The psychiatric and neural effects of L-type calcium channel antagonism: pharmacoepidemiology and experimental medicine studies

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Abstract

L-type calcium channel (LTCC) antagonists are used to manage cardiovascular conditions. However, several factors suggest they may also have therapeutic potential in psychiatry. First, there is evidence for calcium signalling abnormalities in bipolar disorder (BD). Second, there is some evidence, albeit very inconclusive, that LTCC antagonists may have beneficial effects in BD. Third, calcium channel genes are involved in a number of psychiatric conditions. In particular, genome-wide association studies (GWAS) have consistently identified *CACNA1C* as a gene associated with psychiatric disorders including BD. *CACNA1C* codes for the $Ca_V1.2$ alpha subunit, the primary target of LTCC antagonists, and the genomic data have given new impetus to studying whether and how these drugs affect psychiatric or neural phenotypes. This study used two complementary approaches to investigate this issue.

Using a federated network of electronic health records (EHRs), the first part of this thesis aimed to explore the association between LTCC antagonism and psychiatric disorder. Analyses compared LTCC antagonists with other antihypertensives in matched cohorts of patients. Findings demonstrated LTCC antagonists were associated with lower incidence of first-onset psychiatric disorder compared to beta blockers and diuretics, but higher incidence compared to angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs). Follow-up analyses specifically compared brain-penetrant LTCC antagonists with non-penetrant variants (amlodipine and verapamil/diltiazem) and with ARBs. These findings demonstrated that brain-penetrant LTCC antagonists were associated with overall lower incidence of first-onset neuropsychiatric disorder compared to amlodipine, verapamil/diltiazem, and ARBs. However, benefits varied across individual disorders, and indications of residual confounding between groups undermined the interpretation of some of the findings.

The second part of this thesis aimed to examine the broader effects of LTCC antagonism on human brain and behaviour through an exploratory experimental medicine study. The Oxford Study of Calcium Channel Antagonism, Cognition, Mood Instability and Sleep (OxCaMS) compared the effect of 14 days' nicardipine (a brain-penetrant LTCC antagonist) with placebo across various parameters, including measures of mood, cognition, and neural activity, using a randomised, double-blind design. While there was no evidence of an effect of LTCC antagonism on mood instability, behavioural and neural findings suggested LTCC antagonism may shift emotional processing in line with an antidepressant effect. Cognitive evidence indicated that, compared to placebo, LTCC antagonism reduced negative bias through changes in the perception of sad and angry faces, while neural evidence suggested that LTCC antagonism decreased amygdala activity in response to fear. However, neural findings were based on small voxel clusters, and therefore further research is warranted to assess LTCC antagonist effects in the brain.

In summary, these findings offer insights into the possible associations between LTCC antagonists and neuropsychiatric disorder, as well as the effects of these drugs on mood, cognitive function, and neural activity. Several lines of evidence support the potential of brain-selective LTCC antagonists in psychiatry. However further research is required to fully clarify the therapeutic possibilities of LTCC antagonism in the future.

Declaration

An earlier analysis of the datasets in Chapters 2 and 3, and the protocol for the OxCAMS trial presented in Chapters 4-7 have been published:

Colbourne L, Luciano S & Harrison PJ, Onset and recurrence of psychiatric disorders associated with anti-hypertensive drug classes. Transl Psychiatry 11, 319 (2021). doi: 10.1038/s41398-021-01444-1.

Colbourne L, Harrison PJ. Brain-penetrant calcium channel blockers are associated with a reduced incidence of neuropsychiatric disorders. Mol Psychiatry. 2022 Sep;27(9):3904-3912. doi: 10.1038/s41380-022-01615-6.

Atkinson LZ, Colbourne L, Smith A, Harmer CH, Nobre AC, Rendell J, Jones H, Hinds C, Mould A, Tunbridge EM. (2019). The Oxford study of Calcium channel Antagonism, Cognition, Mood instability and Sleep (OxCaMS): study protocol for a randomised controlled, experimental medicine study. Trials 20(1):120. doi:10.1186/s13063-019-3175-0.

The baseline demographic and mood data from the OxCaMS study were also presented in the DPhil thesis of Dr Lauren Z. Atkinson, alongside other cognitive and neuroimaging results. The data included in this thesis were analysed separately and represent my own independent research.

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In accordance with the guidelines, this thesis contains approximately 49,000 words (exclusive of references, appendices, diagrams, and tables), and it contains less than 150 figures.

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List of abbreviations

ACC	Anterior Cingulate Cortex
ACEI	Angiotensin-Converting Enzyme Inhibitor
AHT	Antihypertensive
AIM	Affect Intensity Measure
ALP	Alkaline Phosphatase
ALS-SF	Affective Lability Scale (short form)
ALT	Alanine Transaminase
ANOVA	Analysis of Variance
ARB	Angiotensin Receptor Blocker
ASL	Arterial Spin Labelling
ASRM	Altman Self Rating Mania Scale
ASRS	Attention Deficit Hyperactivity Disorder (ADHD) Self-Report Scale
AUDIT	Alcohol Use Disorders Identification Test
BB	Beta Blocker
BBB	Blood Brain Barrier
BD	Bipolar Disorder
BDNF	Brain-Derived Neurotrophic Factor
BET	Brain Extraction Tool
BIS	Barratt Impulsiveness Scale
BMI	Body Mass Index
BOLD	Blood Oxygen Level Dependant
BP	Blood Pressure
BP-CCB	Brain-Penetrant Calcium Channel Blocker
BPD	Borderline Personality Disorder
Ca^{2+}	Calcium Ions
CBF	Cerebral Blood Flow
CCB	Calcium Channel Blocker
CI	Confidence Interval
Cmax	Maximum Serum Drug Concentration
CREB	cAMP Response Element-Binding Protein
CRF	Clinical Research Facility
CSF	Cerebrospinal Fluid
DAN	Dorsal Attention Network
DHP	Dihydropyridine
DMN	Default Mode Network
ECAT	Emotional Word Categorisation Task
ECG	Electrocardiogram
ECN	Executive Control Network
EEG	Electroencephalography
EHR	Electronic Health Record

EPI	Echo Planar Imaging
EQ-5D	EuroQol 5-dimension Quality of Life Measure
ETB	Emotional Test Battery
EV	Explanatory Variable
FEAT	fMRI Expert Analysis Tool
FERT	Facial Expression Recognition Task
FIX	FMRIB ICA-based Xnoiseifier
fMRI	Functional Magnetic Resonance Imaging
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FNIRT	FMRIB's Non-linear Image Registration Tool
FSL	FMRIB Statistical Library
FWE	Family-Wise-Error
FWHM	Full Width at Half Maximum
GAD-7	General Anxiety Disorder-7 Scale
GLM	General Linear Model
GWAS	Genome-Wide Association Study
Hb	Haemoglobin
HCO	Healthcare Organisation
ICA	Independent Component Analysis
ICD-10	International Classification of Diseases-10
IPL	Inferior Parietal Lobule
KDEF	Karolinska Directed Emotional Faces
KM	Kaplan–Meier
LTCC	L-type Calcium Channel
LTP	Long-Term Potentiation
MADRS	Montgomery-Asberg Depression Rating Scale
McFLIRT	FMRIB Linear Image Registration Tool
MDQ	Mood Disorder Questionnaire
MEG	Magnetoencephalography
MELODIC	Multivariate Exploratory Linear Decomposition into Independent Components
MEQ	Morningness-Eveningness Questionnaire
MINI	Mini-International Neuropsychiatric Interview
MNI	Montreal Neurological Institute
MPFC	Medial Prefrontal Cortex
MRI	Magnetic Resonance Imaging
MSSD	Mean Successive Squared Difference
NART	National Adult Reading Test
NCO	Negative Control Outcome
NIHR	National Institute for Health Research
OFC	Orbitofrontal Cortex
OHBA	Oxford Centre for Human Brain Activity
OxCaMS	Oxford study of Calcium channel antagonism, Mood instability and Sleep

PANAS-SF	Positive And Negative Affect Scale (short-form)
PCC	Posterior Cingulate Cortex
PD	Parkinson's Disease
PE	Parameter Estimate
PET	Positron Emission Tomography
PSM	Propensity Score Matching
QIDS	Quick Inventory of Depressive Symptomatology
Randomise	FSL Randomise Permutation-Testing Tool
RAS	Renin-Angiotensin System
RCT	Randomised Controlled Trial
RMSSD	Root Mean Successive Squared Difference
ROI	Region of Interest
RR	Risk Ratio
rs-fMRI	Resting-State fMRI
RSN	Resting-State Network
RT	Reaction Time
SCI-R	Sleep Condition Indicator
SCNi	Sleep and Circadian Neuroscience Institute
SD	Standard Deviation
SEM	Standard Error of the Mean
SNP	Single Nucleotide Polymorphism
SR	Sustained-Release
SSRI	Selective Serotonin Reuptake Inhibitor
SVC	Small Volume Correction
T3	Triiodothyronine
T4	Thyroxine
TE	Echo Time
TFCE	Threshold-Free Cluster Enhancement
TMS	Transcranial Magnetic Stimulation
TNR	True Negative Ratio
TPR	True Positive Ratio
TR	Repetition Time
tRMSSD	time-adjusted Root Mean Successive Squared Difference
TS	Timothy Syndrome
TSH	Thyroid-Stimulating Hormone
TVA	Theory of Visual Attention
VGCC	Voltage-Gated Calcium Channel
WCC	White Cell Count
WM	White Matter
WoF	'Wheel of Fortune' Gambling Task
YMRS	Young Mania Rating Scale

Chapter 1. General Introduction

1.1 L-type calcium channels and psychiatric disorder

Extensive literature suggests that L-type calcium channels (LTCCs), responsible for regulating calcium entry into cells, play a role in the pathophysiology of psychiatric disorders (Casamassima et al., 2010). LTCC antagonists are drugs which block these channels, and are widely used for cardiovascular indications (Braunwald, 1982). Interest in the psychiatric potential of LTCC antagonists has been linked to various factors, including the role of calcium ions in neuronal function, abnormal calcium signalling observed in psychiatric phenotypes, and the finding that mood stabilisers correct some of these abnormalities. This introduction will begin by examining the early evidence connecting LTCCs to psychiatry. First, the calcium signalling literature, as it relates to psychiatric disorder, will be investigated in more detail. Following this, pharmacological data will ascertain insights from LTCC antagonist studies, and subsequently an examination of recent genomic developments will elucidate the involvement of LTCCs in the genetic basis of psychiatric disorder. By clarifying initial psychiatric interest in calcium disturbances, and emphasising renewed scientific focus in the field, the present chapter aims to lay the groundwork on which this thesis is based.

1.2 Early studies in calcium signalling

Calcium signalling has long been implicated in psychiatric disease. Indeed, the potential role of calcium ions (Ca^{2+}) in bipolar disorder (BD) has been studied for over one hundred years (Weston & Howard, 1922). Early studies hypothesised the involvement of altered intracellular Ca^{2+} in the pathophysiology of BD and depression (Bowden et al., 1988; Crammer, 1977; Dubovsky & Franks, 1983; Jimerson et al., 1979). Specifically, reports demonstrated elevated calcium in the peripheral cells of individuals with BD (Dubovsky et al., 1994). A contemporary systematic review and meta-analysis confirmed a 29% increase in intracellular Ca^{2+} concentration in platelets and lymphocytes of BD patients compared with controls (Harrison, Hall, et al., 2021). These findings were noted in both mania and bipolar depression, but not euthymia, and comparable results were seen in schizophrenia.

Lithium and other mood-altering drugs have been shown to normalise intracellular calcium (Foskett et al., 2007; Schlecker et al., 2006; Warsh et al., 2004; Wasserman et al., 2004). This indicates their effects may be linked to calcium dynamics, and prolonged lithium treatment has been associated with altered calcium metabolism including hyperparathyroidism (Franks et al., 1982). Increased calcium has also been observed in the cerebrospinal fluid (CSF) of BD patients with depression (Jimerson et al., 1979; Levine et al., 1999), whereas patients with

bipolar mania have demonstrated decreased CSF levels (Carman & Wyatt, 1979). In addition, altered calcium signalling has been reported in the stem cells and neurons of patients with BD, in comparison to healthy unaffected controls (Chen et al., 2014; Hahn et al., 2005). Neurons derived from BD lithium responders have shown a reversal in calcium signalling abnormalities, unlike non-responders (Mertens et al., 2015; Stern et al., 2018). Finally, disrupted intracellular calcium signalling has also been demonstrated in neurodevelopmental (Pourtavakoli & Ghafouri-Fard, 2022) and neurodegenerative (Berridge, 2012) disorders, indicating that dysregulated calcium homeostasis may impact diverse brain processes, across multiple psychiatric diagnoses.

1.3 The introduction of LTCC antagonists

In the 1980s, the development of LTCC antagonists for cardiovascular disorders provided a powerful new tool for investigating calcium signalling. LTCC antagonists, also known as calcium channel blockers (terms used interchangeably in this thesis) were licensed for hypertension and angina and were explored as potential treatments for BD and related phenotypes (Dubovsky et al., 1982; Levy & Janicak, 2000).

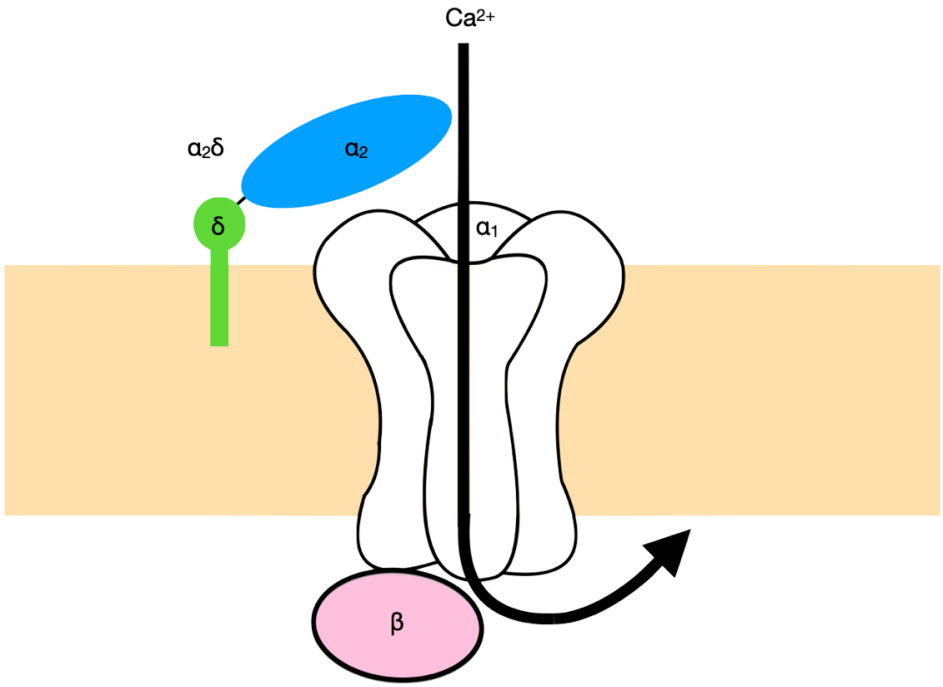
Structure and pharmacology of LTCCs

Voltage-gated calcium channels (VGCCs) are a type of ion channel found in the membranes of excitable cells such as neurons, muscle, and endocrine cells. VGCCs consist of a pore-forming α1 subunit and supporting α2δ and β subunits (see Figure 1.1). The α1 subunit proteins are coded by the *CACNA1x* genes, which include 10 isoforms (see Figure 1.2). Of these, there are four L-type VGCCs (LTCCs), which are known for their long-lasting activation. These comprise $Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$ and $Ca_V1.4$, and are coded by *CACNA1S*, *-C*, *-D* and *-F* respectively (Bhat et al., 2012; Catterall et al., 2005). Only $Ca_V1.2$ and $Ca_V1.3$ are significantly expressed in the brain (Calin-Jageman & Lee, 2008).

CCBs decrease depolarisation-induced Ca^{2+} flux into cells through binding reversibly to the α1 subunit of the LTCC (Casamassima et al., 2010). Calcium influx drives multiple processes, including neurotransmitter release, generation of action potentials and variations in synaptic plasticity, such as long-term potentiation (LTP) (Berridge, 2014). LTCC antagonists have been shown to reduce induction of LTP in rat hippocampus in a dose-dependent manner (Freir & Herron, 2003). LTCCs influence neuronal firing at the postsynaptic membrane (Striessnig et al., 2015) and control Ca^{2+} signalling pathways implicated in excitation-transcription coupling, including the phosphorylation of cAMP response element-binding protein (CREB). CREB can attach to calcium response sites found within brain-derived neurotrophic factor (BDNF),

activating its transcription, and thus influencing various neurobiological processes. These calcium sensitive neural mechanisms, especially CREB and BDNF, have been linked to learning and memory, and other key processes underlying neuropsychiatric disorder (Berridge, 2014; Casamassima et al., 2010; Dolphin, 2016; Moon et al., 2018; Zamponi et al., 2015).

Figure 1.1 Diagram of a voltage-gated calcium channel showing the pore forming α1 subunit and auxiliary β (pink) and α2δ (blue/green) subunits which refine channel function.



In the UK, nine CCBs are currently licenced (Joint Formulary Committee, 2023), belonging to three distinct classes, which include phenylalkylamines (verapamil), benzothiazepines (diltiazem) and dihydropyridines or DHPs (amlodipine, felodipine, isradipine, lercanidipine, nicardipine, nifedipine and nimodipine). In contrast to verapamil which binds directly to the pore, DHPs attach close-by, changing the pore shape and preventing calcium flow (Striessnig et al., 2015). Individual drugs differ in half-life, blood-brain barrier (BBB) permeability and binding affinity (Zamponi et al., 2015), which may affect their psychiatric potential (Harrison, Tunbridge, et al., 2020). It has been suggested that the ability of CCBs to penetrate the brain, influences their occupancy of brain LTCCs, which in turn, is likely to be necessary for CCBs to mediate psychiatric effects. DHPs typically have the highest affinity for LTCCs (Striessnig et al., 2015) and drugs with longer half-lives require fewer daily doses, thus improving acceptability for patients.

Figure 1.2 Calcium channel subtypes and genes

Channel name			L-type			
Channel subtype		$Ca_v1.1$	$Ca_v1.2$	$Ca_v1.3$	$Ca_v1.4$	
Gene		*CACNA1S*	*CACNA1C*	*CACNA1D*	*CACNA1F*	
Channel name	P/Q-type	N-type	R-type		T-type	
Channel subtype	$Ca_v2.1$	$Ca_v2.2$	$Ca_v2.3$	$Ca_v3.1$	$Ca_v3.2$	$Ca_v3.3$
Gene	*CACNA1A*	*CACNA1B*	*CACNA1E*	*CACNA1G*	*CACNA1H*	*CACNA1I*

1.4 Studies of LTCC antagonists in psychiatry

The effects of CCBs on psychiatric phenotypes have been explored in animal models and human subjects. Rodent studies have consistently reported that CCBs, particularly DHPs, are associated with effects resembling antidepressants (Galeotti et al., 2006; Mogilnicka et al., 1987). Nifedipine and other DHPs (including nicardipine, nitrendipine, isradipine, felodipine and nimodipine) have demonstrated antidepressant-like effects in rats and mice on behavioural despair and tail suspension tests (Cohen et al., 1997; Srivastava & Nath, 2000). In a study using rodent behaviour paradigms, simultaneous antidepressant and DHP prescription improved antidepressant efficacy when compared with antidepressant prescription alone (Czyrak et al., 1989). Indeed, antidepressant effects have been demonstrated specifically with DHP compounds. CCBs from other classes including verapamil and diltiazem, as well as amlodipine, which exhibits lower selectivity for DHP binding sites, either showed no activity, or yielded opposite outcomes in the same models of depression (Cohen et al., 1997; Mogilnicka et al., 1988; Srivastava & Nath, 2000). Additionally, pharmacological activation of LTCCs with DHP calcium channel agonists, induced a depressive-like phenotype in mice, as demonstrated by comparable effects on behavioural despair tests (Mogilnicka et al., 1988; Sinnegger-Brauns et al., 2004).

Using rodent models of anxiety, several studies suggest the beneficial impact of CCBs. El Ganouni and colleagues (El Ganouni et al., 1998) found nifedipine and nimodipine produced anxiolytic-like effects in rats, which they hypothesised were mediated by neurotransmitter release. More recently, data in mice (Yoshizawa et al., 2020) have demonstrated similar effects with diltiazem, facilitated by the up-regulation of neurosteroids. Finally, mouse models of Parkinson's disease (PD) have demonstrated that blocking LTCCs with isradipine is neuroprotective for dopaminergic neurones (Chan et al., 2007; Ilijic et al., 2011). This suggests CCBs may theoretically be used as neuroprotective drugs in PD, in addition to potentially wide therapeutic roles in depression, anxiety, emotional learning and cognitive function. However, as later chapters discuss, a randomised controlled trial (RCT) for isradipine in PD did not yield successful results.

The data derived from studies involving human subjects are less convincing. Following the introduction of CCBs over 40 years ago, several case reports and small-scale trials investigated these drugs in patients with BD, depression, and schizophrenia (Deicken, 1990; Garza-Treviño et al., 1992; Giannini et al., 1987; Snedkova et al., 1997). These studies were limited mostly to verapamil, and although early results showed promise (Dubovsky et al., 1982), the data were weak and interest declined (Hollister & Trevino, 1999). In 2016, a systematic review of CCBs in BD concluded no robust findings for or against their use (Cipriani et al., 2016). RCT data were restricted to small trials examining verapamil for mania, and there was no evidence to demonstrate its superiority over placebo (Levy & Janicak, 2000; Yildiz et al., 2015). An absence of high-quality data for other phases of the disorder and alternative CCBs, meant definitive conclusions could not be made for CCBs as a class.

1.5 LTCC genes and the genetic basis of psychiatric disorders

Despite equivocal early findings for CCBs in human subjects, interest in LTCCs as a therapeutic target has grown substantially in recent years (Dubovsky, 2019; Harrison, Tunbridge, et al., 2020). This comes in the context of recent genomic advances, which demonstrate LTCC gene associations with a number of common psychiatric disorders (Als et al., 2023; Ferreira et al., 2008; Mullins et al., 2021; Sklar et al., 2008; Trubetskoy et al., 2022). The earliest genome-wide association studies (GWAS) identified a link between *CACNA1C* and BD (Ferreira et al., 2008; Sklar et al., 2008), suggesting altered calcium signalling may, in part, result from polymorphisms in this gene (Bhat et al., 2012; Casamassima et al., 2010). Subsequent GWAS studies and meta-analyses have confirmed the association between BD and SNPs within the *CACNA1C* locus, with the index SNP being rs1006737 (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2019; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011). Common *CACNA1C* variants have also been implicated in major depression and schizophrenia (Als et al., 2023; Trubetskoy et al., 2022), as well as numerous psychiatric phenotypes including cognition, mood instability and circadian rhythms (Chen et al., 2022; Hindley et al., 2022; Pagani et al., 2016), which suggests transdiagnostic susceptibility across mental disorders.

While GWAS has confirmed genetic associations, the underlying molecular mechanisms remain to be determined. *CACNA1C* is a relatively long gene situated on the short arm of chromosome 12. The common risk SNP rs1006737 is located in a large intron positioned between exons 3 and 4 (Heyes et al., 2015). Thus, the risk allele is non-coding, and is likely to exert its effects through gene regulation and expression, rather than directly disrupting the

structure of $Ca_V1.2$ (as observed with some rare variants, discussed below). There is conflicting evidence in the existing *CACNA1C* literature regarding common risk alleles increasing or decreasing $Ca_V1.2$ channel expression and function. Research has demonstrated both increased (Bigos et al., 2010; Eckart et al., 2016; Yoshimizu et al., 2015) and decreased (Eckart et al., 2016; Gershon et al., 2014; Roussos et al., 2014; Yoshimizu et al., 2015) levels of *CACNA1C* expression, which vary with specific brain regions and cellular systems. This is important because rodent studies demonstrate that changes in *Cacna1c* expression affect psychiatric phenotypes (Moon et al., 2018) and human cell studies indicate that *CACNA1C* risk variants have functional impacts via effects on gene expression (Birey et al., 2017; Yoshimizu et al., 2015). Yoshimizu and colleagues performed quantitative PCR using induced human neurons. They demonstrated increased mRNA expression of *CACNA1C* in neurons homozygous for the rs1006737 risk genotype, compared to non-risk genotypes. This suggests the risk variant is associated with increased LTCC activity, due to increased channel production, and thus demonstrates a functional downstream change associated with the risk genotype. However, a study by Jaffe and colleagues indicates that risk SNPs decrease expression of *CACNA1C* in human hippocampus (Jaffe et al., 2020). Another possible mechanism of *CACNA1C* association with psychiatric disorder could involve alterations in transcript splicing, leading to the production of specific isoforms, rather than an absolute increase or decrease in *CACNA1C* expression (Harrison et al., 2022). This possibility remains unexplored, however the influence of genotype on splicing has been identified as a pivotal factor for other genes associated with psychosis (Gandal et al., 2018; Kleinman et al., 2011; Tao et al., 2014; Xiao et al., 2017; Zhang et al., 2022).

In addition to the common variants found on GWAS, whole genome sequencing has identified rare LTCC variants in patients with neuropsychiatric disorders (Wang et al., 2022). A rare autosomal-dominant disorder known as Timothy Syndrome (TS), presenting with cardiac defects, autism, and cognitive impairment, is caused by a gain-of-function mutation in *CACNA1C* (Splawski et al., 2004) believed to occur partly through changes in splicing (Panagiotakos et al., 2019). Rare and structural variants in *CACNA1C* have also been reported in schizophrenia and autism spectrum disorder (Jiang et al., 2013; Purcell et al., 2014; Song et al., 2018). Although rare variants are not common at a population level, their biological mechanisms often converge with common variants, potentially providing clues to the pathology underlying psychiatric disease.

In summary the molecular mechanisms that underlie how LTCC alleles affect risk are extremely complex. They involve both loss and gain of $Ca_V1.2$ function, and potentially altered splicing, which may collectively contribute to downstream functional changes associated with

psychiatric disorder. While blocking LTCCs would seem logical, if psychiatric disorders were associated with an overproduction of these channels, the genetic evidence is inconclusive and has not definitively shown whether risk alleles result in the generation of either hyperactive or more abundant LTCCs. Nonetheless, genetic findings have provided novel insights into the role of LTCCs in psychiatry. Coupled with the fact LTCCs are druggable, this provides a compelling case for continuing research into the potential psychiatric effects of LTCC antagonists (Harrison et al., 2022; Zamponi et al., 2015).

1.6 A re-evaluation of LTCC antagonists: linking genetics and therapeutics

The genomic developments described above have prompted the re-examination of LTCC antagonists in psychiatry. Exploratory studies (Burdick et al., 2020; Li et al., 2023; Ostacher et al., 2014; Vahdani et al., 2020) are focusing on dihydropyridine CCBs, which have superior BBB penetration and LTCC selectivity compared to CCBs examined in earlier trials (see Section 1.4). Pilot studies have also hypothesised that LTCC genotype could affect CCB response (Li et al., 2023; Ostacher et al., 2014). A pilot investigation of adjunctive isradipine in BD has shown symptomatic improvement in bipolar depression (Ostacher et al., 2014). Over an eight-week study period, BD patients were administered isradipine 2.5 mg, titrated up to 10 mg, once daily. Symptoms of depression were monitored via the Montgomery-Asberg Depression Rating Scale (MADRS), demonstrating improvement in depression severity, and suggesting further exploration of isradipine is warranted. Another pilot study, in bipolar mania, also demonstrated benefits (Li et al., 2023). Patients presenting with acute manic episodes were genotyped for common calcium channel variants and treated with add on nimodipine or nicardipine for 14 days. Add-on treatment was associated with a significant decrease in the Young Mania Rating Scale (YMRS), and SNPs in calcium channel genes were found to be predictors of treatment response. While these studies show promise, potentially linking genetic findings to novel therapeutic targets, well powered RCTs are still required. Research is also needed to investigate the effects of LTCC antagonists on cognitive function and neural activity. Understanding how these drugs may affect cognitive and neural processes is crucial to thoroughly explore their therapeutic potential. These aspects are examined below.

1.7 LTCC antagonists and cognitive function

Impairments in cognitive function are common in *CACNA1C*-associated psychiatric disorders (Millan et al., 2012). Deficits in learning and memory have been observed in BD, depression and schizophrenia, and ongoing research is focusing on the cognitive effects of LTCC antagonists. Animal studies have demonstrated improved cognitive performance following

treatment with CCBs, including enhanced working memory and retention of learning in rats and mice respectively (Levy et al., 1991; Quartermain et al., 2001). LTCCs are also implicated in fear-associated memories (LeDoux, 2000; Singewald et al., 2015), processes which provide valuable insights into mechanisms underlying learning, memory, and emotional processing. In rats, targeted administration of verapamil, directly into the basolateral amygdala, prior to fear conditioning, prevented the formation of long-term fear memories associated with cues (Bauer et al., 2002). Likewise, systemic delivery of nifedipine and nimodipine in mice resulted in impaired cue fear extinction (Cain et al., 2002), but did not affect the acquisition or expression of conditional fear. As well as traditional fear-conditioning, LTCC antagonists have demonstrated ability to disrupt latent inhibition of conditioned fear (McKinney et al., 2008; Tigaret et al., 2021), and fear-potentiated startle (Shinnick-Gallagher et al., 2003), indicating additional effects on alternative types of emotional learning and plasticity. The processes responsible for modifying fear memories remain unclear (Kabir et al., 2017). Cue fear conditioning has been linked to late-phase LTP at thalamic inputs to the amygdala (Pape & Pare, 2010). BDNF, a downstream target of calcium signalling, is one molecule that facilitates LTP in the thalamoamygdala pathway (Meis et al., 2012). BDNF induction in the amygdala is essential for fear memory consolidation (Rattiner et al., 2004) and verapamil has been shown to prevent the induction of BDNF following cue-associated fear conditioning (Ou & Gean, 2007), hinting at a possible mechanism.

There are limited human data on the cognitive effects of CCBs. Small clinical trials indicate benefits on cerebrovascular cognitive impairment (Climent et al., 2013; Hanyu et al., 2007; Tomassoni et al., 2008), and pilot studies have investigated cognitive symptoms in psychosis. A study of adjunctive nifedipine in schizophrenia patients with tardive dyskinesia, found preliminary evidence that LTCC antagonists improve learning and memory. Over a four-week period, patients receiving nifedipine 60 mg daily, in addition to neuroleptic medication, showed improved performance on a rotary pursuit test of procedural learning and a dementia scale assessing general cognitive abilities, compared with patients receiving adjunctive placebo (Schwartz et al., 1997). More recently, a placebo-controlled RCT of isradipine in schizophrenia, demonstrated enhanced verbal memory and attention dysfunction compared with placebo (Vahdani et al., 2020), and an open-label isradipine study demonstrated improved functional capacity in a comparable cohort (Burdick et al., 2020). However, these findings are yet to be confirmed in large scale trials, and future research is required to examine the wider cognitive effects of these drugs in humans.

1.8 LTCC antagonists and neural activity

Little is known about the neural effects of LTCC antagonists, a topic explored in only a few neuromodulation and neuroimaging studies. Research using transcranial magnetic stimulation (TMS) in healthy volunteers, has shown nimodipine interferes with metaplasticity (Wankerl et al., 2010). This suggests impacts on neuronal signalling and synaptic function and highlights the need for further research characterising effects in these pathways. Turning attention to neuroimaging, techniques such as functional magnetic resonance imaging (fMRI) might provide additional insights. However, only one fMRI study has examined the effects of LTCC antagonists in humans. Zink and colleagues (Zink et al., 2020) conducted a study comparing single dose oral nimodipine with matched placebo in healthy subjects. Compared with placebo, nimodipine reduced frontal cortical and parietal cortical activity during a working memory task, without any discernible difference in task performance. Interestingly, there were no observed neural changes during an emotional face matching task. Nonetheless, these findings suggest CCBs, such as nimodipine, produce effects on fMRI blood oxygenation level-dependent (BOLD) signal. BOLD is the primary indicator of neural activity in fMRI. By detecting alterations in regional blood flow and oxygenation levels, BOLD signal is a valuable and sensitive measure of changes in brain activity.

Although limited data are available related to CCBs, fMRI studies have also examined LTCCs in relation to *CACNA1C* risk alleles. This is relevant to LTCC antagonism because *CACNA1C* risk variants are thought to modify the expression and activity of LTCCs, and have been shown to affect neural signalling and connectivity (Gurung & Prata, 2015; Kabir et al., 2017; Romme et al., 2017). Previous genetic imaging studies have demonstrated a correlation between the *CACNA1C* risk allele rs1006737 and brain activity in healthy individuals during task performance. Thus far, findings include increased amygdala activity in response to negative emotional stimuli (Jogia et al., 2011) decreased corticolimbic connectivity on an emotional faces task during fear and happy conditions (F. Wang et al., 2011) and increased prefrontal cortex activation during a working memory task (Bigos et al., 2010). Tests of semantic verbal fluency have been associated with increased activation in the left precuneus and inferior frontal gyrus (Krug et al., 2010), and a memory recall paradigm showed a significant decrease in bilateral hippocampal activation (Erk et al., 2010). Collectively, this literature indicates that the LTCC risk genotype affects neural activity in brain regions associated with emotion and cognitive function. These circuits are also implicated in BD and schizophrenia (Almeida et al., 2010; Whalley et al., 2009) suggesting the effect of *CACNA1C* variation on diverse brain circuits may also reflect underlying mechanisms in psychiatric disorder.

Among these neural findings, change in amygdala activity has been one of the most consistent observations. The amygdala is central to numerous cognitive and behavioural processes, which are relevant to psychiatric disorder. Multiple studies link the rs1006737 risk allele (A) to alterations in the structure and function of the amygdala, both in clinical populations and healthy individuals (Lancaster et al., 2016; Perrier et al., 2011; Tesli et al., 2013; Wessa et al., 2010; Wolf et al., 2014). A 2015 review (Ou et al., 2015) of patients and controls confirmed risk carriers exhibit enhanced amygdala activity to negative stimuli. This was noted especially during facial processing and the effect was proportional to the number of risk alleles (GG < AG < AA). Healthy subjects with AA/AG genotypes demonstrated heightened activity in the right amygdala during a monetary reward task (Wessa et al., 2010) and both BD and healthy control carriers of the risk allele showed increased right amygdala activity during fearful face and negative face matching paradigms (Bigos et al., 2010; Jogia et al., 2011). Increased activity in the amygdala to negative faces is thought to reflect a hypervigilance towards negative information and is seen in a wide range of psychological disorders, including depression and anxiety. This pattern of activity could therefore represent a marker of emotional vulnerability.

In addition to task-based studies, fMRI BOLD signal can also be examined at rest. In fact, combining resting state and task-based fMRI is a valuable approach in pharmacological investigations, as it provides insights into how drugs impact brain function broadly and in specific tasks. Less research has examined *CACNA1C* risk genotypes on neural activity at rest, however one study of adolescent risk carriers, with and without BD, found rs1006737 was associated with differential functional connectivity in the amygdala in youth with BD (Jiang et al., 2023). The study authors proposed this finding, in a brain region related to emotional processing, could represent an intermediate phenotype associated with the *CACNA1C* risk variant in BD. Taken together, these data underscore the significance of the amygdala and indicate the need for further neuroimaging studies to understand how CCBs may influence amygdala function, both at rest and during task performance.

Given the possible effects of CCBs on cerebral blood flow (CBF), exploring these drugs with fMRI may also pose challenges. DHP CCBs induce vasodilation, potentially altering the volume of blood transported to regions of the brain. This could confound the interpretation of the BOLD signal, as distinguishing between drug-induced vascular changes and genuine neural activity could present difficulties. A promising strategy to mitigate against potential vascular confounding factors is to employ simultaneous arterial spin labelling (ASL) scanning, which can correct for cerebral perfusion in drug studies (D. Wang et al., 2011). ASL is a technique which measures CBF non-invasively. It involves magnetically labelling arterial blood

and using MRI to track its distribution. ASL enables the measurement of CBF in physiological units (ml blood/100g tissue/min), both at rest and during activation. Research has demonstrated that ASL exhibits outstanding concurrent validity when compared with CBF measurements obtained through Positron Emission Tomography (PET), the method considered the gold standard in the field (Donahue et al., 2006).

1.9 Pharmacoepidemiology and experimental medicine approaches to studying LTCC antagonists

Despite mounting evidence from preclinical rodent studies and preliminary trials involving patients and healthy controls, significant uncertainty remains surrounding the psychiatric effects of LTCC antagonists. While large clinical trials are awaited, one alternative method for studying LTCCs is pharmacoepidemiology. The increasing availability of electronic health record (EHR) data enables observational studies, which could offer insights into the psychiatric signal associated with CCBs.

Studies assessing patients taking CCBs for cardiovascular conditions have shown individuals with BD and schizophrenia have lower rates of psychiatric inpatient admissions and self-harm whilst taking CCBs (Hayes et al., 2019; Lintunen et al., 2022). Furthermore, studies examining LTCC antagonism in other brain disorders have shown reduced risk of onset of neurodegenerative disease and delirium. A 2019 meta-analysis found CCB use was linked to a 20-30% reduction in Parkinson's disease (PD) risk (Liss & Striessnig, 2019), and EHRs from a US dataset showed CCBs were associated with lower incidence of delirium compared with beta-blockers, but a higher risk compared with renin-angiotensin system drugs (Harrison, Luciano, et al., 2020). These data also revealed CCBs were linked to lower rates of movement and cerebrovascular disorders compared with beta blockers, but higher rates of all neurodegenerative disorders compared with renin-angiotensin system agents (Harrison, Colbourne, et al., 2021). This work, which examined risk of both first-onset and recurrent diagnosis, provides evidence of some CCB benefits in common psychiatric disorders, and suggests the need for further studies using similar designs (see Chapters 2 and 3). An earlier analysis of the data presented in Chapter 2, suggested CCBs are linked to decreased risk of psychotic, affective, and anxiety disorders compared to beta-blockers, yet increased risk compared to angiotensin receptor blockers (Colbourne et al., 2021). These findings are further investigated here, using a larger dataset. Importantly, these investigations did not consider BBB penetrability, which might impact CCB efficacy in psychiatry. To date, only a limited number of studies have explored whether brain-penetrant CCBs (BP-CCBs) exert more pronounced psychiatric effects compared with non-penetrant drugs. Among the initial studies

to consider penetrability were investigations in PD, which indicated a lower risk of disease associated with BP-CCBs in comparison to amlodipine (Lee et al., 2014; Ritz et al., 2010). An earlier analysis of the data presented in Chapter 3 suggested CCBs that permeate the BBB are linked to a reduced risk of neuropsychiatric disorder, particularly first onset, compared to drugs which do not (Colbourne & Harrison, 2022). Again, these discoveries are re-explored here, using an expanded dataset.

Another method for studying LTCC antagonism is experimental medicine. As little is currently known about the potential neural and psychiatric effects of LTCC antagonists, an experimental medicine study may be more appropriate than a clinical trial, with the intention that results may guide forthcoming RCTs. Experimental medicine provides a valuable research approach in psychiatry. Developing new drugs for mental disorders is challenging. Often, promising results from animal models do not translate into efficacy in humans. Experimental medicine aims to bridge the divide between animal and human drug development (Gould & Manji, 2004). Using existing drugs to investigate neural systems, experimental medicine seeks to provide insights into the mechanisms underlying disease (Dawson, 2016). This strategy may hold promise for the investigation of LTCC antagonists. By employing measures that are known to be sensitive to early drug effects, including emotional processing tasks and neuroimaging techniques (see Chapters 5-7 for further discussion), it is hoped this approach might identify new pathways and mechanisms that could lead to pioneering treatments.

Experimental medicine, using analogous non-clinical populations can provide significant value (Harmer, 2008). Rationale for this approach comes from antidepressant studies, which have shown similar drug effects on emotional biases in both clinical and non-clinical populations (Harmer, Bhagwagar, et al., 2003; Harmer, O'Sullivan, et al., 2009; Murphy, Yiend, et al., 2009; Tranter et al., 2009). LTCC risk genes have been implicated in psychiatric phenotypes, such as mood instability, as well as diagnosable disorders. Therefore, studying CCB effects in participants with this phenotype may provide insights into effects in clinical populations.

Mood instability is a common psychiatric phenotype. It has been defined as ‘rapid oscillations of intense affect, with difficulty regulating these oscillations or their behavioural consequences’ (Marwaha et al., 2014). Mood instability is prevalent in many psychiatric disorders with which LTCCs have been genetically linked, including BD, depression and psychotic disorders (Broome, Saunders, et al., 2015; Henry et al., 2008), and recent genomic evidence has independently identified *CACNA1C* risk variants in mood instability (Hindley et al., 2022). The association between mood instability, common psychiatric disorders, and LTCCs suggests a shared molecular pathway. As such, mood instability shows promise as a therapeutic target,

with potential to aid the development of alternative treatments. Furthermore, mood instability occurs in healthy individuals (Marwaha et al., 2013) making it suitable for experimental medicine studies in appropriately selected volunteers (see Chapter 4, section 4.1.1).

Advances in remote monitoring and real time data collection, are enabling researchers to study mood instability in patients' normal environments. One of the first systems to monitor mood prospectively and remotely was launched by the University of Oxford in 2010 (Goodday et al., 2020). The True Colours platform enables patients to complete self-reported ratings of depressive, manic, and other symptoms on a daily or weekly basis, as prompted via text or email reminders (McKnight et al., 2017). This real-time data can then be represented graphically, giving greater clarity over the longitudinal course of patients' symptoms. In addition, developments in digital technologies have enabled the use of devices, such as smartphones and tablets, to record this data. Such devices facilitate ease and convenience over paper-based alternatives, which are typically more time-consuming and often erroneously time-stamped (Faurholt-Jepsen et al., 2019). These devices are also capable of recording the neurobiological correlates associated with mood instability, including neural, physiological, cognitive, and behavioural parameters. These metrics, which encompass heart rate, sleep, movement, and screen-time, include both established markers and novel measures. Applying these rich and detailed outcomes to the study of CCBs, may help inform whether trials of LTCC antagonists are indicated in patients, as well as their likely efficacy and tolerability as a psychiatric treatment.

1.10 Thesis aims and objectives

This thesis aims to explore the role of LTCCs in psychiatric disorder using two distinct research methods: pharmacoepidemiology and experimental medicine. By studying how CCBs affect psychiatric diagnosis, mood, cognitive function, and neural activity, these complementary approaches aim to further our understanding of LTCCs through the investigation of their antagonists.

The first part of this thesis uses electronic health records to evaluate the association between LTCC antagonism and psychiatric disorders. Using data from TriNetX, a large EHR network, Chapter 2 assesses the association of LTCC antagonism with risk of psychiatric disorder onset and recurrence, to establish whether patients treated with CCBs were less likely to experience new-onset or recurrent mood disorder episodes, compared to matched patients treated with other classes of antihypertensive (AHT) drugs. Chapter 3 uses a similar design to quantify the incidence of psychiatric disorders in people treated with BP-CCBs compared to other CCBs

to determine whether brain penetrance has any relationship to psychiatric outcomes. The overall hypothesis was that patients prescribed CCBs would exhibit differential risk of psychiatric disorder in the years following exposure, compared to patients prescribed alternative AHTs. Based on the earlier studies comparing AHTs in psychiatric and neurodegenerative disorders (Colbourne & Harrison, 2022; Colbourne et al., 2021; Harrison, Colbourne, et al., 2021; Harrison, Luciano, et al., 2020), it was predicted CCBs would be linked with a lower risk compared to beta-blockers, and a higher risk compared to renin-angiotensin system (RAS) agents (which include angiotensin-converting enzyme inhibitors and angiotensin receptor blockers). Drawing on previous data regarding brain penetration, it was predicted that benefits of CCBs would be greatest for patients prescribed brain-penetrant drugs.

The second part of this thesis examines the broader effects of LTCC antagonists on human brain and behaviour through an exploratory, multimodal experimental medicine study (Atkinson et al., 2019). The Oxford Study of Calcium Channel Antagonism, Cognition, Mood Instability and Sleep (OxCaMS) aimed to compare the effect of nicardipine (a brain-penetrant DHP LTCC antagonist) with placebo on a wide range of parameters, using a randomised, double-blind design in which all participants were followed for two weeks for baseline measures, after which they were randomised to two weeks' of nicardipine (30mg twice daily) or placebo. Chapters 4, 5, 6 and 7 present the results from OxCaMS, comparing nicardipine and placebo groups both pre and post randomisation. Chapter 4 analyses the physiological and psychological effects of LTCC antagonism, focusing on mood ratings, cognitive tasks and clinical data including blood tests and vital signs. Chapter 5 presents the effect of LTCC antagonism on emotional processing as measured by the Emotional Test Battery (ETB). Chapter 6 explores the effect of LTCC antagonism on resting-state functional connectivity in the brain, and Chapter 7 studies the effect of LTCC antagonism on emotional neural processing, both assessed using fMRI. It was hypothesised that calcium channels play a role in mood instability and blocking these channels would stabilize mood, as measured by the Positive and Negative Affect Schedule, short form (PANAS-SF). Additional hypotheses were also developed. Compared to placebo, it was predicted that nicardipine would influence emotional processing on behavioural tasks, as well as inducing neural effects in brain regions associated with emotion and cognition on fMRI scans. Further detail pertaining to these specific hypotheses are provided in the respective chapters. Investigating whether nicardipine affects mood, cognitive function, neural activity, and physiological parameters could offer evidence for the role of LTCCs in the pathophysiology of mood instability and its constituent phenotypes. Finally, Chapter 8 summarizes the overall findings of this thesis, alongside suggestions for extensions to this work.

Part 1 – TriNetX electronic health records: The association between LTCC antagonism and psychiatric disorders

Chapter 2. The association of LTCC antagonists with psychiatric disorder onset and recurrence

2.1 Introduction

Electronic health records (EHRs) offer a novel approach for investigating LTCC antagonism using existing clinical datasets. While large-scale trials examining CCBs for psychiatric disorder would be required to prove efficacy, pharmacoepidemiology may provide an immediate and accessible alternative for studying the neuropsychiatric associations of these drugs.

2.1.1 Observational studies in pharmacoepidemiology

Large scale observational studies are a powerful source of descriptive data. Although RCTs remain the gold standard for determining drug efficacy, cohort studies offer information on long-term drug effects, including safety, that RCTs cannot provide. Traditionally, large cohort studies take individuals with certain exposures and look for outcomes, by following them up over time. Collecting longitudinal data can be costly and time-consuming, however recent advances in data collection are transforming the way observational studies are performed. EHRs allow researchers to access large-scale data, which may provide insights into the differential risk of psychiatric disorder associated with LTCC antagonists.

The increasing availability of EHR data offers a valuable resource for the study of drugs and their population effects. There are several benefits of EHRs in pharmacoepidemiology. First, EHRs enable large-scale studies by providing access to vast patient datasets. With greater numbers, populations are more diverse, and this improves the generalisability of the data, as well as statistical power. Second, EHRs integrate clinical information with a range of other data including demographic details, comorbidities, laboratory values and healthcare utilisation. This integration not only provides a rich dataset, but also enables researchers to consider a wide range of factors which may influence drug outcomes. Third, EHRs provide longitudinal data on patient populations, thereby allowing the long-term effects of drug exposure to be studied. Finally, EHRs allow treatments to be compared, enabling the relative risks and benefits of different drugs to be considered. Cumulatively these benefits can provide researchers with a better understanding of real-world drug effects, and several EHR studies have started to examine CCBs and psychiatric disorder.

2.1.2 Electronic health record studies examining CCBs

Current data are limited but suggest possible associations between CCBs and psychiatric disorders. A longitudinal study based on a hospital database in Scotland, found in comparison to individuals taking no AHT medication, CCBs were associated with greater risk of admission for mood disorders over a five-year period (Boal et al., 2016). Beta blockers (BBs) were also linked to a higher risk, whereas angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) were linked to a lower risk. A larger follow up study of 1.8 million patients (Shaw et al., 2021) confirmed BB monotherapy was associated with the highest rates of new-onset mood disorders compared to monotherapy with other AHTs, including CCBs. ACEI/ARB monotherapy was associated with the lowest risk.

These findings are supported by Cao and colleagues (Cao et al., 2019), who investigated five AHTs including CCBs, ACEIs, ARBs, BBs, and diuretics. In their study of 180,000 hypertensive patients from an insurance database in China, the lowest rates of depression were seen for patients treated with ARBs followed by CCBs, and the highest rates were for BBs. Data from national registers in Sweden (Hayes et al., 2019) showed patients with serious mental illness, including BD and schizophrenia, had lower rates of inpatient psychiatric admission and self-harm when taking CCBs, compared to when they did not take these drugs. A similar study from a Danish register (Kessing et al., 2020) found decreased incidence of depression with some CCBs over a 10-year period. Kessing and colleagues studied 10 different CCBs, of which amlodipine, verapamil, and verapamil combinations showed decreased risk of developing depression. Most recently, Lintunen and colleagues conducted a study in BD. Using population registers in Finland (Lintunen et al., 2022), the authors found CCBs, in particular DHPs and diltiazem, were associated with reduced risk of psychiatric hospitalization for affective symptoms.

EHR studies have also reported associations between CCBs and neurodegenerative disorders, including Parkinson's Disease (PD). A 2010 study from a Danish national database (Ritz et al., 2010) found patients prescribed centrally acting DHP CCBs were less likely to develop PD than controls. Using a US database, Harrison and colleagues (Harrison, Colbourne, et al., 2021) found lower rates of PD with CCBs compared to BBs, over a two-year follow up period. However, comparable rates were noted between CCBs, renin-angiotensin system agents (ACEIs and ARBs) and diuretics.

2.1.3 Bias and confounding

EHR studies provide many advantages, primarily the large-scale analysis of real-world data, which is fast, inexpensive, and simple to perform. However, these studies are also subject to

limitations. Observational studies are unable to control for confounding and bias as satisfactorily as randomised studies. Randomisation is the best way to match both unknown and known potential confounders between groups, and hence control for selection bias.

A notable limitation of the existing EHR literature is confounding by indication (Brookhart et al., 2010). This results from the non-random allocation of individuals to a particular treatment, often due to unrecognised factors (such as age, sex, and race). Consequently, any differences between the groups may result from these external unconsidered factors, rather than the treatment itself. As confounding by indication cannot be measured directly, it presents a unique challenge to researchers, who must recognise such confounds and account for them in the study design. Many studies, previously examining LTCC antagonists, failed to match or report patient blood pressure, and therefore it is not possible to determine whether outcomes relate to the CCB drugs or the effects of hypertension (Li et al., 2015).

Adjustments for potential confounders are possible in observational studies, but only where confounders are known. Propensity score matching (PSM) is one method which has emerged to enable less biased comparisons between groups. Groups are created to look as though they were randomised to an intervention or control group (D'Agostino, 2007). Essentially, this approach determines the probability (or propensity) of treatment assignment based on observed characteristics at baseline, and then matches treated and non-treated individuals, based on propensity scores (Austin, 2011). This development in data analysis is encouraging for future EHR studies, which examine LTCC antagonism and psychiatric disorder.

2.1.4 Aims

The present analysis aimed to provide stronger statistical evidence for the association of LTCC antagonism with psychiatric disorder. Contrary to previous research, this study looked at both new-onset and recurrent psychiatric disorder episodes. Using a large EHR network, which robustly controlled for confounding, this analysis aimed to establish whether patients treated with CCBs were less likely to experience psychiatric disorders compared to patients treated with other classes of drugs. Where previous studies have been restricted mostly to depression, this research also benefitted from investigating all major ICD-10 mental and behavioural disorder categories. An earlier analysis of the dataset in this chapter has been published (Colbourne et al., 2021). However, significant growth in the EHR network between 2021 and completing this thesis, justified repeating the analyses to reflect the larger cohorts available.

2.2 Methods

2.2.1 TriNetX electronic health records

This retrospective cohort study used TriNetX Analytics, a federated cloud-based EHR network of over 100 million patients from 68 healthcare organisations (HCOs). HCOs included both inpatient and outpatient settings. Over ninety-three percent of HCOs were located in the United States of America and required relevant consents and approvals to submit data to TriNetX. Clinical governance systems ensured patients and HCOs were anonymised and only aggregated data were used (Stapff, 2018). Although identifying details of each HCO were not disclosed, the percentage of patients from each quadrant of the USA was provided. Patients originated from a range of age groups and socioeconomic backgrounds, which included both insured and uninsured individuals. The data were accessed via a browser-based interface and included demographic details, diagnoses, medications, laboratory values and clinical measurements (such as systolic and diastolic blood pressure). Data were typically updated every 24 days and searched for via browser queries. Cohorts were formed using various inclusion and exclusion criteria. They could be matched for known or putative confounders using the TriNetX built-in PSM algorithm, and subsequent outcomes could be compared over set time periods.

2.2.2 TriNetX network data

The TriNetX data source is stored on a physical server located at the company's data centre. The user interface is hosted on a number of virtual appliances, which are linked to a federated network that can send queries to each appliance. Results are then gathered and grouped together. When data are sent to the network, they undergo quality assessment. All new data are compared to a standard template, and 'data cleaning' discards records which fail to meet internal quality standards (see section 2.2.3). All data are governed by the Health Insurance Portability and Accountability Act of 1996 (HIPAA), a US federal law which protects sensitive patient information. HIPAA compliance is accomplished through the de-identification of patient data. A range of data types are stored in the network, including demographic details (coded to HL7 version 3 administrative standards), clinical diagnoses (denoted by ICD-10 codes), clinical procedures (denoted by ICD-10 or Current Procedural Terminology, CPT codes) and laboratory values (denoted by Logical Observation Identifiers Names and Codes, LOINC codes). Although detailed information pertaining to the above categories are available, socioeconomic factors are not represented in detail.

2.2.3 TriNetX network quality control

TriNetX review of data quality uses a recognised approach (Kahn et al., 2016), which includes assessment of conformance (i.e. adhering to agreed standards), completeness (i.e. data presence) and plausibility (i.e. clinical acceptability). Every HCO which joins the network must meet these initial minimum standards, as well as periodic reviews. If any issues are detected, these are discussed with the data provider and settled before data collection may be resumed. Data formatting is also reviewed, to confirm dates and other details have been entered correctly, and any entries missing the basic required information are rejected. Patients require at least one non-demographic detail to be included in the database, and those with only demographic details are removed. Data from different networks are also checked to confirm they can be aggregated successfully. Over time, data volume is monitored to ensure validity.

TriNetX software is also subject to quality control. Software development and testing are carried out independently by different engineers, and all programmes are first tested on mock data. If the software does not generate the expected output, it is adjusted and re-tested as required. Statistical software undergoes additional quality control, where independent codes are assessed in different programming languages (e.g. python and R) and resulting outputs are compared. Again, if differences are observed, software is adjusted and re-tested as needed. All code is also reviewed independently by a second programmer. Overall, the quality control works across three levels, which are unit testing (i.e. testing individual blocks of code), integration testing (i.e. testing components working together), and end-to-end testing (i.e. testing the entire system and final outputs).

2.2.4 Cohorts

Cohorts were created from patients aged 18-90 years. The primary cohort was patients exposed to CCBs for the first time. This cohort was compared to cohorts exposed to other AHTs (diuretics, ACEIs, ARBs and BBs) for the first time. Cohorts excluded any patients with a history of organic psychiatric disorder (including dementia and delirium, F01-F09) or any previous treatment with the AHT of interest.

Two separate analyses were completed. The first analysis included those patients *without* a prior psychiatric diagnosis (i.e. F20-48, including psychotic disorders [F20-29], affective disorders [F30-39] and anxiety disorders [F40-48]) and studied effects of CCBs versus other AHTs on the first diagnosis of these disorders. The second analysis included those patients *with* a prior psychiatric diagnosis and studied the effects of CCBs versus other AHTs on recurrence (i.e. another episode of a previous diagnosis, or a different F20-48 diagnosis). Additional details on ICD-10 codes are provided in section 2.2.6.

The exposure period of interest was two years. Exposure during this time was defined as those patients receiving at least two AHT prescriptions (of a particular class, but not necessarily the same drug) separated by at least two years. All data were collected in October 2023.

2.2.5 Covariates

Earlier studies (Harrison, Colbourne, et al., 2021; Harrison, Luciano, et al., 2020) have demonstrated that cohorts exposed to different AHTs are generally poorly matched for baseline characteristics, such as age, sex, race, and blood pressure. Indeed, this is to be expected given AHT guidelines (Whelton et al., 2018) recommend different prescribing based on these factors. Therefore, a propensity-score matching approach was taken (Colbourne et al., 2021) as used in Colbourne et al (2021). Cohorts were matched at baseline for age, sex, race, blood pressure (most recent systolic and diastolic values), and a number of other factors that could be confounders. These included a history of diabetes, thyroid disease, ischaemic heart disease or cerebrovascular disease, body mass index, prior use of AHTs and other medications, and for the second analysis (patients with a prior psychiatric diagnosis), groups were also matched for history of any F20-48 disorder (see Table 2.1 for detailed ICD-10 diagnostic codes). The method for matching cohorts is described below.

Table 2.1 ICD-10 codes used for propensity score matching.

Category	ICD-10 code(s) and main sub-categories
Propensity score matched characteristics and diagnoses	
Age at Index	
Sex	Male, Female
Race	White, Black or African American, Other or not known
Blood pressure	Most recent systolic and diastolic values
Diabetes mellitus	E08-E13
Thyroid disorders	E00-E07
Ischaemic heart diseases	I20-I25
Cerebrovascular diseases	I60-I69
Body Mass Index (BMI)	
Prior use of antihypertensives	CV700 (Diuretics), CV800 (Ace inhibitors), CV805 (Angiotensin ii inhibitors), CV100 (Beta blockers), CV490 (Antihypertensives, other)
Prior use of other medications	CN600 (Antidepressants), CN700 (Antipsychotics), CN400 (Anticonvulsants), CN300 (Sedatives/hypnotics), CN100 (Analgesics)
History of any psychiatric disorder (recurrent analyses only)	F20-48

2.2.6 Primary outcomes

The main outcomes in this study (see Table 2.2) were ICD-10 psychiatric disorders, including psychotic disorders (F20–F29), mood disorders (F30–F39) [manic episode (F30), bipolar disorder (F31), depressive episode (F32), recurrent depressive disorder (F33)], anxiety disorders (F40–F48), substance use disorders (F10–F18) and sleep disorders (F51 or G47). All outcomes were measured over a two-year period, between 1 and 730 days after the index event. Two years was selected as the best compromise for capturing a sufficient sample size, whilst also ensuring an adequate timeframe for outcomes.

2.2.7 Negative control outcomes

Non-causal association between an exposure and outcome is a significant risk in epidemiological studies (Lipsitch et al., 2010). To mitigate this, negative control outcomes (NCOs) can help recognise bias due to unmeasured confounding (Arnold et al., 2016). NCOs are variables that are not expected to be influenced by the exposure of interest. If an exposure affects the primary outcome but not the NCO, it indicates the association is less likely to be confounded by unrecognised factors. Few epidemiological studies have implemented NCOs in their designs to date. By including NCOs, this study aimed to strengthen the previous evidence. In total, twelve NCOs were measured, which are listed in Table 2.2. They were selected as outcomes with no known association with AHTs, and therefore acted as a type of control for overall health and health care use.

Table 2.2 ICD-10 codes used for outcomes and NCOs.

Outcomes	ICD-10 code(s)	Main sub-categories
Psychotic disorder	F20-F29	F20 (schizophrenia)
Affective disorder	F30-F39	F30 (manic episode), F31 (bipolar disorder), F32 (depressive episode), F33 (recurrent depressive disorder
Anxiety disorder	F40-F48	
Sleep disorder	F51, G47	F51 (sleep disorder not due to a substance or physiological condition), G47 (sleep disorders)
Substance abuse disorder	F10-F19	
Negative control outcomes		
Benign colonic polyp	D12.0	
Cutaneous abscess	L02	
Ganglion	M67.4	
Hallux valgus (acquired)	M20.1	
Hernia	K40-K46	
Ingrowing nail	L60.0	
Onycholysis	L60.1	
Otalgia	H92.09	
Sebaceous cyst	L72.3	
Senile keratosis	L82.1	
Trigger finger	M65.3	
Viral warts	B07	

2.2.8 Statistical analysis

PSM was used to create cohorts with characteristics that were matched at baseline (Austin, 2011). The TriNetX Analytics network uses 1:1 greedy nearest neighbour matching. This approach takes a calliper distance of 0.1 pooled standard deviations of the logit of the propensity score. After matching, characteristics with a standard difference <0.1 between the groups were regarded as matched (Haukoos & Lewis, 2015). Table 2.3 details the TriNetX PSM approach. Comparisons between cohorts (CCBs versus other AHTs) were made with risk ratios (RRs) and 95% confidence intervals.

In addition, Kaplan-Meier analyses were performed to determine the emergence of ICD-10 diagnoses over the two-year outcome period. This statistical approach is valuable in studies where the primary outcome is time-to-onset of a disorder (D'Arrigo et al., 2021). Unlike RRs, which consider the entire two-year period, Kaplan-Meier curves offer valuable data on the progression of outcomes over time. Furthermore, early separation of the Kaplan-Meier curves can serve as an additional method for identifying residual confounding in observational studies given the biological implausibility of rapid outcomes (Mohyuddin & Prasad, 2023). All statistical analyses were completed within the TriNetX network.

Table 2.3 Propensity score matching (PSM) approach used in TriNetX.

Term/Technique	Description	Example
Matching Algorithm	The method of matching subjects in each group, which is based on their propensity scores.	e.g. greedy algorithm, which makes optimal choice at each step
Matching Ratio	The ratio of subjects in the secondary cohort that are matched to subjects in the primary cohort . 1:1 is the most used ratio.	e.g. 1:1, 2:1, 3:1
Nearest neighbour matching	For each subject in the primary cohort, the algorithm finds the subject in the secondary cohort with the nearest propensity score. This can be done with or without replacement.	e.g. matching with replacement describes when a single subject in the secondary cohort is allowed to match with > 1 subject in primary cohort. e.g. matching without replacement describes when a single subject in the secondary cohort is allowed to match with only 1 subject in primary cohort.
Calliper specifications	The maximum value that propensity scores between matched subjects are allowed to differ.	

2.3 Results

At the time of data collection in October 2023, there were over 100 million patient health records on the TriNetx network. From these records, cohorts were created of patients exposed to CCBs versus other AHTs (diuretics, ACEIs, ARBs, BBs). Cohorts were extensively

propensity score matched as per the variables described above. Matching was achieved for all factors (with standard differences < 0.1) except where stated. Risk ratios for onset and recurrence of psychiatric disorders were assessed over a two-year period, and the main results are illustrated in Table 2.5, Table 2.7, and Figure 2.1.

2.3.1 LTCC antagonism and first onset psychiatric disorder

Table 2.4 Baseline characteristics for patients without a prior psychiatric (F20-F48) diagnosis.

	CCB	Diuretic	CCB	ACEI
Number	417,553	417,553	495,845	495,845
Age at index (years)	60.0 (15.4)	60.3 (15.3)	60.0 (15.4)	60.1 (14.4)
Sex (M:F %)	48 : 52	49 : 51	55 : 45	56:44
Race[a] (% W,B,O)	59, 20, 21	59, 19, 22	65, 15, 20	64, 15, 21
Blood pressure	137/79[b]	134/77[b]	135/78	134/78
Prior diuretic (%)	-	-	14	13
Prior ACEI (%)	10	10	-	-
Prior ARB (%)	6	6	2	2
Prior BB (%)	14	14	12	13
Prior other AHT (%)	6	5	6	4
Prior antidepressant (%)	6	6	6	5
Prior antipsychotic (%)	1	1	1	1
Prior anticonvulsant (%)	5	5	5	4
Prior lithium (%)	0	0	0	0

	CCB	ARB	CCB	BB
Number	432,089	432,089	442,694	442,694
Age at index (years)	60.4 (14.5)	60.7 (13.3)	59.3 (15.1)	59.7 (15.2)
Sex (M:F %)	44:56:00	44:56:00	44:56:00	44:56:00
Race[a] (% W,B,O)	70, 11, 19	70, 11, 19	60, 21, 19	60, 21, 19
Blood pressure	135/77	133/78	137/80[c]	133/77[c]
Prior diuretic (%)	13	13	15	14
Prior ACEI (%)	13	14	12	12
Prior ARB (%)	-	-	8	8
Prior BB (%)	15	15	-	-
Prior other AHT (%)	6	5	4	4
Prior antidepressant (%)	7	7	8	10
Prior antipsychotic (%)	1	1	1	2
Prior anticonvulsant (%)	6	5	5	7
Prior lithium (%)	0	0	0	0

Cohorts extensively propensity score-matched as described above. [a]W: white, B: black or African American, O: other or not known. All variables matched except: [b]SD = 0.14 systolic, 0.12 diastolic, and [c]SD = 0.19 systolic, 0.22 diastolic. Abbreviations: ACEI = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, BB = beta blocker, CCB = calcium channel blocker.

These cohorts excluded patients with a history of psychiatric disorder (F20-48) prior to their initial AHT prescription. Cohorts ranged in size from 417,553 for the CCB versus diuretic group, to 495,845 for CCB versus ACEI. Baseline characteristics are displayed in Table 2.4. Despite PSM, cohorts could not be matched for blood pressure in the CCB versus diuretic, or CCB versus BB groups. As noted in the table, the CCB groups had slightly higher blood pressure for both systolic and diastolic readings.

Table 2.5 Diagnostic outcomes for patients without a prior psychiatric (F20-F48) diagnosis. Results in boldface are significant.

Disorder	ICD-10 codes	CCB vs diuretic		CCB vs ACEI	
		% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Psychotic disorder	F20-29	0.38, 0.42	**0.90 (0.84-0.97)**	0.39, 0.37	1.05 (0.98-1.12)
Affective disorder	F30-39	5.78, 6.56	**0.88 (0.87-0.90)**	6.80, 6.22	**1.09 (1.08-1.11)**
Depression	F32,33	5.20, 5.93	**0.88 (0.86-0.89)**	6.15, 5.64	**1.09 (1.07-1.11)**
Bipolar affective disorder	F31	0.36, 0.37	0.99 (0.92-1.06)	0.42, 0.36	**1.15 (1.08-1.22)**
Anxiety disorder	F40-48	7.59, 7.73	**0.98 (0.97-0.99)**	8.69, 7.28	**1.19 (1.18-1.21)**
Sleep disorder	F51,G47	10.61,13.37	**0.79 (0.78-0.80)**	13.76, 11.09	**1.24 (1.23-1.25)**
Substance use disorder	F10-19	8.59, 8.60	0.99 (0.99-1.01)	8.38, 8.38	1.00 (0.99-1.01)
Negative control outcomes		-	**0.84 (0.78-0.89)**		0.96 (0.91-1.02)

Disorder	ICD-10 codes	CCB vs ARB		CCB vs BB	
		% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Psychotic disorder	F20-29	0.46, 0.20	**2.28 (2.10-2.47)**	0.58, 0.56	1.03 (0.98-1.09)
Affective disorder	F30-39	7.53, 5.93	**1.27 (1.25-1.29)**	9.19, 10.24	**0.90 (0.89-0.91)**
Depression	F32,33	6.83, 5.42	**1.26 (1.24-1.28)**	8.13, 9.12	**0.89 (0.88-0.90)**
Bipolar affective disorder	F31	0.47, 0.26	**1.86 (1.73-2.00)**	0.75, 0.91	**0.82 (0.79-0.86)**
Anxiety disorder	F40-48	8.95, 7.57	**1.18 (1.17-1.20)**	8.07, 12.22	**0.66 (0.65-0.67)**
Sleep disorder	F51,G47	13.51,13.69	**0.99 (0.98-0.99)**	12.63, 13.70	**0.92 (0.91-0.93)**
Substance use disorder	F10-19	**9.90, 5.71**	**1.73 (1.71-1.76)**	8.23, 9.50	**0.87 (0.86-0.88)**
Negative control outcomes			1.02 (0.92-1.11)		1.07 (0.98-1.17)

Abbreviations: ACEI = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, BB = beta blocker, CCB = calcium channel blocker.

2.3.1.1 Calcium channel blockers versus diuretics

As detailed in Table 2.5, CCBs were associated with a reduced incidence of psychotic (RR 0.90 [0.84–0.97]) and affective disorders (RR 0.88 [0.87–0.90]), including depression (RR 0.88 [0.86–0.89]), and a marginally reduced incidence of anxiety disorders (RR 0.98 [0.97–0.99]). Sleep disorders (RR 0.79 [0.78–0.80]) were also lower with CCBs compared to diuretics, but there was no difference between groups for BD (RR 0.99 [0.92–1.06]) or substance use disorder (RR 0.99 [0.99–1.01]).

2.3.1.2 Calcium channel blockers versus angiotensin-converting enzyme inhibitors

CCBs were associated with a higher incidence of affective disorders (RR 1.09 [1.08–1.11]), including depression (RR 1.09 [1.07–1.11]) and BD (RR 1.15 [1.08–1.22]), and higher incidences of anxiety (RR 1.19 [1.18–1.21]) and sleep disorders (RR 1.24 [1.23–1.25]). There was no difference between the groups for psychotic disorder (RR 1.05 [0.98–1.12]) or substance use disorder (RR 1.00 [0.99–1.01]).

2.3.1.3 Calcium channel blockers versus angiotensin receptor blockers

CCBs had increased incidence of all disorders (RRs 1.17–2.47), except for sleep disorder (RR 0.99 [0.98–0.99]). Large risk ratios were seen for psychotic disorder (RR 2.28 [2.10–2.47]), BD (RR 1.86 [1.73–2.00]), and substance use disorder (RR 1.73 [1.71–1.76]) in particular.

2.3.1.4 Calcium channel blockers versus beta blockers

CCBs were associated with reduced incidence of all disorders (RRs 0.65–0.93), except for psychotic disorder (RR 1.03 [0.98–1.09]), which showed no difference between the groups. Small risk ratios were seen for BD (RR 0.82 [0.79–0.86]) and anxiety disorder (RR 0.66 [0.65–0.67]) in particular.

2.3.1.5 Kaplan-Meier analyses

Selected results are illustrated with Kaplan–Meier analyses (see Figure 2.2). These graphs show the emergence of psychiatric disorder over time, in cohorts treated with CCBs versus cohorts treated with ARBs and BBs (i.e., AHTs selected with most and least beneficial profiles respectively). Kaplan-Maier curves for first onset CCB versus ARB, and first onset CCB versus BB analyses support the risk ratios reported above. Compared to CCBs, ARBs generally demonstrated lower outcome probabilities for psychiatric disorder, whereas BBs demonstrated greater outcome probabilities. A number of cohort pairs displayed early separation of outcomes during the two-year period, including the CCB versus BB group for first onset psychotic disorder, which showed early separation of the curves followed by a gradual convergence.

2.3.2 LTCC antagonism and recurrent psychiatric disorder

Table 2.6 Baseline characteristics for patients with a prior psychiatric (F20-F48) diagnosis.

	CCB	Diuretic	CCB	ACEI
Number	98,001	98,001	113,370	113,370
Age at index (years)	56.7 (15.6)	57.0 (15.4)	56.6 (15.4)	56.6 (13.6)
Sex (M:F %)	46 : 54	47 : 53	44:56	44:56
Race[a] (% W,B,O)	71, 17, 12	72, 16, 12	76, 13, 11	76, 13, 11
Blood pressure	135/79[b]	131/77[b]	133/78	133/79
Prior diuretic (%)	-	-	20	19
Prior ACEI (%)	16	16	-	-
Prior ARB (%)	8	8	3	3
Prior BB (%)	23	24	22	21
Prior other AHT (%)	11	9	10	8
Prior F20-29 (%)	5	5	4	5
Prior F30-39 (%)	57	59	57	54
Prior F40-48 (%)	58	56	56	53
Prior F10-19 (%)	23	22	21	22
Prior antidepressant (%)	43	42	39	40
Prior antipsychotic (%)	10	9	9	8
Prior anticonvulsant (%)	18	18	18	16
Prior lithium (%)	1	1	1	1

	CCB	ARB	CCB	BB
Number	95,796	95,796	92,591	92,591
Age at index (years)	58.0 (14.2)	58.1 (13.2)	56.7 (14.8)	57.3 (15.1)
Sex (M:F %)	42:58:00	41:59:00	42:58	42:58
Race[a] (% W,B,O)	80, 9, 11	80, 9, 11	69, 19, 12	70, 18, 12
Blood pressure	134/78	132/78	136/80[c]	131/77[c]
Prior diuretic (%)	21	20	18	19
Prior ACEI (%)	24	25	19	19
Prior ARB (%)	-	-	11	10
Prior BB (%)	24	25	-	-
Prior other AHT (%)	12	9	8	7
Prior F20-29 (%)	4	3	5	6
Prior F30-39 (%)	58	59	58	59
Prior F40-48 (%)	56	58	56	54
Prior F10-19 (%)	24	22	21	20
Prior antidepressant (%)	44	45	43	42
Prior antipsychotic (%)	10	8	8	10
Prior anticonvulsant (%)	20	17	16	19
Prior lithium (%)	1	1	1	1

Cohorts extensively propensity score-matched as described above. [a]W: white, B: black or African American, O: other or not known. All variables matched except: [b]SD = 0.16 systolic, 0.14 diastolic, and [c]SD = 0.23 systolic, 0.24 diastolic. Abbreviations: ACEI = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, BB = beta blocker, CCB = calcium channel blocker.

These cohorts included patients with a history of psychiatric disorder (F20-48) prior to their initial AHT prescription. Analyses measured the incidence of another episode of this diagnosis, or a different F20-48 diagnosis, over the two-year period following AHT prescription. Cohorts ranged in size from 92,591 for the CCB versus BB group, to 113,370 for CCB versus ACEI. Baseline characteristics are displayed in Table 2.6. Despite PSM, cohorts could not be matched for blood pressure in the CCB versus diuretic, or CCB versus BB groups. As per the analyses for first onset psychiatric disorder, these CCB groups had slightly higher blood pressure for both systolic and diastolic readings.

Table 2. 7 Diagnostic outcomes for patients with a prior psychiatric (F20-F48) diagnosis. Results in boldface are significant.

Disorder	ICD-10 codes	CCB vs Diuretic		CCB vs ACEI	
		% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Psychotic disorder	F20-29	4.19, 3.75	**1.12 (1.07-1.17)**	3.75, 3.73	1.00 (0.96-1.05)
Affective disorder	F30-39	43.63,45.49	**0.96 (0.95-0.97)**	45.65,45.84	1.00 (0.99-1.01)
Depression	F32,33	38.11,40.75	**0.94 (0.93-0.95)**	40.28,40.79	**0.99 (0.98-0.99)**
Bipolar affective disorder	F31	5.88, 4.96	**1.19 (1.14-1.23)**	5.89, 5.15	**1.15 (1.11-1.18)**
Anxiety disorder	F40-48	46.15,45.21	**1.02 (1.01-1.03)**	47.40,44.17	**1.07 (1.06-1.08)**
Sleep disorder	F51,G47	25.76,28.83	**0.89 (0.88-0.91)**	29.70,26.23	**1.13 (1.12-1.15)**
Substance use disorder	F10-19	21.14,20.17	**1.05 (1.03-1.07)**	20.43,20.17	1.01 (0.99-1.03)
Negative control outcomes			**0.87 (0.81-0.93)**		1.00 (0.94-1.07)

Disorder	ICD-10 codes	CCB vs ARB		CCB vs BB	
		% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Psychotic disorder	F20-29	3.87, 2.03	**1.91 (1.81-2.01)**	3.59, 4.78	**0.75 (0.72-0.79)**
Affective disorder	F30-39	47.78,44.31	**1.08 (1.07-1.09)**	43.30,45.07	**0.96 (0.95-0.97)**
Depression	F32,33	42.40,39.89	**1.06 (1.05-1.07)**	38.06,39.76	**0.96 (0.95-0.97)**
Bipolar affective disorder	F31	6.13, 3.80	**1.61 (1.55-1.68)**	5.09, 6.16	**0.83 (0.80-0.86)**
Anxiety disorder	F40-48	46.93,46.22	**1.02 (1.01-1.03)**	44.01,47.17	**0.93 (0.92-0.94)**
Sleep disorder	F51,G47	30.02,30.62	**0.98 (0.97-0.99)**	26.91,28.03	**0.96 (0.95-0.97)**
Substance use disorder	F10-19	22.71,14.55	**1.56 (1.53-1.59)**	18.64,20.12	**0.93 (0.91-0.94)**
Negative control outcomes			1.01 (0.92-1.10)		1.02 (0.93-1.11)

Abbreviations: ACEI = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, BB = beta blocker, CCB = calcium channel blocker.

2.3.2.1 Calcium channel blockers versus diuretics

For these analyses, the incidence of psychiatric disorders varied depending on the specific diagnosis, and there was no clear pattern for one AHT over the other. Incidence of affective disorders (RR 0.96 [0.95–0.97]), including depression (RR 0.94 [0.93–0.95]) and sleep disorders (RR 0.89 [0.88–0.91]) were lower for CCBs. However, incidence of psychotic disorder (RR 1.12 [1.07–1.17]), BD (RR 1.19 [1.14–1.23]), anxiety disorder (RR 1.02 [1.01–1.03]) and substance use disorder (RR 1.05 [1.03–1.07]) were higher for CCBs compared with diuretics.

2.3.2.2 Calcium channel blockers versus angiotensin-converting enzyme inhibitors

In contrast to the first onset analyses, there was no difference between the groups for affective disorders (RR 1.00 [0.99–1.01]), and CCBs were associated with a marginally lower incidence of depression (RR 0.99 [0.98–0.99]) than ACEI. However, findings for all other disorders were broadly similar to the first onset analyses. This included higher incidence of BD (RR 1.15 [1.11–1.18]), anxiety (RR 1.07 [1.06–1.08]) and sleep disorders (RR 1.13 [1.12–1.15]) for CCBs compared with ACEI and no difference between the groups for psychotic disorder (RR 1.00 [0.96–1.05]) or substance use disorder (RR 1.01 [0.99–1.03]).

2.3.2.3 Calcium channel blockers versus angiotensin receptor blockers

Findings for these cohorts were comparable with the first onset analyses. CCBs had a higher incidence of all disorders (RRs 1.01–2.01), except for sleep disorder (RR 0.98 [0.97–0.99]). And as per first onset analyses, large risk ratios were seen for psychotic disorder (RR 1.91 [1.81–2.01]), BD (RR 1.61 [1.55–1.68]), and substance use disorder (RR 1.56 [1.53–1.59]) in particular.

2.3.2.4 Calcium channel blockers versus beta blockers

This cohort comparison was also broadly similar to the first onset analyses. CCBs were associated with reduced incidence of all disorders (RRs 0.72–0.97), including psychotic disorder (RR 0.75 [0.72–0.79]). In particular, small risk ratios were seen for psychotic disorder RR 0.75 [0.72–0.79]) and BD (RR 0.83 [0.80–0.86]).

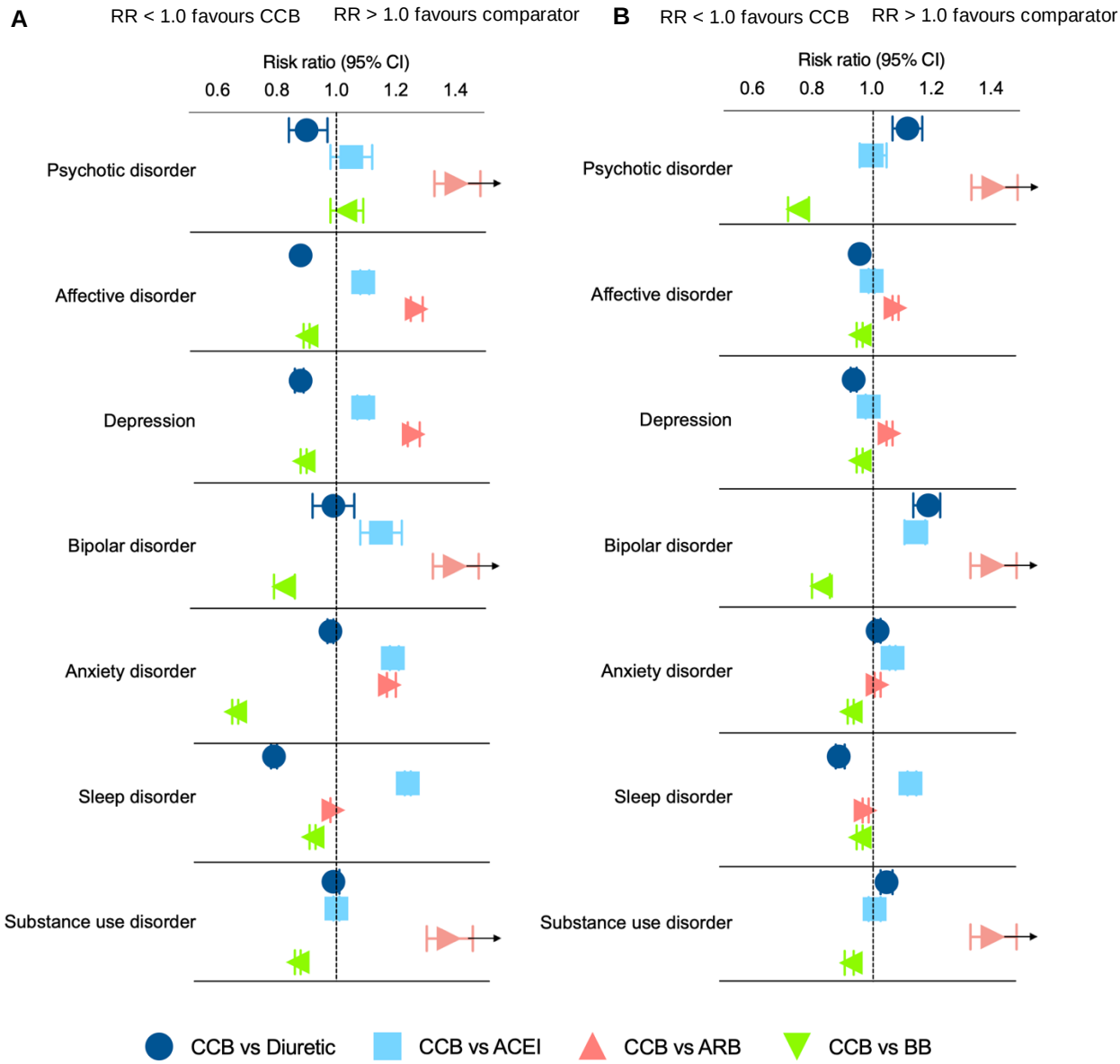
2.3.2.5 Kaplan-Meier analyses

Kaplan-Maier curves for recurrent CCB versus ARB, and recurrent CCB versus BB analyses (see Figure 2.3) support the risk ratios reported above. Compared to CCBs, ARBs generally demonstrated lower outcome probabilities for psychiatric disorder, whereas BBs demonstrated greater outcome probabilities. As for first onset analyses, a number of cohort pairs displayed early separation of outcomes during the two-year period.

2.3.3 Negative control outcomes

Except for the CCB versus diuretic cohorts, incidences of NCOs were similar between the groups (including CCBs versus ACEIs, ARBs and BBs). As shown in Tables 2.5 and 2.7 (and Appendix 2.1, Supplementary Table 2.1), CCBs were associated with a lower incidence of NCOs than diuretics for both the first onset (RR 0.84 [0.78-0.89]) and recurrence analyses (RR 0.87 [0.81-0.93]).

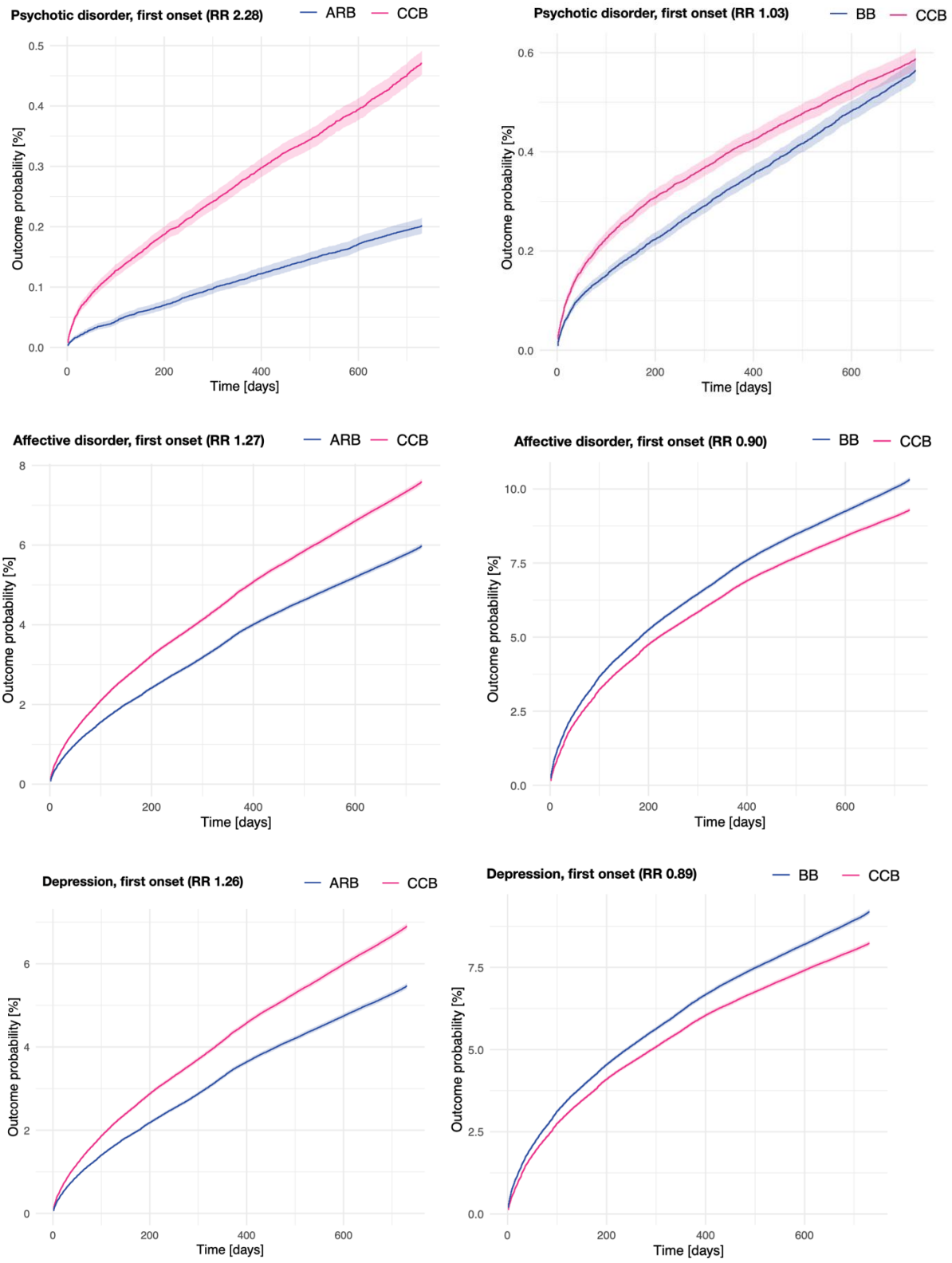
Figure 2.1 Diagnostic outcomes for patients (A) without, and (B) with a prior psychiatric (F20-F48) diagnosis.

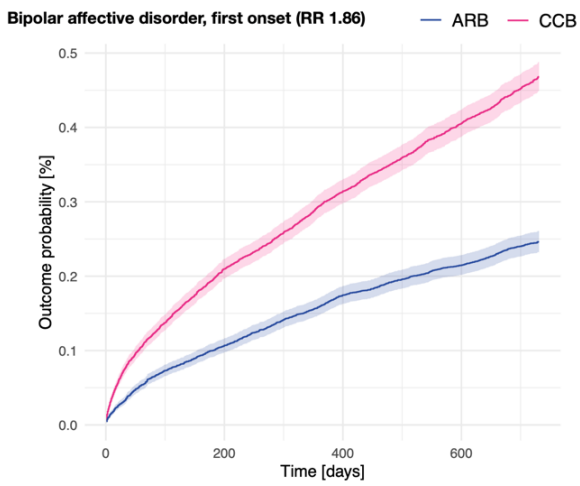


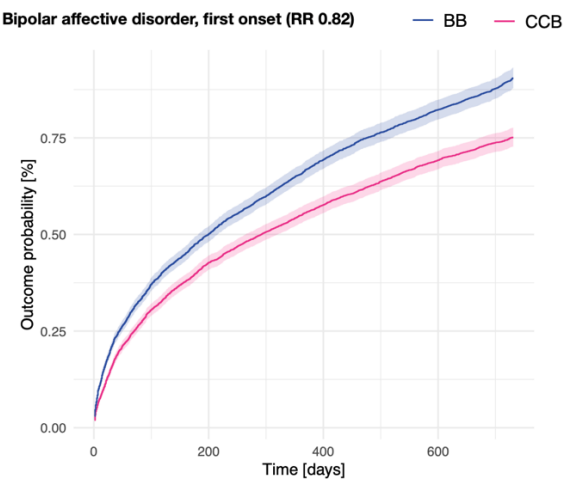
Results are shown as risk ratios with 95% confidence intervals, as per data in Tables 2.5 and 2.7. For illustration purposes, the x axis was adjusted, and some data points are outside the axis limits. CCB versus ARB risk ratios for psychotic disorder, bipolar disorder and substance use disorder for patients with and without prior psychiatric diagnoses are not as pictured. Abbreviations: ACEI = angiotensin-converting enzyme inhibitor, ARB = angiotensin receptor blocker, BB = beta blocker, CCB = calcium channel blocker.

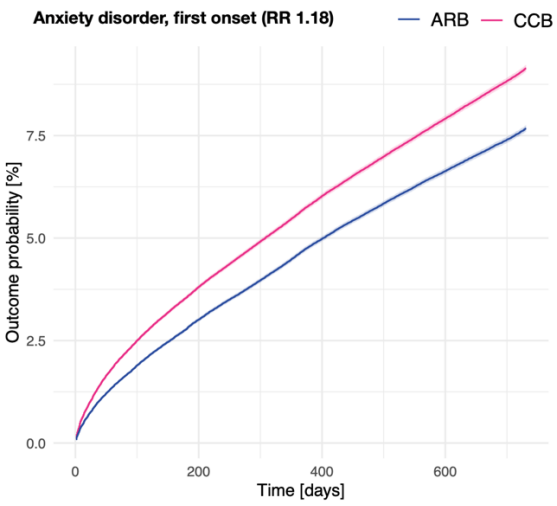
Figure 2.2 Kaplan–Meier curves, emergence of first onset psychiatric disorder for CCB vs ARB, and CCB vs BB.

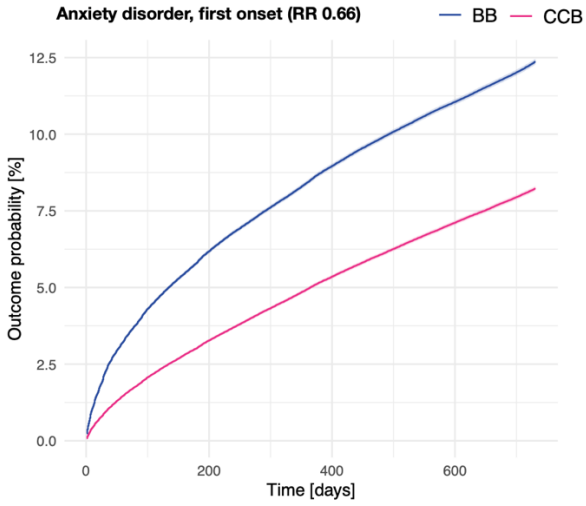
Shaded areas are 95% CIs.

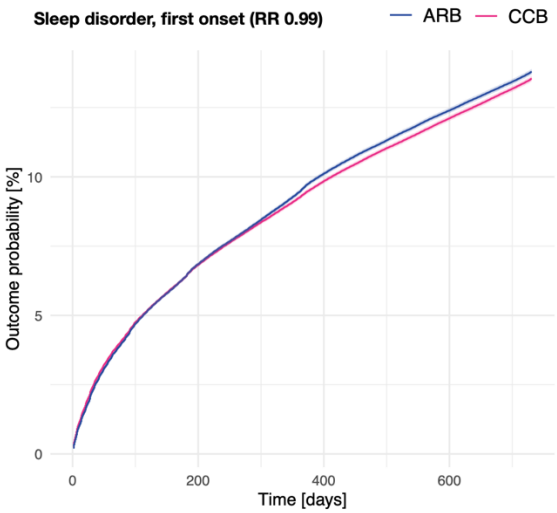


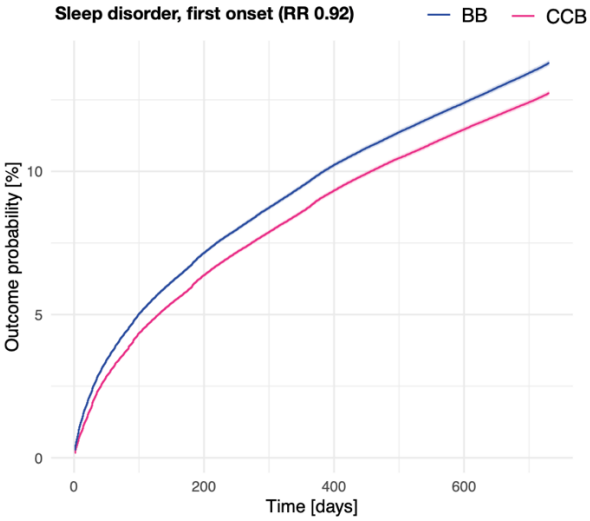

Bipolar affective disorder, first onset (RR 1.86)
ARB
CCB
Outcome probability [%]
0.0
0.1
0.2
0.3
0.4
0.5
0
200
400
600
Time [days]


Bipolar affective disorder, first onset (RR 0.82)
BB
CCB
Outcome probability [%]
0.00
0.25
0.50
0.75
0
200
400
600
Time [days]


Anxiety disorder, first onset (RR 1.18)
ARB
CCB
Outcome probability [%]
0.0
2.5
5.0
7.5
0
200
400
600
Time [days]


Anxiety disorder, first onset (RR 0.66)
BB
CCB
Outcome probability [%]
0.0
2.5
5.0
7.5
10.0
12.5
0
200
400
600
Time [days]


Sleep disorder, first onset (RR 0.99)
ARB
CCB
Outcome probability [%]
0
5
10
0
200
400
600
Time [days]


Sleep disorder, first onset (RR 0.92)
BB
CCB
Outcome probability [%]
0
5
10
0
200
400
600
Time [days]

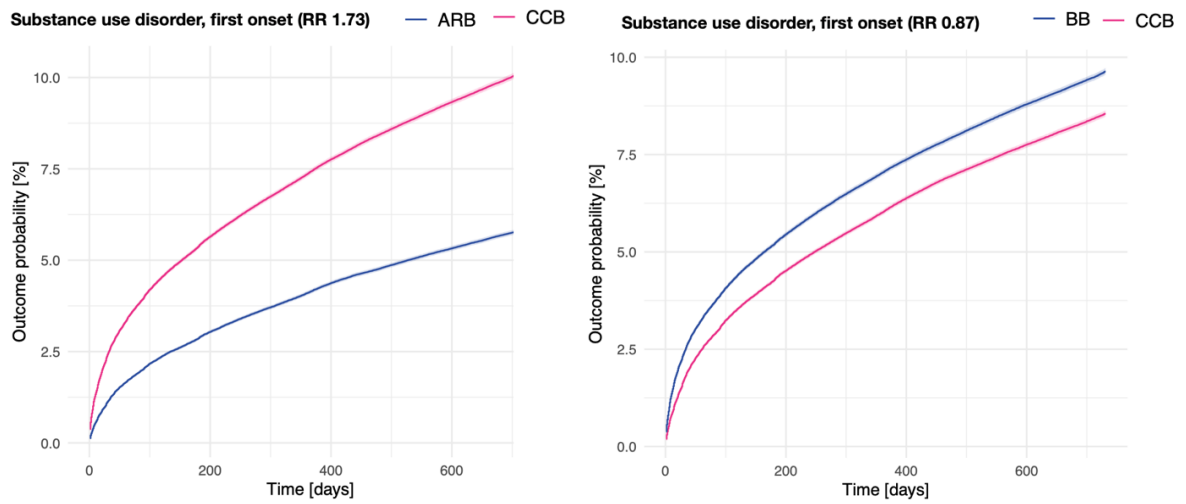
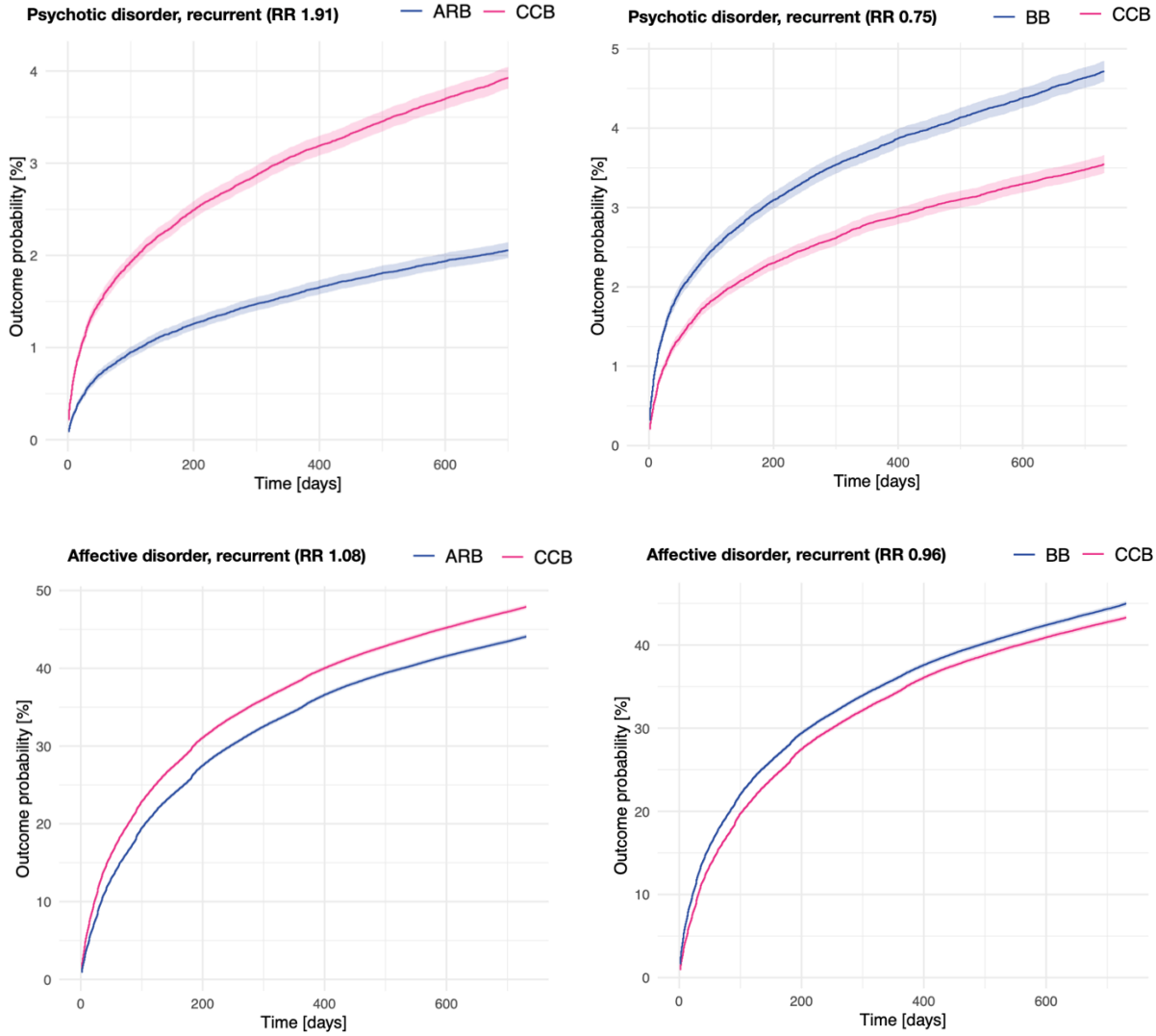
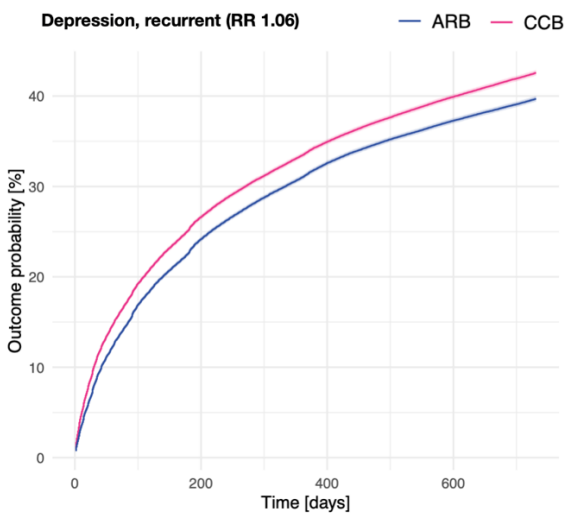
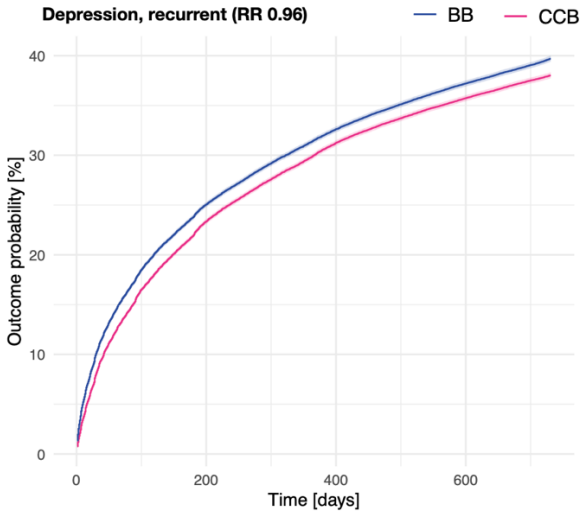


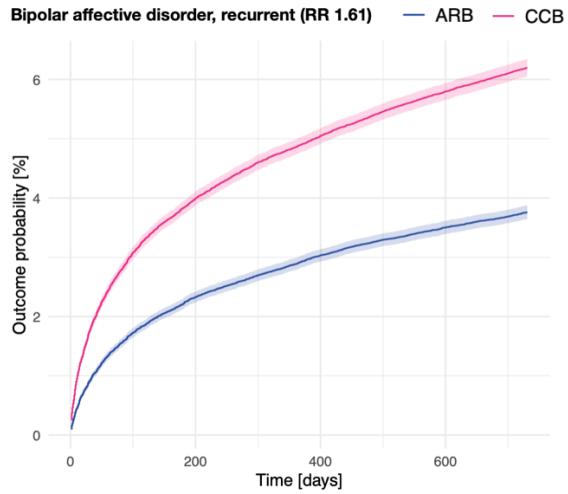
Figure 2.3 Kaplan–Meier curves, emergence of recurrent psychiatric disorder for CCB vs ARB, and CCB vs BB.

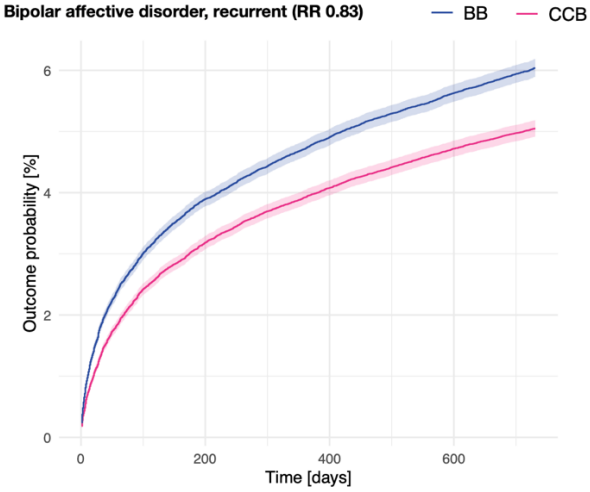
Shaded areas are 95% CIs.

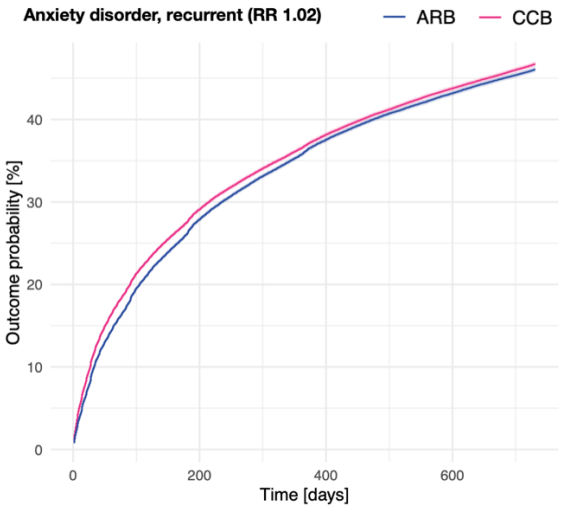


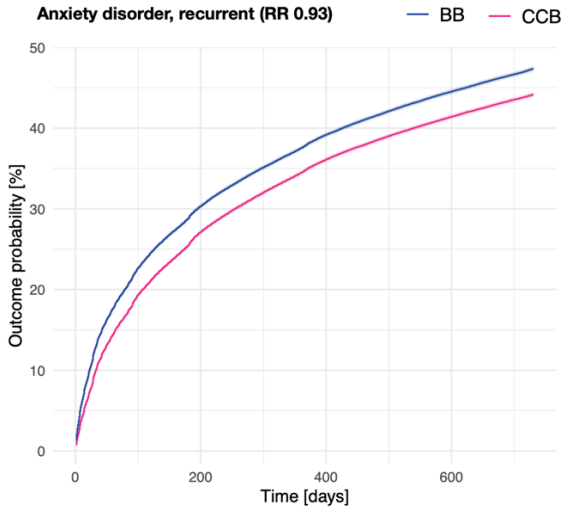

Depression, recurrent (RR 1.06)
ARB
CCB
Outcome probability [%]
Time [days]

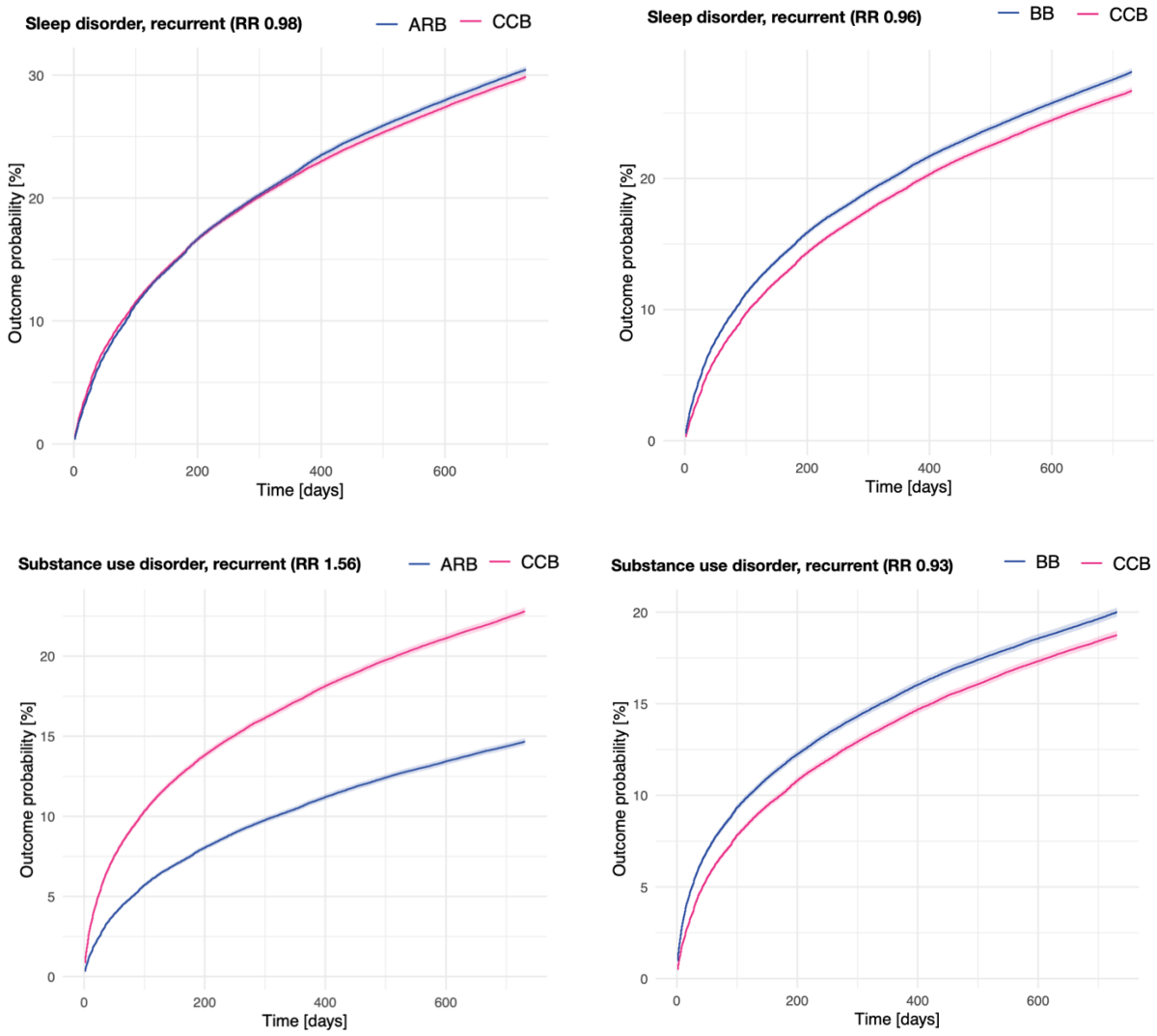

Depression, recurrent (RR 0.96)
BB
CCB
Outcome probability [%]
Time [days]


Bipolar affective disorder, recurrent (RR 1.61)
ARB
CCB
Outcome probability [%]
Time [days]


Bipolar affective disorder, recurrent (RR 0.83)
BB
CCB
Outcome probability [%]
Time [days]


Anxiety disorder, recurrent (RR 1.02)
ARB
CCB
Outcome probability [%]
Time [days]


Anxiety disorder, recurrent (RR 0.93)
BB
CCB
Outcome probability [%]
Time [days]



2.4 Discussion

Existing evidence from EHRs on the association between LTCC antagonism and psychiatric disorder is inconclusive (Boal et al., 2016; Cao et al., 2019; Hayes et al., 2019; Kessing et al., 2020; Lintunen et al., 2022; Shaw et al., 2021). This study, from a federated EHR network, builds on previous research, providing risk estimates for major psychiatric diagnoses in patients treated with CCBs, compared to matched cohorts treated with other AHTs, over a two-year period.

These analyses largely replicated what was found in an earlier dataset (Colbourne et al., 2021). In summary, CCBs were associated with a lower incidence of first onset psychiatric disorder, including depression, compared with BBs and diuretics, and a higher incidence compared with ARBs and ACEIs. The highest risk ratios were seen for CCBs versus ARBs, and the lowest for CCBs versus BBs. Recurrent disorder findings were similar to first onset,

for CCBs versus ARBs and BBs. However, analyses for CCBs versus ACEIs and diuretics differed to first onset results. Here, risk ratios varied with specific disorders, indicating no clear benefit for one AHT class over the other.

These findings are broadly consistent with reports from other datasets. Other studies comparing mood disorder risk in patients prescribed AHTs, have suggested a risk ladder, with ARBs demonstrating the lowest risk, followed by other AHTs (including CCBs), and BBs demonstrating the highest risk (Cao et al., 2019; Shaw et al., 2021). Yet, unlike previous research, mostly limited to depression, the present analyses were the first large-scale data comparing AHTs across all major psychiatric diagnoses. In the current study, CCBs demonstrated higher risk ratios than ARBs for all psychiatric disorders, except sleep. Although incidence differed across diagnoses, with first and recurrent psychotic disorder, including BD, showing considerably larger risk ratios than affective and anxiety disorders, ARBs consistently showed the most advantageous profile for psychiatric diagnoses. These findings were further supported by Kaplan-Maier curves, however the early separation of outcomes may indicate residual confounding factors that were not controlled for in the analysis. Residual confounding is a common limitation in observational studies, which is discussed further below. The magnitude of risk ratios for CCBs versus ARBs were also notable, especially when compared with other cohort pairs. This extends previous data (Boal et al., 2016; Cao et al., 2019; Shaw et al., 2021) and supports the future study of ARBs for brain health. Several theories suggest their possible mechanisms, including the interaction of central angiotensin receptors with the dopamine system (Jackson et al., 2018), and potential anti-inflammatory effects (Kessing et al., 2020). Data from human and preclinical studies have also implicated these receptors in neuropsychiatric phenotypes, such as stress and anxiety (Pulcu et al., 2019; Seligowski et al., 2021; Shekhar, 2014; Stout & Risbrough, 2019).

Differences from ARBs were not the only significant findings. Other cohort pairs also merit individual discussion. CCBs were associated with a lower incidence of all psychiatric disorders compared to BBs, apart from first onset psychotic disorder. Although RRs indicated no difference between CCB and BB groups for this diagnostic outcome (RR 1.03 [0.98-1.09]), the Kaplan-Maier analysis demonstrated early separation of the curves followed by a gradual convergence. This early separation might be attributed to residual confounding (see section 2.2.8 and further discussion below) and it is plausible that with extended follow-up the curves could have intersected, suggesting a lower probability of psychotic disorder for CCBs over the long term. This would align more closely with the outcomes observed for other psychiatric disorders when comparing CCBs with BBs. In particular, lower risk ratios were observed for first onset anxiety disorder and recurrent psychotic disorder including BD. No prior data exist

comparing CCBs with BBs for psychotic and anxiety disorders. However, the above findings support limited research examining these drugs in related psychiatric outcomes. One example comes from population registers in Denmark investigating suicide (Sørensen et al., 2001). Anxiety and psychosis are both significant risk factors for suicide (Huang et al., 2018; Khan et al., 2002), which was found to be higher among individuals prescribed BBs, but not CCBs, compared to the general population (Sørensen et al., 2001).

Whilst there is a lack of previous large-scale EHR data for CCBs and psychotic disorders including BD, a few studies support the use of CCBs in severe mental illness (Hayes et al., 2019; Lintunen et al., 2021). These studies have suggested CCBs are beneficial in reducing risk of psychiatric admission in patients with BD and schizophrenia, but other AHTs were absent from these analyses. Current insights into the mechanisms by which LTCCs mediate neuropsychiatric phenotypes are limited, however several theories exist. Genetic evidence implicates neurobiological consequences of $Ca_V1.2$ and $Ca_V1.3$ dysregulation in the brain (for a summary see Chapter 1, section 1.5), and preclinical studies suggest LTCC channel dysfunction also affects signalling cascades in separate anatomical structures that influence behaviour (Kabir et al., 2017). These complex cascades, which include CREB-regulated networks, amongst other signalling pathways, are briefly outlined in Chapter 1. However, much of this highly complicated relationship, between LTCCs and neuropsychiatric disorder, remains unknown.

Risk ratios for substance use and sleep disorders also differed between CCBs and other AHTs, but no consistent relationship was seen with the F20-48 diagnoses. LTCCs have been implicated in addiction, via their role in synaptic plasticity and molecular neuroadaptation (Casamassima et al., 2010). This study found CCBs were associated with a lower incidence of substance use disorders compared with BBs, yet there was no evidence for the benefit of CCBs over other AHT classes. Except for ACEIs, CCBs were associated with the lowest incidence of sleep disorders. However, these findings may be confounded by differential effects of AHT classes on nocturnal diuresis.

This study has several strengths, including its size, extent of disorders studied and focus on both first onset and recurrent diagnoses. Compared with insurance claim data, this network includes both insured and uninsured patients, and in contrast to surveys, the data captures all patients accessing medical treatment. Since outcomes were similar for first onset and recurrent diagnoses, the data provides additional insights into the nature of AHT effects. First onset analyses discount the possibility that outcomes are confounded by variations in AHT prescribing for individuals with known mental illness, and the recurrent analyses indicate AHT

effects apply broadly similarly to the recurrence of psychiatric disorder as to its initial onset. Overall, the study design represents an improvement on earlier research, which often failed to account for confounding factors. PSM produced cohorts which were successfully matched for age, sex, race, and several other confounding variables. Moreover, NCOs largely demonstrated no difference between CCBs and other AHTs, further supporting the reliability of these results. However, there were some notable exceptions.

Despite PSM, NCOs were less frequent for CCBs than diuretics in both first onset and recurrent analyses. This suggests residual confounding, where for unclear reasons, CCB patients were either generally healthier, or less likely to present for diagnosis. For these cohort pairs, this undermines the causal interpretation that CCBs have a direct effect on psychiatric disorder, and it raises wider doubts over the mechanism of action of CCBs in psychiatry. Although the nature of confounding is unknown, possible causes may be speculated, including socioeconomic determinants of health and concomitant prescribing, amongst other factors. TriNetX does not provide detailed socioeconomic information. Codes related to education, employment and housing problems are non-specific and lack detail. Hence the network may not be sensitive to detecting such differences between the groups. Discrepancies in the prescription of other medication may also play a role. Although some drugs were matched at baseline (including prior AHTs, antidepressants and antipsychotics), TriNetX does not control for these medications during the two-year outcome period, which may contribute another source of confounding. Finally, these cohorts could not be matched for blood pressure at baseline. Therefore, differing hypertension management may have also contributed to the results. Taken together, caution must be exercised when interpreting these analyses. Residual confounding in the dataset requires further investigation, and therefore limited conclusions can be drawn regarding psychiatric risk with CCBs, compared to risk with diuretics.

2.4.1. Limitations

This study has several limitations typical of EHR research (Casey et al., 2016). First, as described in detail above, although attempts to limit confounding were made, it was not possible to prevent in all analyses. Second, cohorts were matched using PSM. This approach can only adjust for measured variables (known or unknown), and to be effective requires substantial overlap of propensity scores between the groups. While this was largely achieved, the PSM approach is typically favoured to control for group imbalances in studies with rare outcomes and multiple confounders, and it is generally accepted that logistic regression is a more powerful technique when the number of outcomes per confounder is increased (Cepeda et al., 2003). Third, with no way of validating psychiatric diagnoses, it is not known how accurate or complete these records are. The EHRs do not provide information on drug

compliance or drug dose. There is also no means of determining when patients change individual drugs within an AHT class, which may be relevant to psychiatric risk (Kessing et al., 2020). The EHRs cannot capture patients who fail to seek treatment, and individuals who present to multiple HCOs may have records missing if a provider is not part of the network. Fourth, the study design relied on two AHT prescriptions, separated by at least two years. Hence, there may not have been continuous prescriptions over this time. Finally, causality cannot be demonstrated through EHR studies, and as such RCTs remain the gold standard for determining the effect of AHT classes on psychiatric disorder risk.

2.5 Conclusion

Previous studies have suggested the therapeutic potential of CCBs for mental disorders, and the possibility of repurposing CCBs as psychiatric agents, especially in BD (Cipriani et al., 2016). More recently, genetic findings have identified new evidence for the role of LTCCs in psychiatry (Heyes et al., 2015), generating fresh interest in these channels as possible drug targets.

This study found different AHT classes are associated with different risks of onset or recurrence of psychiatric disorders. CCB versus diuretic comparisons were affected by residual confounding, which undermined the interpretation of psychiatric outcomes in these cohorts. ARBs demonstrated the most beneficial psychiatric risk profile, and BBs the least, with no compelling evidence to repurpose existing CCBs for psychiatric disorders.

However, closer examination of the CCB cohorts revealed that over ninety percent of patients in these analyses were prescribed either amlodipine, verapamil, or diltiazem. These drugs, which all have relatively low BBB penetrability, accounted for seventy-one, nine, and thirteen percent of prescriptions respectively. Therefore, it is still unknown whether CCBs with superior brain penetrability might have more beneficial effects on brain health and psychiatric disorder. This will be the focus of the next chapter, in which brain-penetrant and non-penetrant CCBs will be compared using the same designs and analyses as described here.

Chapter 3. The association of brain-penetrant LTCC antagonists with psychiatric disorder onset and recurrence

3.1 Introduction

Individual LTCC antagonists vary in their ability to penetrate the BBB. While many enter, several commonly prescribed CCBs do not cross in any significant quantity. It is currently unknown whether BBB-penetration relates to CCB effects on brain health, however such a connection is feasible. The ability of CCBs to cross the BBB is likely to affect their occupancy of brain VGCCs. Although CCB neuropsychiatric effects may be mediated peripherally, it is generally believed they result from central VGCC occupancy (Alves et al., 2019; Nanou & Catterall, 2018; Zamponi et al., 2015). This provides a rationale for investigating whether brain-penetrant CCBs (BP-CCBs) are associated with a reduced incidence of psychiatric diagnoses than CCBs which are non-penetrant. If BP-CCBs demonstrate reduced onset and recurrence of psychiatric disorder, compared with non-penetrant drugs, this may encourage future trials of these compounds.

It is unclear why some CCBs permeate the brain, and others do not. The BBB is a natural membrane that protects the central nervous system from harmful molecules and ensures normal brain function. It regulates drug delivery to the brain, allowing some drugs to cross by passive diffusion, or active uptake through transmembrane protein transporters. However tight epithelial-like junctions in brain capillary endothelium prevent other therapeutic compounds from crossing. To date, there is a paucity of literature comparing BP-CCBs to those which are non-penetrant. An early rodent study examining the potential role of CCBs in depression, reported antidepressant-like effects with the DHPs nifedipine, nicardipine, isradipine, felodipine and nimodipine, but not with amlodipine (Cohen et al., 1997). Other research, limited to Parkinson's disease (PD), found reduced PD risk in patients prescribed BP-CCBs compared to those receiving amlodipine (Lee et al., 2014; Ritz et al., 2010).

Structurally, CCBs belong to three distinct classes; DHPs (including amlodipine, felodipine, isradipine, nicardipine, nifedipine, nimodipine and nisoldipine), phenylalkylamines (verapamil) and benzothiazepines (diltiazem). DHPs share the same general structure, yet within this CCB subgroup there is a spectrum of brain permeability. The data regarding brain penetration (Fridén et al., 2009; Liu et al., 2008) is incomplete, however it is generally accepted that amlodipine, the most commonly prescribed DHP CCB, demonstrates poor to no BBB penetrance. Uchida and colleagues (Uchida et al., 1997) examined mouse brain pharmacokinetics following intravenous injection of amlodipine and other DHPs. Compared to amlodipine, they found three to five times higher brain to plasma concentrations with

nimodipine and isradipine, and twenty times higher brain to heart concentrations with nimodipine, isradipine and nifedipine. These data suggest that amlodipine is taken into the brain less extensively, compared to other DHPs. More recently Siddiqi and colleagues (Siddiqi et al., 2019) screened amlodipine and nine other CCBs. Using drug databases, they ranked BBB penetration based on evidence from the literature, and predicted values calculated from online algorithms (DrugBank, 2024; PubChem, 2024). These rankings aligned with the earlier findings, concluding amlodipine does not cross the BBB, whilst other DHP CCBs do. Although the evidence is incomplete, a range of experimental data further support these findings, which are summarised in Table 3.1.

Table 3.1 Evidence from experimental studies detailing brain penetrance of individual DHP CCBs.

DHP CCB	Findings	Author
Isradipine	Accumulation in brain after intra-peritoneal injection	Supavilai & Karobath 1984
	Extraction from brain after intra-carotid injection	Urien et al 1987
	Brain : plasma ratio 3-5-fold more for isradipine than amlodipine, and brain : heart ratio 20-fold more for isradipine than amlodipine.	Uchida et al 1997
	High performance liquid chromatography showed isradipine present in mouse brain at high concentrations after chronic sub-cutaneous administration. 'Isradipine is bioavailable to the brain…'.	Anekonda et al 2011
	Isradipine crosses BBB based on data from drug databases.	Siddiqi et al 2019
Felodipine	Felodipine crosses BBB based on data from drug databases.	Siddiqi et al 2019
Nicardipine	Extraction from rat brain tissue after intra-carotid injection. '…biologically active amounts of nicardipine may be available…in neurons…'	Grotta et al 1987
	Brain concentration 0.3-0.8 times that in plasma after intra-venous injection	Takakura et al 1992
	65-70% occupancy of brain VGCCs after oral administration of nicardipine in rats.	Amenta et al 2001
	Nicardipine crosses BBB based on data from drug databases.	Siddiqi et al 2019
Nifedipine	Displacement of nitrendipine binding in brain after intra-peritoneal injection of nifedipine.	Schoemaker et al 1983
	Accumulation in brain after intra-peritoneal injection.	Supavilai & Karobath 1984
	Crosses BBB (although nimodipine > nifedipine)	Van den Kerkhoff & Drewes 1985
	High performance liquid chromatography after intra-venous injection. Accumulates in rat brain, concentration dose-related and exceeds that in plasma. '…can easily cross the blood-brain barrier'.	Janicki et al 1988
	High performance liquid chromatography after intra-peritoneal injection in mice, and inhibition of experimental seizures. Concludes nifedipine crosses the blood brain barrier.	Larkin et al 1992
	Brain : heart ratio 20-fold more for nifedipine than amlodipine.	Uchida et al 1997
	Nifedipine crosses BBB based on data from drug databases.	Siddiqi et al 2019
Nimodipine	Gas chromatography. CSF concentration similar to unbound plasma concentration.	Krol et al 1984

	Binding of nimodipine after intra-peritoneal injection. 'Accumulates quickly in the [gerbil] brain…and may be sufficient to fully saturate…even at the lowest dose administered'	Heffez et al 1985
	Crosses BBB (nimodipine > nifedipine)	Van den Kerkhoff & Drewes 1985
	High performance liquid chromatography after intra-peritoneal injection in mice, and inhibition of experimental seizures. Concludes nimodipine crosses the blood brain barrier.	Larkin et al 1992
	Brain : plasma ratio 3-5-fold more for nimodipine than amlodipine, and brain: heart ratio 20-fold more for nimodipine than amlodipine.	Uchida et al 1997
	Nimodipine crosses BBB based on data from drug databases.	Siddiqi et al 2019
Nisoldipine	Nisoldipine crosses BBB based on data from drug databases.	Siddiqi et al 2019

Taken together, the above evidence suggests DHP CCBs can be grouped into two separate classes, those with high BBB penetrability i.e. 'brain-penetrant' and those without i.e. 'non-penetrant'. Amlodipine was categorised as 'non-penetrant', with the other DHPs (including nimodipine, isradipine, felodipine, nifedipine, nicardipine and nisoldipine) grouped as 'brain penetrant'. Primary analyses in this study were limited to DHPs (phenylalkylamines and benzothiazepines were included in secondary analyses for reasons discussed below, see section 3.2.4).

Using this classification, the current chapter utilised EHRs to determine the association of BP-CCBs with neuropsychiatric outcomes. The incidence of first onset and recurrent psychiatric disorders were compared to amlodipine over a two-year exposure period. Propensity score matching (PSM) was implemented to reduce confounding, and negative control outcomes (NCOs) identified any residual bias (Arnold et al., 2016; Lipsitch et al., 2010). Sub-group analyses were performed for males and females and different age groups (less than or more than 60 years) to determine whether there were different effects in these groups. Secondary analyses compared BP-CCBs with phenylalkylamines and benzothiazepines, as well as ARBs. An earlier analysis of the dataset in this chapter has been published (Colbourne & Harrison, 2022). However, significant growth in EHR networks between 2022 and completing this thesis, justified repeating the analyses to reflect the larger cohorts available.

3.2 Methods

3.2.1 Electronic health records

This retrospective cohort study used the TriNetX US Collaborative Network, a federated cloud-based EHR network of over 100 million patients from 61 healthcare organisations (HCOs). As with the TriNetX Analytics Network (Colbourne & Harrison, 2022), HCOs included both

inpatient and outpatient settings, although details were not disclosed. For these repeat analyses, the Collaborative Network was selected due to its larger size. In contrast to the Analytics Network, patients were exclusively from the United States, but otherwise records contained the same information in terms of demographic data, ICD-10 diagnoses, and prescribed medications. Full details of the TriNetX platform are described in Chapter 2 (see section 2.2.1). All data were collected in December 2023.

3.2.2 Cohorts and covariates

Cohort design and covariate selection are described in detail in Chapter 2 (see sections 2.2.4 and 2.2.5). In brief, two cohort types of patients aged 18-90 years, were created. The first included those patients *without* a prior psychiatric or neurodegenerative diagnosis and studied effects of BP-CCBs versus amlodipine on the first episode of these disorders (see section 3.2.3). The second analysis included those patients *with* a prior psychiatric or neurodegenerative diagnosis and studied the effects of BP-CCBs versus amlodipine on recurrence (i.e. another episode of a previous diagnosis, or a different psychiatric or neurodegenerative diagnosis).

As BP-CCB and amlodipine cohorts differed in terms of baseline demographics including age, sex, and blood pressure (data not shown), PSM was used to reduce these differences and other potential confounders (Ali et al., 2019; Austin, 2011). Cohorts were matched at baseline for age, sex, race, blood pressure (most recent systolic and diastolic values), and a number of other factors that could be confounders (see Table 3.2). Full details on how matching was carried out are described in Chapter 2 (see section 2.2.8).

Table 3.2 ICD-10 codes used for propensity score matching.

Category	ICD-10 code(s)	Main sub-categories
Propensity score matched diagnoses		
Diabetes mellitus	E08-E13	
Thyroid disorders	E00-E07	
Ischaemic heart diseases	I20-I25	
Cerebrovascular diseases	I60-I69	
Hypertensive diseases	I10-I16	
Disorders of the respiratory system	J00-J99	
Disorders of the musculoskeletal system	M00-M99	
Problems related to socioeconomic and psychosocial circumstances	Z55-Z65	Z55 (problems related to education and literacy), Z56 (problems related to employment and unemployment), Z57 (occupational exposure to risk factors), Z59 (problems related to housing and economic circumstances)
Body Mass Index (BMI)		

Prior use of AHTs	CV700 (Diuretics), CV800 (Ace inhibitors), CV805 (Angiotensin ii inhibitors), CV100 (Beta blockers), CV490 (Antihypertensives, other)
Prior use of other medications	CN600 (Antidepressants), CN700 (Antipsychotics), CN400 (Anticonvulsants), CN300 (Sedatives/hypnotics), CN100 (Analgesics), CN750 (Lithium salts), CN800 (CNS stimulants)
History of any psychiatric or neurodegenerative disorder (recurrent analyses only)	(See outcome ICD-10 codes below)

3.2.3 Primary outcomes

The outcomes of this study were ICD-10 psychiatric and neurodegenerative disorders as listed in Table 3.3. NCOs were the same as Chapter 2 (see section 2.2.7 and Table 2.2). All outcomes were measured over a two-year period (i.e. between 1 and 730 days after the index event), which required two prescriptions of a BP-CCB or amlodipine at least two years apart. Incidence (%) of patients receiving a diagnosis during the two-year period was measured, and cohorts were compared using risk ratios (RRs) with a 95% confidence interval. Kaplan-Meier analyses were performed to illustrate the emergence of psychiatric diagnoses. Using the TriNetX query builder, separate sub-group analyses were also created for males and females, and patients less than or more than 60 years old. All statistical analyses were conducted within the TriNetX network.

Table 3.3 ICD-10 codes used for diagnostic outcomes.

Category	ICD-10 code(s)	Main sub-categories
Outcomes		
Psychotic disorder	F20-F29	F20 (schizophrenia)
Affective disorder	F30-F39	F30 (manic episode), F31 (bipolar disorder), F32 (depressive episode), F33 (recurrent depressive disorder
Anxiety disorder	F40-F48	
Sleep disorder	F51, G47	F51 (sleep disorder not due to a substance or physiological condition), G47 (sleep disorders)
Substance abuse disorder	F10-F19	
Delirium	F05, R40.0, R41.0	F05 (Delirium due to known physiological condition), R40.0 (Somnolence), R41.0 (Disorientation, unspecified)
Dementia	F01-F03, G30, G31.0, G31.2, G31.83	F01 (Vascular dementia), F02 (Dementia in other disease classified elsewhere), F03 (Unspecified dementia), G30 (Alzheimer's disease), G31.0 (Frontotemporal dementia), G31.2 (Degeneration of nervous system due to alcohol), G31.83 (Dementia with Lewy bodies)
Movement disorder	G20-G26	G20-26 (Extrapyramidal and movement disorders)

3.2.4 Secondary analyses

For the primary analyses, it was hypothesised that any differences in incidence of psychiatric disorder between BP-CCBs and amlodipine could be attributed to some other amlodipine characteristic, such as its VGCC occupancy, rather than solely to its low brain penetrability (Casey et al., 2016). Therefore, a secondary analysis compared BP-CCBs with phenylalkylamines (verapamil) and benzothiazepines (diltiazem). These drugs were not included in the primary analysis for several reasons. Firstly, they differ from dihydropyridines in their pharmacological profiles (Abernethy & Schwartz, 1999; Zamponi et al., 2015), and therefore any disparities between BP-CCBs and these drugs might not simply reflect brain penetrability. Secondly, they are generally prescribed for different reasons. These include arrhythmias and angina, with contraindications in heart failure, and no first line indication for hypertension. Therefore, the probability of confounding by indication is also greater for these analyses. In comparison to DHP data, there is very limited literature on the brain permeability of phenylalkylamines and benzothiazepines. However, it is generally accepted that verapamil and diltiazem do not readily cross the BBB (Bhat et al., 2012; Lee et al., 2014; Siddiqi et al., 2019), and hence they were both included in the 'non-penetrant' group. Finally, BP-CCBs were compared with ARBs, due to their favourable neuropsychiatric profile when compared with CCBs as a class (see Chapter 2, sections 2.3.1.3 and 2.3.2.3). It was hypothesised that the difference between CCBs and ARBs would disappear or decrease when the latter were compared exclusively to BP-CCBs.

3.3 Results

Matching was achieved for all factors (with standard differences < 0.1) except where stated in the tables. Results are illustrated in Figures 3.1-3.2 and Tables 3.4-3.14. Nifedipine was the most widely prescribed BP-CCB, followed by nicardipine and felodpine, which altogether made up over 95% of BP-CCB prescriptions.

3.3.1 Primary analyses

3.3.1.1 BP-CCBs versus amlodipine for first onset neuropsychiatric disorder

These analyses excluded all patients with a previous diagnosis of any outcome of interest. After matching, there were 51,052 patients in each cohort (Table 3.4). As shown in Table 3.5 and Figures 3.1 patients prescribed BP-CCBs had a lower incidence of all diagnoses, although the risk ratios for schizophrenia and movement disorder included 1 reflecting their low

incidence and hence wide confidence intervals. For a first diagnosis of any neuropsychiatric disorder, the relative risk was 12% lower (RR = 0.88 [0.86–0.90]), with an absolute incidence of 20.09% for BP-CCBs and 22.92% for amlodipine. Risk ratios were broadly similar for each individual disorder, ranging from psychotic disorder (RR = 0.79 [0.64-0.97]) to movement disorder (RR = 0.92 [0.83-1.03]). Kaplan-Meier curves illustrating the emergence of first onset neuropsychiatric disorders in BP-CCB versus amlodipine are shown in Figure 3.2.

3.3.1.2 BP-CCBs versus amlodipine for recurrent neuropsychiatric disorder

These analyses only included patients with at least one previous diagnosis of any outcome of interest before first CCB exposure. After matching, there were 22,827 patients in each cohort (Table 3.4). As shown in Table 3.5 and Figure 3.2, results were variable, although no diagnoses were more common in the BP-CCB group. Some diagnoses were more common with amlodipine (e.g. psychotic disorder, RR = 0.85 [0.75-0.95], anxiety disorder, RR = 0.98 [0.95-0.99], sleep disorder, RR = 0.97 [0.94-0.99], and substance use disorder, RR = 0.97 [0.94-0.99]), whilst others showed no difference between BP-CCB and amlodipine groups (e.g. affective disorder, RR = 1.00 [0.98-1.03], delirium, RR = 0.99 [0.89-1.09], dementia, RR = 1.08 [0.97-1.19], and movement disorder, RR = 1.02 [0.94-1.10]). Kaplan-Meier curves illustrating the emergence of recurrent neuropsychiatric disorders in BP-CCB versus amlodipine are shown in Figure 3.2.

Table 3.4 Baseline demographics for matched BP-CCB versus amlodipine cohorts.
A: patients with no prior neuropsychiatric diagnosis. B: patients with a prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis		B: with prior neuropsychiatric diagnosis	
	BP-CCB	Amlodipine	BP-CCB	Amlodipine
Cohort size (n)	51,052	51,052	22,827	22,827
Age at index (y, SD)	58.4 (17.6)	58.9 (16.8)	57.3 (16.5)	57.3 (15.0)
Sex (M:F %)	42 : 58	42 : 58	42 : 58	41 : 59
Race[a] (W,B,O %)	47, 32, 21	47, 33, 20	54, 28, 18	53, 29, 18
Blood pressure	138/77	138/78	138/78	139/80
Body mass index (SD)	30.1 (6.8)	29.8 (6.9)	30.9 (7.2)	30.7 (7.2)
Prior psychotic disorder (%)	0	0	2.8	2.9
Prior affective disorder (%)	0	0	32.2	32.9
Prior anxiety disorder (%)	0	0	31.8	32.1
Prior substance use disorder (%)	0	0	29.9	30.4
Prior sleep disorder (%)	0	0	31.2	31.9
Prior delirium (%)	0	0	4.0	4.2

Prior dementia (%)	0	0	2.6	3.1
Prior movement disorder (%)	0	0	5.5	5.6

Cohorts extensively propensity score-matched as described above. Group B percentages add up to > 100% as patients may have more than one diagnosis. [a]W: white, B: black or African American, O: other or not known. Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

Table 3.5 Outcomes for BP-CCBs versus amlodipine.

Percentage with each diagnosis during exposure period and the risk ratio. A: patients with no prior neuropsychiatric diagnosis. B: patients with a prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis			B: with prior neuropsychiatric diagnosis		
	BP-CCB (%)	Amlodipine (%)	Risk ratio (95% CI)	BP-CCB (%)	Amlodipine (%)	Risk ratio (95% CI)
Psychotic disorder	0.31	0.39	**0.79 (0.64-0.97)**	2.29	2.71	**0.85 (0.75-0.95)**
Schizophrenia	0.10	0.11	0.88 (0.60-1.28)	1.14	1.37	**0.83 (0.70-0.98)**
Affective disorder	5.78	6.68	**0.87 (0.83-0.91)**	31.24	31.13	1.00 (0.98-1.03)
Bipolar disorder	0.36	0.44	**0.80 (0.66-0.98)**	3.79	3.83	0.99 (0.90-1.08)
Major depressive disorder	5.24	5.99	**0.88 (0.83-0.92)**	27.78	27.49	1.01 (0.98-1.04)
Anxiety disorder	6.48	7.67	**0.85 (0.81-0.88)**	29.11	29.84	**0.98 (0.95-0.99)**
Sleep disorder	8.03	9.59	**0.84 (0.80-0.87)**	30.54	31.48	**0.97 (0.94-0.99)**
Substance use disorder	4.78	5.64	**0.85 (0.80-0.89)**	24.23	25.01	**0.97 (0.94-0.99)**
Delirium	0.85	1.03	**0.83 (0.73-0.94)**	3.25	3.3	0.99 (0.89-1.09)
Dementia	0.98	1.12	**0.87 (0.77-0.98)**	3.47	3.23	1.08 (0.97-1.19)
Movement disorder	1.13	1.22	0.92 (0.83-1.03)	5.43	5.35	1.02 (0.94-1.10)
Any of the above	20.09	22.92	**0.88 (0.86-0.90)**	70.64	70.79	0.99 (0.99-1.01)
Negative control outcomes [a]			**0.89 (0.83-0.96)**			**0.93 (0.88-0.99)**

[a] Mean of 12 negative control outcomes. Full details in Table 3.12.
Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

3.3.2 Sub-group analyses

3.3.2.1 BP-CCBs versus amlodipine divided by sex

Analyses comparing BP-CCBs with amlodipine divided by sex are shown in Table 3.6. In patients with no prior neuropsychiatric diagnosis, results were similar in males (RR = 0.90 [0.87-0.94]) and females (RR = 0.86 [0.83-0.89]). In patients with a prior neuropsychiatric diagnosis, there was no difference between BP-CCBs and amlodipine for males (R= 0.99

[0.98-1.02]), but for females, results were variable depending on diagnosis. Psychotic disorder, RR = 0.86 [0.73-0.99], anxiety disorder, RR = 0.95 [0.92-0.98], sleep disorder, RR = 0.94 [0.91-0.98], and substance use disorder, RR = 0.95 [0.90-0.99] were more common with amlodipine, whereas dementia, RR = 1.13 [1.00-1.28] was more common with BP-CCBs.

3.3.2.2 BP-CCBs versus amlodipine divided by age

Analyses comparing BP-CCBs with amlodipine divided by age (under or over 60 years) are shown in Table 3.7. Results demonstrated a trend for lower risk ratios in the younger cohort. For those without a prior neuropsychiatric diagnosis, the overall risk ratio was 0.81 (0.78-0.84) in the under-60s and 0.90 (0.87-0.92) in the over-60s. In those with a prior diagnosis, the equivalent figures were 0.97 (0.95-0.99) and 1.01 (0.99-1.02), the latter reflecting no significant differences between BP-CCBs and amlodipine for any disorder in the older age group.

Table 3.6 BP-CCBs versus amlodipine, subdivided by sex.

A: no prior neuropsychiatric diagnosis				
	Male		Female	
	BP-CCB	Amlodipine	BP-CCB	Amlodipine
Cohort size (n)	21,662	21,662	30,329	30,329
Age at index (y, SD)	61.0 (14.5)	61.4 (13.9)	56.5 (19.3)	56.6 (18.9)
Sex (M:F %)	100:0	100:0	0:100	0:100
Race[a] (W,B,O %)	54, 28, 18	54, 28, 18	44, 36, 20	44, 36, 20
Blood pressure	139/78	141/79	137/77	137/77
Body mass index (SD)	29.6 (5.7)	29.8 (5.6)	30.6 (7.3)	29.9 (7.3)
Outcomes	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Psychotic disorder	0.31, 0.35	0.87 (0.63-1.21)	0.30, 0.44	**0.68 (0.52-0.89)**
Schizophrenia	0.12, 0.09	1.37 (0.76-2.47)	0.08, 0.11	0.76 (0.45-1.27)
Affective disorder	3.98, 4.65	**0.86 (0.78-0.94)**	6.90, 8.08	**0.85 (0.81-0.90)**
Bipolar disorder	0.25, 0.36	**0.69 (0.49-0.98)**	0.42, 0.53	**0.79 (0.63-0.99)**
Major depressive disorder	3.61, 4.11	**0.88 (0.80-0.97)**	6.25, 7.26	**0.86 (0.81-0.91)**
Anxiety disorder	4.47, 5.12	**0.87 (0.80-0.95)**	7.75, 9.30	**0.83 (0.79-0.88)**
Sleep disorder	9.42, 10.38	**0.91 (0.86-0.96)**	7.03, 8.72	**0.81 (0.76-0.85)**
Substance use disorder	5.57, 6.94	**0.80 (0.75-0.86)**	4.12, 4.72	**0.87 (0.81-0.94)**
Delirium	0.97, 1.22	**0.80 (0.67-0.95)**	0.76, 1.08	**0.70 (0.60-0.83)**
Dementia	0.88, 1.01	0.87 (0.72-1.06)	1.02, 1.27	**0.80 (0.69-0.93)**
Movement disorder	1.22, 1.20	1.02 (0.86-1.20)	1.01, 1.23	**0.83 (0.71-0.96)**
Any of the above	19.89, 22.00	**0.90 (0.87-0.94)**	20.02, 23.32	**0.86 (0.83-0.89)**
Negative control outcomes		0.94 (0.87-1.01)		**0.91 (0.84-0.99)**

Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

B: with prior neuropsychiatric diagnosis				
	Male		Female	
	BP-CCB	Amlodipine	BP-CCB	Amlodipine
Cohort size (n)	9,540	9,540	13,378	13,378
Age at index (y, SD)	59.4 (13.6)	59.6 (13.1)	55.8 (18.0)	55.5 (16.5)
Sex (M:F %)	100 : 0	100 : 0	0 : 100	0 : 100
Race[a] (W,B,O %)	61, 25, 14	60, 25, 15	53, 32, 15	53, 32, 15
Blood pressure	139/79	140/80	137/78[b]	138/79[b]
Body mass index (SD)	30.2 (6.3)	30.3 (6.5)	31.4 (7.7)	31.1 (7.6)
Outcomes	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Psychotic disorder	2.52, 2.63	0.96 (0.80-1.14)	2.16, 2.53	**0.86 (0.73-0.99)**
Schizophrenia	1.30, 1.45	0.90 (0.71-1.14)	1.07, 1.10	0.97 (0.77-1.22)
Affective disorder	23.55, 22.99	1.03 (0.97-1.08)	35.98, 36.75	0.98 (0.95-1.01)
Bipolar disorder	2.85, 2.87	0.99 (0.84-1.17)	4.41, 4.44	0.99 (0.89-1.11)
Major depressive disorder	20.74, 19.91	1.04 (0.99-1.10)	32.20, 32.82	0.98 (0.95-1.02)
Anxiety disorder	21.13, 20.89	1.01 (0.96-1.07)	33.88, 35.71	**0.95 (0.92-0.98)**
Sleep disorder	35.03, 34.55	1.01 (0.98-1.05)	27.62, 29.26	**0.94 (0.91-0.98)**
Substance use disorder	27.63, 27.78	0.99 (0.95-1.04)	21.45, 22.67	**0.95 (0.90-0.99)**
Delirium	3.37, 3.62	0.93 (0.80-1.08)	3.21, 3.21	1.00 (0.88-1.14)
Dementia	2.81, 3.23	0.87 (0.74-1.02)	3.87, 3.42	**1.13 (1.00-1.28)**
Movement disorder	6.02, 5.66	1.06 (0.95-1.19)	5.05, 5.14	0.98 (0.89-1.09)
Any of the above	69.73, 69.84	0.99 (0.98-1.02)	70.91, 71.74	0.99 (0.97-1.00)
Negative control outcomes		0.94 (0.86-1.01)		**0.93 (0.87-0.98)**

Cohorts extensively propensity score-matched as described above.[a]W: white, B: black or African American, O: other or not known. All variables matched except: [b]SD = 0.11 diastolic. Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

Table 3.7 BP-CCBs versus amlodipine, subdivided by age.

A: no prior neuropsychiatric diagnosis				
	18-60		61-90	
	BP-CCB	Amlodipine	BP-CCB	Amlodipine
Cohort size (n)	17,877	17,877	37,481	37,481
Age at index (y, SD)	37.2 (10.4)	36.6 (11.7)	68.1 (10.0)	68.2 (10.0)
Sex (M:F %)	31 : 69	34 : 66	46 : 54	47 : 53
Race[a] (W,B,O %)	41, 38, 21	41, 39, 20	51, 29, 20	50, 29, 21
Blood pressure	132/80[b]	134/81[b]	139/75	140/76
Body mass index (SD)	31.0 (7.3)	30.3 (7.7)	29.7 (6.1)	29.9 (6.0)
Outcomes	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Psychotic disorder	0.26, 0.45	**0.57 (0.40-0.82)**	0.34, 0.44	**0.76 (0.61-0.96)**
Schizophrenia	0.12, 0.20	**0.58 (0.34-0.99)**	0.09, 0.09	1.06 (0.66-1.71)
Affective disorder	6.72, 8.30	**0.81 (0.75-0.87)**	5.32, 5.98	**0.89 (0.84-0.94)**
Bipolar disorder	0.51, 0.81	**0.63 (0.48-0.82)**	0.26, 0.26	1.00 (0.76-1.32)
Major depressive disorder	6.11, 7.48	**0.82 (0.76-0.88)**	4.86, 5.45	**0.89 (0.84-0.95)**
Anxiety disorder	8.49, 10.35	**0.82 (0.77-0.88)**	5.59, 6.72	**0.83 (0.79-0.88)**
Sleep disorder	7.11, 9.91	**0.72 (0.67-0.77)**	8.35, 9.46	**0.88 (0.84-0.92)**
Substance use disorder	5.07, 6.71	**0.76 (0.69-0.82)**	4.54, 5.27	**0.86 (0.81-0.92)**

Delirium	0.58, 0.81	**0.72 (0.56-0.92)**	0.99, 1.28	**0.77 (0.67-0.88)**
Dementia	0.06, 0.06	1.00 (0.42-2.40)	1.34, 1.75	**0.77 (0.68-0.86)**
Movement disorder	0.58, 0.88	**0.66 (0.51-0.84)**	1.35, 1.66	**0.81 (0.72-0.91)**
Any of the above	20.12, 24.9	**0.81 (0.78-0.84)**	20.08, 22.37	**0.90 (0.87-0.92)**
Negative control outcomes		**0.85 (0.71-0.99)**		0.92 (0.84-1.00)

B: with prior neuropsychiatric diagnosis				
		18-60		61-90
	BP-CCB	Amlodipine	BP-CCB	Amlodipine
Cohort size (n)	8,914	8,914	16,503	16,503
Age at index (y, SD)	39.1 (10.1)	38.6 (10.8)	66.8 (9.7)	66.9 (9.8)
Sex (M:F %)	33 : 67	35 : 65	46 : 54	55 : 45
Race[a] (W,B,O %)	49, 34, 17	49, 34, 17	58, 25, 17	58, 26, 16
Blood pressure	134/81[c]	136/83[c]	139/76	141/77
Body mass index (SD)	32.3 (7.6)	31.8 (7.8)	30.1 (6.6)	30.3 (6.7)
Outcomes	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Psychotic disorder	2.20, 2.79	**0.79 (0.65-0.95)**	2.26, 2.42	0.93 (0.81-1.07)
Schizophrenia	1.23, 1.26	0.98 (0.76-1.28)	1.03, 1.06	0.97 (0.79-1.20)
Affective disorder	35.18, 36.46	0.97 (0.93-1.00)	28.74, 28.15	1.02 (0.99-1.06)
Bipolar disorder	5.61, 5.90	0.95 (0.84-1.07)	2.61, 2.27	**1.15 (1.01-1.32)**
Major depressive disorder	31.09, 31.43	0.99 (0.95-1.03)	25.72, 25.44	1.01 (0.97-1.05)
Anxiety disorder	36.68, 37.90	0.97 (0.93-1.01)	25.23, 25.75	0.98 (0.94-1.02)
Sleep disorder	26.77, 30.19	**0.89 (0.85-0.93)**	32.24, 31.63	1.02 (0.99-1.05)
Substance use disorder	27.49, 28.74	0.96 (0.91-1.00)	21.15, 21.02	1.01 (0.97-1.05)
Delirium	2.33, 2.73	0.86 (0.71-1.03)	3.60, 3.79	0.95 (0.85-1.06)
Dementia	0.24, 0.26	0.91 (0.51-1.65)	4.91, 5.16	0.95 (0.87-1.05)
Movement disorder	2.79, 2.78	1.00 (0.84-1.19)	6.70, 6.59	1.02 (0.94-1.10)
Any of the above	70.35, 72.56	**0.97 (0.95-0.99)**	70.14, 69.64	1.01 (0.99-1.02)
Negative control outcomes		0.89 (0.74-1.03)		0.97 (0.91-1.03)

Cohorts extensively propensity score-matched as described above. [a]W: white, B: black or African American, O: other or not known. All variables matched except: [b]SD = 0.11 diastolic and [c]SD = 0.15. Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

3.3.3 Secondary analyses

3.3.3.1 BP-CCBs versus verapamil/diltiazem

Analyses comparing BP-CCBs with patients prescribed either verapamil or diltiazem are shown in Tables 3.8 and 3.9. Patients without prior neuropsychiatric diagnoses had lower overall risk ratios in the BP-CCB group (RR = 0.89 [0.87-0.91]). Patients with prior neuropsychiatric diagnoses had marginally lower overall risk ratios in the BP-CCB group (RR= 0.98 [0.97-0.99]). The exception was dementia, for which BP-CCBs showed greater incidence compared with verapamil/diltiazem for analyses both with (RR = 1.17 [1.08-1.27]) and without (RR = 1.19 [1.07-1.34]) prior neuropsychiatric diagnoses.

Table 3.8 Baseline demographics for matched BP-CCB versus verapamil/diltiazem cohorts. A: patients with no prior neuropsychiatric diagnosis. B: patients with a prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis		B: with prior neuropsychiatric diagnosis	
	BP-CCB	Verapamil or diltiazem	BP-CCB	Verapamil or diltiazem
Cohort size (n)	57,522	57,522	32,475	32,475
Age at index (y, SD)	60.6 (17)	61.3 (15.1)	58.9 (15.9)	59.1 (14.3)
Sex (M:F %)	44 : 56	45 : 55	44 : 56	43 : 57
Race[a] (W,B,O %)	54, 24, 22	52, 24, 24	56, 25, 19	55, 26, 19
Blood pressure	138[b]/77	136 [b] /77	140[c]/78	135[c]/77
Body mass index (SD)	29.8 (6.6)	29.9 (6.7)	30.9 (7.1)	31.0 (7.4)
Prior psychotic disorder (%)	0	0	3.2	3.2
Prior affective disorder (%)	0	0	34.1	33.5
Prior anxiety disorder (%)	0	0	33.8	33.9
Prior substance use disorder (%)	0	0	30.9	30.5
Prior sleep disorder (%)	0	0	3.0	3.1
Prior delirium (%)	0	0	4.1	4.1
Prior dementia (%)	0	0	3.6	4.2
Prior movement disorder (%)	0	0	6.1	6.0

Cohorts extensively propensity score-matched as described above. Group B percentages add up to > 100% as patients may have more than one diagnosis. [a]W: white, B: black or African American, O: other or not known. [b]Standard difference = 0.11 (Systolic BP), [c]Standard difference = 0.22 (Systolic BP). Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

Table 3.9 Outcomes for BP-CCBs versus verapamil/diltiazem.

Percentage with each diagnosis during exposure period and the risk ratio. A: patients with no prior neuropsychiatric diagnosis. B: patients with a prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis			B: with prior neuropsychiatric diagnosis		
	BP-CCB (%)	Verapamil or diltiazem (%)	Risk ratio (95% CI)	BP-CCB (%)	Verapamil or diltiazem (%)	Risk ratio (95% CI)
Psychotic disorder	0.29	0.36	0.83 (0.68-1.02)	2.72	2.65	1.03 (0.94-1.13)
Schizophrenia	0.09	0.11	0.87 (0.60-1.26)	1.26	1.13	1.11 (0.97-1.28)
Affective disorder	5.95	6.20	**0.96 (0.92-0.99)**	32.49	31.75	**1.02 (1.00-1.05)**
Bipolar disorder	0.39	0.4	0.97 (0.81-1.17)	3.86	3.70	1.04 (0.97-1.13)
Major depressive disorder	5.37	5.59	0.96 (0.92-1.01)	29.01	28.44	1.02 (0.99-1.05)
Anxiety disorder	6.67	7.48	**0.89 (0.86-0.93)**	29.86	31.03	**0.96 (0.94-0.99)**

Sleep disorder	8.15	11.13	**0.73 (0.71-0.76)**	31.95	37.21	**0.86 (0.84-0.88)**
Substance use disorder	4.65	4.60	1.01 (0.96-1.06)	24.79	25.01	0.99 (0.97-1.02)
Delirium	1.01	1.13	**0.90 (0.80-0.99)**	4.40	4.18	1.05 (0.98-1.13)
Dementia	1.13	0.94	**1.19 (1.07-1.34)**	4.00	3.42	**1.17 (1.08-1.27)**
Movement disorder	1.21	1.36	**0.89 (0.81-0.98)**	5.99	6.09	0.99 (0.93-1.05)
Any of the above	20.36	22.88	**0.89 (0.87-0.91)**	71.84	73.20	**0.98 (0.97-0.99)**
Negative control outcomes [a]			0.98 (0.91-1.04)			**0.94 (0.89-0.99)**

[a] Mean of 12 negative control outcomes. Full details in Table 3.13.
Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

3.3.3.2 BP-CCBs versus ARBs for first onset neuropsychiatric disorder

Analyses comparing BP-CCBs with ARBs are shown in Tables 3.10 and 3.11. Results for patients with no prior neuropsychiatric diagnosis were variable. Some diagnoses were more common in those prescribed BP-CCBs (e.g. psychotic disorder, RR = 1.33 [1.06-1.67], substance use disorder, RR = 1.26 [1.19-1.33], and dementia, RR = 1.28 [1.12-1.50]), whilst others are more common with ARBs (e.g. anxiety disorders, RR = 0.87 [0.83-0.91], sleep disorders, RR = 0.71 [0.68-0.74], and movement disorders, RR = 0.88 [0.78-0.99]). Overall, BP-CCBs were associated with a modestly lower risk than ARBs for any first neuropsychiatric diagnosis (RR = 0.92 [0.90-0.95]).

3.3.3.3 BP-CCBs versus ARBs for recurrent neuropsychiatric disorder

Results for patients with prior neuropsychiatric diagnosis showed no overall difference between BP-CCBs and ARBs (RR = 1.01 [0.99-1.02]), but some disorders were more common with BP-CCBs (e.g. psychotic disorder, RR = 1.28 [1.16-1.42], affective disorder, RR = 1.08 [1.05-1.10], substance use disorder, RR = 1.12 [1.09-1.16], delirium, RR = 1.43 [1.31-1.56] and dementia, RR = 1.31 [1.19-1.44]), and sleep disorder was less common (RR = 0.85 [0.83-0.88]).

Table 3.10 Baseline demographics for matched BP-CCB versus ARB cohorts.

A: patients with no prior neuropsychiatric diagnosis. B: patients with a prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis		B: with prior neuropsychiatric diagnosis	
	BP-CCB	ARB	BP-CCB	ARB
Cohort size (n)	46,229	46,229	25,365	25,365
Age at index (y, SD)	56.6 (18.5)	57.6 (16.6)	56.1 (16.5)	56.6 (14.0)
Sex (M:F %)	42 : 58	43 : 57	44 : 56	44 : 56
Race[a] (W,B,O %)	47, 33, 20	45, 34, 21	52, 31, 17	50, 33, 17
Blood pressure	138/78	137/78	139/79	137/79
Body mass index (SD)	29.7 (6.9)	29.8 (7.0)	30.3 (7.1)	31.0 (7.3)
Prior psychotic disorder (%)	0	0	3.7	3.9
Prior affective disorder (%)	0	0	35.0	35.1
Prior anxiety disorder (%)	0	0	34.2	34.0
Prior substance use disorder (%)	0	0	36.0	36.7
Prior sleep disorder (%)	0	0	29.1	29.7
Prior delirium (%)	0	0	4.2	4.1
Prior dementia (%)	0	0	2.9	4.0
Prior movement disorder (%)	0	0	5.4	5.4

Cohorts extensively propensity score-matched as described above. Group B percentages add up to > 100% as patients may have more than one diagnosis. [a]W: white, B: black or African American, O: other or not known. Abbreviations: ARB = angiotensin receptor blocker, BP-CCB = brain penetrant calcium channel blocker.

Table 3.11 Outcomes for BP-CCBs versus ARBs; percentage with each diagnosis during exposure period and the risk ratio.

A: no prior neuropsychiatric diagnosis. B: with prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis			B: with prior neuropsychiatric diagnosis		
	BP-CCB (%)	ARB (%)	Risk ratio (95% CI)	BP-CCB (%)	ARB (%)	Risk ratio (95% CI)
Psychotic disorder	0.37	0.28	**1.33 (1.06-1.67)**	3.13	2.44	**1.28 (1.16-1.42)**
Schizophrenia	0.12	0.08	1.39 (0.92-2.09)	1.56	1.11	**1.40 (1.21-1.63)**
Affective disorder	5.95	6.09	0.98 (0.93-1.03)	32.90	30.60	**1.08 (1.05-1.10)**
Bipolar disorder	0.37	0.32	1.15 (0.93-1.44)	4.39	3.36	**1.31 (1.20-1.43)**
Major depressive disorder	5.40	5.52	0.98 (0.93-1.03)	29.20	27.28	**1.07 (1.04-1.10)**
Anxiety disorder	6.59	7.58	**0.87 (0.83-0.91)**	30.18	29.54	1.02 (0.99-1.05)
Sleep disorder	7.26	10.23	**0.71 (0.68-0.74)**	28.14	33.03	**0.85 (0.83-0.88)**
Substance use disorder	5.18	4.11	**1.26 (1.19-1.33)**	28.79	25.65	**1.12 (1.09-1.16)**
Delirium	1.04	0.98	1.06 (0.93-1.20)	4.50	3.16	**1.43 (1.31-1.56)**
Dementia	1.05	0.82	**1.28 (1.12-1.50)**	3.80	2.90	**1.31 (1.19-1.44)**
Movement disorder	1.14	1.30	**0.88 (0.78-0.99)**	5.49	5.17	1.06 (0.99-1.14)

Any of the above	20.00	21.64	**0.92 (0.90-0.95)**	71.68	70.85	1.01 (0.99-1.02)
Negative control outcomes [a]			0.94 (0.81-1.07)			0.99 (0.81-1.17)

[a] Mean of 12 negative control outcomes. Full details in Table 3.14. Abbreviations: ARB = angiotensin receptor blocker, BP-CCB = brain penetrant calcium channel blocker.

3.3.4 Negative control outcomes

Incidence of NCOs were lower for BP-CCBs than comparator cohorts, with some differences being significant (see Tables 3.12-3.14). This did not reflect less healthcare use during the exposure period, as the number of clinic visits and hospital admissions in the BP-CCB cohorts were either similar to, or greater than, the comparators (data not shown).

Table 3.12 NCOs for BP-CCBs versus amlodipine.

Percentage with each diagnosis during exposure period and risk ratio. A: patients with no prior neuropsychiatric diagnosis. B: patients with a prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis			B: with prior neuropsychiatric diagnosis		
	BP-CCB (%)	Amlodipine (%)	Risk ratio (95% CI)	BP-CCB (%)	Amlodipine (%)	Risk ratio (95% CI)
Benign colonic polyp	1.50	1.77	**0.85 (0.77-0.93)**	2.22	2.52	**0.88 (0.78-0.99)**
Ganglion	0.31	0.38	**0.80 (0.65-0.98)**	0.57	0.68	0.85 (0.67-1.07)
Hallux valgus	0.53	0.69	**0.77 (0.66-0.91)**	0.90	1.13	**0.80 (0.67-0.96)**
Hernia	3.91	4.05	0.97 (0.91-1.03)	7.47	6.81	**1.10 (1.03-1.17)**
Ingrowing nail	0.49	0.58	**0.84 (0.71-0.99)**	1.04	1.02	1.03 (0.86-1.23)
Sebaceous cyst	0.63	0.73	**0.86 (0.74-0.99)**	1.01	1.23	**0.82 (0.69-0.98)**
Senile keratosis	1.80	1.94	0.93 (0.85-1.01)	2.35	2.41	0.98 (0.87-1.10)
Trigger finger	0.63	0.72	0.88 (0.75-1.02)	1.25	1.39	0.90 (0.77-1.05)
Otalgia	0.83	1.00	**0.83 (0.73-0.95)**	2.06	2.20	0.94 (0.83-1.06)
Onycholysis	0.31	0.27	1.15 (0.92-1.44)	0.46	0.49	0.95 (0.73-1.23)
Viral warts	0.55	0.60	0.92 (0.78-1.08)	0.99	1.01	0.98 (0.82-1.17)
Cutaneous abscess	1.18	1.29	0.91 (0.82-1.02)	2.89	3.08	0.94 (0.84-1.04)
Average			**0.89 (0.83-0.96)**			**0.93 (0.88-0.99)**

Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

Table 3.13 NCOs for BP-CCBs versus verapamil or diltiazem.

Percentage with each diagnosis during exposure period and risk ratio. A: patients with no prior neuropsychiatric diagnosis. B: patients with a prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis			B: with prior neuropsychiatric diagnosis		
	BP-CCB (%)	Verapamil or diltiazem (%)	Risk ratio (95% CI)	BP-CCB (%)	Verapamil or diltiazem (%)	Risk ratio (95% CI)
Benign colonic polyp	1.83	2.02	**0.91 (0.84-0.98)**	2.50	2.92	**0.86 (0.78-0.94)**
Ganglion	0.31	0.34	0.90 (0.73-1.10)	0.56	0.70	**0.81 (0.67-0.98)**
Hallux valgus	0.51	0.54	0.93 (0.79-1.09)	1.05	1.13	0.93 (0.80-1.08)
Hernia	4.28	4.32	0.99 (0.94-1.05)	7.97	8.22	0.97 (0.92-1.02)
Ingrowing nail	0.54	0.49	1.10 (0.94-1.30)	1.17	1.20	0.98 (0.85-1.12)
Sebaceous cyst	0.66	0.78	**0.84 (0.74-0.97)**	1.12	1.29	0.87 (0.76-1.00)
Senile keratosis	2.17	2.24	0.97 (0.90-1.05)	2.70	2.65	1.02 (0.93-1.12)
Trigger finger	0.63	0.71	0.89 (0.77-1.02)	1.25	1.45	**0.86 (0.75-0.98)**
Otalgia	0.91	1.00	0.91 (0.80-1.02)	2.13	2.26	0.94 (0.85-1.05)
Onycholysis	0.32	0.28	1.16 (0.94-1.43)	0.56	0.51	1.10 (0.89-1.35)
Viral warts	0.64	0.63	1.02 (0.88-1.18)	1.08	1.11	0.98 (0.85-1.13)
Cutaneous abscess	1.23	1.12	1.10 (0.99-1.23)	3.15	3.34	0.94 (0.87-1.03)
Average			0.98 (0.91-1.04)			**0.94 (0.89-0.99)**

Abbreviations: BP-CCB = brain penetrant calcium channel blocker.

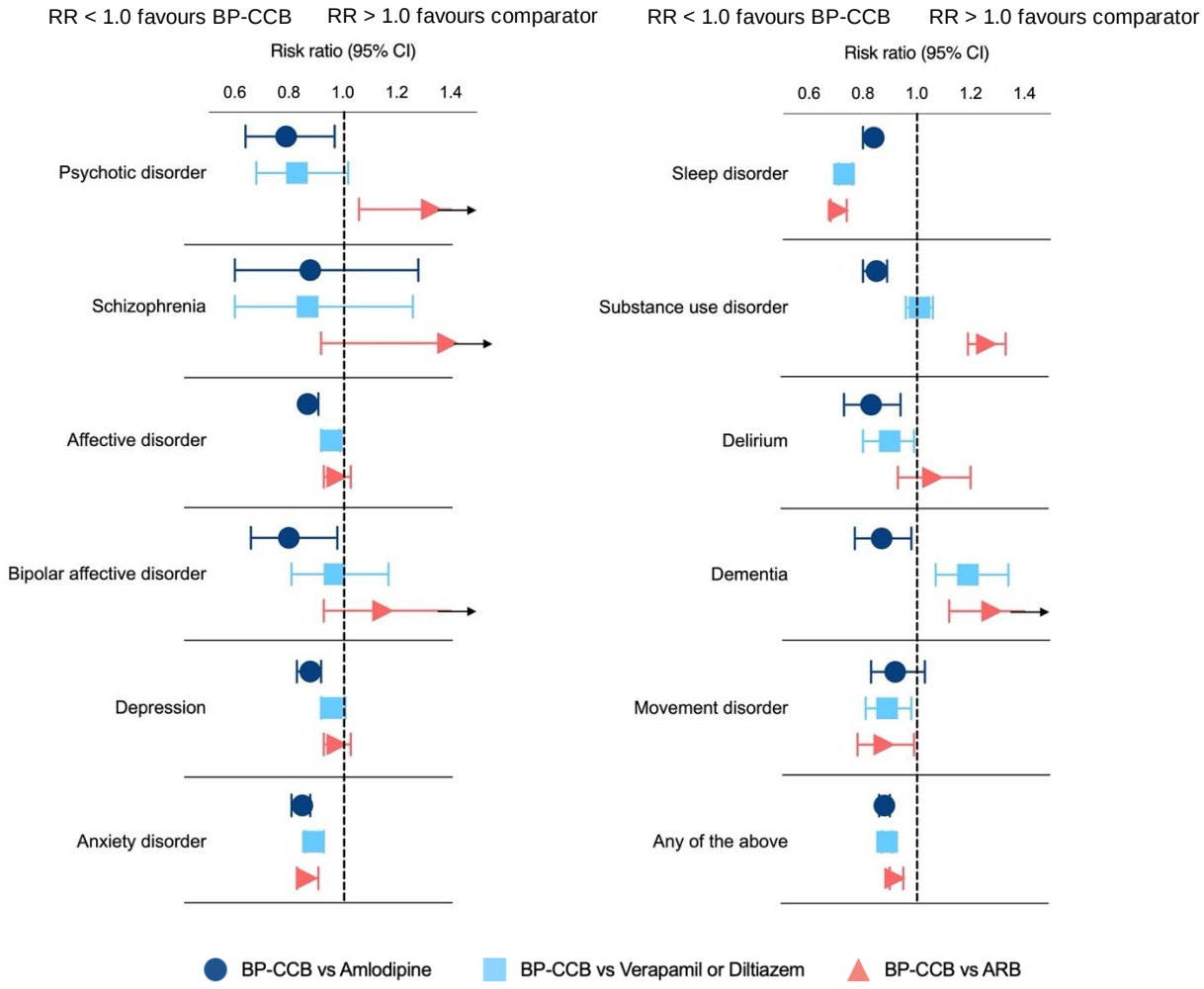
Table 3.14 NCOs for BP-CCBs versus ARBs.

Percentage with each diagnosis during exposure period and risk ratio. A: patients with no prior neuropsychiatric diagnosis. B: patients with a prior neuropsychiatric diagnosis.

	A: no prior neuropsychiatric diagnosis			B: with prior neuropsychiatric diagnosis		
	BP-CCB (%)	ARB (%)	Risk ratio (95% CI)	BP-CCB (%)	ARB (%)	Risk ratio (95% CI)
Benign colonic polyp	1.63	1.56	1.04 (0.94-1.15)	2.41	2.50	0.96 (0.86-1.08)
Ganglion	0.29	0.43	**0.69 (0.56-0.86)**	0.50	0.74	**0.66 (0.53-0.83)**
Hallux valgus	0.48	0.55	0.87 (0.73-1.04)	0.96	1.10	0.87 (0.74-1.04)
Hernia	3.90	3.78	1.03 (0.97-1.10)	7.60	7.21	1.06 (0.99-1.12)
Ingrowing nail	0.54	0.58	0.92 (0.78-1.10)	1.08	1.16	0.93 (0.79-1.09)
Sebaceous cyst	0.60	0.72	**0.83 (0.71-0.98)**	1.04	1.34	**0.78 (0.66-0.91)**
Senile keratosis	1.75	1.91	0.92 (0.84-1.01)	2.32	2.46	0.94 (0.84-1.05)
Trigger finger	0.54	0.84	**0.65 (0.55-0.76)**	1.08	1.57	**0.68 (0.59-0.80)**
Otalgia	0.90	1.15	**0.78 (0.68-0.88)**	2.06	2.11	0.97 (0.86-1.10)
Onycholysis	0.35	0.26	**1.36 (1.08-1.73)**	0.67	0.39	**1.71 (1.34-2.19)**
Viral warts	0.59	0.57	1.04 (0.88-1.23)	1.04	0.92	1.12 (0.94-1.34)
Cutaneous abscess	1.29	1.14	**1.14 (1.01-1.28)**	3.38	2.78	1.21 (1.10-1.34)
Average			0.94 (0.81-1.07)			0.99 (0.81-1.17)

Abbreviations: ARB = angiotensin receptor blocker, BP-CCB = brain penetrant calcium channel blocker.

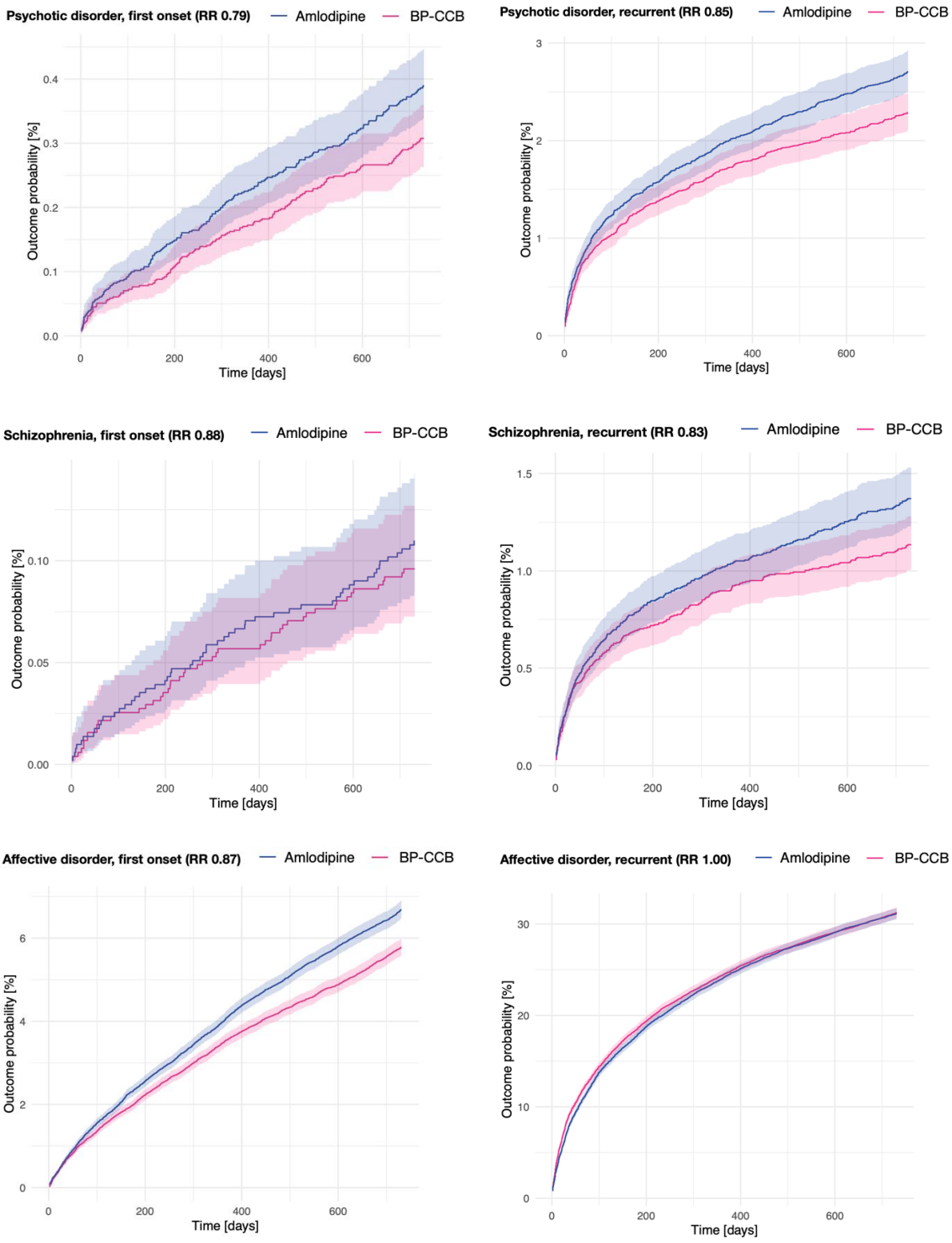
Figure 3.1 Incidence of first onset neuropsychiatric disorder during a two-year exposure period. Results are shown as risk ratios with 95% CIs, as per data in Tables 3.5, 3.9 and 3.11.

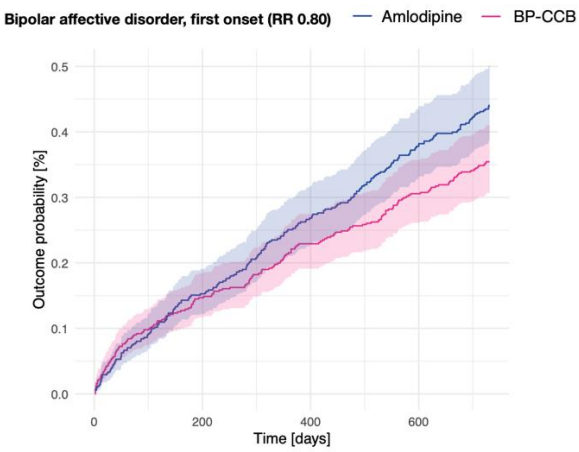


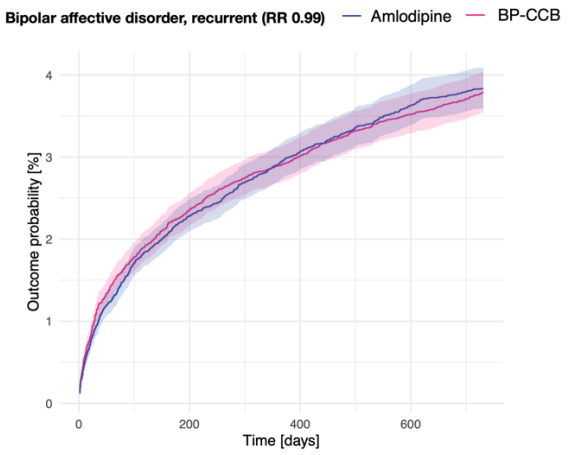
Abbreviations: ARB = angiotensin receptor blocker, BP-CCB = brain penetrant calcium channel blocker.

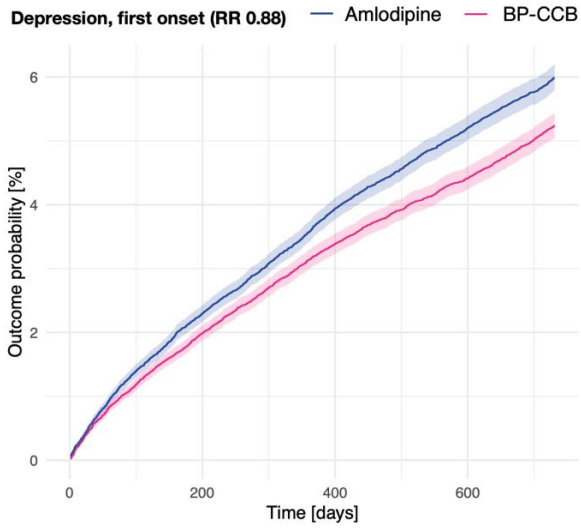
Figure 3.2 Kaplan–Meier curves, emergence of first onset and recurrent neuropsychiatric disorder for BP-CCBs versus amlodipine.

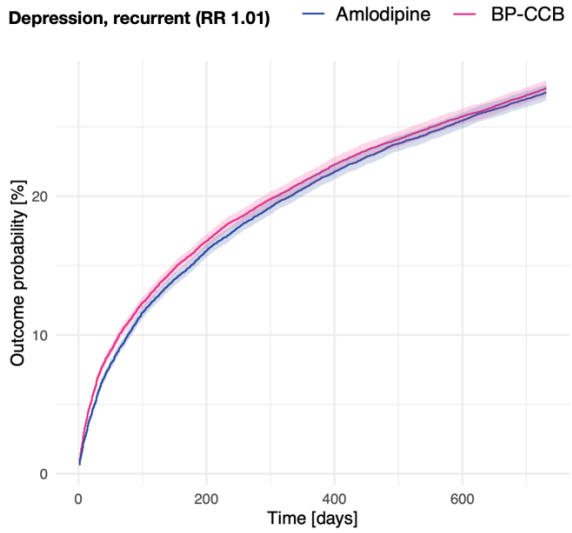
Shaded areas are 95% CIs.

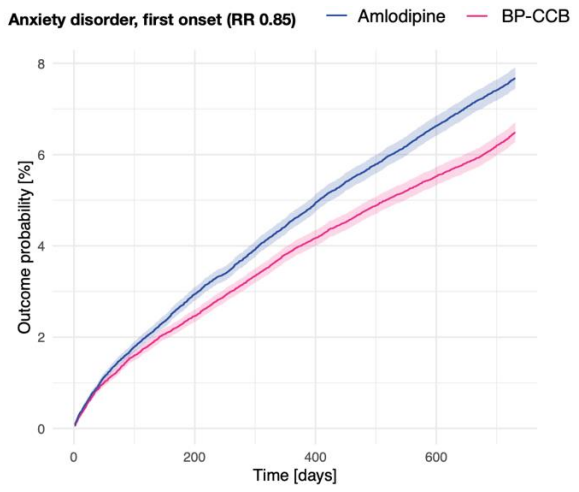


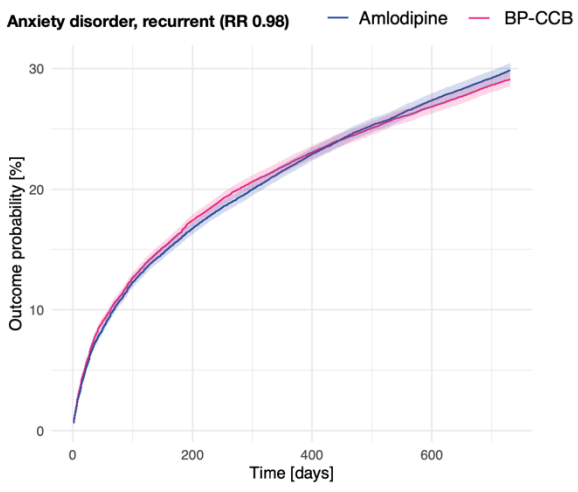

Bipolar affective disorder, first onset (RR 0.80)
Amlodipine
BP-CCB
Outcome probability [%]
0.0
0.1
0.2
0.3
0.4
0.5
0
200
400
600
Time [days]

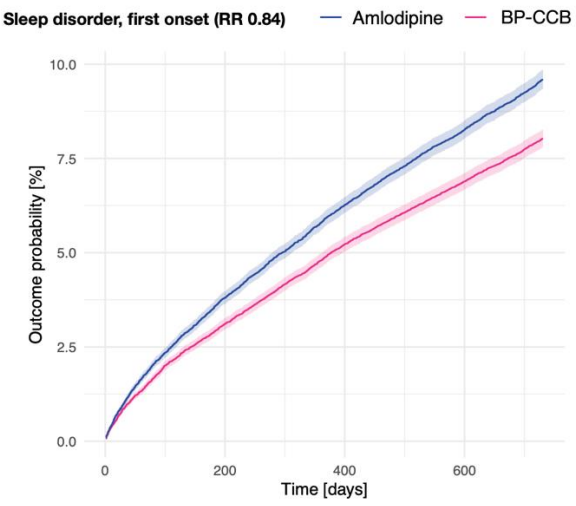

Bipolar affective disorder, recurrent (RR 0.99)
Amlodipine
BP-CCB
Outcome probability [%]
0
1
2
3
4
0
200
400
600
Time [days]

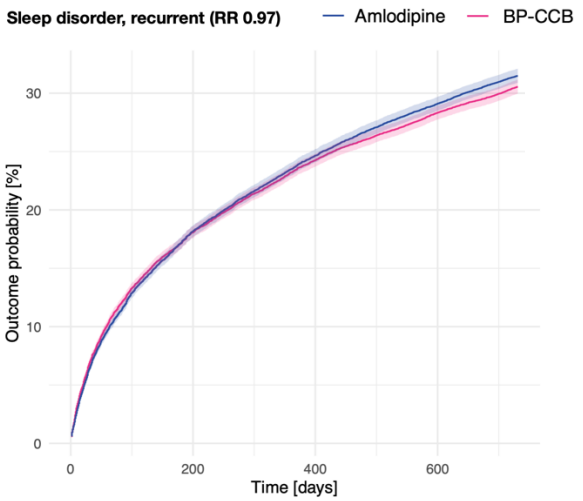

Depression, first onset (RR 0.88)
Amlodipine
BP-CCB
Outcome probability [%]
0
2
4
6
0
200
400
600
Time [days]

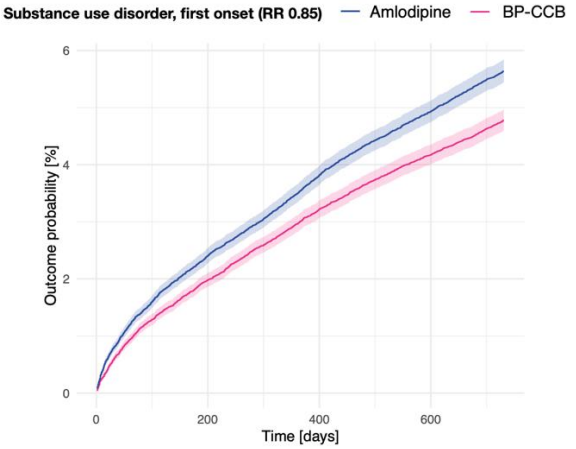

Depression, recurrent (RR 1.01)
Amlodipine
BP-CCB
Outcome probability [%]
0
10
20
0
200
400
600
Time [days]

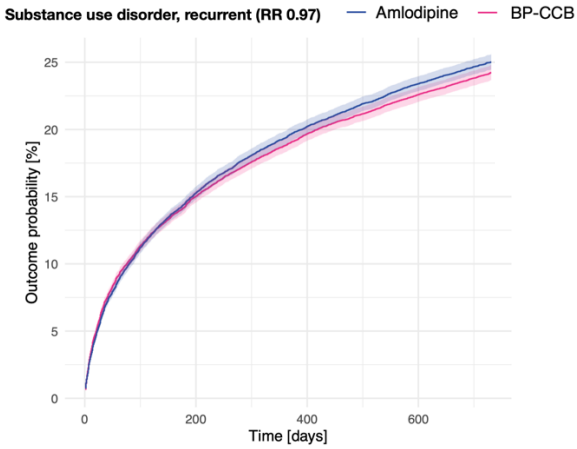

Anxiety disorder, first onset (RR 0.85)
Amlodipine
BP-CCB
Outcome probability [%]
0
2
4
6
8
0
200
400
600
Time [days]

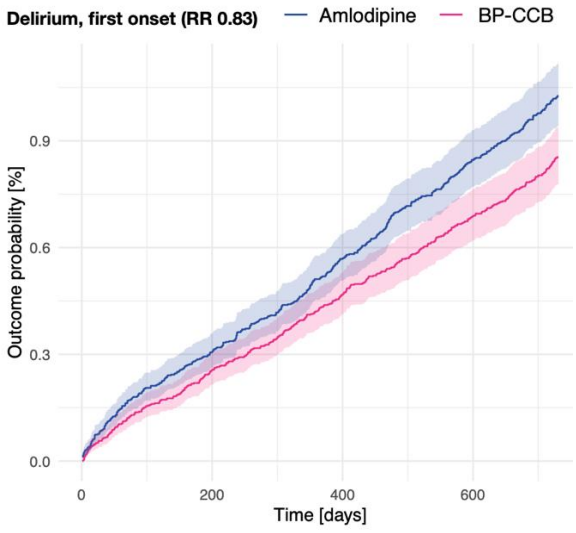

Anxiety disorder, recurrent (RR 0.98)
Amlodipine
BP-CCB
Outcome probability [%]
0
10
20
30
0
200
400
600
Time [days]

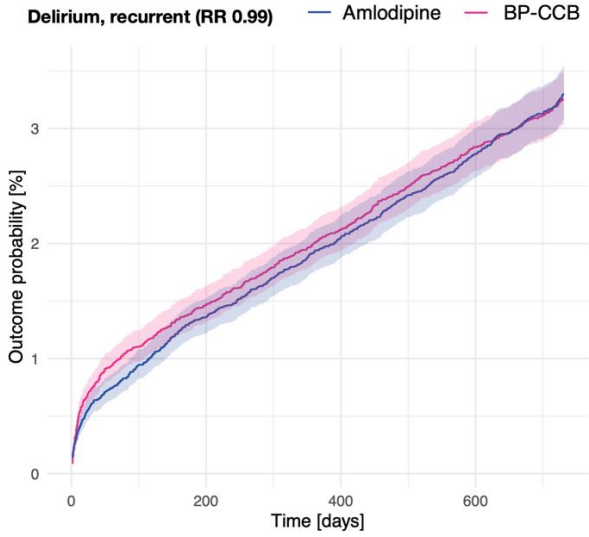

Sleep disorder, first onset (RR 0.84)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]

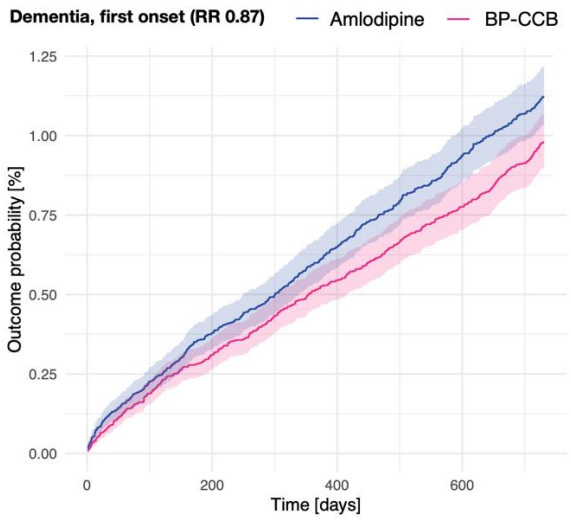

Sleep disorder, recurrent (RR 0.97)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]

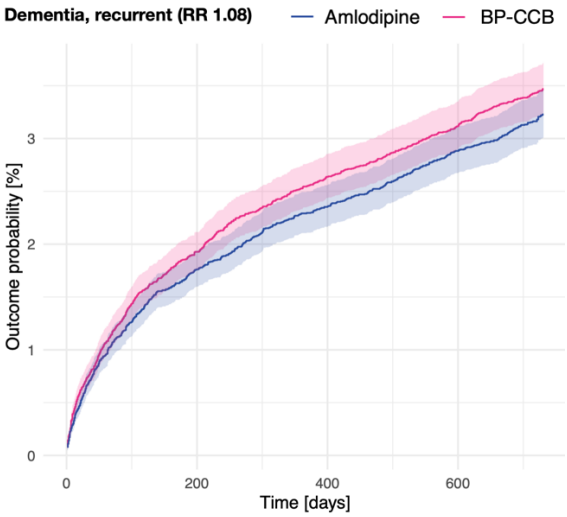

Substance use disorder, first onset (RR 0.85)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]

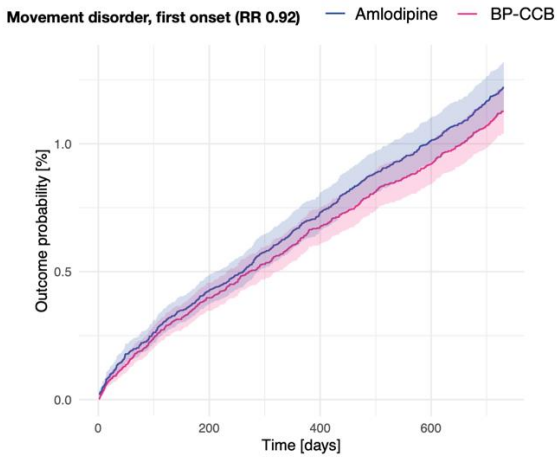

Substance use disorder, recurrent (RR 0.97)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]

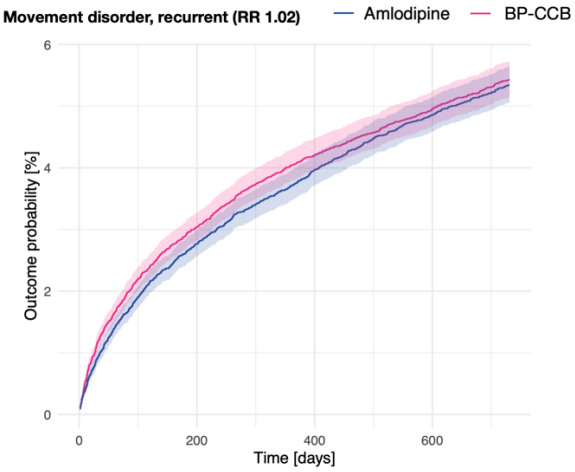

Delirium, first onset (RR 0.83)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]

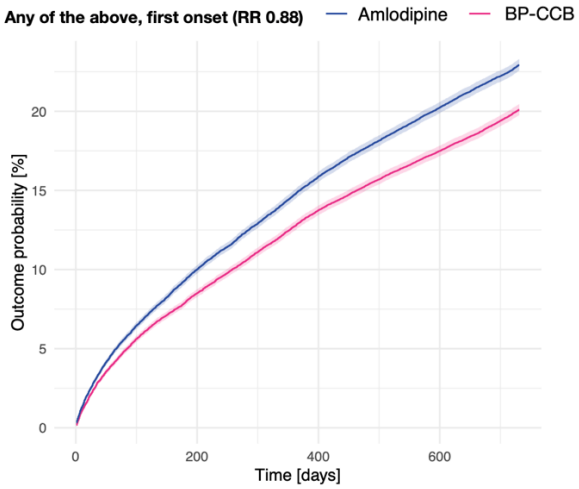

Delirium, recurrent (RR 0.99)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]

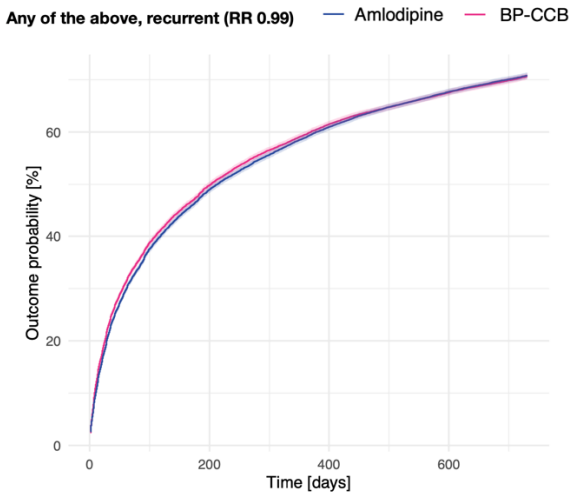

Dementia, first onset (RR 0.87)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]


Dementia, recurrent (RR 1.08)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]


Movement disorder, first onset (RR 0.92)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]


Movement disorder, recurrent (RR 1.02)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]


Any of the above, first onset (RR 0.88)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]


Any of the above, recurrent (RR 0.99)
Amlodipine
BP-CCB
Outcome probability [%]
Time [days]

3.4 Discussion

This study largely replicates what was found in an earlier dataset (Colbourne & Harrison, 2022). Compared with amlodipine, BP-CCBs were associated with lower incidence of first onset neuropsychiatric disorder over a two-year period. Incidence of subsequent disorder, in patients with existing conditions, varied depending on diagnosis. There were no group differences for recurrent neurodegenerative or affective disorders, however other disorders demonstrated lower incidence with BP-CCBs. These first-onset and recurrent results were more marked in women and people under 60 years old. Results for BP-CCBs versus verapamil and diltiazem were similar, with reduced incidence of first onset and recurrent disorder in the BP-CCB group. BP-CCBs also demonstrated lower overall incidence of first onset psychiatric and neurodegenerative disorder compared to ARBs. However, individual risk ratios varied with specific diagnoses, and there were no overall group differences for recurrent disorders. Taken together, these findings suggest BP-CCBs confer neuropsychiatric benefits compared to non-penetrant drugs, and indicate their greatest impact is in reducing the onset of disease, as opposed to influencing disease progression.

A possible explanation of the results is that BP-CCBs exert their positive neuropsychiatric effects through central VGCC binding. VGCCs in the brain are known to have an important role in synaptic plasticity (Alves et al., 2019; Berridge, 2014; Higley & Sabatini, 2012; Nanou & Catterall, 2018), learning and memory, and growing evidence implicates these channels in the pathophysiology of neuropsychiatric disorders (Berridge, 2014; Casamassima et al., 2010; Dolphin, 2016; Harrison, Tunbridge, et al., 2020; Heyes et al., 2015; Zamponi et al., 2015). However, at currently licensed doses, it is unknown whether CCBs reach sufficient VGCC occupancy in the brain to exhibit significant effects (Spedding & Middlemiss, 1985; Triggle, 2007). An fMRI study by Zink and colleagues suggests they may (Zink et al., 2020), however more research is needed (Atkinson et al., 2019).

Although results closely match the earlier dataset (Colbourne & Harrison, 2022), there are a few key differences. In the published primary analysis of first onset neuropsychiatric disorder, incidence of schizophrenia was lower for BP-CCBs versus amlodipine. In contrast, the current study found no difference between the groups. However, the larger psychotic disorder cohorts for both datasets showed lower incidence with BP-CCBs. Differences were also noted for bipolar disorder, which showed equivocal risk in the published dataset, but lower incidence for BP-CCBs in the present study. However again, larger affective disorder cohorts showed lower risk for BP-CCBs compared with amlodipine in both published and present analyses. Risk ratios for first onset movement disorder also differed between the studies. In the published

paper, incidence of movement disorder was lower for BP-CCBs versus amlodipine. In contrast, the current analysis found no difference between the groups. The reason for this is unclear but may be related to disparities between the networks used. It is feasible that the two networks included some HCOs that were different from each other. HCOs in the US Collaborative Network were exclusively in the US. In contrast, while most HCOs in the Analytics Network were in the US (over ninety-three percent), this network also included HCOs in other countries. TriNetX data were aggregated, and HCOs were anonymised. Therefore, it was not possible to determine the proportion of shared HCOs, and this may have contributed to the differences seen between datasets.

Another important difference between this study and the published data is the incidence of NCOs in the primary analyses. Despite extensive use of PSM, NCOs in this dataset were lower for BP-CCBs than amlodipine in both the first-onset and recurrent disorder cohorts. Since NCOs were selected as causally unrelated outcomes, this suggests residual confounding in the main results. Additional group comparisons for visit number and hospital admissions showed BP-CCB and amlodipine rates were similar, or marginally greater, for the BP-CCB groups (data not shown). This refutes the possibility that BP-CCB patients presented less frequently with symptoms, and suggests these patients were more likely to be healthier in general than the amlodipine group. Of particular note, risk ratios for all neuropsychiatric diagnoses showed overlapping confidence intervals with the mean NCOs. This undermines interpretation of the neuropsychiatric findings and raises uncertainty over the mechanism of action of BP-CCBs in neuropsychiatric health. It is not clear what may account for this confounding, but as discussed in Chapter 2, socioeconomic differences must be considered. These subtle yet complex characteristics can be difficult to capture in EHRs, and the way data are combined and anonymised in this particular network means it is not possible to interrogate these factors further. Another explanation could be differing prescribing patterns across HCOs. Although amlodipine is the most commonly prescribed CCB in the network (accounting for over ninety per cent of DHP prescriptions) it is possible individual HCOs preferentially use different CCBs based on local practices. Alternatively, it may reflect patient demand. For example, more educated patients may request prescriptions for newer DHPs after conducting informed research. As TriNetX data are aggregated and anonymised, it is again not possible to explore this further.

The decision to include BP-CCB versus ARB comparisons was driven by existing data reporting the benefits of ARBs over CCBs as a single class. As discussed in Chapter 2, first onset affective and anxiety disorders were found to be more common with CCBs than ARBs (risk ratios 1.27 and 1.18, respectively, see section 2.3.1 for further details). However, the

present analyses showed no difference between BP-CCBs and ARBs for first-onset affective disorders, and a lower incidence of first-onset anxiety disorders. This supports the benefit of BP-CCBs for neuropsychiatric health. The BP-CCB cohort also showed reduced risk of movement disorders, including Parkinson's disease, compared with ARBs. This supports the wider emerging literature indicating the benefits of brain-penetrant AHTs for neurodegenerative disorders compared to non-penetrant equivalents (Jo et al., 2022). BP-CCB versus ARB risk ratios for other neuropsychiatric diagnoses varied depending on disorder. BP-CCBs showed greater risk of first-onset psychosis and dementia, suggesting their benefits on brain health are not consistent. The reason for varying effects of BP-CCBs versus ARBs on specific disorders requires further investigation but may result from how angiotensin receptors are distributed in the brain (Jackson et al., 2018; Jo et al., 2022).

As with any observational data, the present study cannot demonstrate causation. This is particularly pertinent considering recent data studying BP-CCBs for neurodegenerative disorders. Early pharmacoepidemiology and preclinical studies (Kang et al., 2012; Liss & Striessnig, 2019) suggested potential benefits of DHPs in PD, however a recent RCT investigating the BP-CCB isradipine failed to demonstrate delay of early disease progression (Parkinson Study Group STEADY-PD III, 2020). Although these RCT results appear to invalidate the earlier research, the Parkinson Study Group findings raise further questions over the mechanism of these drugs, specifically at which disease stage they might be most effective. As the RCT authors highlight, epidemiological studies of DHP CCBs showed an overall thirty percent reduction in new PD risk, with only a single study demonstrating reduced disease progression (Becker et al., 2008; Marras et al., 2012; Pasternak et al., 2012; Ritz et al., 2010). Therefore, treatment in the prodromal phase of PD, before motor symptoms developed (Lang & Espay, 2018) may have generated more promising RCT results. This has important implications for the current data, as future trials should consider the critical importance of treatment timing, and its potential effect on neuropsychiatric outcomes.

3.4.1 Limitations

In addition to the general challenges faced in EHR research (which are discussed in detail in Chapter 2, see section 2.4.1), there are several limitations specific to this study. First, the simplistic 'brain-penetrant' and 'non-penetrant' categories used do not adequately capture the spectrum of penetrability displayed by CCBs (Fridén et al., 2009; Liu et al., 2008). Brain permeability is not an 'all or nothing' phenomenon.

A further limitation is confounding. Despite thorough PSM, producing cohorts successfully matched for age, sex, race, and other variables, confounding by indication still presents a

concern. Clinical decisions around specific AHT selection are determined by a range of these baseline factors. Although DHP CCBs are recommended as a class, with no distinction between individual drugs, baseline criteria for prescribing other CCBs differ. Individual CCBs can also be used for different diagnostic indications, such as nimodipine for subarachnoid haemorrhage (Liu et al., 2022) and nifedipine for Prinzmetal angina (Goldberg et al., 1979). Verapamil and diltiazem are typically prescribed for angina and arrythmias, and are not indicated as a first line treatment for hypertension. Although network data exist on cohort diagnoses as a whole, this information lacks detail at the individual patient level. Therefore, it is not possible to distinguish CCB indication on a patient-by-patient basis to determine if equivalent comparisons are being made.

As discussed above, NCOs were lower for BP-CCBs compared to amlodipine, in both first-onset and recurrent analyses. The same NCO trends were also seen for recurrent analyses in the BP-CCB versus verapamil and diltiazem cohorts. This suggests extensive residual confounding (Arnold et al., 2016; Lipsitch et al., 2010) across the study, where, for unknown reasons, BP-CCB patients appear to be healthier in general than their comparator cohorts. Residual confounding in any dataset requires further investigation, and in this case undermines interpretation of the neuropsychiatric findings in both the primary and secondary analyses.

3.5 Conclusion

Although the main results show CCBs that easily penetrate the brain are linked to a lower incidence of neuropsychiatric disorder than those that do not, the interpretation of this association is undermined by the possibility of residual confounding. In view of NCO findings, it cannot be excluded that primary (BP-CCB versus amlodipine) and secondary (BP-CCB versus verapamil and diltiazem) analyses are in fact explained by unmeasured variables, rather than a true effect of BP-CCBs. Several theories may account for this confounding, but the nature of these variables remains unknown. Consequently, robust conclusions cannot be drawn from this data regarding psychiatric risk for penetrant versus non-penetrant CCBs. BP-CCB versus ARB analyses were not subject to the same confounding, however this evidence was less compelling. Despite an overall lower incidence of neuropsychiatric disorder with BP-CCBs, first-onset risk ratios varied with specific disorders, suggesting inconsistent benefits on brain health.

It is arguable if further observational studies would provide additional insights given the size and methodological rigour of the present study. Therefore, the following chapters present an exploratory experimental study. The Oxford Study of Calcium Channel Antagonism, Cognition, Mood Instability and Sleep (OxCaMS) was a randomised, placebo-controlled study in healthy volunteers with mood instability. OxCaMS aimed to explore the effect of nicardipine, a BP-CCB, on a wide range of neuropsychiatric parameters to help further characterise any potential psychiatric benefits of BP-CCBs.

Part 2 - OxCaMS: The effect of LTCC antagonism on mood instability, cognitive function, and neural activity

Chapter 4. The mood stabilising, cognitive and physiological effects of LTCC antagonism

4.1 Introduction

Using a range of psychological and physiological parameters, the present chapter investigates the effect of LTCC antagonism in healthy young adults with mood instability. In this way it was intended to examine the role of LTCCs in mood, cognitive function, and neural activity.

While not previously explored for mood instability, LTCC antagonists have been used in bipolar disorder (BD) without clear results. One of the first LTCC antagonists suggested to be beneficial for mania, was verapamil. Several early studies advocated its use, both individually and as an adjunct to lithium (Garza-Treviño et al., 1992; Pal Singh, 2008; Wisner et al., 2002). However, a 2015 network meta-analysis of BD treatments found verapamil was not effective for mania (Yildiz et al., 2015). These findings were supported by a systematic review of CCBs in BD the following year (Cipriani et al., 2016). The latter review, which identified six double-blind RCTs investigating verapamil for acute mania, found no evidence that it is effective. However, an absence of high-quality data for other LTCC antagonists, and different phases of BD, meant definitive conclusions could not be made for CCBs as a class.

With new genetic associations, linking LTCC genes to several common psychiatric disorders (Als et al., 2023; Ferreira et al., 2008; Mullins et al., 2021; Sklar et al., 2008; Trubetskoy et al., 2022), interest in LTCCs as a therapeutic target is increasing. Notable evidence also originates from pharmacoepidemiology studies using electronic health records. These observational data, which are discussed in detail in Chapters 2 and 3, suggest CCBs may have beneficial effects on some neuropsychiatric disorders (Cao et al., 2019; Hayes et al., 2019; Kessing et al., 2020; Lintunen et al., 2022). Data from this thesis indicate CCBs, especially those which penetrate the brain, are associated with a lower incidence of first onset psychiatric diagnosis. However, these findings were affected by residual confounding, which limits any robust conclusions.

Novel clinical studies are investigating newer dihydropyridine (DHP) CCBs. An open-label pilot study of isradipine, has shown efficacy for depressive symptoms in a small number of BD participants (Ostacher et al., 2014). This supports a previous double-blind placebo-controlled

trial, which found nimodipine decreased mood fluctuations in BD (Pazzaglia et al., 1993). However, these results were also based on a small sample, in this case just eight participants. More recently, a double-blind pharmacoimaging study examined the effects of nimodipine on brain activity during an N-back task of working memory (Zink et al., 2020). Using functional magnetic resonance imaging (fMRI), healthy males genotyped for the *CACNA1C* rs1006737 risk allele, were randomised to a single dose of nimodipine or matched placebo. The nimodipine group displayed decreased frontal cortical and parietal cortical activity, with more pronounced frontal cortical decreases in carriers of the risk allele.

CCBs are also being examined as a possible adjunctive treatment for cognitive deficits in schizophrenia. One double-blind placebo-controlled RCT, studied patients with schizophrenia who were randomised to isradipine or matched placebo for six weeks. The isradipine group demonstrated improved verbal memory and attention dysfunction on some aspects of a Stroop test, compared to those taking placebo (Vahdani et al., 2020). Another open-label pilot study examined four-week adjunctive isradipine in schizophrenia patients (Burdick et al., 2020). This study found a positive effect on functional capacity, although no clear benefits on neurocognition were noted.

This cumulative evidence suggests the potentially diverse effects of LTCC antagonists on neuropsychiatric phenotypes and supports the continued evaluation of DHP CCBs for brain health. The current chapter aimed to further investigate LTCC antagonism, by examining CCB effects in healthy young adults with mood instability. Mood instability, which is common across a range of psychiatric disorders, is also prevalent in the general population. It therefore has broad potential as a therapeutic target, and functions as an analogous non-clinical population in this study.

4.1.1 LTCC antagonism and mood instability

Mood instability, which is characterised by rapid and intense fluctuations of affect (Marwaha et al., 2014), is a feature of many psychiatric disorders with which LTCCs have been linked (Broome, Saunders, et al., 2015) (see Chapter 1, section 1.9 for further discussion). As well as characterising numerous psychiatric conditions, such as BD, depression, anxiety, and schizophrenia (Høegh et al., 2020; Patel et al., 2015; Skirrow et al., 2009; Thompson et al., 2011), mood instability is also seen in healthy individuals (Marwaha et al., 2013). This supports the transdiagnostic significance of mood instability and highlights its utility as a possible treatment target. With modern developments in digital technology, mood instability can now be recorded via smartphones and tablets. Such devices can also measure physiological,

cognitive, and behavioural data. These are important correlates of mood instability (Glenn & Monteith, 2014) and could provide a novel approach for studying the wider effects of LTCC antagonism.

4.1.2 LTCC antagonism and the neurobiological correlates of mood instability

Cognitive deficits are common in patients with mood instability (Bourne et al., 2013; Broome, Saunders, et al., 2015), and growing evidence implicates LTCC genes in a range of cognitive functions including working memory, attention (Cosgrove et al., 2017; Heyes et al., 2015; Zhang et al., 2012), reward responsiveness (Lancaster et al., 2014) and emotion recognition (Nieratschker et al., 2015). However, little is currently known about the cognitive effects of LTCC antagonists, beyond the DHP trials described above (Burdick et al., 2020; Vahdani et al., 2020; Zink et al., 2020). Preclinical research has shown enhanced cognitive performance after administration of CCBs in mice (Dudley et al., 2013; Quartermain et al., 2001; Quartermain & Garcia deSoria, 2001), and studies in rats report improved working memory (Levy et al., 1991). Human data is largely limited to small clinical and preclinical trials, which indicate potential benefits on cerebrovascular cognitive impairment (Hanyu et al., 2007; Tomassoni et al., 2008). However, these findings are yet to be confirmed in large scale studies, and future research is required to examine the wider cognitive effects of these drugs in humans.

The underlying neural mechanisms of LTCC antagonists are also uncertain. Neuroimaging techniques, including fMRI (Broome, He, et al., 2015) and magnetoencephalography (MEG) (Baker et al., 2014), are now providing greater insights into the neural correlates of LTCC risk genes. In task-based fMRI, *CACNA1C* rs1006737 alleles have been linked with aberrant activation in several regions of the frontotemporal circuit in carriers both with and without BD (Ou et al., 2015). Studies in healthy risk allele carriers have shown altered fronto-limbic connectivity, including amygdala, prefrontal cortex, hippocampus, medial temporal lobe, and anterior cingulate cortex (Cosgrove et al., 2017; Erk et al., 2010; Paulus et al., 2014; F. Wang et al., 2011), regions linked to emotion regulation and cognitive function. Emotional processing tasks have identified decreased visual-prefrontal and medial frontal gyrus connectivity (Dima et al., 2013; Radua et al., 2013) in adult BD risk allele carriers, and data from young healthy risk allele carriers (aged 13-20 years) have revealed increased amygdala activation when viewing negative (vs neutral) stimuli (Sumner et al., 2015). In addition to task-based changes, *CACNA1C* risk variants in adolescents with BD have also been associated with altered functional connectivity on resting state, particularly in regions linked to emotion, reward, and executive function (Jiang et al., 2023). In this study, BD risk-allele carriers demonstrated positive resting state functional connectivity between the right ACC and the right amygdala,

when compared to BD patients with non-risk variants. The amygdala plays an important role in processing and regulating emotions (Anderson, 2007), and the ACC, with its extensive connectivity to the limbic system and frontal cortex, is considered pivotal for integrating neural pathways involved in regulating emotions (Stevens et al., 2011). Consequently, this indicates *CACNA1C* rs1006737 could be involved in emotion dysregulation associated with BD via effects on resting-state functional connectivity across brain areas that are crucial for emotion regulation. This emerging evidence adds to the current understanding of functional connectivity for *CACNA1C* risk alleles, and highlights potential endophenotypes associated with these risk variants in BD. However further studies, specifically examining the effect of LTCC antagonists on neural circuits are required.

4.1.3 The Oxford study of Calcium channel Antagonism, Cognition, Mood instability and Sleep (OxCaMS)

The increasing evidence described above provides a rationale for reinvestigating LTCC antagonists. The Oxford Study of Calcium Channel Antagonism, Cognition, Mood Instability and Sleep (OxCaMS) was a high-intensity experimental medicine study which aimed to explore the effect of nicardipine sustained release (SR), a brain-penetrant DHP LTCC antagonist, on a range of parameters including cognition, mood, behaviour, brain activity, sleep, and physiology, in healthy young adults with high levels of mood instability.

OxCaMS had a double-blind placebo-controlled design. Participants completed a two-week pre-randomisation phase, when no intervention was given, during which time measures of mood, cognition, neural activity, sleep, and physiology were taken. A two-week randomisation phase followed, where participants were allocated to nicardipine SR (30 mg twice daily) or placebo, and all measures were repeated. This approach enabled both within and between subject comparisons.

Mood instability was selected as an inclusion criterion because it is a core feature of multiple psychiatric disorders where LTCC genes have been implicated. It is also common in the general population providing a pragmatic benefit in terms of study recruitment. Given limited understanding around the impact of LTCC antagonism in psychiatry, an experimental medicine study was deemed more suitable initially than a clinical trial (see Chapter 1, section 1.9 for further discussion). Further rationale for this approach comes from previous psychotropic medication research. For example, antidepressant studies have shown similar effects on positive and negative biases in both clinical and non-clinical populations (Harmer, Bhagwagar, et al., 2003; Harmer, O'Sullivan, et al., 2009; Murphy, Yiend, et al., 2009; Tranter et al., 2009). Therefore, studies of healthy volunteers may provide a valuable model for clinical

drug studies, by facilitating the early phase development of novel medical treatments (Harmer, 2008).

OxCaMS was a multidisciplinary research study, benefiting from the involvement of psychiatrists, data scientists, analysts, and nurses. My specific role within OxCaMS included the day-to-day running of the study, alongside another DPhil student. The parameters that I was particularly interested in were mood instability, cognitive function, and neural activity, assessed using fMRI. My DPhil colleague, focused on MEG data, exploring the impact of LTCC antagonism on oscillatory activity in mood instability. The current chapter explores mood instability, cognitive function, and physiological effects. It was hypothesized that calcium channels have a role in mood stability, and that blocking these channels would stabilize mood in participants with mood instability, as measured using the PANAS-SF. Other outcomes included cognitive, neural, and physiological effects. If nicardipine induced changes on these parameters, it could provide evidence of LTCC involvement in the pathophysiology of mood instability, and its component phenotypes. Although rebranding current LTCC antagonists for neuropsychiatric disorders is unlikely to be suitable, due to their relatively poor selective occupancy in the brain (Zamponi et al., 2015), this would provide a compelling case for developing more selective drugs, with improved efficacy and tolerability in the future.

4.2 Methods

Detailed OxCaMS methods are published in the study protocol (Atkinson et al., 2019), with only the procedures most relevant to this DPhil summarised here. Trial registration information is outlined in Table 4.1 with ethical approval granted by the NHS South Central Oxford C Research Ethics Committee (17/SC/0029, IRAS 213212). A schedule of procedures and timeline/workflow are depicted in Table 4.2 and Figure 4.1 respectively. Study visits were carried out at the National Institute for Health Research (NIHR) Oxford cognitive health Clinical Research Facility (CRF) and Oxford Centre for Human Brain Activity (OHBA) for neuroimaging.

Table 4.1 OxCaMS trial registration details.

Data	Trial Information
Registration ID	ISRCTN33631053; IRAS 213212
Registration date	8th June 2018
Funders	Wellcome Trust, NIHR Oxford Health Biomedical Research Centre, NIHR Oxford cognitive health Clinical Research Facility
Sponsor	University of Oxford
Recruitment country	UK
Condition studied	Mood instability
Intervention	Nicardipine sustained release (30 mg bd) or placebo, for 14 days
Inclusion criteria	Score ≥ 7 on Mood Disorder Questionnaire with evidence of functional impairment. Aged 18–35 years.
Exclusion criteria	Current psychotropic medication. Need for urgent psychiatric treatment. Psychiatric disorder/substance misuse which could compromise safety/data quality.
Study type	Experimental medicine, exploratory study; randomised, double-blind, parallel group; 14 day run-in before allocation.
Date of first enrolment	1st December 2017
Target sample size	40
Outcomes	Mood/cognitive/behavioural variability, BOLD & MEG signals at rest & during task performance, sleep parameters, calcium channels in leukocytes.

Table 4.2 OxCaMS schedule of procedures.

Attendance number	Visit 1	Visit 2	Visit 3	Follow-up
Timeline (days)	0	14	28	
Window (days)		±2	±2	
Informed consent	✓			
Demographics	✓			
Diagnosis (MINI)	✓			
Medical history	✓			
Physical examination	✓	✓		
Vital signs and BMI	✓	✓		
Electrocardiogram (ECG)	✓		✓	
Eligibility assessment	✓	✓		
Alcohol Use Disorders Identification Test (AUDIT)	✓			
Affective Lability Scale (ALS-SF)	✓			
Affect Intensity Measure (AIM)	✓			
ADHD screen	✓			
Morningness-Eveningness Questionnaire (MEQ)	✓			
Barratt Impulsiveness Scale (BIS)	✓			
Neuropsychological tests (TVA, N-back, ETB, Stop-Signal)	✓		✓	
Randomisation		✓		
True Colours (QIDS-SR$_{16}$, ASRM, GAD-7, EQ-5D)	✓ Weekly			
PANAS-SF	✓ Twice daily			
Cognitive tasks (Wheel of fortune, Fractals, Whack-A-T)	✓ Daily			

Actigraphy monitor	✓ Daily (with regular data downloads)			
Concomitant medication check	✓	✓	✓	
Dispensing study drugs and pill count		✓	✓	
Adverse Event check		✓	✓	✓
MEG		✓*	✓	
MRI		✓*	✓	
Sleep Condition Indicator (SCI-R)	✓	✓	✓	
Sleep Diary card commenced	✓	✓		
Sleep Diary card completed & returned			✓	
ePatch**	✓	✓		
Portable blood pressure monitor***		✓		
Blood tests	✓		✓	
Devices returned			✓	
Follow-up call				✓

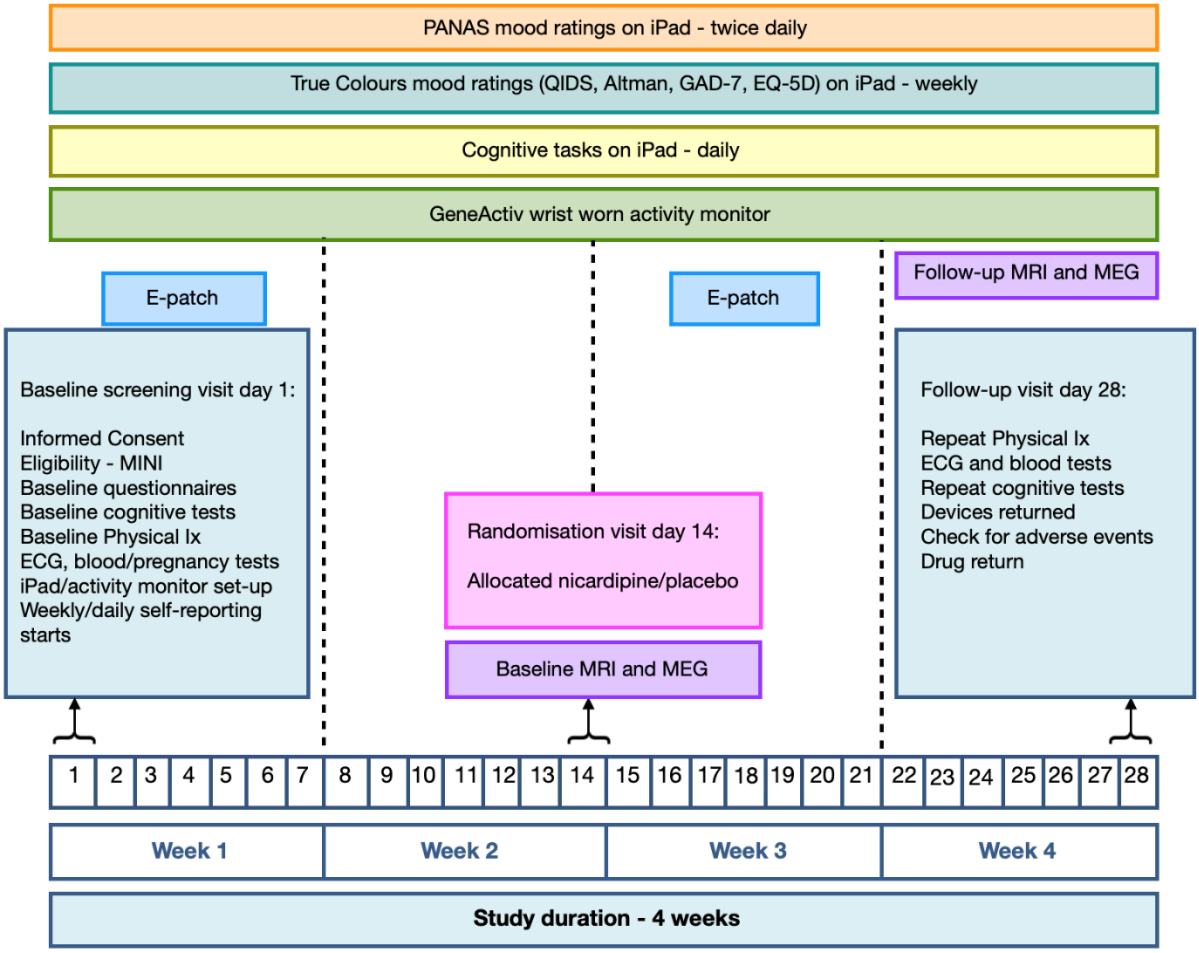
*MEG and MRI scan prior to taking any study medication. ** Wear for 72 hours *** Participants to record blood pressure twice daily throughout two-week randomisation phase.
Abbreviations: ASRM = altman self rating mania scale, BMI = body mass index, ECG = electrocardiogram, EQ-5D = euroqol 5-dimension quality of life measure, ETB = emotional test battery, GAD-7 = generalised anxiety disorder assessment, MEG = magnetoencephalography, MINI = mini-international neuropsychiatric interview, MRI = magnetic resonance imaging, PANAS-SF = positive and negative affect schedule, short form, QIDS-SR = quick inventory of depressive symptomatology, self-report, TVA = theory of visual attention.

4.2.1 Participants

Participants aged 18–35 years, were recruited through (i) advertising, (ii) the Bipolar Disorder Research Clinic at the NIHR Oxford cognitive health CRF, and (iii) the Oxford student sleep survey (Sheaves et al., 2016). This survey is part of a battery of questionnaires sent by the Sleep and Circadian Neuroscience Institute (SCNi) to all Oxford University students at the start of their second term. Only students who completed the questionnaires and consented to further research were contacted.

Participants with high mood instability were identified via the Mood Disorder Questionnaire (MDQ) (Appendix 4.1) (Hirschfeld et al., 2003; Hirschfeld et al., 2000). To meet inclusion criteria participants had to score seven or more on the MDQ with associated functional impairment (Carta et al., 2015) and evidence of several concurrent mood symptoms. This ensured those recruited had experienced significant and persistent instability of mood, with previous work suggesting MDQ scores greater than seven are strongly correlated with current mood variability (Atkinson, unpublished observations). Exclusion criteria (Appendix 4.2) included regular psychotropic medication in the preceding 12 weeks (as required medication was allowed at the discretion of the principal investigator), contraindications to nicardipine, and harmful alcohol or substance use.

Figure 4.1 Overview of OxCaMS timeline/workflow.



4.2.2 Study procedures

OxCaMS was a four-week experimental medicine study. It followed a randomised, double-blind, placebo-controlled design. This included a two-week pre-randomisation phase, when assessments were carried out, followed by randomisation and a further two-week period when assessments were repeated whilst participants took either drug (nicardipine SR 30 mg twice daily) or matched placebo. The target enrolment was 40 participants; 20 receiving nicardipine and 20 receiving placebo.

4.2.2.1 Randomisation

Randomisation used a computer-generated schedule minimising on sex with a 1:1 allocation. It was overseen by a trial manager within the Department of Psychiatry but external to the study group. Dispensing of medication was done by study clinicians, research pharmacists or CRF staff. Participants and researchers remained blind to allocation throughout the study.

4.2.2.2 Drug selection and dosing regimen

Nicardipine was selected as an LTCC antagonist of the DHP class. In terms of structure, it is similar to nifedipine (Amenta et al., 2008). It shows good brain penetration (Amenta et al., 2002; Grotta et al., 1986) and central LTCC binding (Amenta et al., 2002) with a high affinity for $Ca_V1.2$ channels (Lin et al., 2011). It has also demonstrated positive effects in preclinical studies of brain ischemia (Alps et al., 1988), seizures (Gasior et al., 1996) and cerebral ageing (Yamada et al., 1996). In contrast to nifedipine, with its short elimination half-life of approximately two hours, nicardipine is licensed in the UK in a sustained release (SR) formulation. Nicardipine SR capsules typically achieve steady-state within three days. The SR formulation enabled easier twice daily administration and resulted in lower maximal concentration (Cmax) of the medication. This, in turn, reduced side effects related to vasodilation (such as headaches and flushing) and helped maintain blinding. This dosing regimen was implemented in a previous study of normotensive young adults (Sun et al., 1990), where no effects on pulse or blood pressure were found.

4.2.3 Baseline screening and pre-randomisation assessments

Participants who met initial screening criteria were invited for a pre-randomisation visit at the NIHR CRF. During this visit, which took around five hours, participants first provided informed consent, and were then assessed for any current or past psychiatric history using the Mini-International Neuropsychiatric Interview (MINI), version 5.0. This screening tool was administered by a trained clinician to detect any lifetime Axis-1 disorder. Participants meeting criteria for a diagnosis (other than psychosis) were not excluded unless the clinician was of the opinion it would jeopardize safety or data quality. General practitioners were also notified of study involvement on the agreement of participants. It was expected that some participants might meet criteria for a psychiatric diagnosis. In this case appropriate information, signposting and referrals were arranged.

Eligible participants provided demographic details and completed a physical examination involving checks of blood pressure, height, weight, electrocardiogram (ECG), and blood biochemistry. Blood samples included both routine blood tests (full blood count, urea and electrolytes, inflammatory markers, thyroid function) and samples for isolation of lymphocytes to study calcium signalling and gene expression. The latter samples are not reported in this thesis. Female participants had a pregnancy test.

A battery of screening tools were conducted which included the McLean Screening Instrument for Borderline Personality Disorder (Zanarini et al., 2003), Adult Attention Deficit Hyperactivity Disorder (ADHD) Self-Report Scale (ASRS-v1.1), Alcohol Use Disorders Identification Test

(AUDIT) (Saunders et al., 1993), Barratt Impulsiveness Scale (Patton et al., 1995), Affective Lability Scale (short form) (Oliver & Simons, 2004), Affect Intensity Measure (Larsen & Diener, 1987), Morningness-Eveningness Questionnaire (Horne & Ostberg, 1976) and Sleep Condition Indicator (SCI-R) (Espie et al., 2014).

Additionally, a number of cognitive tasks were administered (which were repeated again at study endpoint). These included the N-back (1-, 2-, and 3-back) for working memory (Farrell et al., 2012; Martens et al., 2019), theory of visual attention (TVA) (Bundesen, 1990; Vangkilde et al., 2011) for information processing and attention, two tasks from the emotional test battery (ETB) (Harmer, O'Sullivan, et al., 2009) for facial expression recognition and emotional word categorisation, the stop-signal task (Logan et al., 1997) for impulsivity and response inhibition, and the National Adult Reading Test (NART) (Nelson, 1991) for verbal IQ. Except for the ETB tasks (see Chapter 5), these data are not reported in this thesis. Finally, participants were briefed on daily mood monitoring and cognitive testing via study iPads, which they would complete remotely over the course of the trial.

4.2.4 Longitudinal monitoring

Over the four-week study, participants received prompts from the True Colours online self-management system (https://oxfordhealth.truecolours.nhs.uk/www/en/) to complete mood and cognitive assessments. These short tasks took approximately ten minutes to complete and were recorded automatically. In total, 142 automatic prompts were delivered and if participants failed to complete tasks within one hour, an additional prompt was sent.

The Positive and Negative Affect Schedule, short form (PANAS-SF) was used to record twice daily mood, once in the morning and once in the evening (Appendix 4.3). This self-report scale, which was completed via iPad, included ten items with high validity and reliability (Thompson, 2007; Watson et al., 1988) widely used in healthy volunteers. In addition, True Colours collected weekly clinically validated mood ratings. These included the Quick Inventory of Depressive Symptomatology, self-report (QIDS-SR16) (Rush et al., 2003), Altman Self-Rating Mania Scale (Altman et al., 1997), Generalised Anxiety Disorder assessment (GAD-7) (Spitzer et al., 2006), and the EuroQol 5-dimension (EQ-5D) quality of life measure (EuroQol, 1990). The primary outcome was a reduction in mood instability, analysed using established statistical approaches described below. Alongside mood measures, three daily cognitive tasks were also completed via study iPads. These included 'Whack-A-T' assessing contextual cueing and implicit learning, 'Fractals' assessing reward learning, and 'Wheel of Fortune' (WoF), a simple gambling task. Only data from the WoF game are included in this thesis.

Remote ECG and blood pressure recording were completed via ePatch and portable blood pressure (BP) devices. The ePatch was a small sensor attached to participants' chests. It was fitted twice, first at the pre-randomisation assessment, to be worn for 72 hours, and second at the randomisation visit, where participants were provided with instructions to fit it again three days after starting nicardipine or placebo. These data aimed to determine the effect of nicardipine on heart rate variability but are not reported in this thesis. Portable BP devices recorded daily BP to explore the effect of nicardipine during the randomisation phase. BP results are reported below. Sleep was measured via self-report sleep diaries and ambulatory physiological monitoring. GENEActiv watches collected a range of actigraphy data including information about physical activity and body temperature, as well as timing and duration of sleep. Sleep and actigraphy data however are not included as part of this thesis.

4.2.5 Neuroimaging

Before randomisation, and at the final visit, participants completed functional magnetic resonance imaging (fMRI) and magnetoencephalography (MEG) at OHBA. Neuroimaging aimed to study the effect of LTCC antagonism on neural dynamics. Scanning protocols and procedures were the same pre and post randomisation.

fMRI data were acquired on a Siemens 3T Prisma with a 32-channel head coil. A liquid-crystal display (LCD) projector (BOLDScreen 32; Cambridge Research Systems, Rochester, UK) presented visual stimuli onto a screen which participants could see through a standard mirror attached to the head coil. Eyelink 1000 (an infrared eye tracker) ensured participants remained alert for the duration of the scan, and pulse and respiration were collected using the BIOPAC physiological monitoring system.

The fMRI protocol started with a brief 3-plane localiser, which calibrated the subject's head position and could be mapped onto subsequent scans. This was followed by a ~10 minute resting state scan, where participants were told to keep their eyes open, try not to fall asleep and to think of nothing in particular. The next sequence was the emotional faces (gender discrimination) task (~12 minutes) (Godlewska et al., 2016) where participants were briefed that they would see a series of faces with different emotions, both male and female. They were instructed to ignore the emotions and respond only to the gender of the faces. Immediately following, participants completed the 'wheel of fortune' task (~30 minutes) (not reported in this thesis), followed by a field map (~1 min), T1 structural scan (~6mins) and 3D ASL sequence (~7mins). Arterial spin labelling (ASL) was obtained using a 3D GRASE (hybrid of gradient and spin echo) readout (D. Wang et al., 2011), alongside a calibration image, required for the analysis. The calibration image was acquired using three inversion times

(1000ms, 2000ms and 3650ms) and enabled the estimation of M_0 (the equilibrium magnetisation) by fitting a saturation-recovery curve to the M_0 data from the three inversion times acquired. The ASL sequence was included to aid in distinguishing neural effects of nicardipine from any cerebrovascular effects. ASL analyses were completed by Dr Marieke Martens and are included in Appendix 4.4 for reference.

Analysis of blood oxygenation level dependent (BOLD) fMRI signal used FMRIB Statistical Library (FSL) software. This software comprised tools for structural, functional, and resting state connectivity (Smith et al., 2004) including the fMRI Expert Analysis Tool (FEAT) for task-based data. Resting state was investigated using independent component analysis (ICA). The Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC) tool enabled comparison of neural networks between the nicardipine and placebo groups.

MEG imaging started with a 10-minute resting-state scan, followed by a working-memory task (~ 35 min), visual gamma task (~ 15 min), and gripper task (~ 15 min). MEG analyses, which are not reported in this thesis, are noted for their high temporal resolution, and were used to examine the effects of LTCC antagonism on functional network dynamics.

4.2.6 Post-randomisation assessments

Following the two-week randomisation phase, participants reattended the NIHR CRF for a final visit. During this visit a number of baseline assessments were repeated including physical examination, blood tests, ECG, and the cognitive tasks (with the exception of the NART). Any unused medication, and devices including iPads, ePatch and GENEActiv watches were returned. Participants also answered questionnaires on tolerability and acceptability of longitudinal monitoring. To determine the efficacy of blinding participants were asked to guess whether they had been randomised to nicardipine or placebo.

4.2.7 Statistical analyses and power calculations

Data were first inspected for normality. Outliers were identified using boxplots, and SPSS tests of normality checked for skewness. For normally distributed group data (nicardipine versus placebo), comparisons of longitudinal measures used repeated measures analysis of variance (ANOVA). For simple comparisons between groups, unpaired t tests were used, and for correlations Pearson correlation coefficient was implemented. For data that was not normally distributed, outliers were identified and either non-parametric analyses or data transformation were used. Statistical analyses included both cross-sectional (nicardipine versus placebo) and longitudinal (pre- versus post-exposure to nicardipine) comparisons.

As the first study of its kind, there were no directly comparable data for a power calculation. However, recent findings using lithium in a similar experimental medicine imaging paradigm (Saunders et al., 2016), suggested a sample size of 40 participants (20 nicardipine; 20 placebo) would give more than 90% power to detect a medium effect ($\eta^2 = 0.06$) on the main mood outcomes at a p level of 0.05. This sample size was considered achievable in practice.

4.2.7.1 PANAS-SF

The approach for capturing mood instability was a version of the 'root mean of squared successive difference' (RMSSD). This method has been widely implemented for measuring instability (Sorkin & Clissold, 1987) and has been used previously for longitudinal mood data (Tsanas et al., 2017).

Mood instability differs from variability. Mood variability is the extent an individual's mood has diverged from baseline at a given time. Standard deviation (SD) can be used to quantify the amount of variability in mood scores across a sampling period, but it does not account for temporal succession of the data. In contrast, instability describes the amount of change in a participant's mood from one moment to another. Therefore, instability is better measured by the mean square of successive differences (MSSD) which represents the extent successive mood scores differ from each other. MSSD considers both amplitude changes and temporal dependency between data (Ebner-Priemer & Trull, 2009; Jahng et al., 2008). By using the square-root of MSSD scores (RMSSD), the positive skew of MSSD data is normalised suitable for parametric analyses. The formula for computing RMSSD is:

$$RMSSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N-1}(x_{i+1} - x_i)^2}$$

Here *N* is the number of data points and *xi* is the *i*th data point in the series, with *i*+1 denoting successive data points. In this formula, RMSSD assumes successive data points are equally distributed in time, however this may not be the case for individuals with missing data. Therefore, the RMSSD was altered to take into consideration any elapsed time between data points, giving a time-adjusted RMSSD (tRMSSD) (Taquet et al., 2023):

$$tRMSSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N-1}\left(\frac{x_{i+1} - x_i}{t_{i+1} - t_i}\right)^2}$$

Here *N* is the number of data points, *xi* is the *i*th data point in the series, with *ti* denoting the *i*th timestamp at which the datapoint was submitted and *i*+1 and *t*+1 denoting successive data

points and timestamps, respectively. This means if a participant misses a day of recording, time inflated data variability is avoided. In this particular analysis, tRMSSD +3 was used to ensure data was taken after nicardipine reached steady state (which takes approximately three days), giving 11 days of data in total.

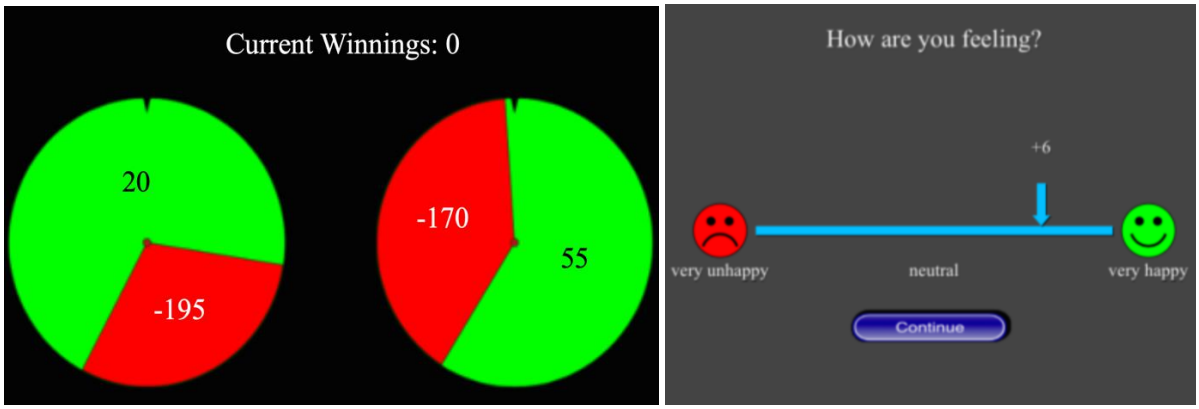
4.2.7.2 Baseline screening, weekly mood ratings and clinical data

Baseline data for nicardipine and placebo groups were compared using independent samples t tests for normally distributed, continuous data and chi-square tests for categorical data. Mean pre and post QIDS, Altman and GAD-7 scores were compared between groups in a repeated-measures ANOVA. Correlations between weekly mood ratings and mood instability (as calculated by the tRMSSD of positive and negative PANAS-SF scores over the four-week trial) were examined with Pearson correlations. Other group comparisons used parametric or non-parametric tests, paired or unpaired, as appropriate. All analyses were undertaken in IBM SPSS Statistics 28.0.

4.2.7.3. Wheel of Fortune (WoF)

The daily WoF task prompted participants to choose between two lottery wheels (Figure 4.2A). Each wheel was divided into green and red segments. The size of the segment represented the probability of winning or losing respectively. The numbers inside each segment specified the amount of money the participant could win or lose, and the aim was to maximise reward over 20 trials. Participants also completed a subjective measure of happiness (Figure 4.2B) before and after each game (ranging from unhappy [-10] to happy [10]).

Figure 4.2 (A) 'Wheel of fortune' (WoF) gambling task. **(B)** WoF iPad happiness scale.



To test for differences in risk taking between the groups, a WoF-Index was calculated, where the higher the index, the greater the risk taking for decision making. Creating the WoF-Index required a number of steps. First the riskiness of each wheel (RV) was calculated using the following formulas:

$$\mathbf{EV} = \boldsymbol{p}(\boldsymbol{x}) + \boldsymbol{q}(\boldsymbol{y})$$

$$\mathbf{RV} = \sqrt{\left[\boldsymbol{p}(\boldsymbol{x} - \mathbf{EV})^2 + \boldsymbol{q}(\boldsymbol{y} - \mathbf{EV})^2\right]}$$

Where, EV is the expected value, p is the probability of winning, q is the probability of losing, x is the amount of potential gain and y is the amount of potential loss. Therefore, RV represents both the probability and reward of winning and losing, and the 'risk' is how much the winning and losing amounts differed from the EV (Burnett et al., 2010; Simioni et al., 2012). The next step was to calculate the difference in RVs between the two wheels for each trial (RV-Diff):

$$\mathbf{RV\text{-}Diff} = \mathbf{RV}_r - \mathbf{RV}_s$$

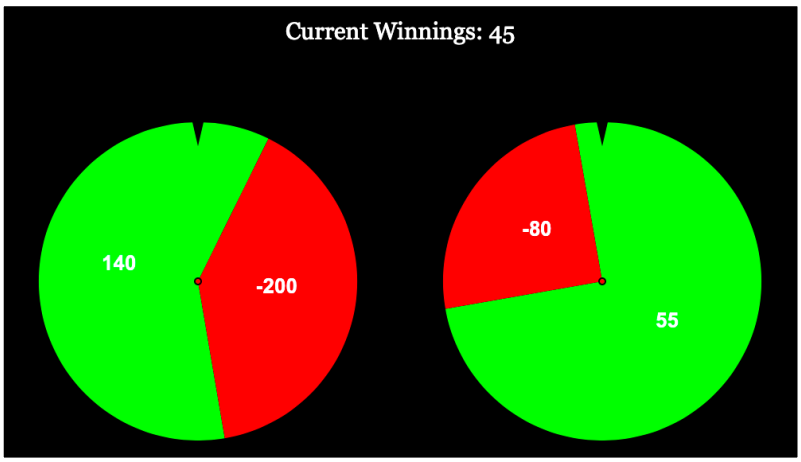
Here, RV_r = RV of riskier wheel (i.e. wheel with larger RV) and RV_s = RV of safer wheel (i.e. wheel with smaller RV). In order to minimise positive skew of RV-Diff values, and more accurately represent the differences in RV-Diffs across trials, absolute RV-Diff values were transformed into fractional ranked values, expressed as percentages (with maximum 100) for each study participant (rRV-Diff). Higher rRV-Diff values represented higher differences in riskiness between wheels, and therefore higher overall risk in the trial.

Choosing a riskier wheel in trials with higher rRV-Diff indicated greater risk taking for decision making. This is because it suggests a propensity to gamble on the wheel with a smaller probability of winning a greater reward (associated with greater possible loss) as opposed to the wheel with lower probability of winning a lesser reward (associated with lower possible loss) (see Figure 4.3). The WoF index was calculated to quantify this risk taking by computing the difference in rRV-Diffs between trials when riskier/safer wheel was chosen:

$$\mathbf{WoF\text{-}Index} = \boldsymbol{M}_j - \boldsymbol{M}_k$$

Here, M_j is the mean of rRV-Diffs of trials when the riskier wheel was chosen, and M_k is the mean of rRV-Diffs of trials when the safer wheel was chosen. Hence a larger WoF-Index indicates greater risk taking in decision making.

Figure 4.3 WoF example trial with high rRV-Diff.

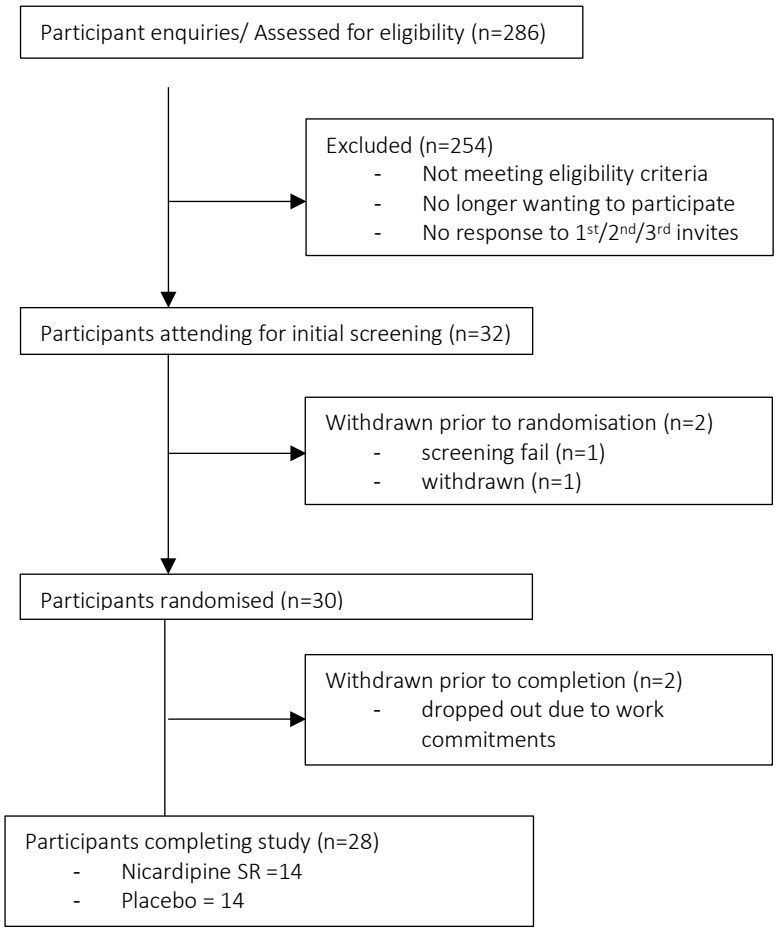


Left wheel has lower probability (60 vs 75%) of greater winnings (140 vs 55 points), and greater probability (40 vs 25%) of higher loss (200 vs 80 points). RVs of left and right wheel respectively are 166.57 and 58.46, hence RV-Diff = 108.11. Ranking this RV-Diff against other trials, gives a rRV-Diff of approximately 95 (out of 100).

4.3 Results

In total, 286 participant enquires were assessed for inclusion via the online screening questionnaire. 254 did not meet the eligibility criteria, chose not to participate, or failed to respond to three invites from the study group, resulting in 32 participants attending for initial screening. One participant failed to meet inclusion criteria at screening, and one was withdrawn due to lack of evidence for mood instability during the pre-randomisation phase. This resulted in 30 individuals being randomised to nicardipine SR (n=15) or placebo (n=15). The study recruitment tree is illustrated in Figure 4.4.

Figure 4.4 OxCaMS study recruitment tree.



4.3.1 Baseline group differences

In terms of demographic details (Table 4.3), groups were well matched for sex (X^2 [1, N=30] = 0.14, p=.71). However, there were baseline differences in age, with nicardipine participants being slightly younger than placebo (24 ± 4 vs. 28 ± 5, *t*(28)=-2.31, *p*=.028, Figure 4.5A).

With regards to baseline screening (Table 4.4), groups were well matched for MDQ scores (10 ± 2 vs. 10 ± 1, t(28)=1.06, p=.297, Figure 4.5B), and there were no baseline differences for AIM (166 ± 23 vs. 151 ± 23, t(28)=1.78, p=.086), AUDIT (7 ± 5 vs. 6 ± 6, t(28)=0.58, p=.566) or SCI (14 ± 7 vs. 14 ± 8, t(28)=-0.12, p=.905). However, there were differences between the groups for Affective Lability Scale (ALS-SF), with nicardipine participants showing higher total scores than placebo (52 ± 10 vs. 44 ± 12, t(28)=2.11, p=.044, Figure 4.6). Baseline differences in the ALS anxiety/depression subscale were most prominent, again with the nicardipine group having higher scores (15 ± 3 vs. 11 ± 4, t(28)=3.00, p=.006, Figure 4.6). There were no group differences for the other ALS subscales, including depression/mania (23 ± 5 vs. 21 ± 6, t(28)=0.99, p=.333, Figure 4.6) and anger (14 ± 4 vs. 11 ± 4, t(28)=1.60,

p=.121, Figure 4.6). BIS total (78 ± 11 vs. 69 ± 9, t(28)=2.44, p=.021, Figure 4.7) and BIS attention subscales (22 ± 4 vs. 19 ± 4, t(28)=2.55, p=.016, Figure 4.7) also differed between the groups, again with higher scores in the nicardipine group compared with placebo. However, the BIS motor (27 ± 5 vs. 25 ± 4, t(28)=1.36, p=.185, Figure 4.7) and non-planning (29 ± 5 vs. 25 ± 6, t(28)=1.83, p=.078, Figure 4.7) subscales showed no group differences.

Although this was a healthy volunteer study, MINI results (Table 4.4) demonstrated a significant proportion met criteria for psychiatric disorder. Eighty-seven percent of nicardipine, and seventy-three percent of placebo participants had a current DSM-IV mental disorder. Mood disorders were most common, but a range of psychiatric diagnoses were noted. Low participant numbers precluded a subgroup analysis.

Figure 4.5 (A) Boxplot of age in years for nicardipine vs placebo. **(B)** Boxplot of MDQ scores (≥7 required for study entry). SEM error bars. *p<.05.

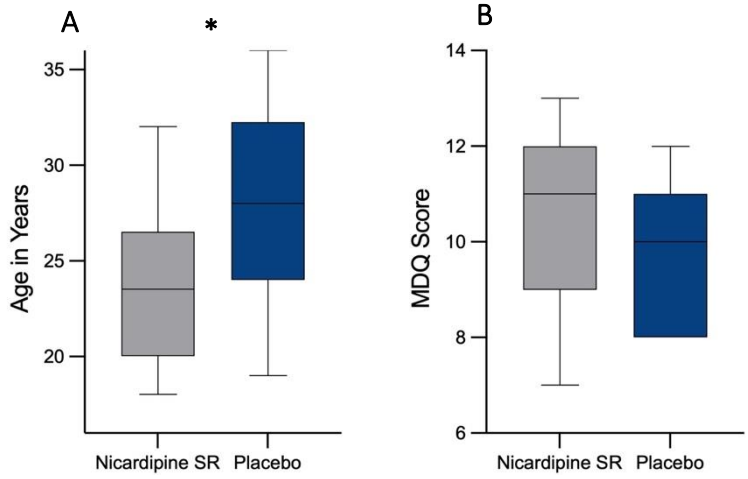


Figure 4.6 Grouped bar plots of Mean ALS-SF scores for nicardipine vs placebo pre-randomisation. SEM error bars. *p<.05 **p<.01.

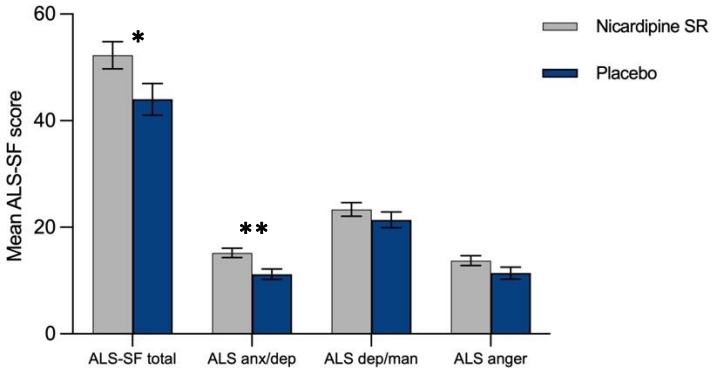


Figure 4.7 Grouped bar plots of Mean BIS scores for nicardipine vs placebo pre-randomisation. SEM error bars. *p<.05.

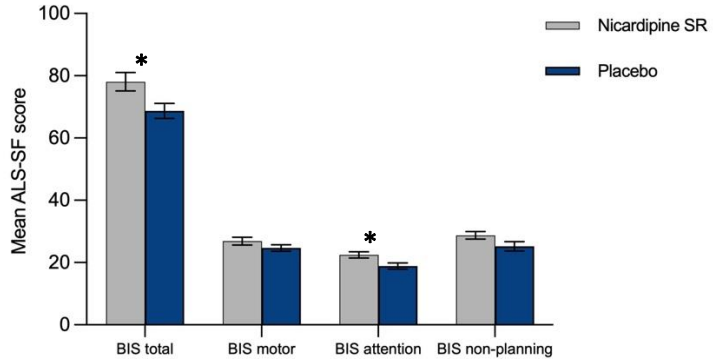


Table 4.3 Baseline demographic details.

	Nicardipine SR	Placebo
	n=15	n=15
Age (mean; SD)	24 (4)*	28 (5)*
Female (n; %)	10 (67%)	9 (60%)
Ethnicity (n; %)		
White British	11 (73%)	7 (47%)
White Other	3 (20%)	4 (27%)
Asian British	0 (0%)	1 (7%)
Asian	0 (0%)	1 (7%)
Hispanic	1 (7%)	0 (0%)
North African	0 (0%)	1 (7%)
Black Caribbean	0 (0%)	1 (7%)
Highest Level of Education (n; %)		
O-Level/GCSE	2 (13%)	2 (13%)
A-Level/AS-Level	4 (27%)	2 (13%)
Undergraduate degree	5 (33%)	4 (27%)
Master's or doctorate degree	4 (27%)	7 (47%)

SD = standard deviation. * p<.05

Table 4.4 Baseline screening.

	Nicardipine SR	Placebo
	n=15	n=15
MDQ score (mean; SD)	10 (2)	10 (2)
Affect Intensity Measure (AIM) (mean; SD)	166 (23)	151 (23)
Affective Lability Scale (ALS-SF) (mean; SD)	52 (10)*	44 (12)*
ALS-SF – anxiety/depression (mean; SD)	15 (3)**	11 (4)**
ALS-SF – depression/mania (mean; SD)	23 (5)	21 (6)
ALS-SF – anger (mean; SD)	14 (4)	11 (4)
Barratt Impulsiveness Scale (BIS) (mean; SD)	78 (11)*	69 (9)*
BIS motor	27 (5)	25 (4)
BIS non-planning	29 (5)	25 (6)

BIS attention	22 (4)*	19 (4)*
AUDIT (median; range)	7 (5)	6 (6)
Sleep Condition Indicator (mean; SD)	14 (7)	14 (8)
MINI DSM-IV Diagnoses – Current (n; %)	13 (87%)	11 (73%)
Major Depressive Episode	9	10
Major Depressive Episode (melancholic features)	5	4
Dysthymia	3	4
Mood Disorder (psychotic features)	1	1
Generalised Anxiety Disorder	5	3
Panic Disorder	8	3
Agoraphobia	5	6
Social Phobia	4	2
Obsessive Compulsive Disorder	3	2
MINI DSM-IV Diagnoses – Lifetime (n; %)	13 (87%)	11 (73%)
Manic Episode	2	3
Hypomanic Episode	11	7
Alcohol Abuse/Dependence	1	2
Substance Abuse/Dependence	0	1

SD = standard deviation. **p<.01, * p<.05

4.3.2 Effects of LTCC antagonism

The physiological, mood stabilising and cognitive effects of nicardipine SR were measured via a range of assessments described above (including longitudinal monitoring and pre-post randomisation testing). After randomisation, two individuals (one nicardipine and one placebo) withdrew from the study. This resulted in 14 participants in each group for the subsequent analyses (except where stated in the tables). Outcomes are presented below, grouped by effect.

4.3.2.1 Physiological effects

Blood samples, pulse, and blood pressure

Physiological data including blood tests, heart rate and blood pressure were collected at baseline and study end point in the clinic. Participants also recorded twice daily measures of heart rate and blood pressure at home throughout the post-randomisation period. All clinical data are illustrated in Table 4.5. Repeated measures ANOVA showed a group-time interaction for diastolic blood pressure (measured in the clinic), with larger reductions for the placebo group following randomisation compared with the nicardipine group ($F_{1,26}$=4.23, p=0.05, η^2 =0.14). There were no other significant group-time differences for the clinical data, indicating mood and cognitive results are unlikely to be confounded by physiological differences between the groups.

The results also confirm moderate to strong correlation between in clinic and at home measures. Post-randomisation systolic BP measured in the clinic correlated positively with at home systolic BP (r=0.74, n=28, p<0.01). Positive correlations were also noted for in clinic and at home diastolic BP (r=0.47, n=28, p=0.01) and heart rate (r=0.51, n=28, p=0.01) (see Figure 4.8).

Table 4.5 Physiological data.

	Nicardipine SR		Placebo	
	Pre (n=14)	Post (n=13)	Pre (n=13)	Post (n=12)
Blood samples				
Serum Cortisol nmol/L (mean, SD)	54.94 (49.96)	45.31 (14.83)	37.86 (30.40)	37.27 (20.18)

	Nicardipine SR		Placebo	
	Pre (n=14)	Post (n=12)	Pre (n=13)	Post (n=13)
Blood samples				
Hb (mean, SD)	143.00 (12.58)	142.58 (14.13)	144.62 (12.80)	139.23 (15.09)
WCC (mean, SD)	5.92 (1.37)	5.86 (1.33)	5.87 (1.17)	5.63 (1.43)
Platelets (mean, SD)	249.43 (54.60)	241.42 (55.88)	268.85 (46.84)	265.38 (43.91)
Bilirubin (mean, SD)	12.93 (5.68)	12.33 (5.02)	14.31 (5.82)	12.38 (4.05)
ALT (mean, SD)	17.93 (8.10)	22.33 (15.59)	22.46 (17.30)	20.31 (11.21)
ALP (mean, SD)	71.14 (23.75)	67.33 (21.65)	65.54 (11.57)	67.23 (14.52)

	Nicardipine SR		Placebo	
	Pre (n=14)	Post (n=12)	Pre (n=14)	Post (n=13)
Blood samples				
Sodium (mean, SD)	139.64 (1.65)	139.67 (1.37)	140.21 (0.89)	140. 69 (1.60)
Potassium (mean, SD)	3.70 (0.25)	3.73 (0.38)	3.84 (0.51)	3.80 (0.34)
Urea (mean, SD)	3.56 (0.90)	3.96 (1.15)	3.93 (1.07)	3.69 (1.06)
Creatinine (mean, SD)	62.14 (11.35)	57.08 (20.44)	63.79 (11.07)	65.77 (10.76)
Albumin (mean, SD)	42.00 (4.80)	40.50 (3.71)	43.36 (2.65)	42.00 (2.55)
T4 (mean, SD)	13.30 (1.29)	12.56 (1.03)	13.41 (1.88)	12.78 (1.41)
T3 (mean, SD)	4.90 (0.55)	4.59 (0.66)	4.63 (0.61)	4.55 (0.55)
Calcium mmol/L (mean, SD)	2.41 (0.12)	2.38 (0.11)	2.44 (0.06)	2.42 (0.09)
Adjusted Calcium mmol/L (mean, SD)	2.35 (0.09)	2.34 (0.09)	2.37 (0.06)	2.36 (0.08)

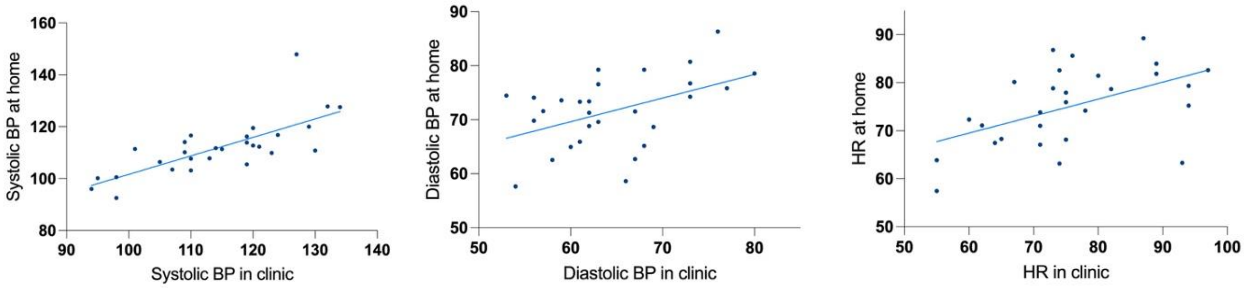
	Nicardipine SR		Placebo	
	Pre (n=13)	Post (n=12)	Pre (n=14)	Post (n=13)
Blood samples				
TSH (mean, SD)	1.65 (0.77)	1.60 (0.71)	1.25 (0.42)	1.36 (0.54)

	Nicardipine SR		Placebo	
	Pre (n=14)	Post (n=14)	Pre (n=14)	Post (n=14)
In clinic physical observations				
Pulse (mean, SD)	75.57 (10.49)	80.86 (11.13)	72.57 (11.05)	70.50 (10.05)
Systolic BP	120.50 (14.59)	115.57 (12.21)	118.93 (13.14)	113.36 (10.54)
Diastolic BP *	67.93 (8.11)	65.07 (7.08)	71.79 (8.19)	64.00 (7.50)

	Nicardipine SR	Placebo
	Post (n=14)	Post (n=14)
At home physical observations		
Mean Pulse (mean, SD)	77.60 (8.19)	72.49 (7.48)
Mean Systolic BP (mean, SD)	112.65 (9.83)	111.24 (12.00)
Mean Diastolic BP (mean, SD)	72.82 (7.11)	70.39 (6.40)
tRMSSD Pulse (mean, SD)	6.09 (3.19)	8.45 (6.73)
tRMSSD Systolic (mean, SD)	6.81 (3.28)	5.66 (2.55)
tRMSSD Diastolic (mean, SD)	4.64 (2.20)	4.74 (3.00)

*Nicardipine SR and placebo significant group-time interaction, $p<.05$. Abbreviations: ALP = alkaline phosphatase, ALT = alanine transaminase, Hb = heamoglobin, T3 = triiodothyronine, T4 = thyroxine, tRMSSD = time-adjusted square root of the mean square of successive differences, TSH = thyroid-stimulating hormone, WCC = white cell count.

Figure 4. 8 Scatter plot of post randomisation in clinic systolic BP, diastolic BP and heart rate by at home measures.



Cerebral blood flow

Data on cerebral blood flow was collected using an Arterial Spin Labelling (ASL) sequence. ASL was included to help distinguish the neural effects of nicardipine from any cerebrovascular effects, which could confound results. ASL analyses were completed by Dr Marieke Martens (see Appendix 4.4 for reference). Although nicardipine was not found to influence global cerebral perfusion, regional perfusion suggested a non-significant reduction with nicardipine compared to placebo. Considering these findings, ASL maps were added as voxel wise regressors in Chapters 6 and 7 of this thesis (OxCaMS resting state and task fMRI). In these chapters, ASL data were included in sensitivity analyses to account for possible perfusion differences between the groups.

4.3.2.2 Mood stabilising effects

Mean mood and mood instability (PANAS-SF)

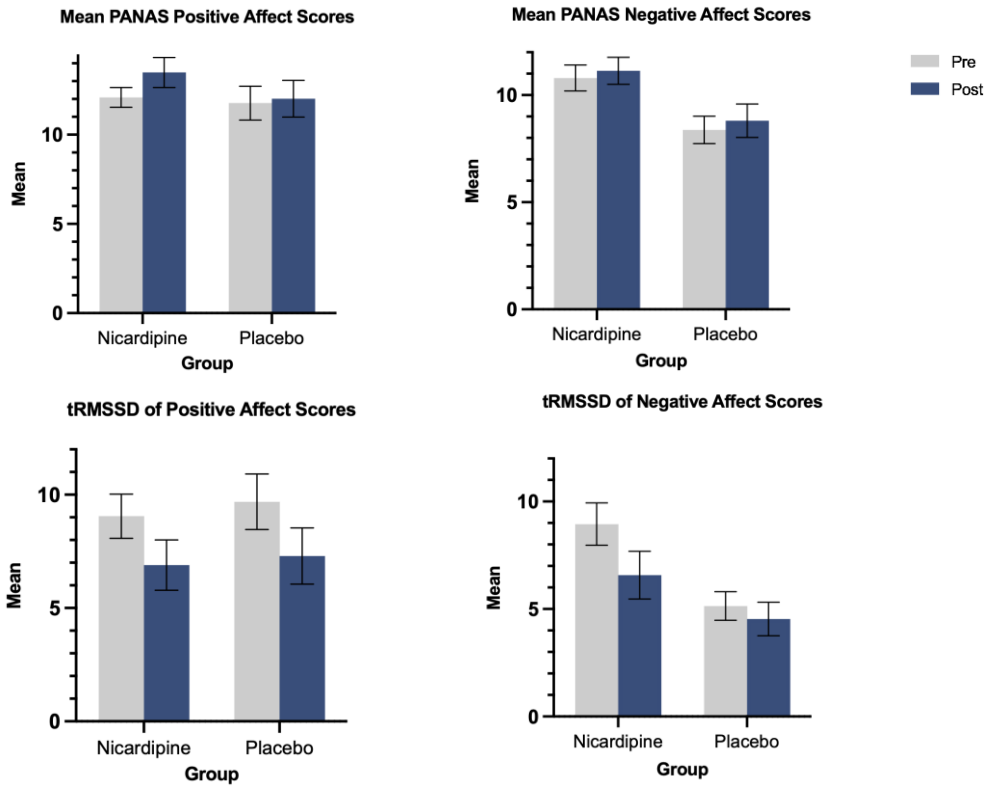
Mean positive and negative mood scores and mood instability are shown in Table 4.6 and Figure 4.9. Repeated-measures ANOVA was used to determine group differences. Mean PANAS scores were stable across the trial for both positive ($F_{1,26} = 1.54$, $p = 0.23$, $\eta^2 = 0.06$) and negative ($F_{1,26} = 0.002$, $p = 0.96$, $\eta^2 = 0.00$) items (Figure 4.9). Positive ($F_{1,26} = 0.01$, $p = 0.92$, $\eta^2 = 0.00$) and negative ($F_{1,26} = 0.50$, $p = 0.49$, $\eta^2 = 0.02$) mood instability also showed no group by time interaction, indicating nicardipine did not reduce mood instability in these particular measures. However, by chance there were baseline differences in negative mood between the groups. The nicardipine group was found to have higher negative affect ($t(26)=2.64$, $p=.01$) and higher negative instability ($t(26)=2.95$, $p=.01$) scores at baseline compared with the placebo group.

Table 4.6 Mean positive and negative affect and instability scores.

	Nicardipine SR (n=14)		Placebo (n=14)	
PANAS-SF questionnaire (mean, SD)	Pre	Post	Pre	Post
Mean positive affect	12.2 (2.3)	13.4 (3.6)	11.8 (3.8)	11.9 (3.9)
Mean negative affect	10.8 (2.5)*	11.3 (2.5)	8.3 (2.5)*	8.8 (3.1)
tRMSSD (instability) positive affect	9.1 (3.6)	6.9 (4.2)	9.7 (4.6)	7.3 (4.6)
tRMSSD (instability) negative affect	9.0 (3.7)*	6.6 (4.1)	5.1 (2.5)*	4.5 (2.9)

*Nicardipine SR and placebo groups significantly different at baseline, $p<.05$.

Figure 4.9 Mean and instability (tRMSSD) of positive and negative affect scores (PANAS). SEM error bars.



Weekly mood ratings

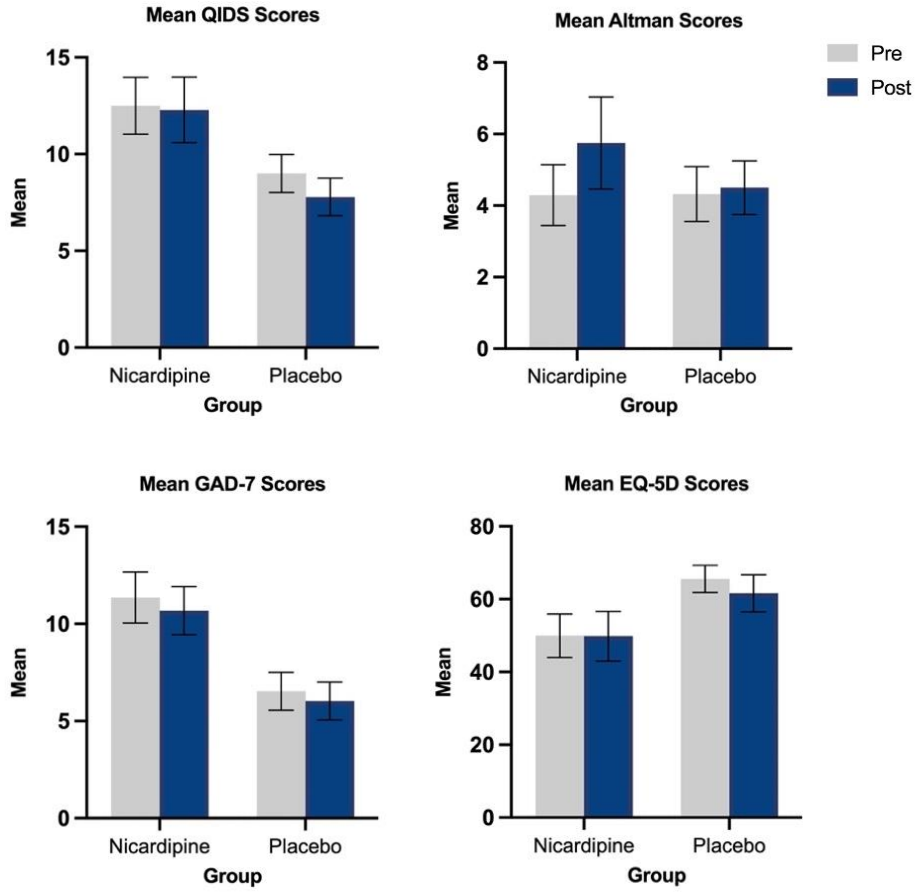
Weekly QIDs, Altman, GAD-7 and EQ-5D scores are shown in Table 4.7 and Figure 4.10. A repeated-measures ANOVA was used to determine group differences. Mean weekly mood ratings were stable across the trial for QIDS ($F_{1,26} = 0.20$, $p = 0.66$, $\eta^2 = 0.01$), Altman ($F_{1,26} = 0.75$, $p = 0.40$, $\eta^2 = 0.03$), GAD-7 ($F_{1,26} = 0.06$, $p = 0.81$, $\eta^2 = 0.002$) and EQ-5D ($F_{1,26} = 0.39$, $p = 0.54$, $\eta^2 = 0.02$) indicating nicardipine did not affect these particular measures. However, as with negative affect (PANAS mean and tRMSSD), there were baseline differences between the groups for QIDS (t(26)=2.42, p=0.02) and GAD-7 (t(26)=2.89, p=0.01) with the nicardipine group showing higher scores than placebo.

Table 4.7 Mean weekly mood rating scores.

	Nicardipine SR (n=14)		Placebo (n=14)	
True Colours weekly questionnaires (mean, SD)	Pre	Post	Pre	Post
QIDS	13.00 (5.49)*	12.62 (5.09)	9.39 (3.09)*	8.29 (3.54)
ASRM	4.29 (2.95)	5.75 (4.46)	4.29 (2.95)	4.50 (2.80)
GAD-7	10.32 (4.46)*	9.71 (4.15)	6.71 (4.34)*	5.86 (3.51)
EQ-5D	49.96 (22.33)	49.82 (25.56)	65.57 (14.01)	61.61 (19.00)

*Nicardipine SR and placebo groups significantly different at baseline, $p<.05$.
Abbreviations: ASRM = altman self rating mania scale, EQ-5D = euroqol 5-dimension quality of life measure, GAD-7 = generalised anxiety disorder assessment, QIDS-SR = quick inventory of depressive symptomatology, self-report.

Figure 4.10 Bar plots of Mean QIDS, Altman and GAD-7 scores for nicardipine vs placebo. SEM error bars.



4.3.2.3 Cognitive effects

Wheel of Fortune (WoF)

The Wheel of Fortune (WoF) was a simple gambling task, included to test for differences in risk taking between the groups. A WoF-Index was calculated, where the higher the index, the greater the risk taking in decision making. WoF data are presented in Table 4.8 and Figure 4.11. Both groups made greater risky choices and fewer safe choices post randomisation. Repeated measures ANOVA showed no significant differences in WoF indices between the two groups ($F_{1,25} = 0.38$, $p = 0.54$, $\eta^2 = 0.02$), indicating nicardipine did not affect risk taking for decision making on this particular task. In addition, correlations between WoF indices and mood ratings (positive and negative PANAS mean and tRMSSD) were examined with Pearson correlations. However, WoF-Index did not correlate with any PANAS mood ratings.

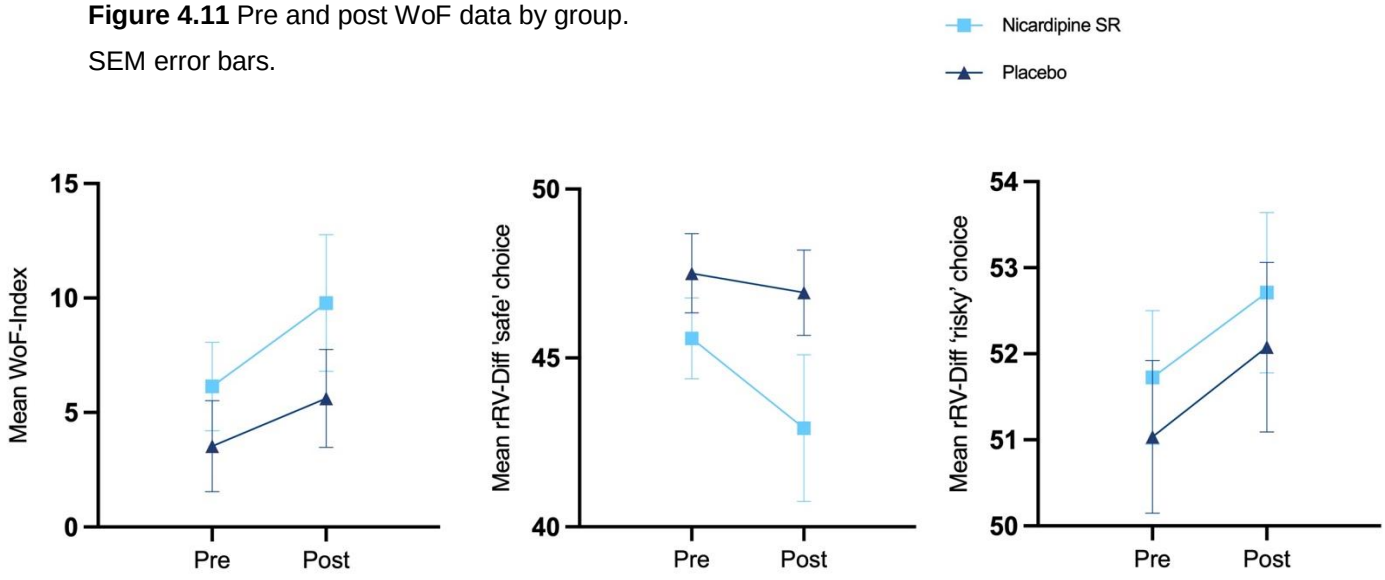
Table 4.8 WoF descriptive statistics.

	Group	N	Minimum	Maximum	Mean	Std. Deviation
WoF-Index (pre)	Nicardipine	14	-7.10	18.08	6.14	7.23
	Placebo	13	-6.97	12.90	3.53	7.15
	Total	27	-7.10	18.08	4.88	7.18
WoF-Index (post)	Nicardipine	14	-8.64	29.09	9.79	11.17
	Placebo	13	-9.12	16.78	5.14	8.05
	Total	27	-9.12	29.09	7.55	9.89
rRV-Diff 'risky' choice (pre)	Nicardipine	14	45.67	55.87	51.73	2.90
	Placebo	13	45.19	54.52	51.04	3.20
	Total	27	45.19	55.87	51.39	3.01
rRV-Diff 'risky' choice (post)	Nicardipine	14	44.56	56.81	52.71	3.48
	Placebo	13	45.12	56.28	52.08	3.55
	Total	27	44.56	56.81	52.41	3.46
rRV-Diff 'safe' choice (pre)	Nicardipine	14	37.24	52.76	45.58	4.47
	Placebo	13	40.79	52.58	47.51	4.22
	Total	27	37.24	52.76	46.51	4.38
rRV-Diff 'safe' choice (post)	Nicardipine	14	27.73	53.19	42.92	8.12
	Placebo	13	39.49	54.24	46.93	4.54
	Total	27	27.73	54.24	44.85	6.83

Abbreviations: rRV-Diff = fractional ranked difference in riskiness of each wheel for each trial, WoF = wheel of fortune.

Figure 4.11 Pre and post WoF data by group.

SEM error bars.



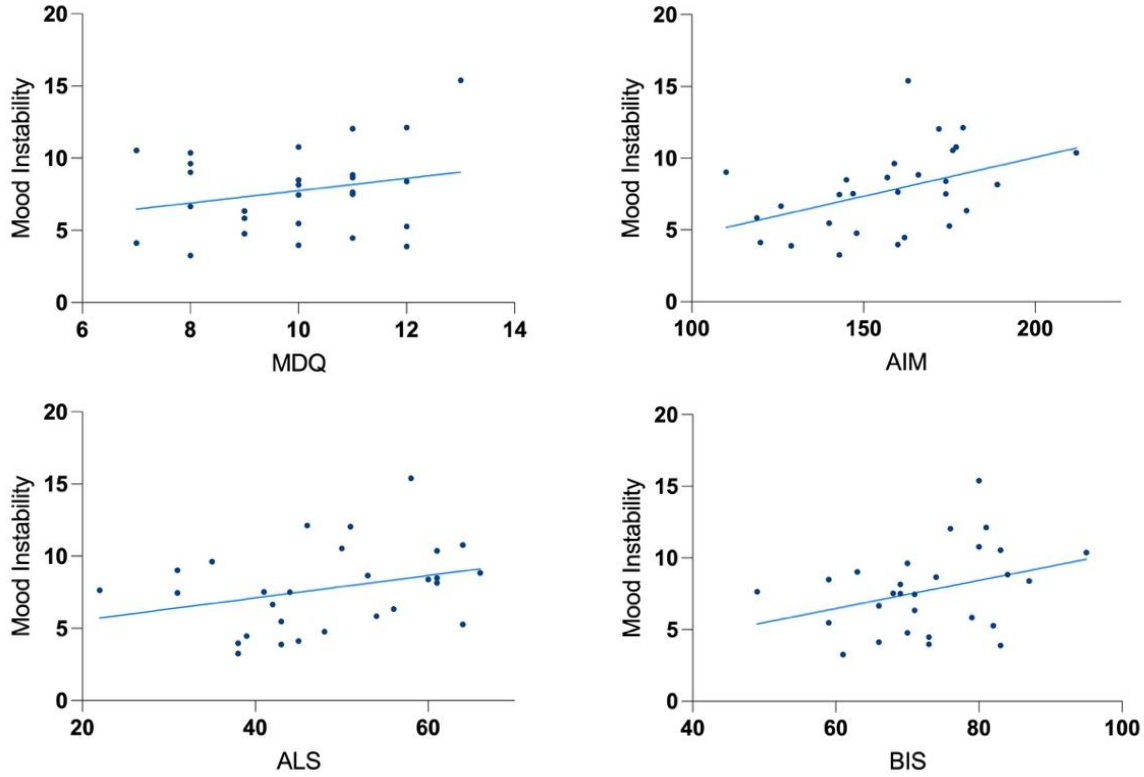
4.3.3 Correlations between mood and exploratory outcomes

Associations between baseline measures, cognitive effects, and mood instability (as calculated by the tRMSSD of positive and negative PANAS-SF scores over the four-week trial) were explored in all participants with *Pearson correlations*. Significant correlations are presented below.

4.3.3.1 Correlations between mood instability and baseline measures

Pre-randomisation negative mood instability (tRMSSD) correlated positively with baseline screening measures including MDQ (r=0.38, n=28, p=0.05), AIM (r=0.43, n=28, p=0.02), ALS (r=0.42, n=28, p=0.03), and BIS (r=0.44, n=28, p=0.02) (Figure 4.12).

Figure 4.12 Scatter plot of MDQ, AIM, ALS, and BIS scores by pre-randomisation negative mood instability (tMRSSD).

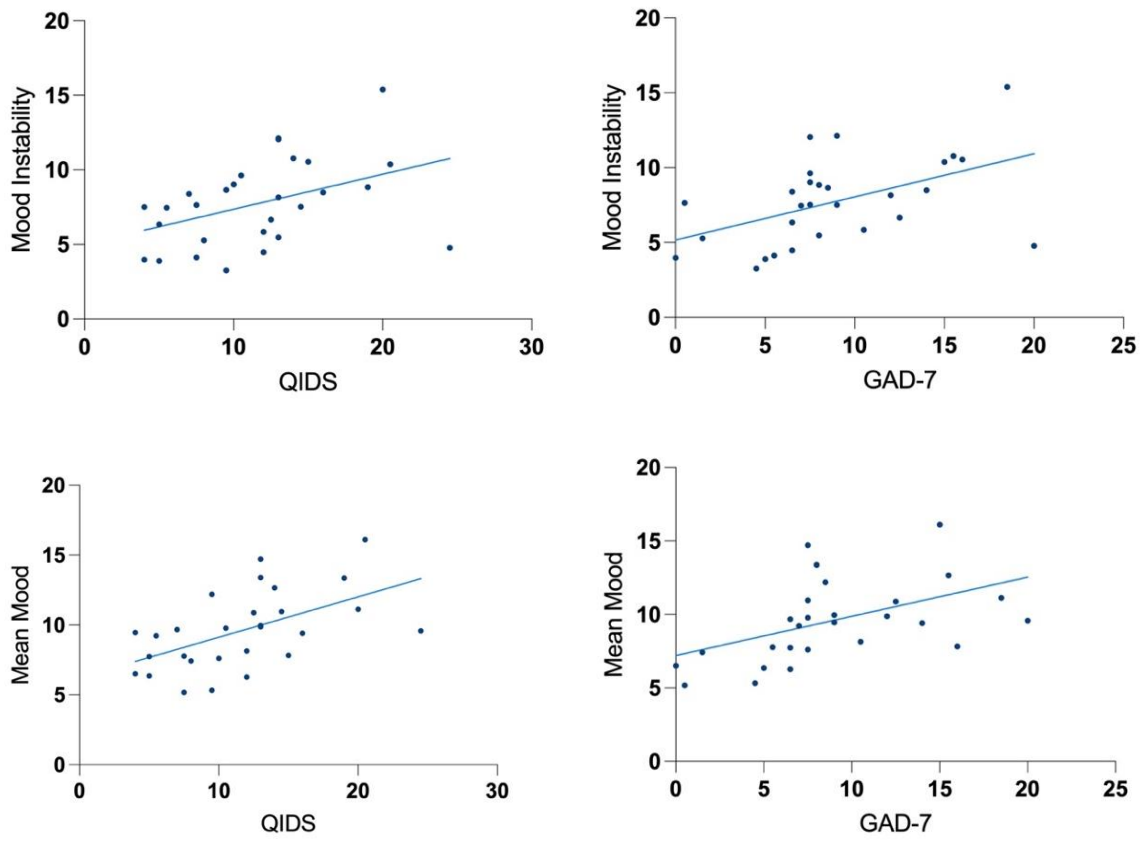


Abbreviations: AIM = affect intensity measure, ALS = affective lability scale, BIS = barratt impulsiveness scale, MDQ = mood disorder questionnaire.

4.3.3.2 Correlations between mood instability and weekly mood ratings

PANAS mean negative mood and negative mood instability (tRMSSD) at baseline correlated positively with pre-randomisation measures of QIDS (r=0.55, n=28, p=0.003, and r=0.42, n=28, p=0.03 respectively) and GAD-7 (r=0.48, n=28, p=0.01, and r=0.50, n=28, p=0.01 respectively) (Figure 4.13). However, PANAS positive baseline mean mood and mood instability did not correlate with any baseline weekly mood ratings.

Figure 4.13 Scatter plot of baseline QIDS and GAD-7 scores by pre-randomisation negative mood instability (tMRSSD) and mean negative mood.



Abbreviations: GAD-7 = generalised anxiety disorder assessment, QIDS-SR = quick inventory of depressive symptomatology, self-report.

4.4 Discussion

This experimental medicine study is the first of its kind to explore LTCC antagonism in subjects with high levels of mood instability. Following fourteen-day nicardipine SR treatment, OxCaMS aimed to establish whether LTCC blockade affects brain function, and to characterise the nature of any such effects. The present chapter compared nicardipine with placebo on a range of parameters and found no significant group differences in overall mood or mood instability. In addition, there were no effects on cognitive function (as measured by a risky decision-making task), physiological measures, or global cerebral perfusion (as assessed by ASL scans).

4.4.1 Nicardipine effects on mood

There was no evidence nicardipine SR had an overall effect on mood, or provided any benefit in reducing mood instability, when compared to placebo. Despite decreases in positive and

negative instability on PANAS-SF in the nicardipine group, these reductions did not significantly differ from those observed in the placebo group. PANAS-SF data were also supported by weekly mood ratings, including QIDS and GAD-7 scores, which correlated positively with mean negative mood and negative mood instability.

Previous mood disorder research has found some evidence for the efficacy of DHP LTCC antagonists. A pilot study of isradipine in BD suggested improved depressive symptoms (Ostacher et al., 2014), and a placebo-controlled trial of nimodipine indicated benefits on affective dysregulation, with reports of decreased mood fluctuations in BD (Pazzaglia et al., 1993). It is unclear why the OxCaMS findings differ. However, one factor to consider is the lower participant numbers than originally outlined in the protocol. The resulting sample may have been insufficiently powered to observe treatment effects in these domains. Additionally, the decline in positive and negative mood instability seen for both nicardipine and placebo participants, may have further reduced power to detect differences between the groups. The basis of this finding is not known but may result, in part, from participants being more aware of their mood through regular self-rating, leading to improved emotional regulation (Bakker & Rickard, 2018).

Participants randomised to nicardipine also had, by chance, greater mean negative affect, and greater negative affective instability, than placebo participants at baseline. This was also seen in the ALS-SF, especially the anxiety and depression subscale, with nicardipine participants scoring higher than placebo. Pre-randomisation group differences are more common when small numbers of participants are studied (Herbert, 2005). Therefore, this may have been mitigated by recruiting more subjects.

4.4.2 Nicardipine effects on cognition

LTCC genes have been implicated in reward processing (Dietsche et al., 2014; Lancaster et al., 2014; Wessa et al., 2010) and risk taking. Rodent studies have shown fewer risk taking behaviours in female *Cacna1c* heterozygous knockout mice (Dao et al., 2010), and studies administering LTCC antagonists in mice have demonstrated reduced impulsivity (Dudley et al., 2013). Unlike these preclinical studies, OxCaMS did not demonstrate effects of nicardipine on risky decision making. The WoF-index showed no significant difference in risk taking for decision making between the groups, with both nicardipine and placebo participants making more risky choices and fewer safe choices post randomisation. The basis of this pattern is uncertain, however it could be a form of habituated response to risky choices seen across multiple trials (Simon & Setlow, 2012).

Risk taking and reward processing are complex multidimensional constructs. The lack of a nicardipine effect on risk taking could be a consequence of task selection. Although nicardipine did not affect risk taking in decision making, as measured by the WoF, nicardipine may affect other aspects of risk taking and reward processing, measured by different tasks. Risk taking was specifically selected as an outcome measure because it is also sensitive to changes in mood (Chou et al., 2007; Yuen & Lee, 2003), enabling correlations with the PANAS-SF questionnaire. Although both mean mood ratings and mood instability were analysed, no significant correlations were found between WOF-Index and PANAS scores, indicating mood instability was not associated with this particular measure of reward processing (H.-J. Lee et al., 2012).

A further consideration of this null result is whether a gambling task associated with tangible reward might have yielded different findings. There is ongoing debate over the impact of hypothetical verses monetary benefits in psychological tasks (Hinvest & Anderson, 2010). Real rewards have been shown to strengthen differences between groups (Bowman & Turnbull, 2003), which might have implications for the current findings. Although nicardipine did not affect this measure of risk taking and reward, these phenotypes remain valuable therapeutic targets. Deficits in risky decision making and reward processing are central features in a number of psychiatric conditions, including BD, depression and schizophrenia (Gold et al., 2008; Ng et al., 2019; Swann et al., 2009; Yechiam et al., 2008), and as such they remain important domains for future LTCC antagonism studies.

4.4.3 Nicardipine effects on physiological measures

No meaningful effects were found for nicardipine on heart rate or blood pressure. This supports previous research demonstrating no effects on vital signs in normotensive young adults (Sun et al., 1990). Early studies in healthy male volunteers have shown nicardipine is quickly absorbed, extensively metabolised, and rapidly excreted, predominantly via the kidneys (Rush et al., 1986). The current data endorse the acceptability of nicardipine use in healthy young adults, indicating the present dosing regimen is well tolerated, with low risk of side effects related to altered vital signs.

Furthermore, no nicardipine effects were seen on routine blood samples, including calcium levels. This is unsurprising given hypocalcaemia is a rare complication of CCB toxicity (DeWitt & Waksman, 2004; Price et al., 2014), and in this study prescribed doses were well within recommended guidelines. Taken together, these findings are important for establishing that primary outcome measures (namely mood and cognition) were not confounded by changes in peripheral blood pressure, heart rate or blood biochemistry (Zink et al., 2020).

In addition, there were no significant effects of nicardipine on cerebral blood flow. The decision to include ASL in this study, was in part to counteract possible confounding. The absence of nicardipine effects on global cerebral perfusion suggests any neural impact of this drug is unlikely to be confounded by cerebrovascular effects. However, ASL data also provide further insights. ASL can act as a proxy measure for the extent drugs get into the brain. The lack of nicardipine effects on cerebral perfusion, suggest the drug may not have penetrated the brain in sufficient amounts to block central LTCCs. Therefore, it is unclear whether results are negative because antagonism of brain LTCCs doesn't have mood and/or cognitive effects, or because inadequate blockade of LTCCs in the brain may have prevented proper testing of the study hypothesis.

4.4.4 Limitations

There are several limitations to this study. As discussed above, the planned recruitment target was not achieved. Although large numbers were screened, only a small percentage met criteria, including the MDQ threshold. Of those considered to have high mood instability, many were already prescribed medication which meant they were not eligible. Therefore, recruitment of this population took longer than expected. Furthermore, the study was intensive, involving multiple in-person visits as well as daily monitoring. Of the participants originally randomised, two withdrew due to work commitments which interfered with study compliance. Therefore, future studies should account for these difficulties and allow for extended recruitment time.

Additional limitations relate to study design. The effect of LTCC antagonism was assessed using a single DHP CCB, and the duration of follow up was limited to two weeks. Future studies may consider analyses of more than one CCB from the DHP class, with longer follow up periods to determine any delayed effects. Furthermore, only patients aged 18-35 years were eligible to take part in this study. This restricted upper age limit may have resulted in findings that are not generalisable to the wider population.

Finally, there are also limitations related to nicardipine. LTCCs exist in both brain and cardiovascular tissue. Nicardipine demonstrates more selective occupancy in the latter, and therefore, side effects including ankle oedema and flushing are commonly reported (Sorkin & Clissold, 1987). In this study, side effects were minimised by using a sustained release formulation. However preferential cardiovascular occupancy limits dosing, and hence the current regimen may not have enabled adequate occupancy in the brain to produce mood and cognitive effects (Uchida et al., 1995). In the short term, future studies may consider

prescribing the higher standard dose of 45 mg BD, or gradually titrating the dose over a longer time. However, developing more selective drugs that target brain LTCCs may expand the therapeutic potential of LTCC antagonists in the longer term.

4.5 Conclusion

Although there was no evidence to support the hypothesis, for effects of nicardipine on mood instability, OxCaMS remains a pioneering experimental medicine trial with some innovative methodological aspects. Employing a high-intensity, multimodal approach, this study explored a wide range of parameters relevant to brain function. In this way, OxCaMS provides a model for investigating the therapeutic potential of genetic discoveries in psychiatry.

In summary, there was no evidence for an effect of nicardipine on these mood, cognitive, or physiological parameters, measured over a two-week period. The reasons for this are unclear. It may be that LTCC antagonism does not result in any significant mood or cognitive effects. However, alternative explanations may include inadequate power, sub-optimal LTCC blockade in the brain, or an insufficient follow-up period for the selected outcome measures. Behavioural parameters may be delayed, and therefore increasing the follow up duration could be one approach to capture effects in these domains.

While specific mood instability data are lacking, mood changes following antidepressant treatment can take four to six weeks (Quitkin et al., 1984; Uher et al., 2011), whereas neural effects and some cognitive parameters are evident more rapidly (Harmer, Goodwin, et al., 2009; Zink et al., 2020). It is plausible that LTCC antagonists could also exhibit a similar pattern, warranting the need for further investigation. The following chapters aim to do this by exploring the effects of nicardipine on other measures of cognition, as well as neural activity, measured both at rest and during task-fMRI.

Chapter 5. The effect of LTCC antagonism on emotional processing

5.1 Introduction

To date, limited studies have investigated the neuropsychological effects of LTCC antagonism in humans. Small pilot trials have examined adjunctive CCBs for cognitive deficits in schizophrenia (Burdick et al., 2020; Vahdani et al., 2020). However, clinically significant effects of CCBs beyond neurocognition in psychotic disorder, remain largely unexplored. Investigation of nicardipine in Chapter 4, demonstrated an absence of mood and cognitive effects, when compared with placebo. However, alternative psychological measures may be more sensitive for detecting group differences, such as subtle emotional change. If effects were established in these domains, this may provide translational applications for LTCC antagonists in psychiatric phenotypes.

Numerous studies have demonstrated shared circuitry underlying mood and anxiety disorders, and negative emotions, such as fear, anger, and sadness (Dalili et al., 2015; Dymond et al., 2015; Warren et al., 2015) . In depression, there is typically a bias toward negative emotions. Studies in subjects with major depression have demonstrated selective attention towards sad expressions (Bourke et al., 2010), and tendency to perceive positive and neutral expressions as sad (Hale et al., 1998; Leppänen et al., 2004). Subjects with social anxiety tend to misidentify neutral faces as angry (Bell et al., 2011), and those with generalised anxiety disorder have shown a bias toward threat-related stimuli (Bradley et al., 1999). Multiple studies have shown more sensitive recognition of fear in individuals with high anxiety compared to controls (Doty et al., 2013; Joormann & Gotlib, 2006; Surcinelli et al., 2006), and understanding the neurobiology of fear extinction has aided in the treatment of anxiety-related disorders (Dymond et al., 2015; Singewald et al., 2015).

Several lines of evidence suggest LTCC antagonism may affect processing of emotional stimuli. On a biochemical level, LTCCs play an important role in neurotransmitter release and synaptic plasticity, processes which are integral for brain function and development (Casamassima et al., 2010; Zamponi et al., 2015), and which have been implicated in emotional regulation (Berridge, 2014; Kabir et al., 2016).

Data from both animal and human studies also demonstrate the role of LTCCs in aspects of emotional processing. In rodents, LTCC antagonists have shown effects on fear-associated memories. While LTCC inhibition with verapamil and nifedipine in the amygdala of rats has shown impaired fear extinction (Davis & Bauer, 2012), mutant mice expressing DHP-insensitive $Ca_V1.2$ did not show impaired fear extinction with nifedipine (Busquet et al., 2008).

This supports the involvement of $Ca_V1.2$ channels in conditioning fear and suggests LTCC antagonism may play a role in modifying mechanisms underlying fear processing. Effects of LTCC antagonism have also been reported in other emotional behaviours including depressive phenotypes. An early rat study demonstrated antidepressant-like effects with nifedipine on a behavioural despair test (Mogilnicka et al., 1987). This was repeated and expanded in a study of nifedipine and other DHP blockers, including felodipine, isradipine, nicardipine, nimodipine and nitrendipine, which demonstrated similar antidepressant effects (Cohen et al., 1997). Mutant mouse models with altered calcium channel gene expression, also provide insights into LTCC function. In comparison with wild-type mice, *Cacna1c* heterozygous knockouts have shown anti-depressive phenotypes on forced swim- and tail suspension tests (Dao et al., 2010; Kabir et al., 2016). These knockout mice also exhibit increased anxiety-like behaviour (Dao et al., 2010), a finding which has been replicated across several studies (Bader et al., 2011; A. S. Lee et al., 2012).

Research investigating the effects of LTCC antagonists on emotional processing in humans is lacking. To date, drug study outcomes have been limited to mood ratings in patients with BD. For example, the open-label isradipine study, demonstrating improved depression scores (Ostacher et al., 2014), and a small double-blind placebo-controlled experiment of nimodipine, which found decreased mood fluctuations on a clinician-administered measure of depression, mania, anger, and anxiety (Pazzaglia et al., 1993). A few studies have explored the impact of *CACNA1C* risk alleles on emotional processing at the behavioural level. These studies are relevant, as both LTCC risk variants and LTCC antagonists alter LTCC activity, potentially producing similar effects. A study of BD patients and healthy controls found *CACNA1C* risk alleles were associated with impaired recognition of emotional faces in BD, but not in healthy participants (Soeiro-de-Souza et al., 2012). However, more recently, a similar task in healthy individuals showed longer reaction times for participants with the rs1006737 SNP compared to those without (Nieratschker et al., 2015). Additional evidence may come from human imaging studies investigating LTCC risk genes. *CACNA1C* risk variants, which alter the function of LTCCs, have been associated with neural networks related to emotional processing (Bigos et al., 2010; Wessa et al., 2010). Patients with the *CACNA1C* rs1006737 genotype have shown increased activity in the hippocampus during an emotional memory task (Bigos et al., 2010). This reinforces the role of altered LTCC channel function in emotional processing and suggests LTCC antagonism may affect similar paradigms.

Collectively, the available data implicate LTCC channels across a range of emotional behaviours and psychiatric phenotypes. However, the specific effect of LTCC antagonism on emotional processing in humans is less clear. Experimental medicine could provide a suitable

way of investigating the mechanism of action of these drugs. This approach has been valuable for determining the early effects of antidepressants. Previous experimental studies have shown that acute administration of drugs with antidepressant profiles can reduce negative biases in healthy controls (Harmer et al., 2017). For example, seven-day citalopram or reboxetine treatment (Harmer et al., 2004) in healthy participants led to emotional biases in the opposite direction of depression, including reduced recognition of fear and increased recall of positive personality descriptors. These findings were observed at one week, which is earlier than the expected timeframe for mood effects, but after drugs have achieved steady-state concentrations. Similar results have been replicated in other studies, with early emotional processing effects also predicting longer term clinical efficacy (Harmer, Goodwin, et al., 2009), typically occurring before any mood improvement (Godlewska & Harmer, 2021; Harmer et al., 2017; Harmer, O'Sullivan, et al., 2009).

Therefore, the present study used an experimental medicine design to investigate the effects of nicardipine in healthy participants, using a battery of validated emotional processing tasks from the Oxford Emotional Test Battery (ETB) (Harmer, Cowen, et al., 2011). Compared with patient groups, this (healthy volunteer) population was deemed easier to recruit, and less likely to be affected by cognitive deficits, which could confound the emotional processing results. It was hoped that investigating these domains with nicardipine might provide valuable translational information about the potential downstream effects of LTCC antagonists on mood and cognition.

5.1.1 Study Aims

Considering the limited data available on neuropsychological effects of CCBs, the principal aim of this study was to comprehensively investigate the short-term (14 day) impact of nicardipine on emotional processing in healthy volunteers with mood instability. To achieve this, emotional processing was measured using two cognitive tasks from the Emotional Test Battery (ETB). The facial expression recognition task (FERT) and emotional word categorisation task (ECAT), which are validated ETB tasks, sensitive to the early effects of antidepressants (Harmer, de Bodinat, et al., 2011). The FERT measures ability to differentiate facial expressions, providing a marker of interpretation bias, and the ECAT assesses response to positive and negative self-referent personality descriptors. Using these paradigms, the primary hypothesis was that nicardipine would demonstrate a shift in emotional processing, comparable to an antidepressant profile, with a preference for positive over negative stimuli.

5.2 Methods

5.2.1 Experimental design and procedure

The ETB, including the FERT and ECAT, was collected as part of the larger OxCaMS trial. Full details on OxCaMS, including study procedures and experimental design are outlined in Chapter 4 (see section 4.2). In brief, the study included a two-week pre-randomisation phase, during which baseline assessments, including FERT and ECAT, were collected. Participants were then randomised to nicardipine SR or placebo, followed by a two-week post-randomisation period, which included follow-up FERT and ECAT to determine the effects of nicardipine versus placebo.

5.2.2 Participants

Thirty individuals were randomised to nicardipine SR or placebo (see Chapter 4, section 4.3.1, for baseline characteristics of randomised groups). Due to work commitments, two participants withdrew after randomisation. Therefore, the final dataset for this analysis consisted of twenty-eight participants, fourteen in the nicardipine group and fourteen in the placebo group (see Table 5.1).

Groups completing the ETB, were well matched for sex (X^2 [1, N=28] = 0.15, p=.70). However, there were baseline differences in age. Nicardipine participants were younger than placebo (23.8 ± 4.2 vs. 28.2 ± 5.1, *t*(26)=-2.52, *p*=.018), which corresponds with baseline characteristics of the larger randomised groups (see Chapter 4, section 4.3.1). There were no significant differences in baseline screening between these groups. However, nicardipine participants had higher negative affect (t(26)=2.64, p=.01) and higher negative instability (t(26)=2.95, p=.01) on pre-randomisation PANAS compared with placebo.

Table 5.1 Baseline characteristics for ETB subgroup.

	Nicardipine SR	Placebo
	n=14	n=14
Age (mean; SD)	23.8 (4.2)*	28.2 (5.1)*
Female (n; %)	9 (64%)	8 (57%)
MDQ score (mean; SD)	10.1 (1.8)	9.8 (1.5)
Affect Intensity Measure (AIM) (mean; SD)	164.0 (22.2)	150.6 (23.6)
Affective Lability Scale (ALS-SF) (mean; SD)	51.9 (10.1)	44.2 (11.9)
Barratt Impulsiveness Scale (BIS) (mean; SD)	76.4 (9.7)	69.4 (9.3)
AUDIT (median; range)	7.5 (5.2)	5.9 (6.4)
Sleep Condition Indicator (mean; SD)	14.2 (7.4)	14.6 (8.0)

	Nicardipine SR	Placebo
PANAS-SF questionnaire (mean, SD)	n=14	n=14
Mean positive affect	12.2 (2.3)	11.8 (3.8)
Mean negative affect	10.8 (2.5)*	8.3 (2.5)*
tRMSSD (instability) positive affect	9.1 (3.6)	9.7 (4.6)
tRMSSD (instability) negative affect	9.0 (3.7)*	5.1 (2.5)*

SD = standard deviation. * Nicardipine SR and placebo groups significantly different at baseline, p<.05. Abbreviations: AUDIT = Alcohol Use Disorders Identification Test, ETB = emotional test battery, PANAS-SF = Positive And Negative Affect Scale (short-form), tRMSSD = time-adjusted Root Mean Successive Squared Difference.

5.2.3 Facial expression recognition task

The FERT, which forms part of the P1vital® Oxford ETB (Harmer, O'Sullivan, et al., 2009), is a validated computerised task used to assess emotional processing. Specifically, the task involves the presentation of six emotions, namely anger, disgust, fear, happy, sad and surprise, as well as a neutral expression, modified from the *Karolinska Directed Emotional Faces* (KDEF) (Lundqvist et al., 1998). Both positive (happy and surprise) and negative (anger, disgust, fear and sad) facial stimuli are presented at a range of intensities (Young et al., 1997), and it is through the depiction of these differing emotions that the FERT investigates participants subtle biases in the perception of facial expressions (Harmer, de Bodinat, et al., 2011). To create this task, facial expressions were morphed between each emotion (100%) and neutral (0%) in 10% increments, creating a range of intensities. In total, there were ten individuals with four examples of each emotion at each intensity level, as well as neutral, giving 250 facial stimuli overall. These were presented in a random order to participants for 500ms, immediately followed by a blank screen. Participants were asked to identify the expression as quickly and as accurately as possible by button press.

The primary outcome measure was accuracy (percentage correct responses), with misclassifications and reaction times (RTs, for correctly identified emotions) also recorded. Misclassifications were investigated in two ways. (i) One method examined the percentage of expressions of one emotion, which were misclassified as another emotion (i.e. positive faces misclassified as negative, negative faces misclassified as positive). (ii) The other method examined which emotions were falsely selected overall. Accuracy, misclassifications, and RTs compared positive, negative and neutral expressions grouped together, as well as comparing expressions individually (Ruzickova et al., 2023).

5.2.4 Emotional word categorisation task

The ECAT (Chan et al., 2008) measures response speed to positive versus negative personality descriptors. Participants were presented with agreeable or disagreeable characteristics and asked whether they would like, or dislike being described in this way. The task included 30 positive (such as 'cheerful') and 30 negative (such as 'dishonest') valanced words (Anderson, 1968), which were randomly presented on a computer screen for 500 ms at a time. On presentation of the words, participants were instructed to press a 'like' or 'dislike' button as quickly and as accurately as possible. Both accuracy and mean RTs were recorded.

5.2.5 Statistical analysis

All analyses were undertaken in IBM SPSS Statistics 28.0. Using boxplots, data were first inspected for normality to exclude outliers greater than three times above or below the interquartile range. FERT and ECAT data were analysed using a mixed-design (split-plot) analysis of variance (ANOVA), with group (nicardipine versus placebo) as the between-subject factor, and valence (emotional face or personality descriptor) and time (pre- and post-administration of nicardipine/placebo) as within-subject factors. Significant interactions were explored with post-hoc analyses including follow-up pairwise tests or repeated measures ANOVA as appropriate. When sphericity was violated, the Greenhouse-Geisser correction was used. However, uncorrected degrees of freedom are reported for clarity. Partial eta squared (η^2) are included as a measure of effect size ($\eta^2 = 0.01$, small effect; $\eta^2 = 0.06$, medium effect; $\eta^2 = 0.14$, large effect). P-values less than 0.05 indicate statistical significance, however trend significance with $p < 0.10$ are also reported. Finally, sensitivity analyses were included to consider the potential effects of differences in age between the groups.

5.3 Results

5.3.1 Facial expression recognition task

A summary of FERT results is presented in Figure 5.1. Across both groups, neutral and positive faces showed the highest accuracy scores, and negative faces showed the lowest accuracy scores. Fear showed the longest RTs, and neutral faces accounted for the greatest percentage of misclassifications. Split-plot ANOVAs investigated significant time-group-valence interactions for accuracy, misclassifications, and RTs, and these were further examined using pairwise tests and repeated measures ANOVAs. Analyses are explored in detail below, with a table of post-hoc comparisons presented in Appendix 5.1.

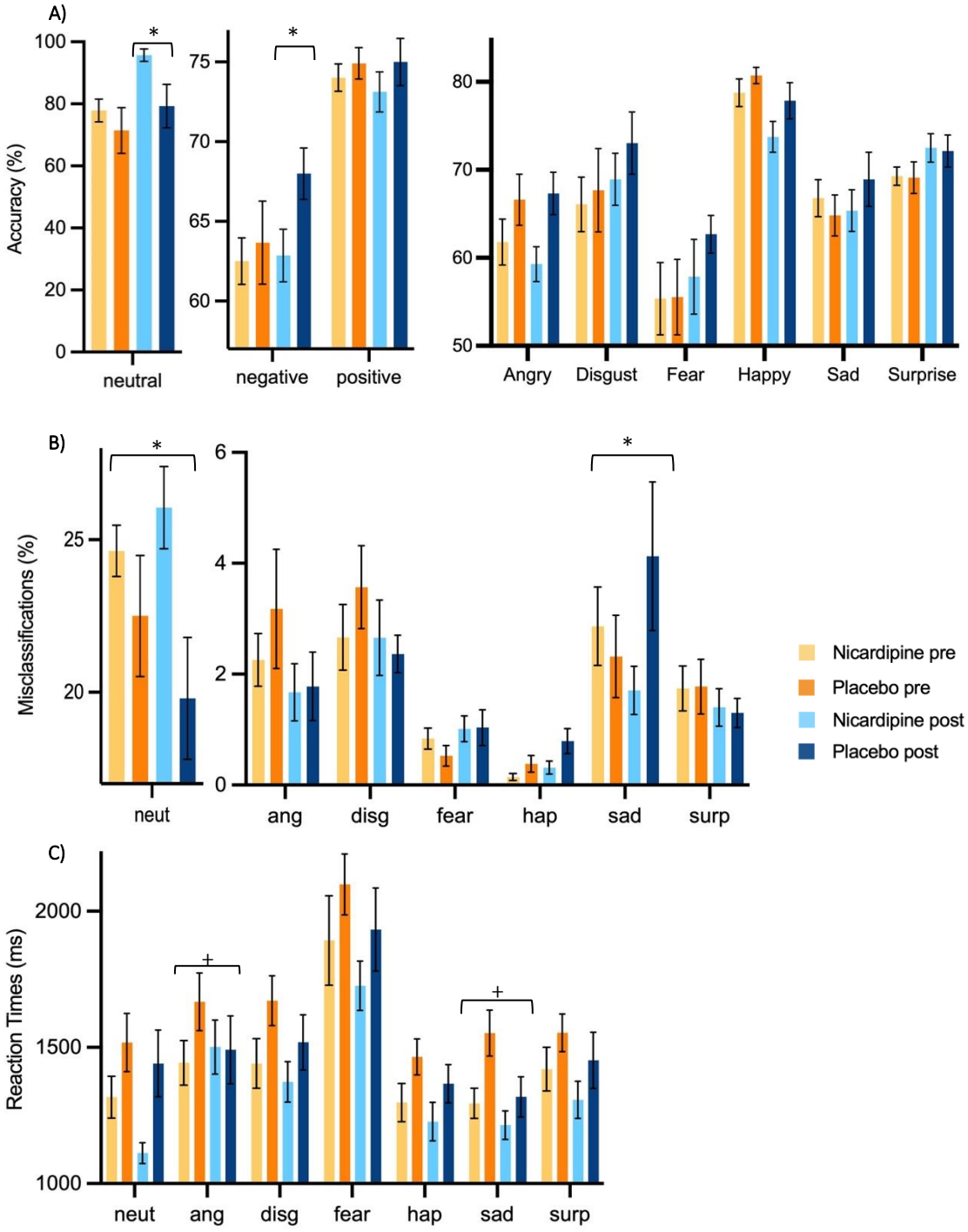


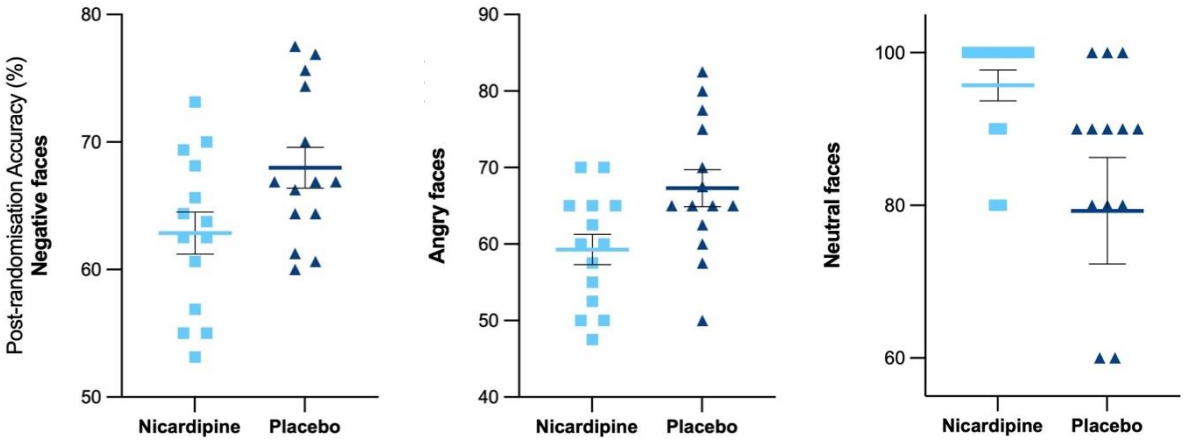
Figure 5.1 Summary of FERT results

(A) FERT accuracy for nicardipine and placebo groups pre and post. Values display % correct responses over range of intensity levels for individual emotions and positive and negative grouped emotions. Note y axis adjusted for individual and grouped accuracy. **(B)** FERT misclassifications overall. Values display % misclassifications for each expression, out of total misclassifications for each participant. Note y axis adjusted for neutral misclassifications. **(C)** FERT average reactions times (RTs), includes only correct responses. Error bars represent SEM. *p <0.05 and + p < 0.10.

5.3.1.1 Accuracy

FERT accuracy showed a significant time-group-valence interaction ($F_{2,52}$ = 4.43, p = 0.03, η^2 = 0.15, Greenhouse-Geisser corrected). The group-time ANOVA did not show a significant interaction ($F_{2,52}$ = 0.96, p = 0.34, η^2 = 0.04, Greenhouse-Geisser corrected) and the group-valence ANOVA showed a trend significant interaction ($F_{2,52}$ = 2.64, p = 0.08, η^2 = 0.09, Greenhouse-Geisser corrected). Follow-up pairwise comparisons revealed the significant time-group-valence interaction was driven by a significant difference in post-randomisation accuracy for negative faces [t(26) = −2.23, p = 0.04, d = -0.84], with the placebo group demonstrating higher negative accuracy compared with the nicardipine group (see Figure 5.2A). Post-hoc analyses of individual emotions, during the post-randomisation phase, demonstrated higher recognition of angry faces [t(26) = −2.57, p = 0.02, d = -0.97] and lower recognition of neutral faces [t(26) = 2.26, p = 0.03, d = 0.85] for placebo versus nicardipine (see Figure 5.2B-C). There was also a significant main effect of time ($F_{2,52}$ = 33.18, p = <0.001, η^2 = 0.56, Greenhouse-Geisser corrected). This was driven by an increase in FERT accuracy from pre to post randomisation for both nicardipine [t(13) = -2.25, p = 0.04, d = -0.58] and placebo [t(13) = -3.45, p = 0.004, d = -0.92] groups.

Figure 5. 2 Individual and mean post-randomisation data for FERT percentage accuracy for (A) negative faces, (B) angry faces, and (C) neutral faces, for nicardipine SR (light blue) and placebo (dark blue). Error bars show SEM.



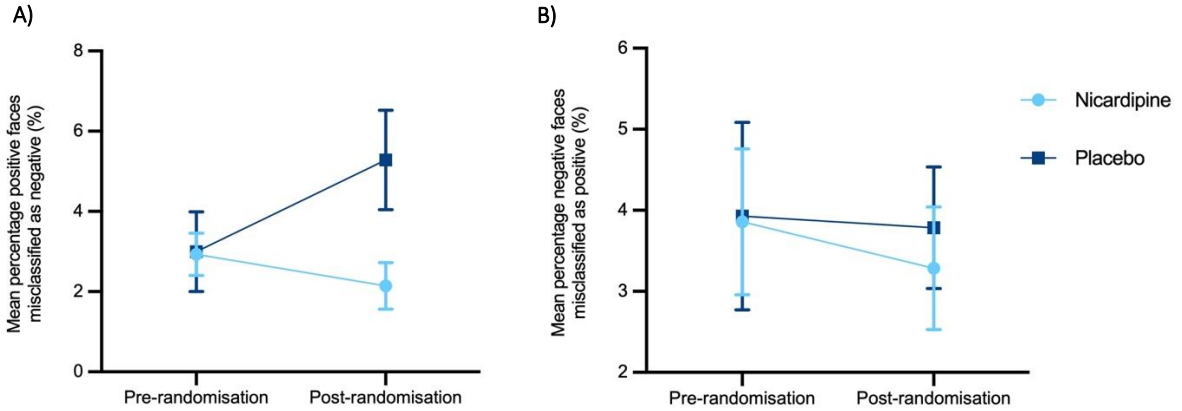
5.3.1.2 Misclassifications

(i) Percentage of expressions of one emotion misclassified as another emotion

There was a significant time-group interaction ($F_{1,26}$ = 7.49, p = 0.01, η^2 = 0.22) for misclassification of positive faces as negative. Nicardipine participants misclassified fewer positive faces as negative after randomisation, whereas placebo participants misclassified more positive faces as negative after randomisation (see Figure 5.3A). No significant time-group interaction ($F_{1,26}$ = 0.14, p = 0.71, η^2 = 0.01) was seen for misclassification of negative faces as positive (see Figure 5.3B).

Figure 5.3 FERT percentage of expressions of one emotion misclassified as another emotion.

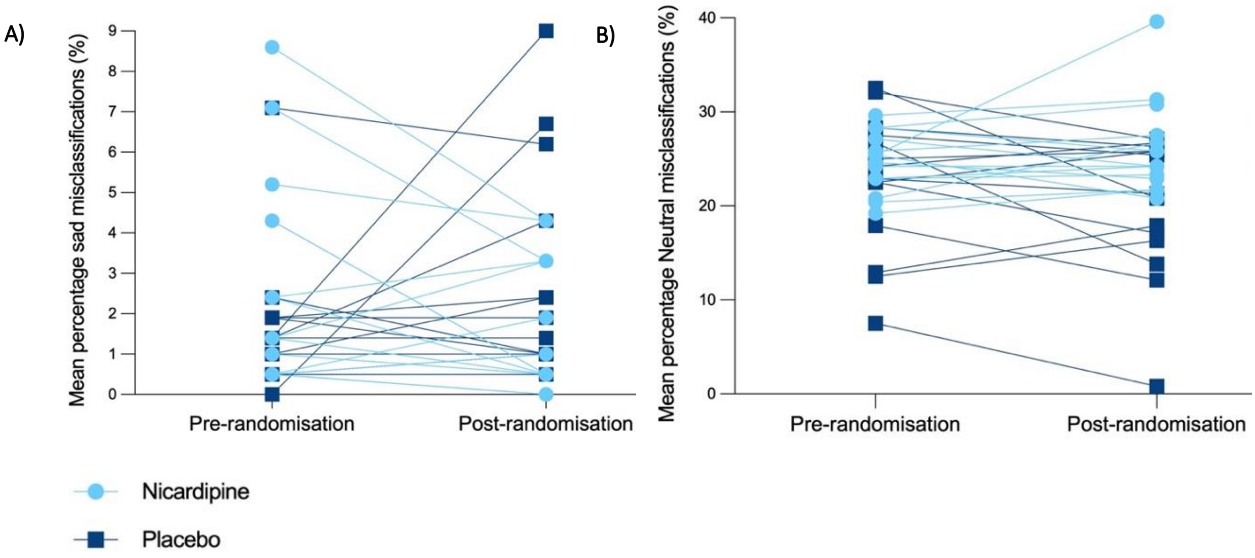
Figure (A) shows positive faces misclassified as negative (negative bias), and Figure (B) shows negative faces misclassified as positive (positive bias), pre- and post-randomisation, for nicardipine SR (light blue) and placebo (dark blue). Error bars show SEM.



(i) <u>Measure of emotions falsely selected overall</u>

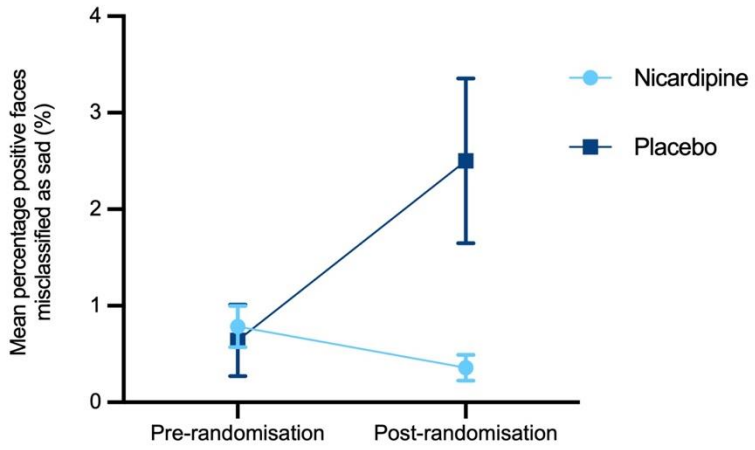
There was a significant time-group-valence interaction for overall percentage misclassifications ($F_{2,52}$ = 4.51, p = 0.04, η^2 = 0.15, Greenhouse-Geisser corrected). Post-hoc analyses of individual emotions showed the interaction was driven by a difference in misclassification for sad faces ($F_{1,26}$ = 7.74, p = 0.01, η^2 = 0.23). After randomisation, participants in the placebo group made more sadness, and less neutral, misclassifications compared with the nicardipine group (see Figure 5.4).

Figure 5.4 Individual participant data for FERT percentage (A) sad and (B) neutral misclassifications pre-and post-randomisation, for nicardipine SR (light blue) and placebo (dark blue).



A follow-up analysis of expressions misclassified as sad, revealed that participants on nicardipine misclassified fewer positive faces as sad after randomisation, whereas participants on placebo misclassified more positive faces as sad after randomisation ($F_{1,26}$ = 11.44, p = 0.002, η^2 = 0.31) (see Figure 5.5).

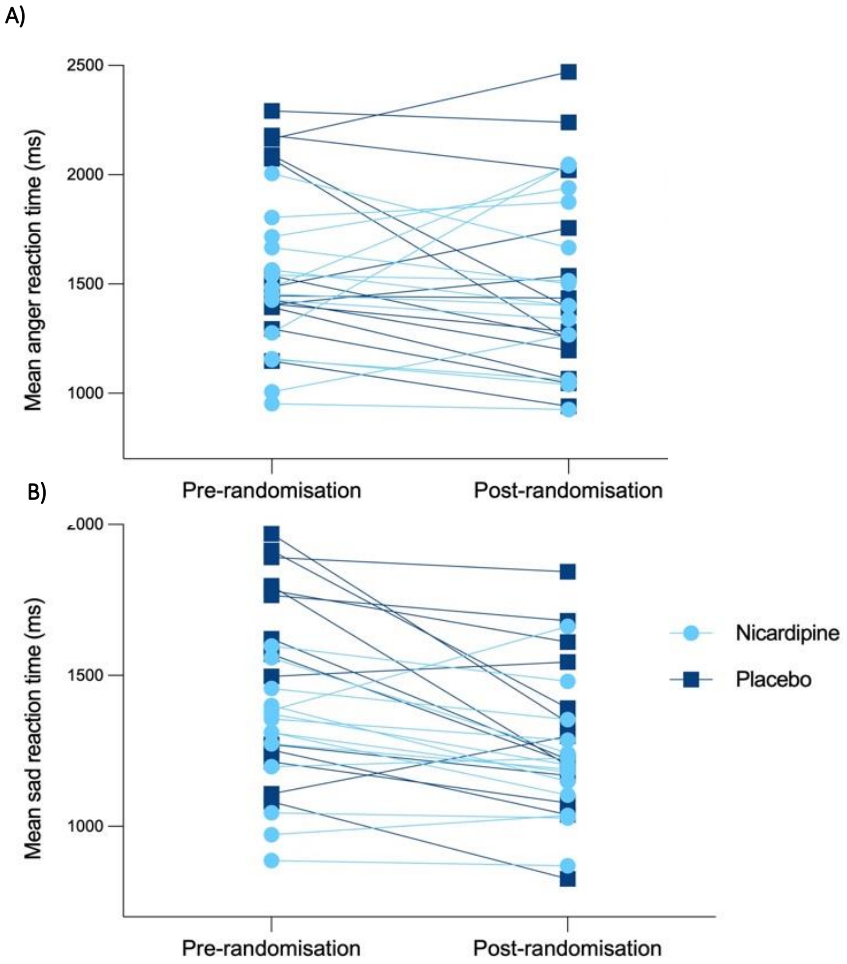
Figure 5.5 FERT percentage positive faces misclassified as sad, pre- and post-randomisation for nicardipine SR (light blue) and placebo (dark blue). Error bars show SEM.



5.3.1.3 Reaction times

FERT RT showed a marginally significant time-group-valence interaction ($F_{2,52}$ = 2.61, p = 0.08, η^2 = 0.09). Post-hoc analyses of individual emotions showed group by time interactions for sad and angry RTs which approached statistical significance. After randomisation, nicardipine RT to angry faces increased whereas placebo decreased ($F_{1,26}$ = 3.99, p = 0.06, η^2 = 0.13) (see Figure 5.6A). Furthermore, RT to sad faces decreased less for nicardipine compared to placebo ($F_{1,26}$ = 4.20, p = 0.05, η^2 = 0.14) (see Figure 5.6B).

Figure 5.6 Individual participant data for A) FERT anger RT and B) FERT sad RT (measured in milliseconds), pre-and post-randomisation, for nicardipine SR (light blue) and placebo (dark blue).



5.3.2 Emotional word categorisation task

There were no significant time-group-valence interactions for the ECAT. Accuracy: $F_{1,26} = 1.74$, $p = 0.20$, $\eta^2 = 0.06$; RT: $F_{1,26} = 0.003$, $p = 0.96$, $\eta^2 = 0.00$ (see Appendix 5.2).

5.3.3 Sensitivity analysis

Results were similar with and without correction for age. As for the uncorrected comparisons, age corrected group-by-time analyses showed a significant difference in misclassification for sad faces between nicardipine and placebo. However, they did not show significant group by time differences for neutral accuracy or neutral misclassifications. Excepting two expressions which approached statistical significance (see Appendix 5.3) all other results remained the same.

5.4 Discussion

The current study examined the effect of 14-day nicardipine SR (30 mg BD) on emotional processing in healthy volunteers, under placebo-controlled conditions. The study aimed to understand the neuropsychological effects of LTCC antagonism, to determine potential benefits in psychiatric phenotypes. The primary hypothesis was that nicardipine would demonstrate a shift in emotional processing (preferential processing of positive vs negative information), comparable to an antidepressant profile.

Findings revealed a significant increase in negative bias in the placebo group. This was characterised by improved recognition of negative facial displays including angry faces. In contrast, the nicardipine group demonstrated improved recognition of neutral faces. The nicardipine group also showed fewer overall misclassifications as sad, fewer positive faces misclassified as sad and fewer positive faces misclassified as negative. Additionally, there was a trend for slower RTs to detect angry and sad faces, compared to placebo. There were no further significant effects on the ETB. These findings may be in line with nicardipine reducing negative bias in emotional processing, and in keeping with an antidepressant effect as hypothesised. However, it is notable that the placebo group was driving the increase in negative accuracy, making it unclear if group differences were due to nicardipine effects or changes in the placebo group.

Decreased negative bias, including reduced recognition of negative faces, has been observed repeatedly in experimental medicine studies of antidepressants. For example, seven-day citalopram or reboxetine treatment reduced anger and fear recognition in healthy controls (Harmer et al., 2004), and similar fear effects have been shown with selective serotonin reuptake inhibitors (SSRIs) in euthymic individuals (Bhagwagar et al., 2004). These findings are further supported by preclinical data, which demonstrate significant reductions in contextual fear expression across a range of animal models treated with SSRIs (Heesbeen et al., 2023). Contemporary theories suggest these positive shifts in emotional processing represent a key mechanism of antidepressant action (Harmer et al., 2017). Studies of depressed patients suggest early positive emotional changes (Godlewska & Harmer, 2021; Harmer, O'Sullivan, et al., 2009; Pringle, Browning, et al., 2011) may contribute to later changes in mood, and predict longer-term treatment response (Godlewska & Harmer, 2021). The effects of antidepressants on negative bias are observed quickly, yet the translation of these effects into improvements in mood requires time as the patient adjusts to a newer, more positive outlook (Harmer, 2008). While more positive perception of social cues might not immediately result in mood changes, it may improve social engagement and interpersonal skills, which over time can enhance mood (Harmer, O'Sullivan, et al., 2009). Consequently,

using a similar model for the investigation of emotional processing in nicardipine may be useful for understanding the therapeutic potential of LTCC antagonism.

In the present study, nicardipine participants were less accurate at identifying angry faces compared with placebo. There were no group differences in the percentage of emotions misclassified as angry, suggesting accuracy differences were not due to non-discriminatory changes in face labelling. Rather, response speed to anger indicated a non-significant increase in reaction time for nicardipine. Although this finding did not meet statistical significance, it is notable in the context of test-retest reliability. Previous use of this task in a repeated design has shown decreased reaction times on subsequent test attempts, for all emotions including anger (Adams et al., 2016). This contrasts with the nicardipine results, which show a trend in the opposite direction. Taken together, this evidence suggests nicardipine reduced overall efficiency at identifying anger, and provides further support for nicardipine inducing a bias away from negative information. Similar anger effects have been reported with acute administration of several antidepressants (Capitão et al., 2015; Harmer et al., 2004). A study examining fluoxetine in young adult volunteers (Capitão et al., 2015) suggested that effects on anger may represent a primary mechanism through which fluoxetine alleviates depressive symptoms. Anger is a key feature of irritability in mood disorders, and growing evidence indicates a possible aetiological role in depression (Leibenluft et al., 2006; Stringaris et al., 2009). More widely, anger has been described as a feature of negative bias in BD, anxiety, schizophrenia and borderline personality disorder (BPD) (Bilderbeck et al., 2017; Davey & Meeten, 2016; Marwick & Hall, 2008; Miskowiak et al., 2019; Potvin et al., 2016; Radimecká et al., 2024), suggesting these findings may have transdiagnostic relevance across multiple psychiatric phenotypes.

Beyond the effects seen in anger, nicardipine also demonstrated changes in the processing of sadness. Decreased misclassification of facial expressions as sad, particularly positive expressions, has been described in several comparable studies as a marker of decreased negative affective bias (De Giorgi et al., 2022; Ruzickova et al., 2023). From a psychopharmacology perspective, drugs showing decreases in negative bias are more consistent with the effects of serotonergic antidepressants, whereas drugs demonstrating increases in positive bias are more typical of noradrenergic drugs (Pringle et al., 2013). Current understanding of how LTCC antagonists might affect emotional processing is limited. LTCC risk genes have been mapped to both psychiatric disorders and their underlying neural circuits. Thus, it is plausible that LTCC antagonists may exert effects on serotonin pathways. Indeed, studies in serotonin-neuron specific *Cacna1c* knockout mice have demonstrated that altered $Ca_V1.2$ function in serotonin neurons disrupts stress-coping behaviour in a forced swim

test (Ehlinger & Commons, 2019). This suggests LTCC channels may play a role in stress behaviour through the serotonergic neurotransmitter system. Considering the involvement of serotonin in emotion regulation, it is possible these channels might also contribute to emotional behaviour via serotonergic mechanisms. Therefore, further research is needed to understand how nicardipine may shift emotional processing, and whether this might translate to clinically significant effects such as mood and cognitive changes.

Finally, it should be highlighted that the present findings are also complicated by changes in the placebo arm, requiring careful interpretation. In the FERT accuracy results, placebo participants demonstrated higher negative accuracy after randomisation. It is uncertain what caused this, however test-retest reliability must again be considered. Repeated use of the FERT in healthy volunteers has shown improved performance one week after baseline (Adams et al., 2016). This is supported by accuracy in the present study, which demonstrated a main effect of time. In general, participants became more accurate between their first and second attempts, suggesting negative accuracy in the placebo group was not unexpected and most likely the result of repeated testing.

5.4.1 Limitations

The present study benefitted from a unique within- and between-subjects design, which allowed baseline comparisons to be made. However, repeated use of the FERT also introduced potential limitations. Although this test battery has been used repeatedly before, such an approach could produce habituation effects on emotional aspects of the task. To address this, the current study used different face images between the first and second visits. Few ETB studies using a within-subject design are available for comparison (Masaki et al., 2016; Ruzickova et al., 2023; Thomas et al., 2016), however a recent examination of the FERT's test-retest reliability (Adams et al., 2016) suggests feasibility of repeat testing. Although performance was better one week after baseline, there were no relative effects on individual emotions. The authors suggested improvements in accuracy and response time were likely due to practice effects, and they concluded that repeat testing over similar time periods was reasonable for future studies.

Even with the randomised double-blind approach, groups differed at baseline. A sensitivity analysis controlled for age, however baseline differences in mood were not controlled for due to the time-varying nature of these covariates (Holmberg & Andersen, 2022). Nicardipine participants were shown to have higher negative instability and higher negative affect compared with the placebo group. Given negative emotional bias is consistently observed in

depressive phenotypes (Peckham et al., 2010) this could have confounded the emotional processing results. It is notable that smaller studies are more prone to such baseline discrepancies, due to random chance. Therefore, future studies may consider a greater sample size or a randomisation schedule minimising on age and/or other covariates.

A general limitation of any FERT analysis is its vulnerability to multiple comparisons (Mayo, 2021), and the subsequent risk of selecting results supporting a particular bias. Although this study aimed to examine multiple facial expressions, the post-randomisation accuracy analyses must be interpreted with caution, due to their post hoc nature. Future studies with a prespecified analysis plan for the post-randomisation data should be considered to replicate these findings more convincingly. Finally, as part of the wider OxCaMS study, this experiment was also subject to the same general limitations discussed in detail in Chapter 4 (see section 4.4.4).

Despite the above limitations, the current study has many strengths. It demonstrates feasibility of using the ETB to screen LTCC antagonists for potential antidepressant effects. Participants were a unique cohort of healthy volunteers, who screened high for mood instability. As such, this was an enriched sample with respect to psychiatric phenotype, yet compared to a clinical sample, less likely to be affected by cognitive deficits that could confound the results.

5.5 Conclusion

The present study suggests nicardipine may reduce negative emotional bias in healthy participants with mood instability. This is consistent with preclinical findings of LTCC antagonists, which have shown effects on emotion processing including emotional memory and conditioned emotional responses. The current findings are in line with an antidepressant effect and suggest nicardipine may reduce negative bias specifically through changes in sad and/or anger perception. This has implications for LTCC antagonists more generally, suggesting one way through which they might mediate clinical effects.

However, further research is required to understand the precise mechanism underlying these changes. Studies exploring neural signal can provide sensitive early biomarkers for psychotropic drug effects (Godlewska et al., 2016). Combined with the current behavioral measures, fMRI techniques may offer novel ways of investigating early changes in emotional processing. This will be the focus of the next chapters, which will examine the potential BOLD response to LTCC antagonism, both at rest and during task-based paradigms.

Chapter 6. The effect of LTCC antagonism on resting-state functional connectivity in the brain

6.1 Introduction

Although previous studies have examined CCBs for mood disorders, little is known about the effect of LTCC antagonism on resting state connectivity. Resting-state fMRI (rs-fMRI) has been widely used to investigate mood disorders, and to understand drug effects, in both patients and healthy controls (McCabe et al., 2011; Taylor et al., 2021). Resting state scans study brain connectivity without external cognitive demands. Brain connectivity is defined as the way in which brain regions communicate and share information with each other (Bijsterbosch et al., 2017). Rs-fMRI examines connectivity by measuring the similarity of blood oxygen level dependent (BOLD) signals in different areas of the brain (Biswal, 2012). If spontaneous fluctuations in the signal are similar, it is likely the areas are sharing information, and hence there is connectivity.

By identifying brain regions with high rates of spontaneous co-activation, rs-fMRI can map resting state networks (RSNs) in the brain. RSNs are cerebral regions demonstrating similarities in their BOLD timeseries acquired during rest (Bijsterbosch et al., 2017). Several RSNs have been reproducibly identified, the most notable of which is the Default Mode Network (DMN). The DMN is a set of brain regions including the precuneus, posterior cingulate cortex (PCC), medial prefrontal cortex (MPFC) and inferior parietal lobule (IPL), which consistently show increased activity during rest, and are thought to contribute to internal cognitive processes. The DMN has been widely studied in mood disorder research, with altered resting state connectivity in the precuneus commonly cited in the literature. For instance, disrupted precuneus connectivity has been found in patients with bipolar II disorder (Gong et al., 2019), and groups of patients with bipolar and unipolar depression have shown decreased functional connectivity between the precuneus and limbic regions compared with healthy controls (Liu et al., 2019). These findings are significant because reduced connectivity in the precuneus has been associated with rumination (Jacobs et al., 2014; Zhu et al., 2017), the repetitive tendency to concentrate on negative thoughts (Nolen-Hoeksema et al., 2008). Hence, it is plausible that impaired functional connectivity in the precuneus could contribute to the rumination observed in BD depression. Importantly, treatment with antidepressants has been shown to reverse some of these alterations in resting state connectivity. For instance, a study in major depressive disorder, showed positive correlations in connectivity between the DMN and left precuneus following administration of citalopram, a finding which was not observed in the placebo group (Dutta et al., 2019). Furthermore, altered functional connectivity

between resting state networks involved in cognitive control, and the left precuneus, has been noted as a predictor of medication response in bipolar depression (Martens et al., 2021).

Another RSN frequently associated with psychiatric disorder is the salience network (Huang et al., 2022; Orliac et al., 2013; Shao et al., 2018). Structurally, the salience network includes the insula and dorsal/pregenual anterior cingulate cortex (ACC). It is responsible for filtering external stimuli and integrating multimodal information (Menon, 2019), and as such is relevant in mood disorders. The literature varies regarding the nature of salience network connectivity in BD. Some studies report increases (Ellard et al., 2019; Li et al., 2018), while others report decreases (Gong et al., 2019; Sheffield et al., 2017). A systematic review by Yoon and colleagues (Yoon et al., 2021) found hypoconnectivity within the salience network in BD patients at rest, whilst a later appraisal reported salience network hyperactivity in BD depression (Claeys et al., 2022). Specifically, Claeys and colleagues reported increased activity of the insula (Liu et al., 2012; Yu et al., 2017), an important node in the salience network (Menon & Uddin, 2010). Given the insula's role in emotional memory and regulation, and the synthesis of cognitive and emotional information (Gu et al., 2013; Schurz et al., 2014), the authors speculated that disrupted insula activity might impede the effective integration of cognitive and emotional information that is commonly observed in BD (Claeys et al., 2022). However, they also noted significant methodological differences across studies, which limited conclusions, and may in part explain inconsistent findings in the literature. Adding to the heterogenous research findings, salience network stability has also been suggested as a marker for euthymia in BD (Syan et al., 2018). Although these diverse findings may reflect differences between the populations studied, including variations in mood episodes, these data hint at the likely complex nature of functional connectivity in mood disorders.

The effect of LTCC antagonists on RSNs has not been investigated before. There is only one previous study examining effects of LTCC antagonism on neural functioning (Zink et al., 2020). In this experiment, single dose nimodipine and matched placebo were studied in healthy male volunteers, genotyped for the *CACNA1C* risk allele rs1006737. Nimodipine significantly reduced frontal cortical and parietal cortical activity on an N-back task of working memory. From this, the authors suggested that nimodipine improves cortical processing efficiency for working memory. They concluded that neural change in these key cognitive regions, which notably include the precuneus, could be a useful biomarker in future CCB studies. However, these inferences related specifically to working memory, and functional connectivity was not investigated.

Extensive fMRI experiments have examined LTCCs through the study of *CACNA1C* risk alleles. This is valuable, because demonstrating *CACNA1C* correlates with functional connectivity may establish a precedent for LTCC antagonists also effecting resting-state fMRI. The rs1006737 risk variant has been linked to abnormal functional connectivity in both BD patients and healthy controls (Janiri et al., 2021; Ou et al., 2015). In BD, risk allele carriers have demonstrated decreased visual-prefrontal (Dima et al., 2013) and medial frontal gyrus (Radua et al., 2013) connectivity during emotional processing tasks. In healthy risk allele carriers, task-based fMRI has shown increased precuneus activity (Kabir et al., 2016; Krug et al., 2010) and task-based functional connectivity has demonstrated altered fronto-limbic connectivity, involving multiple regions (including the amygdala, prefrontal cortex, hippocampus, medial temporal lobe and ACC) (Cosgrove et al., 2017; Erk et al., 2010; Paulus et al., 2014; F. Wang et al., 2011). A resting state study of adolescent risk allele carriers, with and without BD, found rs1006737 was associated with differential functional connectivity in the ACC, amygdala, and orbitofrontal cortex (OFC) (Jiang et al., 2023). This indicates altered connectivity in regions relevant to emotion and cognitive function. However, risk allele carriers with and without BD showed opposite effects, limiting conclusions regarding the direction of functional connectivity in these regions.

The present analysis aimed to examine, for the first time, the effects of nicardipine versus placebo on resting-state functional connectivity in healthy volunteers with mood instability. Average RSN connectivity was investigated using whole brain Independent Component Analysis, an exploratory data-driven technique, to decompose data into different spatial and temporal components. The primary hypothesis was that participants taking nicardipine would exhibit altered functional connectivity, relative to placebo, in networks relevant to emotion and cognition, such as the DMN and salience networks. Any decrease in functional connectivity would correspond to a reduction in temporal correlation of BOLD signal across networks, whereas any increase in connectivity would demonstrate greater temporal correlation of BOLD signal.

Given the literature described above, seed-based analyses were also conducted in the precuneus. This region has shown involvement in mood disorders (Gong et al., 2019; Liu et al., 2019) and is sensitive to drug effects, having previously demonstrated altered DMN connectivity following antidepressant treatment (Dutta et al., 2019). Moreover, emerging task fMRI indicates increased precuneus activity in carriers of the *CACNA1C* risk allele (Krug et al., 2010), adding further rationale for these analyses. Considering the earlier findings in Chapter 5, suggesting an antidepressant-like effect of nicardipine, it was hypothesised that relative to placebo, the nicardipine group would show reduced functional connectivity between

a key area of the DMN, the precuneus, and regions relevant to emotion and cognition, which would be in-keeping with previous SSRI studies (Dutta et al., 2019).

Overall, this chapter hoped to identify neural effects of nicardipine, to advance understanding of LTCC antagonism. In this way, a compelling case for developing more selective drugs, with improved efficacy and tolerability could be provided.

6.2 Methods

6.2.1 Experimental design and procedure

fMRI Resting state data were collected as part of the OxCaMS study. Participants completed two resting state scans, which included one on day 14, before randomisation to nicardipine or placebo, and one on day 21-28, at least seven days after randomisation. The study was designed in this way to allow for 'between subject' (i.e. nicardipine versus placebo) and 'within subject' (i.e. pre- versus post-exposure to nicardipine) analyses.

6.2.2 Participants

Out of thirty participants randomised to OxCaMS (see Chapter 4, section 4.3.1 for baseline characteristics), twenty-eight completed the study and twenty-five had pre- and post-randomisation fMRI scans. ASL calibration images, required to quantify perfusion, were missing for the first subject, resulting in twenty-four participants for the resting state analysis. Of these, twelve were randomised to nicardipine and twelve to placebo. Demographic details are given in Table 6.1 for subjects included in the resting state results.

Groups were well matched for sex (X^2 [1, N=24] = 0.17, p=.68), although nicardipine participants were younger than placebo, resulting in baseline differences in age (23.8 ± 4.4 vs. 27.8 ± 5.4, t(22)=-2.08, p=.048). Nicardipine participants also had higher negative affect (t(22)=2.41, p=.03) and higher negative instability (t(22)=2.83, p=.01) on PANAS at baseline.

Table 6.1 Participant demographics and baseline clinical characteristics

	Nicardipine SR	Placebo
	n=12	n=12
Age (mean; SD)	23.8 (4.4)*	27.8 (5.4)*
Female (n; %)	7 (58%)	6 (50%)
MDQ score (mean; SD)	9.9 (1.9)	9.9 (1.5)
Affect Intensity Measure (AIM) (mean; SD)	162.1 (23.5)	146.8 (22.4)

Affective Lability Scale (ALS-SF) (mean; SD)	52.4 (10.8)	43.6 (11.6)
Barratt Impulsiveness Scale (BIS) (mean; SD)	76.0 (10.4)	69.4 (10.1)
AUDIT (median; range)	7.8 (5.6)	6.1 (6.9)
Sleep Condition Indicator (mean; SD)	15.0 (7.8)	14.8 (8.1)

	Nicardipine SR	Placebo
PANAS-SF questionnaire (mean, SD)	n=12	n=12
Mean positive affect	12.4 (2.4)	12.2 (4.0)
Mean negative affect	10.6 (2.4)*	8.1 (2.7)*
tRMSSD (instability) positive affect	8.6 (3.7)	9.4 (4.9)
tRMSSD (instability) negative affect	8.2 (3.5)*	4.7 (2.4)*

SD = standard deviation. * Nicardipine SR and placebo groups significantly different at baseline, p<.05. Abbreviations: AUDIT = Alcohol Use Disorders Identification Test, PANAS-SF = Positive And Negative Affect Scale (short-form), tRMSSD = time-adjusted Root Mean Successive Squared Difference.

6.2.3 Neuroimaging Protocol/Data acquisition

MRI data was acquired on a Siemens 3T Prisma with a 32-channel head coil (located at the Oxford Centre for Human Brain Activity, OHBA). Eyelink 1000 (an infrared eye tracker) ensured participants remained alert for the duration of the scan, and a pulse meter and respiratory bellows collected physiological data as part of the BIOPAC system.

The MRI protocol started with a brief 3-plane localiser, which calibrated the subject's head position and can be mapped onto subsequent scans. This was followed by a resting state scan (~10 minutes), field map (~1 minute), T1 structural scan (~6 minutes) and 3D ASL sequence (~7 minutes). Full details of the neuroimaging protocol and resting state acquisition are described in Chapter 4 (see section 4.2.5).

6.2.3.1 Resting state fMRI

A gradient-echo EPI sequence was used to complete whole-brain resting-state imaging (TR = 775ms, TE = 37ms, flip angle = 50°, field of view = 216mm, voxel dimension = 2.0 x 2.0 x 2.0mm, acquisition time = 10min 10s). Participants were told to keep their eyes open, try not to fall asleep and to think of nothing in particular. They were presented with a black fixation cross on a white background and an eye tracking camera confirmed that their eyes remained open throughout.

6.2.3.2 Fieldmap and structural fMRI

Distortion correction was performed using an acquired fieldmap (echos at 4.92 ms and 7.38 ms, TR = 482ms, flip angle = 46°). T1-weighted structural MRI scans (TR = 1900ms, TE = 3.96ms, flip angle = 8°, field of view = 256mm, voxel dimension = 1mm isotropic, acquisition time = 7min 21s) were completed at the same time and used for pre-processing of the resting

state analysis. Participants were asked not to fall asleep but were able to close their eyes during this scan.

6.2.4 Data analysis methods

fMRI data were analysed using FMRIB software. The FMRIB Statistical Library (FSL) includes tools for structural, functional and resting state analysis (FMRIB Software Library v6.0; https://fsl.fmrib.ox.ac.uk/fsl) (Jenkinson et al., 2002; Smith et al., 2004). A flow chart, summarising the data analysis methods used, is illustrated in Figure 6.1.

6.2.4.1 Pre-processing

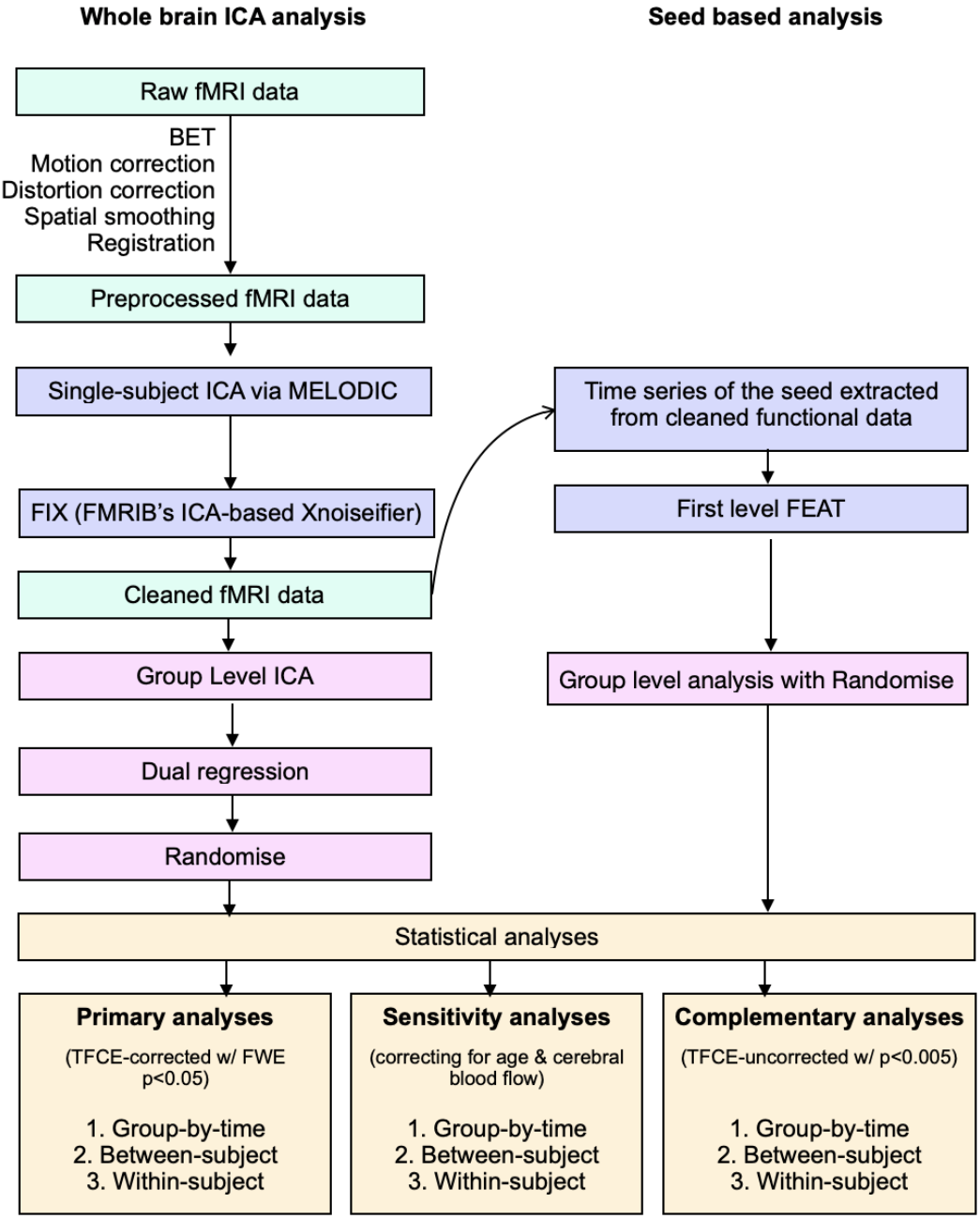
Data pre-processing included multiple steps to reduce unwanted structural variability and to optimise validity of the statistical analyses. Brain extraction of the structural images was completed using FSL's Brain Extraction Tool BET (Smith, 2002), and fieldmaps were prepared by creating the fieldmap rads image and brain extracting the fieldmap magnitude image (Gholipour et al., 2008). Pre-processing and first level analysis of single subject resting state fMRI was carried out using Multivariate Exploratory Linear Decomposition into Independent Components (MELODIC version 3.15, FSL). This included the following steps for each subject:

i) Brain Extraction Tool, BET (Smith, 2002) used to remove non-brain data
ii) FMRIB's Linear Image Registration Tool (McFLIRT) (Jenkinson et al., 2002) used for motion correction
iii) Distortion correction through B0 unwarping (using field-map rads and magnitude images)
iv) Spatial smoothing with a Gaussian kernel of FWHM (full width at half maximum) 5mm
v) Grand-mean intensity normalisation of the 4D dataset by a single multiplicative factor
vi) High pass temporal filtering with a cut-off of 150s

Further resting state pre-processing included masking non-brain voxels, voxel-wise demeaning of the data and normalisation of the voxel-wise variance. Resting state images were registered to their anatomical images using the high contrast functional image and boundary based registration using FLIRT (Jenkinson et al., 2002; Jenkinson & Smith, 2001). The FMRIB non-linear registration tool, FNIRT (Andersson et al., 2007) was used to register from structural to MNI (Montreal Neurological Institute) standard space, with a resampling resolution of 2 mm.

Figure 6.1 Flow chart of resting state data analysis

Pre-processing steps (highlighted in green), first level analyses (purple), higher level analyses (pink), and statistical approach (orange).



Abbreviations: BET = brain extraction tool, FEAT = fMRI expert analysis tool, FWE = family-wise error, ICA = independent component analysis, MELODIC = multivariate exploratory linear decomposition into independent components, TFCE = threshold-free cluster enhancement.

6.2.4.2 Independent Component Analysis

MELODIC Independent Component Analysis (ICA) was used to decompose 4D data into different temporal and spatial components (Griffanti, 2019). ICA is a model-free, non-hypothesis driven approach which produces a set of independent spatial maps and associated time courses. All components were labelled as signal, unknown or noise. ICA denoising was completed to clean the dataset using FMRIB's ICA-based Xnoiseifier (FIX), which regressed out noise components (Griffanti et al., 2017) in two-steps:

i) A sub-group of 20 subjects (ten from each group) were selected at random. Components were manually labelled and checked by an independent researcher taking a conservative approach (i.e. including components where there was any disagreement).

ii) The labels for these 20 subjects were used to create a training dataset which then automatically labelled components as signal, noise or unknown for all subjects.

A LOO test was performed to check the quality of the training dataset and select the best threshold. A threshold of 20 gave a true positive ratio (TPR) of 93.3% (i.e. percentage signal correctly retained) and a true negative ratio (TNR) of 62.7% (i.e. percentage signal correctly removed). Components identified as noise were regressed out.

Following on from this, the pre-processed cleaned data was registered to standard space and temporarily concatenated across subjects, forming a single 4D dataset. A group ICA was run to decompose the data and enable the detection of large-scale patterns of functional connectivity within the subjects. This was limited to 25 components, in line with previous research (Filippini et al., 2009), which were then checked to identify RSNs. Both visual inspection (by two investigators) and comparison to existing published maps (Smith et al., 2009) were used.

Dual regression was completed to allow voxel-wise comparison of resting-state connectivity between the groups. This involves two steps, starting by regressing the group-average spatial maps into the subject's 4D space-time datasets. This produces a set of subject-specific time courses, and then these time courses are regressed into the same 4D dataset, resulting in a set of subject-specific spatial maps. These spatial maps (across groups of subjects) can be compared to look for group differences using FSL's randomise permutation-testing tool (Randomise). Group differences between placebo and nicardipine spatial maps were determined using the voxel-wise general linear model (GLM).

6.2.4.3 Seed based analyses

Seed-based analyses were completed using denoised functional data from the whole brain ICA. Two separate precuneus analyses were completed using masks produced in distinctly different ways. First a mask was created from standard space using the Harvard-Oxford Subcortical Structural Atlas within FSL (Figure 6.2). Second, a mask from MNI coordinates was created, by defining a 10 mm sphere around the peak coordinates (MNI x=48, y=31, z=63) identified in the ICA analysis (Figure 6.3). Masks of an area of white matter (WM) and cerebrospinal fluid (CSF) were also created. Inverse registration maps were used to convert all masks from standard space into each individual's functional space. The mean time series for voxels within the masks was then calculated for each individual.

Seed-based correlations were carried out for each participant using the FMRI Expert Analysis Tool (FEAT) v6.0 The time course from each mask was used as an explanatory variable (EV) to establish which other brain regions positively correlated with this time course. Time courses from white matter (WM) and cerebrospinal fluid (CSF) masks were included as covariates of no interest. Higher-level analyses were run using randomise. Design matrices were created for each seed and imported into randomise to conduct statistical analyses, as described below.

Figure 6.2 Precuneus mask from Atlas
created using Harvard Oxford Subcortical Structural Atlas in FSL.

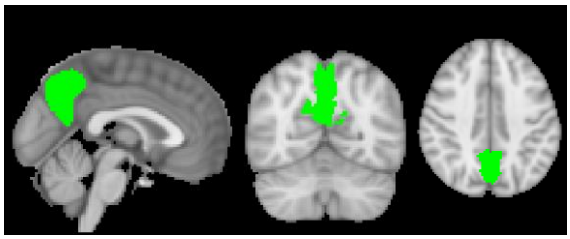
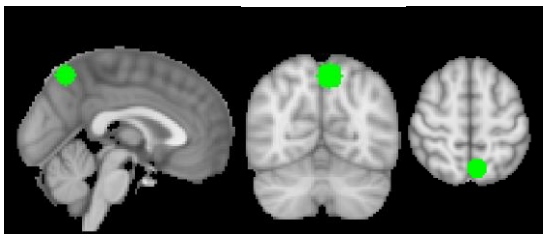


Figure 6.3 Precuneus mask from MNI coordinates
created with a 10mm sphere around peak coordinates (MNI x=48, y=31, z=63).



6.2.4.4 Statistical analyses

Voxel-wise GLM based analyses tested for statistically significant differences between groups using non-parametric permutation testing (5,000 permutations). Clusters were determined using Threshold-Free Cluster Enhancement (TFCE) and a family-wise-error (FWE) corrected

cluster significance threshold of p<0.05. For completeness, TFCE-uncorrected images with p<0.005 were also included (see section 6.2.4.4.3 for further details). The same statistical approach was taken for the whole brain ICA and the seed-based analyses. Due to this study's unique mixed design (including within and between subjects), a range of statistical analyses were conducted, which expanded on standard FSL pipelines.

6.2.4.4.1 Primary analyses

Three separate design matrices were created for primary analyses (see Figure 6.1). First, difference scores between pre and post randomisation were compared between groups, to test for group by time interactions. For these analyses, the following contrasts were used in the GLM: 1. Nicardipine group mean, 2. Placebo group mean, 3. Nicardipine > Placebo, 4. Placebo > Nicardipine. Second, post-randomisation scans were compared between groups to test for between-subject differences. These analyses used the same contrasts as above. Although this is arguably the standard approach taken in the fMRI literature (Capitão et al., 2020; Filippini et al., 2009), these analyses do not capture changes over time within each group. Finally, as an exploratory analysis, pre and post scans within each group were compared to test for within-subject changes over time. For these analyses, the following contrasts were used in the GLM: 1. Pre > Post, 2. Post > Pre.

6.2.4.4.2 Sensitivity analyses

Sensitivity analyses considered the potential effects of age and cerebral blood flow as voxel-wise covariates (see Figure 6.1). These nuisance regressors were included in the GLM to account for baseline differences in age, and subtle group differences in cerebral perfusion as indicated by ASL perfusion maps (see Appendix 4.4). As described previously, ASL was used to distinguish neural effects of nicardipine from effects on the vasculature (D. Wang et al., 2011). Despite no significant effects on global cerebral perfusion, some marginal post-randomisation differences suggested nicardipine may reduce regional perfusion relative to placebo. Thus, ASL was included in the sensitivity analysis to cautiously account for any potential confounding. As for primary analyses, clusters were thresholded using TFCE with a FWE cluster significance threshold of p<0.05. Data were compared using group by time interactions, as well as between- and within-subject analyses.

6.2.4.4.3 Complementary analyses

This thesis also includes TFCE-uncorrected images with p<0.005 (see Figure 6.1). These additional exploratory analyses used lower statistical thresholds, which are in-keeping with complementary analyses used in other DPhil research (Martens, 2019). These complementary analyses were considered useful as they are less prone to type II errors (i.e.

false negatives). Uncorrected images, which do not apply correction for multiple comparisons, may detect functional connectivity that is either undetected or underestimated in TFCE-corrected analyses. However, given the lower statistical threshold, these data must be interpreted with caution.

Activations from primary, sensitivity and complementary analyses are reported using MNI coordinates. Additionally, data are presented in tables and figures, including functional connectivity maps. Graphs depicting BOLD connectivity were created by extracting and plotting individual Parameter Estimate (PE) values from significant clusters.

6.3 Results

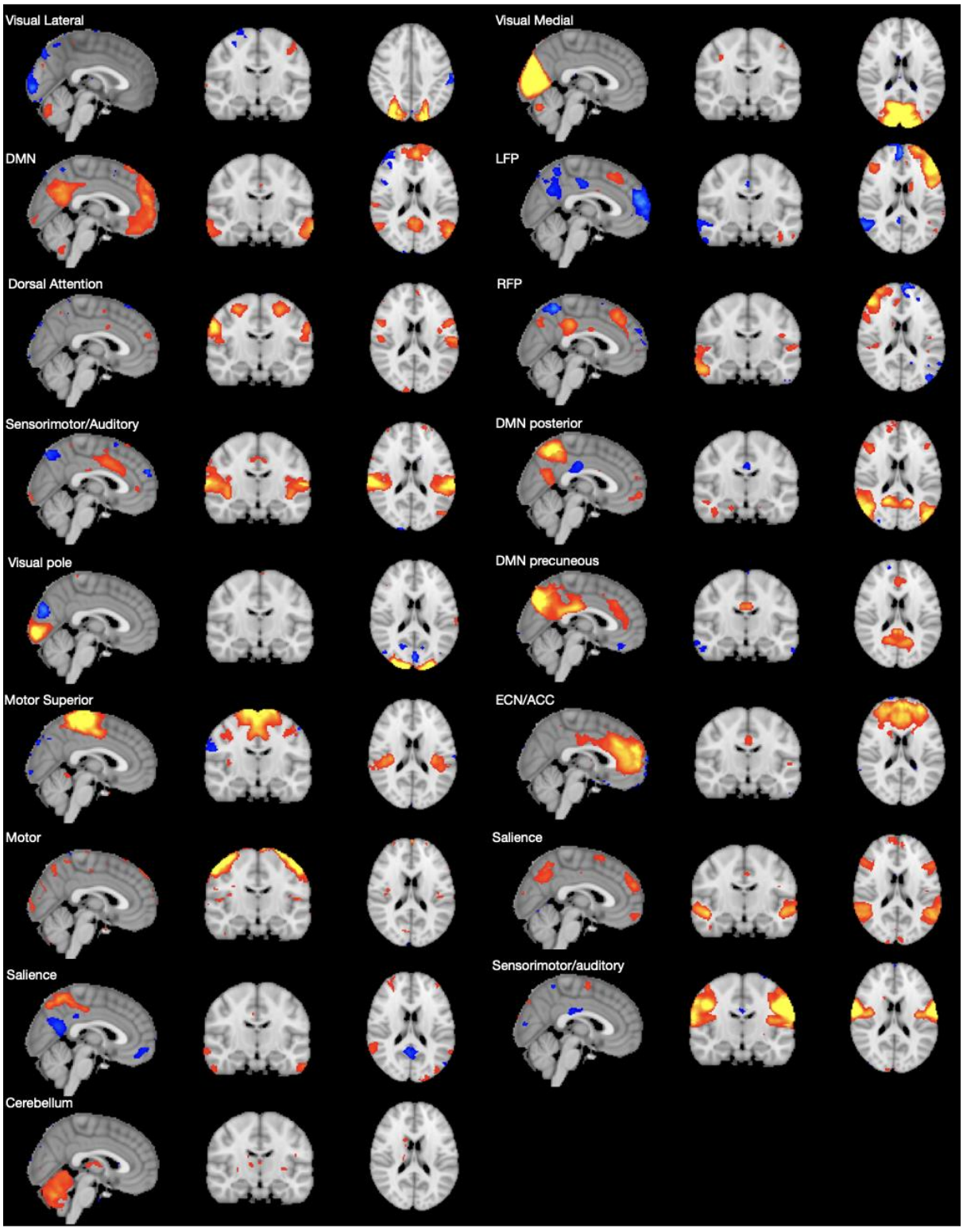
6.3.1 Identifying resting state networks

Independent Component Analysis (ICA) was limited to 25 components, consistent with previous studies (Capitão et al., 2020; Filippini et al., 2009), of which 17 were labelled as RSNs comprising most of the grey matter (Table 6.2 and Figure 6.4). These RSNs matched and overlapped with RSNs previously described (Beckmann et al., 2009; Smith et al., 2009). The remaining 8 components were identified as noise, which included physiological noise (e.g. head motion and cardiac/respiratory function) and scanner artefact.

Table 6.2 RSNs identified by visual inspection

Component number & RSN labelled by visual inspection			
0	Visual Lateral Network	13	Executive Control Network (ECN)/Anterior Cingulate Cortex (ACC)
1	Visual Medial Network	14	Motor network
2	DMN	15	Salience
3	Left Frontoparietal	16	Salience
4	Dorsal attention network	17	Noise
5	Right Frontoparietal	18	Noise
6	Sensorimotor/auditory	19	Sensorimotor/auditory
7	Noise	20	Noise
8	DMN posterior	21	Cerebellum
9	Visual pole	22	Noise
10	Noise	23	Noise
11	DMN Precuneus	24	Noise
12	Motor Superior		

Figure 6.4 RSNs identified (axial, coronal and sagittal slices overlaid onto standard MNI brain).



Left to right, top to bottom: Visual lateral (component 0), Visual medial (component 1), DMN (component 2), Left frontoparietal (component 3), Dorsal attention (component 4), Right frontoparietal (component 5), Senorimotor/auditory (component 6), DMN posterior (component 8), Visual pole (component 9), DMN precuneus (component 11), Motor superior (component 12), Executive Control/Anterior Cingulate Cortex (component 13), Motor (component 14), Salience (component 15), Salience (component 16), Sensorimotor/auditory (component 19), Cerebellum (component 21).

6.3.2 Effect of treatment – Independent Component Analysis

6.3.2.1 Primary analyses

Primary analyses, testing for a group by time interaction, examined whether nicardipine and placebo groups differed in baseline to post-treatment change. For these analyses, there were no differences in RSNs which met the standard level of significance of $p < 0.05$ or trend level of $p < 0.1$. Results for within-subject and between-subject analyses also found no significant RSN differences at $p < 0.05$ or $p < 0.1$.

6.3.2.2 Sensitivity analyses

Sensitivity analyses (see Table 6.3) included age and cerebral blood flow as covariates of interest. Results with correction for these potential effects yielded some significant differences between the groups, which are reported in detail below.

Salience network

A group by time sensitivity analysis revealed significant treatment effects in the salience network (component 15). Functional connectivity between the salience network and the precuneus cortex changed differently for the nicardipine and placebo groups (see Figure 6.5A and Table 6.3). This finding met statistical significance ($p=0.041$) despite comprising only 10 voxels. Uncorrected data were subsequently examined due to the exploratory nature of this analysis (see section 6.3.2.3 and Figure 6.5A). To understand what was driving the difference between groups, paired t-tests were run for nicardipine and placebo participants separately. Despite these post-hoc analyses not meeting significance threshold ($p < 0.1$), parameter estimates were extracted from their custom maps for illustrative purposes, using the significant cluster as a binary mask. Plotting these estimates, to visualise the direction of baseline to post-treatment change in each group, indicated greater connectivity for placebo participants. More specifically, functional connectivity between the salience network and the precuneus cortex increased in the placebo group and decreased in the nicardipine group (from pre to post) (Figure 6.5A).

In addition to the cluster in the precuneus, other small clusters were identified as positively correlated with this RSN (see Figures 6.5B and 6.5C and Table 6.3). Functional connectivity between the salience network and two separate clusters in the inferior frontal gyrus and middle frontal gyrus changed differently (from pre to post treatment) for the nicardipine and placebo groups. Of note, the significant cluster in the inferior frontal gyrus was mostly in the white matter, and the cluster approaching statistical significance in the middle frontal gyrus contained only 6 voxels.

Left frontoparietal network

A group by time sensitivity analysis also revealed significant treatment effects in the left frontoparietal network (component 3) (see Figure 6.6 and Table 6.3). Functional connectivity between the left frontoparietal network and the precentral gyrus changed differently for nicardipine and placebo, with the latter group showing greater increases in functional connectivity. Again, this was a small cluster, which was mostly located in the white matter.

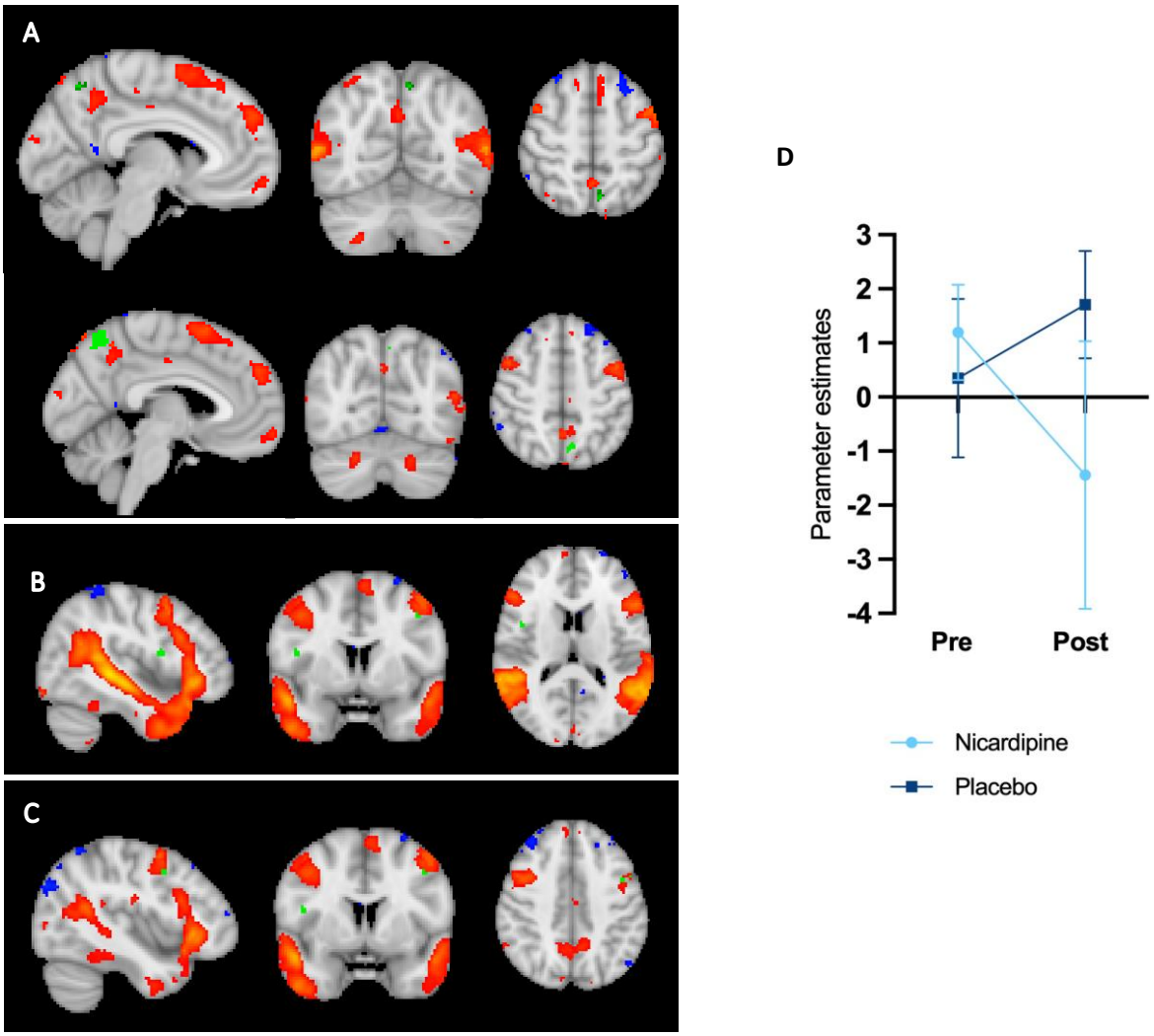


Figure 6.5 Effect of nicardipine versus placebo on salience network; group-by-time analysis. **A)** Functional connectivity between the salience network and the precuneus cortex (cluster highlighted in green) increased in the placebo group and decreased in the nicardipine group (from pre to post). Top figure shows TFCE-corrected data with a FWE cluster significance level of $p<0.05$ ($p=0.041$, peak voxel location: x=48, y=31, z=63, cluster size=10 voxels), and bottom figure shows TFCE-uncorrected data with $p<0.005$ ($p=0.004$, peak voxel location: x=47, y=28, z=61, cluster size=120 voxels). **B)** Functional connectivity between the salience network and the highlighted cluster, partly located in the inferior frontal gyrus, changed differently for the nicardipine and placebo groups. Results are shown for TFCE-corrected data with a FWE cluster significance level of $p<0.05$ ($p=0.039$, peak voxel location: x=22, y=65, z=43, cluster size=9 voxels). **C)** Nicardipine versus placebo differences in functional connectivity

between the salience network and the middle frontal gyrus (cluster highlighted in green) approached statistical significance. Results are shown for TFCE-corrected data with a FWE cluster significance level of p<0.10 (p=0.060, peak voxel location: x=66, y=65, z=57, cluster size=6 voxels). **D)** Functional connectivity between the salience network and the precuneus cortex. Figure shows separate within-subject analyses for nicardipine, and placebo (for illustrative purposes only). Parameter estimates were extracted from their custom maps. Error bars show SEM.

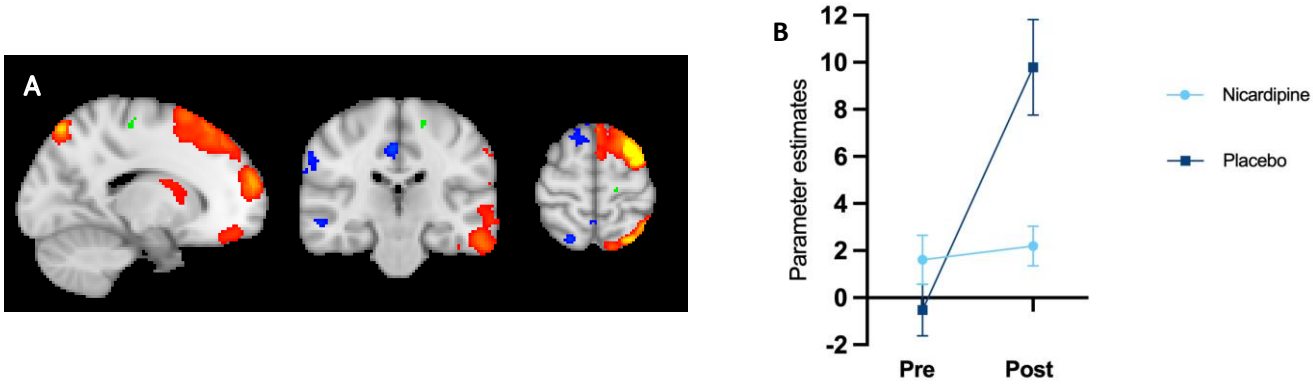


Figure 6.6 Effect of nicardipine versus placebo on left frontoparietal network; group-by-time analysis. **A)** Functional connectivity between the left frontoparietal network and the highlighted cluster, partly located in the precentral gyrus, changed differently for the nicardipine and placebo groups, with the placebo group showing greater increases in functional connectivity. Results are shown for TFCE-corrected data with a family-wise error cluster significance level of p<0.05 (p=0.033, peak voxel location: x=53, y=51, z=67, cluster size=10 voxels). **B)** Functional connectivity between the left frontoparietal network and the precentral gyrus. Figure shows separate within-subject analyses for nicardipine, and placebo (for illustrative purposes only). Parameter estimates were extracted from their custom maps. Error bars show SEM.

Other networks

No further group by time interactions were found in RSN sensitivity analyses. Additional sensitivity analyses explored within-subject changes (pre to post) for each group and between-subject differences at post-treatment. No significant within-subject changes from pre to post were found in the placebo group. In the nicardipine group, sensitivity analysis revealed a treatment effect in the Executive Control Network/Anterior Cingulate Cortex (component 13) which approached statistical significance (see Figure 6.7 and Table 6.3), however this comprised only one voxel. Between-subject sensitivity analyses revealed a significant treatment effect in the motor superior network (component 12) (see Figure 6.8 and Table 6.3). Greater functional connectivity between the superior motor network and the superior frontal gyrus was seen for the placebo group compared to the nicardipine group, post-randomisation. Although this was a larger cluster (69 voxels), most voxels were found in the white matter.

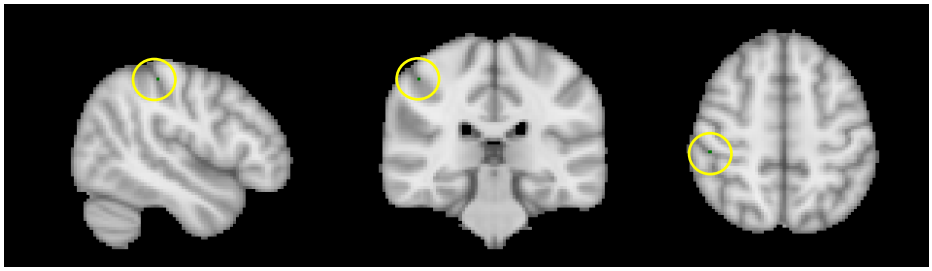


Figure 6.7 Within-subject analyses for executive control network connectivity in the nicardipine group. Functional connectivity between the executive control network and the postcentral gyrus, changed from pre to post randomisation in the nicardipine group. Results are shown for TFCE-corrected data with a FWE cluster significance level of p<0.10 (p=0.090, peak voxel location: x=21, y=48, z=62, cluster size=1 voxel). Data are not plotted in view of small cluster size and p-value approaching statistical significance.

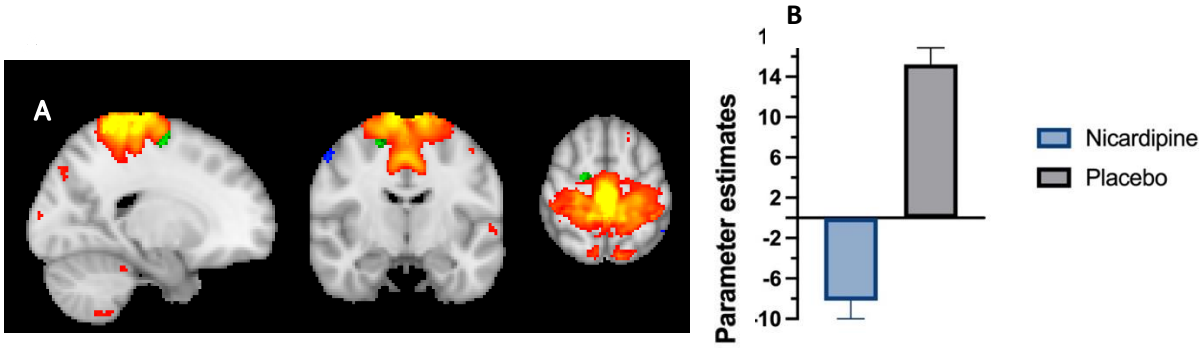


Figure 6.8 Between-subject analyses for motor superior network connectivity.
A) Greater functional connectivity between the superior motor network and the superior frontal gyrus in the placebo group compared to the nicardipine group post-randomisation. Results are shown for TFCE-corrected data with a FWE cluster significance level of p<0.05 (p=0.022, peak voxel location: x=35, y=58, z=65, cluster size=69 voxels). **B)** Functional connectivity between the superior motor network and the superior frontal gyrus. Parameter estimates extracted from the significant cluster in 6.8A. Error bars show SEM.

6.3.2.3 Complementary analyses

For completeness, less rigorous cluster-forming thresholds were examined, for the above sensitivity analyses, as per previous studies (Martens, 2019). Functional connectivity between the salience network and the precuneus cortex was comparable to the TFCE-corrected sensitivity analysis, but a larger cluster was noted. Functional connectivity increased in the placebo group and decreased in the nicardipine group, p=0.004, peak voxel location: x=47, y=28, z=61, cluster size=120 voxels (see Figure 6.5A, bottom figure and Table 6.3 for more details). However, given the lower statistical threshold, this conclusion must be interpreted with caution. All significant clusters in the complementary analyses are presented in Table 6.3. Of note, larger clusters were observed, compared with sensitivity analyses, but otherwise a similar pattern of findings emerged, with significant clusters identified in the same RSNs,

including the frontoparietal and salience networks. This is reassuring as it suggests the sensitivity analyses were not greatly impacted by type II errors (i.e. false negatives).

6.3.3 Effect of treatment – Seed based analysis

Following the ICA, region of interest (ROI) analyses were completed with seeds in the precuneus. As discussed in the introduction (see section 6.1), rationale was based on evidence of precuneus involvement with both LTCC risk genes and psychiatric phenotypes (Gong et al., 2019; Ho et al., 2015; Kabir et al., 2016; Krug et al., 2010; Liu et al., 2019), and previous studies showing this is a region sensitive to drug effects (Dutta et al., 2019).

6.3.3.1 Precuneus mask from Atlas

No significant or trend results were observed for group (between-subject differences post-randomisation), time (within-subject pre to post changes in each group), or group by time interaction, for functional connectivity in the precuneus mask (created using the Harvard-Oxford Subcortical Structural Atlas) at $p < 0.05$ or $p < 0.1$. Sensitivity analyses, correcting for age and cerebral blood flow, and complementary analyses, using uncorrected data, were also examined, however these too found no significant or trend results within the precuneus seed at either $p < 0.05$ or $p < 0.1$, respectively.

6.3.3.2 Precuneus mask from MNI coordinates

No significant or trend results were observed for group (between-subject differences post-randomisation), time (within-subject pre to post changes in each group), or group by time interaction, for functional connectivity in the precuneus mask (created using MNI coordinates) at $p < 0.05$ or $p < 0.1$. Sensitivity analyses, correcting for age and cerebral blood flow, and complementary analyses, using uncorrected data, were also examined, however these too found no significant or trend results within the precuneus seed at either $p < 0.05$ or $p < 0.1$, respectively.

Table 6.3 ICA sensitivity analyses

Detailing effect of nicardipine versus placebo on resting state networks. Age and cerebral blood flow included as covariates. TFCE-corrected and TFCE-uncorrected data shown (for sensitivity and complementary sensitivity analyses respectively).

TFCE-corrected	Cluster	Brain area	Side	Cluster size (voxels)	Voxel max location (x,y,z)	p-value
Component 003 Left Frontoparietal						
nicardipine > placebo[1] *(see Figure 6.6)*	Cluster 1	63% Left cerebral white matter, 36% Left cerebral cortex 32% Precentral gyrus	L	10	53, 51, 67	0.033
Component 012 Motor Superior						
nicardipine > placebo[3] *(see Figure 6.8)*	Cluster 1	62% Right cerebral white matter, 38% Right cerebral cortex 24% Superior frontal gyrus, 11% Precentral gyrus	R	69	35, 58, 65	0.022
Component 013 ECN/ACC						
pre > post[2] *(see Figure 6.7)*	Cluster 1	51% Postcentral Gyrus, 26% Supramarginal Gyrus, anterior division 88% Right Cerebral Cortex	R	1	21, 48, 62	0.090
Component 015 Salience						
nicardipine > placebo[1] *(see Figure 6.5A)*	Cluster 1	65% Left cerebral cortex, 32% Left cerebral white matter 55% Precuneus cortex, 8% Lateral occipital cortex, superior division	L	10	48, 31, 63	0.041
nicardipine > placebo[1] *(see Figure 6.5B)*	Cluster 2	83% Right cerebral white matter, 17% Right Cerebral cortex 7% Inferior frontal gyrus – pars opercularis, 6% Precentral gyrus	R	9	22, 65, 43	0.039
nicardipine > placebo[1] *(see Figure 6.5C)*	Cluster 3	77% Left cerebral cortex, 23% Left cerebral white matter 35% Middle frontal gyrus, 30% Precentral gyrus	L	6	66, 65, 57	0.060

TFCE- uncorrected	Cluster	Brain area	Side	Cluster size (voxels)	Voxel max location (x,y,z)	p-value
Component 003 Left Frontoparietal						
nicardipine > placebo[1]	Cluster 1	100% Left cerebral white matter	L	116	55, 49, 58	0.004
Component 005 Right Frontoparietal						
placebo > nicardipine[1]	Cluster 1	24% Postcentral Gyrus, 22% Central Opercular Cortex, 10% Precentral Gyrus 59% Right Cerebral Cortex, 41% Right cerebral white matter	R	133	15, 60, 43	0.002
placebo > nicardipine[1]	Cluster 2	97% Right cerebral white matter	R	120	24, 50, 52	0.003
Component 009 Visual pole						
nicardipine > placebo[1]	Cluster 1	71% Left cerebral white matter, 21% Left lateral ventricle	L	121	46, 56, 45	0.004
Component 015 Salience						
nicardipine > placebo[1] *(see Figure 6.5A)*	Cluster 1	69% Left cerebral cortex, 31% Left cerebral white matter 65% Precuneus cortex	L	120	47, 28, 61	0.004

[1] Group-by-time interaction

[2] Within-subject differences (pre to post, for nicardipine group)

[3] Between-subject differences at post-treatment

6.4 Discussion

This is the first study to examine the effect of LTCC antagonism on resting-state functional connectivity. RSNs were investigated using two main approaches. First, a whole brain ICA compared average RSN connectivity between the groups, and second, a seed-based method was implemented in the precuneus. It was hypothesised that relative to placebo, the nicardipine group would show altered functional connectivity in networks relevant to emotion and cognition, such as the salience network and DMN. Importantly, unadjusted (primary) and adjusted (sensitivity) analyses (with age and cerebral blood flow as covariates) were performed.

Functional connectivity between the salience network and the precuneus decreased in the nicardipine group from pre to post treatment, whilst increasing in the placebo group. This effect was present between seven and 14 days after starting medication. Whilst the cluster size was relatively small (10 voxels), these findings were replicated in a complementary analysis using uncorrected data, which demonstrated a larger significant cluster (120 voxels).

These results support findings from the MEG resting-state arm of this study, which showed in comparison to placebo, nicardipine was associated with decreases in beta power in two parcels, one in the temporal lobe and one in the parietal lobe including the precuneus (Atkinson, 2020). Although the MEG changes did not survive correction for multiple comparisons across parcels, this pattern may indicate changes in the excitability state of the brain (Routley et al., 2017). Taken together these findings demonstrate, for the first time, effects of nicardipine on precuneus resting state connectivity, and they encourage further investigation in this region.

The precuneus is an important part of the DMN that has unique interactions with the rest of the network. Previous research has shown altered precuneus connectivity in patients with mood disorders. Unmedicated patients with early onset depression have displayed greater precuneus connectivity with regions involved in cognitive control than healthy volunteers, and such connectivity has demonstrated strong associations with symptom severity (Rubart et al., 2022). Within the DMN, the precuneus is involved in a range of highly integrated functions such as self-reflection, emotional reappraisal (Wager et al., 2008) and self-referential thinking, including rumination and worry (Kaiser et al., 2015). Dysregulation of these processes is characterised by exaggerated self-focus, impaired emotion regulation, and persistent rumination, all of which are closely associated with mood disorders, and contribute to the chronicity and severity of symptoms (Dimaggio et al., 2009; Joormann & Gotlib, 2010; Nolen-Hoeksema et al., 2008).

The precuneus has also shown changes in connectivity following psychoactive drug treatment. For instance, a rs-fMRI experiment using intravenous citalopram showed reduced functional connectivity between the precuneus and DMN in healthy controls, which was not observed with placebo (Dutta et al., 2019). This suggests a potential mechanism of action for antidepressant drugs. SSRIs, recognised for their efficacy in treating depressive rumination (Özben et al., 2023), may modulate such symptoms through effects on precuneus connectivity. These findings improve understanding of functional connectivity in the region and may also offer insights into the potential mechanism underlying antidepressant-like effects of nicardipine.

Functional correlations specifically between the precuneus and the salience network have also been demonstrated in previous research. A study exploring connectivity in major depressive disorder and bipolar depression, demonstrated functional hyperconnectivity between the salience network and the precuneus, in depressed patients compared with controls (Todeva-Radneva et al., 2023). The study authors speculated on the clinical significance of these findings. As well as proposing a pathway for internal versus external cognitive bias in depression, these results provide wider insights into the potential direction of precuneus to salience network connectivity in depression and BD.

Several studies demonstrate the importance of the salience network in mood disorders. The salience network is responsible for multiple highly interconnected functions (Schimmelpfennig et al., 2023) including social behaviour, communication, self-awareness and interoception (Nayok et al., 2023), which involve the processing of cognitive, sensory and emotional information. A systematic review by Broome (Broome, He, et al., 2015) found increased functional connectivity in the salience network in clinical populations with affective instability. Disrupted functional connectivity in the salience network and DMN (including the precuneus), was found in patients with bipolar II disorder (Gong et al., 2019) and salience network stability has been shown to reflect a state of remission in BD (Syan et al., 2018). In the current study, the pattern of decreased functional connectivity in the nicardipine group represents altered connectivity between a region involved in self-referential thinking (the precuneus) and a network responsible for interoception, self-awareness, and processing cognitive and affective information (the salience network). It is possible that this pattern reflects a decreased tendency to ruminate following nicardipine administration, as well as diminished focus on interoceptive information, which may have positive clinical implications for BD and related phenotypes. Indeed, it is known that patients with BD show tendency to ruminate during depressive states, as well as increased focus on interoceptive information, which may perpetuate their symptoms

(Nord & Garfinkel, 2022; Perry et al., 2019). Increased interoception might also be linked to affective instability, as research suggests dynamic fluctuations in interoceptive circuitry may impact emotional processing, leading to difficulties in stabilising mood (Perry et al., 2019). Taken together these findings tentatively indicate that functional connectivity between the precuneus and the salience network is relevant to mood disorders, and it may be sensitive to the effects of LTCC antagonism. However, given the absence of behavioural correlates, these hypotheses are speculative, and should be interpreted in the context of several important caveats.

First, the results were nuanced by substantial placebo group changes, which were also driving the group by time interaction. The reason for altered functional connectivity in the placebo group is unclear, however rs-fMRI reliability across repeated tests must be considered. Studies have typically demonstrated robust test-retest reliability for ICA analyses over both short (i.e. less than one hour) and long-term (i.e. over several months) durations (Zuo et al., 2010). However, when scanning sessions are split over different days, the salience network has been shown to be amongst the least reliable (Tozzi et al., 2020), and as such the placebo findings may not be entirely unexpected.

Furthermore, results from the seed-based analyses in the precuneus failed to report the same pattern observed in the ICA results in this region. Generally there is a high level of reproducibility between the approaches (Franco et al., 2013) and therefore it is not certain what might be causing this difference. However, one possibility may result from location differences between the significant ICA cluster and the precuneus seeds. Previous studies have shown that positioning a seed in even a subtly different location, may result in very different outcomes (Cole et al., 2010).

Out with the precuneus finding, several other functional connectivity group differences were reported in the results. Within the salience network, connectivity with two further clusters, in the inferior frontal gyrus and middle frontal gyrus, were noted. However, the significant cluster in the inferior frontal gyrus was mostly in the white matter, and the cluster approaching statistical significance in the middle frontal gyrus contained only 6 voxels. Addressing these findings in the context of the literature, BOLD signal mainly arises from post-synaptic potentials, which occur primarily in the grey matter, as opposed to action potentials in the white matter (Gawryluk et al., 2014). Additionally, despite no minimum recognised cluster size (see Chapter 8, section 8.3 for further discussion), a comparable resting state study investigating medication effects, reported significant clusters ranging from 27 to 41 voxels (Dutta et al., 2019). Similarly, an fMRI study investigating LTCC antagonism reported clusters between 11

and 88 voxels (Zink et al., 2020), although this latter study examined BOLD activation rather than connectivity. Finally, clusters in the left frontoparietal and executive control networks were also limited by size (nine voxels and one voxel respectively), and a cluster in the motor superior network, although larger (69 voxels) was again located mostly in the white matter. It is not clear why the nicardipine group demonstrated decreased functional connectivity in the motor network, compared with placebo. Moon and colleagues (Moon et al., 2018) recently reviewed the role of $Ca_V1.2$ in motor activity, using animal models with variation in *Cacna1c*. These authors reported that dysfunction in *Cacna1c* might result in reduced motor activity, however they suggest further research is necessary.

6.4.1 Limitations

In addition to general considerations of the OxCaMS study, which are discussed in detail in Chapter 4 (see section 4.4.4), there were several limitations specific to the resting state analysis. First, significant findings were only observed in the sensitivity results, with primary analyses demonstrating no significant differences among groups, within subjects, or between groups over time. This suggests the main results were affected by nuisance regressors, namely age and cerebral blood flow. Whilst there is good evidence that age affects RSNs, with a recent study reporting age-related decline affecting functional connectivity across almost every sensory and cognitive network (Varangis et al., 2019), the rationale for including ASL was less persuasive. ASL analyses demonstrated no significant effects of nicardipine on global cerebral perfusion, with only a non-significant reduction in regional perfusion, compared to the placebo group (see Chapter 4, section 4.3.2.1 and Appendix 4.4). This is concerning because the inclusion of ASL was primarily to mitigate against the cerebrovascular effects of nicardipine, which were anticipated to obscure any neural effects across both rs- and task-fMRI. However, as it transpired there were no significant cerebrovascular effects, suggesting nicardipine may not have penetrated the brain to any substantial extent. This in turn warrants further consideration as it prompts wider questions regarding target engagement across all analyses in this study.

A second limitation (which is common to rs-fMRI studies) was the subjective nature of labelling components through the ICA approach. Although this may have introduced discrepancies in the data, attempts were made to mitigate this by using an independent researcher to check labels (Martens et al., 2021), and by creating an automated noise/signal identification algorithm that was “trained” using the study’s own data.

Third, the statistically significant precuneus cluster identified in the salience network sensitivity analysis was only ten voxels. As discussed above, there is no minimum accepted cluster size,

however this cluster appears to fall at the lower end of those reported in similar resting state studies (Dutta et al., 2019; Martens et al., 2021). Minimum cluster size is an important issue, relevant across both imaging chapters of this thesis. As such, the broader implications of fMRI cluster size are further explored in the General Discussion (see Chapter 8, section 8.3).

Finally, traditional ICA and seed-based approaches, employed for these analyses, assume stationary connectivity throughout the scan period (Allen et al., 2014). In reality, connectivity between brain regions and resting state networks varies over time. Therefore, rather than interpreting connectivity as a simple increase or decrease (Cole et al., 2010), future analyses may consider a more dynamic approach that accounts for variability between networks (Chang & Glover, 2010). One of the most commonly used methods is a sliding window analysis which aims to capture switching non-stationarity in resting state functional connectivity (Hansen et al., 2015).

Despite these limitations, the present study also had many strengths. Rs-fMRI is independent of task performance, and the networks identified matched to existing maps (Smith et al., 2009), indicating reliability of the data. Pragmatically the scans were easy to obtain, and they were free from confounders related to task performance. Unique to this study, data were collected for both within and between-subject comparisons. Since this is not a standard fMRI approach, data analysis was complex and additional pipelines were created specifically for these analyses. As these comparisons control for inter-subject variability, sensitivity was increased, compared with typical methods. The study also provided information on rs-fMRI reliability, and the feasibility of using resting state methods in future exploratory drug studies.

6.5 Conclusion

This was the first neuroimaging study investigating the effects of LTCC antagonism on resting state functional connectivity. Studying CCB effects with rs-fMRI is important for developing a deeper understanding of the potential mechanism of LTCC antagonists in neuropsychiatric disorder. In-keeping with the initial hypothesis, there was some evidence that nicardipine decreased functional connectivity between the salience network and the precuneus. However, this finding was nuanced and complicated by unexpected changes in the placebo group. Therefore, it remains to be established if there exist robust rs-fMRI effects with LTCC antagonism. This should form the focus of future work. Functional connectivity must be re-examined to address limitations that may be masking a true treatment change, and furthermore, LTCC antagonism should be studied in task-based fMRI. This will be the basis of the next chapter, which will investigate whether neural markers of emotional processing are modulated by LTCC antagonism during an fMRI faces task.

Chapter 7. The effect of LTCC antagonism on emotional neural processing

7.1 Introduction

LTCC antagonists have been used in BD and related phenotypes, despite limited evidence for their efficacy (Cipriani et al., 2016) or neural effects (Wankerl et al., 2010; Zink et al., 2020). The potential neuropsychological actions of these drugs are poorly understood. Results from Chapter 5, indicate nicardipine SR has early impacts on emotional processing. Compared with placebo, nicardipine decreased negative bias, including reduced recognition of negative faces, and decreased positive face misclassifications (as negative). This suggests nicardipine induced antidepressant-like effects on emotional processing in individuals with mood instability. Further insights may be provided by functional magnetic resonance imaging (fMRI), which is widely used for the study of emotional neural processing. In addition to resting state scans, task-based fMRI is valuable for characterising the mechanisms of drug action (Kotoula et al., 2023). Exposing participants to emotional stimuli and prompting them to perform various tasks in the scanner, allows scientists to study changes in brain activity. By analysing these scans at baseline, and under the influence of pharmacological agents, researchers can improve understanding of drug effects on emotional processing.

The effect of LTCC antagonism on BOLD signal is of interest considering the potential neuropsychiatric effects of these drugs. However, limited fMRI analyses have examined LTCC antagonism in the human brain to date. A 2020 study by Zink and colleagues (Zink et al., 2020) compared the effects of the calcium channel blocker nimodipine versus placebo on neural signal. They found decreased neural activity in frontal and parietal cortices in healthy individuals during the N-back task of working memory, despite no difference in task performance. This suggests nimodipine enhances cortical processing efficiency of working memory information. Notably, the effects were limited to the N-back task, with no changes during an emotional face matching task.

However, these authors previously demonstrated LTCC gene effects on neural circuits related to emotional processing (Bigos et al., 2010). Genetic imaging studies offer a useful way of investigating these channels and may provide clues to the effects of LTCC antagonists. In the Bigos study, individuals with the risk-associated SNP rs1006737 in *CACNA1C* showed increased amygdala activity during an emotional faces task (Bigos et al., 2010), and there is mounting evidence supporting these findings.

Jogia (Jogia et al., 2011) demonstrated increased amygdala activation to fearful faces in healthy rs1006737 carriers, and Wessa (Wessa et al., 2010) showed amygdala changes

during emotional processing in controls with the same gene. Studies in healthy adolescents have also linked *CACNA1C* risk alleles with increased amygdala activation when viewing negative stimuli (Sumner et al., 2015). Other published changes include reduced corticolimbic and frontotemporal functional connectivity during emotional face-processing in healthy risk allele carriers (F. Wang et al., 2011) and decreased ventral prefrontal activation and visual-prefrontal connectivity in BD risk allele carriers (Dima et al., 2013). These changes suggest the psychiatric effects of *CACNA1C* risk alleles focus on neural pathways associated with emotional processing, thus offering a potential mechanism that connects BD to GWAS risk variants. In response to fearful faces, BD risk allele carriers have also demonstrated reduced information transmission from the medial frontal gyrus (Radua et al., 2013). These frontotemporal and corticolimbic areas, including the amygdala, are critical to emotional processing. The prefrontal cortex regulates emotions and cognitive control, while the amygdala acts as a central hub, processing emotional information via complex pathways and neurotransmitter systems. By communicating with various brain regions including the prefrontal cortex, hypothalamus, and brainstem, the amygdala is also responsible for initiating appropriate physiological and behavioural responses to emotional stimuli. Therefore, the observed effects of LTCC risk genes in these regions, support the likely role of $Ca_V1.2$ channels in emotional neural processing (Berger & Bartsch, 2014; Bhat et al., 2012; Kabir et al., 2016), and highlight the amygdala as a region of crucial importance.

Despite this growing research, the specific effects of LTCC antagonism on neural circuits are still poorly understood. Although early evidence for LTCC antagonists in BD is lacking (Cipriani et al., 2016), contemporary studies investigating newer dihydropyridine CCBs show promise in the treatment of depressive and manic symptoms (Li et al., 2023; Ostacher et al., 2014). However, without functional imaging data, it is not possible to characterise the effects of CCBs on neural circuitry relevant to psychiatric disorders. Such knowledge would also help distinguish whether neural effects of LTCC antagonists play a role in improving symptoms, or if such effects are secondary to mood changes. To study this question, fMRI studies are required, investigating LTCC antagonists in a placebo-controlled design.

Neural biomarkers offer sensitive measures of response to pharmacotherapy (Harmer, 2014) and fMRI tasks specifically using emotional faces are well-characterised for probing emotional processing (Capitão et al., 2019; Martens et al., 2023; Rawlings et al., 2010). Early changes in neural processing of emotional faces can predict later clinical effects (Godlewska et al., 2016). For example, antidepressant studies, using the fMRI faces task, have shown acute neural changes with SSRI treatment, especially in brain regions associated with psychiatric disorder, such as the amygdala (Murphy, Norbury, et al., 2009; Rawlings et al., 2010). These

changes can occur rapidly, sometimes following a single administration (Capitão et al., 2019; Martens et al., 2023; Rawlings et al., 2010), and have also been noted in the absence of any behavioural change, suggesting they are direct drug effects. Godlewska and colleagues (Godlewska et al., 2012) studied seven-day escitalopram treatment in depressed patients. Compared to placebo, escitalopram reduced amygdala activation to fear on the faces task, without any associated change in mood. In a follow up escitalopram study, the authors demonstrated early changes in amygdala activation were predictive of later clinical response at six weeks (Godlewska et al., 2016). As such, there is good evidence indicating the fMRI faces task is sensitive to the acute effects of antidepressants on emotional neural processing.

The aim of the present chapter was to employ similar methods for the investigation of LTCC antagonists. The effect of nicardipine SR, 30 mg twice daily for fourteen days, was explored on emotional neural processing measured using an fMRI emotional faces task. Participants were healthy volunteers with high mood instability, recruited into the OxCaMS trial. Considering the earlier findings, reported in Chapter 5, the primary hypothesis was that nicardipine would decrease negative affective neural processing in this task in the absence of subjective mood rating changes. Specifically, it was predicted that participants in the nicardipine group would demonstrate decreased BOLD activation in the amygdala in response to negative emotional stimuli. A primary region-of-interest (ROI) analysis was performed in the amygdala for four key reasons. This area has repeatedly shown altered activation in LTCC gene studies, it is crucially involved in the pathophysiology of multiple psychiatric disorders, it is known to be activated on the emotional faces task (Hariri et al., 2002), and it is sensitive to the early effects of antidepressant drugs (Murphy, Norbury, et al., 2009; Rawlings et al., 2010). For completeness, an exploratory whole-brain analysis was also conducted to identify any additional brain areas affected by nicardipine.

7.2 Methods

7.2.1 Experimental design and procedure

The fMRI faces task was collected as part of the OxCaMS study. As previously described (see Chapter 6, section 6.2.1), participants completed two scans; one on day 14 (pre-randomisation), and one on day 21-28 (post-randomisation).

7.2.2 Participants

There were twenty-four participants for the fMRI faces task, which included twelve in the nicardipine group and twelve in the placebo group. Participants were the same as those completing resting state analyses. Detailed demographic and baseline clinical characteristics are outlined in Chapter 6 (see section 6.2.2).

7.2.3 fMRI emotional faces task

The fMRI emotional faces task (also known as the gender discrimination task) used a block design to investigate the effect of nicardipine on neural response to positive versus negative facial expressions. Participants were rapidly presented with a series of happy, angry, and fearful faces, and were instructed to indicate the gender (male or female) of the face by button press, as quickly and as accurately as possible. The faces used were colour photographs taken from the NimStim database (Tottenham et al., 2009). The task started with a fixation cross (presented for 2900 ms) followed by a face in isolation (presented for 100 ms). In total there were four blocks of the three conditions (each lasting 30 s with ten faces per block). The presentation of blocks was in a fixed order (fearful, happy, angry, repeated four times), and after each block a fixation cross was presented for 30 s. There was no reference made to the face emotion. This task was selected for its sensitivity to emotional biases and antidepressant effects (Capitão et al., 2019; Martens et al., 2023; Rawlings et al., 2010).

7.2.4 fMRI data acquisition and analysis

Details of fMRI data acquisition are as previously described (see Chapter 4, section 4.2.5). fMRI data were analysed using the FMRIB Statistical Library (FSL), which includes tools for fMRI analysis (FMRIB Software Library v6.0; https://fsl.fmrib.ox.ac.uk/fsl) (Jenkinson et al., 2002; Smith et al., 2004).

The fMRI data underwent pre-processing and analysis using the FMRI Expert Analysis Tool (FEAT v6.0), part of FMRIB's Software Library (FSL) www.fmrib.ox.ac.uk/fsl. The pre-processing steps aimed to decrease variability and improve statistical analyses, and included:

i) FMRIB's Linear Image Registration Tool for motion correction (MCFLIRT; (Jenkinson et al., 2002)
ii) Brain Extraction Tool, BET; (Smith, 2002) for deletion of non-brain tissue
iii) Spatial smoothing with a Gaussian kernel of 5 mm FWHM (full- width-half-maximum)
iv) Grand-mean intensity normalisation of the 4D dataset by a single multiplicative factor

v) Temporal high-pass filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 70s)

vi) Registration to standard template; Montreal Neurological Institute (MNI) implemented using FNIRT nonlinear registration (Andersson et al., 2007).

The first-level analysis evaluated the data from each session. Using the general linear model (GLM) with local autocorrelation correction (Woolrich et al., 2001), individual activation maps were created. “Fear”, “happy” and “angry” faces were modelled as three explanatory variables (EVs). Temporal derivatives were incorporated as regressors to increase statistical sensitivity, and variables were modelled by convolving each block with a haemodynamic response function.

The higher-level analysis combined first-level analyses at a group level (nicardipine versus placebo), using FSL’s randomise permutation-testing tool (Randomise). First, design matrices were created for randomise. In addition to standard matrices comparing post-randomisation scans between the groups, and pre-post differences within each group, a more complex design was created to investigate group by time interaction. Difference scores between pre- and post- randomisation (“post-minus-pre”) were calculated, and imported into randomise, to compare groups on their change from baseline to post-treatment. A voxel-wise GLM based analysis tested for statistically significant differences between the groups using non-parametric permutation testing (5000 permutations). Cluster based thresholding was applied using Threshold-Free Cluster Enhancement (TFCE) and a family-wise-error (FWE) corrected cluster significance threshold of $p < 0.05$.

To establish whether the fMRI task engaged regions of the brain associated with happy, angry, and fearful stimuli, blood-oxygen-level-dependent (BOLD) activation in response to happy, angry, and fearful faces was compared with fixation when all participants were combined across the nicardipine and placebo groups. These analyses were run via randomise, using pre-randomisation scans only.

Given the a-priori hypothesis that the amygdala is involved in processing emotional facial stimuli, including fearful and angry expressions (Capitão et al., 2019; Morris et al., 1996; Murphy, Norbury, et al., 2009), an anatomical region of interest analysis was conducted. This targeted analysis, limits the search for significant voxels to a predetermined area of interest, and as such controls for Type I error by reducing the number of statistical tests. Region of interest analyses for the left and right amygdala were completed with anatomical masks created using the Harvard Oxford atlas in FSL. Images were initially inspected visually to

ensure masks were not affected by regions of susceptibility artefact. Subsequently two approaches were taken for region of interest analyses, with differing strengths, using a range of tools:

i) Small volume correction (SVC) is a well-established approach (Poldrack, 2007), which restricts the voxel-wise analysis to a predetermined area and corrects for multiple comparisons only in those voxels. For the SVC analysis, anatomical masks were thresholded at 50%, indicating those voxels with a higher than 50% chance of belonging to the amygdala were included in the mask. 50% was considered an adequate trade-off between inclusivity and specificity.

ii) Region of interest (ROI) analysis, using the featquery tool, was applied to extract mean percentage signal change from the anatomical mask. This approach, which averages signal change across the region, applying a single statistical test, is widely used. However, systematic studies examining the power of this approach, in comparison to the standard SVC analysis are lacking (Poldrack, 2007). To examine the ROI data:
    a. Percentage signal change values were analysed using repeated measures analyses of variance (ANOVAs) in SPSS. Group was the between-subject factor of treatment (nicardipine vs placebo), and time (pre vs post) was the within-subject factor.
    b. Any significant interactions were further explored using post-hoc independent samples t-tests or repeated measures ANOVA as appropriate.

These region of interest analyses improve statistical control by reducing correction for multiple comparisons, to varying extents. In comparison to the SVC method, which adjusts for all voxels in the predetermined region, the ROI approach must only correct for the number of ROIs being tested, thus further improving statistical power. However, by averaging signal across the predefined area, the ROI approach may also present a limitation. For example, a region of interest may exhibit activity that comes from a minority of voxels in that region. Consequently, averaging data across the region could dilute this signal. The SVC approach does not face the same challenge of signal dilution and as such, implementing both the SVC and ROI approaches was considered beneficial for this exploratory investigation.

At the whole-brain level, fearful and angry faces were contrasted with happy. Two additional contrasts of all emotions combined (compared to fixation), and negative (anger and fear) vs positive (happy) emotions were also created, resulting in four emotion contrasts of interest:

(1) anger vs happy, (2) fear vs happy, (3) all emotions (vs fixation), and (4) negative emotions (fear and anger) vs happy. Due to the mixed design (within and between subjects), both primary and exploratory analyses were conducted.

7.2.5 Statistical analyses

7.2.5.1 Primary analyses

Primary analyses tested for between-subject differences, which compared post-randomisation scans between the groups (nicardipine vs placebo), using the following contrasts: 1. Nicardipine group mean, 2. Placebo group mean, 3. Nicardipine > Placebo, 4. Placebo > Nicardipine. This between-subjects design is widely used for the emotional faces task (Capitão et al., 2019; Murphy, Norbury, et al., 2009) and is considered the standard approach in the fMRI literature. Significant brain activations were detected using the Threshold-Free Cluster Enhancement (TFCE) method and a family-wise-error (FWE) correction ($p<0.05$).

7.2.5.2 Secondary analyses

Secondary analyses were also completed looking at:

i) Group by time interaction: Design matrices were created for randomise which determined whether the groups differed in their change from baseline to post-treatment, by comparing difference scores between pre and post randomisation. For this analysis, the following contrasts were used in the GLM: 1. Nicardipine group mean, 2. Placebo group mean, 3. Nicardipine > Placebo, 4. Placebo > Nicardipine.

ii) Within-subject differences: Where significant group by time interactions were identified, follow up analyses examining within-subject differences were conducted. These analyses compared pre and post scans within each group, for nicardipine and placebo respectively, using the following contrasts: 1. Pre > Post, 2. Post > Pre. Of note, within-subject analyses were also conducted independent of interaction for exploratory purposes.

7.2.5.3 Complementary analyses

For completeness, this thesis also presents uncorrected data with $p<0.005$. Inclusion of these analyses, which do not correct for multiple comparisons, is in-keeping with previous research (Martens, 2019) and was deemed useful for this clinical exploratory study (see Chapter 6, section 6.2.4.4.3 for further rationale). However, given the lower statistical threshold, these data must be interpreted with caution.

7.2.5.4 Sensitivity Analyses

The potential effects of age and cerebral blood flow were considered as covariates of interest in the sensitivity analyses. Age was included as a covariate to address baseline differences in the nicardipine versus placebo groups. Cerebral perfusion was included to account for subtle group differences as indicated by ASL (see Chapter 6, section 6.2.4.4.2 and Appendix 4.4). Comparable to primary analyses, clusters underwent thresholding using TFCE with a FWE cluster significance threshold set at $p<0.05$. Data were analysed with group by time interactions, in addition to between- and within-subject differences.

To visualise the results, data are presented in tables and figures. These include brain activation maps and graphs depicting mean BOLD signal change and BOLD activation. BOLD activation graphs were created by extracting and plotting the individual Parameter Estimate (PE) values from their custom maps, using the significant clusters as binary masks.

7.3 Results

Functional MRI Results

7.3.1 Main effect of task

To determine if the task was engaging regions of the brain associated with facial stimuli, BOLD signal was examined in response to all faces across both pre-randomisation groups. Activation was seen in a network of areas including the temporal occipital fusiform cortex, lateral occipital cortex, bilateral amygdala, paracingulate gyrus, central opercular cortex, frontal pole, lateral occipital cortex, frontal orbital cortex, middle frontal gyrus and precentral gyrus (see Figure 7.1 and Table 7.1). These results are consistent with previous published data (Capitão et al., 2019; Rawlings et al., 2010) indicating that the task engages regions of the brain relevant to emotional faces.

Figure 7.1 Main effect of task: Sagittal, coronal, and axial images depicting whole-brain fMRI faces task activations and deactivations.

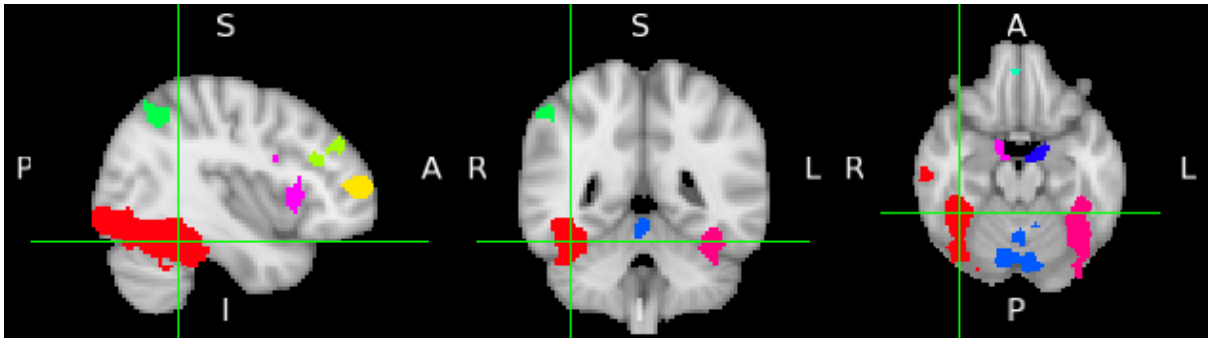


Table 7.1 Peak activation clusters in regions significantly activated by happy, fearful, and angry faces.

Number of voxels	Brain regions	Maximum Z	Peak MNI coordinates (x, y, z)
3184	R Temporal occipital fusiform cortex; inferior temporal gyrus, temporooccipital part	5.90	25, 40, 26
2533	L Lateral occipital cortex, inferior division; occipital fusiform gyrus	5.44	64, 20, 29
1128	R amygdala	4.96	35, 61, 28
1113	L Paracingulate gyrus; cingulate gyrus, anterior division; juxtapositional lobule cortex (formerly supplementary motor cortex); superior frontal gyrus	5.74	48, 68, 60
880	L Central opercular cortex	5.25	68, 61, 43
293	R Frontal pole	4.47	33, 89, 31
285	R Lateral occipital cortex, superior division; superior parietal lobule; angular gyrus	4.96	27, 34, 60
188	L Frontal orbital cortex; inferior frontal gyrus, pars triangularis; insular cortex; frontal operculum cortex	4.83	61, 77, 36
181	R Frontal pole; middle frontal gyrus	4.63	22, 81, 50
131	R Frontal pole	3.94	25, 92, 40
121	L Precentral gyrus	4.06	69, 62, 56

7.3.2 Effect of treatment

Hypothesis driven analysis of amygdala activation

7.3.2.1 Small volume correction (SVC) analysis

SVC Primary analysis

Between-subject differences at post-treatment

Analyses looking at between-subject differences in the left amygdala, found decreased activation to fear (vs happy) in the nicardipine group compared with placebo (see Figure 7.2 and Table 7.2). No significant effects were observed in the anger vs happy, or negative (fear and anger) vs positive (happy) contrasts in the left amygdala. In the right amygdala there were no significant differences for nicardipine versus placebo on any contrasts specified in the methods (i.e., anger vs happy, fear vs happy, negative vs positive).

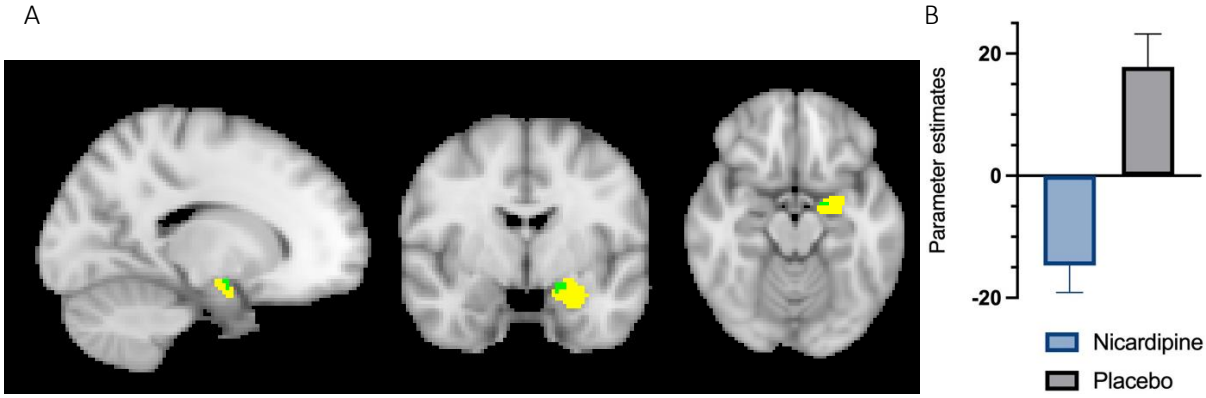


Figure 7.2 Left amygdala SVC results; between-subject differences at post-treatment.
(A) Left amygdala activation in response to **fear > happy** in placebo > nicardipine (no regressors). Sagittal, coronal and axial images (shown at MNI location 53,61,28) depicting significantly increased activation in left amygdala cluster (highlighted in green) in placebo group compared with nicardipine. Areas of significantly increased activity are TFCE corrected with a FWE cluster significance level of $p<0.05$. Mask of left amygdala (highlighted in yellow) created using Harvard-Oxford Atlas. **(B)** BOLD activation to fear (versus happy). Parameter estimates extracted from the cluster in 7.2A. Error bars show SEM.

SVC Secondary analyses

Group by time interaction

Analyses looking at group by time interaction in the left amygdala, found greater reduction in activity to fear (vs happy), from baseline to post-treatment, in the nicardipine group, compared with placebo. This finding was limited to a small cluster of two voxels (see Figure 7.3 and Table 7.2). No significant interactions were observed for anger vs happy, or negative vs positive emotions in the left amygdala. In the right amygdala there were no significant interactions for nicardipine versus placebo on any contrast of interest.

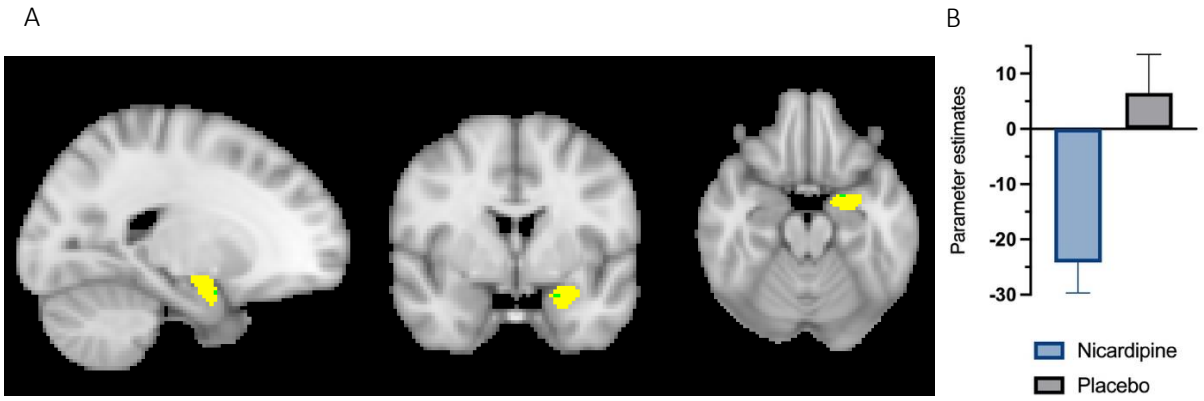


Figure 7.3 Left amygdala SVC results; group by time interaction.

(A) Left amygdala activation in response to **fear > happy** in placebo > nicardipine (no regressors). Sagittal, coronal, and axial images (shown at MNI location 55,63,26) depicting significantly increased baseline to post-treatment activation (highlighted in green) for fear (versus happy) in the placebo group compared with nicardipine. Areas of significantly increased activity are TFCE corrected with a FWE cluster significance level of $p<0.05$. Mask of left amygdala (highlighted in yellow) created using Harvard-Oxford Atlas. **(B)** BOLD activation to fear (versus happy). Parameter estimates extracted from the cluster in 7.3A. Error bars show SEM.

Within-subject differences (pre to post)

Because there was a significant interaction between group and time, follow-up analyses were conducted where change from pre to post was analysed separately within each group. Left amygdala analyses, looking at these within-subject differences over time, found decreased activation to fear (vs happy) after randomization to nicardipine, compared to pre-treatment (see Figure 7.4 and Table 7.2). On the contrary, no significant changes were seen in the placebo group from pre to post. As demonstrated in the between-subject and group by time analyses, no significant differences were observed for anger vs happy, or negative vs positive emotions in the left amygdala. In the right amygdala there were no significant differences for any emotion contrasts.

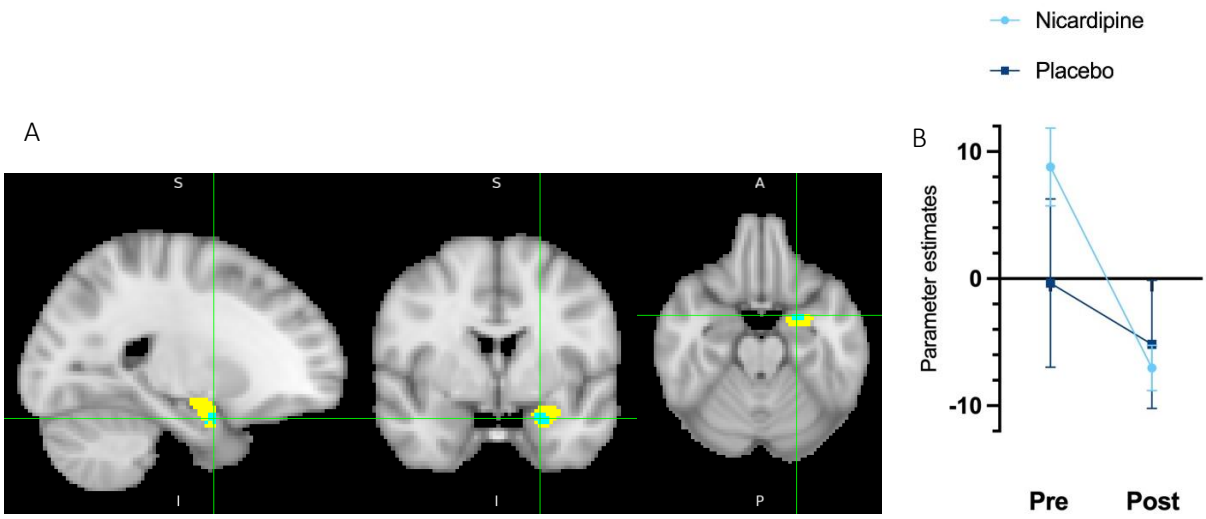


Figure 7.4 Left amygdala SVC results; within-subject differences (pre to post).
(A) Left amygdala activation in response to **fear > happy** in pre > post (no regressors) in the nicardipine group. Sagittal, coronal, and axial images (shown at MNI location 56,63,25) depicting significantly increased activation (highlighted in green) pre-randomisation in left amygdala cluster. Areas of significantly increased activity are TFCE corrected with a FWE cluster significance level of $p<0.05$. Mask of left amygdala (highlighted in yellow) created using Harvard-Oxford Atlas. **(B)** BOLD activation to fear (versus happy) in nicardipine and placebo groups. Parameter estimates extracted from the cluster (as depicted in 7.4A for nicardipine; placebo not shown). Figure B shows separate within-subject analyses for nicardipine, and placebo (illustrative purposes only). Error bars show the SEM.

Table 7.2 Details of activation clusters from SVC analysis.

Analysis	Contrast	Size (voxels)	p-value	MNI	Main region involved in cluster	Regressors
Between-subject differences at post-treatment	Fear > Happy	12	0.019	53,61,28	L amygdala	None
Group-by-time interaction	Fear > Happy	2	0.041	55,63,26	L amygdala	None
Within-subject differences (pre to post)	Fear > Happy	22	0.009	56,63,25	L amygdala	None

SVC Complementary analysis

Data analysed at the lower statistical threshold showed comparable results, with additional activation clusters for grouped negative (fear and anger) versus positive (happy) emotions in the left amygdala. See Table 7.3 for more details. No significant differences were observed for anger vs happy in the left amygdala. In the right amygdala there were no significant results for any of the contrasts of interest.

Table 7.3 Details of activation clusters from SVC Complementary analysis (uncorrected data).

Analysis	Contrast	Size (voxels)	p-value	MNI	Main region involved in cluster	Regressors
Between-subject differences at post-treatment	Fear > Happy	19	0.001	54,60,29	L amygdala	None
	Neg > Pos	4	0.003	53,60,29	L amygdala	None
Group-by-time interaction	Fear > Happy	15	<0.001	58,60,23	L amygdala	None
	Fear > Happy	10	0.002	55,63,26	L amygdala	None
	Fear > Happy	2	0.003	57,64,23	L amygdala	None
	Neg > Pos	2	0.003	58,60,23	L amygdala	None
Within-subject differences (pre to post)	Fear > Happy	42	<0.001	55,63,25	L amygdala	None
	Neg > Pos	23	0.001	58,61,24	L amygdala	None

SVC Sensitivity analysis

Results were similar with and without correction for age and cerebral blood flow. Analyses looking at between-subject differences at post-treatment, found decreased activation to fear (vs happy) and negative (vs positive) faces in the left amygdala for the nicardipine group compared with placebo, when age and ASL maps were included as regressors (see Table 7.4). Group by time interaction for the left amygdala found decreased change in baseline to post-treatment activation in response to fear (vs happy) in the nicardipine group, compared with placebo (when age was included as a regressor). Again, this finding was limited to a small cluster of voxels (see Table 7.4). Analyses looking at within-subject differences, from pre to post in each group separately, found decreased activation to fear (vs happy) and negative (vs positive) faces after randomisation to nicardipine in the left amygdala, when age and ASL maps were included as regressors (see Table 7.4). On the contrary, no significant changes were seen in the placebo group from pre to post. As for the previous SVC analyses, no significant differences were observed for anger vs happy in the left amygdala, and in the right amygdala, there were no significant differences for nicardipine versus placebo on any contrast of interest.

Table 7.4 Details of activation clusters from SVC Sensitivity analysis.

Analysis	Contrast	Size (voxels)	p-value	MNI	Main region involved in cluster	Regressors
Between-subject differences at post-treatment	Neg > Pos	4	0.035	55,60,29	L amygdala	ASL/age

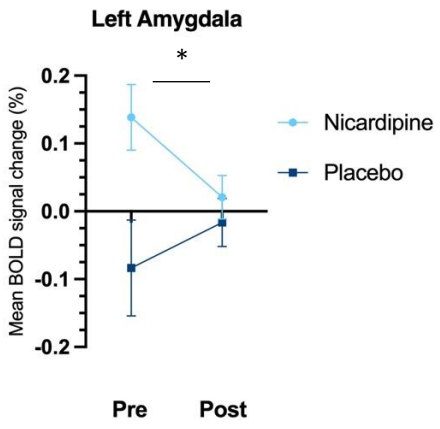
	Neg > Pos	9	0.023	53,60,29	L amygdala	age
	Fear > Happy	7	0.034	53,60,29	L amygdala	age
	Fear > Happy	9	0.025	53,61,28	L amygdala	ASL
Group-by-time interaction	Fear > Happy	2	0.032	55,63,26	L amygdala	age
Within-subject differences (pre to post)	Fear > Happy	20	0.014	56,63,25	L amygdala	ASL/age
	Neg > Pos	11	0.025	58,61,24	L amygdala	ASL/age
	Fear > Happy	22	0.009	56,63,25	L amygdala	age
	Neg > Pos	1	0.044	58,61,24	L amygdala	age
	Fear > Happy	20	0.014	56,63,25	L amygdala	ASL
	Neg > Pos	11	0.025	58,61,24	L amygdala	ASL

7.3.2.2 Region of interest (ROI) analysis

Left Amygdala There was a significant time-group-valence interaction for the left amygdala ($F_{1,14}$ = 1.98, p = 0.02, η^2 = 0.08). Further analysis revealed a significant time-group difference in activation to negative vs positive faces, with the nicardipine group showing decreased activation to negative faces compared with the placebo group (F = 4.30, p = 0.05, η^2 = 0.16) (see Figure 7.5A). This pattern is in-keeping with the SVC results.

Right Amygdala There was a near significant time-group-valence interaction for the right amygdala ($F_{1,14}$ = 1.68, p = 0.06, η^2 = 0.07). Further analysis revealed a near significant time-group difference in activation to negative vs positive faces, with the nicardipine group showing decreased activation to negative faces compared with the placebo group (F = 3.39, p = 0.08, η^2 = 0.13) (see Figure 7.5B).

A



B

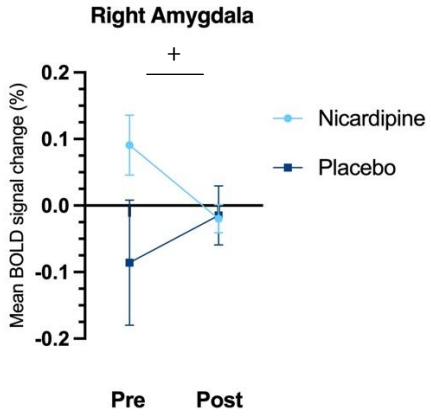


Figure 7.5 Mean BOLD percentage signal change in response to negative > positive faces.
A) Left amygdala ROI results, depicting significant time-group difference. **B)** Right amygdala ROI results, depicting near significant time-group difference. Masks of left and right amygdala created using Harvard-Oxford Atlas. Error bars show SEM, * p < 0.05, + p <0.10.

7.3.2.3 Exploratory whole brain analysis

A whole-brain analysis found no statistically significant between-subject differences, or group by time interaction, for nicardipine versus placebo. However, for exploratory purposes, a within-subject analysis (looking at pre to post differences within each group separately) was conducted, which revealed decreased activation to fear vs happy in the left inferior frontal gyrus after randomisation to nicardipine (TFCE corrected with FWE cluster significance threshold p<0.05). This finding was limited to a small cluster of voxels (see Figure 7.6 and Table 7.5). No significant changes were seen in the placebo group from pre to post., and notably, no significant effects were observed in the anger vs happy, or negative vs positive contrasts.

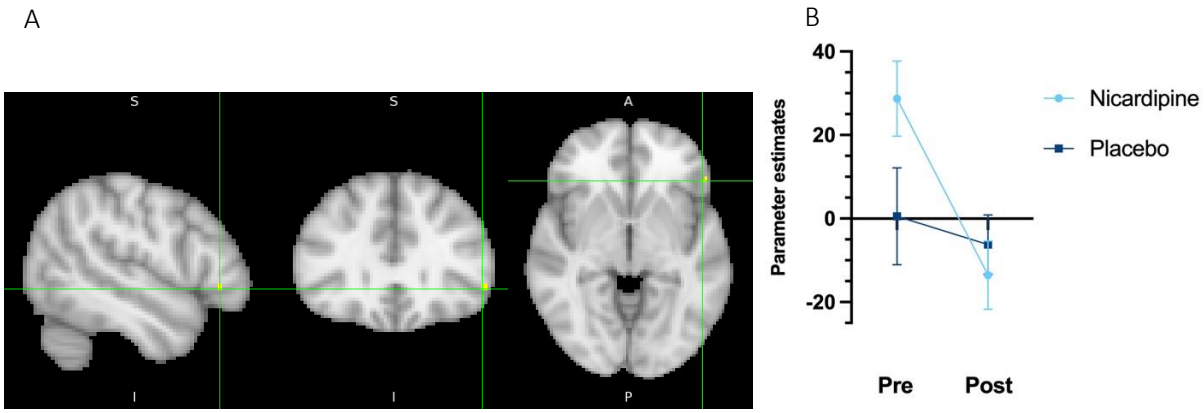


Figure 7.6 Whole brain analysis results; within-subject differences (pre to post).
(A) Whole-brain activation in response to **fear > happy** in pre > post (no regressors) in the nicardipine group. Sagittal, coronal and axial images (shown at MNI location 70,78,34) depicting significantly increased activation pre-randomisation in left inferior frontal gyrus cluster (highlighted in yellow). Areas of significantly increased activity are TFCE corrected with a FWE cluster significance level of p<0.05.
(B) BOLD activation to fear (versus happy) in nicardipine and placebo groups. Parameter estimates extracted from the cluster (as depicted in 7.6A for nicardipine; placebo not shown). Figure B shows separate within-subject analyses for nicardipine, and placebo (illustrative purposes only). Error bars show SEM.

Table 7.5 Details of activation cluster from whole brain analysis

Analysis	Contrast	Size (voxels)	p-value	MNI	Main region involved in cluster	Regressors
Within-subject differences (pre to post)	Fear > Happy	6	0.045	70,78,34	L inferior frontal gyrus	None

Whole brain sensitivity analysis

Results with correction for age and cerebral blood flow showed a similar activation pattern. Between-subject and group by time interaction analyses showed no differences between the groups. For exploratory purposes, a within-subject analysis (looking at pre to post differences within each group separately) was conducted, with age and ASL maps included as regressors. These analyses revealed decreased activation to negative versus positive faces (TFCE corrected with FWE cluster significance threshold p<0.05) in the left frontal orbital cortex (see Figure 7.7 and Table 7.6) after randomisation to nicardipine. On the contrary, no significant changes were seen in the placebo group from pre to post. Furthermore, no significant effects were observed in the fear vs happy or anger vs happy contrasts.

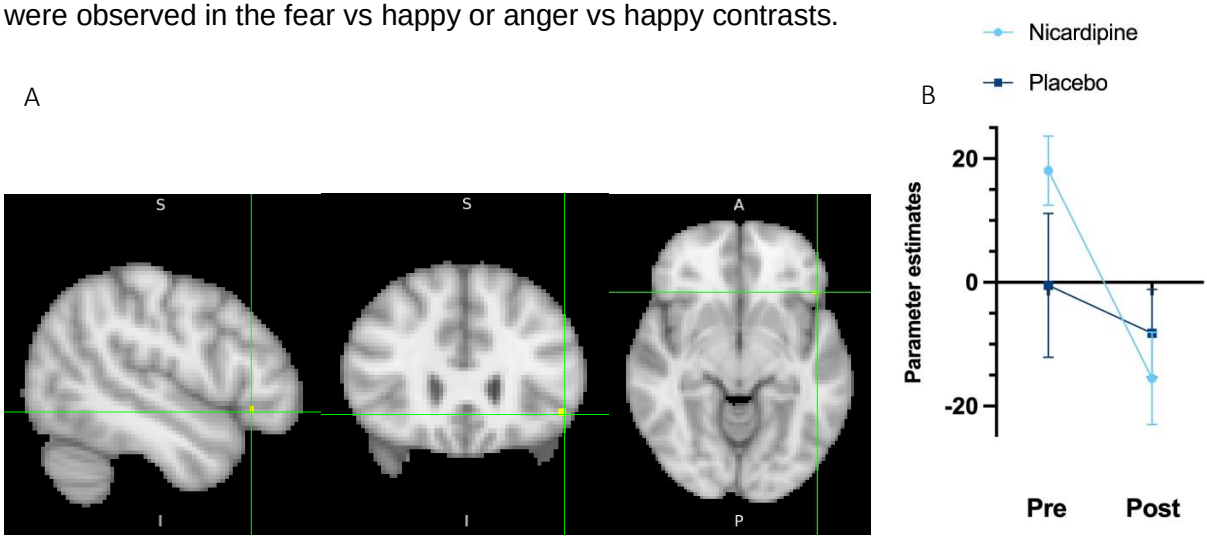


Figure 7.7 Whole brain sensitivity analysis results, within-subject differences (pre to post).
(A) Whole-brain activation in response to **negative > positive** faces in pre > post in the nicardipine group (ASL regressor). Sagittal, coronal and axial images (shown at MNI location 69,76,32) depicting significantly increased activation pre-randomisation in left frontal orbital cortex cluster (highlighted in yellow). Areas of significantly increased activity are TFCE corrected with a FWE cluster significance level of p<0.05. **(B)** BOLD activation to negative (versus positive) faces in nicardipine and placebo groups. Parameter estimates extracted from the cluster (as depicted in 7.7A for nicardipine; placebo not shown). Figure B shows separate within-subject analyses for nicardipine, and placebo (illustrative purposes only). Error bars show SEM.

Table 7.6 Details of activation clusters from whole brain sensitivity analyses

Analysis	Contrast	Size (voxels)	p-value	MNI	Main region involved in cluster	Regressors
Within-subject differences (pre to post)	Neg > Pos	4	0.039	69,76,32	L frontal orbital cortex	ASL/age
	Neg > Pos	4	0.039	69,76,32	L frontal orbital cortex	ASL

7.4 Discussion

This is the first study to examine the effect of LTCC antagonism on emotional neural processing in healthy participants with mood instability. An fMRI faces task compared nicardipine SR (30 mg BD for 14 days) and matched placebo, pre- and post-randomisation, to investigate the effect of nicardipine on neural activity in response to negative and positive facial expressions. The main hypothesis was that nicardipine would decrease negative affective neural processing in the amygdala, the a-priori region of interest, in the absence of change on subjective mood ratings. Study findings suggested a tendency toward decreased activation to negative (fearful) stimuli in the nicardipine group, across multiple analyses. Compared with placebo, nicardipine showed reduced BOLD response to fearful versus happy faces in the left amygdala on the small volume correction (SVC) analysis. This was reinforced by anatomical ROI analyses, which exhibited decreased BOLD signal to negative facial stimuli, in the nicardipine versus placebo group. This difference was significant in the left amygdala and showed a tendency towards significance in the right amygdala, thus indicating effects may be bilateral. As such, findings in the amygdala support the study's hypothesis that nicardipine reduces neural processing of negative emotions.

The observed trend towards reduced activation to negative faces in the nicardipine group, reinforces the decreased negative bias reported in chapter 5, and further supports an antidepressant-like effect of nicardipine. Previous literature in patients and healthy controls, indicates antidepressant drugs have immediate effects on regional brain activity, decreasing negative affective processing and increasing activity associated with positive stimuli (Harmer, Goodwin, et al., 2009; Harmer, Hill, et al., 2003; Harmer et al., 2004; Rawlings et al., 2010). In individuals with depression, such changes have been shown to precede any discernible effects on mood. These changes are thought to contribute to later improvements in mood, by reducing hypervigilance to negative information and helping patients perceive the world in a more positive light over time (Godlewska & Harmer, 2021). This model of emotional neural processing has shown potential in the identification of drugs with antidepressant actions

(Arnone et al., 2009; Pringle, McTavish, et al., 2011), and therefore offers an acceptable approach to testing LTCC antagonists for neuropsychiatric effects.

Like SSRIs (Harmer et al., 2006; Murphy, Norbury, et al., 2009), nicardipine showed decreased amygdala activation to fearful faces, compared with placebo. This supports the earlier behavioural results, where nicardipine displayed a shift in emotional processing on several measures of the FERT, including reduced accuracy for negative faces. The present fMRI findings also align with previous imaging studies in the LTCC literature. The amygdala has repeatedly shown changes in activation in LTCC gene studies, particularly in response to negative (fearful) stimuli (Jogia et al., 2011; Sumner et al., 2015; Wessa et al., 2010). Altered amygdala fear activation is recognised in many neuropsychiatric disorders (Battaglia et al., 2012; Kim et al., 2012; Peluso et al., 2009). Heightened fear response in this region may lead to characteristic features of BD such as emotional volatility and impulsivity (Kim et al., 2012). Additionally, it may contribute to difficulties in emotion regulation, which underlie a wide range of mood disorders (Peluso et al., 2009), along with numerous other psychiatric symptoms, including increased anxiety, hypervigilance to negative cues, and exaggerated emotional responses (Battaglia et al., 2012). As such, elevated fear response in the amygdala appears to be a feature of transdiagnostic significance, and the present nicardipine results may have important implications across multiple psychiatric conditions. Confirmation of these results in future studies would support LTCC antagonism reducing negative bias associated with fear processing in the amygdala, and this could represent a significant mechanism of action of LTCC antagonists, comparable to effects observed with conventional antidepressants.

Although current findings are based on modest cluster sizes, it is encouraging that a consistent pattern of results emerged across various analyses using different methodological approaches. The left amygdala cluster, identified on between-subject SVC analysis, at post-treatment, was limited to 12 voxels. There is little directly comparable evidence with which to evaluate these results, however one other psychiatric fMRI study examining LTCC antagonism in humans reported clusters of comparable size from a working memory paradigm (Zink et al., 2020). In 2020, Zink and colleagues (Zink et al., 2020) found no significant neural effects of nimodipine during an emotional faces task, despite earlier work demonstrating LTCC gene effects on amygdala activity during the same task (Bigos et al., 2010). The reason for these differing results is not clear. However, the nimodipine authors used a different faces task from the present analyses, that was limited to faces of negative valence, and which has not been validated for sensitivity to medication effects (Hariri et al., 2002). In the present study, complementary analyses at the lower statistical threshold (uncorrected p=0.005), and sensitivity analyses correcting for potential confounders, demonstrated comparable results to

the primary analyses. In addition to a pattern of decreased left amygdala activation to fear (vs happy) in the nicardipine group, these analyses also demonstrated significant clusters of decreased activation to grouped negative (vs positive) emotions in this region. As expected, complementary analyses demonstrated larger clusters of significant voxels (due to less stringent statistical thresholds), and sensitivity analyses suggest the primary findings are robust. This implication that the main results are unaffected by confounding variables, further strengthens confidence in the findings.

Alongside the SVC analyses, an additional ROI approach was taken for the amygdala. Implementing both methods was considered beneficial in this exploratory study, due to their complementary strengths and weaknesses. The ROI approach offers improved statistical power by reducing correction for multiple comparisons. Notably, ROI findings for the left amygdala support the SVC results. Interestingly, the ROI analysis also demonstrated a non-significant trend in the right amygdala for decreased activation to negative faces in nicardipine versus placebo. This suggests emotional processing results in the amygdala are potentially bilateral, yet borderline statistical power made it difficult to distinguish true differences between the groups in the SVC analyses. A recent meta-analysis demonstrated amygdala activation is commonly observed bilaterally in response to emotional faces (Ma, 2015). Therefore, these ROI findings are useful for situating the present results within the broader literature.

At the whole-brain level, exploratory analyses revealed a small cluster of decreased activation to fear (vs happy) in the left inferior frontal gyrus (IFG), after randomisation to nicardipine. Notably, whole-brain between-subject, and group by time analyses showed no significant effects, and therefore caution must be taken when interpreting these results. While the IFG has not been investigated in emotional processing as extensively as the amygdala, research indicates its potential involvement in modulating emotional responses (Grecucci et al., 2013). Specifically, the IFG may play a role in cognitive regulation of emotions, including reappraisal and suppression of responses (Ochsner et al., 2012; Wager et al., 2008), as well as understanding the emotions of others, in the context of theory of mind (Hartwright et al., 2016). IFG dysfunction has also been linked to several psychiatric disorders, such as depression and anxiety, which are characterized by deficits in emotion regulation and social cognition (Cha et al., 2016; Rolls et al., 2020). Interestingly, the IFG has also been shown to adjust response bias in a decision-making task (Reckless et al., 2014), indicating a possible role for the IFG in flexible decision-making. It is difficult to interpret the significance of a reduced pattern of activity in response to fear in the IFG. On one hand, it could reflect a dampening effect of nicardipine, leading to a reduced ability to regulate activity in response to negative information or understand the intention of others. On the other hand, it could reflect a reduced response

bias in response to fear, aligning more with the pattern of reduced activation in the amygdala. To provide further clarity, one approach might be to conduct a functional connectivity analysis, such as a psychophysiological interactions (PPI) analysis (Artiach Hortelano et al., 2023; O'Reilly et al., 2012). This fMRI technique can help determine the interaction between brain regions, offering insights into functional connectivity. Taken in this context, the possible impacts of nicardipine on fear processing are complex and may extend beyond effects in the amygdala, potentially influencing various brain regions involved in emotion processing and regulation. Thus, further research is required.

7.4.1 Limitations

There are several considerations for the present analysis. First, the unique design, involving strict selection criteria and intensive participant involvement, posed significant challenges for recruitment and retention. The resulting small groups were limited by statistical power, which may have influenced detection of treatment effects. Small sample sizes in fMRI studies can lead to type II errors (i.e. false negatives). This means genuine neural response to emotional stimuli may have gone undetected or underestimated (Lieberman & Cunningham, 2009). Additionally, false-positive correction methods may underestimate the true false positive rate, raising concerns about the reliability of findings in small-scale studies such as this one (Turner et al., 2018). As this was the first study of its kind, no formal power calculations were carried out to determine ideal sample size. Projected groups of 40 participants (20 nicardipine; 20 placebo), based on a similar experimental medicine imaging paradigm (Saunders et al., 2016), were not achieved and only a sub-group (24 participants) completed the fMRI task. However, groups were comparable to the other psychiatric fMRI study of nimodipine (Zink et al., 2020). The study by Zink et al (2020) detected an effect on BOLD signal in a sample of 30 participants, suggesting the present sample, while suboptimal, is within a comparable range. Nevertheless, future experiments with larger sample sizes are needed to confirm these results.

Second, repeated use of the fMRI faces task may present a limitation. Despite recurrent applications of this task in previous studies (Miskowiak et al., 2018), caution must be taken with serial testing. During the study roll-out, there was only one version of this task available, and therefore the same faces were re-randomised in each session, rather than implementing two distinct iterations. As such, it would be expected that the emotional aspects habituate across multiple sessions, which may render the task less sensitive. This issue is especially relevant to the amygdala, where habituation to recurring stimuli is crucial for regulating appropriate levels of arousal in response to external influences (Breiter et al., 1996; Swartz et al., 2013; Wright et al., 2001).

Third, a potential issue with the ROI results is the direction of mean BOLD signal change. For both the nicardipine and placebo groups, percentage signal change moved closer to the mean post-randomisation. Therefore, it is possible that these effects are accounted for by regression to the mean, rather than a true effect of LTCC antagonism. As few researchers have used this task in pre-post designs, there is limited directly comparable data with which to assess these results. However, they are consistent with the established trend for antidepressants reducing amygdala activation in response to negative emotional stimuli (Capitão et al., 2019; Ma, 2015; Murphy, Norbury, et al., 2009; Rawlings et al., 2010), including reports SSRIs 'normalise' amygdala hyperactivity to fearful faces in depressed patients (Godlewska et al., 2012). Additional research using pre-post treatment designs is required to investigate this further.

Finally, even with careful selection of an LTCC antagonist which is reported to cross the BBB, the dose of nicardipine (30 mg BD) may not have been adequate for sufficient LTCC brain occupancy, thus resulting in limited BOLD signal. This is supported by data showing antihypertensive doses of these drugs do not affect neural function in individuals prescribed such agents for blood pressure control (Striessnig et al., 2014; Zamponi et al., 2015). Therefore, higher doses may be required to see robust effects in the brain (Helton et al., 2005; Zamponi et al., 2015).

7.5 Conclusion

This is the first study to investigate the effect of nicardipine SR versus placebo on neural response to emotional stimuli in healthy participants with mood instability. As predicted, the nicardipine group exhibited decreased amygdala activity to negative versus positive stimuli, compared to the placebo group, across multiple measures. Nicardipine participants showed significantly reduced BOLD activation to fear versus happy in the left amygdala on small volume correction. This finding was supported by anatomical ROI analyses where, compared with placebo, nicardipine demonstrated decreased BOLD signal to negative versus positive faces, a difference that reached significance in the left amygdala and trended towards significance in the right amygdala. Furthermore, exploratory whole brain analyses revealed reduced activation to fear (vs happy) in the left inferior frontal gyrus (IFG) suggesting broader effects of nicardipine on fear processing throughout the brain.

Taken together, these results indicate nicardipine may reduce neural processing in the amygdala in response to negative faces, consistent with previous antidepressant drug findings (Capitão et al., 2019; Ma, 2015; Murphy, Norbury, et al., 2009; Rawlings et al., 2010).

However, whilst this pattern emerged in separate analysis approaches, it corresponded to a small volume of voxels in several of the analyses. Future studies are required, to clarify whether these small clusters resulted from inadequate power or sub-optimal occupancy of brain LTCCs. To address this, a number of approaches may be taken. Firstly, increased drug doses, over and above prescribing limits, may be adequate to see greater effects in the brain (Helton et al., 2005; Zamponi et al., 2015). Alternatively, drug binding studies, for example using magnetoencephalography (MEG), may aid in the evaluation of brain receptor occupancy by measuring magnetic signals consistent with occupancy. The present challenge lies in determining whether LTCC antagonists are effectively penetrating the blood brain barrier, and if so, whether they are binding adequately to LTCCs to induce neural effects. MEG may hold promise in discerning between these possibilities (Lopes da Silva, 2013; Proudfoot et al., 2014), and ongoing research is investigating this as a potential avenue for further exploration.

Chapter 8. General Discussion

Despite the genomic data supporting the trans-diagnostic association between common variants in LTCC genes and psychiatric disease (Als et al., 2023; Ferreira et al., 2008; Trubetskoy et al., 2022), the candidacy of LTCC antagonism as a potential treatment for neuropsychiatric disorder remains poorly understood. This thesis used a combination of pharmacoepidemiology and experimental medicine (including neuroimaging) to characterise the effects of LTCC antagonism. There were two principal objectives. First, to provide more robust statistical evidence regarding the association of LTCC antagonists with psychiatric disorder onset and recurrence, and second to characterise effects of LTCC antagonism on mood instability and its cognitive and neural correlates. It was hoped that these findings would help understand the potential for LTCC antagonists to be repurposed or modified for use in psychiatric disorder and, indirectly, to highlight the role of LTCCs in their pathophysiology.

8.1 Summary of results – TriNetX

The TriNetX study employed a large federated EHR network to determine the association of LTCC antagonism with psychiatric disorder. This study aimed to strengthen the statistical evidence for the association of CCBs with both new-onset and recurrent diagnoses, including all major ICD-10 mental and behaviour disorder categories.

Chapter 2 demonstrated CCBs were associated with a lower incidence of first onset psychiatric disorder compared with beta blockers (BBs) and diuretics. However, residual confounding (as indicated by findings for the NCOs) undermined interpretation of the psychiatric findings in the CCB versus diuretic cohorts. CCBs were associated with a higher incidence of first onset disorders compared with angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACEIs). Recurrent disorder findings were similar for CCB comparisons with ARBs and BBs, but risk ratios for ACEI and diuretic comparisons varied with specific disorders. Overall, it appeared ARBs had the most beneficial profile, yet over ninety percent of patients in the CCB cohorts received amlodipine, verapamil, or diltiazem, which are known for their low blood-brain barrier (BBB) penetration. Therefore, uncertainty remained as to whether CCBs with superior penetrability might offer advantages for psychiatric disorders.

This was explored in Chapter 3, which demonstrated brain-penetrant CCBs (BP-CCBs) were associated with a lower incidence of first onset neuropsychiatric disorder compared with amlodipine. Incidence of recurrent disorders varied with diagnosis, although benefits of BP-CCBs were more pronounced in women and people under 60 years. Comparisons with

verapamil and diltiazem were similar, with lower overall incidence of first-onset and recurrent disorders for BP-CCBs. However, negative control outcomes (NCOs) indicated residual confounding between BP-CCBs and both the amlodipine and verapamil/diltiazem groups, which undermined interpretation of the main findings. BP-CCB versus ARB analyses were not subject to the same confounding, however this evidence was less convincing. In the BP-CCB versus ARB comparisons, an overall lower incidence of first-onset neuropsychiatric disorder with BP-CCBs, suggested their greatest benefits were in reducing disease onset, as opposed to influencing disease progression. However, risk ratios varied depending on diagnosis, potentially suggesting differential involvement of LTCCs in different disorders.

While these pharmacoepidemiological EHR data offer valuable insights, they are observational and thus cannot establish a causal relationship between LTCC antagonism and psychiatric disorders. The following chapters present an exploratory experimental study, which aimed to demonstrate whether a psychiatric 'signal' could be seen with LTCC antagonism.

8.2 Summary of results - OxCaMS

Healthy participants with mood instability were recruited to a randomised, double-blind, placebo-controlled experiment of the BP-CCB nicardipine (SR 30 mg BD) or matched placebo, for 14 days, to determine effects on mood, cognitive function, and neural activity.

Chapter 4 demonstrated no physiological effects of nicardipine, compared with placebo, on measures of pulse, blood pressure or blood biochemistry. General decreases in mood instability, and increases in risky decision making, were noted in both groups. However, there were no group by time interactions for affective instability or risk taking. The physiological findings confirm blood samples and vital signs did not confound the mood or cognitive data. However, the cause of absent nicardipine effects on mood and cognition were unclear. One possibility is that the outcome period may have been insufficient. Though there is a lack of specific data pertaining to mood instability, antidepressant research suggests mood changes take up to six weeks to appear (Quitkin et al., 1984; Uher et al., 2011). Therefore, if LTCC antagonists are comparable, extending follow-up could capture effects in these domains. Alternatively, certain cognitive and neural correlates may be evident more rapidly (Harmer, Goodwin, et al., 2009; Zink et al., 2020), and these were the focus of the next chapters.

Chapter 5 investigated the effect of nicardipine SR on emotional processing using the Emotional Test Battery (ETB). Compared with placebo, the nicardipine group exhibited a decrease in negative bias, indicated by reduced recognition of negative including angry faces and increased recognition of neutral faces, fewer overall misclassifications as sad including

fewer positive faces misclassified as sad, fewer positive faces misclassified as negative, and a trend towards increased RT for angry and sad faces. These findings were in keeping with an antidepressant effect and suggest nicardipine may reduce negative bias specifically through changes in sad and/or anger perception.

Chapter 6 examined the effect of nicardipine on resting-state connectivity. Functional connectivity between the salience network and the precuneus decreased in the nicardipine group from pre to post treatment, whilst increasing in the placebo group. These findings supported the MEG resting-state, which demonstrated decreased beta power in the parietal lobe, including the precuneus, for nicardipine versus placebo (Atkinson, 2020). Together these results indicate the first effects of nicardipine on resting state connectivity in the precuneus. However, caution is warranted in their interpretation as unexpected changes in the placebo group were also driving the group by time interaction on fMRI. Furthermore, MEG changes did not survive correction for multiple comparisons across parcels. The absence of robust statistical evidence from the fMRI and MEG arms of this study may indicate sub-optimal functional occupancy of brain LTCCs by nicardipine (Atkinson, 2020). This is discussed in more detail below (see section 8.4).

Finally, Chapter 7 investigated the effect of nicardipine on neural response to emotional faces. Compared with placebo, the nicardipine group demonstrated decreased amygdala activity to negative (vs positive) stimuli across multiple measures, which is consistent with findings seen in Chapter 5. Nicardipine showed reduced BOLD activation in the left amygdala in response to fear (vs happy) on small volume correction. This was supported by anatomical ROI analyses, where compared with placebo, nicardipine displayed decreased BOLD signal when exposed to negative (vs positive) faces. This difference reached significance in the left amygdala and approached significance in the right amygdala. Overall, these results indicate nicardipine reduced neural processing in response to negative faces, consistent with an antidepressant effect (Capitão et al., 2019; Ma, 2015; Murphy, Norbury, et al., 2009; Rawlings et al., 2010). However, many findings were limited to small volumes of voxels, highlighting the need for cautious interpretation and further investigation. As posited in Chapter 6, a potential explanation for the small clusters was sub-optimal occupancy of brain LTCCs. MEG may hold promise in evaluating LTCC receptor occupancy in the brain, and this is an area of ongoing research, which is detailed in the following discussion (see section 8.4).

8.3 Interpretation and implication of results

The TriNetX and OxCaMS studies made use of pharmacoepidemiology and experimental medicine, two distinct approaches that are useful for evaluating medication effects (Harmer,

Cowen, et al., 2011; Sabaté & Montané, 2023). TriNetX studies examining CCBs as a class demonstrated no convincing evidence to repurpose existing CCBs for psychiatric disorders, and studies looking specifically at BP-CCBs were undermined by likely residual confounding, limiting any robust conclusions. In the OxCaMS study the twice-daily PANAS-SF questionnaire was used to measure change in mood instability. OxCaMS demonstrated no effects of nicardipine on mood instability, relative to placebo, which was disappointing considering increasing evidence of LTCC gene involvement in multiple psychiatric disorders and phenotypes. However, it may be that PANAS-SF had limited utility as a metric for nicardipine, highlighting the importance of more sensitive measures. Recent work characterising affective variability in patients with BD and BPD suggests existing mood ratings lack specificity and are not sensitive to treatment response (Pulcu et al., 2022). These authors assessed a computational model of mood variation, finding it was sensitive to the effects of lithium. Computational models have shown potential for characterising psychiatric phenotypes, thereby advancing our comprehension of psychiatric disorders, and supporting the development of new treatments (Browning et al., 2020). As such, these approaches may have applications for LTCC antagonists, warranting their inclusion in future studies.

In comparison with mood measures, parameters assessing cognitive function were more encouraging. Findings from the ETB facial expression recognition task (FERT) suggested an antidepressant-like effect of nicardipine. This is in-keeping with other studies of dihydropyridine (DHP) CCBs. For example, the pilot study of isradipine in BD patients, demonstrating efficacy for depressive symptoms (Ostacher et al., 2014), and the adjunctive LTCC antagonist study examining isradipine in schizophrenia (Burdick et al., 2020). The latter study found positive outcomes on a Quality-of-Life Scale, including items related to mood and depression, suggesting their findings were consistent with the nicardipine results. As previously discussed, early FERT changes may precede altered mood. Therefore, future studies could consider varying treatment durations to evaluate longer-term mood effects and their correlation with cognitive tasks. Alternatively, it may be that, compared to PANAS, the FERT provides a more sensitive measure of LTCC antagonism, thus emphasizing the need for similar cognitive tasks in future CCB research.

Results from the fMRI analyses suggest potential neural impacts of nicardipine. As far as is known, this is the first study to demonstrate effects of LTCC antagonism on resting state networks and neural processing of emotional stimuli. However, there are several important considerations. Both resting state and task-fMRI revealed small cluster sizes, with significant findings typically comprising fewer than 10-20 voxels. Chapter discussions explored possible explanations, ranging from limited treatment effects to suboptimal occupancy of central

LTCCs. However, statistical power is another important factor to consider. Smaller-than-intended sample sizes suggest the results might have been underpowered. This is particularly evident in fMRI research, where the combination of small subject numbers and the necessity to correct for multiple comparisons can significantly decrease statistical power (Button et al., 2013; Poldrack et al., 2017), especially for between-subject designs. Cremers and colleagues showed that typical fMRI samples of twenty to thirty participants exhibit insufficient power, inadequately capturing true effects, and demonstrating considerable variability in replication attempts (Cremers et al., 2017). During a social cognition task, these authors found a positive correlation between sample size and the number of significant voxels for main effect of task. Therefore, it is conceivable that cluster sizes could have been larger in the current analyses, had greater samples been achieved.

There is no definitive agreement in the literature regarding meaningful cluster size. Certain authors have proposed a minimum of 10 voxels, in the context of uncorrected thresholds ($p<0.005$) (Lieberman & Cunningham, 2009). These researchers argue fMRI has become too cautious in its efforts to reduce Type I errors (i.e. false positives), leading to increased Type II errors (i.e. false negatives). This bias tends to favour large over small effects, which typically correspond to sensory and motor functions as opposed to cognitive and emotional processes. However, the family-wise error (FWE) associated with this method is unknown (Roiser et al., 2016). In fact, a study by Eklund and colleagues (Eklund et al., 2016) estimated the false-positive rate associated with this approach to be between sixty and ninety per cent, thus emphasizing considerable caution. A further challenge is the extensive variation in voxel size across studies. For example, a study presenting a 10-voxel cluster using 2 mm isotropic voxels is 8 times larger than a study using 1 mm isotropic voxels. Voxel dimensions for the current resting state and fMRI faces analyses were 2 x 2 x 2mm. Whilst this voxel size is common, care must be taken when making comparisons to ensure voxel sizes are considered.

To address the issue of power, various approaches may be taken. Apart from increasing sample size, other strategies include multivariate analyses and large-scale data integration. Multivariate and model-based prediction methods combine multiple variables of interest into a single model rather than analysing each brain region separately. This combination allows more efficient use of the data and reduces the need for multiple corrections, thus increasing overall power (Habeck & Stern, 2010). Alternatively, large-scale research integration (Yarkoni et al., 2010) such as meta-analyses and open-data sharing initiatives (Poldrack & Gorgolewski, 2014), can enhance power by aggregating data from multiple studies, and should also be considered in future research designs.

In summary, while TriNetX and OxCaMS data hint at potential effects of LTCC antagonists on brain health, a definitive conclusion regarding efficacy for psychiatric disorder has not been reached. The cognitive data is most compelling, with FERT suggesting a direct impact of nicardipine on the way emotional information is processed, particularly negative faces. Reduced negative bias indicates antidepressant effects, despite an absence of mood change on the PANAS. Altered processing of emotions is a core trait of multiple psychiatric disorders, and LTCCs have been implicated in emotional processing via dysregulated calcium signalling. Specifically negative bias is a characteristic feature of many psychiatric disorders including BD, depression, and schizophrenia, indicating the potential transdiagnostic value of LTCC antagonism. However, fMRI results were less persuasive. Despite consistently demonstrating the same direction of effect, neural findings were restricted to small clusters of voxels. Therefore, it is unclear whether emotional processing effects might be mediated directly, via central LTCC occupancy, or indirectly via some other mechanism (Siddiqi et al., 2019). Additionally, with no discernible impact of nicardipine on mood instability, the cognitive and neural findings introduce uncertainty about potential mechanistic links between emotional processing effects and clinical outcomes. Overall, this lack of sufficient evidence limits conclusions regarding the therapeutic potential of LTCC antagonists, and future implications must be carefully considered.

8.4 Future studies

This thesis enhances current understanding of the effects of LTCC antagonists on brain health, yet it is evident that further research is required. A randomized clinical trial involving patients is the sole definitive method to evaluate the efficacy of LTCC antagonists for psychiatric disorders. However, the OxCaMS and TriNetX findings do not provide a strong incentive to conduct a repurposing trial. The findings raise several concerns.

First, the results from the EHR analyses are insufficiently robust to support a costly clinical trial (Freemantle et al., 2013; Tricklebank et al., 2021). The NCOs, used to identify bias in the TriNetX studies, indicate probable residual confounding between the groups. As a result, it is not possible to ascertain whether any apparent advantage of BP-CCBs is mechanistically related to the drug. This is relevant in the context of previous studies in Parkinson's disease (PD). Clinical trials of isradipine did not delay PD progression (Parkinson Study Group STEADY-PD III, 2020), despite evidence from pharmacoepidemiology and animal studies (Kang et al., 2012; Liss & Striessnig, 2019), thus representing a significant and costly late-stage clinical trial failure. Second, the ideal clinical population for an RCT remains uncertain. Whilst common *CACNA1C* variants were first linked to BD, risk alleles have since been associated with numerous psychiatric disorders and phenotypes (Als et al., 2023; Chen et al.,

2022; Hindley et al., 2022; Pagani et al., 2016; Trubetskoy et al., 2022). Therefore, it is not clear whether BD, depression, schizophrenia, or another disorder altogether, would be most appropriate. Finally, previous literature indicates the critical importance of treatment timing. Research in PD suggests the failure of clinical trials involving LTCC antagonists may be attributed to their administration occurring too late (Lang & Espay, 2018). These authors propose that CCBs could have been more efficacious during the prodromal phase of PD. Consequently, conducting an RCT in healthy participants before the onset of psychiatric disorder might yield greater effects. However, implementing such an approach is impractical, as identifying the prodromal population poses a significant challenge. Additionally, the prolonged duration of monitoring required for the emergence of psychiatric disorder would significantly escalate the costs associated with such a study.

Given these arguments provide insufficient rationale for RCTs of currently licenced LTCC antagonists, it is important to consider what alternative studies might look like. fMRI findings, supported by the ASL data (see Chapter 6, section 6.4.1) and the MEG arm of this study, indicate nicardipine occupancy of brain LTCCs may have been inadequate to produce psychiatric effects (Liu et al., 2008; Middlemiss & Spedding, 1985). This suggestion, coupled with kinetic differences between brain and cardiac LTCCs (Uchida et al., 1995), implies the prescribed dose of 30 mg BD might be insufficient (Cipriani et al., 2016; Zamponi et al., 2015). Future work may consider intensive dose finding studies using electroencephalography (EEG) and/or MEG to evaluate brain electrical signals and/or magnetic fields resulting from neuronal activity, over a range of CCB doses. Such investigations could demonstrate effects consistent with channel occupancy (Wynn et al., 2019). In this way, nicardipine dosage may be determined for optimal target engagement of central LTCCs. However, this approach comes with significant issues, as higher doses may not be tolerated due to side effects, or they may result in participants becoming unblinded to treatment allocation.

In the short term, further work is ongoing using MEG. MEG signals principally arise from the synchronized postsynaptic currents of cortical pyramidal cells (Gross, 2019). $Ca_V1.2$, the LTCC most sensitive to blockade by DHP CCBs, is predominantly situated on the dendrites and soma of postsynaptic neurons (Bhat et al., 2012; Hell et al., 1993). Demonstrating a change in MEG signal with LTCC antagonists may offer an indicator of target engagement. Although neural activity at rest did show some changes induced by nicardipine, these did not remain statistically significant after correction for multiple comparisons (see Chapter 6, section 6.4). It may be that resting-state MEG was not a sensitive enough measure of target engagement, or that we did not have enough statistical power to observe an effect. Instead of measuring spontaneous activation across the whole-brain at rest, it is possible effects could

be detected during cognitive tasks. Cognitive domains where *CACNA1C* risk alleles have already demonstrated effects, such as emotional processing and working memory (Cosgrove et al., 2017; Heck et al., 2014; Thimm et al., 2011) may hold particular promise. Present research is focusing here, and whilst testing has not been stratified by risk allele, MEG studies are currently examining nicardipine effects on attention and working memory.

To date, OxCaMS MEG data recorded during a working memory task, but analysed in the same way as the resting-state scans described above, suggests effects of nicardipine on static spectral power and transient dynamic states (Atkinson, unpublished observations). Relative to placebo, nicardipine has shown decreased power in delta and beta frequency bands (comprising two parcels in the superior frontal gyrus/occipital pole, and two parcels in the occipital cortex/precuneus respectively), and increased power in theta and alpha bands (comprising one parcel in the postcentral gyrus, and four parcels in the occipital cortex/planum temporale/lateral occipital cortex respectively). Hidden Markov Modeling (HMM), which captures time-varying oscillatory dynamics, has also demonstrated decreased fractional occupancy (i.e. proportion of time spent in any brain state) and interval times (i.e. time elapsed between states) in a visual occipital brain state. The longer duration of the working memory task, compared to the resting-state scan, may have provided greater statistical power to observe such effects. Together these results offer preliminary evidence of functional LTCC occupancy in the brain. Confirming target engagement is essential for testing the hypothesis that central LTCC binding leads to neural effects, and therefore further research is warranted to replicate these findings.

Another approach, in the longer term, may be to modify LTCC antagonists to allow them to preferentially occupy LTCCs in the brain. If brain selective CCBs could be developed, which spared VGCCs in the heart, these would be better suited to psychiatric disorders. This may be achievable given VGCC genes encoding the α1 subunit are expressed as multiple isoforms, exhibiting distinct physiological characteristics in the brain compared to the heart (Zamponi et al., 2015). Recent advances in long-range sequencing and bioinformatics enabled Clark and colleagues (Clark et al., 2020) to characterise full-length transcripts of *CACNA1C* in the brain. They showed *CACNA1C*'s transcript profile is unexpectedly diverse in human brain. They identified 38 novel exons and 241 novel transcripts, many of which are relatively abundant and likely to encode channels with altered function. This supports the presence of brain-enriched LTCC isoforms, which could present new drug targets with reduced peripheral side-effects. If also combined with the ability to cross the BBB, these novel treatments targeting brain LTCCs would certainly merit further investigation.

8.5 Broader considerations

The current thesis, which aims to improve understanding of the psychiatric effects of LTCC antagonists, raises several overarching questions worthy of further exploration. In the following sub-sections, a number of these considerations are examined.

First, what is the real relevance of the genomic findings, and why might they make a difference to the value of CCBs as a drug target? A broader examination of the literature reveals that more than half of clinical trials fail due to lack of efficacy (DiMasi et al., 2010; Nelson et al., 2015). The advent of GWAS has enabled the identification of genes associated with a diverse range of medical traits and conditions (Welter et al., 2014). This has the potential to optimise drug target selection (Plenge et al., 2013) and streamline efforts in drug discovery. Recent studies have explored how effective existing genetic evidence is in differentiating between drug successes and failures (Minikel et al., 2024; Nelson et al., 2015). These studies found that choosing genetically supported drug targets could increase success rates in clinical development by more than double, compared to drugs without genetic support. Thus, leveraging GWAS studies linking calcium channel genes to psychiatric syndromes may help identify effective drug targets. One exemplary case of a genotype linked to disease, which has shown efficacy as a drug target is *PCSK9* (Abifadel et al., 2003; Cohen et al., 2005). A gain of function mutation in *PCSK9*, linked with hypercholesterolemia, led to the introduction of monoclonal antibodies used to lower LDL cholesterol (King et al., 2019). However, to date no comparably robust examples have emerged in psychiatry. One of the difficulties with *CACNA1C* is the inconclusive literature regarding SNP effects on LTCC channel expression and function. As previously discussed (see Chapter 1, section 1.5) some studies report *CACNA1C* risk alleles are linked with increased $Ca_V1.2$ function (Bigos et al., 2010; Eckart et al., 2016; Yoshimizu et al., 2015) while other studies report the opposite effect (Eckart et al., 2016; Gershon et al., 2014; Roussos et al., 2014; Yoshimizu et al., 2015). If risk variants consistently increased $Ca_V1.2$ function, this would strongly support the use of CCBs for psychiatric disorder. However, genetic evidence has not definitively linked *CACNA1C* risk alleles with hyperactive channels. This conflicting genetic evidence is relevant for the present thesis, potentially contributing to the difficulties detecting strong conclusive results on behavioural, cognitive, and neural parameters.

A further consideration highlighted by the genomic findings is the issue of polygenicity. Psychiatric disorders are extensively polygenic, characterised by multiple common variants of individually small effect (odds ratios generally < 1.2) (Heyes et al., 2015; Ripke et al., 2014), as well as much rarer variants conferring a large effect in a very small number of patients (Malhotra & Sebat, 2012). As such, *CACNA1C* SNPs represent only a minute proportion of

the numerous risk loci associated with psychiatric disease. Interpreting the underlying mechanisms of these molecular findings will only become more complex as additional risk variants are discovered (Smoller et al., 2019). Significant polygenicity also suggests that patients with the same condition may exhibit distinct genetic profiles that follow separate pathways to the same disorder (Uffelmann et al., 2021). Therefore, efforts translating GWAS findings into potential drug targets must consider the complex interplay of multiple risk loci, and further research is needed to fully understand the implications for LTCC antagonism as a treatment in psychiatry.

Another aspect to consider is the identification of viable therapeutic targets. Given the transdiagnostic nature of LTCC gene associations (Als et al., 2023; Chen et al., 2022; Ferreira et al., 2008; Hindley et al., 2022; Mullins et al., 2021; Pagani et al., 2016; Sklar et al., 2008; Trubetskoy et al., 2022) and the necessity for pharmaceutical companies to pinpoint specific symptoms for drug development, what symptom or syndrome should be the therapeutic target? One possible strategy could be to adopt a broad set of inclusion criteria, for example any individual with mood disorder, or even extending to include patients with any axis 1 diagnosis. As we know from the EHR data (see Chapters 2 and 3) the potential benefits of CCBs are not limited to particular psychiatric conditions, but instead observed across a range of ICD-10 disorders. Therefore, employing an all-encompassing approach may provide the best opportunity for identifying disease targets. However, a review of the wider literature suggests an overinclusive approach may not be successful. Multiple psychopharmacology trials fail from an incomplete understanding of the biological mechanisms underlying mental disorders (Correll et al., 2023). Without this critical comprehension, studies may inadvertently recruit suboptimal populations or employ inadequate outcome measures. Pilot studies like OxCaMS are essential for strengthening understanding and refining the design of future trials. Pilot studies can help identify the most suitable patient cohorts to maximise treatment efficacy, and the outcome measures most sensitive to change. In OxCaMS, mood instability was selected as a target symptom in view of its prevalence across numerous psychiatric disorders associated with LTCC risk genes (Broome, Saunders, et al., 2015). While LTCC antagonism did not yield effects on mood instability, some cognitive outcomes did show promise as measures of CCB effects. Both behavioural and neural data demonstrated that, in comparison with placebo, nicardipine shifted emotional processing akin to an antidepressant effect. Based on this, it could be conjectured that individuals with bipolar or unipolar depression might represent the most appropriate target population, considering the potential advantage of an antidepressant effect within these cohorts. Future studies could consider using emotional processing parameters as outcome measures for these specific populations. This is further supported by research connecting LTCC risk genes to emotion recognition (Nieratschker et

al., 2015) , and emotional processing deficits observed in individuals with unipolar (Krause et al., 2021) and bipolar depression (Marchand et al., 2011; Vizueta et al., 2012). However, there is still a considerable amount of uncertainty, and as such further experimental research is required to confidently determine the most appropriate therapeutic targets before advancing with pharmaceutical development.

A further question concerns whether there is any connection between LTCCs and the evidence for altered calcium signalling in psychiatric disorder (see Chapter 1, section 1.2). Initially, it appears improbable given that the calcium signalling literature primarily focused on non-excitable cells such as lymphocytes and platelets (Harrison, Hall, et al., 2021), whereas LTCCs are typical of excitable cells, such as neurons. However, there is now increasing evidence that LTCCs are also expressed in non-excitable cells (Davenport et al., 2015), including some lymphocytes (Badou et al., 2013; Kotturi & Jefferies, 2005) and glia (Cheli et al., 2016; Sharma & Ping, 2014; Wang et al., 2015). As such, it is possible LTCCs play a role in Ca^{2+} signalling in several cell types. Investigating intracellular Ca^{2+} in glia is of particular interest given their involvement in the pathophysiology of BD (Cotter et al., 2001; Harrison, Colbourne, et al., 2020), and hence it is plausible Ca^{2+} dysregulation in BD may also involve these cells.

Another important consideration is the distribution of $Ca_V1.2$ channels within neural circuits. To make informed predictions on the effect of blocking these channels, it is essential to determine which types of neurons express $Ca_V1.2$, and in which specific neuronal compartments and brain regions. Only once this has been established can we potentially understand any functional effects. Immunohistochemistry techniques have shown $Ca_V1.2$ is highly prevalent in postsynaptic neuron dendrites and soma of the hippocampus, cerebral cortex, and cerebellum (Berger & Bartsch, 2014; Bhat et al., 2012). The main components of the hippocampus and cerebral cortex are excitatory glutamatergic pyramidal cells responsible for information processing, and inhibitory GABAergic (gamma-aminobutyric acid) interneurons that attenuate excitatory signals (Marín, 2012). In humans, the hippocampus comprises around ninety per cent pyramidal cells and ten per cent GABAergic interneurons (Konradi et al., 2011). Although LTCCs are predominantly situated in pyramidal cells (Lacinova et al., 2008; Moosmang et al., 2005) they are also found in interneurons (Xu et al., 2007). Therefore, it is feasible that the expression of these channels in inhibitory neurons, could cancel out the functional effects of LTCCs in excitatory cells (Geier et al., 2011; Rubi et al., 2013). Furthermore, studies conducted in mice have shown that applying LTCC antagonists to ventral hippocampal slice cultures inhibits the growth of inhibitory interneurons and the expression of GABA synthetic enzymes (Jiang & Swann, 2005). This reinforces the significance of LTCC

signalling in these neurons, however additional studies are required to replicate these findings. The presence of $Ca_V1.2$ in the cerebellum is also of interest. This brain structure is largely responsible for motor function and balance, and to a lesser extent cognitive function including attention and language (Koziol et al., 2014). Consequently, it remains uncertain whether blocking channels in this region might contribute to psychiatric effects or to potential side effects, posing an intriguing question for future investigation.

A further consideration relates to other calcium channel genes. The genomic data discussed in this thesis has primarily focused on *CACNA1C*, as these risk variants have provided the most compelling evidence in psychiatry thus far. However, genetic studies also suggest a role for numerous functionally related genes, including *CACNA1D* and *CACNB3* (Heyes et al., 2015; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011), which code for alpha 1D ($Ca_V1.3$) and regulatory β subunits respectively. While $Ca_V1.3$ exhibits a more limited distribution than $Ca_V1.2$, these channels still account for approximately ten per cent of LTCCs in the brain (Hell et al., 1993; Sinnegger-Brauns et al., 2009), and gain of function mutations in *CACNA1D* have been linked with autism spectrum disorder (Heyes et al., 2015). In rodent studies *CACNB3* risk alleles have shown impaired working memory, decreased anxiety behaviours, and heightened aggression (Murakami et al., 2007), which suggests their involvement in a number of behavioural phenotypes relevant to psychiatric disorders such as BD and schizophrenia. With these growing genetic discoveries, the task of translating them into effective treatments will be one of the key challenges for future research.

VGCCs also serve as targets for the gabapentinoid drugs, gabapentin and pregabalin. Gabapentinoids bind to α2δ subunits, and genes encoding these auxiliary subunits have also been associated with psychiatric disorders (Andrade et al., 2019). The majority of pharmacological and gating characteristics of VGCCs are governed by the α1 subunit, however auxiliary β and α2δ subunits assist channel targeting to the plasma membrane and refine channel function (Catterall, 2011; Catterall et al., 2005; Dolphin, 2012; Striessnig et al., 2014). Alongside CCBs, gabapentinoids represent another established class of drugs that target these channels and demonstrate effects on psychiatric phenotypes. Currently, gabapentinoids are used for various conditions such as sleep disorders, epilepsy, and pain (Hong et al., 2022). This evidence of druggability by two existing drug classes, combined with the extensive genetic evidence, suggests significant potential for the further investigation of LTCCs in psychiatry.

One last consideration extends to the design of future neuroimaging studies. Traditional resting-state approaches, used in these analyses, assume connectivity remains constant

throughout the scan period. However, contemporary theories suggest connectivity varies dynamically over time (Hutchison et al., 2013; Long et al., 2023). Therefore, forthcoming studies could consider measuring temporal stability of dynamic resting-state networks (Cavanna et al., 2018; Preti et al., 2017; Shunkai et al., 2023). These approaches may be more sensitive to change in the context of affective stability. Recent work in our department has demonstrated differing patterns of dynamic functional connectivity (dFNC) in participants with high versus low mood instability (Panchal, 2020). Specifically, individuals with low mood instability were found to occupy fewer resting meta-states. Therefore, similar approaches may be of value to investigate the effect of LTCC antagonism in these cohorts. Finally, future fMRI studies could also include paradigms measuring further phenotypes relevant to mood instability. In addition to emotional processing studied here, other cognitive functions such as risky decision making and reward responsiveness have been linked to LTCC risk genes (Lancaster et al., 2014), and as such may be valuable future outcome measures.

8.6 Conclusion

By examining a range of pharmacoepidemiology, behavioural and neuroimaging data, this thesis explored the effects of LTCC antagonism on psychiatric phenotypes, mood, cognitive function, and neural activity. EHR data suggested brain penetrant CCBs might offer advantages over other AHTs in reducing onset of psychiatric disease. However, this observational data was affected by likely residual confounding which limited conclusions. In the experimental medicine study, contrary to the hypothesis, there was no evidence supporting effects of nicardipine on mood instability. Combined cognitive and neural evidence suggested nicardipine may shift emotional processing in line with standard antidepressant drugs. Behavioural techniques indicated nicardipine reduced negative bias through changes in the perception of sad and/or angry faces, while neuroimaging techniques suggested nicardipine may reduce neural processing in the amygdala in response to fear. However, in the absence of effects on mood instability, these findings raise uncertainty regarding which effects, if any, could be mechanistically related to clinical effects.

Furthermore, while neural patterns of decreased activation to negative stimuli emerged in several separate analysis approaches, each finding corresponded to a small volume of voxels. Additional research is required to clarify these small clusters, and whether they may have resulted from underpowered analyses or sub-optimal occupancy of LTCCs in the brain. Whilst recruitment challenges led to fewer participants than intended, converging evidence suggests current LTCC antagonists may not be effective in their ability to occupy central LTCCs. As

such there is currently no compelling evidence to repurpose existing CCBs for psychiatric disorders.

However, the development of more brain-selective drugs may offer greater therapeutic potential for LTCC antagonism in the future. Considering the recent identification of numerous LTCC isoforms, which are enriched in the brain, including isoforms sensitive to DHP CCBs this is likely to be achievable, and marks a potential next phase in LTCC antagonism research.

Appendices

Appendix 2.1 Negative Control Outcomes

Supplementary Table 2.1a – NCOs for patients without a prior psychiatric (F20-F48) diagnosis.

	CCB vs diuretics		CCB vs ACEI		CCB vs ARB		CCB vs BB	
	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Benign colonic polyp	1.22, 1.59	**0.77 (0.74-0.79)**	1.46, 1.66	**0.88 (0.85-0.91)**	1.59, 1.50	**1.06 (1.03-1.10)**	1.50, 1.36	**1.11 (1.07-1.15)**
Ganglion	0.38, 0.42	**0.90 (0.84-0.97)**	0.38, 0.41	**0.93 (0.88-0.99)**	0.39, 0.45	**0.88 (0.82-0.94)**	0.46, 0.38	**1.21 (1.13-1.29)**
Hallux valgus	0.41, 0.46	**0.89 (0.84-0.95)**	0.45, 0.47	0.95 (0.90-1.01)	0.47, 0.48	0.99 (0.93-1.06)	0.53, 0.45	**1.17 (1.10-1.24)**
Hernia	3.83, 4.56	**0.84 (0.82-0.86)**	4.65, 3.99	**1.17 (1.14-1.19)**	4.73, 4.02	**1.18 (1.15-1.20)**	3.67, 4.57	**0.80 (0.79-0.82)**
Ingrowing nail	0.38, 0.48	**0.79 (0.74-0.85)**	0.42, 0.47	**0.90 (0.85-0.96)**	0.48, 0.46	1.04 (0.98-1.11)	0.44, 0.46	0.96 (0.91-1.03)
Sebaceous cyst	0.62, 0.79	**0.79 (0.75-0.83)**	0.69, 0.78	**0.88 (0.84-0.92)**	0.75, 0.74	1.00 (0.95-1.05)	0.79, 0.69	**1.14 (1.09-1.20)**
Senile keratosis	1.92, 2.32	**0.83 (0.81-0.85)**	2.20, 2.43	**0.91 (0.88-0.93)**	2.27, 2.50	**0.91 (0.88-0.93)**	2.22, 1.86	**1.19 (1.16-1.23)**
Trigger finger	0.66, 0.69	0.97 (0.92-1.02)	0.67, 0.72	**0.93 (0.89-0.98)**	0.68, 0.91	**0.75 (0.72-0.79)**	0.75, 0.71	**1.06 (1.01-1.12)**
Otalgia	0.92, 0.96	**0.95 (0.91-0.99)**	0.98, 0.95	1.04 (1.00-1.08)	0.98, 1.01	0.97 (0.93-1.01)	1.10, 1.00	**1.10 (1.06-1.15)**
Onycholysis	0.13, 0.20	**0.67 (0.61-0.75)**	0.16, 0.17	0.92 (0.83-1.01)	0.18, 0.17	1.09 (0.99-1.21)	0.17, 0.18	0.98 (0.89-1.08)
Viral warts	0.58, 0.67	**0.87 (0.82-0.92)**	0.65, 0.63	1.02 (0.98-1.08)	0.66, 0.66	1.01 (0.96-1.06)	0.73, 0.55	**1.32 (1.26-1.39)**
Cutaneous abscess	1.03, 1.36	**0.76 (0.73-0.79)**	1.25, 1.22	1.02 (0.98-1.06)	1.39, 1.06	**1.32 (1.27-1.37)**	1.14, 1.34	**0.85 (0.82-0.88)**
Average		**0.84 (0.78-0.89)**		0.96 (0.91-1.02)		1.02 (0.92-1.11)		1.07 (0.98-1.17)

Supplementary Table 2.1b – NCOs for patients with a prior psychiatric (F20-F48) diagnosis.

	CCB vs diuretics		CCB vs ACEI		CCB vs ARB		CCB vs BB	
	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)	% in each cohort	Risk ratio (95% CI)
Benign colonic polyp	1.57, 2.01	**0.78 (0.73-0.84)**	1.83, 2.07	**0.89 (0.84-0.94)**	2.02, 2.02	1.00 (0.94-1.06)	1.79, 1.75	1.02 (0.95-1.09)
Ganglion	0.76, 0.73	1.04 (0.93-1.15)	0.78, 0.75	1.05 (0.95-1.15)	0.79, 0.90	**0.88 (0.80-0.97)**	0.84, 0.73	**1.15 (1.04-1.28)**
Hallux valgus	0.81, 0.91	**0.89 (0.81-0.98)**	0.87, 0.86	1.01 (0.93-1.10)	0.89, 0.97	0.91 (0.83-1.00)	0.97, 0.87	**1.12 (1.02-1.23)**
Hernia	6.03, 7.22	**0.84 (0.81-0.86)**	7.35, 5.93	**1.24 (1.20-1.28)**	7.54, 6.52	**1.16 (1.12-1.20)**	5.57, 7.10	**0.78 (0.76-0.81)**
Ingrowing nail	0.85, 0.98	**0.87 (0.80-0.96)**	0.92, 0.90	1.02 (0.94-1.11)	1.07, 0.96	**1.12 (1.03-1.22)**	0.89, 0.88	1.01 (0.92-1.11)
Sebaceous cyst	1.03, 1.26	**0.81 (0.75-0.88)**	1.09, 1.23	**0.89 (0.82-0.96)**	1.23, 1.27	0.97 (0.89-1.05)	1.11, 1.06	1.05 (0.96-1.14)
Senile keratosis	2.43, 2.84	**0.86 (0.81-0.90)**	2.62, 2.68	0.98 (0.93-1.03)	2.74, 2.98	**0.92 (0.87-0.97)**	2.64, 2.39	**1.11 (1.04-1.17)**
Trigger finger	1.22, 1.22	1.01 (0.93-1.09)	1.18, 1.24	0.96 (0.89-1.03)	1.32, 1.64	**0.81 (0.75-0.87)**	1.30, 1.29	1.01 (0.93-1.09)
Otalgia	2.23, 2.38	**0.94 (0.88-0.99)**	2.40, 2.24	**1.08 (1.02-1.13)**	2.42, 2.42	1.00 (0.95-1.06)	2.41, 2.26	**1.07 (1.01-1.13)**
Onycholysis	0.22, 0.31	**0.72 (0.60-0.85)**	0.27, 0.31	**0.85 (0.73-0.99)**	0.33, 0.31	1.04 (0.89-1.22)	0.25, 0.30	0.85 (0.72-1.02)
Viral warts	1.08, 1.16	0.93 (0.85-1.01)	1.21, 1.15	1.05 (0.97-1.13)	1.12, 1.13	0.99 (0.91-1.08)	1.17, 0.96	**1.23 (1.12-1.34)**
Cutaneous abscess	2.14, 2.78	**0.77 (0.73-0.81)**	2.55, 2.49	1.02 (0.97-1.08)	2.86, 2.18	**1.31 (1.24-1.39)**	2.12, 2.61	**0.81 (0.77-0.86)**
Average		**0.87 (0.81-0.93)**		1.00 (0.94-1.07)		1.01 (0.92-1.10)		1.02 (0.93-1.11)

Appendix 4.1 The MDQ questionnaire

A score of 7 or more items for question 1, 'yes' for question 2 and 'moderate' or 'serious' for question 3 is a positive screen and sensitive to possible BD.

	YES	NO
1. Has there ever been a period of time when you were not your usual self and...		
...you felt so good or so hyper that other people thought you were not your normal self or you were so hyper that you got into trouble?	○	○
...you were so irritable that you shouted at people or started fights or arguments?	○	○
...you felt much more self-confident than usual?	○	○
...you got much less sleep than usual and found you didn't really miss it?	○	○
...you were much more talkative or spoke much faster than usual?	○	○
...thoughts raced through your head or you couldn't slow your mind down?	○	○
...you were so easily distracted by things around you that you had trouble concentrating or staying on track?	○	○
...you had much more energy than usual?	○	○
...you were much more active or did many more things than usual?	○	○
...you were much more social or outgoing than usual, for example, you telephoned friends in the middle of the night?	○	○
...you were much more interested in sex than usual?	○	○
...you did things that were unusual for you or that other people might have thought were excessive, foolish, or risky?	○	○
...spending money got you or your family into trouble?	○	○
2. If you checked YES to more than one of the above, have several of these ever happened during the same period of time?	○	○
3. How much of a problem did any of these cause you – like being unable to work; having family, money or legal troubles; getting into arguments or fights? *Please circle one response only.*		
No Problem Minor Problem Moderate Problem Serious Problem		

Appendix 4.2 OxCaMS Inclusion and Exclusion criteria

Inclusion Criteria
• Willing and able to give informed consent to participate in the study • Male or female • Aged 18 – 35 • Significant mood instability (defined as a score of ≥7 as measured by the Mood Disorder Questionnaire (MDQ)) • No indication that urgent psychiatric treatment is required • Pre-treatment tests including renal, cardiac and liver function acceptable for the initiation of treatment with nicardipine • Willing and able to comply with the study requirements • Willing to allow his/ her General Practitioner, if appropriate, to be notified of his/her participation in the study.
Exclusion Criteria
• Contraindication(s) to nicardipine (as documented in the Summary of Product Characteristics for Cardene) • History or current axis 1 disorder, if in the opinion of the investigator it will compromise safety or affect data quality. • Regular psychotropic drug use within the last 12 weeks. Recent 'as required' use of psychotropic medication may be permitted at the investigators discretion, if it will not compromise safety or affect data quality. • Currently taking any other medication or herbal extracts that would affect study results or safety (e.g. St. John's Wort). • Judged to be at significant immediate risk of suicide/self-harm • Clinically significant alcohol (14 units for women, 21 units for men) or substance use • Requiring urgent treatment for an acute mood episode such that placebo would be inappropriate • Female and pregnant, lactating or planning a pregnancy during the course of the study • Female of child-bearing potential not willing to use effective contraception • Participation in a research study involving an investigational medicinal product in the past 12 weeks • Individuals who are lactose intolerant (due to Nicardipine containing lactose). **Plus:** Participants who have a pacemaker, non-MR-compatible metal implant, or any other contraindication for MRI or MEG brain scanning will be excluded from the corresponding brain scanning element(s) of the study Individuals who are not willing to consume gelatine (due to drug and placebo capsules being made of gelatine). * No participants will be withdrawn from effective medication or treatment for the purposes of this study

Appendix 4.3 The PANAS-SF scale

Q1. Upset
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q2. Hostile
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q3. Alert
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q4. Ashamed
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q5. Inspired
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q6. Nervous
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q7. Determined
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q8. Attentive
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q9. Active
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Q10. Afraid
0. Very slightly/Not at all
1. A little
2. Moderately
3. Quite a bit
4. Extremely

Appendix 4.4 Arterial Spin Labelling (ASL) statistical methods and results

ASL analyses were completed by Dr Marieke Martens, Senior *Postdoctoral* Researcher at the University of Oxford. ASL data are included as regressors in some sensitivity analyses described in Chapters 6 and 7. Therefore, details of the ASL statistical methods and results are included here for reference.

Appendix 4.4.1 ASL methods

ASL data were analysed using FSL v6.0 tools (https://fsl.fmrib.ox.ac.uk/fsl*). Resting perfusion maps underwent distortion and motion correction.* Global grey matter and global white matter perfusion were calculated in units of mL/100g tissue/min using the Oxford_ASL tool, which is part of the Bayesian Inference for Arterial Spin Labelling (BASIL) software (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BASIL) (Chappell et al., 2009). The Anatomical Processing Script, FSL_Anat (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/ fsl_anat) was implemented to pre-process each subject's T1 structural image (which included brain extraction and registration to standard space via FLIRT [FMRIB's Linear Image Registration Tool] and FNIRT [FMRIB's Non-linear Image Registration Tool]). Perfusion images for each participant were also registered to standard space via calibration and structural images and warps generated by FSL_Anat. Next, Gaussian smoothing of 2.35 mm was applied to all images to match functional data, and data were analysed using the voxel-wise general linear model (GLM) permutation non-parametric testing (with 5000 permutations) via randomise (the FSL tool for non-parametric permutation testing). Data were corrected for multiple comparisons (cluster-based TFCE thresholding with a family-wise error (FWE)-corrected significance threshold of $p<0.05$). This created spatial maps which characterised the between-subject/group differences, allowing different analyses to be run including both between group and within group comparisons.

Appendix 4.4.2 ASL results

25 participants completed both pre and post randomisation ASL scans. Global grey matter and global white matter perfusion were calculated in units of mL/100g tissue/minute. Results are shown below in Supplementary Table 4.4.2.1 and a repeated-measures ANOVA was used to determine group differences. There was no significant group-time interaction for global grey matter ($F_{1,22} = 0.25$, $p = 0.63$) or global white matter ($F_{1,22} = 0.09$, $p = 0.77$) perfusion. Therefore, nicardipine did not appear to influence global cerebral perfusion.

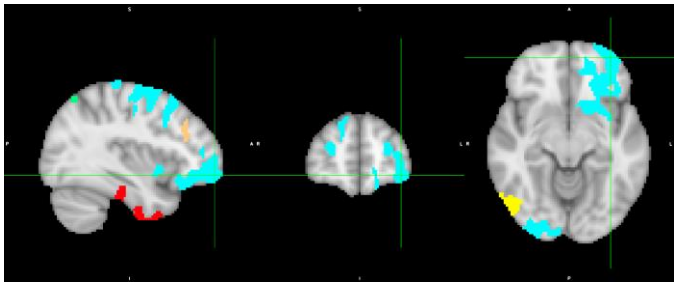
Regional perfusion was subsequently analysed using both smoothed and non-smoothed data. Again, there were no significant results at $p<0.05$ threshold. However, some brain areas

showed trend significance at p<0.1 threshold, when nicardipine and placebo post-randomisation scans were compared (see Supplementary Table 4.4.2.2). Although no firm conclusions can be made from these data, these regional results suggest nicardipine may reduce cerebral perfusion compared to placebo. Therefore, it was determined that ASL perfusion maps could be added as voxel wise regressors in the resting state and task fMRI analyses to account for any possible differences in perfusion between the groups.

Supplementary Table 4.4.2.1. ASL data for global cerebral perfusion.

	Nicardipine SR		Placebo	
	Pre (n=13)	Post (n=12)	Pre (n=13)	Post (n=13)
ASL scans	12 pre-post comparisons		12 pre-post comparisons	
Global grey matter perfusion (mean, SD)	48.0 (8.2)	46.7 (7.8)	52.2 (10.3)	52.7 (12.8)
Global white matter perfusion (mean, SD)	14.2 (3.6)	14.0 (3.6)	15.1 (3.0)	14.6 (3.5)

Supplementary Figure 4.4.2.1. ASL data for regional cerebral perfusion.



Supplementary Table 4.4.2.2. ASL data for regional cerebral perfusion. Nicardipine SR verses placebo comparison for post-scans only.

	Brain area (Placebo > Nicardipine SR)*	Cluster size (voxels)	MNI max (x,y,z)	t-score	p-value
Cluster 10 (light blue, Supp. Figure 4.4.2.1)	L Fp extending to OFC, IC, L putamen, NAc, CAU, L PAC, ACC, mPFC, R Fp, SFG, MFG, R LOC, occipital pole	15072	-36,56,-10	3.38	0.08
Cluster 9	L SPL, extending to PoCG, LOC	1274	-10,-54,72	3.08	0.09
Cluster 8	L TF, TP, ITG. Separate PHG, HP cluster.	759	-30,2,-48	2.75	0.09
Cluster 7	Bilateral PCN extending to cuneal cortex, SPL.	460	0, -76, 50	3.27	0.09
Cluster 6	R LOC	377	48,-70,-12	3.27	0.09
Cluster 5	L WM extending into IC and MFG	231	-28,18,18	3.09	0.09

Cluster 4	L PCN	69	-8, -50, 40	2.55	0.10
Cluster 3	L FP and MFG extending to SFG	39	-26,36,44	2.36	0.10
Cluster 2	L LOC / IPL	25	-32, -88, 28	2.59	0.10
Cluster 1	Mainly WM but on border of R PAC .	12	14, 46, 24	2.26	0.10

*Age included as a nuisance regressor. Abbreviations: ACC = anterior cingulate cortex, CAU = caudate, Fp = frontal pole, HP = hippocampus, IC = insular cortex, IPL = inferior parietal lobule, ITG = inferior temporal gyrus, LOC = lateral occipital cortex, MFG = middle frontal gyrus, mPFC = medial prefrontal cortex, NAc = nucleus accumbens, OFC = orbitofrontal cortex, PAC = paracingulate gyrus, PCN = precuneus cortex, PHG = parahippocampal gyus , PoCG = post-central gyrus, SPL = superior parietal lobule, SFG = superior frontal gyrus, TF = temporal fusiform cortex, TP = temporal pole, WM = white matter

Appendix 5.1 FERT and ECAT emotional processing measures from the ETB.

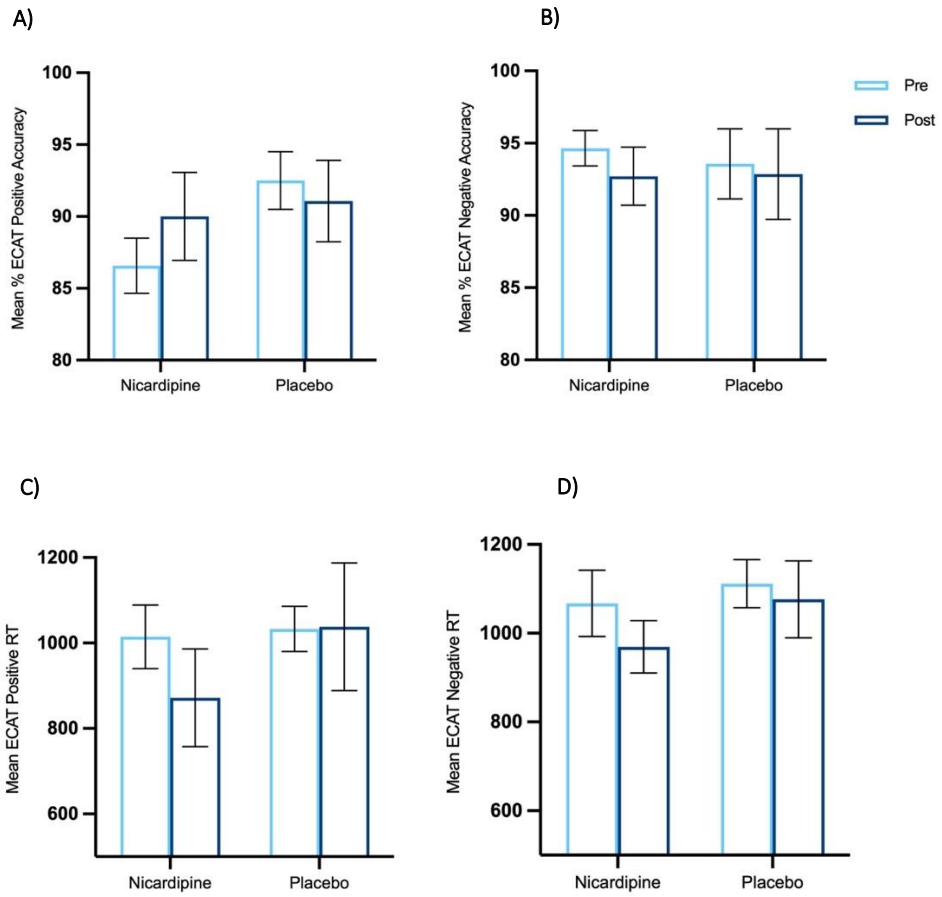
Facial Expression Recognition Task (FERT)								
	Mean (SD)				Post-hoc analyses			
	Pre		Post		t-test[1]		ANOVA[2]	
	Nicardipine	Placebo	Nicardipine	Placebo	p-value	Sig.[3]	p-value	Sig.[3]
Accuracy (%)								
Grouped emotions								
Positive	74.0 ± 3.2	74.9 ± 3.7	73.1 ± 4.7	75.0 ± 5.5	0.34	ns	0.66	ns
Negative	62.5 ± 5.5	63.7 ± 9.7	62.9 ± 6.2	68.0 ± 6.0	0.04	*	0.09	+
Individual emotions								
Anger	61.8 ± 9.8	66.6 ± 10.9	59.3 ± 7.4	67.3 ± 9.0	0.02	*	0.37	ns
Disgust	66.1 ± 11.6	67.7 ± 17.1	68.9 ± 11.1	73.0 ± 13.2	0.38	ns	0.45	ns
Fear	55.4 ± 15.4	55.5 ± 16.0	57.9 ± 15.9	62.7 ± 8.0	0.32	ns	0.38	ns
Happy	78.8 ± 5.9	80.7 ± 3.5	73.8 ± 6.6	77.9 ± 7.7	0.14	ns	0.42	ns
Sad	66.8 ± 7.9	64.8 ± 8.7	65.4 ± 8.9	68.9 ± 11.5	0.37	ns	0.17	ns
Surprise	69.3 ± 3.9	69.1 ± 6.7	72.5 ± 6.0	72.1 ± 6.8	0.88	ns	0.96	ns
Neutral	77.9 ± 13.7	71.4 ± 27.7	95.7 ± 7.6	79.3 ± 26.2	0.03	*	0.05	+
Misclassifications (%)								
Grouped emotions								
Positive	0.9 ± 0.8	1.1 ± 1.1	0.9 ± 0.7	1.0 ± 0.8	0.50	ns	0.84	ns
Negative	2.2 ± 0.9	2.4 ± 1.5	1.7 ± 1.1	2.3 ± 1.7	0.31	ns	0.34	ns
Individual emotions								
Anger	2.3 ± 1.8	3.2 ± 4.0	1.7 ± 1.9	1.8 ± 2.3	0.90	ns	0.33	ns
Disgust	2.7 ± 2.2	3.6 ± 2.8	2.7 ± 2.5	2.4 ± 1.3	0.70	ns	0.21	ns
Fear	0.8 ± 0.7	0.5 ± 0.7	1.0 ± 0.9	1.0 ± 1.2	0.96	ns	0.39	ns
Happy	0.1 ± 0.2	0.4 ± 0.6	0.3 ± 0.4	0.8 ± 0.8	0.07	+	0.30	ns
Sad	2.9 ± 2.6	2.3 ± 2.8	1.7 ± 1.6	4.1 ± 5.0	0.10	+	0.01	*
Surprise	1.7 ± 1.5	1.8 ± 1.8	1.4 ± 1.3	1.3 ± 1.0	0.82	ns	0.81	ns
Neutral	24.6 ± 3.1	22.5 ± 7.4	26.1 ± 5.0	19.8 ± 7.5	0.02	*	0.04	*
Reaction time (ms)								
Grouped emotions								
Positive faces	1359 ± 270	1509 ± 219	1267 ± 238	1410 ± 301	0.18	ns	0.92	ns
Negative faces	1518 ± 331	1747 ± 329	1454 ± 261	1565 ± 377	0.37	ns	0.21	ns
Individual emotions								
Anger	1443 ± 307	1668 ± 394	1502 ± 371	1491 ± 465	0.95	ns	0.06	+
Disgust	1441 ± 339	1671 ± 342	1373 ± 277	1519 ± 378	0.26	ns	0.44	ns
Fear	1893 ± 616	2099 ± 417	1727 ± 339	1933 ± 569	0.26	ns	1.00	ns

Happy	1297 ± 261	1465 ± 249	1228 ± 265	1367 ± 262	0.17	ns	0.74	ns
Sad	1294 ± 207	1552 ± 315	1215 ± 197	1318 ± 275	0.26	ns	0.05	+
Surprise	1420 ± 301	1553 ± 258	1307 ± 255	1452 ± 388	0.25	ns	0.91	ns
Neutral	1317 ± 287	1518 ± 385	1112 ± 142	1441 ± 443	0.01	*	0.40	ns
Emotional Categorisation Task (ECAT)								
Accuracy (%)								
Positive	86.4 ± 7.2	92.5 ± 7.5	90.7 ± 11.7	91.1 ± 10.6	0.93	ns	0.26	ns
Negative	94.6 ± 4.6	93.6 ± 9.1	93.2 ± 7.7	92.9 ± 11.7	0.93	ns	0.77	ns
Reaction time (ms)								
Positive	1023 ± 280	1033 ± 197	955 ± 284	1045 ± 261	0.39	ns	0.57	ns
Negative	1064 ± 279	1112 ± 204	952 ± 229	1076 ± 324	0.25	ns	0.53	ns

[1] Independent sample t-tests explored differences in post-randomisation accuracy, misclassifications, and RTs, for nicardipine versus placebo groups.
[2] Repeated measures ANOVAs explored group by time differences in accuracy, misclassifications, and RTs, for nicardipine versus placebo groups.
[3] Nicardipine SR versus placebo *p<0.05, +p<0.10, n.s. = non-significant.

Appendix 5.2 Emotional word categorisation task

Supplementary Figure 5.2.1. ECAT A) mean percentage accuracy for positive descriptors. B) mean percentage accuracy for negative descriptors. C) mean RT for positive descriptors. D) mean RT for negative descriptors, pre-and post-randomisation, for nicardipine SR and placebo. Error bars show SEM.



Appendix 5.3 Sensitivity Analysis

Supplementary Table 5.3.1. FERT group-time interactions for accuracy, RT, and misclassifications, with and without age as a regressor. Facial expressions presented individually and grouped together as positive and negative emotions. **Bold** values indicate analyses where covarying for age changed the statistical significance of the p-value. *p <0.05 and + p < 0.10.

		Accuracy			Reaction Time			Misclassifications		
		F	p-value	η^2	F	p-value	η^2	F	p-value	η^2
Positive	no regressors	0.20	0.66	0.01	0.01	0.92	0.00	0.04	0.84	0.00
	age regressor	0.54	0.47	0.02	0.42	0.52	0.02	0.20	0.66	0.01
Negative	no regressors	3.14	**0.09+**	0.11	1.67	0.21	0.06	0.94	0.34	0.04
	age regressor	2.15	**0.16**	0.08	1.59	0.22	0.06	0.01	0.93	0.00
Anger	no regressors	0.84	0.37	0.03	3.99	0.06+	0.13	0.98	0.33	0.04
	age regressor	0.29	0.59	0.01	3.20	0.09+	0.11	1.96	0.17	0.07
Disgust	no regressors	0.58	0.45	0.02	0.62	0.44	0.02	1.66	**0.21**	0.06
	age regressor	0.82	0.37	0.03	0.82	0.37	0.03	3.30	**0.08+**	0.12
Fear	no regressors	0.80	0.38	0.03	0.00	1.00	0.00	0.75	0.39	0.03
	age regressor	0.60	0.45	0.02	0.01	0.93	0.00	0.14	0.72	0.01
Happiness	no regressors	0.68	0.42	0.03	0.11	0.74	0.00	1.11	0.30	0.04
	age regressor	0.30	0.59	0.01	0.32	0.58	0.01	0.68	0.42	0.03
Sadness	no regressors	1.95	0.17	0.07	4.17	0.05+	0.14	7.74	0.01 *	0.23
	age regressor	1.19	0.29	0.05	5.33	0.03*	0.18	6.42	0.02 *	0.20
Surprise	no regressors	0.00	0.96	0.00	0.01	0.91	0.00	0.06	0.81	0.00
	age regressor	0.34	0.57	0.01	0.30	0.59	0.01	0.02	0.90	0.00
Neutral	no regressors	4.26	**0.05+**	0.14	0.74	0.40	0.03	4.59	**0.04***	0.15
	age regressor	2.38	**0.14**	0.09	0.41	0.53	0.02	1.92	**0.18**	0.07

Supplementary Table 5.3.2. ECAT group-time interactions for accuracy and RT, with and without age as a regressor. There was no change in statistical significance of p-values when correcting for age.

		Accuracy			Reaction Time		
		F	p-value	η^2	F	p-value	η^2
Positive	no regressors	1.41	0.25	0.05	0.36	0.56	0.01
	age regressor	1.34	0.26	0.05	0.04	0.84	0.002
Negative	no regressors	0.10	0.76	0.004	0.44	0.51	0.02
	age regressor	2.18	0.15	0.08	0.13	0.73	0.01

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