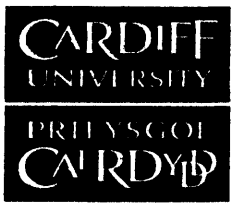

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An Analysis of Motivational Processes in Mice with a Deletion of the GluR-1 AMPA Receptor Subunit.

A Thesis Submitted for the Degree of Doctor of Philosophy at Cardiff University

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December 2005

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DECLARATION

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PUBLICATIONS

Johnson, A.W., Bannerman, D., Rawlins, N., Sprengel, R., & Good, M (2005). Impaired outcome-specific devaluation of instrumental responding in mice with a targeted deletion of the AMPA receptor GluR-1 subunit. *Journal of Neuroscience. 25(9)*: 2359-65.

CONFERENCES & PRESENTATIONS

Johnson, A.W. An examination of motivational processing in gene-targeted mice lacking the GluR-1 subunit of the AMPA receptor. Presented at the Molecular and Cellular Cognition Society 4th Annual Meeting, October 2005, Washington, D.C.

Johnson, A.W. & Good, M. Disruption of reinforcer representations in GluR-1$^{-/-}$ mice. Presented at the 9th Annual Associative Learning Symposium, March 2005, Gregynog, Wales.

Johnson, A.W., Bannerman, D., Rawlins, N., Sprengel, R. & Good, M. Mice with a targeted deletion of the GluR-1 subunit of the AMPA receptor show impaired processing of the affective/motivational value of a primary reinforcer. Presented at Neuroscience 34th Annual Meeting, October 2004, San Diego, California.

Johnson, A.W., Bannerman, D., Rawlins, N., Sprengel., R. & Good, M. Impaired contextual and cued fear conditioning in mice with a targeted deletion of the GluR-A subunit. Presented at Neuroscience 34th Annual Meeting, October 2004, San Diego, California.

Johnson, A.W. & Good, M. Fear conditioning impairments in GluR-1$^{-/-}$ mice. Presented at the 8th Annual Associative Learning Symposium, April 2004, Gregynog, Wales.

ACKNOWLEDGEMENTS

The undertaking of writing this thesis, and the practicality of its completion are due to the help and encouragement of a number of colleagues, friends and family members- without which this task would not have reached fruition.

Firstly, I express my sincere gratitude to my supervisor and mentor Dr. Mark Good. For his guidance, patience and humor have influenced both this thesis and my academic career.

Secondly, I thank all my work colleagues and friends, Anna, Duncan, Guillaume, Kate, Laura, Pete, Peter, and Steve. Thanks for a memorable three years, including winning at football, loosing at pool and making friends with the locals.

Finally, I would like to pay tribute to my family, my mother, Giusi, for proving me with the enthusiasm necessary to seek my goals, my father, Victor, for teaching me the power of commitment, and to both of them for being excellent parents and role models. Also, I must thank my sister, Lidia, for providing me with that needed dose of reality and helping me realise what 'being family' really means. Last, but not least, I must thank my partner, Melissa, without your help through my darkest times I simply would not have been able to continue through and accomplish what seemed impossible.

For Sarah

Short Abstract

The experiments in this thesis evaluated the proposal that GluR-1$^{-/-}$ mice display impairments in affective and motivational processes (Mead & Stephens, 2003a). The introductory experiments examined sensorimotor and affective aspects of behaviour in GluR-1$^{-/-}$ and wild-type control mice (Chapter 2). These studies attempted to evaluate any performance-based behavioural impairment which may have interfered with learning. Chapter 3 assessed BLA-dependent learning on a Pavlovian fear conditioning paradigm (e.g., LeDoux, Sakaguchi & Reis, 1986). The simple nature of this learning task, and the large body of evidence implicating the amygdala in this form of learning provided an opportunity to examine the influence of the GluR-1 mutation on emotional learning (Maren, 2000a; Cardinal et al., 2002). Chapters 4 and 5 made use of separate Pavlovian and instrumental preparations which characterised different affective and sensory-specific associatively activated outcome representations (Blundell, Hall & Killcross, 2001; Balleine, Dickinson & Killcross, 2003; Corbit & Balleine, 2005). The results are discussed in respect to a failure of GluR-1$^{-/-}$ mice to attribute affective and motivational incentive value to the sensory-specific properties of a US; an account which furthers that proposed by Mead and Stephens.

Long Abstract

The development of gene-targeted knock-out technology allows for the manipulation of the genetic makeup of an organism by specific targeting of selected genes. This contemporary research tool allows one to investigate the neurobiological mechanisms underlying *in-vivo* behavioural change (Capecchi, 1989). The experiments in this thesis evaluated the proposal that mice with a targeted deletion of the GluR-1 AMPA receptor subunit display impairments in affective and motivational processes closely allied to functions supported by the basolateral amygdala (Mead & Stephens, 2003a). The activation of these receptors accounts for basal excitatory synaptic transmission and many forms of synaptic plasticity such as long-term potentiation (Zamanillo et al., 1999), which is thought to underlie learning and memory. Moreover, they are also potential targets for therapies for CNS disorders such as epilepsy, Alzheimer's disease and drug addiction.

The introductory experiments examined sensorimotor and affective aspects of behaviour in GluR-1 mutant mice. (Chapter 2). These studies attempted to evaluate whether deletion of the GluR-1 subunit would result in any non-mnemonic impairments. Chapter 3 assessed BLA-dependent learning on a Pavlovian fear conditioning paradigm (e.g., LeDoux, Sakaguchi & Reis, 1986). The simple nature of this learning task, and the large body of evidence implicating the amygdala in this form of learning provided an opportunity to examine the influence of the GluR-1 mutation on emotional learning (Maren, 2000a; Cardinal et al., 2002). Chapters 4 and 5 made use of Pavlovian and instrumental preparations to characterise affective and sensory-specific associatively

activated outcome representations in mutant mice; using paradigms which have previously been shown to be sensitive to BLA damage in rats (Blundell, Hall & Killcross, 2001; Balleine, Dickinson & Killcross, 2003; Corbit & Balleine, 2005). Whilst in agreement with motivational disturbances following deletion of the GluR-1 receptor (Mead & Stephens, 2003a) these results suggest a more specific interpretation of the GluR-1 syndrome indicative of a failure to attribute motivational incentive value to the sensory-specific elements of an outcome.

Chapter 1.

1.1 *An Overview of the General Introduction.*

The ability to learn and store information is thought to occur via changes in synaptic efficacy (Collingridge & Bliss, 1993; Shors & Matzel, 1997; Nicoll & Malenka, 1999). Long-term potentiation (LTP) has been posited as a model of the cellular process that underlies activity-dependent changes in synaptic efficacy (Bliss & Lomø, 1973). Gene-targeted mice provide a powerful means of elucidating gene function *in vivo* and the relationship between LTP mechanisms and memory (Stephens, Mead & Ripley, 2002; Austin et al., 2004). The main aim of this thesis is to evaluate the hypothesis proposed by Mead and Stephens (2003a) that mice with a targeted deletion of the AMPA receptor subtype GluR-1 show impaired learning mediated by the amygdala.

The purpose of this introduction is to provide a critique of mechanisms which underlie synaptic plasticity in learning and memory. I will begin by summarising the key developments which have led to the emergence of a cellular and molecular neuroscience, including the discovery of LTP and its cellular mechanisms (Bliss & Lomø, 1973) and the development of genetically altered mice which allow one to map specific genes to both synaptic plasticity and animal behaviour (Kandel & Squire, 2000). Specifically, this review will focus on the role of NMDA and AMPA receptors in plasticity and their role in learning and memory. In later sections, I will focus on the current debate regarding the behavioural phenotype resulting from a targeted deletion of an AMPA receptor subtype, GluR-1 (Zamanillo et al., 1999), and will concentrate on the hypothesis that GluR-1 knock-out (KO) mice show impairments in motivational learning mediated by the amygdala (Mead & Stephens, 2003a).

1.2 *Emergence of a Cellular and Molecular Neuroscience: The Development of LTP as a Model of Learning and Memory.*

Modern day neuroscience was founded on two fundamental advances: the *neuron doctrine* and the *ionic hypothesis*. The neuron doctrine was established by the Spanish anatomist Santiago Ramon y Cajal (1906), who provided evidence that the brain is composed of discrete neuronal cells, and that these neurons likely serve as separate signalling units. In the same period, the British physiologist Sir Charles Sherrington proposed that contact between neurons occurs at specific sites, called synapses (Sherrington, 1897).

By the 1930s, Hodkin (1937) discovered that the *action potential* (the brief electrical impulse that provides the basis for conduction of information along the axon of a neuron) gives rise to local current flow on its advancing edge. That is, in its stable state, a neuron has a *resting potential* which develops into a action potential (following either excitatory or inhibitory postsynaptic potentials) transmitting information from the cell body of the neuron to its terminal buttons (Carlson, 2001). In the late 1940s a breakthrough occurred (Hodkin & Huckley, 1939; Hodkin, Huxley & Katz, 1952) whereby the resting potential was explained in terms of movement of three specific ions: potassium (K^+), sodium (Na^+) and chloride (Cl^-), each moving through the ion channels in the axonal membrane. Accordingly, the ionic hypothesis was born which unified a large body of descriptive data and offered the first realistic premise that the nervous system could be explained in terms of physicochemical principles common to all cell biology.

During the 1960s and 1970s neuroscientists identified many amino acids as chemical transmitters, including glutamate, GABA, serotonin, dopamine and

norepinephrine (Kandel & Squire, 2000). By the late 1980s it became clear that synaptic actions were mediated by two class of receptors: ionotropic and metabotropic. Ionotropic receptors contain a binding site for a neurotransmitter and an ion channel that directly opens when a molecule of the neurotransmitter exposes the binding site, allowing ions (e.g., Na^+ and K^+) to flow into the cell. In contrast, metabotropic receptors (so-called due to their expansion of metabolic energy; Carlson, 2001) contain a binding site for a neurotransmitter. However, here the binding of the neurotransmitter initiates intracellular metabolic events and leads only *indirectly* by way of G-protein-coupled 'second messengers' to the gating of ion channels (Lefkowitx, 2000).

As previously mentioned, networks of neurons work not in a process of combined unitization (Cajal, 1906) but rather communicate with one another at specialised synaptic junctions (Sherrington, 1897). These ideas were refined in the late 1940s by Donald Hebb, who proposed a *coincidence-detection* rule in which the synapse linking two cells strengthened if the pre- and postsynaptic cells became co-active at the same time. In 1973, a long-lasting synaptic plasticity of the kind postulated by Hebb was discovered in the hippocampus (Bliss & Lomø, 1973), a key brain structure involved in learning and memory processes in both human and non-human mammals (Correll & Scoville, 1965, Squire, 1992; Good, 2002). Bliss and Lomø stimulated axons in the perforant path (the main input pathway to the dentate gyrus from the entorhinal cortex; Carlson, 2001) of the rabbit, which resulted in a long-term increase in the magnitude of excitatory postsynaptic potentials (EPSPs). The tetanisation led to an increase in synaptic efficacy in the perforant pathway such that later stimulation created larger EPSPs in the granule cells of the dentate gyrus, a phenomenon known as *long-term potentiation* (LTP; Bliss & Lomø, 1973). Although

persistent, the increase in synaptic strength seen in LTP can be reversed by differing patterns of neuronal activity; a process which leads to *long-term depression* (LTD; Lynch, Gribkoff & Deadwyler, 1976).

1.31 *Physiological Classifications of LTP.*

Synaptic potentiation can be categorised on the basis of whether or not its induction requires the *N*-methyl-D-aspartate (NMDA) glutamate receptor. Three main categories of NMDA receptor-dependent synaptic plasticity [which is blocked in the presence of NMDA antagonists such as 2-amino-5-phosphonopentanoate (AP5; Collingridge, Kehl & McLennan, 1983)] have been characterised on the basis of the rate of decay of the excitatory postsynaptic potential (EPSP): (1) Short-term potentiation which occurs for only 30-60 min (Lovinger, Wong, Murakami & Routtenberg, 1987); (2) Early LTP which occurs for a duration less than 3-4 h (E-LTP; also known as LTP1 & LTP2) and is subserved by persistent kinase activation; and (3) late LTP (L-LTP; also known as LTP3) which has a time constant of several days and is only expressed if the animal is unanaesthetised at the time of induction (Jeffery, Abraham, Dragunow & Mason, 1990) and is dependent on protein synthesis and altered gene expression (Dineley et al., 2001).

LTP occurs in many pathways, not just in the dentate gyrus of the hippocampus where it was first observed (Bliss & Lomø, 1973), but also in the prefrontal cortex, piriform cortex, entorhinal cortex, motor cortex, visual cortex, thalamus and amygdala (Gerren & Weinberger, 1983; Clugnet & LeDoux, 1990; Aroniadou & Tyler, 1991; Baranyi, Szente & Woody, 1991; Lynch, Larson, Staubli & Granger, 1991). It should be noted, however, that the most commonly studied form of

persistent modification of synaptic transmission is NMDA receptor dependent LTP at glutamatergic synapses within the hippocampus (Malenka & Nicoll, 1999). Due to the vast array of research examining this form of LTP, and the functional implication of the hippocampus in learning and memory (Scoville & Milner, 1957; Squire, 1992; Good, 2002) the following section will focus predominantly on examination of hippocampal NMDA-dependent LTP.

1.32 *Induction of NMDA Dependent LTP.*

For the purpose of this review, induction is defined as the physiological mechanisms which occur up to and including the influx of Ca^{2+} into the postsynaptic cell (Malenka & Nicoll, 1999). It is generally regarded that the triggering of LTP requires the activation of glutamatergic neurotransmitters located on the post-synaptic cell (Malenka & Nicoll, 1999). If a single stimulus is applied to the Schaffer collateral-commissural pathway (low-frequency transmission stimulated from CA3-CA1 hippocampal regions; Bliss & Collingridge, 1993), an EPSP develops that is mediated predominantly by glutamatergic neurotransmission. The evoked EPSP can be blocked by the antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; Davies & Collingridge, 1989), which is specific to α-amino-3-hydroxy-5-methy-4-isoxazole-propionic acid (AMPA) receptors. AMPA receptors are heteromeric assembles composed of up to four subunits GluR1-4 (Gasic & Hollman, 1992; Hollman & Heinemann, 1994). When the Schaffer collateral-commissural pathway is stimulated it also activates inhibitory GABAergic interneurons, via glutamatergic synapses (Davies & Collingridge, 1989). Two GABAergic receptors have been identified, $GABA_A$, which is ionotropic and controls a Cl^- channel, and $GABA_B$, which is

metabotropic and controls a K^+ channel (Thompson, 2000). Low-frequency stimulation leads to a biphasic inhibitory postsynaptic potential (IPSP) which restrains the EPSP (Bliss & Collingridge, 1993). The initial segment of the IPSP is caused independently by the activation of $GABA_A$ receptors and is then supplemented by the activation of $GABA_B$ receptors (Mott & Lewis, 1992). The NMDA receptor contributes relatively little to the synaptic response evoked during low frequency transmission (Collingridge, Herron & Lester, 1988). NMDA receptors comprise of assemblies of NR1 and NR2 subunits. NR2 subunits are composed of one of four separate sub-types (NR2A-D; e.g., Collingridge, Issac & Wang, 2004). During low frequency transmission NMDA receptors are inactive, as they are blocked by magnesium ions (Mg^{2+}) which prevent Ca^{2+} from entering the cell. The IPSP that develops hyperpolarises the post-synaptic neuron which enhances the blocking of NMDA receptor channels by Mg^{2+} (Collingridge et al., 1988).

The contribution of NMDA receptors to synaptic transmission alters significantly in response to high-frequency input (Bliss & Lynch, 1988; Gustafsson & Wigstrom, 1988; Martin, Grimwood, Morris 2001). During high-frequency stimulation the post-synaptic membrane becomes depolarised, which removes the Mg^{2+} ion from the NMDA ion channel. Consequently, glutamate activates the NMDA receptor and results in the influx of Na^{2+} and Ca^{2+} into the postsynatpic cell (10:1 ratio of Na^{2+}:Ca^{2+}; Bliss & Collingridge, 1993). The NMDA receptor is believed to be a critical substrate for the induction of LTP, and this view is supported by studies using NMDA antagonists which can act (a) on the receptor (e.g., AP5; Harris & Cotman, 1986), (b) in the channel (e.g., MK-801, Coan, Saywood & Collingridge, 1989), or (c) at the allosteric glycine site (e.g., 7-chlorokynurenic acid; Bashir, Tam &

Collingridge, 1990) all of which block the induction of LTP in the hippocampal Schaffer collateral-commissural pathway.

The above section implies that activation of NMDA receptors are an essential component for the induction of LTP. Furthermore, during high-frequency transmission there is considerably less GABA released per impulse which leads to a transfer in the balance from inhibition to excitation (Davies, Starkey, Pozza & Collingridge, 1991). The reduction in inhibition allows for greater activation of the NMDA receptor system, which further contributes to the state of depolarisation, consequently reducing the level of Mg^{2+} block. The long duration of synaptic conductance means that NMDA receptor-mediated EPSPs summate very efficiently and effectively during high-frequency stimulation (Bliss & Lynch, 1988; Gustafsson & Wigstrom, 1988; Bliss & Collingridge, 1993; Larkman & Jack; 1995; Martin, Grimwood & Morris, 2001).

Following depolarisation, the subsequent rapid influx of postsynaptic Ca^{2+} is the critical trigger for LTP (Teyler & DiScenna, 1987; Nicoll, Kauer & Malenka, 1988; Nicoll & Malenka; 1995). For example, preventing the rise in postsynaptic Ca^{2+} with Ca^{2+} chelators, [molecules which bind with Ca^{2+} making it insoluble (e.g., EGTA; Lynch, Larson, Kelso, Barrionuevo & Schottler, 1983)] blocks LTP, whereas directly raising the amount of postsynaptic Ca^{2+} by photolysis (light sensitive activation) of caged Ca^{2+} can mimic LTP (e.g., Yang, Tang & Zucker, 1999). Advances in calcium imaging technology have led to the ability to perform combined electrophysiology-imaging experiments in individual neurons (Koester and Sakmann, 1998). Imaging studies have demonstrated that increases in Ca^{2+} occur within the dendritic spine following NMDA receptor activation (Regher & Tank, 1990; Yuste & Denk, 1995; Ismailov, Kalikulov, Inoue, & Friedlander, 2004). Research suggests that

a short-lasting (1- to 3-s) threshold level of Ca^{2+} must be reached in order to trigger LTP (Malenka, Lancaster & Zucker, 1992; Malenka & Nicoll, 1999).

Ca^{2+} influx results in the triggering of signal transduction mechanisms via calcium-sensitive kinases. Several different Ca^{2+} sensitive enzymes have been proposed to play a role in converting the induction signal (entry of Ca^{2+} through the NMDA channel) into persistent modifications of synaptic strength. These include the protease calpain (Oliver, Baudry & Lynch, 1989), phosphatases such as calcineurin (Halpain & Greengard, 1990), and in particular protein kinases such as protein kinase C, A and type II calcium/calamodium-dependent kinases (CaMKII; Malenka et al., 1989).

In summary, the available evidence indicates that under low frequency transmission (or during a stable action potential) normal synaptic functioning is governed by both AMPA receptors and $GABA_A$ interneurons, each producing biphasic EPSPs and IPSPs respectively (Gustafsson & Wigstrom, 1988). However, following high-frequency stimulation, NMDA receptors are activated by simultaneous depolarisation of the postsynaptic cell and the action of glutamate. The subsequent Ca^{2+} influx activates several Ca^{2+} sensitive enzymes. The discussion will now turn to the mechanisms which underlie the expression of changes in synaptic efficacy following LTP induction.

1.33 *Expression of LTP: Signal Transduction Mechanisms and AMPA Receptors.*

Perhaps the most intriguing property of CaMKII, which make it such a viable candidate in the mechanism of LTP, is that besides acting on other molecules, CaMKII can act on itself by attaching a phosphate group to a particular location- the

286^{th} amino acid, a threonine molecule (Thr^{286}; Lisman, Malenka, Nicoll & Malinow, 1997). This process is known as *autophosphorylation*, and once accomplished, it renders CaMKII activity independent of Ca^{2+} (Lisman et al., 1997). Autophosphorylation has been implicated as a necessary component in order for the induction of LTP to take place (Giese, Fedorov, Filipkowski & Silva, 1998). Thus, to determine whether autophosphorylation of CaMKII was required for LTP, Giese et al., (1998) substituted Thr^{286} with alanine (a nonessential amino acid used to build proteins). This point mutation was introduced into the αCaMKII gene that blocked the autophosphorylation of Thr^{286} of the kinase without affecting its CaM-dependent activity. The resulting mutant mice had no NMDA receptor-dependent LTP in the hippocampal CA1 area, suggesting that the autophosphorylation of Thr^{286} appears necessary for LTP. However, as yet the effects of altering the background strain have not been reported.

A further important piece of evidence implicating CaMKII in LTP is that it can directly phosphorylate AMPA receptors (Barria, Muller, Derkach, Griffith & Soderling, 1997). Here, phosphorylation of AMPA receptors increased the sensitivity of these receptors to glutamate; that is, AMPA phosphorylation appeared to be catalysed by Ca^{2+}. Furthermore, its phosphrous-32 peptide map (a radioactive isotope used to detail peptide structure; ^{32}P) was the same as that of GluR-1, indicating that this subunit was the site of phosphorylation on AMPA receptors. Thus, GluR-1 can be phosphorylated on Ser^{831} (located at the intracellular C-terminus; Dingledine, Borges, Bowie & Traynelis, 1999) by CaMKII (Barria et al., 1997), and the phosphorylation of GluR-1 by CaMKII increases the single-channel conductance of homeric GluR-1 AMPA receptors (Derkach, Barria & Soderling, 1999). Moreover, since an increase in AMPA single-channel conductance also occurs during LTP (Benke, Luthi, Isaac &

Collingridge, 1998), one potential mechanism underlying LTP expression is CaMKII phosphorylation of the GluR-1 subunit (Malenka & Nicoll, 1999). Support for this idea is provided by the finding that mice with a targeted deletion of the GluR-1 subunit, showed no LTP in the hippocampal CA1 region (Zamanillo et al., 1999; Malenka & Nicoll, 1999).

Several other protein kinases have been implicated in the expression of LTP (Malenka & Nicoll, 1999). For instance, protein kinase C (PKC) activity is increased following hippocampal LTP (Akers, Lovinger, Colley, Linden & Routtenburg, 1986); although, PKC activation is important for the persistence of LTP rather than for the initial potentiation (Colley, Sheu & Routtenberg, 1990). Activation of PKC [via forskolin or phorbol-12,13-di-butryate (PDBu) stimulation] causes a large increase in miniature excitatory postsynaptic potentials (mEPSP's; Carroll, Nicoll & Malenka, 1998). However, only PDBu caused large increases in mEPSP amplitude. This result was consistent with previous findings, in that direct application of the catalytic component of PKC was found to enhance mEPSP amplitude (Wang, Salter& McDonald, 1994). Thus, it has been suggested that PKC plays an analogous role in the persistence of LTP, as that of CaMKII. PKC inhibitors block LTP expression (Colley et al., 1990) and PKC application can enhance mEPSP's (Carroll et al., 1998). In addition, both CaMKII and PKC phosphorylate the GluR-1 AMPA subunit at Ser^{831} (Barria et al., 1997).

Cyclic adenosine 3',5'-monophosphate (cAMP)- dependent protein kinase (PKA; e.g., Makhinson, Chotiner, Watson & O'Dell, 1999) has also been suggested to facilitate CaMKII activity indirectly by decreasing competing protein phosphate activity of inhbitor-1, an endogenous protein phosphate inhibitor (Blitzer et al., 1998).

In addition, PKA has been assigned a role in the phosphorylation of GluR-1 at Ser^{845}, rather then phosphorylating at Ser^{831} [as with CaMKII and PKC (Barria et al., 1997)].

Finally, as was previously described, entry of Ca^{2+} through NMDA receptors initiates signal transduction mechanisms via phosphorylation of protein kinases to mediate the expression of LTP (e.g., CaMKII; Silva et al., 1992). The negative counterpart to LTP, long-term synaptic depression (LTD) involves dephosphorylation of AMPA receptors, which reduces their sensitivity to glutamate (Lee, Kameyama, Huganir & Bear, 1998). LTD produces a persistent dephosphorlytion of the GluR1 subunit of AMPA receptors for PKA (at Ser^{845}), PKC and CaMKII (at Ser^{831}); suggestive that the dephosphorylation of AMPA receptors is necessary for the expression of LTD (Lee et al., 1998, 2000).

1.34 *Postsynaptic Changes in LTP: A key role for AMPA receptors.*

Early endeavours assessing postsynaptic expression measured the responses generated by direct application of glutamate agonists. Initial studies found no increase in the sensitivity to L-glutamate for up to a 30 min post tetanisation (Lynch, Gribkoff & Deadwyler, 1976). However, this may have been due to problems associated with the uptake and non-specific actions of L-glutamate. Consequently when AMPA was used as an agonist, a slow onset increase in sensitivity was detected which began over a few minutes, however it took an hour or more to reach asymptote (Davies, Lester Reymann & Collingridge, 1989), suggesting the involvement of these receptors in the persistence of the potentiated response (Malenka & Nicoll, 1999).

Quantitative analysis of the size and frequency of miniature synaptic events at the neuromuscular junction has proven to be an effective measurement in determining

the locus responsible for a change in synaptic strength. A change in miniature excitatory postsynaptic current (mEPSC) frequency is thought to reflect a presynaptic change, whereas a change in size reflects a postsynaptic modification (Manabe, Renner & Nicoll, 1992). The postsynaptic events are due to the spontaneous secretion (exocytosis) of individual presynaptic vesicles, each containing multimolecular packets of transmitter termed *quanta* (Katz,1952). If one assumes that the amount of L-glutamate in each vesicle is relatively fixed, an increase in the size of the mEPSC would reflect an increase in the function or number of AMPA receptors, i.e., it would indicate a postsynaptic change (Nicoll & Malenka, 1999; Malenka & Nicoll, 1999). In fact, such an increase occurs both during LTP (Manabe, Renner & Nicoll, 1992) and following brief applications of NMDA or strong depolarising voltage pulses (Kauer, Malenka & Nicoll, 1988; Wyllie, Manabe & Nicoll, 1994), clearly suggesting postsynaptic modification.

Minimal stimulation experiments have shown that LTP is typically associated with a decrease in synaptic failures (Skrede & Malthe-Sorenssen, 1981; Feasley, Lynch & Bliss, 1986), which relate to the release probability of quanta. Generally, a decrease in failures has been interpreted as an increase in the probability of transmitter release and therefore as indicating that LTP is associated with an increase in transmitter release. Theses results are inconsistent with the postsynaptic hypothesis suggested by the majority of findings. However, one can resolve these issues when considering a recent finding suggesting the existence of postsynaptically silent synapses. For instance, if some synapses lacked functional AMPA receptors, then any failure could be due to a failure to detect released transmitter, rather than a failure to release transmitter (Issac, 2003). Experimental support for the idea that glutamatergic synapses exhibit NMDA receptor mediated responses in the absence of an AMPA

receptor component came from a study comparing the variability of AMPA- and NMDA-receptor mediated synaptic transmission (Kullmann, 1994). Observation of unitary NMDA receptor mediated transmission soon followed (Issac, Nicoll & Malenka, 1995; Liao, Hessler & Malinow, 1995). This was achieved through the identification of NMDA receptors that had no detectable AMPA component while the postsynaptic cell was in a stable non-polarised state. Following LTP induction, stimuli that previously showed no evidence of an AMPA EPSC component evoked AMPA EPSCs. This suggests that a proportion of synapses contain NMDA receptors but not functional AMPA receptors, that is until LTP-inducing stimuli activate these silent synapses (Issac et al., 1995). This finding suggests a mechanism of AMPA receptor cycling following the induction of LTP, a finding which has been supported through recent trafficking experiments (Shi, Hayashi, Petralia, Zaman, Wenthold, Svoboda & Malinow,1999; Hayashi, Shi, Esteban, Piccini, Poncer & Malinow, 2000; Shi, Hayashi, Esteban & Malinow, 2001). One way this constitutive recycling may take place is through the rapid insertion of AMPA receptors in postsynaptic regions following LTP induction.

In an ingenious study, Shi and colleagues (Shi et al., 1999) monitored the distribution of AMPA receptors at high resolution in living neurons. This was achieved through 'tagging' a green fluorescent protein (GFP) to the extracellular amino terminus of GluR-1 AMPA subunits. Shi et al then expressed the recombinant GluR-1-GFP receptor in living, organotypic hippocampal slice cultures. Through the use of two-photon laser scanning microscopy they monitored the distribution over the time-course of LTP expression. Prior to the induction of LTP, surprisingly few GluR-1-GFP receptors were located in dendritic spines where most excitatory synapses are located. Rather, the majority were located on the dendritic trees. However, following

tetanic stimulation, GluR-1-GFP rapidly distributed in the dendrites, and into the synaptically rich dendritic spines. These results suggest that AMPA receptors can be rapidly recruited to spines in response to LTP induction (Malinow, 2003).

In a related study, Hayashi et al., (2000) assessed whether these redistributed receptors were contributing to increases in synaptic efficacy. Through the use an electrophysiological 'tag', Hayashi and colleagues monitored the function of the ionotropic receptors. With this electrophysiological assay, Hayashi et al., (2000) again showed that recombinant AMPA receptors were delivered from the dendritic tree to the synapses following LTP induction. In addition, and consistent with the well-documented role of CaMKII as a key mediator of LTP, co expression of CaMKII and GluR-1-GFP resulted in the delivery of recombinant receptors. However this process was not dependent on the phosphorylation of GluR1 at Ser^{831} by CaMKII (Barria et al., 1997), but was dependent on an intact PDZ binding site.

Finally, detailed trafficking of each AMPA subunit revealed subunit-specific roles (Shi, Hayashi, Esteban & Malinow, 2001). Thus, dependent on the receptor stochiometry, there were two different AMPA receptor synaptic delivery mechanisms. Potentiation of synaptic transmission was dependent on the delivery of GluR-1 and CaMKII to synapses, whereas the maintenance of synaptic transmission required AMPA receptors containing GluR-2 to be constitutively swapped with existing AMPA receptors. Similar rules were also defined for the heteromeric AMPA receptors containing a mixture of subunits. For instance, to establish the induction of changes in the efficacy of plasticity, GluR-1 and GluR-2 heteromers would be delivered to synapses; whereas the maintenance of this plasticity would rely on GluR-2 and GluR-3 heteromers to replace the existing synaptic AMPA receptors independent of neuronal activity (Shi, 2001).

According to this model postulated by Shi and colleagues, synaptic transmission is normally maintained by recycling a relatively constant number of GluR-2 receptors in synaptic regions. However stimuli inducing plasticity (e.g., induction of LTP; Bliss & Lomø, 1973) immediately cause a net addition of GluR-1 containing AMPA receptors, which may eventually be replaced by GluR-3 containing AMPA receptors, resulting in a long-lasting increase in synaptic transmission (Shi et al., 2001). This model is consistent with the observed LTP impairment seen in GluR-1 knock-out mice (Zamanillo et al., 1999). However it should be noted that in the trafficking studies mentioned above, the researchers used organtypic hippocampal slices from postnatal 5- to 8-day-old rats. It has been suggested that constitutive AMPA receptor cycling may be less apparent in older animals where the receptor kinetics may be relatively more stable (personal communication, from Graham Collingridge to Alex Johnson, Cardiff University School of Medicine, June 9th, 2005).

1.35 *Properties of LTP: A Model of the Cellular Mechanisms of Memory.*

In general, LTP is identified by three fundamental properties: *cooperativity*, *input-specificity* and *associativity* (Gustafsson & Wigstrom, 1988). Cooperativity relates to the intensity threshold which mediates LTP induction, and is reflected by the inability to induce LTP following weak intensity tetanisation (McNaughton, Douglas & Goodard, 1978). The cooperativity threshold follows the need for depolarisation to reduce the level of Mg^{2+} block on the NMDA channel. Therefore, weak stimuli which activate only a few fibres fail to induce LTP (Bliss and Lomø, 1973; Bliss & Collingridge, 1993; Malenka & Nicoll, 1999), whereas strong stimuli which activate in synchrony result in depolarisation which spreads between synapses

to enhance the unblocking of NMDA channels (Gustafsson & Wigstrom, 1988; Martin, Grimwood & Morris, 2000).

That LTP is input specific is shown by the fact that other inputs that are not active at the time of the tetanus do not share in the potentiation induced in the tetanized pathway (Andersen, Sundberg, Sveen, & Wigstrom, 1977). Input specificity is explained by the need for the presynaptic cell to provide a concentration of L-glutamate that is sufficient to stimulate adequate numbers of NMDA receptors (Gustafsson, Asztely, Hanse & Wigstrom, 1989). Finally, the associativity aspect of LTP reflects the fact that the concurrent stimulation of weak and strong synapses to a given neuron strengthens the weak inputs (McNaughton, Douglas & Goodard, 1978; Urban & Barrionuevo, 1996). Associativity can be explained in a similar way to cooperativity, except that the required depolarisation is provided by a different set of fibres. Thus, when a weakly stimulated input causes the release of glutamate, it nevertheless fails to depolarize the postsynaptic cell to relieve the Mg^{2+} block. When neighbouring inputs are strongly stimulated, they provide the associative depolarization necessary to relieve the block. LTP induced by the pairing of synaptic input with depolarization may work similarly; the synaptic input releases glutamate, while the coincident depolarization relieves the Mg^{2+} block of the NMDA receptor (Bliss & Collingridge, 1993).

1.4 Properties of LTD.

Up to this point LTP has been the predominant focus of discussion. However, I will now briefly summarise the phenomena associated with LTD. Similar to LTP, a variety of stimulation patterns are capable of inducing long-term changes in synaptic

depression (Artola & Singer, 1993). LTD has been observed to take place at both active and inactive synapses (Linger, 1994). That is, there are two distinctive forms of LTD: (1) Heterosynaptic LTD, which is a long-lasting decrease in synaptic strength induced when strong postsynaptic activity occurs in the absence of presynaptic stimulation (Dunwiddie & Lynch, 1978); and (2) homosynaptic LTD which is induced when presynaptic activity occurs with moderate postsynaptic activity (Dudek & Bear, 1992). LTD is considered to be established if the slope of the EPSP is stable and below baseline levels for 30 to 60 min (Staubi & Ji, 1996). Some experimental protocols result in a depression of synaptic transmission that has a shorter duration (5-20 min; Frégnac, Smith & Friedlander, 1990). Analogous with STP, this form of LTD is usually referred to as short-term depression (STD), consistent with this analogy, STD can still occur in the presence of protein kinase inhibitors (e.g., H7; Hrabetova & Sacktor, 1996; c.f. Lovinger et al., 1987).

In respect to heterosynaptic LTD, an input system undergoes LTD even when inactive if other inputs are strongly activated. This form of LTD has been observed in the hippocampus (Dunwiddie & Lynch, 1978) and in the neocortex (Artola & Singer, 1993). The induction protocols require strong postsynaptic activation and hence high-frequency stimulation of input, such that the signal has to spread from the site of activation to the synapses that undergo depression (Linden, 1994). Several studies have suggested that synaptic activation has to induce strong depolarisation in order to produce heterosynaptic LTD (Staubi & Ji, 1996). Further, it is usually prevented by blockade of NMDA receptors (using AP5) and is facilitated by reducing GABAergic inhibition (e.g., Bradler & Barrionuevo, 1989). Since its induction requires strong postsynaptic activation, stimulus conditions suitable for LTD can induce LTP of the stimulated afferents (Lynch, Gribkoff & Deadwyler, 1976; Staubli & Ji, 1996).

Moreover, heterosynaptic LTD is not input specific (unlike homosynaptic LTD; Dudek & Bear, 1992).

Homosynaptic LTD occurs where activity in the modified pathway contributed to its depression (Linden & Connor, 1995). Dudek and Bear (1992) highlighted a clear dissociation in the induction protocols required for LTP and homosynaptic LTD. The authors stimulated the Schaffer collateral-commissural pathway with 900 pulses of electrical current, delivered at a range of rates from 1 to 50 Hz. They reported an induction of LTP using frequencies above 10 Hz, whereas those below 10 Hz produced LTD. Both of these effects were dependent on NMDA receptors (Dudek & Bear, 1992).

As with LTP, associative LTD has been demonstrated (Stanton & Sejnowski, 1989; Normann et al., 2000) when presynaptic activity occurs explicitly out of phase with strong postsynaptic activity (Linden & Connor, 1995). Although associative LTD is referred to as homosynaptic, it should be noted that associative LTD requires presynaptic activity occurring in the absence of postsynaptic activity; unlike homosynaptic LTD which depends on presynaptic activity combined with moderate postsynaptic activity (Artola & Singer, 1993). Furthermore, LTD is input-specific in that it is confined to synapses that are active during induction (Dudeck & Bear, 1992).

LTD is blocked in the presence of NMDA antagonists (e.g., AP5; Dudek & Bear, 1992). However, 3-((RS)-2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) an antagonist that binds with high affinity to conventional NMDA receptors subtypes, but not to atypical subtypes that are relatively independent of voltage-dependent Mg^{2+}-blockade, blocks LTP whilst leaving LTD intact (Hrabetova & Sacktor, 1997). It was therefore postulated that LTP and LTD may be mediated by different NMDA receptor subtypes, a suggestion supported by the recent finding that

antagonists which differentially block the NR2A and NR2B NMDA receptor subtypes, differentially effect LTP and LTD (Massey et al., 2004). Here, application of the NR2B specific antagonist Ro 25-6981 blocked LTD; whereas application of the NR2A antagonist NVP-AAM077 blocked the expression of LTP (Massey et al.).

As demonstrated by Shi and colleagues (Shi et al., 1999, 2001; Hayashi et al., 2000), the volume of AMPA receptors in postsynaptic regions increases following LTP induction. Conversely, LTD induction involves a decrease in the number of AMPA receptors at postsynaptic regions (Carroll, Lissin, Zastrow, Nicoll & Malenka, 1999). Immunocytochemical analysis revealed a decrease in the number of AMPA receptors at synaptic regions following LTD. Furthermore, this process may involve internalisation of the AMPA receptor subunits GluR-1 and GluR-2 (Ashby et al., 2004). Ashby et al., used pH-sensitive GFP to visualise surface-expressed GluR-2 in real-time at individual synapses, the authors reported a marked decrease in the number of heteromeric AMPA receptors following LTD. Consistent with these findings it has also been reported that AMPA receptors are slowly removed from synapses following NMDA exposure (which induced chem-LTD; Eshlers, 2000).

In summary, homosynaptic LTD takes place at inputs whose activation mediates the induction of the modification (Dudek & Bear, 1992) and heterosynaptic LTD occurs when LTD is manifested at inputs that are inactive at the time of induction (Dunwiddie & Lynch, 1978). Homosynaptic LTD is input-specific (Dudek & Bear, 1992) and can be associative (Stanton & Sejnowski, 1989; Normann et al., 2000), although alternate mechanisms mediate homosynaptic LTD and associative LTD (Artola & Singer, 1993). Furthermore, homosynaptic LTD shares many functional similarities with LTP. However these properties seem to be at an opposite end of a continuum to that seen in LTP; such that LTD is correlated with

dephosphorylation (Lee et al., 1998, 2000) and a reduction in the number of AMPA receptors present at postsynaptic regions (Carroll et al., 1999; Ashby et al., 2004).

Thus far I have described the proposed mechanisms for synaptic plasticity hypothesis. However, the mechanisms underlying synaptic plasticity are far from fully understood and there are a number of unresolved issues. Not least is the ongoing debate concerning the pre- or post-synaptic mechanism of LTP expression (Nicoll & Malenka, 1999). I now will briefly summarise other controversial issues which at present are still unresolved and fuel the debate as to whether LTP and LTD are valid physiological models of the neural mechanisms underlying learning and memory.

1.5 *Critique of Synaptic Plasticity.*

1.51 *Physiological Evidence.*

LTP (and LTD) have attracted considerable attention since the first reported discovery over 30 years ago (Bliss & Lomø, 1973; Gustafsson & Wigstrom, 1988; Bliss & Collingridge, 1993; Martin, Grimwood & Morris, 2000). However, a serious impediment to determining the role of LTP in learning and memory relates to the confusion regarding its definition. Some researchers focus on the role of NMDA-dependent forms of LTP, despite the numerous instances in which long-lasting changes occur independent of NMDA function, such as mossy-fibre LTP (Harris & Cotman, 1986; Bliss & Collingridge, 1993). Thus, defining LTP based on its NMDA dependence may be unnecessarily limiting, and misleading with regard to LTP and memory (Shors & Matzel, 1997).

Further complications arise when one examines the use of the term input-specificity, which is often used to describe very different phenomena. According to some authors, the notion of specificity limits the locus of LTP to synapses (e.g., Dunwiddie & Lynch, 1993). However the modifications that are induced following LTP are very rarely specific to the synapse. Furthermore, there is no reason for a plasticity mechanism underlying memory to be limited to synapses (Shors & Matzel, 1997). However, a necessary component for any Hebbian based model of learning is that changes will be restricted to those synapses that are active during induction (Bliss & Collingridge, 1993; Malenka & Nicoll, 1999). In contrast to this hypothesis, changes accompanying the induction of LTP are apparent when one examines the spread of messenger RNA (mRNA) from an ipsilateral to contralateral side following LTP induction (Smirnova et al., 1993; Castren et al., 1993). Thus, mRNA levels are increased (via transcription) in response to the induction of LTP in regions that fail to exhibit enhanced synaptic efficacy. In a related example, LTP was induced following unilateral tetanisation, once again there was a bilateral increase, this time in the binding affinity of AMPA receptors. Therefore, in contrast to input-specificty (Dunwiddie & Lynch, 1993) these data indicate that some effects of LTP are not confined to the synapses active during the induction, and are inconsistent with the proposed model of NMDA-dependent LTP (Bliss & Lomø, 1973; Bliss & Collingridge, 1993).

When viewed from an integrated brain systems approach these non-specific effects may provide evidence that LTP has physiological relevance. For instance, it has been suggested that LTP can spread to synapses on neighbouring neurons by a diffusible NO signal (Madison & Schuman, 1991; Barinaga, 1994). Similarly, theoretical frameworks based on dopaminergic activity have been shown to alter

synaptic activity based on non-specific plastic modifications (Monatgue, Dayan & Sejnowsk, 1996). However, hippocampal LTP is dominated by the view that memory is limited to synapses themselves or to synapses active during tetenisation, rather than these non-specific LTP-like processes (Shors & Matzel, 1997).

1.52 *Behavioural Evidence.*

In the following section, I have limited the discussion of the behavioural relevance of synaptic plasticity to hippocampal function and focus on spatial memory tasks conducted in a watermaze. The reasons for this are that up to this point the review has focussed on NMDA-dependent LTP which has been extensively studied in the hippocampus – a structure that is critically involved in spatial memory in rodents and consequently has been adopted as the main experimental preparation in the study of the pharmacological and genetic basis of memory (Bliss & Lomø, 1973; Bliss & Collingridge, 1993; Nicoll & Malenka, 1999; Morris et al., 2003). Theoretical characterisations of the role of LTP in learning are understandably linked to current views of the role of the hippocampus in learning and memory. Thus, rival theories include, but are not limited to a role for LTP in spatial and cognitive mapping (O'Keefe & Nadel, 1978; O'Keefe, 1993); episodic memory (memory for learning episodes; Tulving, 1983; Morris & Frey, 1997); the acquisition of conjunctive and configural representations (Sutherland & Rudy, 1989; O'Reilly & McClelland, 1994); and declarative and relational memory (encoding information about the perceptual and behavioural structure of experience; Squire, 1992; Shapiro & Eichenbaum, 1999). In all of these examples, a large number of authors conclude that the evidence supports a role for LTP in learning and memory.

One of the first studies investigating the role of LTP in memory process was conducted by Morris and colleagues (Morris, Anderson, Lynch & Baudry, 1986). Rats received a chronic intracerebroventricular (ICV) infusion of the potent NMDA antagonist AP5 into the ventricle surrounding the hippocampus, and were assessed on their ability to perform in the Morris water maze a task which assesses reference memory ability in rodents (Morris, 1981). In this procedure, rats were first trained in a circular pool (filled with opaque water) to locate a visible cued platform. Following this training, the cued platform was switched with a submerged platform which was located in a fixed position across trials. Morris et al. reported an impairment in reference memory following AP5 infusion. It should be noted that AP5 treated rats showed an increase in escape latency on the first three-trial block, prior to the point when learning normally occurs (Shors & Matzel, 1997). This suggests that infusion of AP5 may have resulted in aberrant sensory or motivational processes which, in turn, may have interfered with learning.

Similar results have been shown using ICV AP5 administration across a range of doses comparable to that which induces LTP induction impairment in in-vitro preparations (Davies, Butcher & Morris, 1992). Here, rats were given 12 trials on non-spatial training over 3 d where the platform was moved on each trial. This pretraining was used to allow the animals to practice swimming prior to commencing reference memory acquisition. Minipumps were then surgically implanted and the rats were given AP5 infusions prior to each spatial training session. Rats were released at various locations in the watermaze, however, the platform remained in a fixed position. Following 5 d of training (with 3 trials per day), the researchers attempted to evoke in-vivo LTP in each individual rat. The results indicated that AP5 blocked both spatial learning and LTP in a dose-dependent manner (Davies et al., 1992).

Unfortunately, the researchers failed to implement any basic sensorimotor tasks (e.g., measures of locomotor activity; Cain, Saucier, Hall, Hargreaves & Boon, 1996) to assess whether any of the learning impairments could be explained by generalised behavioural disturbances.

Similar results have been reported through the use of alternate NMDA antagonists. For instance, Bannerman, Butcher, Good and Morris (1997) administered chronic intracerebroventricular infusion of the glycine site antagonist 7-chlorokynurenate (7CK; Bashir, Tam & Collingridge, 1990). Initially, rats were given 1 d of non-spatial pretraining which consisted of six trials where the platform was moved on a trial-to-trial basis. On completion, the minipumps were surgically implanted and 1-2 μl of 7CK were infused into the lateral ventricle prior to fixed-platform location training which was conducted over three days and followed by a probe trial. Bannerman et al. (1997) reported impaired performance during the acquisition of the spatial reference memory task in 7CK treated rats. However, in contrast to the other studies, the authors also reported motor impairments in the 7CK treated animals, including pronounced thigmotaxis (time at the side walls), difficulty in climbing onto and staying on the platform and a slower righting reflex. Immediately following the probe test, individual rats were anesthetised, and an attempt was then made to induce LTP. In contrast to expectations, LTP was induced in all of the drug treated rats and the authors argued that low tissue levels of 7CK may have accounted for this result (Bannerman et al., 1997).

The above result is troubling on two counts. Firstly, if one assumes that the behavioural impairment is learning, and not performance based, then a clear discrepancy between learning impairment and LTP is evident. Secondly, if the spatial learning impairment reflected a non-specific behavioural deficit induced by infusion

of 7CK, then this study supports the idea that the previously reported 'learning' deficits (Morris et al., 1986; Davis et al., 1992) are consequently the result of aberrant motor abilities induced by infusion of NMDA antagonists. Cain, Saucier, Hall, Hargreaves and Boon (1996) used concentrations of NMDA antagonists similar to the previous studies (infusions ranging between 1 to 2 μl of AP5; Morris et al., 1986; Davis et al., 1992; Bannerman et al., 1997). Cain et al., (1996) conducted a range of behavioural assays to determine the extent of disruption induced by NMDA infusion. It was found that AP5 infusion caused a range of sensorimotor deficits in behaviours which were required for maze performance. For example, a water-filled alley with a wire mesh ladder leading from the water to a goal platform was used. Rats were placed at one end and swam to the ladder at the other end. This task assessed the ability of rats to swim and escape the pool. There were increases in swim and escape time in AP5 treated rats. Impairments such as these correlated with acquisition impairments in both the hidden and visible platform versions of the water maze task (Morris et al., 1986; Bannerman et al., 1997; Davis et al., 1992). The authors suggest that drug-induced sensorimotor disturbances contributed to poor acquisition scores in AP5 treated rats (Cain et al., 1996).

More recently, a delay-dependent impairment has been noted in a matching-to-place water maze task following IPV infusion of AP5 into the hippocampus (Steele & Morris, 1999). Prior to surgery rats were given pre-training on a delayed-matching to sample task. Over 9 d, rats were given 4 daily trials to find an escape platform hidden in a new location each day, with the memory interval (ITI) varying from 15 sec to 2 hrs between trials 1 and 2, but always remaining at 15 s for the remaining trials. Following pre-training, guide cannulae were surgically implanted into the hippocampus. Intrahippocampal AP5 infusion caused a delay-dependent deficit in

memory of the last location visited in the water maze. That is, when the trial ITI was 15 s the AP5 treated rats did not differ from controls, whereas a clear deficit was apparent at longer ITIs (20 min to 2h). The authors reported "some indications of unsteadiness on the platform on day 1, but no obvious differences between the groups thereafter" (1999, pp. 123). At face-value then, this experiment supports the idea that NMDA-dependent LTP underlies matching-to-place spatial memory. However there are several factors which question the acceptance of this result. Firstly, although behavioural impairments (even if limited to the first day) were noted, no attempt to assess sensorimotor ability was undertaken. Therefore (however unlikely), one cannot rule out sensorimotor disturbances contributing to the learning impairment. Secondly, a cohort of hippocampal lesioned rats were also examined in the matching-to-sample paradigm. Unlike the AP5 treated rats, however, the lesioned group failed to show the delay-dependent effect in this task as these rats were impaired at all ITI intervals. This suggests that NMDA independent processes in the hippocampus were governing the ability of AP5 treated rats to acquire the matching-to-sample task in the short ITI.

In stark contrast to the impairments noted in the above studies, some researchers have reported no impairment in rats treated with NMDA antagonists using the water maze task (Saucier & Cain, 1995; Bannerman et al., 1995; Hoh, Beiko, Boon, Weiss & Cain; 1999). For instance, rats trained to learn the location of a hidden platform showed no impairment following infusion of the NMDA antagonist CGS19755 (CGS; Lehmann et al., 1988; Hoh et al., 1999) during acquisition of the watermaze task. Additionally, following training, Hoh and colleagues assessed whether LTP could be induced in-vivo following CGS infusion. It was reported that CGS blocked LTP both in the dentate gyrus and CA1 regions. Thus, the initial place-response training prevented the effects of the antagonist from manifesting a learning

impairment in drug treated rats. These results suggest that although hippocampal NMDA-dependent LTP might contribute to the water maze task, this putative mechanism of memory may not be essential for learning behavioural strategies or multiple platform locations (Shors & Matzel., 1997; Hoh et al., 1999).

To conclude this section, it has been suggested that NMDA-dependent LTP is a critical component of the neural mechanism underlying memory processes in general (Morris et al., 1986). In the literature, the contribution of LTP to specific memory processes reflects the theoretical function assigned to the hippocampus (O'Keefe & Nadel, 1978; Tulving, 1983; Morris & Frey, 1997; Sutherland & Rudy, 1989; O'Reilly & McClelland, 1994). In all of these cases, the research groups conclude that the evidence supports a role for LTP in learning and memory. This has been based largely on findings obtained from antagonism of the NMDA receptor (Shors & Matzel, 1997). However, the case supporting such a mechanism is made less compelling when one examines the inconsistent findings reported by various research groups. Although several studies report deficits in water maze learning following infusion of NMDA antagonists (Morris et al., 1986; Davis et al., 1992; Steele & Morris, 1999), their interpretation is often confounded by evidence of drug-induced non-specific side effects (Shors & Matzel, 1997). Based on the data reviewed here, LTP cannot be unequivocally accepted as a model of learning and memory.

1.53 *Coda.*

There are several possible conclusions that may be drawn in relation to the validity of the mechanisms of LTP as a model of the physiological processes supporting learning and memory. The most extreme conclusion is that LTP represents

neither an information-processing device nor a memory mechanism. Alternatively, the mechanisms of LTP play a critical role in the processing of sensory information necessary for the establishment of stable memories and that it is induced in response to environmental stimuli as well as the organism's response to those stimuli. The lack of conclusive evidence indicating a necessary contribution of NMDA receptor-dependent LTP as a model of memory, as well as recent evidence to the contrary, is viewed by some to be a sufficient caveat to warrant a complete reassessment of the synaptic memory hypothesis (Shors & Matzel, 1997). However, while many neuroscientists have found evidence to challenge the LTP-hypothesis, this phenomenon continues to hold interest as a model of the cellular basis of learning and memory (Malinow, 2003). The continued interest in the contributions of LTP mechanisms to learning reflects, at least in part, the advent of more specific molecular techniques that promised a more selective method of manipulating synaptic plasticity and potentially memory. I shall now go on to briefly describe some pertinent findings in this area.

1.6 *The use of Gene-targeted mice to study LTP and Behaviour.*

The literature suggests that multiple forms of regulation, induction and maintenance of synaptic plasticity exist. While the field has begun to assemble the types of induction and maintenance mechanisms necessary for plasticity to occur, the advent of gene-targeting techniques has provided a novel means of investigating the neurological and psychological mechanisms which underlie behavioural change (Stephens, Mead & Ripley, 2002). As we shall see this methodology is not without its own complications. To date, the most readily tractable mammalian species for genetic

manipulation is the mouse (*Mus musculus)*. This is predominantly due to the commonly shared factors between mouse and man including; behavioural development, body topography, physiology, behaviour, disease and genetic homology (99 percent of mouse genes are identical to those of humans). Moreover, the mouse genome supports targeted mutagenesis in specific genes by homologous recombination in embryonic stem cells (ES), allowing for specific genes to be identified and altered (Capecchi, 1989).

1.61 *Limitations in the use of Gene-targeted mice.*

Although providing both a high degree of genetic specificity, as well as the ability to analyse pharmacologically tractable agents, it should be acknowledged that the use of conventional KO mice is fraught with potential problems. For example, for the vast majority of KO's used to examine LTP, learning and memory, each cell in these mice lacks the targeted gene of interest (Gerlai, 2000). As a consequence of this whole-brain deletion, it can be problematic for one to implicate region-specific neural impairments with behaviour (Winder & Schramm, 2001). However it should be possible to use refined behavioural techniques that engage specific learning systems, and that offer opportunities to evaluate non-specific effects of gene manipulation on performance, to characterise the learning deficit. In addition, each cell carries the mutated DNA for the life span of the animal. As such, it can be difficult to determine whether a particular behavioural phenotype observed in the adult animal is a consequence of developmental abnormalities, or, a compensatory event provoked by the genetic manipulation (Gingrich & Hen, 2000).

The background strain on which the genetic manipulation is made can also contribute to the observed phenotype (Gerlai, 1996). Several studies have demonstrated that different inbred strains of mice perform idiosyncratically according to the background strain (Crawley et al., 1997; Owen et al., 1997), and that the magnitude and duration of LTP elicited by different protocols varies dependent on the strain used (Nguyen, Abel, Kandel & Bourtchouladze, 2000). To compound this problem, in many cases genetically modified mice are bred as hybrids from different strains, resulting in a variable background from animal to animal (Crawley, 1996). The two most common strains used in targeted experiments are the substrains of 129 mice for the embryonic stem cell line, and C57BL/6J for the blastocytes. The choice of these two strains is based on technical success. However, problems may arise due to the unusual behaviours which can be seen in these strains. For example, some 129 substrains (e.g., 129/J & 129/SvJ) have an incomplete and missing corpus callous (Livy & Wahlsten, 1997) and perform poorly on learning and memory tasks (Crawley et al., 1997), whereas C57BL/6J mice show an unusual propensity to self-administer drugs of abuse, including cocaine (Grahame, Philips, Burkhart-Kasch & Cunningham, 1995) and alcohol (Philips & Crabbe, 1991). The unique traits of 129 and C57BL/6J mice are indicative of a more general problem for the interpretation of behavioural phenotypes of mutations, arsing from these background strains (Schram & Winder, 2001). Behavioural neuroscientists are encouraged to consult with the literature and examine the behavioural phenotype of the background strain, prior to attributing causality specific to the gene of interest. Hence, genetic background should be as carefully controlled as any other experimental variable (Crawley & Paylor, 1997; Crawley et al., 1997; Schram & Winder, 2001; Stephens, Mead & Ripley, 2002).

Conditional KO's have been developed which are designed to restrict the effects of the mutation to a specific period of adulthood, thus avoiding the complications which may arise due to genetic compensatory mechanisms occurring during development (Crawley et al., 1997). These KO's make use of Cre/LoxP mediated recombination (Sauer, 1993), allowing for more appropriate controls in behavioural experiments. Nevertheless the use of either traditional KO techniques or conditional KO's provides a contemporary tool with which to assess the role of specific genes in physiological processes of learning and memory, such as LTP. The following section will evaluate studies of KO mice used to examine the relationship between LTP and behaviour. However, given the plethora of studies on mutations that influence synaptic plasticity, the following review will focus on mutations related specifically to the NMDA and AMPA receptor function, as these receptors have been assigned a primary role in the mechanisms of synaptic plasticity (Collingridge & Bliss, 1993).

1.62 *NR-1 KO mice.*

The expression of the NMDA receptor subunit NR1 (also known as GluRζ) has been investigated using homologous recombination in ES cells. Studies using these KO's have demonstrated that expression of the NR1 subunit is essential for normal NMDA receptor function, somatosensory map formation and neonatal survival (Forrest et al., 1994; Iwasato et al., 1997; Adams, Vaccari & Corriveau, 2004). Earlier studies using the NR-1 KO (herein NR-$1^{-/-}$) were plagued with developmental difficulties, such that NR-$1^{-/-}$ mice died within 8-15 hours after birth, indicating a vital neonatal developmental function for the NMDA receptor (e.g.,

Forrest et al., 1994). These mice showed complete loss of NR-1 levels throughout the brain, in addition to reduced levels of NR2B. The NR-1 deletion also resulted in a dramatic loss of the NMDA-induced rise in intracellular Ca^{2+} and a complete loss of the formation of whisker-related barrel patterns in the brainstem (Li et al., 1994). Due to their very short life expectancy any behavioural assessment was precluded.

In order to overcome the problems associated with reduced life expectancy, NR-1^{neo} mice have been developed. These mice express around 5 to 10 % of the normal levels of NR-1, which whilst allowing a deficiency in CNS NMDA receptors, also allows survival through to adulthood (e.g., Möhn, Gainetdinov, Caron & Koller, 1999). These mice show hyperactivity when introduced to a novel context and various social-behavioural deficits such as lower levels of social investigation and abnormal sexual function (Rampon et al., 2000). Moreovèr, Möhn et al., (1999) demonstrated that the aforementioned locomotor and behavioural deficits are attenuated following treatment with clozapine, an antipsychotic drug used to treat schizophrenia. Thus, the NR-1^{neo} mice support a model in which decreased NMDA receptor expression leads to behavioural changes; which are readily treatable via the use of antipsychotic drugs. (Mohn et al., 1999).

An alternate NR-$1^{-/-}$ mouse has also been developed, allowing for the restriction of NR-1 deletion to the CA1 region of the hippocampus (NR-1-$CA1^{-/-}$; Tsien, Huerta & Tonegawa, 1996). These NR-1-$CA1^{-/-}$ mice have been assessed in a variety of physiological and behavioural paradigms. For instance, STP, LTP or LTD cannot be induced in the CA1 region. At a behavioural level these mice display deficits in the spatial reference memory version of the Morris water maze task in comparison to wild-type control group (Tsien, Huerta & Tonegawa, 1996). Also, these mice show deficits in both contextual fear memory consolidation when the NR-

1-CA1$^{-/-}$ is switched off soon after learning (Cui et al., 2004). Here, during the conditioning episode, mice were placed in a experimental chamber, and received foot shocks paired with the conditioning context and an auditory cue (e.g., Fanselow, 1980). During this period the NR-1 subunit in the CA-1 region was fully functional. After training, the mice were not tested for 7 months and were given *dox*-containing food pellets during either the first 7 or all of the 30 days of the seven month retention period. This interval was chosen as previous computational analysis suggested that the consolidation processes (where fear memories are consolidated from hippocampal regions to neocortical regions; Kim & Fanselow, 1992) may be dependent on the periodic reactivation of NMDA receptors in the hippocampus (Wittenberg et al., 2002). Only dox treatment for 30 days allowed for the transient deletion of NR-1-CA1$^{-/-}$. Following treatment, only the mice who had a complete block of NR-1-CA1$^{-/-}$ (i.e., 30 days exposure to dox containing pellets) showed deficits in the consolidation of contextual fear. This deficit did not reflect a generalised performance deficit as these mice showed normal levels of locomotor activity and cerebellar coordination (Cui et al., 2004). Interestingly, NR-1-CA1$^{-/-}$ mice showed normal consolidation of conditioned fear to the auditory cue which had previously been paired with shock. Unfortunately, it is unclear as to whether the fear elicited by presentation of the tone was the result of the conditioning episode or due to unconditioned reactions to the cue, i.e., sensitisation to the tone (Harris, 1943).

In contrast to CA1 specific NR-1$^{-/-}$ mice, NR1-CA3$^{-/-}$ mice (deletion of NR-1 restricted to the CA3 region of the hippocampus) showed normal spatial reference memory in the Morris water maze (Nakazawa et al., 2002). Nevertheless, an impairment was reported when, in a probe test, the mice were presented with only a fraction of the cues which had been previously used during training. This deficit in

pattern completion was assessed through the removal of three out of the four extramaze cues in the Morris water maze. However, the authors only removed the cues which were more proximal to the platform; therefore it is possible that the KO mice would have been able to find the platform location if only the more proximal extramaze cue had remained; thus questioning the degree of impairment in pattern completion for NR1-CA3$^{-/-}$ mice. Finally, in this same report NMDA-dependent LTP was essentially absent in the CA3 region (Nakazawa et al., 2002).

In a follow-up to their original study, Nakazawa et al., (2003) report that NR1-CA3$^{-/-}$ mice were impaired in a delayed-matching-to-place task (DMP) when they were required to discover the novel location of a hidden platform in a water maze. The protocol used was similar to that reported by Steele and Morris (1999). In total there were 12 days of DMP training, with four trials per day and an ITI of 5 min between each trial. Each day the mice were trained with a novel location to find a hidden platform. After completion of the training phase, the mice were divided into two groups for testing. One group of mice continued with the same protocol which lasted for 4 d (i.e., searching for the novel platform location during each session). A second group of mice also underwent DMP, however, the platform location was the same as that experienced 4 days earlier (i.e., for mice for whom the platform location was the same on day 13 test day as it had been on day 9 pretraining day). NR1-CA3$^{-/-}$ mice were impaired in the DMP task when they were required to rapidly encode the spatial representations of the novel platform location in the environment. In contrast, if NR1-CA3$^{-/-}$ mice had previously experienced the locations of the hidden platform, then they showed comparable levels of escape latency as control mice.

In summary, studies using brain wide NR-1 KO's have demonstrated that expression of the NR1 subunit is essential for normal NMDA receptor function,

somatosensory map formation and neonatal survival (Forrest et al., 1994; Iwasato et al., 1997; Adams, Vaccari & Corriveau, 2004). Further, it is suggested that NR-1 receptors (and by implication the hippocampus) serve complementary but computationally distinct roles in regions CA1 and CA3. Thus, NR-1 in region CA1 seems to be critical for the formation of spatial reference memory (Tsien et al., 1996), whereas NR-1 in region CA3 seems to be particularly important in pattern completion ability (Nakazawa et al., 2002) and the processing of rapid one-trial memory (Nakazawa et al., 2003).

1.63 *NR-2 KO mice.*

All four NR-2 subunits (also known as GluRε) have been targeted and deleted to produce KO mice. Studies examining mice with whole-brain deletion of the NR2-A subunit (NR2-$A^{-/-}$, or GluRε1) have suggested that these mice show normal development (Sakimura et al., 1995). These NR2-$A^{-/-}$ mice show reduced NMDA-dependent induction of LTP in regions CA3 and CA1. However, these LTP deficits are overcome through the use of stronger tetenisation protocols, suggesting that the deficits are attributable to a decrease in Ca^{2+} influx in NR2-$A^{-/-}$ mice (Kiyama et al., 1998). Kiyama et al have suggested that the NR-2 subunit of the NMDA receptor channel is a determinant of thresholds for hippocampal LTP.

Behaviourally, NR2-$A^{-/-}$ mice show deficits in water maze learning reflected by an increased escape latency to locate a hidden platform in the maze (Sakimura et al., 1995). However, the same mice also took longer to reach a visible-platform during the first block of trials in the non-spatial water maze task (Sakimura et al., 1995), suggesting that possible sensorimotor impairments may have interfered with water

maze learning. It has also been reported that NR2-A$^{-/-}$ mice display deficits in contextual, but not cued (auditory) fear learning (Kiyama et al., 1998). In respect to the latter finding, the authors failed to present any data regarding a control for any unconditioned inhibition elicited by presentation of the tone. It should also be noted that the deficits reported in contextual fear conditioning were only apparent when the chamber exposure time (prior to foot shock delivery) was shortened in the conditioning stage. Finally, the researchers assessed freezing following shock presentation at various time intervals (ranging from 20 s to 6 min) during a 9 min conditioning session. KO mice showed lower levels of freezing during this stage than control mice and in the context retention test these mutant mice showed lower levels of responding during the first 3 min of testing. Subsequently, both groups of mice showed comparable levels of freezing to the context. The authors suggested that the contextual learning of the NR2-A$^{-/-}$ mice is impaired under short chamber exposure times and indicate that the threshold for contextual learning increases in KO mice (Kiyama et al., 1998). However, it has been previously reported that NR2-A$^{-/-}$ mice are hyperactive (Sakimura et al., 1995). Thus, the lower levels of immediate freezing may reflect immediate high levels of activity when initially introduced to a novel context, rather than any increase in the contextual threshold of learning.

As with mice lacking the NR-1 subunit, NR2-B$^{-/-}$ mice die within 1 d of birth (Kutsuwada et al., 1996), suggesting that the NR2-B (also known as GluRε2) subunit is essential for neonatal development. However the mice can survive for a short duration (p10) by hand feeding, making it possible to examine the effect of the disruption of the gene on synaptic plasticity but not on behaviour. In the hippocampus of the mutant mice, NMDA-dependent LTP and LTD was abolished, suggesting that the NR2-B plays a critical role in both neuronal pattern formation and synaptic

plasticity (Kutsuwada et al., 1996). Since global NR2-B$^{-/-}$ and NR-1$^{-/-}$ mice die shortly after birth and show similar synaptic disruptions, this suggests that the glycine binding site found on both of these subunits is integral for the development and functioning of NMDA receptors (Kutsuwada et al., 1996; Sprengel et al., 1998).

The NR2-C subunit (or GluRε3), would appear to have a less modulatory role in NMDA receptor functioning than the NR2-B and NR-1 subunits. The NR2-C subunit is primarily expressed during brain development and has been shown to exist in complexes with NR1 and NR2 subunits (Ebralidze, Rossi, Tonegawa & Slater, 1996). As yet little is known about the NR2-C subunit, but from research carried out with the NR2-C$^{-/-}$ mice, it appears that the subunit is involved in dendrite spine maturation (Das et al., 1998), and contributes to the functional heteromeric stochiometry at the mossy fibre synapse and extra synaptic sites during development (Ebralidze et al., 1996). At a behavioural level, these mice have only been assessed in an open field environment (Kadotani et al., 1996). It was reported that NR2-C$^{-/-}$ mice showed comparable levels of locomotor activity as controls. However, the amount of vertical activity as assessed by rearing behaviour was decreased in these KO mice. The authors suggest that this observed decrease in rearing behaviour was as a consequence of decreased muscle strength in KO mice (Kadotani et al., 1996).

Studies examining mice with a targeted deletion of the NR2-D subunit (or GluRε4) have revealed relatively few deleterious effects. The NR-2D subunit is mainly expressed from embryonic day 13 through P14 in the mouse brain (Wantanabe, Inoue, Sakimura & Mishina, 1992). Therefore it is suggested that the deletion of this subunit affects the maturation of the brain (Miyamoto et al., 2002). However, NR2-D$^{-/-}$ mice show normal development and no impairments in hippocampal LTP or LTD (Okabe et al., 1998). Behaviourally, these mice show

reduced spontaneous activity as assessed in an 1 hr open-field test (Ikeda et al., 1995) due to reduced levels of locomotor activity and rearing behaviour. In this task, immediately following the 1 hr open field test two novel bars were positioned in the central regions of the open field to assess spontaneous activity evoked by the novel objects. Both control and NR2-D$^{-/-}$ mice showed greater activity around the novel bars in the central area, although the time spent in the central area was shorter for mutant mice (Ikeda et al., 1995). Finally, the mice were placed in an elevated plus-maze (EPM) to assess unconditioned anxiety-like behaviour (Lister 1987). In brief, the EPM consists of two adjacent open and two adjacent enclosed arms, emanating from a common central platform. Generally, anxious rodents will spend significantly more time in the enclosed dark arms than in the exposed open arms. The fact that NR2-D$^{-/-}$ mice showed comparable latencies in each compartment as control mice revealed that both groups of mice showed similar behaviour to the EPM task.

In contrast, more recently it was reported that NR2-D$^{-/-}$ mice spent significantly more time exploring the open arms and had more entries into the open arms than control mice (Miyamoto et al., 2002). As a further assessment of anxiety-related behaviour, mice were introduced to a interconnected light-dark box which consisted of two compartments; a transparent box with a white floor, and a black box with a black floor. Mice were initially placed in the black box and the time spent in each component was measured. Consistent with the results from the EPM, mutant mice spent significantly more time in the white box than wild-type mice. This result contradicts with that found in the Ikeda et al., (1995) study. One possible explanation for this discrepancy may be the procedural differences adopted for the two studies. For example, the relative time in which mice were placed in the EPM (20 min; Ikeda et al., 1995; and 5 min; Miyamoto et al., 2002) could have generated higher levels of

habituation in the former case, in turn producing increased exploration of the open arms in control mice. Consistent with this idea is the observation in the Ikeda et al. study that mutant mice (mean duration spent in open arms; 200 s) displayed more time in the open arms when compared to controls (mean duration spent in open arms; 150 s). An alternative explanation to the discrepancy reported by the two studies may be attributable to the difference in the age of the mutant mice used i.e., 3-month-old mice (Miyamoto et al., 2002), compared to 26-day-old mice (Ikeda et al., 1995). That is, considering that disruption of the NR-2D subunit may affect the maturation of the brain, the function of the CNS in the mutant mice may be more stable at 3 months than at 4 weeks. However, additional studies are required to examine whether NR-2D$^{-/-}$ mice display altered sensitivity to stress in an age-dependent manner.

In summary, the NR2-A subunit appears necessary for the determination for thresholds in hippocampal LTP (Kiyama et al., 1998). At a behavioural level these mice show an impairment in water maze learning (Saikimura et al., 1995); although the effects of sensorimotor deficits interfering with maze learning cannot be ruled out. Additionally, these mice show normal cued, but impaired contextual fear conditioning, such that the threshold for contextual learning increases in NR-2A$^{-/-}$ mice (Kiyama et al., 1998). Whether hyperactivity influences this effect warrants further investigation. In contrast, the NR-2B subunit (as with the NR-1 subunit) appears critical for synaptic plasticity and neuronal development, suggesting that the glycine binding site is critical for the development and functioning of NMDA receptors (Kustawada et al., 1996; Sprengel et al., 1998). As yet, less is known about the functions and relevance of the NR2-C subunit to synaptic plasticity and behaviour. However, it has been reported that the subunit may be involved in dendritic spine maturation (Ebralidze et al., 1996; Kadotani et al., 1996). Finally, NR-2D$^{-/-}$ mice

show normal development, reduced spontaneous activity and are less-anxious (Ikeda et al., 1995; Miyamoto et al., 2002).

As mentioned previously, NMDA receptors are critically involved in the induction of the LTP. Interestingly, however, investigations with KO mice suggest that not all NMDA subunits are necessary in order for induction to take place. That is, NR-1, NR-2A and NR-2B (Forrest et al., 1994; Kiyama et al., 1998; Kutsuwada et al., 1996) subunits are required for LTP induction, whilst NR-2C and NR-2D subunits are not (Ebralidze et al., 1996; Okabe et al., 1998). Similarly, investigations with NMDA KO's have suggested a putative role for these subunits in various learning and memory tasks, which prior to the development of this technology had not been identified, such as the involvement of NR-1 receptor in contextual fear conditioning (Kiyama et al., 1998). Taking into consideration the suggested role of AMPA receptors in the expression following the induction of LTP (Bliss & Collingridge, 1993), similar insights into the role of specific subunits in various aspects of LTP, learning and memory have been reported recently through the development of AMPA KO mice.

1.64 *GluR-1 KO mice.*

Gene-targeted mice lacking the GluR-1 subunit (GluR-$1^{-/-}$, or GluR-$A^{-/-}$) exhibit normal development, life expectancy, fine structure of neuronal dendrites and synapses (Zamanillo et al., 1999), reduced levels of aggression (Vekovischeva et al., 2004) and elevated levels of locomotor activity (Vekovischeva et al., 2001; Bannerman et al., 2004). However, the disruption of GluR-1 has profound effects on the subcelluar distribution of the GluR-2 subunit (Zamanillo et al., 1999). In the

absence of the GluR-1 receptor, the expression of the GluR-2 subunit is redirected (due to compensatory mechanisms) to the cell body layer in the hippocampus rather then being found principally in the dendritic layers (Zamanillo et al., 1999; Mack et al., 2001). Additionally, an increased number of GluR-2/3 subunit neuronal cell bodies have been observed following immunostaining of sections in the basolateral nuclei of the amygdala in GluR-$1^{-/-}$ mice (Mead & Stephens, 2003a). In 3-month-old adult GluR-$1^{-/-}$ mice, associative high-frequency stimulation LTP is absent in CA3-CA1 synapses (Zamanillo et al., 1999). However, late-onset low-frequency theta-burst LTP is present in GluR-$1^{-/-}$ mice (Hoffman, Sprengel & Sakmann, 2002). Further, an additional LTP form which is independent of GluR-1 phosphorylation is operative in mice under 3 weeks of age (Lee et al., 2003; Jensen et al., 2003). It is suggested that GluR-1 independent LTP is related to the establishment of hippocampal synaptic connectivity before the hippocampus becomes functionally important (Jensen et al., 2003).

The first behavioural studies conducted with GluR-1 mutant mice showed that GluR-$1^{-/-}$ mice were not impaired in a spatial reference memory task in the water maze (Zamanillo et al., 1999). Mice were trained to find a hidden platform over a series of 13 d with 4 trials per day. In a subsequent probe test, both control and GluR-$1^{-/-}$ mice spent the majority of time in the training quadrant. This finding was subsequently replicated in a series of experiments assessing spatial memory in GluR-$1^{-/-}$ mice (Reisel et al., 2002). Spatial reference memory was assessed in both the water maze (using the same protocols as Zamanillo et al., 1999) and the appetitivly-motivated elevated Y-maze task and consistent with previous findings, GluR-$1^{-/-}$ mice showed no impairments in spatial reference memory. As a further test for non-spatial reference memory, the mice were given a visual discrimination task in a T-maze

which consisted of a start arm and two identical goal arms. Mice were trained to discriminate between two visually discriminable goal arms (light grey vs. black and white striped). The reward was available on one of the coloured goal arms only. After 10 sessions (4 trials per day) all mice reliably acquired the visual discrimination (Reisel et al., 2002). However, GluR-1$^{-/-}$ mice showed a profound spatial working memory impairment in T-maze non-matching-to-place task (NMTP; Rawlins & Olton, 1982). Unlike controls, GluR-1$^{-/-}$ mice were profoundly impaired on this task even after 10 sessions of training. This working memory impairment was also observed in a Y-maze version of this task (Reisel et al., 2002).

More recently, this dissociation in spatial memory performance has been investigated using a six arm, radial arm maze (Schmitt, Deacon, Seeburg, Rawlins & Bannerman, 2003). In this task, three out of the six arms were always baited with milk, but during the session the milk was not replaced. When one reward was collected, mice were transferred back to the start arm and had to update a representation of the location of (the now depleted) food in the maze. This procedure allowed a within-subjects, within-trial assessment of spatial working memory and spatial reference memory (Schmitt et al., 2003). GluR-1$^{-/-}$ mice displayed more reference memory errors (defined as entries into arms that had never been baited); more working memory correct errors (defined as entries into the arm which had previously been correct but had been previously visited on that trial); and more working memory incorrect errors (defined as repeated entries into the arm which had never been baited). Thus, this deficit in spatial reference memory errors is in contrast to the previous reports (Zamanillo et al., 1999; Reisel et al., 2002). However, the authors suggested that the working memory component of this task had interfered with reference memory performance (Schmitt et al., 2003).This idea was consistent

with a second experiment wherein a separate group of mice were given a reference memory version of this task, i.e., guillotine doors prevented the mice from re-entering a previously visited arm. In this version comparable numbers of errors were committed by both groups (Schmitt et al., 2003).

Schmitt et al., (2004a) also examined the effects of GluR-1 deletion on a conditional discrimination task. This task used inserts in the T-maze to indicate which goal arm contained a food reward. When the inserts were restricted to the start-arm, GluR-$1^{-/-}$ mice were unable to acquire the contingency. However, when the inserts were present throughout the maze, mice were able to learn the contingency as the cue was present at the time when the animal experienced the place-reward association. These results suggested to Schmitt et al. that GluR-1 dependent plasticity is required for encoding spatial and temporal contexts associated with a particular event. Moreover, it was suggested that GluR-1 dependent synaptic plasticity contributes to a memory system in rodents for encoding both the spatial and temporal contexts (the where and the when components of episodic memory) associated with a particular event. This hypothesis reflects current interest in the role of the hippocampus in episodic memory processes and more specifically that the hippocampus encodes information about the spatio-temporal context in which events occur (Eichenbaum & Fortin, 2003).

Finally, spatial reference memory has been assessed in a novel paddling pool escape task (Schmitt et al., 2004b). In this task mice were trained to escape from a circular pool filled with water to a depth of 2.5 cm; hence, only the underside of the belly of the mice became wet. The perimeter of the pool contained 12 holes arranged equidistantly around the circumference of the pool; corresponding to a 12 h clock face. Eleven of these tubes were sealed with black plugs, while one was open and

allowed the mouse to escape the pool via a pipe which led to the animals home cage. Initially, mice were given non-spatial pretraining in a smaller version of the pool which was designed to train the mice to paddle in water and make their escape through the pipe. Following training, mice were exposed to the paddling pool and were trained to escape from the water by finding the single fixed location exit pipe at one of the 12 positions. On each trial, the mouse was placed in the centre of the pool facing one of three randomly selected positions on the perimeter wall. The time taken to find the real exit and the number of false exits was recorded. On completion of training, a probe test was conducted to assess the reliance on intramaze or extramaze cues. This was achieved by rotating the pool by 120°, such that 1 o'clock was repositioned with the same extramaze cues that 5 o'clock had previously occupied. The exit were exchanged so that the exit pipe now occupied the same position as the extramaze cues; but any intramaze cues occupied a new position. The authors reported a small initial impairment in the GluR-1$^{-/-}$ mice, but by session five both groups showed comparable levels of escape latency. However, it should be noted that the performance of the control mice did not alter from sessions 2 to 7, suggesting that the test may not have been sufficiently sensitive to reveal any differences between the groups (i.e., a floor or ceiling effect). In respect to the number of errors performed during training, both groups of mice performed a similar number of errors. Finally, the results from the probe trial suggested that both sets of mice were using extramaze cues around the experimental room to acquire the reference memory task, as evidenced by the equivalent performance between the final training session and the probe trial (Schmitt et al., 2004b).

As a result of the aforementioned behavioural result, Bannerman and colleagues posited that GluR-1$^{-/-}$ mice possess a dissociation in spatial memory

abilities. That is, GluR-1$^{-/-}$ mice show normal spatial reference memory ability; in addition to a specific and enduring spatial working memory impairment (Bannerman 2004; Reisel et al., 2002; Schmitt et al., 2003; Schmitt et al 2004a;b;). It has been suggested that the early and late forms of hippocampal LTP may contribute differently to memory processes (Zamanillo et al., 1999; Hoffman, Sprengel & Sakmann, 2002) and may therefore have functional implications for hippocampal information processing in GluR-1$^{-/-}$ mice (Hoffman et al., 2002; Reisel et al., 2002; Schmidtt et al., 2003). Early-onset LTP is absent in these mice, but late-onset, GluR-1 independent LTP is present. One possibility, therefore is that the latter form of LTP may suffice to underpin the learning of fixed, stimulus-reward contingencies (Reisel et al., 2002; Olton & Papas, 1979). However, this working-memory deficit hypothesis has not been investigated outside the spatial domain. Therefore, as yet one cannot determine whether the sensitivity of GluR1 mutant mice to working memory paradigms is restricted to spatial tasks, or also non-spatial working memory procedures.

According to Bannerman and colleagues, reference memory refers to tests in which the information required for successful performance remains consistent from trial-to-trial (Honig 1978; Rawlins, 1985). That is, the correct response to a given stimulus is the same each time that the stimulus is presented throughout the experiment. In contrast, the cardinal feature of working memory procedures is the inherent flexible stimulus response contingency. That is, specific stimulus information is only valid for one trial of an experiment and not for subsequent trials (Honig, 1978; Olton & Papas, 1972).

1.65 *Instrumental Learning in GluR-1$^{-/-}$ mice.*

The studies discussed above suggest that the GluR-1 mutation has deleterious effects specifically on (spatial) working memory tasks. In conflict with this hypothesis however, is evidence that GluR-1$^{-/-}$ mice are impaired on selective instrumental learning tasks. These tasks cannot easily be categorised *a priori* in terms of a spatial working versus reference memory distinction. Thus, Mead and Stephens (2003a) reported that GluR-1$^{-/-}$ mice were capable of forming a Pavlovian association between a conditioned stimulus (CS) and the delivery of reward (US) and showed normal Pavlovian approach. In this task, mice were given sessions where food delivery (the US) followed the brief illumination of two flashing lights and the onset of a tone. Both GluR-1 mutant and control mice showed higher levels of magazine responding as a function of increased CS-US pairings (Mead & Stephens, 2003a). To assess the ability of the cue to evoke Pavlovian conditioned approach, one of the cue lights was replaced with an infrared detector which measured the number of nose-poke entries. Rates of nose-poke entries increased during CS presentation compared to the ITI. Furthermore, in a separate test, a Pavlovian CS augmented instrumental responding for the same outcome in both KO and control mice (Pavlovian-to-instrumental transfer; PIT). To assess PIT, mice were trained to respond on a lever for the same outcome which had previously been delivered in the Pavlovian conditioning stage. Following acquisition, the previously trained CS was presented and the rates of responding during the CS and ITI were measured. CS presentation augmented responding to the lever for both groups of mice (Mead & Stephens, 2003a). These tasks appear to conform to the definition of a reference memory procedure and the absence of an impairment is consistent with the working memory hypothesis.

However, when the mutant mice were required to learn a novel response to gain access to presentations of a CS that had been previously paired with food (conditioned reinforcement; Mackintosh, 1974, pp.89-90) or respond under a second-order schedule of reinforcement, GluR-1$^{-/-}$ mice were impaired relative to controls (Mead & Stephens, 2003a). To assess the ability of a CS to act as a conditioned reinforcer (CDR), two levers were introduced into the operant chambers. Responding on one lever resulted in the presentation of the CDR (which had previously been used in the above Pavlovian conditioning preparation; cue conditions and locations exactly as during conditioning phase), whereas performance on the other lever had no consequences. Control mice showed a greater number of lever presses to the lever associated with CDR presentation, compared to mutant mice who displayed low levels of responding to both levers. These procedures do not appear to conform to the definition of a working memory procedure and thus the deficits in these tasks cannot be easily explained by the working memory hypothesis without additional assumptions. Indeed, the results suggest an alternative hypothesis of the GluR-1 syndrome. The result suggests that the cue had not acquired conditioned reinforcing properties for GluR-1 mutant mice following Pavlovian conditioning (Mackintosh, 1974; Mead & Stephens, 2003a).

In the second-order operant responding task mice were trained to lever-press for milk. Initially, each lever press caused the delivery of food which was preceded and accompanied by the presentation of the cue. After increases in ratio of lever presses to food delivery (e.g., 10 lever presses produced food delivery), the schedule was switched to a second-order schedule in which lever presses caused the presentation of the cue only. Under this schedule, the mice had to evoke the presentation of the cue a given number of times (set by the criterion) before food

delivery was presented. GluR-1$^{-/-}$ mice were less competent in acquiring responding under this second-order schedule (Mead & Stephens, 2003a). This pattern of behaviour (with deficits in conditioned reinforcement and second-order conditioning) mimics that seen in rats with lesions of the basolateral amygdala (BLA; Hatfield, Han, Conley, Gallagher & Holland, 1996; Cardinal et al., 2002). Interestingly, an increased number of GluR-2/3 subunit neuronal cell bodies was observed in the BLA in GluR-1$^{-/-}$ mice (Mead & Stephens, 2003a). This may suggest compensatory over-expression of GluR-2/3 following GluR-1 deletion, which could theoretically alter the ability of the synapse to show plasticity and would therefore be expected to interfere with BLA-dependent learning and memory processes (Mead & Stephens, 2003a). The authors concluded that the GluR-1 subunit in the BLA was critical for processing the motivational properties of reward value. Nevertheless, GluR-1$^{-/-}$ mice remained sensitive to some aspects of reward, as assessed by their normal performance in Pavlovian conditioning, approach and PIT. This raises the interesting question of what specific aspects of motivational processes are impaired by the GluR-1 deletion, and questions the nature of the disruption seen in the outcome encoding in these mice. Further theoretical discussion of the procedures used by Mead and Stephens will be reserved until Chapter 3.

One interpretation of the range of behavioural tests sensitive to GluR-1 deletion is that deficits reflect impairments generated by two different neural systems. According to the working memory hypothesis proposed by Bannerman and colleagues one systems reflects disruption to hippocampal processing. The second account proposed by Mead and Stephens, is that GluR-1 deletion compromises functions supported by the BLA. The latter hypothesis will form the main focus of this thesis.

1.66 *GluR-2 KO mice.*

The GluR-2 subunit controls Ca^{2+} permeability of the AMPA receptor (Dingeldine et al., 1999), and loss of the subunit results in receptors with higher Ca^{2+} permeability and causes a nine-fold increase in Ca^{2+} permeability (in response to kainate application), and a two-fold increase in LTP in the hippocampal CA1 region (Jia et al., 1996; Gerlai et al., 1998). GluR-$2^{-/-}$ mice are smaller than wild-type controls and have a higher mortality rate (Jia et al., 1996). Behaviourally these animals show reduced exploration and decreased frequency of visits to a novel object in an open field environment. In addition, these mutant mice show impaired motor coordination, as assessed in a rotorod test (Jia et al., 1996). This sensorimotor impairment might explain the impaired spatial reference memory ability in these mice reported by Gerlai et al., (1998). Here, mice were assessed in both a spatial and non-spatial learning test. In the spatial reference memory task mice received 6 d of hidden platform training (three trials per day), followed by a probe trial (no platform present). Following a 3 d resting period mice were given 3 d non-spatial visible platform training. In both the hidden- and visible platform tasks, GluR-$2^{-/-}$ mice showed an increase in latency to escape the water maze compared to control animals (Gerlai et al., 1998). Eye-closure reflex and motor performance on the rotarod were also abnormal in the KO mice. Thus, GluR-$2^{-/-}$ mice may suffer from an overall non-specifically increased excitability that may alter cognitive functions ranging from stimulus processing, motivation, motor function and learning (Gerlai et al., 1998).

More recently, Mead and Stephens (2003b) have investigated the effects of GluR-2 subunit deletion in stimulus-reward learning. Adopting procedures based on those used for the study of GluR-$1^{-/-}$ mice (Mead & Stephens, 2003a), the authors

reported normal acquisition of Pavlovian conditioning in GluR-2$^{-/-}$ mice. In a subsequent test the cue used in Pavlovian conditioning was capable of maintaining a novel instrumental lever-press response i.e., was capable of acting as a conditioned reinforcer. Interestingly these mice displayed enhanced responding for the cues; a result that the authors suggest is indicative of attributing enhanced motivational value to the CDR (Mead & Stephens, 2003b). This result is consistent with the facilitated levels of hippocampal LTP seen in GluR-2$^{-/-}$ mice (Jia et al., 1996). It should be noted, however, that a trend for KO mice to be hyperactive has been reported (Gerlai et al., 1998). As such, enhanced responding in GluR-2 mutant mice may be indicative of hyperactivity, rather than any enhanced learning process.

Furthermore, the targeted deletion of the GluR-2 subunit resulted in impairments in conditioned approach and PIT. The deficit seen in conditioned approach and PIT could not be attributed to a sensory deficit in KO mice, as these mice could use the cue as a discriminative stimulus for predicting food delivery (i.e., normal Pavlovian conditioning). This pattern of behaviour mimics that seen after lesions of the central nucleus (CeN) of the amygdala (Cardinal et al., 2002). CeN lesions abolish amphetamine-induced potentiation of responding for conditioned reinforcement (Robledo et al., 1996) which is also impaired in GluR-2$^{-/-}$ mice. Thus, Mead and Stephens (2003b) suggested that GluR-2 subunits in the CeN are critical for encoding the formation of stimulus-reward associations for PIT and conditioned approach; although they are not involved in the synaptic processes underlying the motivational value of the CS.

To summarise, mice with a specific deletion of the GluR-2 AMPA receptor subunit have provided insights into the necessity of these receptors for LTP and

behaviour. Deletion of the GluR-2 subunit causes a facilitation of LTP (Jia et al., 1996) and impairments in spatial and non-spatial reference memory (Gerlai et al., 1998). In addition, GluR-2 mutant mice show impairments in conditioned approach and PIT, mimicking a behavioural pattern seen in rats with CeN lesions (Cardinal et al., 2002; Mead & Stephens, 2003b). It should be noted that AMPA subunit KO's have highlighted a rather important finding in the context of the synaptic plasticity hypothesis of memory. GluR-1 deletion impairs hippocampal LTP induction and spatial working memory (e.g., Zamanillo et al., 1999), whereas GluR-2 deletion results in the facilitation of LTP and a disruption in spatial memory (Gerlai et al., 1998). The fact that both an enhancement and impairment in LTP produces the same behavioural phenotype questions the validity of a simple LTP and learning hypothesis (Shors & Matzel, 1997). However, it should be noted that a facilitation of LTP, might occlude LTD, which could explain the noted deficit in GluR-$2^{-/-}$ mice (Abraham, 1997). Alternatively, such a finding might reflect a lack of understanding of how animals solve spatial navigation tasks. Thus the locus of the impairment might be independent of any hippocampal or LTP-like process.

1.67 *Coda.*

The evidence summarised above indicates that: (1) The phosphorylation of the GluR-1 subunit is the locus for the phosphorylation of AMPA receptors (Barria et al., 1997); (2) GluR-1 receptors are critically involved in the constitutive recycling of AMPA receptors from the dendritic tree to synaptic regions following LTP induction (Shi et al., 1999; Hayashi et al., 2000; Shi et al., 2001); (3) GluR-1 receptors are critical for LTP in the hippocampus (Zamanillo et al., 1999); (4) juvenile GluR-1

independent LTP may be related to the establishment of proper hippocampal synaptic connectivity, which occurs before the hippocampus becomes functionally important, possibly indicating normal physiological development following GluR-1 deletion (Jensen et al., 2003); (5) GluR-1$^{-/-}$ mice show normal development and task-specific deficits (Zamanillo et al.,1999; Reisel et al., 2002; Schmidtt et al., 2003; 2004a,b; Bannerman et al., 2004). Two psychological hypotheses have been proposed to explain the GluR-1 mutant behavioural phenotype. The first characterises the deficit as an impairment in spatial working processes that may also be symptomatic of a deficit in memory for the spatio-temporal context in which events occur (Reisel et al., 2002; Schmidtt et al., 2003; 2004a,b). The second hypothesis asserts that deletion of the GluR-1 subunit disrupts motivational processes supported by the amygdala (Mead & Stephens, 2003a).

Collectively these experiments indicate that GluR-1$^{-/-}$ mice are extremely interesting at both a physiological and behavioural level, not least because of the fact that dissociable behavioural impairments are revealed by the mutation (Zamanillo et al., Reisel et al., 2002; Schmidtt et al., 2003; 2004a,b; Bannerman et al., 2004; Mead & Stephens, 2003a). For the purpose of this thesis, I will attempt to further elucidate the nature of the dysfunction suggested by these distinct (not necessarily exclusive) hypotheses of the GluR-1 dependent behavioural phenotype; concentrating predominantly on the disruption of amygdala function proposed by Mead and Stephens.

1.7 *The Amygdala.*

Prior to introducing the literature concerning the psychological functions of the amygdala, I will first briefly review the anatomy and physiology of this system. The amygdala is composed of a topographic almond-shaped structure which is located in the medial temporal lobe. This structure is divided into subdivisions that have extensive internuclear and intranuclear connections distinguished on the basis of cytoarchitectonics, histochemistry and the connections they make (Krette & Price, 1978). In the rodent, the amygdala is comprised of several functionally and anatomically distinct nuclei, that include the lateral (LA), basal (B), basomedial and central (CeN) (Brodal 1957; Pitkänen, 2000). In general, the amygdala nuclei are divided into three groups. Firstly, there is the basolateral complex (BLA) which is composed of LA, B and basomedial sub-systems. The second groups the superficial or cortical-like group, which includes the cortical nuclei and nucleus of the lateral olfactory tract. Finally, the centromedial group which is comprised of the phylogenetically simpler medial nuclei and CeN.

1.71 *Extrinsic Amygdala Projections: Afferent Connections.*

The amygdala receives inputs from all modalities; somatosensory, visual, auditory, olfactory, gustatory and visceral (Figure 1.1; Pitkänen, 2000).

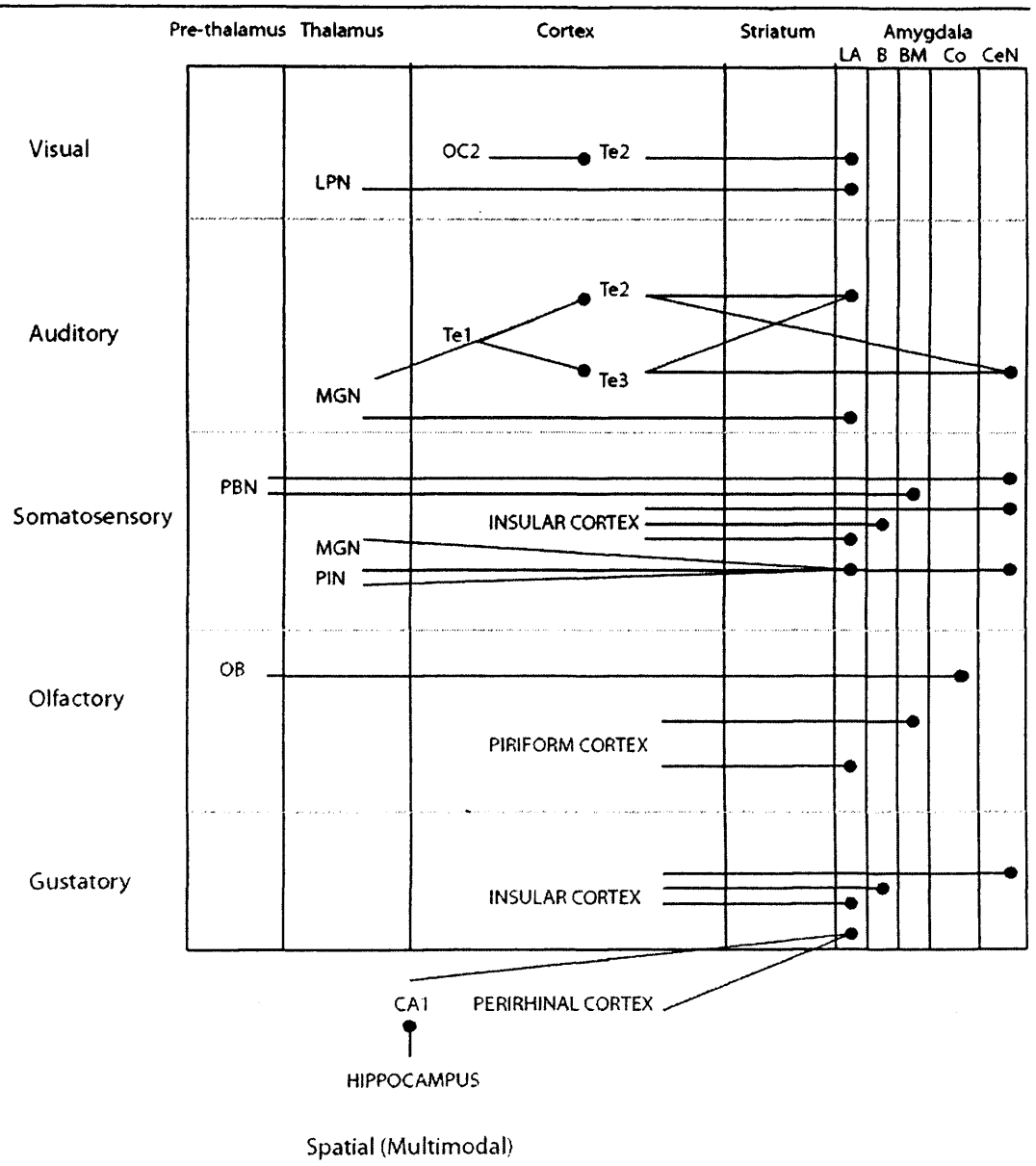

Figure 1.1. *Summary of the sensory inputs into the separate amygdala nuclei.*

This figure is based on anatomical track tracing studies in rats (Pitkänen, 2000; Sah et al., 2003). Where: LPN = lateral posterior nucleus; OC2 = occipital cortex; Te = temporal cortex; MGN = medial geniculate nucleus; PBN = parabrachial nucleus; PIN = posterior intralaminar nucleus; OB = olfactory bulb; LA = lateral amygdala; B = basolateral nucleus; BM = basomedial nucleus; Co = cortical nucleus; CeN = central nucleus.

Somatosensory inputs arise directly from primary somatosensory areas. Most afferents reach the amygdala via the dysgranular parietal insular cortex in the parietal lobe (Shi & Cassel, 1998). These projections target the LA, B and CeN (Pitkänen, 2000). Anterograde tracers revealed strong labelling in the dorsolateral subdivision of the LA, whilst in the B the afferent connections are not segregated (Shi & Cassel, 1998). Moreover, somatosensory information also projects from the pontine parabrachial nucleus and thalamic nuclei, the medial portion of the medial geniculate and the posterior internucleur nucleus (PIN); which has been reported to receive nociceptive information (Bennard, Peschanski & Besson, 1989; Sah et al., 2003).

Visual and auditory information reaches the amygdala from cortical and subcortical areas. For visual information, cortical projections to the amygdala originate from high-order cortical and thalamic visual areas (Shi & Davis, 2001). Cortical projections arise in area Oc2 of the visual cortex and project via the auditory cortex Te2; collectively terminating in the dorsal subdivision of the LA and the lateral subdivision of the amygdala (Sah et al., 2003); whilst subcortically visual information is transmitted via the posterior thalamus (Shi & Cassel, 1998). Similarly, for auditory information anterograde tracers have revealed that the primary auditory cortex (area Te1) has no direct projections to the amygdala; rather, it is area Te3 and Te2 which projects directly to the LA and CeN (Pitkänen, 2000; Sah et al., 2003). Subcortically, auditory information is transmitted via the thalamic medial geniculate nucleus to the LA (LeDoux, Farb & Ruggiero, 1990).

Olfactory projections arise from the primary olfactory cortex as well as the main and accessory olfactory bulbs (Shi et al., 2003). The main olfactory bulbs projects from the nucleus of the lateral olfactory tract, anterior cortical nucleus, and the periamygdaloid cortex. The accessory olfactory bulb projects from the bed

nucleus of the accessory olfactory tract, the medial nucleus and the posterior cortical amygdala (Pitkänen, 2000). Moreover, the piriform cortex and anterior olfactory nucleus have projections to the LA, B and accessory basal nuclei (Shi et al., 2003). The dorsal endopiriform nucleus additionally projects to all cortical nuclei of the amygdala as well as the nucleus of the lateral olfactory tract, the periamygdaloid cortex and the medial amygdala (Pitkänen, 2000). Thus, the amygdala has extensive afferent connections with all regions of the olfactory nuclei (Sah et al., 2003).

Gustatory and visceral primary areas in the anterior and posterior insular cortices provide strong projections to the dorsal subdivision of LA, posterior B, and CeN (Pitkänen, 2000). Moreover, afferent connections from the thalamic gustatory nucleus terminate in the LA, B, and accessory basal nuclei (Sah et al., 2003), and those from the parabrachial nucleus, which receives projections from the nucleus of the solitary tract, target the accessory basal nuclei (Bernard, Alden & Besson, 1993). As with visual and auditory information, visceral and gustatory information arrive from both cortical and subcortical inputs to converge in the amygdala (Pitkänen, 2000).

Finally, there are several loci with which direct multi-modal information reaches the amygdala. These include the hippocampal formation, perirhinal cortex and the prefrontal cortex. The hippocampal formation, consisting of the hippocampus proper, the dentate gyrus, the subicular complex and the entorhinal cortex, communicates its information via reciprocal connections with the separate amygdala nuclei (McDonald, 1998; Maren, 1999). Specifically for the hippocampus proper afferent connections project from the CA1 region (and subicular complex) to the LA, B, accessory basal and CeN; although no afferent connections project from the dentate gyrus to the amygdala nuclei (Pitkänen, 2000). Similarly, the perirhinal cortex

transmits multi-modal information predominantly to the LA, although projections from the ventral portion of the perirhinal cortex to the BL and projections from the dorsal portion to the accessory basal have also been described (Pitkänen, 2000; Bucci et al., 2001).

In summary, the amygdala receives somatosensory, visual, auditory, gustatory, visceral and multi-modal information from a range of cortical and subcortical structures. Finally it should be noted that the LA, medial and CeN receive substantial inputs from the hypothalamus; whilst for brain stem inputs (including the midbrain, pons and medulla oblongata; Carlson, 2001), the CeN is a major target. In contrast, other amygdala nuclei receive few or no inputs from this area (Cardinal et al., 2002).

1.72 *Extrinsic Amygdala Projections: Efferent Connections.*

The amygdala projects to numerous cortical, hypothalamic and brain stem regions (Figure 1.2). The LA has substantial efferent connections with the infralimbic cortex, perirhinal cortex, the entorhinal cortex and the hippocampus proper (via CA1 and subiculum; Sah et al., 2003). Similarly the B sends substantial projections to the infralimbic cortex, anterior cingulate cortex, insular cortex, entorhinal cortex, piriform cortex and the hippocampus proper via CA1, CA3 and parasubiculum and subiculum (Petrovich, Canteras & Swanson, 2001). Additionally, the B has strong efferent connections with the striatum and basal forebrain including the bed nucleus of the stria terminalis (BNST) and the nucleus accumbens (McDonald, 1991). In addition, the B has a well-defined projection to the medial dorsal nucleus of the thalamus and the lateral hypothalamus (Pitkänen, 2000). That is, the BLA receives and transmits information to a range of cortical and subcortical structures.

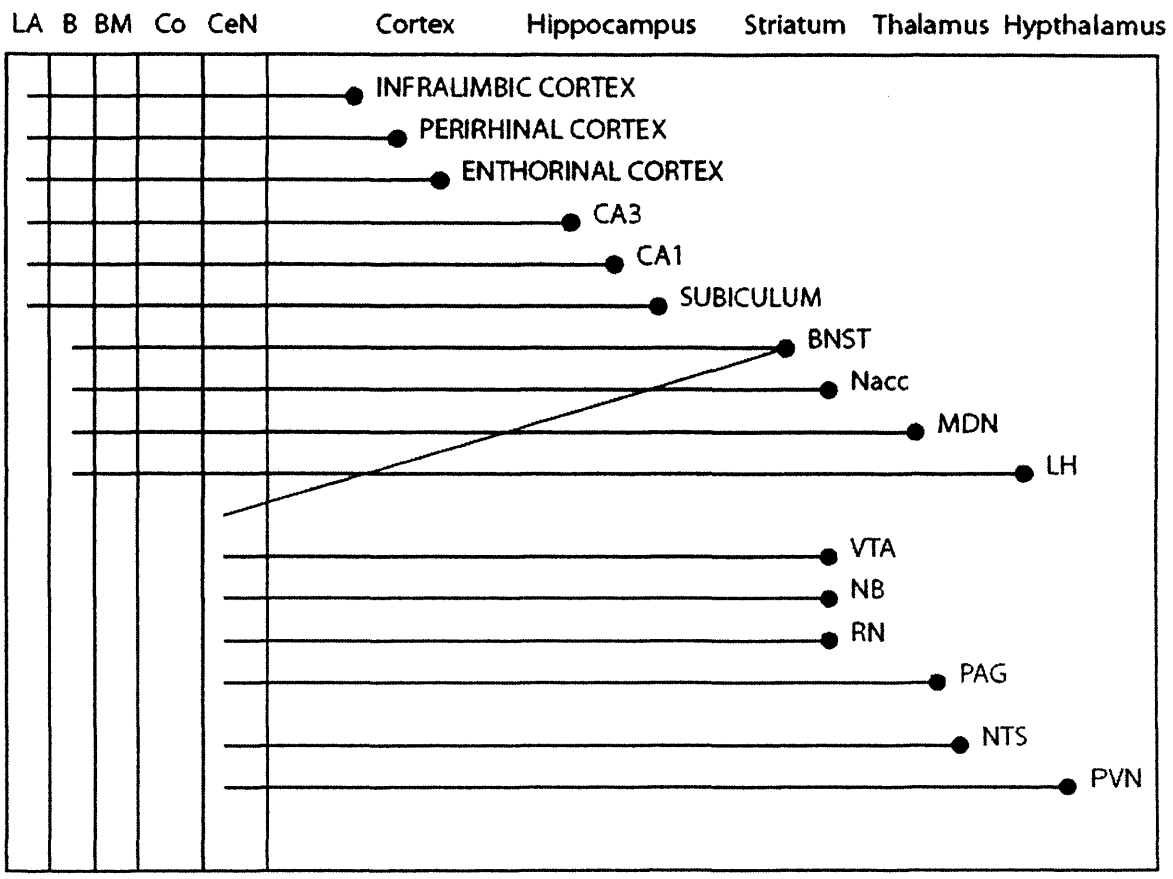

Figure 1.2. *Summary of efferent projections from the amygdala nuclei.*

This figure is based on the anatomical studies in rats. Where: Nacc = nucleus accumbens; MDN = medial dorsal nucleus; LH = lateral hypothalamus; VTA = ventral tegmental area; NB = nucleus basalis; RN = raphae nuclei; PAG = periaqeuductal gray; NTS = nucleus of the solitary tract; PVN = paraventricular nucleus. ; LA = lateral amygdala; B = basolateral nucleus; BM = basomedial nucleus; Co = cortical nucleus; CeN = central nucleus.

In contrast, the CeN is viewed as a controller of the brain stem; as such it orchestrates behavioural, autonomic and endocrine responses to a range of hypothalamic and brain stem structures (Cardinal et al., 2002). The CeN and the BNST have strong projections to ascending monoaminergic and cholinergic neuron

groups. These include but are not limited to the dopaminergic substantia nigra, ventral tegmental area (VTA), the noradrenergic locus coeruleus, the cholinergic nucleus basalis and the serotonergic raphae nuclei (Sah et al., 2003). Each of these separate systems innervate large regions of the forebrain and medial temporal lobe structures as well as providing inputs to the amygdala. Additionally, the medial subdivision of the CeN has strong projections to the hypothalamus, BNST and also to the brain stem (Sah et al., 2003). Projections to the brain stem include the periaqeuductal gray; the parabrachial nucleus; and the nucleus of the solitary tract (NTS).

The hypothalamus projects to autonomic cell groups in the brain stem and spinal cord. Efferents from the lateral subdivision of the CeN and from nuclei related with the olfactory system in the amygdala also project to these areas. The ventromedial nucleus (involved in reproductive behaviour) is also innervated by nuclei related to the olfactory system in the amygdala, particularly the medial nucleus, posterior basal nucleus, and posterolateral cortical nucleus. The medial nucleus also sends projections to the hypothalamic neuroendocrine zone, mainly to the anterior paraventricular nucleus (Dayas, Buller & Day, 1999).

1.73 *Intrinsic Amygdala Circuitry.*

The intrinsic circuitry detailed in this section is adapted from that reported by Pitkänen (2000) using tract tracing studies. The LA sends extensive projections to the B and the accessory basal nuclei and the capsular part of the CeN. However, the CeN is the only region where these connections are not reciprocal. It should be noted that most reciprocal projections in the LA terminate in the ventrolateral subdivisions of the LA (Shi et al., 2003). The presence of the LA to medial subdivision has been suggested to be a site for integration of sensory information (LeDoux, 2000).

The B and accessory basal nuclei have extensive inter-, in addition to, intranuclear connections. The B projects to both the LA and the CeN. However, the largest of these projections is to the medial subdivision of the CeN. Since the hypothalamic and brain stem projections from the amygdala terminate in the CeN, it is assumed that the B has also a role in controlling output of the amygdaloid complex (Shi et al., 2003).

The accessory basal projects to the LA and CeN. However, only connections with the LA are reciprocal. The intrinsic CeN connections are largely afferent in nature, with connections from the LA, B and accessory basal; whilst no efferent connections from the CeN to the LA or B regions exist (Pitkänen, 2000). Thus, the BLA has both strong efferent and afferent internuclear and intranuclear connections; whilst the CeN connections are mostly afferent in nature.

1.74 *BLA Intrinsic Circuitry and GluR-1.*

The BLA contains two major classes of neurons; predominantly GABAergic non-pyramidal interneurons; and pyramidal principal projection neurons (McDonald, 1996). Studies of the localisation of GluR-1 distribution in the BLA, identified marked immunoreactivity in a specific sub-population of non-pyramidal interneurons (McDonald, 1992; McDonald, 1996; Mahanty & Sah, 1998). Consistent with the presence of AMPA receptors at these interneurons, NMDA-independent LTP can be evoked at these synapses (Mahanty & Sah, 1998). Moreover, BLA interneurons display a high permeability to Ca^{2+} indicating AMPA receptors lacking the GluR-2 subunit. Since GluR-2 is absent in most local circuit GABAergic neurons in the BLA, this cell class-specific difference in subunit stochiometry has implications for the response properties as well as selective vulnerability of neurons within this region of the amygdala (He et al., 1999). Due to the high-calcium permeability of non-GluR-2 containing AMPA receptors, these receptors are more vulnerable to glutamate-induced toxicity (Dingeldine et al., 1999).

In the normally functioning BLA, AMPA receptors (non-GluR-2) mediate the excitability of GABAergic interneurons (via a disynaptic NMDA-independent LTP process; Mahanty & Sah, 1998). Further, these GABAergic interneurons control the GABAergic pyramidal projection neurons via a $GABA_A$ mediated process (Rainnie, Asprodini & Shinnick- Gallagher, 1991) involving NMDA and AMPA (GluR-2 containing) receptors (Mahanty & Sah, 1998). However, in response to GluR-1 subunit deletion, it is quite possible that BLA interneurons exhibit a profound reduction in their excitability, with a consequent disruption on firing patterns of BLA pyramidal output neurons, to which they normally provide an inhibitory control

(Rainnie et al., 1991; Mead & Stephens, 2003a). This could result in aberrant synaptic processing both within the local-circuitry, and through outputs of the BLA. That the deletion of GluR-1 interferes with synaptic activity in the BLA was supported by the upregulation of GluR2/3 subunits in the BLA of GluR-1$^{-/-}$ mice; further, no such changes were reported in the CeN of these mice (Mead & Stephens, 2003a).

Thus, it is suggested that as a result of aberrant synaptic processes in the BLA, GluR-1$^{-/-}$ mice display a specific deficit on tasks which require the mice to encode the motivational properties of an associatively activated outcome. In support of this proposition, it has been reported that GluR-1$^{-/-}$ mice show impairments in conditioned reinforcement and second-order operant responding (Mead & Stephens, 2003a); tasks that are sensitive to lesions to the BLA (Hatfield, Han, Conley, Gallagher & Holland, 1996; Everitt, Cardinal, Hall, Parkinson & Robbins, 2000; Cardinal et al., 2002).

1.8 *Experimental Aims.*

Prior to the assessment of BLA-dependent behaviour in GluR-1$^{-/-}$ mice, it was necessary to compare GluR-1$^{-/-}$ and wild-type control mice in a set of tests designed to assess sensorimotor and general affective aspects of behaviour which are described in Chapter 2. These studies simply attempted to evaluate the extent of non-specific deficits in mutant mice that may compromise interpretation of the behavioural syndrome.

Chapter 3 assessed the effects of GluR-1 deletion on contextual and cued fear conditioning; a task that is acknowledged as highly sensitive to lesions of the BLA (e.g., LeDoux, Sakaguchi & Reis, 1986). The simple nature of this learning task, and the large body of evidence implicating the amygdala in this form of learning provided

an opportunity to examine the influence of the GluR-1 mutation on emotional learning (Maren, 2000a; Cardinal et al., 2002). According to the BLA deficit hypothesis I predicted that GluR-1 mutant mice would show impairments in both cued and contextual fear conditioning. In contrast the working memory hypothesis would predict no impairment, as the procedure can be characterised as a reference memory procedure.

Chapters 4 and 5 made use of separate Pavlovian and instrumental preparations which characterised different affective and sensory-specific associatively activated outcome representations; using paradigms which have previously been shown to be sensitive to BLA damage in rats (Blundell, Hall & Killcross, 2001; Balleine, Dickinson & Killcross, 2003; Corbit & Balleine, 2005).

Chapter 2.

2.1 Introduction.

The purpose of this experimental chapter is to provide an assessment of sensorimotor and affective behaviours in GluR-1$^{-/-}$ mice. Despite the molecular specificity that can be achieved by genetic engineering, analysis of the resulting mutant can be nonetheless difficult to interpret. Thus, an impairment on a particular learning task in mutant mice may be attributed to deficits in, for example, synaptic plasticity processes or interactions with background genes and/or other non-specific perturbations related to the mutation (Crawley et al., 1997). As such, the compensatory changes triggered by this disruption may induce sensorimotor impairments which could interfere with learning and memory processes.

In respect to sensorimotor capabilities, it has been reported that GluR-1$^{-/-}$ mice show subtle differences in their behaviour following systematic assessment of sensorimotor and affective behaviour (Bannerman et al., 2004). Consistent with Vekovischeva et al., (2001), Bannerman and colleagues reported that mutant mice were consistently hyperactive and displayed impaired motor coordination as assessed on the accelerating rotarod, the multiple static rod and the horizontal bar task. Interestingly, the authors reported that GluR-1$^{-/-}$ mice were more anxious then their wild-type counterparts. This was assessed by a test for hyponeophagia. Mutant mice took significantly longer to start drinking a novel sweetened milk solution in a novel context. However, in a black-white alley test mutant mice showed comparable entries into the white compartment as controls (Bannerman et al., 2004). The fact that GluR-1$^{-/-}$ mice appeared to be more anxious when assessed through hyponeophagia contrasts with the postulated BLA-impairment in GluR-1$^{-/-}$ mice (Mead & Stephens, 2003a). That is, rats and mice with lesions to this region are generally reported to be

less anxious (e.g., Dunn & Everitt, 1988). Unfortunately, the fact that mutant mice were hyperactive may have increased the latency to contact and consume the milk. We therefore cannot conclude with any certainty that GluR-1 mutant mice show increased anxiety relative to WT control mice.

The aim of the current experiments was to assess basic sensorimotor and affective behaviours in mice with a targeted deletion of the GluR-1 AMPA subunit. Experiment 1 examined basic sensorimotor capabilities using a simple habituation task. GluR-1$^{-/-}$ mice were initially exposed to a novel context where the level of locomotor activity could be assessed using an automated procedure (c.f., Vekovischeva et al., 2001; Bannerman et al.., 2004). In the second stage of the experiment, a visual cue was presented to the mice over several sessions. This procedure provided a gross assessment of visual acuity in mutant mice through the initiation and habituation of the orienting response (OR; Sokolov, 1963). The OR also provided a measure of whether GluR-1 mutant mice modified the degree of processing (or attention) directed towards the visual stimulus following non-reinforced exposure (Swan & Pearce, 1988). This simple test therefore provided an assessment of a number of fundamental sensorimotor and information processing resources in GluR-1 mutant mice.

In order to make contact with extant data on the GluR-1 mutant mouse, Experiment 2, attempted to replicate the spatial working memory deficit reported by Bannerman and colleagues using the T-maze non-matching-to-position task (Reisel et al., 2002). Experiments 3 and 4, examined the claim by Bannerman et al., (2004) that GluR-1$^{-/-}$ mice are more anxious then their wild-type counterparts. Mice were tested on the elevated plus-maze (EPM; Experiment 3). Normal rodents avoid open elevated arms of the maze and spend more time in the enclosed arms of the maze

(Montgomery, 1955). In Experiment 4, we examined food neophobia in mutant mice (Experiment 4), using a two-choice preference test to determine whether food avoidance reflected a non-specific deficit in contacting food in an open arena (see also, Dunn & Everitt, 1988).

2.2 Experiment 1.

The present experiment was designed to simply provide an initial assessment of the behavioural phenotype in GluR-1$^{-/-}$ mice in terms of basic sensorimotor and information processing mechanisms. Mice were placed in a novel environment (operant chamber) and locomotor activity elicited by the context was examined over 6 sessions. The mice then received phasic presentations of a novel visual stimulus. To determine the extent to which the cue was processed I monitored the initiation and the habituation of the orienting response elicited by the cue over ten sessions of training. This procedure provided a basic measure of visual information processing and a measure of memory processes in terms of both short (within-session) and long-term (between-session) habituation of the OR (Mackintosh, 1974; Wagner, 1981)

2.2.1 Method.

Subjects and Breeding.

The experiment was conducted in two replications with naïve wild-type GluR-1 controls (n = 20) and GluR-1$^{-/-}$ mice (n = 20). Mice were bred in the Department of Experimental Psychology at the University of Oxford and transferred to the School of

Psychology, Cardiff University for behavioural testing at 6 months of age. In all the experiments mentioned in this thesis the GluR-1 mutant mice and wild-type controls were derived and genotyped at Oxford University. ES cells from the 129/SV background strain were electroporated with the targeting vector (the GluR-1 gene). The transfected ES were then selected using neomycin screening, and were subsequently injected into C57BL6 blastocytes. The chimeric offspring were then backcrossed with C57BL6 mice. Finally, this produced heterozygous male and female GluR-$1^{+/-}$ mice, which were subsequently mated producing wild-type (approx. 25%), KO (approx. 25%) and heterozygous (approx. 50%) mice. Mice were genotyped at approximately 9 weeks after birth. All behavioural analysis was carried out using batchmates with matching ages. All GluR-$1^{-/-}$ and wild-type mice were male littermates. In Experiment 1, mice were individually housed on a 12 h light:`dark schedule (lights on at 07:00 h), in plastic cages with wood shaving and bedding. All behavioural testing took place during the light phase between 09:00h and 17:00h. All experiments were conducted under the auspices of the U.K. Home Office Project license held by Dr. Mark Good and Personal license held by the author.

Apparatus.

The mice were exposed to stimuli through the use of a pair of Colbourn operant chambers (internal dimensions: 18 cm wide x 17 cm deep x 21 cm high; supplied by Colbourn Instruments). Each chamber was housed in a sound-attenuating box. Each chamber had two aluminium walls, a transparent Perspex wall and a Perspex door that served as a fourth wall. The two side aluminium walls were divided into three sections, allowing for the introduction of additional stimuli. The ceiling was

also aluminium, and contained an infrared detector measuring the animals' movement (Colbourn Instruments; Model H24-61MC; set to mouse sensitivity) positioned above a hole in the roof panel. The activity monitor recorded the change in position of the subjects' infrared body heat signature. The infrared monitor was capable of detecting both lateral and vertical (rearing) movements (see www.Colbourn.com for further information). This system calculates the animals movement in 'movement units'; each unit corresponding to whether movement was detected during a 20 millisecond period. This method was used to provide a measure of locomotor activity (see also Barnes & Good, 2004). The chambers received ambient illumination from a house light operated at 24V located on the middle section at the top of the right hand side wall. The light stimulus was located on the far section at the bottom of the right hand side wall, and was elevated 5 cm from the floor of the chamber. The light could be presented either at a constant rate or at a pulsed rate (alternating .25 s on and .25 s off). The mouse's behaviour was video recorded using a camera located at the rear of the chamber behind the transparent wall and attached to a Goodmans or Panasonic VCR, for chambers 0 and 1 respectively.

Procedure.

Stage 1: Context Habituation.

The mice were first habituated to the experimental context before the introduction of the visual target stimulus. The mice were placed in to the operant chambers for 20 minute periods for a total of 6 days.

Stage 2: Light Habituation.

On day seven, mice were given one 30-min light habituation session per day for a total of 10 days during which the orienting behaviour of the mice was measured. No stimuli were presented for the first 6 minutes to allow for the mice to accustom themselves to the context. Each mouse then received 10 presentations of a visual stimulus. For half of the mice the visual cue was a constant illumination of a stimulus light, whereas for the remaining half of the mice the visual cue was pulsed light (2 sec on and 2 sec off). Each cue was presented for 30-seconds, with a 120 s fixed inter-trial interval (ITI).

Scoring.

During presentations of the light stimulus, we scored whether an OR occurred. An OR was defined as the tip of the mouse's snout moving in close proximity (5cm) to the light. This was scored by drawing two identical reference frames around the light source on the monitor for each chamber. The number of occasions on which the animals' snout entered in to the reference frame was scored during each light presentation. Video scoring was conducted by AJ and a second observer, who were "blind" with respect to the genotype and group of the animal. Pearsons correlation conducted on the scores between the two observers revealed a high degree of inter-rate reliability between the two observers, Pearsons ($r = 0.91$, $r^2 = 0.87$, $p<0.01$).

Statistical Analysis.

For all experiments in this thesis statistical calculations were carried out using analysis of variance (CLR ANOVA, v2.0, Clear Lake Research Incorporated, USA). Prior to calculation of the analysis of variance the data were checked to ensure that the assumptions of ANOVA were not violated. The interactions involving groups were analysed using tests of simple main effects and the pooled error term. Where appropriate, group differences were evaluated using the Newman-Keuls post-hoc comparison test. A type 1 error rate of $p<.05$ was adopted for all statistical tests.

2.2.3 Results.

Context Habituation.

Figure 2.1.1 shows the automated activity levels recorded during the six sessions of habituation to the context. Initially GluR-1$^{-/-}$ mice demonstrated more locomotor activity in the novel environment compared to wild-type controls. However, by the fourth session of testing, both GluR-1$^{-/-}$ and control mice displayed similar levels of activity. This interpretation was confirmed by a three-way mixed ANOVA with genotype and session as factors. The ANOVA revealed no main effect of genotype ($F_{(1,38)} = 1.76$, $p>0.05$); a main effect of session ($F_{(5,190)} = 66.8$, $p<0.01$) and a significant interaction between these factors, ($F_{(5,190)} = 5.13$, $p <0.01$). Tests of simple main effects revealed significant differences between the groups on session 1 ($F_{(1,77)}= 7.57$, $p < 0.01$) and session 3 ($F_{(1,77)} = 5,43$, $p < 0.05$) There were no significant differences between the groups on sessions 2 and 4-6 (max F session 2,

$F_{(1,77)} = 3.10$, $p>0.05$). Thus, consistent with previous research (Vekovischeva et al., 2001; Bannerman et al., 2004), GluR-1$^{-/-}$ mice displayed higher levels of locomotor activity in a novel context. However, this hyperactivity in GluR-1$^{-/-}$ mice was transient, such that mutant mice showed habituation of exploratory activity and by the end of training showed comparable levels of locomotor activity to control mice.

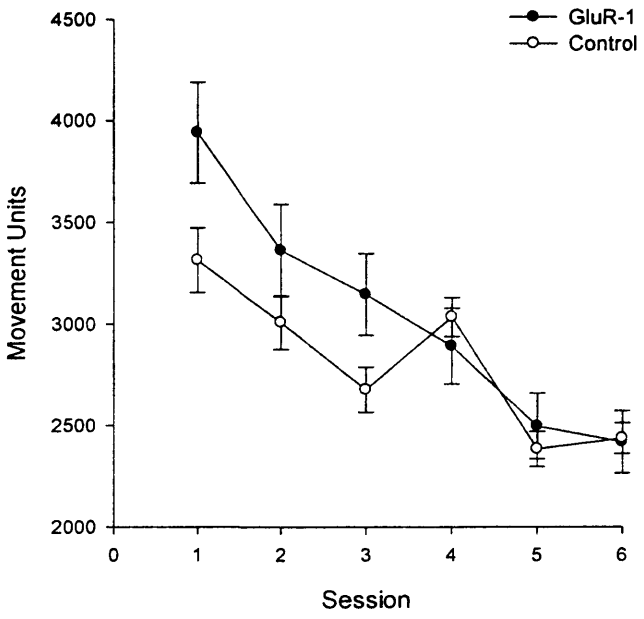

Figure 2.1.1 *Context Habituation: Locomotor activity scores.* Mean locomotor activity during the six sessions of context habituation. Closed circles = GluR-1$^{-/-}$ locomotor activity scores; open circles = wild-type control locomotor activity scores. Error bars equal standard error of the mean.

Habituation of the orienting response to a localised visual stimulus.

Figure 2.1.2a shows the overall percentage of OR's to a light during the ten sessions of habituation. In general, both GluR-1$^{-/-}$ and control mice showed a steady

decline across sessions in the percentage of trials with an OR to the light. A two-way ANOVA confirmed this observation and revealed a non-significant main effect of genotype ($F_{(1,38)} = 3.54$, $p>0.05$), a main effect of session ($F_{(9,342)} = 16.7$, $p<.01$), and a significant interaction between these factors ($F_{(9,342)} = 2.82$, $p < 0.01$). Tests of simple main effects revealed significant differences between the groups on sessions 7-10 (smallest F-value; session 8, $F_{(1,150)}=3.95$, $p < 0.05$). Thus, GluR-1$^{-/-}$ mice initially showed a level of orienting that was comparable to control mice. However, the OR declined across sessions at a faster rate in GluR-1$^{-/-}$ mice than in control mice.

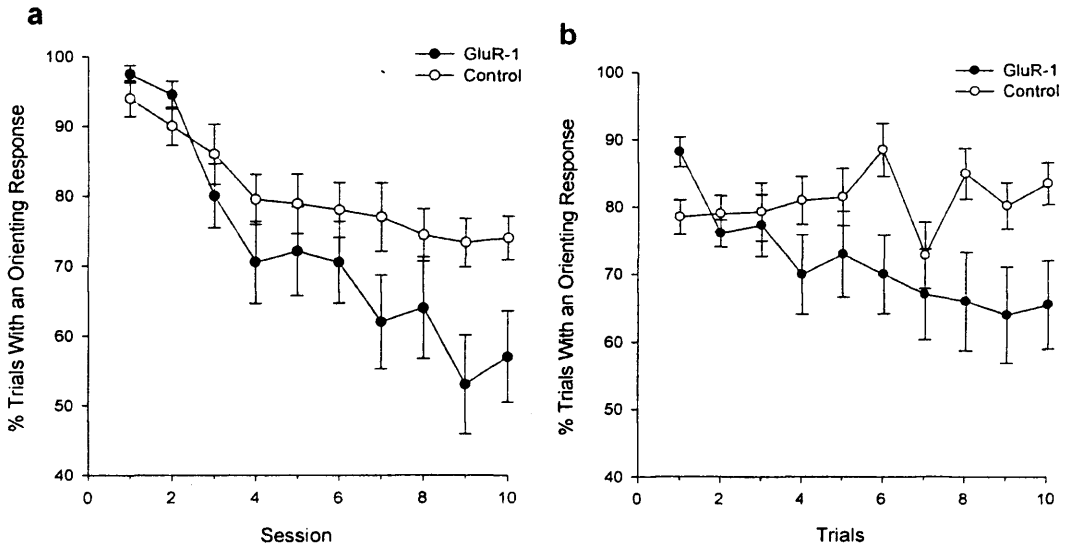

Figure 2.1.2 *Habituation of the orienting response to a localised visual stimulus.* **(a)** Mean percentage of trials in which an orienting response occurred. **(b)** Mean percentage of orienting responses occurring on each particular trial collapsed across all sessions. Closed circles = GluR-1$^{-/-}$ mice; open circles = wild-type control mice. Error bars indicate standard error of the mean

To determine whether the difference in the rate of decline of the OR reflected increased locomotor activity in GluR-1$^{-/-}$ mice, activity during the first 6 minutes of acclimatisation to the conditioning chamber, prior to the first stimulus presentation, was examined (Table 2.1.3). This period was analysed to provide a measure of locomotor activity that was not influenced by behaviour generated by the light source in control and mutant mice. The overall mean level of locomotor activity averaged across exposure session was 610 (SE 45.46) units for GluR-1$^{-/-}$ mice and 701 (SE 48.65) units for control mice. An ANOVA with group and session as factors revealed no significant main effect of group ($F_{(1,38)}$= 1.88, p>0.10), a main effect of session, ($F_{(9,342)}$= 2.25, p<.01) and no significant interaction between these factors (F<1). Thus a gross increase in locomotor activity would seem to be an unlikely explanation for the more rapid decline in the OR in GluR-1$^{-/-}$ mice.

SESSION	WILD-TYPE CONTROL	GLUR-1$^{-/-}$
1	759 (52.3)	702 (49.9)
2	736 (59.5)	635 (52.1)
3	742 (48.1)	623 (48.3)
4	693 (37.8)	603 (50.5)
5	749 (44.2)	611 (48.2)
6	675 (37.9)	606 (43.7)
7	698 (47.5)	601 (39.3)
8	682 (39.1)	593 (49.2)
9	660 (44.4)	570 (47.7)
10	620 (37.2)	556 (44.3)

Table 2.1.3 *Locomotor activity scores prior to stimulus presentation during OR stage.* Mean locomotor activity scores prior to presentation of visual cue for wild-type control (left panel) and GluR-1$^{-/-}$ mice (right panel). Values represent group means followed by standard error in parentheses.

Subsequent analysis examined how the animals' behaviour changed across stimulus presentations within each training session. Figure 2.1.2.b shows the percentage stimulus presentations accompanied by an OR, averaged across all sessions. Inspection of this figure revealed that GluR-1$^{-/-}$ and control mice initially displayed high levels of orienting toward the light. However, as the number of stimulus presentations increased, GluR-1$^{-/-}$ mice displayed a more rapid decline in orienting toward the light than control mice.

In order to evaluate these differences, a two-way ANOVA was conducted, with factors of genotype and trial. This revealed a non-significant main effect of genotype ($F_{(1,38)} = 3.54$, $p>0.05$), trial ($F_{(9,342)} = 3.80$, $p<.01$), and, importantly an interaction between the two factors ($F_{(9,342)} = 5.65$, $p<.0001$). Analysis of simple main effects revealed a main effect of genotype on trials 6 and 8-10 (smallest F-value; trial 2, $F_{(1,100)} = 7.14$, $p<.01$). The analysis indicates that GluR-1$^{-/-}$ mice showed a more rapid decline in the OR evoked by a visual stimulus within sessions.

2.2.4 Discussion.

Consistent with previous findings (Veckovisheva et al., 2001; Bannerman et al., 2004) the experiment clearly demonstrated that young GluR-1$^{-/-}$ mice were hyperactive in response to a novel environment (Figure 2.1.1). Nevertheless, the difference between the groups was ameliorated by extended exposure to the context. However, GluR-1$^{-/-}$ mice showed normal levels of initial orienting to a novel visual stimulus and a more rapid within-session and between-session decline in the orienting response compared to control mice. Two main conclusions may be drawn from this pattern of results. First, the initial sensitivity to the presentation of a novel visual cue

is intact in GluR-1$^{-/-}$ mice. Therefore, mutant mice do not appear to suffer from any gross visual impairment. Second, the results suggest that the mechanisms underlying habituation of the OR are facilitated in GluR-1$^{-/-}$ mice. Before I consider the implications of these results for memory processes in GluR-1$^{-/-}$ mice I will first describe a theory of habituation that has provided an informative framework for understanding habituation in normal animals (Honey & Good 2000a).

Wagner's standard operating procedures (SOP; Wagner, 1981) assumes that the presentation of a stimulus will activate a memory of itself that consists of a set of elements. When the stimulus is unexpected, or novel, a proportion of a cue's elements move from an inactive state (I) to a primary state of activation in memory, referred to as the A1 state. The representation then decays to a secondary state of activation, the A2 state, from where the representation decays to the original inactive state (I). The theory asserts that when a proportion of a cue's elements are in the A1 state, the stimulus can readily evoke its unconditioned response and may enter into associations with other representations also in the A1 state. However, when the elements of a representation are in the A2 state, they (1) cannot move into the A1 state and (2) are less capable of evoking an unconditioned response. Elements in the A2 state eventually decay into the inactive state. The A1 state has been allied to the "focus of attention" or "rehearsal", whereas the A2 has been allied to a short-term memory store and the inactive state to long-term memory. That is, SOP evaluates the representational activity that may be produced directly by a stimulus, or indirectly via associative connections between the CS and a US. The model assumes that when a stimulus is presented, its presentation activates a proportion of elements from the inactive long-term store, to the A1 state, which will subsequently decay first to the A2 state and then back to the inactive state. The decay rates from the two active states

differ, such that decay is greater from the A1 to the A2 state (pd1) than from A2 to the inactive state (pd2). Similarly, the capacity of the A1 state, governs the number of nodes which may be concurrently active, such that the capacity of the A1 node is less than that of the A2 node.

According to Wagner (1981), habituation (the decline in an unconditioned response) occurs as a result of an increasing number of a cue's representational elements entering into the A2 state. This occurs as a result of priming the cue's representational elements into the A2 state following either a recent presentation of the cue (self-generated priming) or via associations formed between the context and the target cue (context or associative priming). According to Wagner, as the association between the context and target cue increases in strength, the context successfully primes more of the target cues elements from the inactive state to the A2 state of activation. As a result, a proportion of the target cues elements are already in the A2 state of activation at the time the cue is presented and fewer cue elements are available for activation in the A1 state. As a target cue becomes better predicted by the context, more of its elements are provoked into the A2 state and the magnitude of the unconditioned response (OR) declines.

In respect to the previous experiment, SOP could account for the facilitated habituation noted in the mutant group, as either an increase in decay rate between A1 and A2, or a limited capacity to maintain elements in the A1 state. Thus, at the start of training, initial presentation of the visual cue will elicit a majority of elements in the A1 state. However, following repeated presentation, the visual cue will evoke more of its elements in the A2 state. One could suggest this is consistent with habituation noted by both groups, resulting in fewer OR's directed toward the cue (Figure 2.1.2). If however, the decay rate between A1 and A2 (i.e., pd1 & pd2) is greater in the

mutant mice, then SOP would predict a reduction in OR's directed to the cue, in the KO group compared to controls. Similarly, decreasing the capacity of the A1 state, would reduce the attention paid to the visual cue, consequently resulting in a reduction of OR elicited by the cue in mutant mice. Although we are unable to discriminate between these accounts, it is interesting to note that GluR-$1^{-/-}$ mice show impaired induction of hippocampal long-term synaptic potentiation (Zamanillo et al., 1999). The disruption to the mechanisms underlying LTP in GluR-$1^{-/-}$ may provide a physiological substrate for a putative rapid decay of information from the A1 state of activation in mutant mice. However, this suggestion is clearly speculative as the precise relationships between the synaptic mechanisms underlying LTP and learning remain to be fully characterised (Shors & Matzel, 1997).

The present study has revealed a facilitated pattern of habituation of cue processing in GluR-$1^{-/-}$ mice. Previous studies have shown that GluR-$1^{-/-}$ mice are particularly sensitive to spatial working memory paradigms; although acquisition of spatial reference memory tasks appears to be unimpaired (Reisel et al., 2002; Schmitt et al., 2003, 2004b). The habituation task used in the present study is more readily characterised as a reference memory procedure (Olton & Papas, 1972). However, an impairment in spatial working memory may also be explained in terms of a disruption to A1 and/or A2 state processing. For example, published tests of spatial working memory in GluR-$1^{-/-}$ mice have used a procedure involving repeated presentations of sample cues. For example, in the T-maze alternation task, used by Reisel et al., (2002), mice were required to avoid the most recently visited of two familiar arms. The ability to discriminate between two recently visited arms may rely on the proportion of elements of each goal arm representation present in the A1 state. For example, exposure to a recently visited arm will promote its elements in the A2 state

(self-generated priming). The relatively novel arm, on the other hand, may provoke relatively more of its elements in the A1 state and thus elicit a stronger approach response. This proactive interference would be expected to mediate the familiarity of the cues associated with the novel and recently visited arm. As such, for the novel arm one would expect the performance weightings (W1) associated with the A1 elements to be greater in respect to the performance weightings (W2) associated with the A2 elements. In contrast, for the recently visited arm one would expect W2 to be greater than W1. However, if the GluR-1 mutation results in a more rapid decay of elements from the A1 state into the A2 state; or representational elements are maintained longer in the A2 state before decaying into the I state, the discrimination between two familiar arms may be more difficult. That is, proactive interference could be expected to interfere with spatial working memory performance in GluR-$1^{-/-}$ mice. This analysis is of course speculative, but it does lead to the prediction that if GluR-$1^{-/-}$ mice are sensitive to the effects of proactive interference than a longer ITI may improve performance in mutant mice by allowing any elements in the A2 state to decay into the I state.

In summary, the present study has shown that mice with a targeted deletion of the GluR-1 receptor are initially hyperactive on exposure to a novel context. However, locomotor activity declined rapidly to a level shown by control mice. GluR-$1^{-/-}$ mice also showed normal levels of orienting to a novel cue. The OR, however, declined more rapidly following subsequent presentations of the cue in mutant mice, both within and between training sessions. This change in the modulation of the OR suggests that the processing of visual information by mutant mice is facilitated. One potential explanation for this pattern of deficits in mutant mice may be derived from Wagner's SOP model and suggests that the interaction between A1 and A2 states may

be impaired. For example, decay of information from the A1 state (the focus of attention) to the A2 state may occur more rapidly in mutant mice. Experiment 2 examined one implication of this analysis for the GluR-1 spatial working memory deficit.

2.3 Experiment 2.

Bannerman and colleagues (Reisel et al., 2002) showed that GluR-1 mutant mice are particularly sensitive to spatial working memory tasks, showing enduring deficits in T-maze NMTP. Experiment 1 revealed that mutant mice showed a more rapid decline in the OR elicited by a visual stimulus and it was suggested that this may reflect disruption to memory processes characterised by Wagner's SOP theory of habituation. Here I investigated the possibility that Wagner's memory processes may also offer an explanation for the sensitivity of GluR-$1^{-/-}$ mice to spatial working memory. Thus, the ability to discriminate between two familiar arms may rely on the proportion of elements of each goal arm representation present in the A1 state. For example, exposure to a recently visited arm will promote more activity of its representational elements in the A2 state (i.e., self-generated priming). On the other hand, relatively more of the representational elements of the arm that has been seen least recently may be evoked into the A1 state as more of its elements may have decayed into the inactive state with time. This may then contribute to a stronger approach response. If the GluR-1 mutation results in a more rapid decay of elements from the A1 state into the A2 state; or representational elements are maintained longer in the A2 state before decaying into the I state, the discrimination between two familiar arms will be impaired. Effectively, this impairment can be conceived of as

increased proactive interference. Moreover, during NMTP rodents need to maintain a representation of the last turn made, in order to accurately approach the unsampled arm during the choice stage (Dudchenko, 2001). Consistent with a disruption to working memory systems (e.g., increased pd1 & pd2 decay) a failure to maintain this representation would result in disturbances in NMTP performance.

I hypothesised that an increase in the interval between trials would allow more of the representational elements associated with each arm to decay into the inactive state. This may then increase the number of representational elements of the "non-matching" arm evoked into the A1 state. To the extent that the OR reflects these memory processes, the results of Experiment 1 showed that the level of orienting evoked on the initial trials of a session, following a 24 hour interval, was comparable in mutant and wild type mice. The current experiment examined whether a short or a long intertrial interval interacted with the performance of GluR-1$^{-/-}$ mice on the T-maze spatial working memory task. The maze consisted of a start arm and two identical goal arms. Each trial consisted of a sample run and a choice run. In the sample run, mice were placed at the distal end of the start arm and forced either to the left or right goal arm to collect milk reward. Once retrieved, the mice were placed back at the start arm and were then given the choice of the two goal arms. Reward was available in the previously unsampled goal-arm.

2.3.1 Method.

Subjects and Apparatus.

The mice were those used in the previous experiment and were maintained on a restricted feeding schedule at 85% of their ab-libitium baseline weight. Spatial working memory was assessed in a T-maze which was elevated 90 cm from the ground. The maze itself consisted of a start arm (47 cm × 10 cm) and two identical goal arms (35 cm × 10 cm) surrounded by a 10 cm high wall. Food wells were located 3 cm from the end of each goal arm. The experimental room contained various prominent extramaze cues and was illuminated by two ceiling-mounted fluorescent lights. Prior to the initiation of each trial a 70% alcohol solution was wiped on each arm of the maze in order to remove any olfactory cues that remained from previous trials.

Procedure.

Prior to commencing the session equal numbers of GluR-$1^{-/-}$ and control mice were randomly assigned into one of two groups; GluR-$1^{-/-}$ mice trained in short ITI condition (n = 10); wild-type control mice trained in short ITI condition (n = *10)*; GluR-$1^{-/-}$ mice trained in long ITI condition (n = 10); wild-type control mice trained in long ITI condition (n = 10). Mice were transported into the experimental room individually prior to the start of the session. Each trial consisted of a sample run followed by a choice run. On the sample run, the mice were forced down either the left or right goal arms by restricting access to the arm using a black Perspex guillotine

door at the choice point. The mice received equal numbers of left and right turns throughout the session, with no more than two consecutive turns in the same direction. An individual 20 mg food pellet (Noyes precision pellets, Formula A1; Research Diets, New Brunswick, NJ) was placed in the food well of the sample arm. Once retrieved, the mouse was placed, facing the experimenter at the end of the start arm. The interval between the sample run and the choice run was no longer than 20 s and the mouse was rewarded for choosing the previously unsampled arm. The ITI was approximately 10 min in the short ITI condition and 2 hrs in the long ITI condition. Each session consisted of 4 trials and the mice received a total of 40 trials.

2.3.2 Results.

Figure 2.2.1 shows the mean percentage of correct responses during acquisition of the T-maze non-matching to-sample task. Wild-type mice readily learned the reinforcement contingencies. In contrast, GluR-1$^{-/-}$ mice, irrespective of the ITI condition (10 min versus 2 hours), remained at chance levels of responding throughout testing. In order to confirm this impression a three-way mixed ANOVA was conducted with a between-subjects factors of genotype and ITI (long versus short) and a within-subject factor of session (1-10). The analysis revealed a main effect of genotype ($F_{(1,12)}$ = 9.753, p<.01) and session ($F_{(9,108)}$ = 2.653, p<.01), no effect of ITI ($F_{(1,12)}$ = 1.623, p>0.27) and no interaction terms approached significance (largest F value; genotype × ITI interaction, $F_{(1,12)}$ = 1.878, p>0.19). The analysis reflected a failure in mutant mice to readily alternate from trial-to-trial; moreover, the ITI condition did not interact with the deficit in GluR-1 mutant mice.

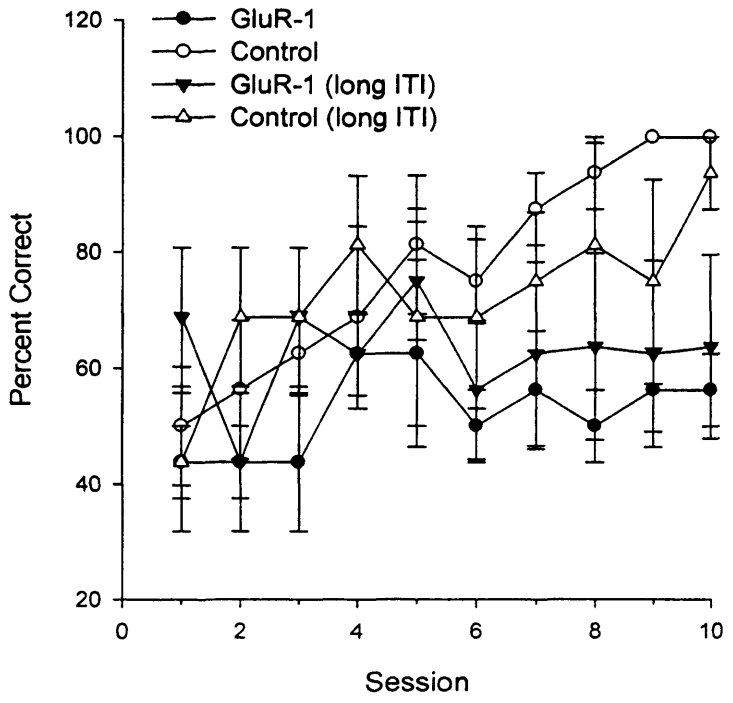

Figure 2.2.1. *Percentage of correct responses in the t-maze non-matching-to-sample task.* Closed circles = GluR-1$^{-/-}$ mice trained with short ITI (10 min); open circles = wild-type control mice trained with short ITI (10 min); closed triangles = GluR-1$^{-/-}$ mice trained with long ITI (2 hours); open triangles = wild-type control mice trained with long ITI (2 hours). Error bars indicate standard error of the mean.

2.3.3 Discussion.

Consistent with previous research (Resiel et al., 2002) GluR-1$^{-/-}$ mice in the present experiment showed a profound deficit in spatial working memory on the T-maze NMTP task. The ITI condition had no effect on the acquisition of the task for either control, or, more importantly GluR-1 mutant mice.

It is generally agreed that normal rodents use extramaze landmarks and a representation of the last turn made in order to solve the T-maze alternation task

(Dudchencko, 2001). Attributing a similar strategy to the control mice in the current experiment, Wagner's SOP model could account for the successful acquisition of this strategy via the ability to discriminate between recent presentations of two familiar arms, reflecting different proportions of representational elements in the A1 and A2 memory states. Thus, during the sample phase the mouse is forced down one arm of the T-maze. At this stage one would expect a proportion of elements in the A1 state to be activated, coding for example, the extramaze cues. During the choice phase, the mouse is given the choice between the two arms. Due to exposure of one of the two arms in the sample phase, one would expect a proportion of the elements associated with the sample arm to have decayed into the A2 state, whilst the least recently visited arm may provoke relatively more of its elements into the A1 state and thus elicit a stronger approach or exploratory response (via proactive interference).

If following GluR-1 deletion, the rodent is left with a disruption to this system such as an increase in decay rates between pd1 and pd2, or maintenance of elements in the A2 state- one would predict that mutant mice would not be able to use this proactive interference to facilitate discrimination between the two arms (i.e., sample versus choice). Additionally, it could be suggested that any disruption to working memory processes, such as an increase in the decay rates of pd1 and pd2, may interfere with the response strategy in mutant mice.

To summarise, I suggested that increasing the duration of the ITI should allow more representational elements of both arms to decay from the A2 state into the inactive state and enhance the discriminability between the sample arm and the correct arm at the choice test. However, no improvement in performance was noted in GluR-1 mutants in the long ITI condition relative to control mice or mutant mice trained in the short ITI condition (see Figure 2.1.3). This would seem to suggest that

an explanation of the GluR-1 T-maze learning impairment in terms of Wagner's SOP model of habituation is inappropriate. Nevertheless, these findings do serve to replicate the findings of Bannerman and colleagues and confirm the phenotype of GluR-$1^{-/-}$ mice in this laboratory.

2.4 Experiment 3.

Experiment 3 examined the claim by Bannerman et al., (2004) that GluR-1 mutant mice are more anxious than their wild-type counterparts. As noted earlier, the increase in latency of GluR-$1^{-/-}$ mice to drink a reward in a novel environment reported by Bannerman et al., (2004) may have been attributable to the hyperactivity shown by the GluR-1 mutant mice in the novel environment. A commonly used test of anxiety is the elevated plus maze (EPM). The EPM is composed of two exposed open arms, and two enclosed arms. In this task animals are placed on the interconnecting central platform and their entries into exposed and enclosed arms is monitored for a short period (usually 5 min). This measurement provides an assessment of neophobia or fear generated by the open and enclosed arms of the maze (Montgomery, 1955). If GluR-1 mutant mice are more anxious than wild-type control mice we would predict that mutant mice would show less activity in the open arms of the plus maze. In contrast, if GluR-$1^{-/-}$ mice show a BLA lesion phenotype (Mead & Stephens, 2003a) we would predict the opposite pattern of results (e.g., Dunn & Everitt, 1988).

2.4.1 Method.

Subjects.

The GluR-1$^{-/-}$ (n = 9) and wild-type (n = 10) control mice were naïve mice obtained from the Department of Experimental Psychology, University of Oxford at 6 months of age. All mice were housed in conditions identical to those stated previously.

Apparatus.

The EPM consisted of two adjacent open arms (8 cm × 50 cm) and two adjacent enclosed arms (8 cm × 50 cm × 10 cm) with a connecting central platform (8 cm × 8 cm) and was elevated 90 cm from the floor. The floor of the maze was painted white. The maze was located in an experimental room where a variety of visual cues (e.g. benching, racks and posters, etc.) were displayed on and along each of the 4 walls of the testing room. Two ceiling-mounted fluorescent lights illuminated the experimental room. During the EPM paradigm, the mice were video tracked using a Noldus Ethovision 3.0 interface (Noldus Information Technology, Wageningen, The Netherlands). The system recorded the distance travelled by the subjects and the time spent in defined zones (each arm of the EPM).

Procedure.

To assess more specifically fear generated to the EPM, rather than to the experimenter (e.g., through initial handling), mice were handled for 5 min each day for 2 weeks (Linden et al., 2005). On the test day, the mice were brought into the experimental room individually in their holding cages. Prior to commencing the session, the experimenter wiped the arms of the maze with a 70 % alcohol (in distilled water) solution to remove olfactory odours. Each mouse was placed in the centre of the maze facing one of the enclosed arms, the location of which was counterbalanced across groups. Once the mouse was placed in the EPM, the experimenter left the room and the behaviour of the mouse was video recorded for 5 min.

Scoring.

The following parameters were recorded by the experimenter: number of open and closed arm entries and the time spent in each of the arms. An arm entry was defined as a mouse having entered an arm of the maze with all four legs. The parameters were recorded by AJ and a second observer, who were unaware of the genotype of each animal. The inter-rater reliability between the two observers was high (>90%) as suggested by Pearsons correlation conducted on the scores between the two observers. This revealed a high degree of inter-rate reliability between the two observers, Pearsons ($r = 0.84$, $r^2 = 0.79$, $p<0.05$).

2.4.2 Results

The results from the EPM are detailed in Table 2.3.1 Firstly, in consideration of the time spent in the exposed and enclosed arms, only wild-type control mice showed a significant preference for the enclosed arms. In contrast, GluR-1$^{-/-}$ mice seemed to display a preference for the exposed arm. In order to confirm this impression a two-way ANOVA was conducted with a between subjects factor of genotype (wild-type and GluR-1$^{-/-}$) and a within-subject factor of arm (enclosed versus exposed). The analysis revealed no main effect of genotype ($F_{(1,17)}$ = 2.64, $p>0.12$) or arm ($F<1$). However, an interaction between these two factors was noted ($F_{(1,17)}$ = 5.22, $p<0.05$). Simple main effects analysis revealed a main effect of genotype for both the enclosed ($F_{(1,18)}$ = 4.03, $p<0.05$) and exposed ($F_{(1,18)}$ = 6.28, $p<0.05$) arms, with only wild-type ($F_{(1,17)}$ = 3.76, $p<0.05$) but not GluR-1$^{-/-}$ mice ($F_{(1,17)}$ = 1.72, $p>0.2$) showing a preference for the enclosed arms.

Secondly, in respect to the number of entries recorded from video scoring, consistent with the automated tracking data, there was a general trend for wild-type control mice to show more entries in to the enclosed arms than the exposed arms, whilst the mutant group failed to show a clear preference (Table 2.3.1). In order to confirm this impression a two-way mixed ANOVA was conducted with factors of genotype and arm. This revealed no main effect of genotype ($F<1$) or arm ($F_{(1,17)}$ = 2.03, $p>0.17$) and a significant interaction between these two factors ($F_{(1,17)}$ = 17.13, $p<0.001$). Main effects analysis was conducted which revealed a preference for the enclosed ($F_{(1,28)}$ = 5.42, $p<0.05$), but not the exposed ($F_{(1,28)}$ = 3.79, $p>0.06$) arms. Similarly, control mice showed a clear preference for the enclosed arm ($F_{(1,17)}$ = 16.3, $p<0.01$), whilst GluR-1$^{-/-}$ mice failed to show any preference for either arm ($F_{(1,17)}$ = 3.5, $p>0.08$).

Parameter	Wild-type control	GluR-1$^{-/-}$
Automated Measures		
Total Enclosed (seconds)	149.42 (11.87)	99.71 (20.06)
Total Exposed (seconds)	84.18 (11.72)	146.2 (24.87)
Recorded Measures		
Enclosed Arm Entries	7.77 (0.65)	7.74 (1.25)
Exposed Arm Entries	5.77 (1.09)	7 (1.01)

Table 2.3.1. *Automated and recorded measures during plus-maze anxiety test.*

Values represent group means followed by standard error in parentheses.

2.4.3 Discussion.

The aim of the current experiment was to help establish whether GluR-1$^{-/-}$ mice displayed a differing pattern of exploratory activity in the EPM (Ward & Stephens, 1998). In contrast to Bannerman et al., (2004), GluR-1$^{-/-}$ mice displayed reduced levels of anxiety in terms of increased exploration of the open arms of the maze (Table 2.3.1) relative to control mice. However it should be noted that the propensity for GluR-1$^{-/-}$ mice to display hyperactivity (Experiment 1; Bannerman et al., 2004) could potentially confound the observed results. That is, if the mutants were simply hyperactive then one might expect activity to be more evenly spread amongst the two type of arms. This was not the case as they showed a trend in preference for the open arms. It should be noted that an atyptical EPM was used. Therefore, although this maze has not been validated for its ability to evoke neophobia or fear, that control

mice showed a clear preference for the enclosed arm would suggest that the open arm was sufficient to induce a state of fear or neophobia. The findings from Experiment 2 are consistent with the proposal by Mead and Stephens (2003a) that GluR-1 mutant mice show impaired BLA function. To the extent that the mutant mice are less fearful (of open arms) then their proclivity to occupy the open arms is entirely consistent with the Mead and Stephens hypothesis.

2.5 Experiment 4.

The previous experiment suggests that GluR-1$^{-/-}$ mice are less anxious in the EPM (Table 2.3.1). Previously it had been reported that GluR-1$^{-/-}$ mice showed greater hyponeophagia than control mice in a novel food (Bannerman et al., 2004). This finding suggests that mutant mice are more anxious although we noted that the tendency for GluR-1$^{-/-}$ mice to be hyperactive may have interacted with the measurement of food preference. We examined this issue in the present experiment by using a two choice procedure in which we would measure consumption of a novel versus a familiar food in an open field environment. Based on the previous experiment and in contrast with the findings of Bannerman et al., (2004) I would predict that GluR-1$^{-/-}$ mice would not show a preference for a familiar over a novel food in an open field environment to the same degree as control mice.

2.5.1 Method.

Subjects and Apparatus.

The mice used in the current experiment were the same as those used in Experiment 3. All mice were housed in conditions identical to those mentioned previously. In addition mice were placed on food-withdrawal until all mice weighed below 85% of their *ab-libitium* baseline weight. Once achieved, mice underwent a test for neophobic reaction to novel food; conducted in an open-field with dimensions (100 cm × 100 cm × 20 cm). The maze was divided into 16 squares each 20 cm × 20 cm in size. Prior to commencing the test session for each mouse, 70% alcohol was wiped across the maze in order to remove fecal body and olfactory cues. Crushed lab chow pellets were used as the familiar food and crushed honey-nut loops were used as the novel food.

Procedure.

In order to ensure sensitivity to differences within and between groups, the mice received two 20-min habituation exposure sessions to the open-field conducted over two days. On day 3, 5 grams of each food type (novel & familiar) was placed in to separate food bowls. The mice were then brought into the experimental room individually in their housing cages. Each of the separate food bowls were placed next to one another in the centre of the open-field. The mouse was then placed into one of the four corners of the open field (counterbalanced across groups), at which point the experimenter left the room and the mouse received a 30-min consumption test. At the

end of the session, the mouse was placed back in to its holding cage and the remaining food was collected and weighed.

2.5.2 Results.

Behavioural Results.

The results from the 30-min consumption test are shown in Figure 2.4.1. In general, wild-type mice showed a clear preference to consume the familiar lab chow over the novel honey nut loops, whereas GluR-1$^{-/-}$ mice showed no preference for either outcome. A two-way mixed ANOVA with a between subject factor of group and a within-subject factor of food type (familiar versus novel) was conducted. The analysis revealed no main of group (F<1), a main effect of food type ($F_{(1,17)}$ = 8.29 p<0.05) and a significant interaction between the two factors ($F_{(1,17)}$ = 9.87, p<0.01). Simple main effects analysis revealed a consumption preference for the familiar food for control ($F_{(1,34)}$ = 17.2, p<0.01) mice, whereas GluR-1$^{-/-}$ mice showed no preference for consuming either reward type (F<1).

That the effect was not due to differences in overall consumption was supported by the finding that on average wild-type control consumed 0.56 grams (S.E. 0.13), whilst GluR-1$^{-/-}$ mice consumed 0.58 grams of reward (S.E. 0.20). An ANOVA revealed no group differences in overall consumption during the 30-min neophobia test (F<1).

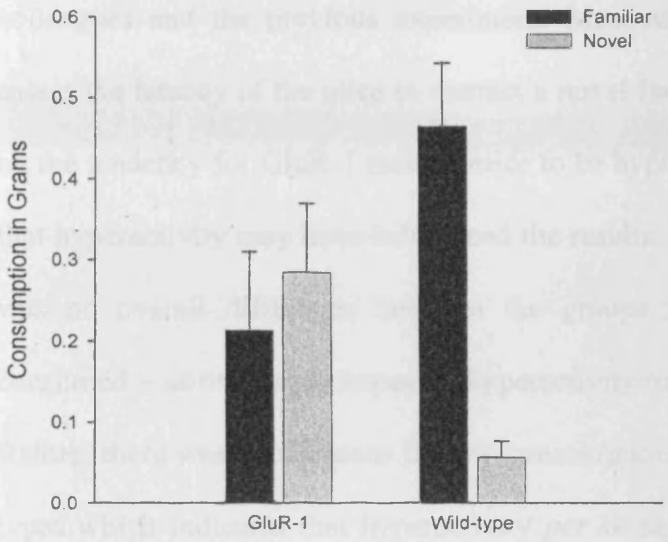

Figure 2.4.1. *Test of neophobic reaction to food.* Consumption of familiar lab-chow (black bars) and novel honey nut loops (grey bars) during a 30 min food preference test. Error bars indicate standard error of the mean.

2.5.3 Discussion.

The results from the neophobia test suggest that GluR-1 mutant mice show reduced neophobia to novel food. (Figure 2.4.1). This phenotype is consistent with that seen in BLA-lesioned rats (Dunn & Everitt, 1988; Rollins et al., 2001). When viewed in the context of the results from Experiment 3, the results suggest that GluR-$1^{-/-}$ mice show an attenuation of neophobia to novel foods in an open field and show reduced behavioural measures of anxiety in an elevated plus maze. These results are consistent with the hypothesis that mutant mice show a behavioural phenotype similar to that seen in BLA-lesioned rats (Mead & Stephens, 2003a). Furthermore, the results contrast directly with those reported previously by Bannerman et al., (2004). There

are, however, key procedural differences between the test used by Bannerman and colleagues and the previous experiment. Most notably, the test of hyponeophagia assess the latency of the mice to contact a novel food and may have been confounded by the tendency for GluR-1 mutant mice to be hyperactive, therefore it was suggested that hyperactivity may have influenced the results. In contrast, in Experiment 4, there was no overall difference between the groups in terms of the amount of food consumed – as one might expect if hyperactivity resulted in fewer contacts with food. Rather, there was a difference in how consumption was distributed over the two food types which indicates that hyperactivity *per se* cannot explain the pattern of results reported in the present study. However, one cannot rule out an explanation of the results based on increased palatability to the novel food, or failure to discriminate between the two food types in mutant groups. A choice test will be included in further experiments to evaluate the latter of these possibilities (Experiment 7). Even in consideration of these issues, the overall results from the experiments 3 and 4 in this chapter support the proposal by Mead and Stephens (2003a) that GluR-$1^{-/-}$ mice show impairments in processes supported by the BLA.

2.6 Chapter Discussion.

The previous experiments have attempted to quantify sensorimotor and affective behaviours in mice following targeted deletion of the GluR-1 AMPA subunit. Previous findings suggested that GluR-$1^{-/-}$ mice were hyperactive and displayed impaired motor coordination (Vekovischeva et al., 2001; Bannerman et al., 2004). Therefore the aim of Experiment 1 was to assess whether GluR-$1^{-/-}$ mice displayed any non-mnemonic sensorimotor performance deficits induced by the

mutation. Consistent with previous research, GluR-1$^{-/-}$ mice were transiently hyperactive and showed aberrant habituation of the OR to a visual stimulus.

This phenotype was discussed in relation to Wagner's SOP model (Wagner, 1981) whereby it was suggested that: (1) the GluR-1 mutation resulted in an increased decay rate between the A1 and A2 states; or (2) the representational elements may be maintained longer in the A2 state. I hypothesised that increasing the ITI might allow more of the elements representing the non-matching arm to be primed in to the A1 state in both GluR-1$^{-/-}$ and control mice. In turn one would assume that this would benefit discrimination between a recently exposed arm and an arm visited some time ago. Interestingly, the ITI duration had no effect on the acquisition of the task in control mice; perhaps reflecting a ceiling effect in performance. Nevertheless, the ITI duration did not influence performance of the GluR-1 mutant mice who showed little or no improvement in performance of the task across the period of training. Although this result does not support the hypothesis that GluR-1$^{-/-}$ mice may show aberrant memory processing in terms of Wagner's SOP model of habituation, they allow one to conclude that the mice show an appropriate behavioural phenotype in our hands. Discussion of the relationship between the OR and the anxiety deficits will be reserved until the general discussion chapter (Chapter 6).

Mead and Stephens (2003a) proposed that mice lacking the GluR-1 subunit displayed a behavioural phenotype mimicking that seen following lesions to the BLA. However, BLA-lesioned rats are generally less anxious than normal animals (LeDoux, 2000). In contrast to this finding, GluR-1$^{-/-}$ mice were reported to be more anxious than control mice (Bannerman et al., 2004). Experiment 2 assessed this issue using the plus-maze paradigm (Montgomery 1955; Lister 1990; Ward & Stephens, 1998). In contrast to the finding from Bannerman and colleagues, GluR-1$^{-/-}$ mice displayed

more open arm entries and spent more time in the open arms than controls, therefore suggesting a less anxious phenotype in the mutant group. In the final experiment in this chapter a food neophobia paradigm was used to assess anxiety in an attempt to replicate Bannerman et al., (2004) but in addition control for the potentially confounding influence of hyperactivity. Consistent with the hypothesis proposed by Mead and Stephens (2003a) and similar to the results obtained from BLA-lesioned rats (Burns & Everitt, 1988; Rollins et al., 2001), GluR-1$^{-/-}$ mice displayed an attenuation of neophobia toward the novel food (Experiment 4). Thus, experiments 3 and 4 suggests a phenotype indicative of BLA dysfunction in the GluR-1 mutant and provide support for the hypothesis proposed by Mead and Stephens (2003a).

Chapter 3.

3.1 Introduction.

Conditioned fear is a hypothetical construct that is used to explain behavioural changes that occur in rats when an initially neutral stimulus (CS) is paired with an aversive foot shock stimulus (US). Following contemporaneous CS-US pairings the CS evokes a representation of the US allowing for the expression of a CR. In Pavlovian fear conditioning, animals learn to associate a CS (e.g., tone) with an aversive US (e.g., foot shock) that reflexively activates unconditioned fear responses (Rescorla, 1967). Following subsequent pairings the CS comes to elicit various CRs that share similar characteristics to innate fear responses. In rats, species-typical fear CR measures include freezing (Blanchard & Blanchard, 1969) enhancement of musculature reflexes (Brown, Kashir & Farber, 1951), analgesia (Fanselow, 1986), alterations in autonomic activity (e.g., increased blood pressure rates; Kapp, Frysinger, Gallagher & Haselton, 1979) and 22kHz ultrasonic vocalisation (USV; Blanchard, Blanchard, Augullan & Weiss, 1991).

In recent years, the analysis of the neural circuitry of fear conditioning has implicated the amygdala (e.g., LeDoux, Sakaguchi & Reis, 1986), and the hippocampus (e.g., Kim & Fanselow, 1992) in the processing of emotive stimuli. Moreover, considerable progress in the techniques used to identify the neural circuitry of fear conditioning have allowed for the opportunity to analyse the synaptic and molecular components which also govern the formation and storage of fear memories (Miserendino, Sannes, Melia & Davis, 1990). In brief there is considerable evidence to support a role for the amygdala in fear conditioning. According to the hypothesis proposed by Mead and Stephens (2003a), GluR-1 mutant mice should be impaired in both contextual and cued fear conditioning. In contrast, according to the working

memory hypothesis fear conditioning can be described as a reference memory procedure, as the contingencies between the CS and the US remain consistent across training. Therefore, according to the working memory hypothesis proposed by Bannerman and colleagues (e.g., Riesel et al., 2002) GluR-1 mutant mice should condition normally. Before presenting the results from this study, I will briefly overview the literature regarding the contribution of the amygdala and the hippocampus to fear conditioning as both structures have been implicated in this form of learning and are implicated in the GluR-1 phenotype. This in turn will provide a conceptual framework in which the pattern of results from the present can be interpreted.

3.1.1 *The Amygdala.*

In general, selective lesions of the BLA produce severe modality-independent deficits in both the acquisition and expression of Pavlovian fear conditioning (Davis, 1992; LeDoux, 2000; Maren, 2001). The LA but not the B is necessary for fear conditioning (Amorapanth, Nader & LeDoux, 2000; Nader et al., 2001). Lesions to the B attenuate the acquisition of instrumental avoidance (Amorapanth et al., 2000; Killcross, Robbins & Everitt, 1997). Consistent with this view either selective neurotoxic or electrolytic lesions of the BLA severely attenuate the acquisition of fear conditioning to both contextual and discrete visual or auditory CS's when conducted prior to conditioning (LeDoux, Cicchetti, Xagoraris & Romanski, 1990; Sananes & Davis, 1992; Fanselow & LeDoux, 1999; Huff & Rudy, 2004; Vazdarjanova, Cahill, McGaugh, 2001; Desmedt, Marighetto, Garcia & Jaffard, 2003; Nader, Aajidishad, Amorapanth & LeDoux, 2001), shortly after conditioning (Campeau & Davis,1995;

Maren, Aharonov & Fanselow, 1996), or up to one month post-conditioning (Maren et al., 1996). Interestingly, pre-training BLA-lesioned rats will show conditional freezing to contextual, but not acoustic stimuli, when given 10 times the number of footshocks required in order to produce asymptotic fear responding (Maren, 1998, 1999a). These results suggest that systems outside the BLA mediate some (contextual) forms of learning following overtraining. The locus of this area is unknown, although the superior colliculus (Dean, Mitchell & Redgrave, 1988), cerebellar vermis (Supple, Leaton & Fanselow, 1987), and the midbrain periaqueductal gray (Bandler & Shipley, 1994), are involved in generating defensive responses and may be able to mediate BLA-independent contextual fear conditioning following overtraining (Maren, 1999a). This same training regime does not mediate savings when lesions are conducted post-training (Maren, 1999a), suggesting a role for the BLA in associative processing underlying fear conditioning (Maren, 2000).

In contrast, the CeN is viewed by some as the terminus for the generation of learned responses in the amygdala (Goosens & Maren, 2001). Following electrolytic and neurotoxic lesions of the CeN, profound deficits in the acquisition and expression of conditioned fear have been reported (Hitchcock & Davis, 1986; Nader et al., 2001; Goosens & Maren, 2001). Further, lesions in structures efferent to the CeN can produce autonomic deficits. For instance, lesions of the lateral hypothalamus produce deficits in cardiovascular fear responses (LeDoux, Iwata, Cicchetti & Reis, 1988), whereas lesions to the periaqueductal grey produce deficits in somatic conditional fear responses (Amorapanth, Nader & LeDoux, 1999). A parsimonious explanation of these findings is that, during fear conditioning, complex information about environmental stimuli is carried to the BLA (via sensory thalamus, sensory neocortex and hippocampus; Figure 1.1) where CS-US associations takes place. The CeN

provides the output pathway through which these associations gain access to appropriate responses. This is known as a serial model of BLA and CeN function (LeDoux, 2000).

This model would suggest that learning about aversive Pavlovian CS-US associations would not be possible following lesions of the BLA (Koo, Han & Kim, 2004). However, certain forms of learned fear have been shown to survive following fear conditioning, specifically contextual fear conditioning, assessed by the degree of fear responding to the conditioned context (Selden, Everitt, Jarrard & Robbins, 1991). Similarly, savings of contextual fear responding have been reported in pre-training BLA lesioned rats after a footshock was delivered in a context where they had already received two footshocks 24 h earlier, compared to lesioned rats that had received this earlier conditioning in a different context (Cahill, Vazdarjanova & Setlow, 2000). Furthermore, if rats are trained on two levers for food reward, one of which intermittently produces a CS followed by presentation of shock, then BLA-lesioned rats are still able to produce an inhibition of response during the CS presentations (i.e., shown normal conditioned suppression; Killcross, Robbins & Everitt, 1997). Nevertheless these same animals were unable to bias their response away from the lever which produced the CS and shock (i.e., impaired instrumental avoidance).

These data support the idea the BLA-lesioned rats are still able to acquire CRs, and therefore supports a parallel processing view of the amygdala (Cardinal et al., 2002). This view promotes the idea that representations stored in, or communicated through the BLA and CeN mediate behaviour via separate afferent and efferent pathways. Consistent with this parallel processing view, the CeN also receives sensory input from cortical and thalamic regions (Turner & Herkenham, 1991;

McDonald, 1998), which could support association formation independent of the BLA (Cardinal et al., 2002).

However, it should be noted that the previously postulated role for the CeN in conditioned fear (Hitchcock & Davis, 1986; Nader et al., 2001; Goosens & Maren, 2001) may have reflected damage to fibres of passage coursing through the CeN. Consistent with this view, Koo, Han and Kim (2004) adopted myelin staining procedures to confirm that the fibres of passage were left undamaged in the CeN-lesioned group. Here, following neurotoxic lesions of the CeN animals acquired conditioned fear responses to an auditory CS and to the context that had been previously paired with shock, although a moderate impairment in conditioned freezing to a tone was observed. In contrast, rats with electrolytic CeN lesions failed to show freezing behaviour to either the cue or context (Koo et al., 2004). These data suggest that the expression of conditioned fear involves parallel BLA projections, some of which course through the CeN enroute to downstream response structures (i.e., not actively making use of the CeN). Specifically, it was suggested by Koo and colleagues that multi-modal contextual information passes through the CeN (via LA and B pathway); whereas simple modality specific CS information engages two parallel processes, one of which relies on the CeN, whilst the other makes use of projection neurons coursing through the CeN (Koo et al., 2004).

Indeed stronger forms of this hypothesis have been posited, suggesting that amygdala processes modulate the formation and storage of memory in other brain regions (Cahill, Weinberger, Roozendaal & McGaugh, 1999; Weinberger, Javid & Lepan, 1993). For instance, during auditory fear conditioning the medial geniculate nucleus (MGN) displays associative single-unit activity during fear learning (McEchron, McCabe, Green, Llabre, & Schneiderman, 1995). Moreover, the MGN

exhibits LTP (Gerren & Weinberger, 1983) and fear conditioning induces synaptic plasticity in the MGN (McEchron et al., 1996). It has been suggested that amygdala afferents such as the MGN or cortical areas (Cahill et al., 1999) are the critical regions of CS-US learning during Pavlovian fear conditioning. This is in contrast to the serial model of amygdala functioning, which suggests that CS-US information is gathered in the BLA, and then sent to the CeN for generation of fear CRs (Nader & LeDoux, 1997).

In summary, some researchers have suggested that during Pavlovian fear conditioning the BLA acts as the site where stimulus-outcome association takes place, whilst the CeN provides the output pathway through which the associations gain access to appropriate responses (serial model; LeDoux, 2000; Nader & LeDoux, 1997). However, the fact that some forms of fear conditioning still occur in the presence of BLA lesions (Selden et al., 1991; Killcross et al., 1997; Vazdarjanova & McGaugh, 1998) suggests an additional parallel process which allows BLA and CeN to affect certain behaviour through separate afferent and efferent pathways (Cardinal et al., 2002). Similarly, it has been suggested that although the BLA governs cued and contextual Pavlovian CSs (Sannes & Davis, 1992; LeDoux, 2000), the neural pathways which process these types of stimuli with shock are different (Koo et al., 2004), some of which course through the CeN enroute to downstream response structures (i.e., not actively making use of the CeN; Koo et al., 2004). Stricter forms of this model suggest that structures other than the amygdala govern the encoding of CS-US information during fear conditioning (Cahill et al., 1999).

3.1.2 *Pharmacological manipulations of the Amygdala.*

As with lesion studies (Sananes & Davis, 1992; Fanselow & LeDoux, 1999; Huff & Rudy, 2004; Vazdarjanova, Cahill, McGaugh, 2001; Desmedt, Marighetto, Garcia & Jaffard, 2003; Nader, Aajidishad, Amorapanth & LeDoux, 2001) temporary inactivation of the BLA prevents both the acquisition and expression of fear conditioning. The BLA contains two major classes of neurons: (1) predominantly GABAergic non-pyramidal interneurons: (2) pyramidal principal projection neurons (McDonald, 1996). Marked GluR1 immunoreactivity is found in nonpyramidal GABAergic neurons, whereas pyramidal cells exhibit only light GluR1 immunoreactivity (McDonald, 1996). Inactivation of BLA neurons with muscimol, a $GABA_A$ receptor agonist, prevents the acquisition and expression of fear conditioning (Helmsetter & Bellgowan, 1994; Wilensky, Schafe & LeDoux, 1999). $GABA_A$ is an inhibitory ionotropic receptor which governs interneuron stability. Therefore, due to the expression of GluR-1 on GABAergic interneurons in the BLA, one would anticipate application of musicmol to interfere with GluR-1 dependent processing in normal animals. Moreover, muscimol only blocks conditioning when infused prior to training. Immediate post-training infusions of muscimol have no effect on conditioning (Wilensky et al., 1999) suggesting that synaptic activity in the BLA is necessary during learning (Maren, 2000).

NMDA receptors are involved in the induction of LTP (Bliss & Collingridge, 1993) in the amygdala (Huang & Kandel, 1998). The first demonstration of the involvement of NMDA receptors in the fear mechanism involved the use of intra-BLA infusion of APV. This prevented the acquisition of conditioned fear to a visual CS in a fear potentiated startle paradigm (Miserandino, Sananes, Melia & Davis,

1990). The involvement of these receptors in conditioned fear was later revealed in a series of experiments examining conditioned fear; where intra-BLA infusion severely attenuated the acquisition (Fanselow & Kim, 1994; Maren, Aharonov, Stote & Fanselow, 1996; Goosens & Maren, 2003) and expression (Maren et al., 1996; Lee, Chooi, Brown & Kim, 2001) of conditioned fear to both contextual and discrete CSs.

As previously mentioned, there is still debate regarding the locus of CS-US information following fear conditioning (LeDoux, 2000; Maren, 2000; Koo et al., 2004; Cahill et al., 1999). In an attempt to address this issue, Goosens and Maren (2004) examined the effects of BLA or CeN NMDA receptor inactivation during fear conditioning. Here, APV infusion into the BLA or CeN during conditioning attenuated any fear learning. However, following conditioning, rats underwent further trials in which no APV was administered. CeN, but not BLA, treated rats elicited a facilitation of learning compared to non-shocked controls for both contextual and auditory fear during a separate one-trial (one US presentation) per session testing regime (Goosens & Maren, 2004). The authors suggest that NMDA receptors in both the CeN and BLA are critical for CS-US learning, but the BLA is able to retain some aspects of aversive information, unlike the CeN.

Once the induction of LTP has taken place, the expression of this induction is reliant upon AMPA receptors (Collingridge & Lømo, 1993). Pre-training infusions of the potent AMPA antagonist CNQX impairs both the acquisition and expression of fear potentiated startle (Walker & Davis, 1997). Similarly, infusion of the AMPA facilitating drug 1-(quinoxolin-6-ylcarbonyl)piperidine (BDP-12) facilitated the acquisition of auditory fear conditioning in a dose-dependent manner (Rogan, Staubli, LeDoux, 1997b). Here, rats were given IP injections of BDP-12 prior to each of the conditioning and testing sessions. Over the course of 2d of training (2 CS-US pairings

per session), the level of freezing acquired by drug-treated rats surpassed that of controls. However, on the testing day (extinction session; day 3) the level of asymptotic freezing of both groups of rats was identical. The finding that the drug accelerates acquisition but does not affect the level of acquired conditioned fear parallels the effect of the drug on LTP, suggesting that common mechanisms may govern fear conditioning and LTP (Rogan et al., 1997b). Consistent with these findings *in-vivo* (Quirk, Repa & LeDoux, 1995; Quirk, Armony & LeDoux, 1997; Rogan et al., 1997a) and *in-vitro* (McKernan & Shinnick-Gallagher, 1997) studies have found increases in synaptic potentials in the LA following fear conditioning. That ionotropic (AMPA or NMDA) receptors are involved in this plasticity is supported by the latter study where the effect was ameliorated following application of CNQX or APV (McKernan & Shinnick-Gallagher, 1997).

Similarly it has been found that induction of LTP at synapses linking the thalamic input and the LA synapses potentiates auditory evoked potentials in the LA pathway; a site critical for auditory fear conditioning (Rogan & LeDoux, 1995). Thus, the increase in auditory evoked potentials in the LA by both fear conditioning (Quirk et al., 1995, 1997; Rogan et al., 1997a; McKernan & Shinnick-Gallagher, 1997) and LTP (Rogan & LeDoux, 1995) collectively suggests that LTP-like processes contribute to the acquisition of auditory fear conditioning in the amygdala (Chapman et al., 1990; Miserendino et al., 1990; Kim et al., 1991). Interestingly, mice lacking Ras-GRF show abnormal LTP in the BLA and are impaired in a range of emotional conditioning tasks including Pavlovian contextual and cued fear conditioning (Brambilla et al., 1997). Ras activity appears necessary to generate LTP and is the downstream effector of CaMKII (Zhu et al., 2002).

The role of protein kinases in fear conditioning has been assessed using a range of inhibitors. For instance, intra-BLA administration of H7 (a potent inhibitor for protein kinases PKA and PKC) attenuated long-term context and cued conditional fear in a dose dependent manner (Goosens, Holt & Maren, 2000). However (consistent with the parallel view of amygdala-fear processing; Koo et al., 2004) infusion into the CeN failed to produce any deficits in cued or contextual fear conditioning. Finally, another group of rats were given post-training infusion of H7 immediately prior to a retention test. Here, H7 treated rats showed normal freezing to the context, suggesting that the impairment in learning was not the result of performance deficits induced by H7 infusion (Goosens et al., 2000). These findings suggest that protein kinase activation in the BLA is required for the acquisition of contextual and cued fear memories, and further the possibility that LTP-like processes are involved in fear memory consolidation.

To summarise, the disturbances to the synaptic mechanisms governing BLA plasticity either by muscimol (Helmsetter & Bellgowan, 1994; Wilensky, Schafe & LeDoux, 1999), APV (Fanselow & Kim, 1994; Maren, Aharonov, Stote & Fanselow, 1996; Goosens & Maren, 2003) or CNQX (Walker & Davis, 1997) block the acquisition and expression of conditioned fear. Consistent with the importance of this region in fear conditioning; either *in-vivo* (Rogan et al., 1997a) or *in-vitro* (McKernan & Shinnick-Gallagher, 1997) recordings have indicated LTP-like increases following Pavlovian fear conditioning. Interestingly, a recent investigation using injections of viral vectors into the LA of young rats, evoked impairments in both plasticity and auditory fear conditioning (Rumpel, LeDoux, Zador & Malinow, 2005). Here, the authors used sRNA to block synaptic GluR-1 incorporation in a quarter of the neurons in the LA. This caused impairments in synaptic transmission in infected neurons and

an impairment in auditory fear conditioning in treated rats. Thus, together with the reviewed lesion and pharmacological findings, any mutation which could potentially interfere with synaptic GluR-1 activity in the BLA, would be expected to interfere with the acquisition and expression of conditioned fear (Rumpel et al., 2005).

3.1.3 *The Hippocampus.*

The first demonstrations that the hippocampus was involved in contextual fear conditioning came from studies using electrolytic lesions to the dorsal hippocampus (DH). These lesions resulted in the attenuation of contextual fear responding, whilst leaving discrete cue (auditory) conditioning intact (Kim & Fanselow, 1992; Philips & LeDoux, 1992). The effects of pretraining lesions of the hippocampus are dependent on the lesion technique and site of lesion, such that electrolytic lesions to the dorsal hippocampus (DH; Maren, Aharonov, Fanselow, 1997) and fibre-sparing excitotoxic lesion to the VH or to both the DH and VH impair contextual fear conditioning (Richmond et al., 1999). However, fibre-sparing neurotoxic lesions to just the DH do not impair contextual fear conditioning (Maren et al., 1997; Rudy, Barrientos & O'Reilly, 2002). Furthermore, pretraining VH lesions usually produce deficits in the acquisition of auditory fear conditioning (Bannerman et al., 2003).

Post-training lesions of the hippocampus, like that seen in human amnesia (Squire & Zola-Morgan, 1991) exhibit a temporal dependence, such that fear conditioning was only impaired when made within 1 month after training (Kim & Fanselow, 1992). Similarly, electrolytic lesions to the DH disrupts contextual fear conditioning when given 1 day following training. However if damage is induced 50 days post-training no impairment is seen (Anagnostaras, Maren & Fanselow, 1999).

Here, the same rats expressed fear when training occurred 50 days prior to surgery, but not when surgery was conducted 1 d after the final training session. Thus, although initial reports indicated that electrolytic DH lesions conducted prior to fear conditioning result in impairment (Maren et al., 1997; Kim & Fanselow, 1992; Philips & LeDoux., 1992) it has since been suggested that this is not the case for neurotoxic lesions of the DH when conducted prior to training (Maren et al., 1997; Rudy, Barrientos & O'Reilly, 2002); although, post-training DH lesions attenuate contextual fear responding (Maren et al., 1997). These findings have led a number of researchers to conclude that the DH is involved in forming configural or conjunctive representations of the environment during fear conditioning (see below for discussion).

The differential effects of pre- and post-training lesions of the DH suggest the existence of alternate strategies for acquiring contextual fear representations (Maren et al., 1997; Maren, 2001; Rudy et al., 2004). It has been suggested that intact rats make use of a hippocampus-dependent 'unified representation' (Anagnostaras, Gale & Fanselow, 2001). The idea that the hippocampus binds together many sensory features of an episode to form a unified representation has long been proposed (Marr , 1971; O'Keefe & Nadel, 1978). Consistent with this view, normal rats have been shown to use a configural strategy to acquire contextual fear conditioning (Rudy & O'Reilly, 1999). Here, preexposure to the conditioning context, but not the individual features of this context, improved contextual fear conditioning (see also Amat et al., 2004). In addition, generalisation gradients to other similar contexts were weakened following preexposure to the conditioning context. These observations suggest a configural process of pattern completion to the conditioning context following preexposure (Rudy & O'Reilly, 1999). Once normal animals acquire a unified

representation of the conditioning context, fear memories then become dependent on an intact hippocampus (Anagnostaras et al., 2001). In contrast, rats with pre-training lesions of the hippocampus do not use a configural unified strategy, rather they make use of a hippocampus-independent elemental solution. Thus, during conditioning individual elements of the context become weakly paired with the shock. However, in test, these individual elements summate to elicit strong hippocampal-independent contextual fear (Anagnostaras et al., 2001). Consistent with this idea, Rudy et al., (2004) have posited a two-process model of contextual fear conditioning. In this model, conditioning is supported by associations linking the hippocampus-dependent conjunctive representations to the amygdala. Here, context features activate cortical representations which are reciprocally connected to the hippocampus. At the same time, as a consequence of shock, the learned associative connections between the hippocampus and the amygdala are strengthened. Thus, when the hippocampus is damaged the conjunctive representation is no longer available to support contextual fear conditioning. In contrast, hippocampus-independent contextual fear representations are governed by individual context features that activate cortical representations which link the individual feature representations to the amygdala (Rudy et al., 2004). As with the proposed unified and elemental models (Anagnostaras et al., 2001) both strategies can be used to acquire contextual fear conditioning, although only the configural or learned conjunctive representation requires hippocampal involvement and is proposed to dominate over the unified (Anagnostaras et al., 2001) or feature representations (Rudy et al., 2004) in normal animals.

However these models still cannot account for the impairments seen in pretraining electrolytic DH (Maren et al., 1997). The observed deficits seen in these

animals appear to be the result of damage to the fibres of passage which connect the ventral subiculum and nucleus accumbens. Consistent with this view, electrolytic or neurotoxic damage to the ventral subiculum, a major afferent of the nucleus accumbens (McDonald, 1996), produces fear conditioning deficits (Maren, 1999). Similarly, the proposed models also have difficulty in accounting for pre-training contextual fear conditioning deficits seen following excitotoxic VH or complete hippocampal lesions (e.g., Richmond et al., 1999). One suggestion focuses on the possibility that VH and complete damage to the hippocampus results in hyperactivity, such that these lesions might induce a predisposition towards hyperactivity sufficient to alter conditioned freezing to context (Good & Honey, 1997; Richmond et al., 1999). Since hyperactivity is proposed to directly disrupt freezing, accordingly one would expect an activity-freezing correlation (Anagnostaras et al., 2001). However, no such correlation was reported when activity was measured in the same conditioning chambers as freezing (Maren, Anagnostaras & Fanselow, 1998). Importantly, it should be noted that the authors used DH-lesioned rats only. Therefore one cannot discount the possibility that VH or complete hippocampal lesions induce hyperactivity that could disrupt freezing behaviour.

Finally, although it has been shown that the hippocampus is critical for the consolidation of fear to a context, but not to a discrete tone (Selden et al., 1991; Kim & Fanselow, 1992; Phillips & LeDoux, 1992), this is not necessarily the case in all experimental preparations (McEchron et al., 1998; Quinn et al., 2002). For instance, in Pavlovian trace conditioning, a stimulus free trace interval is inserted into the experimental protocol between the CS (e.g., tone) and US (e.g., shock), such that it forms a non-contiguous relationship between the two stimuli (Mackintosh, 1974, pp.57). In contrast to delay conditioning, where the CS presentation is extended until

US delivery, and standard Pavlovian conditioning, trace conditioning requires an intact hippocampus (Quinn et al., 2002). Here, post-training excitotoxic DH lesions produce a marked deficit in auditory trace fear conditioning. These findings indicate a critical role of the DH in both the acquisition (McEchronen al., 1998), consolidation and expression of trace fear conditioning to an auditory CS; indicating a mnemonic role for the DH (Quinn et al., 2002). Similar findings have been shown with pre-training excitotoxic DH lesions (McEchron et al., 1998).

In summary, the effects of pre-training lesions of the hippocampus on fear conditioning are dependent on the lesion technique and site of lesion, such that electrolytic lesions to the DH (Maren, Aharonov, Fanselow, 1997), fibre-sparing excitotoxic lesion to the VH or to both the DH and VH (Richmond et al.,. 1999) impair contextual fear conditioning. However, fibre-sparing neurotoxic lesions to just the DH do not impair contextual fear conditioning (Maren et al., 1997; Rudy, Barrientos & O'Reilly, 2002). Post-training DH lesions, however, attenuate contextual fear responding (Maren et al., 1997). These retrograde findings have led a number of researchers to conclude that the DH makes an important contribution to the processing of contextual information during fear conditioning (Fanselow, 2000; Maren 2001).

3.1.4 *Pharmacological manipulations of the Hippocampus.*

In addition to the role of the hippocampus in encoding contextual representations, inactivation studies have suggested a role for contextual retrieval of fear memory. For instance, the context-specific effects of latent inhibition (LI; Lubow & Moore, 1959) are attenuated following DH inactivation with muscimol (Holt &

Maren, 1999). Here, rats were given 5 d preexposure either in a context that would later be used for fear conditioning, or a different context. Following preexposure those rats who were subsequently shocked in the pre-exposed context showed less freezing to the tone than either rats preexposed and tested in a different context or non-preexposed rats. This context-specific effect of LI (Hall & Minor, 1984) was ameliorated following pre-training muscimol infusion into the DH, suggesting that the DH is necessary for contextual memory retrieval in LI (Holt & Maren, 1999).

Consistent with a role of the DH in contextual memory retrieval, the DH supports the acquisition of a unified memory representation of a context which it then associates with shock (Amat, Higgins, Barrientos & Rudy, 2004). Rudy and colleagues examined whether DH inactivation prevented the context pre-exposure-facilitation effect (CPFE). In this task rats who are given a short pre-exposure session condition to a greater degree than rats who are immediately shocked in a novel context (e.g., Fanselow, 1990). Intra-DH muscimol infusion prior to either pre-exposure, shock, or the extinction test caused an attenuation of the CPFE (Amat et al., 2004). These results are consistent with the previous findings (Holt & Maren, 1999), in that the DH seems necessary for the acquisition and retrieval of contextual memories associated with shock. However, in the same experiment [(and consistent with the lesion literature (Maren et al., 1997; Richmond et al., 1999; Rudy et al., 2002)] muscimol infusion did not impair the acquisition of contextual fear conditioning when injected prior to standard (non-preexposed) contextual fear conditioning. This finding has subsequently been repeated (Maren & Holt, 2004).

However, DH inactivation targeted at glutamatergic synapses results in a wider range of observable impairments. For instance, it has been observed that pre-training infusion of the NMDA antagonist MK-801 (which blocks the pore of the

NMDA channel; Coan, Saywood & Collingridge, 1989) results in an attenuation of fear conditioning, whilst leaving auditory fear conditioning intact (Bast, Zhang & Feldon, 2003). Thus, MK-801 impairs the formation but not the expression of contextual fear conditioning; a finding which has been observed following APV infusion in rats (Kim, DeCola, Fernandez & Fanselow, 1991; Kim, Fanselow, DeCola, Fernandez, 1992; Young, Bohenek & Fanselow, 1994; Fanselow, Kim, Yipp & Oca, 1994) and mice (Stiedl, Birkenfeld, Palve & Spiess, 2000).

Sanders and Fanselow (2003) reported that intra-DH APV infusions, prior to fear conditioning (in context A), prevented the context fear conditioning deficits (under APV) when trained in a subsequent discriminatively different context (context B). That is, consistent with APV effects reported previously in the water maze (Hoh et al., 1999) pre-training prevented the deficit in context fear normally induced by APV infusion. This saving was limited to the subsequent conditioning phase (context B), as APV-treated rats showed impairments in freezing to the conditioned context (context A) in a test phase carried out 24h after the initial pre-training. Which aspects of the pre-training prevented the deficit are unknown; however, it was suggested that NMDA-independent hippocampal processes allowed for the retention of some aspects of the conditioning procedure in the pre-training phase (Sanders & Fanselow, 2003). Similarly, as with the noted reference memory impairment (e.g., Bannerman et al., 1995) one cannot rule out the possibility of sensorimotor impairment contributing to the observed deficit in contextual fear conditioning following APV infusion. For instance, hyperactivity may have contributed to the apparent deficit, as drug-treated rats were capable of freezing, although at a reduced level (shown in the test phase in context A). That is, since freezing requires an absence of movement other than

respiration (Blanchard & Blanchard, 1971), drug-induced hyperactivity may interfere with this behaviour.

The literature is rather more complicated when examining inactivation studies of the VH. For instance, some have reported pre-training muscimol infusions impair contextual fear conditioning and spare auditory fear conditioning (Bast, Zhang & Feldon, 2001), whereas others have reported the opposite effect, i.e., impaired auditory fear conditioning and spared contextual fear conditioning (Maren & Holt, 2004). However, procedural differences may have accounted for the apparently contradictory sets of data. For instance, in the Zhang et al., (2001) study, rats were exposed to ten auditory CS-US parings, where the CS was 30 s and the intensity of the US was 0.5 mA. However, in the Maren and Holt study, rats received 5 CS-US pairings, where the auditory CS was presented for 10 s and the US intensity was set at 1 mA. It should be noted that the level of US intensity can affect the associative strength of the CR developed (e.g., Rescorla & Wagner, 1972). Additionally, in the Zhang et al., (2001) study the context extinction test occurred prior to the cued extinction test. The effects of context-specificity on extinction are well-documented (Bouton, 1993). For instance, if conditioning to a cue is conducted in one context (A) and extinction of that cue is conduced in a second context (B), then when the cue is returned to the original context (A) responding returns to the cue. This context-specificity of extinction would suggest that in the Zhang et al., (2001) study, during context extinction, second-order extinction processes may have also extinguished the cue. It may be that if the authors had counterbalanced the order of tests (context vs. tone) then conditioning to tone may have been evident. These procedural differences highlight the difficulty in interpreting the findings from the aforementioned inactivation studies.

Targeted inactivation of glutamatergic activity in the VH has also been assessed. In general, pre-training infusion of MK-801 into the VH results in an impairment in contextual fear conditioning, whilst leaving auditory fear conditioning intact (Zhang, Bast & Feldon, 2001). These results suggest that NMDA-dependent processes mediate fear conditioning to context in the VH. Similar to that seen in the DH (Bast et al., 2003), administration of NMDA resulted in a deficit to both context and cued fear conditioning. As previously noted, lesions of the VH results in hyperactivity (Richmond et al., 1999). Similarly, following MK-801 infusion, elevated levels of locomotor activity have been noted (Zhang et al., 2001). Thus, hyperactivity may have interfered with the ability of VH-treated animals to acquire a freezing response to the cue. No articles have been published to date examining the effects of AMPA receptor manipulations on hippocampus-dependent fear learning.

Finally, hippocampal place cells have also suggested a role for the hippocampus in contextual fear conditioning. Thus, when a rat explores an environment, hippocampal place cells establish specific firing locations in that environment (O'Keefe & Dostrovsky, 1971; Wilson & McNaughton, 1993). Similarly, the stability of hippocampal place cells is disrupted by NMDA receptor blockade (Kentros et al., 1998). Further, hippocampal place cells can remap their preferred firing location within a spatial environment, even when the environment itself is unchanged (Wood, Dudchenko, Robitsek & Eichenbaum, 2000). Recently, it has been reported that fear conditioning causes DH place cells to remap (or alter) their preferred firing locations in their environment (Moita, Rosis, Zhou, LeDoux & Blair, 2004). It was also reported that contextual fear conditioning caused significantly more remapping than auditory fear conditioning, suggesting that place cells in the DH remap when the context becomes the best predictor of shock (Moita et al, 2004).

In a previous study, hippocampal place cells acquired CS evoked responses following auditory conditioning (Moita et al., 2003). Here, both place cells and theta cells were recorded. Place cell activity is generated by excitatory pyramidal neurons, whereas theta activity is characteristic of inhibitory interneurons (e.g., Rack, 1973). It was reported that hippocampal neurons (place and theta cells) acquired responses to the auditory CS following fear conditioning. Aversive conditioning caused place cells to acquire CS-evoked responses which were mediated by the cells' place-specific firing. In addition, theta cells exhibited enhanced CS-evoked responses following auditory fear conditioning (Moita et al., 2003). These results support the view that CS-US convergence within the hippocampus may drive the acquisition of responses of hippocampal cells to the auditory CS. These findings support the view that the hippocampus contributes to context-specific mèmory formation during associative learning (Moita et al, 2003).

In summary, GABAergic synaptic plasticity in the DH seems necessary for the retrieval of contextual memories associated with shock (Holt & Maren, 1999). Additionally, NMDA-dependent synaptic plasticity fulfils a role in the acquisition, but not expression of contextual fear responses (Kim, DeCola, Fernandez & Fanselow, 1991; Kim, Fanselow, DeCola, Fernandez, 1992; Young, Bohenek & Fanselow, 1994; Fanselow, Kim, Yipp & Oca, 1994). The role of the VH following muscimol infusion is somewhat contradictory, with some researchers reporting impairments in auditory cued fear conditioning and sparing of contextual conditioning (Bast et al., 2001) while others report the reverse (Maren & Holt, 1999). In order to fully ascertain the role of GABAergic activity in this region, it is argued that consistency between experimental procedures is necessary. Further, NMDA-dependent activity in the VH seems necessary for the acquisition of contextual fear conditioning (Zhang et al., 2001b).

Finally, studies examining place cells have suggested a role for the hippocampus in contextual fear (Moita et al., 2004) and possibly auditory processing (Moita et al., 2003). Thus, collectively the hippocampus seems necessary for the acquisition of contextual fear conditioning (but see, Maren & Holt, 1999). Interestingly, synaptic plasticity in the BLA has been induced by *in-vivo* stimulation of the hippocampal formation, and lesions of both of these structures impaired contextual fear (Maren & Fanselow, 1995). As such, the authors suggest that the connections following plasticity between the two regions are critically involved in the acquisition of contextual fear. Thus, one would expect that a mutation interfering with plasticity in the hippocampus (Zamanillo et al., 1999) would consequently disrupt this form of learned fear.

3.1.5 *Coda.*

In the current series of experiments I assess the effects of GluR-1 deletion of the acquisition of contextual and cued Pavlovian fear conditioning. The above review has revealed the neural and synaptic circuits involved in Pavlovian fear condition. The published research suggests that GluR-1$^{-/-}$ mice display aberrant synaptic impairments in the Schaffer collateral-commissural pathway (Zamanillo et al., 1999). Therefore one would predict impairments in contextual fear conditioning (Kim & Fanselow, 1992). However, Bannerman and colleagues have posited a specific spatial working memory (but not spatial reference memory) impairment (Resiel et al., 2002) in GluR-1 mutant mice. Since reference memory refers to fixed stimulus-outcome contingencies, under this hypothesis one would assume Pavlovian fear conditioning to tone and context should be normal. Finally, a behavioural pattern similar to that seen

in rats with lesions of the BLA has been suggested (Mead & Stephens, 2003*a*). If this is so, one might expect an impairment in both contextual and cued Pavlovian fear conditioning (Koo et al., 2004).

3.2 Experiment 5.

Previous work indicates that lesions of the BLA produce severe modality-independent deficits in both the acquisition and expression of Pavlovian fear conditioning (Davis, 1992; LeDoux, 2000; Maren, 2001). Additionally, pre-training electrolytic lesions to the DH (Maren, Aharonov, Fanselow, 1997), fibre-sparing excitotoxic lesions to the VH or total hippocampal lesions (Richmond et al., 1999) impair contextual fear conditioning. Here I assess Pavlovian fear conditioning using either auditory or visual stimuli to ensure that any noted deficit could not be attributable to a specific impairment in one sensory domain. Additionally, a cohort of GluR-3$^{-/-}$ mice were included for comparison. Gene-targeted deletion of the GluR-3 subunit results in a reduction in the levels of GluR-1, GluR-2 and GluR-4 in the cerebellum but not in the hippocampus (Sanchi-Segura et al., in press). This downregulation of subunits, essentially results in an overall reduction of AMPA receptors in the cerebellum. Consistent with these findings GluR-3$^{-/-}$ mice show normal hippocampal LTP (Meng, Zhang & Jai, 2003), therefore these GluR-3 mutant mice provide an interesting comparison in investigating the role of LTP in hippocampal-dependent (i.e., contextual fear conditioning) learning and memory processes and provided a control for non-specific effects associated with the production of the GluR mutation.

3.2.1 Method.

Subjects.

The experiment was conducted in two replications with naïve wild-type GluR-1 controls (n = 13), GluR-$1^{-/-}$ (n = 19), wild-type GluR-3 controls (n = 15) and GluR-$3^{-/-}$ (n = 20) mice. The mice were bred in the Department of Experimental Psychology at the University of Oxford and transferred to the School of Psychology, Cardiff University for behavioural testing at 12 months of age. Subjects were individually housed on a 12 h light: dark schedule (lights on at 07:00 h), in plastic cages with wood shaving and bedding. All behavioural testing took place during the light phase between 09:00h and 17:00h.

Apparatus.

The apparatus used in the experiment was identical to that used in Experiment 1. However, the floor of the grids were connected to a Colbourn precision regulated animal shocker (model number H13-16).

Procedures.

Stage 1: Conditioning.

The assignment of mice was counterbalanced for experimental groups and operant chambers, such that equal numbers of wild-type control and GluR-1$^{-/-}$ mice were conditioned in one of the two experimental chambers. On day 1, all the mice were transferred to their assigned operant chamber and received a 12 min conditioning session. After an initial acclimatization period of 6 min followed by a 30 s pre-CS period, half the mice (equal numbers of control and mutant mice) received three presentations of an auditory tone (80dB; 30s) followed by foot shock (0.4 mA, 2s) which occurred during the final 2s of the cue presentation. The remaining mice received the same conditioning contingency, however the CS was a 30s constant visual cue. Presentations were separated by 2 min ITI and mice were removed from the chamber 30 s after the last shock presentation. The grid floor was wiped with 70% alcohol between each subject. On completion of conditioning, 2d of testing followed. For half of the mice, the cue test was conducted on day 2 and the context test was conducted on day 3, whereas for the remaining half this order was reversed.

Stage 2: CS and Context Retention Test.

The contextual cues in the operant chamber were altered for the CS test. The aluminium walls were replaced by black and white striped alternating pattern using Perspex inserts and the grid floor was covered by Perspex solid floor with a black and white checkerboard pattern, which was covered with a thin layer of sawdust prior to

commencing the test session. Additionally a vanilla scented odour cube was placed inside each of the two sound attenuating chambers, attached (using small Velcro pads) to the inner wall of the Perspex door. The cube (measuring approximately, 4.6 cm^3) contained a vanilla scented pad (Dale Air Ltd, Lancashire, England), and 6 small holes on one side of the cube allowing the odour to diffuse into the chamber. During the CS test, the mice were placed in this novel chamber and locomotor activity and freezing behaviour were monitored. The first 6 min measured exploratory activity elicited by the novel context, after which the CS was presented continuously for 8 min. For the context test the chambers were arranged in their original configuration which had previously been used in the conditioning stage. Mice were placed in the chamber for 8 min, and their freezing behaviour and locomotor activity was scored. As in the conditioning stage, the floors of the chambers were wiped with 70% alcohol solution between each subject.

Scoring.

During the fear conditioning procedures, the mouse's tendency to freeze was scored. Observations were carried out using a time-sampling procedure; whereby every 5 s, each mouse was judged as either freezing or active. Freezing was defined as the absence of visible movement, except for respiration (Blanchard & Blanchard, 1971). Scoring began 10 s after the mouse was placed in the chamber. From this observation, a percentage freezing score was calculated by dividing the number of intervals the subject was judged to be freezing by the total number of observations. All scoring was conducted by two observers who were "blind" in respect to the critical aspects of the experimental manipulation (i.e., genotype of the mouse and

experimental condition). The interrater reliability between the two observers who used this scoring procedure was high, Pearsons ($r = 0.88$, $r^2 = 0.79$, $p<0.05$).

3.2.2 Results.

Locomotor Activity: Conditioning stage acclimatisation period.

There were no main effects or interactions involving the two groups of wild-type control mice from the GluR-1 and GluR-3 background strains (all F values <1), so the results from the two groups are herein combined for all wild-type control mice. In general, rodents show greater levels of conditioning to auditory rather than visual cues (e.g., Kim et al., 1996), therefore the activity levels during the conditioning stage (and for subsequent analysis), are shown separately for the light conditioned (Figure 3.5.1a) and tone conditioned (Figure 3.5.1b) mice. During the pre-stimulus period all mice displayed similar levels of baseline activity. In order to confirm this observation, separate two-way ANOVAs were conducted on the activity levels prior to stimulus presentation, with a between subject factor of genotype (GluR-1, GluR-3 and control), and a within subject factor of time bin (1-12). In respect to mice conditioned with the visual cue (3.5.1a), the ANOVA revealed no main effect of genotype ($F<1$), or time bin ($F_{(11,330)} = 1.17$, $p>0.3$), although a significant genotype × time bin interaction was revealed ($F_{(22,330)} = 1.86$, $p<0.05$). Simple main effects analysis conduced on the significant interaction revealed no main effect of genotype at any time bin (largest F value; bin 9, $F_{(2,77)} = 2.72$, $p>0.07$) with only GluR-$1^{-/-}$ ($F_{(11,330)} = 2.05$, $p<0.05$) but not GluR-$3^{-/-}$ ($F_{(11,330)} = 1.356$, $p>0.1$) or wild-type control mice ($F_{(11,330)} = 1.48$, $p>0.1$) showing evidence of changes in locomotor activity during this period. In order

to evaluate whether any group differences were evident during this acclimatisation period *post-hoc* Newman-Keuls analysis was adopted, which revealed no overall activity differences between any genotype ($p>0.05$).

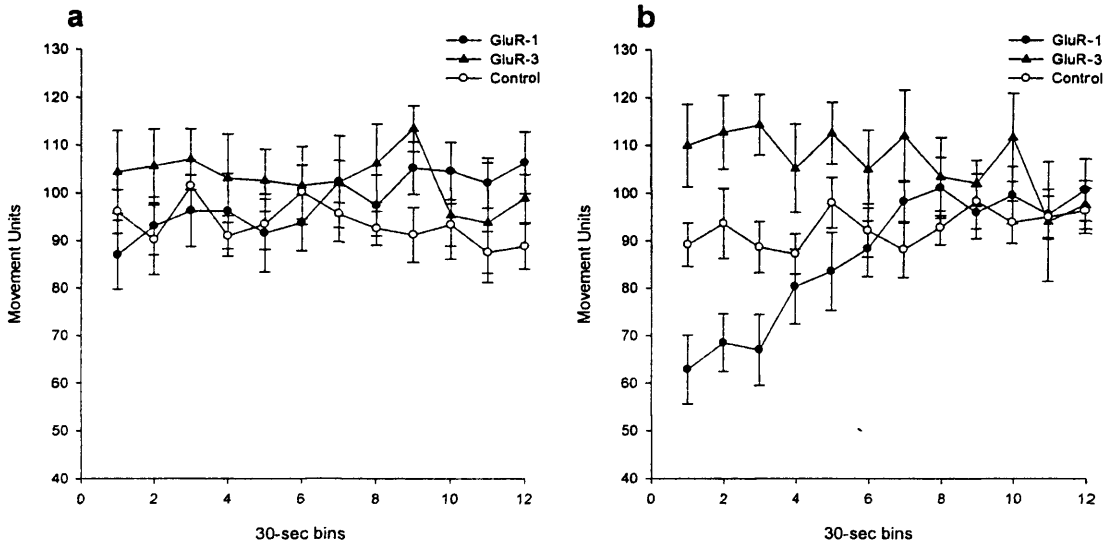

Figure 3.5.1. *Mean locomotor activity scores during acclimatisation period.* **(a)** Mean locomotor activity scores during the 6-min acclimatisation period (in 30-sec bins) prior to the presentation of the stimuli for mice conditioned with the visual cue. **(b)** Mean locomotor activity scores during the 6-min acclimatisation period (in 30-sec bins) prior to the presentation of the stimuli for mice conditioned with the auditory cue. Closed circles = GluR-$1^{-/-}$ mice; closed triangles = GluR-$3^{-/-}$ mice; open circles = wild-type control mice. Error bars indicate standard error of the mean.

A similar analysis adopted for mice in the tone condition (Figure 3.5.1b) revealed no main effect of genotype ($F_{(2,29)} = 2.43$, $p>0.1$) a main effect of time bin ($F_{(11,319)} = 2.58$, $p<0.01$) and a significant interaction between the two factors ($F_{(22,319)} = 4.16$, $p<0.001$). Main effects analysis revealed a significant effect of time bin at bins 1-3 and 5 (smallest F value; bin 5, $F_{(2,64)} = 3.25$, $p<0.05$) with GluR-$1^{-/-}$ ($F_{(11,319)} = 7.46$, $p<0.01$) but not GluR-$3^{-/-}$ ($F_{(11,319)} = 1.61$, $p>0.09$) or control mice ($F<1$) showing evidence of changes in locomotor activity during this period. *Post-hoc* Newman-Keuls analysis revealed no overall differences between groups during this period ($p>0.05$).

Locomotor Activity: CS conditioning.

The activity levels during each 30s, CS presentation in the conditioning stage are shown separately for mice conditioned to the light (Figure 3.5.2a) or tone cues (Figure 3.5.2b). In general, GluR-$1^{-/-}$ mice from both conditions failed to show any evidence of activity level suppression following continued CS-shock pairings. In respect to mice conditioned with the visual cue, a two-way mixed ANOVA with factors of genotype and time bin revealed a main effect of genotype ($F_{(2,30)} = 9.05$, $p<0.001$) and time bin ($F_{(2,60)} = 27.2$, $p<0.0001$) and a significant interaction between the two factors ($F_{(4,60)} = 9.13$, $p<0.0001$). Main effects analysis revealed a main effect of genotype at bins 2 and 3 (smallest F value; bin 2, $F_{(2,62)} = 5.77$, $p<0.01$) with both GluR-$3^{-/-}$ ($F_{(2,58)} = 30.5$, $p<0.001$) and control ($F_{(2,58)} = 24.5$, $p<0.0001$) but not GluR-$1^{-/-}$ mice ($F<1$) showing evidence of activity suppression during the conditioning

trials. *Post-hoc* Newman-Keuls analysis revealed GluR-$1^{-/-}$ differed from both GluR-$3^{-/-}$ and control groups ($p<0.01$); no other group differences were revealed ($p>0.05$).

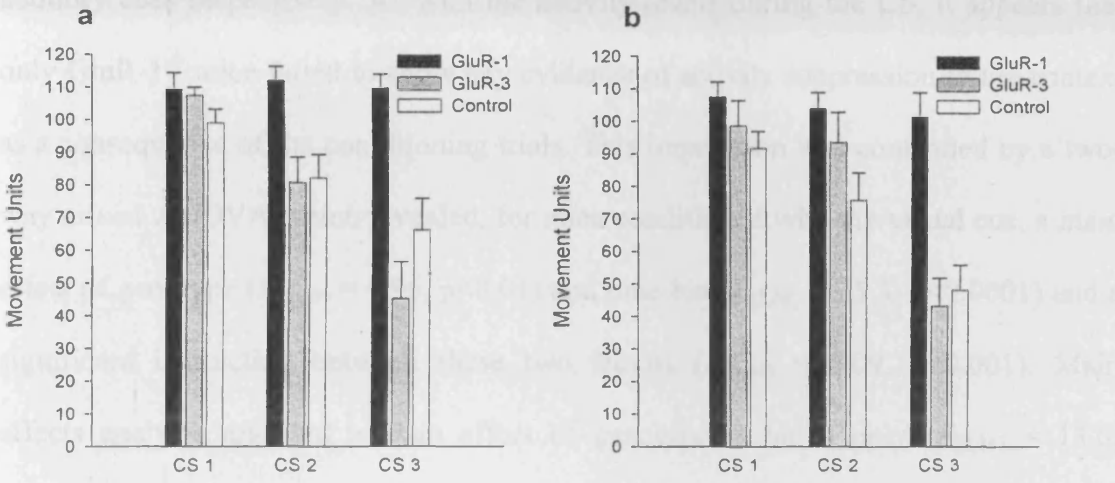

Figure 3.5.2 *Mean locomotor activity scores during conditioning stage.* **(a)** Mean activity scores during 30 s presentations of light CS. **(b)** Mean activity scores during 30 s presentations of tone CS. Black bars = GluR-$1^{-/-}$ mice; grey bars = GluR-$3^{-/-}$ mice; white bars = wild-type control mice. Error bars indicate standard error of the mean.

Similar analysis applied to mice conditioned with the auditory stimulus, revealed a main effect of genotype ($F_{(2,29)} = 7.21$, $p<0.01$) and time bin ($F_{(2,58)} = 37.2$, $p<0.001$) and a significant interaction between these two factors ($F_{(4,58)} = 7.85$, $p<0.0001$). Main effects analysis revealed a main effect of genotype at bins 2 and 3 (smallest F value; bin 2, $F_{(2,55)} = 3.52$, $p<0.05$) with GluR-$3^{-/-}$ ($F_{(2,58)} = 30.5$, $p<0.0001$) and control ($F_{(2,58)} = 24.5$, $p<0.0001$) but not GluR-$1^{-/-}$ mice ($F<1$) showing activity suppression following conditioning. *Post-hoc* Newman-Keuls comparisons revealed that GluR-$1^{-/-}$ mice differed from both GluR-$3^{-/-}$ and control mice ($p<0.01$).

Locomotor Activity: Context Conditioning.

The mean levels of activity in the 30 s periods prior to the CS presentation are displayed in Figures 3.5.3a and 3.5.3b for mice conditioned with the visual and auditory cues respectively. As with the activity levels during the CS, it appears that only GluR-1$^{-/-}$ mice failed to show any evidence of activity suppression to the context as a consequence of the conditioning trials. This impression was confirmed by a two-way mixed ANOVA which revealed, for mice conditioned with the visual cue, a main effect of genotype ($F_{(2,30)} = 4.96$, $p<0.01$) and time bin ($F_{(2,60)} = 25.3$, $p<0.0001$) and a significant interaction between these two factors ($F_{(4,60)} = 6.09$, $p<0.001$). Main effects analysis revealed a main effect of genotype at bin 3 only ($F_{(2,56)} = 13.6$, $p<0.001$), with both GluR-3$^{-/-}$ ($F_{(2,60)} = 22.9$, $p<0.001$) and control ($F_{(2,60)} = 13.2$, $p<0.001$) but not GluR-1$^{-/-}$ ($F<1$) showing evidence of activity level suppression. *Post-hoc* Newman-Keuls revealed a group differences between GluR-1$^{-/-}$ and all other groups ($p<0.01$). No other differences were revealed ($p>0.05$).

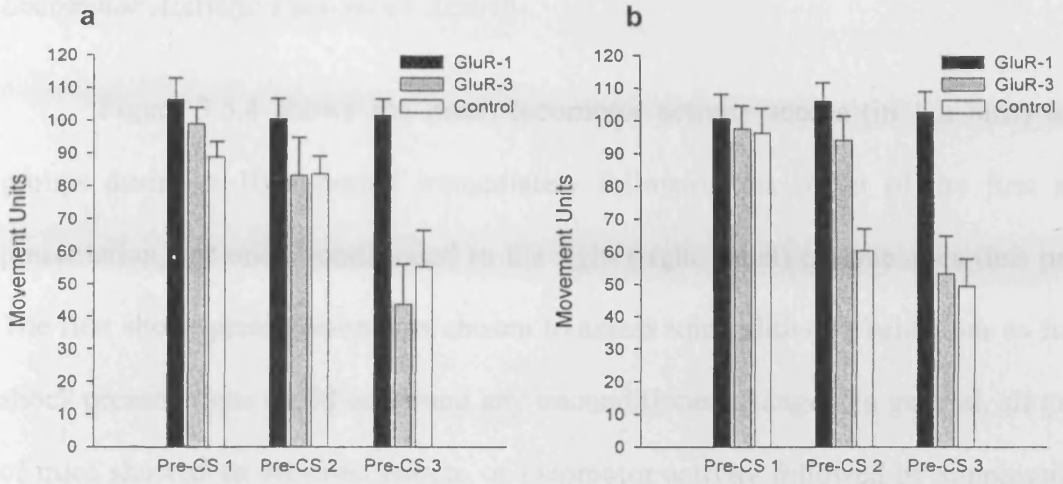

Figure 3.5.3. *Mean locomotor activity scores during conditioning stage.* **(a)** Mean activity scores during 30 s Pre-CS period for mice conditioned with the visual cue. **(b)** Mean activity scores during 30 s Pre-CS period for mice conditioned with the auditory cue. Black bars = GluR-$1^{-/-}$ mice; grey bars = GluR-$3^{-/-}$ mice; white bars = wild-type control mice. Error bars indicate standard error of the mean.

A similar analysis conducted on the data obtained from mice conditioned with the auditory cue revealed a main effect of genotype ($F_{(2,29)} = 6.436$, $p<0.01$) and time bin ($F_{(2,58)} = 25.02$, $p<0.0001$) and a significant interaction between the two factors ($F_{(4,58)} = 10.02$, $p<0.0001$). Simple main effects analysis conducted on the interaction revealed a main effect of genotype at bins 2 and 3 (smallest F value; bin 2, $F_{(2,52)} = 8.62$, $p<0.02$) with GluR-$3^{-/-}$ ($F_{(2,58)} = 19.8$, $p<0.001$) and control ($F_{(2,58)} = 30.5$, $p<0.001$) but not GluR-$1^{-/-}$ mice ($F<1$) showing evidence of suppression across the conditioning session. *Post-hoc* Newman-Keuls comparisons confirmed this trend revealing significant differences between GluR-$1^{-/-}$ mice and all other groups only ($p<0.001$).

Locomotor Activity: Post-shock Activity.

Figure 3.5.4 shows the mean locomotor activity scores (in 1 s bins) for all groups during a 10 s period immediately following the offset of the first shock presentation, for mice conditioned to the light (right panel) or tone cues (left panel). The first shock presentation was chosen to assess unconditioned behaviour as further shock presentations could confound any unconditioned changes. In general, all groups of mice showed an elevated pattern of locomotor activity followed by suppression of this activity immediately following shock presentation. However, the degree of suppression appears lower in GluR-1$^{-/-}$ mice compared to the other experimental groups. In order to evaluate these differences separate ANOVA's were conducted with a between subjects factor of genotype and a within subject factor of time bin. For mice in the light conditioned groups (Figure 3.5.4a), this analysis revealed no main effect of genotype ($F_{(2,29)} = 4.52$, $p>0.05$) a main effect of time bin ($F_{(9,261)} = 6.91$, $p<0.01$) but no interaction between these two factors ($F_{(18,261)} = 1.62$, $p>0.05$). Similar analysis employed for mice conditioned with the auditory cue (Figure 3.5.4b) revealed no main effect of genotype ($F_{(2,28)} = 2.45$, $p>0.1$), a main effect of time bin ($F_{(9,252)} = 5.727$, $p<0.0001$) but no interaction between these two factors ($F<1$).

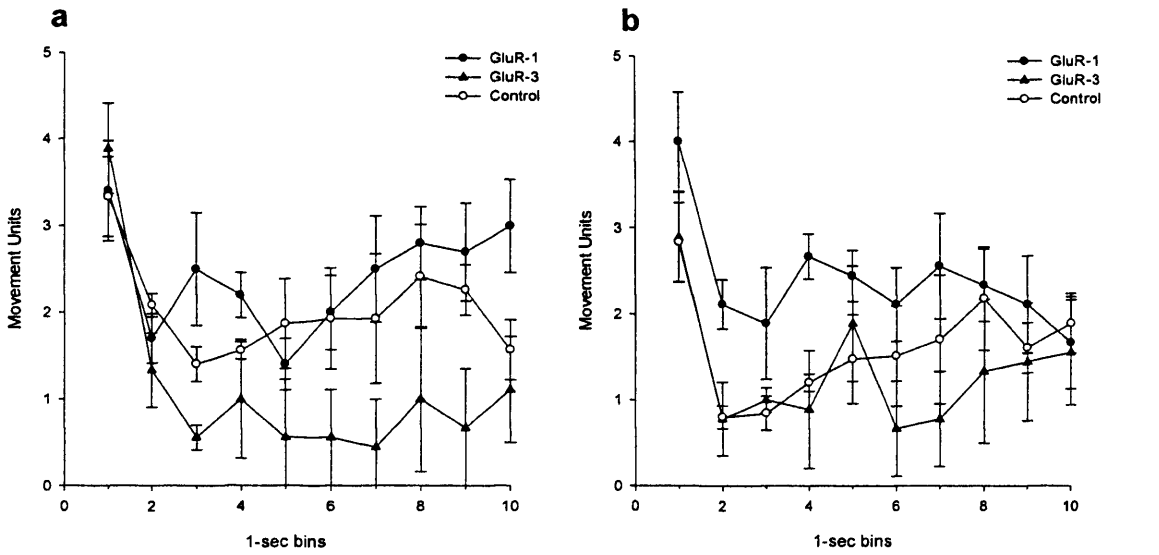

Figure 3.5.4. *Unconditioned response to shock.* **(a)** Mean infrared locomotor scores during the first 10 s period of ITI following the offset of the first foot shock in mice conditioned with the visual cue. **(b)** Mean infrared locomotor scores during the first 10 s period of ITI following the offset of the first foot shock in mice conditioned with the auditory cue. **(c)** Closed circles = GluR-$1^{-/-}$ mice; closed triangles = GluR-$3^{-/-}$ mice; open circles = wild-type control mice. Error bars equal standard error of the mean.

Unconditioned response to the tone.

One possible explanation for the aforementioned conditioning impairment in GluR-$1^{-/-}$ mice may relate to a sensory deficit in processing the CS. Normally novel auditory CS presentation, will itself evoke a UR in animals (e.g., Harris, 1943). In order to assess whether GluR-$1^{-/-}$ mice were sensitive to the tone presentation we calculated the mean locomotor activity scores in 0.5 sec time bins for the last two second of the ITI and first two seconds of the first CS tone presentation (Figure 3.5.5). In order to determine whether the mice showed an unconditioned reaction to the novel

tone (which usually takes the form of inactivity), a two-way ANOVA with genotype, phase (ITI versus CS) and time bin as factors was conducted on the locomotor activity scores. Analysis of the activity date revealed no main effect of genotype ($F_{(2,28)}$ =2.52, $p > 0.09$). However, there was a main effect of phase ($F_{(1,28)} = 13.58$, $p < 0.01$), a main effect of time bin ($F_{(1,28)} = 14.16$, $p<.01$), and a significant interaction between these factors ($F_{(3,84)} = 3.54$, $p< 0.05$). Test of simple main effects revealed a significant effect of time bin only during the CS period ($F_{(3,84)}$ =7.83, $p < 0.001$) that reflected a gradual rise in locomotor activity during the CS. These changes in activity did not vary as a function of genotype ($F_{(6,84)} = 1.49$, $p > 0.10$). The analysis suggests, therefore, that all groups were able to detect the presentation of the Tone CS.

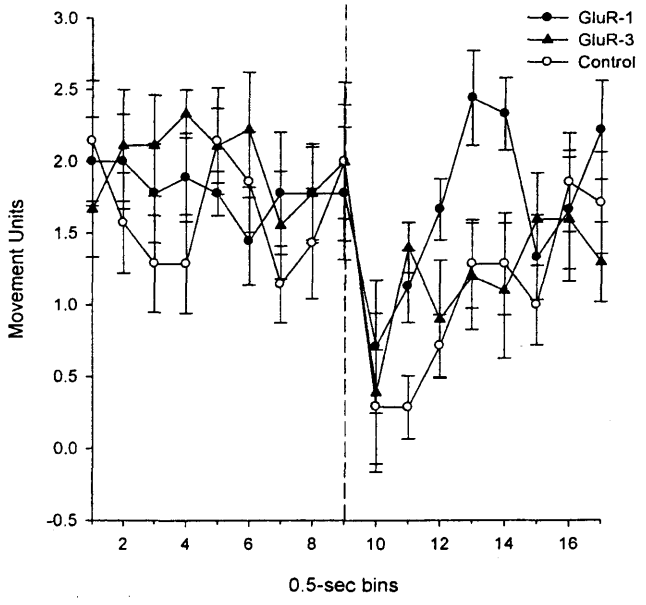

Figure 3.5.5. *Unconditioned response to tone.* Mean locomotor activity scores in (0.5 sec bins) immediately prior to and post initial presentation of the auditory cue. Dashed line indicates cue presentation. Closed circles = GluR-$1^{-/-}$ mice; closed triangles = GluR-$3^{-/-}$ mice; open circles = GluR-$3^{-/-}$ mice. Error bars indicate standard error of the mean.

CS Retention Test: Locomotor Activity.

Figure 3.5.6 shows the locomotor activity levels for mice in the light (left panel) and tone conditions (right panel) separately. In general, all mice showed similar levels of activity to the novel context prior to the presentation of the CS. This impression was confirmed by separate two-way ANOVA's, on the activity data prior to stimulus presentation. For mice in the light condition (Figure 3.5.6a) the analysis revealed a main effect of time bin only ($F_{(11,319)} = 31.763$, $p<.0001$), all other factors ($F<1$). Similarly, for mice in the tone condition (Figure 3.5.6b), a main effect of time bin was revealed ($F_{(11,330)} = 2.172$, $p<.02$), with no main effect of genotype ($F_{(2,30)} = 1.522$, $p>0.2$) or interaction ($F<1$).

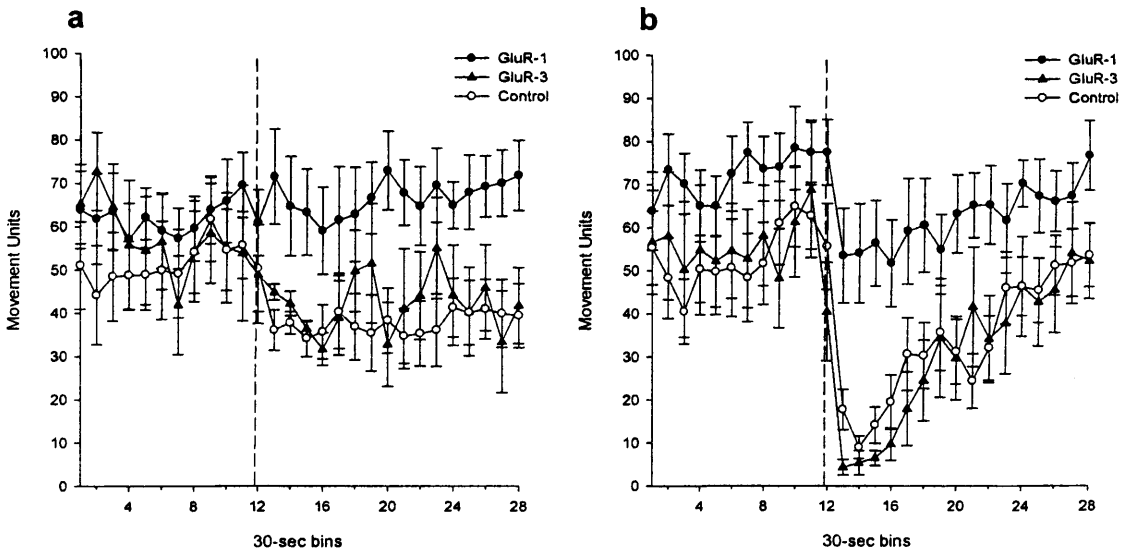

Figure 3.5.6. *CS retention test: Locomotor activity scores.* **(a)** Mean infrared locomotor activity scores during the visual CS retention test for mice conditioned with the visual cue. **(b)** Mean infrared locomotor activity scores during the auditory CS retention test for mice conditioned with the auditory cue. Dashed line indicates presentation of the CS. Closed circles = GluR-$1^{-/-}$ mice; closed triangles = GluR-$3^{-/-}$ mice; open circles = wild-type control mice. Error bars indicate standard error of the mean.

The activity monitor was used to assess both the level of activity and also provide an independent means of assessing freezing behaviour i.e., due to activity suppression. As an assessment of validity, the Pearson's correlation coefficient was calculated for locomotor activity and freezing behaviour of all animals during the CS retention test. This revealed Pearson's correlation of r = -0.77 (r^2 = 0.61, $p<0.05$) indicating a negative correlation between the two measures, which suggests that the locomotor activity responses provided an independent measure of freezing behaviour in the cue test.

Inspection of Figure 3.5.6 shows that on presentation of the CS, GluR3$^{-/-}$ and control mice conditioned to the light (left panel) and the tone (right panel) showed suppression of locomotor activity. However, GluR-1$^{-/-}$ mice in both conditions failed to show any evidence of activity suppression during the CS presentation. In order to confirm this impression, separate two-way ANOVA's were conducted on the locomotor activity scores during stimulus presentation. The analysis conducted on mice that had previously been conditioned with the visual cue (Figure 3.5.6a), revealed a main effect of genotype ($F_{(2,29)}$ = 3.350, p<.05), although no other main effects or interaction terms reached significance (all F's<1). *Post-hoc* Newman-Keuls comparisons revealed a significant difference between the GluR-1$^{-/-}$ group and all other mice (p<0.01); no other differences were revealed. For mice in the tone condition (Figure 3.5.6b), this analysis revealed a main effect of genotype ($F_{(2,30)}$ = 6.187, p<.01), time bin ($F_{(15,450)}$ = 14.811, p<.0001) and a significant interaction between these two factors ($F_{(2,450)}$ = 1.910, p<.05). Analysis of simple main effects carried out on the significant interaction, followed by *post*-hoc Newman-Keuls comparisons, revealed a main effect of genotype at bins 13-18 and 20-22 (smallest F value; bin 22, $F_{(2,71)}$ = 4.806, p<.05), with control and GluR-3$^{-/-}$ mice differing from GluR-1$^{-/-}$ mice in the tone condition (p<0.01).

CS Retention test: Freezing Behaviour.

Figure 3.5.7 shows the mean percentage of observations on which a freezing response was recorded for mice conditioned to the light (left panel) and the tone (right panel) conditions separately. In general, all mice showed relatively low levels of freezing during exploration of the novel context prior to the presentation of the CS. In

order to confirm this observation, separate ANOVA's were conducted on the freezing scores prior to stimulus presentation. For all mice in tone (Figure 3.5.7a) and light conditions (Figure 3.5.7b), the analysis revealed no main effects of genotype nor interactions involving this factor (all F's<1).

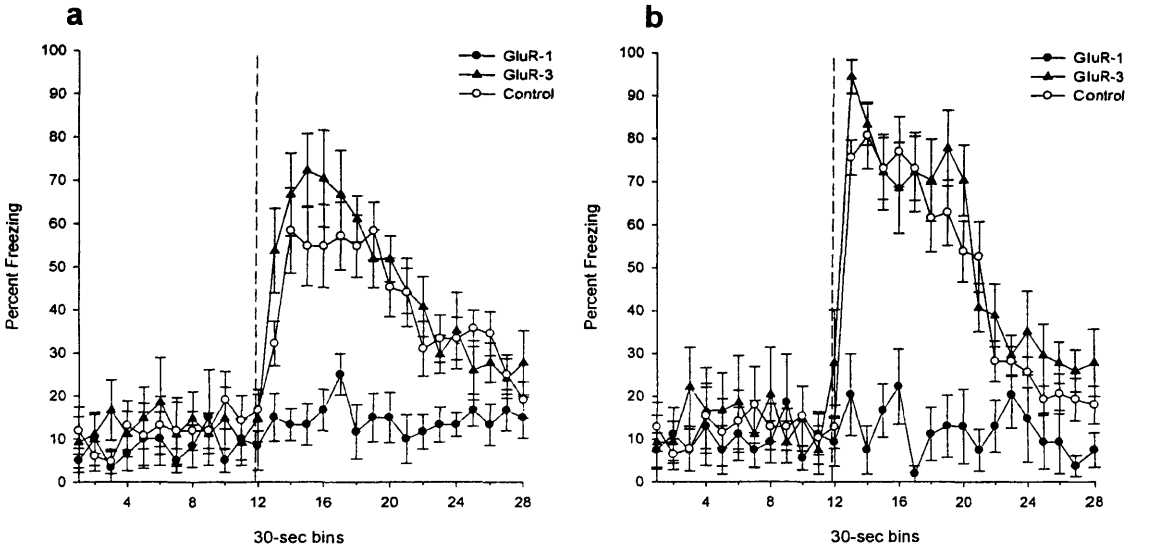

Figure 3.6.7. *CS retention test: Freezing behaviour.* **(a)** The mean percentage of observations with a freezing response during the visual CS retention test for mice conditioned with the visual cue. **(b)** The mean percentage of observations with a freezing response during the auditory CS retention test for mice conditioned with the auditory cue. Dashed line indicates presentation of the CS. Closed circles = GluR-$1^{-/-}$ mice; closed triangles = GluR-$3^{-/-}$ mice; open circles = wild-type control mice. Error bars indicate standard error of the mean.

Following presentation of the CS (Figure 3.6.7) the level of freezing was significantly attenuated for GluR-$1^{-/-}$ mice compared to the other two groups. For mice in the light condition (Figure 3.6.7a) the analysis revealed a main effect of genotype ($F_{(2,30)}$ = 26.149, p<.001) and of time bin ($F_{(15,450)}$ = 7.448, p<.001) and a

significant interaction between these two factors ($F_{(30,450)}$ = 2.230, p<.001). Main effects analysis carried out on the significant genotype × time bin interaction revealed a main effect of genotype at bins 12-21 (smallest F value; bin 21, $F_{(2,319)}$ = 4.460, p<.02), with GluR-3$^{-/-}$ ($F_{(15,450)}$ = 6.265, p<.001) and control mice ($F_{(15,450)}$ = 5.357, p<.01) displaying high levels of freezing to the light cue, compared to GluR-1$^{-/-}$ mice (F<1). *Post-hoc* Newman-Keuls comparisons revealed a significant difference between GluR-1$^{-/-}$ mice and all other groups (p<0.01); no other differences were revealed. For mice in the tone condition (Figure 3.6.7b), the analysis revealed a main effect of genotype ($F_{(2,28)}$ = 33.428, p<.001) and time bin ($F_{(15,420)}$ = 23.798, p<.001) and a significant interaction between these two factors ($F_{(30,420)}$ = 5.696, p<.001). Analysis of simple main effects carried out on the significant interaction revealed a main effect of genotype at bins 13-17 (smallest F; bin 17, $F_{(2,205)}$ = 3.560, p<.05), with both GluR-3$^{-/-}$ ($F_{(15,420)}$ = 14.926, p<.001) and control ($F_{(15,420)}$ = 22.655, p<.001), but not GluR-1$^{-/-}$ mice (F<1) showing high levels of freezing behaviour throughout the extinction test. *Post-hoc* Newman-Keuls comparisons revealed significant differences between GluR-1$^{-/-}$ and all other mice (p<0.01); no other differences were reported. Overall these results suggests that GluR-1$^{-/-}$ mice showed no evidence of freezing to a previously conditioned auditory or visual cue; whereas GluR-3$^{-/-}$ mice show a similar propensity to freeze as control mice.

Context Retention test: Locomotor Activity.

Once again, locomotor suppression was used to provide an independent means of assessing suppression of activity i.e., freezing during the context test. Correlational analysis comparing the freezing and locomotor activity data for all groups of mice

during the context retention test revealed a negative correlation between the two factors, Pearson's r = -0.86 (r^2 = 0.75, p <0.05). Therefore, this indicates that the automated activity measure was sensitive to response suppression elicited by the conditioned context.

Figure 3.6.8 shows the locomotor activity levels for all mice when reexposed to the conditioning context for mice in the light (left panel) and tone conditions (right panel). In general, GluR-$1^{-/-}$ mice showed the highest levels of activity when reexposed to the previously shocked context. Separate two-way ANOVA's were conducted for all mice in the tone and light conditions. For mice in the light conditions (Figure 3.6.8a), the analysis revealed a main effect of genotype ($F_{(2,30)}$ = 3.459, p<.05), no main effect of time bin ($F_{(15,450)}$ = 1.202, p>0.2), but a significant interaction between these two factors ($F_{(30,450)}$ = 2.080, p<.001). Tests of simple main effects carried out on the significant interaction followed by *post*-hoc Newman-Keuls analysis revealed a main effect of genotype at bins 5,8-12 and 14-16 (smallest F value; bin 15, $F_{(2,53)}$ = 3.385, p<.05); with overall differences between GluR-3 and GluR-1 mice only (p<.05).

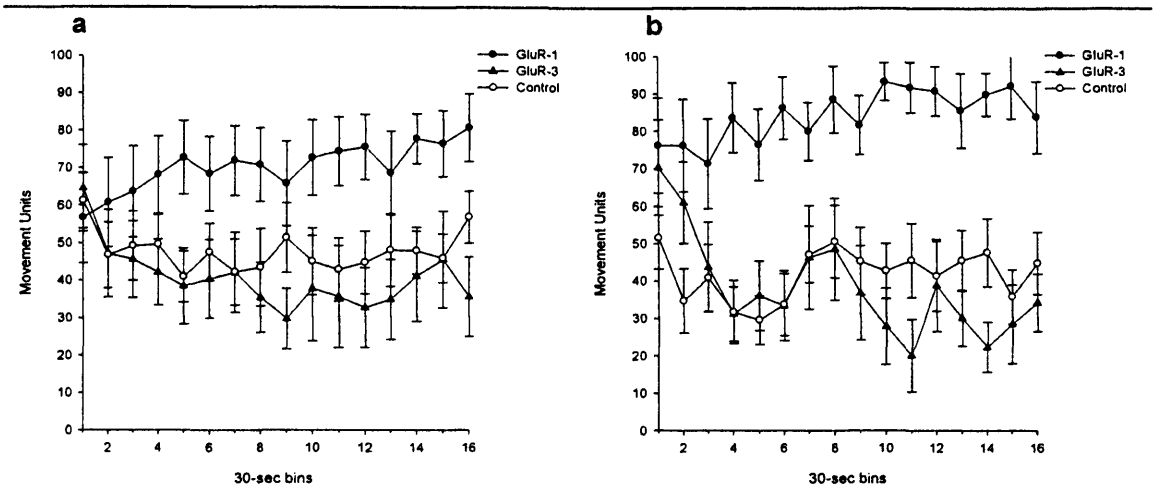

Figure 3.6.8. *Context retention test: Locomotor activity scores.* **(a)** Mean infrared locomotor activity scores during the context retention test for mice conditioned with the visual cue. **(b)** Mean infrared locomotor activity scores during the context retention test for mice conditioned with the auditory cue. Closed circles = GluR-$1^{-/-}$ mice; closed triangles = GluR-$3^{-/-}$ mice; open circles = wild-type control mice. Error bars indicate standard error of the mean.

Similarly, the analysis conducted on the activity scores for mice in the tone condition (Figure 3.6.8b) revealed a main effect of genotype ($F_{(2,29)}$ = 12.270, p<.002), and time bin ($F_{(15,435)}$ = 2.107, p<.01), and a significant interaction between these two factors ($F_{(30,435)}$ = 3.213, p<.001). In order to evaluate the nature of the significant interaction, tests of simple main effects were carried out followed by *post-hoc* Newman-Keuls analysis. This revealed a main effect of genotype at bins 2-16 (smallest F value; bin 3, $F_{(2,69)}$ = 3.394, p<.05), with control mice and GluR-$3^{-/-}$ mice differing from GluR-$1^{-/-}$ mice (p<.05). Thus, in comparison to GluR-$3^{-/-}$ and wild-type control mice, GluR-$1^{-/-}$ mice displayed little evidence of locomotor activity suppression to the conditioned context.

Context Retention test: Freezing Behaviour.

Figure 3.6.9 shows the mean percentage of observations in which a freezing response was observed for mice in light (left panel) and tone (right panel) conditions. Inspection of this figure shows that GluR-1$^{-/-}$ mice failed to show control levels of freezing to the conditioned context. In order to confirm this impression, separate two-way ANOVA's were conducted for mice in light (Figure 3.6.9a) and tone conditions (Figure 3.6.9b). For mice conditioned with the visual cue the analysis revealed a main effect of genotype ($F_{(2,30)} = 20.251$, $p<.001$) and time bin ($F_{(15,450)} = 3.390$, $p<.05$), but no interaction between these factors was revealed ($F_{(30,420)} = 1.186$, $p>0.8$). *Post-hoc* Newman-Keuls comparisons revealed a difference between GluR-1$^{-/-}$ mice and all other genotypes ($p<.01$). For mice in the tone condition, analysis revealed a main effect of genotype ($F_{(2,28)} = 19.396$, $p<.001$) and time bin ($F_{(15,420)} = 4.634$, $p<.001$) and a significant interaction between these two factors ($F_{(30,420)} = 1.996$, $p<.01$). Tests of simple main effects carried out on the significant interaction followed by *post-hoc* Newman Keuls revealed a main effect of genotype during bins 3-6 and 8-16 (smallest F value; bin 16, $F_{(2,175)} = 5.328$, $p<.01$), with GluR-3$^{-/-}$ and control mice differing from GluR-1$^{-/-}$ mice ($p<.01$). Thus, in comparison to GluR-3$^{-/-}$ and control mice these results suggest that GluR-1$^{-/-}$ mice displayed little evidence of freezing to the previously conditioned context.

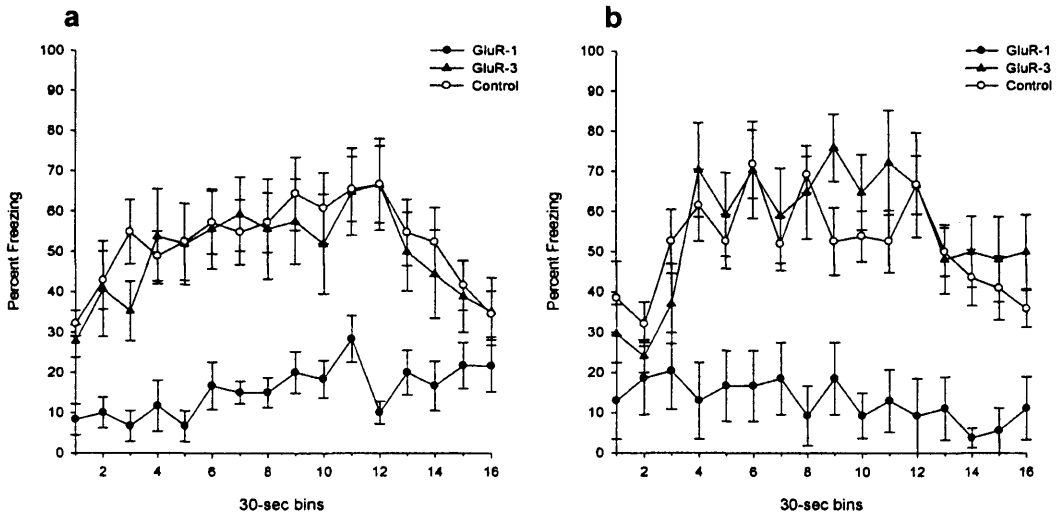

Figure 3.6.9. *Context retention test: Freezing behaviour.* **(a)** The mean percentage of observations with a freezing response during the context retention test for mice conditioned with the visual cue. **(b)** The mean percentage of observations with a freezing response during the context test for mice conditioned with the auditory cue. Closed circles = GluR-$1^{-/-}$ mice; closed triangles = GluR-$3^{-/-}$ mice; open circles = wild-type control mice. Error bars indicate standard error of the mean.

3.3.3 Discussion.

GluR-$1^{-/-}$ mice displayed a robust deficit in conditioned freezing to a tone CS, a light CS and to the experimental context, relative to wild-type controls. The deficit was evident as a reduction in freezing and as differences in an automated measure of locomotor activity during the CS and context retention tests. The deficit was unlikely to be due to differences in baseline activity levels. That is, hyperactivity could be expected to interfere with the mouses' ability to freeze, however prior to presentation of the cues all mice displayed a similar level of activity, therefore this account is unlikely. Similarly, all mice displayed a similar unconditioned response to either the

tone CS or the footshock US, indicating sensitivity to these stimuli. Additionally, the impaired conditioned freezing to the visual cue was unlikely to reflect a gross visual impairment as in Experiment 1 GluR-1$^{-/-}$ mice were clearly sensitive to presentations of a similar light source. It must be noted, that the fact that hyperactivity was not noted in GluR-1$^{-/-}$ mice in the present experiment contrasts with other studies (Vekovischeva et al., 2001; Bannerman et al., 2004; Experiment 1). However due to limitations with the availability of mice during the period of conducting the experiment, the mice used in Experiment 5 were 12 months of age (in contrast to previous experiments where mice had a maximum age of 6 months). Typically in mice, age-related cognitive deficits develop from 18 through 22 months of age (Forster et al., 1996). Furthermore, several studies have investigated the levels of ionotropic receptor distribution in aging. In general, middle-aged animals (10 months) display only a decrease in NMDA sites in the cortex and hippocampus, whilst in aged mice (30 months) a decline in both NMDA and AMPA sites have been noted (Magnusson & Cotman, 1993). Therefore an explanation based on differential age-related impairments in GluR-1$^{-/-}$ mice seems unlikely. Additionally, the results from the current experiment suggest that the hyperactivity phenotype is attenuated with age in GluR-1$^{-/-}$ mice; although the reason for this is not immediately obvious. Nevertheless, similar findings have been noted with other mutant mice (e.g., R6/2 mice; Li, Popovic & Brundin, 2005). In contrast, GluR-3$^{-/-}$ mice revealed only a mild and transient deficit in freezing behaviour that was evident after the first tone-footshock pairing during the conditioning day but was absent after subsequent tone-footshock pairings and during both the tone CS and context extinction tests. Similarly, no reported deficits were noted for GluR-3$^{-/-}$ mice in the light condition.

In contrast to Experiment 1, GluR-1 mutant mice failed to show any evidence of habituation to the CS throughout the extinction test. This finding might suggest that the previous fear conditioning session interfered with habituation processes in GluR-$1^{-/-}$ mice. However, the results from Experiment 1 suggest significant increases in habituation occur in mutant mice from sessions 3 onwards, therefore it would be of interest to examine whether this would still be the case following fear conditioning.

Of interest to the current set of data, SOP (Wagner, 1981) assumes that the A1 and A2 process each has its own behavioural consequence. That is, if the UR to a stimulus produces a behavioural sequence of first one behaviour followed by another, it is possible that this corresponds to a theoretical processing sequence of A1- followed by A2- dependent responding (Wagner & Brandon, 1989). A prototypical example of a UR in which the A1 and A2 processes appear to be associated with these dissociable behaviours is that noted using fear conditioning. Thus, as previously mentioned, the immediate primary reaction to footshock is agitation and hyperactivity (Fanselow, 1980). This would be akin to an A1-dependent response (Wagner, 1981). Consequently, following further conditioning, this is then followed by a secondary CR, i.e., freezing. In consideration of the previous discussion (pp.76) a failure to emit a freezing response could be interpreted an impairment in the mechanisms controlling the A2 memory system such as a disruption in the movement of elements from the inactive state to the A2 state. Obviously this analysis is speculative, however, it provides a further example that SOP can be used to interpret data following deletion of the GluR-1 receptor.

These results are in agreement with the suggestion that GluR-$1^{-/-}$ mice display a phenotype mimicking that seen in BLA-lesioned animals (Mead & Stephens, 2003a; Koo et al., 2004). Consistent with this view, GluR-$1^{-/-}$ mice (Experiment 5) displayed

impairments in conditioned fear to both a punctuate CS and to the experimental context. Additionally, all groups of mice showed a similar phenotype in response to the initial shock presentation. That is, the typical burst of activity followed by suppression of activity was noted for all mice following the initial shock presentation (Fanselow, 1980; Figure 3.2.5). This result suggests that GluR-1$^{-/-}$ mice were sensitive to the presentation of the unconditioned stimulus. These results are also consistent with the findings reported by Rumpel et al., (2005) who demonstrated the importance of the GluR-1 AMPA subunit for conditioned contextual and cued freezing using sRNA technology.

The pattern of results from this experiment are difficult to interpret in terms of the spatial working-memory hypothesis proposed by Bannerman and colleagues (Riesel et al., 2002). In its strictest form, this hypothesis would predict normal Pavlovian learned fear in mutant mice, as the procedure matches the characterisation of a reference memory procedure (see, Olton & Papas, 1979). However, it could be argued that GluR-1 dependent hippocampal spatial working memory may contribute to the encoding of the conditioning events in terms of episodic memory processes; the temporal encoding of events (what happened when; Eichenbaum & Fortin, 2003). That is, during fear conditioning acquisition is rapid and occurs in several short trials. Consistent with the results from Experiment 5, GluR-1 dependent synaptic plasticity may underlie the ability to encode both when and where events happened. Bannerman and colleagues have postulated a similar theory to account for the disturbance in conditional learning following deletion of the GluR-1 subunit (Schmitt et al., 2004a). Nevertheless, given the putative role of the hippocampus in processing only contextual information, this hypothesis might only apply to the results from contextual fear conditioning (Maren et al., 1997). As conditioned fear to either

auditory or visual CSs was also impaired, it suggests that the most parsimonious explanation of the deficit in GluR-1 mutant mice is in terms of impaired BLA function.

Nevertheless, inferring such regional specificity when using whole-brain deleted GluR-$1^{-/-}$ mice can be problematic. It is necessary therefore to examine the anatomy of fear conditioning circuits in the brain, in the context of the present experiment. Obviously, the site most readily implicated in the fear conditioning is the amygdala (LeDoux, 2000). From a neural systems approach, the BLA has strong reciprocal connections with the medial prefrontal cortex, where it receives inputs from all modalities; somatosensory, visual, auditory, olfactory, gustatory and visceral (Figure 1.1; Pitkänen, 2000). However, a direct role in fear conditioning per se is not suggested for the prefrontal cortex (Morgan et al., 1993). Rather, this region seems necessary for the consolidation of extinction processes related to fear conditioning (Quirk et al., 2000). Furthermore, it appears that protein synthesis-dependent mPFC activation is required for consolidation processes to take place (Santini et al., 2004). Consistent with this idea, it has also been reported that the recall of extinction is associated with plasticity in the mPFC (Milad & Quirk, 2002). Interestingly, medial prefrontal cortex lesions produce deficits in spatial NMTP on the T-maze, suggestive that damage to this region renders rats unable to hold task-related information 'on-line' in a manner that allows guidance of forthcoming actions, i.e., a working-memory impairment (Shaw & Aggleton, 1993; Dias & Aggleton, 2000; Corbit & Balleine, 2003; but see, Schmitt et al., 2003). It would be unsurprising if more than just one learning and memory system syndrome were disrupted – if only because learning systems do not exist in complete isolation. Nevertheless, by employing suitable

behavioural techniques it should be possible to discriminate the effects of the GluR-1 mutations on distinct learning systems.

As already alluded to, the BLA receives multi-modal information from the hippocampus proper and the perirhinal cortex (Sah et al., 2003). However, each of these regions have been implicated in the acquisition of contextual, rather than cued fear (Kim & Fanselow, 1992; Maren et al., 1997; Bucci et al., 2000; but see Lindquist, Jarrard & Brown, 2004). Although, it has been noted that the $GABA_A$ agonist muscimol infused into the VH disrupts auditory fear (Maren & Holt, 2004), others have reported no disruption (Bast, Zhang & Feldon, 2001). As previously discussed, procedural consistency is required prior to assigning a role for the VH in auditory fear conditioning.

Similarly the BLA receives multi-modal information from the subicular complex (Sah et al., 2003). Lesions of the ventral subiculum region interfered with the acquisition and expression of both cued, and to a lesser degree, contextual freezing (Maren, 1999b). It has been suggested that the locus of this impairment lies with the nucleus accumbens since the ventral subiculum is a major afferent to this region. Consistent with this idea, lesions of the fornix, the tract through which subiculo-accumbens fibres travel, also impairs contextual fear conditioning (Maren & Fanselow 1997, Phillips & LeDoux 1995). Additionally, it has been noted that pharmacological inactivation of the accumbens produces selective deficits in the acquisition of contextual fear conditioning (Haralambous & Westbrook 1999, Westbrook et al 1997; see also Riedel et al 1997). However, in the absence of direct evidence implicating the accumbens in discrete cued fear conditioning, it is unlikely that an accumbens-dependent impairment mediates the fear conditioning deficit in GluR-$1^{-/-}$ mice.

Additionally, the projections from the BLA to the BNST are suggested to play an important role in the expression of fear CRs (Fendt et al., 2003). A main target of the BNST is the periaqueductal gray (PAG) which has been implicated in the expression of freezing behaviour (Bandler & Shipley, 1994). It must be conceded that an impairment based on expression of freezing cannot be completely ruled out. In order to explore this issue further it would be interesting to carry out summation or retardation tests with an aversively conditioned CS in GluR-1 mutant mice to determine the extent to which the CS has gained associative strength (Mackintosh, 1974, pp.97). For instance, one could establish the extent to which the aversive CS (shock paired cue) suppresses CR to an established appetitive CS, when both are presented in compound.

Finally, the CeN of the amygdala has been implicated in fear conditioning circuitry (Hitchcock & Davis, 1986; Nader et al., 2001; Goosens & Maren, 2001; but see, Koo et al., 2004). However, it is of interest to note that following deletion of the GluR-1 subunit, no upregulation of GluR-2/3 subunits has been noted in the CeN. Moreover, GluR-$1^{-/-}$ mice are normal on tasks which require integrity of this region (e.g., PIT). Therefore, it is suggested that synaptic processes in the CeN of GluR-1 mutant mice remain intact following deletion of this AMPA subunit (Mead & Stephens, 2003a).

3.24 *Implications of Current Experimental Findings.*

The previous experiment in this chapter has highlighted a specific and enduring impairment in Pavlovian fear conditioning in GluR-$1^{-/-}$ mice. Consistent with the BLA-dysfunction hypothesis (Mead & Stephens, 2003a), GluR-$1^{-/-}$ mice

displayed a profound impairment in CS (both auditory and visual) and contextual fear conditioning (Experiment 5). However, these results are difficult to explain in respect to the hippocampal-dysfunction and working memory hypothesis (Zamanillo et al., 1999; Reisel et al., 2002). Rats with hippocampal lesions show an impairment in conditioning to the context and not the CS (Kim & Fanselow, 1992). Taken together, these results support the hypothesis of aberrant BLA processing in GluR-1$^{-/-}$ mice. However, they fail to further the claim suggested by Mead and Stephens regarding the nature of the dysfunction in mutant mice.

It has been argued previously that an inability to form an association between a CS and the affective values of a US such as footshock could account for the deficit in conditioned freezing in BLA lesioned animals (Everitt et al., 2003). Everitt and colleagues have suggested that the immediate, unconditioned reaction to footshock is increased locomotor activity induced by agitation, jumping and escape response. Thus, during conditioning trials there is no freezing response to coincide with the presence of the CS and thus it is not possible for CS-UR associations to be formed. The freezing response appears only after the initial burst of locomotor activity that follows the shock, which is believed to represent a US-specific conditioned response, possibly as the result of a conditioned association between the shock and the experimental context. Under this view, the impairment noted in GluR-1$^{-/-}$ mice reflects an inability to form or access a representation of the affective or motivational properties of the US, in this case footshock. The inability to form an association between the CS and the affective value of the US could therefore disrupt the performance of the US-specific conditioned freezing response.

This view is entirely consistent with that proposed by Mead and Stephens (2003a), in that GluR-1 mutant mice are unable to attribute affective properties to a

cue. However, as previously alluded to, this hypothesis still leaves in question the nature of the dysfunction induced by the GluR-1 mutation. Thus, the presentation of a US can be seen as possessing both sensory and affective components. Referring the reader to Figure 3.3.1, US presentation activates a node or nodes representative of its specific sensory properties (link 1). For instance, if the US was a shock presentation, than this representation would code information regarding the duration of the shock and its effects on the animals sensory receptors. If the US was food, this representation would code information on the visual properties of the food including its taste and texture. This sensory specific US node can then form an association with a representation of the CS (link 3). Additionally, presentation of the US activates a node or nodes representing the affective properties (link 2). Here, if the US was foot shock, this representation would code information regarding the negative aversive nature of the shock, whilst if the US was food delivery this representation would encode the positive rewarding aspects of food delivery. Similarly, the affective US node can form an association with a representation of the CS (link 4). Additionally, each of the sensory and affective nodes can be assumed to elicit its own characteristic response. Finally, links are also formed between the various US nodes. Such a link would allow the sensory properties that characterise a food type to evoke an affective state.

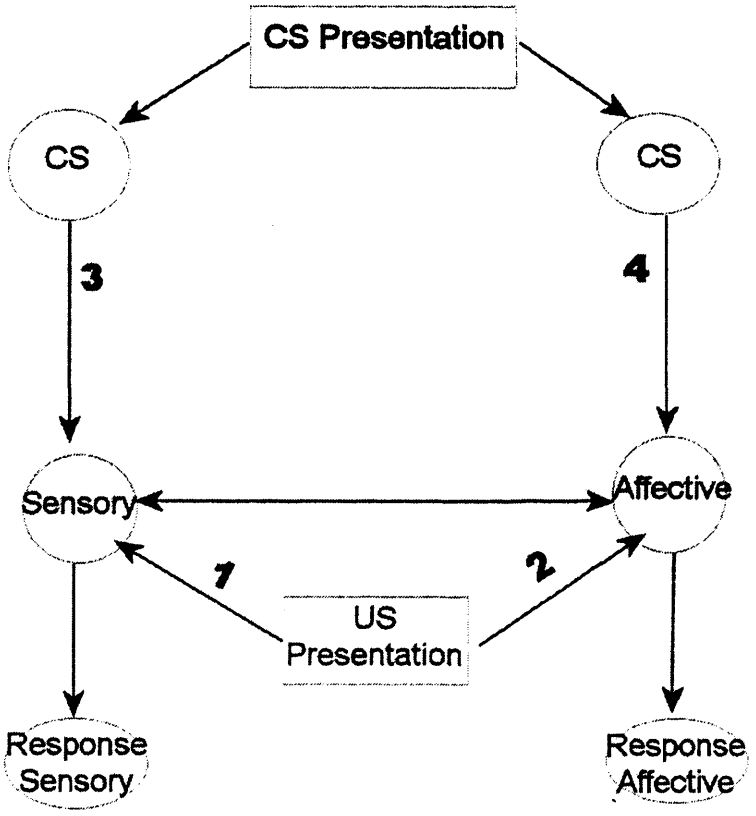

Figure 3.3.1. *Simplified associative structure of stimulus processing.* This model is based on the view that US presentation evokes separate functionally distinct representations which may form during conditioning (Konorski, 1967; Dickinson & Dearing, 1978; Brandon & Wagner, 1989).

3.25 *Coda.*

The findings from the present experiment are consistent with the suggestion that GluR-1$^{-/-}$ mice are impaired in processing and learning about the motivational properties of rewards. However, the precise nature of the impairment remains unclear. Fear conditioning (Experiment 5), second-order conditioning and conditioned reinforcement do not discriminate between the influence of sensory-specific features of a US and the general motivational properties of the US on performance (Gewirtz & Davis, 2000). I will now consider this issue more systematically in Chapter 4.

Chapter 4.

4.1 Introduction.

It has been previously reported that mice lacking the GluR-1 subunit of the AMPA receptor are capable of forming a Pavlovian association between a CS and the delivery of a reward (US) and show normal Pavlovian conditioned approach responses to the site of food delivery (Mead & Stephens, 2003a). In addition, a Pavlovian CS augmented instrumental responding for the same outcome in both GluR-$1^{-/-}$ and control mice (Pavlovian-to-instrumental transfer; PIT). However, when required to learn a novel instrumental response to obtain presentations of a CS (conditioned reinforcement; Mackintosh, 1974), or respond under a second-order schedule of reinforcement, GluR-$1^{-/-}$ mice were impaired relative to control mice. Mead and Stephens (2003a) suggested that the GluR-1 deletion disrupted processing of the motivational properties of a US carried out by the BLA.

Typically, in a conditioned reinforcement procedure the stimulus must exert its effects because of its association with primary reinforcement. This association with primary reward endows the stimulus with sufficient acquired motivational significance (conditioned incentive motivation; Mackintosh, 1974) to support the acquisition of novel instrumental responding. According to Mackintosh a number of factors must be controlled in order to reach this conclusion. Firstly, the stimulus must be shown to exert its effects because of its previous association with primary reward. Secondly, it must be shown that instrumental responding is not maintained by any previous or current association with primary reward. Thirdly, the increase in subsequent instrumental responding must be dependent on the contingency between responding and presentation of the conditioned reinforcer. In respect to the first two points, these factors are controlled by testing in the absence of primary reward

delivery. In respect to the latter point, it could be argued that augmented instrumental responding (following stimulus presentation) may be attributed to a general increase in the level of arousal or general activity. Thus, a commonly used method to establish that conditioned reinforcement reinforces a specific response, is to measure choice behaviour. Typically, in this procedure two actions are introduced, only one of which delivers contingent presentation of the conditioned reinforcer following response (Mackintosh, 1974). These findings suggest that during pairing of the stimulus with primary reward, the pairing transfers conditioned affective/motivational properties to the stimulus, allowing the now conditioned cue to act as a reinforcer in its own right.

Similarly, during second-order conditioning, the first-order CS acquires motivational significance, such that its pairing with a second-order CS, during a second phase, enables the CR associated with the first-order CS to become elicited by the second-order CS (Holland & Rescorla, 1975). Specifically, there are three proposed mechanisms for the acquisition of responding using a second-order schedule. Firstly, animals may form associations between the second-order CS and the first-order CS, via an associative chain (i.e., stimulus-stimulus associations; S-S; Rescorla, 1975). Secondly, animals may form associations by way of direct associations between the second-order CS and the representation of reward evoked following first-order CS presentation (Ross, 1986). Finally, animals may form S-R associations between the second-order CS and the response evoked by the first-order CS presentation (Holland & Rescorla, 1975). The degree to which each of these associations mediate second-order conditioning depends on experimental parameters, the ability of the first-order CS to evoke a response during first- and second-order CS presentation, and the similarity between the first and second-order CSs (Rescorla, 1973, 1982).

4.1.1 *The Amygdala and Reward Representation.*

BLA-lesioned animals show a deficit in conditioned reinforcement (Cador, Robbins & Everitt, 1989; Cador & Robbins, 1989), which suggested that the first-order CS used failed to acquire affective/motivational significance. Similarly, BLA lesions prevent the acquisition of second-order conditioning (Hatfield et al., 1996). It was suggested that lesions attenuated light-food pairings in the first phase from endowing the light with conditioned reinforcement value. In a subsequent experiment, Hatfield and colleagues reported that BLA-lesions attenuated the acquisition of positive incentive value as assessed in a reinforcer devaluation procedure. Here, when the food pellets (which had served as a US) were devalued through pairings of the food pellets with LiCl, the CR to the first-order CS was unaffected in the lesion group. In contrast, the control group showed sensitivity to post-training devaluation of the US. At the same time, there was no evidence that BLA lesions interfered with food-toxin associations, as both groups consumed less of the food paired with the toxin. The insensitivity to revaluation of the reinforcer suggested that BLA-lesions attenuated the ability of these animals to retrieve a representation of the current motivational and incentive value of the US (Hatfield et al., 1996). The conclusion provided by Hatfield et al. is similar to that proposed by Everitt and colleagues. That is, BLA lesions impair the associative learning process that gives a CS access to the motivational value of the associated USs (Everitt & Robbins, 1992). Thus, single-outcome devaluation procedures provide evidence to suggest that animals acquire information about the nature of the outcome generated by their responding (Adams & Dickinson, 1981). However, this representation can have separate affective and sensory components. Affective components relate to motivational states, such that,

when the food is paired with the toxin, the food reward changes from a highly favourable positive affective state to a negative affective state. It is the change in affective state, that is the arousing aspects of motivation, which are affected following reinforcer devaluation (Dickinson & Dearing, 1978). Additionally, reward devaluation by LiCl injection also works by reducing the palatability of the specific reward via the direct sensory-specific features of reward (Holland, 1990; Balleine, 2001). However the devaluation procedure adopted by Hatfield et al. fails to discriminate between the sensory and affective components of US processing (as only a single outcome was devalued; Figure 3.3.1).

The sensory-specific outcome devaluation paradigm requires the animal to discriminate between the motivationally relevant sensory features of multiple outcomes in order to influence instrumental performance appropriately. An outcome-specific reinforcer devaluation procedure has been used to examine the nature of the dysfunction in reward encoding following BLA lesions (Balleine, Dickinson & Killcross, 2003). Animals were trained to perform two separate actions, lever pressing and chain pulling, each reinforced with a different outcome. Although the BLA lesioned rats were able to discriminate between the two actions, they were unable to selectively modify their performance when only one of the outcomes was devalued by a satiety treatment. This deficit in outcome encoding was manifested as a dysfunction in choice performance after devaluation and insensitivity to the degradation of the instrumental contingency (Balleine et al., 2003). It should be noted that in order for reinforcer devaluation to influence performance, consummatory contact with the outcome whilst the rodent is in a non-deprived or satiated state is required; a process which has been termed 'incentive learning' (Balleine & Dickinson, 1991).

4.1.2 *GluR-1$^{-/-}$ Mice and Reward Representation.*

The impairment in conditioned reinforcement and second-order conditioning in GluR-1$^{-/-}$ mice was suggested to reflect an inability of mutant mice to associate a cue with the affective/motivational properties of a US (Mead & Stephens, 2003a). In the Mead and Stephens study GluR-1$^{-/-}$ mice remained sensitive to at least some aspects of reward, as evidenced by their unimpaired performance in Pavlovian and instrumental conditioning tasks (Pavlovian approach and PIT). This raises the question of the nature of the reward representation disrupted by the GluR-1 mutation. The representation of a US includes both sensory and affective properties of reinforcement and CSs or actions may form associations with both of these features of a reward representation (Kamin, 1968; Wagner & Brandon, 1989; Balleine 2001; Figure 3.3.1). Recent research has provided evidence that lesions of the BLA in rats disrupt the formation of representations involving the sensory properties of a US and their incentive value (Blundell, et al., 2001; Balleine, et al., 2003). Thus, if rats are trained on tasks where different outcomes become associated with different stimuli and/or responses, BLA lesions attenuate the ability to mediate responding following either; presentation of stimuli which had previously signalled different reward types (outcome-specific PIT; Blundell et al., 2001; Corbit & Balleine, 2005), or post-conditioning changes in incentive value of one of the two outcomes (outcome-specific reinforcer devaluation; Balleine et al., 2003). Second-order conditioning and conditioned reinforcement procedures (Mead & Stephens, 2003) do not discriminate between the influence of sensory-specific features of reinforcement and the general motivation/affective properties of reward on performance (c.f., Holland & Rescorla, 1975; Stanhope, 1992; see Gewirtz & Davis, 2000 for a review).

4.1.3 *Current Experiments.*

The current set of experiments examines whether mice with a targeted deletion of the GluR-1 subunit are able to use a Pavlovian or instrumental (Experiment 6 and 7) signal to gain access to the current sensory-specific incentive value of a primary reward. Experiment 6 assessed outcome-specific devaluation in a runway task, in order to determine whether GluR-$1^{-/-}$ mice could use either an instrumental or Pavlovian signal (provided by the contextual extramaze cues associated with each outcome) to gain access to the current incentive value of reward in order to adjust appropriate response behaviour. Devaluation was achieved using a sensory-specific satiety treatment in which the mice were prefed one of the two outcomes prior to test. Experiment 7 more specifically assessed instrumental goal-directed behaviour in GluR-$1^{-/-}$ mice using an instrumental nose poke procedure in an operant chamber. This paradigm was loosely based on experiments which had been conducted to assess outcome-specific encoding in BLA-lesioned rats (Blundell et al., 2001; Balleine et al., 2003).

4.2 Experiment 6.

It has previously been reported that GluR-$1^{-/-}$ mice are not impaired in spatial reference memory as assessed in the elevated Y-maze (Reisel et al., 2002). In this task, mice are trained to travel from one of two start arms, to a target arm, where reward is made available. The target arm is defined according to its given spatial location relative to the room cues. In an effort to make contact with these studies I used a reference memory procedure, where mice were trained to traverse the runway

from a start arm to the goal-box in order to receive sucrose (from one start arm goal-box route) and pellet reward (from the other start arm goal-box route). Reward may become associated with a response or place (location) depending on the nature of the strategy adopted by the animal (Restle, 1957; Dudchenko, 2001). Therefore, outcome-specific devaluation should serve to reduce the proclivity for responding (mediated by place-reward or response-reward representations) to an arm associated with the devalued reward. Unlike the t-maze alternation task, this procedure has no working memory component, therefore according to working-memory hypothesis (Reisel et al., 2002) one would expect no impairment in GluR-1$^{-/-}$ mice. However, in respect to that suggested by Mead and Stephens (2003a), one would expect any dysfunction in attributing affective/motivational properties to the cue to manifest itself as an impairment in inhibiting responding following devaluation.

4.2.1 Method.

Subjects.

Experiment 6 was conducted in two replications with 6-month-old GluR-1$^{-/-}$ (n =10) and wild-type (n =10) mice. The mice were derived and transported in exactly the same fashion as in previous experiments. Mice were housed two or three to a cage under a light-dark 12:12 cycle (lights on 07.00-19.00). Prior to the start of training mice were reduced to 85% of their ad libitum weights and the mice weighed between 25-30 gm at the start of the experiment.

Apparatus.

The plus maze consisted of 4 arms, which were 8 cm wide, 50 cm long and 10 cm high. The floor of the maze was made of wood and had been painted white. The surrounding walls were made of clear Perspex. The guillotine doors used to block the start and goal arms were made of opaque black Perspex. Each arm contained a circular food well sunk into the floor at the end of the runway. In each goal box a pair of infrared photo beam sensors were located on either side of the food well and were used to time the latency of the mice to traverse the runway from the start box to the goal box. The latency data were recorded using an IBM compatible PC using Colbourn Instruments Graphic State Notation package (Colbourn Instruments, Allentown, PA, USA). The maze was elevated 90 cm from the floor. Two ceiling-mounted fluorescent lights illuminated the experimental room. A variety of visual cues (e.g. benching, racks and posters, etc.), were displayed on and along each of the 4 walls of the testing room.

Behavioural Training.

Stage 1: Plus-maze acquisition.

During each experimental phase, the plus maze was used as a simple runway. This effectively created two runways, each with a start box at one end and a goal arm at the other. One reinforcer was assigned to one goal box and the remaining reinforcer to the alternative goal box. The rewards were individual 20 mg food pellets (Noyes precision pellets, Formula A1; Research Diets) and 0.1 ml 20% (wt/vol) sucrose

solution. The allocation of goal box and reward type was fully counterbalanced for both the GluR-$1^{-/-}$ and control mice. Once each animal was allocated to its specific goal box it was continually trained with this contingency. Following two days of habituation, which involved 20 min exposure to the plus maze and the rewards, mice were then trained to traverse each runway, from the start arm to the goal arm. Entries into the goal boxes were rewarded with access to one of the two outcomes. During each of the ten days of training, each mouse received two training session, one in each of the alternate runways. These sessions were separated from one another by approximately 4 hours. Each session consisted of 10 trials. The order in which the animals received exposure to each runway, either in the morning or afternoon was counterbalanced across days and within groups. The arms of the maze were wiped down with 70 % alcohol solution between each run in the apparatus in order to remove olfactory odours.

Stage 2: Outcome-specific devaluation and extinction test.

Following this stage of training, the mice received an outcome devaluation test. This was achieved by prefeeding the mice with one of the two outcomes for 120 min in the home cages. Consumption of the outcome was expected over time to induce a progressive reduction in food deprivation, while consummatory contact with the outcome provided the opportunity for incentive learning about the reduction in palatability of the outcome (Balleine & Dickinson, 1998). The allocation of the reward for the devaluation treatment was counterbalanced within each group. Immediately following the devaluation treatment, the mice received a series of test trials carried out in extinction. Half the mice (equal numbers of GluR-$1^{-/-}$ and control

mice) were tested initially on the devalued goal arm; whereas the remaining half were tested on the non-devalued goal arm. Mice were allowed to traverse each runway for a total of 18 trials. However, those animals that failed to complete a single run within 2 min were considered to have extinguished responding and testing was discontinued. On completion of testing on one goal arm, mice were immediately tested on the alternative goal arm.

4.2.2 Results.

Plus-maze training.

Figure 4.6.1a shows the latencies to transverse the runway from the start arm to the goal box for both GluR-1$^{-/-}$ and control mice. During training all mice showed a reduction in latency to retrieve both types of reinforcement. This impression was confirmed by a three-way mixed ANOVA with a between-subjects factor of genotype, and within-subject factors of reinforcer type (sucrose vs. pellet) and session (1-10). The analysis revealed no main effect of genotype ($F_{(9,26)}$ = 3.064, p>0.08) or reinforcer (F<1), and a main effect of session ($F_{(9,26)}$ = 3.936, p< 0.01); however no interactions were noted (F<1).

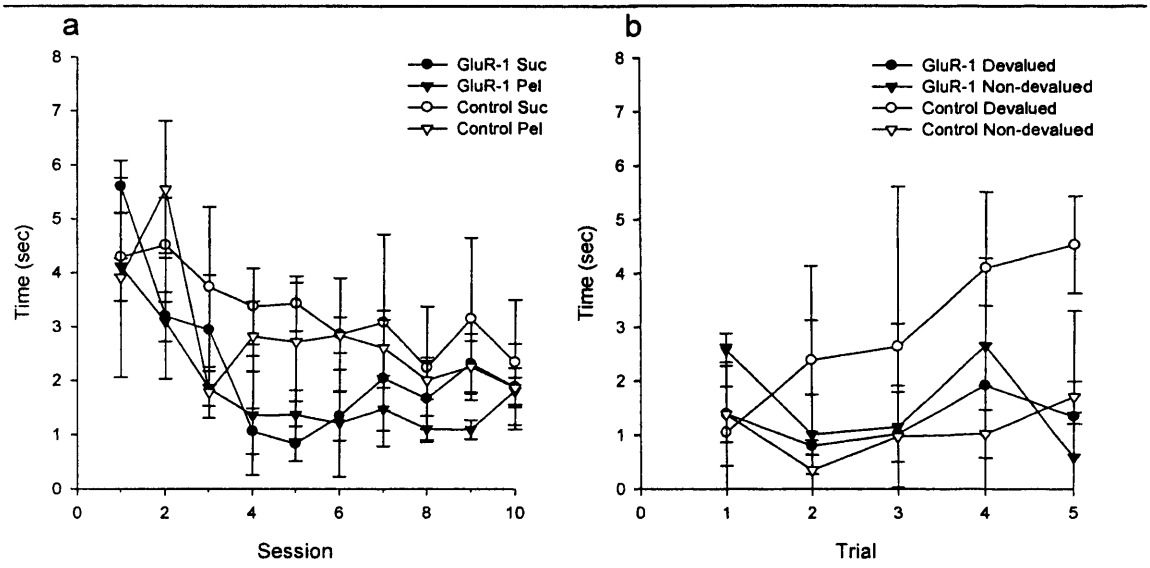

Figure 4.6.1. *Plus maze training and test latencies.* **(a)** Mean latencies to traverse the runway from the start arm to the goal box during plus maze training. **(b)** Mean latencies to traverse the runway from the start arm to the goal box during the outcome devaluation extinction test. Closed circles = GluR-1$^{-/-}$ mice trained to traverse runway for sucrose reward; closed triangles = GluR-1$^{-/-}$ mice trained to traverse runway for pellet reward; open circles = wild-type control mice trained to traverse runway for sucrose reward; open triangles = wild-type control mice trained to traverse runway for pellet reward. Error bars indicate standard error of the mean.

Outcome devaluation: extinction test.

The results of the extinction test for GluR-1$^{-/-}$ and control mice are shown in Figure 4.6.1b. During testing an increasing number of control mice failed to complete a trial within the allotted time period as the extinction test proceeded. Therefore, to provide a meaningful comparison with mutant mice only the first 5 trials of the extinction test were analysed. Up to this stage, all of the control mice successfully

completed each trial. Inspection of Figure 4.6.1b suggests that control mice showed a gradual increase in latency to reach the goal box associated with the devalued reward, relative to the non-devalued goal box. In contrast, GluR-1$^{-/-}$ mice failed to show any evidence of this discrimination.

In order to evaluate these differences, a three-way mixed ANOVA was conducted with factors of genotype, box (associated with either devalued or non-devalued reward) and trial. This revealed no overall effect of genotype ($F_{(1,24)}$ = 1.223, p> 0.28), or box ($F_{(1,24)}$ = 2.283, p>0.2) and no effect of trial ($F_{(4,24)}$ = 1.69, p>0.16). There was, however, a significant genotype × box interaction ($F_{(1,24)}$ = 4.408, p<.05). No other interactions approached significance (F's<1). An analysis of simple main effects revealed that the control mice showed a significantly longer latency to reach the devalued goal box than the non-devalued box ($F_{(1,24)}$ = 6.518, p<0.05). In contrast, there was no significant difference between latencies to enter the two goal boxes in GluR-1$^{-/-}$ mice (F <1).

Survival curves were used to establish whether GluR-1$^{-/-}$ mice eventually reduced running speeds (and therefore extinguished responding) to the box associated with the devalued reward (Figure 4.6.2a) or non-devalued reward (Figure 4.6.2b) Survival curves plot the results of experiments where the outcome is an end point. This end point can only occur one time per subject. In respect to the current analysis this end point was the trial where mice were considered to have extinguished responding (i.e., failure to complete a run within allocated 2 min interval). This assessment also allowed one to explore the possibility that the results from this experiment could be accounted for via differences in the rates of extinction.

Firstly, in reference to Figure 4.6.2a, all control mice had extinguished responding to the goal box associated with the devalued reward by the 12th trial

(median trial 8). In contrast, GluR-1$^{-/-}$ mice continued responding to this goal box until the 18th trial (median 13.5). To evaluate this impression, the Mantel-Haenszel logrank test was used to compare the survival curves testing the null hypothesis that the survival curves are identical in their overall populations. This analysis revealed differences between the two sets of data; chi-square, ($\chi^2(1,N = 20) = 7.254$, $p<0.01$), indicating that control mice extinguished responding to the devalued arm in fewer trials than did the mutant group.

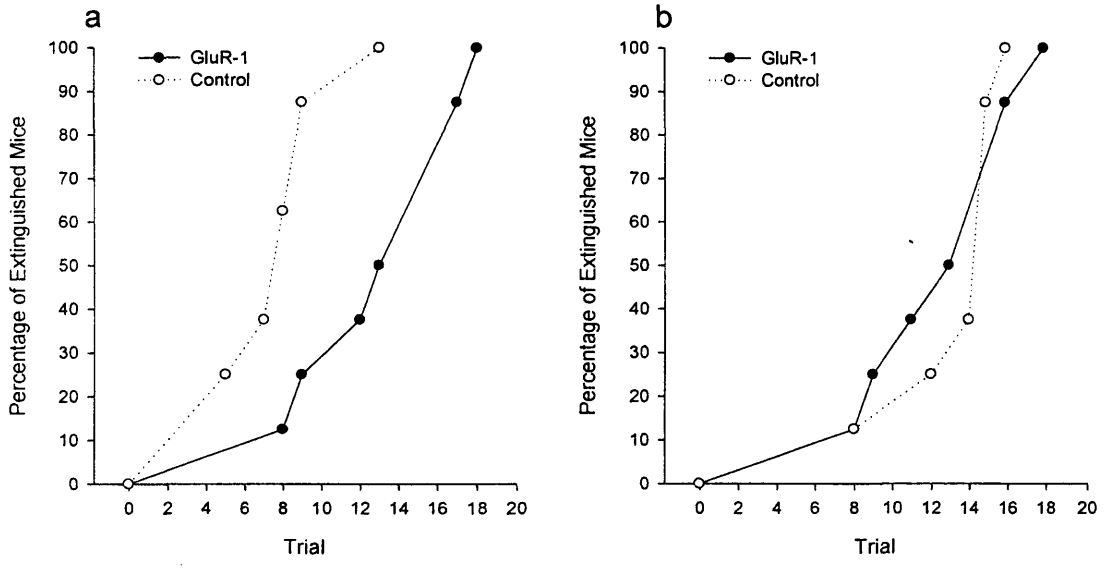

Figure 4.6.2. *Plus maze devaluation extinction test: Survival Curves.* **(a)** Percentage of mice that extinguished responding on each trial to the goal box associated with the devalued reward. **(b)** Percentage of mice that extinguished responding on each trial to the goal box associated with the non-devalued reward. Closed circles = GluR-1$^{-/-}$ mice extinction; open circles = wild-type control mice extinction

Further assessment evaluated extinction to the non-devalued goal box (Figure 4.2.6b). Here, both groups of mice appeared to show a similar pattern of extinction to this non-devalued goal box. Control mice extinguishing responding by trial 16

(median 15) and all GluR-1$^{-/-}$ mice extinguishing responding by trial 18 (median 14). The Mantel-Haenszel logrank test revealed that these two samples of data did not differ in their overall populations; chi square, ($\chi^2(1,N = 20) = 0.6466$, $p>0.42$). Thus, both groups of mice took similar number of trials to extinguish responding to the non-devalued arm, suggesting that extinction rates to the non-devalued arm were similar between the two groups.

4.2.3 Discussion.

Mice were trained to retrieve two different reinforcers from two different goal boxes in a plus maze. Following devaluation induced by an outcome-specific satiety treatment, control mice showed an increase in latency to enter the goal box associated with the devalued outcome compared with the non-devalued goal-box. That this change was gradual rather than immediate could have reflected the fact that at each start arm the extramaze (contextual) and intramaze cues associated with both goal-boxes are visible. Not until the mouse reaches the goal-arm, do the cues associated with each specific goal-box become present. Once present, the cue-reward association (and therefore the devaluation treatment) becomes effective in respect to attenuating further responding to the goal-arm.

It is worth noting that despite the impairment noted above there are however several issues which remain unresolved. Firstly, the results from the training stage suggest a non-significant trend in latency to traverse the runway in the mutant group (Figure 4.6.1.a). This result could suggest better performance in the KO group, compared to controls. However, with better performance one would still have expected to see a devaluation effect in the mutant group. Alternatively, it is possible

that GluR-1$^{-/-}$ mice failed to show a devaluation effect due to perseveration. However such behaviour would be expected to interfere with extinction during the test stage (e.g., Morgan, Schulkin & LeDoux, 2003). That both groups displayed a similar pattern of extinction to the non-devalued goal box, suggests that the failure to suppress approach behaviour to the devalued goal box in KO mice, could not be accounted by perseveration in these mice.

Secondly, although this form of locomotor task is readily characterised as instrumental (Dickinson & Balleine, 1994), it has been noted that such forms of maze behaviour may be explicable in terms of Pavlovian approach to stimuli associated with the outcome (Hershberger, 1986; Dickinson, 1994). For instance, the normal relationship between approach behaviour and reward was assessed by Herschberger (1986) using a 'looking glass' runway. Chicks were required to learn the contingency of running away from the reward in order to receive reward. Even after 100 min of exposure to the reversed contingency, chicks failed to learn the inverse looking glass relationship, suggesting therefore that the approach response was mediated by Pavlovian representations which were insensitive to the response-outcome contingency. Thirdly, the plotted survival curves suggest that this result is unlikely to be explained by differences in the rates of extinction, as extinction to the non-devalued arm were similar for both groups (4.6.2b). Finally, the deficit in GluR-1 mutant mice could be explained by an inability of the mice to simply discriminate between the outcomes. This issue will be addressed in Experiment 7.

4.3 Experiment 7.

In Experiment 7 I assessed goal-directed behaviour using an instrumental biconditional discrimination learning paradigm. The mice were trained to perform different actions, each associated with a different reward. The current value of one of the rewards was then changed, and the propensity of the animal to subsequently perform the action associated with the devalued outcome was examined (Balleine and Dickinson, 1991, 1998; Balleine et al., 2003). The use of such a paradigm allows assessment of goal-directed behaviour (Balleine & Dickinson, 1994) which is defined as the concurrent ability of the rodent to form a representation of the response-outcome contingency, in addition to forming a representation of the outcome as a goal (Cardinal et al., 2002).

4.3.1 Method.

Subjects.

The mice were derived and transported in exactly the same fashion as in Experiment 6. Mice were housed two or three to a cage under a light-dark 12:12 cycle (lights on 07.00-19.00). Prior to the start of training mice were reduced to 85% of their ad libitum weights and weighed 25-30 gm at the beginning of the experiment. Experiment 7 was conducted with experimentally naïve age matched GluR-1$^{-/-}$ (n =12) and wild-type (n=12) mice and were maintained under the same schedule of food deprivation as described in Experiment 6.

Apparatus.

Instrumental conditioning was carried out in six, identical, standard operant chambers (Med Associates, St. Albans, VT, USA), housed in sound attenuating boxes. The dimensions of the chambers were 15-cm-wide, 12-cm-high and 14-cm-deep. The chambers were made from clear polycarbonate, and the front and back of the chambers were fabricated from stainless steel. The floor was a standard grid floor, with 20 stainless steel rods, each with a diameter of 2.5 mm, arranged with centres 5 mm apart. The chambers were fitted with two nose-poke manipulanda each 10 mm in diameter, and located at identical heights (15 mm) on the left-hand and right-hand side of the front wall. Each nose-poke manipulanda contained a yellow stimulus light located at the rear of the recessed hole and a photo beam sensor to monitor nose-poke entries. Located between the two manipulanda was a trough-type dual pellet/dipper dispenser, into which either 0.1 ml liquid reward or food pellets could be delivered. This modular unit featured a 2.5 cm x 2.5 cm receptacle opening with a photo beam inside. A speaker was mounted to the outside of the chamber, on the wall opposite the nose-poke manipulanda. The speaker was connected to a 3KHz tone generator. A heavy-duty clicker module was also mounted on this wall, and could be switched on and off to emit a 10 Hz train of clicks. The tone and click-train were measured and matched to emit a sound level of approximately 80 dB. A 28V, 100 mA house light was mounted at the top-centre of the inner wall. An IBM-compatible computer equipped with Med-PC software (Med Associates, St. Albans, VT, USA) controlled and recorded all stimuli and responses.

Behavioural Training.

Stage 1: Magazine training.

Each animal was assigned to one of six operant chambers, and thereafter was always trained in that chamber; the assignment of each chamber was counterbalanced between groups. At the start of the session, the house light came on and remained on during the session. Throughout training, the rewards were either a single 20 mg Noyes food pellets or 0.1 ml 20% sucrose solution. Mice were trained to collect food rewards for two days, with two 20 min sessions per day. The rewards were delivered on a random time (RT) 60 s schedule. Magazine entry during this training session was recorded. Half the mice (equal numbers of GluR-$1^{-/-}$ and control mice) were trained to collect food pellets in the morning session, and half were trained to collect sucrose solution. In the afternoon session, mice received identical training with the alternative reward. The next day, the order of training was reversed, so that each mouse received each reward for one morning and afternoon session

Stage 2:Nose-poke training.

After magazine training, the mice were initially trained to respond on the nose-poke manipulanda during two 20-min sessions with a continuous schedule of reinforcement. The mice received two separate training sessions on each manipulandum separately with background illumination provided by a house light. However due to low levels of responding by both groups of mice after two sessions of

training, the house light was turned off to enhance the salience of the manipulanda lights.

On the next 4 days of training, each session was 20 min long and the mice received two training sessions each day, one in the morning and the second, approximately 4 hours later, in the afternoon. Response-outcome assignment was counterbalanced within each group. Throughout training mice were given two daily separate sessions (separated by 3-4 hours) on each manipulandum, one on the right nose-poke and the other on the left nose-poke, with the action that was trained first on each day alternating from one day to the next. During the training phase, both nose-poke manipulanda were present, but only the active manipulandum was illuminated. Mice were initially trained to respond for 2 d with a continuous schedule of reinforcement. If animals did not complete 20 nose-poke responses, they underwent an additional training session immediately following the session on that nose-poke manipulandum. In total, 3 wild-types and 1 GluR-1$^{-/-}$ mouse required this additional training session. Following this session all mice acquired the 20 nose-poke criterion and therefore proceeded to the next stage of training. In order to increase the overall rate of nose-poke responding in each session, the schedule of reinforcement was made progressively leaner. Thus, the mice were first transferred to a fixed ratio 5 schedule, during which every 5th nose-poke response resulted in the delivery of reward (fixed response-5; FR-5 schedule) which was then advanced to a FR-15 schedule for the penultimate and final instrumental training sessions respectively. Mice that failed to complete 50 nose-poke responses during the final day of instrumental training were excluded and did not proceed to the discrimination phase of training. In total four control mice and one GluR-1$^{-/-}$ mouse failed to reach this criterion and were subsequently excluded from the remainder of the experiment; leaving a total of 8

control mice and 11 GluR-1$^{-/-}$ mice to continue to the biconditional discrimination training stage.

Stage 3: Biconditional stimulus-response-reinforcer training.

In the discrimination phase, which lasted 14 d, each session was 30 min long, and consisted of 10 alternating 2 min presentations of either a 3 kHz tone (at 80 dB), or a 10 Hz train of clicks (at 80 dB), with an inter-trial interval (ITI) of 1 min. During the discrimination training stage, both nose-poke manipulanda were illuminated. The assignment of the subjects to the biconditional stimulus-response-outcome discriminations was counterbalanced. For half the mice in each group activation of the right nose-poke manipulanda during presentations of the tone resulted in the delivery of food pellets, whilst activation of the left manipulanda during presentations of the clicker resulted in the delivery of sucrose solution. For the remaining mice in each group, the stimulus-response-outcome assignments were reversed. During the ITI period, reward was not available.

The first discriminative stimulus (S^D) presented in each training session was determined by the computer using a pseudorandom sequence that ensured the animals received 5 presentations of each S^D in each 30 min session. For the first two days of training reward delivery was available on a continuous schedule of reinforcement. On day 3, the mice were trained on a random ratio (RR) 5 schedule. This constant probability schedule approximates to a fixed probability of reward for the first response in each second; thus a RR 5 schedule approximated to a probability of 0.2. This contingency continued for the following session, after which the reinforcement contingencies were altered to a RR 10 schedule (probability of 0.1). An increment in

the RR schedule then occurred every 2 d according to the following sequence: 15, 20, 25 and 30. Thus, during the final 2 d of discrimination training, reward delivery was made available on a RR 30 schedule. A RR schedule was chosen rather than a random interval (RI), as the former but not the latter appears to be controlled by knowledge about the instrumental contingency that encodes specific properties of the reward (Dickinson, Nicholas & Adams, 1983).

Stage 4: Biconditional discrimination extinction test.

On completion of training, mice received a test session conducted in extinction to examine whether performance was governed by within session reinforcement contingencies or whether the mice had learned the appropriate instrumental contingencies. The procedure was identical to that used for the training session, but no rewards were delivered. Following the extinction test, mice received four days of retraining on the original discrimination. For the first two days of training reward delivery was available on a continuous schedule of reinforcement. On day 3, the mice were trained on a RR 15 schedule. Finally, on day 4 mice were trained on a RR 30 schedule; during which asymptotic performance was re-established. This was assessed by comparing the levels of responding during day 4 reacquisition session with that from the final day of training prior to the initial extinction session.

Stage 5: Outcome devaluation and extinction test.

The outcome devaluation test was conducted on the day after the final reacquisition session. This was achieved by prefeeding the mice with one of the two

outcomes for 120 min in their home cages located in the holding room. The allocation of devaluation treatment to each mouse was counterbalanced for the stimulus (tone vs. clicker), for the response (left nose-poke manipulandum vs. right nose-poke manipulandum), and for the outcome (pellet vs. sucrose). Immediately following the devaluation treatment, the mice received an extinction session where the procedure was identical to that described above. Finally, after the extinction test, the mice were placed back into their holding cages and were administered a 30 min choice test, in which both outcomes were present. Food pellets were presented in a dish (5cm × 5 cm) located at one end of the home cage. A bottle containing the sucrose reward was located at the opposite end of the cage. The amount of fluid and food consumed was obtained by weighing the containers before and after each choice test.

4.3.2 Results.

Acquisition of biconditional discrimination.

Figure 4.7.1a shows the mean rates of responding to the correct and incorrect nose-pokes across training for GluR-1$^{-/-}$ and control mice. Correct responding was defined as nose pokes that occurred in the appropriate manipulandum during an S^D presentation (i.e., the manipulandum associated with the correct stimulus-response-reinforcer contingency). Incorrect responding was defined as nose-pokes in the manipulandum that was not appropriate for the specific S^D (i.e., the manipulandum associated with non-reward). Inspection of Figure 4.7.1a suggests that there was a tendency for GluR-1$^{-/-}$ mice to respond at a higher rate than controls, with both groups of mice showing higher levels of nose-poke responding to the correct nose-poke

manipulandum compared to the incorrect manipulandum by the end of training. A three-way mixed ANOVA was conducted with a between-subjects factor of genotype, and within-subject factors of session (1-14) and response type (correct vs. incorrect). This analysis indicated that overall level of responding was not significantly different between the two groups ($F_{(1,36)} = 2.881$, $p > 0.10$). However, there was a main effect of session ($F_{(13,936)} = 10.430$, $p<0.0001$) and response type ($F_{(1,72)} = 4.864$, $p<0.01$) and a significant interaction between these two factors ($F_{(13,936)} = 6.826$, $p<0.01$). In order to investigate the nature of this interaction, an analysis of the simple main effects was conducted and revealed a significant effect of correct versus incorrect responding on sessions 8 to 14, (smallest F-value; session 8, $F_{(1,118)} = 4.537$, $p<0.05$).

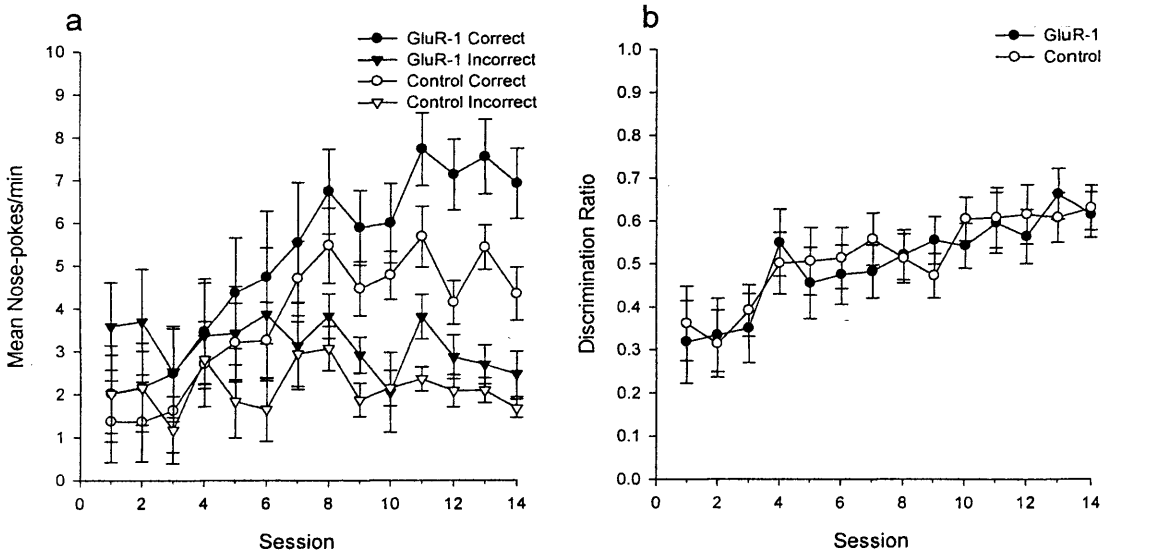

Figure 4.7.1. *Acquisition of biconditional discrimination.* **(a)** Mean rates of responding (in responses per minute) to the correct and incorrect nose-poke manipulanda (collapsed across S^D's) during acquisition of the biconditional discrimination. Closed circles = GluR-$1^{-/-}$ mice correct nose-poke responding; closed triangles = GluR-$1^{-/-}$ mice incorrect nose-poke responding; open circles = wild-type

control mice correct nose-poke responding; open triangle = wild-type control mice incorrect nose-poke responding. **(b)** Discrimination ratio scores during acquisition of the biconditional discrimination. Closed circles = GluR-1$^{-/-}$ mice discrimination scores; open circles = wild-type control discrimination scores. Error bars indicate standard error of the mean.

To ensure that group differences in acquisition of the biconditional discrimination were not obscured by the apparent differences in response rates, the data were transformed into a discrimination ratio. Discrimination ratios were calculated by dividing the mean rate of responding (in responses per minute, rpm, Figure 4.7.1b) to the reinforced stimuli (correct nose-poke responding) by the mean rate of responding to reinforced and non-reinforced stimuli (correct and incorrect nose-poke responding). A ratio that exceeds 0.5 indicates that correct responding to the nose-poke manipulandum was greater than responding to the incorrect nose-poke manipulandum. A two-way ANOVA was conducted on the discrimination scores, with genotype and session as factors, and revealed a significant main effect of session ($F_{(13,221)} = 12.554$, $p<0.001$). However, there was no main effect of genotype or interaction between these two factors (all F's <1).

Extinction test: Discrimination ratio scores.

The results of the extinction test are presented in Figure 4.7.2a and show the discrimination ratios for GluR-1$^{-/-}$ and control mice for the last session of training and during the extinction test. Both groups of mice maintained the discrimination indicating that performance during acquisition was not dependent on cues provided by

the delivery of rewards within a session. In order to confirm this impression a two-way ANOVA was conducted, with a between-subject factor of genotype and a within-subject factor of phase (training vs. extinction session). The analysis revealed a main effect of phase ($F_{(1,17)} = 45.595$, $p<0.01$), reflecting generally higher rates of responding during the extinction test. In addition, no effect of genotype ($F_{(1,17)} = 3.871$, $p > .05$), or interaction ($F<1$) between these factors was revealed.

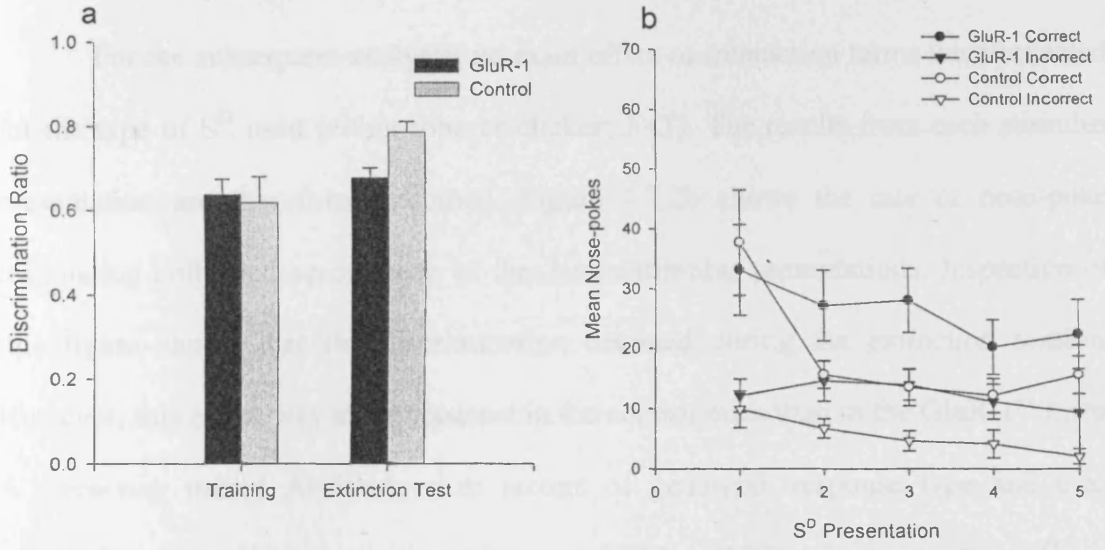

Figure 4.7.2. *Biconditional training and extinction test.* **(a)** Discrimination ratio scores from the final training session (left columns) and from the extinction test (right columns). Black bars = discrimination scores for GluR-$1^{-/-}$ mice; grey bars = discrimination scores for wild-type control mice.**(b)** Mean nose-poke responding during 2-min presentations of the discriminative stimuli. Closed circles = total nose-poke response to the correct nose-poke manipulanda during discriminative stimuli presentation for GluR-$1^{-/-}$ mice; closed triangles = total nose-poke response to the incorrect nose-poke manipulanda during discriminative stimuli presentation for GluR-$1^{-/-}$ mice; open circles = total nose-poke response to the correct nose-poke manipulanda during discriminative stimuli presentation for wild-type control mice;

closed triangles = total nose-poke response to the incorrect nose-poke manipulanda during discriminative stimuli presentation for wild-type control mice. Error bars equal standard error of the mean.

Extinction test: Response rates.

For the subsequent analysis; no main effect or interaction terms were revealed for the type of S^D used (either tone or clicker; $F<1$). The results from each stimulus presentation are therefore combined. Figure 4.7.2b shows the rate of nose-poke responding collapsed across each of the 2-min stimulus presentations. Inspection of this figure shows that the discrimination declined during the extinction session. However, this effect was more apparent in the control mice than in the GluR-$1^{-/-}$ mice. A three-way mixed ANOVA, with factors of genotype, response type and trial, revealed a main effect of genotype ($F_{(1,72)} = 6.143$, $p<0.02$), of response type ($F_{(1,72)} = 23.528$, $p<0.01$) and of trial ($F_{(4,288)} = 10.889$, $p<0.01$). In addition, a significant genotype × trial ($F_{(4,288)} = 3.246$, $p<0.05$) and response type × trial ($F_{(4,288)} = 5.099$, $p<0.01$) interaction was revealed. However, no further interactions approached significance (all F's<1). Simple main effects analysis conducted on the significant genotype × trial interaction revealed a main effect of genotype at bins 2-4 (smallest F value; bin 4, $F_{(1,194)} = 4.055$, $p<0.05$), with both GluR-$1^{-/-}$ ($F_{(4,288)} = 3.239$, $p<0.02$) and control mice ($F_{(4,288)} = 9.852$, $p<0.01$) showing a decline in performance across the extinction session. Examination of the response type × trial interaction revealed a significant effect of response type at each trial (smallest F value; bin 4, $F_{(1,194)} =$

5.263, p=0.05), with a progressive decline across the session in correct responses ($F_{(4,288)}$ = 15.073, p<0.01), but not incorrect responses ($F_{(4,288)}$ = 1.324, p>0.10). These results indicate that although GluR-1$^{-/-}$ mice responded at a higher rate than control mice during extinction, both groups maintained the discrimination and showed a reduction in the rate of responding to the correct nose poke during the extinction session.

Outcome devaluation: extinction test.

Figure 4.7.3a shows the mean rates of correct nose-poke responding from control and mutant mice during the devaluation extinction test. The results are collapsed across S^D's during each 2-min stimulus presentation. In general, control mice showed a differential level of responding in the presence of cues associated with either devalued or non-devalued outcomes. Control mice responded at a lower rate during cue presentations associated with the devalued outcome and maintained higher levels of responding to the cue associated with the non-devalued outcome. In contrast, GluR-1$^{-/-}$ mice failed to show this differential level of responding to either cue. This impression was confirmed by an ANOVA with a between subject-factor of genotype, and within-subject factors of cue-presentation (devalued or non-devalued) and trial (1-5); which revealed a non-significant main effect of group ($F_{(1,17)}$ =3.62, p>0.05), of cue presentation, (F<1), and a non-significant interaction between these factors (F<1). There was a main effect of trial, ($F_{(4,68)}$ = 4.81, p < 0.01) an interaction of cue-presentation with trial, ($F_{(4,68)}$ = 5.81, p < 0.01) and, importantly, a significant three-way interaction of group, cue-presentation and trial, ($F_{(4,68)}$ =3.88, p < 0.01).

To interpret the three-way interaction, separate ANOVA's were conducted for each genotype, with within-subject factors of cue presentation (either devalued or

non-devalued) and S^D trial. For control mice the analysis revealed a main effect of cue presentation ($F_{(1,7)}$ =10.67 p<0.05), a main effect of trial, ($F_{(4,28)}$=3.66, p < 0.02) and a significant interaction between these factors, ($F_{(4,28)}$ = 8.41, p < 0.01). Tests of simple main effects showed that there was a significant difference between responding during the devalued versus non-devalued cue presentations on bin 1 ($F_{(1,7)}$=9.45, p < 0.05) and on bin 2 ($F_{(1,7)}$=15.57, p <0.01); although there were no significant differences in nose-poke responding during bins 4 or 5 (maximum F value; bin 4, $F_{(1,7)}$=1.50, p >0.18). A similar analysis carried out on the data from GluR-$1^{-/-}$ mice showed no significant main effect of cue presentation, (F<1), of trial, ($F_{(4,40)}$ = 1.93, p > 0.12), nor interaction between these factors, (F<1). An additional analysis with genotype and trial as factors confirmed that the rate of nose-poke responding during the cue associated with the devalued outcome differed significantly between the two groups ($F_{(1,17)}$ = 7.02, p < 0.05). There was no main effect of trial, (F<1), nor a significant interaction of this factor with group, ($F_{(4,68)}$ = 1.91, p >0.10). Thus, control mice showed a clear devaluation effect, as shown by their differential nose-poke responding during presentations of cues associated with the devalued and non-devalued outcomes. In contrast, the devaluation treatment failed to alter nose-poke responding in GluR-$1^{-/-}$ mice. They showed comparable levels of performance during presentation of each cue irrespective of its association with the devalued and non-devalued outcomes.

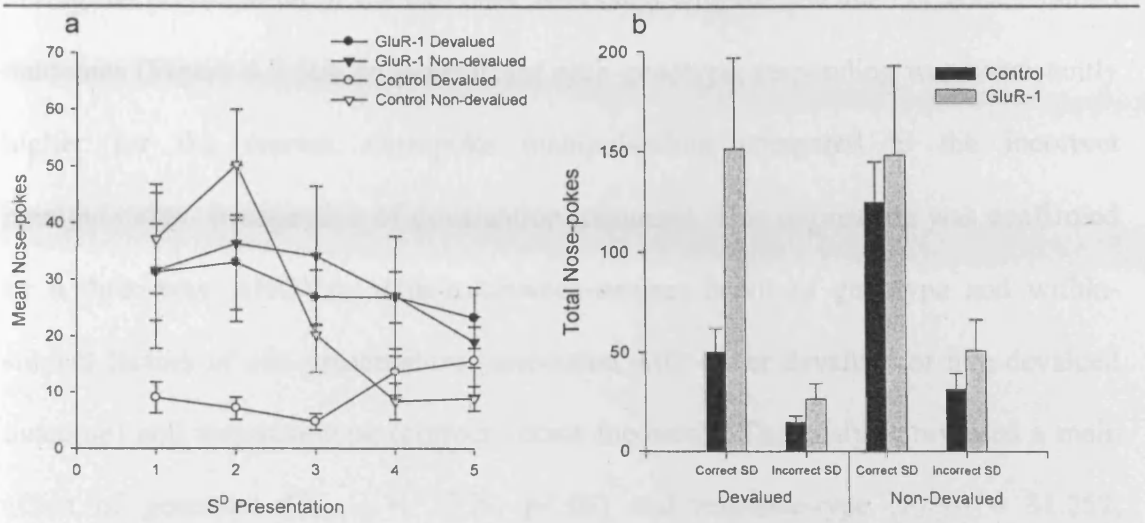

Figure 4.7.3. *Outcome devaluation extinction test results.* **(a)** Mean nose-poke responses during presentation of auditory stimuli associated with devalued or non-devalued rewards. Closed circles = mean nose-poke responses in the presence of the cue associated with the devalued reward for GluR-1$^{-/-}$ mice; closed triangles = mean nose-poke responses in the presence of the cue associated with the non-devalued reward for GluR-1$^{-/-}$ mice; open circles = mean nose-poke responses in the presence of the cue associated with the devalued reward for wild-type control mice; open triangles = mean nose-poke responses in the presence of the cue associated with the non-devalued reward for wild-type control mice. **(b)** Total nose-poke responses to the correct and incorrect nose-poke manipulanda during presentation of auditory stimuli associated with devalued and non-devalued rewards. Black bars = wild-type control mice total responses; grey bars = GluR-1$^{-/-}$ mice total responses. Error bars equal standard error of the mean.

In order to establish whether both groups of mice maintained the discrimination I calculated the rate of correct and incorrect nose-poke responses

during the presentation of the two cues associated with the devalued or non-devalued outcomes (Figure 4.7.3b). In general, for each genotype, responding was consistently higher for the correct nose-poke manipulandum compared to the incorrect manipulandum irrespective of devaluation treatment. This impression was confirmed by a three-way ANOVA, with a between-subject factor of genotype and within-subject factors of cue-presentation (associated with either devalued or non-devalued outcome) and response-type (correct versus incorrect). The analysis revealed a main effect of genotype ($F_{(1,17)}$ = 7.729, $p<.05$) and response-type ($F_{(1,17)}$ = 31.252, $p<.0001$); no other main effects or interaction terms approached significance (largest F value; genotype × response type interaction, $F_{(1,17)}$ = 2.18, $p>0.1$). Thus, both groups of mice maintained the discrimination; although responding to the correct nose-poke was lower during cue-presentations associated with the devalued outcome for control mice only.

Choice Test.

Figure 4.7.4 shows the results of the reward choice test for GluR-$1^{-/-}$ and control mice. Both groups consumed more of the non-devalued outcome then the devalued outcome. A two-way ANOVA confirmed this observation, revealing no main effect of genotype ($F_{(1,17)}$ = 1.991, $p>0.05$), but a main effect of food choice (devalued vs. non-devalued; ($F_{(1,17)}$ = 2.358, $p<0.05$). Additionally, no interaction between the two factors was found ($F<1$). Thus, the absence of a devaluation effect in the GluR-$1^{-/-}$ mice cannot be attributed to a failure of these mice to discriminate between the two outcomes. Finally, the lack of devaluation effect in the GluR-1 mice cannot be attributed to any difference in the number of pellets or the amount of

sucrose solution consumed by the two groups during the specific satiety treatment. Control animals consumed on average 1.29 and 1.18 grams of pellets and sucrose respectively. GluR-1$^{-/-}$ mice consumed on average 1.72 and 1.09 grams of pellets and sucrose respectively during the prefeeding phase. Subsequent analysis of the means revealed no significant differences between the groups (F<1).

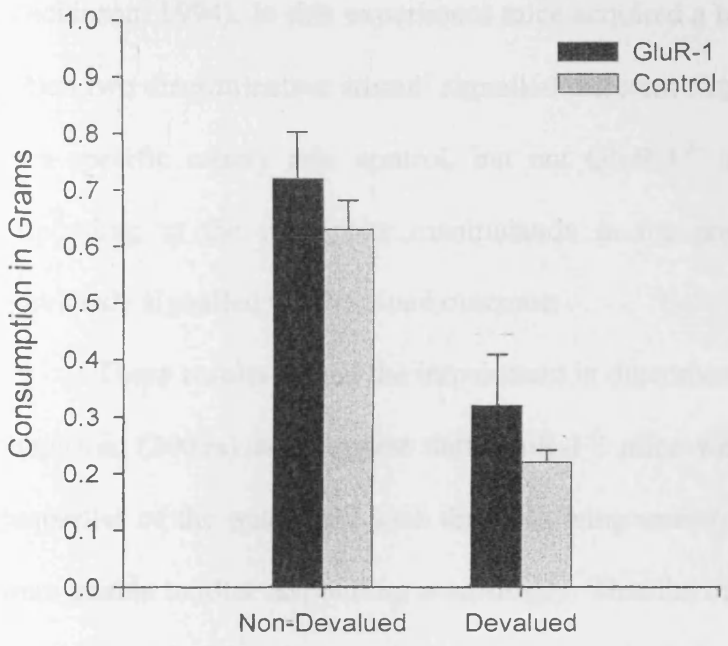

Figure 4.7.4. *Food preference choice test.* Consumption in grams of the devalued and non-devalued outcomes during the 30-min choice test. Black bars = consumption for GluR-1$^{-/-}$ mice; grey bars = consumption for wild-type control mice. Error bars equal standard deviation of the mean.

4.3.3 Discussion.

This experiment examined outcome encoding using an instrumental design based on other tasks which have assessed outcome-specific encoding in BLA-lesioned rats (Blundell, Hall & Killcross, 2001; Balleine, Dickinson & Killcross 2003). Only through instrumental actions can one directly assess goal-directed behaviour (Dickinson & Balleine, 1994); defined as an ability of the animal to encode (1) the response-outcome contingency, and (2) a representation of the outcome as a goal (Dickinson, 1994). In this experiment mice acquired a biconditional discrimination in which two discriminative stimuli signalled different response-outcome contingencies. In a specific satiety test, control, but not GluR-1$^{-/-}$ mice showed a lower rate of responding to the nose-poke manipulanda in the presence of the S^D which had previously signalled the devalued outcome.

These results extend the impairment in outcome-encoding reported by Mead & Stephens, (2003a) and suggest that GluR-1$^{-/-}$ mice were insensitive to the specific properties of the outcomes, such that following sensory-specific satiety mutant mice were unable to alter responding accordingly. That the impairment was due to aberrant processing of associatively activated US-sensory representations was supported by the finding that mutant mice were clearly able to discriminate between the two rewards in a subsequent choice test immediately following instrumental devaluation.

Alternative Explanations: Experimental Caveats.

It is worth noting that despite the specific impairment noted above, there are however several experimental caveats which require discussion. Firstly, one could

question the use of nose-poke responding as an index of instrumental response. This behavioural response requires the mouse to approach the manipulandum in order to emit a response; it could be argued that such a response is indicative of Pavlovian approach behaviour (Hershberger, 1986). However, it has been demonstrated that rats can be trained to omit a previously trained nose-poke response in order to collect reward (Aaron & Throne, 1975). That such a response can be omitted reflects the capacity of the animal to represent R-O contingency and contrasts with how one would anticipate a purely Pavlovian animal would respond (Hershberger, 1986; Dickinson, 1994).

On a related issue, rather than our instrumental paradigm assessing response-outcome associations (and hence goal-direct action), it is possible that a more specific association involving an interaction of stimulus-outcome (S-O) and stimulus-response (S-R) representations governed performance in Experiment 7. That is, it could be argued that during training the manipulanda (i.e., the nose-pokes) elicited different Pavlovian responses due to their differential association with the two rewards. These Pavlovian responses could reflect different internal representations appropriate for each instrumental response manipulandum, mediated by an S–R mechanism. For instance, Pavlovian S-O cues closely associated with the outcome generated by one response (governed by contextual cues associated with a specific manipulandum) may form a strong association with that outcome, whereas contextual cues associated with the other response will be associated with the other outcome.

Given these assumptions, a S–R account (based on stimulus-outcome [S-O] Pavlovian influences) of reward devaluation can be formulated by supposing that devaluing one reward reduces the corresponding Pavlovian response, thereby reducing any internal representation controlling the instrumental response (Donahoe,

Palmer & Burgos, 1997; Hall, 2002). However, this view fails to recognise the fact that reward devaluation effects can be observed when animals are trained to perform different actions on a single manipulandum. For example, Dickinson, Campos, Varga and Balleine, (1996) trained rats to press a vertically suspended pole in one direction for food pellets and in the other for the starch solution. Applying the S-R view to this task, one would predict that any Pavlovian responses elicited by the pole should exert equivalent control over both instrumental responses. That is, the devaluation will affect both actions equally. In contrast to this hypothesis, Dickinson et al., (1996) found that performance of the action that, in training, had delivered the devalued food was selectively reduced (Dickinson et al., 1996). This finding renders one alternative account of instrumental outcome devaluation effect, based on a reduction in the impact of Pavlovian excitatory cues on performance, much less plausible.

In summary, the previous experiment assessed goal-directed outcome encoding in GluR-1$^{-/-}$ mice. My results support the view that, similar to BLA-lesioned rats (Balleine et al., 2003), mutant mice are unable to use an instrumental signal to gain access to the current incentive value of a primary reward. Additionally, in consideration of the theoretical discussion presented above, such a deficit is attributable to an incapacity of GluR-1$^{-/-}$ mice to form a response-outcome representation based on the current sensory-specific incentive value of the primary reward.

4.4 Chapter Discussion.

Mead and Stephens (2003a) suggested that the GluR-1 deletion disrupted processing of the motivational properties of a US performed by the BLA. However, the tasks used to support this claim were limited in their ability to characterise the specific nature of the impairment in processing US value. The experiments reported in this chapter attempted to make use of contemporary paradigms related to BLA-mediated processing (Blundell et al., 2001; Balleine et al., 2003; Corbit & Balleine, 2005). Thus, although GluR-$1^{-/-}$ mice were able to acquire the Pavlovian (Experiment 6) and instrumental (Experiment 7) discrimination, their performance was unaffected by a manipulation that changed the motivational value of a specific reward.

It is worth noting that despite the specific impairment noted above, these mice clearly remained sensitive to some aspects of the reinforcing properties of outcomes and suggests that GluR-1 mutant mice were still able to engage a general reinforcement learning mechanism – one possibly mediated by S-R representations. Interestingly, if normal rats are given extended training on a continuous schedule of reinforcement and then subsequent receive a devaluation treatment, responding is no longer sensitive to outcome devaluation (Adams, 1982). Recently an analysis of the effects of overtraining has been developed on the basis of two different learning mechanisms; one mechanism involving S-R learning and the other R-O learning. As already mentioned, the available evidence indicates that the devaluation effect relies on R-O representations (Dickinson, Campos, Varga & Balleine, 1996). It is plausible therefore that GluR-$1^{-/-}$ mice acquired the conditional discrimination using a general S-R reinforcement mechanism. A similar claim has been posited for rats with BLA lesions (e.g., Balleine et al., 2003) and suggests a parallel between the behavioural

effects of GluR-1 deletion and cell loss in the BLA. In order to explore the boundaries of this parallel further, the next section will briefly review the effects of BLA lesions on instrumental learning. In addition, as a phenotype based on hippocampal dysfunction has also been reported in GluR-1$^{-/-}$ mice (Zamanillo et al., 1999; Riesel et al., 2002), I will also consider the effects of hippocampal lesions on instrumental learning before presenting the next experimental chapter.

4.4.1 *The Amygdala and Instrumental Learning.*

Rats with damage to the BLA are clearly sensitive to some aspects of reward value as shown by their acquisition of conditioned responding using various Pavlovian and instrumental paradigms (Hatfield et al., 1997; Killcross et al., 1997). However, the CR expressed by BLA-lesioned animals seem to lack the flexibility of that seen in normal animals (Cardinal et al., 2002). As previously mentioned, rats with damage to the BLA are impaired in Pavlovian or instrumental revaluation procedures (Hatfield et al., 1996; Balleine et al., 2003). Similarly, BLA-lesioned rats fail to show a CR to a second-order tone CS when it is paired with a previously rewarded first-order light CS (Hatfield et al., 1996). Hatfield and colleagues attributed the deficit to an inability of lesioned rats to adjust CRs following changes in the current value of the reinforcer. This interpretation could be used to explain a range of deficits in BLA-lesioned rats such as that observed in conditioned reinforcement paradigms. In this task, rats with selective BLA lesions are impaired in their acquisition of new responses. That is, they fail to respond on the conditioned reinforced lever in order to obtain presentations of a CS which had previously been paired with reward (Cador et al., 1989; Burns et al., 1993). The deficit in responding for the cue (conditioned

reinforcer) reflects an inability of BLA-lesioned rats to attribute affective properties to a cue (Everitt, Cardinal, Hall, Parkinson & Robbins, 2000), similar to the effect seen in second-order conditioning (Hatfield et al., 1996).

As already mentioned, however, a more detailed interpretation has been recently postulated (Balleine et al., 2003). Thus, if rats are trained on an outcome-specific reinforcer devaluation procedure (as adopted for Experiment 7), rats with lesions to the BLA are unable to discriminate between different reward types as reflected by a dysfunction in choice performance followed outcome devaluation and a insensitivity to the degradation of the instrumental contingency. These results provide further evidence consistent with the view that a failure of R-O learning is lesioned animals is mediated by a failure to discriminate between a representation of the two rewards (Balleine et al., 2003).

Consistent with this view BLA-lesions attenuate performance of the DOE and outcome-specific PIT (Blundell et al., 2001; Corbit & Balleine, 2005). The DOE refers to the improved conditional discrimination performance when consistent different outcomes are used during discrimination training (Trapold, 1970). BLA lesions eliminate the DOE which suggests that BLA-lesioned rats are unable to make use of the unique properties of the different rewards to facilitate acquisition of the discrimination i.e., are insensitive to their sensory-specific properties (Blundell et al., 2001). That the BLA lesioned rats were unable to make use of the US-sensory representations was supported by the additional finding that these same rats were unable to differentially respond in the presence of cues which had previously signalled different reward type (i.e., were impaired in the outcome-specific PIT). However, these same animals remained sensitive to some aspects of reward value as evidenced by non-specific transfer during cue presentations in the PIT test phase and

the successful acquisition of the conditional discrimination (Blundell et al., 2001); a finding which has subsequently been replicated (Corbit & Balleine, 2005). BLA-lesions impair the ability of animals to form associatively activated US-sensory representations, whilst leaving US-affective representations intact (Blundell et al., 2001; Corbit & Balleine, 2005).

The view that the BLA is involved in representing sensory aspects of motivationally-significant stimuli leads to the prediction that BLA lesions should be without effect on post-conditioning changes in reinforcer value if initial learning involves only the use of sensory aspects of otherwise neutral events. To examine this possibility, Blundell et al., (2003) exposed rats to a devaluation procedure in which the rats were trained to press one lever to receive sucrose pellets and another lever to receive food pellets. The rats were then pre-fed one of the reinforcers until satiated. Sham-lesioned rats displayed attenuated lever pressing for the devalued reinforcer, whereas the BLA-lesioned rats failed to display differential response rates on the two levers. However, a second experiment revealed that BLA-lesioned rats were sensitive to devaluation if assessed in a sensory preconditioning task. Here, the rats received pairings of two flavours, followed by aversive conditioning with one of the flavours. The neutral flavour that had been paired with the now revalued flavour was avoided in favour of a neutral cue that had been paired with a non-devalued flavour. This indicates that the BLA is unlikely to play a role in neutral stimulus-stimulus learning (Blundell et al., 2003).

Thus far the deficit in rats with BLA lesions appears to be best characterized as a deficit in encoding the sensory-specific aspects of motivationally significant stimuli (Blundell et al., 2001, 2003; Balleine et al., 2003; Corbit & Balleine 2005). Furthermore, evidence suggests that these sensory aspects of rewarding events play an

important role in the hedonic evaluation of nutritive instrumental outcomes on the basis of their palatability (Balleine, 2001; Berridge, 2001). Hence, to use a recently used classification, it would seem that the BLA is involved in aspects of "liking" rather than "wanting" (Simbayi, Bokes, Burton, 1986; Berridge & Robinson, 1998; Berridge, 2001; Blundell & Killcross, 2002; Balleine et al., 2003). From this perspective, wanting refers to appetite, the willingness to eat food and the reinforcement power of food. In contrast, liking is indexed by orofacial response measures of palatability; which reflect changes in their liking for specific tastes (Berridge & Robinson, 1998). Previous traditional views of incentive motivation viewed 'wanting' and 'liking' to be identical. However, 'wanting' and 'liking' are in fact dissociable and have different neural substrates. This dissociation was noted following manipulations of the mesolimbic dopamine system (e.g., Berridge et al., 1989). This revealed changes in motivated behaviour for the reward, such as reductions in instrumental performance and consumption of rewards- i.e., wanting. However, these same animals were capable of showing affective facial expressions in response to taste, which Berridge and colleagues suggests indicates a 'liking' of the reward (Berridge & Robinson, 1998). In respect to BLA-lesioned rats, an inability to encode the associatively activated sensory-specific elements of a US (e.g. taste) would be expected to prevent the latter of these two systems from functioning (Balleine et al., 2003).

Using this heuristic it becomes possible for one to relate other behavioural dysfunctions following BLA lesions, such as deficits in CS-potentiated feeding. In CS-potentiated feeding paradigms, during training rats are rewarded with food delivery during CS presentations. Following training, rats are satiated with the reward, after which the ability of the CS to promote feeding is assessed. In BLA-lesioned rats

the CS fails to promote feeding (Holland, Hatfield & Gallagher, 2001; Holland & Gallagher, 2003; Holland, Petrovitch & Gallagher, 2001). This failure could reflect modulation of some aspect of an appetitive motivational state, and might involve alterations in the palatability ("liking") of the food reinforcer (Holland & Gallagher, 2003). Additionally, the inability of the CS to promote feeding may reflect a similar inability of CS-US reward conditioning to endow the CS with positive incentive value, as such CS presentation fails to elicit feeding behaviour. However, this suggestion has yet to be investigated (Holland & Gallagher, 2003).

In contrast to the lesions data (Blundell et al., 2001; Balleine et al., 2003), infusion of the NMDA antagonist AP5 into the BLA has been shown to impair the acquisition of lever-press responding (Baldwin, Holahan, Sadeghian & Kelly, 2000). Here, the authors reported a learning deficit in lever-press responding where rats had to learn to chose between a correct or an incorrect lever-press to receive presentations of a stimulus light followed by reward. Although this finding seems somewhat contradictory, it is worth noting that the authors did not measure the extent to which AP5 defused throughout the BLA or indeed adjacent structures. Furthermore, the experimental protocol adopted may have been examining more than simply instrumental learning, since following responding on the correct lever, a 3 s stimulus light preceded reward delivery. Therefore, it is possible that responding was dependent on the conditioned reinforcing properties of the stimulus light; a task which is sensitive to lesions of the BLA (Mackintosh, 1974; Cador et al., 1989; Burns et al., 1993). Unfortunately, the authors failed to assess whether the stimulus light had acquired any CRf properties. Thus, the involvement of NMDA-dependent activation in the BLA requires more stringent examination.

Finally, the BLA has also been implicated in intolerance to the delay of reinforcement (Winstanley, Theobald, Cardinal & Robbins, 2004). Here, rats were trained to lever-press for food reward and to nose-poke into the magazine recess for presentation of the levers. The onset of the houselight indicated to the rats that the trial had commenced, at which point each rat was given 10 s to nose-poke in the magazine recess. If responding failed, the trial commenced once again following an ITI. Following nose-poke responding two levers were presented to the rats. Responding on one lever was rewarded with an small immediate reward and responding on the other lever resulted in the delivery of a large immediate reward. As the session progressed the delay to the large reward increased as a function of time, such that by the fifth block of trials rats had to wait for 60 s until food was delivered following a response on the large reward lever (Winstanley et al., 2004). Normally, rats would respond to the large reward lever initially in the session, and then transfer their response to the small reward lever following increases in the delay-to-reward. However, BLA-lesioned rats although initially preferring the large reward, readily responded on the small reward lever to a greater extent than sham-lesioned rats (Winstanley et al., 2004). The authors suggest that the increase in impulsive choice may have reflected an impairment in using representations of the incentive value to guide responding. Thus, to continue responding on the large reward lever, the rats were required to maintain a representation of the reward following increases in the delay to reinforcement. As such, the delay may have functioned as a conditioned reinforcer (Winstanley et al., 2004, see Garrud, Goodall & Mackintosh, 1974). However, recently it has been postulated that the BLA is involved in the acquisition of timing (Blundell & Kirkpatrick, in press). Therefore, one could suggest that BLA-lesioned rats would have difficulty in learning about changes in temporal contingency.

As a consequence lesioned rats would be expected to respond more on the short-delay small-reward lever, in comparison to the long-delay lever where a maintained representation of the delay to reward is required.

The CeN is thought to be involved in controlling the general motivational influence of reward-related events. CeN lesions attenuate the acquisition and maintenance of the conditioned-dependent enhancement of OR's paired with food; however, they had no effect on the acquisition of conditioned behaviour directed towards the food cup, nor did they prevent the ability of post-conditioning changes in US value to reduce first-order food-cup responses (Gallagher, Graham & Holland, 1990; Hatfield et al., 1996; Holland & Gallagher, 1999).

That the CeN modulatory function extends to more general changes in processing of a CS was supported by a series of experiments examining the CeN with blocking and unblocking paradigms. (Holland & Gallagher, 1993). In blocking acquisition of CR's to one element (X) of a compound CS (AX) paired with a US is lessened due to prior conditioning of the other element (A) with the same US (Kamin, 1968). A disruption to blocking can take place (i.e., unblocking) if the value of a US is reduced when the AX compound is introduced. Whilst control rats will show substantial conditioning to X using this unblocking paradigm, CeN lesions attenuate this unblocking (Holland & Gallagher, 1993). Since unblocking is believed to reflect an incremental attentional process (Mackintosh, 1975) it is suggested that the CeN is also involved in these incremental changes in attentional modulation (Holland & Gallagher, 1993). These results suggest that the CeN is involved not only in the conditioning-dependent modulation of ORs to CSs paired with food (Gallagher et al., 1990; Holland & Gallagher, 1999), but also in the engagement of incremental changes in attentional modulation (Holland & Gallagher, 1993; Hatfield et al., 1996).

That the CeN is involved in modulating incremental changes in attention was supported by a follow-up investigation examining the role of the CeN in the performance of a well-learned selective attention task (Holland, Han & Gallagher, 2000). Here, rats were trained on a multiple choice reaction task, where subjects were required to nose-poke into one of three ports; guided by the brief illumination of one of the three ports. Rats with CeN lesions performed comparably with controls in many aspects of the experiment. However, a reduction in the target duration (from 0.5 to 0.25 s), or an introduction of variability in the duration of the ready signal [duration between house light activation (i.e., initiation of trial) and target presentation] produced response deficits in CeN-lesioned groups. Consistent with previous findings (Gallagher et al., 1990; Holland & Gallagher, 1993; Hatfield et al., 1996) the authors suggest that the CeN is involved in a wide range of attentional processes including the acquisition of new learning and those involved in directing action (Holland et al., 2000)

Additionally intra-CeN infusion of the competitive AMPA antagonist NBQX similarly blocks conditioning-dependent changes in rearing/orienting responses to a CS (McDannald, Kerfoot, Gallagher & Holland, 2004). However, control rats who were infused with vehicle in training (during light→ food pairing), showed evidence of rearing responses to the visual CS in a subsequent test session under NBQX. These results suggest that the CeN is not involved in the expression of conditioning-dependent orienting behaviour (McDannald et al., 2004). To confirm this prediction, a different squad of rats underwent post-training CeN lesions. As suggested, these rats still orientated toward the visual CS in the test sessions following surgery. The authors postulated that the memory for this conditioned-orienting behaviour may be stored in regions efferent to the CeN such as the substantia nigra pars compacta;

further they suggest that the CeN enhances orienting behaviour to predictors of behaviourally significant events such as food by potentiating nigrostriatal systems (McDannald et al., 2004).

On a similar note, infusion of the NMDA antagonist AP5 into the CeN and the posterior lateral striatum (efferent to the CeN) resulted in impaired instrumental conditioning (Andrzejewski, Sadeghian & Kelley, 2004). Here, rats were given pre-training infusions of AP5; which attenuated instrumental lever-press learning for sucrose delivery. Unlike the effects of NBQX; AP5 not only attenuated acquisition; but also attenuated expression of the learnt response (Andrzejewski et al., 2004). However, it must be noted that in a separate consumption test AP5 resulted in an reduction in the amount of sucrose consumed during test, suggesting that disturbances in general motivational arousal may have contributed to the so-called 'learning deficit'.

Furthermore, CeN lesions effects similar to those reported by McDannald et al. have been observed in a discriminative autoshaping paradigm (Parkinson, Robbins & Everitt, 2000). Here, illumination of a screen on one side of an operant chamber was followed by food delivery, while illumination of the other side was non-reinforced. Normally, rats learn to approach both the screen and the food-recess when the food-screen is illuminated, an effect termed autoshaping (Browns & Jenkins, 1968). Parkinson et al., (2000) found that rats with CeN lesions approached the food cup normally but failed to approach the visual CS. Autoshaping is believed to reflect the acquisition of incentive motivational significance to the visual CS (e.g., Holland 1977; Tomie, 1966). According to this view, the visual CS acquires incentive value as a result of learning, and rats approach the CS as they would with the reward itself. Under this perspective one would assume that BLA rather than CeN lesions would

interfere with autoshaping. However, BLA-lesioned rats show normal-autoshaping acquisition (Parkinson et al.,2000; Blundell et al., 2003). Thus, one could suggest that autoshaping reflects changes in CeN-dependent motivational arousal, rather than mediated by specific incentive features of reward (Corbit & Balleine, 2005).

Finally, as previously mentioned BLA lesions attenuate outcome-specific PIT (e.g., Blundell et al., 2001). In contrast, lesions of the CeN impairs general instrumental transfer (Blundell et al., 2001; Hall, Parkinson, Connor, Dickinson & Everitt, 2001; Holland & Gallagher, 2003; Corbit & Balleine, 2005). Thus, CeN lesions abolish the general motivational influences of Pavlovian stimuli, whilst leaving the specific effects of the cues intact. Corbit & Balleine, (2005) trained rats on an instrumental paradigm in which responding on two different levers resulted in the delivery of two different rewards (O1 and O2). Subsequently, in the Pavlovian training stage during CS1 presentations, O1 was delivered, while during CS2 presentations, O2 was delivered. A third CS (CS3) was presented where a novel outcome was delivered (O3). In the PIT test stage carried out in extinction, the authors used a within-subject measure to evaluate both the outcome-specific PIT effect (i.e., selective responding in the presence of cues which had previously signalled different reward types) and the general PIT effect (through presentation of CS3). Rats with lesions of the CeN showed an outcome-specific PIT. However, the same rats did not show a general PIT effect. Thus, the CeN appears necessary for controlling general motivational/affective arousal properties of reward-related events (Corbit & Balleine, 2005).

In summary, the CeN attenuates conditioning-dependent ORs; down-shifts in US value mediating unblocking; Pavlovian autoshaping; well-learned selective attention task and general PIT (Blundell et al., 2001; Hall, Parkinson, Connor,

Dickinson & Everitt, 2001; Holland & Gallagher, 2003; Corbit & Balleine, 2005). These results suggest a role for the CeN in mediating attentional modulation via controlling the general motivational influence following reward. Further, to use a commonly used heuristic, the CeN reflects top-down attentional control which refers to the capture of attention on the basis of goals and expectations of the observer (e.g., learned expectancies that allow the agent to predict other, behaviourally significant events). This is in contrast to bottom-up processing by intrinsic physical properties of a stimulus (e.g., sudden onset or high intensity; McDannald et al., 2004). These findings are interesting when one examines CeN function from a neural systems approach. For instance, the expression of conditioned orienting appears to be mediated by the CeN dopamine input to the striatum. Inactivation (using 6-OHDA) of the dopaminergic amygdalo-nigrostriatal pathway produces a similar attenuation of conditioned orienting to a visual cue (Han, McMahan, Holland & Gallagher, 1997).

4.4.2 *The Hippocampus and Instrumental Learning.*

One of the earliest studies of the involvement of the hippocampus in instrumental learning used bilateral hippocampal stimulation which disrupted the acquisition and extinction of instrumental responding (Correll, 1957). Subsequent studies made use of the recording of electrical activity in hippocampal neurons during instrumental learning. These studies provided evidence that the hippocampus was active during instrumental learning (Konorski, Santibanez & Beck, 1968; Reymann, Shvyrkov & Grinchenko, 1977; Bartel & Uryvaev, 1980). Clark and Isaacson (1965) provided the first demonstration that damage to the hippocampus impaired instrumental learning. In their study, pre-training electrolytic hippocampal lesions

impaired performance on a differential reinforcement of low-rates of responding schedule.

Other studies showed that pre-training hippocampal lesions did not attenuate instrumental appetitive conditioning following delayed-reinforcement (Port, Curtis, Inoue, Briggs & Seybold, 1993). Here, training consisted of daily sessions in which lever-press responding was autoshaped, such that responding was reinforced with food pellets following a 5 s delay. Consistent with previous findings (e.g., Rawlins et al., 1983) both hippocampal- and sham-lesioned rats acquired the instrumental response over a similar period. Further, response-rates between the two groups were comparable (Port et al., 1993). However, in contrast to control rats, Davenport and Holloway (1980) found that hippocampal-lesioned rats trained to lever-press for food continued to press at high rates when the causal relationship between response and outcome was removed and reward was shifted from a random interval to a random time schedule. Davenport and Holloway suggested that without an intact hippocampus, instrumental responding is governed by S-R associations. It should be noted, however, that the hippocampal lesioned groups were also hyperactive which may have interacted with performance on the schedules.

It has also been reported that excitotoxic hippocampal lesions facilitate progressive ratio (PR) responding (Schmelzeis & Mittleman, 1996). Here, the PR paradigm was implemented under the assumption that PR breakpoints reflected the degree of effort an animal would exert in order to obtain reward (Hodos, 1961). Generally, breakpoints are viewed as rate-free measures of a reinforcers strength because they have no direct relationship to response rate and because there are only minimal temporal constraints on responding (Hodos, 1961). Complete hippocampal- and sham-lesioned rats were trained to obtain reward whereby systematic increases in

the value of fixed-ratio responding would follow the delivery of reward. During the course of training, breakpoints were assessed for each animal. This predetermined time interval separated responses which exceeded some predetermined length. The results showed that hippocampal lesions produced long-lasting changes in the dynamics of PR responding (i.e., increase in PR breakpoints). This finding may reflect lesion-induced increases in the rewarding or incentive motivational properties of the delivered food pellets (Schmelzeis & Mittleman, 1996). However, it should be noted that consistently throughout training lesioned animals showed evidence of hyperactivity which may have accounted for the noted increase in PR breakpoints of lesioned rats. Schmelzeis and Mittleman argued against such a claim in noting an increase in breakpoints manifested by increases in reward palatability. However, even through the activity of lesioned animals seemed dependent on the palatability of the reward, the fact this paradigm uses higher response rates as a signal for reflecting increases in the perceived hedonic value of reward causes one to question whether such a task would be sensitive to such manipulations in hyperactive hippocampal-lesioned animals.

With respect to the neural basis of instrumental conditioning, recent evidence suggests that declarative memory is dependent on a fully-functional hippocampus (e.g., Squire, 1992; Good 2000). Further, some authors suggest that R-O associations are encoded in declarative memory (e.g., Squire & Zola-Morgan, 1996) and suggest therefore, that R-O learning is dependent on an intact hippocampus (Corbit & Balleine, 2000). Corbit and Balleine examined this possibly using two tasks that are directly sensitive to R-O manipulations, reinforcer devaluation and contingency degradation. Interestingly, DH-lesioned rats remained sensitive to post-training revaluation of the outcome. However, these same rats were insensitive to degradation

of the R-O contingency (Corbit & Balleine, 2000). The authors suggested that hippocampal lesioned rats were sensitive to adventitious R-O relationships. However, lesioned rats were unable to discriminate between causal R-O relations compared to non-contingent reward delivery. The influence of non-contingent outcome delivery relies on the ability of rats to represent Pavlovian contextual influences on reward delivery (Dickinson, 1994). Therefore, it was suggested that the deficit in hippocampal lesioned rats reflected an inability to form context-outcome representations resulting in a impairment in contiguous versus non-contiguous R-O information (Corbit & Balleine, 2000).

Although this interpretation seems to reflect an involvement of the DH in R-O contingency representations, it should be noted that the authors used pre-training electrolytic lesions to induce hippocampal cell loss. This lesion technique inevitably causes damage to the fibres of passage which could have influenced the behavioural phenotype. In a follow-up study, Corbit, Ostlund and Balleine (2002) examined this possibility. Using a variety of lesion techniques and disconnection procedures, including both electrolytic and neurotoxic lesions, the authors reported that the entorhinal cortex (which has afferent connections with the DH; Totterdell & Meredith, 1997) was the region critically involved in detecting changes in R-O contingency (Corbit et al., 2002).

Interestingly, a facilitation rather than impairment has been reported following delayed-reinforcement following complete excitotoxic hippocampal lesions (Cheung & Cardinal, 2005). Here, rats were trained to lever-press for reward with a variety of delays (0, 10 or 20 s) between response and the subsequent delivery of the reward. Normal rats are sensitive to the contiguity between response and outcome delivery such that increases in delays reduces instrumental performance (Dickinson, Watt &

Griffiths 1992). However, hippocampal-lesioned rats responded at a lower-rate than control rats prior to the introduction of a delay. In contrast, response rates were higher than sham-lesioned controls following introduction of the delay. Thus, the deleterious effect of delays was evident in the lesioned group, possibly due to an inability of lesioned rats to form context-outcome representations. As such lesioned rats would not be distracted from mistakenly attributing non-scheduled context-outcome representations with reward delivery and consequently causing an attenuation of responding (Dickinson, 1994; Cheung & Cardinal, 2005).

In summary, it has been suggested that the hippocampus is involved in attributing hedonic impact to reward delivery (Schmelzeis & Mittleman, 1996); although whether response-rates interfered with the results remains a possibility. As mentioned in the Chapter 3, the hippocampal formation seems necessary for an animal to gain an accurate representation of their environment. As such, when contextual information mediates responding it is expected that this encourages hippocampal involvement. Impairments are manifested when the context is required to disambiguate a causal relationship between action and outcome from a noncontingent schedule in which an outcome occurs relatively frequently but independently of their behaviour (Corbit & Balleine, 2000; Corbit et al., 2002). Alternatively, facilitations have been noted when the context distracts the animal from forming a causal relationship between action and outcome (Cheung & Cardinal, 2005).

4.4.3 *Coda.*

The previous discussion highlights the dissociable influence of the amygdala and hippocampus governing instrumental behaviour. It is clear that the BLA is implicated in attributing incentive value to outcomes in order to mediate instrumental performance. The results from this chapter are in agreement with that posited by Mead and Stephens (2003a) suggesting that GluR-1$^{-/-}$ mice display a phenotype mimicking that seen in BLA-lesioned animals. As such, the following chapter attempted to further the examination into the nature of the disturbance in outcome-encoding manifested by targeted deletion of the GluR-1 subunit. This involved the use of procedures which had been previously used to assess both BLA dysfunction (Blundell et al., 2001; Corbit & Balleine, 2005) and variations in outcome encoding (Balleine et al., 1995; Corbit & Balleine, 2003).

Chapter 5.

5.1 Introduction.

In the previous chapter, it was suggested that GluR-1$^{-/-}$ mice are impaired in forming associatively activated US-sensory representations to gain access to current incentive value of an outcome. Despite the specific impairment noted in chapter 4, GluR-1$^{-/-}$ mice were clearly sensitive to some aspects of the reinforcing impact of outcome delivery. Similarly, Mead and Stephens (2003a) reported that GluR-1$^{-/-}$ mice were sensitive to CS presentations as assessed in a PIT paradigm. In this procedure animals are separately trained to perform an instrumental response to receive reward, while during alternate sessions animals learn that a Pavlovian stimulus signals the delivery of the same reward. In a test phase, the degree of responding to the instrumental manipulandum is assessed during presentations of the Pavlovian CS (e.g., Lovibond, 1983). Typically, animals respond more during the CS presentation than during a baseline period. Dickinson (1994) suggested that the influence (of the Pavlovian cue) on performance is engaged when the stimulus and action share common outcomes and operates by reinstating the conditions under which the action was conditioned. That is, the transfer reflects some learned motivational/affective influence over the instrumental response.

Interestingly, it has been suggested that transfer can influence performance through a second route (Holland, 2004; Corbit & Balleine, 2005). Colwill and Rescorla (1988) gave rats Pavlovian training in which a stimulus signalled the delivery of a particular reward. For half the rats, this reward was a food pellet, whereas for the remaining rats this reward was sucrose solution. In separate instrumental training phases, rats were trained to respond on one manipulandum (e.g., chain pull) for pellets and on a second (e.g., lever press) for sucrose solution. In the

transfer stage, the CS augmented responding to the manipulandum that had been reinforced by the same reward more than one that had been reinforced by the different reinforcer. This suggests that potentiation can also be reinforcer specific, that is mediated by the ability of stimuli to activate the memory of the sensory-specific features of the reward associated with a specific action (Dickinson, 1994; Corbit & Balleine, 2005).

Studies carried out with BLA lesioned animals have provided evidence for two dissociable processes mediating PIT. For instance, BLA lesions do not impair performance on the single-outcome PIT (Hall et al., 2001; Holland & Gallagher, 2003). Thus, BLA animals are able to augment responding via some learned motivational/affective influence of the Pavlovian cue over the instrumental response. In contrast, BLA lesions attenuate performance when trained on a multiple-outcome reinforcer specific version of the PIT (Blundell et al., 2001; Balleine et al., 2005; Corbit & Balleine, 2005). In these tasks, rats were trained to associate Pavlovian cues and instrumental responses with different rewards. In the test phase, BLA lesions did not prevent the augmentation of responding during cue presentation (i.e., general PIT), however these lesions abolished the reinforcer specificity of that enhancement. It has been suggested that these results reflect the inability of CSs to activate a memory of the sensory-specific properties (i.e., their unique properties) of a US following BLA damage (Blundell et al., 2001).

There is a striking parallel between the effects of BLA lesions and the deficits observed in GluR-1$^{-/-}$ mice on certain tasks that index the motivational properties of reward (Mead & Stephens, 2003a). Like BLA lesioned rats, GluR-1$^{-/-}$ mice seem unable to form a representation of associatively activated outcome representations (Experiment 6 & 7) but are nevertheless able to represent some general affective

quality of reward revealed by the single outcome PIT task. However, if the above analysis is correct, one would predict that mutant mice would be impaired on the reinforcer-selective version of PIT (Blundell et al., 2001). Experiment 8 evaluated this prediction using an outcome-specific PIT task that provided simultaneous examination of both a general and outcome specific Pavlovian-instrumental transfer effect.

5.2 Experiment 8.

5.2.1 Method.

Subjects.

Experiment 8 was conducted with age matched GluR-1$^{-/-}$ (n =11) and wild-type (n =11) mice, which were bred in the Department of Experimental Psychology at the University of Oxford and transferred to Cardiff University for behavioural testing at 12 months of age [for details of genetic construction, breeding and subsequent genotyping, Zamanillo et al., (1999)]. Mice were housed two or three to a cage under a light-dark cycle (lights on 07.00-19.00). Prior to the start of training mice were reduced to 85% of their ab-libitum weights and weighed 25-30 gm at the beginning of the experiment. All testing took place during the light phase between 9:00 am and 5:00 pm.

Apparatus.

The apparatus was identical to that described in Experiment 7.

Procedure.

Stage (1): Magazine Training.

Each animal was assigned to one of six operant chambers, and thereafter was always trained in that chamber. Throughout training, the rewards were either a single 20 mg Noyes food pellets or 0.1 ml 20% sucrose solution. Mice were trained to collect rewards for two days, with two 20 min sessions per day. The rewards were delivered on a random time (RT) 60 s schedule. Magazine entry during this training session was recorded. Approximately half the mice (6 GluR-1$^{-/-}$ and 6 control mice) were trained to collect food pellets in the morning session, and approximately half (7 GluR-1$^{-/-}$ and 7 control mice) were trained to collect sucrose solution. In the afternoon session, the mice received magazine training with the alternative reward. The next day, the order of training was reversed so that each mouse received each reward for one morning and afternoon session.

Stage 2: Pavlovian and instrumental training.

This procedure was based on methodology developed for rats (Blundell et al., 2001). Instrumental and Pavlovian training sessions were conducted on separate days, with instrumental sessions conducted on days 3,5,7,9,11,13, 15 and 17 and Pavlovian

sessions conducted on days 4,6,8,10,12,14,16 and 18. Initially, the mice received training on instrumental nose-poke responding. Each session lasted 20 min, and there were two sessions per day. At the start of each session one of the two nose-poke manipulandum was illuminated and responding was reinforced on a random interval (RI) 30 s schedule. This schedule was implemented to maintain consistency in respect to the parameters used by Blundell et al., (2001). The mice were trained in separate A.M. and P.M. sessions to respond on each of the two nose-poke manipulanda. The two rewards were earned by responding on a specific manipulandum. To encourage initial responding on the first session, each manipulandum was smeared with a small quantity of the relevant reward. The response-outcome contingency was fully counterbalanced across groups, such that, for approximately half of the mice in each group the right nose-poke manipulandum was reinforced by food pellets and responding on the left nose-poke manipulandum was reinforced by sucrose solution (7 GluR-1$^{-/-}$ and 7 control mice). These contingencies were reversed for the remaining animals (6 GluR-1$^{-/-}$ and 6 control mice).

The mice then received Pavlovian training sessions. Each session lasted 30 min, and there was only one session per day. During these sessions the nose-poke manipulanda were not activated, i.e., were present but not illuminated. Each session comprised 10, alternating 2 min presentations of either a 3 kHz tone (at 80 dB), or a 10 Hz train of clicks (at 80 dB), with an inter-trial interval (ITI) of 1 min. The first auditory stimulus presented in each training session was determined by the computer using a pseudorandom sequence that insured the animals received equal numbers of each trial type in each session. During each stimulus, one of the two rewards (either food pellets or sucrose solution) was delivered on a RT 30 s schedule. The reward which was delivered was different for each of the two auditory stimuli, and was fully

counterbalanced across groups. The instrumental and Pavlovian training sessions were presented an alternating schedule, culminating in the final Pavlovian training session on day 18.

Stage 3: Pavlovian-to-instrumental transfer tests.

After the final training session, there was a test session which lasted 30 min and comprised 10, alternating 2 min presentations of either a tone, or click-train, with an ITI of 1 min. As with Pavlovian training, the first auditory stimulus presented was determined by the computer. Therefore, in total there were 5 presentations of each CS randomly presented to each subject. For the entirety of the test session both nose-poke manipulanda were activated but no rewards were delivered.

5.2.2 Results.

Instrumental training.

Figure 5.8.1 shows the rate of nose-poke responding during the instrumental training phase. Inspection of this figure reveals that both groups of mice showed increases in the rates of responding as a function of training. In order to confirm this impression a three-way ANOVA was conducted with a between-subject factor of genotype and within-subject factors of session (1-9) and nose-poke response type (sucrose versus pellet reward). The analysis revealed a main effect of session, ($F_{(8,160)}$ = 3.035, $p<.001$), no effect of genotype ($F_{(1,20)}$ = 1.019, $p>0.32$) and no effect of

response type ($F_{(8,160)}$ = 1.232, p>.71). Additionally no interaction terms approached significance (all F's <1).

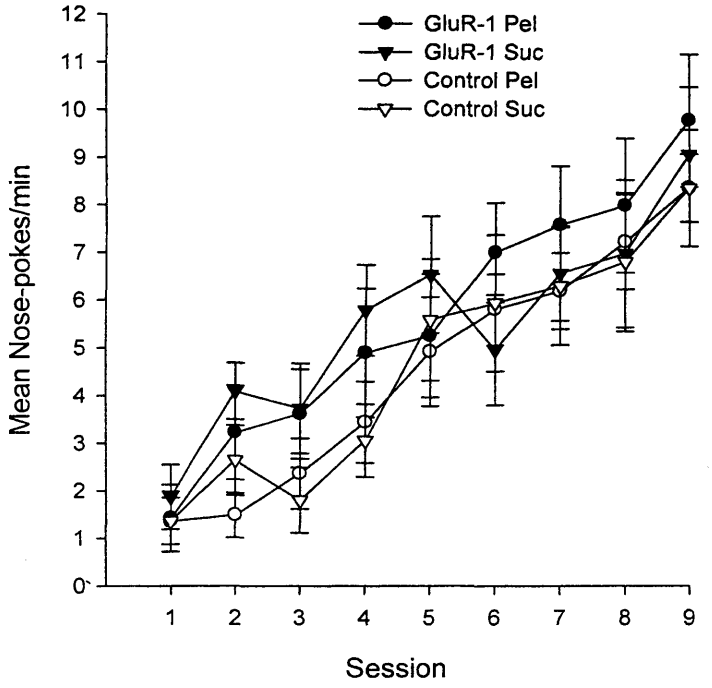

Figure 5.8.1. *Instrumental training results.* Mean rates of responding (in responses per minute) for pellet and sucrose reward. Closed circles = GluR-1$^{-/-}$ mice nose-poke responding for pellet reward; closed triangles = GluR-1$^{-/-}$ mice nose-poke responding for sucrose reward; open circles = wild-type mice nose-poke responding for pellet reward; open triangles = wild-type mice nose-poke responding for sucrose reward. Error bars indicate standard error of the mean.

Additionally, there was no difference in the overall amount of rewards obtained during the instrumental training phase for GluR-1$^{-/-}$ or control mice. On average GluR-1$^{-/-}$ mice received 14.6 (S.E. 1.56) pellets per session and 14.9 (S.E. 1.94) sucrose deliveries per session, whilst control mice received 17.7 (S.E. 1.82) pellets per session and 16.7 (S.E. 2.40) sucrose deliveries per session. An analysis of

these means revealed no significant differences or interactions between the groups (all $F's<1$).

Pavlovian training.

During the Pavlovian training stage, mice progressively made more magazine entries during the CS (Figure 5.8.2a). In order to evaluate the level of magazine responding during training, a three-way mixed ANOVA was conducted, with a between-subjects factor of genotype and within-subject factors of session (1-9) and CS (tone and clicker). The analysis revealed no main effect of genotype ($F_{(1,20)} = 2.93$, $p>0.1$), a main effect of session ($F_{(8,160)} = 2.38$, $p<0.05$), no effect of CS type ($F<1$) or interactions approaching significance (largest F value; session × CS type interaction, $F_{(8,160)} = 1.522$, $p>0.15$). The number of magazine entries during the ITI period is shown in Figure 5.8.2b. In general, responding during the ITI period was relatively similar throughout training. In order to confirm this impression a two-way mixed ANOVA was conducted with factors of genotype and session. The analysis revealed no main effect of genotype ($F_{(1,21)} = 3.87$, $p>0.05$), no main effect of session or interaction between the two factors (both $F's<1$).

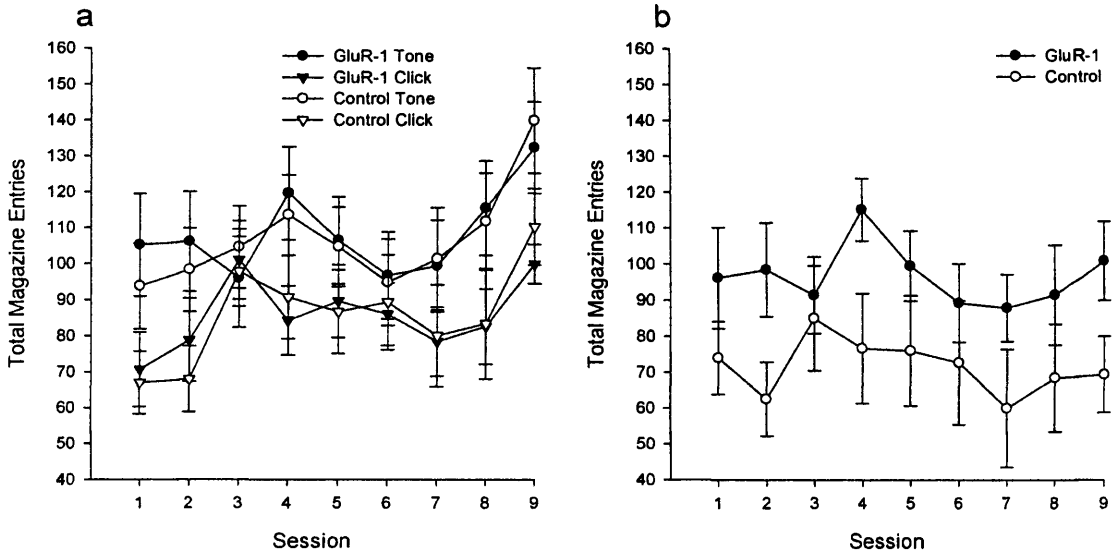

Figure 5.8.2. *Pavlovian training stage: Magazine entries.* **(a)** Total number of magazine entries during presentation of auditory stimuli. Closed circles = GluR-1$^{-/-}$ mice magazine entries during tone presentations; closed triangles = GluR-1$^{-/-}$ mice magazine entries during clicker presentations; open circles = wild-type control mice magazine entries during tone presentations; open triangles = wild-type control mice magazine entries during clicker presentations. **(b)** Total number of magazine entries during the ITI period where no stimuli were presented. Closed circles = GluR-1$^{-/-}$ mice magazine entries during the ITI period; open circles = wild-type control mice. Error bars indicate standard error of the mean.

In order to ensure sensitivity to group differences in acquisition, discrimination ratios were calculated by dividing the mean rate of magazine entry during the CS period by the mean rate of responding during both the CS and the ITI. A ratio that exceeds 0.5 indicates that magazine entry was more frequent in the CS period compared to the ITI (no stimulus presented) period. Figure 5.8.3 shows the discrimination scores during the tone and clicker presentations across each of the nine

Pavlovian training sessions. In general, both groups of mice acquired the discrimination and reached similar asymptotic performance. However, there was a tendency for the GluR-1$^{-/-}$ to acquire the discrimination at a slower rate than control mice. A three-way ANOVA was conducted with genotype, session (1-9) and stimulus type (tone or clicker) as factors and revealed a main effect of genotype ($F_{(1,20)}$ = 8.729, p<.01), session ($F_{(8,160)}$ = 21.538, p<.0001) and a significant interaction between these two factors ($F_{(8,160)}$ = 3.313, p<.01). No other main effects or interaction terms approached significance (largest F value; stimulus type, $F_{(1,20)}$ = 1.884, p>0.18). Simple main effects analysis was carried out on the significant genotype × session interaction and revealed a main effect of genotype at sessions 4 through to 8 (smallest F value; session 4, $F_{(1,154)}$ = 4.496, p<.05) with both GluR-1$^{-/-}$ ($F_{(8,160)}$ = 7.222, p<.0001) and control ($F_{(8,160)}$ = 17.629, p<.001) mice showing an increases in magazine entry during CS presentations across training sessions.

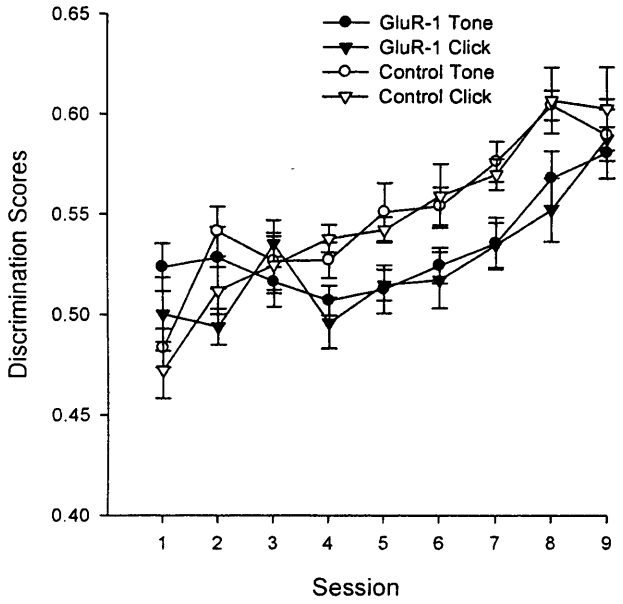

Figure 5.8.3. *Pavlovian training stage: Discrimination ratio scores.* Mean discriminations scores for the Pavlovian training stage following presentation of the tone and clicker CS. Closed circles = GluR-1$^{-/-}$ mice discrimination scores during tone presentations; closed triangles = GluR-1$^{-/-}$ mice discrimination scores during clicker presentations; open circles = wild-type control mice discrimination scores during tone presentations; open triangles = wild-type control mice discrimination scores during clicker presentations. Error bars indicate standard error of the mean.

PIT test stage.

Figure 5.8.4a shows the mean rate of nose-poke responding during the CS and during the ITI baseline period. Inspection of this figure shows that both groups of mice elicited higher levels of nose-poke responding during the CS compared to the ITI baseline period. A two-way mixed ANOVA was conducted on the response data (in RPM) with a between subject factor of genotype and a within-subject factor of

period (CS versus ITI). This analysis revealed no main effect of genotype ($F<1$) but a significant main effect of period ($F_{(1,20)} = 41.869$, $p<.0001$), and no significant interaction between these two factors ($F<1$). Thus, both groups of mice showed an equivalent general transfer effect of a Pavlovian CS on instrumental responding. In order to establish that responding during the CS and baseline ITI period did not differ between the groups, separate ANOVA's were conducted on response levels during CS and ITI periods. The analysis revealed that responding did not differ during these periods between groups (all F's<1).

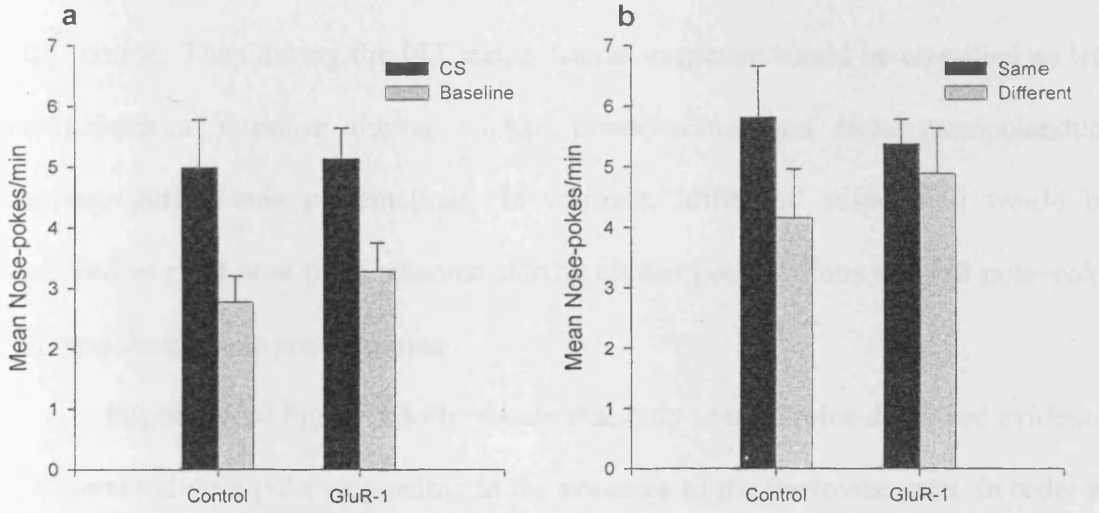

Figure 5.8.4. *PIT test stage results.* **(a)** Mean rates of responding (in responses per minute) during the CS and baseline ITI period in the PIT test stage. Black bars = responding during presentation of the clicker and tone periods; grey bars = responding during the baseline ITI period. **(b)** Mean rates of 'same' and 'different' responses (in responses per minute). Black bars = 'same' responding that had been previously reinforced by the food-type associated with the CS being presented; grey bars = 'different' responding on the alternate manipulanda. Error bars indicate standard error of the mean.

In order to determine whether the Pavlovian CSs had specific effects on instrumental responses associated with the same outcomes, the levels of left and right nose-poke responding evoked by presentation by each auditory stimulus was calculated (Figure 5.8.4b). In reference to Figure 5.8.4b a 'same' nose-poke response is the instrumental action that had been reinforced by the same US that was paired with the specific CS. The 'different' nose-poke response refers to an instrumental action that was associated with a different US. For example, during the instrumental training stage, responding on the left nose-poke manipulandum resulted in pellet delivery and responding on the right manipulandum resulted in sucrose delivery. In addition, during the Pavlovian stage, the clicker was paired with pellet and the tone with sucrose. Then during the PIT test, a 'same' response would be classified as left manipulandum response during clicker presentations and right manipulandum response during tone presentations. In contrast, 'different' responding would be classified as right nose-poke response during clicker presentations and left nose-poke response during tone presentations.

Inspection of Figure 5.8.4b reveals that only control mice displayed evidence of differential nose-poke responding in the presence of the Pavlovian cues. In order to confirm this observation a two-way ANOVA was conducted with factors of genotype and response type (same versus different). No main effect of genotype ($F<1$) was revealed, a main effect of response type ($F_{(1,20)} = 7.410$, $p<.02$), and no interaction between these factors ($F_{(1,20)} = 2.105$, $p>0.1$).

In order to assess whether variability in response rates masked the observed trend for differential responding by control mice, elevation scores were calculated (c.f., Blundell et al., 2003) and are shown in Figure 5.8.5a, for same and different responses. The rate of responding on the specific nose-poke (i.e., same or different)

was divided by the rate of responding on that response manipulandum during the ITI (baseline) period. If there was no increase in the rate of responding during presentation of the CS, the elevation ratio would be 1 or less. Inspection of Figure 5.1.5a shows that control mice displayed an enhanced level of 'same' nose-poke responding, whilst GluR-1$^{-/-}$ mice show no evidence of differential responding. A two-way ANOVA, with genotype and response type (same versus different) as factors, revealed no main effect of genotype (F<1) or response type ($F_{(1,20)}$ = 3.706, p>0.6). However, a significant interaction between the two factors was observed ($F_{(1,20)}$ = 5.619, p<.05). Analysis of simple main effects revealed a main effect of response type for control ($F_{(1,20)}$ = 10.149, p<.01) but not GluR-1$^{-/-}$ mice (F<1). Thus, only control mice displayed evidence of differential responding during presentations of Pavlovian cues which had previously signalled different reward types; i.e., a reinforcer-selective PIT effect.

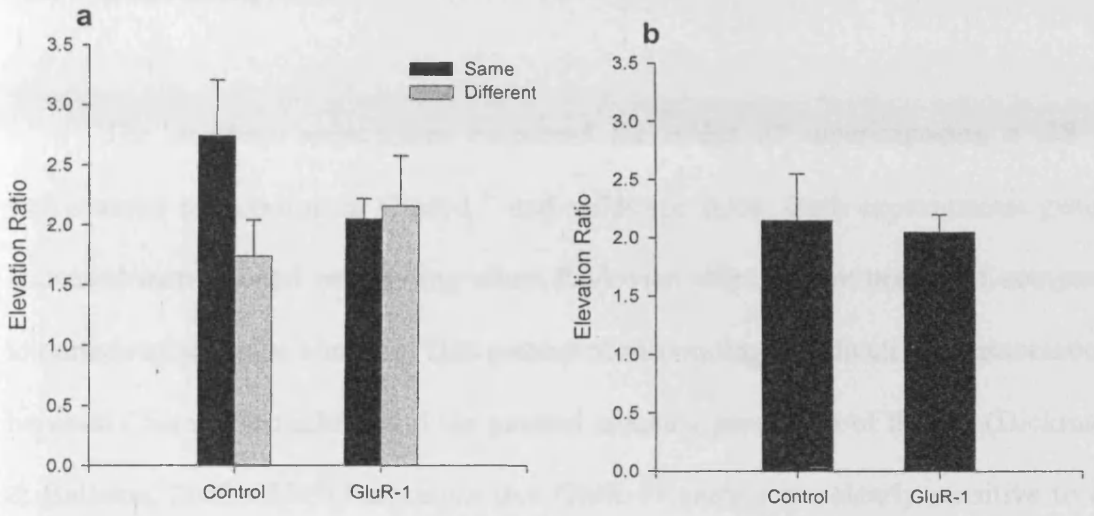

Figure 5.8.5. *PIT test stage elevation scores.* **(a)** Group mean elevation ratios for 'same' and 'different' responses. Black bars = elevation scores for 'same' responses; grey bars = elevation scores for 'different' responses. **(b)** Group mean elevation ratios averaged across 'same' and 'different' responses. Error bars indicate standard error of the mean.

Finally, the general elevation of responding following stimulus presentation (i.e., averaged across same and different responses; Figure 5.8.5b), did not differ between the groups, (F<1) and indicates that both groups showed a similar level of augmented instrumental responding during presentation of the Pavlovian CSs.

5.2.3 Discussion.

The previous experiment examined the effect of superimposing a CS on instrumental responding in GluR-1$^{-/-}$ and wild-type mice. Both experimental groups increased instrumental responding when Pavlovian stimuli were presented compared to periods of stimulus absence. This pattern of responding is indicative of associations between CS representations and the general arousing properties of the US (Dickinson & Balleine, 2002). The observation that GluR-1$^{-/-}$ mice were clearly sensitive to the general arousing properties of Pavlovian stimuli is consistent with the findings reported by Mead and Stephens, (2003a). Furthermore, these results suggest that GluR-1 mutant mice are not governed simply by an S-R learning mechanism as in Chapter 4. That is, one would expect a purely S-R driven rodent to approach the magazine during CS presentation (since the response in the Pavlovian stage was magazine approach), a response that would interfere with rather than enhance nose-poke responding during test (Dickinson, 1994; Hall, 2002).

However, in contrast to wild-type control mice, GluR-1$^{-/-}$ mice did not show an outcome-specific form of PIT. This suggests that the representation in GluR-1$^{-/-}$ mice reflects a more general motivational process which does not include a detailed sensory representation of reward (Holland, 2004). This deficit in outcome-specific PIT parallels that seen in BLA-lesioned rats (Blundell et al., 2001; Corbit & Balleine, 2005) and is consistent with the hypothesis of aberrant BLA processing proposed by Mead and Stephens (2003a).

Thus a clear behavioural phenotype seems to be emerging in GluR-1$^{-/-}$ mice- these mice are unable to encode and update the relationship between sensory-specific aspects of reward and current incentive value in order to mediate performance. The

deficit in KO mice does not reflect a gross impairment in discriminating between reward types, as food preference behaviour in these mice following devaluation is normal (Experiment 7). Moreover, these mice are still able to mediate instrumental performance based on associatively activated affective/motivational representations. Importantly, the hypothesis that GluR-1$^{-/-}$ mice are unable to associatively evoke US-sensory representations, whilst showing relatively normal US-affective associations, suggests that these mice should remain sensitive to the effects of changes in motivational state when instrumental performance is *not* governed by incentive learning. This prediction was examined in Experiment 9.

5.3 Experiment 9.

Balleine (1992) noted that following instrumental training, an attenuation of instrumental responding following outcome devaluation required the rats to experience the outcome in the non-deprived state (that is it required incentive learning to take place). Interestingly, however, magazine responding (as opposed to instrumental responding) was attenuated following a shift in motivational state (e.g., from deprived to non-deprived), without the need for incentive learning. In an attempt to mimic the situation where response followed magazine approach, Balleine, Garner, Gonzalez and Dickinson (1995) examined performance of animals trained on a heterogeneous chain of actions (i.e., R1→ R2→ O). Balleine et al. noted that actions proximal to outcome delivery (R2 responses) were directly sensitive to shifts in motivational state, whereas suppression of R1 actions required the animal to experience the outcome in the devalued state (i.e., incentive learning was required).

These results were suggested to reflect the variations in outcome encoding associated with each manipulandum (Balleine et al., 1995; Balleine, 2001).

Interestingly, one suggestion offered by Balleine and colleagues was that, in the heterogeneous chain, the performance of actions proximal to outcome delivery were influenced by excitatory (S-O) associations. This was supported by the finding that following PIT training, the stimulus paired with the same outcome as that used during instrumental chain training facilitated R2 responding, when the stimulus was presented in the heterogeneous chain setting (Corbit & Balleine, 2003a). Additionally, the finding that R1 actions were sensitive to devaluation via incentive learning, was consistent with the view that the R1 action was encoded by an instrumental R-O association (Corbit & Balleine, 2003a). These results suggest that R2 actions were influenced by the excitatory nature of Pavlovian cue presentation. In contrast, responding to the R1 action was mediated by specific changes in the value of the reward induced by incentive learning. Thus, responding to the distal action is governed by motivational influences which determine the emotional reactions associated with the consequence of performance, i.e., the reward (Corbit & Balleine, 2003a; Balleine, 2001).

The above findings indicate that R2 actions are mediated by representations governed by the current affective state of the animal. As discussed previously, the evidence from the reinforcer devaluation and the outcome specific PIT tasks suggests that GluR-1$^{-/-}$ mice are sensitive to the general motivational/arousal properties of reward. If this analysis is correct then it leads to the prediction that GluR-1$^{-/-}$ mice should be able to adjust responding to the R2 action following changes in motivational state on a heterogeneous chain schedule. However, because R1 actions are governed by changes in the current incentive value of an outcome (Balleine et al.,

2995; Corbit & Balleine, 2003a) one would predict no effect of incentive learning on the level of R1 responding in GluR-$1^{-/-}$ mice.

5.3.1 Method.

Subjects.

Experiment 9 was conducted with experimentally naïve age matched GluR-$1^{-/-}$ (n =9) and wild-type (n =10) mice. Mice were bred in the Department of Experimental Psychology at the University of Oxford and transferred to Cardiff University for behavioural testing at 6 months of age. Prior to the start of training mice were reduced to 85% of their ab-libitum weights and weighed 25-30 gm at the beginning of the experiment. All testing took place during the light phase between 9:00 am and 5:00 pm. All experiments were undertaken under the auspices of Home Office personal and project licences.

Apparatus.

Instrumental training was conducted in the same operant chambers as detailed in Experiment 7. However, the two instrumental manipulanda were changed. Each chamber was fitted with one nose-poke manipulandum, and one retractable lever. The position of the two manipulanda was counterbalanced in respect to the central magazine recess. The lever contained a blue LED located just above the lever which was switched on when the lever was activated and retracted out. The nose-poke manipulandum was the same as that described in Experiment 7.

Behavioural Training.

Stage 1: Magazine training.

Each animal was assigned, in a counterbalanced fashion, to one of six operant chambers, and thereafter was always trained in that chamber. Throughout training, the reward was a single 20 mg Noyes sucrose food pellets (Noyes precision pellets, Formula A; Research Diets, New Brunswick, NJ). Mice were trained to collect food rewards for one 40 min session, during which the reward was delivered on a RT 120 s schedule. Magazine entry during this training session was recorded.

Stage 2: Instrumental acquisition.

Following training mice received 10, 30-min instrumental training sessions, with one training session per day. The first response was designated R1 whilst the second was designated R2. The designation of response type was fully counterbalanced within each group. For the first session of training, mice were trained to respond on R2 manipulandum only; thus the R1 manipulandum was either inactive (for the nose-poke) or retracted (for the lever). In this session, reward delivery was made available on a continuous schedule of reinforcement in order to promote responding. The second instrumental training session was conduced on the on the following day. In this session 30-min session, both the R1 and R2 manipulanda were activated and the delivery of food pellets was maintained on a continuous schedule of reinforcement. However, scheduled pellets were delivered contingent on the

performance of R2, given that an R1 action had been executed once. No other constraints were placed on the R1 and R2 relation. On day 3, the reward delivery schedule was altered to a random ratio (RR) schedule of 5. The RR 5 schedule was maintained until training day 5, when the schedule was increased to 15. On day 8 the schedule was increased to 25, and for the final 2 days the RR schedule was set to 30. Such a schedule was implemented to gradually promote acquisition of the instrumental chain of actions.

On the final training session mice that failed to complete 50 responses (either nose-poke responses or lever presses), to the R1 response, were subsequently excluded from the experiment. This criterion was chosen as previous pilot data suggested that responding below 50 nose-pokes would be insensitive to any suppression changes following devaluation treatment. Due to the task demands this resulted in a total of 6 wild-type control mice and 6 GluR-$1^{-/-}$ mice that achieved criterion.

Stage 3: Heterogeneous chain test stage.

After the final training session all mice were given ad-libitum access to standard lab-chow food that evening from 5 P.M. to 9 A.M. The test stage involved two separate test sessions. Prior to commencing the first test stage, mice were weighed to guarantee that they had gained weight due to overnight food consumption. Following this, half the mice (three from each experimental group) underwent incentive learning treatment, which involved exposing the mice to the sucrose pellet reward for 2hr immediately prior to the start of the extinction test. Thus, half the mice experienced a change in motivational shift (from hunger to satiety; NON-DEP

condition); whereas the remaining half (three from each experimental group) experienced the reward while in the non-deprived state (i.e., provided an opportunity for incentive learning to take place; referred to as the INC condition). Immediately following food exposure all mice underwent an extinction test session where responding to the R1 and R2 action was measured over a 30 min period.

After completion of this first stage of testing, mice underwent a further 4 d of instrumental retraining. Each session was identical to the previous training regime although the RR schedule was maintained at 30. On completion, the mice reached similar asymptotic levels of performance compared to the final session of acquisition. In the evening prior to the second test stage, mice were once again given ad-libitum access to food from 5 P.M. to 9 A.M. However, the next day, those mice that had previously undergone incentive learning treatment, only experienced the change in motivational state. In contrast, those mice that had previously experienced the change in motivational shift, underwent the incentive learning treatment. Finally, mice underwent an extinction test session identical to the previous extinction test session.

Stage 4: Choice test.

On completion of testing mice were maintained on a food-restricted protocol. The following day, mice were given 2 h prefeeding, with either the lab chow or the food pellet. Following prefeeding, each mouse was given a choice test. Lab chow was presented in a dish (5cm × 5cm) located at one end of the home cage, whereas the sucrose food pellets were located in a dish at the adjacent end of the home cage. The amount of food consumed was determined by weighing the containers before and after each choice test.

Stage 5: Chain Acquisition.

Finally, to establish that the mice acquired the instrumental chain, all groups of mice were given a session of retraining on the instrumental chain on a RR-30 schedule. On completion, mice were given a 10-min choice extinction test in which the two response manipulanda were available, but no rewards were delivered. The probabilities of performing R1 after R2 and performing R2 after R1 were calculated as a function of time in 1-s bins after performing each of the two actions.

5.3.2 Results.

Chain Acquisition.

Figure 5.9.1 shows the acquisition of responding (in RPM) for the distal action (R1; 5.9.1a) and the proximal action (R2; 5.9.1b) across the nine sessions of chain acquisition. In general, all mice increased their level of responding to both distal and proximal actions as a function of training. The response rates were consistently higher for the proximal action. In order to confirm this impression a three-way mixed-ANOVA was conducted; with a between-subject factor of genotype (GluR-$1^{-/-}$ and wild-type control); and within-subject factors of session (1-9) and response type (R1 and R2). The analysis revealed no main effect of genotype ($F<1$), a main effect of session ($F_{(8,80)} = 7.88$, $p<.0001$), and a main effect of response type ($F_{(1,10)} = 11.696$, $p<.01$). No interactions approached significance (largest F value; response × session, $F_{(8,80)} = 1.89$, $p>0.7$).

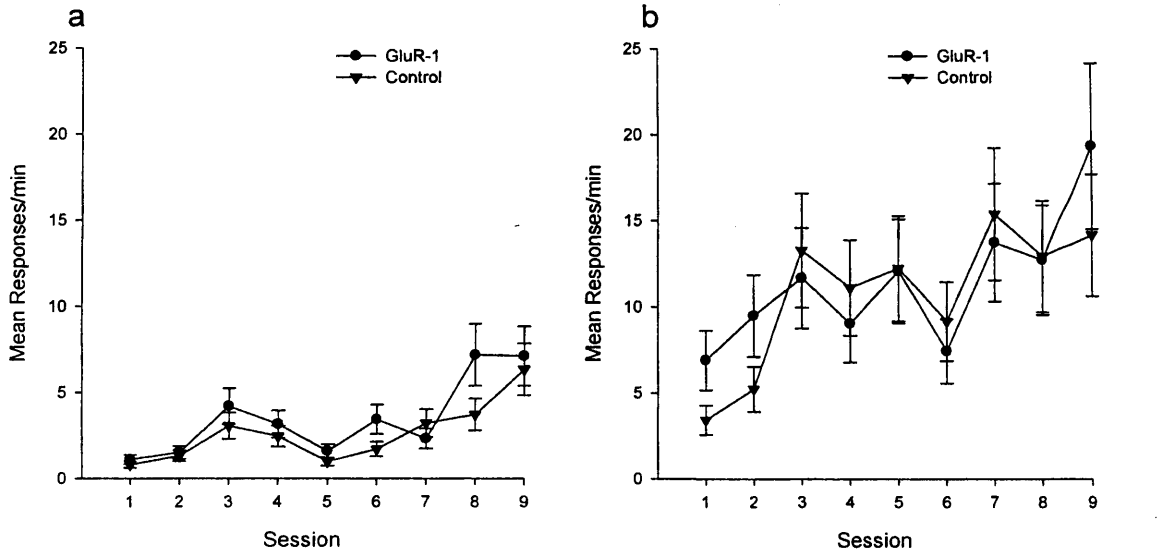

Figure 5.9.1. *Acquisition of the heterogeneous instrumental chain.* **(a).** Mean rates of responding during the training stage for the distal R1 action. **(b)** Mean rates of responding during the training stage for the proximal R2 action. Closed circles = GluR-1$^{-/-}$ mice nose-poke responding; close triangles = wild-type control mice responding. Error bars equal standard error of the mean.

Motivational shift and Incentive Learning Test.

The levels of R1 and R2 responding prior to each test session are shown in Figure 5.9.2a. In general, response rates during the final training sessions prior to test were similar for both groups of mice (Figure 5.9.2a). In order to confirm this impression a three-way mixed ANOVA was conducted, with a between-subject factor of genotype, and within subject factors of session (pre-test 1 versus pre-test 2) and response type (R1 versus R2). The analysis revealed a main effect of genotype ($F_{(1,10)}$ = 7.72, $p<0.05$), no main effect of session ($F<1$), a main effect of response type ($F_{(1,10)}$ = 14.6, $p<0.01$) and a significant genotype × response type interaction ($F_{(1,10)}$ = 8.19, $p<0.05$). No further interaction terms reached significance (all F's<1). Main

effects analysis carried out on the significant genotype × response type interaction revealed a significant difference in the rates of responding, with both groups of mice responding more to the proximal R2 action than the R1 action ($F_{(1,18)} = 15.3$, $p<0.01$). Additionally, GluR-1$^{-/-}$ mice responded more to the R2 action compared to control mice ($F_{(1,10)} = 22.3$, $p<0.01$) , with both groups of mice responding equivalently to the R1 distal action ($F<1$). Importantly, however, these results suggest that within-group response rates during each session prior to the first and second test stages were similar.

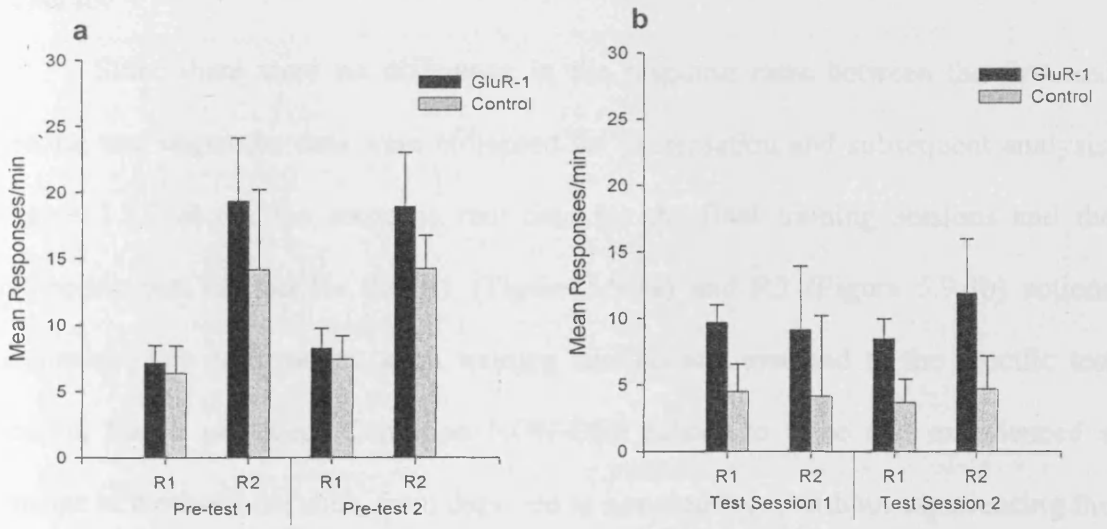

Figure 5.9.2. *Pre-test and test R1 and R2 responding.* **(a)** Comparison of response levels (in RPM) to the distal (R1) and proximal (R2) actions, during the final training sessions prior to the first test session (pre-test 1) and prior to the second test session (pre-test 2). Error bars indicate standard error of the mean. **(b)** Comparison of response levels (in RPM) to the distal (R1) and proximal (R2) actions, during the two extinction sessions. Black bars = GluR-1$^{-/-}$ mice response levels; grey shaded bars = control mice response levels. Error bars equal standard error of the mean.

Figure 5.9.2b shows the rates of R1 and R2 responding during the two separate test stages. Inspection of this figure revealed that responding did not differ as a function of test order (test session 1 versus test session 2). In order to confirm this impression a three-way mixed ANOVA was conducted with a between-subject factor of genotype and within-subject factors of test session (1^{st} versus 2^{nd}) and response type. The analysis revealed a main effect of genotype ($F_{(3,18)} = 4.51$, $p<0.05$), but no other main effects or interaction terms approached significance (all F's<1). These results suggest that response levels did not differ across the first and second test sessions.

Since there were no difference in the response rates between the first and second test stages the data were collapsed for presentation and subsequent analysis. Figure 5.9.3 shows the response rate data for the final training sessions and the extinction test session for the R1 (Figure 5.9.3a) and R2 (Figure 5.9.3b) actions separately. For each mouse, each training session was matched to the specific test session that it preceded. Condition NON-DEP relates to mice that experienced a change in motivational shift, from deprived to non-deprived, without experiencing the reward in this changed motivational state. Condition INC relates to mice that experienced a change in motivational shift, but also experienced the reward in this non-deprived state (i.e., allowing incentive learning to take place; Balleine, 2001). Inspection of the R1 response (Figure 5.9.3a) suggests that wild-type control mice following incentive learning treatment (Condition INC) displayed evidence of response suppression in the test session compared to training. In contrast, inspection of the R2 response data (Figure 5.9.3b) suggests that, in general, all mice showed

lower levels of responding to the R2 action during the test stage compared to the previous training stage.

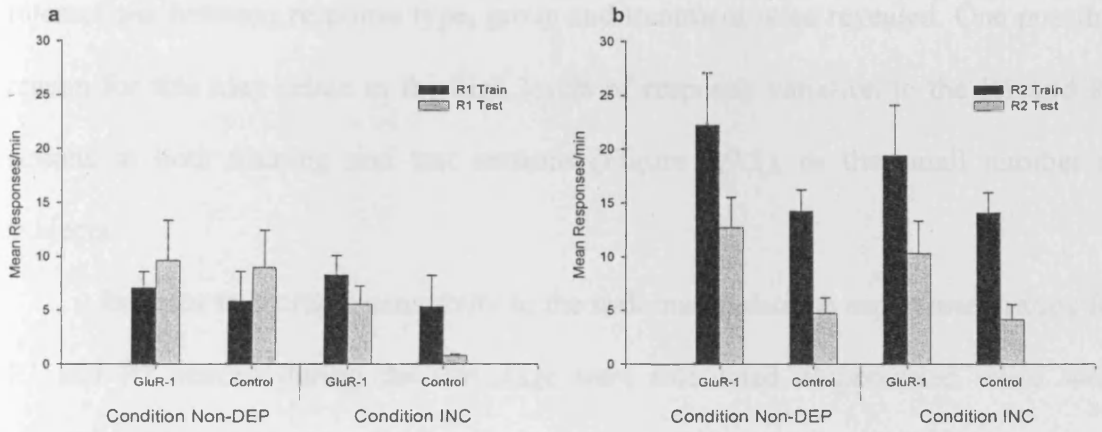

Figure 5.9.3. *Training and test R1 and R2 responses following a shift in deprivation and incentive learning treatment.* **(a)** Mean rate of responding (in RPM) on the distal R1 action during the final training and subsequent test session for all mice following a change in deprivation (Condition Non-DEP) and following incentive learning treatment (Condition INC). **(b)** Mean rate of responding (in RPM) on the proximal R2 action during the final training and subsequent test session for all mice following a change in deprivation (Condition Non-DEP) and following incentive learning treatment (Condition INC). Black bars = response rate during the final training session prior to specific test session; grey bars = response rate during the test session following the previous day training session. Error bars indicate standard error of the mean.

In order to confirm this impression a four-way mixed-ANOVA was conducted with a between-subject factor of genotype and within-subject factors of treatment (condition NON-DEP versus condition INC); response type (R1 versus R2) and phase (training versus test). The analysis revealed a main effect of genotype ($F_{(1,10)}$ = 10.37, p<.01), treatment ($F_{(1,10)}$ = 13.16, p<0.05), response type ($F_{(1,10)}$ = 4.87, p<0.05) and

phase ($F_{(1,10)}$ = 4.84, p<0.05). Additionally, a significant three-way interaction was revealed, group × response × phase ($F_{(1,10)}$ = 10.8, p<0.01). Unfortunately, no interactions between response type, group and treatment were revealed. One possible reason for this may relate to the high levels of response variation to the R1 and R2 actions in both training and test sessions (Figure 5.9.3), or the small number of subjects.

In order to increase sensitivity to the task manipulations suppression ratios for R1 and R2 actions during the test stage were calculated. Suppression ratios were calculated by dividing the mean rate of responding (in responses per minute) during the test stage by the mean rate of responding during both the test and training stage. A ratio below 0.5 indicates that responding was lower in the test session than in the training session (Figure 5.9.4). Inspection of this figure revealed that during the test stage the proximal response, R2, was responded to at a consistently lower rate compared to the training stage for mice in both the NON-DEP (Figure 5.9.4a) and INC conditions (Figure 5.9.4b). Interestingly, following the incentive learning treatment (i.e., INC condition), only wild-type control mice displayed any evidence of suppression to the distal R1 response (Figure 5.9.4b). In order to confirm this impression a three-way mixed ANOVA was conducted with factors of genotype, treatment and response type. The analysis revealed no main effect of genotype ($F_{(1,10)}$ = 1.77, p>0.2), treatment ($F_{(1,10)}$ = 1.86, p>0.2) or response type ($F_{(1,10)}$ = 3.11, p>0.1). There was, however, a significant treatment × response type interaction ($F_{(1,10)}$ = 10.31, p<0.01), and, importantly, a significant three-way group × treatment × response type interaction ($F_{(1,10)}$ = 5.99, p<0.05).

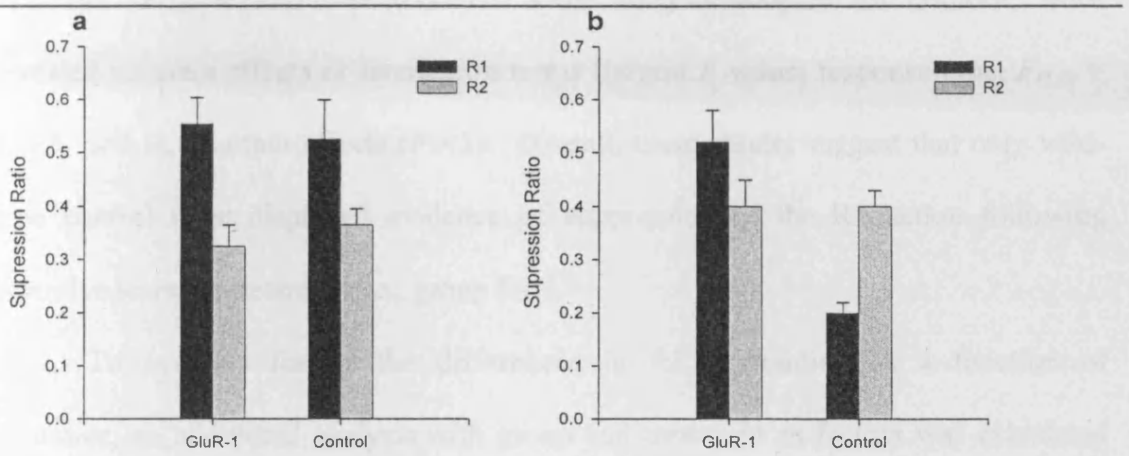

Figure 5.9.4. *Heterogeneous chain of actions test stage.* **(a)** Mean suppression ratio scores of response rates to the proximal and distal actions during the extinction test compared to the final training session for mice that experienced a change in deprivation state without experiencing the reward in the non-deprived state (Condition NON-DEP). **(b)** Mean suppression ratio scores of response rates to the proximal and distal actions in the extinction test session compared to the final training session for mice that experienced the reward in the non-deprived state (Condition INC). Black bars = responding on the distal R1 action; grey bars = responding on the proximal R2 action. Error bars indicate standard error of the mean.

To interpret the three-way interaction, separate two-way ANOVAs were conducted for each group, with within-subject factors of treatment and response type. For wild-type control mice, this analysis revealed no main effect of treatment ($F_{(1,5)}$ = 5.662, p>0.06), or response-type (F<1). However, there was an interaction between these two factors ($F_{(1,5)}$ = 11.449, p<.02). Analysis of simple main effects revealed a main effect of response type for control mice while non-deprived (condition NON-DEP; $F_{(1,5)}$ = 16.603, p<.02) and following incentive learning treatment (condition INC; $F_{(1,5)}$ = 6.663, p<.05). Additionally, R1 responding differed as a function of

treatment ($F_{(1,5)}$ = 9.811, p<.05). The same analysis adopted for GluR-1$^{-/-}$ mice revealed no main effects or interaction terms (largest F value; response-type, $F_{(1,5)}$ = 3.038, p>0.1), all other effects (Fs<1). Overall, these results suggest that only wild-type control mice displayed evidence of suppression of the R1 action following incentive learning treatment i.e., group INC.

To evaluate further the differences in R1 responding as a function of treatment, an additional analysis with group and treatment as factors was calculated for the distal action. This analysis conducted on the R1 response, revealed no main effect of genotype ($F_{(3,18)}$ = 2.16, p>0.12), a main effect of treatment ($F_{(1,18)}$ = 9.575, p<.01) and a genotype × treatment interaction ($F_{(3,18)}$ = 3.101, p<.05). Simple main effects analysis carried out on the significant interaction revealed a main effect of genotype for mice following incentive learning treatment (condition INC; $F_{(3,35)}$ = 5.066, p<.01), but not following a shift in motivational state (condition NON-DEP; F<1), with wild-type control ($F_{(1,18)}$ = 13.358, p<.01) displaying differences in R1 responding as a function of treatment. However, GluR-1$^{-/-}$ mice (F<1) failed to show any difference in R1 responding while under non-deprived (Condition NON-DEP) or incentive learning treatment (Condition INC). This result further supports a suppression of responding, following incentive learning treatment, to the R1 action for control mice only. A similar analysis adopted for the R2 responses revealed no main effect of genotype (F<1), no main effect of treatment ($F_{(1,18)}$ = 1.35, p>0.26) and no interaction between the two factors (F<1). These result suggest that R2 responding was similar for all groups irrespective of treatment.

In summary, all mice displayed evidence of response suppression to the R2 action in conditions NON-DEP and INC. That is, R2 responding was similar independent of treatment regime. However, only the wild-type control group,

following the incentive learning treatment (condition INC) displayed evidence of a reduction in performance to the distal R1.

Finally, to confirm that the mice acquired the instrumental chain, the relationship between the two actions was assessed in the 10 min choice extinction test. This analysis was based on that previously reported by Balleine and colleagues (Balleine et al., 1995; Corbit & Balleine, 2003a). Figure 5.9.5 displays the probability of performing the proximal action (R2) after the distal action (R1) i.e., the training contingency [p(R2 after R1)] and the probability of performing the distal action after the proximal action [p(R1 after R2)], as a function of time in 1 s bins after performing each of the two responses.

Inspection of Figure 5.9.5 revealed that there was a high probability of performing R2 after R1 [p(R2 after R1)]. However, the performance of R1 seems not to have relied upon the previous performance of R2 [p(R1 after R2)]. In order to confirm this impression a three-way mixed ANOVA was conducted on the data with between-subject factors of genotype and within-subject factors of probability [p(R2 after R1 versus p(R1 after R2)] and time bin (1-10). Analysis of the data revealed no main effect of genotype ($F_{(1,10)} = 1.21$, $p>0.3$); a main effect of probability ($F_{(1,10)} = 247.24$, $p<0.0001$) a main effect of time bin ($F_{(9,90)} = 36.67$, $p<0.0001$) and a significant three-way interaction between these factors ($F_{(9,90)} = 2.68$, $p<0.01$).

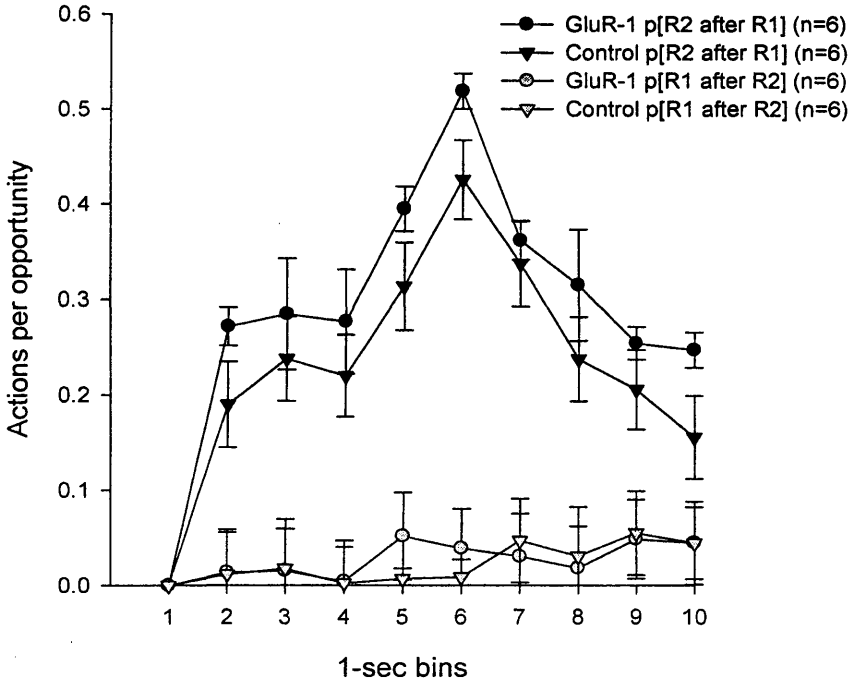

Figure 5.9.5. *Chain acquisition test.* The probability of performing one response in the heterogeneous chain as a function of time since performing the other response. This measure is calculated as actions per opportunity for both components of the chain. p(R2 after R1) relates to the probability of performing R2 after the performance of the R1 response. p(R1 after R2) relates to the probability of performing one R1 response after the performance of the R2 response. Error bars indicate standard error of the mean.

In order to evaluate the nature of the interaction, separate two-way within-subject ANOVAs were conducted for control and GluR-1$^{-/-}$ mice with factors of probability [p(R2 after R1 versus p(R1 after R2)] and time bin. For control mice this revealed a main effect of probability ($F_{(1,5)}$ = 178.887, p<.0001); and time bin ($F_{(9,45)}$ = 15.626, p<.001) and a significant interaction between the two factors ($F_{(9,45)}$ = 15.653, p<.0001). Main effect analysis revealed a main effect of response probability at bins 2 through to 10 (smallest F value; bin 1, $F_{(1,5)}$ =13.423, p<.01). The same

analysis carried out on GluR-1$^{-/-}$ mice revealed a main effect of probability ($F_{(1,5)}$ = 99.36, p<.0002) and time bin ($F_{(9,45)}$ = 23.087, p<.0001) and a significant interaction between the two factors ($F_{(9,45)}$ = 22.085, p<.0001). Simple main effects analysis revealed a main effect of probability for bins 2 through to 10 (smallest F value; bin 2, $F_{(1,5)}$ = 23.105, p<.001). Thus, for mice in each group, the probability of responding on the R2 action was directly related to previous response on the R1 action. In contrast, this probability relationship was not seen for R1 actions following a previous R2 response. Therefore, all mice learned the R1-R2 contingency in order to receive reward.

Choice Test.

To establish that failure of GluR-1$^{-/-}$ mice to attenuate R1 responding following satiety could not be attributable to a failure to discriminate between the reward used in the experimental setting and the standard lab chow (used to change deprivation state in the mice), a choice test was conducted following satiety of either the reward pellet or lab chow (Figure 5.9.6). In general, both groups of mice showed a preference for the non-devalued food compared to the sated devalued food type in the 30-min consumption test. In order to confirm this impression, a two-way ANOVA was conducted with a between-subjects factor of genotype and a within-subjects factor of food type (devalued vs. non-devalued). The analysis revealed a main effect of food type ($F_{(1,10)}$ = 7.78, p<0.05), no other effects or interactions were revealed (all F's<1).

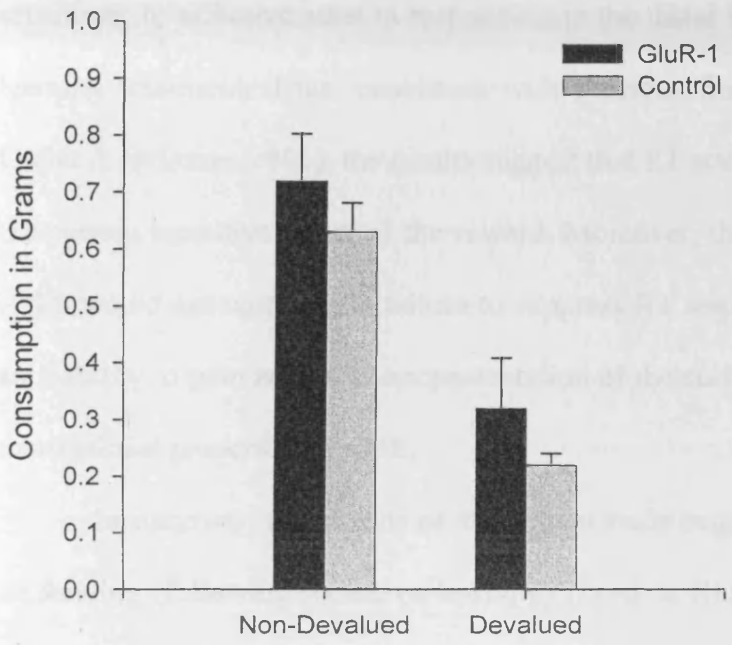

Figure 5.2.7. *Food preference choice test.* Consumption in grams during 30-min choice test where mice had access to either a previously devalued or non-devalued food type. Black bars = consumption of devalued food; grey bars = consumption of non-devalued food. Error bars indicate standard error of the mean.

5.4.3 Discussion.

Experiment 9 examined responding on a heterogeneous chain of actions following a change in motivational shift (i.e., from deprived to non-deprived; condition NON-DEP) or incentive learning treatment (i.e., consummatory contact with the outcome whilst in the non-deprived state; condition INC). These results revealed several interesting findings. Firstly, all mice displayed an equivalent drop in instrumental responding following a shift in motivational state. This is consistent with previous research showing that the proximal R2 action is directly sensitive to the

affective state of the animal. Secondly, only wild-type control mice displayed sensitivity to affective state in responding to the distal R1 action following incentive learning treatment. Thus, consistent with previous findings (Balleine et al., 1995; Corbit & Balleine, 2003), the results suggest that R1 actions are under the influence of the current incentive value of the reward. Moreover, the results from Experiments 7 and 8 would account for the failure to suppress R1 responding (in GluR-$1^{-/-}$ mice) as an inability to gain access to a representation of the current sensory-specific incentive motivational properties of a US.

In summary, the results of the present study suggest that the impairment in R1 responding (following incentive learning) noted in GluR-$1^{-/-}$ mice was mediated by the inability of these mice to reduce performance to an action associated with a reduction in value of a specific reward. In addition, these results also suggest that responses on instrumental chain of actions in mice are mediated by different representational systems; the proximal R2 action being mediated by changes in general motivational state; whereas R1 actions may be mediated by (outcome-specific) incentive value.

It is important to note that there remains some debate regarding the precise nature of the representations governing instrumental actions in a heterogeneous chain schedule. It has been suggested that the R1 response is governed by instrumental R-O associations, while the R2 response is governed by Pavlovian S-O associations (Balleine, 2001) or S-R associations susceptible to Pavlovian influences (Balleine et al., 1995). That R1 is mediated by R-O associations is supported by the previous experiments (Experiment 7) and previous studies (Balleine et al., 1995; Corbit & Balleine, 2003) by the evidence that this action is directly sensitive to an incentive learning treatment. However, one cannot fully rule out a more specific Pavlovian

influence associated with the manipulandum. Furthermore, the view that R2 responses are governed by Pavlovian S-O associations is supported by evidence that single-outcome PIT effects can be revealed and are specific to the R2 action; although the same manipulation has no effect on the distal R1 action (Corbit & Balleine, 2003). This selective elevation in performance was observed only in the presence of the stimulus paired with the same outcome as that used in the instrumental chain, providing evidence against an S-R account (Balleine et al., 1995). It would be expected that any appetitive stimulus would elevate S-R mediated responding. However, stimuli paired with different outcomes from those used in the chain failed to augment R2 responding (Corbit & Balleine, 2003).

Nevertheless, this view does not make clear why different responses in a heterogeneous chain should be differentially sensitive to incentive learning treatment. It has been suggested that the relative temporal relation between the links of a behavioural chain, rather than the chain contingency itself, critically determines the motivational control of performance (e.g., Balleine et al., 1995). According to this analysis, the distal (R1) action acquires discriminative control (Colwill & Rescorla, 1990a) over the action possibly acting as an occasion setter for the proximal (R2) action (Holland, 1986). Thus, discriminative control augments responding via a hierarchical S-(R-O) associative structure (Colwill & Rescorla, 1988, 1990b). If it is anticipated that the R1 action acts as the S^D (Balleine et al., 1995), the analogous relationship to that proposed by Colwill and Rescorla would yield a R1-(R2-O) relationship. In this respect only the proximal, and not the distal action, enters into a direct relation with the outcome. Thus such responses (i.e., R2) would be directly sensitive to the immediate effects of current motivational state (Balleine et al., 1995; Corbit & Balleine, 2003).

Balleine (2001) has proposed an alternative explanation; arguing that the components of the heterogeneous chain are associated with different sensory features of an outcome. Thus, the proximal (R2) action is associated with the most salient features of the outcome that are directly related to the motivational structures that mediate the biological significance of the outcome. According to this account, the R2 response overshadows the distal action for association with this feature; and as a consequence the distal action is associated with the more diffuse less salient features which are not themselves related to the nutritive system. The nutritive systems is modulated by hunger; hence explaining why a shift in motivational state directly affects R2 responding. Incentive learning in this context occurs via a feed-forward projection between the R1 action and emotional feedback generated once consummatory contact in the non-deprived state has taken place (Balleine, 2001; Corbit & Balleine, 2003).

To summarise, responding on a heterogeneous chain of actions is an interesting phenomenon which allows one to investigate the motivational control of instrumental action. Regardless of the merits denoted by the above perspectives, it is clear that actions proximal to outcome delivery seem to be represented by a different representation system (Pavlovian influences) to that supporting distal actions (incentive influences).

Chapter 6.

6.1 General Discussion.

6.1.1 *Summary of findings.*

The experiments in chapter 2 were designed to assess sensorimotor and affective processes in GluR-1$^{-/-}$ mice. Specifically, Experiment 1 examined whether mutant mice suffered from any non-specific performance deficits that may have influenced learning. Consistent with previous findings (Vekovischeva et al., 2001; Bannerman et al., 2004) GluR-1$^{-/-}$ mice displayed a hyperactive phenotype but remained sensitive to a visual cue. However, in comparison to controls, mutant mice showed a faster within-session and between-session decrement in the magnitude of the orienting response evoked by a visual target. It was suggested, therefore, that the mechanisms underlying habituation were disrupted in GluR-1$^{-/-}$ mice. Additionally, the hypothesis of spatial working memory deficits was supported as an enduring impairment in a NMTP t-maze task in Experiment 2.

The remaining experiments in chapter 2 showed that GluR-1$^{-/-}$ mice displayed a behavioural phenotype similar to that reported in BLA-lesioned rats. That is, mutant mice showed less anxious behaviour, as evidenced by a lack of preference for either the enclosed or exposed arms during anxiety plus maze testing (Experiment 3), and an attenuation of the neophobic reaction to a novel food (Experiment 4). These findings supported the hypothesis proposed by Mead and Stephens (2003a) that at least one component of the behavioural phenotype in GluR-1 mutant mice reflects impaired BLA function.

Chapter 3 examined whether the parallels between the BLA lesion and GluR-1 behavioural phenotype extended to fear conditioning. Interestingly, GluR-1$^{-/-}$ mice

displayed impairments in conditioned freezing to both contextual and cued (auditory and visual) stimuli (Experiment 5). This finding was consistent with the view that GluR-1 mutant mice were unable to form or access a representation of the affective or motivational properties of the footshock US (Everitt et al., 2003). However, these findings did not inform the precise nature of the US representation impaired in GluR-$1^{-/-}$ mice. More specifically, a US can form representations (with a CS) in terms of both general affective and its sensory-specific motivational properties (Figure 3.3.1; Brandon & Wagner, 1989). An implication of the BLA hypothesis of the GluR-1 phenotype was that the latter of these two representations should be sensitive to the mutation; this was subsequently assessed in chapter 4.

The experiments in chapter 4 examined the capability of GluR-$1^{-/-}$ mice to use a Pavlovian or instrumental signal to gain access to a sensory-specific representation of a primary reward (US) to modulate responding following devaluation of a specific US. In the plus-maze devaluation task (Experiment 6), GluR-$1^{-/-}$ mice displayed progressively shorter latencies to traverse the runway during training, indicating that these mice were sensitive to the reinforcing properties of reward. However, once sensory-specific satiety was induced, only control mice showed an attenuation of responding to the devalued goal-box. These results suggested that mutant mice were unable to retrieve the current incentive value of the primary reward in order to update the place-reward association and thus attenuate responding accordingly. Experiment 7 further assessed this impairment in an operant setting.

In this experiment mice acquired a biconditional discrimination in which two discriminative stimuli (S^D) signalled different response-outcome contingencies. In a specific satiety test, control, but not GluR-$1^{-/-}$, mice showed a lower rate of responding to the nose-poke manipulanda in the presence of the S^D which had

previously signalled the devalued outcome. This phenotype is consistent with that seen in rats with BLA lesions (Balleine et al., 2003) and suggests that GluR-1$^{-/-}$ mice were insensitive to the specific properties of the outcomes. Experiment 8 further evaluated outcome encoding in mutant mice using an outcome-specific PIT paradigm, where two reinforcers were associated with two different stimuli and two different actions. In the test phase of this experiment, mutant mice were unable to differentially respond in the presence of cues which had previously signalled different reward types. This suggests that they were unable to guide their behaviour using the unique properties of each reward. They were, however, sensitive to the general arousing properties of Pavlovian cues as evidenced by an overall increase in instrumental performance during cue presentation (Experiment 8).

Collectively, the results suggest GluR-1$^{-/-}$ mice are unable to retrieve information regarding the current incentive value of an associatively activated sensory-specific US representation. However, these mice are clearly able to use aspects of affective and motivational features of reward value in order to mediate responding. The notion that GluR-1$^{-/-}$ mice show normal US-affective associations led to the prediction that mutant mice should be able to adjust instrumental responding which is governed by changes in the general affective state of the animal without the need for consummatory contact with the reward (i.e., incentive learning). Experiment 9 sought to examine this possibility using responses which are understood to be mediated by differential representational structures according to their proximity to outcome delivery i.e., R1⇨R2⇨O1. (Balleine, 1992; Balleine et al., 1995; Corbit & Balleine, 2003a). The results revealed an equivalent drop in instrumental responding on the proximal R2 action for all groups of mice following a shift in motivational state. This is consistent with the idea that the R2 action is directly sensitive to the

affective state of the animal. In contrast, only wild-type control mice displayed sensitivity in responding to the distal R1 action following incentive learning treatment. Moreover, this impairment may have reflected an inability for GluR-1$^{-/-}$ mice to retrieve US-sensory representations and was supported by evidence of an impaired outcome-specific devaluation effect in the KO animals (Experiment 7).

Collectively, the findings from the outcome-specific devaluation and PIT, and from the heterogeneous chain experiments suggest that the underlying impairment in motivational processing in GluR-1 mutant mice results from an impairment in associatively accessing a representation of the sensory-specific incentive motivational properties of a US. This phenotype has been suggested to underlie disturbances in reward processing for BLA-lesioned rats (Blundell et al., 2001; Blundell & Killcross, 2002; Balleine et al., 2003). The discussion below focuses on an evaluation of this hypothesis in respect to GluR-1$^{-/-}$ mice.

6.1.2 *Psychological and Theoretical Implications.*

It has previously been reported that GluR-1$^{-/-}$ mice show deficits in second-order operant conditioning and conditioned reinforcement (Mead & Stephens, 2003a). Stephens and colleagues interpreted the failure in these paradigms as "attributable to the *gria1* (GluR-1) KO mice not having attributed affective properties to the cue" (pp.1046). In general, the results from the experiments in this thesis are in agreement with such a disturbance in affective/motivational processing. However, it should be noted that the hypothesis postulated by Mead and Stephens cannot explain why mutant mice are unable to use the specific feature of an outcome in order to mediate performance (impaired outcome-specific PIT and devaluation), whilst at the same

time being sensitive to the reinforcing impact of reward delivery (e.g., acquisition of biconditional discrimination). Therefore, consistent with contemporary research (e.g., Blundell et al., 2001; Killcross & Blundell, 2002; Balleine et al., 2003) the results gathered from this thesis suggest a more specific interpretation of the impairment. That is, in the outcome-specific devaluation and PIT tasks, mutant mice were required to use the differential representation of outcomes in order to mediate performance on the basis of changes in incentive value of one of the outcomes (reinforcer devaluation), or on presentation of discrete cues which had previously signalled the delivery of each specific outcome (outcome-specific PIT). These results suggest that the underlying disturbance in motivational processing reflects an inability of KO mice to use a signal to gain access to the current sensory-specific value of a US. This suggested impairment is depicted pictorially in Figure 6.1.1.

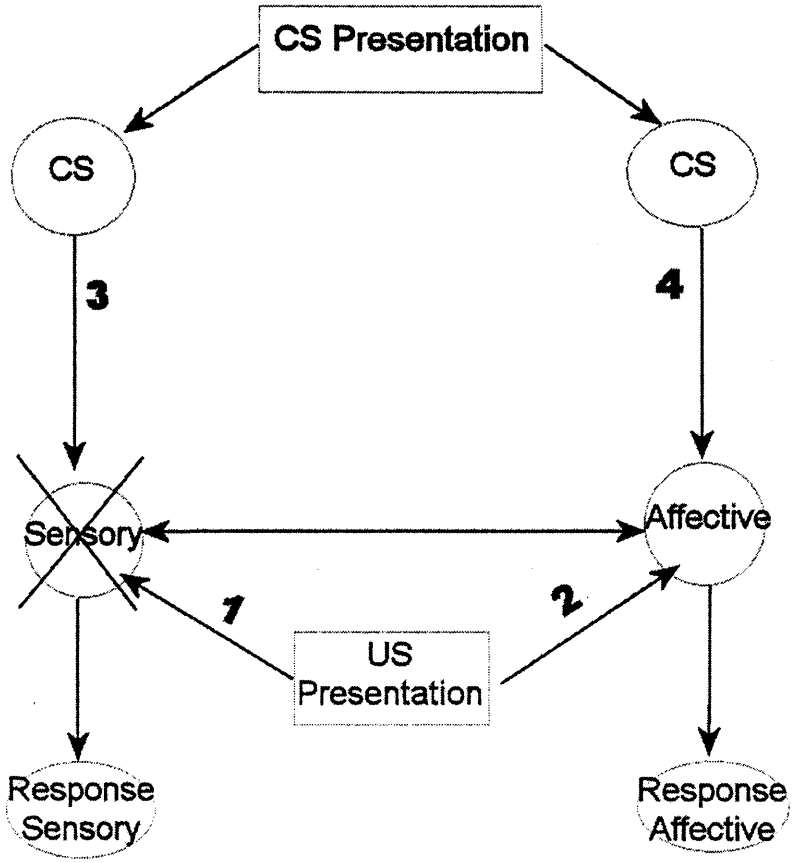

Figure 6.1.1. *An illustrative example of the suggested nature of the associatively-activated disturbance in stimulus processing for GluR-1$^{-/-}$ mice.* The affective system (mediating links 2 & 4) relates to motivational states induced by the US. These can be either positive for rewarding events, or negative for punishing events. Positive states would be expected to increase preparatory activity and promote responding whilst negative states would be expected to inhibit responding. The sensory system (normally mediating links 1 & 3) relates to the specific properties of the US. This representation carries information regarding the specific features of a reward (e.g., taste, size & nutritive value) and together with the affective system allows changes in value of the US to mediate performance. It is the sensory system (as indexed by the cross) which is not capable of supporting sensory-specific associatively activated event representations in GluR-1$^{-/-}$ mice.

This analysis focuses on discussion centred on ideas derived from Konorski (1967) and the affective extension of standard operating procedures (AESOP; Wagner & Brandon, 1981). Animals can distinguish between the representation of motivational and sensory attributes of governing US representations. Thus, in respect to the Figure 6.1.1 the affective system relates to motivational states induced by the US, and thus reflects a sensitivity of mutant mice to the affective properties of US presentation (links 2 & 4). However, it is suggested that mutant mice are unable to mediate performance based on associatively activated sensory-specific US value (links 1 & 3). This is, in contrast to an impairment in forming sensory representations *per se*, since GluR-1$^{-/-}$ mice were clearly able to discriminate between the rewards (Experiments 7 & 9) which suggests that they were sensitive to the sensory properties of neutral stimuli (Blundell et al., 2003).

This analysis depicted in Figure 6.1.1 can account for the results of majority of the experimental findings contained in this thesis. For instance, in respect to the instrumental discrimination (Experiment 7), during presentation of the S^D's, mice were reinforced when making a correct response during a specific stimulus. Each time this process occurred, one would expect that in GluR-1$^{-/-}$ mice each specific stimulus becomes associated with a specific response mediated by the affective system (links 2 & 4). However, during devaluation (via sensory-specific satiety) the incentive value of the outcome changes, from a highly positive (due to previous consummatory contact in the deprived state), to a negative state (due to satiation with reward). In test, the presentation of the S^D associated with the devalued reward would normally evoke the affective (link 4) and sensory (link 3) systems to consequently inhibit responding to the now devalued cue. However, if the sensory system is disturbed, the mouse is

unable to use the CS as a signal to retrieve information regarding the specific features of outcome, therefore an attenuation of devaluation would be expected.

Similarly, in the PIT task the positive affective value of the CSs was acquired due to the pairing of each cue with reward (via S-O associations). Thus, during the test stage stimulus presentation augmented responding (via the affective system) causing an increase in preparatory activity and to changes in strength, but not direction, of Pavlovian modulation of instrumental performance (Dickinson & Dearing, 1978). However, in respect to reinforcer-selective PIT, each CS presentation (in the test stage) normally results in a representation forming between the CS and the sensory-specific value of the reward US (link 3). Thus, CS presentation would evoke a state of arousal (mediated by the affective system) which would be associated with the specific outcome (mediated by the sensory system), and consequently augment performance on the direction of Pavlovian modulation of instrumental responding (Dickinson & Dearing, 1978). A failure of the sensory system to mediate performance based on the association of a representation of the specific features of the US would be expected to prevent any differential levels of responding in the presence of cues associated with different rewards.

In respect to the instrumental chain of actions it would be suggested that each response becomes differentially mediated by the affective and sensory systems. That is, in control animals responding on the R1 action was only attenuated when preceded by incentive learning. This suggests that the R1 action is associated with a specific representation of the outcome as a goal, and that the R1 response is sensitive to the R-O contingency (Balleine et al., 1995; Balleine, 2001; Corbit & Balleine, 2003a). Therefore, it could be suggested that the impoverished representation of the outcome (due to the disrupted sensory-system) would impair the ability of mutant mice to form

a representation of the outcome as a goal. As such, GluR-1$^{-/-}$ mice would be unable to modify their instrumental performance in any selective manner, when required to recognise a change in the value between the performance of an action (R1) and the delivery of a specific reward. In respect to the proximal response, one would suggest that the sensitivity of the R2 action to indirect changes in motivational shift would be mediated by the affective system in mutant mice. As such a change in deprivation state would be expected to attenuate responding on the proximal action.

Unfortunately this account has difficultly in interpreting the impairments noted in fear conditioning. That is, following pairing with the shock, one would expect the CS presentation to evoke a representation of the aversive negative features of the US and hence induce freezing behaviour to the CS. In contrast to this prediction, mutant mice showed little evidence of freezing to either contextual or discrete cues which had previously been paired with shock. However, it has been argued that the freezing response elicited by the cue is thought to represent a US-specific conditioned response (Everitt et al., 2003). Therefore, in reference to Figure 6.1.1, it is possible that mutant mice lacked the ability to freeze (i.e., a performance deficit). Alternatively, one could suggest that the specific UR to shock reflects an association with the sensory specific components of the US (Konorski, 1967). Therefore any failure to freeze, in mutant mice, could be mediated by a failure to attribute the specific features of the US (with the aversive affective features), and therefore this impoverished representation could potentially fail to develop into associations with the CS.

6.1.3 *Future Directions: An analysis of mediated acquisition effects.*

The previous discussion has highlighted that mediated performance (i.e., reinforcer devaluation) is attenuated in mutant mice by an inability to use a signal (e.g., CS) to gain access to the associatively activated sensory-specific US representation. It would be of interest, therefore, to examine whether this connection is reciprocal. That is, if one was to devalue the CS (by associating it with an aversive consequence; e.g., LiCl), would any CS-mediated learning occur to reduce consumption or performance toward a previously associated US. Interestingly, Holland (1981; 1990) noted that a CS activated representation can mediate new learning about the US, even in the absence of that US. For instance, Holland (1998) initially paired an auditory CS with a food pellet US. Then the animals received CS-toxin (LiCl) pairings. The results suggested that CS-LiCl did not mediate CR during CS presentation. However, if the animals were given subsequent consumption tests with the US (in the absence of the CS), then those animals previously trained on the CS-LiCl contingency consumed fewer food pellets then control rats who had received unpaired presentations of the CS and toxin. Holland (1998) termed this phenomenon 'mediated acquisition', indicating that a CS-activated representation of food substituted for the food itself in the acquisition of the aversion. However, this effect was only evident during limited CS-US exposure (16 pairings); the effect failed to develop following extensive CS-US exposure (28 or 48 pairings).

Holland suggested two explanations of this effect, each of which would assume a different prediction if GluR-1$^{-/-}$ mice are used. Thus, the effects seen by Holland may be explained in terms of popular learning theory proposed by Pearce and Hall (1980). Within this theory, the associability of a CS declines as it becomes a

better predictor of its consequences, thus the CS-activated US representation was mediated by the associability of the CS during the limited CS-US exposure. Since changes in CS associability are mediated by the central nucleus of the amygdala (Holland & Gallagher, 1999) one would predict that mutant animals would acquire this CS-mediated devaluation. An alternative view posited by Holland was that mediated learning effects may be due to the CS's activation of perceptual processes normally evoked by the US. That is, as mentioned above, the CS forms direct associations with the sensory representation of the US (Wagner & Brandon, 1989). Since GluR-1$^{-/-}$ mice are unable to make use of a signal (in this case the CS) to gain access to the associatively activated US representations, one would predict, under this perspective an impairment in mutant mice. Moreover, if an impairment were noted, the finding would suggest not only are GluR-1$^{-/-}$ mice unable to use a signal (e.g., CS) to gain access to the updated incentive value of a US to mediate performance (e.g., following devaluation), but also this same disrupted mechanism prevents the establishment of an aversion (induced by association with the CS) updating the sensory-specific value of the US (i.e., mediated acquisition).

Finally, as an assessment in examining whether mediated acquisition and performance overlap, one could examine a procedure developed by Holland (1990a). Thirsty rats were trained to receive pairings of CS1 and CS2 with differentially-flavoured sucrose solutions. One solution was then paired with LiCl. Finally, CS1 and CS2 were presented when rats were consuming unflavoured sucrose. Presentation of the CS associated with the devalued flavour evoked consummatory response of rejection normally seen when rats are forced to consume unpleasantly-flavoured substances (Grill & Holland, 1978; Holland, 1990a). These results suggest that under some circumstances, animals apparently tasted the flavour US in the presence of a

signal for that flavour, but in the absence of that flavour itself. Assuming the orofacial rejection response is US-specific (Konorski, 1967), one would not expect such a response to be evoked in mutant mice using Holland's procedure. That is, the rejection response is expected to be associated with the specific feature of the US (e.g., its taste). Therefore on the basis of the analysis presented above, the presentation of a CS would be ineffective in retrieving this representation in GluR-$1^{-/-}$ mice.

6.1.4 *Neural Implications.*

(1) Evidence of Amygdala Involvement.

The behavioural data from the previous experiments are broadly consistent with a proposal of basolateral amygdala nuclei dysfunction following genetic manipulations of the GluR-1 AMPA receptor subunit. This conclusion is based on the variety of tasks which have differed in terms of their procedural requirements. Initially amygdala dysfunction was suggested by the tasks which were designed to assess disturbances in motivational and affective behaviour (Chapter 2). For instance, GluR-$1^{-/-}$ mice showed a reduction in neophobic reaction usually present on exposure to a novel food source (Experiment 4). As previously alluded to, the most commonly espoused behavioural phenotype following BLA-dysfunction is a reduction in levels of expressed fear (LeDoux, 2000). Entirely consistent with this proposal, GluR-$1^{-/-}$ mice showed a reduction in expressed fear to both contextual and cued CS's which had previously been paired with an aversive shock (Experiment 5). Postulating however, that GluR-$1^{-/-}$ mice are simply less fearful then their control counterparts is

somewhat limiting when considering the impairments noted in outcome-specific devaluation (Experiment 6,7 & 10) and PIT (Experiment 8). Consistent with findings in this thesis, BLA-lesioned rats show impairments in outcome-specific devaluation and PIT due to an inability to form associatively activated representations of a sensory-specific nature in order to adjust behaviour accordingly (Blundell et al., 2001; Killcross & Blundell, 2002; Balleine et al., 2003; Corbit & Balleine, 2005). Adopting a similar perspective for GluR-1$^{-/-}$ mice would explain the deficit in fear conditioning as an inability of mutant mice to process the sensory-specific incentive value of an aversive US.

Interestingly, the fear conditioning circuitry had been well defined especially for the auditory modality (LeDoux, 2000). However, similar principles appear to hold for visual and multimodal conditioning (Shi & Davis, 2001). The BLA receives efferent information from a range of cortical and subcortical structures, although it is the auditory cortex and the external capsule, which have been implicated in the auditory fear conditioning circuitry (Sah et al., 2003). Principle and local circuit neurons of the BLA (specifically the LA) receive a symmetrical afferent input of these two pathways (Szinyei et al., 2000) as well as nociperceptive information from the brain stem (Romanski et al., 1993). Afferent information from the basal forebrain and hippocampus also converge on the LA (Stork & Pape, 2002). Therefore, the BLA seems well situated to support an integratory role of CS-US information during Pavlovian fear conditioning. Thus, consistent with that reported (Experiment 5), impairments in the synaptic processing in this region (Mead & Stephens, 2003a) would be expected to impair Pavlovian fear conditioning in GluR-1$^{-/-}$ mice.

At a cellular level, the BLA contains two classes of neurons: (1) GABAergic GluR-1 containing non-pyramidal interneurons and; (2) pyramidal projections

neurons containing low-levels of GluR-1 (McDonald, 1996; Mahanty & Sah, 1998). Following deletion of the GluR-1 subunit, it has been observed that compensatory mechanisms result in the upregulation of GluR-2/3 subunits in the BLA (Mead & Stephens, 2003a). Thus, following GluR1 deletion BLA neurons might manifest a disruption in firing patterns of pyramidal projection neurons, to which they normally provide an inhibitory control (Rainnie et al.,1991; Mead & Stephens, 2003a). Such a disturbance could produce an increase in GABA function of projection neurons. Consistent with this position, the elicitation of conditioned fear in mice is associated with a reduction of extracelluar GABA levels in the amygdala (Stork et al.,. 2002). Similarly, heterozygous null mutant mice for the γ2-subunit of the $GABA_A$ receptor show a reduction in synaptic clustering of $GABA_A$ receptors and deficits in GABA-mediated transmission. Interestingly, these same mutants display an enhanced conditioning to anxiety related cues as assessed in a variety of tasks including anxiety plus maze and fear conditioning (Crestani et al., 1999). Collectively, these results implicate decreases in GABA release following Pavlovian fear conditioning (Wilensky et al., 1999).

That deletion of the GluR-1 subunit results in aberrant synaptic processing in the BLA is supported by the recent finding that mutant mice show impairments in LTP in this region. Reisel and colleagues (personal communication, from Daniel Reisel to Alex Johnson, Oslo University, Centre for Molecular Biology and Neuroscience, February 2004) assessed cortical and thalamic in-vitro LTP in GluR-$1^{-/-}$ mice. Both forms of cellular LTP were impaired in comparison to the matched control group. That auditory evoked potentials in the LA have been noted in-vivo following fear conditioning and in-vitro following LTP induction suggests that LTP-like processes in this region contribute to the acquisition of conditioned fear (Chapman et

al., 1990; Quirk et al., 1995; 1997; Rogan et al.., 1997a; McKernan & Shinnick-Gallagher, 1997). Finally, Malinow and colleagues (Rumpel et al., 2005) used sRNA to block synaptic GluR-1 incorporation in a quarter of the neurons in the LA. The viral vector caused impairments in synaptic transmission in infected neurons and an impairment in auditory fear conditioning in treated rats. In consideration of the behavioural and cellular data, collectively these findings are highly suggestive of behavioural phenotype of BLA dysfunction induced by deletion of the GluR-1 subunit.

(2) Evidence of Hippocampal Involvement.

Although the present program of work has provided support for BLA-dysfunction hypothesis in GluR-1 mutant mice, it must be conceded that facets of the experimental data are also consistent with a hippocampal impairment. For instance, the initial task reported in this thesis examined sensorimotor functioning to determine whether any performance deficits could detrimentally mediate other affective and cognitive behaviours. My results indicated that GluR-$1^{-/-}$ mice were sensitive to a visual cue (as indexed by OR evoked by the cue), although mutant mice showed faster between session and within session decrement in the magnitude of OR elicited by the cue. These results were explained in terms of Wagner's SOP model (1981). Interestingly, this framework has been discussed in reference to hippocampal-lesioned rats (Honey & Good, 2000a,b). In the Honey and Good (2000a) study rats were given three days training on which one auditory prime (e.g., click train) preceded the presentation of a visual cue (e.g., constant light), whilst another auditory prime (e.g., tone) preceded the presentation of a second visual cue (e.g., pulsed light). Following

training, during the test trial (day 4) one of the auditory primers preceded the presentation of both auditory cues. It was expected that presentation of the auditory primer would result in a proportion of the associated target visual cue elements to manifest in the A2 state. As such, if an unannounced presentation of the alternate visual cue occurs, one would expect these elements to manifest in the A1 state; accordingly a primed target should evoke less responding than an unprimed target (Wagner, 1981; Honey & Good, 2000a,b). However, in the hippocampal-lesioned group, priming resulted in a preference for the primed target i.e., the opposite trend to that observed in control rats. It was suggested that in the lesioned group the visual target lacked intensity, as such a target would only provoke a small number of elements in the A1 state. Thus the proportion of elements that are activated on a primed target trial could exceed the proportion activated when the target is unannounced (Doneghan, 1981; for review, see, Honey & Good, 2000b). Honey and Good suggested that the visual target could lack intensity via; (1) a disruption in the movement of elements from the inactive state to the A2 state; (2) a more rapid decay from the A1 state to the A2 state. The fact that a theoretical perspective can be used to explain behaviour in both GluR-1$^{-/-}$ mice (Experiment 1) and hippocampal-lesioned rats, suggests to some degree that similar associative structures may be governing behaviour in both groups of animals.

Consistent with hippocampal impairment GluR-1$^{-/-}$ mice also displayed a profound and enduring impairment in spatial working memory (Experiment 2) as assessed in the t-maze NMTP procedure. This result was consistent with that previously reported by Bannerman and colleagues (e.g., Reisel et al., 2002) and is consistent with the view that mutant mice displayed a phenotype mimicking that seen in hippocampal-lesioned rats (e.g., Aggleton et al., 1986). Furthermore, it is also

possible that the deficits in conditioned freezing to the experimental context for GluR-$1^{-/-}$ mice (Experiment 6) could reflect a dysfunction in hippocampal processes (Kim & Fanselow, 1992; Philips & LeDoux, 1992). Thus, in line with the suggestion proposed by Bannerman and colleagues one could postulate that the hippocampal memory system could be involved in the encoding of specific events or episodes (Eichenbaum & Fortin, 2003). This could play a role in the acquisition of rapid contextual information associated with the aversive US. As such, aberrant hippocampal processes evident in GluR-$1^{-/-}$ mice (Zamanillo et al., 1999) could be sufficient to induce an impairment in contextual fear. It should be noted, however, that this proposal cannot explain the deficits seen in conditioned fear to both the auditory and visual CS's following conditioning.

Evidence of hippocampal involvement also becomes more tentative when one considers the other experimental data reported in this thesis. Tasks which are dependent on outcome-specific sensory US representations have revealed no impairments following hippocampal lesions in rats (e.g., reinforcer devaluation; Corbit & Balleine, 2000; Corbit et al., 2002). Furthermore, it should be noted that hippocampal lesions have been associated with an increase in progressive-ratio responding, and although this interpretation has been questioned, the authors suggested that this effect reflected an increase in attributing hedonic value to reward (Schmelzeis & Mittleman, 1996). However, since both hippocampal-lesioned rats and GluR-$1^{-/-}$ mice display a hyperactive phenotype, attributing increases in hedonic value using rates of responding as the dependent measure is somewhat difficult to interpret.

Bannerman and colleagues have suggested that GluR-1-dependent synaptic plasticity contributes to a memory system in rodents for encoding both the spatial and temporal contexts (the where and the when) associated with a particular event

(Schmitt al., 2004b). This idea is suggestive of what is known as hippocampal-dependent episodic memory; a concept linked to Tulving's to theory of human declarative memory. However, Tulving (1983, pp. 1) doubted whether animals have the same ability to remember events "from a different time and in a different place" as that of humans. However, it has been suggested that rodent hippocampal memory system may provide support for memory for episodes (the where and the when) associated with a particular event (Morris et al., 2003; Eichenbaum and Fortin, 2003).

In respect to episodic memory, one could suggest that the devaluation effect requires some form of episodic-type memory. Thus, during the devaluation treatment (induced by satiety) this process requires the mouse to integrate knowledge of the instrumental contingencies acquired during initial training with some representation of the status of the outcome as a goal following the devaluation treatment. Not only would this process bear similarities to working memory in which the animal requires information that is useful for a period (Olton & Papas, 1979), but would also potentially require an intact 'episodic-like' system. Although this proposal could potentially explain the devaluation effect, its explanatory power with respect to the outcome-specific PIT effect is rather limiting and a more parsimonious and tractable hypothesis has been provided.

6.1.5 *Unification of a hippocampal and BLA dysfunction I GluR-1 mutant mice: The emotional tag hypothesis.*

The hippocampus is considered to lay a central role in the formation of explicit/declarative memories types of memories (Squire, 1992; Good, 2000). The hippocampus receives and projects multimodal information from a range of cortical and subcortical structures. As such, the hippocampus is ideally situated to put a specific event into a proper context. That is, it integrates together multiple events that occur during an experience, and consolidates them into a long-term memory (Richter-Levin, 2004). In respect to reference memory, GluR-$1^{-/-}$ mice posses the ability to form such a representation gradually over a course of a series of sessions in order to mediate further choice performance. Consider, however, what may occur when this experience involves the generation of an emotional response. That is, according to some researchers emotional cues (e.g., changes in incentive value of an outcome) can seek to modulate information into enhanced memories by strengthening plasticity in regions such as the hippocampus, via emotional mediators such as the BLA (Richter-Levin & Akirav, 2003; Richter-Levin, 2004).

Evidence supporting an amygdala modulation of the hippocampus has been reported during the examination of stressful events. The effects of the amygdala on hippocampus-dependent tasks are suggested to be mediated by the stress hormone norepinephrine (NE) and glucocorticods (GLUC). As such, post-training intra-amygdala infusions of NE produced dose-dependent enhancement of memory storage for several tasks including spatial tasks (McGaugh, 2000). Similarly, lesions to the BLA block the memory-enhancing effects of post-training intrahippocampal injections of corticosterone or glucocorticoid receptors agonists on a spatial learning

task in the water maze (Roozendaal, 2000). Collectively, these results suggest that the amygdala modulates stress-related hippocampal activity by way of interaction with GLUC.

At a cellular level there is evidence to suggest BLA-hippocampal neural interactions potentially are mediated by emotional evaluation. It has been shown that BLA activation reinforced the induction and maintenance of dentate gyrus LTP and the transformation from early-LTP into a lasting potentiation (Akirav & Richter-Levin, 1999). Furthermore, a single exposure to moderate stress facilitated LTP in basal amygdala but did not affect DG LTP, whilst stress re-exposure inhibited long-lasting LTP in the DG (Vouimba et al., 2004). Furthermore, electrolytic lesions of the amygdala effectively blocked the adverse physiological and behavioural effects of tailshock and restraint stress, without effecting the increase in corticosterone secretion to stress (Kim, Lee, Han & Packard, 2001). Additionally these authors reported hippocampal slices from stressed animals exhibited impaired LTP relative to slices from unstressed control animals, whereas hippocampal slices from stressed animals with amygdala lesions exhibited normal LTP (Kim et al., 2001). Collectively these results suggest that the synaptic modulation of the hippocampus occurs via the emotional mediator, the BLA, which when primed can modulate synaptic plasticity in the hippocampus (Akirav & Richter-Levin, 1999). Thus, the above findings suggest that these readily dissociable properties of amygdala (Mead & Stephens, 2003a) and hippocampus (Reisel et al., 2002) dysfunction are not mutually exclusive factors.

In respect to the emotional tag hypothesis one could relate the working memory deficit (Reisel et al., 2002) to the inability of GluR-1$^{-/-}$ mice to associatively retrieve the current sensory-specific value of the US. For instance, during outcome-specific PIT the CS would be expected to activate a representation of the current

motivational (emotional) value of the sensory-specific properties of the US, to allow Pavlovian cues to modulate the direction of instrumental performance. One could suggest that this representation could be transmitted to the hippocampus (via the BLA), and therefore require this representation to be maintained in working memory; that is, a failure of working memory processing could mediate the disturbance in outcome-specific encoding (Corbit & Balleine, 2003).

6.1.6 *Future Directions: Examining the selective disruption in associatively activated event representations.*

The results from the appetitivly-motivated tasks contained in this thesis have suggested that GluR-1$^{-/-}$ mice show a profound deficit in using associatively activated sensory-specific event representations in order to mediate instrumental performance. This phenotype mimics that seen in BLA-lesioned rats (Blundell et al., 2000; Blundell & Killcross, 2002; Balleine et al., 2003). There are, therefore, several experiments which could also be implemented for examining this phenotype further in GluR-1$^{-/-}$ mice. These include, CS-potentiated feeding, the DOE, conditioned suppression and tests of impulsivity (Killcross et al., 1997; Blundell, Hall & Killcross, 2001; Holland, Hatfield & Gallagher, 2001; Winstanley, Theobald, Cardinal & Robbins, 2004). The benefits of these experiments have been discussed in Chapter 4. However I would like conclude with the use of a task which could dissociate between hippocampal and BLA dysfunction in GluR-1$^{-/-}$ mice.

6.1.7 *Using the DOE to dissociate the effects of hippocampal and BLA dysfunction in meditating performance in mutant mice.*

The results in this thesis have suggested that there are at least two dissociable phenotypes governing behaviour in GluR-1$^{-/-}$ mice. The first, relates to the profound impairment in hippocampal-dependent spatial working memory capabilities as suggested by Bannerman and colleagues. The second relates to aberrant processing of BLA-dependent motivational properties of US value as suggested by Mead and Stephens. Thus, one way to dissociate these processes would be through the use of a task which improves performance in one domain of behaviour (e.g., working memory representations) via the representation of an alternate form of memory (e.g., outcome representations). In fact the DOE can increase the accuracy of memory-based performance across long-delays resulting in improved task performance (Savage, Buzzetti & Ramirez, 2004). In this task hippocampal and sham-lesioned rats were examined in the DOE autoshaping procedure where rats were segregated into one of two conditions. A consistent condition (group DOP), where one lever was consistently paired with O1; whereas a second lever was paired consistently with O2. For rats in the second condition, O1 and O2 reinforcement was equally available following presentation of each lever (group NOP). In this autoshaping procedure rats were not required to press either lever during presentation to receive reward during this phase of training. However, if the subject pressed the lever during presentation, the lever was retracted and followed by reinforcement. Gradually rats responded on the levers at which point a matching to sample phase was implemented. Here, a sample lever was introduced, and rats were required to respond on this lever. This caused the presentation of a cue light which remained on until a magazine nose-poke

occurred. Following nose-poke responding in the magazine recess, both levers were presented and reward was delivered for responding on the previously sampled lever. Hippocampal-lesioned rats acquired this task, although an impairment in the rate of acquisition was noted (Savage et al., 2004). After this stage of training a delayed version of this MTS task was implemented where a delay was given between the sample and choice test. At this stage of the task, hippocampal animals were initially impaired compared to controls. However, savings were seen late in training in the DOE hippocampal group. This contrasted with the hippocampal NOP group where the impairment in performance was enduring. It was suggested that the savings in MTS in the DOE hippocampal group reflected the ability of lesioned animals to use the unique properties of the rewards to guide responding during the delayed MTS phase (Savage et al., 2004). It would be expected that GluR-1$^{-/-}$ mice mediated by a purely hippocampal impairment would gradually acquire the delayed version of the DOE. However, if as the experiments in this thesis suggest, mutant mice are unable to use the unique properties of rewards to guide performance, then one would expect no savings in this setting. Furthermore, it could be suggested that the results indicating hippocampal (e.g., Experiment 1 & 2) and amygdala (e.g., Experiment 5 & 8) dysfunction may be dependent on the type of task implemented. That the MTS DOE procedure assess both these regions in the same instrumental experimental setting, would allow a within-task demonstration of the influence of these regions in modulating instrumental performance in GluR-1$^{-/-}$ mice.

6.1.8 *Coda.*

The results of the previous experiments have suggested that targeted deletion of the GluR-1 subunit of the AMPA receptor results in a phenotype similar to that seen in BLA-lesioned rats (Blundell et al., 2001; Killcross & Blundell, 2002; Balleine et al., 2003). That is, mutant mice seem unable to associate the current incentive value of a particular commodity, and use this association to influence and direct behaviour. This finding furthers that reported by Mead and Stephens, as it would suggest that the underlying feature modulating the dysfunction in conditioned reinforcement and second-order conditioning impairment, reflects a failure to associate the sensory-specific incentive features of the US with the cue. Thus, further examination of this phenotype is warranted. This can be achieved by adapting tasks which have been shown to be sensitive to insults of the basolateral nuclei (Killcross et al., 1997; Blundell, Hall & Killcross, 2001; Holland, Hatfield & Gallagher, 2001; Winstanley, Theobald, Cardinal & Robbins, 2004). Moreover, it is of interest to use contemporary learning theory principles to evaluate further the underlying associative structure modulating the impairment in outcome-specific encoding. I have attempted to suggest one possible avenue this research may take via the examination of mediated acquisition effects (Holland, 1990a; Holland, 1998).

Interestingly, the results from this thesis could be implicated into the mechanisms underlying drug addiction (Stephens et al., 2002). That is, the incentive value of a reward (e.g., drug) can be transferred via a process of second-order conditioning in which secondary rewards (e.g., environment where drugs are experienced) initiate drug seeking behaviour when the drug itself is not immediately available (Carter & Tiffany, 1999). Of course this idea is only speculative, however

the development of region specific (i.e., BLA) GluR-1 selective antagonists could potentially induce a disruption to the cues which mediate relapse.

Nonetheless, as the deletion of the GluR-1 receptor is brain wide, it is clear that there are other neural systems mediating dysfunction. Specifically, the results from Chapter 2 were in agreement with the working memory phenotype suggested by Bannerman and colleagues. It is of necessity now to examine further this dysfunction hypothesis by assessing the contribution of hippocampal working memory to the processing of outcome encoding. Firstly, it is necessary to determine that the working memory dysfunction occurs outside the spatial domain. Secondly, one could implement tasks which are sensitive to both working memory and outcome encoding disturbances as that suggested for the MTS DOE procedure.

Finally, as a general point, a proportion of these experiments noted in this thesis are novel in respect of their application to mice. Obviously, with the birth of the genetically modified mouse, the development of tasks (based on contemporary learning theory) which are sensitive to higher-order learning processes is an advantage to all who seek to evaluate the relationship between genes and behaviour.

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