert changes on each other in a way that amplifies and maintains an action potential. This theory was first proposed by Donald Hebb, who wrote, " When axon A excites cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic changes take place in one or both cells so that A's efficiency, as one of the cells firing B.

increased” (Hebb 1949). This idea is now thought of as Hebb’s rule. This is also the basis for the concept of synaptic plasticity.

Learning and memory can take on many forms and both have been studied extensively by cognitive neuroscientists and psychologists. Learning is defined as when an organism alters its behavior in response to stimuli from the environment (Kandel and Schwartz 1982, and Squire, Knowlton, and Musen 1993). Memory stores that learned information for future retrieval. Whether this recall of an event is for self-defense, mating, or obtaining food, an obvious selective advantage is clear. The ability of an organism to alter behavior through learning is the result of neurological changes regarded as behavioral plasticity.

For the purpose of this study, two types of learning are of particular importance. These two types of learning have been classified as, nonassociative and associative learning. Nonassociative learning can result from repeated exposure to one stimulus. This could either be in the form of habituation, when repeated exposure to non-harmful stimuli decreases the reaction, or in the form of sensitization when there is an increase in response to various stimuli, not just the original one (Kandel and Schwartz 1982 and Squire et al. 1993). Memory based on association requires learning the relationship between stimuli (Kandel and Schwartz 1982 and Squire et al. 1993). Fear conditioning, used in this experiment, is a good example of associative learning. A subject is given a noxious stimulus, an electric shock, paired with a harmless stimulus, a tone. The animal soon learns to respond to the harmless stimuli (tone), as if it would be the painful one (electric shock). Both these types of learning require neurons to alter cellular activity to

accommodate the synaptic input of the novel stimuli. This neuroplasticity, as it is called, is put into motion by the actions of gene regulation and protein synthesis.

Episodic memory is a form of associative memory requiring the observer to take a snapshot of the entire scene and then use the unique elements of that event for future recall. Episodic memory involves the association of spatial cues with non-spatial cues (Squire 1992, Squire and Knowlton 1995, and Tulving, Hayman, and MacDonald 1991). This then would constitute the entire context of events that led to the experience. Association of stimuli by the hippocampus is hypothesized to allow for recall of the event to occur (Squire et al. 1993). The theory is that association would allow subjects to reactivate pathways in the neocortex involved in the original episode with only a stimulus from a partial cue of the event. The possibility of this occurring in the present experiment made it important to eliminate all potential partial cues from the tone only task discussed below. A partial cue in this task may cause the subject to recall the entire event, producing a false positive result. Another interesting aspect of stimuli association is that the hippocampus and neocortex must integrate stored information in such a way as to allow for overlapping of that information without that storage coming at the expense of losing other memories already stored (Rolls and Treves 1998). In this way an intertwined network of stimuli association is created that becomes useful for survival. A perfect example of this attribute in action, involving spatial memory, is the ability of a person to travel many different paths in a given area and form a spatial arrangement of the entire region based on those individual journeys. It would then become possible for that

individual to speculate on distances and terrain of paths not yet taken, again a selective advantage can be seen here with respect to hunting and food gathering.

The hippocampus serves as an excellent model for the study of neuroplasticity (Rolls and Treves 1998) and the subsequent role that neuroplasticity has in memory and learning (figure 3). Information enters the hippocampus through the perforant fiber pathway and travels to the dentate gyrus. The axons of the dentate gyrus send the signal down the mossy fiber pathway to synapse within the CA3 region (Huang et al. 1994). From here, information is sent to the CA1 region via the Schaffer collateral fiber pathway (figure 4). The hippocampus not only exhibits LTP in most major synaptic pathways, but there may be different mechanisms in specific regions of the hippocampus to achieve LTP (Rolls and Treves 1998 and Squire et al. 1993). For example, the pyramidal cells of the CA1 region are cooperative in an associative capacity, meaning that a weak synaptic excitation could induce an LTP if paired with a stronger synaptic input at the postsynaptic dendrite.

Bilateral damage to the hippocampus and areas of the temporal lobe produce amnesia in both humans and mice. This amnesia is anterograde, persons remember events prior to the hippocampal damage, but have trouble creating memories of events that have occurred after the damage (Baddeley and Warrington 1970 and Kesner and Novak 1982). The fact that damage to the hippocampus causes anterograde amnesia suggests that long-term storage of memory is not a function of the hippocampus. Rather it may function as the initial matrix in which new memories are formed for future neocortical acquisition. Once pathways are established in the neocortex for long term

storage, there may still remain a dependence for sometime on the hippocampus to recall those memories, however as those memories become reinforced through associated pathways, the need for the hippocampus diminishes (Squire et al. 1993). Though the hippocampus may not have a role in the long-term storage of memory, it does appear to be required for certain types of memory. In humans, this includes short-term declarative memory, which is the ability to verbally recall visual and spatial events. In contrast to this is a non-hippocampal specific form of memory called procedural memory (Zola-Morgan and Squire 1984). Procedural memory is the ability to know how to do a task without being able to explain how it is recalled. Riding a bicycle is an example of procedural memory. A person recalls how to maintain balance while pedaling, but fails to recall verbally how that balance was accomplished. It is obvious that an animal cannot respond verbally about events experienced, however behavioral analysis that targets hippocampal specific function can be useful in assessing memory in non-human subjects. In other words, the response of animals, such as fear and anxiety can be quantified for analysis of memory and learning. This study used a hippocampal specific contextual memory and learning task. Animals were required here to use the multiple cues of the environment, and associate them to recall the event, in this case a mild foot shock.

EphinA5-IgG delivery and behavioral testing

This study used a classical fear conditioning paradigm to evaluate contextual learning and memory in the hippocampus of mice (Aiba, Chen, Herrup, Rosenmund, Stevens and Tonegawa 1994, and Squire and Zola-Morgan 1991). Damage to the

hippocampus has been shown to cause mice to perform poorly in tasks that require exploration, as is required in configural learning (Aiba et al. 1994). Contextual memory uses configural learning to create complex multiple cue associations within a given environment. A fear conditioning chamber was used to deliver a conditioned stimulus (CS), given in the form of a tone cue, accompanied by an unconditioned stimulus (US), a mild shock (Davis, Walker, and Lee 1997 and LeDoux 1995). This allows the experimenter to select what the test subject will use in training as the CS. The single cue fear response of the mice is considered a non-hippocampal function; without this CS, the test subject would select their own single cue within the conditioning chamber, and contextual testing would be impossible. The fear response behavior in mice is evaluated as the amount of freezing observed in mice after training and testing sessions (Milner et al. 1998). Following training, the mice are given time to store the fear conditioning into memory. The training sessions are designed to test hippocampal function a week after treatment.

Hippocampal dysfunction was generated by using ketamine to induce retrograde amnesia. This was done in an attempt to see if it was possible to rescue the memory of *in vivo* subjects by infusing the immunoadhesin Ephrin-A5-IgG. An immunoadhesin molecule is a genetically engineered chimeric fusion protein that joins the Fc region of the human IgG molecule with the binding domain of the molecule of interest (figure 2) (Chamow and Ashkenazi 1996). In this study, that molecule of interest is Ephrin-A5. This ligand and its receptor Eph-A5 are believed to facilitate neural and behavioral plasticity (Gerlai et al. 1997). Before it was possible to estimate the effects of Ephrin-

A5-IgG, it was necessary to determine the amount of retrograde amnesia induced by the anesthesia ketamine. This evaluation was used to set a foundation for quantifying the results of this study. The discovery of retrograde amnesia was based on the attempts to use osmotic mini-pumps to deliver agents in a previous investigation. The fact that memory was being effected by the pre-surgical administration of anesthesia itself, made it a necessity to establish how much loss could be expected. In an attempt to study the effects of Ephrin-A5, it became imperative to see the severity of retrograde amnesia in both operative and non-operated mice. The first control groups received anesthesia only, without the administration being followed by a surgical procedure. The next group underwent the same conditions, however they received the surgery, with an osmotic mini-pump delivering phosphate buffer saline (PBS) only.

The osmotic mini-pumps delivered equal hourly doses of solution over a seven-day period, after which time the subjects were tested. This consisted of isolating the CS to a context other than the conditioning chamber. This was used to verify that functional use of non-hippocampal tasks was still working to the levels established in previous studies. The important test came when mice were reintroduced to the fear condition chamber without the CS or the US. The amount of freezing an animal did in response to this testing of hippocampal function was evaluated and compared. The question this study hoped to answer was whether the delivery of Ephrin-A5-IgG ameliorates retrograde amnesia induced by the anesthesia ketamine.

MATERIALS AND METHODS

Animals and Housing

This experiment used (11-12) week old C57BL/6 male mice. Strain selection was based on a previous evaluation that determined C57BL/6 responded to fear conditioning with a higher amount of freezing due to a possible lower threshold for pain (Gerlai et al 1997). The mice were housed in groups of 10, in plastic containers measuring (45x24x15cm), on sawdust bedding. The groups of mice were housed in the same room at a temperature of 20 ± 1 C° and had a light/dark cycle of 12hr/12hr respectively (lights turned on at 6:00 am). Food and water were available *ad lib*. All mice received the same handling condition before and after training. The mice were returned to the community housing when treatment and/or surgery were complete. Ephrin-A5-IgG and CD4-IgG treated subjects shared the same plastic housing containers. The non-operated mice were always separated from those that had undergone surgery, for fear that the former would harm the latter before they had fully recovered.

Fear Conditioning

The methods for fear conditioning have been described in detail elsewhere (Gerlai et al. 1997, and Gerlai et al. 1998). Briefly, the paradigm had three phases: a training phase, a context-dependent test, and a cue-dependent test. Training and testing of the mice were performed in the Gemini active/passive avoidance fear conditioning apparatus (San Diego Instruments). The chamber consisted of three black acrylic and one transparent side wall. The floor of the chamber had metal bars that delivered the (US)

mild electric shock. A 10W light bulb illuminated the chamber through a white acrylic ceiling. Placing the apparatus in a sound-attenuating hood reduced auditory stimulus. The transparent wall allowed the subject's behavior to be monitored by video camera; this decreased the amount of possible fear elicited by direct observation. The testing had duration of six minutes. At 160 seconds an 80dB, 2900 Hz tone cue was activated for 20 seconds. A 1 second 0.7 mA electric shock was delivered through the floor bars at 179 seconds, tone and shock was turned off at 180 seconds. A second tone started at 220 seconds with the shock delivered at 239 seconds, at 240 seconds tone and shock was turned off. The third and last tone was turned on at 280 seconds, with shock delivery at 299 seconds, at 300 seconds tone and shock was turned off (see figure 5A for timeline of procedure). The recording of each session ended at 360 seconds. Each mouse was tested in a room isolated from its group so that the other subject could not habituate to the tone prior to training. Those subjects undergoing treatment were given 90 minutes to store the learning of the fear-conditioning chamber into memory before being anesthetized, as described above.

Seven days after training and treating the mice, they were tested for contextual memory, and non-hippocampal specific behavioral responses. The contextual memory task is sensitive to hippocampal lesions (Gerlai et al. 1998, Kim and Fanselow 1992, and Phillips and LeDoux. 1992) or to mutation-induced hippocampal dysfunction (Abeliovich, Paylor, Chen, Krim, and Tonegawa 1993 and Aiba et al. 1994), and was tested by reintroducing the mice to the fear-conditioning chamber. The testing sessions lasted 6 minutes, and were recorded by video camera, as was done in training. Neither a

cue nor a shock was given at anytime during the 6 minutes of reintroduction. To prevent the subjects from using olfactory senses as a single cue during testing, the fear conditioning chamber was cleaned before each mouse was reintroduced. The cue only tested was carried out in a container of similar dimensions to the fear-conditioning chamber but visual, olfactory, and tactile cues were different (Gerlai 1998). A tone was presented over 6 minutes in the same time intervals and duration as was presented to the subjects in training. The tone cue task is insensitive to hippocampal damage or dysfunction and is often used as a control situation for performance factors or general, associative, learning ability (Kim and Fanselow 1992 and Phillips and LeDoux 1992). The cue only test was also recorded on video.

The evaluation of the results was based on the active behavior recorded on the video camera and later replayed and quantified by an observer blind to the treatment grouping. The fear response in mice is identified by the amount of freezing the mouse does when presented with the learned stimuli it associates with danger (Blanchard and Blanchard 1969, Fanselow 1986). The number and duration of freezing events that occurred was measured and plotted for each minute of training and testing. The fear-conditioning chamber also had a photocell detector that would detect motion within the chamber and information was sent to an event recorder (Noldus 1991). The event recorder was used in the case of inconclusive results on video. The observation of results by video camera seemed more reliable, as we were able to identify other fear responses that would not have been shown on the event recorder. Fear induced behaviors also include tail rattling and body elongation.

Experiment 1: induction of retrograde amnesia

The effect of anesthetic agents injected into the peritoneum (i.p.) 90 minutes after training was tested in this series of experiments. Combinations of the drugs (ketamine, xylazine, and acepromazine) required for surgical anesthesia were used. Ketamine is thought to induce both anesthesia and amnesia (Kovalev, Sazin, and Kuleshove 1990, and Saha, Chugh, Sankaranarayanan, and Datta 1990) by functionally disrupting neuronal activity via affecting a range of neurotransmitter systems and receptors including the glutaminergic (N-methyl-D-aspartate receptor, NMDA-R) (Avis, Burton, Berry, and Lodge 1983, Collingridge and Bliss 1987, and Oshima and Richards 1998), GABAergic, serotonergic, adrenergic and dopaminergic systems (Plumb 1995). Several of these neurotransmitter systems and receptors have been implicated in memory but perhaps the most thoroughly studied receptor of all in this respect is NMDA-R, as mentioned above, a calcium channel that is a key player in LTP as a coincidence detector of converging stimuli (Davis, Butcher, and Morris 1992, and Morris et al. 1986).

The analgesic effect of ketamine is insufficient in rodents for surgery. Concurrent application of sedatives xylazine (alpha-2-adrenergic agonist) and acepromazine (phenothiazine derivative) is advised (Plumb 1995). Ketamine was used together with these drugs in all surgeries. Neither acepromazine nor xylazine alone is expected to induce amnesia (Plumb 1995).

Mice were randomly assigned in five groups. Group 1 received surgical anesthesia (i.p. injection of ketamine (9 ml/kg body weight), xylazine (4.5 ml/kg body weight), acepromazine (0.9 ml/kg body weight) in PBS) 30 hours before training to test whether

anesthesia has a lasting anterograde effect on learning and memory. Mice in groups 2, 3, and 4 were all injected with different agents 90 minutes post-training. This timing was based on preliminary experiments and on findings from a study using tetrodotoxin-induced hippocampal lesions (Lorenzini et al. 1996). Mice of group 2 received surgical anesthesia identical to that of group 1 but 90 minutes after training. This treatment is expected to induce retrograde amnesia. Mice in group 3 received an i.p. injection of xylazine (4.5 ml/kg body weight) and acepromazine (0.9 ml/kg body weight) in PBS but not ketamine. Mice in group 4 received saline injection only and served as a control group for injection and handling, and group 5 mice received no injection.

Experiment 2: stereotaxic surgery and its effect on memory

The second experiment tested whether stereotaxic surgery involving canulation and intrahippocampal infusion of vehicle, PBS, impairs behavioral performance compared to non-operated mice. Mice were trained and anesthetized 90 minutes after training, as described above. Briefly, using a stereotaxic frame mice received bilateral intrahippocampal implantation of canulae at position anterior to posterior (AP) -1.5 , medial to lateral (ML) ± 1.8 , as measure in mm from the bregma and dorsal to ventral (DV) 1.8 mm from the flat skull surface. Canulae were connected to a pair of ALZET 1007D micro-osmotic pumps (ALZET brain infusion kit, ALZA, Palo Alto, California, USA: volume 99 μ l; pumping rate: 0.52 μ l/h; delivery period ~ 7 days). All mice survived the operation, remained active, ate well, were groomed, and exhibited no signs of discomfort. Stereotaxic surgery and intrahippocampal infusion of PBS or CD4-IgG (see below) led to no gross neuroanatomical lesion, edema, or

inflammation as ascertained by magnetic resonance imaging (MRI) and immunostaining for various neuronal and astrocytic-marker (Gerlai et al. 1998 and unpublished observation).

Experiment 3: intrahippocampal infusion of immunoadhesins, ephrinA5-IgG and CD4-IgG, and their effect on memory

The third experiment was performed to test the effect of ephrinA5-IgG and EphA5 receptor agonist immunoadhesin. Eph A5 receptor is expressed abundantly in CA1-3 and dentate neurons of the hippocampal formation (Gao et al. 1998, Gerlai et al. 1997, Gerlai et al. 1999). EphrinA5-IgG, or a control, inactive immunoadhesin CD4-IgG (Gao et al. 1998, and Meima et al. 1997), was infused into the hippocampus in a manner identical to the PBS infusion explained above (both as 0.5 mg/ml solution, 99-μl volume per hippocampus, in artificial cerebrospinal fluid (ACSF) pH 7.4 containing 124 mM NaCl₂, 3mM KCl, 2.4 mM MgSO₄, 1.25 mM KH₂PO₄, 26 mM NaHCO₃, 10 mM D-glucose). As previously shown, immunoadhesin infusion leads to homogeneous immunoadhesin distribution in the entire hippocampus affecting only a small cortical area around the canula insertion site but not infrahippocampal areas (Gerlai et al. 1998).

For behavioral testing, all mice were trained first and 90 minutes later received surgical anesthesia. Stereotaxic brain surgery was started 10 minutes after full anesthesia. The micro-osmotic pumps were primed so that infusion started approximately 2 hours after mice recovered from anesthesia. Context and cue tests were performed 7 days later.

In addition, two more groups of mice were trained and infused with ephrinA5-IgG or CD4-IgG as described above. These animals were tested not 7 days but 17 days after the start of immunoadhesin infusion. As the 1007 D ALZET micro-osmotic pump delivers its content in 7 days, these mice had been without ephrinA5-IgG infusion for 10 days by the time of behavioral tests.

STATISTICAL ANALYSIS

Relative duration of freezing was calculated for each 1-minute interval of the training, context test, and cue test sessions. This data was statistically analyzed using SYSTAT 5.2 for Macintosh. Two-way repeated measure variance analysis (ANOVA) was employed to test the effect of treatment (anesthesia vs. control, or anesthesia with PBS infusion vs. control, or anesthesia with CD4-IgG infusion vs. anesthesia with ephrinA5-IgG) and time (six 1 minute intervals of the session, the repeated measure factor). In case of significant interaction, post hoc multiple comparison tests such as Tukey Honestly Significant Difference (HSD) tests were applied.

RESULTS

Experiment 1: anesthesia causes a severe impairment in the context test

Experiment 1 was performed to test the effects of post-training surgical anesthesia and to induce retrograde amnesia in fear conditioning. The five groups of mice showed identical freezing before shock presentation during training (figure 5A) and all mice were active (data not shown) suggesting lack of fear in the shock chamber or to tone alone.

Upon repeated shock presentation, all mice responded with increasing amounts of freezing (figure 5A). ANOVA showed no treatment effect ($F(4, 51) = 1.15, P > 0.34$), a significant time effect ($F(2, 255) = 380.80, P < 0.0001$), and a non-significant time X treatment interaction ($F(5, 255) = 1.15, P > 0.30$). This demonstrates that first, pre-training anesthesia (group 1) did not affect performance, and second, mice that received different post-training treatment responded differently.

In the context test conducted 7 days later, significant group differences were observed (figure 5B); ANOVA treatment effect ($F(4, 51) = 9.35, P < 0.0001$), time effect ($F(5, 225) = 1.50, P > 0.20$), treatment X time interaction ($F(5, 255) = 0.98, P > 0.48$). Post hoc Tukey's HSD test confirmed that mice receiving surgical anesthesia 90 minutes post-training froze significantly less ($P < 0.01$) compared to mice from the other four treatment groups, and that the latter four groups were not different from each other ($P > 0.05$). This suggests that, first surgical anesthesia has a significant amnesic effect when applied 90 minutes post-training. Second, xylazine and acepromazine alone do not affect performance. Third, i.p. injection of saline, and the pain and handling associated with it, has no adverse effect on memory performance in the context task.

In the cue only test, ANOVA showed a significant treatment effect ($F(4, 51) = 3.23, P < 0.02$), a significant time effect ($F(5, 255) = 290.02, P < 0.0001$), and significant treatment X time interaction ($F(5, 255) = 2.61, P > 0.001$). However, post hoc Tukey's HSD test revealed no difference ($P > 0.05$) between treatment groups for the first five intervals of the session. Before receiving the tone signal, i.e. during the first two intervals, all mice were active (data not shown) and exhibited almost no freezing (figure

5C). Upon tone presentation all mice froze, a response that became robust after repeated tone presentation. It is noteworthy that the reduced freezing at the last interval of the cued task in the surgical anesthesia group is reminiscent of the cue task performance of DBA/2, an inbred mouse strain suffering from hippocampal dysfunction (Bliss and Collingridge 1993, Gerlai et al. 1996, Gerlai et al. 1997, and Logue, Paylor, and Wehner. 1997), which suggests that accelerated extinction of freezing in the cued task may be associated with hippocampal dysfunction (Bliss and Collingridge 1993 and Gerlai et al. 1996).

In conclusion, mice that underwent post-training surgical anesthesia exhibited pronounced amnesia in the context task yet showed unimpaired response to tone in the cued task. Mice that received the analgesics xylazine and acepromazine, but not ketamine, or mice that received saline injection only were unimpaired on either task.

Experiment 2: anesthesia and subsequent PBS infusion causes impairment in the context test

Experiment 2 investigated the effect of the stereotaxic surgical procedure. Mice infused with PBS exhibit impairment identical to those receiving surgical anesthesia alone (figures 5 and 6). ANOVA for comparison of PBS infused and control mice in training (figure 6A) showed no significant treatment effect ($F(1, 25) = 0.454$, $P > 0.50$), or treatment X time interaction ($F(5, 125) = 0.207$, $P > 0.950$) and revealed a significant time effect ($F(5, 125) = 184.325$, $P < 0.0001$). In the context test, PBS infused mice exhibited a significantly decreased level of freezing, compared to control, an impairment

that remained unchanged throughout the test (ANOVA, treatment $F(1,24) = 20.253$, $P < 0.0001$; time $F(5, 120) = 1.848$, $P > 0.10$; treatment X time interaction $F(5, 120) = 0.735$, $P > 0.590$). In the cue test, both PBS infused and control mice were active before tone cue (data not shown) and responded to tone with equivalent, robust freezing (figure 6C). Although ANOVA revealed a treatment effect bordering significance ($F(1, 23) = 4.336$, $P = 0.05$), a significant time effect ($F(5, 115) = 186.726$, $P < 0.0001$) and a significant treatment X time interaction ($F(5, 115) = 4.892$, $P < 0.0001$). Tukey's HSD demonstrated a difference ($P < 0.05$) only at the last interval ($P > 0.05$ for all other intervals).

Experiment 3: intrahippocampal infusion of ephrinA5-IgG ameliorates anesthesia induced retrograde amnesia

Experiment 3 investigated whether ephrinA5-IgG can ameliorate the amnesic effects of surgical anesthesia. Previously, this study has shown that intrahippocampal ephrinA5-IgG infusion leads to quantitatively increased EphA5 phosphorylation in the hippocampus compared to CD4-IgG infusion or sham, confirming that ephrinA5-IgG is capable of activating the endogenous EphA5 receptor *in vivo* (Gerlai et al. 1998, Gerlai et al. 1999, and Grant 1996). This study also looked at whether chronic infusion of ephrinA5-IgG leads to compensatory responses at the gene expression level. Analysis of message levels of all known ephrinA ligands and EphA receptors revealed no differences between ephrinA5-IgG infused and uninfused hippocampal tissue as measured by real time quantitative RT-PCR (unpublished observations, for procedures see Ref. Winer et al.

1999). The behavior of ephrinA5-IgG infused mice was compared to mice receiving the inactive immunoadhesin, CD4-IgG. All mice were trained, operated, and tested in an identical manner as described for PBS group in experiment 2.

In training, all mice performed similarly (figure 7A; ANOVA group effect $F(1, 43) = 0.10$, $P > 0.75$; time effect $F(5, 215) = 369.93$, $P < 0.0001$; group X time interaction $F(5, 215) = 1.17$, $P > 0.30$). In the context test, ephrinA5-IgG infused mice exhibited an increased fear response (figure 7B). ANOVA revealed a significant treatment effect ($F(1, 43) = 6.39$, $P < 0.02$). A significant time effect $F(5, 215) = 2.32$, $P < 0.05$, and no significant treatment X time interaction ($F(5, 215) = 0.32$, $P > 0.85$). Although the performance improvement in the ephrinA5-IgG infused group was robust and significant, freezing levels of these mice did not reach those exhibited by normal uninfused and non-anesthetized mice (compare figures 7B and 6B, and also see figure 5B). ANOVA for comparison of context test performance of ephrinA5-IgG infused mice from experiment 3 and non-anesthetized non-infused mice from experiment 2 showed significant treatment ($F(1, 34) = 17.81$, $P < 0.0001$), time ($F(5, 170) = 3.05$, $P < 0.05$) and treatment X time interaction ($F(5, 170) = 0.73$, $P > 0.60$). In the cue test (figure 7C) ANOVA showed no significant differences between the CD4-IgG and the ephrinA5-IgG infused mice (treatment $F(1, 43) = 1.57$, $P > 0.20$). All mice were active before tone cue presentation and responded to tone with a robust increase of freezing (ANOVA time $F(5, 215) = 188.41$, $P < 0.0001$; treatment X time interaction $F(5, 265) = 0.38$, $P > 0.85$) suggesting that the improvement seen in the context test in mice infused with ephrinA5-IgG was specific to that task.

Comparison of another group of ephrinA5-IgG and CD4-IgG infused mice tested 17 days after training and anesthesia showed similar results (figures 8A-C). ANOVA revealed no group differences during training (treatment $F(1, 18) = 0.08$, $P > 0.75$; treatment X time interaction $F(5, 90) = 0.47$, $P > 0.75$; time $F(5, 90) = 151.16$, $P < 0.0001$). In the context test, ephrinA5-IgG infused mice exhibited significantly increased freezing compared to their CD4-IgG infused control counterparts throughout the test session (treatment $F(1, 18) = 1.72$, $P < 0.05$; treatment X time interaction $F(5, 90) = 2.01$, $P > 0.08$; time $F(5, 90) = 2.00$, $P > 0.08$), but cued task performance was not different between the two groups (treatment $F(1, 18) = 1.23$, $P > 0.25$; time $F(5, 90) = 92.69$, $P < 0.0001$; treatment X time interaction $F(5, 90) = 0.74$, $P > 0.50$). Thus, ephrinA5-IgG infused after anesthesia for 7 days had a lasting effect on context dependent fear responses at least up to 17 days from the start and 10 days after the end of infusion.

CD4-IgG and PBS infusion did not alter retrograde amnesia induced by anesthesia

Although experiments 1, 2, and 3 were carried out separately and the subjects were only randomized within experiment, the three groups of mice that received surgical anesthesia and were tested 7 days later (surgical anesthesia group in experiment 1, PBS group in experiment 2, CD4-IgG group in experiment 3) exhibited virtually identical performance in all phases of fear conditioning. Most importantly, they were all impaired to the same degree in the context task. ANOVA revealed no significant difference between these groups (treatment $F(2, 39) = 0.331$, $P > 0.70$; time $F(5, 195) = 0.579$, $P >$

0.70; treatment X time interaction $F(10, 195) = 1.188, P > 0.30$). As anesthetized mice were not different from mice anesthetized and infused with PBS, it can be inferred that stereotaxic surgery and intrahippocampal cannulation did not contribute to anesthesia induced retrograde amnesia. Furthermore, as intrahippocampal infusion of CD4-IgG had no effect on behavioral performance when compared to PBS infusion or anesthesia only, it can be inferred that this immunoadhesin was a neutral agent, a conclusion that will be important in the interpretation of ephrinA5-IgG effects.

DISCUSSION

In this study, post-training behavioral performance deficits were induced resembling retrograde amnesia by applying i.p. injection of surgical anesthetics 90 minutes after training in fear conditioning. The impairment was robust in the contextual task conducted 7 days after training but only in the injection containing ketamine. The impairment could not be attributed to altered performance factors in training, including sensitivity to electrical shock, motivation, attention, or perception because the anesthesia was applied after training. It is also unlikely that the anesthesia affected perception or motor performance per se during the context test as it was applied 7 days before the test and pharmacokinetic studies suggest that the anesthetic drugs are metabolized within hours (Rose et al. 1993). The unaffected cued task performance also argues against a generalized performance deficit. Furthermore, when anesthesia was applied 30 hours before training, it was ineffective and led to no amnesia or performance deficits in subsequent training and tests. Lastly, when administered 6 hours after acquisition and 48

hours before memory test, ketamine amnesia was shown to have no effect on behavioral performance in a passive avoidance learning paradigm similar in performance factors and learning requirements to fear conditioning (Lorenzini, Baldi, Bucherelli, Sacchetti, and Tassoni 1996). In summary, these results suggest that first, anesthesia has no lasting (30 hours or more) effects on general behavioral performance per se, and second, anesthesia must be applied within a specific time window (less than 6 hours) following training in order for it to induce impaired performance measured at a later time. It can be concluded therefore that impaired behavioral performance, induced by 90 minutes post-training anesthesia, in these experiments was indeed due to cognitive impairments associated with retrograde amnesia. It is uncertain why surgical anesthesia led to a robust context specific performance deficit while sparing cued performance, but our results agree with observations in the human literature showing that anesthetics often allow the formation of simple memories but disrupt complex, explicit, memories (for review see Andrade 1996) that are thought to be dependent upon hippocampal function (Squire et al. 1993). Nevertheless, it is possible that the contextual test, being a more complex task, is more sensitive to detect cognitive dysfunction than a single cue association task and the apparently hippocampal specific nature of our findings is an artifact of the experimental design. Irrespective of the exact neuroanatomical locale of the action of the anesthesia, however, the results clearly demonstrate that the impaired response observed in the contest task is not due to deficits in trivial performance factors.

EphrinA5-IgG ameliorates retrograde amnesia

The most important finding of this study is the demonstration of the ameliorative effects of ephrinA5-IgG on anesthesia induced retrograde amnesia. EphrinA5-IgG infused mice were previously found to exhibit increased EphA5 phosphorylation levels (Chamow and Ashkenazi 1996, Gerlai et al. 1998, and Gerlai et al. 1999), and in the present study showed significantly improved freezing responses, in the context task, compared to their CD4-IgG infused control counterparts 7 days after surgical anesthesia. As anesthesia was applied after training and ephrinA5-IgG infusion was started after anesthesia, we infer that the effects of ephrinA5-IgG could not have been due to altered acquisition. Furthermore, as training was carried out uniformly on all mice, ephrinA5-IgG and CD4-IgG infused mice should not differ in general motivation. It is also unlikely that the improvement exhibited by ephrinA5-IgG infused mice is due to overall enhancement in performance because freezing in the cued task was unaltered. Lastly, the performance improvement is unlikely to be a result of direct effects of ephrinA5-IgG idiosyncratic performance factors at the time of context testing because the effects of ephrinA5-IgG lasted as long as 17 days after training, i.e. 10 days after the end of infusion. By this time there was no, or very little, biologically active ephrinA5-IgG available in the hippocampus of the infused mice (binding affinity of ephrinA5-IgG diminishes significantly with time, decreasing to 7% of the original after 10 days at 37°C) (Gerlai et al. 1998, and Chamow and Ashkenazi 1996), and infusion from the 1007D micro-osmotic pump stops after 7 days). Thus, improved performance is more likely to

be due to neurobiological changes triggered by ephrinA5-IgG during the 7 days post-acquisition infusion period.

It is noteworthy that CD4-IgG infused mice performed similarly to mice infused with physiological saline or to mice that were anesthetized and not operated on. Therefore, this study confirms the conclusion that CD4-IgG plays no role in behavioral function (Gerlai et al. 1998) in fear conditioning, and the difference between ephrinA5-IgG and CD4-IgG infused mice is due to the ameliorative effect of the former immunoadhesin. Taken together, these findings suggest that ephrinA5-IgG infusion counteracted anesthesia induced retrograde amnesia and led to improved cognitive function.

Retrograde amnesia was reversed by ephrinA5-IgG infusion in a context specific manner implying that ephrinA5-IgG acting through EphA tyrosine kinase receptors had a hippocampal dependent effect. Although it is possible that EphA receptors are uniquely involved in hippocampal dependent plastic changes because they are most strongly expressed in the brain region (Gao et al. 1998 and Gerlai et al. 1999) or because they mediate LTP that is strongly associated with hippocampal learning processes (Bliss and Collingridge 1993), their seemingly hippocampal specific effect may be an artifact of the present experimental design. Firstly, the effects of ephrinA5-IgG infusion may have remained undetected in the cue dependent (non-hippocampal) task because there was no impairment to ameliorate (ceiling effect associated with the good performance) in this task. Secondly, ephrinA5-IgG was directly infused into the hippocampus and although some cortical areas proximal to the canula insertion site were infused in addition to the

hippocampus (Chamow and Ashkenazi 1996, Gerlai et al. 1998, and Gerlai et al. 1999), the rest of the brain remained uninfused and is expected to exhibit no alterations.

The molecular and neurobiological mechanisms underlying ephrinA5-IgG action are not known. EphrinA5-IgG is unlikely to have inhibited the action of surgical anesthetics directly. Firstly, EphA receptors and their ligands have no known binding sites that could interact with ketamine, xylazine, or acepromazine. Second, ephrinA5-IgG could not physically interact with these anesthetic drugs as they were not present in the hippocampus at the same time: infusion of ephrinA5-IgG was started about an hour after anesthesia was over.

The potential substrate of ephrinA5-IgG action may be molecular mechanisms coupled to LTP (Gao et al. 1998, Gerlai et al. 1997, and Gerlai et al. 1999). EphA receptors contain a cytoplasmic sequence motif, YEPD, which mediates binding src kinases, including src and fyn (Ellis, Kasmi, Ganju, Walls, Panayotou, and Reuth 1996). Fyn is involved in the phosphorylation of NMDA-R (Grant et al. 1996), a key player in LTP (Morris et al. 1986 and Sakimura, Kutsuwada, Ito, Manabe, Takayama, Kushiya, Yagi, Aizawa, Inoue, and Sugiyama 1995), and fyn null mutant mice exhibit impaired spatial learning and blunted hippocampal LTP (Grant et al. 1992). Src also modulates NMDA-R function (Ungerer et al. 1991) and plays a crucial role in LTP (Lu, Roder, Davidow, and Slater, 1998). LTP, and NMDA-R itself, have been implicated in memory consolidation (Flood, Baker, and Davis 1990, Izquierdo and Medina 1984, Kim 1992, Ungerer, Mathis, Melan, and de Berry 1991, and Walker and Gold 1997). Thus, src kinase mediated synaptic plasticity may be a potential substrate of EphA action. This

suggestion is in line with observed acute effects of EphA modulation on LTP in vitro (Gao et al. 1998). However, it may not fully account for the observations of the present paper because of the potentially different time scale of changes underlying NMDA-R mediated LTP and memory consolidation. A more plausible explanation for potential mechanisms underlying our behavioral observations is the micro-structural hypothesis of EphA action (Gerlai et al. 1999). Synaptic structural changes, such as perforated synapses, have been implicated in the induction and maintenance of LTP (Edwards 1995, and Geinisman, de Toledo-Morrell, Heller, Rossi, and Parshell 1993). Actin, a structural element of cytoskeleton present in dendrites, axons, and synapses (Fifkova and Delay 1982, Hayashi, Ishikawa, Ye, He, Takata, and Kohama 1996, Landis and Reese 1983, and Westrum, Jones, Gray, and Barron 1980), itself plays a role in synaptic plasticity (Allison, Gelfand, Spector, and Craig 1998, Hayashi et al. 1996, Plumb 1995, Ter-Margarian and Djamgoz 1992, and Wang Zheng, and Poo 1996). Importantly, EphA activation by ephrinA5-IgG has shown to lead to perturbation of actin polymerization (Meima et al. 1997). Furthermore, EphA receptors and their ligands were recently found to contain PDZ recognition motifs and to interact with PDZ proteins involved in clustering of cytoskeletal proteins, receptors, and ion channels at synaptic sites (Torres, Firestein, Dong, Staudinger, Olson, and Huganir 1998), processes that again may have a dramatic effect on neural plasticity. The hypothesis that EphA receptors are involved in microstructural processes in the adult mammalian brain has gained further support by our recent findings showing transcriptional changes, mediated by EphA receptors, in genes of tubulin and MAP2 in the adult mouse hippocampus (Gerlai et al. 1999). Both tubulin

(Mileusnic, Rose, and Tillson 1980, Rose 1993, and Scholey, Bullock, and Rose 1992) and MAP2 (Quinlan and Halpain 1996) have been implicated in cytoskeletal processes associated with neural plasticity. Thus, EphA receptors may influence neural plasticity via mediating cytostructural mechanisms that allow formation and/ or maintenance of stable memory.

It is unclear how ephrinA5 ligands mediate this process. In the adult brain, ephrin-A ligands are expressed at very low level (Gerlai et al. 1999) implying that perhaps a considerable proportion of the EphA receptor molecules are normally not activated. It is plausible that induction of expression of the ligands is a primary process that leads to receptor activation, however, this has not been investigated. It is also possible that proper clustering of the GPI anchored membrane bound ephrin-A ligands underlines receptor activation (at least two ligands need to be in close proximity to induce receptor dimerization and initiate the autophosphorylation process (Chamow and Ashkenazi 1996). However, again no evidence is available to confirm the validity of this suggestion. Interestingly, activity dependent induction of EphA receptors (EphA4 and EphA5) has been recently demonstrated in the hippocampus (Moreno-Flores and Wandosell 1999) suggesting that transcriptional regulation of the receptors and/or ligands may mediate plastic changes. Whether this observation is relevant for learning and memory is uncertain because the authors of the study used excitotoxins for activation (Moreno-Flores and Wandosell 1999).

Clearly, this study has raised numerous mechanistic questions. However, irrespective of the exact molecular mechanisms associated with EphA receptor tyrosine

kinase function, these results represent the first pieces of evidence for the involvement of EphA receptor tyrosine kinases in memory in the mammalian brain. These findings open a new avenue into the functional analysis of this large receptor protein kinase family and perhaps imply novel targets for therapeutic intervention in human brain and behavioral disorders.

References:

- Abeliovich, A., Paylor, R., Chen, C., Krim, J., and Tonegawa, S. (1993) PKC γ mutant mice exhibit mild deficits in spatial and contextual learning. *Cell*. 75, 1263-1271.
- Andrade, J. (1996) Investigations of hypesthesia: Using anesthetics to explore relationships between consciousness, learning, and memory. *Consciousness and Cognition*. 5: 562-580.
- Aiba, A., Chen, C., Herrup, K., Rosenmund, C., Stevens, C., and Tonegawa, S. (1994) Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell*. 79, 365-375.
- Allison, D. W., Gelfand, V. I., Spector, I., and Craig, A. M. (1998) Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons, Differential attachment of NMDA versus AMPA receptors. *Journal of Neuroscience*. 18, 2423-2436.
- Avis, N. A., Burton, N. R., Berry, S. C., and Lodge, D. (1983) The disassociative anesthetics, ketamine and phencyclidine selectively reduce excitation of central mammalian neurons by N-methyl-D-aspartate. *British Journal of Pharmacology*. 79, 565-575.
- Baddeley, A. D. and Warrington, E. K. (1970) Amnesia and the distinction between long and short-term memory. *Journal of Verbal Learning Verbal Behavior*. 9, 176-89.
- Blanchard, R. J. and Blanchard, D. C. (1969) Crouching as an index of fear. *Journal of Comparative Physiology and Psychology*. 81, 281-290.
- Bliss, T. V. P. and Lomo, T. (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *Journal of Physiology*. 232, 331-356.

Bliss, T. V. P., and Collingridge, G. L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*. 361, 34-39.

Brandon, E. P., Idzerda, R. L., and McKnight, S. G. (1997) PKA isoforms, neural pathways, and behavior: making the connection. *Current Opinions in Neuroscience*. 7, 397-403.

Baudry, M. (1998) Synaptic plasticity and learning and memory 15 years of progress. *Neuroscience of Learning and Memory*. 70, 113-118.

Chamow, S. and Ashkenazi, A. (1996) Immunoadhesin: principles and applications. *Trends in Biotechnology*. 14, 52-60.

Collingridge, G. L. and Bliss, T. V. P. (1987) NMDA receptors, their role in long-term potentiation in the hippocampus. *Trends in Neuroscience*. 10, 288-293.

Cremer, H., Lange, R., Christoph, A., Plomann, M., Vopper, G., Roes, J., Brown, R., Baldwin, S., Kraemer, P., and Scheff, S. (1994) Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. *Nature*. 367, 455-9.

Davis, M., Walker, D. L., and Lee, Y. (1997) Amygdala and bed nucleus of the stria terminalis: differential roles of fear and anxiety measured with the acoustic startle reflex. *Research Society of London Biological Sciences*. 352, 1675-1687.

Davis, S., Butcher, S. P., and Morris, R. G. M. (1992) The NMDA receptors antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intercerebral concentrations comparable to those that block LTP in vitro. *Journal of Neuroscience*. 12, 21-34.

Edwards, F. A. (1995) LTP-a structural model to explain the inconsistencies. *Trends in Neuroscience*. 18, 250-255.

Eichenbaum, H., Stewart, C., and Morris, R. G. M. (1990) Hippocampal representation in place learning. *Journal of Neuroscience*. 10, 3531-3542.

Ellis, C., Kasmi, F., Ganju, P., Walls, E., Panayotou, G., and Reuth, A. D. (1996) A juxtamembrane autophosphorylation site in the Eph family receptor tyrosine kinase, Sek, mediates high affinity interaction with p59fyn. *Oncogene*. 12, 1727-1736.

Fanselow, M. S. (1986) Associative vs. topographical accounts of immediate shock freezing deficit in rats: implications for the response selection rules governing species-specific defensive reactions. *Learning and Motivation*. 17, 16-39.

Fifkova, E. and Delay, R. J. (1982) Cytoplasmic actin in neuronal processes as a possible mediator of synaptic plasticity. *Journal Cell Biology*. 95, 345-350.

Flanagan, J.G. and Vanderhaeghan, P. (1998) The ephrins and Eph receptors in neural development. *Annual Review Neuroscience*. 21, 309-45.

Flood, J. F., Baker, M. L., and Davis, J. L. (1990) Modulation of memory processing by glutamic acid receptor agonists and antagonists. *Brain Research*. 521, 197-202.

Friedman, G. C. and O'Leary, D. D. M. (1996) Eph receptor tyrosine kinases and their ligands in neural development. *Current Opinions in Neurobiology*. 6, 127- 33.

Gao, P-P., Yu, Y., Cerretti, P., Dreyfus, C., and Zhou, R. (1999) Ephrin-dependent growth and pruning of hippocampal axons. *Proceedings of the National Academy of Science*. 96, 4073-4077.

Gao, W-Q., Shinsky, N., Armanini, M., Moran, P., Zheng, L., and Mendoza-Ramirez, J. (1998) Regulation of hippocampal synaptic plasticity by the tyrosine kinase receptor REK7/EphA5, and its ligand, AL1/ephrinA5. *Molecular and Cellular Neuroscience*. 11, 247-59.

Geinisman, Y., de Toledo-Morrell, F., Heller, R. E., Rossi, M., and Parshall, R. F. (1993) Structural synaptic correlate of long-term potentiation, formation of axospinous synapses with multiple, completely partitioned transmission zones. *Hippocampus*. 3, 435-446.

Gerlai, R. (1996) Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? *Trends in Neuroscience*. 19, 177-181.

Gerlai, R., Shinsky, N., Caras, I., Gao, W-Q., Philips, H., and Winslow, J. (1997) The receptor tyrosine kinase REK7, and its ligand, AL1, are involved in neural plasticity in vivo: a behavioral and electrophysiological study in mice. *Society of Neuroscience Abstract*. 97:11.

Gerlai, R. (1998a) Contextual learning and cue association in fear conditioning in mice: a strain comparison and a lesion study. *Behavioural Brain Research*. 95, 191-203.

Gerlai, R., Cairns, B., Van Bruggenn, N., Moran, P., Shih A., Caras, I., Sauer, H., Philips, H., and Winslow, J. (1998) Protein targeting in the analysis of learning and memory: a potential alternative to gene targeting. *Experimental Brain Research*. Nov. 123, 24-35.

Gerlai, R. and Clayton, N. Analyzing hippocampal function in transgenic mice: an ethological perspective. (1999) *Trends in Neuroscience*. 22 (2), 47-51.

- Gerlai, R., Shinsky N., Shih A., Williams P., Winer J., and Armanini M. (1999) Regulation of learning by EphA receptors: a protein targeting study. *Journal of Neuroscience*. 19 (21), 9538-49.
- Grant, S. G. N., O'Dell, T. J., Karl, K. A., Stein, P. L., Soriano, P., and Kandel, E. R. (1992) Impaired long term potentiation, spatial learning, and hippocampal development in *fyn* mutant mice. *Science*. 258, 1903-1910.
- Grant, S. G. and Silva, A. (1994) Targeting learning. *Trends in Neuroscience*. 17, 71-5.
- Grant, S.G.N. (1996) Analysis of NMDA receptor mediated synaptic plasticity using gene targeting: roles of Fyn and FAK non-receptor tyrosine kinases. *Journal of Physiology*. 90, 337-8.
- Gustafsson, B. and Wigstrom, H. (1986) Hippocampal long-lasting potentiation produced by pairing single volleys and brief conditioning tetani evoked in separate afferents. *Journal of Neuroscience*. 6, 1575-82.
- Hayashi, K., Ishikawa, R., Ye, L., He, X. L., Takata, K., and Kohama, K. (1996) Modulatory role of debrin on the cytoskeleton within dendritic spines in the rat cerebral cortex. *Journal of Neuroscience*. 16, 7161-70.
- Hebb, D. O. (1949) The organization of behavior: a neuropsychological theory. New York. Wiley.
- Huang, Y., Li, X., and Kandel, E. R. (1994) cAMP contributes to mossy fiber LTP by initiating both covalently mediated early phase and macromolecular synthesis-dependent late phase. *Cell*. 79, 69-79.
- Impey, S., Mark, M., Villacres., E. C., Poser, S., Chavkin, C., and Storm, D., R. (1996) Induction of CRE-mediated gene expression by stimuli that generate long lasting LTP in the area of the CA1 of the hippocampus. *Neuron*. 16, 973-982.
- Izquierdo, I. and Medina, J. (1984) Role of amygdala, hippocampus and entorhinal cortex in memory consolidation and expression. *Brazilian Journal of Medical and Biological Research*. 26, 573-89
- Kartsounis, F. Rudge, P., and Stevens, J. M. (1995) Bilateral lesions of the CA1 and CA2 fields of the hippocampus are sufficient to cause a severe amnesic syndrome in humans. *Journal of Neurology, Neurosurgery and Psychiatry*. 59, 95-98.
- Kandel, E. R. and Schwartz J. H. (1982) Molecular biology of learning: modulation of transmitter release. *Science*. 218, 433-443.

- Kandel, E. R. (1989) Genes, nerve cells, and remembrance of things past. *Journal Neuropsychiatry*. 1, 103-125.
- Kesner, R. P. and Novak, J. M. (1982) Serial position curve in rats: role of the dorsal hippocampus. *Science*. 218, 173-75.
- Kim, J. J. and Fanselow, M. S. (1992) Modality-specific retrograde amnesia of fear. *Science*. 256, 275-677.
- Kovalev, G. V., Sazin, V. A., and Kuleshove, I. P. (1990) Pharmacological correction of ketamine-impaired spatial memory of rats. *Bulletin of Experimental Biology and Medicine*. 110, 618-20.
- Landis, D. M. D. and Reese, T. S. (1983) Cytoplasmic organization in cerebellar dendrite spines. *Journal of Cell Biology*. 97, 1169-78.
- Logue, S. F., Paylor, R., and Wehner, J. M. (1997) Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behavioral Neuroscience*. 111, 104-113.
- LeDoux, J. E. (1995) Emotion: clues from the brain. *Annual Review Psychology*. 46, 209-264.
- Lorenzini, C. A., Baldi, E., Bucherelli, C., Sacchetti, B., and Tassoni, G. (1996) Role of dorsal hippocampus in acquisition, consolidation, and retrieval of rat's passive avoidance response: tetrodotoxin functional inactivation study. *Brain Research*. 730, 32-9.
- Lu, Y. M., Roder, J. C. Davidow J., and Slater, M. W. (1998) Src activation in the activation of long term potentiation in CA1 hippocampal neurons. *Science*. 279, 1363-7.
- Meima, L., Kljavin, I. J., Shih, A., Winslow, J. W., and Caras, I. W. (1997) AL-1-induced growth cone collapse of rat cortical neurons is correlated REK7 expression and rearrangement of the actin cytoskeleton. *European Journal of Neuroscience*. 9, 177-88.
- Mileusnic, R., Rose, S. P. R., and Tillson, P. (1980) Passive avoidance learning results in region-specific changes in concentration of and incorporation into colchicines-binding proteins in the chick forebrain. *Journal of Neurochemistry*. 34, 1007-15.
- Milner, B., Squire, R., and Kandel, E. (1998) Cognitive neuroscience and the study of memory. *Neuron*. 20, 445-468.
- Moreno-Flores, M. T. and Wandosell, F. (1999) Up-regulation of Eph tyrosine kinase receptors after excitotoxic injury in adult hippocampus. *Neuroscience*. 91, 193-201.

- Morris, R. G. M., Anderson, E., Lynch, G. S., and Baudry, M. (1986) Selective impairment of learning and blockade of long-term potentiation by a N-methyl-D-aspartate receptor antagonist, AP5. *Nature*. 319, 774-6.
- Nicoll, R. A., Kauser, J. A., and Malenka, R. C. (1988) The current excitement in long-term potentiation. *Neuron*. 1, 97-103.
- Noldus, L. P. J. (1991) The Observer: a software system for collection and analysis of observational data. *Behavioral Research Methods*. 23, 415-29.
- Orioli, D. and Klein, R. (1997) The eph receptor family: axonal guidance by contact repulsion. *Trends in Genetics*. 13, 354-9.
- Oshima, E. and Richards, C. D. (1998) An in vitro investigation of the action of ketamine on excitatory synaptic transmission in the hippocampus of the guinea pig. *European Journal of Pharmacology*. 148, 25-33.
- Paylor, R., Tracy, R., Wehner J., and Rudy, J. W. (1994) DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning. *Behavioral Neuroscience*. 108, 1-8.
- Pasquale, E. B. (1997) The Eph family of receptors. *Current Opinions in Cell Biology*. 9, 608-15.
- Phillips, R. G. and LeDoux, J. E. (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear but not auditory fear conditioning. *Behavioral Neuroscience*. 106, 274-85.
- Plumb, D. C. (1995) Veterinary drug handbook, second ed. Ames: Iowa State University Press. 722.
- Quinlan, E. M., and Halpain S. (1996) Postsynaptic mechanisms for bi-directional control of MAP2 phosphorylation by glutamate receptors. *Neuron*. 16, 357- 68.
- Rolls, E. T. and Treves, A. (1998) Neural networks and brain function. Oxford University Press. 7-135.
- Rose, P. R. S. (1993) Synaptic plasticity, learning and memory. In: Baudry M., Thompson R. F., and Davis J. L. editors. Synaptic plasticity. Cambridge, USA: MIT Press. 209-29.
- Saha, N., Chugh, Y., Sankaranarayanan, A., and Datta, H. Interactions of verapamil and diltiazem with ketamine: effects on memory and sleep time in mice. *Methods and Findings in Experimental Clinical Pharmacology*. 12, 507- 11.

- Sakimura, K., Kutsuwada, T., Ito, I., Manabe, T., Takayama, C., Kushiya, E., Yagi, T., Aizawa, S., Inoue, Y., and Sugiyama H. (1995) Reduced hippocampal LTP and spatial learning in mice lacking NMDA receptor ϵ 1 subunit. *Nature*. 373, 151-5.
- Scholey, A., Bullock, S., and Rose, P. R. S. (1992) Passive avoidance learning in the young chick results in time- and locus-specific elevations of alpha-Tubulin immunoreactivity. *Neurochemistry International*. 21, 343-50.
- Squire, L. R. (1986) Mechanisms of memory. *Science*. 232: 1612-1619.
- Squire, L. R. and Cave, C. B. (1991) The hippocampus, memory, and space. *Hippocampus*. 1, 329-40
- Squire, L. R. and Zola- Morgan, S. (1991) The medial temporal lobe memory system. *Science*. 253, 1380-86.
- Squire, L. R. (1992) Declarative and non-declarative memory: multiple brain systems supporting learning and memory. *Journal of Cognitive Neuroscience*. 4, 323-43.
- Squire, L. R., Knowlton, B., and Musen, G. (1993) The structure and organization of memory. *Annual Review Psychology*. 44, 453-95.
- Squire, L. R. and Knowlton, B. (1995) Memory, hippocampus, and brain systems. Ch. 53, pp. 825-837 in *The Cognitive Neuroscience*, ed. M.S. Gazzangia. MIT press: Cambridge, Mass.
- Ter-Margarian, A. and Djamgoz, M. B. (1992) Cytochalasin inhibits light-dependent synaptic plasticity of horizontal cells in teleretina. *Neuroscience Letters*. 147, 131-5.
- Tessier-Lavigne, M. (1995) Eph receptor tyrosine kinase axon repulsion, and the development of topographic maps. *Cell*. 82, 345-8.
- Tonegawa, S., Li Y., Erzurumlu, R. S., Jhaveri, S., Chen, C., and Goda, Y. (1995) Knockout technology for the analysis of learning and memory, and neural development. *Progress in Brain Research*. 106, 3-14.
- Torres, R., Firestein B. L., Dong, H., Staudinger, J., Olson, E. N., and Huganir, R. L. (1998) PDZ proteins bind, cluster, and synaptically colocalized with Eph receptors and their ephrinA ligands. *Neuron*. 21, 1453-63.
- Tsein, R. W. and Malinow, R. (1990) Long term potentiation: presynaptic enhancement following postsynaptic activation of Ca-dependent protein kinases. *Cold Spring Symposium for Quantitative Biology*. 55, 147-159.

- Tulving, E., Hayman, C. A. G., and MacDonald, C. (1991) Long-lasting perceptual priming and semantic learning in amnesia: a case experiment. *Journal of Experimental Psychiatry Learning Memory Cognition*. 17, 595-617.
- Ungerer, A., Mathis, C., Melan, C., and de Berry, J. (1991) The NMDA receptor antagonists, CCP and g-L-glutamyl-L-aspartate, selectivity block post-training improvement of performance in a Y-maze avoidance learning task. *Brain Research*. 549, 59-65.
- Villacres, E. C., Wong, S. T., Chavkin, C., and Storm, D. R. (1998) Type I Adenylyl-cyclase mutant mice have impaired long term potentiation. *Journal of Neuroscience*. 18(9), 3186-3194.
- Walker, D. L. and Gold, P. E. (1997) NMDA receptors: substrates or modulators of memory formation. *Behavioural Brain Science*. 20: 634.
- Wang, X. H., Zheng, J. Q., and Poo, M. M. (1996) Effect of cytochalasin treatment on the short-term synaptic plasticity at developing neuromuscular junctions in frogs. *Journal of Physiology*. 491: 187-95.
- Westrum, L. E., Jones D. H., Gray, E. G., and Barron, J. (1980) Microtubules, dendritic spines and spine apparatus. *Cell Tissue Research*. 208, 71-181.
- Wilson, M. and Tonegawa, S. (1997) Synaptic plasticity, place cells and spatial memory: study with second generation knockouts. *Trends in Neuroscience*. 20 (3): 102- 106.
- Winer, J., Shinsky, N., Gerlai, R., and Williams, P. M. (1999) Application of real-time RT-PCR for quantification of gene expression. In: Crusio W. E., and Gerlai R. *Recombinant DNA techniques in behavioral neuroscience*. Amsterdam: Elsevier. 237-48.
- Winslow, J. W., Moran, P., Valverde, J., Shih, A., Yaun, J. Q., and Wong, S. C. (1995) Cloning of Al-1, a ligand for an eph related tyrosine kinase receptor involved in axon bundle formation. *Neuron*. 14, 973-81.
- Zhou, R. (1998) The Eph family receptors and ligands. *Pharmacology Therapeutics*. 77, 151-81.
- Zola-Morgan, S. and Squire, L. R. (1984) Preserved learning in monkeys with medial temporal lesions: sparing of motor and cognitive skills. *Journal of Neuroscience*. 4, 1072-85.

FIGURE LEGENDS

Figure 1. Demonstration of how recombinant technology used to create transgenic mice may create problems with flanking alleles of different strains of mice. The red band on the chromosome represents the region of interest. Most embryonic cell lines used to create transgenic mice are from the 129 strain of mouse. This strain of mice is known to perform poorly in spatial behavioral tasks. These 129 mice are then crossbred with the C57/BL6 strain of mice for genomic stability.

Figure 2. An immunoadhesin molecule.

Figure 3. Anatomic structure of a human hippocampus.

Figure 4. Pathway that stimuli travels, forward and reverse, to and from the neocortex.

Figure 5. Surgical anesthesia causes significant retrograde amnesia in fear conditioning. Relative duration of freezing of C5BL/6 mice is shown for 1 minute. recording intervals. Error bars represent S. E. M. Sample sizes (n) are also indicated. The timing of delivery of the three tone signals is shown as solid lines (training and cue test). During training the tone signals were paired with shock (arrows), a, acepromazine; x, xylazine; k, ketamine. A: performance at training. No significant differences were found between the five groups. Ninety minutes after training, mice were injected with different agents as

indicated. B: context test performed seven days after training. Surgical anesthesia (a + x + k) administered post-training caused significant impairment. The other four groups were not different from each other. C: cue test performed seven days after training. No differences were found between any groups until the last interval.

Figure 6. Surgical anesthesia with intrahippocampal phosphate buffered saline (PBS) infusion causes significant retrograde amnesia in fear conditioning. Relative duration of freezing of C57BL/6 mice is shown for 1 minute recording intervals. Error bars represent S.E.M. sample sizes (n) are also indicated. The timing of delivery of three tone signals is shown as solid lines (training and cue test). During training, the tone signals were paired with shock (arrows). Training. No significant differences were found between the two groups. Ninety minutes after training, a subset of mice underwent surgical anesthesia and started to receive intrahippocampal infusion of PBS. The rest of the mice (non-operated) served as control and received no anesthesia or infusion. B: Context test performed seven days after training. PBS infused mice were significantly impaired compared to non-operated control. C: Cue test performed seven days after training. No differences were found between the groups until the last interval.

Figure 7. Intra hippocampal ephrinA5-IgG infusion significantly ameliorates surgical anesthesia induced retrograde amnesia in fear conditioning. Relative duration of freezing of C57BL/6 mice is shown for 1 minute recording intervals. Error bars represent S.E.M. Sample sizes (n) are also indicated. The timing of delivery of the three tone signals is

shown as solid lines (training and cue test). During training, the tone signals were paired with shock (arrows). A: Training. No significant differences were found. Ninety minutes after training all mice underwent surgical anesthesia and bilateral intrahippocampal infusion of either CD4-IgG or ephrinA5-IgG. Mice were tested seven days later in the context (B) and the cue test (C). EphrinA5-IgG infused mice exhibited improved freezing response to context compared to control but cued task performance was not altered.

Figure 8. Intrahippocampal ephrinA5-IgG infusion significantly ameliorated surgical anesthesia induced retrograde amnesia in fear conditioning and the effects last for 17 days. All mice were trained and treated as described in figure 6 (also see methods) except that they were tested in the context and cue test 17 days after training. Relative duration of freezing of C57BL/6 mice is shown for 1 minute recording intervals. Error bars represent S.E.M. Sample sizes (n) are also indicated. The timing of delivery of three tone signals is shown as solid lines (training and cue test). During training the tone signals were paired with shock (arrows). A: Training. No differences were seen. B: Context test. Mice infused with ephrinA5-IgG exhibited significantly improved freezing performance compared to those infused with control CD4-IgG. C: Cue test. No differences were found.

Figure 1

Figure 2

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8