

*Investigating the role of AMPA α 1 in
an animal model of
post-traumatic stress disorder (PTSD)*

EUGENE HAMLYN

B.Pharm

Dissertation submitted in partial fulfillment of the requirements for the degree

MAGISTER SCIENTIAE

in the

SCHOOL OF PHARMACY (PHARMACOLOGY)

at the

NORTH-WEST UNIVERSITY (POTCHEFSTROOM CAMPUS)

SUPERVISOR: PROF. B. H. HARVEY

CO-SUPERVISOR: PROF. L. BRAND

POTCHEFSTROOM

November 2008

ABSTRACT

Post-traumatic stress disorder (PTSD) is a severe anxiety disorder affecting cognitive function. 1 in 4 individuals exposed to a life-threatening event may develop PTSD, which is characterised by symptoms of hyperarousal, avoidance and intrusions. Although treatment is effective in most cases, the response is far from satisfactory. It is now clear that novel drug treatment and a better understanding of the neurobiology of PTSD are necessary if we are to realise a better response and treatment outcome in these patients. Glutamatergic pathways play an important role in cognition, while recent studies have emphasized a causal role for glutamate in PTSD, and of the potential value of glutamate receptor modulators in treating the disorder. Stress-related elevation in glutamate exerts detrimental effects on cognition, especially via activation of the N-methyl-D-aspartate (NMDA) receptor, and has been implicated in PTSD associated cognitive deficits. Recently, the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptor has been found to exert a modulatory action on NMDA receptor function. Ampakines are positive allosteric modulators of the AMPA receptor, and have demonstrated beneficial effects in animal models of learning as well as antidepressant action, and to improve short-term memory in humans. The aims of this study were firstly to study the effects of the ampakine, *Org 26576*, on spatial memory performance in healthy male Sprague-Dawley rats. Secondly, since PTSD is associated with pronounced deficits in cognition, we studied the ability of *Org 26576* to modify stress-evoked spatial memory deficits in rats subjected to single prolonged stress (SPS), a putative animal model of PTSD. In both cases, neuroreceptor studies were performed to determine any relationship between hippocampal and cortical NMDA receptor binding characteristics and effects on spatial memory performance.

After exposure of the animals to either normal handling or SPS conditions, spatial memory performance was assessed using a 5 day memory acquisition and consolidation protocol in a modified version of the Morris water maze (MWM). Experimental and control groups both received either saline (1ml/kg i.p.) or Org 26576 at incremental doses of 1, 3 or 10 mg/kg intraperitoneally twice daily for 12 days. Separate groups of animals were used for the neuroreceptor studies, except that behavioural testing was not performed. 24hrs after drug treatment discontinuation, the animals were sacrificed and frontal cortex and hippocampus removed for NMDA receptor binding analysis.

In normal rats, Org 26576 3 mg/kg and 10 mg/kg exerted a short-lasting reduction in escape latency on day 1, but which lost prominence over the subsequent training days. Org 26576 1, 3 and 10 mg/kg, however, significantly improved spatial memory retrieval on day 5. No changes in frontal cortical or hippocampal NMDA receptors were observed. Contrary to expected, rats subjected to SPS failed to express noteworthy deficits in spatial memory as previously described. Treatment of SPS-exposed animals with Org 26576 did not significantly alter spatial learning evident in SPS animals on day 1 of acquisition training, as well as on subsequent training days. Org 26576 1 mg/kg increased spatial memory retrieval compared to the unstressed saline control, but not compared to the SPS group. Org 26576 only at a dose of 1 mg/kg decreased cortical, but not hippocampal NMDA receptor density (B_{max}) in SPS animals versus unstressed but not saline treated SPS animals. No changes in receptor affinity (K_d) were noted.

Org 26576 therefore improves early initial spatial learning in healthy rats, but exerts a lesser effect on memory consolidation over the remainder of the training period. However, Org 26576 significantly improves retrieval of spatial memory without simultaneous changes in frontal cortical and hippocampal NMDA receptor binding. Org 26576 thus may benefit both short-term and long-term memory processes in normal animals without effects on limbic NMDA receptor binding, and provides a rationale for testing in conditions that present with cognitive disturbances. However, the SPS model failed to engender marked deficits in spatial memory performance;

this result ultimately complicated the interpretation of the combined stress-drug treatment studies. Studies in healthy animals therefore conclude that Org 26576 is an effective agent to enhance long-term memory processes and should be investigated further for its possible application in disorders of cognition. Although the value of Org 26576 in an animal model of PTSD were inconclusive, further studies in SPS and other PTSD models, as well as models of relevance for schizophrenia, Alzheimer's disease and depression, are encouraged.

Keywords: *AMPAkine, AMPA receptor, frontal cortex, hippocampus, learning, memory, Morris water maze, NMDA receptor, Org 26576, PTSD, SPS*

OPSOMMING

Posttraumatische stresversteuring (PTSV) is 'n erge angsversteuring wat kognitiewe werking beïnvloed. 1 uit 4 individue wat 'n lewensbedreigende ervaring gehad het, kan PTSV ontwikkel met simptome soos hiperopgewektheid, ontwyking en intrusies. Hoewel behandeling in die meeste gevalle effektief is, is die respons glad nie bevredigend nie. Nuwe middels en 'n beter begrip van die neurobiologie van PTSV is nodig as ons 'n beter respons en uitkoms op die behandeling van hierdie pasiënte wil bereik. Glutaminergiese weë speel 'n belangrike rol in kognisie, terwyl onlangse studies die oorsakende rol van glutamaat in PTSV en die moontlike waarde van moduleerders van die glutamaatreseptor vir die behandeling van die versteuring beklemtoon het. Toename in die vlakke van glutamaat vanweë stres oefen veral deur aktivering van die N-metiel-D-aspartaat (NMDA)-reseptor 'n nadeling effek op kognisie uit. Onlangs is gevind dat die α -amino-3-hidroksi-5-metiel-4-isoskasoalpropionsuur (AMPA)-tipe glutamaatreseptor 'n modulerende effek op die NMDA-reseptor uitoefen. Ampakiene is positiewe allosteriese modulatore van die AMPA-reseptor en toon in diërmodelle goeie effekte op leer asook op depressie en verbeter korttermyngeheue in mense. Die doel van hierdie studie was eerstens om die effekte van die ampakien, Org 26576, op ruimtelike geheue van gesonde manlike Sprague-Dawley-rotte te bepaal. Omdat PTSV met uitgesproke gebreke in kognisie gepaardgaan, het ons tweedens die vermoë bestudeer van Org 26576 om gebrekkige ruimtelike geheue by rotte, wat ontstaan het as gevolg van blootstelling aan eenmalige langdurige stress (ELS) te verbeter. ELS is 'n voorgestelde diërmodel vir PTSV. In albei gevalle is neuroreseptorstudies gedoen om 'n verwantskap tussen NMDA-reseptorbinding in die hippokampus en korteks en effekte op ruimtelike geheue te bepaal.

Na blootstelling van die diere aan óf normale hantering óf ELS-toestande is ruimtelike geheue beoordeel deur 'n protokol van leer en konsolidasie oor 5 dae deur 'n

gemodifiseerde weergawe van die Morriswaterdoolhof (MWD) te gebruik. Eksperimentele en kontrolegroepe het albei óf soutoplossing (1 ml/kg ip) óf *Org 26576* teen inkrementele dosisse van 1, 3 of 10 mg/kg ip twee keer per dag vir 12 dae gekry. Afsonderlike groepe diere, waarop gedragstoetse nie gedoen is nie, is vir die neuroreseptorstudies gebruik. 24 uur na staking van die middels is die diere gedood en die frontale korteks en hippokampus vir ontleding van NMDA-reseptorbinding verwyder.

In normale rotte het *Org 26576* teen 3 en 10 mg/kg op dag 1 'n kortstondige afname in ontsnapvermoë veroorsaak, maar hierdie effek het in die daaropvolgende dae verminder. *Org 26576* teen 1, 3 en 10 mg/kg het ruimtelike geheue op dag 5 egter beduidend verbeter. Geen veranderings in NMDA-reseptore in die korteks of hippokampus is waargeneem nie. Anders as verwag, het rotte wat aan ELS blootgestel was, geen noemenswaardige gebreke in ruimtelike geheue vertoon soos voorheen beskryf is nie. Behandeling van diere met *Org 26576* na ELS het ruimtelike leer wat op dag 1 en daaropvolgende dae van opleiding sigbaar was, nie beduidend beïnvloed nie. *Org 26576* teen 1 mg/kg het ruimtelike geheue verbeter vergeleke met soutoplossing sonder ELS, maar nie vergeleke met die ELS-groep nie. *Org 26576* het slegs teen 'n dosis van 1 mg/kg NMDA-reseptordigtheid (B_{maks}) in die korteks verlaag, maar nie in die hippokampus nie van ELS-diere teenoor ongestresde diere, maar nie dié wat soutoplossing gekry het nie. Geen verandering in reseptoraffiniteit (K_d) is waargeneem nie.

Org 26576 verbeter vroeë ruimtelike leer dus in gesonde rotte, maar het 'n swakker effek op geheue in die res van die opleidingsperiode. *Org 26576* verbeter ruimtelike geheue egter beduidend sonder gelyktydige veranderings in NMDA-reseptorbinding in die frontale korteks en hippokampus. *Org 26576* kan korttermyn- en langtermyngeheue in normale diere dus verbeter sonder effekte op limbiese NMDA-reseptorbinding en verskaf 'n toetsmodel vir toestande van kognitiewe versteurings. Die ELS-model kon egter geen merkbare gebreke in ruimtelike geheue veroorsaak nie; dit het die interpretasie in die studie van stres saam met behandeling gekompliseer. Studies met gesonde diere het dus aangetoon dat *Org 26576* 'n

effektiewe middel is om langtermyngeheue te verbeter en kan verder vir moontlike gebruik vir versteurings in kognisie ondersoek word. Hoewel die waarde van *Org 26576* in 'n diemodel van PTSV twyfelagtig is, word verdere studies met ELS en ander PTSV-modelle, asook modelle van belang vir skisofrenie, Alzheimer se siekte en depressie aangemoedig.

Sleutelwoorde: *AMPAkien, AMPA-reseptor, frontale korteks, hippokampus, leer, geheue, Morriswaterdoolhof, NMDA-reseptor, Org 26576, PTSV, ELS*

ACKNOWLEDGEMENTS

Aan God al die eer

I could not have done this on my own and I wish to thank the following people for their contributions:

- My parents, Kevin and Sarie, for 25 years of unconditional love and support. Thanks for always being there for me. You made this possible and I am blessed to have such wonderful parents. I love you very much.
- Charlene, my fiancée and the love of my life. You kept me going through the tough times. Thanks for your love, support and patience throughout this study. I can't wait spending every second of my life with you. I love you more than you can imagine.
- My brother, Morné, for all your love, support and encouragement. Thanks for being such a wonderful brother.
- Wilmarie, thanks for always being there for "*one more cup of coffee*". You are an amazing friend.
- All of my family and friends not mentioned by name, thank you.
- A special thanks to everyone at Patria – my home away from home. Thanks for the friendship, support and all the good times.
- †Dr. Leonard Haasbroek, for his support and care as family doctor and friend throughout the years.
- My supervisor, Prof. Brian Harvey, for your excellent guidance, support and advice. Thank you for sharing your passion and knowledge.
- My co-supervisor, Prof. Linda Brand, for your assistance with the neuroreceptor studies and throughout my study.
- Schering-Plough for the donation of compound Org 26576.
- Mr. C. Bester, Mr. P. Bronkhorst, Mrs. A. Fick, and all the personnel of the Animal Research Centre at North-West University for their guidance in the animal studies.
- Biomedical Resource Unit, University of Kwazulu Natal for providing the animals.
- North-West University and National Research Foundation (NRF) for funding.

TABLE OF CONTENTS

Abstract.	i
Opsomming.	iv
Acknowledgements.	vii
Table of Contents.	ix
List of Figures.	xviii
List of Abbreviations.	xxiii
Chapter 1: Introduction	1
1. Problem Statement.	1
2. Project Aims.	7
3. Project Layout.	8
3.1. Animal Models of PTSD.	8
3.1.1. <i>Single prolonged stress (SPS).</i>	<i>9</i>
3.1.2. <i>Morris water maze (MWM) and spatial memory.</i>	<i>9</i>
4. General points.	10
5. Hypothesis and expected results.	11
6. References.	12

Chapter 2: Literature Overview

22

1. Introduction.	22
2. Characteristics of PTSD.	28
2.1. Intrusions.	28
2.2. Hyperarousal.	28
2.3. Avoidance.	29
2.4. Cognitive impairment.	29
3. Diagnostic criteria for PTSD.	29
4. Aetiology of PTSD.	31
5. Quality of life in PTSD.	32
6. Pathophysiology of PTSD.	32
7. Neuroanatomy of PTSD.	34
7.1. Limbic system.	34
7.1.1. Hypothalamus.	35
7.1.2. Hippocampus.	37
7.1.3. Amygdala.	39
7.1.4. Prefrontal cortex (PFC).	40
8. Stress response.	41
9. Neurochemistry of PTSD.	45
9.1. Amino acids.	46
9.1.1. Glutamate and glutamate receptors.	47
9.1.1.1. NMDA receptors.	48
9.1.1.2. AMPA receptors.	50
9.1.1.3. NMDA-AMPA receptor interactions.	52

9.1.2. GABA.	53
9.2. Monoamines.	54
9.3. Acetylcholine (ACh).	56
10. Drug treatment in PTSD.	57
10.1. Antidepressants.	58
10.1.1. Selective serotonin reuptake inhibitors (SSRIs).	58
10.1.2. Tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs).	59
10.1.3. Atypical antidepressants.	59
10.1.4. Other medications.	60
10.2. Glucocorticoids.	61
11. Learning and memory.	62
11.1. Types of memory.	63
11.1.1. Explicit and implicit memory.	63
11.2. Brain regions involved in memory: Relevance for PTSD.	64
11.3. Neurobiology of memory.	67
11.3.1. Synaptic plasticity.	69
11.3.2. Long-term potentiation (LTP).	69
12. Animal models of relevance for PTSD.	70
12.1. Introduction.	70
12.2. Validity of animal models.	71
12.2.1. Face validity.	72
12.2.2. Construct validity.	72
12.2.3. Predictive validity.	72

13. Animal models of PTSD.	73
13.1. Time-dependent sensitization (TDS).	73
13.2. Single prolonged stress (SPS).	75
14. Ampakines.	76
15. Conclusion.	81
16. Project aims and objectives.	82
17. References.	84

Chapter 3: Article

125

The ampakine, *Org 26576*, bolsters spatial memory performance in rats without changes in cortical and hippocampal NMDA receptor binding

Introduction.	125
Abstract.	127
1. Introduction.	128
2. Materials and methods.	130
2.1. Animals.	130
2.2. Drug treatment.	130
2.3. Experimental design.	131
2.4. Behavioural testing.	131
2.4.1. <i>Morris water maze.</i>	<i>131</i>
2.5. Neuroreceptor studies.	133
2.6. Statistical analysis.	134

3. Results.	135
3.1. Effect of Org 26576 on spatial memory performance.	135
3.1.1. Memory consolidation (acquisition training).	135
3.1.2. Memory retrieval (Probe trial).	136
3.1.3. Cued trial.	137
3.2. Effect of Org 26576 on NMDA receptor binding.	137
3.2.1. Frontal cortical NMDA receptor binding and response to drug treatment.	137
3.2.2. Hippocampal NMDA receptor binding and response to drug treatment.	138
4. Discussion.	138
5. Conclusion.	144
Acknowledgements.	144
References.	144
Legends to Figures.	153
Figures.	154

Chapter 4: 161

Effects of Org 26576 treatment on spatial memory performance and on hippocampal - frontal cortical NMDA receptors in rats subjected to single prolonged stress

1. Introduction.	161
2. Materials and methods.	164
2.1. Animals.	164

2.2. Drug treatment.	165
2.3. Experimental design.	165
2.4. Behavioural testing.	167
2.4.1. <i>Morris water maze</i>	167
2.5. Neuroreceptor studies.	169
2.6. Statistical analysis.	170
 3. Results.	 171
3.1. Effect of <i>Org 26576</i> on spatial memory performance. ...	171
3.1.1. <i>Memory consolidation (acquisition training)</i>	171
3.1.2. <i>Memory retrieval (Probe trial)</i>	174
3.1.3. <i>Cued trial</i>	175
3.2. Effect of <i>Org 26576</i> on NMDA receptor binding.	176
3.2.1. <i>Frontal cortex NMDA receptor binding and response to drug treatment</i>	176
3.2.2. <i>Hippocampal NMDA receptor binding and response to drug treatment</i>	177
 4. Discussion.	 178
5. Conclusions.	183
References.	184
 Chapter 5: Conclusion	 191
References.	198

Addendums 202

Addendum I.203

Instructions to Authors *Neuropharmacology*

1.	Guide for Authors.	203
2.	The Neuroscience Peer Review Consortium.	203
3.	Type of manuscripts.	205
3.1	Research Papers.	205
3.2	Mini-Reviews.	205
4.	Online submission of papers.	205
4.1	Once you are ready to submit.	206
4.2	Transfer of Copyright.	206
4.3	Experimental procedures.	207
4.4	Style.	207
4.5	Abbreviations.	208
4.6	Title page.	208
4.7	Summary.	208
4.8	Keywords.	208
4.9	Introduction.	208
4.10	Methods.	209
4.11	Results.	209
4.12	Discussion.	209
4.13	Acknowledgements.	209

4.14	References.	210
4.15	Illustrations.	210
4.16	Tables.	211
4.17	Multimedia files.	211
4.18	Proofs.	212
4.19	Disclaimer.	212

Article submitted for publication in *Behavioural Pharmacology*...214

The ampakine, Org 26576, bolsters early spatial reference memory and retrieval in the 5-day Morris water maze task: A dose-ranging study in rats

Introduction.	214
Abstract.	216

Introduction.	217
---------------------------	------------

Methods.	218
----------------------	------------

Animals.	218
----------------------	------------

Experimental design and drug treatment.....	219
--	------------

Behavioural testing.	219
----------------------------------	------------

<i>Morris water maze.</i>	<i>219</i>
--------------------------------	------------

Statistical analysis.	221
-----------------------------------	------------

Results.	221
----------------------	------------

Effect of Org 26576 on spatial memory performance.	221
--	------------

<i>Memory consolidation (acquisition training).</i>	<i>221</i>
--	------------

Table of Contents

<i>Memory retrieval (Probe trial).</i>	<i>222</i>
<i>Cued trial.</i>	<i>223</i>
Discussion.	223
Acknowledgements.	227
References.	228
Legends to Figures.	233
Figures.	234

LIST OF FIGURES

Chapters 2 & 4:

Figure 1: Subtypes of glutamate receptors, indicating subunit composition and ligand binding sites (Meador-Woodruff & Kleinman, 2002).....	26
Figure 2: Anatomy of the human brain (top) and rat brain (bottom). (Genetic Science Learning Center, 2008).....	34
Figure 3: HPA-axis response to stress, indicating the negative feedback system mediated by peripheral cortisol on hypothalamic and pituitary function. (Adapted from Schimmer & Parker, 2008).....	36
Figure 4: a) Location of the medial temporal lobe and b) the hippocampus and amygdala. (Medical Care Corporation, 2008; Posit Science Corporation, 2008).....	38
Figure 5: The pathways of fear (Dubuc, 2008). Refer to the text for a detailed description.....	41
Figure 6: The stress response with normal recovery in healthy individuals (a) and in susceptible individuals that later develop a maladaptive response (b) [McEwen, 2004]. Refer to the text for detailed description.....	43 & 44
Figure 7: Synthesis of glutamate and GABA (Palmada & Centelles, 1998).....	46
Figure 8: The NMDA receptor protein complex indicating the relevant binding sites within and outside the ion channel (redrawn from Kalia et al., 2008).....	49
Figure 9: The structure of the AMPA receptor and its functioning (Lynch, 2004). Refer to text for detailed discussion.....	51

Figure 10: NMDA-AMPA receptor cross-talk. (Tomita et al., 2005). Refer to text for detailed description.....	52
Figure 11: Brain regions implicated in PTSD.....	65
Figure 12: Signaling pathways during LTP (Mayford, 2007) Refer to description in the text.....	67
Figure 13: Structure of the AMPA receptor potentiator aniracetam. (Wikipedia contributors, 2008a).....	79
Figure 14: Chemical structures of ampakines CX516, CX546 and CX614 (Lynch, 2006).....	79
Figure 15: Structures of ampakines a) LY503430 and b) LY404187. (Wikipedia contributors, 2008b) and (Wikipedia contributors, 2008c).....	80
Figure 16: Outline of the Morris water maze study. Animals received either saline or <i>Org</i> 26576 at doses of 1, 3 or 10 mg/kg administered intraperitoneally twice daily for a total of 12 days. Morris water maze testing began on day 8, with the final training and memory retrieval tests performed on day 12.....	167
Figure 17: Effect of treatment with saline and <i>Org</i> 26576 (1, 3 and 10 mg/kg i.p. twice daily) in the acquisition phase of spatial learning in the Morris water maze. The escape latency over the five acquisition training days is expressed as mean $\pm$ S.E.M. of 10 animals. Data sets are the average of 4 separate learning sessions performed on each day of training.....	172
Figure 18: Effects of saline and <i>Org</i> 26576 (1, 3 and 10 mg/kg i.p. twice daily) on spatial memory performance on days 1-5 (Figures 18 a-e) of acquisition training. Data represent the means $\pm$ S.E.M. of 10 animals.....	173
Figure 19: (a) Percentage time in target zone and (b) time in target quadrant on day 5 during the probe trial to access memory retrieval in animals receiving saline or <i>Org</i> 26576 at dosages of 1, 3 and 10 mg/kg (i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Tukey test).....	175

Figure 20: (a) Escape latency and (b) swim speed during the cued trial of the Morris water maze in animals receiving saline or <i>Org 26576</i> (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals.....	176
Figure 21: (a) NMDA receptor density (B_{max}) and (b) NMDA receptor affinity (K_d) in the frontal cortex of animals receiving saline or <i>Org 26576</i> (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 6 animals. (* $P<0.05$, Tukey test).....	177
Figure 22: (a) NMDA receptor density (B_{max}) and (b) NMDA receptor affinity (K_d) in the hippocampus of animals receiving saline or <i>Org 26576</i> (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 6 animals.....	178
Figure 23: NMDA-AMPA receptor cross-talk. (Tomita et al., 2005). Refer to Chapter 2 (Section 9.1.1.3.) for detailed description.....	182
Table 1: Prescribing in PTSD (Nash & Nutt, 2007).....	58

Chapter 3 – Article:

Figure 1: Outline of the Morris water maze study. Animals received either saline or <i>Org 26576</i> at doses of 1, 3 or 10 mg/kg administered intraperitoneally twice daily for a total of 12 days. Morris water maze testing began on day 8, with the final training and memory retrieval tests performed on day 12.....	154
Figure 2: Effect of treatment with saline and <i>Org 26576</i> (1, 3 and 10 mg/kg i.p. twice daily) in the acquisition phase of spatial learning in the Morris water maze. The escape latency over the five acquisition training days is expressed as mean $\pm$ S.E.M. of 10 animals. Data sets are the average of 4 separate learning sessions performed on each day of training.....	155
Figure 3: Effects of saline and <i>Org 26576</i> (1, 3 and 10 mg/kg i.p. twice daily) on spatial memory performance on days 1-5 (Figures 3 a-e) of acquisition training. Data represent the means $\pm$ S.E.M. of 10 animals (* $P<0.05$, Dunnett's test).....	156

Figure 4: (a) Percentage time in target zone and (b) time in target quadrant on day 5 during the probe trial to access memory retrieval in animals receiving saline or <i>Org</i> 26576 at dosages of 1, 3 and 10 mg/kg (i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Dunnett's test).....	157
Figure 5: (a) Escape latency and (b) swim speed during the cued trial of the Morris water maze in animals receiving saline or <i>Org</i> 26576 (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals.....	158
Figure 6: (a) NMDA receptor density (B_{max}) and (b) NMDA receptor affinity (K_d) in the frontal cortex of animals receiving saline or <i>Org</i> 26576 (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 6 animals.....	159
Figure 7: (a) NMDA receptor density (B_{max}) and (b) NMDA receptor affinity (K_d) in the hippocampus of animals receiving saline or <i>Org</i> 26576 (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 6 animals.....	160

Article submitted for publication in *Behavioural Pharmacology*:

Figure 1: Effect of treatment with saline and <i>Org</i> 26576 (1, 3 and 10 mg/kg i.p. twice daily) in the acquisition phase of spatial learning in the Morris water maze. The escape latency over the five acquisition training days is expressed as mean $\pm$ S.E.M. of 10 animals. Data sets are the average of 4 separate learning sessions performed on each day of training.....	234
Figure 2: Effects of saline and <i>Org</i> 26576 (1, 3 and 10 mg/kg i.p. twice daily) on spatial memory performance on days 1-5 (Figures 3 a-e) of acquisition training. Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Dunnett's test).....	235
Figure 3: (a) Percentage time in target zone and (b) time in target quadrant on day 5 during the probe trial to access memory retrieval in animals receiving saline or <i>Org</i> 26576 at dosages of 1, 3 and 10 mg/kg (i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Dunnett's test).....	236

Figure 4: (a) Escape latency and (b) swim speed during the cued trial of the Morris water maze in animals receiving saline or <i>Org 26576</i> (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals.....	237
--	------------

LIST OF ABBREVIATIONS

ACTH	adrenocorticotrophic hormone / adrenocorticotropin
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
BDNF	brain-derived neurotrophic factor
EPSCs	excitatory postsynaptic currents
GR	glucocorticoid receptors
HPA-axis	Hypothalamic-pituitary-adrenal axis
KA	kainic acid
LTP	long-term potentiation
MR	mineralocorticoid receptors
MWM	Morris water maze
NMDA	N-methyl-D-aspartate
PFC	prefrontal cortex
PTSD	Post-traumatic stress disorder
SPS	Single prolonged stress
SSRIs	selective serotonin reuptake inhibitors
TDS	Time-dependent sensitization

CHAPTER 1

INTRODUCTION

1. Problem Statement

Post-traumatic stress disorder (PTSD) is a disorder that may follow a life-threatening event and is categorized as an anxiety disorder according to the Diagnostic and Statistical Manual of Mental Disorders – 4th Edition Text Revision (DSM-IV-TR), published by the American Psychiatry Association (APA, 2000). Natural disasters (Liu, 2008), war (Cardozo et al., 2004, Turner et al., 2003), motor vehicle accidents (Kessler et al, 1995) or any kind of sexual, physical or emotional abuse (Kaminer et al., 2008) can lead to PTSD. Traumatic events can have long-term physiological and/or psychological sequelae (Ray, 2008) that persist long after the passing of the emotional stressor. PTSD, together with phobias are the most common psychiatric illnesses (Becker et al., 2007; Michael, et al., 2007; Yehuda, 2002) and the lifetime prevalence for PTSD is between 1.3% and 7.8% (Davidson, et al., 1991; Kessler, et al., 1995). Studies have shown Vietnam veterans and female rape victims have higher lifetime prevalence for PTSD of around 30% (Kulka et al., 1990; Resnick et al., 1993). It is important to note that not everybody exposed to a traumatic event will develop the chronic symptoms of PTSD (Breslau et al., 1998). Indeed, between 20-30% (Green, 1994) of traumatised patients go on to develop PTSD, such that the mechanisms of resilience versus susceptibility have become the focus of many studies on the neurobiology of PTSD. Apart from the broad symptoms of anxiety and avoidance, PTSD is also associated with diverse cognitive impairments (Bremner, 2005; Vasterling et al., 2002), including increased fear memory related to the trauma

(Michael et al., 2005; Rothbaum & Davis, 2003) but diminished explicit memory function (Weber et al., 2005; Elzinga & Bremner, 2002).

Despite evidence for a possible causal role of monoamines and altered hypothalamic pituitary stress axis function in PTSD, the illness remains highly treatment resistant. Consequently, there is a need for improved drug treatments coupled with deeper knowledge of the neurobiology of PTSD.

Pre-clinical and clinical studies have highlighted the important role for glucocorticoids in the stress response and indeed in the development of PTSD (Baker et al., 1999; Liberzon, et al., 1999a; Yehuda et al., 2000). However, PTSD has been associated with raised (Liberzon et al., 1999b), unchanged (Baker et al., 1999) as well as low cortisol (Yehuda et al., 2000) which has complicated the acceptance of a simple glucocorticoid hyperfunction hypothesis. What is very clear is that glucocorticoids exert a profound effect on cognitive performance (Het et al., 2005). Yehuda found PTSD patients to have low cortisol levels in the presence of high catecholamines (Yehuda, 1997), giving rise to the idea that low cortisol levels mediates the lack of control on monoamine and other responses in PTSD patients, leading to symptoms of hyperarousal and cognitive changes.

Glucocorticoids are involved in the stress-related activation of noradrenaline in the hippocampus, prefrontal cortex and amygdala (Ferry et al., 1999). The noradrenergic system, on the other hand, acts as the arousal and alerting system of the body and plays an important role in the amygdala where it is involved in conditioned fear responses and facilitation of fear memory retrieval (Nemeroff et al., 2006; Debeic & LeDoux, 2004). The natural response of the body to acute stress is the release of noradrenaline in the corticolimbic regions (Cecchi et al., 2002). Regions of the brain involved in integrating the response to anxiety, i.e. hippocampus, amygdala, cortex, periaqueductal gray (PAG), hypothalamus and all corticolimbic regions are highly innervated with noradrenergic pathways (Tanaka et al., 2000; Schatzberg & Schildkraut, 1995). However, noradrenaline and adrenaline play a central role in the stress-induced activation of the HPA-axis (Herman et al., 2003; Ziegler & Herman,

2002), suggestive of a positive loop between the two. The brainstem noradrenergic system, particularly the locus coeruleus (LC), is the main innervating pathway to the paraventricular nucleus (PVN), which in turn produces corticotrophin-releasing factor (CRF) responsible for the release of adrenocorticotrophic hormone (ACTH). The LC is activated by stressors and is responsible for the modulation of the hypothalamic-pituitary-adrenal (HPA) axis response to stimuli (Douglas, 2005). Hyperarousal symptoms are presumably the cause of sensitization of noradrenaline (Southwick et al., 1997). Maladaptive noradrenergic responses to stress may contribute to the aetiology of stress-related psychiatric disorders such as PTSD, depression and other anxiety disorders (Sullivan et al., 1999; Schatzberg & Schildkraut, 1995). Evidence for the crucial role for noradrenaline in the development of PTSD is the evidence that administering beta-adrenoceptor blockers, such as propranolol shortly or immediately after the traumatic event may significantly reduce the development of the illness (Pitman et al., 2002).

Dopamine (DA) has a central role in the encoding of memories for arousing, stressful and fearful memories (Southwick et al., 1999). The high density of DA D1 receptors and high dopaminergic innervations in the prefrontal cortex (PFC) is crucial for working memory function (Lidow et al., 1991). Optimal DA levels are crucial for functional working memory performance with hypo- or hyperdopamine levels resulting in cognitive and working memory impairments (Mattay et al., 2000). The dopaminergic pathways in the mesolimbic region play an important role in fear and anxiety states (Millan, 2003), with elevated dopaminergic levels associated with PTSD symptoms such as emotional numbing and hypervigilance (Bremner, 1999). There is also evidence that decreased dopaminergic activity in the frontal cortex plays a role in how the person develops effective coping strategies to dealing with trauma (Harvey et al., 2006; Pezze & Feldon, 2004; Pani et al., 2000), contributing to hyper-vigilance and greater susceptibility to trauma. Furthermore, dopamine transporter single nucleotide polymorphisms have been documented in PTSD (Segman et al., 2002), further contributing towards a causal role in the illness. DA has also been associated with anxiety and depression (Mathews et al., 1980; McCrae

& Costa, 1989; McCracken et al., 1992), which are very often co-morbid with PTSD and as such complicate its adequate treatment and outcome.

The role of serotonin in anxiety and stress-related disorders is well-established, especially given the wide-spread clinical use of serotonergic agents in illnesses like general anxiety, panic, depression, PTSD and obsessive compulsive disorder (Graeff et al., 1997). The development and function of the serotonergic regulatory system and the HPA-axis are interconnected (Laplante et al., 2002), being extremely sensitive to any early adverse lifetime experience (Weinstock, 2001). Clinical studies have revealed that offspring of anxious or depressed mothers have a higher risk for developmental of neurobehavioral (Field et al., 2004a) and physiological disturbances (Field et al., 2004b). The serotonergic system is involved in anxiety regulation (Klaassen, 2002) and therefore considered in the pathophysiology of cognitive impairments associated with PTSD. Animals exposed to restraint stress or electric shocks have shown increased serotonin (5-HT) turnover in the prefrontal cortex, nucleus accumbens, amygdala and lateral hypothalamus (Inoue et al., 1994). Activation of 5-HT_{2A} receptors in the hippocampus and amygdala generate the anxiogenic effect of 5-HT, while the expression of aversive events is subdued by activation of hippocampal 5-HT_{1A} receptors (Graeff et al., 1993). 5-HT_{1A} and 5-HT_{2A} receptor expression are under direct control of stress and glucocorticoids (Lopez et al., 1998). Chronic stress has been found to mediate a decrease in 5-HT_{1A} and 5-HT_{2A} receptors in the hippocampus and cerebral cortex respectively (Watanabe et al., 1993), while serotonin release seems to be differently released following acute stress and re-stress, being enhanced in the hippocampus immediately post trauma, but suppressed following exposure to a reminder of the prior stressor (Harvey et al., 2006). Importantly, serotonin released in the hippocampus following stress (Hajos-Korcsok et al., 2003) has been linked to the further release of the excitatory transmitter, glutamate, which has been causally related to hippocampal atrophy in PTSD (Sapolsky, 2000b).

PTSD patients are more vulnerable to experience atrophy of the hippocampus as a combined result of heightened release of glutamate (Yehuda, 1998) and increased hippocampal glucocorticoid receptor sensitivity (Sapolsky, 2000b). Glucocorticoids selectively increase glutamate levels in this brain region causing hippocampal damage and further elevation of glutamate levels (Sapolsky, 2000a). The hippocampus is important for its regulation of glucocorticoid release through its inhibition of the HPA-axis (Bremner et al., 1999). Damage to the hippocampus will therefore disrupt the negative feedback onto the HPA-axis and increases the exposure of the hippocampus to further toxic levels of glucocorticoids (Sapolsky, 2000a, b). Deficits in declarative memory and reduced hippocampal volume, as shown by magnetic resonance imaging (MRI), have been related to excessive release of cortisol over a long period of time (Starkman et al., 1992). Consequently, stress and the ensuing release of glucocorticoids and glutamate may lead to excitotoxic effects on hippocampal neurons (Sapolsky, 2000a), eventually leading to damage and shrinkage of the hippocampus. Recent work has now begun to consider the value of using HPA-axis and glucocorticoid modulators in the treatment and prevention of PTSD, including glucocorticoid antagonists (Yan et al., 2002; Wolff et al., 1993) as well as cortisol itself (Aerni et al., 2004; Schelling et al., 2004).

Glutamates, and particularly the N-methyl-D-aspartate (NMDA) receptor, have in recent years been found to play a significant role in the aetiology of PTSD (Chambers et al., 1999). The NMDA receptor plays an important role in the memory process, with over-stimulation of the NMDA receptor leading to the formation of deeply rooted emotional memories (McCaslin & Oh, 1995). However, deficiency of glutamate-NMDA signalling that may follow incapacitation of the hippocampus with long-standing PTSD is also associated with compromised memory performance, especially of explicit memory functions relating to short-term and spatial memory capabilities. The NMDA receptor also plays a role in fear extinction (Ledgerwood et al., 2003, 2004, 2005), also compromised in PTSD leading to ongoing fear-driven memories.

The NMDA receptor is a member of the ionotropic family of glutamate receptors, such that sustained activation leads to excessive Ca^{2+} release that directly contributes to cell toxicity and cell death (Tymianski et al., 1993; Olney et al., 1977). However, glutamate receptors comprise three other families, viz. α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainic acid (KA; Hollmann & Heinemann, 1994) and metabotropic (mGlu) receptors (Du et al., 2004; Zarate et al., 2003). The physiological and clinical importance of these receptors has in recent years received a great deal of interest. One important result of this work has been the realization that the AMPA and NMDA receptors enjoy a closely knit relationship (Michaelis, 1998), especially with AMPA receptors involved in recruiting voltage-gated NMDA receptor ion channels (Tanaka et al., 2000), while repetitive NMDA receptor activation results in increased AMPA receptor trafficking within the membrane (Shi et al., 1999; Song and Huganir, 2002). This mutual interaction represents an important component in long-term potentiation (LTP) and the laying down of memory (Granger et al., 1993; Staubli et al., 1994). The benefits of this mutual interaction provide a possible means of addressing a long-standing problem in psychotropic drug development, namely how to rationally utilize glutamate signalling as a drug target. Targeting NMDA receptors as a pharmacological approach to treating neuropsychiatric illnesses that involve excessive glutamatergic activity has met with many problems due to the severity of side effects that full antagonism of these receptors is associated with (Wong et al., 1986; Anis et al., 1983). This has prompted the idea that targeting AMPA receptors may be an alternate approach to bolster/maintain normal functioning of the NMDA receptor without evoking excessive glutamatergic activity or under activity.

Animal models are useful in studies of neuropsychiatric illnesses. The animal models are designed to simulate human disorders under controlled circumstances, observing symptoms associated with the disorder as they develop and to use these as a means to study novel drug treatments (Yehuda & Antelman, 1993). Different animal models have been designed to reflect the core symptoms of a human disorder. A robust animal model of PTSD should therefore be able to reflect long-term biological and

behavioural changes following a relatively short stressor (Yehuda & Antelman, 1993). However, conditioned responses by animals towards stimuli related to the trauma should not be the only response created by the model. Animals should be sensitized by the trauma so that subsequent stimuli unrelated to the original trauma will provoke an excessive behavioural or physiological response (Charney et al., 1993; Pitman et al., 1993). Animal models are categorized in order to meet this criteria, but also to address issues of validity, specifically face (similarity of behavioural response to that seen in the human disorder), predictive (similar response, or not, to typical drug treatments used in the human disorder) and construct (close association between the underlying neurobiology between the animal model and the human disorder) validity. Brief exposure to electric shocks, encountering a predator or odour of a predator and combinations of different stressors in a single session such as single prolonged stress (SPS) and time-dependent sensitization (TDS) are examples of models used. In this project, the SPS model will be used and is described below.

2. Project Aims

Since PTSD presents with diverse cognitive changes, as has been alluded to earlier, but especially a decrement in explicit memory performance (e.g. spatial memory), this study will evaluate the role of AMPA-NMDA receptor interactions during cognitive processing of a spatial memory task, firstly in unstressed healthy animals and then in animals that have undergone prior exposure to SPS. SPS will be applied as previously described by Takahashi et al (2006) and Kohda et al (2007).

Given the exciting prospects for AMPA receptor directed therapy in stress-related disorders such as anxiety (Da Cunha et al., 2008) and depression (Mackowiak et al., 2002), we have initiated the first pre-clinical study evaluating the possible benefits of AMPA receptor modulation in an animal model of PTSD. Ampakines are molecular compounds that bind to AMPA receptors, without agonist or antagonist effects, potentiating AMPA receptor transmission. In the present study, the effects of *Org*

26576 (generic name withheld for proprietary reasons, refer to **section 4 - General points** for full explanation) on spatial memory performance in healthy and stressed animals will be studied using a dose response analysis.

Considering the important role of NMDA and AMPA receptors in cognition, and particularly that these two receptors are mutually interactive with regards glutamate signalling, stress evoked changes in NMDA receptor binding in the frontal cortex and hippocampus will be determined in normal healthy animals following treatment with various doses of *Org 26576*, viz. 0 (saline), 1, 3 and 10 mg/kg administered for 12 days. This will be done in order to more closely relate drug dose to behavioural and neuroreceptor change. Thereafter, these same dosages will be applied in a similar dose response manner to animals exposed to SPS in order to determine whether changes in NMDA receptor binding are associated with any abilities of the ampakine to reverse stress-induced spatial memory deficits following exposure of the animals to SPS. The same spatial memory and neuroreceptor parameters will be assessed.

3. Project Layout

3.1. Animal models of PTSD

Two existing animal models of PTSD, viz. TDS (Harvey et al., 2003) and SPS (Kohda et al., 2007), have been found to evoke a marked suppression of spatial memory performance in the Morris water maze. In earlier work, we had demonstrated that SPS and TDS evoke qualitatively different behavioural and neuroendocrine responses (Harvey et al., 2006). For this study, only SPS will be used as previously described (Kohda et al., 2007). Once a dose response is established in non-stressed animals, the SPS model will be used for all subsequent ampakine dose-response studies and associated effects on behaviour and regional brain neuroreceptor changes.

3.1.1. Single prolonged stress (SPS)

In the SPS model, animals are exposed to a prolonged session of acute stress involving sequential exposure to restraint, underwater stress and exposure to ether vapours (see Khan & Liberzon, 2004; Liberzon et al., 1997; Takahashi et al., 2006; Harvey et al., 2006; Kohda et al., 2007). The animals are either left undisturbed for another 7 days (Liberzon et al., 1999a; Takahashi et al., 2006), with biobehavioural determination performed on day 14, or biobehavioural tests are performed on or after day 7 post SPS (Yamamoto et al., 2008; Imanaka et al., 2006; Kohda et al., 2007). For the purposes of this study, all biobehavioural tests will be carried out on day 7 post stress.

3.1.2. Morris water maze (MWM) and spatial memory

The MWM is used to study pathological processes and the effect of drug treatment response in the laying down and expression of spatial learning and memory in rodents (Brandeis et al., 1989; McNamara & Skelton, 1993). In this paradigm, the animals are learned to navigate a swimming pool that contains a submerged platform by using spatial navigational cues surrounding the pool (Morris, 1981). After a series of training sessions over a period of days, the animals will show a reduced latency in locating the platform, unless spatial memory is compromised by a pathological process, such as stress, or a drug that has been administered. However, just as spatial memory can be adversely affected by pharmacological means; it can also be bolstered by appropriate drug treatment. Such an agent may have particular importance as a novel drug candidate for the treatment of disorders of cognition, such as Alzheimer's disease, depression and as emphasized in the present study, PTSD. This study will examine both possibilities, namely whether the ampakine can bolster normal Morris water performance in rats, and whether it can do so in animals prior exposed to a PTSD-like paradigm.

4. General points

The study was performed at North-West University (NWU), School of Pharmacy, Pharmacology Department in collaboration with an international pharmaceutical company. The names of the company and the compound used for the study have been withheld for confidentiality reasons, a request made by the aforementioned company, until all the experimental data and discussion have undergone internal company review. Please note the following points from the legal agreement between the NWU and the undisclosed pharmaceutical company:

Article 4 - Confidentiality

4.1 NWU agrees to maintain the Confidential Information in the strictest confidence and will ensure that:

- (i) the Confidential Information will be used only for the purpose of the research undertaken on the Material;
- (ii) the Confidential Information will not be disclosed to any third party; and
- (iii) that researchers, employees and (sub)contractors (as applicable) will only be given access to the Confidential Information on a need to know basis.

The publication of the data in any form, including a dissertation, thesis or congress presentation, is prohibited until it has been approved by the company. Such approval will be undertaken after examination of the dissertation, but prior to its publication.

The company has given permission to submit the dissertation in article format to the **three examiners**, under the following conditions:

- The name of the company or compound must not be mentioned.
- No reference to either the company name or the compound name is made.

The dissertation will be written and submitted in the article format for thesis/dissertation submission, as approved by the North-West University. The article format consists of an introductory chapter, a literature overview chapter and one chapter (Chapter 3) containing a full length article for submission to a peer-review, accredited neuroscience or pharmacology journal, followed by subsequent chapters or addendums not intended for publication. The article chapter will be prepared in the house-style and instructions to the authors of the selected journal. The manuscript will begin with the title, contributing authors and affiliations on a separate page, followed by an Abstract and a list of keywords. Thereafter the main body of the manuscript will follow including Introduction, Methods, Results, Discussion, Acknowledgements, Bibliography and Legends to Figures. As per the journal submission format, all figures are provided at the end of the paper and annotated as Figure 1 onwards, with Tables annotated in roman numerals and similarly placed at the back of the manuscript.

5. Hypothesis and expected results

We hypothesize that SPS will result in NMDA receptor binding changes and that this will be associated with spatial memory deficits in rats. Chronic treatment with *Org 26576* may indeed have beneficial effects on spatial memory in unstressed animals, possibly involving regional hippocampal and cortical NMDA receptor changes. *Org 26576* should significantly attenuate stress-induced memory deficits in stressed animals, together with associated changes of NMDA receptors in the above-mentioned brain regions.

6. References

- Aerni, A., Traber, R., Hock, C., Roozendaal, B., Schelling, G., Papassotiropoulos, A., Nitsch, R. M., Schnyder, U., DeQuervain, D. J., 2004. Low-dose cortisol for symptoms of posttraumatic stress disorder. *Am J Psychiatry* 161, 1488–1490.
- American Psychiatric Association., 2000. Diagnostic and statistical manual of mental disorders (4th ed., text revision). Washington, D.C.: American Psychiatric Press.
- Anis, N. A., Berry, S. C., Burton, N. R., Lodge, D., 1983. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-D-aspartate. *Brit J Pharmacol* 79, 565-575.
- Baker, D. G., West, S. A., Nicholson, W. E., Ekhtor, N. N., Kasckow, J. W., Hill, K. K., et al., 1999. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. *Am J Psychiatry* 156, 585–588.
- Becker, E. S., Rinck, M., Turke, V., Kause, P., Goodwin, R., Neumer, S., Margaf, J., 2007. Epidemiology of specific phobia subtypes: findings from the Dresden mental health study. *Eur Psychiatry* 22, 69-74.
- Brandeis, R., Brandys, Y., Yehuda, S., 1989. The use of the Morris water maze in the study of memory and learning. *Int J Neurosci* 48, 29–69.
- Bremner, J. D., 1999. Does stress damage the brain. *Biol Psychiatry* 45, 797-805.
- Bremner, J. D., 2005. Effects of traumatic stress on brain structure and function, relevance to early responses to trauma. *J Trauma Dissociation* 6, 51-68.
- Breslau, N., Kessler, R. C., Chilcoat, H. D., Schultz, L. R., Davis, G. C., Andreski, P., 1998. Trauma and posttraumatic stress disorder in the community: The 1996 detroit area survey of trauma. *Arch Gen Psychiat* 55, 626-632.
- Cardozu, B. L., Bilukha, O. O., Crawford, C. A., Shaikh, I., Wolfe, M. J., Gerber, M. L., et al., 2004. Mental health, social functioning and disability in postwar Afghanistan. *J Amer Med Assoc* 292, 575–584.

- Cecchi, M., Khoshbouei, H., Javors, M., Morilak, D. A., 2002. Modulatory effects of norepinephrine in the lateral bed nucleus of the stria terminalis on behavioral and neuroendocrine responses to acute stress. *Neuroscience* 112, 13–21.
- Chambers, R. A., Bremner, J. D., Moghaddam, B., Southwick, S. M., Charney, D. S., Krystal, J. H., 1999. Glutamate and post-traumatic stress disorder: Toward a psychobiology of dissociation. *Semin Clin Neuropsychiatry* 4, 274-281.
- Charney, D. S., Deutch, A. Y., Krystal, J. H., Southwick, S. M., Davis, M., 1993. Psychobiologic mechanisms of posttraumatic stress disorder. *Arch Gen Psychiat* 50, 294–305.
- Da Cunha, I. C., Lopes, A. P. F., Steffens, S. M., Ferraz, A., Vargas, J. C., De Limab, T. C. M., Neto, J. M., Paschoalini, M. A., Faria, M. S., 2008. The microinjection of AMPA receptor antagonist into the accumbens shell, but not into the accumbens core, induces anxiolysis in an animal model of anxiety. *Beh Brain Res* 188, 91–99.
- Davidson, J. R. T., Hughes, D., Blazer, D. G., George, L. K., 1991. Post-traumatic stress disorder in the community: an epidemiological study. *Psychol Med* 21, 713-721.
- Debeic, J., Ledoux, J. E., 2004. Disruption of reconsolidation but not consolidation of auditory fear conditioning by adrenergic blockade in the amygdala. *Neuroscience* 267–272.
- Douglas, A. J., 2005. Central noradrenergic mechanisms underlying acute stress responses of the hypothalamo-pituitary-adrenal axis: adaptations through pregnancy and lactation. *Stress* 8, 5–18.
- Du, J., Quiroz, J., Yuan, P., Zarate, C., Manji, H. K., 2004. Bipolar disorder: Involvement of signaling cascades and AMPA receptor trafficking at synapses. *Neuron Glia Biology* 13, 231-243.
- Elzinga, B. M., Bremner, J. D., 2002. Are the neural substrates of memory the final common pathway in posttraumatic stress disorder (PTSD)? *J Affective Dis* 70, 1-17.
- Ferry, B., Roozendaal, B., McGaugh, J. L., 1999. Role of norepinephrine in mediating stress hormone regulation of long-term memory storage: a critical involvement of the amygdala. *Biol Psychiat* 46, 1140-1152.
-

- Field, T., Diego, M., Dieter, J., Hernandez-Reif, M., Schanberg, S., Kuhn, C., et al., 2004a. Prenatal depression effects on the fetus and the newborn. *Infant Behav Dev* 27, 216-229.
- Field, T., Diego, M., Hernandez-Reif, M., Vera, Y., Gil, K., Schanberg, S., et al., 2004b. Prenatal maternal biochemistry predicts neonatal biochemistry. *Int J Neurosci* 114, 933-945.
- Graeff, F. G., Silveira, M. C., Nogueira, R. L., 1993. Role of amygdale and periaqueductal gray in anxiety and depression. *Behav Brain Res* 58, 123-131.
- Graeff, F. G., Viana, M. B., Mora, P. O., 1997. Dual role of 5-HT in defence and anxiety. *Neurosci Biobehav Rev* 21, 791-799.
- Granger, R., Staubli, U., Davis, M., Perez, Y., Nilsson, L., Rogers, G. A., Lynch, G., 1993. A drug that facilitates glutamatergic transmission reduces exploratory activity and improves performance in a learning-dependant task. *Synapse* 15, 326-329.
- Green, B. L., 1994. Psychological research in traumatic stress: an update. *J Trauma Stress* 7, 343-361.
- Hajós-Korcsok, É., Robinson, D. D., Yu, J. H., Fitch, C. S., Walker, E., Merchant, K. M., 2003. Rapid habituation of hippocampal serotonin and norepinephrine release and anxiety-related behaviors, but not plasma corticosterone levels, to repeated footshock stress in rats. *Pharmacol Biochem Be* 74, 609-616.
- Harvey, B. H., Naciti, C., Brand, L., Stein, D. J., 2003. Endocrine, cognitive and hippocampal/cortical 5HT_{1A/2A} receptor changes evoked by a time-dependent sensitisation (TDS) stress model in rats. *Brain Res* 983, 97-107.
- Harvey, B. H., Brand, L., Jeeva, Z., Stein, D. J., 2006. Corticalhippocampal monoamines, HPA axis changes and aversive behavior following stress and re-stress in an animal model of post-traumatic stress disorder. *Physiol Behav* 87, 881-890.
- Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., Cullinan, W. E., 2003. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitaryadrenocortical responsiveness. *Front Neuroendocrinol* 24, 151–180.
-

- Het, S., Ramlow, G., Wolf, O. T., 2005. A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrino* 30, 771–784.
- Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. *Annu Rev Neurosci* 17, 31-108.
- Imanaka, A., Morinobu, S., Toki, S., Yamawaki, S., 2006. Importance of early environment in the development of post traumatic stress disorder-like behaviors. *Behav Brain Res* 173, 129-137.
- Inoue, T., Tsuchiya, K., Koyama, T., 1994. Regional changes in dopamine and 5-hydroxytryptamine activation with various intensity of physical and psychological stress in rat brain. *Pharmacol Biochem Behav* 49, 911-920.
- Kaminer, D., Grimsrud, A., Myer, L., Stein, D. J., Williams, D. R., 2008. Risk for post-traumatic stress disorder associated with different forms of interpersonal violence in south africa. *SocSci Med* 67, 1589-1595.
- Kessler, R., Sonnega, A., Bromet, E., Hughes, M., Nelson, C., 1995. Posttraumatic stress disorder in the National Comorbidity survey. *Arch Gen Psychiat* 52, 1048–1060.
- Khan, S., Liberzon, I. 2004. Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. *Psychopharmacology* 172, 225-29.
- Klaassen, T., 2002. Serotonergic studies in affective disorders. Thesis. Universitaire Pers Maastricht, Maastricht.
- Kohda, K., Harada, K., Kato, K., Hoshino, A., Motohashi, J., Yamaji, T., Morinobu, S., Matsuoka, N., Kato, N., 2007. Glucocorticoid receptor activation is involved in producing abnormal phenotypes of single-prolonged stress rats: a putative Post-Traumatic Stress Disorder model. *Neuroscience* 148, 22-33.
- Kulka, R. A., Schlenger, W., Fairbank, J., Hough, R., Jordan, B., Marmar, C., Weiss, D., 1990. Trauma and the Vietnam War Generation. New York, Brunner/Mazel, p. 322.
- Laplanche, P., Diorio, J., Meaney, M. J., 2002. Serotonin regulates hippocampal glucocorticoid receptor expression via a 5-HT₇ receptor. *Brain Res Dev Brain Res* 139, 199-203.
-

- Ledgerwood, L., Richardson, R., Cranney, J., 2003. Effects of D-cycloserine on extinction of conditioned freezing. *Behav Neurosci* 117, 341–349.
- Ledgerwood, L., Richardson, R., Cranney, J., 2004. D-cycloserine and the facilitation of extinction of conditioned fear: consequences for reinstatement. *Behav Neurosci* 118, 505–513.
- Ledgerwood, L., Richardson, R., Cranney, J., 2005. D-cycloserine facilitates extinction of learned fear: effects on reacquisition and generalized extinction. *Biol Psychiat* 57, 841–847.
- Liberzon, I., Krstov M, Young EA., 1997. Stress-restress: Effects on ACTH and fast feedback. *Psychoneuroendocrino* 22, 443–453.
- Liberzon, I., López, J. F., Flagel, S. B., Vázquez, D. M., Young, E. A., 1999a. Differential regulation of hippocampal glucocorticoid receptor mRNA and fast feedback: relevance to post-traumatic stress disorder. *J Neuroendocrinol* 11, 11–17.
- Liberzon, I., Taylor, S. F., Amdur, R., Jung, T. D., Chamberlain, K. R., Minoshima, S., et al., 1999b. Brain activation in PTSD in response to trauma-related stimuli. *Biol Psychiat* 45, 817–826.
- Lidow, M. S., Goldman-Rakic, P. S., Gallager, D. W., Rakic, P., 1991. Distribution of dopaminergic receptors in the primate cerebral cortex: quantitative autoradiographic analysis using [3H]raclopride, [3H]spiperone and [3H]SCH23390. *Neuroscience* 40, 657–671.
- Liu, A., 2008. A short DSM-IV screening scale to detect posttraumatic stress disorder after a natural disaster in a Chinese population. *Psychiat Res* 159, 376–381.
- Lopez, J. F., Chalmers, D. L., Little, K. Y., 1998. Regulation of serotonin 1A, glucocorticoid and mineralocorticoid receptors in the rat and human hippocampus: implications for neurobiology of depression. *Biol Psychiat* 43, 547–573.
- Mackowiak, M., O'Neill, M. J., Hicks, C. A., Bleakman, D., Skolnick, P., 2002. An AMPA receptor potentiator modulates hippocampal expression of BDNF: an in vivo study. *Neuropharmacology* 43, 1–10.
- Mathews, R. J., Ho, B. T., Kralik, P., Taylor, D., Claghorn, J., 1980. MAO, DBH and COMT; the effect of anxiety. *J Clin Psychiat* 41, 25–28.
-

- Mattay, V. S., Callicott, J. H., Bertolino, A., Heaton, I., Frank, J. A., Coppola, R., et al., 2000. Effects of dextroamphetamine on cognitive performance and cortical activation. *Neuroimage* 12, 268–275.
- McCaslin, P. P., Oh, S. 1995., Nitric oxide and glutamate receptors. In: Stone, T. W., (Ed), CNS neurotransmitters and neuromodulators: glutamate. CRC Press, New York, pp. 159-179.
- McCrae, R. R., Costa, P. T., 1989. Reinterpreting the Myers–Briggs type indicator from the perspectives of the five-factor model of personality. *J Personal* 57, 17–40.
- McCraken, I. M., Zaifert, C., Gross, R. T., 1992. The pain anxiety symptoms scale: development and validation of a scale to measure fear of pain. *Pain* 50, 67–73.
- McNamara, R. K., Skelton, R. W., 1993. The neuropharmacological and neurochemical basis of place learning in the Morris water maze. *Brain Res Rev* 18, 33–49.
- Michael, T., Ehlers, A., Halligan, S. L., 2005. Enhanced priming for trauma-related material in posttraumatic stress disorder. *Emotion* 5, 103-112.
- Michael, T., Zetsche, U., Margraf, J., 2007. Epidemiology of anxiety disorders. *Psychiatry* 6, 136-142.
- Michaelis, E. K., 1998. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. *Prog Neurobiol* 54, 369–415.
- Millan, M., 2003. The neurobiology and control of anxious states. *Prog Neurobiol* 70, 83–244.
- Morris, R. G. M., 1981. Spatial localization does not require the presence of local cues. *Learn Motiv* 12, 239-260.
- Nemeroff, C. B., Bremner, J. D., Foa, E. B., Mayberg, H. S., North, C. S., Stein, M. B., 2006. Posttraumatic stress disorder: a state-of-the-science review. *J Psychiatr Res* 40, 1–21.
- Olney, J. W., Rhee, V., Gubareff, T. D., 1977. Neurotoxic effects of glutamate on mouse area postrema. *Brain Res* 120, 151–157.
-

- Pani, L., Porcella, A., Gessa, G. L., 2000. The role of stress in the pathophysiology of the dopaminergic system. *Mol Pharmacol* 5, 14–21.
- Pezze, M. A., Feldon, J., 2004. Mesolimbic dopaminergic pathways in fear conditioning. *Prog Neurobiol* 74, 301–320.
- Pitman, R. K., Orr, S. P., Shalev, A. Y., 1993. Once bitten, twice shy: beyond the conditioning model of PTSD. *Biol Psychiat* 33, 145–146.
- Pitman, R. K., Sanders, K. M., Zusman, R. M., Healy, A. R., Cheema, F., Lasko, N. B., et al., 2002. Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biol Psychiat* 51, 189–192.
- Ray, S. L., 2008. Evolution of posttraumatic stress disorder and future directions. *Arch Psychiat Nurs* 22, 217–225.
- Resnick, H. S., et al., 1993. Prevalence of civilian trauma and posttraumatic stress disorder in a representative national sample of women. *J Consult Clin Psychol* 61, 984–991.
- Rothbaum, B. O., Davis, M., 2003. Applying learning principles to the treatment of post-trauma reactions. *Ann NY Acad Sci* 1008, 112–121.
- Sapolsky, R. M., 2000a. The possibility of neurotoxicity in the hippocampus in major depression: A primer on neuron death. *Biol Psychiat* 48, 755–765.
- Sapolsky, R. M., 2000b. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiat* 57, 925–935.
- Schatzberg, A. F., Schildkraut, J. J. 1995., Recent studies on norepinephrine systems in mood disorders. In: Bloom, F. E., Kupfer, D. J., (Eds), *Psychopharmacology: the fourth generation of progress*. Raven Press, Ltd., New York, pp. 911–920.
- Schelling, G., Kilger, E., Roozendaal, B., de Quervain, D. J., Briegel, J., Dagge, A., et al., 2004. Stress doses of hydrocortisone, traumatic memories, and symptoms of posttraumatic stress disorder in patients after cardiac surgery: A randomized study. *Biol Psychiat* 55, 627–633.
- Segman, R. H., Cooper-Kazaz, R., Macciardi, F., Goltser, T., Halfon, Y., Dobroborski, T., 2002. Association between the dopamine transporter gene and posttraumatic stress disorder. *Mol Psychiat* 7, 903–907.
-

- Shi, S. H., Hayashi, Y., Petralia, R. S., Zaman, S. H., Wenthold R., Svoboda K., Malinow R., 1999. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* 284, 1811-1816.
- Song, I., Huganir R. L. 2002. Regulation of AMPA receptors during synaptic plasticity. *Trends in Neurosciences* 25, 578-589.
- Southwick, S. M., Krystal, J. H., Bremner, J. D., Morgan, C. A. 3rd., Nicolaou, A. L., Nagy, L. M., Johnson, D. R., Heninger, G. R., Charney, D. S., 1997. Noradrenergic and serotonergic function in posttraumatic stress disorder. *Arch Gen Psychiat* 54, 749–758.
- Southwick, S. M., Bremner, J. D., Rasmusson, A., Morgan, C. A. 3rd., Arnsten, A., Charney, D. S., 1999. Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiat* 46, 1192-1204.
- Starkman, M. N., Gebarski, S. S., Berent, S., Schteingart, D. E., 1992. Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's Syndrome. *Biol Psychiat* 32. 756 –765.
- Stäubli, U., Perez, Y., Xu, F. B., Rogers, G., Ingvar, M., Stone-Elander, S., Lynch, G., 1994. Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. *Proc Nat Acad Sci USA* 91, 11158-11162.
- Sullivan, G. M., Coplan, J. D., Kent, J. M., Gorman, J. M., 1999. The noradrenergic system in pathological anxiety: a focus on panic with relevance to generalized anxiety and phobias. *Biol Psychiat* 46, 1205–1218.
- Tanaka, M., Yoshida, H., Emoto, H., Ishii, H., 2000. Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: basic studies. *Eur J Pharmacol* 405, 397–406.
- Takahashi, T., Morinobu, S., Iwamoto, Y., Yamawaki, S., 2006. Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats, *Psychopharmacology (Berl)* 189, 165–173.
- Turner, S. W., Bowie, C., Dunn, G., Shapo, L., Yule, W., 2003. Mental health of Kosovan Albanian refugees in the UK. *Brit J Psychiat* 182, 444–448.
- Tymianski, M., et al., 1993. Secondary Ca²⁺ overload indicates early neuronal injury which precedes staining with viability indicators. *Brain Res* 607, 319–323.
-

- Vasterling, J. J., Duke, L. M., Brailey, K., Constans, J. L., Allain, A. N., Sutker, P. B., 2002. Attention, learning, and memory performances and intellectual resources in Vietnam veterans, PTSD and no disorder comparisons. *Neuropsychology* 16, 5-14.
- Watanabe, Y., Sakai, R. R., McEwan, B. S., 1993. Stress and antidepressant effects on hippocampal and cortical 5HT1A and 5HT2A receptors and transport sites for serotonin. *Brain Res* 615, 87-94.
- Weber, D. L., Clark, C. R., McFarlane, A. C., Moores, K. A., Morris, P., Egan, G. F., 2005. Abnormal frontal and parietal activity during working memory updating in post-traumatic stress disorder. *Psychiat Res* 140, 27-44.
- Weinstock, M., 2001. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. *Prog Neurobiol* 65, 427-451.
- Wolff, D. J., Datto, G. A., Samatovicz., 1993. The dual mode of inhibition of calmodulin-dependent nitric oxide synthase by antifungal imidazole agents. *J Biol Chem* 268, 9430-9436.
- Wong, E. H., Kemp, J. A., Priestley, T., Knight, A. R., Woodruff, G. N., Iversen, L. L., 1986. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc Natl Acad Sci USA* 83, 7104-7108.
- Yamamoto, S., Morinobu, S., Fuchikami, M., Kurata, A., Kozuru, T., Yamawaki, S., 2008. Effects of single prolonged stress and D-Cycloserine on contextual fear extinction and hippocampal NMDA receptor expression in a rat model of PTSD. *Neuropsychopharmacol* 33, 2108-2116.
- Yan, Z., Rafferty, B., Caldwell, G. W., Masucci, J. A., 2002. Rapidly distinguishing reversible and irreversible CYP450 inhibitors by using fluorometric kinetic analysis. *Eur J Drug Metab Pharmacokinet* 27, 281-287.
- Yehuda, R., Antelman, S. M., 1993. Criteria for rationally evaluating animal models for posttraumatic stress disorder. *Biol Psychiat* 33, 479-486.
- Yehuda, R. 1997., Sensitization of the hypothalamic-pituitary-adrenal axis in posttraumatic stress disorder. In: Yehuda, R., McFarlane, A. C., (Eds), *Psychobiology of Posttraumatic Stress Disorder*. The New York Academy of Sciences, New York, pp. 57-75.
-

- Yehuda, R. 1998., Neuroendocrinology of trauma and posttraumatic stress disorder. In: Yehuda, R., (Ed), Psychological trauma. American Psychiatric Press, Washington, DC, pp. 97-131.
- Yehuda, R., Bierer, L. M., Schmeidler, J., Aferiat, D. H., Breslau, I., Dolan, S., 2000. Low cortisol and risk for PTSD in adult offspring of Holocaust survivors. *Am J Psychiat* 157, 1252-1259.
- Yehuda, R., 2002. Post-traumatic stress disorder. *N Engl J Med* 346, 108-114.
- Zarate, C. A., Du, J., Quiroz, J., Gray, N. A., Denicoff, K. D., Singh, J., 2003. Regulation of cellular plasticity cascades in the pathophysiology and treatment of mood disorders: Role of the glutamatergic system. *Ann NY Acad Sci* 1003, 273-291.
- Ziegler, D. R., Herman, J. P., 2002. Neurocircuitry of stress integration: anatomical pathways regulating the hypothalamo-pituitary-adrenocortical axis of the rat. *Integr Comp Biol* 42, 541-551.

CHAPTER 2

LITERATURE OVERVIEW

1. Introduction

Post-traumatic stress disorder (PTSD) is a severe anxiety disorder that may follow a traumatic event. Characteristics of PTSD include hyperarousal, avoidance, intrusions and cognitive dysfunction, while recent studies have noted that if not adequately treated, the disorder gets progressively worse over time (Johnsen et al., 2002). Brain imaging studies in patients with PTSD have indicated that the illness is associated with atrophy of the hippocampus, a brain region especially important in regulating the stress response and that also is critical in working/declarative memory function (Bremner, 1999; Elzinga & Bremner, 2002). Evidence has suggested that the severity of memory dysfunction in PTSD is correlated with the degree of hippocampal shrinkage (Bremner, 1999), while successful drug treatment is associated with improvements in both mnemonic function and return of hippocampal volume to control values (Vermetten et al., 2003). PTSD can be directly or indirectly related to a state of disorganized memory. Importantly, general declarative memory functions as well as explicit information about the trauma are compromised while at the same time non-declarative memory relating to involuntary recollection of the trauma is bolstered (Elzinga & Bremner, 2002).

Pre-clinical and clinical studies have highlighted the important role for glucocorticoids in the stress response and indeed in the development of PTSD (Baker et al., 1999; Liberzon et al., 1999a; Yehuda et al., 2000). However, PTSD has been associated

with raised (Liberzon et al., 1999b), unchanged (Baker et al., 1999) as well as low cortisol (Yehuda et al., 2000) which has complicated the acceptance of a simple glucocorticoid hyperfunction hypothesis. While there remains uncertainty as to whether hippocampal shrinkage reflects a consequence of stress, or whether a smaller hippocampus predisposes to PTSD (Gilbertson et al., 2002), the undeniable evidence for regional brain shrinkage in PTSD has been instrumental in strengthening the glucocorticoid hypothesis of PTSD, this especially since stress-evoked hypercortisolemia mediates the release of the excitotoxic transmitter, glutamate (Sapolsky, 2000b). This has raised a great deal of interest in the putative role of neurodegenerative mechanisms in PTSD, especially of cell-survival mediators linked to over-stimulation of the glutamate N-methyl-D-aspartate (NMDA) receptor.

Earlier pre-clinical (Harvey et al., 2004a; Adamec et al., 1998) as well as clinical (Heresco-Levy et al, 2002; Chambers et al., 1999; Dawson & Dawson, 1995) studies have indicated not only the involvement of the NMDA receptor in PTSD, but also of the promise that the NMDA receptor may hold as a novel and useful pharmacological target in treating the disorder. However, the complex composition and regulation of the NMDA receptor (see Huggins & Grant, 2005 for review) has complicated the development of safe and effective drug candidates. The NMDA receptor complex is a tetramer with two NR1 and two NR2 subunits (Dingledine et al., 1999). Glycine or D-serine bind to the NR1 subunit and glutamate binds to the NR2A/B subunit. NMDA receptor activation requires binding of glutamate and glycine/D-serine (Paoletti & Neyton, 2007). Simultaneous depolarization of the ion-channel by AMPA or kainate receptors, thereby relieving the Mg^{2+} block, will result in NMDA receptor ion channel opening and the influx of Na^{+} and Ca^{2+} and the efflux of K^{+} . The NMDA receptor complex also has two binding sites within the ion channel, namely the sigma (σ) site binding Mg^{2+} and the phencyclidine (PCP) site (Du et al., 2006). These multiple regulatory sites provide various options for pharmacological manipulation of NMDA receptor function. However, the manner of NMDA receptor regulation requires careful consideration.

Thus, for example, dizocilpine (MK-801; Wong et al., 1986) and PCP (Anis et al., 1983), which fully block NMDA receptors through binding in the ion permeation channel (Mori et al., 1992; Kashiwagi et al., 2002), prevent further physiological functioning of the NMDA receptor gated ion channel, such that the cells are deprived of essential Ca^{2+} entry needed for normal neuronal functioning. The result is that these agents are troubled by serious side effects that preclude their general clinical use. Adverse effects include hallucinations, central nervous system (CNS) depression and coma; possibly even death (Lipton & Chen, 2004). Moreover, excessive NMDA blockade can also induce neurodegeneration of pyramidal neurons (Olney et al., 1999; Olney & Farber, 1995) through excessive release of glutamate (Moghaddam et al., 1997; Adams & Moghaddam, 1998), leading to cognitive and behavioural disturbances (Olney & Farber, 1995; Moghaddam et al., 1997; Adams & Moghaddam, 1998). Memantine, on the other hand, only blocks the channel under conditions of excessive NMDA-ion channel opening (Bormann, 1989; Blanpied et al., 1997), such that physiological glutamatergic activity essential for normal neuronal functioning is allowed to continue. This property makes the drug much less toxic and thus more clinically usable (Lipton & Chen, 2004). Consequently, memantine has found use in neurological disorders characterized by excessive glutamate activity, such as Alzheimer's disease (AD; Cosman et al., 2007). Another promising approach to NMDA receptor modulation is through partial agonism. The antituberculosis drug, D-cycloserine, acts as a partial NMDA receptor agonist, cross-reacting with the glycine site in the NMDA ion channel (Hood et al., 1989). In this way, in the presence of low glycine levels, D-cycloserine facilitates NMDA receptor functioning with up to 60% of the efficacy of glycine or D-serine, while NMDA receptor functioning might be reduced by between 40% and 50% when glycine binding is maximal (Emmett et al., 1991; Hood et al., 1989; Watson et al., 1990). Indirect stimulation of the high affinity glycine binding site of the NMDA receptor complex therefore represents a less problematic way of enhancing NMDA glutamate receptor function (D'Souza et al., 1995).

Recently, preclinical and clinical studies have suggested that modulation of the NMDA ion channel by targeting the zinc binding site within the channel has important psychotropic actions in animals and humans. Indeed, zinc has antidepressant actions in humans and similar actions in animals (Nowak, 2001; Nowak et al., 2005). This action may, along the same lines as the above-mentioned NMDA channel modulators, have potential in treating other neuropsychiatric illnesses involving excessive glutamate function. However, no studies to date have investigated the value of zinc in treating PTSD. Considering the above, it can be concluded that NMDA receptor modulation by targeting zinc, using open-channel blockers, or by indirect stimulation of the high affinity glycine binding site, are attractive mechanisms for targeting the NMDA receptor.

However, glutamate receptors comprise three other families, namely α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainic acid (KA; Hollmann & Heinemann, 1994) and metabotropic (Du et al., 2004; Zarate et al., 2003) receptors (*refer to **figure 1***), the physiological and clinical importance of which are now becoming clear. Of importance here is that AMPA and NMDA receptors enjoy a closely knit relationship (see Rao & Finkbeiner, 2007 for review; Michaelis, 1998), such that targeting AMPA receptors may be an alternate approach to bolster/maintain normal functioning of the NMDA receptor without evoking excessive glutamatergic activity. One such approach that has recently been developed are the ampakines, compounds that potentiate AMPA transmission without having agonist or antagonist effects at the AMPA binding site (Suppiramaniam et al., 2001; Lynch & Gall, 2006). Rather, these allosteric modulators act by enhancing AMPA-receptor-mediated neurotransmission by reducing desensitization of the ion channel (Lynch & Gall, 2006) such that following the release of glutamate; they stabilize the receptor in its channel-open state. This prolongs current flow through the receptor complex leading to an enhanced and sustained synaptic response (Lynch & Gall, 2006) resulting in the strengthening of NMDA receptor activity (Tanaka et al., 2000; Tomita et al., 2005).

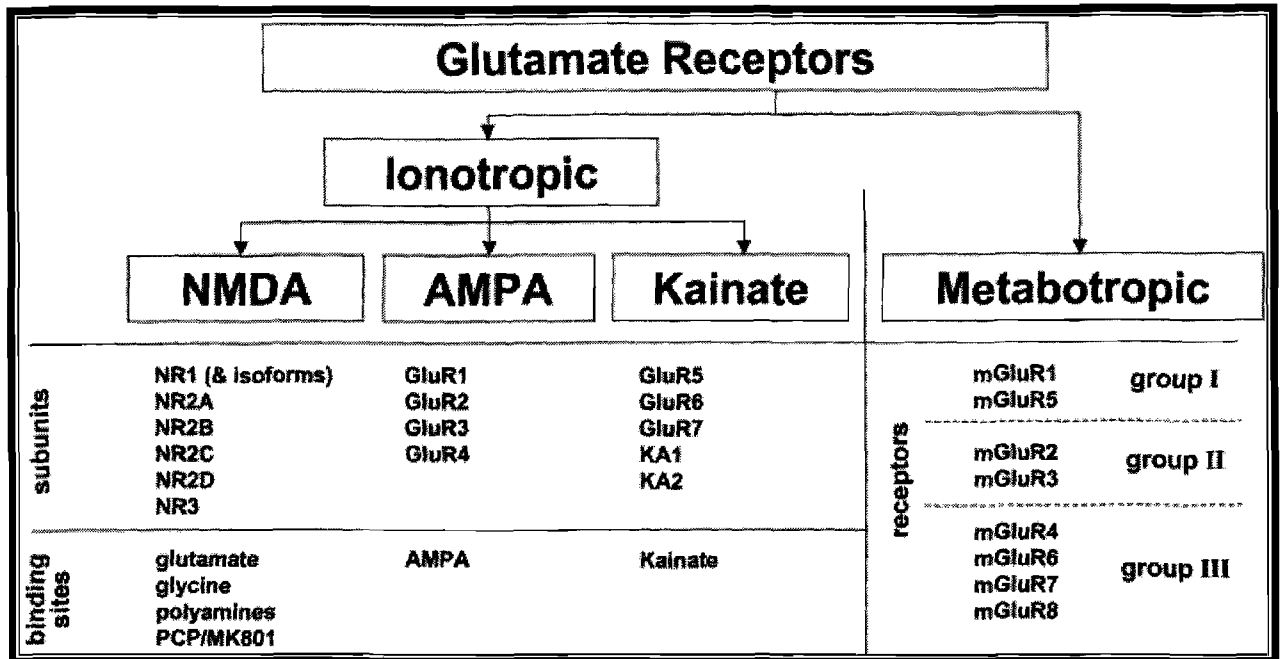


Figure 1: Subtypes of glutamate receptors, indicating subunit composition and ligand binding sites (Meador-Woodruff & Kleinman, 2002).

Using ampakines as a pharmacological treatment modality for PTSD, or any other neuropsychiatric disorder, implies that they will be used to increase glutamate activity in conditions where hypoglutamatergia prevails. However, depression, and possibly PTSD, has been more associated with increased glutamate activity, requiring that pharmacological treatment attenuates rather than bolsters function at glutamatergic synapses (Skolnick 1999; Berman et al., 2000; Zarate et al., 2003). Considering that ampakines essentially bolster NMDA receptor activity, the use of positive AMPA receptor allosteric modulators to treat PTSD would seem to be contradictory. Nevertheless, ampakines have demonstrated antidepressant-like effects in rodents using the forced swim test and tail suspension test (Bai et al., 2001; Li et al., 2003), while they too have shown synergistic effects with clinically effective antipsychotics in the treatment of schizophrenia (Johnson et al., 1999). Importantly, ampakines are able to stimulate memory-dependent processes in animals (Rivadulla et al., 2001). Since cognitive disturbances are an important component of depression, this action may contribute to their antidepressant effects. Although not yet established, this same action may offer benefit in treating the cognitive disturbances evident in PTSD.

The AMPA receptor is also involved in anxiety, with antagonists of this receptor found to possess anxiogenic-like effects (Karcz & Liljequist, 1995). More importantly, ampakines have been shown to increase the levels of brain-derived neurotrophic factor (BDNF) *in vitro* (Legutko et al., 2001) and *in vivo* (Mackowiak et al., 2002; Dicou et al., 2003). Stress, and indeed depression, has been associated with a reduction in BDNF possibly via the actions of dysregulated cortisol, while antidepressant treatments are known to reverse these effects (Nibuya et al., 1995; Chen et al., 2001). Increases in BDNF are now more and more being realized as a critically important molecular mechanism of action of antidepressants (Alt et al., 2006).

Attempts to reconcile the antidepressant effects resulting from NMDA receptor blockade and/or AMPA receptor potentiation have led to the hypothesis that perhaps the effects of NMDA receptor block may at least partly be mediated by indirect enhancement of glutamatergic tone at AMPA receptors, which exerts a subsequent "toning down" of NMDA receptor function. Indeed it has been shown that pre-treatment with NBQX [2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline], an AMPA receptor antagonist, attenuates both NMDA receptor antagonism-induced antidepressant-like behaviour and regulation of GluR1 AMPA receptor phosphorylation (Maeng et al., 2008). These same authors conclude that NMDA antagonists might exert rapid antidepressant-like effects by enhancing AMPA relative to NMDA throughput in critical neuronal circuits. This evidence strongly suggests that ampakines may represent a novel approach to treating depression, but also other mental illnesses characterised by deficits in cognition. The remainder of this chapter will discuss the growing importance of glutamate in stress-related disorders, with special interest in the development, clinical presentation, neurobiology and treatment of PTSD.

2. Characteristics of PTSD

PTSD is an anxiety disorder affecting cognitive function, with intrusive memories considered as the hallmark symptom (Holmes, Grey, & Young, 2005). The main characteristics of PTSD are discussed below.

2.1. Intrusions

Individuals with PTSD experience symptoms like flashbacks, nightmares or feelings of distress when they encounter reminders of the traumatic event (APA, 2000). Flashbacks occur when memories of the trauma recur causing distress to the patient. The re-experiencing symptoms often result in patients seeking psychological help (Steil & Ehlers, 2000). In PTSD, traumatic re-experiencing is reconsolidated into memory, such that intrusive recollections of the trauma persist long after the initial trauma (Brewin, 2001; Michael et al., 2005 a, b). These intrusions are also often sensory cues related to the original trauma, e.g. petrol fumes, smoke, alarm whistles, metallic taste etc. These sensory cues take the form of visual, auditory, olfactory and gustatory “flashbacks” that enhance fear memory consolidation (Brand et al., 2008).

2.2. Hyperarousal

High levels of anxiety cause specific symptoms of PTSD, including irritability, outbursts of anger, concentration problems, disrupted sleeping patterns and increased startle (APA, 2000). The sympathetic nervous system (SNS) is activated during stress and its arousal results in increased heart rate and respiration, raised blood pressure, dilated pupils and cold, pale skin.

2.3. Avoidance

PTSD patients avoid thinking about the traumatic event and reminders of the event are avoided. Decreased concentration and amnesia are also experienced (APA, 2000).

2.4. Cognitive impairment

PTSD is characterized by cognitive impairment and in particular working memory dysfunction (Weber et al., 2005). Working memory is defined as short-term or recent memory. This system stores and manages information required to carry out complex cognitive tasks such as learning, reasoning and comprehension (Baddeley, 1998). Persistent interference by traumatic memories may be the reason for the poor performance of PTSD patients in working memory tests (Vasterling et al., 1998, 2002).

3. Diagnostic criteria for PTSD

Individuals exposed to a life-threatening event and that go on to develop symptoms of intrusions, hyperarousal and avoidance are candidates for the diagnosis of PTSD. The American Psychiatry Association has revised the diagnostic criteria for PTSD in the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR). The diagnostic criteria, as described below, have been reproduced from the DSM-IV-TR (APA, 2000):

DSM-IV-TR Criteria for PTSD

A. The person has been exposed to a traumatic event in which both of the following were present:

- the person experienced, witnessed, or was confronted with an event or events that involved actual or threatened death or serious injury, or a threat to the physical integrity of self or others.
- the person's response involved intense fear, helplessness, or horror.

B. The traumatic event is persistently re-experienced in one (or more) of the following ways:

- recurrent and intrusive distressing recollections of the event, including images, thoughts, or perceptions
- recurrent distressing dreams of the event
- acting or feeling as if the traumatic event were recurring (includes a sense of reliving the experience, illusions, hallucinations, and dissociative flashback episodes, including those that occur on awakening or when intoxicated)
- intense psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event
- physiological reactivity on exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event

C. Persistent avoidance of stimuli associated with the trauma and numbing of general responsiveness (not present before the trauma), as indicated by three (or more) of the following:

- efforts to avoid thoughts, feelings, or conversations associated with the trauma
- efforts to avoid activities, places, or people that arouse recollections of the trauma
- inability to recall an important aspect of the trauma
- markedly diminished interest or participation in significant activities
- feeling of detachment or estrangement from others
- restricted range of affect (e.g., unable to have loving feelings)
- sense of a foreshortened future (e.g., does not expect to have a career, marriage, children, or a normal life span)

D. Persistent symptoms of increased arousal (not present before the trauma), as indicated by two (or more) of the following:

- difficulty falling or staying asleep
- irritability or outbursts of anger
- difficulty concentrating
- hypervigilance
- exaggerated startle response

E. Duration of the disturbance (symptoms in Criteria B, C, and D) is more than 1 month.

F. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

4. Aetiology of PTSD

Aetiology is the study of the causes or origin, as well as the factors which produce or predispose toward a disorder or disease.

The primary causal factor for PTSD is the exposure to a life-threatening traumatic event. However, evidence for neurobiological deficits may be present in individuals at risk of PTSD (Gurvits et al., 2000). It has for example been suggested that a smaller hippocampus, rather than stress-induced hippocampal shrinkage, may play a more prominent role in developing PTSD (Gilbertson et al., 2002). Similarly, a causal role for glucocorticoids and a malfunctioning hypothalamic-pituitary-adrenal (HPA)-axis in PTSD are widely accepted, although its exact contribution remains an area of intense research (Baker et al., 1999; Liberzon et al., 1999a; Yehuda et al., 2000). Aversive memories are also believed to play an important role in the pathology and symptomatology of PTSD (Brewin, 2001; Michael et al., 2005a, b), contributing to repeated re-experiencing of the event.

5. Quality of life in PTSD

The impact of an anxiety disorder influences not only the general well-being of the patient, but also the patient's social activities (Cuthbert, 2002; Yehuda, 2002). A study by Leserman and colleagues found that patients seeking medical help, specifically for pelvic pain, often have a history of a lifetime trauma (Leserman et al., 2006). Indeed, a definite correlation exists between physical health problems of the patient and symptoms of hyperarousal and avoidance (Woods & Wineman, 2004).

Comorbidity, or the presence of one or more disorder/disease in addition to the primary disorder, is common amongst individuals with PTSD. Irritable bowel syndrome (IBS) is a very common gastro-intestinal problem, with about one-third of PTSD patients diagnosed with the syndrome (Dobie et al., 2004; Irwin et al., 1996). Sleep disorders too are highly prevalent in PTSD. In fact 50% to 70% of PTSD patients experience disturbed sleeping patterns (Leskin et al., 2002), with 40% to 50% of PTSD patients experience insomnia (Ohayon et al., 2000). Affective disorders, anxiety disorders, depression, alcohol (Brady et al., 2000b), and substance abuse are frequently associated with PTSD patients (Kessler et al., 1995; Breslau et al., 1998; Creamer et al., 2001). Major depression is comorbid in 30% to 50% of patients suffering from PTSD (Shalev, 2001).

6. Pathophysiology of PTSD

Pathophysiology is the study of functional changes associated with or resulting from a condition, for instance PTSD.

Structural brain changes, especially of the hippocampus, is one of the main functional changes caused by increased levels of glucocorticoids and repeated stressful situations (McEwan, 2000; Sapolsky, 1992). Imaging studies have established functional relationships between the hippocampus, amygdala and frontal cortex of individuals with PTSD (Shin et al., 2006; Bremner, 2005). Magnetic resonance

imaging (MRI) studies in PTSD patients following different forms of trauma have shown significant decreases in hippocampal volume (Villarreal et al., 2002; Stein et al., 1997; Bremner et al., 1997). Damage to the hippocampus will have a profound negative effect on the homeostasis of the stress response, particularly through disruption of negative feedback onto the HPA-axis resulting in increased exposure of the hippocampus to toxic levels of glucocorticoids (Sapolsky 2000a, b). The functional inability of an abnormal hippocampus may result in a maladaptive reaction to a stress response and development of PTSD over time. However, while some studies have described decreased hippocampal volumes (Bremner et al., 1995b; Stein et al., 1997), other studies did not (Pederson et al., 2004; Schuff et al., 2001). In fact, separate studies have concluded that a smaller hippocampus may predispose to the development of chronic PTSD (Gilbertson et al., 2002; Bonne et al., 2001). Thus, hippocampal shrinkage may be a cause and not an effect of PTSD.

However, other structural brain changes have been found in PTSD, including significantly smaller anterior cingulate cortex and amygdala volumes (Karl et al., 2006). Functional neuroimaging studies in PTSD patients have revealed increased activity in the prefrontal cortex (PFC), amygdala and hippocampus (Francati et al., 2007; Nemeroff et al., 2006; Hull, 2002). The increased activity of the amygdala has been associated with retrieval of aversive memories (Bremner, 2007) and enhanced activation of the sympathetic nervous system (McGaugh & Roozendaal, 2002). Thus, PTSD can be viewed as an illness of the limbic system, involving the hippocampus, hypothalamus, the amygdala and the PFC. Their neuroanatomy will be discussed below.

7. Neuroanatomy of PTSD

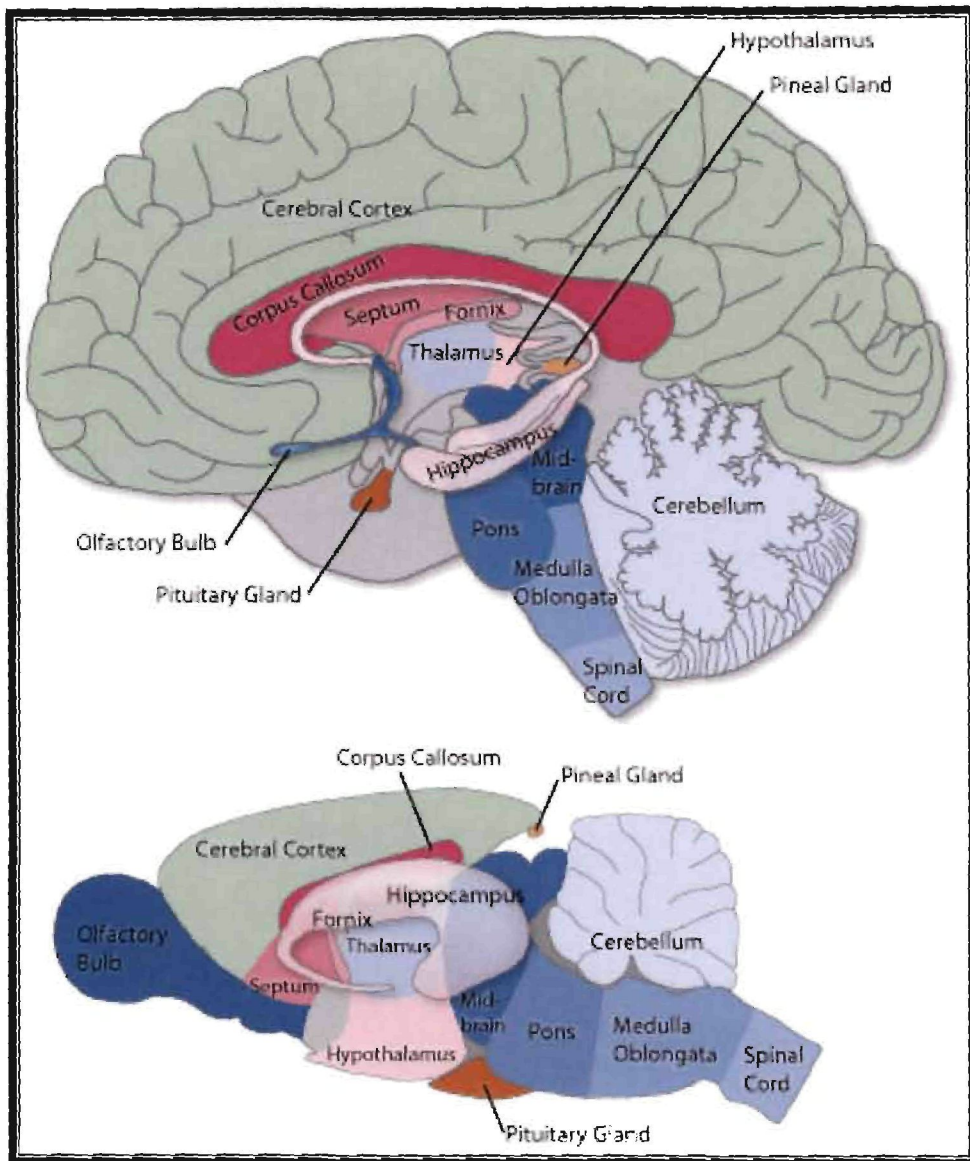


Figure 2: Anatomy of the human brain (top) and rat brain (bottom). (Genetic Science Learning Center, 2008).

7.1. Limbic system

The limbic system regulates survival behaviour and emotional responses, and includes various subcortical brain structures, but particularly the hypothalamus, hippocampus and amygdala. The endocrine system and autonomic nervous system

(ANS) are in close relationship with the limbic system, a diffuse area of the brain also known as the emotional brain due to its important contributions towards emotion, behaviour and long-term memory. Because of this association, disorders of the limbic system most often presents with anxiety, moodiness, irritability and clinical depression (Amen & Routh, 2003). The limbic brain structures are closely connected to the PFC, such that the limbic-cortical loop provides an important regulatory component over the expression of behavioural responses to environmental events (Grace, 2000).

7.1.1. Hypothalamus

The hypothalamus is responsible for integrating endocrine functioning, autonomic responses and behaviour (Saper, 1997; Swaab, 1997) via the hypophysis (pituitary gland). It secretes various releasing or inhibiting hormones to control anterior and posterior pituitary gland hormone secretion. These include corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), gonadotropin-releasing hormone (GnRH), growth hormone releasing hormone (GHRH), somatostatin and dopamine that together modulate various anterior lobe function, such as secretion of growth hormone (GH), adrenocorticotropin (ACTH), thyroid stimulating hormone (TSH), prolactin (PRL), luteinizing hormone (LH) and follicle stimulating hormone (FSH). On the other hand, antidiuretic hormone (ADH or vasopressin) and oxytocin (OXT) are secreted by the posterior pituitary after being synthesized by neurons in the supraoptic nuclei (SON) and paraventricular nuclei (PVN; Campbell, 2008). OXT inhibits the release of ACTH (Legros, 2001).

The hypothalamus controls various central effects such as stress-related behaviour and mood, appetite, respiration and cardiovascular regulation (Lehnert et al., 1998). The hypothalamus is situated below the thalamus and above the brain stem (midbrain, pons, medulla oblongata and spinal cord) (*refer to **figure 2***) where hypothalamic-releasing hormones are synthesized and secreted (Bartness et al.,

2001; Buijs & Kalsbeek, 2001). CRH is one such hormone, and functions to regulate the anterior lobe of the pituitary gland. CRH stimulates the secretion of ACTH from the pituitary gland which acts on the adrenal cortices to produce glucocorticoid hormones, the most important being cortisol. The production of CRH and ACTH are inhibited by negative feedback actions of the glucocorticoids on the hypothalamus and the pituitary (Reul & de Kloet, 1985). Arginine vasopressin (AVP), expressed by a part of the CRH neurons in the hypothalamic paraventricular nucleus (PVN), strongly potentiates the release of ACTH (Gillies et al., 1982; Rivier & Vale., 1983). The increase of ACTH during acute stress is caused by CRH, while AVP is responsible for increased ACTH release in chronic stress (Scott & Dinan, 1998).

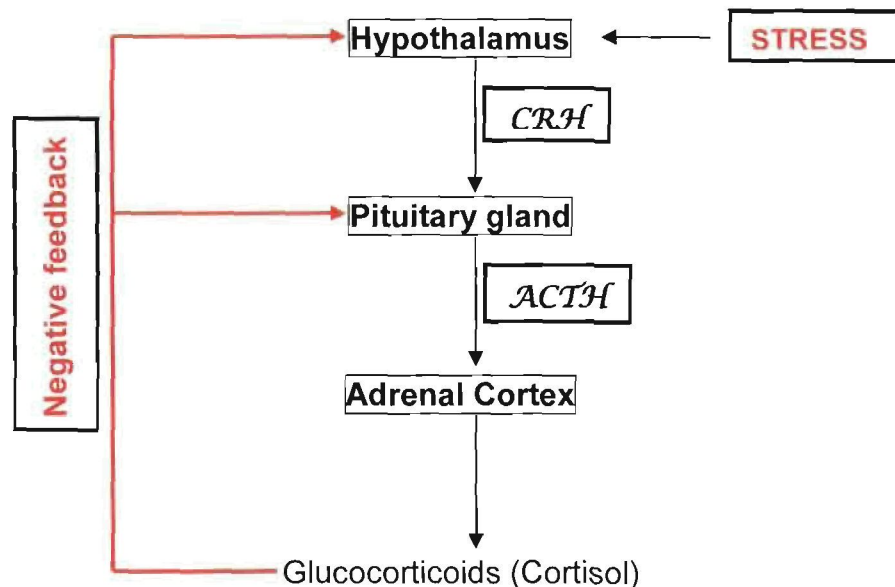


Figure 3: HPA-axis response to stress, indicating the negative feedback system mediated by peripheral cortisol on hypothalamic and pituitary function. (Adapted from Schimmer & Parker, 2008).

The HPA-axis controls the reactions to stress and regulates mood and emotion. The medial parvocellular region of the PVN controls the HPA-axis (Antoni, 1986; Whitnall, 1993). Studies have shown PTSD patients have increased CRH levels in cerebrospinal fluid (CSF; Baker et al., 1999; Bremner et al., 1997b) while diurnal plasma cortisol levels are decreased (Bremner et al, 2003) indicating the presence of enhanced HPA-axis negative feedback. Chronic stress in animals results in the

inability of animals to terminate the glucocorticoid response to stress (Sapolsky et al., 1984a, b) and a maladaptive feedback response of the HPA-axis by glucocorticoids (Young et al., 1990) (*refer to figure 3*).

Stressful events have effects on several of the hypothalamic hormones contributing to the symptoms observed in PTSD. Song and co-workers revealed significantly higher serum GH and cortisol levels amongst PTSD patients (Song et al., 2008). PTSD patients also have elevated plasma levels of AVP (De Kloet et al., 2008), which are correlated with avoidance symptoms in these patients. The secretion of PRL and OXT is also altered during stress (Freeman et al., 2000; Wotjak et al., 2001), while the reproductive system too is suppressed; CRH prevents the release of hypothalamic luteinizing hormone-releasing hormones (LHRH) and LH-induced ovulation and sperm release is inhibited by cortisol and other glucocorticoid hormones. The testes and ovaries are directly inhibited by glucocorticoids, interfering with the production of male and female sex hormones (Swaab, 2003).

7.1.2. Hippocampus

The hippocampus is one of the most important brain structures for learning and memory (Burgess et al., 2002; Eichenbaum & Cohen, 2001) and is part of the forebrain, located in the medial temporal lobe (*refer to figure 4a*). The hippocampus is formed by two folded layers of neurons, the cornu ammonis (CA) and the dentate gyrus (DG). The cornu ammonis is further divided into the CA1 and CA2/3 regions (see Yushkevich et al., 2008 for review). The hippocampus is important for memory functions such as encoding of declarative memory (specifically episodic memory) (Eichenbaum & Cohen, 2001). The hippocampus also plays a key role in both encoding and retrieval of long-term memory (Squire et al., 2004; Frankland & Bontempi, 2005). The hippocampus plays a time-limited role in the consolidation of new memories into stable long-term memory (Wittenberg & Tsien, 2002). Thus, in for example animals that have undergone training of a memory task, lesions of the

hippocampus made a day after training causes significant retrieval impairment, but when made weeks after learning, lesioning has no effect (Takehara et al., 2003).

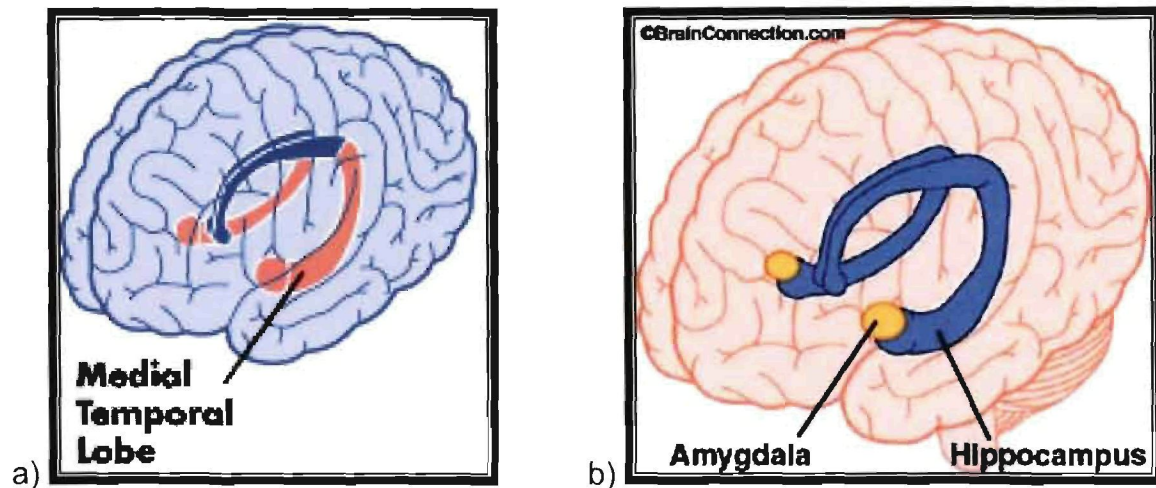


Figure 4: a) Location of the medial temporal lobe and b) the hippocampus and amygdala. (Medical Care Corporation, 2008; Posit Science Corporation, 2008).

As has been alluded to earlier, PTSD is associated with atrophy of the hippocampus with consequent memory deficits, such as fragmentation of memories and trauma related amnesia (Elzinga & Bremner, 2002). The hippocampus contains a high concentration of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) regulating the HPA-axis by glucocorticoid negative feedback (Herman et al., 2005; Jacobson & Sapolsky, 1991). Hippocampal damage is associated with disruption of this negative feedback system and an increased exposure of the hippocampus to toxic levels of glucocorticoids (Sapolsky 2000a, b). Lupien and colleagues (1998) revealed that hippocampal atrophy was associated with higher basal cortisol levels and a greater increase of cortisol levels over time (Lupien et al., 1998).

Cortisol levels are at its lowest between 20:00 and 02:00, increasing as time progresses until the highest level is reached shortly after awakening. Cortisol levels decrease during the day in the absence of external stressors (Pruessner et al., 1997). GR's are involved in the recovery after stress and enables the system to respond to future encounters; any deviation from this natural rhythm will mean excessive GR receptor exposure during periods normally associated with lower circulating cortisol

levels (De Kloet & Derijk, 2004). The MR/GR dual receptor system is crucial for maintaining homeostasis during stress responses through the regulation of the HPA-axis negative feedback system (De Kloet, 2000). Dysfunctioning of these receptors during stress will result in disruption of the homeostasis and may result in psychiatric disorders such as depression (Sapolsky, 2000b; Yehuda, 2001). MR's are activated at low levels of glucocorticoids and are associated with stress-induced hippocampal changes and impairment of neurogenesis (Gass et al., 2000). The sensitivity of GR's are increased in the presence of low cortisol levels, resulting in enhanced negative feedback and further lowering of cortisol levels (Yehuda, 2003) (*refer to **figure 3***). Cognitive performance is remarkably compromised by excessive glucocorticoids (Het et al, 2005; Sauro et al, 2003), and mediated by their atrophic effects on the hippocampus (Lupien et al., 1998). For example in patients with Cushing's disease, where plasma cortisol levels are very high, there is a significant degree of cognitive impairment as well as reduction in hippocampal volume (see Harvey et al, 2003 for review; Starkman et al., 1992).

7.1.3. Amygdala

The amygdalae are two almond shaped structures located on either side of the thalamus at the lower end of the hippocampus (*refer to **Figure 4b***). The amygdala controls emotional memories (Phelps & LeDoux, 2005) and coordinates the response of the human body to fear (Öhman, 2005). The amygdala responds to emotional stimuli and sends impulses to the hypothalamus, activating the SNS (McGaugh & Roozendaal, 2002).

The role of the amygdala in the formation and retrieval of aversive memories highlights its importance in the development of PTSD symptoms (Bremner, 2007; Shin et al, 2006). The amygdala modulates memory-related processing in the hippocampus and frontal cortex (Kilpatrick & Cahill, 2003; Richter-Levin & Akirav, 2000), modulating consolidation of hippocampal-dependent memories through

activation of adrenergic receptors in the basolateral amygdala (McGaugh & Roozendaal, 2002). Studies of traumatic reminder exposure as measured with positron-emission tomography (PET) or single photon-emission computed tomography (SPECT) have revealed increased functioning of the amygdala in PTSD (Liberzon et al., 1999b; Shin et al., 2004; Rauch et al., 1996). A correlation between increased amygdala functioning and decreased medial prefrontal cortex (mPFC) functioning with traumatic reminders, indicates failure by the mPFC to inhibit the amygdala (Shin et al., 2004), possibly explaining the escalation in classical PTSD symptoms.

7.1.4. Prefrontal cortex (PFC)

The PFC is vital for working memory functions (Curtis & D'Esposito, 2003; Kane & Engle, 2002). The mPFC also plays an important role in inhibiting the amygdala, such that any impairment of the mPFC will lead to enhanced activation and functioning of the amygdala (Shin et al., 2004). Indeed, neuroimaging studies have shown abnormal working memory systems in PTSD patients (Shaw et al., 2002; Clark et al., 2003) as well as impaired PFC functioning together with an overactive amygdala (Bremner, 2002). Exposure to stress increases catecholamines in the PFC (Goldstein et al., 1996), with NA-mediated stimulation of $\alpha 1$ receptors in the PFC resulting in impaired PFC functioning (Birnbaum et al., 1999). In agreement with this, the $\alpha 1$ adrenoreceptor inhibitor prazosin strengthens the inhibitory actions of the PFC on the amygdala and is effective in reducing PTSD symptoms, such as intrusive thoughts and flashbacks (Raskind et al., 2000, 2003; Taylor & Raskind, 2002; Taylor et al., 2006). On the other hand, $\alpha 2A$ adrenoreceptor stimulation plays a role in cognitive enhancement (see Arnsten et al., 1996 for review). However, it is not only adrenergic signaling that plays a role in PFC functioning, but also glutamate, glycine, dopamine (DA), serotonin (5-HT) and acetylcholine (ACh), which all need careful consideration when studying the neurobiological basis of PFC function (see Arnsten & Robbins, 2002 for review).

8. Stress response

The limbic system functions in a close relationship with the SNS and parasympathetic nervous system (PNS). Indeed, patients with PTSD present with various anomalies of the autonomic nervous system, including increased skin conduction (measuring conductivity of sweat released upon emotional stimuli), increased heart rate, raised blood pressure and anxiety (Yehuda & Harvey, 1997). The limbic system responds to a traumatic threat by releasing various hormones including activation of the SNS, which serve to prepare the body for the fight or flight reaction (Rothschild, 1996). Acute stress activates the HPA-axis, resulting in the release of adrenaline and glucocorticoids from the adrenal gland (Jacobson & Sapolsky, 1991). These stress hormones not only are important in the immediate preparation of the body for dealing with the perceived threat, but also in the facilitation and consolidation of strong emotional memories in the hippocampus and amygdala (Reul & de Kloet, 1985).

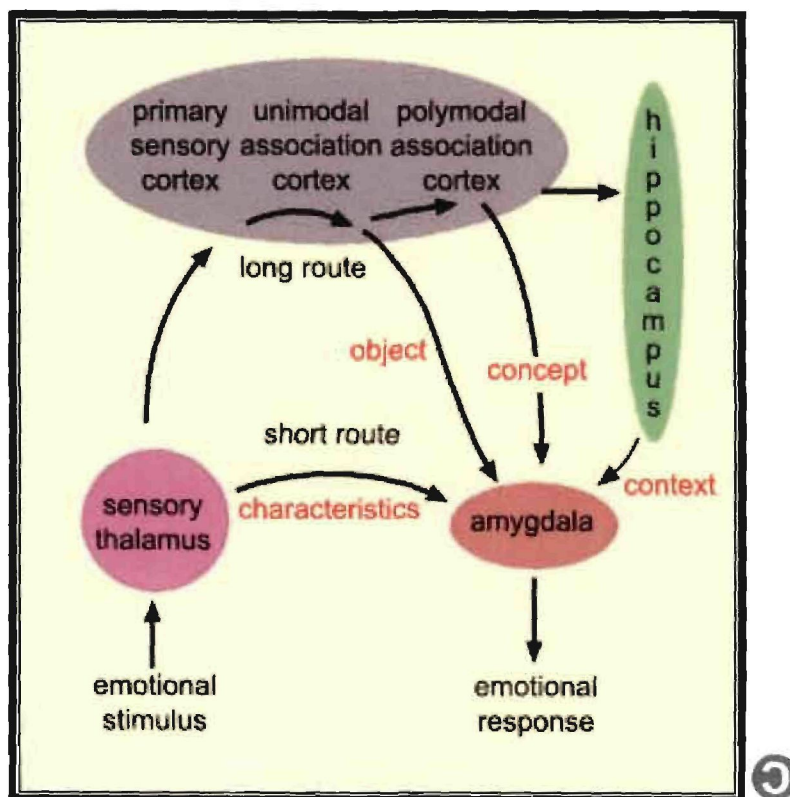


Figure 5: The pathways of fear (Dubuc, 2008). Refer to the text for a detailed description.

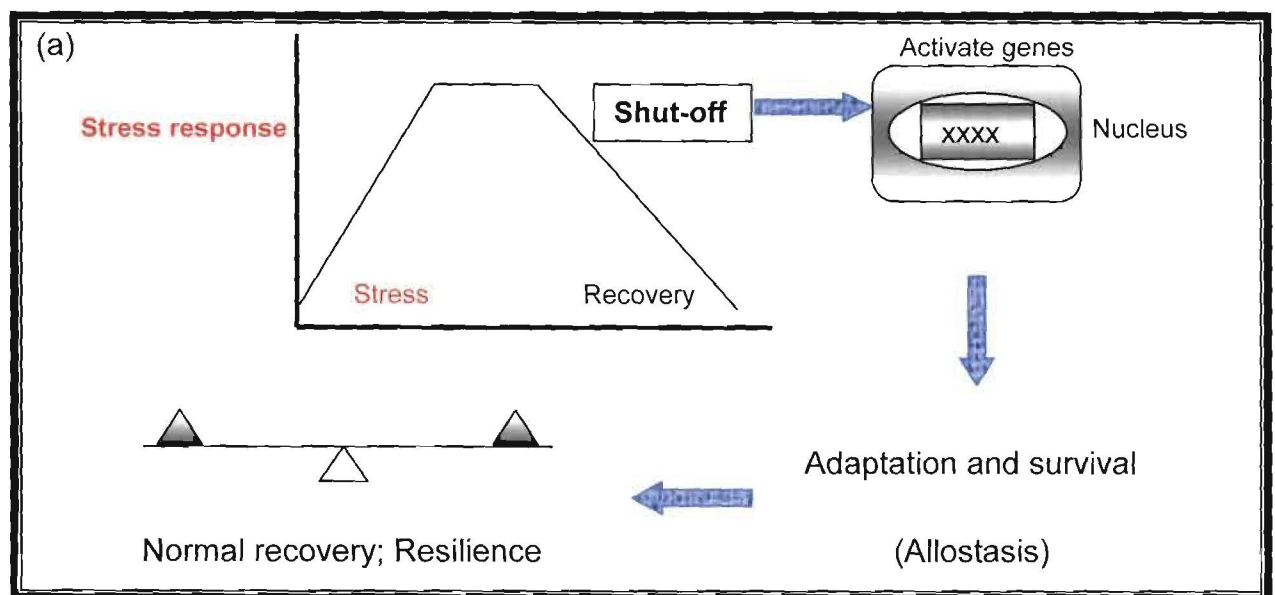
Stimuli received by the brain during a fearful event are directed to the thalamus (*refer to figure 5*). Information can now be sent via either the thalamo-amygdala pathway (short route; LeDoux, 1990) or the thalamo-cortico-amygdala pathway (long route; Adolphs, 2002; LeDoux, 1998). The thalamo-amygdala pathway is a sub-cortical pathway that provides a fast and rough impression of the situation without involvement of cognition. In this way, the amygdala is activated generating an emotional response before a full perception of the stimuli is performed (Armony & LeDoux, 2000). Information following the thalamo-cortico-amygdala pathway involves cortical cognitive processing of the event, and is aimed at distinguishing whether it is a real threat, before it reaches the amygdala (Adolphs, 2002). These two stimuli are then compared within the context of explicit memory stored in the hippocampus. The hippocampus encodes the context associated with prior aversive experiences (Dubuc, 2008).

Abnormalities in any of these interactive brain structures may produce fear-related symptoms associated with psychotic and anxiety disorders (Rauch et al., 2000; Williams et al., 2004). Impaired PFC functioning, an overactive amygdala (Bremner, 2002; Rauch et al., 2000) as well as deficits in the declarative memory process (Shaw et al., 2002; Clark et al., 2003) provide a unique neuro-anatomical basis for the bio-behavioural changes that are seen in PTSD patients.

Chronic stress and prolonged exposure to elevated glucocorticoid levels and dysfunction of the HPA-axis are known to be contributing pathologies for major depressive disorder and PTSD (Reagan & McEwen, 1997). However, the manner of HPA-axis involvement is different, with depression characterised by a hypercortisolemia (De Winter et al., 2003), while PTSD appears ambiguous in this regard with raised (Liberzon et al., 1999b), unchanged (Baker et al., 1999) as well as low cortisol (Yehuda et al., 2000) levels documented. The manner in which animals deal with stress are determined by the HPA-axis and the monoaminergic-sympathetic nervous system (Heuser & Lammers, 2003). However, it is the nature and duration of the stressor that ultimately determines the neuropsychiatric illness that eventually develops (De Kloet, 2003; De Kloet et al., 2005; McEwen, 2002, 2004).

Psychiatric illnesses are the result of maladaptive responses to stress, where individual vulnerability to stress plays a pre-emptive role in determining the impact of the stressor on body and mind, and the later development of an anxiety or mood disorder (Kendler et al., 2001). Central to this is dysregulation of stress hormones and failed termination of the activated HPA-axis response following cessation of the stressor (De Kloet et al, 2005; McEwen, 2004). McEwen (McEwen & Wingfield, 2003) has proposed a process of allostasis and allostatic load that determines how an individual deals with a stressful condition and whether the person will succumb to the stressor and go on to develop psychopathology.

This process is described in **figure 6a-b**.



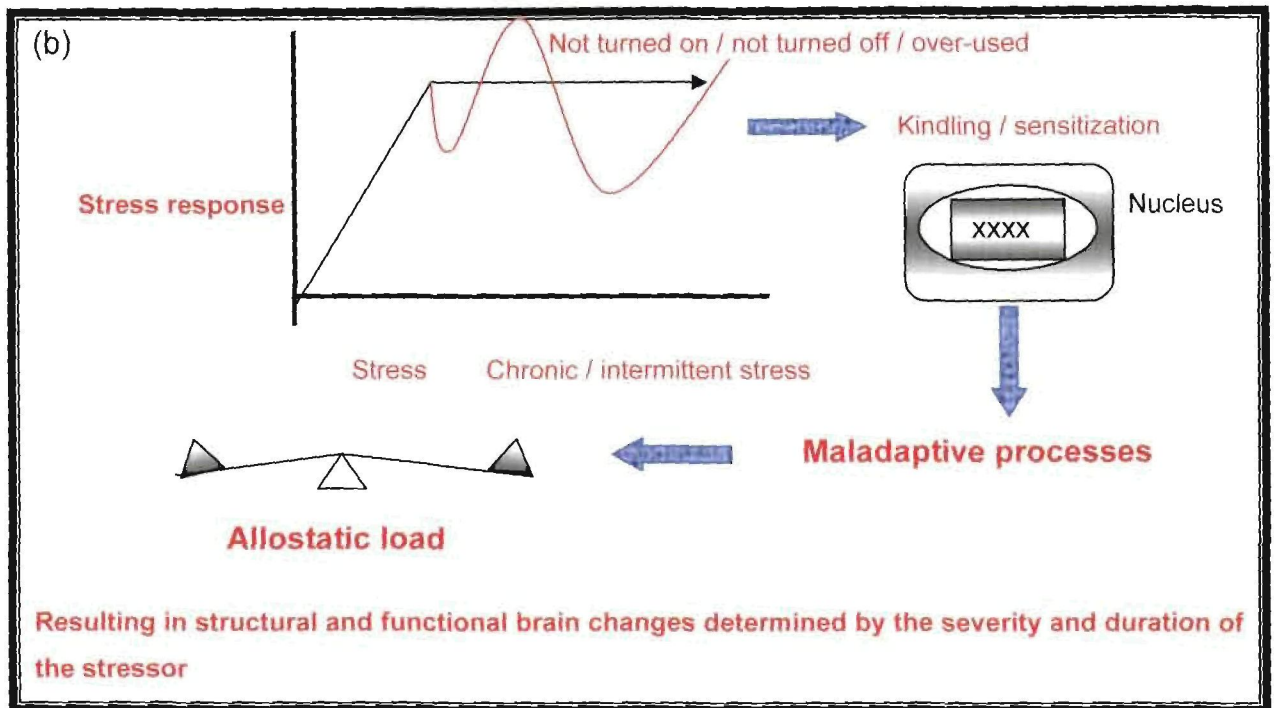


Figure 6: The stress response with normal recovery in healthy individuals (a) and in susceptible individuals that later develop a maladaptive response (b) [McEwen, 2004]. Refer to the text for detailed description.

Following exposure to a severe aversive event, the body initiates a series of adaptive mechanisms that allow the individual to cope. This is a natural response and is referred to as allostasis. Allostasis describes the biological and psychological processes involved in acquiring a learned adaptive response necessary for survival in the face of adversity. This involves mounting an adequate response to stress and thereafter initiating such a natural shut-off once the stressor has passed. However, individual susceptibility to stress, which may be ascribed to a variable combination of gene-environmental considerations, will prevent such a natural beneficial response. The result is that the stress response is either not turned on, or not turned off or in fact over-used, and is termed allostatic load. This excessive “loading” over time eventually leads to maladaptive responses and a lack of coping that ultimately culminates in stress-related illnesses such as PTSD. A great deal of research is now being focussed on the biological underpinnings of *resilience*, the ability of a person to

absorb stress, and *susceptibility*, the state of being vulnerable to stress and liable to develop a stress-related disorder (McEwen & Wingfield, 2007).

As suggested in **figure 6**, successful handling of an environmental stressor requires the appropriate shutting off of the stress response following cessation of the aversive event via negative feedback inhibition of cortisol on the HPA axis (Heuser & Lammers, 2003; De Kloet et al., 2005). Failure to do this, for example if the HPA-axis response to stress is not turned on, is not switched off or is over-used, will lead to an ongoing stress response that is inappropriate, culminating in a maladaptive response and eventually psychopathology. In PTSD this is typically seen as hyperarousal behaviours, such as irritability, outbursts of anger, concentration problems, disrupted sleeping patterns and increased startle (APA, 2000). Apart from the maladaptive behaviours that develop, maladaptive neuroendocrine responses are also observed, including hyper- and hypocortisolemia (Liberzon et al., 1999b; Yehuda et al., 2000) as well as altered levels of monoamines and other neurotransmitters (Goldstein et al., 1996). Clearly, stress and stress-related disorders are characterised by unique neurochemical profiles. The neurochemistry of PTSD will now be described.

9. Neurochemistry of PTSD

Stressful responses involve a cascade of neurotransmitter-receptor interactions. However, it should be recognised that PTSD is not simply an anxiety disorder that develops after exposure to an acute severe stressor, but rather is a bio-behavioural syndrome that follows an initial severe trauma but that is perpetuated through continued re-experiencing via flashbacks as well as sensory and other contextual reminders of the original trauma. These cues essentially drive a maladaptive response in the absence of the original trauma (Charney et al., 1995). Extensive research has studied the effects of acute stress on glutamate, gamma aminobutyric acid (GABA), NA, DA and 5-HT release in the hippocampus and other brain regions involved in the stress response (Harvey et al., 2006). However, recent evidence

suggests that re-exposure to a stressful situation, which represents a hallmark characteristic of PTSD, elicits a different neuroendocrine response that may underlie the development of unique behavioural pathology (Harvey et al, 2006). Stress and restress also has important effects on the glutamate-GABA systems (Harvey et al, 2004b). Together, acute stress-evoked changes on select monoamines and glutamate, and subsequently modified with re-experience as in PTSD, will together mediate the behavioural pathology as well as other diverse changes typical of the disorder, such as gradual degenerative effects on the hippocampus. It is thus necessary to review the effects of acute stress in the presence and absence of reminders on brain neurochemistry, as this may tell a tale that is unique to PTSD.

9.1. Amino acids

Glutamate and GABA are synthesized in the brain from α -ketoglutarate, with glutamine as the common precursor for the synthesis of both glutamate and GABA (refer to **figure 7**).

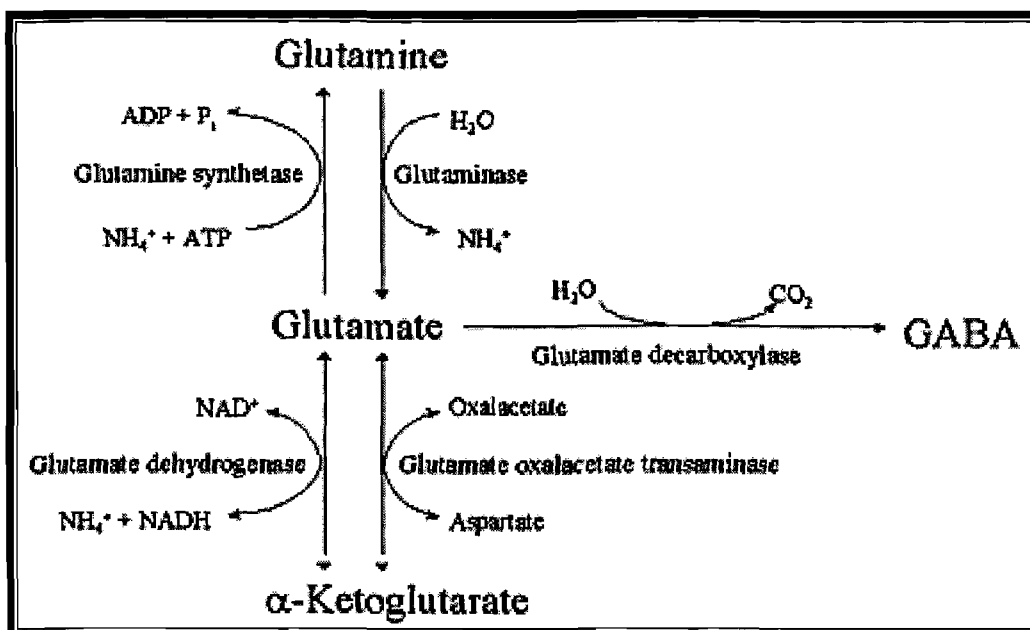


Figure 7: Synthesis of glutamate and GABA (Palmada & Centelles, 1998).

9.1.1. Glutamate and glutamate receptors

Glutamate is the main excitatory amino acid in the brain (Nieuwenhuys, 1994) and plays an important role in memory and learning (Riedel et al., 2003). However, regulation of glutamate levels between a narrow physiological window is critical, since too low levels of glutamate negatively affect many important brain processes mediating neurotransmission and memory formation, while too high levels are severely excitotoxic (McEwen, 1999; Patel & Li, 2003). Stress and glucocorticoids increase the concentration of glutamate in the hippocampus (Sapolsky, 2000a) becoming excitotoxic. Damage to the hippocampus after trauma, caused by elevated glucocorticoid levels, will result in a further elevation of glutamate levels (Sapolsky, 2000a) thereby potentiating neurotoxic effects. It is therefore important to note that both hypocortisolemia and hypercortisolemia are associated with deficits in memory function (De Kloet, 2003). Excessive glutamate has been suggested to play a causal role in a number of neurodegenerative disorders such as AD (Cosman et al., 2007), amyotrophic lateral sclerosis (ALS) (Van den Bosch et al., 2006) and Parkinson's disease (PD; Przedborski et al., 2005). However, glutamate is also involved in fear responding (Davis & Myers, 2002; Walker et al., 2002) and anxiety (Kessler et al., 1995; Moghaddam, 1993). Indeed, pre-clinical studies have revealed an important role for glutamate in the stress response in analogous animal models of PTSD. Thus, single prolonged stress (SPS) in rats induces a significant increase in acoustic startle response that is blocked by topiramate, an ant glutamate drug used for the treatment of epilepsy (Khan & Liberzon, 2004). In animals subjected to SPS plus re-stress, a marked suppression of hippocampal GABA levels was noted, together with a significantly reduced NMDA receptor density ($B_{\max}$) (Harvey et al, 2004b). Interestingly, however, is that studies performed on mice have found that a fully functional glutamate NMDA receptor is necessary in the stress response (Miyamoto et al, 2002), suggesting that glutamate is indeed necessary for the normal physiological response to stress, but that if left unchecked, excessive release of glutamate can be detrimental.

Glutamate receptors are divided into the ionotropic receptor family, including the N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (KA) receptors (Dingledine et al., 1999; Bleakman & Lodge, 1998; Hollmann & Heinemann, 1994), and metabotropic receptors (Du et al., 2004; Zarate et al., 2003). While ionotropic receptors are ligand-linked ion channels, metabotropic receptors are G-protein coupled (Javitt, 2004). Clinical studies (Heresco-Levy et al., 2002; Yun et al., 1997) have provided evidence for the possible involvement of NMDA receptors in PTSD (Chambers et al., 1999). Little however, is known regarding the possible role of the AMPA receptor in PTSD, or of its value as a potential therapeutic target.

9.1.1.1. NMDA Receptors

The NMDA receptor ion channel contains four subunit proteins, namely two NR1 and two NR2 subunits (Dingledine et al., 1999). The NMDA receptor channel pore can only open if glutamate and glycine bind to the receptor, thereby allowing the passage of monovalent (Na^+ and K^+) and divalent cations (Ca^{2+}) through the NMDA receptor ion channel (Paoletti & Neyton, 2007). The channel pore consists of a binding site within for Mg^{2+} , which after binding at resting membrane potential will block the ion flow through the channel (*refer to **figure 8***). Depolarization of the membrane results in the release of Mg^{2+} and consequently promoting ion permeability through the channel. Maximum current flow through the NMDA receptor ion channel is only possible if both actions, depolarization of the postsynaptic neuron and presynaptic release of glutamate, take place (Wolosker, 2006). NMDA receptors present with two glycine-binding subunits and two glutamate-binding subunits (Hollmann & Heinemann, 1994), with both glycine (or D-serine) and glutamate binding being needed to facilitate full functional opening of the ion channel. The NR2 subunits contain sites of action for polyamines, zinc and protons (Perin-Dureau et al., 2002; Rachline et al., 2005). The sigma site (holding Mg^{2+}) and the phencyclidine (PCP) site

are additional sites inside the NMDA receptor gated ion channel (Du et al., 2006) (refer to **figure 8**).

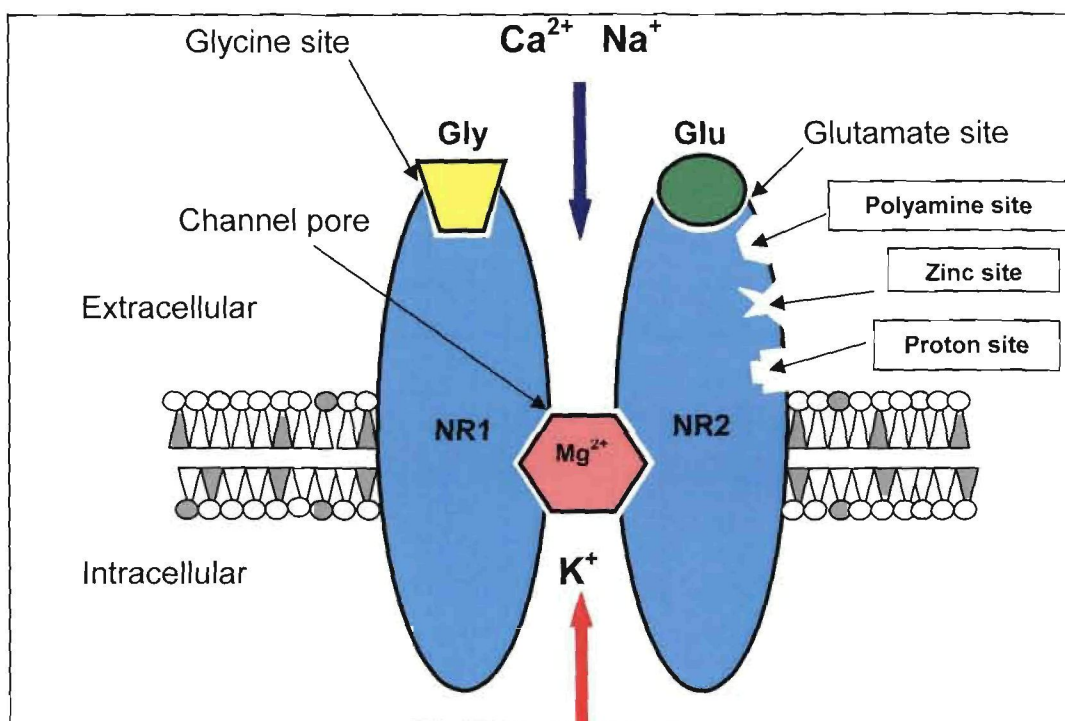


Figure 8: The NMDA receptor protein complex indicating the relevant binding sites within and outside the ion channel (redrawn from Kalia et al., 2008).

An important down-stream messenger following activation of the NMDA receptor is the activation of nitric oxide (NO) synthase (NOS). High concentrations of NOS are found in the brain regions involved in the modulation of anxiety and defensive behaviour (Vincent & Kimura, 1992), while raised NOS activation has been demonstrated in an animal model of PTSD (Harvey et al, 2004b; 2005). Like glutamate, NO has important physiological functions in the brain, including memory and neuromodulation, while it also has neuroprotective actions (Contestabile et al., 2003). However, excessively raised NO has also been linked to neurotoxicity (Yun et al., 1997; Contestabile et al., 2003). Evidence for the activation of NO producing neurons in the amygdala and hypothalamus by stressful stimuli is important (Krukoff & Khalili, 1997); since these regions are preferentially activated in PTSD patients after exposure to trauma reminders (see Stam, 2007 for review). Although more

definitive studies are needed in chronic PTSD, clinical studies have described altered NO in patients with acute stress disorder (Yeh et al, 2002).

9.1.1.2. *AMPA Receptors*

AMPA receptors mediate fast excitatory postsynaptic currents (EPSC's) in most of the brain synapses (Malenka & Nicoll, 1999) and play a critical role in the development and expression of memory-related long-term potentiation (LTP; see **section 11.3.2** for further discussion). AMPA receptors are tetrameric structures containing four subunits (GluR1-GluR4). A single subunit has two domains forming a V structure and a dimer is formed by two adjacent subunits (*refer to **figure 9***). Thus, a tetrameric receptor consists of two dimers. Transmitters binding to a subunit cause a domain of a subunit to move towards the other domain, trapping the transmitter. Tension is created in the transmembrane zone of the subunit and the ion channel is opened (Lynch, 2004). The process of deactivation refers to the reopening of the two domains (V structure) resulting in the release of the transmitter and closing of the channel (Lynch, 2004). Desensitization is a physiological condition where the transmitter is trapped by the domains that remain closed (V structure) and the channel closes (Sun et al., 2002; Jin et al., 2005).

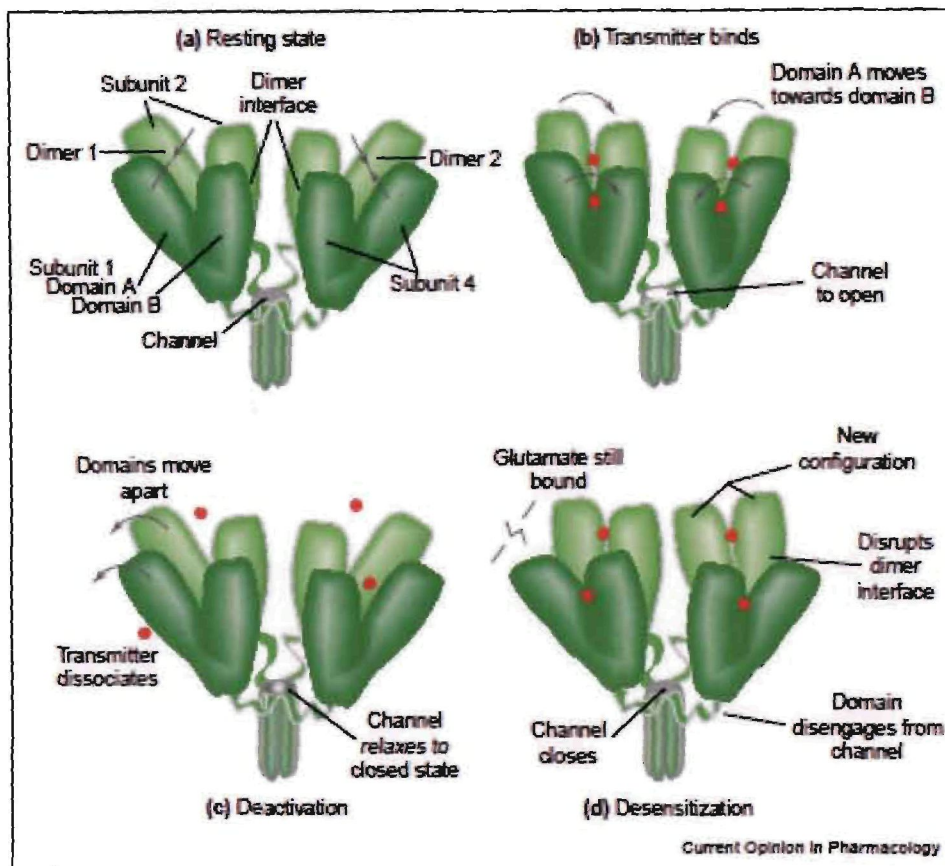


Figure 9: The structure of the AMPA receptor and its functioning (Lynch, 2004). Refer to text for detailed discussion.

The GluR2 subunit determines Ca^{2+} permeability of the AMPA receptors, with receptors lacking the GluR2 subunit being permeable to Ca^{2+} (Seeburg et al., 2001). The regulation of AMPA receptors is a key factor during synaptic functioning. Both LTP and long-term depression (LTD) are dependent upon either increased or decreased AMPA receptor functioning (Collingridge et al., 2004; Malenka & Bear, 2004). The sensitivity of AMPA receptors is determined by the activity of Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII), which is influenced by protein kinase A (PKA) and protein kinase C (PKC; Shobe, 2002).

9.1.1.3. NMDA-AMPA Receptor interaction

AMPA receptors are involved in recruiting voltage-gated NMDA receptor ion channels (Tanaka et al., 2000), while repetitive NMDA receptor activation results in increased AMPA receptor trafficking within the membrane (**figure 10**; Shi et al., 1999; Song & Huganir, 2002). This mutual interaction represents an important component in LTP and the laying down of memory (Granger et al., 1993; Staubli et al., 1994a). NMDA receptor activation and the resulting increase in calcium release activate CaMKII which phosphorylates the GluR1 subunit of the AMPA receptor, and as such enhances channel conductance of AMPA receptors to promote LTP (refer to **figure 12**).

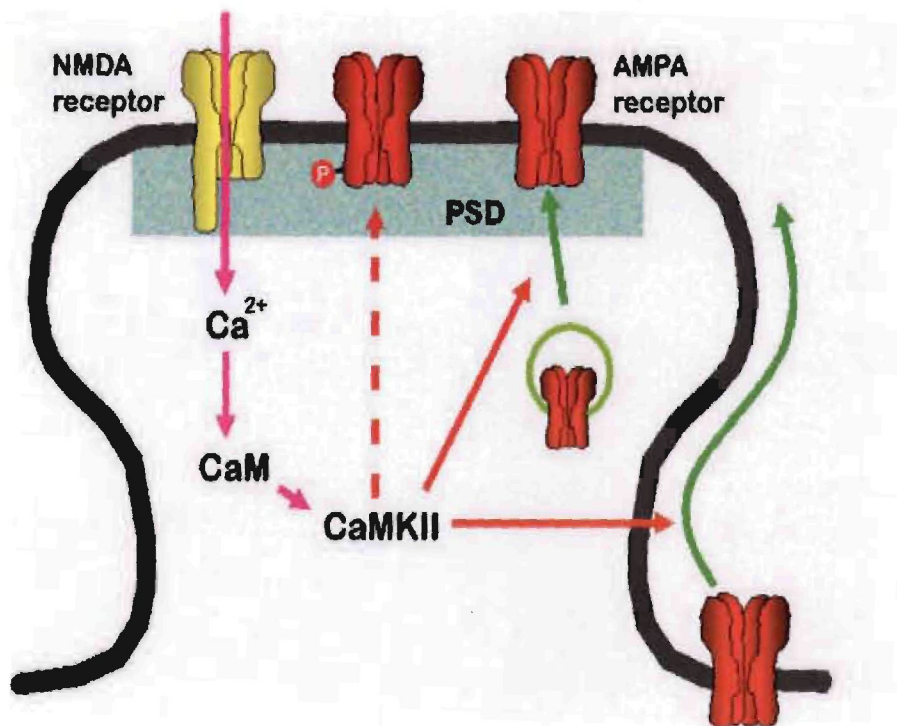


Figure 10: NMDA-AMPA receptor cross-talk. (Tomita et al., 2005). Refer to text for detailed description.

Stargazin, a protein associated with AMPA receptors, is the CaMKII substrate that promotes AMPA receptor trafficking into the postsynaptic density (PSD; Tomita et al., 2005). AMPA receptor activation provides the necessary depolarization for reversing

the magnesium-dependent block of NMDA receptors (Nowak et al., 1984), thereby allowing Ca^{2+} to enter the cell (Burnashev et al., 1995; Iino et al., 1997) with the exit of K^+ via the open NMDA receptor channels.

AMPA receptor stimulation, like that observed following activation of the NMDA receptor (see **Section 9.1.1.1.**), also leads to activation of NOS (Zhou et al., 2006). However, NMDA and AMPA/kainic acid receptors appear to exert differential effects on NO production, with AMPA antagonists blocking kainic acid-induced nitrite accumulation (Radenovic & Selakovic 2005). Importantly, the increase in NO-cGMP response to AMPA receptor activation is prevented by NMDA receptor blockade but bolstered by augmentation of endogenous glutamatergic tone (Ryder et al., 2006). Similarly, a complex association between NMDA receptor activation and the phosphorylation of NOS determines the functioning of the AMPA receptor (Rameau et al., 2007). These data clearly illustrate the complex yet important interaction that exists between these two signalling pathways.

9.1.2. GABA

Gamma aminobutyric acid (GABA) is the principle inhibitory neurotransmitter in the brain (McCormick, 1989) and occupies a central role in regulating the stress response (Shiah & Yatham, 1998). GABA receptors are divided into ionotropic GABA_A and metabotropic GABA_B receptors (Sivilotti & Nistri, 1991). NMDA receptor stimulation through glutamate stimulates GABA interneurons to release GABA (Nutt, 2000), maintaining homeostasis with the simultaneous release of the neurotransmitters. GABA is also an important negative regulator of glutamate (Yamada et al., 1999). The homeostatic response with a fine balance between glutamate and GABA needs to be maintained to prevent excessive glutamate levels, resulting in excitotoxicity (Nutt, 2000).

The hippocampus, amygdala, lateral septum and periaqueductal gray (PAG) are corticolimbic structures highly innervated with GABAergic neurones (Cherubini &

Conti, 2001; Mody, 2001). The parvocellular region of the paraventricular nucleus (mpPVN) is highly innervated with GABA receptors, with GABA being the main inhibitory neurotransmitter in the regulatory control of CRH neurons (Herman et al., 2003). GABAergic neurones inhibit the release of neurotransmitters involved in anxiogenic actions, such as cortic limbic NA and 5-HT (Möhler et al., 2002; Shekhar et al., 2002). Activation of GABA_B receptors by GABA results in inhibition of glutamate release, preventing excessive NMDA receptor activation (Kisch, 2002).

Vaiva and colleagues (Vaiva et al., 2004) found GABA plasma levels to be low after a traumatic event, correlating with abnormally low GABA levels found in people with panic disorders (Goddard et al., 2004). In an animal model of PTSD, Harvey and colleagues (2004b) found a significant reduction in hippocampal GABA levels after stress and re-stress. That reduced inhibitory GABA was found together with altered NMDA receptor density and elevated NOS activity emphasises that stress may disinhibit excitatory activity in the brain by comprising inhibitory GABA pathways. GABA has an important role in regulating normal affective state, being significantly reduced in depression (Shiah & Yatham, 1998). With PTSD being associated with reduced GABA levels, it comes as no surprise that depression is often comorbid with PTSD. Inhibition of GABA-reuptake with tiagabine (Taylor, 2003) and agonism of the GABA_B receptor with baclofen (Drake et al., 2003) reduces the characteristic symptoms of PTSD, specifically hyperarousal and avoidance symptoms (Drake et al., 2003).

9.2. Monoamines

Elevated levels of 5-HT and NA play an important role in the genesis of anxiety as observed in chronically stressed animals (Kirby et al., 1997; Kirby & Lucki, 1997; Rosario & Abercrombie, 1999). It is now well recognised that PTSD is associated with elevated noradrenergic tone (Geraciotti et al., 2001) through the stimulation of post-synaptic alpha- and beta-adrenoceptors resulting in increased cardiovascular

and other autonomic responses (see Ramos & Arnsten, 2007 for review), as well as central effects, of which increased arousal and anxiogenesis are the most important within the context of anxiety disorders (Bremner et al., 1996). A role for presynaptic auto-inhibitory alpha-2 adrenoceptors also has been suggested (Arnsten, 1998). Stressful events also result in increased synaptic 5-HT levels that may precipitate or worsen anxiety, by activation of 5-HT_{1A} receptors in the hippocampus (Connor & Davidson, 1998). Clinical studies in patients with combat-related PTSD have revealed an association between decreased 5-HT levels and heightened anxiety (Spivak et al., 1999). Altered cortical 5-HT_{2A} and hippocampal 5-HT_{1A} receptor binding, together with deficits in spatial memory, are evident in an animal model of PTSD (Harvey et al., 2003), while it has been suggested that elevated levels of 5-HT contribute to the deleterious effects of stress on spatial memory and cognition (Harvey et al., 2004a).

Again focussing on stress evoked by an analogous animal model of PTSD, studies have shown significantly raised 5-HT levels 7 days after a severe acute stress and reduction of 5-HT levels 1 hour after restress, suggesting that a severe stressor may evoke a maladaptive 5-HT_{1A} response to later stressors and also to attenuate the HPA-axis response (Harvey et al., 2006). Since stress and re-stress evoke qualitatively different monoamine responses, these authors have suggested that different approaches to treatment should be considered depending on when treatment is initiated, i.e. immediately after trauma or some time later, since the changing profile of monoaminergic responses over time and re-experience will determine successful treatment response. Similarly, diverse changes in NA and DA following stress and re-stress highlights that stress and re-experience exert differing time dependent changes on monoamine levels in the cortex and hippocampus (Harvey et al., 2006).

Regarding the role of DA in stress and PTSD, overactivation of mesolimbic dopamine transmission exacerbates the fear response, while its inhibition reduces conditioned fear (Pezze & Feldon, 2004). Increased plasma DA levels have indeed been described in trauma victims (Spivak et al., 1999). Pre-clinical evidence suggests a

causal role for frontal cortex DA dysfunction in anxiety-like behaviour evoked by stress and re-stress, and which also may contribute to the intrusive thoughts and diminished extinction of trauma-related memory that are characteristic features of PTSD (Harvey et al., 2006; Pezze & Feldon, 2004; Seamans & Yang, 2004). Elevated levels of DA in PTSD may explain the rationale for using neuroleptics in PTSD (Albucher & Liberzon, 2002), as will be discussed later.

9.3. Acetylcholine (ACh)

Acetylcholine (ACh) is an excitatory neurotransmitter implicated in arousal and cognitive processing in the brain (Picciotto et al., 2002). It is synthesized in nerve terminals from acetyl coenzyme A and choline by choline acetyltransferase and released following opening of a voltage dependent calcium channel (Meyer & Quenzer, 2004). ACh then binds to muscarinic receptors (G-protein facilitated) or nicotinic receptors (ionotropic). The muscarinic 1-receptor (M1) is the most abundant muscarinic receptor sub-type in the hippocampus and PFC (Levey et al., 1991). The cholinergic basal forebrain complex provides cholinergic innervation throughout the brain, penetrating the cortex and hippocampus (Vaucher et al., 1997; Sarter & Bruno, 2000). Nicotine induces the release of DA in all the major DA systems, while the functioning of the nigrostriatal DA system is modulated by ACh through nicotinic acetylcholine receptors (Lichtensteiger et al., 1982).

ACh has been shown to enhance LTP in many areas of the brain including the hippocampus (Huerta & Lisman, 1995) and cortical areas (Cheong et al., 2001). Acute stress facilitates long-lasting changes in cholinergic gene expression, while inhibitors of the acetylcholine-hydrolysing enzyme acetylcholinesterase may induce psychopathologies that are reminiscent of PTSD (Kaufer et al., 1998). In a rodent model of PTSD, Brand and colleagues (2008) have demonstrated distinct cortical and hippocampal muscarinic receptor involvement during stress-induced conditioned taste aversion learning in rats. These authors conclude that associative sensory-

related memories linked to aversive events can perpetuate aversive responses in the absence of the initial stressor, a typical characteristic of PTSD (Brand et al, 2008). Moreover, this response involves both hippocampal and frontal cortical M1 receptors.

10. Drug treatment in PTSD

Research have shown that although the treatment of PTSD with behavioural psychotherapy (non-drug treatment) and selective serotonin reuptake inhibitors (SSRIs) are effective, response is still far from satisfactory (Bisson & Andrew, 2007; Bisson et al., 2007; Brunello et al., 2001; Davidson et al., 2004; Ipser et al., 2006). Patients not responding to SSRI treatment can be treated with tricyclic antidepressants (TCAs) or monoamine oxidase inhibitors (MAOIs) to help reduce intrusions. Nefazodone and venlafaxine have been used in patients refractory to SSRIs as an alternative to the TCAs and MAOIs. Monotherapy with atypical antipsychotics or as add-on therapy have been studied for treating patients with prominent flashbacks (Asnis et al., 2004). Please refer to **table 1** for an overview of pharmacotherapeutic options in PTSD.

Treatment guidelines for drug treatment in PTSD according to the National Institute for Health and Clinical Excellence (NICE) are as follows (NICE, 2005):

- Paroxetine and mirtazapine are first choice treatment in primary care.
- Amitriptyline and phenelzine are used in secondary care.
- If a patient failed to respond in first-line treatment, the dosage should be increased within the recommended range.
- If further drug treatment is considered, augmentation with olanzapine can be considered.
- Patients responding positively to drug treatment should continue treatment for at least 12 months. Medication should be withdrawn gradually.

The only drugs currently licensed in the United Kingdom for treatment of PTSD are paroxetine and sertraline (NICE, 2005). It must be clearly stated that these are the *only registered drugs* currently licensed for treating PTSD and that health professionals use guidelines (*refer to **table 1***) and their own discretion when prescribing medication for PTSD, making it difficult to find clinical proof of the efficacy for most of the drugs used in the treatment of PTSD.

Table 1: Prescribing in PTSD (Nash & Nutt, 2007).

First-line treatment	Second line treatment	Other treatments
Paroxetine (20–50 mg daily)	Amitriptyline (150–200 mg daily)	Carbamazepine Lamotrigine Venlafaxine
Sertraline (50–200 mg daily)	Phenelzine (30–60 mg daily)	Augmenting agents
	Mirtazapine (30–45 mg daily)	Olanzapine Risperidone

10.1. Antidepressants

10.1.1. Selective serotonin reuptake inhibitors (SSRIs)

SSRIs prevent reuptake of serotonin in synapses, thereby increasing synaptic levels of 5-HT in the brain, which has been directly implicated in their antidepressant activity (Iversen, 2000; Schloss & Williams, 1998). SSRIs are the treatment of choice for PTSD, although for many patients treatment remains inadequate (Albucher & Liberzon, 2002; Hageman et al., 2001; Schoenfeld et al., 2004). Clearly changes in the 5-HT system are not solely responsible for the development or expression of PTSD (see Stam, 2007 for review), while drugs that target only 5-HT will inevitably have shortfalls in clinical efficacy.

Clinical studies have confirmed the efficacy of paroxetine (Stein et al., 2001) and sertraline (Brady et al., 2000a) in decreasing symptoms of PTSD. Connor and colleagues investigated the effects of fluoxetine in PTSD patients using doses of up to 60 mg per day over a period of 12 weeks (Connor et al., 1999). These authors found fluoxetine to be an effective treatment of PTSD, producing marked improvement in intrusive, hyperarousal and avoidance symptom subcategories (Connor et al., 1999; Martenyi et al., 2002a, b; Van der Kolk et al., 1994).

10.1.2. Tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs)

TCAs and MAOIs are effective in reducing symptoms of PTSD (Davidson et al., 1990; Davidson, 1997; Kosten et al., 1991). TCAs mode of action, and efficacy in treating PTSD, has been directly ascribed to the inhibition of the reuptake of NA and 5-HT which potentiates their action in the brain. MAOIs inhibit the enzyme monoamine oxidase (MAO) which is responsible for the metabolism of various neurotransmitters. MAO type A metabolises Adr, NA and 5-HT, while DA is metabolised by MAO type B (Sweetman, 2008); consequently potentiating the effects of these neurotransmitters and relieving symptoms associated with PTSD.

However, due to side effects and compliance issues, TCAs such as amitriptyline and imipramine are prescribed as second line treatment of PTSD (Nash & Nutt, 2007). Similarly, while the MAOI, phenelzine, reduces intrusive memories (nightmares and flashbacks; Frank et al., 1988), it is plagued by side effects and other risk factors that compromise treatment outcome.

10.1.3. Atypical antidepressants

Mirtazapine (Remeron), a NA'ergic and specific 5-HT'ergic antidepressant (Puzantian, 1998), and venlafaxine (Efexor), a 5-HT and NA reuptake inhibitor (SNRI;

Morton et al., 1995), enhance the effects of NA and 5-HT. Mirtazapine has been used in an open label trial and has been shown promise in the treatment of PTSD (Chung et al., 2004). Venlafaxine too has been used in clinical trials with positive results in the short-term management of PTSD symptoms (Davidson et al., 2006).

10.1.4. Other medications

Clinical studies have indicated that anticonvulsants and antiglutamatergic agents, such as carbamazepine, valproic acid and lamotrigine can be beneficial in PTSD (Lipper et al., 1986; Fesler, 1991; Hertzberg et al., 1999). These latter studies have realised the great potential in targeting glutamatergic pathways as possible novel therapeutic targets in treating PTSD. Indeed, one such promising group of compounds are the ampakines, the subject of this dissertation. Ampakines are discussed in detail in **section 14** of this chapter. Typical antipsychotics such as risperidone have also found use in the treatment of SSRI-resistant PTSD patients (Stein et al., 2000). Risperidone may be especially useful in suppressing vivid nightmares and flashbacks (Stein et al., 2000), while augmentation with risperidone appears to be particularly useful in treating patients not responding to sertraline alone (Rothbaum et al., 2008).

Propranolol, a beta-adrenergic blocker, effectively opposes the effects of stress hormones on fear conditioning (McGaugh, 2004). Pitman and colleagues describe a reduction of trauma-related responses after receiving propranolol within 6 hours of a traumatic event (Pitman et al., 2002). However, noradrenergic mechanisms modulate the consolidation of conditioned learning only within a couple of hours of the initial trauma, thereafter attenuation of fear conditioning by propranolol will not be possible (Ji et al., 2003). The alpha-2 blocker clonidine may also be useful to suppress excessive noradrenergic activity in PTSD (Stein et al., 2000).

Other possible treatment options, albeit unsubstantiated in clinical studies, include opioid antagonists, certain benzodiazepines and serotonergic antagonists (see Stein et al., 2000 for review).

10.2. Glucocorticoids

Glucocorticoids are hormones released from the adrenal cortex during stress. Their purported effects on the consolidation of fear memory on the one hand, and their negative effects on explicit memory on the other, have attracted interest in recent years as possible strategies in the treatment and prevention of PTSD. Studies in both humans and animals have shown enhanced consolidation of new memories after a single administration of glucocorticoids (Kuhlmann & Wolf, 2006; Roozendaal, 2000). Contradictory to these studies, De Quervain and colleagues found impaired memory retrieval after glucocorticoid administration (De Quervain et al., 1998). Glucocorticoid administration may indeed be beneficial for the treatment of patients with PTSD (Aerni et al., 2004; Soravia et al., 2006), the key objective being to reduce the excessive retrieval of aversive memories in these patients (Aerni et al., 2004). In agreement with this suggestion, low-dose cortisol (10 mg/day) administration in PTSD patients for one month, during a 3 month observation period, has been found to reduce excessive aversive memory retrieval (Aerni et al., 2004). Similarly, Schelling and colleagues reported a lower incidence of PTSD symptoms in patients receiving hydrocortisone during the perioperative phase of cardiac surgery (Schelling et al., 2004). Thus, while cortisol may indeed play a causal role in the post stressor sequelae in trauma victims, glucocorticoid treatment may be beneficial in patients with PTSD. This provides direct support for the theory that PTSD represents a hypocortisolemic state arising from hypersensitivity of the inhibitory elements of the HPA-axis (Yehuda et al., 2000), resulting in an inability to shut off the stress response.

While hypocortisolemia may be an important pathological construct of PTSD, it is noteworthy that preclinical studies using the glucocorticoid synthesis blocker, ketoconazole (KCZ), effectively blocks stress-evoked NOS activation (Harvey et al.,

2004b). This observation thus implies that stress induced elevation of glucocorticoids and the subsequent release of neurotoxic molecules such as glutamate and NO (Sapolsky, 2000a; McEwen, 1996, 1999) may play an early role in the response to stress, and possibly in establishing hippocampal atrophy. KCZ inhibits both adrenal steroid synthesis (Yan et al., 2002) and prevents the formation of NO (Wolff et al., 1993). Finally, CRF antagonists have anxiolytic-like effects (Okuyama et al., 1999) and may be beneficial in the treatment of PTSD through its actions on the HPA-axis.

11. Learning and memory

The formation of learning and memory can be attributed to activity-dependent changes in the efficacy of synapses. Hebb formulated a learning rule and proposed that strengthening of synaptic connections is the result of presynaptic activity triggering action potentials in the postsynaptic neuron (Hebb, 1949). Memory is defined as the ability to store information and the subsequent recalling of the information. Learning consists of three general phases i.e. the initial learning (acquisition), molecular stabilization of long-term memory (consolidation) and the recall of the original learning (retrieval; Bruel-Jungerman et al., 2007; Izquierdo et al., 2004). The learning process and consolidation of trauma-related memories are facilitated by the increased secretion of glucocorticoids acting on hippocampal glucocorticoid receptors (Reul and de Kloet, 1985) and glutamate activating hippocampal NMDA receptors (see Riedel et al., 2003 for review). Memory consolidation is a slow process where a memory trace is converted into a more permanent form by connections between the medial temporal lobes and neocortical areas (McGaugh, 2000) and uses NMDA, AMPA and dopaminergic D1 receptors (Bernabeu et al., 1997) in effecting this response.

Both NMDA and AMPA receptors are required for spatial learning (Malinow & Malenka, 2002). AMPA receptors are necessary for both immediate memory and

consolidation of long-term memories (Izquierdo et al., 2007). NMDA receptors are linked to consolidation of memories, while the retrieval of spatial memories is the function of AMPA receptors (see Riedel et al., 2003 for review). PTSD can be directly or indirectly related to a state of disorganized memory. At the same time, both memory and the neuropathology of PTSD (as reviewed above) involve important changes in glutamatergic function. Importantly, general declarative memory functions as well as explicit information about the trauma are compromised in PTSD while at the same time non-declarative memory relating to involuntary recollection of the trauma is bolstered (Elzinga & Bremner, 2002). In order to better understand the complex cognitive disturbance that is PTSD, it is necessary to review and discuss the concept of learning and memory.

11.1. Types of memory

Memories can be divided into different categories: spatial memory, non-spatial memory, representational memory, configural memory, declarative memory, procedural memory and episodic memory (Eichenbaum, 2001; Eichenbaum et al., 1993; Zola et al., 2000). Memory processes are divided according to contents and time, with working memory and short-term memory dissociated from long-term memory (Baddeley, 1990; Shallice & Warrington, 1970). Temporal persistence is the main criterion to classify memories, with short-term memory lasting for seconds to minutes, while long-term memory can last for years (Corkin, 1984; Squire, 1987). The functioning of memory can be divided into explicit (declarative) and implicit (non-declarative) memory processes (Squire & Zola-Morgan, 1991), and will be described in more detail below.

11.1.1. Explicit and implicit memory

The process of memory involved to consciously remember and regenerate events and facts is referred to as declarative or explicit memory (Eichenbaum, 2001; James,

1890). Considering PTSD, explicit information of the traumatic event involving the senses, emotions and any physiological reactions experienced during the trauma are related to explicit memory (Elzinga & Bremner, 2002). Declarative memory can be divided further into episodic memory and semantic memory (Tulving, 1972). Episodic memory has a specific spatial context and stores information of one's own experience on what, where and when a specific situation occurred, with semantic memory being memory of general knowledge, facts and concepts about the world (Tulving, 2001; 2005). Retrieval of semantic memory occurs automatically, while episodic memory requires conscious recollection (Tulving, 1972).

Spatial memory is defined as memory based on spatial information and is dependant on the hippocampus (O'Keefe & Nadel, 1978). Object-location memory is essential for daily activities and operates together with spatial memory as a system (Hasher & Zacks, 1979). Object-location memory consists of three processes: object processing, spatial-location processing and binding of objects to location (Postma et al., 2004).

Implicit memory refers to memory of an event resulting from or influenced by emotions or behaviour, without any conscious memory (Graf & Schacter, 1985). The implicit memory process in individuals with PTSD may trigger information of the traumatic event unconsciously and be responsible for re-experiencing.

11.2. Brain regions involved in memory: Relevance for PTSD

As has been mentioned earlier (*refer to Section 7 – Neuroanatomy of PTSD*), the brain regions most implicated in PTSD are the hippocampus, amygdala and frontal cortex (*refer to figure 11*). All these regions play either a direct or indirect role in the consolidation and recollection of fear and/or explicit memory.

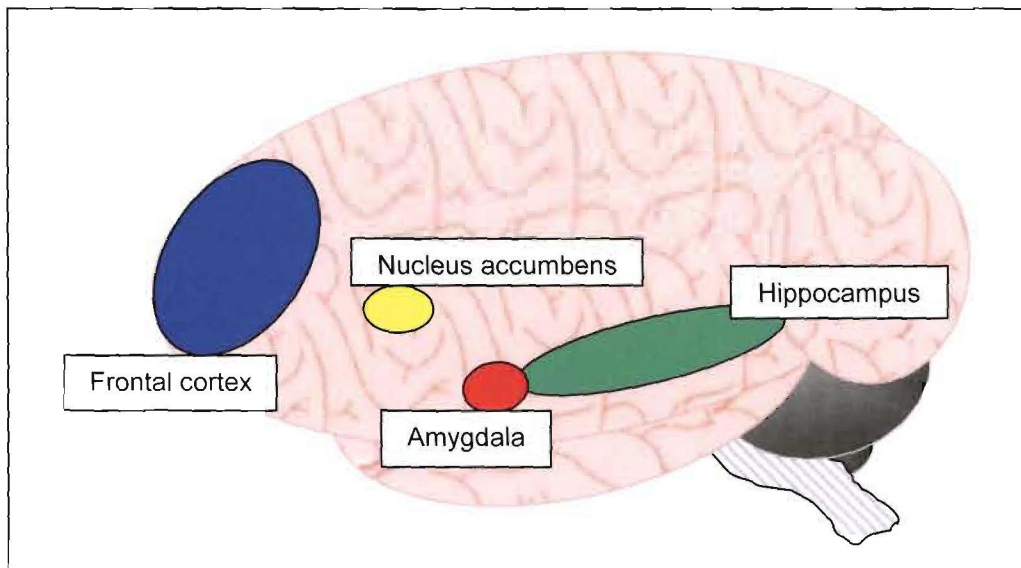


Figure 11: Brain regions implicated in PTSD.

The hippocampus plays a critical role in remembering spatial (Burgess et al., 2002; Morris et al., 1982) and non-spatial events (Agster et al., 2002; Fortin et al., 2002), and as has been previously highlighted, this brain region and indeed spatial memory, is severely compromised in patients with PTSD. The high concentrations of NMDA and AMPA receptors, while playing an important role in hippocampal-mediated learning and memory (Izquierdo & Medina, 1997), at the same time make the hippocampus vulnerable to excitotoxicity (De Groot et al., 2001). As a direct result of elevated levels of glucocorticoids released during stress; damage to the hippocampus is inevitable if left unchecked (McEwen et al., 1992), resulting in short-term memory deficits (see Bremner et al., 1995a for review).

Damage to medial temporal structures such as the hippocampus; but also the parahippocampal gyrus, perirhinal cortex and entorhinal cortex, or the diencephalon, results in impaired episodic memory function and amnesic syndrome. The syndrome is characterized by forgetfulness for new information (anterograde amnesia) and some previously acquired information (retrograde amnesia), while general knowledge retrieval is not affected (Parkin & Leng, 1993). Episodic information is stored in the neocortex (Sakai & Miyashita, 1993) and the frontal cortex is activated during retrieval of episodic memory (Buckner & Tulving, 1995).

Impaired PFC functioning is characteristic of PTSD and is also associated with working memory dysfunction. The PFC, specifically the dorsolateral (Bailey & Mair, 2004) and ventromedial regions (Akirav & Maroun, 2006) are associated with working memory processing. Accessing of semantic knowledge is dependant on interactions between several cortical areas, with the left temporal regions storing semantic knowledge (De Renzi et al., 1987; Hart & Gordon, 1990) and the left frontal cortex retrieving the semantic knowledge (Janowsky et al., 1989; Milner, 1982; Wiggs et al., 1999). The right-sided occipitoparietal and prefrontal areas are involved in spatial working memory (Smith & Milner, 1981, 1984), while the left parietal and temporal areas are activated by object working memory (Smith et al., 1995). Neuro-imaging studies have indicated that the amygdala is overactive in PTSD patients and the basolateral amygdala (BLA) plays a central role in emotion and memory (Davis & Whalen, 2001; Charney, 2003). The amygdala is sensitive to fear provoking stimuli (Phelps & LeDoux, 2005) and an overreactive amygdala may be the reason for the enhanced retrieval of fear memories and associated intrusive memories experienced by PTSD patients.

Finally, the nucleus accumbens is innervated by several limbic structures such as the ventral hippocampus, basolateral amygdala and the mPFC (Groenewegen et al., 1999), and integrates these limbic and cortical inputs. Traumatic experiences are processed and stored consciously as explicit memories by the hippocampus and PFC, while the amygdala unconsciously stores implicit memories which are emotionally driven. Reminders of the traumatic experience will unconsciously activate the amygdala and consequently result in the activation of the SNS. Refer to **section 8 – Stress response** for a detailed description of the pathways of fear.

11.3. Neurobiology of memory

Excitatory glutamate receptors are situated in the principal brain regions involved in memory consolidation and retrieval. It is especially the pre- and postsynaptic ionotropic and metabotropic groups of receptors, whose functions include neuronal communication and signal processing (Storm-Mathisen et al., 1983, 1995), that are involved. These receptors interact with cAMP dependent protein kinase A (PKA) and the mitogen-activated protein kinase (MAPK), two inter-related signal transduction pathways that play a key role in learning and memory (Houslay, 2006; Stork & Schmitt, 2002).

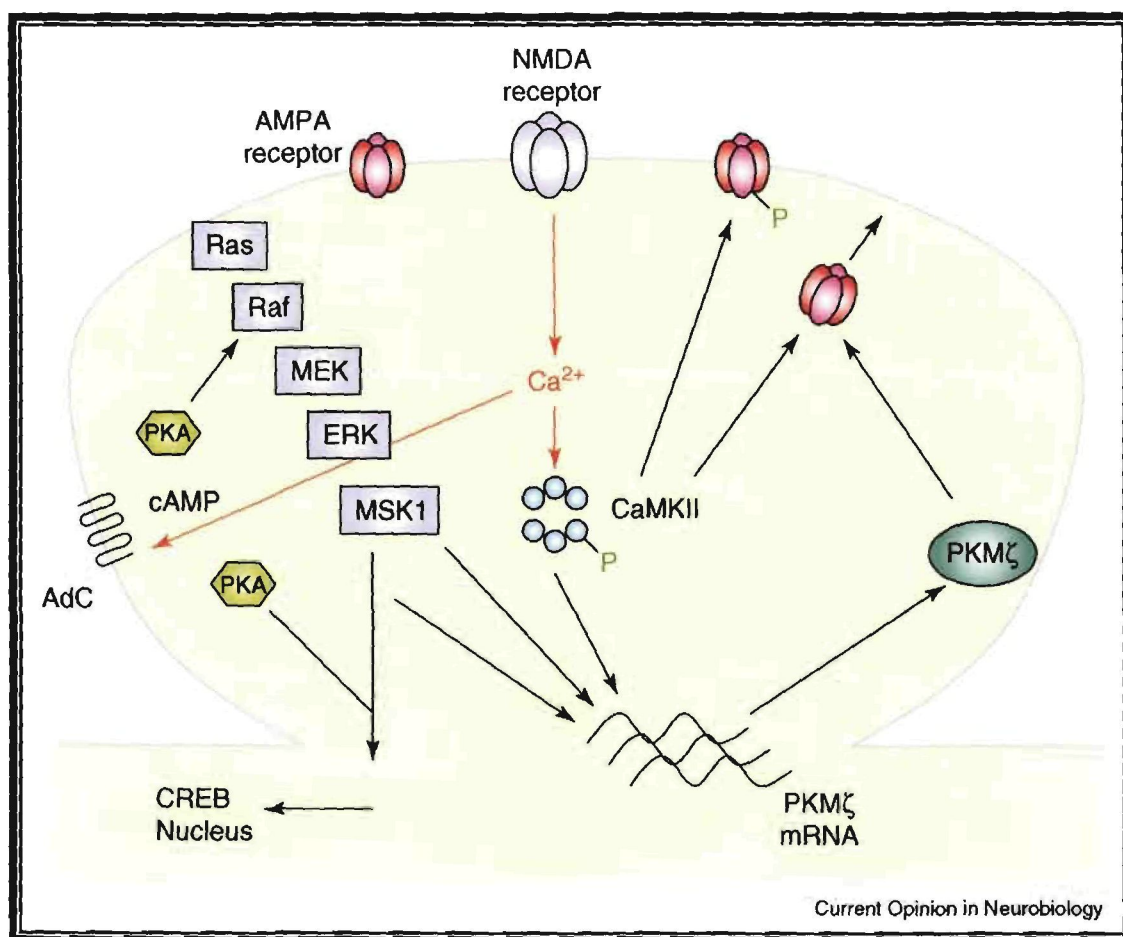


Figure 12: Signaling pathways during LTP (Mayford, 2007) Refer to description in the text.

ERK (extracellular signal-regulated kinase) is abundant in brain regions involved in learning and memory such as the neocortex, hippocampus, amygdala, striatum and cerebellum (Flood et al., 1998; Ortiz et al., 1995; Fiore et al., 1993). ERK 1 and ERK 2 are part of the MAPK family that plays an important role in signal transduction pathways (*refer to **figure 12***). Other kinases include c-Jun N-terminal kinase/stress-activated protein kinases (JNK/SAPK), p38 MAPK, ERK 3 and ERK 5 (English et al., 1999; Schaeffer & Weber, 1999; Seger & Krebs, 1995). ERK signaling pathways are important for neuronal functioning and the consecutive phosphorylation of three kinases forms the basis of the ERK cascade: Raf-1 being the first kinase, phosphorylating mitogen-activated ERK-activating kinase (MEK), which in turn activates ERK through phosphorylation of both a threonine and tyrosine residue (English et al., 1999; Seger & Krebs, 1995).

PKA is a calcium sensitive second messenger activated by the influx of Ca^{2+} upon NMDA receptor activation (see Selcher et al., 2002 for review) (*refer to **figure 12***). MAPK is one of the major down-stream effectors of PKA (Sananbenesi et al., 2003) and is the link between the activation of PKA and protein kinase C (PKC) and cyclic adenosine monophosphate (cAMP) response element binding protein (CREB); see Sweatt, 2001 for review). The activated PKA translocates to the nucleus and phosphorylates CREB and the phosphorylated CREB then binds to DNA and regulates gene transcription (Silva et al., 1998).

CREB is a transcription factor which is distributed widely in the brain. Calcium, neurotrophin and cytokine signals induce CREB activation (Carlezon et al., 2005; Lonze & Ginty, 2002). Phosphorylation of CREB, induced by elevated cAMP or membrane depolarization, activates CREB-dependent transcription (Sheng et al., 1991; Gonzalez & Montminy, 1989) which plays an essential role in long-lasting forms of synaptic plasticity and long-term memory (see Carlezon et al., 2005 for review; Yin et al., 1995).

11.3.1. Synaptic plasticity

Synaptic plasticity is the variable efficacy of neurotransmission at synapses and a substrate of memory (Martin et al., 2000). Glutamate NMDA and AMPA receptors are necessary for the induction of several forms of synaptic plasticity, such as LTP and LTD (Malenka & Bear, 2004; Cooke & Bliss, 2006; Liu & Zukin, 2007). The AMPA receptors are responsible for most of the inward current during low-frequency synaptic transmission, while NMDA receptor channels are blocked by magnesium (Mg^{2+}). The postsynaptic membrane needs to be sufficiently depolarized for the Mg^{2+} block to be relieved, resulting in Ca^{2+} and Na^{+} entering through NMDA channels (Popoli et al., 2000). Ca^{2+} influx through NMDA receptors is responsible for the activation of the above-mentioned signalling cascades, with the amount of postsynaptic calcium determining whether LTP or LTD is induced (Cummings et al., 1996; Yang et al., 1999). Protein kinases and protein phosphatases in the near vicinity of NMDA receptors enable synaptic plasticity to occur (Husi et al., 2000), while protein synthesis and gene transcription are required in certain forms of synaptic plasticity involved in memory storage, to stabilize synaptic changes over a period of time (Adams & Dudek, 2005).

11.3.2. Long-term potentiation (LTP)

Long-term potentiation is a process in how memories are laid down (Malenka & Nicoll, 1999) and is a form of synaptic plasticity describing an increase in chemical strength of a synapse, lasting from minutes to several days. The induction of LTP is boosted by increasing excitatory transmission (Staubli et al., 1994a). Enhanced depolarization in unblocking NMDA receptors results in the formation of LTP. The influx of Ca^{2+} through NMDA receptor channels resulting in LTP has been studied extensively (Lisman et al., 2003) and is considered as the key event for the triggering of LTP (Popoli et al., 2000) (*refer to **figure 12***). The primary initiating signal in LTP is thought to be Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII). The kinase is

activated by the NMDA-receptor based Ca^{2+} signal (Ouyang et al., 1999). LTP is divided into an early phase (without protein synthesis) of approximately 1-2 hours and a late phase requiring protein synthesis (Popoli et al., 2000). CaMKII and PKC are accountable for different phases of LTP. The AMPA-type glutamate receptor GluR1 has been identified as a key target substrate for CaMKII (see **figure 12**). The action of AMPA receptors are potentiated by direct phosphorylation by CaMKII while the insertion of new AMPA receptors into the membrane is indirectly mediated by CaMKII (see **figures 10 & 12**; Hayashi et al., 2000). The PKA and ERK pathways are activated by the Ca^{2+} signal causing the production of cAMP through Ca^{2+} -dependent adenylyl cyclase (AdC) (Kandel, 2001). Transcriptional changes are stimulated through phosphorylation of CREB required for LTP and memory formation (Frank & Greenberg, 1994; Yin et al., 1994). Ampakines promote the formation of LTP by preventing or slowing down deactivation and desensitization of AMPA receptors (Lynch, 2002; see **Section 14**), thus making them suitable as memory-enhancing drugs (Lynch, 1998; Staubli et al., 1994a, b).

12. Animal models of relevance for PTSD

12.1. Introduction

Animal models of PTSD have utilized intense stressors, aversive challenges, and situational reminders of a traumatic event in an attempt to model long-term effects on behavioural, autonomic, and hormonal responses seen in humans with PTSD (Uys et al., 2003). Different types of stressors i.e. acute, chronic, controllable, escapable, physiological or psychological are used to induce biobehavioural changes in animals (Yehuda & Antelman, 1993). However, no animal model is an exact replica of the human disorder and for an animal model to be taken seriously; it needs to fulfil certain validation criteria that describes the accuracy and robustness with which it simulates the human condition at levels of behaviour, neurobiological underpinnings and treatment response. Often it is necessary to combine two models to achieve the

best outcome. During consideration of relevant animal models, it is important to differentiate between factors influencing the manifestation of the disorder and those essential for induction of the disorder (Yehuda & Antelman, 1993).

The criteria for the evaluation of PTSD as identified by Yehuda and Antelman (1993) are reproduced below:

- The stressor should be capable of producing PTSD-like symptoms in a dose-dependent manner.
- The stressor should produce biological alterations that persist over time or become more pronounced with the passage of time.
- The stressor should induce biobehavioural alterations that have the potential for bidirectional expression.
- Inter-individual variability in response to a stressor should be present either as a function of experience, genetics or an interaction of the two.

12.2. *Validity of animal models*

Animal models should be developed in such a way that behavioural and neurobiological features can be studied. The intended purpose of the model is a critical consideration during the development of the animal model and determines the criteria that the model must satisfy to establish its validity. Validation of animal models is very important and determines whether the model can be taken seriously. Animal models are assessed on three levels of validity: face validity, construct validity and predictive validity (Geyer & Markou, 1995), which are discussed in more detail below.

12.2.1. Face validity

The face validity of an animal model refers to its similarity with the clinical condition, the ideal being that the condition or specific features of the human condition, such as behavioural symptoms, are closely reproduced in the animal model (Willner, 1986; Swerdlow & Sutherland, 2006).

12.2.2. Construct validity

Construct validity reflects the close association of the known pathology of a human disorder and the presence of similar pathology in the animal model. Consequently, there should be close resemblance in neurochemical and other pathological alterations between the animal model and the human clinical condition (Geyer & Markou, 1995).

12.2.3. Predictive validity

Predictive validity is the ability to predict changes in the human condition based upon the outcomes of the animal model (Geyer & Markou, 1995) and is of great importance in drug development to screen drugs for therapeutic potential in humans (Willner, 1986). Thus, drug treatment modalities that are selectively effective in the human condition should show equal selectivity in the model, and is regarded as an essential criterion for the validity of an animal model (Geyer & Markou, 1995). Animal models that can make predictions of the human condition based on the performance of the animal model have predictive validity.

13. Animal models of PTSD

Animal models of PTSD include stressors such as inescapable footshock (Pynoos et al., 1996), underwater stress (Cohen et al., 2004), social confrontations (Stam et al., 2000), exposure to predators (Cohen et al., 2003; Adamec et al., 1999), time-dependant sensitization (TDS; Harvey et al., 2003), and single prolonged stress (SPS; see Liberzon et al., 1997; Khan & Liberzon, 2004; Takahashi et al., 2006; Harvey et al., 2006; Kohda et al., 2007). Since type and duration of the stressor will have significant implications for the expression of neuro-endocrine and behavioural changes in the animals; the applied stressors need to be relevant for the animal being used. Moreover, the stressor needs to simulate a severely stressful event in the life of the animal. Thus, for example the above models have varyingly used somatosensory stressors (such as restraint), inescapable or psychological stressors (such as underwater swimming or predator exposure) or complex stress-stimuli (such as that evoked by exposure to drugs or chemicals). For the purpose of this study, only the SPS and TDS models will be discussed in detail.

13.1. Time-dependant sensitization (TDS)

The concept of sensitization is important in the neuro-development of PTSD, and is emphasized by repeated trauma over time by stress and restress in the TDS model. TDS stress evokes distinct cognitive and aversive behaviours, as well as hormonal disturbances, resembling PTSD that present with significant validity with respect to its application in PTSD research, as outlined below:

Face validity: TDS stress evokes spatial memory deficits, increased anxiety and decreased levels of plasma corticosterone, as seen in clinical studies of PTSD (Harvey et al., 2003; 2006). Recently, Brand and co-workers (2008) have demonstrated that TDS stress strengthens conditioned taste aversion learning in rats, thus indicating that associative sensory-related memories linked to aversive events

can perpetuate aversive responses in the absence of the initial stressor, a typical characteristic of PTSD.

Construct validity: TDS stress evokes HPA-axis abnormalities, as in PTSD (Yehuda & Antelman, 1993), including increased sensitivity to negative glucocorticoid feedback (Liberzon et al., 1997; Liberzon et al., 1999a; Harvey et al., 2003). Given the general acceptance of the prominent role of the 5-HT_{1A}ergic system in the neurobiology of anxiety and stress disorders, including PTSD (Agrypolous et al., 2000), it is of importance that regionally specific changes in 5-HT receptor binding in the hippocampus (5-HT_{1A}) and prefrontal cortex (5-HT_{2A}) have been found following TDS stress (Harvey et al., 2003). The hippocampus and prefrontal cortex are also involved in memory and stress responsiveness. With the well-established role for altered monoamines in PTSD, TDS has been found to evoke regional brain monoamine changes in rats exposed to this stress paradigm (Harvey et al., 2006). Considering the more recent revelation concerning the role of excitatory and inhibitory transmission in PTSD; that TDS stress induces marked changes in GABA levels and the binding properties of hippocampal glutamate NMDA receptors, is of significance (Harvey et al., 2004b). The glutamate NMDA pathways in the hippocampus are critical in memory function (Heresco-Levy & Javitt, 1998). The reduction of the NMDA receptor density after stress-restress (Harvey et al., 2004a) suggests that cognitive deficits observed in the TDS model (Harvey et al., 2003) may be associated with reduced NMDA receptor density, thus providing construct validity for the model.

Predictive validity: Studies performed by Harvey and colleagues (Harvey et al., 2004a; Korff, 2007) have demonstrated that the SSRI, fluoxetine, attenuates TDS-induced spatial memory deficits and hypocortisolemia. Moreover, the steroid synthesis inhibitor, ketoconazole, has similarly been found to reverse TDS effects on spatial memory (Brand et al., 2004), as well as TDS-induced activation of NOS (Harvey et al., 2004b). The reversal of SPS-associated memory changes with fluoxetine or ketoconazole, confirm the predictive validity of the model.

13.2. Single prolonged stress (SPS)

SPS evokes distinct cognitive and aversive behaviours, as well as hormonal disturbances, resembling PTSD that present with significant validity with respect to its application in PTSD research, as outlined below:

Face validity: Takahashi et al (2006) found SPS produced enhanced HPA-axis negative feedback, leading to low levels of plasma cortisol, similar to the effect seen in PTSD patients. Enhanced negative feedback, a neuroendocrine abnormality of PTSD, and changes in glucocorticoid and mineralocorticoid receptors have also been found to be induced in animals exposed to SPS (Liberzon et al., 1999a). Another typical symptom of PTSD, exaggerated acoustic startle (Shalev et al., 1992; Orr & Lasko, 1995), is heightened by SPS in rats (Khan & Liberzon, 2004). SPS induces contextual freezing in rodents which is consistent with PTSD patients showing enhanced anxiety and fear in response to stimuli not related to previous trauma. Recently Kohda et al (2007) demonstrated that SPS induces pronounced spatial memory deficits in rodents, as well as increasing contextual fear conditioning. Both the latter behavioural changes represent core symptoms of PTSD and hence support the face validity of the model. Finally, SPS induces the development of impaired extinction of fearful memory (Yamamoto et al., 2008), a major symptom in PTSD (Bremner et al., 2005; Milad et al., 2006).

Construct validity: Reversal of SPS-induced acoustic startle with topiramate, a blocker of voltage-dependent sodium channels (Taverna et al., 1999) and AMPA/kainate receptors (Gibbs et al., 2000), has particular importance for the putative role of AMPA receptors in PTSD. This evidence for construct validity is particularly important for the objectives of the current study. SPS stress is associated with various aspects of altered glutamatergic signalling, including increased hippocampal levels of glycine-transporter 1 (Gly-T1) and vesicle-associated membrane protein2 (VAMP2) (Iwamoto et al., 2007) and upregulation of NMDA receptors (Yamamoto et al., 2008). The higher levels of Gly-T1 may be the cause for the development of impaired extinction of fearful memory. Moreover, SPS-induced

impairment in fear extinction is prevented by D-cycloserine, a partial agonist of the glycine site in the NMDA ion channel (Yamamoto et al., 2008).

Predictive validity: Chronic administration of paroxetine reduces the elevated fear response evoked by SPS (Takahashi et al., 2006), confirming predictive validity for the model, while increased acoustic startle induced by SPS is blocked by the anticonvulsant, topiramate (Khan & Liberzon, 2004). Anti-glutamate drugs have found use in the treatment of PTSD (see **Section 10.1.4.**)

The above data indicate that SPS and SPS plus reminders (TDS stress) may be useful in studying the biobehavioural effects of novel drug treatments and their possible relevance in the treatment of PTSD. However, it is interesting that despite their similarities in validity, recent work that has attempted to examine the importance of either re-experience (TDS) or acute severe stress alone (SPS) as a contributor to the development of PTSD, has noted that SPS and TDS stress evoke qualitatively different endocrine, behavioural and monoamine responses (Harvey et al, 2006).

14. Ampakines

Ampakines describe a group of compounds that potentiate AMPA receptor transmission through allosteric modulation and not through any agonist or antagonist effects of their own (Suppiramaniam et al., 2001; Lynch & Gall, 2006). Deactivation and desensitization of AMPA receptors are slowed by ampakines (Lynch, 2002). By reducing desensitization of the ion channel (Lynch & Gall, 2006), thereby stabilizing the receptor in its channel-open state following the release of glutamate, ampakines effectively prolong current flow through the receptor complex leading to an enhanced and sustained synaptic response (Lynch & Gall, 2006). However, ampakines from each category bind to different sites of the AMPA receptor and have diverse affects on the excitatory post-synaptic potential (EPSP; Arai et al., 2002), as well as different effects on LTP (Arai et al., 2004).

The first AMPA receptor potentiators were benzamide compounds, with the first ampakines derived from aniracetam. Currently several classes of AMPA receptor potentiators have been reported. The classes include **pyrrolidones** i.e. piracetam and aniracetam; **benzothiazides** i.e. cyclothiazide; **benzoylpiperidines** i.e. CX516 (Ampalex) and CX546, and the more recent **biarylpropylsulfonamides** i.e. LY392098, LY404187 and LY503430 (O'Neill et al., 2004). The ampakines, Org26576 and Org24448 represent a structurally distinct chemical series derived from the first generation ampakine, CX516 (Ampalex; Jordan et al., 2005), and are chemically related to aniracetam (refer to **figure 13**).

Studies have shown that ampakines have beneficial effects in animal models of learning (Lebrun et al., 2000) as well as animal models of depression (Li et al., 2003; O'Neill et al., 2004; Jordan et al., 2005). Ampakines also improve short-term memory in humans (Ingvar et al., 1997).

Pharmaceutical companies have developed several ampakine compounds, starting with CX516, which was found to increase AMPA-mediated excitatory postsynaptic potential responses and decreases AMPA receptor deactivation and desensitization (Arai et al., 1996). The compound was researched under the brand name Ampalex as a possible treatment for AD and dementia (U.S. National Library of Medicine, 2008), and successfully demonstrated an improvement in performance in some memory tasks (Lynch et al., 1996, 1997). Later, Knapp and colleagues (2002) investigated the effects of CX516 and second generation ampakines, CX691 and CX731, in an animal model of depression. Their results suggested that these ampakines may have antidepressant activity (Knapp et al., 2002).

CX546 has been used in animal studies as a proposed treatment for schizophrenia (Lipina et al., 2007), while it has also demonstrated significant efficacy in reversing respiratory depression (Ren et al., 2006). Farampator, otherwise known as CX691 or Org24448, has been researched as a potential treatment for schizophrenia and AD. Clinical studies showed that the drug improved short-term memory, although episodic memory was impaired (Wezenberg et al., 2007). Mechanistically, ampakine CX614

slows deactivation of AMPA receptors, contributing to its cognitive enhancing properties (Arai & Lynch, 1998). However, CX614 also increases levels of BDNF (Lauterborn et al., 2003) and may thus have potential as a treatment for neurodegenerative disorders. Refer to **figure 14** for structures of the ampakines CX516, CX546 and CX614.

The biarylpropylsulfonamide class (LY404187 and LY503430) reduces desensitization of the AMPA receptor ion channel, resulting in enhancement of AMPA receptor-mediated neurotransmission (refer to **figure 15**). These AMPA receptor modulators have potential as a treatment for AD, schizophrenia, depression and Parkinson's disease (O'Neill et al., 2004). The ampakine LY503430 also increased levels of BDNF in animal models of Parkinson's disease (O'Neill, 2005; Murray, 2003).

Ampakine compounds are designated as either low impact or high impact compounds. The ampakines, CX614 and CX929, are high impact ampakine compounds binding to the cyclothiazide binding site and activating the expression of BDNF. On the other hand, CX717, CX701, Org24448 and Org26576 bind to a different modulatory site on the AMPA receptor complex and are referred to as low impact ampakine compounds. The low impact ampakine compounds do not significantly enhance BDNF, but remain effective in strengthening synaptic connectivity (Cortex Pharmaceuticals, 2008).

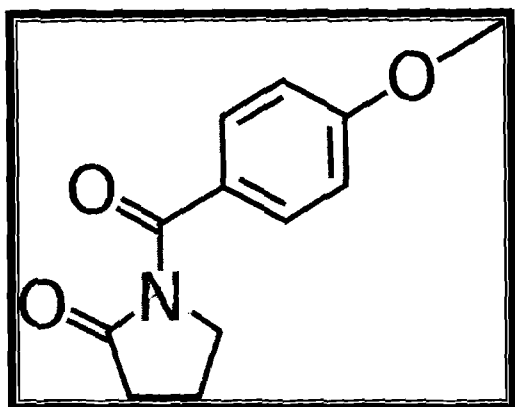


Figure 13: Structure of the AMPA receptor potentiator aniracetam. (Wikipedia contributors, 2008a).

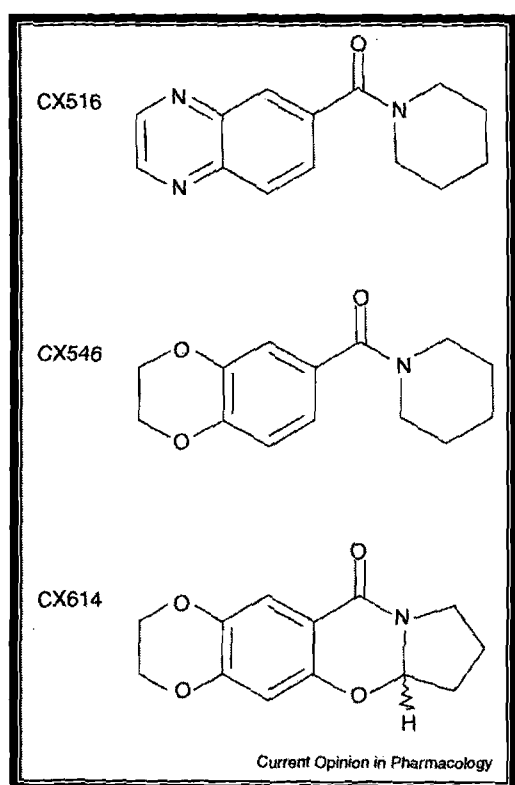


Figure 14: Chemical structures of ampakines CX516, CX546 and CX614 (Lynch, 2006).

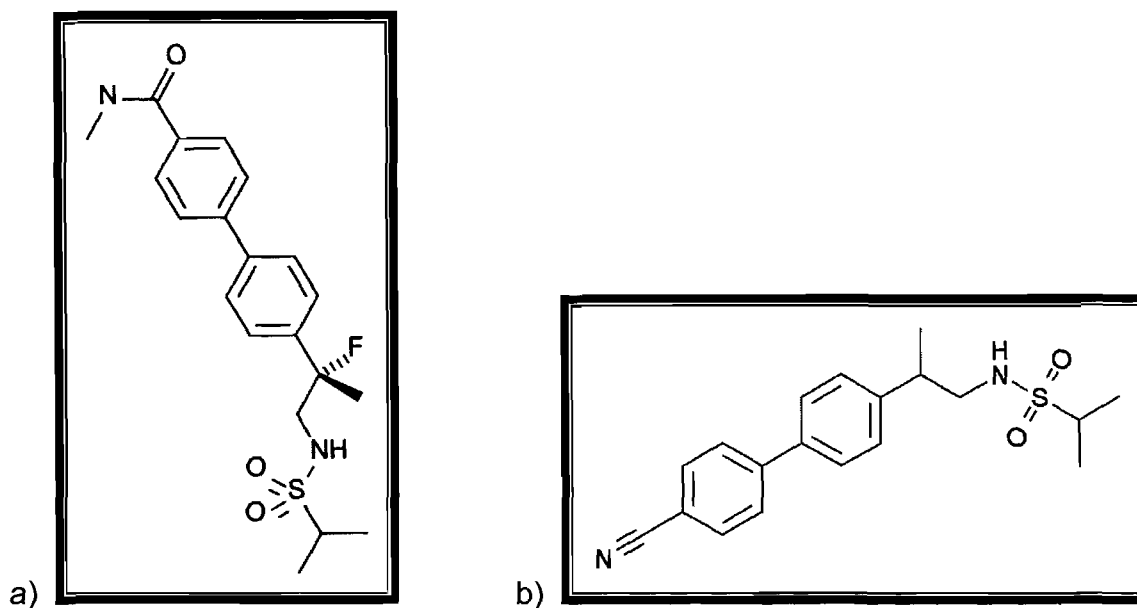


Figure 15: Structures of ampakines a) LY503430 and b) LY404187.

(Wikipedia contributors, 2008b) and (Wikipedia contributors, 2008c).

The ampakines, Org26576 and Org24448, increase local cerebral glucose metabolism in the mouse in various regions of the thalamus, cortex, dentate gyrus and hippocampus (Jordan et al, 2005), thus providing an anatomical basis for the use of these agents in disorders where the conservation and improvement of cognitive functioning are required, such as schizophrenia, AD and depression. In support of their possible use in depression, increased striatal AMPA receptor binding has been reported in suicide victims, while others have noted a decrease in AMPA-receptor-directed MAPK signaling pathways in brain of depressed suicide victims (Alt et al., 2006). The MAPK pathway plays an important regulatory role in the expression of BDNF, now being increasingly recognized as an important biomolecular target for antidepressant action (Alt et al., 2007). Furthermore, increased synaptic 5-HT levels that follow antidepressant treatments are known to mediate changes in phosphorylation of important signaling proteins, particularly dopamine and cAMP regulated phosphoprotein (DARPP) and the GLU_{R1} subunit of the AMPA receptor. Actions on the latter will favor increased channel conductance, with a resulting effect on mood (Alt et al, 2006).

While a possible role for AMPA receptors in depression is evident, its role in PTSD is less defined. However, a recent paper has provided evidence that emotional experiences enhances learning via NA-mediated phosphorylation of GluR1-containing AMPA-receptors and as such enhances AMPA trafficking (Hu et al, 2007). Consequently, increased AMPA receptor activity and function provides a molecular mechanism for how emotion enhances learning and memory.

Thus, ampakines may have clinical usefulness in the treatment of stress-related illnesses where excessive neurodegenerative mechanisms are involved by enhancing cognitive deficits and promoting cell proliferation. These effects may follow one or more of the following mechanisms:

- 1) By attenuating deactivation and/or desensitisation of AMPA receptors.
- 2) By expanding cortical networks by increasing transmission.
- 3) By bolstering NMDA activity and facilitating LTP.
- 4) By up-regulating BDNF (Witkin & Eiler, 2006; Lynch, 2004).

15. Conclusion

PTSD is characterized by hyperarousal, intrusions, avoidance and cognitive dysfunction; symptoms that have a great impact on the well-being of the patient and interfering substantially with daily life. Although current drug treatment is effective, the response is still far from satisfactory and improved drugs, and new drug targets, are urgently needed.

Ampakine studies up to date have indicated their potential as novel drug candidates in the treatment of various neuropsychiatric disorders, but most notably in illnesses characterised by deficits in cognitive function. Indeed, animal studies have indicated treatment possibilities in major depression, PD, AD and schizophrenia. Clinical

studies have shown synergistic effects in combination with antipsychotics, and have demonstrated an ability to improve short-term memory.

Animal models play an important role in new drug discovery and also in identifying specific drug treatments for certain behavioural and biochemical correlates of human illness. The term “PTSD model” has become fashionable to describe many different animal studies where behavioural alterations are induced by stress. However, only few of these truly reflect the human disorder. Novel investigation into the neurobiology and treatment of PTSD requires valid animal modelling with clearly defined criteria. These criteria seem to be addressed in the SPS model, which will be used in the current study.

16. Project aims and objectives

Aims: The AMPA receptor plays a critical role in memory, while clinical and pre-clinical evidence supports the possible value of AMPA receptor modulators (ampakines) in the treatment of various neuropsychiatric disorders where deficits in cognition are evident. This study represents the first dose-dependent evaluation of whether an ampakine may improve spatial memory in rodents, and particularly whether it can reverse explicit memory deficits evoked in an animal model of PTSD.

Moreover, with AMPA-NMDA receptor interactions playing a decisive role in LTP and hence memory formation, this study will explore whether any observed benefits in explicit memory induced by the ampakine are accompanied by changes in frontal cortical and hippocampal NMDA receptor binding.

Finally, with the above effects established, the study will then explore whether the ampakine can reverse or attenuate stress-induced cognitive dysfunction and NMDA receptor changes induced by a pathological state, in this case the SPS model of PTSD.

Objection 1: To investigate the dose-dependent effects of a novel ampakine, *Org 26576*, on explicit memory performance in normal rats, and whether any behavioural changes are accompanied by regional brain NMDA receptor binding changes (manuscript prepared for submission to *Behavioural Pharmacology*??; Chapter 3).

Objective 2: To investigate the dose-dependent effects of a novel ampakine, *Org 26576*, on stress-evoked changes in explicit memory performance following exposure of rats to SPS, a putative animal model of PTSD, and whether any behavioural changes following drug treatment are accompanied by regional brain NMDA receptor binding changes (Chapter 4).

17. References

- Adamec, R. E., Kent, P., Anisman, H., Shallow, T., Merali, Z., 1998. Neural plasticity, neuropeptides and anxiety in animals--implications for understanding and treating affective disorder following traumatic stress in humans. *Neurosci Biobehav Rev* 23, 301-318.
- Adamec, R. E., Burton, P., Shallow, T., Budgell, J., 1999. NMDA receptors mediate lasting increases in anxiety-like behaviour produced by the stress of predator exposure--implications for anxiety associated with posttraumatic stress disorder. *Physiol Behav* 65, 723-737.
- Adams, B., Moghaddam, B., 1998. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* 18, 5545-5554.
- Adams, J. P., Dudek, S. M., 2005. Late-phase long-term potentiation: getting to the nucleus. *Nat Rev Neurosci* 6, 737-743.
- Adolphs, R., 2002. Neural systems for recognizing emotion. *Curr Opin Neurobiol* 12, 169-177.
- Aerni, A., Traber, R., Hock, C., Roozendaal, B., Schelling, G., Papassotiropoulos, A., Nitsch, R. M., Schnyder, U., DeQuervain, D. J., 2004. Low-dose cortisol for symptoms of posttraumatic stress disorder. *Am J Psychiat* 161, 1488-1490.
- Agrypolous, S. V., Sandford, J. J., Nutt, D. J., 2000. The psychobiology of anxiolytic drugs. Part 2: Pharmacological treatments of anxiety. *Pharmacol Therap* 88, 213-227.
- Agster, K. L., Fortin, N. J., Eichenbaum, H., 2002. The hippocampus and disambiguation of overlapping sequences. *J Neurosci* 22, 5760-5768.
- Akirav, I., Maroun, M., 2006. Ventromedial prefrontal cortex is obligatory for consolidation and reconsolidation of object recognition memory. *Cerebral Cortex* 16, 1759-1765.
- Albucher, R. C., Liberzon, I., 2002. Psychopharmacological treatment in PTSD: a critical review. *J Psychiat Res* 36, 355-367.
- Alt, A., Nisenbaum, E. S., Bleakman, D., Witkin, J. M., 2006. A role for AMPA receptors in mood disorders. *Biochem Pharmacol* 71, 1273-1288.
-

- Alt, A., Weiss, B., Ornstein, P. L., Gleason, S. D., Bleakman, D., Stratford, R. E. Jr., Witkin, J. M., 2007. Anxiolytic-like effects through a GLUK5 kainate receptor mechanism. *Neuropharmacology* 52, 1482-1487.
- Amen, D. G., Routh, L. C., 2003. *Healing anxiety and depression*. New York, NY US: The Berkley Publishing Group.
- American Psychiatric Association., 2000. Diagnostic and statistical manual of mental disorders (4th ed., text revision). Washington, D.C.: American Psychiatric Press.
- Anis, N. A., Berry, S. C., Burton, N. R., Lodge, D., 1983. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-D-aspartate. *Brit J Pharmacol* 79, 565-575.
- Antoni, F. A., 1986. Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. *Endocr Rev* 7, 351–378.
- Arai, A., Kessler, M., Ambros-Ingerson, J., Quan, A., Yigiter, E., Rogers, G., Lynch, G., 1996. Effects of a centrally active benzoylpyrrolidine drug on AMPA receptor kinetics. *Neuroscience* 75, 573–585.
- Arai, A., Lynch, G., 1998. AMPA receptor desensitization modulates synaptic responses induced by repetitive afferent stimulation in hippocampal slices. *Brain Res* 799, 235–242.
- Arai, A. C., Xia, Y-F., Rogers, G., Lynch, G., Kessler, M., 2002. Benzamide-type AMPA receptor modulators form two subfamilies with distinct modes of action. *J Pharmacol Exp Ther* 303, 1075-1085.
- Arai, A. C., Xia, Y-F., Suzuki, E., 2004. Modulation of AMPA receptor kinetics differentially influences synaptic plasticity in the hippocampus. *Neuroscience* 123, 1011-1024.
- Armony, J. L., LeDoux, J. E., 2000. How danger is encoded: toward a systems, cellular, and computational understanding of cognitive–emotional interactions in fear. In: Gazzaniga, M. S., (Ed), *The cognitive neurosciences*. Cambridge, MA: The MIT Press, pp. 1067–1079.

- Arnsten, A. F., Steere, J. C., Hunt, R. D., 1996. The contribution of alpha 2-noradrenergic mechanisms of prefrontal cortical cognitive function. potential significance for attention-deficit hyperactivity disorder. *Arch Gen Psychiat* 53, 448-455.
- Arnsten, A. F. T., 1998. Catecholamine modulation of prefrontal cortical cognitive function. *Trends Cogn Sci* 2, 436-447.
- Arnsten, A. F. T., Robbins, T. W. 2002. Neurochemical modulation of prefrontal cortical function in humans and animals. In: Stuss, D. T., Knight, R. T. (Eds), *In Principles of Frontal Lobe Function*. NewYork: Oxford University Press, pp. 51-84.
- Asnis G. M., Kohn, S. R., Henderson, M., Brown, N. L., 2004. SSRIs versus non-SSRIs in post-traumatic stress disorder: an update with recommendations. *Drugs* 64, 383-404.
- Baddeley, A. D., 1990. *Human Memory: theory and practice*. Boston: Allyn and Bacon.
- Baddeley, A., 1998. Recent developments in working memory. *Curr Opin Neurobiol* 8, 234-238.
- Bai, F., Li, X., Clay, M., Lindstrom, T., Skolnick, P., 2001. Intra- and interstrain differences in models of "behavioral despair". *Pharmacol Biochem Behav* 70, 187-192.
- Bailey, K. R., Mair, R. G. 2004. Dissociable effects of frontal cortical lesions on measures of visuospatial attention and spatial working memory in the rat. *Cerebral Cortex* 14, 974-985.
- Baker, D. G., West, S. A., Nicholson, W. E., Ekhtor, N. N., Kasckow, J. W., Hill, K. K., et al., 1999. Serial CSF corticotropin-releasing hormone levels and adrenocortical activity in combat veterans with posttraumatic stress disorder. *Am J Psychiat* 156, 585-588.
- Bartness, T. J., Song, C. K., Demas, G. E., 2001. SCN efferents to peripheral tissues: implications for biological rhythms. *J Biol Rhythms* 16, 196-204.
- Berman, D. E., Hazvi, S., Neduva, V., Dudai, Y., 2000. The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste:
-

- Activation of ERK1-2 and formation of a memory trace. *J Neurosci* 20, 7017-7023.
- Bernabeu, R., Bevilacqua, L., Ardenghi, P., Bromberg, E., Schmitz, M., Bianchin, M., et al., 1997. Involvement of hippocampal D1/D5 receptor-cAMP signaling pathways in late memory consolidation. *Proc Nat Acad Sci USA* 94, 7041-7046.
- Birnbaum, S.G., Gobeske, K. T., Auerbach, J., Taylor, J. R., Arnsten, A. F., 1999. A role for norepinephrine in stress-induced cognitive deficits: alpha-1-adrenoceptor mediation in prefrontal cortex. *Biol Psychiat* 46, 1266-1274.
- Bisson, J., Andrew, M., 2007. Psychological treatment of post-traumatic stress disorder (PTSD). *Cochrane Database of Systematic Reviews*, (3) CD003388.
- Bisson, J. I., Ehlers, A., Matthews, R., Pilling, S., Richards, D., Turner, S., 2007. Psychological treatments for chronic post-traumatic stress disorder. Systematic review and meta-analysis. *Br J Psychiat* 190, 97-104.
- Blanpied, T. A., Boeckman, F. A., Aizenman, E., Johnson, J. W., 1997. Trapping channel block of NMDA-activated responses by amantadine and memantine. *J Neurophysiol* 77, 309-323.
- Bleakman, D., Lodge, D., 1998. Neuropharmacology of AMPA and kainate receptors. *Neuropharmacology* 37, 1187-204.
- Bonne, O., Brandes, D., Gilboa, A., Gomori, J. M., Shenton, M. E., Pitman, R. K., Shalev, A. Y., 2001. Longitudinal MRI study of hippocampal volume in trauma survivors with PTSD. *Am J Psychiat* 158, 1248-1251.
- Bormann, J., 1989. Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. *Eur J Pharmacol* 166, 591-592.
- Brady, K., Pearlstein, T., Asnis, G. M., et al., 2000a. Efficacy and safety of sertraline treatment of posttraumatic stress disorder: a randomized controlled trial. *JAMA* 283, 1837-1844.
- Brady, K. T., Killeen, T. K., Brewerton, T., Lucerini, S., 2000b. Comorbidity of psychiatric disorders and posttraumatic stress disorder. *Journal of Clinical Psychiatry* 61, 22-32. In: Duke, L. M., Vasterling, J. J. (Eds), *Neuropsychology of PTSD: biological, cognitive, and clinical perspectives*. Guilford Press, NY, pp. 3-24.
-

- Brand, L., Naciti, C., Stein, D. J., Harvey, B. H., Cognitive dysfunction and serotonin receptor changes evoked by stress-restress are reversed by the steroid synthesis inhibitor, ketoconazole. *Int J Neuropsychopharmacol*. 2004;7(suppl 1):S459 (published abstract).
- Brand, L., Groenewald, I., Stein, D. J., Wegener, G., Harvey, B. H., 2008. Stress and re-stress increases conditioned taste aversion learning in rats: possible frontal cortical and hippocampal muscarinic receptor involvement. *Eur J Pharmacol* 586, 205-211.
- Bremner, J. D., Randall, P. R., Capelli, S., Scott, T., McCarthy, G., Charney, D. S., 1995a. Deficits in short-term memory in adult survivors of childhood abuse. *Psychiatry Res* 59, 97–107.
- Bremner, J. D., Randall, P., Scott, T. M., Bronen, R. A., Seibyl, J. P., Southwick, S. M., Delaney, R. C., McCarthy, G., Charney, D. S., Innis, R. B., 1995b. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. *Amer J Psychiat* 152, 973–981.
- Bremner, J. D., Krystal, J. H., Southwick, S. M., Charney, D. S., 1996. Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. *Synapse* 23, 28–38.
- Bremner, J. D., Randal, P., Vermetten, E., Staib, L., Bronen, R. A., Mazure, C., et al., 1997a. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse – a preliminary report. *Biol Psychiat* 41, 23–32.
- Bremner, J. D., Licinio, J., Darnell, A., Krystal, J. H., Owens, M. J., Southwick, S. M., et al., 1997b. Elevated CSF corticotropin-releasing factor concentrations in posttraumatic stress disorder. *Am J Psychiat* 154, 624–629.
- Bremner, J. D., 1999. Does stress damage the brain. *Biol Psychiat* 45, 797-805.
- Bremner, J. D., 2002. Neuroimaging studies in post-traumatic stress disorder. *Curr Psychiat Rep* 4, 254–263.
- Bremner, J. D., Vythilingam, M., Anderson, G., Vermetten, E., McGlashan, T., Heninger, G., et al., 2003. Assessment of the hypothalamic–pituitary–adrenal axis over a 24-hour diurnal period and in response to neuroendocrine challenges in women with and without childhood sexual abuse and posttraumatic stress disorder. *Biol Psychiat* 54, 710–718.
-

- Bremner, J. D., Vermetten, E., Schmahl, C., Vaccarino, V., Vythilingam, M., Afzal, N., Grillon, C., Charney, D. S., 2005. Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. *Psychol Med* 35, 791–806.
- Bremner, J. D., 2007. Does stress damage the brain? In Kirmayer, L. J., Lemelson, R., Barad, M., Kirmayer, L. J., Lemelson, R., Barad, M., (Eds), *Understanding trauma: Integrating biological, clinical, and cultural perspectives*. Cambridge University Press, NY US, New York, pp. 118-141.
- Breslau, N., Kessler, R. C., Chilcoat, H. D., Schultz, L. R., Davis, G. C., Andreski, P., 1998. Trauma and posttraumatic stress disorder in the community: The 1996 detroit area survey of trauma. *Arch Gen Psychiat* 55, 626-632.
- Brewin, C. R., 2001. A cognitive neuroscience account of posttraumatic stress disorder and its treatment. *Behav Res Ther* 39, 373–393.
- Bruel-Jungerman, E., Davis, S., Laroche, S., 2007. Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* 13, 492–505.
- Brunello, N., Davidson, J. R., Deahl, M., Kessler, R. C., Mendlewicz, J., Racagni, G., Shalev, A. Y., Zohar, J., 2001. Posttraumatic stress disorder: diagnosis and epidemiology, comorbidity and social consequences, biology and treatment. *Neuropsychobiol* 43, 150–162.
- Buckner, R. L., Tulving, E., 1995. Neuroimaging studies of memory: Theory and recent PET results. In: Boller, F., Grafman, J., (Eds), *Handbook of Neuropsychology* Vol. 10. Elsevier Amsterdam, pp. 439-466.
- Buijs RM, Kalsbeek A., 2001. Hypothalamic integration of central and peripheral clocks. *Nat Rev Neurosci* 2, 521–526.
- Burgess, N., Maguire, E. A., O'Keefe, J., 2002. The human hippocampus and spatial and episodic memory. *Neuron* 35, 625-641.
- Burnashev, N., Zhou, Z., Neher, E., Sakmann, B., 1995. Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. *J Physiol* 485, 403-418.
- Campbell, A., 2008. Attachment, aggression and affiliation: The role of oxytocin in female social behavior. *Biol Psychol* 77, 1-10.
-

- Carlezon, W. A., Jr., Duman, R. S., Nestler, E. J., 2005. The many faces of CREB. *Trends Neurosci* 28, 436–445.
- Chambers, R. A., Bremner, J. D., Moghaddam, B., Southwick, S. M., Charney, D. S., Krystal, J. H., 1999. Glutamate and post-traumatic stress disorder: Toward a psychobiology of dissociation. *Semin Clin Neuropsychiat* 4, 274-281.
- Charney, D. S., Deutch, A. Y., Southwick, S. M., Krystal, J. H., 1995. Neural circuits and mechanisms of post-traumatic stress disorder. In: Friedman, M. J., Charney, D. S., Deutch, A. Y., (Eds), *Neurobiological and Clinical Consequences of Stress: from normal adaptation to PTSD*. Lippincott-Raven, Philadelphia, pp. 271-287.
- Charney, D. S., 2003. Neuroanatomical circuits modulating fear and anxiety behaviors. *Acta Psychiatr Scand Suppl* 417, 38–50.
- Chen, B., Dowlathahi, D., MacQueen, G. M., Wang, J. F., Young, L. T., 2001. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. *Biol Psychiat* 50, 260-65.
- Cheong, M. Y., Yun, S. H., Mook-Jung, I., Joo, I., Huh, K., Jung, M. W., 2001. Cholinergic modulation of synaptic physiology in deep layer entorhinal cortex of the rat. *J Neurosci Res* 66, 117-121.
- Cherubini, E., Conti, F., 2001. Generating diversity at GABAergic synapses. *Trends Neurosci* 24, 155–162.
- Chung, M. Y., Kyung, H. M., Yong, J. J., Sung, S. K., Wan, C. K., Eun, M. J., 2004. Efficacy and tolerability of mirtazapine and sertraline in Korean veterans with posttraumatic stress disorder: a randomized open label trial. *Hum Psychopharmacol* 19, 489–494.
- Clark, C. R., McFarlane, A. C., Morris, P., Weber, D. L., Sonckilla, C., Shaw, M. E., Marcina, J., Tochon-Danguy, H. J., Egan, G. F., 2003. Cerebral function in posttraumatic stress disorder during verbal working memory updating: a positron emission tomography study. *Biol Psychiat* 53, 474–481.
- Cohen, H., Zohar, J., Matar, M., 2003. The relevance of differential response to trauma in an animal model of posttraumatic stress disorder. *Biol Psychiat* 53, 463–473.
-

- Cohen, H., Zohar, J., Matar, M.A., Zeev, K., Loewenthal, U., Richter-Lewin, G., 2004. Setting apart the affected: the use of behavioural criteria in animal models of posttraumatic stress disorder. *Neuropsychopharmacol* 29, 1962–1970.
- Collingridge, G. L., Isaac, J. T. R., Yu Tian Wang., 2004. Receptor trafficking and synaptic plasticity. *Nat Rev Neurosci* 5, 952-962.
- Connor, K. M, Davidson, J. R. T., 1998. The role of serotonin in posttraumatic stressdisorder:neurobiology and pharmacotherapy. *CNS Spectr* 3, 43–51.
- Connor, K. M., Sutherland, S. M., Tupler, L. A., Malik, M. L., Davidson, J. R. T., 1999. Fluoxetine in posttraumatic stress disorder. Randomized, double blindstudy. *Br J Psychiat* 175:17–22.
- Contestabile, A., Monti, B., Contestabile, A., Ciani, E., 2003. Brain Nitric Oxide and Its Dual Role in Neurodegeneration / Neuroprotection: Understanding Molecular Mechanisms to Devise Drug Approaches. *Curr Med Chem* 10, 2147-2174.
- Cooke, S. F., Bliss, T. V., 2006. Plasticity in the human central nervous system. *Brain* 129, 1659–1673.
- Corkin, S., 1984. Lasting consequences of bilateral medial temporal lobectomy: clinical course and experimental findings in H.M. *Semin Neurol* 4, 249–259.
- Cortex Pharmaceuticals, 2008. <http://www.cortexpharm.com/> (Accessed on [21 Sep. 2008]).
- Cosman, K. M., Boyle, L. L., Porsteinsson, A. P., 2007. Memantine in the treatment of mild-to-moderate Alzheimer's disease. *Exp Opin Pharmac* 8, 203-214.
- Creamer, M., Burgess, P., McFarlane, A. C., 2001. Post-traumatic stress disorder: findings from the Australian National Survey of Mental Health and Well-being. *Psychol Med* 31, 1237-1247.
- Cummings, J. A., Mulkey, R. M., Nicoll, R. A., Malenka, R. C., 1996. Ca²⁺ signaling requirements for long-term depression in the hippocampus. *Neuron* 16, 825–833.
- Curtis, C. E., D'Esposito, M., 2003. Persistent activity in the prefrontal cortex during workingmemory. *Trends Cogn Sci* 7, 415–423.
-

- Cuthbert, B. N., 2002. Social anxiety disorder: trends and translational research. *Biol Psychiat* 51, 4 – 10.
- Davidson, J. R. T., Kudler, H., Smith, R., et al., 1990. Treatment of posttraumatic stress disorder with amitriptyline and placebo. *Arch Gen Psychiat* 47, 259–266.
- Davidson, J. R. T., 1997. Biological therapies for posttraumatic stress disorder: an overview. *J Clin Psychiat* 58, 29–32.
- Davidson, J. R. T., Stein, D. J., Shalev, A. Y., Yehuda, R., 2004. Posttraumatic stress disorder: acquisition, recognition, course, and treatment. *J Neuropsychiat Clin Neurosci* 16, 135–147.
- Davidson, J. R. T., Baldwin, D., Stein, D. J., Kuper, E., Benattia, I., Ahmed, S, Pedersen, R., Musgnung, J., 2006. Treatment of posttraumatic stress disorder with venlafaxine extended release: a 6-month randomized controlled trial. *Arch Gen Psychiat* 63, 1158–1165.
- Davis, M., Whalen, P. J., 2001. The amygdala: vigilance and emotion. *Mol Psychiat* 6, 13–34.
- Davis, M., Myers, K. M., 2002. The role of glutamate and Gamma-Aminobutyric acid in fear extinction: clinical implications for exposure therapy. *Biol Psychiat* 998–1007.
- Dawson, V. L., Dawson, T. M. 1995., Physiological and toxicological actions of nitric oxide in the central nervous system. In: Ignarro, L., Murad, F. (Eds). Nitric oxide: biochemistry, molecular biology, and therapeutic implications. Academic Press, London, pp. 323–330.
- De Groot, D. M., Bierman, E. P., Bruijnzeel, P. L., Carpentier, P., Kulig, B. M., Lallement, G., et al., 2001. Beneficial effects of TCP on soman intoxication in guinea pigs: seizures, brain damage and learning behaviour. *J Appl Toxicol* 21, S57–65.
- De Kloet, E. R., 2000. Stress in the brain. *Eur J Pharmacol* 405, 187–198.
- De Kloet, E. R., 2003. Hormones, brain and stress. *Endocr Regul* 37, 51–68.
- De Kloet, E. R., Derijk, R., 2004. Signaling pathways in brain involved in predisposition and pathogenesis of stress-related disease: Genetic and kinetic factors affecting the MR/GR balance. *Ann NY Acad Sci* 1032, 14–34.
-

- De Kloet, E. R., Joels, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 6, 463–475.
- De Kloet, C. S., Vermetten, E., Geuze, E., Wiegant, V. M., Westenberg, H. G., 2008. Elevated plasma arginine vasopressin levels in veterans with posttraumatic stress disorder. *J Psychiatr Res* 42, 192–198.
- De Quervain, D. J., Roozendaal, B., McGaugh, J. L., 1998. Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature* 394, 787–790.
- De Renzi, E., Liotti, M., Nichelli, P., 1987. Semantic amnesia with preservation of autobiographical memory: a case report. *Cortex* 23, 575–597.
- De Winter, R., van Hemert, A., DeRijk, R., Zwinderman, K., Frankhuijzen-Sierevogel, A., Wiegant, V., et al., 2003. Anxious-retarded depression: relation with plasma vasopressin and cortisol. *Neuropsychopharmacol* 28, 140–147.
- Dicou, E., Rangon, C., Guimiot, F., Spedding, M., Gressens, P., 2003. Positive allosteric modulators of AMPA receptors are neuroprotective against lesions induced by an NMDA agonist in neonatal mouse brain. *Brain Res* 970, 221–225.
- Dingledine, R., Borges, K., Bowie, D., Traynelis, S. F., 1999. The glutamate receptor ion channels. *Pharmacol Rev* 51, 7–61.
- Dobie, D. J., Kivlahan, D. R., Maynard, C., Bush, K. R., Davis, T. M., Bradley, K. A., 2004. Posttraumatic stress disorder in female veterans. Association with self-reported health problems and functional impairment. *Arch Intern Med* 164, 394–400.
- Drake, R. G., Davis, L. L., Cates, M. E., Jewell, M. E., Ambrose, S. M., Lowe, J. S., 2003. Baclofen treatment for chronic posttraumatic stress disorder. *Ann Pharmacol* 37, 1177–1181.
- D'Souza, D. C., Charney, D. S., Krystal, J. H. 1995. Glycine site agonists of the NMDA receptor: a review. *CNS Drug Rev* 1, 227–260.
- Du, J., Quiroz, J., Yuan, P., Zarate, C., Manji, H. K., 2004. Bipolar disorder: Involvement of signaling cascades and AMPA receptor trafficking at synapses. *Neuron Glia Biology* 13, 231–243.

- Du, J., Machado-Vieira, R., Maeng, S., Martinowich, K., Manji, H. K., Zarate, C. A., 2006. Enhancing AMPA to NMDA throughput as a convergent mechanism for antidepressant action. *Drug Discovery Today: Therapeutic Strategies* 3, 519-526.
- Dubuc, B., 2008. <http://www.thebrain.mcgill.ca> (Accessed on [23 Jul. 2008]).
- Eichenbaum, H., Otto, T., Cohen, N. J., 1993. Two functional components of the hippocampal memory system. *Beh Brain Sci*, 1–50.
- Eichenbaum, H., 2001. The hippocampus and declarative memory: cognitive mechanisms and neural codes. *Beh Brain Res* 127, 199–207.
- Eichenbaum, H., Cohen, N. J., 2001. From conditioning to conscious recollection: Memory systems of the brain. New York, NY US: Oxford University Press.
- Elzinga, B. M., Bremner, J. D., 2002. Are the neural substrates of memory the final common pathway in posttraumatic stress disorder (PTSD)? *J Affect Disorders* 70, 1-17.
- Emmett, M. R., Mick, S. J., Cler, J. A., Rao, T. S., Iyengar, S., Wood, P. L., 1991. Actions of D-cycloserine at the N-methyl-D-aspartate-associated glycine receptor site in vivo. *Neuropharmacology* 30, 1167–1171.
- English, J., Pearson, G., Wilsbacher, J., Swantek, J., Karandikar, M., Xu, S., et al., 1999. New insights into the control of MAP kinase pathways. *Exp Cell Res* 253, 255–270.
- Fesler, F. A., 1991. Valproate in combat-related posttraumatic stress disorder. *J Clin Psychiat* 52, 361–364.
- Fiore, R. S., Bayer, V. E., Pelech, S. L., Posada, J., Cooper, J. A., Baraban, J. M., 1993. p42 mitogen-activated protein kinase in brain: prominent localization in neuronal cell bodies and dendrites. *Neurosci* 55, 463–472.
- Flood, D. G., Finn, J. P., Walton, K. M., Dionne, C. A., Contreras, P. C., Miller, M. S., et al., 1998. Immunolocalization of the mitogen-activated protein kinases p42MAPK and JNK1, and their regulatory kinases MEK1 and MEK4, in adult rat central nervous system. *J Comp Neurol* 398, 373–392.
- Fortin, C., Couture, E., 2002. Short-term memory and time estimation: Beyond the 2-second 'critical' value. *Can J Exp Psychol*, 56, 120-127.

- Francati, V., Vermetten, E., Bremner, J. D., 2007. Functional neuroimaging studies in posttraumatic stress disorder: review of current methods and findings. *Depress Anxiety* 24, 202–218.
- Frank, J. B., Kosten, T. R., Giller, E. L. Jr., Dan, E., 1988. A randomized clinical trial of phenelzine and imipramine for posttraumatic stress disorder. *Am J Psychiat* 145, 1289–1291.
- Frank, D. A., Greenberg, M. E., 1994. CREB: a mediator of long-term memory from mollusks to mammals. *Cell* 79, 5–8.
- Frankland, P. W., Bontempi, B., 2005. The organization of recent and remote memories. *Nat Rev Neurosci* 6, 119–130.
- Freeman, M. E., Kanyicska, B., Lerant, A., Nagy, G., 2000. Prolactin: structure, function, and regulation of secretion. *Physiol Rev* 80, 1523–1631.
- Gass, P., Kretz, O., Wolfer, D. P., Berger, S., Tronche, F., Reichardt, H. M., Kellendock, C., Lipp, H-P., Schmid, W. Sxhittz, G. 2000. Genetic disruption of mineralocorticoid receptor leads to impaired neurogenesis and granule cell degeneration in the hippocampus of adult mice. *EMBO Reports* 1, 447–451.
- Genetic Science Learning Center, "Learn.Genetics," *Learn.Genetics*, 2008. <<http://learn.genetics.utah.edu/>> (20 November 2008)
- Geraciotti, T. D., Baker, D. G., Ekhtator, N. N., West, S. A., Hill, K. K., Bruce, A. B., Schmidt, D., Rounds-Kugler, B., Yehuda, R., Keck, P. E., Kasckow, J. W., 2001. CSF norepinephrine concentrations in posttraumatic stress disorder. *Amer J Psychiat* 158, 1227–1230.
- Geyer, M. A., Markou, A. 1995., Animal models of psychiatric disorders. In: Bloom, F. E., Kupfer, D. J., (Ed), *Psychopharmacology: the fourth generation of progress*. Raven Press, New York, pp. 787–798.
- Gibbs, J. W., Sombati, S., DeLorenzo, R. J., Coulter, D. A., 2000. Cellular actions of topiramate: blockade of kainate-evoked inward currents in cultured hippocampal neurons. *Epilepsia* 41, 10–16.
- Gilbertson, M. W., Shenton, M. E., Ciszewski, A., Kasai, K., Lasko, N. B., Orr, S. P., Pitman, R. K., 2002. Smaller hippocampal volume predicts pathologic vulnerability to psychological trauma. *Nat Neurosci* 5, 1242–1247.
-

- Gillies, G. E., Linton, E. A., Lowry, P. J., 1982. Corticotropin releasing activity of the new CRF is potentiated several times by vasopressin. *Nature* 299, 355–357.
- Goddard, A. W., Mason, G. F., Appel, M., Rothman, D. L., Gueorguieva, R., Behar, K. L., et al., 2004. Impaired GABA neuronal response to acute benzodiazepine administration in panic disorder. *Amer J Psychiat* 161, 2186–2193.
- Goldstein, L. E., Rasmusson, A. M., Bunney, B. S., Roth, R. H., 1996. Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *J Neurosci* 16, 4787–4798.
- Gonzalez, G. A., Montminy, M. R., 1989. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* 59, 675–680.
- Grace, A. A., 2000. Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Res Brain Res Rev* 31, 330–341.
- Graf, P., Schacter, D. L., 1985. Implicit and explicit memory for new associations in normal and amnesic subjects, *J Exper Psychol Learn Mem Cogn* 11, 501–518.
- Granger, R., Staubli, U., Davis, M., Perez, Y., Nilsson, L., Rogers, G. A., Lynch, G., 1993. A drug that facilitates glutamatergic transmission reduces exploratory activity and improves performance in a learning-dependant task. *Synapse* 15, 326–329.
- Groenewegen, H. J., Wright, C. I., Beijer, A. V., Voorn, P., 1999. Convergence and segregation of ventral striatal inputs and outputs. *Ann NY Acad Sci* 877, 49–63.
- Gurvits, T. V., Gilbertson, M. W., Lasko, N. B., Tarhan, A. S., Simeon, D., Macklin, M. L., Orr, S. P., Pitman, R. K., 2000. Neurologic soft signs in chronic posttraumatic stress disorder. *Arch Gen Psychiat*, 57, 181–186.
- Hageman, I., Andersen, H. S., Jørgensen, M. B., 2001. Post-traumatic stress disorder: a review of psychobiology and pharmacotherapy. *Acta Psychiatrica Scandinavica* 104, 411–22.
- Hart, J. Jr., Gordon, B., 1990. Delineation of single-word semantic comprehension deficits in aphasia with anatomical correlation. *Ann Neurol* 27, 226–231.

- Harvey, B. H., Naciti, C., Brand, L., Stein, D. J., 2003. Endocrine, cognitive and hippocampal/cortical 5HT_{1A/2A} receptor changes evoked by a time-dependent sensitisation (TDS) stress model in rats. *Brain Res* 983, 97-107.
- Harvey, B. H., Naciti, C., Brand, L., Stein, D. J., 2004a. Serotonin and stress: Protective or malevolent actions in the biobehavioral response to repeated trauma? *Ann NY Acad Sci* 1032, 267-272.
- Harvey, B. H., Oosthuizen, F., Brand, L., Wegener, G., Stein, D. J., 2004b. Stress-restress evokes sustained iNOS activity and altered GABA level and NMDA receptors in rat hippocampus. *Psychopharmacology* 175, 494-502.
- Harvey, B. H., Bothma, T., Nel, A., Wegener, G., Stein, D. J., 2005. Involvement of the NMDA receptor, NO-cyclic GMP and nuclear factor K- β in an animal model of repeated trauma. *Hum Psychopharmacol* 20, 367-373.
- Harvey, B. H., 2006. Adaptive plasticity during stress and depression and the role of glutamate-nitric oxide pathways. *S Afr Psychiat Rev* 9, 132- 139.
- Harvey, B. H., Brand, L., Jeeva, Z., Stein, D. J., 2006. Cortical/hippocampal monoamines, HPA axis changes and aversive behavior following stress and re-stress in an animal model of post-traumatic stress disorder. *Physiol Behav* 87, 881-890.
- Hasher, L., Zacks, R.T., 1979. Automatic and effortful processes in memory. *J Exp Psychol Gen* 108, 356–388.
- Hayashi, Y., Shi, S. H., Esteban, J. A., Piccini, A., Poncer, J. C., Malinow, R., 2000. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* 287, 2262-2267.
- Hebb, D. O., 1949. *The organization of behavior; a neuropsychological theory*. Oxford England: Wiley.
- Heresco-Levy, U., Javitt, D. C., 1998. The role of N-methyl-D-aspartate (NMDA) receptor-mediated neurotransmission in the pathophysiology and therapeutics of psychiatric syndromes. *European Neuropsychopharmacology: J Eur Coll Neuropsychopharmacol* 8, 141-152.
- Heresco-Levy, U., Kremer, I., Javitt, D. C., Goichman, R., Reshef, A., Blamaru, M., Cohen, T., 2002. Pilot-controlled trial of D-cycloserine for the treatment of post-traumatic stress disorder. *Int J Neuropsychopharmacol* 5, 301-307.
-

- Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., Cullinan, W. E., 2003. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary–adrenocortical responsiveness. *Front Neuroendocrin* 24, 151–180.
- Herman, J. P., Ostrander, M. M., Mueller, N. K., Figueiredo, H., 2005. Limbic system mechanisms of stress regulation: hypothalamo–pituitary–adrenocortical axis. *Prog Neuropsychopharm Biol Psychiat* 29, 1201–1213.
- Hertzberg, M. A., Butterfield, M. I., Feldman, M. E., et al., 1999. A preliminary study of lamotrigine for the treatment of posttraumatic stress disorder. *Biol Psychiat* 45, 1226–1229.
- Het, S., Ramlow, G., Wolf, O. T., 2005. A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrinol* 30, 771–784.
- Heuser, I., Lammers, C.L., 2003. Stress and the brain. *Neurobiol Aging* 24, 69-76.
- Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. *Annu Rev Neurosci* 17, 31-108.
- Holmes, E. A., Grey, N., Young, K. A. D., 2005. Intrusive images and “hotspots” of trauma memories in posttraumatic stress disorder: An exploratory investigation of emotions and cognitive themes. *J Beh Ther Exp Psychiat* 36, 3-17.
- Hood, W. F., Compton, R. P., Monahan, J. B., 1989. D-cycloserine: a ligand for the N-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics. *Neurosci Lett* 98, 91–95.
- Houslay, M. D., 2006. A RSK(y) Relationship with Promiscuous PKA. *Sci STKE* p. pe32.
- Hu, H., Real, E., Takamiya, K., Kang, M. G., LeDoux, J., Huganir, R. L., Malinow, R., 2007. Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. *Cell* 131, 160-173.
- Huerta, P. T., Lisman, J. E., 1995. Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. *Neuron* 15, 1053-1063.
- Huggins, D. J., Grant, G. H., 2005. The function of the amino terminal domain in NMDA receptor modulation. *J Mol Graphics Modelling* 23, 381-388.
-

- Hull, A. M., 2002. Neuroimaging findings in post-traumatic stress disorder. Systematic review. *Br J Psychiatr* 181, 102–110.
- Husi, H., Ward, M. A., Choudhary, J. S., Blackstock, W. P., Grant, S. G., 2000. Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nat Neurosci* 3, 661–669.
- Iino, M., Ciani, S., Tsuzuki, K., Ozawa, S., Kidokoro, Y., 1997. Permeation properties of Na⁺ and Ca²⁺ ions through the mouse epsilon2/zeta1 NMDA receptor channel expressed in *Xenopus* oocytes. *J Membr Biol* 155, 143–56.
- Ingvar, M., Ambros-Ingerson, J., Davis, M., Granger, R., Kessler, M., Rogers, G. A., Schehr, R. S., Lynch, G., 1997. Enhancement by an ampakine of memory encoding in humans. *Exp Neurol* 146, 553–559.
- Ipsier, J., Seedat, S., Stein, D. J., 2006. Pharmacotherapy for post-traumatic stress disorder - a systematic review and meta-analysis. *S Afr Med J* 96, 1088–1096.
- Irwin, C., Falsetti, S. A., Lydiard, R. B., Ballenger, J. C., Brock, C. D., Brener, W., 1996. Comorbidity of posttraumatic stress disorder and irritable bowel syndrome. *J Clin Psychiat* 57, 576–578.
- Iversen, L., 2000. Neurotransmitter transporters: fruitful targets for CNS drug discovery. *Mol Psychiat* 5, 357–362.
- Iwamoto, Y., Morinobu, S., Takahashi, T., Yamawaki, S., 2007. Single prolonged stress increases contextual freezing and the expression of glycine transporter 1 and vesicle-associated membrane protein 2 mRNA in the hippocampus of rats. *Progr Neuro-Psychopharmacol Biol Psychiat* 31, 642–651.
- Izquierdo, I., Medina, J. H. 1997. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiol Learn Mem* 68, 285–316.
- Izquierdo, I., Cammarota, M., Medina, J. H., Bevilaqua, L. R., 2004. Pharmacological findings on the biochemical bases of memory processes: a general view. *Neural Plast* 11, 159–189.
- Izquierdo, L. A., Barros, D. M., da Costa, J. C., Furini, C., Zinn, C., Cammarota, M., et al., 2007. A link between role of two prefrontal areas in immediate memory and in long-term memory consolidation. *Neurobiol Learn Mem* 88, 160–166.

- Jacobson, L., Sapolsky, R., 1991. The role of the hippocampus in feedback regulation of the hypothalamic–pituitary–adrenocortical axis. *Endocr Rev* 12, 118–134.
- James, W., 1890. *The Principles of Psychology*, 1918 ed. New York: Holt.
- Janowsky, J. S., Shimamura, A. P., Kritchevsky, M., Squire, L. R., 1989. Cognitive impairment following frontal lobe damage and its relevance to human amnesia. *Behav Neurosci* 103, 548-560.
- Javitt, D. C., 2004. Glutamate as a therapeutic target in psychiatric disorders. *Mol Psychiat* 9, 984-997.
- Ji, J. Z., Wang, X. M., Li, B. M., 2003. Deficit in long-term contextual fear memory induced by blockade of beta-adrenoceptors in hippocampal CA1 region. *Eur J Neurosci* 17, 1947–1952.
- Jin, R., Clark, S., Weeks, A. M., Dudman, J. T., Gouaux, E., Partin, K. M., 2005. Mechanism of positive allosteric modulators acting on AMPA receptors. *J Neurosci* 25, 9027-9036.
- Johnson, S. A., Luu, N. T., Herbst, T. A., Knapp, R., Lutz, D., Arai, A., et al., 1999. Synergistic interactions between AMPAKINES® and antipsychotic drugs. *J Pharmacol Exp Ther* 289, 392-397.
- Johnsen, B. H., Eid, J., Laberg, J. C., Thayer, J'F., 2002. The effect of sensitization and coping style on post-traumatic stress symptoms and quality of life: two longitudinal studies. *Scand J Psychology* 43, 181-188.
- Jordan, G. R., McCulloch, J., Shahid, M., Hill, D. R., Henry, B., Horsburg, K., 2005. Regionally selective and dose-dependent effects of the ampakines Org 26576 and Org 24448 on local cerebral glucose utilisation in the mouse as assessed by ¹⁴C-2-deoxyglucose autoradiography. *Neuropharmacology* 49, 254-264.
- Kalia, L. V., Kalia, S. K., Salter, M. W., 2008. NMDA receptors in clinical neurology: Excitatory times ahead. *Lancet Neurol* 7, 742-755.
- Kandel, E. R., 2001. The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 294, 1030-1038.
- Kandel, E. R., 2001. The molecular biology of memory storage: A dialogue between genes and synapses. *Science* 294, 1030-1038.
-

- Kane, M. J., Engle, R. W., 2002. The role prefrontal cortex in working-memory capacity, executive attention, and general fluid intelligence: an individual-differences perspective. *Psychon Bull Rev* 9, 637–671.
- Karcz-Kubicha, M., Liljequist, S., 1995. Evidence for an anxiogenic action of AMPA receptor antagonists in the plus-maze test. *Eur J Pharmacology* 279, 171-177.
- Karl, A., Schaefer, M., Malta, L. S., Dörfel, D., Rohleder, N., Werner, A., 2006. A meta-analysis of structural brain abnormalities in PTSD. *Neurosci Biobehav Rev* 30, 1004-1031.
- Kashiwagi, K., Masuko, T., Nguyen, C. D., Kuno, T., Tanaka, I., Igarashi, K., Williams, K., 2002. Channel blockers acting at N-methyl-D-aspartate receptors: differential effects of mutations in the vestibule and ion channel pore. *Mol Pharmacol* 61, 533-545.
- Kaufer, D., Friedman, A., Seidman, S., Soreq, H., 1998. Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature* 393, 373-377.
- Kendler, K. S., Thornton, L. M., Gardner, C. O., 2001. Genetic risk, number of previous depressive episodes, and stressful life events in predicting onset of major depression. *Am J Psychiat* 158, 582-586.
- Kessler, R., Sonnega, A., Bromet, E., Hughes, M., Nelson, C., 1995. Posttraumatic stress disorder in the National Comorbidity survey. *Arch Gen Psychiat* 52, 1048–1060.
- Khan, S., Liberzon, I., 2004. Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. *Psychopharmacology* 172, 225-229.
- Kilpatrick, L., Cahill, L., 2003. Amygdala modulation of parahippocampal and frontal regions during emotionally influenced memory storage. *NeuroImage* 20, 2091-2099.
- Kirby, L. G., Allen, A. R., Lucki, I., 1995. Regional differences in the effects of forced swimming on extracellular levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. *Brain Res* 682, 189–196.
- Kirby, L. G., Chou-Green, J. M., Davis, K., Lucki, I., 1997. The effects of different stressors on extracellular 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. *Brain Res* 760, 218–230.
-

- Kirby, L. G., Lucki, I., 1997. Interaction between the forced swimming test and fluoxetine treatment on extracellular levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in the rat. *J Pharmacol Exp Ther* 282, 967–976.
- Kisch, E., 2002. The role of GABA in the pathogenesis and treatment of anxiety and other neuropsychiatric disorders. http://www.vcu-cme.org/gaba/gaba2_22.pdf (Accessed on [17 Nov. 2008]).
- Knapp, R. J., Goldenberg, R., Shuck, C., Cecil, A., Watkins, J., Miller, C., Crites, G., Malatynska, E., 2002. Antidepressant activity of memory-enhancing drugs in the reduction of submissive behavior model. *Eur J Pharmacol* 440, 27-35.
- Kohda, K., Harada, K., Kato, K., Hoshino, A., Motohashi, J., Yamaji, T., Morinobu, S., Matsuoka, N., Kato, N., 2007. Glucocorticoid receptor activation is involved in producing abnormal phenotypes of single-prolonged stress rats: a putative Post-Traumatic Stress Disorder model. *Neuroscience* 148, 22-33.
- Korff, A., 2007. Extended characterisation and validation of an animal model of post traumatic stress disorder: Behavioural, molecular and pharmacological studies. PhD thesis, North-West University, Potchefstroom, South Africa.
- Kosten, T. R., Frank, J. B., Dan, E., et al., 1991. Pharmacotherapy for posttraumatic stress disorder using phenelzine or imipramine. *J Nerv Ment Dis* 179, 366–370.
- Krukoff, T.L., Khalili, P., 1996. Stress-induced activation of nitric oxide-producing neurons in the rat brain. *J Comp Neurol* 377, 509-519.
- Kuhlmann, S., Wolf, O. T., 2006. Arousal and cortisol interact in modulating memory consolidation in healthy young men. *Behav Neurosci* 120, 217–223.
- Lauterborn, J. C., Truong, G. S., Baudry, M., Bi, X., Lynch, G., Gall, C. M., 2003. Chronic elevation of brain-derived neurotrophic factor by ampakines. , 307, 297–305.
- Lebrun, C., Pillière, E., Lestage, P., 2000. Effects of S 18986-1, a novel cognitive enhancer, on memory performances in an object recognition task in rats. *Eur J Pharmacol* 401, 205–212.
- LeDoux, J. E., 1990. The emotional brain. New York: Simon & Schuster.
- LeDoux, J. E., 1998. The Emotional Brain: the mysterious underpinnings of emotional Life. Touchstone, New York.
-

- Legros, J. J., 2001. Inhibitory effect of oxytocin on corticotrope function in humans: are vasopressin and oxytocin ying-yang neurohormones? *Psychoneuroendocrin* 26, 649–655.
- Legutko, B., Li, X., Skolnick, P., 2001. Regulation of BDNF expression in primary neuron culture by LY392098, a novel AMPA receptor potentiator. *Neuropharmacology* 40, 1019–27.
- Lehnert, H., Schulz, C., Dieterich, K., 1998. Physiological and neurochemical aspects of corticotropin-releasing factor actions in the brain: the role of the locus coeruleus. *Neurochem Res* 23, 1039–1052.
- Leserman, J., Zolnoun, D., Meltzer-Brody, S., Lamvu, G., Steege, J. F., 2006. Identification of diagnostic subtypes of chronic pelvic pain and how subtypes differ in health status and trauma history (includes discussion). *Am J Obst Gynecol* 195, 554–561.
- Leskin, G. A., Woodward, S. H., Young, H. E., Sheikh, J. I., 2002. Effects of comorbid diagnoses on sleep disturbance in PTSD. *J Psychiat Res* 36, 449–452.
- Levey, A. I., Kitt, C. A., Simonds, W. F., Price, D. L., Brann, M. R., 1991. Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J Neurosci* 11, 3218–3226.
- Li, X., Witkin, J. M., Need, A. B., Skolnick, P., 2003. Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell Mol Neurobiol* 23, 419–430.
- Liberzon, I., Krstov, M., Young, E. A., 1997. Stress-restress: Effects on ACTH and fast feedback. *Psychoneuroendocrin* 22, 443–453.
- Liberzon, I., López, J. F., Flagel, S. B., Vázquez, D. M., Young, E. A., 1999a. Differential regulation of hippocampal glucocorticoid receptor mRNA and fast feedback: relevance to post-traumatic stress disorder. *J Neuroendocrin* 11, 11–17.
- Liberzon, I., Taylor, S. F., Amdur, R., Jung, T. D., Chamberlain, K. R., Minoshima, S., et al., 1999b. Brain activation in PTSD in response to trauma-related stimuli. *Biol Psychiat* 45, 817–826.
- Lichtensteiger, W., Hefti, F., Felix, D., Huwyler, T., Melamed, E., Schlumpf, M., 1982. Stimulation of nigrostriatal neurones by nicotine. *Neuropharmacology* 21, 963–968.
-

- Lipina, T., Weiss, K., Roder, J., 2007. "The ampakine CX546 restores the prepulse inhibition and latent inhibition deficits in mGluR5-deficient mice". *Neuropsychopharmacology* 32, 745–756.
- Lipper, S., Davidson, J. R., Grady, T. A., et al., 1986. Preliminary study of carbamazepine in post-traumatic stress disorder. *Psychosomatics* 27, 849–854.
- Lipton, S. A., Chen, H-SV., 2004. Paradigm shift in neuroprotective drug development: clinically tolerated NMDA receptor inhibition by memantine. *Cell Death Differ* 11, 18-20.
- Liu, S. J., Zukin, R. S., 2007. Ca^{2+} -permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends Neurosci* 30, 126–134.
- Lisman, J., Lichtman, J. W., Sanes, J. R., 2003. LTP: perils and progress. *Nat Rev Neurosci* 4, 926-929.
- Liu, S. J., Zukin, R. S., 2007. Ca^{2+} -permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends Neurosci* 30, 126-134.
- Lonze, B. E., Ginty, D. D., 2002. Function and regulation of CREB family transcription factors in the nervous system. *Neuron* 35, 605–623.
- Lupien, S. J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N. P., Thakur, M., McEwen, B. S., Hauger, R. L., Meaney, M. J., 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat Neurosci* 1, 69–73.
- Lynch, G., Kessler, M., Rogers, G., Ambros-Ingerson, J., Granger, R., Schehr, R. S., 1996. Psychological effects of a drug that facilitates brain AMPA receptors. *Int Clin Psychopharmacol* 11, 13–19.
- Lynch, G., Granger, R., Ambros-Ingerson, J., Davis, C. M., Kessler, M., Schehr, R., 1997. Evidence that a positive modulator of AMPA-type glutamate receptors improves delayed recall in aged humans. *Exp Neurol* 145, 89– 92.
- Lynch, G., 1998. Memory and the brain: unexpected chemistries and a new pharmacology. *Neurobiol Learn Memory* 70, 82–100.
- Lynch, G., 2002. Memory enhancement: the search for mechanism based drugs. *Nat Neurosci* 5, 1035-1038.
-

- Lynch, G., 2004. AMPA receptor modulators as cognitive enhancers. *Curr Opin Pharmacology* 4, 4-11.
- Lynch, G., 2006. Glutamate-based therapeutic approaches: ampakines. *Curr Opin Pharmacol* 6, 82–88.
- Lynch, G., Gall, C. M., 2006. Ampakines and the threefold path to cognitive enhancement. *Trends Neurosci* 29, 554-562.
- Mackowiak, M., O'Neill, M. J., Hicks, C. A., Bleakman, D., Skolnick, Phil., 2002. An AMPA receptor potentiator modulates hippocampal expression of BDNF: an in vivo study. *Neuropharmacology* 43, 1-10.
- Maeng, S., Zarate, C. A., Du, J., Schloesser, R. J., McCammon, J., Chen, G., 2008. Cellular mechanisms underlying the antidepressant effects of ketamine: Role of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological Psychiatry* 63, 349-352.
- Malenka, R. C., Nicoll, R. A., 1999. Long-term potentiation - a decade of progress? *Science* 285, 1870-1874.
- Malenka, R. C., Bear, M. F., 2004. LTP and LTD: an embarrassment of riches. *Neuron* 44, 5–21.
- Malinow, R., Malenka, R. C., 2002. AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci* 25, 103–126.
- Martenyi, F., Brown, E. B., Zhang, H., Prakash, A., Koke, S. C., 2002a. Fluoxetine versus placebo in posttraumatic stress disorder. *J Clin Psychiatry* 63, 199–206.
- Martenyi, F., Brown, E. B., Zhang, H., Koke, S. C., Prakash, A., 2002b. Fluoxetine v. placebo in prevention of relapse in post-traumatic stress disorder. *Br J Psychiatry* 181, 315–320.
- Martin, S. J., Grimwood, P. D., Morris, R. G. M., 2000. Synaptic plasticity and memory: An evaluation of the hypothesis. *Annu Rev Neurosci* 23, 649-711.
- Mayford, M., 2007. Protein kinase signaling in synaptic plasticity and memory. *Curr Opin Neurobiol* 17, 313–317.
- McCormick, D. A., 1989. GABA as an inhibitory neurotransmitter in human cerebral cortex. *J Neurophysiol* 62, 1018– 1027.
-

- McEwen, B. S., Gould, E. A., Sakai, R. R., 1992. The vulnerability of the hippocampus to protective and destructive effects of glucocorticoids in relation to stress. *Brit J Psychiat: Suppl* 15, 18-23.
- McEwen, B. S., 1996. Gonadal and adrenal steroids regulate neurochemical and structural plasticity of the hippocampus via cellular mechanisms involving NMDA receptors. *Cell Mol Neurobiol* 16, 103–116.
- McEwen, B. S., 1999. Stress and hippocampal plasticity. *Annu Rev Neurosci* 22, 105–122.
- McEwen, B. S., 2000. Effects of adverse experiences for brain structure and function. *Biol Psychiat* 48, 721–731.
- McEwen, B. S., 2002. Protective and damaging effects of stress mediators: the good and bad sides of the response to stress. *J Clin Endocrinol Metab* 51, 2–4.
- McEwen, B. S., Wingfield, J. C., 2003. The concept of allostasis in biology and biomedicine. *Horm Behav* 43, 2–15.
- McEwen, B. S., 2004. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci* 1032, 1–7.
- McEwen, B. S., Wingfield, J. C., 2007. Allostasis and allostatic load Encyclopedia of Stress, pp. 135-141.
- McGaugh, J. L., 2000. Memory – a century of consolidation. *Science* 287, 248–251.
- McGaugh, J. L., Roozendaal, B., 2002. Role of adrenal stress hormones in the forming of lasting memories in the brain. *Curr Opin Neurobiol* 12, 205-210.
- McGaugh, J. L., 2004. The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Ann Rev Neurosci* 27, 1-28.
- Meador-Woodruff, J. H. Kleinman, J. E. 2002., Neurochemistry of schizophrenia: Glutamatergic abnormalities. In: Davis, K. L., et al., (Eds), *Neuropsychopharmacology: The fifth generation of progress*, pp. 717-728.
- Medical Care Corporation. 2008. <http://www.mccare.com/content/memory> (Accessed on [13 Oct. 2008]).
-

- Meyer, J. S., Quenzer, L. F., 2005. *Psychopharmacology: Drugs, the brain, and behavior*. Sunderland, MA US: Sinauer Associates pp. 555.
- Michael, T., Ehlers, A., Halligan, S. L., 2005a. Enhanced priming for trauma related material in posttraumatic stress disorder. *Emotion* 5, 103–112.
- Michael, T., Ehlers, A., Halligan, S. L., Clark, D. M., 2005b. Unwanted memories of assault: what intrusion characteristics are associated with PTSD? *Behav Res Ther* 43, 613–628.
- Michaelis, E. K., 1998. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. *Progr Neurobiol* 54, 369-415.
- Milad, M. R., Rauch, S. L., Pitman, R. K., Quirk, G. J., 2006. Fear extinction in rats: Implications for human brain imaging and anxiety disorders. *Biol Psychol* 73, 61-71.
- Milner, B., 1982. Some cognitive effects of frontal lobe lesions in man. *Philos T Roy Soc London* 298, 211-226.
- Miyamoto, Y., Yamada, K., Noda, Y., Mori, H., Mishina, M., Nabeshima, T., 2002. Lower sensitivity to stress and altered monoaminergic neuronal function in mice lacking the NMDA receptor epsilon 4 subunit. *J Neurosci* 22, 2335-2342.
- Mody, I., 2001. Distinguishing between GABAA receptors responsible for tonic and phasic conductances. *Neurochem Res* 26, 907–913.
- Moghaddam, B., 1993. Stress preferentially activates extraneuronal levels of excitatory amino acids in the prefrontal cortex: a comparison to hippocampus and basal ganglia. *J Neurochem* 60, 1650 –1657.
- Moghaddam, B., Adams, B., Verma, A., 1997. Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 17, 2921-2927.
- Möhler, H., Fritschy, J. M. Rudolph, U., 2002. A new benzodiazepine pharmacology. *J Pharmacol Exp Ther* 300, 2-8.
- Mori, H., Masaki, H., Yamakura, T., Mishina, M., 1992. Identification by mutagenesis of a Mg²⁺ block site of the NMDA receptor channel. *Nature* 358, 673-675.
-

- Morris, R. G. M., Garrud, P., Rawlins, J. N. P., O'Keefe, J., 1982. Place navigation in rats with hippocampal lesions. *Nature* 297, 681–683.
- Morton, W. A., Sonne, S. C., Verga, M. A., 1995. Venlafaxine: Structurally unique and novel antidepressant. *Ann Pharmacother* 29, 387-395.
- Murray, T. K., 2003. LY503430, a novel alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor potentiator with functional, neuroprotective and neurotrophic effects in rodent models of Parkinson's disease. *J Pharmacol Exp Ther* 306, 752-62.
- Nash, J., Nutt, D., 2007. Psychopharmacology of anxiety. *Psychiatry* 6, 143-148.
- Nemeroff, C. B., Bremner, J. D., Foa, E. B., Mayberg, H. S., North, C. S., Stein, M. B., 2006. Posttraumatic stress disorder: a state-of-the-science review. *J Psychiatr Res* 40, 1–21.
- Nibuya, N., Morinobu, S., Duman, R. S., 1995. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* 15, 7539-7547.
- NICE., 2005. *Guideline No 26: Post traumatic stress disorder in adults and children in primary, secondary care*. London and Leicester: Gaskilland British Psychological Society.
- Nieuwenhuys, R., 1994. The neocortex: An overview of its evolutionary development, structural organization and synaptology. *Anat Embryol (Berlin)* 190, 307-337.
- Nowak, K. M., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307, 462-465.
- Nowak, G., 2001. Does interaction between zinc and glutamate system play a significant role in the mechanism of antidepressant action? *Acta Pol Pharm Drug Res* 58, 73–75.
- Nowak, G., Szewczyk, B., Pilc, A., 2005. Zinc and depression: an update. *Pharmacol Rep* 57, 713–718.
- Nutt, D. J., 2000. The psychobiology of Posttraumatic stress disorder. *J Clin Psychiat* 61, 24-29.
-

- Ohayon, M. M., Shapiro, C. M., Psych, M., Kennedy, S. H., 2000. Differentiating DSM-IV anxiety and depressive disorders in the general population: Comorbidity and treatment consequences. *Can J Psychiat*, 45, 166-172.
- Öhman, A., 2005. The role of the amygdala in human fear: Automatic detection of threat. *Psychoneuroendocrin* 30, 953-958.
- O'Keefe, J., Nadel, L., 1978. The hippocampus as a cognitive map. Oxford: Clarendon Press.
- Okuyama, S., Chaki, S., Kawashimi, N., Suzuki, Y., Ogawa, S., Nakazato, A., et al., 1999. Receptorbinding, behavioral, and electrophysiological profiles of nonpeptide corticotropin-releasingfactor subtype 1 receptor antagonists CRA1000 and CRA1001. *J Pharmacol Exp Ther* 289, 926–935.
- Olney, J. W., Farber, N. B., 1995. Glutamate receptor dysfunction in schizophrenia. *Arch Gen Psychiat* 52, 998-1007.
- Olney, J. W., Newcomer, J. W., Farber, N. B., 1999. NMDA receptor hypofunction model of schizophrenia. *J Psychiat Res* 33, 523-533.
- O'Neill, M. J., Bleakman, D., Zimmerman, D. M., Nisenbaum, E. S., 2004. AMPA receptor potentiators for the treatment of CNS disorders. *Curr Drug Targets CNS Neurol Disord* 3, 181-194.
- O'Neill, M. J., 2005. LY503430: pharmacology, pharmacokinetics, and effects in rodent models of Parkinson's disease. *CNS Drug Rev* 11, 77-96.
- Orr, S. P., Lasko, N. B., 1995. Physiologic responses to loud tones in Vietnam veterans with posttraumatic stress disorder. *J Abn Psychology* 104, 75-82.
- Ortiz, J., Harris, H. W., Guitart, X., Terwilliger, R. Z., Haycock, J. W., Nestler, E. J., 1995. Extracellular signal-regulated protein kinases (ERKs) and ERK kinase (MEK) in brain: regional distribution and regulation by chronic morphine. *J Neurosci* 15, 1285–1297.
- Ouyang, Y., Rosenstein, A., Kreiman, G., Schuman, E. M., Kennedy, M. B., 1999. Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *J Neurosci* 19, 7823-7833.

- Palmada, M., Centelles, J. J., 1998. Excitatory amino acid neurotransmission. pathways for metabolism, storage and reuptake of glutamate in brain. *Front Bioscie: A Journal and Virtual Library* 3, d701-718.
- Paoletti, P., Neyton, J., 2007. NMDA receptor subunits: function and pharmacology. *Curr Opin Pharmacol* 7, 39–47.
- Parkin, A. J., Leng, N. R. C., 1993. Neuropsychology of the amnesic syndrome. Hillsdale, NJ England: Lawrence Erlbaum Associates, Inc.
- Patel, M., Li, Q. Y., 2003. Age dependance of seizure-induced oxidative stress. *Neurosci* 118, 431-437.
- Pederson, C. L., Maurer, S. H., Kaminski, P. L., Zander, K. A., Peters, C. M., Stokes-Crowe, L. A., Osborn, R. E., 2004. Hippocampal volume and memory performance in a community-based sample of women with posttraumatic stress disorder secondary to child abuse. *J Trauma Stress* 17, 37–40.
- Perin-Dureau, F., Rachline, J., Neyton, J., Paoletti, P., 2002. Mapping the binding site of the neuroprotectant ifenprodil on NMDA receptors. *J Neurosci* 22, 5955–5965.
- Pezze, M. A., Feldon, J., 2004. Mesolimbic dopaminergic pathways in fear conditioning. *Prog Neurobiol* 74, 301–320.
- Phelps, E. A., LeDoux, J. E., 2005. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron* 48, 175-187.
- Picciotto, M. R., Alreja, M., Jentsch, J. T. 2002., Acetylcholine. In: Davis, K. L., Charney, D., Coyle, J. T., Nemeroff, C., (Eds), Neuropsychopharmacology: The fifth generation of progress. *Am Coll Neuropsychopharmacol*, pp. 3-14.
- Pitman, R. K., Sanders, K. M., Zusman, R. M., Healy, A. R., Cheema, F., Lasko, N. B., et al., 2002. Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biol Psychiat* 51, 189–192.
- Popoli, M., Brunello, N., Perez, J., Racagni, G., 2000. Second messenger regulated protein kinases in the brain: Their functional role and the action of antidepressant drugs. *J Neurochem* 74, 21–33.
- Posit Science Corporation. 2008. <http://www.BrainConnection.com> (Accessed on [22 Oct. 2008]).
-

- Postma, A., Kessels, R. P. C., Van Asselen, M. 2004., The neuropsychology of object location memory. In: Allen, G., (Ed), *Remembering Where: Advances in understanding spatial memory*. Lawrence Erlbaum Associates, Inc., Mahwah, NJ, pp. 191-215.
- Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von-Auer, K., Jobst, S., Kaspers, F., Kirschbaum, C., 1997. Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sci* 61, 2539–2549.
- Przedborski, S., Ischiropoulos, H., 2005. Reactive oxygen and nitrogen species: weapons of neuronal destruction in models of Parkinson's disease. *Antioxid Redox Signal* 7, 685-693.
- Puzantian, T., 1998. Mirtazapine, an antidepressant. *Am J Health-Syst Pharm* 55, 44–9.
- Pynoos, R. S., Ritzmann, R. F., Steinberg, A. M., Goenjian, A., Prisecaru, I., 1996. A behavioral animal model of posttraumatic stress disorder featuring repeated exposure to situational reminders. *Biol Psychiat* 39, 129-134.
- Rachline, J., Perin-Dureau, F., Le, G. A., Neyton, J., Paoletti, P., 2005. The micromolar zinc-binding domain on the NMDA receptor subunit NR2B. *J Neurosci* 25, 308–17.
- Radenovic, L., Selakovic, V., 2005. Differential effects of NMDA and AMPA/kainate receptor antagonists on nitric oxide production in rat brain following intrahippocampal injection. *Brain Res Bull* 67, 133-141.
- Rameau, G. A., Tukey, D. S., Garcin-Hosfield, E. D., Titcombe, R. F., Misra, C., Khatri, L., Getzoff, E. D., Ziff, E., 2007. Biphasic Coupling of Neuronal Nitric Oxide Synthase Phosphorylation to the NMDA Receptor Regulates AMPA Receptor Trafficking and Neuronal Cell Death. *J Neurosci* 27, 3445-3455.
- Ramos, B. P., Arnsten, A. F. T., 2007. Adrenergic pharmacology and cognition: Focus on the prefrontal cortex. *Pharmacol Ther* 113, 523-536.
- Rao, V. R., Finkbeiner, S. 2007. NMDA and AMPA receptors: old channels, new tricks. *Trends Neurosci* 30, 284-291.
- Raskind, M. A., Dobie, D. J., Kanter, E. D., Petrie, E. C., Thompson, C. E., Peskind, E. R., 2000. The alpha1-adrenergic antagonist prazosin ameliorates combat
-

- trauma nightmares in veterans with posttraumatic stress disorder: a report of 4 cases. *J Clin Psychiat* 61, 129–133.
- Raskind, M. A., Peskind, E. R., Kanter, E. D., Petrie, E. C., Radant, A., Thompson, C., et al., 2003. Prazosin reduces nightmares and other PTSD symptoms in combat veterans: a placebo-controlled study. *Am J Psychiat* 160, 371–373.
- Rauch, S. L., van der Kolk, B. A., Fisler, R. E., Alpert, N. M., Orr, S. P., Savage, C. R., et al., 1996. A symptom provocation study of posttraumatic stress disorder using positron emission tomography and script-driven imagery. *Arch Gen Psychiat* 53, 380–387.
- Rauch, S. L., Whalen, P. J., Shin, L. M., McInerney, S. C., Macklin, M. L., Lasko, N. B., Orr, S. P., Pitman, R. K., 2000. Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study. *Biol Psychiat* 47, 769–776.
- Reagan, L. P., McEwen, B. S., 1997. Controversies surrounding glucocorticoid-mediated cell death in the hippocampus. *J Chem Neuroanat* 13, 149–167.
- Ren, J., Poon, B. Y., Tang, Y., Funk, G. D., Greer, J. J., 2006. Ampakines alleviate respiratory depression in rats. *Am J Resp CritiCare Med* 174, 1384–1391.
- Reul, J. M. H., de Kloet, E. R., 1985. Two receptor systems for corticosterone in rat brain: micro distribution and differential occupation. *Endocrinology* 117, 2505–2511.
- Richter-Levin, G., Akirav, I., 2000. Amygdala-hippocampus dynamic interaction in relation to memory. *Mol Neurobiol* 22, 11–20.
- Riedel, G., Platt, B., Micheau, J., 2003. Glutamate receptor function in learning and memory. *Beh Brain Res* 140, 1–47.
- Rivadulla, C., Sharma, J., Sur, M., 2001. Specific roles of NMDA and AMPA receptors in direction-selective and spatial phase-selective responses in visual cortex. *J Neurosci* 21, 1710–1719.
- Rivier, C., Vale, W., 1983. Interaction of corticotropin-releasing factor and arginine vasopressin on adrenocorticotropin secretion in vivo. *Endocrinology* 113, 939–942.
- Roosendaal, B., 2000. 1999 Curt P. Richter award. Glucocorticoids and the regulation of memory consolidation. *Psychoneuroendocrin* 25, 213–238.
-

- Rosario, L. A., Abercrombie, E. D., 1999. Individual differences in behavioural reactivity: correlation with stress-induced norepinephrine efflux in the hippocampus of Sprague–Dawley rats. *Brain Res Bull* 48, 595–602.
- Rothbaum, B. O., Killeen, T. K., Davidson, J. R., Brady, K. T., Connor, K. M., Heekin, M. H., 2008. Placebo-controlled trial of risperidone augmentation for selective serotonin reuptake inhibitor-resistant civilian posttraumatic stress disorder. *J Clin Psychiat* 69, 520-525.
- Rothschild, B., 1996. Applying the Brakes: Theory and Tools for Understanding, Slowing Down and Reducing Autonomic Nervous System Activation in Traumatized Clients, Paper presented at the Tenth Scandinavian Conference for Psychotherapists working with Traumatized Refugees, 24-26 May 1996, Ibo, Finland.
- Ryder, J. W., Falcone, J. F., Manro, J. R., Svensson, K. A., Merchant, K. M., 2006. Pharmacological Characterization of cGMP Regulation by the Biarylpropylsulfonamide Class of Positive, Allosteric Modulators of AMPA Receptors. *J Pharmacol Exp Ther* 319, 293-298.
- Sakai, K., Miyashita, Y., 1993. Memory and imagery in the temporal lobe. *Curr Opin Neurobiol* 3, 166-170.
- Sananbenesi, F., Fischer, A., Schrick, C., Spiess, J., Radulovic, J., 2003. Mitogen-activated protein kinase signaling in the hippocampus and its modulation by corticotropin-releasing factor receptor 2: a possible link between stress and fear memory. *J Neurosci* 23, 11436–11443.
- Saper, C. B. 1997., The hypothalamus. In: Paxinos, G., (Ed), The human nervous system, part 1. Elsevier, Amsterdam, pp. 39-137.
- Sapolsky, R., Krey, L., McEwen, B., 1984a. Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. *Proc Natl Acad Sci USA* 81, 6174–6177.
- Sapolsky, R. M., Krey, L. C., McEwen, B. S., 1984b. Stress down-regulates corticosterone receptors in a site-specific manner in the brain. *Endocrinology* 114, 287–292.
- Sapolsky, R. M., 1992. Stress, the aging brain, and the mechanism of neuron death. Cambridge, MA: MIT Press; pp. 1-423.
-

- Sapolsky, R. M., 2000a. The possibility of neurotoxicity in the hippocampus in major depression: A primer on neuron death. *Biol Psychiat* 48, 755-765.
- Sapolsky, R. M., 2000b. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiat* 57, 925-935.
- Sarter, M., Bruno, J. P., 2000. Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents, *Neuroscience* 95, 933-952.
- Sauro, M. D., Jorgensen, R. S., Pedlow, C. T., 2003. Stress, glucocorticoids, and memory: a meta-analytic review. *Stress* 6: 235-245.
- Schaeffer, H. J., Weber, M. J., 1999. Mitogen-activated protein kinases: Specific messages from ubiquitous messengers. *Mol Cell Biol* 19, 2435-2444.
- Schelling, G., Kilger, E., Roozendaal, B., de Quervain, D. J., Briegel, J., Dagge, A., et al., 2004. Stress doses of hydrocortisone, traumatic memories, and symptoms of posttraumatic stress disorder in patients after cardiac surgery: A randomized study. *Biol Psychiat* 55, 627-633.
- Schimmer, B. P., Parker, K. L., 2008. "Chapter 59. Adrenocorticotrophic hormone; adrenocortical steroids and their synthetic analogs; inhibitors of the synthesis and actions of adrenocortical hormones" (Chapter). Brunton LL, Lazo JS, Parker KL: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th Edition: <http://www.accessmedicine.com/content.aspx?aID=954143>. (Accessed on [20 Nov. 2008]).
- Schloss, P., Williams, D. C., 1998. The serotonin transporter: a primary target for antidepressant drugs. *J Psychopharmacol* 12, 115-121.
- Schoenfeld, F. B., Marmar, C. R., Neylan, T. C., 2004. Current Concepts in Pharmacotherapy for Posttraumatic Stress Disorder. *Psychiat Serv* 55, 519-531.
- Schuff, N., Neylan, T. C., Lenoci, M. A., Du, A. T., Weiss, D. S., Marmar, C. R., Weiner, M. W., 2001. Decreased hippocampal N-acetylaspartate in the absence of atrophy in posttraumatic stress disorder. *Biol Psychiat* 50, 952-959.
-

- Scott, L. V., Dinan, T. G., 1998. Vasopressin and the regulation of hypothalamic-pituitary-adrenal axis function: Implications for the pathophysiology of depression. *Life Sci* 62, 1985-1998.
- Seamans, J. K., Yang, C. R., 2004. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Prog Neurobiol* 74, 1–58.
- Seeburg, P. H., Single, F., Kuner, T., Higuchi, M., Sprengel, R., 2001. Genetic manipulation of keydeterminants of ion flow in glutamate receptor channels in the mouse. *Brain Res* 907, 233-243.
- Seger, R., Krebs, E. G., 1995. The MAPK signaling cascade. *FASEB Journal* 9, 726–735.
- Selcher, J. C., Weeber, E. J., Varga, A. W., Sweatt, J. D., Swank, M., 2002. Protein kinase signal transduction cascades in mammalian associative conditioning. *Neuroscientist* 8, 122–131.
- Shalev, A. Y., Rogel-Fuchs, Y., 1992. Auditory startle reflex in post-traumatic stress disorder patients treated with clonazepam. *Isral J Psychiat Rel Sci* 29, 1-6.
- Shalev, A. Y. 2001., What is posttraumatic stress disorder? *Journal of Clinical Psychiatry* 62, 4-10. In: Duke, L. M., Vasterling, J. J. (Eds), *Neuropsychology of PTSD: biological, cognitive, and clinical perspectives*. Guilford Press, NY, pp. 3-24.
- Shallice, T., Warrington, E. K., 1970. Independent functioning of verbal memory stores: a neuropsychological study. *Q J Exp Psychol* 22, 261–273.
- Shaw, M. E., Strother, S. C., McFarlane, A. C., Morris, P., Anderson, J., Clark, C. R., Egan, G. F., 2002. Abnormal functional connectivity in posttraumatic stress disorder. *NeuroImage* 15, 661–674.
- Shekhar, A., Katner, J. S., Sajdyk, T. J., & Kohl, R. R. (2002). Role of norepinephrine in the dorsomedial hypothalamic panic response: An in vivo microdialysis study. *Pharmacol Biochem Be* 71, 493-500.
- Sheng, M., Thompson, M. A., Greenberg, M. E., 1991. CREB: A Ca(2+)-regulated transcription factor phosphorylated by calmodulin dependent kinases. *Science* 252, 1427–1430.
-

- Shi, S. H., Hayashi, Y., Petralia, R. S., Zaman, S. H., Wenthold R., Svoboda K., Malinow R., 1999. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* 284, 1811-1816.
- Shiah, I. S., Yatham, L. N., 1998. GABA function in mood disorders: an update and critical review. *Life Sci* 63, 1289–1303.
- Shin, L. M., Orr, S. P., Carson, M. A., Rauch, S. L., Macklin, M. L., Lasko, N. B., et al., 2004. Regional cerebral blood flow in the amygdala and medial prefrontal cortex during traumatic imagery in male and female vietnam veterans with PTSD. *Arch Gen Psychiat* 61, 168-176.
- Shin, L. M., Rauch, S. L., Pitman, R. K., 2006. Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Ann NY Acad Sci* 1071, 67-79.
- Shobe, J., 2002. The Role of PKA, CaMKII, and PKC in Avoidance Conditioning: permissive or instructive? *Neurobiol Learn Mem* 77, 291–312.
- Silva, A. J., Kogan, J. H., Frankland, P. W., Kida, S., 1998. CREB and memory. *Ann Rev Neurosci* 21, 127–148.
- Sivilotti, L., Nistri, A., 1991. GABA receptor mechanisms in the central nervous system. *Prog Neurobiol* 36, 35–92.
- Skolnick, P., 2008. AMPA receptors: A target for novel antidepressants? *Biol Psychiat* 63, 347-348.
- Smith, M. L., Milner, B., 1981. The role of the right hippocampus in the recall of spatial location. *Neuropsychologia* 19, 781–793.
- Smith, M. L., Milner, B., 1984. Differential effects of frontal-lobe lesions on cognitive estimation and spatial memory. *Neuropsychologia* 22, 697–705.
- Smith, E. E., Jonides, J., Koeppe, R. A., Awh, E., Schumacher, E. H., Minoshima, S., 1995. Spatial versus object working memory: PET investigations. *J Cogn Neurosci* 7, 337–356.
- Song, I., Huganir R. L., 2002. Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci* 25, 578-589.
- Song, Y., Zhou, D., Wang, X., 2008. Increased serum cortisol and growth hormone levels in earthquake survivors with PTSD or subclinical PTSD. *Psychoneuroendocrin* 33, 1155-1159.
-

- Soravia, L. M., Heinrichs, M., Aerni, A., Maroni, C., Schelling, G., Ehler, U., Roozendaal, B., de Quervain, D. J., 2006. Glucocorticoids reduce phobic fear in humans. *Proc Natl Acad Sci USA* 103, 5585–5590.
- Spivak, B., Vered, Y., Graff, E., Blum, I., Mester, R., Weizman, A., 1999. Low platelet poor plasma concentrations of serotonin in patients with combat-related posttraumatic stress disorder. *Biol Psychiat* 45, 840–845.
- Squire, L. R., 1987. Memory and brain. New York, NY US: Oxford University Press.
- Squire, L. R., Zola-Morgan, S., 1991. The medial temporal lobe memory system. *Science* 253, 1380–1386.
- Squire, L. R., Clark, R. E., Bayley, P. J. 2004., Medial temporal lobe function and memory. In: Gazzaniga, M. S., Gazzaniga, M. S., (Eds), The cognitive neurosciences (3rd ed.). MA US: MIT Press, Cambridge, pp. 691–708.
- Stam, R., 2000. Posttraumatic Stress Disorder, Evolving Pathophysiological Models of Functional GI Disorders. Health Education Alliance, New York, pp. 92–95.
- Stam, R., 2007. PTSD and stress sensitisation: A tale of brain and body: Part 1: Human studies. *Neurosci Biobehav Rev* . *Neurosci Biobehav Rev* 31, 530–557.
- Starkman, M. N., Gebarski, S. S., Berent, S., Schteingart, D. E., 1992. Hippocampal formation volume, memory dysfunction and cortisol levels in patients with Cushing's Syndrome. *Biol Psychiat* 32, 756 –765.
- Staubli, U., Perez, Y., Xu, F. B., Rogers, G., Ingvar, M., Stone-Elander, S., Lynch, G., 1994a. Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. *Proc Nat Acad Sci USA* 91, 11158–11162.
- Staubli, U., Rogers, G., Lynch, G., 1994b. Facilitation of glutamate receptors enhances memory. *Proc Nat Acad Sci USA* 91, 777–781.
- Steil, R., Ehlers, A., 2000. Dysfunctional meaning of posttraumatic intrusions in chronic PTSD. *Behav Res Ther* 38, 537–558.
- Stein, M. B., Koverola, C., Hanna, C., Torchia, M. G., McClarty, B., 1997. Hippocampal volume in women victimized by childhood sexual abuse. *Psychol Med* 27, 951–959.
-

- Stein, D. J., Seedat, S., van der Linden, G., Kaminer, D. 2000., In: Post-traumatic Stress Disorder: Diagnosis, management and Treatment, (Eds), Nutt, D., Davidson, J. R. T., Zohar, J., Martin Dunitz, Ltd, London, UK, pp. 131-146.
- Stein, D. J., Hewett, K., Oldham, M., et al., 2001. Paroxetine in the treatment of PTSD. Presented at the 7th World Congress of Biological Psychiatry, Berlin, Germany.
- Stork, P. J. S., Schmitt, J. M., 2002. Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. *Trends Cell Biol* 12, 258-266.
- Storm-Mathisen, J., Leknes, A. K., Bore, A., Vaaland, J. L., Edminson, P., Haug, F. M. S., Ottersen, O. P., 1983. First visualisation of glutamate and GABA in neurones by immunocytochemistry. *Nature* 301, 517-520.
- Storm-Mathisen, J., Danbolt, N. C., Ottersen, O. P. 1995., Localisation of glutamate and its membrane transport proteins. In: Stone, T. W., (Ed), CNS Neurotransmitters and Neuromodulators. Boca Raton, FL: CRC Press, pp. 1-18.
- Sun, Y., Olson, R., Horning, M., Armstrong, N., Mayer, M., Gouaux, E., 2002. Mechanism of glutamate receptor desensitization. *Nature* 417, 245–253.
- Suppiramaniam, V., Bahr, B. A., Sinnarajah, S., Owens, K., Rogers, G., Yilma, S., et al., 2001. Member of the ampakine class of memory enhancers prolongs the single channel open time of reconstituted AMPA receptors. *Synapse (New York, N.Y.)* 40, 154-158.
- Swaab, D. F. 1997., Neurobiology and neuropathology of the human hypothalamus. In: Bloom, F. E., Hökfelt, T., (Eds), The primate nervous system, part I. Elsevier, Amsterdam, pp. 39–137.
- Swaab, D. F. 2003., The human hypothalamus, basic and clinical aspects. Part I: Nuclei of the hypothalamus. In: Aminoff, M. J., Boller, F., Swaab, D. F., (Eds), Handbook of Clinical Neurology, vol. 79. Elsevier, Amsterdam, 476 pp.
- Sweatt, J. D., 2001. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem* 76, 1–10.
-

- Sweetman, S. C. (Ed), 2008., *Martindale: The Complete Drug Reference* 35. [online] London: Pharmaceutical Press <<http://www.medicinescomplete.com/>> (Accessed on [10 Nov. 2008]).
- Swerdlow, N. R., Sutherland, A. N., 2006. Preclinical models relevant to Tourette syndrome *Adv Neurol* 99, 69-88.
- Takahashi, T., Morinobu, S., Iwamoto, Y., Yamawaki, S., 2006. Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats, *Psychopharmacology (Berl)* 189, 165–173.
- Takehara, K., Kawahara, S., Kirino, Y., 2003. Time-dependent reorganization of the brain components underlying memory retention in trace eyeblink conditioning. *J Neurosci* 23, 9897-9905.
- Tanaka, H., Grooms, S. Y., Bennett, M. V. L., Zukin, R. S., 2000. The AMPAR subunit GluR2: still front and center-stage. *Brain Res* 886, 190-207.
- Taverna, S., Sancini, G., Mantegazza, M., Franceschetti, S., Avanzini, G., 1999. Inhibition of transient and persistent Na⁺ current fractions by the new anticonvulsant topiramate. *J Pharmacol Exp Ther* 288, 960-968.
- Taylor, F., Raskind, M. A., 2002. The alpha1-adrenergic antagonist prazosin improves sleep and nightmares in civilian trauma posttraumatic stress disorder. *J Clin Psychopharmacol* 22, 82–85.
- Taylor, F. B., 2003. Tiagabine for posttraumatic stress disorder: a case series of 7 women. *J Clin Psychiat* 64, 1421–1425.
- Taylor, F., Lowe, K., Thompson, C., McFall, M. M., Peskind, E. R., Kanter, E. D., et al., 2006. Daytime prazosin reduces psychological distress to trauma specific cues in civilian trauma posttraumatic stress disorder. *Biol Psychiat* 59, 577–581.
- Tomita, S., Bredt, D. S., Nicoll R. A., 2005. CaMKII substrates in synaptic plasticity. *Cellscience Rev* 2. <http://www.cellscience.com> (Accessed on [15 May 2008]).
- Tulving, E. 1972., Episodic and semantic memory. In: Tulving, E., Donaldson, W., (Eds), *Organization of Memory*. Academic Press, New York, pp. 381-403.
- Tulving, E., 2001. Episodic memory and common sense: How far apart? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 356, 1505-1515.
-

- Tulving, E. 2005., Episodic memory and autonoesis: Uniquely human? In: Terrace, H. S., Metcalfe, J., Terrace, H. S., Metcalfe, J., (Eds), The missing link in cognition: Origins of self-reflective consciousness. NY US: Oxford University Press, New York, pp. 3-56.
- U.S. National Library of Medicine, "CX516," 2008. <http://clinicaltrials.gov> (Accessed on [20 Nov. 2008]).
- Uys, J. D. K., Stein, D. J., Daniels, W. M. U., Harvey, B. H., 2003. Animal models of anxiety disorders. *Curr Psychiatry Rep* 5, 274–281.
- Vaiva, G., Thomas, P., Ducrocq, F., Fontaine, M., Boss, V., Devos, P., Rascle, C., Cottencin, O., Brunet, A., Laffargue, P., Goudemand, M., 2004. Low posttrauma GABA plasma levels as a predictive factor in the development of acute posttraumatic stress disorder. *Biol Psychiat* 55, 250-254.
- Van den Bosch, L., Van Damme, P., Bogaert, E., Robberecht, W., 2006. The role of excitotoxicity in the pathogenesis of amyotrophic lateral sclerosis. *Biochimica et Biophysica Acta* 1762, 1068-1082.
- Van der Kolk, B. A., Dreyfuss, D., Michaels, M., Shera, D., Berkowitz, R., Fisler, R., et al., 1994. Fluoxetine in posttraumatic stress disorder. *J Clin Psychiat* 55, 517–522.
- Vasterling, J. J., Brailey, K., Constans, J. I., Sutker, P. B., 1998. Attention and memory dysfunction in posttraumatic stress disorder. *Neuropsychology* 12, 125–133.
- Vasterling, J. J., Duke, L. M., Brailey, K., Constans, J. L., Allain, A. N., Sutker, P. B., 2002. Attention, learning, and memory performances and intellectual resources in Vietnam veterans, PTSD and no disorder comparisons. *Neuropsychology* 16, 5-14.
- Vaucher, E., Borredon, J., Bonvento, G., Seylaz, J., Lacombe, P., 1997. Autoradiographic evidence for flow-metabolism uncoupling during stimulation of the nucleus basalis of Meynert in the conscious rat. *J Cerebr Blood-Flow Metab* 17, 686-694.
- Vermetten, E., Vythilingam, M., Southwick, S. M., Charney, D. S., Bremner, J. D., 2003. Long-term treatment with paroxetine increase verbal declarative memory and hippocampal volume in post-traumatic stress disorder. *Biol Psychiat* 54, 693-702.
-

- Villareal, G., Hamilton, D. A., Petropoulos, H., Driscoll, I., Rowland, L. M., Griego, J. A., et al., 2002. Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. *Biol Psychiat* 52, 119–125.
- Vincent, S. R. Kimura, H., 1992. Histochemical mapping of nitric oxide synthase in the rat brain. *Neurosci* 46, 755-784.
- Walker, D. L., Ressler, K. J, Lu, K. T., Davis, M., 2002. Facilitation of Conditioned Fear Extinction by Systemic Administration or Intra-Amygdala Infusions of D-Cycloserine as Assessed with Fear-Potentiated Startle in Rats. *J Neurosci* 22, 2343-2351.
- Watson, G. B., Bolanowski, M. A., Baganoff, M. P., Deppeler, C. L., Lanthorn, T. H., 1990. D-cycloserine acts as a partial agonist at the glycine modulatory site of the NMDA receptor expressed in *Xenopus* oocytes. *Brain Res* 510, 158 –160.
- Weber, D. L., Clark, C. R., McFarlane, A. C., Moores, K. A., Morris, P., Egan, G. F., 2005. Abnormal frontal and parietal activity during working memory updating in post-traumatic stress disorder. *Psychiat Res: Neuroimaging Section*, 140, 27-44.
- Wezenberg, E., Jan Verkes, R., Ruigt, G. S. F., Hulstijn, W., Sabbe, B. G. C., 2007. Acute effects of the amphetamine stimulant on memory and information processing in healthy elderly volunteers. *Neuropsychopharmacol* 32, 1272-1283.
- Whitnall, M. H., 1993. Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system. *Prog Neurobiol* 40, 573–629.
- Wiggs, C. L., Weisberg, J., Martin, A., 1999. Neural correlates of semantic and episodic memory retrieval. *Neuropsychologia* 37, 103-118.
- Wikipedia contributors, "Aniracetam," *Wikipedia, The Free Encyclopedia*, 2008a. <http://en.wikipedia.org/w/index.php?title=Aniracetam&oldid=252095135> (Accessed on [11 Nov. 2008]).
- Wikipedia contributors, "LY-503,430," *Wikipedia, The Free Encyclopedia*, 2008b. <http://en.wikipedia.org/w/index.php?title=LY-503,430&oldid=252096488> (Accessed on [11 Nov. 2008]).
-

- Wikipedia contributors, "LY-404,187," *Wikipedia, The Free Encyclopedia*, 2008c. <http://en.wikipedia.org/w/index.php?title=LY-404,187&oldid=252096019> (Accessed on [11 Nov. 2008]).
- Williams, L. M., Das, P., Harris, A. W., Liddell, B. B., Brammer, M. J., Olivieri, G., Skerrett, D., Phillips, M. L., David, A. S., Peduto, A., Gordon, E., 2004. Dysregulation of arousal and amygdala – prefrontal systems in paranoid schizophrenia. *Am J Psychiat* 161, 480–489.
- Willner, P., 1986. Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. *Prog Neuropsychopharmacol Biol Psychiat* 10, 677–690.
- Witkin, J. M., Eiler, W. J. A. II., 2006. Antagonism of metabotropic glutamate group II receptors in the potential treatment of neurological and neuropsychiatric disorders. *Drug Devel Res* 67, 757–769.
- Wittenberg, G. M., Tsien, J. Z., 2002. An emerging molecular and cellular framework for memory processing by the hippocampus. *Trends Neurosci* 25, 501–505.
- Wolff, D. J., Datto, G. A., Samatovicz., 1993. The dual mode of inhibition of calmodulin-dependent nitric oxide synthase by antifungal imidazole agents. *J Biol Chem* 268, 9430–9436.
- Wolosker, H., 2006. D-serine regulation of NMDA receptor activity. *Science's STKE*: 356, pe41-pe41.
- Wong, E. H., Kemp, J. A., Priestley, T., Knight, A. R., Woodruff, G. N., Iversen, L. L., 1986. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc Natl Acad Sci USA* 83, 7104–7108.
- Woods, S. J., Wineman, M., 2004. Trauma, posttraumatic stress disorder symptom clusters, and physical health symptoms in post abused women. *Archives of Psychiat Nurs* 18, 26–34.
- Wotjak, C. T., Naruo, T., Muraoka, S., Simchen, R., Landgraf, R., Engelmann, M., 2001. Forced swimming stimulates the expression of vasopressin and oxytocin in magnocellular neurons of the rat hypothalamic paraventricular nucleus. *Eur J Neurosci* 13, 2273–2281.
- Yamada, J., Saitow, F., Satake, S., Kiyohara, T., Konishi, S., 1999. GABA(B) receptor-mediated presynaptic inhibition of glutamatergic and GABAergic
-

- transmission in the basolateral amygdala. *Neuropharmacology* 38, 1743–1753.
- Yamamoto, S., Morinobu, S., Fuchikami, M., Kurata, A., Kozuru, T., Yamawaki, S., 2008. Effects of single prolonged stress and D-cycloserine on contextual fear extinction and hippocampal NMDA receptor expression in a rat model of PTSD. *Neuropsychopharmacology* 33, 2108–2116.
- Yan, Z., Rafferty, B., Caldwell, G. W., Masucci, J. A., 2002. Rapidly distinguishing reversible and irreversible CYP450 inhibitors by using fluorometric kinetic analysis. *Eur J Drug Metab Pharmacokinet* 27, 281–287.
- Yang, S. N., Tang, Y. G., Zucker, R. S., 1999. Selective induction of LTP and LTD by postsynaptic $[Ca^{2+}]_i$ elevation. *J Neurophysiol* 81, 781–787.
- Yeh, C.B., Leckman, James F., Wan, F., Shiah, I., Lu, R., 2002. Characteristics of acute stress symptoms and nitric oxide concentration in young rescue workers in Taiwan. *Psychiatry Res* 112, 59–68.
- Yehuda, R., Antelman, S. M., 1993. Criteria for evaluating animal models of post-traumatic stress disorder. *Biol Psychiat* 33, 479–486.
- Yehuda, R., Harvey, H. 1997., Relevance of neuroendocrine alterations in PTSD to cognitive impairments of trauma survivors. In: Read D, Lindsay, S., (Eds), *Recollections of trauma: scientific research and clinical practice*. Plenum Press, NewYork, pp. 221–252.
- Yehuda, R., Bierer, L. M., Schmeidler, J., Aferiat, D. H., Breslau, I., Dolan, S., 2000. Low cortisol and risk for PTSD in adult offspring of Holocaust survivors. *Am J Psychiat* 157, 1252–1259.
- Yehuda, R., 2001. Are glucocorticoids responsible for putative hippocampal damage in PTSD? How and when to decide. *Hippocampus* 11, 85–89.
- Yehuda, R., 2002. Post-traumatic stress disorder. *N Engl J Med* 346, 108–114.
- Yehuda, R., 2003. Hypothalamic-pituitary-adrenal alterations in PTSD: are they relevant to understanding cortisol alterations in cancer? *Brain Behav Imm* 17, S73–S83.
- Yin, J. C. P., Wallach, J. S., Del Vecchio, M., Wilder, E. L., Zhou, H., Quinn, W. G., Tully, T., 1994. Induction of a dominant-negative CREB transgene specifically blocks long-term memory in *Drosophila*. *Cell* 79, 49–58.
-

- Yin, J. C. P., del Vecchio, M., 1995. CREB as a memory modulator: induced expression of a dCREB2 activator isoform enhances long-term memory in *Drosophila*. *Cell* 81, 107–115.
- Young, E. A., Akana, S., Dallman, M. F., 1990. Decreased sensitivity to glucocorticoid fast feedback in chronically stressed rats. *Neuroendocrinology* 51, 536–542.
- Yun, H. Y., Dawson, V. L., Dawson, T. M., 1997. Nitric oxide in health and disease of the nervous system. *Mol Psychiatry* 2, 300-310.
- Yushkevich, P. A., 2008. A high-resolution computational atlas of the human hippocampus from postmortem magnetic resonance imaging at 9.4 T. *NeuroImage* (Article In Press).
- Zarate, C. A., Du, J., Quiroz, J., Gray, N. A., Denicoff, K. D., Singh, J., 2003. Regulation of cellular plasticity cascades in the pathophysiology and treatment of mood disorders: Role of the glutamatergic system. *Ann NY Acad Sci* 1003, 273-291.
- Zhou, Y., Zhou, L., Chen, H., Koliatsos, V. E., 2006. An AMPA glutamatergic receptor activation - nitric oxide synthesis step signals transsynaptic apoptosis in limbic cortex. *Neuropharmacology* 51, 67-76.
- Zola, S. M., Squire, L. R., Teng, E., Stefanacci, L., Buffalo, E. A., Clark, R. E. 2000. Impaired recognition memory in monkeys after damage limited to the hippocampal region. *J Neurosci* 20, 451–463.

CHAPTER 3

ARTICLE

Article for submission for publication in *Neuropharmacology*

Introduction

This chapter presents the full-length manuscript for submission to *Neuropharmacology*, published by Elsevier. The manuscript is presented in the required format prescribed by *Guide for Authors*, as per the journal website:

http://www.elsevier.com/wps/find/journaldescription.cws_home/279/authorinstructions

The complete “Guide for Authors” can be viewed and/or down-loaded from the above web link. Thus, the manuscript will begin with the title, contributing authors and affiliations on a separate page, followed by an Abstract (of no more than 250 words) and a list of up to 6 keywords. Thereafter the main body of the manuscript will follow including Introduction, Methods, Results, Discussion, Acknowledgements, Bibliography and Legends to Figures. As per the journal submission format, all figures are provided at the end of the paper and annotated as Figure 1 onwards, with Tables annotated in roman numerals and similarly placed at the back of the manuscript.

The ampakine, *Org 26576*, bolsters spatial memory performance in rats without changes in cortical and hippocampal NMDA receptor binding

Eugene Hamlyn, Linda Brand, Mohammed Shahid[#] and Brian H. Harvey^{*}

Unit for Drug Research and Development, Division of Pharmacology, School of Pharmacy, North-West University, Private Bag X6001, Potchefstroom, South Africa, 2520, and [#]Schering-Plough, Newhouse, Lanarkshire, UK.

^{*}Corresponding author: Tel: +27 17 299 2238; Fax: +27 18 299 2225; e-mail: brian.harvey@nwu.ac.za

Funding and grants: The authors would like to acknowledge the South African Medical Research Council (BHH) and the National Research Foundation (BHH, EH; grant number 2073038) for financial support. There is no financial relationship, or conflict of interest, with these funding agencies.

Abstract

Ampakines have demonstrated beneficial effects on cognition in select animal models of learning, and which may be ascribed to recruitment of voltage-gated NMDA receptor ion channels. However, their ability to modify long-term spatial memory tasks has not been studied. This will lend credence to their possible value in treating disorders of cognition. We evaluated the actions of chronic *Org 26576* administration on spatial memory performance in the Morris water maze in male Sprague-Dawley rats at doses of 0 (saline control), 1, 3 and 10 mg/kg b.d. over 12 days via intraperitoneal injection. We also investigated the effects of treatment on hippocampal and frontal cortical NMDA receptor radioligand binding. *Org 26576* exerted a dose and time-dependent bolstering of spatial learning, with dosages of 3 and 10 mg/kg *Org 26576* significantly enhancing learning on day 1. Globally, escape latency decreased significantly as the training days progressed in the saline and *Org 26576* treated groups, indicating that significant learning had taken place in all the treatment groups. At the end of the learning period, all doses of *Org 26576* significantly improved spatial memory retrieval without confounding effects in the cued version of the task. Interestingly, none of the drug treatments induced a noteworthy change in NMDA receptor binding density or affinity in the brain regions studied. *Org 26576* therefore enhances spatial memory acquisition in rats without concomitant changes in limbic NMDA receptor binding, and may have clinical utility in treating disorders characterized by deficits in cognitive performance.

Keywords: *Org 26576, AMPA receptor, NMDA receptor, learning, spatial memory, Morris water maze*

1. Introduction

AMPA receptors, one of a group of four subtypes of glutamate receptors in the brain, including N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainic acid and metabotropic receptors, play a critical role in mediating fast excitatory postsynaptic currents (Malenka and Nicoll, 1999). AMPA receptors also are involved in recruiting voltage-gated NMDA receptor ion channels (Tanaka et al., 2000). NMDA receptor activation in turn results in the calcium-dependent activation of calmodulin kinase II which phosphorylates the GluR1 subunit of the AMPA receptor, thereby enhancing AMPA channel conductance (Merril et al., 2005; Liu et al., 2004; Lee et al., 2000). The latter provides the necessary depolarization for reversing the magnesium-dependent block of NMDA receptors (Nowak et al., 1984). Together, the NMDA and AMPA receptors play a pivotal role in memory-related long-term potentiation (LTP, Granger et al., 1993; Staubli et al., 1994a).

Considering these actions, pharmacologic manipulation of NMDA and AMPA receptors has realised increasing interest in novel drug development for treating illnesses characterised by disturbed cognition (O'Neill et al., 2004). However, the complex composition and regulation of the NMDA receptor, involving multiple regulatory sites for glutamate, glycine and zinc, amongst others (see Kalia et al., 2008 for review), has complicated the development of safe and effective drug candidates for the treatment of these disorders. Moreover, full NMDA blockers, such as dizocilpine (MK-801; Wong et al., 1986) and phencyclidine (PCP; Anis et al., 1983) that fully abrogate physiological functioning of the NMDA receptor gated ion channel, are plagued by serious side effects including hallucinations, central nervous system depression and coma (Lipton and Chen, 2004) and neurodegenerative phenomena (Olney et al., 1999; Olney and Farber, 1995; Moghaddam et al., 1997; Adams and Moghaddam, 1998). However, agents that modulate the NMDA receptor but yet maintain normal physiological NMDA ion channel opening, such as the partial glycine site agonist, D-cycloserine (Hood et al., 1989) and the open channel blocker,

memantine (Bormann, 1989; Blanpied et al., 1997), have distinct clinical utility (Kushner et al., 2007; Hoffman et al., 2006). Since AMPA receptors play an important role in recruiting NMDA receptors, targeting AMPA receptors may be an alternate approach to bolster/maintain normal functioning of the NMDA receptor without non-physiological disruption of glutamatergic activity.

Ampakines are a group of positive allosteric modulators of the AMPA receptor that, without having any agonist or antagonist effects of their own, retard deactivation and desensitization of AMPA receptors (Lynch, 2002). In this way, they exert diverse effects on the excitatory postsynaptic potential (EPSP) (Arai et al., 2002), particularly an enhanced synaptic response by prolonging current flow through the AMPA receptor complex. In humans, ampakines have demonstrated an improvement in various memory tests (Ingvar et al., 1997; Lynch et al., 1996). Pre-clinical studies have found that AMPA receptor potentiators, such as LY404187, increase local cerebral activity as measured by glucose metabolism or MRI in select regions of the rodent brain involved in learning and memory, particularly the cortex and hippocampus (Fowler et al., 2004; Jones et al., 2005), further supporting a rationale for their use in disorders of cognition. Thus, ampakines improve retention scores in the radial maze (Staubli et al., 1994b), improve olfactory-related memory (Larson et al., 1995) and facilitate memory in aged rats (Granger et al., 1996). The ampakines CX516 and S-18986-1 have been found to enhance short-term memory (Hampson et al., 1998) and episodic memory (Lebrun et al., 2000), respectively. However, no studies to date have evaluated the efficacy of ampakines to bolster learning of long-term memory tasks such as spatial memory.

In this study, we have investigated more fully the cognitive effects of the ampakine, *Org 26576*, in rats by assessing explicit memory performance in the Morris water maze. In order to confirm a dose-relationship, the behavioural studies will be performed over a dosage range of 1, 3 and 10 mg/kg administered twice daily over a period of 12 days. Moreover, since evidence now concurs that ampakines may act to bolster AMPA-NMDA receptor cross talk, we have also assessed whether effects on

spatial memory are possibly associated with long-term changes in frontal cortical and/or hippocampal NMDA receptor binding following chronic *Org 26576* treatment.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, weighing 220-250 g and provided by the Biomedical Resource Unit, University of Kwazulu Natal, were grouped five rats per cage and housed in identical cages at the Animal Research Centre, North-West University. The rooms were maintained at constant conditions of temperature ($21\pm5^{\circ}\text{C}$), relative humidity ($50\pm5\%$) with free access to food and water. Full spectrum cold white light (350-400 lux) was provided over a 12 hour light/dark cycle. Positive air pressure was maintained in the facility with air filtration 99.7 % effective for a particle size of 2 micron and 99.9 % for a particle size of 5 micron. All animals were handled according to the code of ethics in research, training and testing of drugs as laid down by the Animal Ethics Committee of the North-West University (Ethics number: NWU-00016-07-A6).

2.2. Drug treatment

Org 26576 was freshly prepared daily by dissolving in Sabax Saline 0.9%, and administered intraperitoneally (i.p.) twice daily at 08:00 and 17:00. Dosages were obtained from in-house propriety data courtesy of the Schering-Plough.

2.3. Experimental design

For the *behavioural analyses*, four groups of animals (n=10 rats per group) received either saline (1 ml/kg i.p.) or *Org 26576* in incremental dosages of 1, 3 or 10 mg/kg i.p. b.d., in the same injection volume as above, for 12 days. Drug treatment was initiated 7 days prior to Morris water maze training, continuing throughout the 5 day spatial memory learning and assessment period. During this period, the animals received the first injection approximately half an hour before the first trial started (08:00) and then again at 17:00. The dose response study was performed in two separate sessions spaced 2 weeks apart.

The treatment protocol is outlined in **figure 1**.

Insert Figure 1

For the *neuroreceptor studies*, separate groups of animals were used, although essentially as described for the behavioural protocol above (see **figure 1**). In this group, no behavioural testing was carried out. The animals were sacrificed 24hrs after drug treatment discontinuation, with the appropriate brain tissues dissected out for the receptor binding assays.

2.4. Behavioural testing

2.4.1. Morris water maze

In order to assess spatial memory performance, a form of explicit memory, a modified version of the Morris water maze was used (Morris, 1981). One day prior to training, animals were habituated for 60 s in the pool to avoid initial stress associated with the Morris water task. The animal was placed in a 1.8 m diameter pool (wall height 0.65 m; water depth 0.275 m) and encouraged to escape the maze by locating an olive-green coloured escape platform submerged 2 cm below the water surface. Learning was assisted with extra maze cues, with training taking place over 5 days.

Confirmation that the animal had learned the location of the platform was assessed by removing the platform from the pool and monitoring the search strategy of the animal as it searched to locate the platform where it was previously located. The pool, constructed from a dark olive green plastic to prevent visibility of the hidden platform, was divided into four quadrants and labelled N-E-S-W. A digital video camera was used to collect the swim activities in the four quadrants, which were subsequently analyzed using AccuTrack digital tracking software (AccuScan Instruments Inc., Columbus, OH 43228, USA). The protocol is discussed in detail below.

After receiving either saline or *Org 26576* injections for the designated period, the animals were placed into cages and moved to a room adjacent to the pool and allowed to acclimatize for 30 minutes. Acquisition training took place over a period of 5 days. Each rat underwent 4 training trials each day with a 30 minute inter-trial interval on day 1 and a 15-20 minute inter-trial interval for days 2 to 5. Each animal was placed on the platform for 30 seconds to orientate before the first trial on day 1. The rat was placed, tail first with its head facing the wall, in a quadrant not containing the platform, with video-recording and timing started as soon as the rat entered the water. The animal was allowed to swim for 60 seconds. If the animal failed to locate the submerged platform in this time, it was assisted onto the platform and allowed to orientate for 30 seconds. The rat was removed and dried before being returned to the cage. The procedure was repeated in the other quadrants over subsequent days with each rat doing 20 training trials over the 5 days (memory consolidation period). The extent of memory retrieval was assessed in the probe trial, which was initiated 120-150 minutes after the last trial on day 5. In this test, the platform was removed and the animal allowed to swim for 60 seconds. The time that the animal spent in the target quadrant and the target zone (immediate area where the platform was previously located) is an indication of the degree of memory retrieval that had taken place after learning. There was a 30 minute recovery time for the animal between the probe trial and cued trial. The cued trial involved attaching a 30 cm long white ruler to the platform in the original quadrant, with the animal then allowed to swim for

a maximum of 60 seconds. The cued trial controls for purely spatial memory deficits and that vision and locomotor impairment are not confounding variables.

Swim parameters were analyzed using the AccuTrack Software system. The parameters used during acquisition training (day 1 to day 5) included escape latency, target zone entrance latency, time spent in target zone and ratio time in target zone. Percentage time in target zone and time in target quadrant were assessed during the probe trial (day 5). Escape latency time and swim speed were used for the visible platform (cued) trial.

2.5. Neuroreceptor studies

NMDA receptor binding characteristics were determined in the frontal cortex and hippocampus of rats following the various treatment doses of *Org 26576*. Rats received either saline or *Org 26576* as described earlier and sacrificed by decapitation 24hrs after the last drug dose. Total hippocampus and frontal cortices, including dorsal, lateral, prefrontal and medial cortex, were dissected out on an ice-cooled dissection slab, and the tissue snap frozen in liquid nitrogen and stored at -86°C until neuroreceptor analysis.

Tissues were pooled for the determination of NMDA receptor binding, with 2 cortices or 2 hippocampi pooled per assay and performed in triplicate. The tissue was homogenized in 30 volumes of cold HTS buffer (5 mM HEPES / 4.5 mM Tris buffer, pH 7.4) with an Ultra-Turrax (6 seconds) and the suspension centrifuged (48 000 xg for 15 minutes at 4°C). After the resulting supernatant was decanted, 25 ml of buffer was added. A glass-Teflon homogenizer was then used to resuspend the homogenate after which it was again centrifuged. The pellet was finally re-suspended in 60 volumes of HTS buffer (1 g tissue in 60 ml) and the homogenate kept on ice.

The following was added to the test tubes to define the total binding: 300 µl homogenate, 50 µl 300 µM glycine, 50 µl 100µM L-glutamate, 50 µl buffer and 50 µl

[³H]-MK-801 in a concentration range of 0.7 – 25 nM. To determine non-specific binding, the following was added to the test tubes: 300 µl homogenate, 50 µl 300 µM glycine, 50 µl 100 µM L-glutamate, 50 µl 300 µM MK801 and 50 µl [³H]-MK-801 of the concentration range in each tube. Each tube was then vortexed and thereafter incubated in a shaking bath for 90 minutes at 25°C. The contents of each tube were transferred to a Whatman GF/B filter, pre-soaked with ice-cold buffer, on the Hoeffler apparatus and the labelled membranes were harvested by rapid vacuum filtration. The filters were rinsed with 4 ml ice-cold buffer and placed in scintillation vials containing 3 ml Filter Count® scintillation cocktail and radioactivity determined by scintillation counting using a Packard Tri-carb 4660 scintillation spectrometer. A standard curve was generated by adding 10 µl of each radioligand concentration to a clean filter and adding 3 ml scintillation cocktail to determine each standard of the radioligand concentration series. The radioactivity on the filters was then determined by scintillation counting. NMDA receptor density was expressed in fmol/mg protein, with protein routinely assayed using the Bradford assay.

2.6. Statistical analysis

GraphPad PRISM version 5 (GraphPad Software, San Diego, USA) was used for all graphical presentations and either PRISM or SAS System (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. The acquisition training data was analyzed using a two way analysis of variance (ANOVA) with repeated measures followed with Tukey multiple comparison tests. Memory consolidation data was analyzed using one way ANOVA with subsequent Dunnett's *post hoc* analysis. Neuroreceptor data were analyzed using a one way ANOVA, followed by post-hoc Dunnett's tests, if required. Statistical significance are defined as $P < 0.05$.

3. Results

3.1. Effect of *Org 26576* on spatial memory performance

3.1.1. *Memory consolidation (acquisition training)*

Spatial learning revealed significant overall trial ($F=10.41$, $P<0.001$) and treatment effects ($F=3.25$, $P=0.024$) on the escape latencies in animals treated with saline or *Org 26576* (1, 3 & 10 mg/kg) during the training sessions (**figure 2**). A two way repeated measure ANOVA revealed a significant overall treatment $\times$ time interaction for latency to escape the water maze ($F=2.60$, $P=0.002$). Two way ANOVA with repeated measures of day 1 to day 5, with treatment and trial as variable, revealed a significant effect ($F=253.75$, $P<0.001$, **figure 2**) for escape latency during acquisition training in animals treated with either saline or one of the doses of *Org 26576*. There was also a significant global effect over the 5 days ($F=109.35$, $P<0.001$), confirming that learning took place over the acquisition training period (**figure 2**).

Insert Figure 2

That escape latency between the different groups from day 2 to day 5 were non-significantly different indicates that all the groups learned the task (**figure 2**, $P>0.05$).

Dunnett's *post-hoc* across group comparisons on each training day and using escape latency as parameter was then used to establish a day by day account of how the different treatments performed. On day 1, it is clear that animals receiving 3 mg/kg ($P<0.001$, Dunnett's, **figure 3a**) and 10 mg/kg ($P<0.05$, Dunnett's, **figure 3a**) dosages of *Org 26576* showed significantly improved spatial memory acquisition, as evinced by a significant decrease in escape latency time compared to saline treated animals. The 1 mg/kg dose similarly was numerically lower than saline treated animals, but failed to reach significance.

Insert Figure 3a to 3e

Post-hoc analysis of learning on day 2 using the Dunnett's multiple comparison tests revealed a very similar profile as that on day 1, but less emphatic. Thus, acquisition on day 2 showed no significant treatment effect, although as on day 1, the 3 mg/kg dosage demonstrated a strong tendency towards decreasing escape latency (**figure 3b**) vs. saline. Learning on days 3 to 5 showed no noteworthy differences evident between the different treatment groups (**figure 3c-e**).

3.1.2. Memory retrieval (Probe trial)

In order to confirm the presence and extent of learning at the end of the learning period, animals treated with either saline or one of the doses of *Org 26576* were subjected to a probe trial performed 120-150min after the last training session on day 5. One way ANOVA revealed a significant effect of treatment across the groups [$F(3,36)= 4.412$, $P=0.01$, (**figure 4a**)] using percentage time spent in target zone as end point. Subsequent Dunnett's *post-hoc* analysis revealed that 1, 3 and 10 mg/kg *Org 26576* significantly increased time spent in the target zone compared to the saline treated control group ($P<0.05$, Dunnett's, **figure 4a**).

Insert Figures 4a and 4b

Using an additional swim parameter as end point measure in the probe trial, viz. time in target quadrant, one way ANOVA revealed a significant effect of treatment across the groups ($F= 3.838$, $P=0.018$, **figure 4b**). Subsequent *post-hoc* analysis revealed that, while 1 mg/kg and 3 mg/kg *Org 26576* evoked a numerical increase in time spent in the target quadrant but failing to separate from saline treated control animals (Dunnett's, **Figure 4b**), 10 mg/kg *Org 26576* resulted in a significantly greater time spent in the target quadrant compared to saline treated control animals ($P<0.05$, Dunnett's, **Figure 4b**).

3.1.3. Cued trial

In order to exclude deficits in visual acuity and locomotor function as possible confounding variables in interpreting how the animals learned to orientate, navigate and escape the water maze, animals treated with either saline or one of the doses of *Org 26576* were subjected to a cued (visible platform) trial. This test was performed 30 min after the probe trial on day 5 using swimming speed and escape latency time as primary end point parameters.

One way ANOVA failed to reveal any significant effect of treatment ($F= 1.197$, $P=0.326$, **figure 5a**) in the cued visible platform assessment performed on day 5, using escape latency time as end point. Using swim speed as end point, one way ANOVA similarly failed to reveal any significant effect of treatment ($F= 2.094$, $P=0.118$, **figure 5b**). Subsequent *post-hoc* analysis of both parameters revealed no effect of the three doses of *Org 26576* on swim speed or on escape latency compared to saline treated animals (Dunnett's, $P > 0.05$, **Figures 5a and 5b**).

Insert Figures 5a and 5b

3.2. Effect of *Org 26576* on NMDA receptor binding

3.2.1. Frontal cortical NMDA receptor binding and response to drug treatment

One way ANOVA revealed no significant differences in frontal cortical NMDA receptor densities [$F(3,8)=0.7848$, $P=0.5352$, (**figure 6a**)] or in the K_d values [$F(3,8)=0.8451$, $P=0.5068$, (**figure 6b**)] of animals treated with the various dosages of *Org 26576* compared to saline treated animals.

Insert Figure 6a and 6b

3.2.2. Hippocampal NMDA receptor binding and response to drug treatment

Org 26576 at all dosages tested failed to reveal any significant changes in the hippocampal NMDA receptor densities [$F(3,8)=2.021$, $P=0.1896$, (**figure 7a**) or K_d values [$F(3,8)=0.3939$, $P=0.7609$, (**figure 7b**)], compared to that of saline-treated animals.

Insert Figure 7a and 7b

4. Discussion

In the present study we have examined the behavioural effects of chronic *Org 26576* treatment, at various dosages, on spatial memory acquisition in male Sprague-Dawley rats, as assessed with the Morris water maze. In addition, we determined whether effects on explicit learning involved long term changes in NMDA receptor binding in the rat frontal cortex and hippocampus. *Org 26576* was found to bolster spatial learning in a unique manner, with dosages of 3 and 10 mg/kg, but not 1 mg/kg, significantly enhancing learning on day 1 and to a lesser extent on day 2 of training (**figure 3a-b**). Thereafter its effects did not separate from vehicle over subsequent training days (**figure 3c-e**). Globally, however, escape latency decreased significantly in both the saline and *Org 26576* treated groups as the training days progressed so that at the end of the 5 day training period, a significant but equal degree of learning had taken place in all drug and saline treated groups (**figure 2**). Of interest was that, despite the apparent dose-dependent bolstering of learning evident only in the early stages of training, all doses of *Org 26576* significantly increased spatial memory retrieval in the probe trial on day 5 (**figure 4a-b**). Treatment with *Org 26576* therefore significantly improves spatial memory learning in normal rats. These effects were observed in the absence of undue effects in the visible platform version of the Morris water maze (**figure 5a-b**). Moreover, neuroreceptor analysis of frontal cortical (**figure 6a-b**) and hippocampal (**figure 7a-b**) NMDA receptor binding

revealed that receptor density ($B_{\max}$) and affinity (K_d) in both regions studied remained unchanged following all of the applied treatment dosages.

Autoradiographic quantification of AMPA receptors has confirmed its heterogeneous distribution throughout the brain, including the hippocampus and cortex (Monaghan et al., 1984). Furthermore, ampakines evoke cerebral glucose metabolism changes in various brain regions associated with cognitive functioning, but in particular regions of the cortex and hippocampus (Fowler et al., 2004; Jones et al., 2005). Subsequent studies have found that ampakines improve performance in a variety of cognitive tasks (Black, 2005). Various studies have demonstrated an ability of ampakines to improve short-term memory in humans (Ingvar et al., 1997; Lynch et al., 1996), while in animals ampakines have demonstrated beneficial effects in a range of learning and memory tests (Staubli et al., 1994b; Larson et al., 1995; Granger et al., 1996; Hampson et al., 1998). Recently, Bloss and colleagues have demonstrated an improvement in spatial memory performance in aged rats using S18986 (Bloss et al., 2008), an ampakine that has earlier been found to bolster cognitive functioning in healthy rats using a one-trial object recognition test (Lebrun et al., 2000). Our results with *Org 26576* are thus consistent with these findings. However, the aforementioned studies used short-term memory tasks such as the Y-maze, while the Morris water maze as applied in the current study measures long-term memory.

From a therapeutic perspective these data are potentially interesting for illnesses like schizophrenia, Alzheimer's disease and depression. Indeed, deficits in cognition represent a core symptom of depression (Castaneda et al., 2008), while animal studies have noted the antidepressant-like effects of ampakines (Li et al., 2003; Knapp et al., 2002). Studies in post-mortem brain of depressed patients have found an increase in AMPA receptor binding in the striatum of suicide victims (Freed et al., 1993), as well as a decrease in AMPA-receptor-directed mitogen-activated protein kinase (MAPK) signalling (Alt et al., 2006), thus supporting a role for altered AMPA receptor function in depression. Moreover, emotion enhances learning via noradrenaline mediated phosphorylation of GluR1-containing AMPA-receptors and as such enhances AMPA receptor trafficking (Hu et al., 2007). This latter study

provides a molecular mechanism for how emotion enhances learning and memory via an AMPA receptor-mediated event. AMPA receptors are involved in recruiting voltage-gated NMDA receptor ion channels (Tanaka et al., 2000) and together play a pivotal role in memory-related long-term potentiation (LTP, Granger et al., 1993; Staubli et al., 1994a).

Depression, however, has been more associated with increased glutamate activity (see Paul and Skolnick, 2003 for review), requiring that pharmacological treatment should attenuate rather than bolster function at glutamatergic synapses (Skolnick, 2008; Zarate et al., 2006; Berman et al., 2000), which are contrary to the glutamatergic actions of the ampakines. The effects of NMDA receptor block may at least partly be mediated by indirect enhancement of glutamatergic tone at AMPA receptors, due to “toning down” of NMDA receptor function. Indeed it has been shown that pre-treatment with the AMPA receptor antagonist, NBQX [2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline], attenuates both NMDA receptor antagonism-induced antidepressant-like behaviour and regulation of GluR1 AMPA receptor phosphorylation (Maeng et al., 2008). These same authors conclude that NMDA antagonists might exert rapid antidepressant-like effects by enhancing AMPA relative to NMDA throughput in critical neuronal circuits. This evidence suggests that ampakines may represent a promising novel approach to treating depression by targeting NMDA-AMPA balance involved in regulating cognition.

Learning a spatial navigational task such as the Morris water maze displays three levels of learning, namely initial learning, consolidation and retrieval (Bruehl-Jungman et al., 2007). Interestingly, our behavioural data suggests that, especially with the 3 mg/kg and 10 mg/kg doses, *Org 26576* exerts an initial reduction in escape latency, suggesting potentiation of *initial learning* on day 1 but which loses prominence over the vehicle treated group over the subsequent training days (*refer to figure 2 and figure 3a-e*). This phenomenon may be more representative of a short-term memory benefit, as was observed in earlier studies (Lebrun et al., 2000; Bloss et al., 2008). Thus, it may be argued that *Org 26576* increases AMPA receptor functioning to initiate early phase LTP, an event that lasts between 1-2 hours.

Indeed, AMPA receptor mediated LTP in the hippocampus specifically targets short term memory processes (Shi et al., 1999; Takahashi et al., 2003). This response involves MAPK and cAMP-dependent protein kinase A (PKA) signalling (Wang et al., 2004) culminating in postsynaptic membrane depolarisation and activation of NMDA receptor-initiated Ca^{2+} signalling.

Org 26576 treatment at all doses failed to separate from the saline treated group on subsequent days, in fact with all doses found to perform equal to that of vehicle, eventually attaining the same degree of spatial memory acquisition on day 5 (*refer to figure 2 and figure 3c-e*). *Org 26576* therefore does not significantly alter spatial memory *consolidation* in the Morris water maze task. This gradual loss of separation from saline treated animals over training days 1-5 may be attributable to changes in the postsynaptic NMDA:AMPA ratio. The ratio of relative activities of NMDA and AMPA receptors before LTP is 1:1 (Rao and Finkbeiner, 2007). Binding of *Org 26576* to the AMPA receptors and subsequent increasing of AMPA receptor activity; results in NMDA receptor activation that promotes the post-synaptic delivery of AMPA receptors, leading to early phase LTP. The NMDA:AMPA ratio is decreased as a result of the post-synaptic insertion of AMPA receptors (Rao and Finkbeiner, 2007). It is clear that learning is significantly enhanced during early phase LTP, as supported by our results showing that *Org 26576* (3 and 10 mg/kg) significantly enhanced spatial performance during the initial learning phase.

Persistent changes in synaptic transmission strength are important for long term memory formation (Martin et al., 2000). In the current study, results from day 3 to day 5, a period in which memory consolidation takes place, indicate that the effects of *Org 26576* declines over the subsequent training days (*refer to figures 3c-e*). A possible explanation for this declining effect may be due to synaptic scaling, a process where synaptic AMPA receptors are removed, and which takes place during late phase LTP resulting in synaptic NMDA receptor currents being potentiated (Watt et al., 2004). Consequently, learning will ensue. Indeed, the significant decrease in escape latency observed in all four *Org 26576*- and saline-treated groups over the 5

training days indicates that significant learning has indeed taken place in all the treatment groups (refer to **figure 2**).

However, all three dosages of *Org 26576* (1, 3 and 10 mg/kg) were significantly superior to saline treatment in the probe trial (**figure 4a**), with animals receiving *Org 26576* spending significantly more time in the target quadrant where the platform was located previously. These data confirm the drug's superior ability to bolster memory *retrieval*. These results indicate that *Org 26576* initiates and maintains processes involved in retrieval of long-term memory, in this case spatial memory. While NMDA receptors are more associated with consolidation of memories, retrieval of spatial memories is the function of AMPA receptors (see Riedel et al., 2003 for review). AMPA receptors also regulate short term changes in synaptic strength, while NMDA receptors are responsible for the regulation of genes for long term maintenance of these synaptic changes. The fact that all the doses of *Org 26576* demonstrated superior efficacy than the saline group during the probe trial suggests the activation or maintenance of memory processes throughout the 12 days of treatment.

Advantages of the present study over earlier assessments of ampakine effects on mnemonic function is the employment of a long-term memory task, which has allowed us to identify differential abilities of *Org 26576* to target specific behavioural components of spatial memory acquisition, namely on initial learning, on memory consolidation and on memory retrieval. Moreover, this question has been addressed using a three-tiered dose response analysis. Finally, the use of a sub-acute dosage regimen, and using a long-term memory-dependent task, has allowed a better extrapolation to the human condition where deficits in cognition are evident and subsequently treated over an extended period.

Despite the noteworthy improvement in learning processes observed during early stages of acquisition, and more robustly on day 5 in the probe trial, these behavioural changes were not associated with significant changes in hippocampal or frontal cortical NMDA receptor binding. Interactions between the hippocampus and frontal cortex play a critical role in learning and memory. Short-term memory processes are

dependent on the hippocampus and these unstable short-term memories are consolidated into more stable long-term memories in the cortex (Squire et al., 2004; Wiltgen et al., 2004). Although *Org 26576* at 10 mg/kg dosage engendered a decrease in cortical NMDA receptor density (**figure 6a**), this change failed to reach statistical significance. No associated changes in receptor affinity were noted (**figure 6b**). In similar fashion, the 3 mg/kg dosage numerically decreased the NMDA receptor density in the hippocampus, although again narrowly missing significance (**figure 7a**). Here too there were no noteworthy effects on NMDA receptor affinity (**figure 7b**). Activation of AMPA receptors are reported to increase the recruitment of NMDA receptors (Tanaka et al., 2000). Thus, there is the expectancy that *Org 26576* should increase NMDA receptor density, possibly supporting (and explaining) its beneficial effects on mnemonic function. However, our binding data in the frontal cortex and hippocampus suggest a decrease (albeit not significantly) in NMDA receptor density with the 10 and 3 mg/kg dose, respectively. This response, however, may reflect a decrease in NMDA:AMPA ratio due to post-synaptic insertion of AMPA receptors (Derkach et al., 2007). This observation may underscore the effects on spatial memory performance on day 1 (refer to **figure 3a**), especially since short-term memory processes are dependent upon the hippocampus (Izquierdo et al., 2002). *Org 26576* may therefore facilitate short-term and long-term memory processes by altering NMDA:AMPA receptor balance in the hippocampus and frontal cortex. It should however be borne in mind that the cognitive enhancing effects of ampakines are very likely not restricted to effects on one transmitter or signalling system in these brain regions. Indeed, ampakines have been found to affect cholinergic (Rosi et al., 2004), and noradrenergic release (Lockhart et al., 2000), as well as neurotrophin expression (Lockhart et al., 2007), all of which may contribute to the behavioural responses depicted in the current study.

5. Conclusions

This study has for the first time assessed the efficacy of an ampakine to bolster acquisition of a long-term memory task, providing evidence for a unique action involving the various phases of learning, and by implication, the processes of LTP. *Org 26576* significantly improves early initial learning in the Morris water maze, as well as significantly improves spatial memory retrieval. These behavioural effects were not associated with simultaneous effects on frontal cortical and hippocampal NMDA receptor binding. This study therefore provides evidence that *Org 26576* may be an effective agent to enhance both short-term as well as long-term memory processes, attributes that warrant further investigation into its use in major depression, Alzheimer's disease and post-traumatic stress disorder.

Acknowledgements

The authors would like to acknowledge the South African Medical Research Council (BHH) and the National Research Foundation (BHH, EH; grant number 2073038) for financial support. There is no financial relationship, or conflict of interest, with these funding agencies.

References

- Adams, B., Moghaddam, B., 1998. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* 18, 5545-5554.
- Alt, A., Nisenbaum, E. S., Bleakman, D., Witkin, J. M., 2006. A role for AMPA receptors in mood disorders. *Biochem Pharmacol* 71, 1273-1288.

- Anis, N. A., Berry, S. C., Burton, N. R., Lodge, D., 1983. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-D-aspartate. *Brit J Pharmacol* 79, 565-575.
- Arai, A. C., Xia, Y-F., Rogers, G., Lynch, G., Kessler, M., 2002. Benzamide-type AMPA receptor modulators form two subfamilies with distinct modes of action. *J Pharmacol Exp Ther* 303, 1075-1085.
- Berman, D. E., Hazvi, S., Neduva, V., Dudai, Y., 2000. The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste: Activation of ERK1-2 and formation of a memory trace. *J Neurosci* 20, 7017-7023.
- Black, M. D., 2005. Therapeutic potential of positive AMPA modulators and their relationship to AMPA receptor subunits. A review of preclinical data. *Psychopharmacology (Berl)* 179, 154-163.
- Blanpied, T. A., Boeckman, F. A., Aizenman, E., Johnson, J. W., 1997. Trapping channel block of NMDA-activated responses by amantadine and memantine. *J Neurophysiol* 77, 309-323.
- Bloss, E. B., Hunter, R. G., Waters, E. M., Munoz, C., Bernard, K., McEwen, B. S., 2008. Behavioral and biological effects of chronic S18986, a positive AMPA receptor modulator, during aging. *Exp Neurol* 210, 109-117.
- Bormann, J., 1989. Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. *Eur J Pharmacol* 166, 591-592.
- Bruel-Jungerman, E., Davis, S., Laroche, S., 2007. Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* 13, 492-505.
-

- Castaneda, A. E., Tuulio-Henriksson, A., Marttunen, M., Suvisaari, J., Lönqvist, J., 2008. A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *J Aff Dis* 106, 1-27.
- Derkach, V. A., Oh, M. C., Guire, E. S., Soderling, T. R., 2007. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat Rev Neurosci* 8, 101–113.
- Fowler JH, Whalley K, Murray T, O'Neill MJ, McCulloch J., 2004. The AMPA receptor potentiator LY404187 increases cerebral glucose utilization and c-fos expression in the rat. *J Cereb Blood Flow Metab* 24, 1098-1109.
- Freed, W. J., Dillon-Carter, O., Kleinman, J. E., 1993. Properties of [3H]AMPA binding in postmortem human brain from psychotic subjects and controls: increases in caudate nucleus associated with suicide. *Exp Neurol* 121, 48–56.
- Granger, R., Staubli, U., Davis, M., Perez, Y., Nilsson, L. Rogers, G. A., Lynch, G., 1993. A drug that facilitates glutamatergic transmission reduces exploratory activity and improves performance in a learning-dependant task. *Synapse* 15, 326-329.
- Granger, R., Deadwyler, S. A., Davis, M., Moskovitz, B., Kessler, M., Rogers, G., Lynch, G., 1996. Facilitation of glutamate receptors reverses an age-associated memory impairment in rats. *Synapse* 22, 332-337.
- Hampson, R. E., Rogers, G., Lynch, G., 1998. Facilitative effects of the ampakine CX516 on short-term memory in rats: Enhancement of delayed-nonmatch-to-sample performance. *J Neurosci* 18, 2740-2747
- Hofmann, S. G., Meuret, A. E., Smits, J. A., Simon, N. M., Pollack, M. H., Eisenmenger, K., 2006. Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. *Arch Gen Psychiat* 63, 298 –304.
-

- Hood, W. F., Compton, R. P., Monahan, J. B., 1989. D-cycloserine: a ligand for the N-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics. *Neurosci Lett* 98, 91-95.
- Hu, H., Real, E., Takamiya, K., Kang, M. G., LeDoux, J., Huganir, R. L., Malinow, R., 2007. Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. *Cell* 131, 160-173.
- Ingvar, M., Ambros-Ingerson, J., Davis, M., Granger, R., Kessler, M., Rogers, G. A., Schehr, R. S., Lynch, G., 1997. Enhancement by an ampakine of memory encoding in humans. *Exp Neurol* 146, 553–559.
- Izquierdo, L. A., Barros, D. M., Vianna, M. R., Coitinho, A., deDavid e Silva, T., Choi, H., Moletta, B., Medina, J. H., Izquierdo, I., 2002. Molecular pharmacological dissection of short- and long-term memory. *Cell Mol Neurobiol* 22, 269–287.
- Jones, N., O'Neill, M. J., Tricklebank, M., Libri, V., Williams, S. C., 2005. Examining the neural targets of the AMPA receptor potentiator LY404187 in the rat brain using pharmacological magnetic resonance imaging. *Psychopharmacology* 180, 743-751.
- Kalia, L.V., Kalia, S. K., Salter, M. W., 2008. NMDA receptors in clinical neurology: excitatory times ahead. *Lancet Neurol* 7, 742–755.
- Knapp, R. J., Goldenberg, R., Shuck, C., Cecil, A., Watkins, J., Miller, C., Crites, G., Malatynska, E., 2002. Antidepressant activity of memory-enhancing drugs in the reduction of submissive behavior model. *Eur J Pharmacol* 440, 27-35.
- Kushner, M. G., Kim, S. W., Donahue, C., Thuras, P., Adson, D., Kotlyar, M., McCabe, J., Peterson, J., Foa, E., 2007. D-cycloserine augmented exposure therapy for obsessive-compulsive disorder. *Biol Psychiat* 62, 835– 838.
-

- Larson, J., Lieu, T., Petchpradub, V., LeDuc, B., Ngo, H., Rogers, G. A., Lynch, G., 1995. Facilitation of olfactory learning by a modulator of AMPA receptors. *J Neurosci* 15, 8023-8030.
- Lebrun, C., Pillière, E., Lestage, P., 2000. Effects of S18986-1, a novel cognitive enhancer, on memory performance in an object recognition task in rats. *Eur J Pharmacol* 401, 205-212.
- Lee, H-K., Barbarosie, M., Kameyama, K., Bear, M. F., Huganir, R. L., 2000. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405, 955-959.
- Li, X., Witkin, J. M., Need, A. B., Skolnick, P., 2003. Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell Mol Neurobiol* 23, 419-430.
- Lipton, S. A., Chen, H-SV., 2004. Paradigm shift in neuroprotective drug development: clinically tolerated NMDA receptor inhibition by memantine. *Cell Death Differ* 11, 18-20.
- Liu, L., Wong, T. P., Pozza, M. F., Lingenhoehl, K., Wang, Y., Sheng, M., Auberson, Y. P., Wang, Y. T., 2004. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science* 304, 1021-1024.
- Lockhart, B., Iop, F., Closier, M., Lestage, P., 2000. (S)-2,3-dihydro-[3,4]cyclopentano-1,2,4-benzothiadiazine-1,1-dioxide: (S18986-1) a positive modulator of AMPA receptors enhances (S)-AMPA-mediated [3H] noradrenaline release from rat hippocampal and frontal cortex slices. *Eur J Pharmacol* 401, 145-153.
- Lockhart, B. P., Rodriguez, M., Mourlevat, S., Peron, P., Catesson, S., Villain, N., Galizzi, J. P., Boutin, J. A., Lestage, P., 2007. S18986: a positive modulator of AMPA-receptors enhances (S)-AMPA-mediated BDNF mRNA and protein expression in rat primary cortical neuronal cultures. *Eur J Pharmacol* 561, 23-31.
-

- Lynch, G., 2002. Memory enhancement: the search for mechanism based drugs. *Nat Neurosci* 5, 1035-1038.
- Lynch, G., Kessler, M., Rogers, G., Ambros-Ingerson, J., Granger, R., Schehr, R. S., 1996. Psychological effects of a drug that facilitates brain AMPA receptors. *Int Clin Psychopharmacol* 11, 13–19.
- Maeng, S., Zarate, C. A., Du, J., Schloesser, R. J., McCammon, J., Chen, G., 2008. Cellular mechanisms underlying the antidepressant effects of ketamine: Role of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiat* 63, 349-352.
- Malenka, R. C., Nicoll, R. A., 1999. Long-term potentiation - a decade of progress? *Science* 285, 1870-1874.
- Martin, S. J., Grimwood, P. D., Morris, R. G. M., 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Ann Rev Neurosci* 23, 649-711.
- Merrill, M. A., Chen, Y., Strack, S., Hell, J. W., 2005. Activity-driven postsynaptic translocation of CaMKII. *Trends Pharmacol Sci* 26, 645–653.
- Moghaddam, B., Adams, B., Verma, A., 1997. Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 17, 2921-2927.
- Monaghan, D. T., Yao, D., Cotman, C. W., 1984. Distribution of [3H]AMPA binding sites in rat brain as determined by quantitative autoradiography. *Brain Res* 324, 160-164.
- Morris, R. G. M., 1981. Spatial localization does not require the presence of local cues. *Learn Motiv* 12, 239-260.
-

- Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307, 462-465.
- Olney, J. W., Farber, N. B., 1995. Glutamate receptor dysfunction in schizophrenia. *Arch Gen Psychiat* 52, 998-1007.
- Olney, J. W., Newcomer, J. W., Farber, N. B., 1999. NMDA receptor hypofunction model of schizophrenia. *J Psychiat Res* 33, 523-533.
- O'Neill, M. J., Bleakman, D., Zimmerman, D. M., Nisenbaum, E. S., 2004. AMPA receptor potentiators for the treatment of CNS disorders. *Curr Drug Targets CNS Neurol Disord* 3, 181-194.
- Paul, I. A., Skolnick, P., 2003. Glutamate and depression: clinical and preclinical studies. *Ann NY Acad Sci* 1003, 250-272.
- Rao, V. R., Finkbeiner, S. 2007. NMDA and AMPA receptors: old channels, new tricks. *Trends Neurosci* 30, 284-291.
- Riedel, G., Platt, B., Micheau, J., 2003. Glutamate receptor function in learning and memory. *Beh Brain Res* 140, 1-47.
- Rosi, S., Giovannini, M. G., Lestage, P. J., Muñoz, C., Corte, L.D., Pepeu, G., 2004. Abstract S 18986, a positive modulator of AMPA receptors with cognition-enhancing properties, increases ACh release in the hippocampus of young and aged rat. *Neurosci Lett* 361, 120-123.
- Shi, S. H., Hayashi, Y., Petralia, R. S., Zaman, S. H., Wenthold, R. J., Svoboda, K., Malinow, R., 1999. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* 284, 1811-1816.
- Skolnick, P., 2008. AMPA receptors: A target for novel antidepressants? *Biol Psychiat* 63, 347-348.
-

- Squire, L. R., Stark, C. E., Clark, R. E., 2004. The medial temporal lobe. *Annu Rev Neurosci* 27, 279–306.
- Staubli, U., Perez, Y., Xu, F. B., Rogers, G., Ingvar, M., Stone-Elander, S., Lynch, G., 1994a. Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. *Proc Nat Acad Sci USA* 91, 11158-11162.
- Staubli, U., Rogers, G., Lynch, G., 1994b. Facilitation of glutamate receptors enhances memory. *Proc Nat Acad Sci USA* 91, 777-781.
- Takahashi, T., Svoboda, K., Malinow, R., 2003. Experience strengthening transmission by driving AMPA receptors into synapses. *Science* 299, 1585-1588.
- Tanaka, H., Grooms, S. Y., Bennett, M. V. L., Zukin, R. S., 2000. The AMPAR subunit GluR2: still front and center-stage. *Brain Res* 886, 190-207.
- Wang, J. Q., Tang, O., Parekar, N. K., Liu, Z., Samdani, S., Choe, E. S., Yang, L., Mao, L., 2004. Glutamate signaling to Ras–MAPK in striatal neurons: mechanisms for inducible gene expression and plasticity. *Mol Neurobiol* 29, 1–14.
- Watt, A. J., Sjöström, P. J., Häusser, M., Nelson, S. B., Turrigiano, G. G., 2004. A proportional but slower NMDA potentiation follows AMPA potentiation in LTP. *Nat Neurosci* 7, 518-524.
- Wiltgen, B. J., Brown, R. A., Talton, L. E., Silva, A. J., 2004. New circuits for old memories: the role of the neocortex in consolidation. *Neuron* 44, 101–108.
- Wong, E. H., Kemp, J. A., Priestley, T., Knight, A. R., Woodruff, G. N., Iversen, L. L., 1986. The anticonvulsant MK-801 is a potent N-methyl-D-aspartate antagonist. *Proc Natl Acad Sci USA* 83, 7104-7108.
-

Zarate, C. A., Du, J., Quiroz, J., Gray, N. A., Denicoff, K. D., Singh, J., 2003. Regulation of cellular plasticity cascades in the pathophysiology and treatment of mood disorders: Role of the glutamatergic system. *Ann NY Acad Sci* 1003, 273-291.

Legends to Figures

Figure 1: Outline of the Morris water maze study. Animals received either saline or *Org 26576* at doses of 1, 3 or 10 mg/kg administered intraperitoneally twice daily for a total of 12 days. Morris water maze testing began on day 8, with the final training and memory retrieval tests performed on day 12.

Figure 2: Effect of treatment with saline and *Org 26576* (1, 3 and 10 mg/kg i.p. twice daily) in the acquisition phase of spatial learning in the Morris water maze. The escape latency over the five acquisition training days is expressed as mean $\pm$ S.E.M. of 10 animals. Data sets are the average of 4 separate learning sessions performed on each day of training.

Figure 3: Effects of saline and *Org 26576* (1, 3 and 10 mg/kg i.p. twice daily) on spatial memory performance on days 1-5 (Figures 3 a-e) of acquisition training. Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Dunnett's test).

Figure 4: (a) Percentage time in target zone and (b) time in target quadrant on day 5 during the probe trial to access memory retrieval in animals receiving saline or *Org 26576* at dosages of 1, 3 and 10 mg/kg (i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Dunnett's test).

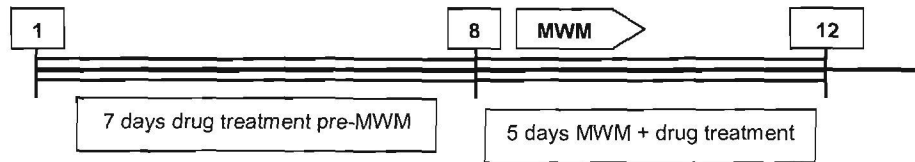
Figure 5: (a) Escape latency and (b) swim speed during the cued trial of the Morris water maze in animals receiving saline or *Org 26576* (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals.

Figure 6: (a) NMDA receptor density (B_{max}) and (b) NMDA receptor affinity (K_d) in the frontal cortex of animals receiving saline or *Org 26576* (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 6 animals.

Figure 7: (a) NMDA receptor density (B_{max}) and (b) NMDA receptor affinity (K_d) in the hippocampus of animals receiving saline or *Org 26576* (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 6 animals.

Figures

- Animals receiving drug treatment



- Animals receiving saline treatment

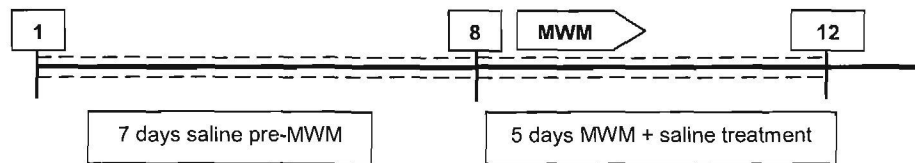


Figure 1

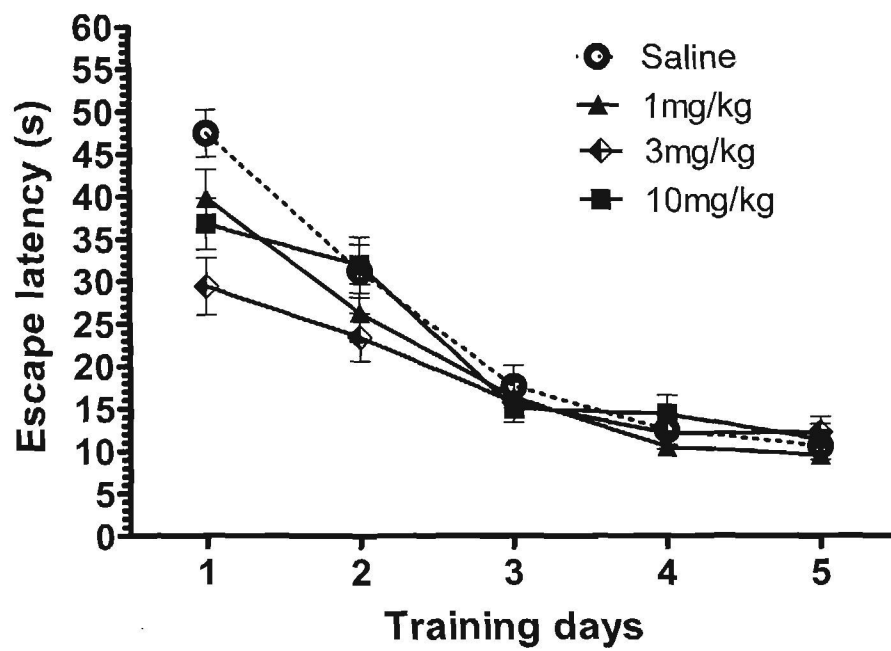


Figure 2

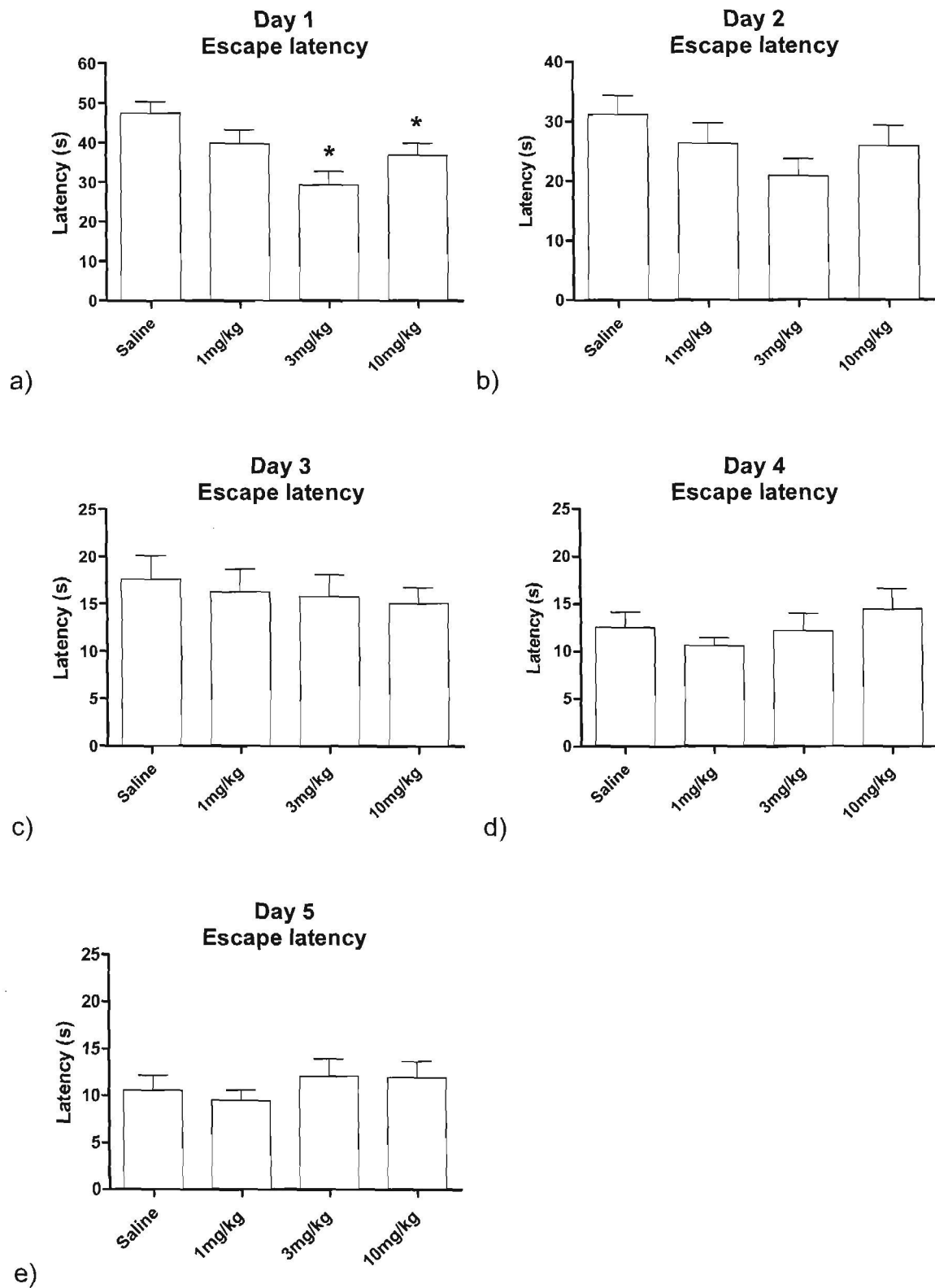


Figure 3 (a-e)

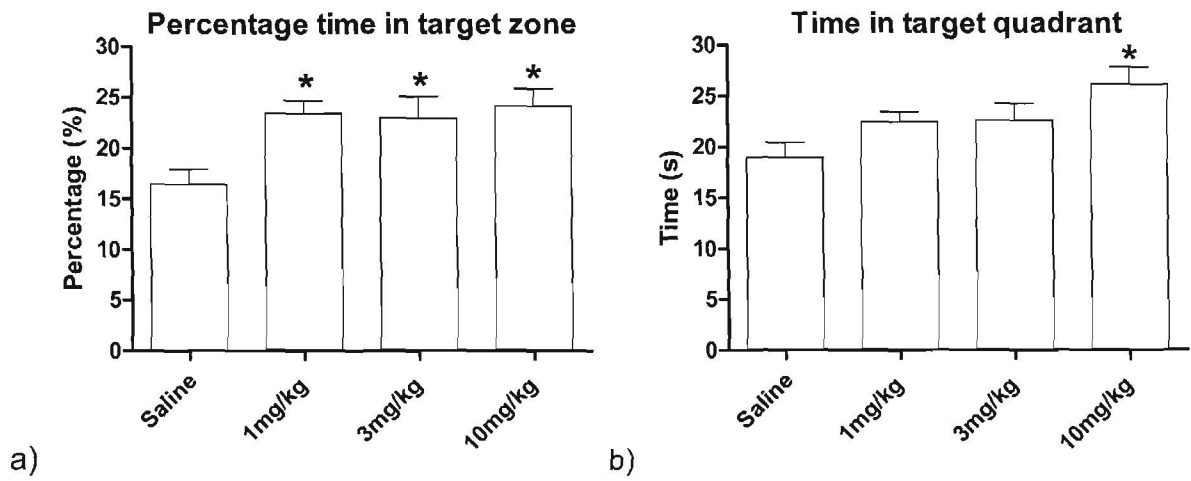


Figure 4 (a-b)

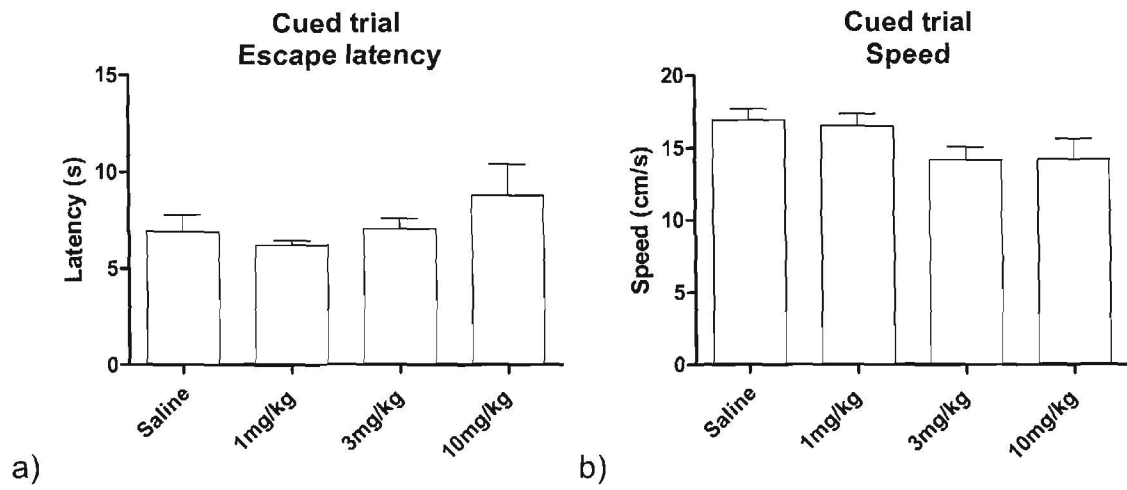


Figure 5 (a-b)

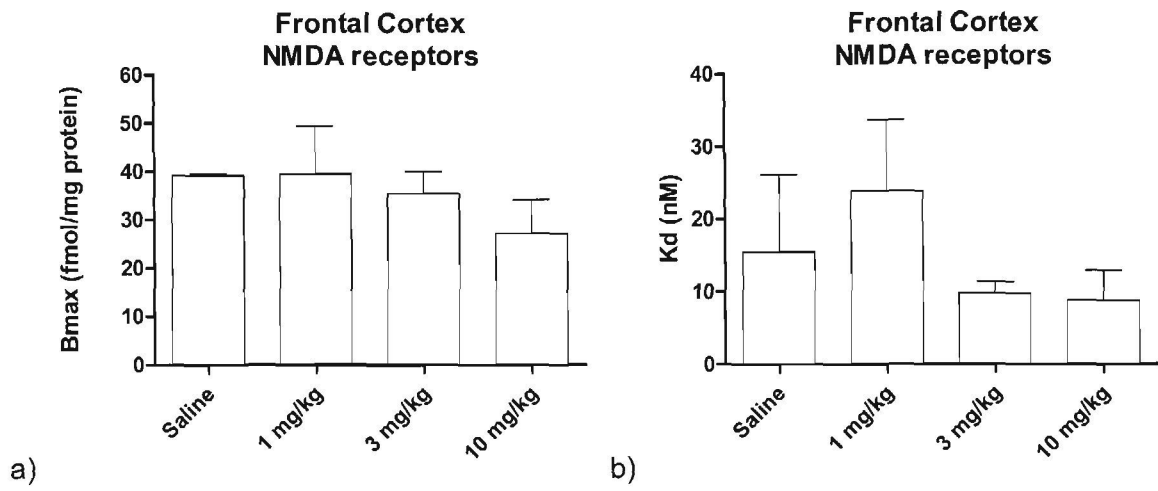


Figure 6 (a-b)

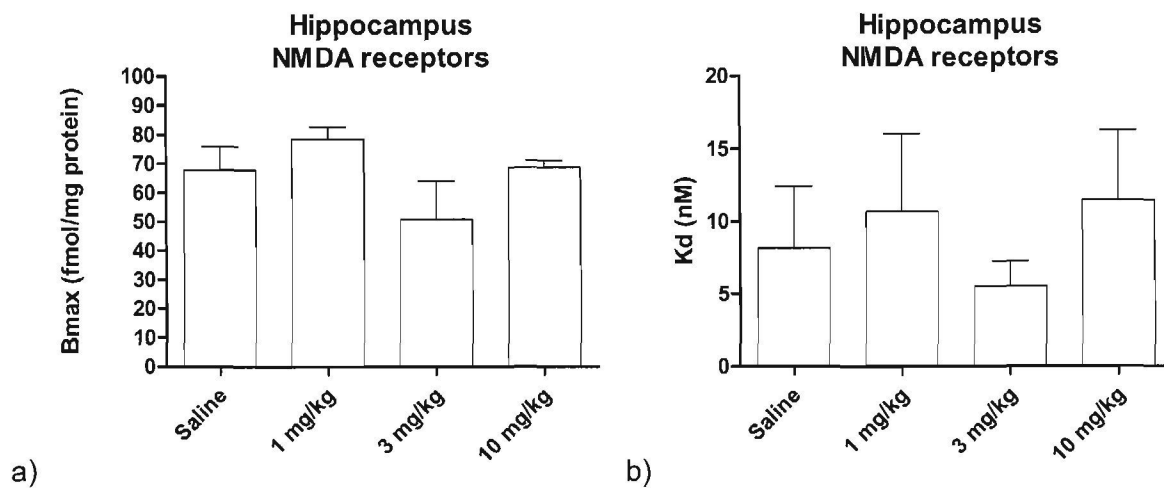


Figure 7 (a-b)

CHAPTER 4

Effects of Org 26576 treatment on spatial memory performance and on hippocampal - frontal cortical NMDA receptors in rats subjected to single prolonged stress

1. Introduction

Post-traumatic stress disorder (PTSD) is a severe anxiety disorder that may follow a traumatic event. Characteristics of PTSD include hyperarousal, avoidance, intrusions and cognitive dysfunction (*refer to **Chapter 2 – Section 2***). Animal models of PTSD simulate a severely stressful event in the life of the animal. Thus, for example the single prolonged stress (SPS)-model has varyingly used somatosensory stressors (such as restraint), inescapable or psychological stressors (such as underwater swimming or predator exposure) or complex stress-stimuli (such as that evoked by exposure to drugs or chemicals). SPS evokes distinct cognitive (Kohda et al., 2007) and aversive behaviours (Yamamoto et al., 2008), as well as hormonal disturbances (Takahashi et al., 2006), that resemble PTSD; while SPS-induced fear response has also shown response to treatment with the SSRI, paroxetine (Takahashi et al., 2006). When viewing the validation criteria for an animal model of PTSD (*see **Chapter 2 - Section 12***), these data confirm that SPS presents with significant face, predictive and construct validity with respect to PTSD.

AMPA receptors, one of a group of four subtypes of glutamate receptors in the brain, including N-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainic acid and metabotropic receptors, play a critical role in

mediating fast excitatory postsynaptic currents (Malenka & Nicoll, 1999). AMPA receptors also are involved in recruiting voltage-gated NMDA receptor ion channels (Tanaka et al., 2000), which together play a pivotal role in memory-related long-term potentiation (LTP, Granger et al., 1993; Staubli et al., 1994a). NMDA receptor activation in turn results in an increase in calcium release, the activation of calmodulin kinase II which phosphorylates the GluR1 subunit of the AMPA receptor, which then enhances channel conductance of AMPA receptors to promote LTP (Merril et al., 2005; Liu et al., 2004; Lee et al., 2000). AMPA receptor activation provides the necessary depolarization for reversing the magnesium-dependent block of NMDA receptors (Nowak et al., 1984), thereby allowing calcium to enter the cell (Burnashev et al., 1995; Iino et al., 1997) with the exit of potassium via the open NMDA receptor channels. Autoradiographic quantification of AMPA receptors has confirmed its heterogeneous distribution throughout the brain, including the hippocampus and cortex (Monaghan et al., 1984). Furthermore, ampakines evoke cerebral glucose metabolism changes in various brain regions associated with cognitive functioning, but in particular regions of the cortex and hippocampus (Jordan et al., 2005). Subsequent studies have found that ampakines improve performance in a variety of cognitive tasks (Black, 2005), thus providing impetus for the use of these agents in disorders where the conservation and improvement of cognitive functioning are required, such as schizophrenia, Alzheimer's disease and depression.

Considering these actions, pharmacologic manipulation of NMDA and AMPA receptors has realised increasing interest for novel drug development for treating illnesses characterised by disturbed cognition (O'Neill et al., 2004). However, the complex composition and regulation of the NMDA receptor (see Kalia et al., 2008 for review and **Chapter 2 - Section 9.1.1.1.**), has complicated the development of safe and effective drug candidates for the treatment of these disorders. Moreover, full antagonism of the NMDA receptor is plagued by serious side effects that preclude their general clinical use (Lipton & Chen, 2004; Olney et al., 1999; Olney & Farber, 1995; Moghaddam et al., 1997; Adams & Moghaddam, 1998). However, indirect modulation of the NMDA receptor that allows normal physiological NMDA ion channel

function, e.g. with D-cycloserine or memantine (Hood et al., 1989; Bormann, 1989; Blanpied et al., 1997) has distinct clinical utility (Kushner et al., 2007; Hoffman et al., 2006). Since AMPA receptors play an important role in recruiting NMDA receptors, targeting AMPA receptors may be an alternate approach to bolster/maintain normal functioning of the NMDA receptor without evoking excessive glutamatergic activity.

Ampakines are positive allosteric modulators of the AMPA receptor (see **Chapter 2 – Section 14**; Lynch, 2002) that enhance synaptic response by prolonging current flow through the AMPA receptor complex. In humans, Ampakines have been found to improve various measures of memory (Ingvar et al., 1997; Lynch et al., 1996), while pre-clinical studies have shown that Ampakines improve retention scores in the radial maze (Staubli et al., 1994b), improve olfactory-related memory (Larson et al., 1995) and facilitate memory in aged rats (Granger et al., 1996). The Ampakines CX516 and S-18986-1 have been found to enhance short-term memory (Hampson et al., 1998) and episodic memory (Lebrun et al., 2000), respectively. In an earlier study, we have provided the first behavioural evidence confirming that Ampakines are effective in bolstering learning of a long-term spatial memory task in rodents (see **Chapter 3**).

Post-traumatic stress disorder (PTSD) is a severe anxiety disorder that may follow a traumatic event. Characteristics of PTSD include hyperarousal, avoidance, intrusions and cognitive dysfunction, while recent studies have noted that if not adequately treated, the disorder gets progressively worse over time (Johnsen et al., 2002). Brain imaging studies in patients with PTSD have indicated that the illness is associated with atrophy of the hippocampus, a brain region especially important in regulating the stress response and that also is critical in working/declarative memory function (Bremner, 1999; Elzinga & Bremner, 2002). PTSD can be directly or indirectly related to a state of disorganized memory. Importantly, general declarative memory functions as well as explicit information about the trauma are compromised while at the same time non-declarative memory relating to involuntary recollection of the trauma is bolstered (Elzinga & Bremner, 2002). Earlier studies in animal models of PTSD have found that SPS (Kohda et al., 2007) as well as SPS plus re-experience (i.e. TDS

stress; Harvey et al., 2003) engender profound deficits in spatial memory performance in rats. Moreover, recent evidence in both SPS and TDS models have confirmed the evidence for disturbances in glutamatergic signalling in the brains of animals subjected to either SPS (Khan & Liberzon, 2004) and TDS stress (Harvey et al., 2004 b; 2005). Since AMPA receptors may modify NMDA receptor signalling (see Rao & Finkbeiner, 2007 for review; see **Chapter 2 - Section 9.1.1.3.**), the present study will investigate spatial memory performance of rats exposed to normal environmental conditions as well as after exposure to SPS, and whether chronic treatment with the Ampakine, Org 26576, is able to modify stress-induced changes in cognitive performance. As described in **Chapter 3**, Org 26576 will be administered over a dosage range of 1, 3 and 10 mg/kg administered twice daily over a period of 12 days. Since AMPA receptor activation increases the recruitment of NMDA receptors, we will also assess whether effects on spatial memory are possibly associated with long-term changes in frontal cortical and/or hippocampal NMDA receptor binding following chronic Org 26576 treatment.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats, weighing 220-250 g and provided by the Biomedical Resource Unit, University of Kwazulu Natal, were grouped five rats per cage and housed in identical cages at the Animal Research Centre, North-West University. The rooms are kept at constant conditions of temperature (21 ± 5 °C), relative humidity (50 ± 5 %) with free access to food and water. Full spectrum cold white light (350-400 lux) was provided over a 12 hour light/dark cycle. Positive air pressure was maintained in the facility with air filtration 99.7 % effective for a particle size of 2 micron and 99.9 % for a particle size of 5 micron. All animals were handled according to the code of ethics in research, training and testing of drugs as laid down by the Animal Ethics Committee of the North-West University. The study has been approved

by the local institutional review board for the use of experimental animals at North-West University, Potchefstroom. Ethics number: NWU-00016-07-A6.

2.2. Drug treatment

The drugs were freshly prepared daily. Org 26576 was dissolved in Sabax® Saline 0.9 % according to the weight of the animal (1 ml/kg) and was administered intraperitoneally (i.p.) twice daily for 12 days at dosages of 1, 3 or 10 mg/kg. Dosages were obtained from in-house propriety data courtesy of the manufacturer.

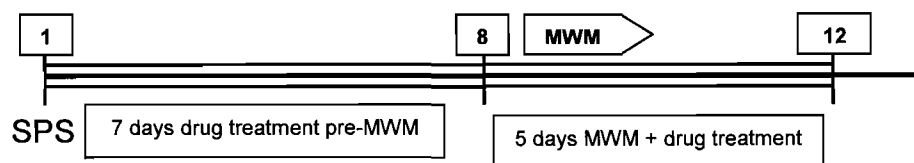
2.3. Experimental design

Single prolonged stress (SPS) model: We employed a well-validated animal model of PTSD, viz. the single prolonged stress model, or SPS. This model offers similar relevance with respect to PTSD as does the time-dependent sensitisation (TDS) model that has previously been used in our group (Harvey et al., 2003, 2004a; 2004b; 2005; 2006; Brand et al., 2008), but is less tedious and less time consuming. Since the SPS model had demonstrated noteworthy face, construct and predictive validity for PTSD, it was selected as the model of choice for this project (*refer to Chapter 2 - Section 13* for full description of SPS and TDS). SPS has previously been established in our laboratory (Harvey et al., 2006). Animals are exposed to a prolonged session of acute stress involving sequential exposure to restraint, underwater stress and exposure to ether vapours (see Khan & Liberzon, 2004; Liberzon et al., 1997; Takahashi et al., 2006; Harvey et al., 2006; Kohda et al., 2007). The animals are then left undisturbed for a week (Liberzon et al., 1999; Takahashi et al., 2006), with biobehavioural determinations performed on 7 days after SPS (Yamamoto et al., 2008; Imanaka et al., 2006; Kohda et al., 2007). Briefly, animals were exposed to single prolonged stress that consists of sequential exposure to a somatosensory stressor (restraint), an inescapable or psychological stressor (forced

swimming and underwater trauma), followed by a complex stress-stimuli evoked by exposure to diethyl ether (Harvey et al., 2006; Brand et al., 2008). All stress procedures started at 09:00. Rats were placed in a Perspex® restrainer for 2 hours with the tail-gate adjusted to keep the rat well contained without impairing circulation to the limbs. Immediately thereafter, the rats were individually placed in 18 cm of ambient water (25°C) in a cylindrical perspex® swim tank, and allowed to swim for 20 min before being held under water with a metal net for 40 s. During forced swimming, the depth of the water is adjusted to allow the animal to keep its nose above the water by using its tail as support. Following this, each rat was then immediately exposed to 0.8 ml of 100 % ether vapours in a 5 l sealed plastic container until loss of consciousness ($\pm$ 4 min) and then immediately removed. The animal was returned to its home cage, dried and allowed to recover over a period of 7 days.

For the behavioural analyses, four groups of animals (n=10 rats per group) received either saline (1 ml/kg) intraperitoneally (i.p.) or Org 26576 at incremental dosages of 1, 3 or 10 mg/kg i.p. administered twice daily at 08:00 and 17:00 over a period of 12 days starting on day 1 of the SPS procedure. Org 26576 injections were prepared in the same injection volume as saline. Treatment began 7 days prior to Morris water maze training and continuing throughout the training and assessment period, a total of 12 days. During the 5 days of Morris water maze testing, the animals received the first injection approximately half an hour before the first trial started (08:00) and then again at 17:00. The dose response study was performed in two separate sessions spaced 2 weeks apart. The treatment protocol is outlined in **figure 16**.

▪ Animals receiving drug treatment



▪ Animals receiving saline treatment

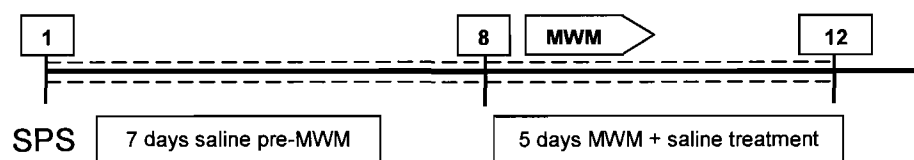


Figure 16: Outline of the Morris water maze study. Animals received either saline or *Org* 26576 at doses of 1, 3 or 10 mg/kg administered intraperitoneally twice daily for a total of 12 days. Morris water maze testing began on day 8, with the final training and memory retrieval tests performed on day 12.

For the neuroreceptor studies, separate groups of four ($n=10$ rats per group) received either saline (1 ml/kg) intraperitoneally (i.p.) or *Org* 26576 as described above. The animals were sacrificed 24hrs after drug treatment discontinuation without behavioural testing, and brain tissue dissected for the receptor binding assays.

2.4. Behavioural testing

2.4.1. Morris water maze

In order to assess spatial memory performance, a form of explicit memory, a modified version of the Morris water maze was used (Morris, 1981). One day prior to training, animals were habituated for 60 s in the pool to avoid initial stress associated with the Morris water task. The animal was placed in a 1.8 m diameter pool (wall height 0.65 m; water depth 0.275 m) and encouraged to escape the maze by locating an olive-green coloured escape platform submerged 2 cm below the water surface. Learning was assisted with extra maze cues, with training taking place over 5 days.

Confirmation that the animal had learned the location of the platform was assessed by removing the platform from the pool and monitoring the search strategy of the animal as it searched to locate the platform where it was previously located. The pool, constructed from a dark olive green plastic to prevent visibility of the hidden platform, was divided into four quadrants and labelled N-E-S-W. A digital video camera was used to collect the swim activities in the four quadrants, which were subsequently analyzed using AccuTrack digital tracking software (AccuScan Instruments Inc., Columbus, OH 43228, USA). The protocol is discussed in detail below.

After receiving either saline or *Org 26576* injections for the designated period, the animals were placed into cages and moved to a room adjacent to the pool and allowed to acclimatize for 30 minutes. Acquisition training took place over a period of 5 days. Each rat underwent 4 training trials each day with a 30 minute inter-trial interval on day 1 and a 15-20 minute inter-trial interval for days 2 to 5. Each animal was placed on the platform for 30 seconds to orientate before the first trial on day 1. The rat was placed, tail first with its head facing the wall, in a quadrant not containing the platform, with video-recording and timing started as soon as the rat entered the water. The animal was allowed to swim for 60 seconds. If the animal failed to locate the submerged platform in this time, it was assisted onto the platform and allowed to orientate for 30 seconds. The rat was removed and dried before being returned to the cage. The procedure was repeated in the other quadrants over subsequent days with each rat doing 20 training trials over the 5 days (memory consolidation period). The extent of memory retrieval was assessed in the probe trial, which was initiated 120-150 minutes after the last trial on day 5. In this test, the platform was removed and the animal allowed to swim for 60 seconds. The time that the animal spent in the target quadrant and the target zone (immediate area where the platform was previously located) is an indication of the degree of memory retrieval that had taken place after learning. There was a 30 minute recovery time for the animal between the probe trial and cued trial. The cued trial involved attaching a 30 cm long white ruler

to the platform in the original quadrant, with the animal then allowed to swim for a maximum of 60 seconds. The cued trial controls for purely spatial memory deficits and that vision and locomotor impairment are not confounding variables.

Swim parameters were analyzed using the AccuTrack Software system. The parameters used during acquisition training (day 1 to day 5) included escape latency, target zone entrance latency, time spent in target zone and ratio time in target zone. Percentage time in target zone and time in target quadrant were assessed during the probe trial (day 5). Escape latency time and swim speed were used for the visible platform cued trial.

2.5. Neuroreceptor studies

NMDA receptor binding characteristics were determined in the frontal cortex and hippocampus of rats following the various treatment doses of *Org 26576*. Rats receiving either saline or *Org 26576* (1, 3 or 10 mg/kg) treatment were sacrificed by decapitation 24hrs after the last dose. Total hippocampus and frontal cortices, including dorsal, lateral, prefrontal and medial cortex, were dissected out on an ice-cooled dissection slab, and the tissue snap frozen in liquid nitrogen and stored at -86°C until neuroreceptor analysis. Tissues were pooled for the determination of NMDA receptor binding, with 2 cortices or 2 hippocampi pooled per assay and performed in triplicate. The tissue was homogenized in 30 volumes of cold HTS buffer (5 mM HEPES / 4.5 mM Tris buffer, pH 7.4) with an Ultra-Turrax (6 seconds) and the suspension centrifuged (48 000 xg for 15 minutes at 4°C). After the resulting supernatant was decanted, 25 ml of buffer was added. A glass-Teflon homogenizer was then used to resuspend the homogenate after which it was again centrifuged. The pellet was finally re-suspended in 60 volumes of HTS buffer (1 g tissue in 60 ml) and the homogenate kept on ice.

The following was added to the test tubes to define the total binding: 300 μ l homogenate, 50 μ l 300 μ M glycine, 50 μ l 100 μ M L-glutamate, 50 μ l buffer and 50 μ l [3 H]-MK-801 in a concentration range of 0.7 – 25 nM. To determine non-specific binding, the following was added to the test tubes: 300 μ l homogenate, 50 μ l 300 μ M glycine, 50 μ l 100 μ M L-glutamate, 50 μ l 300 μ M MK801 and 50 μ l [3 H]-MK-801 of the concentration range in each tube. Each tube was then vortexed and thereafter incubated in a shaking bath for 90 minutes at 25°C. The contents of each tube were transferred to a Whatman GF/B filter, pre-soaked with ice-cold buffer, on the Hoeffler apparatus and the labelled membranes were harvested by rapid vacuum filtration. The filters were rinsed with 4 ml ice-cold buffer and placed in scintillation vials containing 3 ml Filter Count® scintillation cocktail and radioactivity determined by scintillation counting using a Packard Tri-carb 4660 scintillation spectrometer. A standard curve was generated by adding 10 μ l of each radioligand concentration to a clean filter and adding 3 ml scintillation cocktail to determine each standard of the radioligand concentration series. The radioactivity on the filters was then determined by scintillation counting. NMDA receptor density was expressed in fmol/mg protein, with protein routinely assayed using the Bradford assay.

2.6. Statistical analysis

GraphPad PRISM version 5 (GraphPad Software, San Diego, USA) was used for all graphical presentations and either PRISM or SAS System (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. The acquisition training data was analyzed using a two way analysis of variance (ANOVA) with repeated measures followed with Tukey multiple comparison test. Memory consolidation data and data from the probe trial were analyzed using one way ANOVA with subsequent Tukey *post hoc* analysis, while the cued trial was analysed with one way ANOVA with Dunnett's *post hoc* tests. Neuroreceptor data were analyzed using one way ANOVA followed by the Tukey test. Statistical significance are defined as $P < 0.05$.

3. Results

3.1. Effect of Org 26576 on spatial memory performance

3.1.1. Memory consolidation (acquisition training)

Spatial learning revealed significant overall trial ($F=19.71$, $P<0.001$) and treatment effects ($F=3.23$, $P=0.014$) on the escape latencies in animals subjected to SPS treatment with saline or Org 26576 (1, 3 and 10 mg/kg), compared to unstressed saline control, during the 5 day training sessions (**figure 17**). A two way repeated measure ANOVA revealed a significant overall treatment $\times$ time interaction for latency to escape the water maze ($F=1.79$, $P=0.028$). Two way ANOVA with repeated measures of day 1 to day 5, with treatment and trial as variable, revealed a significant effect ($F= 387.13$, $P<0.001$, **figure 17**) for escape latency during acquisition training in animals subjected to SPS treated with either saline or one of the doses of Org 26576. There was also a significant global effect over the 5 days, comparing each day with day 1 of training ($F=148.25$, $P<0.001$), confirming that learning took place for all the groups over the acquisition training period (**figure 17**).

That escape latency between the different groups from day 2 to day 5 were non-significantly different indicates that all the groups learned the task ($P>0.05$, **figure 17**).

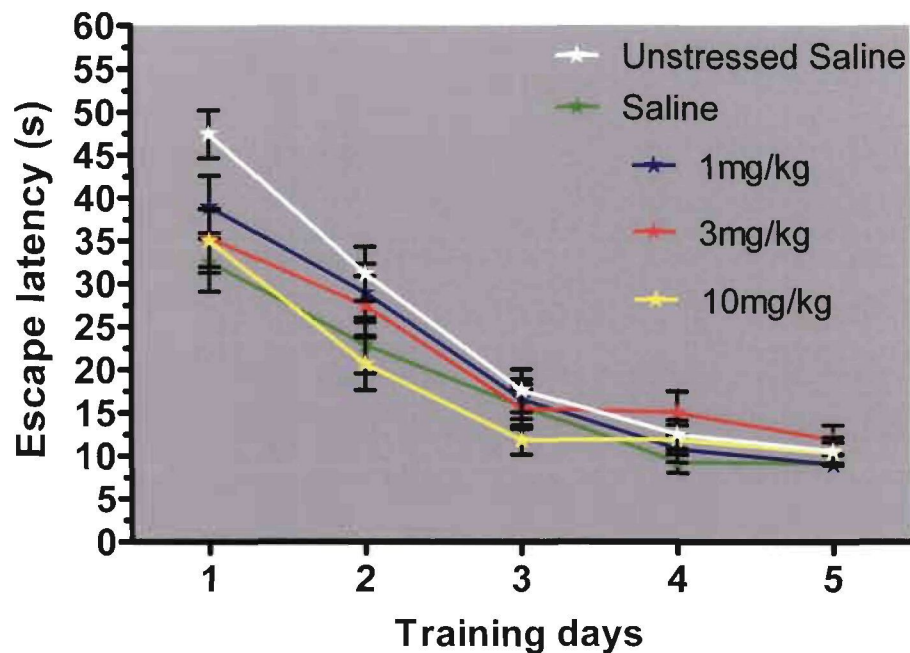


Figure 17: Effect of treatment with saline and Org 26576 (1, 3 and 10 mg/kg i.p. twice daily) in the acquisition phase of spatial learning in the Morris water maze. The escape latency over the five acquisition training days is expressed as mean $\pm$ S.E.M. of 10 animals. Data sets are the average of 4 separate learning sessions performed on each day of training.

Tukey *post-hoc* across group comparisons on each training day, and using escape latency as parameter, was then used to establish a day by day account of how the different treatment groups performed following exposure to SPS compared to the saline-treated unstressed group, and how the drug-treated groups compared against animals subjected to SPS plus saline. On day 1, all the groups exposed to SPS, including saline and Org 26576 treated groups, had a numerically shorter escape latency time than unstressed saline-treated animals ($P > 0.05$, Tukey, **figure 18a**), suggesting a tendency to locate the hidden platform quicker than saline-treated control animals. However, SPS-exposed animals receiving 3 mg/kg ($P > 0.05$, Tukey, **figure 18a**) and 10 mg/kg ($P > 0.05$, Tukey, **figure 18a**) of Org 26576 showed a similar degree of learning on day 1 as did the saline-treated SPS animals.

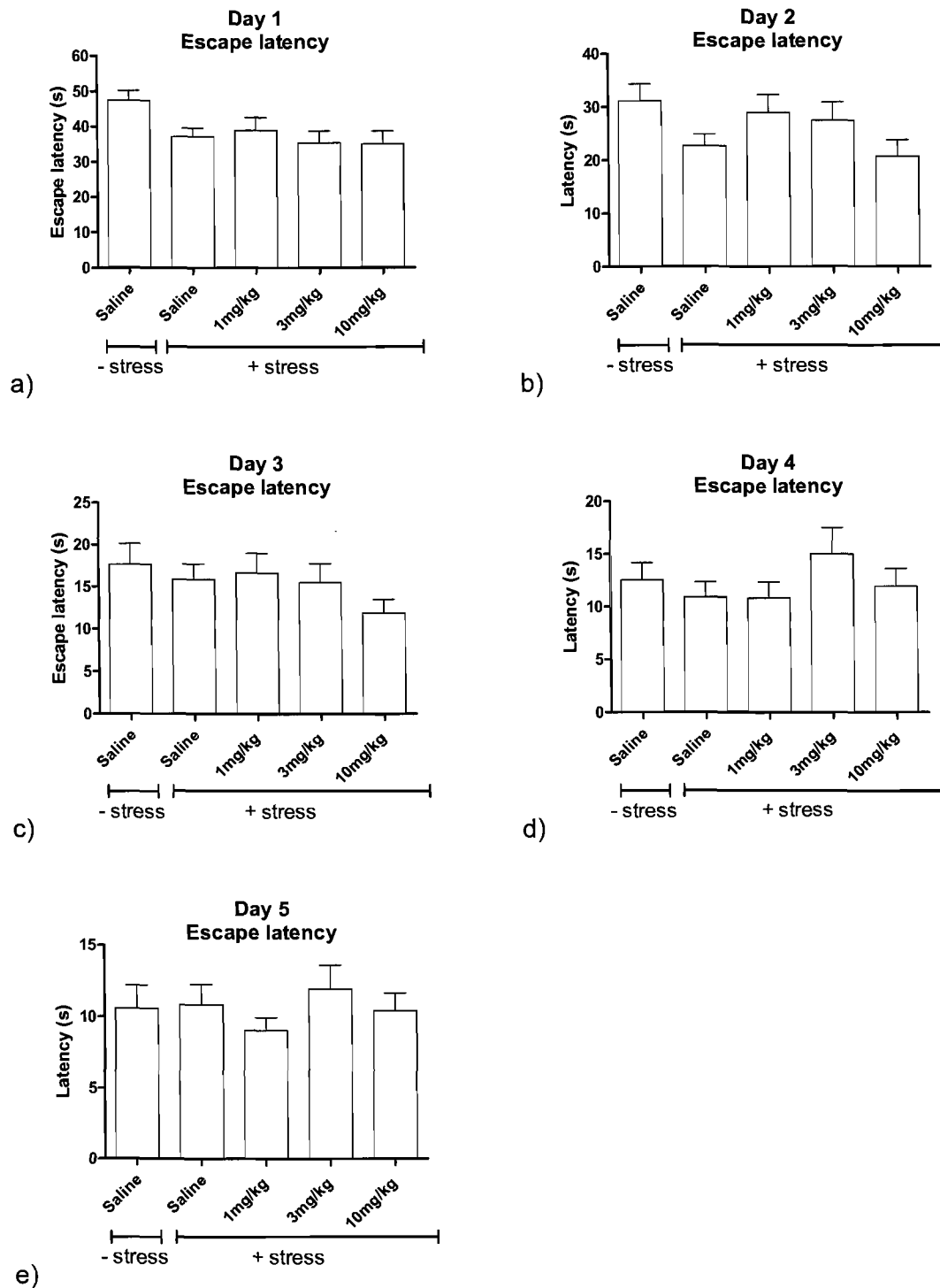


Figure 18: Effects of saline and Org 26576 (1, 3 and 10 mg/kg i.p. twice daily) on spatial memory performance on days 1-5 (Figures 18 a-e) of acquisition training. Data represent the means $\pm$ S.E.M. of 10 animals.

Post-hoc analysis of learning on days 2-5 failed to identify any significant treatment related effects ($P>0.05$; **figure 18b-e**), implying that SPS failed to modify learning during the training period compared to the unstressed saline treated group. Moreover, drug treatment in SPS animals also did not differ from the SPS plus saline group. Thus, none of the treatments were able to modify stress-induced changes in spatial learning on day 2 compared to unstressed saline treated animals or compared to SPS plus saline treated animals.

3.1.2. Memory retrieval (Probe trial)

In order to confirm the presence and extent of learning at the end of the learning period, stressed animals treated with either saline or one of the doses of Org 26576 were subjected to a probe trial performed 120-150min after the last training session on day 5. One way ANOVA did not reveal a significant effect of treatment across the groups [$F(4,55)= 2.381$, $P=0.063$, (**figure 19a**)] using percentage time spent in target zone as end point. Subsequent Tukey post-hoc analysis however revealed that 1 mg/kg Org 26576 significantly increased time spent in the target zone compared to the unstressed saline treated control group ($P<0.05$; Tukey, **figure 19a**) but missing significance compared to the SPS-saline treated group ($P>0.05$, Tukey, **figure 19a**). All other doses of Org 26576 did not separate significantly from unstressed control or SPS-saline treated groups ($P>0.05$; Tukey, **figure 19a**).

Using an additional swim parameter as end point measure in the probe trial, viz. time in target quadrant, one way ANOVA failed to reveal a significant effect of treatment across the groups [$F(4,55)= 0.6540$, $P=0.627$, **figure 19b**].

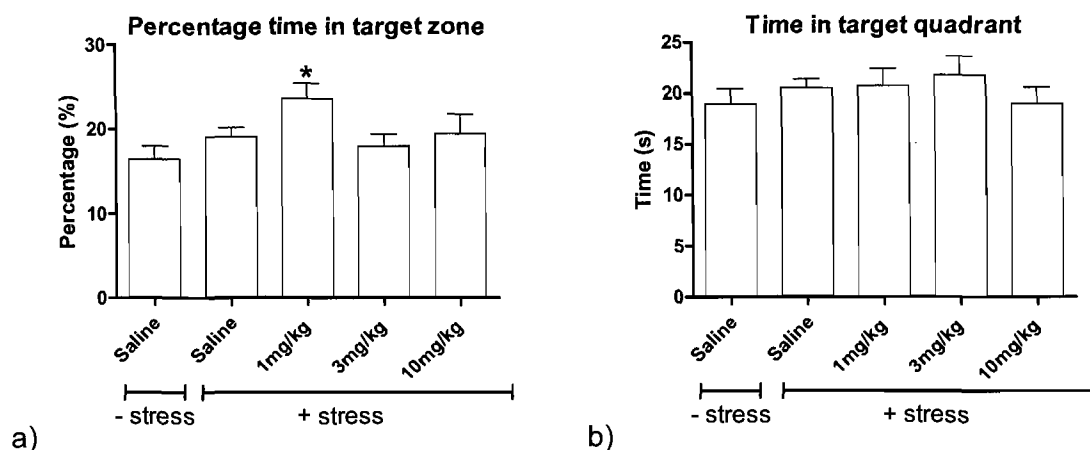


Figure 19: (a) Percentage time in target zone and (b) time in target quadrant on day 5 during the probe trial to access memory retrieval in animals receiving saline or Org 26576 at dosages of 1, 3 and 10 mg/kg (i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Tukey test).

3.1.3. Cued trial

In order to exclude deficits in visual acuity and locomotor function as possible confounding variables in interpreting how the animals learned to orientate, navigate and escape the water maze, animals treated with either saline or one of the doses of Org 26576 were subjected to a cued (visible platform) trial. This test was performed 30 min after the probe trial on day 5 using swimming speed and escape latency time as primary end point parameters.

One way ANOVA failed to reveal any significant effect of treatment ($F = 0.849$, $P = 0.501$, **figure 20a**) in the cued visible platform assessment performed on day 5, using escape latency time as end point. Using swim speed as end point, one way ANOVA similarly failed to reveal any significant effect of treatment ($F = 0.593$, $P = 0.669$, **figure 20b**).

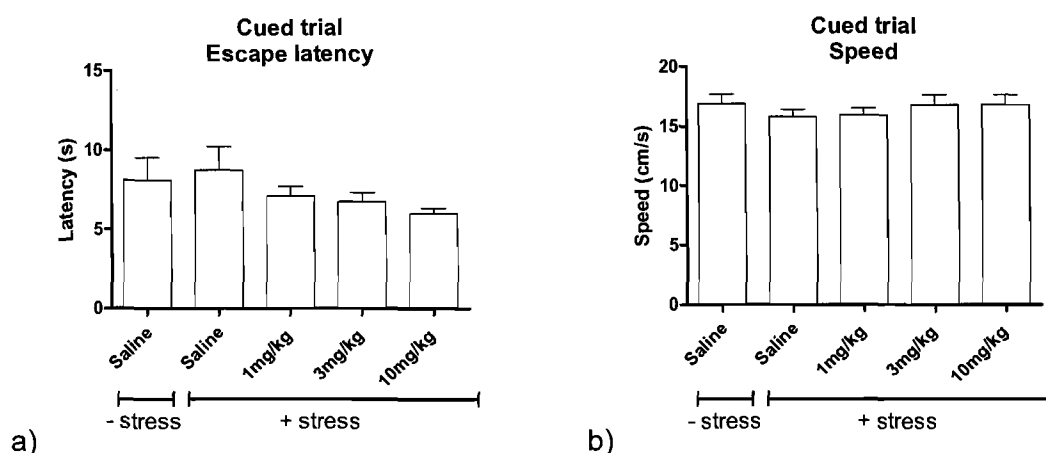


Figure 20: (a) Escape latency and (b) swim speed during the cued trial of the Morris water maze in animals receiving saline or Org 26576 (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals.

3.2. Effect of Org 26576 on NMDA receptor binding

3.2.1. Frontal cortex NMDA receptor binding and response to drug treatment

One way ANOVA revealed a significant effect of treatment in frontal cortical NMDA receptor density [$F(4,10)=5.083$, $P=0.017$, (**figure 21a**)]. Tukey *post-hoc* test performed across the various groups revealed that SPS induced a reduction in NMDA receptor density but not significantly ($P>0.05$, Tukey, **figure 21a**). A significant reduction in frontal cortical NMDA receptor density was observed in animals treated with 1 mg/kg Org 26576 ($P<0.05$; Tukey, **figure 21a**), but not in animals receiving 3 and 10 mg/kg Org 26576 compared to unstressed animals ($P>0.05$; Tukey, **figure 21a**). However, none of the Org 26576 treated SPS groups were markedly different from SPS-saline treated animals ($P>0.05$; Tukey, **figure 21a**). With regard to NMDA receptor affinity (K_d), no significant differences in the K_d values were evident across the groups [$F(4,10)=0.439$, $P=0.778$, (**figure 21b**)].

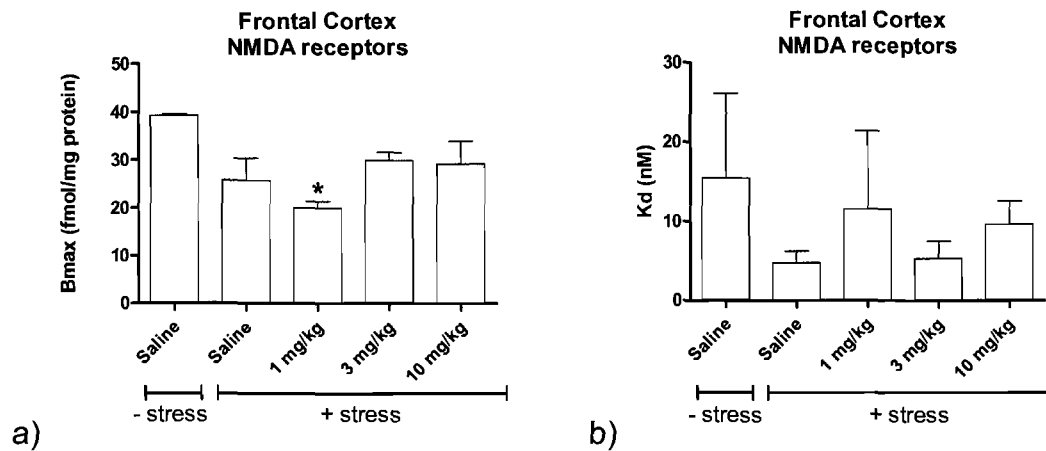


Figure 21: (a) NMDA receptor density (B_{max}) and (b) NMDA receptor affinity (K_d) in the frontal cortex of animals receiving saline or Org 26576 (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 6 animals. (* $P < 0.05$, Tukey test).

3.2.2. Hippocampal NMDA receptor binding and response to drug treatment

No evidence for differences in hippocampal NMDA receptor density [$F(4,10)=1.549$, $P=0.262$, **figure 22a**] or K_d values [$F(4,10)=0.317$, $P=0.861$, (**figure 22b**)] were evident across the groups.

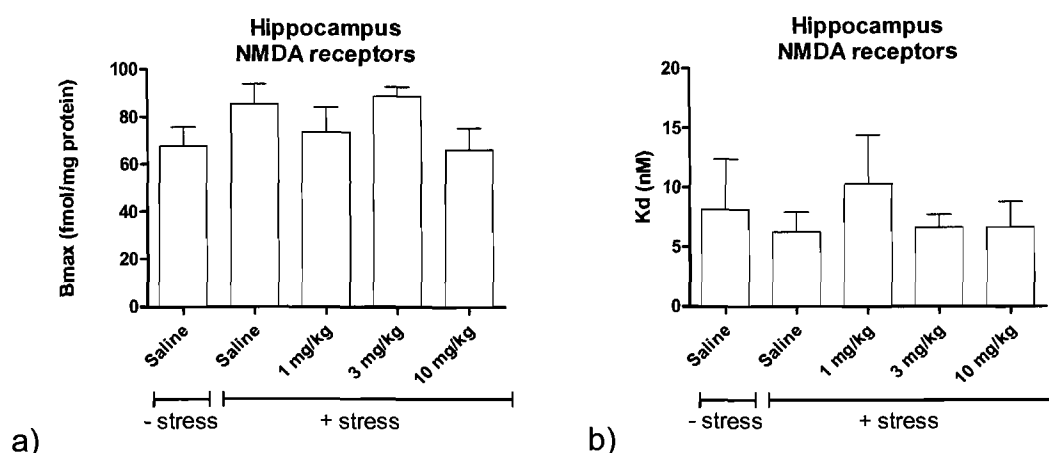


Figure 22: (a) NMDA receptor density ($B_{\max}$) and (b) NMDA receptor affinity (K_d) in the hippocampus of animals receiving saline or *Org 26576* (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 6 animals.

4. Discussion

In the present study we have examined the behavioural effects of chronic *Org 26576* treatment, at various dosages, on spatial memory acquisition in male Sprague-Dawley rats subjected to SPS, as assessed with the Morris water maze. In an earlier study (see **Chapter 3**), we described the effects of various doses of *Org 26576* on spatial memory performance in healthy, unstressed rats. In that study, it became clear that *Org 26576* exerts a bolstering of early phase learning of the task, but its strengthening of spatial memory retrieval was especially noteworthy (see **Chapter 3** for extended detail). In animals prior exposed to SPS (the present study), SPS was found to marginally but not significantly reduce escape latency time on day 1 of training, hinting that stress may have tended to enhance early phase learning of the task (**figure 18a**). Concomitant administration of *Org 26576* at all three doses (1, 3 and 10 mg/kg) maintained this tendency, but again failed to reach significance versus unstressed animals. More importantly, however, ampakine treatment was no different to that of SPS saline treated groups. These data together suggest that SPS tended to enhance spatial learning on day 1 of acquisition training, and was unchanged by *Org*

26576 treatment (**figure 18a**). Globally, escape latency decreased significantly in both the unstressed saline and SPS (Org 26576 and saline) treated groups as the training days progressed, so that at the end of the 5 day training period, a significant but equal degree of learning had taken place in all drug and saline treated groups (see **figure 17**). Interestingly, despite a lack of effect of Org 26576 to alter escape latency during acquisition training (**figure 18a**), the 1 mg/kg dose (but not the other doses) significantly increased spatial memory retrieval in the probe trial on day 5 compared to unstressed animals (**figure 19a**). However, this change did not reach significance compared to SPS treated animals (**figure 19a**). These effects were observed in the absence of undue effects in the visible platform version of the Morris water maze (**figure 20a-b**). Although SPS did not significantly alter cortical or hippocampal NMDA receptor density or affinity (**figures 21-22**), Org 26576 treatment at a dose of 1 mg/kg, but not at other doses, significantly decreased cortical NMDA receptor density (B_{max}) (**figure 21a**) compared to unstressed animals, without effects on receptor affinity (K_d) (**figure 21b**). However, this response was similar and not significantly different to that evoked by SPS-saline treated animals (**figure 21a**). Hippocampal NMDA receptor density and affinity remained unchanged following all of the applied treatment dosages.

What is most unexpected from the above-described results is that SPS failed to evoke a significant effect on spatial memory performance. As previously described, SPS (Kohda et al., 2007) and SPS plus re-stress (TDS stress; Harvey et al., 2003) have both been found to engender a marked attenuation of spatial memory performance in rats, a response that is in line with the symptoms of cognitive dysfunction described in PTSD (Weber et al., 2005). Further, SPS did not evoke changes in cortical or hippocampal NMDA receptor binding, which is also contrary to earlier papers describing either SPS plus restress (TDS stress; Harvey et al., 2004a; Harvey et al., 2005) or SPS (Yamamoto et al., 2008) to evoke changes in NMDA receptor expression or NMDA-mediated signalling. This would suggest that the anticipated stress response evoked by SPS was not as robust as was expected.

Possible reasons for this are described elsewhere in this dissertation (see **Chapter 4 – Shortcomings of this study**). This unfortunately does not allow for accurate interpretation of how Org 26576 performed in the model, although some interesting observations can be made.

The ampakines, Org26576 and Org24448, have been found to increase local cerebral glucose metabolism in the mouse thalamus, cortex, dentate gyrus and hippocampus (Jordan et al., 2005). This data provides an anatomical basis for the use of these agents in disorders where the conservation and improvement of cognitive functioning are required, such as schizophrenia, Alzheimer's disease and depression. Animal studies have indeed noted the antidepressant-like effects of ampakines (Li et al., 2003), while studies in post-mortem brain of depressed patients have found an increase in AMPA receptor binding in the striatum of suicide victims (Freed et al., 1993), as well as a decrease in AMPA-receptor-directed mitogen-activated protein kinase (MAPK) signalling (Alt et al., 2006), thus supporting a role for altered AMPA receptor function in depression. Moreover, emotion enhances learning via noradrenaline mediated phosphorylation of GluR1-containing AMPA-receptors and as such enhances AMPA receptor trafficking (Hu et al., 2007). This latter study provides a molecular mechanism for how emotion enhances learning and memory via an AMPA receptor-mediated event, and is of particular relevance to PTSD. AMPA receptors are involved in recruiting voltage-gated NMDA receptor ion channels (Tanaka et al., 2000) and together play a pivotal role in memory-related long-term potentiation (LTP, Granger et al., 1993; Staubli et al., 1994a).

In our earlier work (**Chapter 3**), we found that Org 26576 displayed an ability to improve the learning of a spatial navigational task by differentially modulating three levels of learning, namely initial learning, consolidation and retrieval (Bruehl-Jungerman et al., 2007). Briefly, Org 26576 bolstered early phase learning in the Morris water maze, but without pronounced effects on memory consolidation through the subsequent training days, but at the end of the training period, all doses tested were found to significantly increase spatial memory retrieval (see **Chapter 3 for full**

description and discussion). In the present study, 3 mg/kg and 10 mg/kg Org 26576 exerted a tendency to induce a short-lasting reduction in escape latency, although this observation was in the presence of SPS. Since this response was no different to that of SPS-saline treated animals, further interpretation of these data would amount to speculation. However, it does hint that the ampakine exerts more pronounced effects during initial learning on day 1, corresponding to that described in our earlier study (**Chapter 3**). The fact that the SPS-saline group also decreased escape latency to a similar degree as did the Org 26576 treated groups, implies that Org 26576 is not responsible for the initial short-term improvement of memory on day 1 (**figure 18a**). Indeed, stress via the acute secretion of corticosteroids, can induce bi-directional effects on memory (Kuhlmann and Wolf, 2006; De Quervain et al., 1998), namely an improvement or impairment in memory. Improvement usually occurs immediately or shortly after the aversive event to aid memory of the event and so to enhance survival and coping mechanisms, while impairment of memory ensues with chronically raised glucocorticoids, probably involving glutamate-mediated damage to the hippocampus (Sapolsky, 2000). MWM testing provokes a stress response in rodents (Engelmann et al., 2006), so that an immediate improvement in navigational skills on day 1 may be bolstered by stress-induced release of corticosterone. Moreover, SPS also induces a marked elevation in corticosterone (Harvey et al., 2006) which, together with a sustained elevation in corticosterone induced by forced swimming in the MWM on days 2-5, may explain the early benefits in navigational skills noted on day 1 of training, and the later loss of these benefits from days 2-5 of training. Indeed, this loss of spatial performance is already noticeable as early as day 2, but which gets progressively weaker over the ensuing training days.

However, the gradual diminution of learning over training days 1-5 may also be attributable to changes in the postsynaptic NMDA:AMPA ratio (Rao & Finkbeiner, 2007). SPS induced a noteworthy trend to reduce cortical but not hippocampal NMDA receptor density, which was further significantly strengthened by 1 mg/kg Org 26576 compared to unstressed animals. Activation of AMPA receptors are reported

Interactions between the hippocampus and frontal cortex play a critical role in learning and memory. Short-term memory processes are dependent on the hippocampus and these unstable short-term memories are consolidated into more stable long-term memories in the cortex (Squire et al., 2004; Wiltgen et al., 2004). The non-significant effects on short-term memory on day 1 (**figure 18a**) is supported by the non-significant effects in hippocampal NMDA receptor binding (**figure 22a-b**), especially since short-term memory processes are dependent upon the hippocampus (Izquierdo et al., 2002). The ability of Org 26576 1 mg/kg to bolster long-term spatial memory retrieval (**figure 19a**) was associated with decreased frontal cortical NMDA receptor density (**figure 21a**), which may imply the presence of elevated neurotransmitter levels, in this case glutamate. Org 26576 1 mg/kg may therefore facilitate long-term memory processes by altering NMDA:AMPA receptor balance in the frontal cortex. It should however be borne in mind that the cognitive enhancing effects of ampakines are very likely not restricted to effects on one transmitter or signalling system in these brain regions. Indeed, ampakines have been found to effect cholinergic (Rosi et al., 2004), and noradrenergic release (Lockhart et al., 2000), as well as neurotrophin expression (Lockhart et al., 2007), all of which may contribute to the behavioural responses depicted in the current study.

5. Conclusions

As demonstrated in **Chapter 3**, the data described in this chapter imply that Org 26576 may facilitate short-term and long-term memory processes in stressed animals by altering NMDA:AMPA receptor balance in the frontal cortex, although the latter was not definitively demonstrated. Although further studies in PTSD animal models are needed, this work provides some evidence that Org 26576 may be an effective agent to enhance long-term memory processes and may prove to be effective in the treatment of the cognitive disturbances seen in PTSD.

References

- Adams, B., Moghaddam, B., 1998. Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* 18, 5545-5554.
- Alt, A., Nisenbaum, E. S., Bleakman, D., Witkin, J. M., 2006. A role for AMPA receptors in mood disorders. *Biochem Pharmacol* 71, 1273-1288.
- Black, M. D., 2005. Therapeutic potential of positive AMPA modulators and their relationship to AMPA receptor subunits. A review of preclinical data. *Psychopharmacology (Berl)* 179, 154-163.
- Blanpied, T. A., Boeckman, F. A., Aizenman, E., Johnson, J. W., 1997. Trapping channel block of NMDA-activated responses by amantadine and memantine. *J Neurophysiol* 77, 309-323.
- Bormann, J., 1989. Memantine is a potent blocker of N-methyl-D-aspartate (NMDA) receptor channels. *Eur J Pharmacol* 166, 591-592.
- Brand, L., Groenewald, I., Stein, D. J., Wegener, G., Harvey, B. H., 2008. Stress and re-stress increases conditioned taste aversion learning in rats: possible frontal cortical and hippocampal muscarinic receptor involvement. *Eur J Pharmacol* 586, 205-211.
- Bremner, J. D., 1999. Does stress damage the brain. *Biol Psychiat* 45, 797-805.
- Bruel-Jungerman, E., Davis, S., Laroche, S., 2007. Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* 13, 492-505.
- Burnashev, N., Zhou Z, Neher E, Sakmann B., 1995. Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. *J Physiol* 485, 403-418.
- De Quervain, D. J., Roozendaal, B., McGaugh, J. L., 1998. Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature* 394, 787-790.
- Derkach, V. A., Oh, M. C., Guire, E. S., Soderling, T. R., 2007. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat Rev Neurosci* 8, 101-113.
-

- Elzinga, B. M., Bremner, J. D., 2002. Are the neural substrates of memory the final common pathway in posttraumatic stress disorder (PTSD)? *J Affective Dis* 70, 1-17.
- Engelmann, M., Ebner, K., Landgraf, R., & Wotjak, C. T. (2006). Effects of morris water maze testing on the neuroendocrine stress response and intrahypothalamic release of vasopressin and oxytocin in the rat. *Horm Behav* 50, 496-501.
- Freed, W. J., Dillon-Carter, O., Kleinman, J. E., 1993. Properties of [3H]AMPA binding in postmortem human brain from psychotic subjects and controls: increases in caudate nucleus associated with suicide. *Exp Neurol* 121, 48-56.
- Granger, R., Staubli U, Davis M, Perez Y, Nilsson L, Rogers GA, Lynch G., 1993. A drug that facilitates glutamatergic transmission reduces exploratory activity and improves performance in a learning-dependant task. *Synapse* 15, 326-329.
- Granger, R., Deadwyler, S. A., Davis, M., Moskovitz, B., Kessler, M., Rogers, G., Lynch, G., 1996. Facilitation of glutamate receptors reverses an age-associated memory impairment in rats. *Synapse* 22, 332-337.
- Hampson, R. E., Rogers, G., Lynch, G., 1998. Facilitative effects of the ampakine CX516 on short-term memory in rats: Enhancement of delayed-nonmatch-to-sample performance. *J Neurosci* 18, 2740-2747.
- Harvey, B. H., Naciti, C., Brand, L., Stein, D. J., 2003. Endocrine, cognitive and hippocampal/cortical 5HT_{1A/2A} receptor changes evoked by a time-dependent sensitisation (TDS) stress model in rats. *Brain Res* 983, 97-107.
- Harvey, B. H., Naciti, C., Brand, L., Stein, D. J., 2004a. Serotonin and stress: Protective or malevolent actions in the biobehavioral response to repeated trauma? *Ann NY Acad Sci* 1032, 267-272.
- Harvey, B. H., Oosthuizen, F., Brand, L., Wegener, G., Stein, D. J., 2004b. Stress-restress evokes sustained iNOS activity and altered GABA level and NMDA receptors in rat hippocampus. *Psychopharmacology* 175, 494-502.
- Harvey, B. H., Bothma, T., Nel, A., Wegener, G., Stein, D. J., 2005. Involvement of the NMDA receptor, NO-cyclic GMP and nuclear factor K- β in an animal model of repeated trauma. *Hum Psychopharmacol* 20, 367-373.
-

- Harvey, B. H., Brand, L., Jeeva, Z., Stein, D. J., 2006. Cortical/hippocampal monoamines, HPA axis changes and aversive behavior following stress and re-stress in an animal model of post-traumatic stress disorder. *Physiol Behav* 87, 881-890.
- Hofmann, S. G., Meuret, A. E., Smits, J. A., Simon, N. M., Pollack, M. H., Eisenmenger, K., 2006. Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. *Arch Gen Psychiat* 63, 298 –304.
- Hood, W. F., Compton, R. P., Monahan, J. B., 1989. D-cycloserine: a ligand for the N-methyl-D-aspartate coupled glycine receptor has partial agonist characteristics. *Neurosci Lett* 98, 91-95.
- Hu, H., Real, E., Takamiya, K., Kang, M. G., LeDoux, J., Huganir, R. L., Malinow, R., 2007. Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. *Cell* 131, 160-173.
- Iino, M., Ciani S, Tsuzuki K, Ozawa S, Kidokoro Y., 1997. Permeation properties of Na⁺ and Ca²⁺ ions through the mouse epsilon2/zeta1 NMDA receptor channel expressed in *Xenopus* oocytes. *J Membr Biol* 155, 143-56.
- Imanaka, A., Morinobu, S., Toki, S., Yamawaki, S., 2006. Importance of early environment in the development of post traumatic stress disorder-like behaviors. *Behav Brain Res* 173, 129-137.
- Ingvar, M., Ambros-Ingerson, J., Davis, M., Granger, R., Kessler, M., Rogers, G. A., Schehr, R. S., Lynch, G., 1997. Enhancement by an ampakine of memory encoding in humans. *Exp Neurol* 146, 553–559.
- Izquierdo, L. A., Barros, D. M., Vianna, M. R., Coitinho, A., deDavid e Silva, T., Choi, H., Moletta, B., Medina, J. H., Izquierdo, I., 2002. Molecular pharmacological dissection of short- and long-term memory. *Cell Mol Neurobiol* 22, 269–287
- Johnsen, B. H., Eid, Jarlc; Laberg, Jon Christian; Thayer, Julian' F., 2002. The effect of sensitization and coping style on post-traumatic stress symptoms and quality of life: two longitudinal studies. *Scand J Psychology* 43, 181-88.
- Jordan, G. R., McCulloch, J., Shahid, M., Hill, D. R., Henry, B., Horsburg, K., 2005. Regionally selective and dose-dependent effects of the ampakines Org 26576 and Org 24448 on local cerebral glucose utilisation in the mouse as assessed by ¹⁴C-2-deoxyglucose autoradiography. *Neuropharmacology* 49, 254-264.
-

- Kalia, L.V., Kalia, S. K., Salter, M. W., 2008. NMDA receptors in clinical neurology: excitatory times ahead. *Lancet Neurol* 7, 742–755.
- Khan, S., Liberzon, I., 2004. Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. *Psychopharmacology* 172, 225-229.
- Kohda, K. Harada, K.; Kato, K.; Hoshino, A.; Motohashi, J.; Yamaji, T.; Morinobu, S.; Matsuoka, N.; Kato, N., 2007. Glucocorticoid receptor activation is involved in producing abnormal phenotypes of single-prolonged stress rats: a putative Post-Traumatic Stress Disorder model. *Neuroscience* 148, 22-33.
- Kuhlmann, S., Wolf, O. T., 2006. Arousal and cortisol interact in modulating memory consolidation in healthy young men. *Behav Neurosci* 120, 217–223.
- Kushner, M. G., Kim, S. W., Donahue, C., Thuras, P., Adson, D., Kotlyar, M., McCabe, J., Peterson, J., Foa, E., 2007. D-cycloserine augmented exposure therapy for obsessive-compulsive disorder. *Biol Psychiat* 62, 835– 838.
- Larson, J., Lieu, T., Petchpradub, V., LeDuc, B., Ngo, H., Rogers, G. A., Lynch, G., 1995. Facilitation of olfactory learning by a modulator of AMPA receptors. *J Neurosci* 15, 8023-8030.
- Lebrun, C., Pillière, E., Lestage, P., 2000. Effects of S18986-1, a novel cognitive enhancer, on memory performance in an object recognition task in rats. *Eur J Pharmacol* 401, 205-212.
- Lee, H-K., Barbarosie, M., Kameyama, K., Bear, M. F., Huganir, R. L., 2000. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405, 955–959.
- Li, X., Witkin, J. M., Need, A. B., Skolnick, P., 2003. Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell Mol Neurobiol* 23, 419-430.
- Liberzon, I., Krstov M, Young EA., 1997. Stress-restress: Effects on ACTH and fast feedback. *Psychoneuroendocrin* 22, 443-453.
- Liberzon, I., López, J. F., Flagel, S. B., Vázquez, D. M., Young, E. A., 1999. Differential regulation of hippocampal glucocorticoid receptor mRNA and fast feedback: relevance to post-traumatic stress disorder. *J Neuroendocrin* 11, 11-17.
-

- Lipton, S. A., Chen, H-SV., 2004. Paradigm shift in neuroprotective drug development: clinically tolerated NMDA receptor inhibition by memantine. *Cell Death Differ* 11, 18-20.
- Liu, L., Wong, T. P., Pozza, M. F., Lingenhoehl, K., Wang, Y., Sheng, M., Auberson, Y. P., Wang, Y. T., 2004. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science* 304, 1021–1024.
- Lockhart, B., Iop, F., Closier, M., Lestage, P., 2000. (S)-2,3-dihydro-[3,4]cyclopentano-1,2,4-benzothiadiazine-1,1-dioxide: (S18986-1) a positive modulator of AMPA receptors enhances (S)-AMPA-mediated [3H] noradrenaline release from rat hippocampal and frontal cortex slices. *Eur J Pharmacol* 401, 145-153.
- Lockhart, B. P., Rodriguez, M., Mourlevat, S., Peron, P., Catesson, S., Villain, N., Galizzi, J. P., Boutin, J. A., Lestage, P., 2007. S18986: a positive modulator of AMPA-receptors enhances (S)-AMPA-mediated BDNF mRNA and protein expression in rat primary cortical neuronal cultures. *Eur J Pharmacol* 561, 23-31.
- Lynch, G., Kessler, M., Rogers, G., Ambros-Ingerson, J., Granger, R., Schehr, R. S., 1996. Psychological effects of a drug that facilitates brain AMPA receptors. *Int Clin Psychopharmacol* 11, 13–19.
- Lynch, G., 2002. Memory enhancement: the search for mechanism based drugs. *Nat Neurosci* 5, 1035-1038.
- Malenka, R. C., Nicoll, R. A., 1999. Long-term potentiation - a decade of progress? *Science* 285, 1870-1874.
- Merrill, M. A., Chen, Y., Strack, S., Hell, J. W., 2005. Activity-driven postsynaptic translocation of CaMKII. *Trends Pharmacol Sci* 26, 645–653.
- Moghaddam, B., Adams, B., Verma, A., 1997. Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 17, 2921-2927.
- Monaghan, D. T., Yao, D., Cotman, C. W., 1984. Distribution of [3H]AMPA binding sites in rat brain as determined by quantitative autoradiography. *Brain Res* 324, 160-164.
-

- Morris, R. G. M., 1981. Spatial localization does not require the presence of local cues. *Learn Motiv* 12, 239-260.
- Nowak, K. M., Bregestovski, P., Ascher, P., Herbet, A., Prochiantz, A., 1984. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307, 462-465.
- Olney, J. W., Farber, N. B., 1995. Glutamate receptor dysfunction in schizophrenia. *Arch Gen Psychiat* 52, 998-1007.
- Olney, J. W., Newcomer, J. W., Farber, N. B., 1999. NMDA receptor hypofunction model of schizophrenia. *J Psychiat Res* 33, 523-533.
- O'Neill, M. J., Bleakman, D., Zimmerman, D. M., Nisenbaum, E. S., 2004. AMPA receptor potentiators for the treatment of CNS disorders. *Curr Drug Targets CNS Neurol Disord* 3, 181-194.
- Rao, V. R., Finkbeiner, S. 2007. NMDA and AMPA receptors: old channels, new tricks. *Trends Neurosci* 30, 284-291.
- Riedel, G., Platt, B., Micheau, J., 2003. Glutamate receptor function in learning and memory. *Beh Brain Res* 140, 1-47.
- Rosi, S., Giovannini, M. G., Lestage, P. J., Muñoz, C., Corte, L.D., Pepeu, G., 2004. Abstract S 18986, a positive modulator of AMPA receptors with cognition-enhancing properties, increases ACh release in the hippocampus of young and aged rat. *Neurosci Lett* 361, 120-123.
- Sapolsky, R. M., 2000. The possibility of neurotoxicity in the hippocampus in major depression: A primer on neuron death. *Biol Psychiat* 48, 755-765.
- Squire, L. R., Stark, C. E., Clark, R. E., 2004. The medial temporal lobe. *Annu Rev Neurosci* 27, 279-306.
- Staubli, U., Perez, Y., Xu, F. B., Rogers, G., Ingvar, M., Stone-Elander, S., et al., 1994a. Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. *Proc Nat Acad Sci USA* 91, 11158-11162.
- Staubli, U., Rogers, G., Lynch, G., 1994b. Facilitation of glutamate receptors enhances memory. *Proc Nat Acad Sci USA* 91, 777-781.
-

- Takahashi, T., Morinobu, S., Iwamoto, Y., Yamawaki, S., 2006. Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats, *Psychopharmacology (Berl)* 189, 165–173.
- Tanaka, H., Grooms, S. Y., Bennett, M. V. L., Zukin, R. S., 2000. The AMPAR subunit GluR2: still front and center-stage. *Brain Res* 886, 190-207.
- Tomita, S., Bredt, D. S., Nicoll R. A., 2005. CaMKII substrates in synaptic plasticity. *Cellscience Rev* 2. <http://www.cellscience.com> (Accessed on [15 May 2008]).
- Weber, D. L., Clark, C. R., McFarlane, A. C., Moores, K. A., Morris, P., Egan, G. F., 2005. Abnormal frontal and parietal activity during working memory updating in post-traumatic stress disorder. *Psychiat Res: Neuroimaging Section*, 140, 27-44.
- Wiltgen, B. J., Brown, R. A., Talton, L. E., Silva, A. J., 2004. New circuits for old memories: the role of the neocortex in consolidation. *Neuron* 44, 101–108.
- Yamamoto, S., Morinobu, S., Fuchikami, M., Kurata, A., Kozuru, T., Yamawaki, S., 2008. Effects of single prolonged stress and D-cycloserine on contextual fear extinction and hippocampal NMDA receptor expression in a rat model of PTSD. *Neuropsychopharmacology* 33, 2108-2116.

CHAPTER 5

CONCLUSION

Post-traumatic stress disorder (PTSD) is a disorder that may follow a life-threatening event and is categorized as an anxiety disorder (APA, 2000). Traumatic events can have long-term physiological and/or psychological sequelae (Ray, 2008) that persist long after the passing of the emotional stressor. PTSD, together with phobias, are the most common of psychiatric illnesses (Becker et al., 2007; Michael, et al., 2007; Yehuda, 2002) and the lifetime prevalence for PTSD is between 1.3 % and 7.8 % (Davidson, et al., 1991; Kessler, et al., 1995). It is important to note that not everybody exposed to a traumatic event will develop the chronic symptoms of PTSD, with 20-30 % of exposed individuals eventually developing the illness (Green, 1994). Novel drug treatment and a better understanding of the neurobiology of PTSD are necessary in order to raise our expectations of treatment and to improve response.

The evidence for neurodegenerative phenomena in especially the hippocampus of patients with PTSD (Elzinga & Bremner, 2002) has emphasised the possible causal role of excitotoxic pathways in PTSD. Indeed, recent pre-clinical (Harvey et al., 2004a; Khan & Liberzon, 2004) and clinical (Cosman et al., 2007) studies have realised the importance of glutamatergic transmission in the neurobiology and treatment of the disorder. However, direct modulation of the NMDA ion channel has been problematic due to the risk of severe adverse events (Lipton & Chen, 2004; Olney et al., 1999; Olney & Farber, 1995), and has prompted investigators to consider other mechanisms with which to target dysfunctional glutamatergic signalling (Blanpied et al., 1997; D'Souza et al., 1995). Recent work has revealed a close mutual interaction between the NMDA and AMPA receptors (see Rao &

Finkbeiner, 2007 for review; Michaelis, 1998), one in particular being that AMPA receptor stimulation may recruit NMDA receptors (Tanaka et al., 2000), thus providing a means of modulating NMDA receptor signalling with less potential for adverse events (Lynch & Gall, 2006; Suppiramaniam et al., 2001). Due to the involvement of NMDA and AMPA receptors in memory and LTP (Malinow & Malenka, 2002), such an approach may hold great importance for novel drug development for disorders of cognition, such as depression, Alzheimer's disease and PTSD.

In the present study we have examined the behavioural effects of chronic *Org 26576* treatment, at various dosages, on spatial memory acquisition in healthy male Sprague-Dawley rats. Thereafter, we investigated this agent under the same treatment conditions in male Sprague-Dawley rats subjected to a PTSD-like stress paradigm, viz. single prolonged stress or SPS. In both instances, we assessed spatial memory performance in the Morris water maze. In addition, we determined whether SPS evoked changes on explicit learning involved long term changes in NMDA receptor binding in the rat frontal cortex and hippocampus, two brain regions that are intimately involved in the processing and laying down of explicit memory, and whether chronic *Org 26576* treatment could reverse these neuroreceptor changes.

Using healthy animals, *Org 26576* 3 mg/kg and 10 mg/kg was found to exert a short-lasting reduction in escape latency, suggesting potentiation of initial learning on day 1 but which loses prominence over the subsequent training days. *Org 26576* treated animals did not show any marked benefit over saline treated controls during the memory consolidation phase of the test (i.e. days 2-5 of training), yet all doses of *Org 26576* significantly improved spatial memory retrieval. However, despite these beneficial effects on explicit memory, *Org 26576* at all three dosages applied was devoid of any significant effects on hippocampal or frontal cortical NMDA receptor binding. We can thus conclude that *Org 26576* significantly improves spatial memory learning in normal rats without any changes in frontal cortical or hippocampal NMDA receptors. These data hold great importance for disorders of cognition such as schizophrenia, Alzheimer's disease, depression and PTSD. Indeed, deficits in cognition represent a core symptom of depression (Castaneda et al., 2008), while

animal studies have noted the antidepressant-like effects of ampakines (Li et al., 2003; Knapp et al., 2002).

Advantages of the above described study over earlier assessments of ampakine effects on mnemonic function is the employment of a long-term memory task, which has allowed us to target specific behavioural components of spatial memory acquisition, namely initial learning, memory consolidation and memory retrieval, and to access the abilities of *Org 26576* to modify these critical stages of memory acquisition and recall. Moreover, the three-tiered dose response analysis has highlighted subtle evidence for a U-shaped response to *Org 26576*, showing that 3 mg/kg doses may have some superiority over lower and higher doses in augmenting short term initial learning. Finally, the use of a sub-acute dosage regimen, and using a long-term memory-dependent task, has allowed a better extrapolation to the human condition where deficits in cognition are evident and subsequently treated over an extended period. This study has for the first time assessed the efficacy of an ampakine to bolster acquisition of a long-term memory task, providing evidence for a unique action involving the various phases of learning, and by implication, the processes of LTP. Although these behavioural effects were not associated with simultaneous effects on frontal cortical and hippocampal NMDA receptor binding, one needs to consider the possible contribution of other signalling pathways involved in memory, such as acetylcholine (Cheong et al., 2001), noradrenaline (Berridge & Waterhouse, 2003) and BDNF (Bekinschtein et al., 2008). Clearly, further studies in this regard are needed. This study therefore provides evidence that *Org 26576* may be an effective agent to enhance both short-term as well as long-term memory processes, attributes that warrant further investigation into its use in major depression, Alzheimer's disease and PTSD. These novel data thus prompted the investigation of *Org 26576* in animals subjected to SPS, an animal model of PTSD. The rationale of this being to access the ability of *Org 26576* to reverse stress-induced cognitive deficits that have previously been observed following SPS (Kohda et al., 2007).

In the stress study SPS was observed to induce a non-significant trend towards improving early learning on day 1 of training, although this response was completely lost over the subsequent training period (days 2-5). This was an unexpected observation, since we had expected SPS to compromise learning in the Morris water maze. However, this subtle bolstering of learning may be ascribed to stress-induced elevation in corticosterone evoked by combined SPS and MWM training. Furthermore, that SPS did not evoke a sustained worsening of spatial memory consolidation as well as memory retrieval made it impossible to access the ability of *Org 26576* treatment to reverse stress-evoked deficits in spatial memory performance, as has been previously described for SPS (Kohda et al., 2007) and for SPS plus re-stress (Harvey et al., 2003). *Org 26576* treatment also did not alter stress-induced changes in spatial learning on day 1 of acquisition training, with dosages of 3 and 10 mg/kg, but not 1 mg/kg, evoking a similar increase in learning as did SPS. Globally, a significant but equal degree of learning had taken place in all the groups. *Org 26576* 1 mg/kg, however, managed to significantly increase the time spent in the target zone in the probe trial compared to the unstressed saline control, but not compared to SPS animals. Since SPS failed to induce a worsening of spatial memory, the significance of these data is speculative. Cortical NMDA receptor density was also found to be reduced following SPS, but not significantly so, suggesting that the subtle bolstering of spatial memory retrieval on day 5 in stressed animals may involve a concomitant decrease in specifically cortical but not hippocampal NMDA receptors. No apparent changes in NMDA receptor affinity were observed. Moreover, with the marked effect of 1 mg/kg *Org 26576* observed in SPS animals on memory retrieval on day 5 (the probe test), frontal cortical NMDA receptor density at this same time point was also significantly decreased, with no effects noted on NMDA receptor affinity (K_d), suggesting that improvements in memory retrieval were associated with reduced NMDA receptor density. Again, hippocampal NMDA receptor density and affinity remained unchanged.

Although assessment of glutamate release and/or regional brain glutamate accumulation are needed to confirm it, these data may suggest that the improvement

in memory retrieval induced by 1 mg/kg *Org 26576* is related to an increase in glutamate release, culminating in the observed reduction in receptor density. However, the improvement in memory in SPS animals, as well as the reduced cortical NMDA receptor density observed following 1 mg/kg *Org 26576* was not significantly different to that induced by SPS plus saline. Moreover, as alluded to earlier, SPS failed to evoke a meaningful effect on spatial memory, making it difficult to reconcile the observed effects of SPS on NMDA receptor binding to behavioural changes in the Morris water maze. In healthy animals *Org 26576* alone also failed to modify NMDA receptor binding in a significant manner, while at the same time bolstered spatial memory retrieval. These data would indicate a dissociation between ampakine effects on NMDA receptor binding and spatial memory, as assessed in SPS challenged animals. Further studies using SPS, or another PTSD model, are needed to reappraise these data.

Interactions between the hippocampus and frontal cortex play a critical role in learning and memory. Short-term memory processes are dependent on the hippocampus and these unstable short-term memories are consolidated into more stable long-term memories in the cortex (Squire et al., 2004; Wiltgen et al., 2004). Activation of AMPA receptors are reported to increase the recruitment of NMDA receptors (Tanaka et al., 2000). Thus, there is the expectancy that *Org 26576* should increase rather than decrease NMDA receptor density, thereby supporting (and explaining) its beneficial effects on pneumatic function observed especially after administration in healthy animals. However, in both healthy and stressed animals, our binding data in the frontal cortex reveal a decrease in NMDA receptor density with the 1 mg/kg dose with limited to no change in the hippocampus. *Org 26576* therefore may more target cortical as opposed to hippocampal NMDA receptors as part of its pro-cognitive actions. Although this response may reflect a decrease in NMDA:AMPA ratio due to post-synaptic insertion of AMPA receptors (Derkach et al., 2007), further detailed studies in this regard are needed to confirm these suggestions.

Primary observations of this study:

- *Org 26576* bolsters early phase learning in the Morris water maze.
- *Org 26576* bolsters spatial memory retrieval in the Morris water maze.
- *Org 26576* associated benefits in spatial memory are not associated with changes in frontal cortical or hippocampal NMDA receptor binding.

Shortcomings of the study:

One of the principle objectives of this study was to assess the ability of an ampakine to reverse stress-induced cognitive deficits. In order to increase the validity and relevance of the data with respect to PTSD, we employed a well-validated animal model of PTSD, viz. the single prolonged stress model, or SPS. This model offers similar relevance with respect to PTSD as does the time-dependent sensitisation (TDS) model that has previously been used in our group (Harvey et al., 2003, 2004a; 2004b; 2005; 2006; Brand et al., 2008), but is less tedious and less time consuming. Since the SPS model had demonstrated noteworthy face, construct and predictive validity for PTSD, it was selected as the model of choice for this project. However, as has been outlined above, the model failed to evoke the desired behavioural response, and eventually nullified much of the drug treatment data. There were, however, important reasons for this, which are outlined below:

- A severe national power grid crisis lasting a number of weeks occurred during January and February 2008, resulting in many power-related complications throughout the year. Although the initial crisis occurred before the experiments began, the resultant effects on the environmental regulation of the animal facility experienced over the year may have been a contributing factor.
- These variable environmental conditions the animals were subjected to, may have adversely affected the behavioural patterns of the animals and in this way contributed towards the failure of the SPS-model.

Future studies:

- The current study has confirmed the potential of using ampakines in disorders of cognition. However, as was the original intention of this work, assessment of the cognitive benefits of *Org 26576* in a pathological animal model that is characterised by deficits in cognition are now needed to take these novel and exciting data further. Thus, future studies should include dose response studies, as performed in Chapter 3, in validated animal models of schizophrenia, PTSD, depression and Alzheimer's disease. Indeed, earlier studies have used either pathological or drug-induced animal models presenting with memory deficits to investigate the role of ampakines on cognition (i.e. Zajackowski & Danysz, 1997; Vaglenova et al., 2008; Woolley et al., 2008).
- Determine the effects of *Org 26576* on AMPA receptors using autoradiographic quantification, which will enable us to investigate more accurately the interactions between AMPA- and NMDA receptors.
- Determine the effect of *Org 26576* on cell signalling molecules and proteins involved in memory, eg. ERK, CREB, BDNF, NO.
- Investigate the effects of *Org 26576* on fear memory in an animal model of PTSD.
- The SPS-model has proven to be a robust animal model of PTSD. Therefore future studies using the SPS-model should be undertaken, provided that power and environmental constraints at the animal housing facility are given urgent attention and actions are taken to upgrade facilities to the required standard.

Concluding, *Org 26576* has demonstrated an ability to improve short-term –and long-term memory processes and may thus be a putative novel drug candidate for further study and use as a cognitive enhancing drug in neuropsychiatric conditions

associated with cognitive impairment. Although unsubstantiated in the present study, the results from the effects of Org 26576 in the SPS animal model of PTSD has revealed the possibility for investigating Org 26576 further in pre-clinical and possibly clinical studies as a possible drug treatment in PTSD.

References

- American Psychiatric Association., 2000. Diagnostic and statistical manual of mental disorders (4th ed., text revision). Washington, D.C.: American Psychiatric Press.
- Becker, E. S., Rinck, M., Turke, V., Kause, P., Goodwin, R., Neumer, S., Margaf, J., 2007. Epidemiology of specific phobia subtypes: findings from the Dresden mental health study. *Eur Psychiatry* 22, 69-74.
- Bekinschtein, P., Cammarota, M., Katche, C., Slipczuk, L., Rossato, J.I., Goldin, A., Izquierdo, I., Medina, J.H., 2008. BDNF is essential to promote persistence of long-term memory storage. *Proc Natl Acad Sci USA* 105, 2711–2716.
- Berridge, C. W., Waterhouse, D., 2003. The locus coeruleus-noradrenergic system: modulation of behavioural state and state dependent cognitive processes. *Brain Res Rev* 42, 33-84.
- Blanpied, T. A., Boeckman, F. A., Aizenman, E., Johnson, J. W., 1997. Trapping channel block of NMDA-activated responses by amantadine and memantine. *J Neurophysiol* 77, 309-323.
- Brand, L., Groenewald, I., Stein, D. J., Wegener, G., Harvey, B. H., 2008. Stress and re-stress increases conditioned taste aversion learning in rats: possible frontal cortical and hippocampal muscarinic receptor involvement. *Eur J Pharmacol* 586, 205-211.
- Castaneda, A. E., Tuulio-Henriksson, A., Marttunen, M., Suvisaari, J., Lönqvist, J., 2008. A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *J Aff Dis* 106, 1-27.

- Cheong, M. Y., Yun, S. H., Mook-Jung, I., Joo, I., Huh, K., & Jung, M. W., 2001. Cholinergic modulation of synaptic physiology in deep layer entorhinal cortex of the rat. *J Neurosci Res* 66, 117-121.
- Cosman, K. M., Boyle, L. L., Porsteinsson, A. P., 2007. Memantine in the treatment of mild-to-moderate Alzheimer's disease. *Exp Opin Pharmacol* 8, 203-214.
- Davidson, J. R. T., Hughes, D., Blazer, D. G., George, L. K., 1991. Post-traumatic stress disorder in the community: an epidemiological study. *Psychol Med* 21, 713-721.
- Derkach, V. A., Oh, M. C., Guire, E. S., Soderling, T. R., 2007. Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat Rev Neurosci* 8, 101-113.
- D'Souza, D. C., Charney, D. S., Krystal, J. H. 1995. Glycine site agonists of the NMDA receptor: a review. *CNS Drug Rev* 1, 227-260.
- Elzinga, B. M., Bremner, J. D., 2002. Are the neural substrates of memory the final common pathway in posttraumatic stress disorder (PTSD)? *J Aff Dis* 70, 1-17.
- Green, B. L., 1994. Psychological research in traumatic stress: an update. *J Trauma Stress* 7, 343-361.
- Harvey, B. H., Naciti, C., Brand, L., Stein, D. J., 2003. Endocrine, cognitive and hippocampal/cortical 5HT_{1A/2A} receptor changes evoked by a time-dependent sensitisation (TDS) stress model in rats. *Brain Res* 983, 97-107.
- Harvey, B. H., Naciti, C., Brand, L., Stein, D. J., 2004a. Serotonin and stress: Protective or malevolent actions in the biobehavioral response to repeated trauma? *Ann NY Acad Sci* 1032, 267-272.
- Harvey, B. H., Oosthuizen, F., Brand, L., Wegener, G., Stein, D. J., 2004b. Stress-restress evokes sustained iNOS activity and altered GABA level and NMDA receptors in rat hippocampus. *Psychopharmacology* 175, 494-502.
- Harvey, B. H., Bothma, T., Nel, A., Wegener, G., Stein, D. J., 2005. Involvement of the NMDA receptor, NO-cyclic GMP and nuclear factor K- β in an animal model of repeated trauma. *Hum Psychopharmacol* 20, 367-373.
- Harvey, B. H., 2006. Adaptive plasticity during stress and depression and the role of glutamate-nitric oxide pathways. *S Afr Psychiatry Rev* 9, 132- 139.
-

- Kessler, R., Sonnega, A., Bromet, E., Hughes, M., Nelson, C., 1995. Posttraumatic stress disorder in the National Comorbidity survey. *Arch Gen Psychiat* 52, 1048–1060.
- Khan, S., Liberzon, I., 2004. Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. *Psychopharmacology* 172, 225-229.
- Knapp, R. J., Goldenberg, R., Shuck, C., Cecil, A., Watkins, J., Miller, C., Crites, G., Malatynska, E., 2002. Antidepressant activity of memory-enhancing drugs in the reduction of submissive behavior model. *Eur J Pharmacol* 440, 27-35.
- Kohda, K., Harada, K., Kato, K., Hoshino, A., Motohashi, J., Yamaji, T., Morinobu, S., Matsuoka, N., Kato, N., 2007. Glucocorticoid receptor activation is involved in producing abnormal phenotypes of single-prolonged stress rats: a putative Post-Traumatic Stress Disorder model. *Neuroscience* 148, 22-33.
- Li, X., Witkin, J. M., Need, A. B., Skolnick, P., 2003. Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell Mol Neurobiol* 23, 419-430.
- Lipton, S. A., Chen, H-SV., 2004. Paradigm shift in neuroprotective drug development: clinically tolerated NMDA receptor inhibition by memantine. *Cell Death Differ* 11, 18-20.
- Liu, S. J., Zukin, R. S., 2007. Ca²⁺-permeable AMPA receptors in synaptic plasticity and neuronal death. *Trends Neurosci* 30, 126–134.
- Lynch, G., Gall, C. M., 2006. Ampakines and the threefold path to cognitive enhancement. *Trends Neurosci* 29, 554-562.
- Malinow, R., Malenka, R. C., 2002. AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci* 25, 103–126.
- Michael, T., Zetsche, U., Margraf, J., 2007. Epidemiology of anxiety disorders. *Psychiatry* 6, 136-142.
- Michaelis, E. K., 1998. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. *Prog Neurobiol* 54, 369-415.
- Olney, J. W., Farber, N. B., 1995. Glutamate receptor dysfunction in schizophrenia. *Arch Gen Psychiat* 52, 998-1007.
- Olney, J. W., Newcomer, J. W., Farber, N. B., 1999. NMDA receptor hypofunction model of schizophrenia. *J Psychiat Res* 33, 523-533.
-

- Rao, V. R., Finkbeiner, S. 2007. NMDA and AMPA receptors: old channels, new tricks. *Trends Neurosci* 30, 284-291.
- Ray, S. L., 2008. Evolution of posttraumatic stress disorder and future directions. *Arch Psychiat Nurs* 22, 217-225.
- Squire, L. R., Clark, R. E., Bayley, P. J., 2004. Medial temporal lobe function and memory. In Gazzaniga, M. S., Gazzaniga, M. S., (Eds), *The cognitive neurosciences* (3rd ed.). Cambridge, MA US: MIT Press, pp. 691-708.
- Suppiramaniam, V., Bahr, B. A., Sinnarajah, S., Owens, K., Rogers, G., Yilma, S., et al., 2001. Member of the ampakine class of memory enhancers prolongs the single channel open time of reconstituted AMPA receptors. *Synapse (N.Y.)* 40, 154-158.
- Tanaka, H., Grooms, S. Y., Bennett, M. V. L., Zukin, R. S., 2000. The AMPAR subunit GluR2: still front and center-stage. *Brain Res* 886, 190-207.
- Vaglenova, J., Pandiella, N., Wijayawardhane, N., Vaithianathan, T., Birru, S., Breese, C., Suppiramaniam, V., Randal, C., 2008. Aniracetam reversed learning and memory deficits following prenatal ethanol exposure by modulating functions of synaptic AMPA receptors. *Neuropsychopharmacol* 33, 1071-1083.
- Wiltgen, B. J., Brown, R. A., Talton, L. E., Silva, A. J., 2004. New circuits for old memories: the role of the neocortex in consolidation. *Neuron* 44, 101-108.
- Woolley, M. L., Waters, K. A., Gartlon, J. E., Lacroix, L. P., Jennings, C., Shaughnessy, F., Ong, A., Pemberton, D. J., Harries, M. H., Southam, E., Jones, D. N., Dawson, L. A., 2008. Evaluation of the pro-cognitive effects of the AMPA receptor positive modulator, 5-(1-piperidinylcarbonyl)-2,1,3-benzoxadiazole (CX691), in the rat. *Psychopharmacology (Berl)*. Sep 16. [Epub ahead of print]. DOI 10.1007/s00213-008-1325-2
- Yehuda, R., 2002. Post-traumatic stress disorder. *N Engl J Med* 346, 108-114.
- Zajackowski, W., Danysz, W., 1997. Effects of D-cycloserine and aniracetam on spatial learning in rats with entorhinal cortex lesions. *Pharmacol Biochem Be* 56, 21-29.

ADDENDUMS

Addendum I:

Instructions to Authors for submission to *Neuropharmacology*

Article submitted for publication in *Behavioural Pharmacology*

The ampakine, Org 26576, bolsters early spatial reference memory and retrieval in the 5-day Morris water maze task: A dose-ranging study in rats

ADDENDUM I

INSTRUCTIONS TO AUTHORS

http://www.elsevier.com/wps/find/journaldescription.cws_home/279/authorinstructions

1. Guide for Authors

NEUROPHARMACOLOGY

2. The Neuroscience Peer Review Consortium

Neuropharmacology is a member of the Neuroscience Peer Review Consortium (NPRC). The NPRC has been formed to reduce the time expended and, in particular, the duplication of effort by, and associated burden on reviewers involved in the peer review of original neuroscience research papers. It is an alliance of neuroscience journals that have agreed to accept manuscript reviews from other Consortium journals. By reducing the number of times that a manuscript is reviewed, the Consortium will reduce the load on reviewers and Editors, and speed the publication of research results.

If a manuscript has been rejected by another journal in the Consortium, authors can now submit the manuscript to Neuropharmacology and indicate that the referees' reports from the first journal be made available to the Editors of Neuropharmacology.

N.B. Only manuscripts which were first submitted to another journal after the 1st January 2008 are eligible for the NPRC scheme.

It is the authors' decision as to whether or not to indicate that a set of referee's reports should be forwarded from the first journal to Neuropharmacology. If an author does not wish for this to happen, the manuscript can be submitted to Neuropharmacology without reference to the previous submission. No information will be exchanged between journals except at the request of authors. However, if the original referees' reports suggested that the paper is of high quality, but not suitable for the first journal, then it will often be to an author's advantage to indicate that referees' reports should be made available.

Authors should revise the original submission in accordance with the first journal's set of referee reports, reformat the paper to Neuropharmacology's specification and submit the paper to Neuropharmacology with a covering letter describing the changes that have been made, and informing the Editors that they are happy for referees' reports to be forwarded from the first Consortium journal. Authors will be asked upon submission to Neuropharmacology the title of the first journal submitted to and the manuscript ID that was given by that journal. The editorial office of Neuropharmacology will request the referees' reports from the first journal.

The Editors of Neuropharmacology will use forwarded referees' reports at their discretion. The Editors may use the reports directly to make a decision, or they may request further reviews if they feel such are necessary.

Visit <http://nprc.incf.org> for a list of Consortium journals, as well as further information on the scheme.

US National Institutes of Health (NIH) voluntary posting ("Public Access") policy

Elsevier facilitates author response to the NIH voluntary posting request (referred to as the NIH "Public Access Policy"; see <http://www.nih.gov/about/publicaccess/index.htm>) by posting the peer-reviewed author's manuscript directly to PubMed Central on request from the author, 12 months after formal publication. Upon notification from Elsevier of acceptance, we will ask you to confirm via e-mail (by e-mailing us at NIHauthorrequest@elsevier.com)

that your work has received NIH funding and that you intend to respond to the NIH policy request, along with your NIH award number to facilitate processing. Upon such confirmation, Elsevier will submit to PubMed Central on your behalf a version of your manuscript that will include peer-review comments, for posting 12 months after formal publication. This will ensure that you will have responded fully to the NIH request policy. There will be no need for you to post your manuscript directly with PubMed Central, and any such posting is prohibited.

Exceptions: It is the policy of Elsevier that authors need not obtain permission in the following cases only: (1) to use their original figures or tables in their future works; (2) to make copies of their papers for use in their classroom teaching; and (3) to include their papers as part of their dissertations.

3. Type of manuscripts

3.1 Research Papers.

Full papers can be of any length. However, authors should be as succinct as possible and excessively verbose manuscripts may be returned without review.

3.2 Mini-Reviews.

Short, timely reviews will be by invitation. However suggestions for reviews are most welcome, and should be sent to the Chief Editor.

4. Online submission of papers

It is now possible to submit your manuscript to the Neuropharmacology Editorial office electronically.

Before submitting, it is essential that you refer to the Elsevier Artwork Guidelines: <http://www.elsevier.com/artworkinstructions>

4.1 Once you are ready to submit:

1. Select 'Submit online to this journal' option from the 'Journal Services' on the <http://www.elsevier.com/journals>'.
2. Follow prompts online. Please note that at each stage of the submission process it is possible to go back a step, save the submission to continue later or remove/change any information already entered.
3. The submission tool will generate a PDF file to be used for the reviewing process.
4. You will receive confirmation of your submission, and further progress of your paper at every stage of its review period thereafter, via e-mail.

4.2 Transfer of Copyright

Elsevier will mail the corresponding author a "Transfer of Copyright" agreement once the paper has been received in production. All authors must sign the agreement before the article can be published. This transfer agreement enables Elsevier Ltd to protect the copyrighted material for the authors, but does not relinquish the author's proprietary rights. The copyright transfer covers the exclusive rights to reproduce and distribute the article, including reprints, photographic reproductions, microform or any other reproductions of similar nature and translations, and includes the right to adapt the article for use in conjunction with computer systems and programs, including reproduction or publication in machine-readable form and incorporation in retrieval systems. Authors are responsible for obtaining from the copyright holder permission to reproduce any figures for which copyright exists.

4.3 *Experimental procedures*

All animal experiments should be carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, the European Communities Council Directive of 24 November 1986 (86/609/EEC) or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the authors should clearly indicate in the manuscript that such guidelines have been followed.

Manuscripts should be accompanied by a statement that all efforts were made to minimise animal suffering, to reduce the number of animals used, and to utilise alternatives to in vivo techniques, if available.

Authors are advised to consult A fair press for animals [New Scientist (1992) 1816: 1830] before preparing their manuscript. The Editors reserve the right to reject papers if there is doubt whether suitable procedures have been used.

4.4 *Style*

Manuscripts, including references and figure legends, should be typed double-spaced on one side of A4 paper (206x294 cm) or equivalent, with margins no less than 2.5 cm. Type should be no smaller than 12 point. All typed pages should be numbered consecutively, starting with the title page. Either U.K. or U.S. spelling may be used, but must be consistent throughout.

With the understanding that these suggestions will not necessarily be followed, authors should suggest at least four competent reviewers in their field, being sure to include current e-mail addresses. These suggestions should be stated on a separate file titled Possible Reviewers.

4.5 *Abbreviations.*

Abbreviations should be kept to a minimum. All abbreviations should be written in full when first used and the abbreviation given in parentheses.

4.6 *Title page.*

This should contain a brief, but informative, title, a running title (not exceeding 40 characters), the names and addresses of the authors and a list of keywords or phrases. The author for correspondence, with telephone, Fax numbers and E-mail address, should be clearly indicated on this page. The manuscript should normally be produced using the following headings. One additional level of sub-headings may also be used.

4.7 *Summary.*

The second page should consist of a short summary (not exceeding 250 words) which should be readily accessible to the non-specialist and contain the important points of the paper.

4.8 *Keywords.*

Authors should provide up to six keywords, to appear just underneath the summary section. The keywords will be used for indexing purposes.

4.9 *Introduction.*

The third page should start with a succinct account of why the work was performed. Long historical introductions should not be used. The suggested word limit for Introduction is 500 words. A statement accessible to a lay audience indicating the

potential benefit of the work to man or animals should be included. If any of the work contained in the manuscript has been published previously in the form of an abstract this must be referenced.

4.10 Methods.

Sufficient detail is required to enable others to repeat the experiments. Where animals are involved full descriptions of all analgesic, anaesthetic and surgical procedures must be stated. Where abbreviations are used in place of long chemical names the full chemical name should be provided in this section. Details of any statistical analyses performed should be given. SI units should be used.

4.11 Results.

The results should be fully illustrated. Negative findings should also be noted to avoid unnecessary replication by others.

4.12 Discussion.

This should be as concise as possible; the suggested word limit is 1,500 words. Its main function should be to discuss the results in context with the current state of the field.

4.13 Acknowledgements.

These should be as brief as courtesy allows.

4.14 References.

These should not normally exceed 40. The reference list must be arranged alphabetically according to the surname of the first author, and chronologically if several papers by the same author(s) are referenced. Neuropharmacology's reference style is included in EndNote version 7 and above. If you have an earlier version of EndNote, you can download the style from the EndNote website at <http://www.endnote.com/support/enstyles.asp>

Davies, J., Francis, A. A., Jones, A. W., Watkins, J. C., 1981. 2-Amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. *Neuroscience Letters* 21, 77-81.

Ascher, P., Johnson, J. W. 1989., The NMDA receptor, its channel, and its modulation by glycine. In: Watkins, J. C., Collingridge, G. L., (Eds), *The NMDA Receptor*. IRL Press at Oxford University Press, Oxford, pp. 109-121.

Papers that have been accepted for publication may be cited as 'In press', and a photocopy of the manuscript must be provided. Papers submitted or in preparation should be referred to as (unpublished observations/personal communications) within the text, if absolutely necessary.

In the text, references should be given as: Smith (1964) or (Smith, 1964). In the case of multiple authorship, et al. should be used throughout, i.e. Smith et al. (1964). If works published by the same author(s) in the same year are cited, they should be distinguished by the letters a, b, c, etc.

4.15 Illustrations.

For hard-copy submission manuscripts, two sets of figures in the form of high quality photographs, line drawings or laser prints should be sent to the Editorial Office together with the figure legends typed double-spaced on separate sheets. The figures should be lettered and clearly identified on the reverse with authors, figure

numbers and orientation. Additional sets of figures should be included in the copies of the manuscript. These may be high quality photocopies and, where possible, the legend should be included with each figure (single-spaced if necessary) to aid the refereeing process.

All colour figures in accepted manuscripts will be charged at the rate of USD 300 for the first page of color per manuscript, followed by USD 200 for each subsequent page. Interactive Reports will be published on the web and in the print journal free of charge.

4.16 Tables.

These should be kept to a minimum and should be self explanatory without reference to the text. Data presented in tables should not reproduce that presented in figures or the text.

A suitable location for the placement of tables and figures should be indicated in the text.

4.17 Multimedia files.

Neuropharmacology is now able to accept electronic supplementary material to support and enhance your scientific research. Supplementary files offer additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets and sound clips. Any files supplied will be published online alongside the electronic version of your article in Elsevier web products, including ScienceDirect. In order to ensure that your submitted material is directly usable, please ensure that data files are provided in one of our recommended file formats. Full details can be accessed on www.elsevier.com/authors

When supplementary files are supplied, an additional 'supplementary' figure list should also be submitted. Any supplementary material that is not directly referred to from within the text of your manuscript should be referred to via use of a footnote to the article title. In addition, it is also recommended that a short description is provided for each supplementary file supplied. When published online, the descriptive texts will appear as captions alongside links to the relevant supplementary files, an example layout of online supplementary material can be viewed via the following link: <http://www.elsevier.com/artworkinstructions>

Please note that any supplementary material supplied is subject to the normal peer review process.

4.18 Proofs

For all accepted manuscripts, page proofs will be sent to the corresponding author (or the first-named author) for checking. Corrections to the proofs must be restricted to printer's errors. Any substantial alterations other than these may be charged to the author. Authors are particularly requested to return their corrected proofs as quickly as possible in order to facilitate rapid publication. Please note that authors are urged to check their proofs carefully before return, since late corrections cannot be guaranteed for inclusion in the printed journal. Reprints and copies of the issue (at a specially reduced rate) can be ordered on the form which will accompany the proofs. These should be returned to: Elsevier Ltd, Bampfylde Street, Exeter, EX1 2AH, U.K.

4.19 Disclaimer:

Whilst every effort is made by the publishers and editorial board to see that no inaccurate or misleading data, opinion or statement appears in this journal, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the sole responsibility of the contributor or advertiser

concerned. Accordingly, the publishers, the editorial board and editors and their respective employees, officers and agents accept no responsibility or liability.

ARTICLE

BEHAVIOURAL PHARMACOLOGY

Article submitted for publication in *Behavioural Pharmacology*

Introduction

This chapter presents the full-length manuscript submitted to *Behavioural Pharmacology*, published in association with the European Behavioural Pharmacology Society. The manuscript is presented in the required format prescribed by *Guidance for Authors*, as per the journal website:

<http://www.editorialmanager.com/bpharm/>

The complete "Guidance for Authors" can be viewed and/or down-loaded from the above web link.

The ampakine, Org 26576, bolsters early spatial reference memory and retrieval in the 5-day Morris water maze task: A dose-ranging study in rats

Eugene Hamlyn, Linda Brand, Mohammed Shahid[#] and Brian H. Harvey^{*}

Unit for Drug Research and Development, Division of Pharmacology, School of Pharmacy, North-West University, Private Bag X6001, Potchefstroom, South Africa, 2520, and [#]Schering-Plough, Newhouse, Lanarkshire, UK.

^{*}Corresponding author: Tel: +27 17 299 2238; Fax: +27 18 299 2225; e-mail: brian.harvey@nwu.ac.za

Funding and grants: The authors would like to acknowledge the South African Medical Research Council (BHH) and the National Research Foundation (BHH, EH; grant number 2073038) for financial support. There is no financial relationship, or conflict of interest, with these funding agencies.

Abstract:

Ampakines have demonstrated beneficial effects on cognition in select animal models of learning. However, their ability to modify long-term spatial memory tasks has not been studied. This will lend credence to their possible value in treating disorders of cognition. We evaluated the actions of sub-chronic *Org 26576* administration on spatial reference memory performance in the 5-day Morris water maze task in male Sprague-Dawley rats at doses of 0 (saline control), 1, 3 and 10 mg/kg b.d. over 12 days via intraperitoneal injection. *Org 26576* exerted a dose and time-dependent bolstering of spatial learning, with dosages of 3 and 10 mg/kg *Org 26576* significantly enhancing learning on day 1. Globally, escape latency decreased significantly as the training days progressed in the saline and *Org 26576* treated groups, indicating that significant and equal learning had taken place in all the treatment groups. However, at the end of the learning period, all doses of *Org 26576* significantly improved spatial memory retrieval without confounding effects in the cued version of the task. *Org 26576* offers early phase spatial memory benefits in rats, particularly enhancement of reference memory retrieval. These results support its possible utility in treating disorders characterized by deficits in cognitive performance.

Keywords: *Org 26576, AMPA_kine, AMPA receptor, learning, spatial reference memory, Morris water maze, rat*

Introduction

α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors play a critical role in mediating fast excitatory postsynaptic currents in the brain (Malenka and Nicoll, 1999). Through their recruitment of voltage-gated NMDA receptor ion channels (Tanaka et al., 2000), and subsequent calcium-dependent activation of calmodulin kinase II and the phosphorylation of the GluR1 subunit of the AMPA receptor, this mutual interaction between AMPA and NMDA receptors culminates in an increase in AMPA channel conductance (Merril et al., 2005; Liu et al., 2004; Lee et al., 2000), and plays a pivotal role in memory-related long-term potentiation (LTP, Granger et al., 1993; Staubli et al., 1994a; Riedel et al., 2003). It is not surprising then, that pharmacologic manipulation of NMDA and AMPA receptors has realised increasing interest in novel drug development for treating illnesses characterised by disturbed cognition (O'Neill et al., 2004). Since AMPA receptors play an important role in recruiting NMDA receptors, targeting AMPA receptors with the aim of *bolstering/maintaining* normal functioning of the NMDA ion channel offers an attractive choice that is less problematic than actions directed fully at the NMDA receptor and its associated risks (Lipton and Chen, 2004; Olney et al., 1999; Moghaddam et al., 1997; Adams and Moghaddam, 1998).

Ampakines are a group of positive allosteric modulators of the AMPA receptor that, without having any agonist or antagonist effects of their own, retard deactivation and desensitization of AMPA receptors (Lynch, 2002). In this way, they exert diverse effects on the excitatory postsynaptic potential (EPSP) (Arai et al., 2002), particularly an enhanced synaptic response by prolonging current flow through the AMPA receptor complex. In humans, ampakines have demonstrated an improvement in various memory tests (Ingvar et al., 1997; Lynch et al., 1996). Pre-clinical studies have found that AMPA receptor potentiators, such as LY404187, increase local cerebral activity in select regions of the rodent brain involved in learning and memory, particularly the cortex and hippocampus (Fowler et al., 2004; Jones et al., 2005). Similarly, *Org 26576* produces dose-dependent AMPA receptor mediated increases in local cerebral glucose use in the anteroventral and laterodorsal thalamus, cingulate cortex, dentate gyrus and CA3 subfield of the hippocampus in mice (Jordan

et al, 2005). These data provide an anatomical basis suggestive of its possible use in the treatment of conditions presenting with deficits in cognition. In line with this, ampakines improve retention scores in the radial maze (Staubli et al., 1994b), improve olfactory-related memory (Larson et al., 1995) and facilitate memory in aged rats (Granger et al., 1996). The ampakines CX516 and S-18986-1 have been found to enhance short-term memory (Hampson et al., 1998) and episodic memory (Lebrun et al., 2000), respectively. However, to-date no studies have evaluated the efficacy of ampakines to bolster learning of long-term memory tasks such as spatial reference memory. It is also unknown whether such a response may be dose-related.

In this dose-ranging study, we have investigated the pro-cognitive effects of the ampakine, *Org 26576*, in rats by assessing explicit memory performance in the 5-day Morris water maze task. In order to confirm a dose-relationship, the behavioural studies will be performed over a dosage range of 0 (vehicle control), 1, 3 and 10 mg/kg administered twice daily over a period of 12 days (Jordan et al, 2005).

Methods

Animals

Male Sprague-Dawley rats, weighing 220-250 g and provided by the Biomedical Resource Unit, University of Kwazulu Natal, were grouped five rats per cage and housed in identical cages at the Animal Research Centre, North-West University. The rooms were maintained at constant conditions of temperature ($21\pm5^{\circ}\text{C}$), relative humidity ($50\pm5\%$) with free access to food and water. Full spectrum cold white light (350-400 lux) was provided over a 12 hour light/dark cycle. Positive air pressure was maintained in the facility with air filtration 99.7 % effective for a particle size of 2 micron and 99.9 % for a particle size of 5 micron. All animals were handled according to the code of ethics in research, training and testing of drugs as laid down by the Animal Ethics Committee of the North-West University (Ethics number: NWU-00016-07-A6).

Experimental design and drug treatment

Org 26576, kindly donated by Schering-Plough, Newhouse, Lanarkshire, UK, was freshly prepared daily by dissolving in saline 0.9%, and administered intraperitoneally (i.p.) twice daily at 08:00 and 17:00. Dosages used in the study followed that described by Jordan and colleagues (2005), where the drug was described as evoking a dose-dependent activation of brain regions in mice known to be involved in memory and cognition. For the behavioural analyses, four groups of animals (n=10 rats per group) received either saline (1 ml/kg i.p.) or Org 26576 in incremental dosages of 1, 3 or 10 mg/kg i.p. b.d., in the same injection volume as above, for 12 days. Drug treatment was initiated 7 days prior to Morris water maze training, continuing throughout the 5 day spatial memory learning and assessment period. During this period, the animals received the first injection approximately half an hour before the first trial started (08:00) and then again at 17:00. The dose response study was performed in two separate sessions spaced 2 weeks apart.

Behavioural testing

Morris water maze

In order to assess spatial reference memory performance, a form of explicit memory, a modified version of the Morris water maze was used (Morris, 1981). One day prior to training, animals were habituated for 60 s in the pool to avoid initial stress associated with the Morris water task. The animal was placed in a 1.8 m diameter pool (wall height 0.65 m; water depth 0.275 m) and encouraged to escape the maze by locating an olive-green coloured escape platform submerged 2 cm below the water surface. The platform surface was constructed of mesh to assist climbing. The testing room was well-lit and enriched with visual extra-maze stimuli to assist learning, with training taking place over 5 days. Confirmation that the animal had learned the location of the platform was assessed by removing the platform from the pool and monitoring the search strategy of the animal as it searched to locate the platform where it was previously located. The pool, constructed from a dark olive

green plastic to prevent visibility of the hidden platform, was divided into four quadrants and labelled N-E-S-W. A digital video camera was used to collect the swim activities in the four quadrants, which were subsequently analyzed using AccuTrack digital tracking software (AccuScan Instruments Inc., Columbus, OH 43228, USA). The protocol is discussed in detail below.

After receiving either saline or Org 26576 injections for the designated period, the animals were placed into cages and moved to a room adjacent to the pool and allowed to acclimatize for 30 minutes. Acquisition training took place over a period of 5 days. Each rat underwent 4 training trials each day with a 30 minute inter-trial interval on day 1 and a 15-20 minute inter-trial interval for days 2 to 5. Each animal was placed on the platform for 30 seconds to orientate before the first trial on day 1. The rat was placed, tail first with its head facing the wall, in a quadrant not containing the platform, with video-recording and timing started as soon as the rat entered the water. The animal was allowed to swim for 60 seconds. If the animal failed to locate the submerged platform in this time, it was assisted onto the platform and allowed to orientate for 30 seconds. The rat was removed and dried before being returned to the cage. The procedure was repeated in the other quadrants over subsequent days with each rat doing 20 training trials over the 5 days (memory consolidation/acquisition period). The extent of memory retrieval was assessed in the probe trial, which was initiated 120-150 minutes after the last trial on day 5. In this test, the platform was removed and the animal allowed to swim for 60 seconds. The time that the animal spent in the target quadrant and the target zone (immediate area where the platform was previously located) is an indication of the degree of memory retrieval that had taken place after learning. There was a 30 minute recovery time for the animal between the probe trial and cued trial. The cued trial involved attaching a 30 cm long white ruler to the platform in the original quadrant, with the animal then allowed to swim for a maximum of 60 seconds. The cued trial controls for purely spatial memory deficits and that vision and locomotor impairment are not confounding variables.

Swim parameters were analyzed using the AccuTrack Software system. The parameters used during acquisition training (day 1 to day 5) included escape latency,

target zone entrance latency, time spent in target zone and ratio time in target zone. Percentage time in target zone and time in target quadrant were assessed during the probe trial (day 5). Escape latency time and swim speed were used for the visible platform (cued) trial.

Statistical analysis

GraphPad PRISM version 5 (GraphPad Software, San Diego, USA) was used for all graphical presentations and either PRISM or SAS System (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. The acquisition training data was analyzed using a two way analysis of variance (ANOVA) with repeated measures followed with Tukey multiple comparison tests. Memory consolidation data was analyzed using one way ANOVA with subsequent Dunnett's *post hoc* analysis.

Results

Effect of Org 26576 on spatial memory performance

Memory consolidation (acquisition training)

Spatial learning revealed significant overall trial ($F=10.41$, $P<0.001$) and treatment effects ($F=3.25$, $P=0.024$) on the escape latencies in animals treated with saline or Org 26576 (1, 3 & 10 mg/kg) during the training sessions (**figure 1**). A two way repeated measure ANOVA revealed a significant overall treatment $\times$ time interaction for latency to escape the water maze ($F=2.60$, $P=0.002$). Two way ANOVA with repeated measures of day 1 to day 5, with treatment and trial as variable, revealed a significant effect ($F=253.75$, $P<0.001$, **figure 1**) for escape latency during acquisition training in animals treated with either saline or one of the doses of Org 26576. There was also a significant global effect over the 5 days ($F=109.35$, $P<0.001$), confirming that learning took place over the acquisition training period (**figure 1**). That escape

latency between the different groups from day 2 to day 5 were non-significantly different indicates that all the groups learned the task (**figure 1**, $P>0.05$).

Dunnett's *post-hoc* across group comparisons on each training day and using escape latency as parameter was then used to establish a day by day account of how the different treatments performed. On day 1, it is clear that animals receiving 3 mg/kg ($P<0.001$, Dunnett's, **figure 2a**) and 10 mg/kg ($P<0.05$, Dunnett's, **figure 2a**) dosages of Org 26576 showed significantly improved spatial memory acquisition, as evinced by a significant decrease in escape latency time compared to saline treated animals. The 1 mg/kg dose similarly was numerically lower than saline treated animals, but failed to reach significance. *Post-hoc* analysis of learning on day 2 using the Dunnett's multiple comparison tests revealed a very similar profile as that on day 1, but less emphatic. Thus, acquisition on day 2 showed no significant treatment effect, although as on day 1, the 3 mg/kg dosage demonstrated a strong tendency towards decreasing escape latency (**figure 2b**) vs. saline. Learning on days 3 to 5 showed no noteworthy differences evident between the different treatment groups (**figure 2c-e**).

Memory retrieval (Probe trial)

In order to confirm the presence and extent of learning at the end of the learning period, animals treated with either saline or one of the doses of Org 26576 were subjected to a probe trial performed 120-150min after the last training session on day 5. One way ANOVA revealed a significant effect of treatment across the groups [$F(3,36)=4.412$, $P=0.01$, (**figure 3a**)] using percentage time spent in target zone as end point. Subsequent Dunnett's *post-hoc* analysis revealed that 1, 3 and 10 mg/kg Org 26576 significantly increased time spent in the target zone compared to the saline treated control group ($P<0.05$, Dunnett's, **figure 3a**). Using an additional swim parameter as end point measure in the probe trial, viz. time in target quadrant, one way ANOVA revealed a significant effect of treatment across the groups ($F=3.838$, $P=0.018$, **figure 3b**). Subsequent *post-hoc* analysis revealed that, while 1 mg/kg and 3 mg/kg Org 26576 evoked a numerical increase in time spent in the target quadrant

but failing to separate from saline treated control animals (Dunnett's, **Figure 3b**), 10 mg/kg Org 26576 resulted in a significantly greater time spent in the target quadrant compared to saline treated control animals ($P < 0.05$, Dunnett's, **Figure 3b**).

Cued trial

In order to exclude deficits in visual acuity and locomotor function as possible confounding variables in interpreting how the animals learned to orientate, navigate and escape the water maze, animals treated with either saline or one of the doses of Org 26576 were subjected to a cued (visible platform) trial. This test was performed 30 min after the probe trial on day 5 using swimming speed and escape latency time as primary end point parameters. One way ANOVA failed to reveal any significant effect of treatment ($F = 1.197$, $P = 0.326$, **figure 4a**) in the cued visible platform assessment performed on day 5, using escape latency time as end point. Using swim speed as end point, one way ANOVA similarly failed to reveal any significant effect of treatment ($F = 2.094$, $P = 0.118$, **figure 4b**). Subsequent *post-hoc* analysis of both parameters revealed no effect of the three doses of Org 26576 on swim speed or on escape latency compared to saline treated animals (Dunnett's, $P > 0.05$, **Figures 4a-b**).

Discussion

Autoradiographic quantification of AMPA receptors has confirmed its heterogeneous distribution throughout the brain, especially regions of importance for memory functions (Monaghan et al., 1984). Indeed, ampakines evoke cerebral glucose metabolism changes in the hippocampus and cortex (Fowler et al., 2004; Jones et al., 2005), while Org 26576 has been found to produce dose-dependent AMPA receptor mediated activation of the anteroventral and laterodorsal thalamus, cingulate cortex, dentate gyrus and CA3 subfield of the hippocampus (Jordan et al, 2005). Subsequent pre-clinical (Black, 2005) and clinical (Ingvar et al., 1997; Lynch

et al., 1996) studies have found that ampakines improve performance in a variety of cognitive tasks. In animals ampakines have demonstrated beneficial effects in a range of learning and memory tests (Staubli et al., 1994b; Larson et al., 1995; Granger et al., 1996; Hampson et al., 1998; Lebrun et al., 2000). Recently, Bloss and colleagues (2008) demonstrated an improvement in spatial memory performance in aged rats using S18986, an ampakine that has earlier been found to bolster cognitive functioning in healthy rats using a one-trial object recognition test (Lebrun et al., 2000). Our results with *Org 26576* are thus consistent with these findings. However, the aforementioned studies have used short-term memory tasks such as the Y-maze, while the 5-day Morris water maze task, as applied in the current study, effectively measures the early consolidation/acquisition *and* retrieval of long-term memory. Indeed, the latter test provides an assessment of various levels of learning,

From a therapeutic perspective, the above-mentioned data are potentially interesting for illnesses like schizophrenia, Alzheimer's disease and depression. Indeed, deficits in cognition represent a core symptom of depression (Castaneda et al., 2008), while animal studies have noted the antidepressant-like effects of ampakines (Li et al., 2003; Knapp et al., 2002). Studies in post-mortem brain of depressed patients have found an increase in AMPA receptor binding in the striatum of suicide victims (Freed et al., 1993), as well as a decrease in AMPA-receptor-directed mitogen-activated protein kinase (MAPK) signalling (Alt et al., 2006), thus supporting a role for altered AMPA receptor function in depression. Moreover, emotion enhances learning via noradrenaline mediated phosphorylation of GluR1-containing AMPA-receptors and as such enhances AMPA receptor trafficking (Hu et al., 2007). This latter study provides a molecular mechanism for how emotion enhances learning and memory via an AMPA receptor-mediated event.

However, illnesses like depression and schizophrenia appear to have separate roles for glutamate in their neurobiology. While schizophrenia encompasses more a role for reduced glutamate function (Olney et al., 1999) that could benefit from ampakine treatment, depression is viewed as a disorder of elevated glutamate activity (see Paul and Skolnick, 2003 for review), requiring that pharmacological treatment should attenuate rather than bolster function at glutamatergic synapses (Skolnick, 2008;

Zarate et al., 2003; Berman et al., 2000). Nevertheless, the effects of NMDA receptor block may at least partly be mediated by indirect enhancement of glutamatergic tone at AMPA receptors, due to “toning down” of NMDA receptor function. Indeed it has been shown that pre-treatment with an AMPA receptor antagonist attenuates both NMDA receptor antagonism-induced antidepressant-like behaviour and regulation of GluR1 AMPA receptor phosphorylation (Maeng et al., 2008). These same authors conclude that NMDA antagonists might exert rapid antidepressant-like effects by enhancing AMPA relative to NMDA throughput in critical neuronal circuits. In the end, the ensuing changes in NMDA:AMPA balance will play a pivotal role in memory-related long-term potentiation (LTP, Granger et al., 1993; Staubli et al., 1994a), suggesting that ampakines may represent a promising novel approach to treating cognitive deficits in stress-related illnesses involving disturbances in glutamate.

Learning a spatial navigational task such as the Morris water maze displays three levels of learning, namely initial learning, consolidation or acquisition and retrieval (Brüel-Jungfermann et al., 2007). Interestingly, our behavioural data suggests that 3 mg/kg and 10 mg/kg doses of Org 26576, but not 1mg/kg, exerts an initial reduction in escape latency (Figure 2a, 2b), suggesting a bolstering of *initial learning* on day 1 but which loses prominence over the subsequent training days (Figure 2c-e). This phenomenon may be more representative of a short-term memory benefit, as was observed in earlier short-term memory studies (Lebrun et al., 2000; Bloss et al., 2008). Org 26576 therefore does not significantly alter spatial memory *consolidation* compared to vehicle treated animals (Figure 1). Since AMPA receptor mediated LTP in the hippocampus specifically targets short term memory processes (Shi et al., 1999; Takahashi et al., 2003), this suggests that Org 26576 increases AMPA receptor functioning to initiate early phase LTP, an event that lasts between 1-2 hours. In early phase LTP, AMPA receptors are rapidly inserted into post-synaptic membranes, while in a similar fashion, NMDA and AMPA receptor insertion also occurs during early phase LTP *in vivo* (Williams et al., 2007). From day 3 to day 5, a period in which memory consolidation/acquisition takes place, the effects of Org 26576 declines over the subsequent training days, possibly due to synaptic scaling, a process where synaptic AMPA receptors are removed, and which takes place during late phase LTP resulting in synaptic NMDA receptor currents being potentiated (Watt

et al., 2004). Consequently, all four Org 26576- and saline-treated groups maintain an equal degree of learning over the 5 day training period. Thus, early phase LTP and its resultant effects on spatial memory are significantly enhanced by Org 26576 at selected doses. This response is possibly driven by more pronounced AMPA-mediated signalling, thereby explaining its ability to enhance spatial performance during the initial learning phase.

However, all three dosages of Org 26576 (1, 3 and 10 mg/kg) were significantly superior to saline treatment in the probe trial (Figure 3a-b) without undue effects in the visible platform version of the Morris water maze, thus confirming the drug's superior ability to bolster memory *retrieval*. During late phase LTP, as it occurs in the dentate gyrus *in vivo*, there is an associated increase in the levels of NMDA complex proteins 2 days after LTP induction (Williams et al, 2003) which will lead to increased cognitive processing, which concurs with our observations. These results therefore indicate that Org 26576 initiates and maintains subcellular processes involved in retrieval of long-term memory, possibly via bolstering of late emergent synaptic NMDA receptor currents (Watt et al., 2004).

Concluding, this study has used a 3-tier dose response design to assess the efficacy of an ampakine to bolster acquisition of a long-term memory task, providing evidence for a unique action involving the various phases of spatial learning, and by implication, the processes of LTP. As predicted by earlier 14C-2-deoxyglucose autoradiography studies (Jordan et al, 2005), Org 26576 significantly improves early initial learning in the Morris water maze, as well as significantly improves spatial memory retrieval 5 days later. Together with earlier studies that have demonstrated the favourable benefits of ampakines on short term memory tasks, this study provides evidence that Org 26576 may be an effective agent to enhance both short-term as well as long-term memory processes, attributes that warrant its further investigation in Alzheimer's disease, major depression, schizophrenia and other stress-related illnesses. Further studies are needed to investigate if and how Org 26576 alters NMDA-AMPA signalling in the postsynaptic density over a specific learning period.

Acknowledgements

The authors would like to acknowledge the South African Medical Research Council (BHH) and the National Research Foundation (BHH, EH; grant number 2073038) for financial support. There is no financial relationship, or conflict of interest, with these funding agencies.

References

- Adams B, Moghaddam B (1998). Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* 18: 5545-5554.
- Alt A, Nisenbaum E S, Bleakman D, Witkin J M (2006). A role for AMPA receptors in mood disorders. *Biochem Pharmacol* 71: 1273-1288.
- Arai AC, Xia Y-F, Rogers G, Lynch G, Kessler M (2002). Benzamide-type AMPA receptor modulators form two subfamilies with distinct modes of action. *J Pharmacol Exp Ther* 303: 1075-1085.
- Berman DE, Hazvi S, Neduva V, Dudai Y (2000). The role of identified neurotransmitter systems in the response of insular cortex to unfamiliar taste: Activation of ERK1-2 and formation of a memory trace. *J Neurosci* 20: 7017-7023.
- Black MD (2005). Therapeutic potential of positive AMPA modulators and their relationship to AMPA receptor subunits. A review of preclinical data. *Psychopharmacology (Berl)* 179: 154-163.
- Bloss E B, Hunter RG, Waters E M, Munoz C, Bernard K, McEwen BS (2008). Behavioral and biological effects of chronic S18986, a positive AMPA receptor modulator, during aging. *Exp Neurol* 210: 109-117.
- Bruel-Jungerman E, Davis S, Laroche S (2007). Brain plasticity mechanisms and memory: a party of four. *Neuroscientist* 13: 492-505.
- Castaneda AE, Tuulio-Henriksson A, Marttunen M, Suvisaari J, Lönnqvist J (2008). A review on cognitive impairments in depressive and anxiety disorders with a focus on young adults. *J Aff Dis* 106: 1-27.
- Fowler JH, Whalley K, Murray T, O'Neill MJ, McCulloch J (2004). The AMPA receptor potentiator LY404187 increases cerebral glucose utilization and c-fos expression in the rat. *J Cereb Blood Flow Metab* 24: 1098-1109.
-

- Freed WJ, Dillon-Carter O, Kleinman JE (1993). Properties of [3H]AMPA binding in postmortem human brain from psychotic subjects and controls: increases in caudate nucleus associated with suicide. *Exp Neurol* 121: 48–56.
- Granger R, Staubli U, Davis M, Perez Y, Nilsson L, Rogers GA, Lynch G (1993). A drug that facilitates glutamatergic transmission reduces exploratory activity and improves performance in a learning-dependant task. *Synapse* 15: 326-329.
- Granger R, Deadwyler SA, Davis M, Moskovitz B, Kessler M, Rogers G, Lynch G (1996). Facilitation of glutamate receptors reverses an age-associated memory impairment in rats. *Synapse* 22: 332-337.
- Hampson RE, Rogers G, Lynch G (1998). Facilitative effects of the ampakine CX516 on short-term memory in rats: Enhancement of delayed-nonmatch-to-sample performance. *J Neurosci* 18: 2740-2747
- Hu H, Real E, Takamiya K, Kang MG, LeDoux J, Huganir RL, Malinow R (2007). Emotion enhances learning via norepinephrine regulation of AMPA-receptor trafficking. *Cell* 131: 160-173.
- Ingvar M, Ambros-Ingerson J, Davis M, Granger R, Kessler M, Rogers GA, Schehr RS, Lynch G (1997). Enhancement by an ampakine of memory encoding in humans. *Exp Neurol* 146: 553–559.
- Jones N, O'Neill MJ, Tricklebank M, Libri V, Williams SC (2005). Examining the neural targets of the AMPA receptor potentiator LY404187 in the rat brain using pharmacological magnetic resonance imaging. *Psychopharmacology* 180: 743-751.
- Jordan GR, McCulloch J, Shahid M, Hill DR, Henry B, Horsburgh K (2005). Regionally selective and dose-dependent effects of the ampakines Org 26576 and Org 24448 on local cerebral glucose utilisation in the mouse as assessed by 14C-2-deoxyglucose autoradiography. *Neuropharmacology* 49: 254-64.
-

- Knapp RJ, Goldenberg R, Shuck C, Cecil A, Watkins J, Miller C, Crites G, Malatynska E (2002). Antidepressant activity of memory-enhancing drugs in the reduction of submissive behavior model. *Eur J Pharmacol* 440: 27-35.
- Larson J, Lieu T, Petchpradub V, LeDuc B, Ngo H, Rogers GA, Lynch G (1995). Facilitation of olfactory learning by a modulator of AMPA receptors. *J Neurosci* 15: 8023-8030.
- Lebrun C, Pillière E, Lestage P (2000). Effects of S18986-1, a novel cognitive enhancer, on memory performance in an object recognition task in rats. *Eur J Pharmacol* 401: 205-212.
- Lee H-K, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405: 955-959.
- Li X, Witkin JM, Need AB, Skolnick P (2003). Enhancement of antidepressant potency by a potentiator of AMPA receptors. *Cell Mol Neurobiol* 23: 419-430.
- Lipton SA, Chen H-SV (2004). Paradigm shift in neuroprotective drug development: clinically tolerated NMDA receptor inhibition by memantine. *Cell Death Differ* 11: 18-20.
- Liu L, Wong TP, Pozza MF, Lingenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT (2004). Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science* 304: 1021-1024.
- Lynch G (2002). Memory enhancement: the search for mechanism based drugs. *Nat Neurosci* 5: 1035-1038.
- Lynch G, Kessler M, Rogers G, Ambros-Ingerson J, Granger R, Schehr RS (1996). Psychological effects of a drug that facilitates brain AMPA receptors. *Int Clin Psychopharmacol* 11: 13-19.
- Maeng S, Zarate CA, Du J, Schloesser RJ, McCammon J, Chen G (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: Role of α -
-

amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiat* 63: 349-352.

Malenka R C, Nicoll RA (1999). Long-term potentiation - a decade of progress? *Science* 285: 1870-1874.

Merrill MA, Chen Y, Strack S, Hell JW (2005). Activity-driven postsynaptic translocation of CaMKII. *Trends Pharmacol Sci* 26: 645-653.

Moghaddam B, Adams B, Verma A (1997). Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *J Neurosci* 17: 2921-2927.

Monaghan DT, Yao D, Cotman CW (1984). Distribution of [3H]AMPA binding sites in rat brain as determined by quantitative autoradiography. *Brain Res* 324: 160-164.

Morris RGM (1981). Spatial localization does not require the presence of local cues. *Learn Motiv* 12: 239-260.

Olney JW, Newcomer JW, Farber NB (1999). NMDA receptor hypofunction model of schizophrenia. *J Psychiat Res* 33: 523-533.

O'Neill M J, Bleakman D, Zimmerman DM, Nisenbaum ES (2004). AMPA receptor potentiators for the treatment of CNS disorders. *Curr Drug Targets CNS Neurol Disord* 3: 181-194.

Paul IA, Skolnick P (2003). Glutamate and depression: clinical and preclinical studies. *Ann NY Acad Sci* 1003: 250-272.

Riedel G, Platt B, Micheau J (2003). Glutamate receptor function in learning and memory. *Beh Brain Res* 140: 1-47.

- Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R (1999). Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* 284: 1811-1816.
- Skolnick P (2008). AMPA receptors: A target for novel antidepressants? *Biol Psychiat* 63: 347-348.
- Staubli U, Perez Y, Xu FB, Rogers G, Ingvar M, Stone-Elander S, Lynch G (1994a) Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. *Proc Nat Acad Sci USA* 91:11158-11162.
- Staubli U, Rogers G, Lynch G (1994b). Facilitation of glutamate receptors enhances memory. *Proc Nat Acad Sci USA* 91: 777-781.
- Takahashi T, Svoboda K, Malinow R (2003). Experience strengthening transmission by driving AMPA receptors into synapses. *Science* 299: 1585-1588.
- Tanaka H, Grooms SY, Bennett MVL, Zukin RS (2000). The AMPAR subunit GluR2: still front and center-stage. *Brain Res* 886: 190-207.
- Watt AJ, Sjöström PJ, Häusser M, Nelson SB, Turrigiano GG (2004). A proportional but slower NMDA potentiation follows AMPA potentiation in LTP. *Nat Neurosci* 7: 518-524.
- Williams JM, Guévremont D, Kennard JT, Mason-Parker SE, Tate WP, Abraham WC (2003). Long-term regulation of N-methyl-D-aspartate receptor subunits and associated synaptic proteins following hippocampal synaptic plasticity. *Neuroscience* 118: 1003-13.
- Williams JM, Guévremont D, Mason-Parker SE, Luxmanan C, Tate WP, Abraham WC (2007). Differential trafficking of AMPA and NMDA receptors during long-term potentiation in awake adult animals. *J Neurosci* 27: 14171-8.
- Zarate CA, Du J, Quiroz J, Gray NA, Denicoff KD, Singh J (2003). Regulation of cellular plasticity cascades in the pathophysiology and treatment of mood disorders: Role of the glutamatergic system. *Ann NY Acad Sci* 1003: 273-291.
-

Legends to Figures

Figure 1: Effect of treatment with saline and Org 26576 (1, 3 and 10 mg/kg i.p. twice daily) in the acquisition phase of spatial learning in the Morris water maze. The escape latency over the five acquisition training days is expressed as mean $\pm$ S.E.M. of 10 animals. Data sets are the average of 4 separate learning sessions performed on each day of training.

Figure 2: Effects of saline and Org 26576 (1, 3 and 10 mg/kg i.p. twice daily) on spatial memory performance on days 1-5 (Figures 3 a-e) of acquisition training. Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Dunnett's test).

Figure 3: (a) Percentage time in target zone and (b) time in target quadrant on day 5 during the probe trial to access memory retrieval in animals receiving saline or Org 26576 at dosages of 1, 3 and 10 mg/kg (i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals (* $P < 0.05$, Dunnett's test).

Figure 4: (a) Escape latency and (b) swim speed during the cued trial of the Morris water maze in animals receiving saline or Org 26576 (1, 3 and 10 mg/kg i.p. twice daily). Data represent the means $\pm$ S.E.M. of 10 animals.

Figures

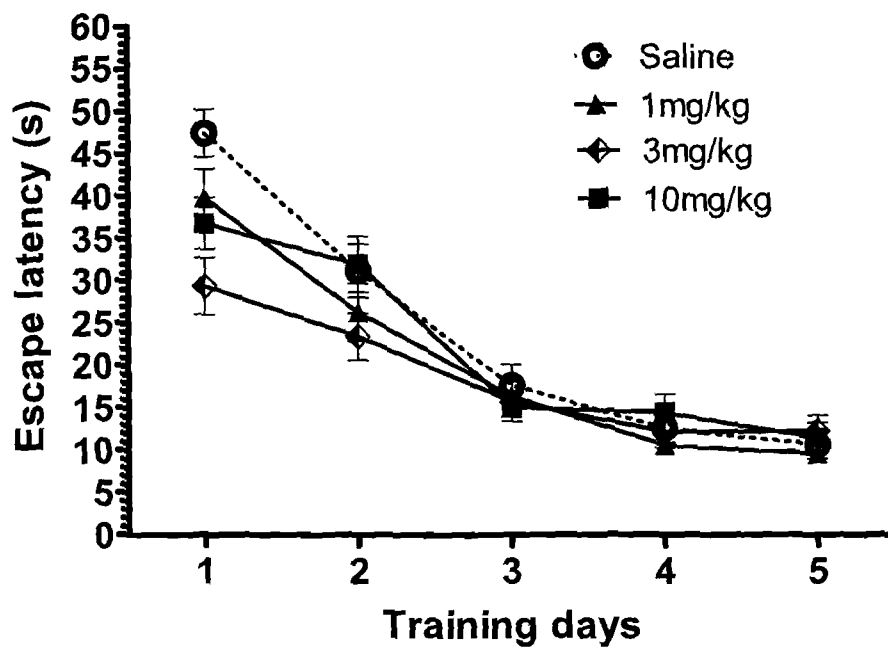


Figure 1

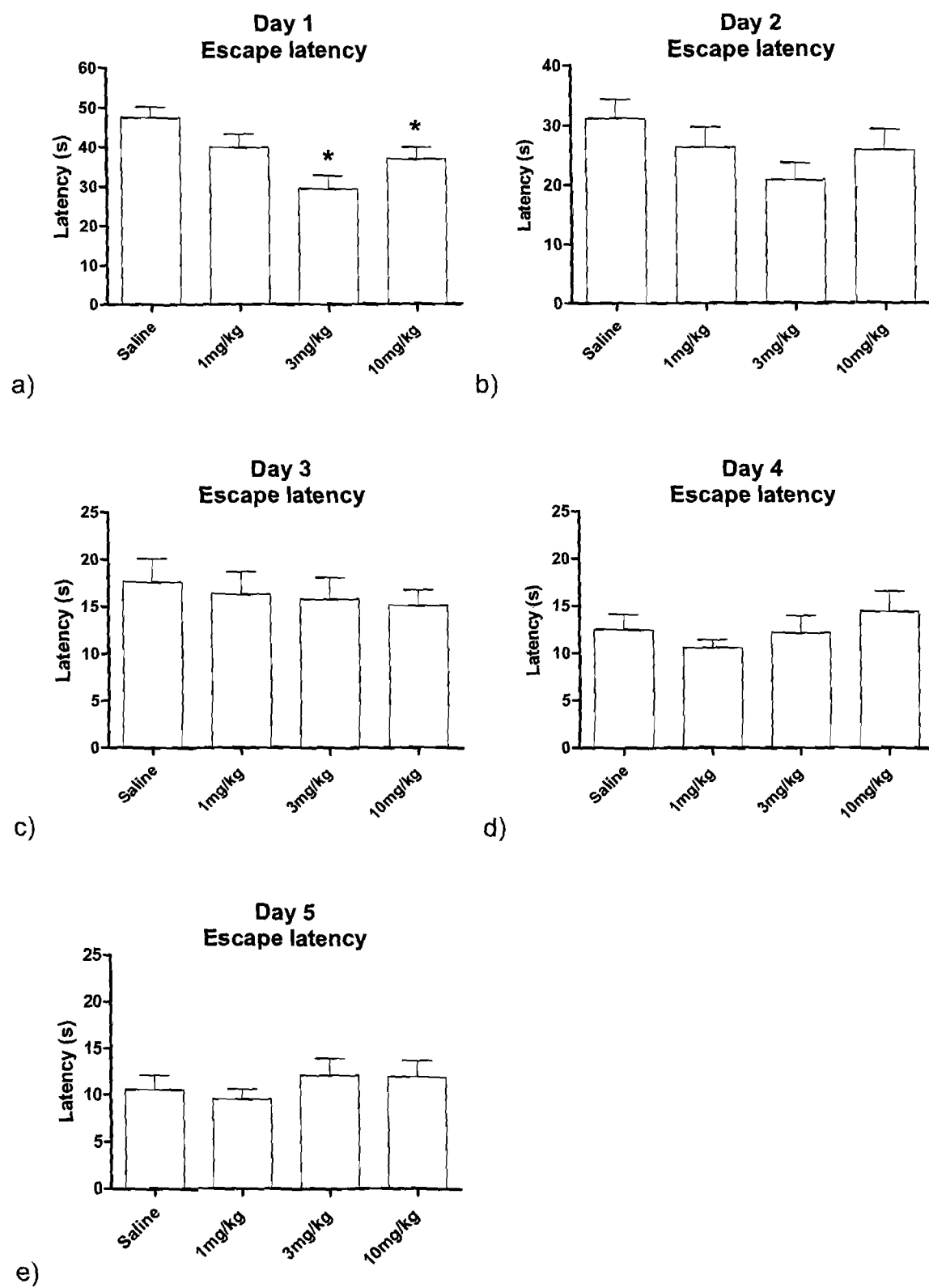


Figure 2 (a-e)

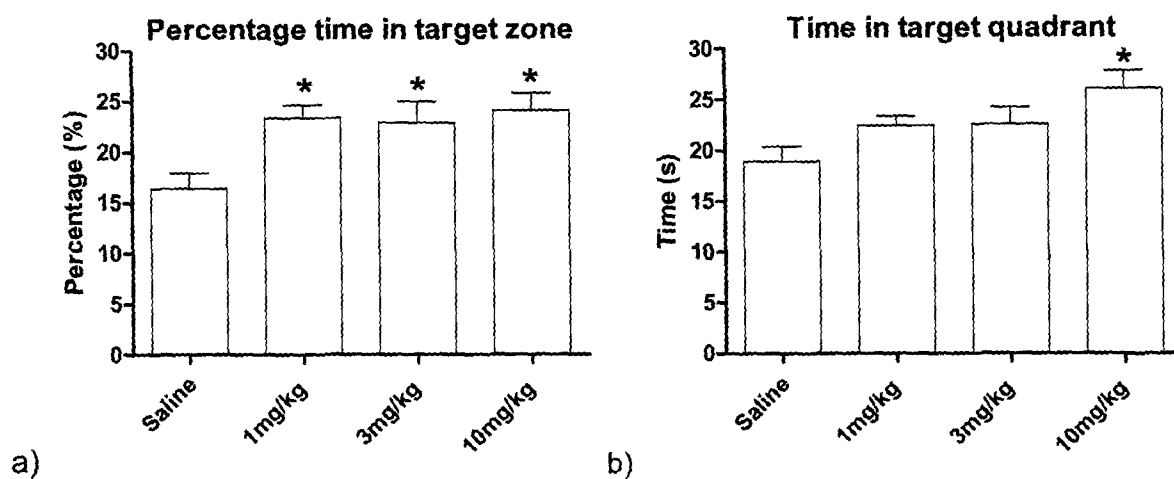


Figure 3 (a-b)

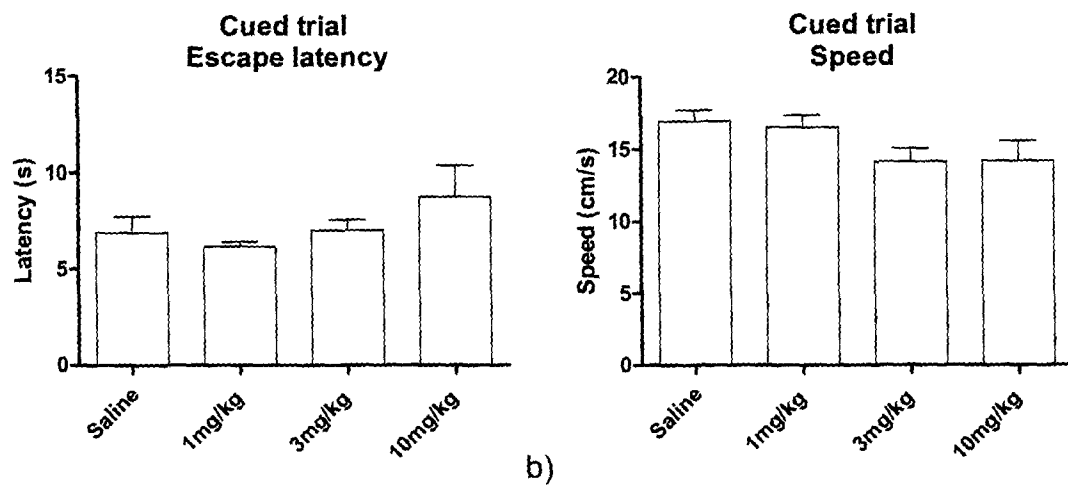


Figure 4 (a-b)