UNIVERSITY OF CRETE

SCHOOL OF MEDICINE

Neurophysiology and Behavior Lab

Master Thesis

Modeling of firing pattern-specific neuronal circuits of the prefrontal cortex: Functional effects of Schizophrenia-linked polymorphisms in CACNA1C gene.

MARGELI MYRTO

Assistant Professor: KYRIAKI SIDIROPOULOU

HERAKLION 2018

Η παρούσα μεταπτυχιακή εργασία πραγματοποιήθηκε στο «Εργαστήριο Νευροφυσιολογίας και Συμπεριφοράς» του τμήματος Βιολογίας του Πανεπιστημίου Κρήτης.

Examination Committee

Kyriaki Sidiropoulou
Assistant Professor of Neurophysiology, Dept of Biology, University of Crete

Maria Papadopouli
Professor of Computer Science, University of Crete

Panayiota Poirazi
Research Director at IMBB-FORTH

Ευχαριστίες

Θα ήθελα πρώτα, να ευχαριστήσω πολύ την καθηγήτρια μου, Κυριακή Σιδηροπούλου, για την καθοδήγηση που μου έδωσε κατά τη διάρκεια της εκπόνησης της μεταπτυχιακής μου εργασίας. Οι γνώσεις, οι ιδέες της, η υπομονή και η αισιοδοξία που έδειξε, ήταν καθοριστικής σημασίας για την ολοκλήρωση της εργασίας μου.
Έπειτα, θα ήθελα να ευχαριστήσω τους φίλους και την οικογένεια μου, για την κατανόηση, τη ψυχολογική υποστήριξη και συμπαράσταση. Ιδιαίτερα ευχαριστώ, την Ελισάβετ Δρούλου, φίλη και συμφοιτήτρια μου, που ήταν πάντα εκεί για να μοιραστούμε κοινούς προβληματισμούς και ανησυχίες και να δώσουμε ώθηση η μία στην άλλη.
Τέλος, θα ήθελα να ευχαριστήσω μέσα από την καρδιά μου, την υπεύθυνη του μεταπτυχιακού μας προγράμματος, κα. Κυριακή Θερμού για την αδιάκοπη προσπάθεια της να είναι δίπλα σε όλους τους φοιτητές του προγράμματος, σαν πραγματική δασκάλα, μέντορας και φίλη.

List of Abbreviations

Ad: Adapting

AP: Action potential

DA: Dopamine

CNS: Central nervous system

dlPFC: dorsolateral PFC

ISIs: Interspike Intervals

IS: irregular spiking

FF: Firing frequency

FS: fast spiking

mPFC: medial prefrontal cortex

nAd:non-adapting

PA: Persistent activity

PFC: Prefrontal cortex

Pyr: pyramidal neuron

RS: regular spiking

SCZ: Schizophrenia

SNPs: Single Nucleotide Polymorphisms

List of Figures

List of Tables

Περίληψη

Η σχιζοφρένεια αποτελεί μία από τις πολυπλοκότερες ψυχιατρικές νόσους. Οι κλινικές της εκδηλώσεις εμπίπτουν σε ένα ευρύ φάσμα της ανθρώπινης “πνευματικής” δραστηριότητας και προκύπτουν από διαταραχές σε ποικίλους γνωσιακούς τομείς. Τον όρο “σχιζοφρένεια”, εισήγαγε τον 19ο αιώνα, ο ελβετός ψυχίατρος Eugen Bleuler, αναδεικνύοντας ως κεντρικό γνώρισμα της νόσου, την σχάση (“splitting”) των ψυχονοητικών λειτουργιών. Σύγχρονα, θεωρητικά γνωσιακά μοντέλα υιοθετούν και εμπλουτίζουν αυτή τη θεώρηση, υποστηρίζοντας ότι η ελλιπής νοητική απαρτίωση, οδηγεί στην εμφάνιση των συμπτωμάτων της νόσου.

Η ετερογένεια των συμπτωμάτων, αμφισβητεί την κατηγοριοποίηση της σχιζοφρένειας ως μια ενιαία κλινική οντότητα και οδηγεί σε μια διαστατική περιγραφή της νόσου, σε επιμέρους συναφή σύνδρομα, ή αλλιώς ενδοφαινοτύπους. Η ανεύρεση των υποκείμενων παθοφυσιολογικών μηχανισμών, καθίσταται εξαιρετικά δύσκολη αφού προϋποθέτει την αποσαφήνιση του νευροβιολογικού υποστρώματος πολλών, διακριτών εγκεφαλικών λειτουργίων. Για αυτό το λόγο, προτίνεται σα μεθοδολογία διερεύνησης των αιτιοπαθογενετικών μηχανισμών της νόσου, η ανά σύμπτωμα μελέτη. Στην παρούσα εργασία, γίνεται ανασκόπηση των θεωρητικών προσεγγίσεων που προσπαθούν να ερμηνεύσουν την εκδήλωση του κάθε, επιμέρους ενδοφαινοτύπου. Παράλληλα, γίνεται αναφορά σε ευρήματα νευροαπεικονιστικών και νευροχημικών μελετών που υποστηρίζουν την εμπλοκή συγκεκριμένων νευρωνικών κυκλωμάτων στη διεκπεραίωση των φυσιολογικών νοητικών λειτουργιών και υπογραμμίζουν την τροποποιημένη δραστηριότητα αυτών των κυκλωμάτων, στην περίπτωση ελλειμάτων και παθολογικών διαφοροποίησεων.

Τα αίτια της σχιζοφρένειας δεν έχουν ακόμα αποσαφηνιστεί, αλλά η εμφάνιση της νόσου έχει συσχετιστεί με ποικιλία γενετικών και περιβαλλοντικών παραγόντων κινδύνου. Τα τελευταία χρόνια, η γενετική συνιστώσα της ασθένειας έχει έρθει στο προσκήνιο, λόγω των συνεχώς αυξανόμενων γενετικών ευρημάτων, τα οποία προέρχονται από μελέτες GWAS. Οι μελέτες αυτές, αναδεικνύουν διαφορές στη συχνότητα εμφάνισης συγκεκριμένων Μονήρων Νουκλεοτιδικών Πολυμορφισμών (SNPs), μεταξύ υγιών και πάσχοντων από σχιζοφρένεια. Τα γονίδια που φέρουν αυτούς τους -συσχετιζόμενους με τη νόσο- πολυμορφισμούς, κωδικοποιούν πρωτεΐνες που αφορούν στην ντοπαμινεργική, γλουταματεργική νευροδιαβίβαση και άλλες που ασκούν έλεγχο σε συναπτικο επίπεδο. Οι περισσότερες από αυτές τις πρωτεΐνες, έχουν προηγουμένως εμπλεχθεί στην παθοφυσιολογία της νόσου. Σε αυτό το πλαίσιο, αξίζει να αναφερθεί ότι ένα από τα πιο

συνεπή ευρήματα, είναι οι συσχετιζόμενοι με τη σχιζοφρένεια SNPs στο CACNA1C γονίδιο, το οποίο κωδικοποίει το L-type Ca^{2+} Cav 1.2 ιοντικό δίαυλο, συγκεκριμένα την α1 υπομονάδα του.

Στη συνέχεια, τονίζεται ο κυρίαρχος ρόλος του προμετωπιαίου φλοιού στη διαμεσολάβηση των γνωσιακών λειτουργιών και παρατίθενται επιχειρήματα, που συνηγορούν στην εμπλοκή της διαταραγμένης λειτουργίας του, στην παθοφυσιολογία της νόσου. Η περιοχική, λειτουργική υποδιαίρεση του προμετωπιαίου φλοιού, σκιαγραφείται μέσω μιας ανασκόπησης της δομικής και λειτουργικής συνδεσιμότητάς του. Επίσης, γίνεται αναφορά σε ενδείξεις περεταίρω λειτουργικής κατάτμισής του, μέσα στην ίδια περιοχή, σε επίπεδο στιβάδας. Τα εν λόγω ευρήματα, αναδεικνύουν την οργάνωση διακριτών, όσον αφορά στα χαρακτηριστικά γνωρίσματα, πυραμιδικών νευρωνικών πληθυσμών, σε μικροκυκλώματα, γεγονός που εγείρει υποψίες για λειτουργική εξειδίκευση αυτών των κυκλωμάτων.

Η μνήμη εργασίας, μια γνωσιακή λειτουργία που υποστηρίζεται κυρίως από το νευρωνικό υπόστρωμα του προμετωπιαίου φλοιού, παρουσιάζει ελλείματα στους σχιζοφρενείς ασθενείς. Τελευταία, αυτά τα ελλείματα έχουν συσχετιστεί με την εύρεση κάποιων από τους προαναφερθέντες SNPs, στο γονιδιώμα των ασθενών. Κυτταρικό αντίστοιχο, αυτής της γνωσιακής λειτουργίας, είναι η παραμένουσα δραστηριότητα που επιδεικνύουν οι πυραμιδικοί νευρώνες του προμετωπιαίου φλοιού. Αυτή η δραστηριότα, θεωρείται ότι επάγεται λόγω της ενσωμάτωσης των πυραμιδικών νευρώνων, σε δίκτυα συγκεκριμένης συνδεσμολογίας και συνεπώς, πρόκειται για μια ιδίοτητα δικτύου.

Ανατρέξαμε σε βιβλιογραφία που υποστηρίζει ότι διακριτά νευρωνικά δίκτυα, που αποτελούνται από ξεχωριστούς πυραμιδικούς υποπληθυσμούς, εμφανίζουν διαφοροποίησεις της παραμένουσας δραστηριότητας. Οι ξεχωριστοί αυτοί πυραμιδικοί υποπλήθυσμοι, φαίνεται να διαφοροποιούνται ως προς την εγγενή διεγερσιμότητά τους. Το ερώτημα που τίθεται, λοιπόν, είναι το κατά πόσο αυτή η διαφοροποίηση των ιοντικών μηχανισμών των κυττάρων, συμβάλλει ώστε διακριτά νευρωνικά δίκτυα να συμμετέχουν με διαφορετικό τρόπο τόσο στην επαγωγή και διατήρηση της παραμένουσας δραστηριότητας, όσο και στην διαμόρφωση της εκλεκτικότητας ως προς το ερέθισμα, που αυτή επιδεικνύει, πάντα στο πλαίσιο της φυσιολογικής, λειτουργικής εξειδίκευσης του εγκεφάλου.

Μεταβολές της εγγενούς διεγερσιμότητας των νευρώνων, μπορεί επίσης, να επέρχονται λόγω μεταβολής της ποσότικής έκφρασης ενός γονιδιού που κωδικοποιεί έναν τύπο ιοντικού διαύλου. Αυτή η υπόθεση, φέρνει στην επιφάνεια το δεύτερο ερώτημα. Στο πλαίσιο μιας “παθογόνου”

παραλλαγής της γονιδιακής αλληλουχίας, μπορεί διαφοροποιήσεις στο επίπεδο της λειτουργίας του νευρώνα, να καταλήξουν σε τροποποίηση μιας ιδιότητας δικτύου και εν τέλει, σε τροποποίηση γνωσιακών ικανοτήτων που διαμεσολαβούνται από αυτήν την ιδίοτητα;

Στην παρούσα εργασία, ακολουθήσαμε μια υπολογιστική προσέγγιση, ώστε να μελετήσουμε αν διακριτά, ως προς το πρότυπο πυροδότησης, νευρωνικά δίκτυα του προμετωπιαίου φλοιού αλλά και παραλλαγές αυτών των δικτύων, οι οποίες προσομοιώνουν τις λειτουργικές επιδράσεις των SNPs στο CACNA1C γονίδιο, επιδεικνύουν διαφοροποιημένες ιδιότητες της παραμένουσας δραστηριότητας.

Abstract

Schizophrenia constitutes one of the most complex psychiatric diseases. Its clinical manifestations emerge through disturbances in diverse cognitive domains, covering for a wide range of human mental activity. Eugen Bleuler, a leading exponent of the association psychology of the 19th century, introduced the term “schizophrenia”, and conceptualized the disease, as a “splitting/ fragmentation of psychic functions”. This influential concept of disruption of the, normally, unified cognitive processes, has been adopted and enriched by contemporary theoretical cognitive models, trying to interpret the pathophysiology of this disease.

Linking the symptoms of schizophrenia with their underlying pathophysiological mechanisms, is extremelly challenging, as it necessitates the delineation of high order-cognitive functions’ neural substrate. This study, begins with a theoritical account of the impaired brain processes, underlying the emergence of SCZ symptomatology and a review of the supporting evidence. A symptom-based approach, is suggested as a methodology of studying complex psychiatric diseases pathophysiology and the reasons for which, working memory deficits, are on focus is explained.

The occurrence of schizophrenia, has been associated with a variety of distinct genetic and environmental risk factors, but in the last years, the genetic component of the disease has been on the scene. The continuously expanding, genetic findings of Genome Wide Association Studies, provide us with associations of SNPs in multiple genes, with susceptibility risk. SCZ related genes, encode for proteins, previously implicated in the pathophysiology of the disease. In this context, SCZ-associated SNPs in CACNA1C gene, which encodes for the L-type Ca^{2+} ,Cav 1.2 channel, appears to be one of the most consistent findings.

The prevalent role of prefrontal cortex, in the mediation of cognitive functions is underlined and the implication of its impairement in the pathophysiology of SCZ, is extensively argued. PFC’s segregation in functional subdivisions, on a large-scale level, is depicted with an overview of its functional and structural connectivity. Furthermore, its functional segregation, on a between and intra-layer level, is inferred by presenting evidence, which suggest the formation of subcircuits with distinct, regarding their features, pyramidal neuronal populations.

Working memory, is a cognitive function, mediated predominantly by the neural substrate of PFC. Its impairement is a concistent finding in SCZ and lately, is associated with the presence of SCZ-related SNPs. The cellular correlate of working memory, persistent activity, is mainly observed in prefrontal

pyramidal neurons and it is considered, an emergent property of their incorporation in networks. From the relevant literature review, we picked out evidence, suggesting that this network property is differentially mediated by distinct, pyramidal subpopulations, in the context of normally specialized brain function. The first question that arises, concerns the specific way these subpopulations contribute to the induction, maintenance and stimulus-selectivity of this network property. An attribute of normal brain specialization is the documented differentiation of intrinsic excitability of prefrontal, pyramidal neurons. This brings us to the second question; is this differentiation of intrinsic excitability and its consequent impact to the emergent network function, a common attribute between normal, brain specialization and the kind of pathologic state, imposed by the functional effects of SCZ-related SNPs?

We undertook a computational approach to study whether, the firing-pattern specific PFC subcircuits and CACNA1C variants of these subcircuits, exhibit differentiated properties of persistent activity.

Table of Contents

1. INTRODUCTION

1.1 Schizophrenia and Schizophrenia Spectrum

Schizophrenia spectrum includes many, diverse psychotic disorders, such as schizophrenia, which lasts for at least 6 months and the active-phase persists at least one month, brief psychotic disorder, schizophreniform disorder, schizotypal personality disorder, schizoaffective disorder, substance/medication-induced psychotic disorder, catatonia and delusional disorder (American Psychiatric Association 2013).

This classification is not important only to clinicians, as the subentities of the spectrum exhibit different leading symptoms in dinstict combinations, severity, duration, drug resistance and prognosis, making it reasonable to infer that in some level, they are underlined by distinct neurobiological mechanisms. This should be taken into account in studies with human subjects, so that inferences and conclusions could be driven from, as possible homogenous groups.

The schizophrenia spectrum disorders are defined by one or more of the following key features, seperated in positive; delusions, hallucinations, disorganized thinking (speech), grossly disorganized or abnormal motor behavior (including catatonia), and negative symptoms (American Psychiatric Association, 2013).

1.1.1 Phenotype and theoretical account of the impaired brain processes.

In my view, the complexity of psychiatric disorders and especially schizophrenia, necessitates the decomposition of the clinically integrated phenotype of a disease, in endophenotypes of its distinct symptoms. Pathophysiological mechanisms mediating the emergence of a symptom, from altered structural markers to disrupted cellular signaling pathways, are easier to be found, if we know where to look for.

This section of introduction, aims in highlighting this point of view and drawing an outline of the impaired brain processes of the main psychotic symptoms, which constitute multilateral phenomena, defined experientially.

Positive Symptoms

Hallucinations, one of the cardinal positive symptoms of SCZ and a concequence of disrupted perception, are experiences of any sensory modality (with most common, the auditory ones), that occur without the existence of an external stimulus. Without an impairement localised to the

cortical areas, supporting the specific modality e.g auditory, visual cortex, hallucinations are concidered to arise from disruption of higher cognitive functions, such as reality monitoring, a process defined as the ability to judge and discriminate between real and imagined information, or else, from internal or external sourses (Buda et al. 2011). Many theoritical models of brain function, propose that perception of a sensation, is an optimal estimation of the external inputs, and its formation is based on predictive coding (Friston 2016). A prerequisite for perceiving a sensory stimulus-induced experience, is the computation of precision-weighted prediction errors. This computational process is accomplished by comparing "bottom-up" inputs from sensory organs and "top-down" predictions, based on knowledge imprinted on learned synaptic connections, from higher-level regions (McClelland & Rumelhart 1981;Maia & Cleeremans 2005) . In other words, these models, suggest a way that cognition, influences or "shapes" perception.

Structural and functional connectivity studies investigate the brain structural and functional markers, respectively, which predict and may underlie the occurrence of hallucinations. Indicatively, neuroimaging research, using structural MRI scans and automated whole-brain analyses methods, has implicated the medial anterior prefrontal cortex (mPFC) and the absence or reduced length of a specific anteromedial PFC brain structure, the paracingulate sulcus (PCS) in hallutination status (Buda et al. 2011;Garrison et al. 2015) , while, other studies examined differences in global measures of cortical gyrification or sulcation (Cachia et al. 2014). Reduced local gyrification index, observed in the mPFC regions surrounding the PCS, may represent diminished connectivity between the mPFC and both proximal and distal brain regions, such as cortical regions involved in processing sensory representations (Garrison et al. 2015). Futhrmore, other functional neuroimaging studies, make efforts to "capture the symptom", investigating functional connectivity during an actual experience of a hallucination and report increased coupling among the auditory, language and striatal regions (Ćurčić-Blake et al. 2017).

The second pathognomonic symptom of SCZ and other psychotic disorders, is *delusions*. Delusions are fixed, false beliefs, that are resistant in change, when conflicting evidence is presented. Their content concerns a variety of themes (e.g., persecutory, referential, religious, grandiose) and their emergence implies disruption of brain processes that mediate inference processing of external reality.

Contingency learning disruption, co- occurs with a tendency to update internal representations of the environment, based on incidental violations of its regularities and together they lead to the installation of delusions (Vinckier et al. 2016). A mechanism that attributes salience to actual

violations of regularities and attenuates the salience of expected sensory evidence, seems to underlie the way we form assosiations and representations of our environment. Scizophrenic patients, accredit overwhelming significance to details, probably because of a blunted process of salience attenuation. As a result, they experience a perplexed reality, for which they need an explanation. Compensatory prevalence of priorly formed high-level beliefs may explain the formation of aberrant assosiations about the causes of sensory input.

The brain processes considered to mediate learning and assosiation formation, are the following: prediction-error (differences between expected and actual outcomes) signaling, attentional allocation, assignement of salience in external and internal inputs, and working memory. One, some or all of them can be blunted, producing a defficient pattern of learning and that is the reason, that the study, of even a single symptom instead of a whole disease phenotype, can be extremely challenging.

There is extended litterature; human functional neuroimaging, human and animal behavioral studies, biologically inspired computational modeling approaches, about the neural architecture of learning and association formation. Indicatively, prediction error-mediated learning of associations, consists the basis of reinforcement learning theories and most of the studies, use probabilistic learning tasks in order to elucidate how prediction error signaling may be deranged, resulting to deficits in representations of expected value (Corlett et al. 2007; Gold et al. 2008;Gradin et al. 2011).

The incentive/motivational salience hypothesis of Berridge and Robinson (Berridge & Robinson 1998) ,

based on considerable evidence of phasic dopamine signals, encoding prediction error (independently of the quality of the outcome;rewarding or aversive) extends the previous theories that considered firing of dopamine neurons, just as a mediator of reward. This theory accomplishes to embedd the aberrant prediction errors, in the pathophysiologic formation of both positive and negative symptoms (described later). The confirmed, mesolimbic hyperdopaminergic state of the schizophrenic brain, may contribute to encoding of non-salient events as attention-wothing, giving rise to positive symtoms, while psychomotor poverty may be induced by abnormal reward prediction, resulting in lack of motivation (Deserno et al. 2013).

Given of the strong implication of reward system, in outcome-based learning, it is important to mention its circuit elements; the mesolimbic, mesocortical, and nigrostriatal dopamine systems, forming the mesocorticolimbic system. Prevalent structures, regarding their role in coding predictions, prediction's outcome and updating predictions, are prefrontal areas (ventromedial PFC, anterior cingulate cortex (ACC), ventral striatum (vStr) and ventral tegmental area (VTA).

Studies exploring functional alterations in these neural components of the reward circuit, during prediction-error mediated reinforcing learning, demonstrate an altered lateral PFC haemodynamic response occuring at the presentation of feedback to a prediction, in schizophrenic patients, indicative of an inability to distinguish between violations and fullfilment of expectancies (Corlett et al. 2007). Furthermore, many of the other substructures of the reward circuit, are found to have modified functionality; reduced prediction error signals were observed in midbrain dopaminergic neurons (VTA), the caudate, thalamus, insula and amygdala-hippocampal complex (Gradin et al. 2011).

Reward processing and reward prediction error, are mediated by dopamine, in both humans and animals and a mesolimbic dopamine abnormality is confirmed in schizophrenia, either as a primary neurochemical abnormality or a secondary consequence of hypofrontality. Therefore the midbrain VTA and substantia nigra dopamine neurons, have been under extended study, and abnormal responses of these neuronal populations in fMRI studies, associate with blunted discrimination between motivationally salient and neutral stimuli (Murray et al. 2008). Schizophrenic patients, not displaying normal differential activation between expected and unexpected outcomes, was correlated with exaggerated ventral striatal responses to expected rewards and attenuated responses to unexpected outcomes. This finding suggest the key role of aberrant activity in the vStr, in the neural circuitry mediating the differentiation of expected and unexpected feedback in schizophrenia (Morris et al. 2012).

Most of these findings of a blunted reward-processing system, in schizophrenic patients, are accompanied with a correlation with the severity of psychotic symptoms and specifically, with the occurrence of delusions, giving credit to the possibility that this system constitutes the neural substrate of delusions.

Additonal evidence for this possibility, is provided by the spacial coincidence of the cortical/subcortical areas that exhibit structural and functional alterations, in linkage with the emergence of delusions. These findings come from studies that explore structural connectivity of the brain, in the basis of occurrence of delusions, independently of the functional integrity of reinforcement learning processes (Zhu et al. 2016; Whitford et al. 2009).

Fractional anisotropy (FA), gray matter volume (GMV) and cerebral blood flow (CBF) are estimated with diffusion, structural and perfusion MRIs, respectively, The results of this kind of studies, present evidence about an altered structural integrity of the areas under investigation, such as reduced cerebral blood flow in ACC(Anterior Cingulate Cortex), dorsolateral and medial PFC, being positively correlated with delusions (Zhu et al. 2016). Although, conflicting evidence exists; other studies

reporting negative or no correlation of delusions with impaired structural connectivity (Spalletta et al. 2013).

This inconsistency in findings of structural connectivity, suggests that mulfunction in the neural circuit, implicated in belief formation, is not driven from excessive alterations in brain volume, resting metabolism or blood flow in the circuit's areas. In support of that, functional neuroimaging of the implicated brain areas, when subjects are performing an experimentally controlled task or when they experience the symptom, appears the only technique, reporting distinguishable findings between patients and healthy subjects. Therefore, it is plausible that pathologic function of this circuit, relates with an impaired reconfiguration of functional brain networks based on the momentary demands of internal and external environment, as acute task or stimulus effects. This is also consistent with the transient nature of both delusions and hallucinations.

Given all the aforementioned evidence, in positive symtomatology, a blunted reward-processing system equals with a system that attributes salience in expectancies feedback, necessary for extracting regularities, attend their violations, updating internal representations and in total, forming beliefs. In the other hand, in negative symtomatology; disruption of the reward system has a more explicit effect, inducing blunted coding of reward predictions, resulting in motivational deficits (avolition, anhedonia). Being a system with anatomical dipersion and different neurochemical components, a challenging question, is what part of it starts to mulfunction.

Negative symtoms

Negative symptoms are particularly prominent in schizophrenia and include diminished emotional expression, avolition, alogia, anhedonia, and asociality (American Psychiatric Association 2013). Avolition, is defined as a decrease in motivated, self-initiated goal-directed activities and anhedonia is the disrupted ability to experience pleasure. Numerous studies, have been focuced in identifying the neural correlate of negative symtoms, integrating them in a syndrome labelled "psychomotor poverty". Reward brain circuitry, responsible for reward processing is again on focus, this time bearing a straightforward relationship with the symptoms; diminished hedonic experience (anhedonia), reflecting a disrupted estimation of the rewarding outcome and motivational deficits (avolition), reflecting blunted coding of the expected rewarding value. In schizophrenia, in contrast with major depressive disorder (MD), both, avolition and anhedonia are mostly related to reward prediction, and prediction error processing rather than, to an attenuated reward experience; this being consistent with uncoupling of "wanting" and "liking" (Barch and Dowd 2010).

In translational psychiatry research, there is extended literature that links the functional neuroanatomy and neurochemistry of the reward system with behavioral paradigms, in humans and in a range of mammalian species (Gold et al. 2008). This system has cortical and subcortical components, as mentioned above; midbrain dopaminergic cell groups of the ventral tegmental area (VTA) and substantia nigra, striatum and prefrontal cortex areas are reciprocally interconnected. A mesolimbic dopamine abnormality is profound and consists the primary target of current pharmacological treatment approach for schizophrenia, with antipsychotics blocking the dopamine receptor D2. The role of dopamine in this system is well studied; short-term firing of mesolimbic dopaminergic neurons, trigger phasic dopamine signals, which encode prediction errors (Gradin et al. 2011). Prefrontal cortical afferents, modify the phasic component of these dopaminergic neurons in sustained, "background" tonic dopamine release, which influences the background level of dopamine receptor stimulation and thus the responsivity of the system to dopamine in these sites. In schizophrenics, an altered prefrontal cortical activity is proposed to reduce tonic dopamine release, leading to dysregulation of the intensity of the phasic dopamine response (Grace 1991). These evidence suggest the potential role of PFC, as regulator of the reward system and implicate its impairement in the pathogenesis of negative symptoms. In this direction, many neuroimaging and pharmacological studies, such as the ketamine model of psychosis, support the derivative nature of dopamine levels imbalance and subcortical areas' performance, considering as primary cause, an altered pattern of PFC neurons activity, (mediated by NMDA hypofunction (Jackson et al. 2004)) , termed "hypofrontality". In addition, phenomenological resemblance of negative symptoms to the clinical features, observed in patients with prefrontal injury, raise the suspicion that negative syndrom results from altered frontal lobe function and in particular, altered connectivity of fronto-striato-thalamic system.

Cognitive deficits

Cognitive deficits, are accessed with neuropsychological tests of executive function. Executive functions comprise a set of high-order functions, that render human beings capable of having an adapting behavior toward their internal and external enviroment; design and carry out plans, learn and obey social rules, solve complex problems, being multitasking, integrate temporospacial perception etc. Executive functions, traditionally include divided and sustained attention, set-shifting, cognitive flexibility, motor planning, temporal organization, behavioral inhibition, rule learning, emotional regulation and working memory (WM) (Orellana & Slachevsky 2013).

Schizophrenia, is a common disease, affecting approximately 1% of the population and is accompanied by significant psychosocial deterioration. Impaired executive functions constitute an important source of the disease's morbidity, as patients face difficulties in performing activities of daily living. Many neuropsychological studies, accessing schizophrenic patients' performance in a range of behavioral tasks, report deficits in a range of cognitive domains as verbal memory, abstraction, working memory, flexible control and capacity to optimize behavior(Nestor et al. 1998) . Clinical manifestations of deranged executive cognitive functions, correspond to either a "dysexecutive syndrome" or a "self-regulatory disorder" (SRD) (Stuss & Alexander 2000).

Neural correlates of different executive fuctions, are different cortico-subcortical or corticocortical neural networks, such as the prefronto-striato-thalamic, prefronto-temporal, prefronto-thalamo-cerebellar, and prefronto-parietal neural network (Orellana & Slachevsky 2013). These networks include proximal and distal cortical and subcortical regions, in direct and indirect connection with the PFC. This observation postulates that dysfunction of the PFC and its altered connectivity with other regions, may be central to the pathophysiology of the disease.

PFC anatomy and connectivity, give us insight for its intervention in mediating cognitive functions, and will be discussed later in the introduction section. As it will be elaborated, discrete regional subdivisions of PFC, are involved in different subcircuits, which support distinct neural processes.

Many of the executive functions, rely on coordinated information processing in a circuit consisting of PFC and mediodorsal nucleus of thalamus. There is evidence coming for diffusion-weighted imaging with probabilistic tractography analysis, which report reduced prefrontal-thalamic anatomical connectivity in schizophrenic patients (Giraldo-Chica et al. 2018) and impaired working memory performance.

In literature and clinical practice, cognitive deficits, in terms of executive functions, are most of the times, mentioned and accessed seperately from positive and negative symptoms or only in connection with the negative syndrom. In my view, this is a conventional dissection of symptomatology, as disturbances in diverse cognitive domains, underlie all the spectrum of schizophrenic phenotype.

Cognitive functions "shape" perception, are responsible of inferential thinking, fluency of thought and speech, organization of thought and language, motor planning, emotional regulation and expression, ability to initiate and complete goal-oriented behavior. Disruption of the implicated cognitive mechanisms give rise to the respective symptoms; hallucinations, delusions, alogia, formal thought disorder (Cavelti et al. 2018), catatonia, flat affect, avolition, all observed in SCZ. Symptoms

or endophenotypes, are the pathological, or else not functional, aspects of the outcome of many dinstict normal neural processes.

For understanding psychotic symptoms, beyond clinical description, I believe that we should identify the disruted cognitive mechanisms which induce each symtom's emergence and then, delineate the neural processes mediating each cognitive mechanism. We can point out the most frequently implicated neural processes and decipher their neurobiological substrate, look for an impaired activity of the specific neuronal populations and try to understand the causative factors of its impairment.

Working memory and schizophrenia

Working memory (WM), is included in the set of exexutive functions and there are many reasons for its special attribute. Its impairement is one of the most consistent findings in neuropsychological acessments of schizophrenic patients (Callicott et al. 2003;Driesen et al. 2008; Perlstein et al. 2003;Giraldo-Chica et al. 2018;Barch et al. 2003; Park & Holzman 1992) and fMRI studies link this deficit with dorsolateral PFC (dlPFC) altered activity, suggesting that this specific region, constitutes WM's main neural substrate.

Eventhough, WM deficit, is not pathognomonic of SCZ, as it is encountered in many other neuropsychiatric diseases, there is evidence of specificity in the underlying abnormal PFC activation, during performance of WM tasks, between SCZ and major depression (MD) patients. Schizophrenic patients failed to show activation of right dlPFC in contrast with MD patients, suggestive of a more severe PFC activation deficit in SCZ (Barch et al. 2003).

Furthermore, working memory is a neural process that plays a determinant role in the functional integrity of many other cognitive mechanisms and thus, working memory's deficits crutially influence their disruption and as a concequence, the emergence of distinct symptoms. For example, the integrity of reinforcing learning mechanism, a cognitive mechanism implicated in formation of beliefs and motives, depends on the undisrupted working memory function. Holding "online" short term representation of cues and values, mediated by working memory, is essential for prediction error signalling and updating of the learned representations. Encoding of rewarding events or predictions, blunted in anhedonia and avolition, discriminating between salient and non-salient events, blunted in delusions, and between internal and external representations, blunted in hallucinations, all necessitate the normal function of working memory.

Without, in any way, implicating that is the core deficit from which all the symptomatology arise, working memory is an important neural process, that may influence the intensity of performance of

the SCZ-impaired cognitive mechanisms. Evidence of this specific cognitive deficit, leading to reinforcement learning abnormalities in schizophrenia has been reported (Deserno et al. 2013), although most consistently is assosiated with the schizophrenic thought disorder.

Finally, family studies indicate a relation of deficits in WM to genetic susceptibility for schizophrenia. Data aquired from fMRI studies of individuals, facing a greater genetic risk for SCZ e,g ciblings of SCZ patients, provide arguments for a primary physiological abnormality in dlPFC's function, even when a cognitive defficiency is not manifested (Callicott et al. 2003). These data, infer that risk for SCZ is increased, with the inheritance of alleles, which are implicated in the development of an inefficient prefrontal information processing.

Therefore, identifying the neural substrates and cellular correlates of intermediate phenotypes, such as working memoty deficits, provides hints that may guide the research for susceptibility genes in complex psychiatric disorders. In reverse, it is important to investigate which are the functional effects of known mutations or polymorphisms of SCZ-susceptibility genes in the cellular substrate of SCZ-related cognitive mechanisms; persistent activity (PA) in the case of WM.

1.1.2 Genes and Environment: "Two-Hit Hypothesis"

The etiology of schizophrenia, as in most psychiatric disorders, remains unknown. Decades of research into

the pathogenesis of schizophrenia have not concluded in a single candidate mechanism but istead, disease's emergence has been associated with a variety of distinct genetic and environmental risk factors. Given the prevalent role of PFC dysfunction in disease's phenotype, it comes as no surprise that the exposure to schizophrenia risk factors, analyzed in this section, coincides with critical neurodevelopmental stages of this region of the brain.

"Two-Hit hypothesis"

"Two-hit hypothesis model" is a disease model, which as described in (Bayer et al. 1999) , applies the best framework, in order to access the origins of complex diseases, proven to have both genetic and environmental components, such as neuropsychiatric disorders but also cancer. It offers a well-

founded way to integrate genetic, developmental, and environmental factors that contribute to vulnerability and finally, emergence of a multifactorial disease. Most of neuropsychiatric diseases, share this common pattern of numerous risk factors being linked with their origin, indicating that more than one mechanism, is involded in their pathogenesis. This statement seems plausible, accounting for both brain's resilience and redundancy of regulatory and compensatory mechanisms, which sustain proper cells' functions and the complexity of the phenotypes produced. In schizophrenia, "two-hit hypothesis" proposes that the "first hit" comes at the perinatal period and may be genetic or environmental. It seems to disrupt some aspect of brain's development process and establishes a state of increased vulnerability, that renders the "second environmental hit", considered to occur in adolescence, sufficient to induce the symptomatology of the disease. One generic mechanism that might be pertrubated and fits the time table of the model, is cell-cell signaling pathways, initially involved in induction, differentiation and formation of the CNS, and later, determined by experience in the ongoing maintenance of CNS neurons and circuits (Maynard et al. 2001). Although, all cortical cerebral regions exhibit an experience-dependent modification of organization and function, they are differentialy influenced by experience an thus, they present discrete level of susceptibility in pre- and postnatal disturbing factors (Kolb et al. 2012; Gao et al. 2012).

All cortical areas share the common stages of development **(Figure 1);** neurons are born, proliferate, migrate to their appropriate cortical position, mature, form synapses and develop glia which forms both myelin and other supporting cells. At birth, cortex is completely formed, regarding its cellular architecture and basic structure of cellular elements (Fuster 2008).

Four features of cerebral development are very important for understanding the reason for which PFC is a better candidate, as a target of "two hit hypothesis" of schizophrenia. In general, it appears more susceptible to external effects during both the formation of its basic circuitry and during its modification in adolescence.

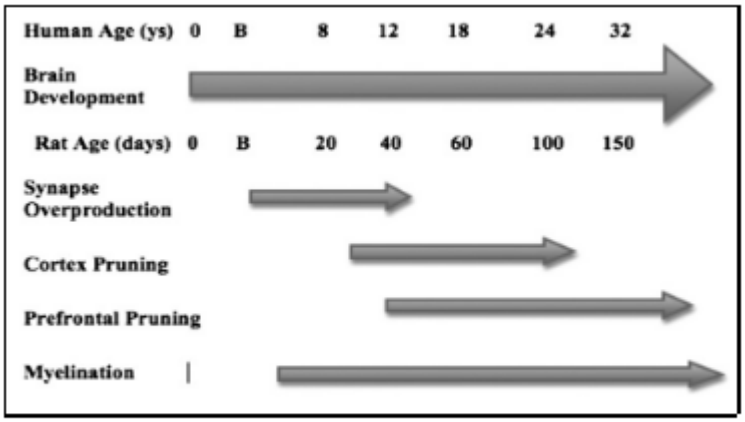


Figure 1. Time line of human development (adapted from Kolb et al. 2012).

First, a common trait in all cortex's regions, is the perinatal overproduction of neurons and their synapses. Dendritic spine density constitutes an indicator of the number of excitatory synapses on a neuron and is 2-3 fold greater in childhood than in adulthood. Sensory regions reach their peak

synapse density around 1 year, while some PFC regions, reach theirs in 5 years or later (Petanjek et al. 2011). This process appears to be dictated by a caudal to-rostral gradient, with posterior (sensory) regions peaking sooner than more anterior (PFC) ones and results in a greatest overproduction of spines is in the PFC.

Second, this overproduction of synapses is followed by a synapse elimination process, called synaptic prunning, beginning in late childhood, and in the PFC, it continues till the third decade of life, as PFC shows the slowest development rate of all the other cortical regions (Elston et al. 2009). This prolonged period of synapse elimination in the PFC results in increased weight of environmental influences in puberty. Experience-depedent neural activity "sculps" the synapses and determines the consolidation of specific neuronal subcircuits, mediated through synaptic plasticity processes.

Third, PFC exhibits a delayed development of myelin, as well, reaching its full development in late adolescence, apparently coinciding with maturation of the cognitive functions of the prefrontal cortex. Myelin consists the physical basis of connections between brain regions and in this context, extended studies, enriched with magnetic resonance imaging techniques, especially DTI (Diffusion Tensor Imaging) and cytochemical techniques, link the conceptualization of schizophrenia as a disorder of disrupted connectivity (Friston & Frith 1995; Friston 2016) , with findings of impaired myelination (Flynn et al. 2003;Davis et al. 2003; Peters & Karlsgodt 2015). Finally, an additional element, that links the development of PFC with the onset of SCZ in late adolescence, is the substantial developmental changes that, GABA neurons in the middle layers of PFC, undergo at this specific period of life (Lewis et al. 1999).

Now, having pointed out the time sequence of specific developmental processes and stages in the cortex and especially in PFC, we can better understand the effect of genetic and environmental factors coinciding with each of them; early morphogenesis, synapse formation, synapse elimination and myelin formation.

Environment

The environmental component of the disease appears, as we mentioned in two dinstict and critical phases of CNS development; first, during endometrial, perinatal and early-life period and second, during adolescense.

The first piece of evidence, for the contribution of prenatal enviromental stress comes from epidimiological studies, which associate an elevated risk for manifestation of the disease with multiple enviromental factors. Adverse life events occuring during pregnancy, such as war, famine (St

Clair et al. 2005; Susser et al. 1996) and season of birth (births in the winter-spring months) (Mortensen et al. 1999), both increase schizophrenia risk, with the latter, possibly explained due to the occurence of winter viral infections (Brown and Derkits 2010) . Epidimiologic literature was just the beginning; now, the effect of enironmental prenatal and early-life stress, together with the epigentic contribution, is widely investigated with neurodevelopmental animal models of schizophrenia (Ratajczak et al. 2013); models of maternal immune activation (Holloway et al. 2013), models created by pharmacological intervention, such as MAM--methylooxymethanol acetate model (Chalkiadaki et al. 2018; Lodge 2013) and prenatal stress, maternal deprivation, isolation rearing, maternal malnutrition models. All these models are validated by disruption in PPI (prepulse inhibition), submitted in a large range of behavioral tasks and tested with molecular, cytohistologic techniques. This gives us the opportunity, to assess seperately the effect of each environmental risk factor, associated with SCZ, to follow up the neurodevelopment progress of a "vulnerable" substrate and finally, to reveal intriguing findings, indicating behavioral, neurochemical, and neurophysiologic abnormalities consistent with observations in schizophrenia.

During adolescence, exposure to sensory stimuli, hormones, parent-child relationships, stress, and psychotropic drugs, may enbody the "2nd hit". Giving credit to the importance of adolescence, as a time period of dynamic remodeling of the neural circuits, many studies have concentrated in elucidating the impact, of different environmental factors in behavior. Exposure to chronic physical and emotional stress and the involvement of CRF (corticotropin releasing factor) and HPA (hypothalamic–pituitary–adrenal) axis is studied and long-term changes in behavior have been identified, mainly depression-like and anxiety traits (Yohn & Blendy 2017). The validated long-term impact of adolescent stress pardigms, combined with genetic-SCZ-predisposed animal models may produce a more consistent SCZ-like phenotype. Other studies focus on specific alterations produced by adolescent exposure in psychotropic drugs, such as altered PFC GABAergic transmission and dysregulation of subcortical dopamine function and investigate if they , finally, induce the psychotic-like phenotype (Miller et al. 2018; Renard et al. 2018; Renard et al. 2017; Zamberletti et al. 2014).

Genetics

The genetic component of the disease was early identified from adoption, family, and twin studies that provide strong evidence of high heritability estimates. However, it was, soon, understood that the genetic contribution can not solely account for the disease, as the concordance rate of schizophrenia, even in the case of monozygotic twins is about 50% (Cardno and Gottesman 2000),

leaving space for non-inherited risk factors to further explain the emergence of the disease phennotype.

As with environmental risk factors, epidimiological studies gave their place to more elaborate methods of investigating heritability. Schizophrenia does not show clear Mendelian inheritance and the genetic analysis of this type of disorders, involve a variety of different strategies. Historically, the strategy followed for schizophrenia was to detect risk alleles for a few candidate genes (Heyes et al. 2015). The selection of the candidate genes was based on the hypothesis of disruption of monoaminergic systems, in grounds of the efficacy of drugs such as amphetamine, to mimic some symptoms of schizophrenia and the fact that administration of D2 receptor antagonists remains the guideline-suggested treatment of the disease. The early candidate gene studies did not lead to identification of risk alleles, partly because of the use of small samples, inadequate to give statistical power to the findings (Heyes et al. 2015).

An other linkage analysis approach, intended to identify rare gene mutations, that run into families, in which the disorder seems to be inherited. In this way, the gene DISC1 was identified, due to its association with a chromosomal translocation breakpoint (Millar et al. 2000). However, this type of studies, gives limited insight in the etiology of common psychiatric disorders with complex polygenic nature, as SCZ (~1% prevalence in general population). After decades of frustration, genetic research of psychiatric diseases turned in to a common disease/common variant (CD/CV) model, which considers that predisposition for these diseases, is emerging through the inheritance of a combination set of numerous common variants. Given that common variants (**Figure 2**) predispose to illness, only in combination with other alleles, the CD/CV hypothesis was the foundation of genome-wide association studies (GWAS); as using very large samples, seemed the only efficient way in order to detect an increase in allele frequency in disease cases versus healthy subjects (Mitchell 2011).

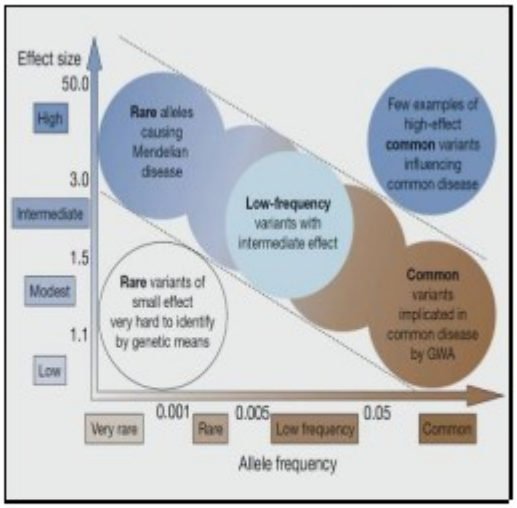


Figure 2. Adapted from McCarthy MI, Abecasis GR, Cardon LR, et al. Genome- wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genetics.* 2008;9:356-369. Copyright© Nature Publishing Group 2008.

Now, GWAS are widely employed for examining the frequencies of common genetic variants represented by single nucleotide polymorphisms (SNPs) and have successfully identified a number of associations, surpassing genome-wide levels of significance (Li et al. 2017; Ripke et al. 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014).

SNPs are found throughout the genome and most of them are predictably, located in non-coding regions. Implication of an SNP related to a disease, is characterized by ambiguity in the matter of actually representing the disease-associated alteration, or just being in linkage disequilibrium with the variant causing the functional effect. Although, the effect size of individual SNPs, associated with common psychiatric disorders, is small (odds ratio <1.2), collectively, common alleles answer for a large fraction of the genetic liability to develop psychiatric disorders. Specifically for schizophrenia, a comprehensive GWAS computed this fraction to at least 32% of the variance in liability to develop the disease, in the population studied (Ripke et al., 2013).

1.2 Genes related to SCZ

One of the most important GWAS, concerning the identification of inherited genetic variants, related to the occurrence of schizophrenia, was produced after the creation of Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC). Assuming that sample size is one of the most important limiting factors in applying GWAS to schizophrenia, they focused in accomplishing the integration of all available schizophrenia samples, with published or unpublished GWAS genotypes, into a single, systematic analysis,. They gathered 36,989 cases and 113,075 control samples and the results of the analysis, pointed out at least 108 independent genomic loci that exceed genome-wide significance (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014) .

Three key findings of this and other precedent and subsequent GWAS for SCZ, are reported. First, although risk variants ranged in frequency from common to extremely rare, it has been estimated that half to a third of the genetic risk of SCZ, is indexed by common alleles.

Second, in consistency with exome sequency findings and previously mentioned hypotheses, most of associated common variants detected by GWAS, seem to exert their effects through altering gene expression, not protein structure. This is postulated, according to the identified location of the most of the associated SNPs, in introns or intergenic regions. Their location, within or proximal to the regulated gene, contains sites for binding of regulatory factors, which determine the proper tissue-specific and temporal expression of genes. Promoter and enhancer regions or regulation regions of alternative splice variant expression, are part of the cis-regulatory elements of the gene.

Third, the reported findings can be seperated on those giving a potential for new insights in the pathogenesis of SCZ and those which are in support of leading, till now, pathophysiological

hypotheses of the etiology of schizophrenia. In the latter category, consistent with the dopamine hypothesis and target of all, currently effective antipsychotic drugs, is the association made with the DRD2 (dopamine receptor D2) gene, whereas the implication of many genes, e.g GRM3, GRIN2A, SRR, GRIA1, involved in glutamatergic neurotransmission and synaptic plasticity, is consistent with the glutamate hypothesis. The more generic hypothesis, suggesting synaptic dysfunction in the basis of emergence of a wide spectrum of psychiatric disorders, finds support in the highlighted association of CACNA1C, CACNB2 and CACNA1I genes (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014) , which encode voltage-gated calcium channel subunits and are considered synapse-associated proteins of great importance.

An interesting fact, suggesting convergence at a broad functional level between studies of common and rare genetic variation, is that the previously independent implication of genes encoding calcium channels, and proteins involved in glutamatergic neurotransmission and synaptic plasticity, in schizophrenia, comes from studies of rare genetic variation.

Finally, it is a worth- mentioning finding, that implicated gene variants did not concern, solely, genes expressed in brain. Associated genes, which are expressed in tissues mediating the immune response, argue for the previously, reported link between the immune system and schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014).

1.2.1 Calcium Channel Genes associated with Neuropsychiatric Disorders

Given that depolarization-induced calcium entry into neurons, is primarily mediated by voltage-gated calcium channels, these channels are, generally, acknowledged to be of critical importance to brain function, while their inappropriate expression or dysfunction, gives rise to a variety of neurological diseases.

Voltage-gated channels are permeable to calcium cations and their opening allows calcium influx, along its electrochemical gradient, resulting in a localised elevation of intracellular calcium to a micromolar range, in great contrast with normal resting conditions, where its concentration lies in the 100 nM range.

In turn, this elevation triggers many calcium-dependent processes, including gene transcription, hormone secretion, spontaneous pacemaker activity in some neurons, neurotransmitter release, activation of calcium-dependent enzymes and neurite outgrowth. As a consequence, beside the profound electrogenic role, of these channels in cell's intrinsic exitability and proper synaptic function, their pattern of expression and functional integrity affects a wide range of intracellular processes critical for the generalised cellular function (Simms & Zamponi 2014).

There is currently a great deal of interest in voltage-gated calcium channels, regarding the identification of SNPs in calcium channel genes as risk alleles in a spectrum of psychiatric disorders (Cross-disorder group of Psychiatric Genomics Consortium, 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). As mentionned before, among the highlighted findings of the GWA study that identified the 108 SCZ- associated loci, three calcium channel gene SNPs were identified as risk factors with genome-wide levels of significance; SNPs in CACNA1C, CACNB2 and CACNA1I genes. In particular, the common allele associations identified in *CACNA1C* (SNP rs1006737 and other SNPs in linkage disequilibrium with this SNP), are highly reproduced in many studies, rendering CACNA1C, a gene that codes for the a1C subunit of the Ca v 1.2 voltage-dependent L-type calcium channel, one of the most consistent genetic findings related to SCZ.

It is important to point out that some identified SNPs are linked with a specific susceptibility to either bipolar disorder (BD) or SCZ, in contrast with CACNA1C and CACNB2 genes, whose SNPs are found to confer susceptibility to a wider spectrum of psychiatric disorders. In a five disorder meta-analysis (Cross-Disorder Group of the Psychiatric Genomics Consortium 2013), shared risk loci effects, were identified, on five major psychiatric disorders; autism spectrum disorder, attention deficit-hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia. In addition, this meta-analysis, compiled the list of SNPs in voltage-gated calcium channel genes with the a1 subunit genes CACNA1C, CACNA1D, CACNA1E, CACNA1S, a2d subunit genes CACNA2D2 and CACNA2D4, and b subunit gene CACNB2.

The challenge emerging, from the expanding psychiatric genetic findings, is their translation into altered physiological function and identification of its contribution, in the wider pathology of the disease.

In order to understand how susceptibility genes, that code for voltage-gated calcium channels, may relate to neuropsychiatric diseases, it is necessary to flash back to the classification of these channels, their different properties and functions, as well as their distribution and interaction with other proteins.

1.2.2 Calcium Channels – Classification, distribution, function

Voltage-gated calcium channels exhibit great diversity of subtypes, in grounds of multiple channel subunits and alternative splicing. They are subgrouped in two major categories; high voltage-activated (HVA) channels, activanted in response to large membrane depolarizations and low voltage-activated (LVA) channels, whose openning is triggerd by smaller voltage changes. HVA channels are heteromultimeric protein complexes, consisting of a coassembly of a pore-forming α1 subunit, as well as, auxiliary β and α2δ subunits and in some cases,γ subunits. In contrast, LVA channels lack these auxillary subunits, being functional with only the presence of α1 subunit (Simms & Zamponi 2014). This pore-forming subunit, mainly determines the channel's kinetics, voltage dependence, single-channel conductance and pharmacology, while the other auxilliary subunits also influence these parameters, with their significant role in channel trafficking and interaction with other proteins (Heyes et al. 2015). The further branching of calcium channels in distinct subtypes, is dependent of the multiple different CACNA1 genes, that encode the a1 subunit (**Figure 3**). There are three major families of calcium channel α1 subunits; each family is characterized by its pharmacollogically-induced inhibition by distinct antagonists.

Cav1 family (L-type)

The Cav1 channel family includes three L-type channels, present in the brain, named Cav1.1 ,Cav1.2, Cav1.3 and a skeletal muscle isoform, named Cav1.4. These channels share their slow voltage-dependent gating characteristics and are inhibited by a variety of dihydropyridine (DHP) antagonists (Heyes et al. 2015).

Cav 1.2**,** encoded by CACNA1C gene, is expressed throughout the brain and it is the predominant channel in ventricular cardiac muscle, while Cav 1.3**,** encoded by CACNA1D gene exhibits a more restricted distribution in the brain and is located in sinoatrial node in the heart. Despite its limited distribution, Cav 1.3, plays an important role in the brain; considered to induce the pacemaker activity in brain dopaminergic neurons (Liu et al., 2014) and also, implicated in synaptic pruning during development (Hirtz et al., 2012). Both processes have been discussed before in introduction, as their potential disruption may lead to pathogenesis of psychiatric disorders. Given that, the identification of CACNA1D, as a susceptibility gene for SCZ and other disorders, suggests that the functional effects of variants of this gene, need further consideration.

Although, Cav 1.2 and Cav 1.3 L-type channels exhibit differential distribution, functions and properties, such as, activation of Ca V 1.3 at lower voltage thresholds, both have a postsynaptic role in dendritic signalling, their function is linked with a process termed excitation–transcription coupling (Wheeler et al., 2012) and also, both exhibit SCZ-associated SNPs.
Finally, Cav 1.4, encoded by CACNA1F, is located, mainly in the retina.

Cav2 family

The Cav2 channel family consists of three members Cav2.1, Cav2.2, and Cav2.3. Members of this family have, mainly, neuronal distribution and they are all blocked by some peptide toxins, such as ω-agatoxin IVA.
P and Q type channels, emerge from Cav 2.1, through alternative splicing and assembly with specific auxilliary subunits and they are both encoded by CACNA1A gene (Simms & Zamponi 2014). They are found throughout the brain, located in presynaptic terminals of mature central neural system (CNS) and considered to be the prevalent calcium channels involved in neurotransmission. Their substantial role in neurotransmitter release, both in mature and inhibitory synapses, may explain the fact of not being implicated in psychiatric disorders. Mutations in CACNA1A gene, result in disorders, such as spinocerebellar ataxia, episodic ataxia and epilepsy.
Cav 2.2, is the molecular equivalent of the N-type calcium channels and it is encoded by CACNA1B. Their pattern of expression, alters throughout the development; they play an important role in neurotransmission and are widely distributed, in CNS early in development and in peripheral nervous systems (PNS) (Heyes et al. 2015). Both, Cav 2.1/Cav 2.2 channels, mediate presynaptic transmitter release, and have a temporal and tissue -specific pattern of expression.
Cav 2.3, corresponds to the residual R-type calcium current, recorded after pharmacological block of N-type, P/Q-type, and L-type channels and is encoded by CACNA1E. Its distribution concerns many brain regions including the hippocampus, both pre- and post-synaptically (Parajuli et al., 2012).

Cav3 family (T-type)

The Cav3 channel family includes three members Cav3.1, Cav3.2, and Cav3.3, encoded by CACNA1G, CACNA1H, CACNA1I respectively. They are distinguidhed by their low-voltage gating and their relative resistance to inhibition by cadmium ions, which block all HVA channels. In brain, they are widely distributed, with high expression in the thalamus (Heyes et al. 2015). They have an important contribution in neuronal excitability and pacemaker activity (Perez-Reyes, 2003) indicated

by their postsynaptic location, and in some synapses, they are found to regulate neurotransmitter's release. CACNA1H gene, has reported SCZ-related allele variants.

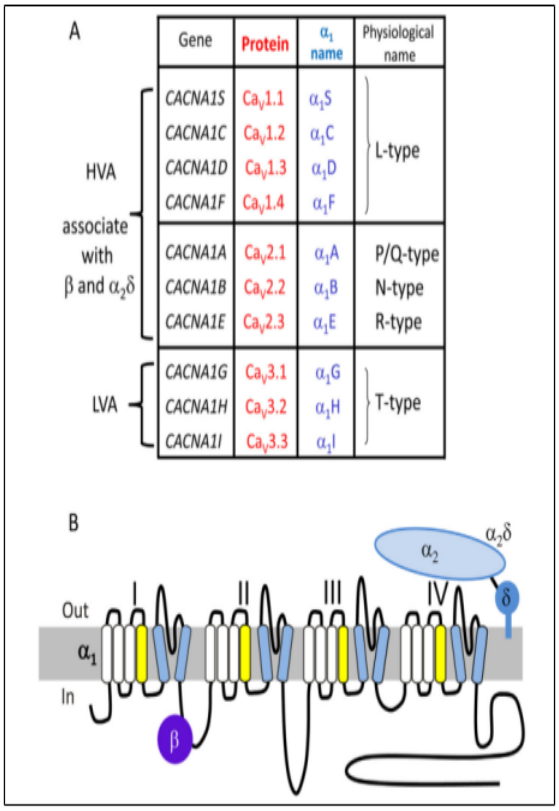


Figure 3. Calcium channel a1 , b , and a2d subunits, and their topology.
(A) Nomenclature of calcium channel subunits, including gene name, initial names of cloned a1 subunits, rationalised protein names (CaV nomenclature), and names used in physiological discovery of the channels. HVA, LVA = classical definition of channels as high- or low-voltage-activated.
(B) Calcium channel a1 ,b and a2d subunit topology. The a1 subunit has 24 transmembrane segments, comprising four homologous domains, labelled I–IV. Each domain has six transmembrane segments (S1–S6), including the S4 voltage sensor (yellow), and the S5–S6 pore-forming segments (blue). (figure and text of the figure adapted from (Heyes et al. 2015) .)

1.2.3 SCZ-associated SNPs in CACNA1C gene

Given that SNPs found in CACNA1C, the gene coding for the α1C subunit of the Ca v 1.2 voltage-dependent L-type calcium channel, is one of the most consistent genetic findings related to SCZ, this section is focused in reporting the SNPs in this particular gene and refers to studies investigating their functional effects.

CACNA1C is located on the short arm of chromosme 12p13.3 and consists of at least 55 exons. The association signal identified in the CACNA1C gene is concentrated in a large intron between exons 3 and 4, which contains several, related to neuropsychiatric disorders, SNPs in linkage disequilibrium (Ripke et al., 2013). The SCZ – associated SNPs in CACNA1C gene, emrged from the literature review I have done, are the following; rs1006737 (the SNP can be either G or A, with A being the risk variant and occurring in the general population with a frequency of about 0.33) (Hamshere et al., 2013), rs2007044 (Schizophrenia Working Group of the Psychiatric Genomics Consortium,2014), rs1024582 (Bhat et al. 2012), rs4765905 (Hamshere et al., 2013).

However, there is still lack of available data, concerning the functional consequences of these SNPs. The functional effects of a genetic variant can be assessed in many levels; determine if there is an increase or decrease in gene expression or a gain or loss of function of the calcium channel gene products, then given of an alteration, in either direction, examine what are its consequences in neuron's excitability, synaptic mechanisms, signaling pathways etc, then assess the impact to the network of interconnected neurons, bearing the variant channels etc. The answer in the question of whether this effect is constant throughout development, and in what specific tissues or brain regions is occuring,, may drive our research for altered structural or functional connectivity in specific regions. Finally, the ultimate goal is to link the identified risk variants at these gene loci with altered performance on measures of cognition, underlying specific cognitive or behavioral effects.

In the first level of assesment, I described; given the location of reported SNPs, in a non-coding region of the gene, the assumption made is that they do not directly interfere with the structure-functional properties of Ca v 1.2 (e.g channel kinetics). The most probable effect of an intronic variation, is the regulation of expression of the protein product. Till now, there is no clear consensus concerning the effect of the implicated SNPs to the expression level of calcium channel Cav 1.2. Indicatively, for the rs1006737 risk allele, there are reports of an observed small increase in Ca V 1.2 mRNA , in a study of post-mortem human brain samples from dorsolateral prefrontal cortex of

control subjects (Bigos et al., 2010) and in a study comparing induced neurons derived from skin fibroblasts, from subjects with homozygous AA containing allele. In the later study, neurons also exhibited increased calcium current density for 10/11 risk allele lines analysed (Yoshimizu et al., 2015). However, the same study reports, that there was lower CACNA1C mRNA levels and lower calcium current density in one of the cell lines analysed. This ambiguity is a consistent finding with other studies that identified both increased and reduced expression of CACNA1C in the presence of the rs1006737 risk allele (Bigos et al., 2010; Roussos et al., 2014; Gershon et al., 2014)

For rs2007044, a specific interaction of an enhancer region, with the proximal CACNA1C gene promoter, is reported and appears to result in association of the risk variant SNP with reduced CACNA1C gene expression (Roussos et al. 2014).

In the subsequent level of functional consequences of CACNA1C variants, assumptions can be made on the grounds of these channels' distribution and function. Cav 1.2, as mentionned before, plays both a modulatory role in dendritc processing, being located postsynaptic on dendritic spines and an important role in coupling neuronal activity to gene transcription.

Human functional studies, show contradictory results in their effort to identify an association between genetic risk variants and altered brain function or structure and cognitive performance. Indicatively, CACNA1C risk variant rs1006737 is found associated with reduction of bilateral hippocampal activation during episodic memory recall (Erk et al. 2010), with a significant decrease in activity in the dorsolateral prefrontal cortex and a decrease inconnectivity with the medial temporal lobe during working memory tasks (Paulus et al., 2014), whereas the opposite result was reported in a previous study (Bigos et al., 2010). Furthermore, it was associated in a dose-dependent manner with blunted reward responsiveness (Lancaster et al. 2014) and with working memory deficits in schizophrenic rs1006737 carriers (Zhang et al., 2012). Finally, there is evidence of CACNA1C risk variant rs2007044 being associated with working memory deficits due to dysconnectivity between the right DLPFC and several cortical regions (Cosgrove et al. 2017).

1.3 **Prefrontal Cortex (PFC)**

A glance in PFC's neuroanatomy is essential in order to understand, models of its function. Its great connectivity, is the reason for being considered on the top of an hierarchical brain network

.

1.3.1 Anatomical connectivity

Prefrontal cortex, is the name given to the cortex that covers the anterior pole of the brain; it lies in front of limbic cortex on the orbital and medial surfaces and in front of motor cortex on the lateral surface. Anatomically, PFC is defined, as the part of the cerebral cortex that receives afferent projection fibers from the mediodorsal nucleus of the thalamus, although, it actually receives them from a variety of other cortical and subcortical regions (Fuster 2008). Its boundaries are marked by distinctive morphological attributes: arcuate sulcus, inferior precentral fissure and the anterior curvature of the cingulate. In humans, PFC exhibit the maximum relative size (with respect to brain size or body weight), in an across-species comparison. It actually, constitutes nearly one-third of the entire neocortex and for many, it is the area of the brain that distinguishes human from the other primates, as its phylogenetically more evolved.

PFC, can be divided in a manner of functional localization. Its subdivisions and their extent vary, in litterature, but I will refer to a common one that sections PFC in the following way; **medial** prefrontal cortex (mPFC), with further subdivisions; dorsomedial and ventromedial PFC, which encompasses the anterior cingulate cortex (ACC) ,the infralimbic cortex and prelimbic cortex, **lateral (lPFC) ,** further divided in the ventrolateral and

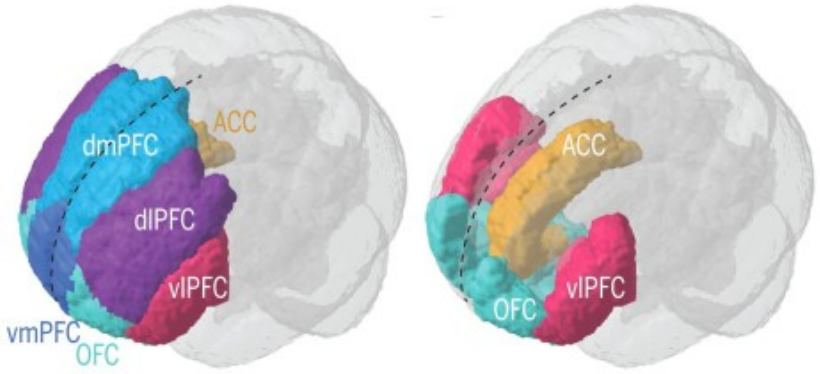


Figure 4 . Functional division of PFC (adapted from Carlén 2017)

dorsolateral prefrontal cortex,the **orbitofrontal** cortex**,** and the **caudal** prefrontal cortex, which includes the frontal eye fields (FEF) (Murray et al. 2016). Each of these sections of prefrontal cortex,

has been implicated in a distinct functional field, based on its connectivity with other cortical or subcortical areas and findings of neuroimaging and lession studies.

Almost all the prefrontal connections with other brain areas, are reciprocal; with structures sending afferent fibers to the prefrontal cortex, receiving its effernt fibers. The PFC, is connected with other cortices of association, but not with primary sensory or motor cortices; fibers from assosiation areas, implicated in sensory functions, converge on, both the lateral and orbitomedial aspects of the prefrontal cortex; a pattern of multimodal convergence. All prefrontal areas receive, directly or indirectly, afferent projections from some structures, such as; the hippocampus (Rosene & Van Hoesen 1977) and thalamus (Preuss & Goldman-Rakic 1987). Reduced thalamo-cortical connectivity in combination with augmented thalamic connectivity with somatosensory and occipital cortices and correlation with impaired cognitive functions, is widely reported, in schizophrenia (Woodward & Heckers 2016; Giraldo-Chica et al. 2018). Thalamic projections dispersed to all the prefrontal cortex areas, may include specificity, as they are part of a parallel organization of functional loops between specific midbrain, striatum and PFC areas.

However,as mentioned before, different regions of the prefrontal cortex exhibit discrete sets of reciprocal connections, making ostensible, a different topological pattern of connectivity, for orbitomedial and lateral areas. Connections of functional regions, give us an insight of their role in behavior.

Ventral PFC receives afferents from the brainstem and the hypothalamus, that integrate information about the awareness level, arousal status, internal environment. Specifically, the ventromedial PFC, receives inputs from the DLPFC and core regions of dicrete neural processes, such as: amygdala for emotional processing, hippocampus for long-term memory consolidation and evocation, and temporal association cortex for coplex visual processing. The orbital and medial prefrontal cortex with their connections with the aforementioned structures and structures of the limbic system e.g ventral striatum, form a complex interconnected system, composed of phylogenetically old, early developing structures, considered the anatomical substrate for emotional, instinctive, and affect-modulated behavior and attentional control. Superomedial prefrontal region, an additional subdivision, if lessioned, results in an apathetic phenotype, characterized by decreased initiative (Stuss & Levine 2002).

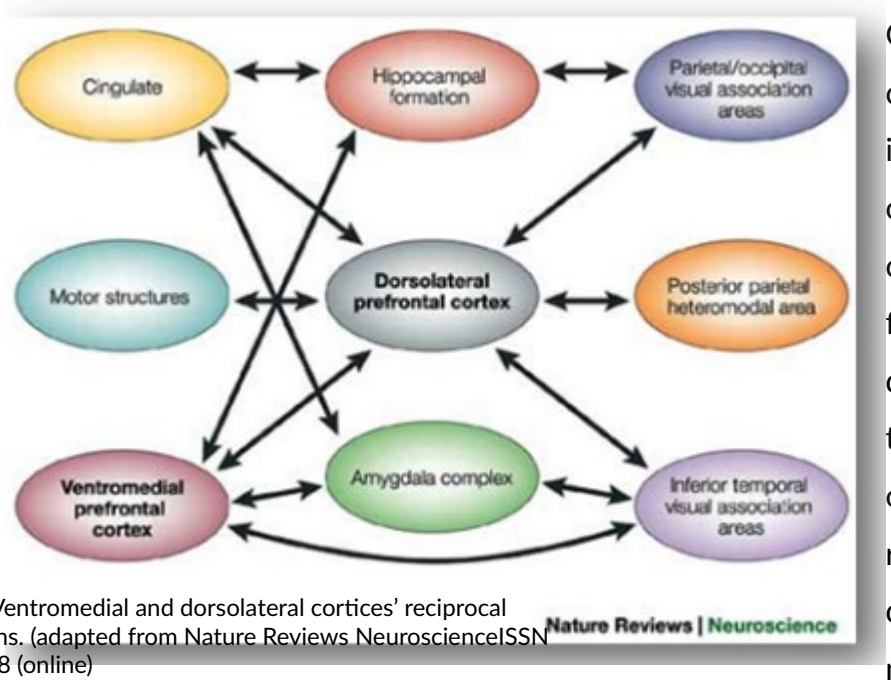


Figure5 . Ventromedial and dorsolateral cortices' reciprocal connections. (adapted from Nature Reviews NeuroscienceISSN 1471-0048 (online)

On the other hand, lateral prefrontal cortex, is a newer system of interconnected structures and is considered the substrate of goal conceptualization, cognitive/behavior flexibility and action-planning. The dorsolateral PFC , being on the top of the hierarchy of interconnected areas of motor function, it maintains reciprocal connections with the downstream brain regions involved in motor control (supplementary motor area (SMA), and premotor cortex), action-monitoring (cingulum bundle) and complex processing of sensory stimuli (temporal and parietal association cortices). It is also connected with the basal ganglia by reentrant connective loops that convey through the lateral thalamus and the cerebellum, constituting the upper stage of the perception–action cycle (Barbas 2000). Furthermore, dlPFC, is considered, functionally specialized in processing visuospatial information in working memory, encoding mental representations of coordinates within the spatial domain(Goldman-Rakic, 1995). In accordance with that, deficits in function of dlPFC correlating with deficits in performance of working memory tasks, is one of the most consistent findings, in studies of schizophrenic patients(Barch et al. 2003; Park & Holzman 1992; Collins et al. 2014) .

Each of the broader divisions of PFC; orbital,medial and lateral, is interconnected with the other two and with itself (Jacobson & Trojanowski 1977). Prefrontal limbic cortices, namely, orbital and medial, send widespread efferent projections, originating from their deep layers and terminating in the upper layers of lateral cortex, suggesting a feedback-type of communication. On the other hand, lateral prefrontal cortex, is installating a feedforward communication pattern with limbic areas, issuing efferent fibers from its upper layers, to the deep layers of orbital and medial cortex (Barbas 2000) .

Although, an overlapp, surely, exists, functional segregation is observable in PFC, an area considered to operate as the "central executive" of the brain, exerting supervisory control over all the other mental resources, integrating information from them and driving "decision making" (Goldman-Rakic, 1995). This constitutes an important feature of the dynamics of large-scale networks; a balance between opposing tendencies toward functional segregation and integration. A

hypothesis, is that different processing streams, with an area of PFC being at the summit of each of them, are finally producing an integrated high-order function, when a combination of these core regions become task-engaged, across many different types of cognitive tasks. This process might be mediated through coherent phase-synchronized activity between neuronal populations interconnecting these areas, facilitating their mutual communication and modulating their interaction strength (Fries, 2015).

1.3.2 Layers

On the continuum of the region-specific differences; lateral prefrontal cortex (lPFC), is called " isocortex " because it is a typical 6-layered neocortex with structural uniformity, or "frontal granular cortex", in grounds of its characteristic morphological feature; a well-marked internal granular layer (layer IV) whereas, cortex of medial and orbital prefrontal regions, is called " agranular, " due to a restricted or nonexistent granular layer and eminent deeper layers (V and VI). Beside the region-specific differences, laminar and columnar organization is observed across all regions of the prefrontal cortex. Layer borders are defined based on cytoarchitectural features, such as cell density and cell soma size.

A layer-specific organization is observable, as each layer may be characterized by a distinct neuronal type or a specific connection with other cortical or subcortical regions. Their functional and structural specificity may be a reflection of the temporal specificity of their development; deeper layers IV-VI, develop earlier and at a faster pace than the more superficial ones, II/III. Furthermore, there is evidence that the refinement and differentiation of pyramidal neurons in layer III continues until puberty (Fuster 2008;Mrzljak et al. 1990).

Layer I, the molecular layer, is particularly wide in the prefrontal cortex compared with other cortical regions and almost devoid of cell bodies, except from a few interneuron types, dispersed between dendrites and afferent axon collaterals of deeper layers.

Layer II, is called external granular layer, due to its granular appearance. It contains, mainly small, densely-packed pyramidal neurons, but also, most of interneurons are located in layer II and superficial layer III (Arteaga et al. 2015) . **Layer III**, is called the external pyramidal cell layer and the neurons located deeper in this layer, are typically larger than those located more superficially. Cells in **layers II/III**, resemble regarding the origin of their afferents; their basal and proximal apical dendrites receive inputs from layer IV cells and also local-circuit excitation, while their distal apical dendrites receive inputs from other cortical areas and nonspecific thalamic inputs (Spruston 2008). They, also have in common, the target of their efferent projections; their axons project locally to other neurons within the same cortical area, as well as, to other cortical areas, therefore, considered to mediate intracortical communication.

Layer IV , as above-mentioned is refined in lateral PFC but restricted or absent in medial and orbital cortex. It is termed the internal granular cell layer, and it contains many, small spherical neurons. It has a great significance, as it is the main recipient of input from the thalamus and it is less clear how cortical circuits of agranular cortices, are organized without its presence.

Layer V, the internal pyramidal cell layer, contains mainly pyramidal-shaped cells, larger than those in superficial layers. Pyramidal neurons in this layer, constitute the main output pathway of the cortex, projecting to other cortical areas and to subcortical structures.

Layer VI is heterogeneous, regarding the morphology of the cells it includes and in grounds of that, is named polymorphic or multiform layer. It folds in the white matter and feedbacks to the thalamus, influencing its output to neocortex. The main dendrites of this layer's neurons, often are oblique, horizontally orientated or inverted, in contrast with, layer V pyramidal neurons, whose apical dendrites, all are ascending, pointing towards the pia (van Aerde & Feldmeyer 2015).

Cortical circuit serial processing, based on its organization, has been under extensive study, mainly in sensory cortices, such as the visual and somatosensory cortices. The suggested processing model of cortical circuit, is described in this way; the main thalamic input is send to layer IV, from which is forwarded to supragranular layers II/III, where, it is integrated with input from other cortical areas (Feldmeyer et al. 2002). Neurons of layer II/III projrct to the layer V pyramidal neurons, which mainly drive subcortical structures. They also, innervate layer VI pyramidal neurons, which projecting to the thalamus, modulate the synaptic input that neocortex receives.

Even if, this processing model does not fit exactly, in how neurons in PFC layers communicate, we can safely infer that cortical processing, across all the cortex, depends on the interaction between neurons in different cortical layers and areas.

In different cortical layers, but even in the same layer, there are distinct pyramidal subtypes based either on their morphology or electrophysiological characteristics, such as, their firing pattern. Between layers of the cortex, one considerable morphological difference of pyramidal neurons, is that layer V pyramidal neurons have longer apical dendrites than layer II/III pyramidal neurons (**Figure 6**) (Spruston 2008) ,while a difference in their firing pattern, is documented in still unpublished electrophysiological data of my lab, with pyramidal neurons of layer II, exhibiting a principally adaptive firing pattern, in contrast with the non adapting firing pattern of layer V neurons.

In the same layer, many studies report a link between distinct neuronal subtypes and their targeted projection area. In the study of Morishima & Kawaguchi, corticostriatal pyramidal neurons of layer V , in rat frontal cortex, form subpopulations with distinct synaptic connectivity and morphology based on their subcortical targets. The two projection cell types (corticopontine (CPn) cells and crossed corticostriatal (CCS) cells) exhibit different morhology of their apical tufts and the researchers reported directional and domain-dependent preferences in their synaptic connectivity (Morishima & Kawaguchi 2006). Both distinct morphology and intrinsic excitability , is reported in two cell types with different subcortical targets (corticothalamic and and corticostriatal neurons) in layer V of mouse cortex. These subtypes also exhibit a unique set of hyperpolarizing and depolarizing afterpotentials (Hattox & Nelson 2007). Finally, layer V pyramidal cells in rat frontal cortex, projecting to distinct subcortical areas were assosiated with three subtypes of pyramidal neurons, classified with regard to their firing patterns (Otsuka & Kawaguchi 2008). Having evidence for the existence of between layer and intra-layer, distinct pyramidal subpopulations and an association of their existence with differences in subcortical projection areas, the question is if these different pyramidal neuron subtypes, based either on their morphology or firing pattern, can form distinct sub-networks. Studies revealing differences in pyramidal cell types and their intralaminar connections (Morishima & Kawaguchi 2006;Dembrow et al. 2010;Wang et al. 2006) suggest that they can form subnetworks, depending on pyramidal cell type. In addition, both in vivo and ex vivo studies, argue that subpopulations of prefrontal pyramidal neurons, can be segregated into distinct subcircuits based on their long-range projection targets and that these subpopulations, are influenced by neuromodulators such as acetylcholine, noradrenaline, serotonin, and adenosine, in a discrete way (Dembrow et al. 2010;Dembrow & Johnston 2014).

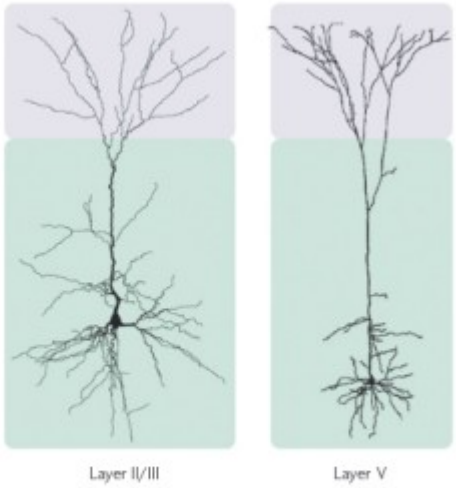


Figure6 The structures of pyramidal neurons from different cortical areas (adapted from Spruston 2008)

1.3.3 Neurons

Pyramidal neurons and interneurons are the two generic types of neurons in the PFC. Pyramidal neurons comprise the 70-80% of cortical neurons and they constitute the excitatory units of the cortex, releasing glutamate as a neurotransmitter. On the other hand, interneurons comprise the 20-25% of neuronal cells of the cortex, constituting its inhibitory units and they release GABA, as a neurotransmitter. In this section, there is an overview of the basic subtypes of these cells and theirs characteristics, concerning the morphology and intrinsic excitability.

Pyramidal neurons

Pyramidal neurons, are concidered a principal computational element of the cerebral circuit. A pyramidal cell, consists of the following compartments; a soma (cell body), a single axon and two dendritic trees; the basal dendritic tree and a large apical dendritic tree. These compartments exhibit characteristic morphology. Their soma has a triangular, pyramidal shape, after which they take their name. The basal and apical dendrites, origin from the base and the apex of the soma, respectively. The basal dendritic tree is composed of multiple, relatively short dendrites that extend radially from the soma, while the apical tree consists of a single apical trunk, which branches along its length at various angles. At its top, the several, thinner dendritic sections form the apical tuft. The morphology of the apical side branches and the apical tuft, presents a great variability among different pyramidal neurons of different layer and cortical regions(Spruston 2008). Dendrites are characterized by the existence of a great number of dendritic spines, which receive most of the

excitatory inputs (EPSPs) of the cell, through the AMPA and NMDA receptors. They also, receive inhibitory signals through activation of GABAa and GABAb receptors, but most of the inhibitory inputs are received at the cell's soma and axon. Dendritic spines are increasing the receptive surface area of the neuron and together its ability to process and integrate a large amount of information.

Beside the morphological characteristics, the electrophysiological features of pyramidal neurons vary. Differences in intrinsic excitability and other active properties of pyramidal neurons, results in distinct firing responses to the same input signals (depolarizing current pulses). In respect to these distinct firing patterns, pyramidal neurons are classified in 2 main types: Intrinsic Bursting (IB) and Regular Spiking (RS) neurons (Connors & Gutnick 1990).

Intrinsic bursting (IB) neurons, are characterized by an initial burst of action potentials (APs) followed by single spikes, occuring at a steady rate. Within a burst, AP's amlitude is decreasing, for each successive spike.

On the other hand, RS pyramidal neurons, can be further categorized as **Adapting (Ad)**, whose interspike interval in response to a constant current step increases during the stimulation and **non-Adapting (nAd)**, whose firing rate is steady during the whole stimulation. One of the possible explanations for the observed firing patterns, is the dinstict set of intrinsically generated afterhyperpolarizations (AHPs) and afterdepolarizations (ADPs), following the initiation of a spike. In its turn, this different set of AHPs and ADPs, comes as a result of the heterogeneity in morphology and active electrical properties of pyramidal neurons, formed by a distinct set of ionic channels in the neuron's membrane. The exact differences between these subclasses of pyramidal neurons, have not yet been elucidated. Parameters as, the field span of apical and basal dendrites, total dendritic length, number of branches, soma size, combination set of a channels, each channel's expression level , kinetics and distribution on the membrane, may differ. Furthermore, the functional role of this differentiation of pyramidal neurons, also remains unclear. Beside the cause of the emergennce of these differentiated pyramidal neuron subtypes, it is really interesting to investigate the functional effect of its existence in a network, since there is evidence that they, actually can form distinct sub-networks, that project to different subcortical areas, such as the pons or the striatum, suggesting that they might serve distinctive functional role.

Spike frequency Adaptation

Spike frequency adaptation constitutes a phenomenon that is considered to be mediated by a variety of biophysical mechanisms . These mechanisms can be clustered in two major categories; events that lead to inactivation or delay of depolarizing currents and activity- dependent mechanisms that activate slow hyperpolarizing or shunting currents (Benda and Herz 2003).

In the first category, depolarizing currents are inactivated, mainly, due to an increase of the refractory period of the membrane. After initiation of an action potential, Na+ channels, which mediate the depolarization of the cell membrane, render to an inactivated state, during which a second stimulus is incabable (absolute refractory period) to initiate an action potential, or needs to be greater than the first one in order to reach the initiation threshold (relative refractory period). The process of Na+ channels regaining their ability to open in response to stimulus, depends to their activation/inactivation kinetics and to the time needed for the membrane to repolarize and retun to its resting state voltage value. A decrease in K+ channels' conductance, which are responsible for the repolarization of the membrane, can cause a delay in the de-inactivation of Na+ channels, which in turn, leads to an increase of the interspike interval of the cell.

The second category consists of currents which hyperpolarize the membrane, either voltage-dependent ones, such as, the non-inactivating potassium current, and the slow delayed rectifier potassium current, or spike-dependent after-hyperpolarization currents; slow and fast Ca2+ /voltage activated currents.

Interneurons

Cortical interneurons compose the 20–25% of neocortical neurons, they are mostly inhibitory and they have diverse morphological, physiological, molecular and synaptic features (Markram et al. 2004). They can ,easily, be discerned from the pyramidal cells, as they exhibit distinctive attributes: First, interneurons form GABAergic, Grays's type II, symmetrical, axo-somatic, axo-dendritic and axo-axonal synapses with pyramidal neurons or other types of interneurons, while pyramidal neurons form glutamatergic, primarily axodendritic synapses, which are classified as Gray's I assymetrical synapses. . Second, interneurons are identified as 'local circuit neurons'; their axonal projections do not typically project in distant brain areas, they rather arborize ,where their cell bodies and dendrites are located, within a cortical column and can project laterally across columns

(Letinic et al. 2002). Third, the absense of dendritic spines; the majority of mature interneurons have aspiny dendrites (Markram et al. 2004). Finally, all these discrete features underline their distinct functional role inside a brain network; pyramidal neurons and their networks support information processing and constitute the major sources of output from any brain region, whereas interneurons, are considered to govern ensemble activity, enhancing cortical circuits' performance (Sohal et al. 2009).

As mentioned above, interneurons show outstanding variability in their features; in grounds of the great diversity of electrophysiological, molecular and biochemical e.g receptor expression pattern or their co-transmitter content and morphological characteristics, their classification in distinct groups has been challenging. There are many types of classification, upon different kind of features and I will make a report of the most known and used;

i) Classification in terms of the target-compartment of inhibition, is the most generic one, as it branches interneurons in those who target the dendritic cell compartment and those who primarily affect the perisomatic and axonic region of neurons. The reasoning of this classification, is that inhibition in different cell compartments is functionally distinct; dendritic tree is the site of termination of most excitatory afferents of pyramidal neurons and the site, where short- or long-term plastic changes take place. Thus, dendritic inhibitory interneurons are designed to influence the effect of excitatory inputs and in this way, controlling the emergence of plasticity. On the other hand, the perisomatic domain has an integrative role, being the place of summation of postsynaptic potentials arriving from all dendritic branches, a process that determines the final outcome. Perisomatic inhibitory interneurons, innervating somata, proximal dendrites or axon initial segments, control the output generation from large pyramidal neuronal populations and they seem to be responsible for rhythmically synchronizing pyramidal cell activity at gamma (30-100 Hz) frequencies (Sohal et al. 2009).

ii) Structure-based classification: morphologically, interneurons can differ in parameters, such as the number or laminar distribution of dendrites and the arborization pattern of axon. In grounds of morphological diversity, the following subtypes have been pointed out; basket cells, chandelier cells (Ch), Martinotti cells (M), bipolar cells, double bouquet cells (DB), and bitufted cells.

Basket cells are the most abundand, comprising the 50% of all inhibitory interneurons and specifically, target the somata and proximal dendrites of pyramidal neurons and interneurons. They have a basket-like appearance and regarding their calcium-binding proteins (CaBPs), they typically express parvalbumin (PV) or calbindin (CB).

Chandelier cells have chandelier- like appearance, they form axon-axonic synapses and have the same expression pattern of CaBPs, with the one reported in basket cells.

Martinotti cells ,are typically located in layer II-VI but project their axons towards layer I, inhibiting the tuft dendrites of pyramidal neurons, providing a source of cross-columnar inhibition. They always express somatostatin (SOM), but never found to express parvalbumin or VIP (vasoactive intestinal polypeptide).

Bipolar cells are small cells with narrow bipolar or bitufted dendrites. They form synapses only with a restricted number of cells, projecting mainly on the basal dendrites of pyramidal neurons. They typically express calretinin (CR) and VIP and they have an inside group variability regarding their neurotransmitter, using GABA or VIP. When releasing only VIP, they have an excitatory effect (Markram et al. 2004).

Double bouquet cells present a bitufted dendritic morphology and they are dendritic-targeting cells. Their CaBPs expression pattern is unique, as they typically express CB, have the ability to express CR and CB together and can also express VIP or cholecystokinine (CCK), but not PV, SOM or NPY (neuropeptide Y) (Cauli et al. 1997;Markram et al. 2004))

Bitufted cells are similar to bipolar and to double bouquet cells. They are targeting the dendritic compartment and they can express CB, CR, NPY, VIP, SOM, CCK, but not PV (Markram et al. 2004)

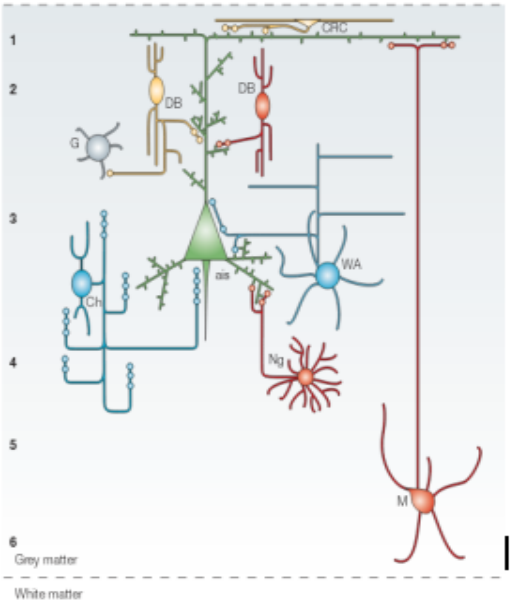


Figure 2-7 Morphological and biochemical features of subpopulations of cortical GABA neurons in the dorsolateral prefrontal cortex.
The diagram illustrates the calcium-binding proteins — parvalbumin (blue), calbindin (red) and calretinin (yellow) — and the locations of inhibitory synaptic inputs to a pyramidal neuron (green) by different morphological classes of cortical GABA (γ-aminobutyric acid) neurons. The chandelier (Ch) and wide arbor (WA) or basket neurons provide inhibitory input to the axon initial segment (ais) and the cell body proximal dendrites, respectively, of pyramidal neurons. By contrast, the calbindin-expressing double bouquet (red DB), neurogliaform (Ng) and Martinotti (M) neurons tend to provide inhibitory inputs to the distal dendrites of pyramidal neurons. Finally, calretinin-expressing (yellow) DB and Cajal–Retzius cell (CRC) appear to target both pyramidal cell distal dendrites and other GABA (G) neurons. 1–6, layers of dorsolateral prefrontal cortex. Figure and text figure, adapted from (Lewis et al. 2005)

iii) Classification in terms of diverse electrophysiological features, such as firing pattern and the ability for short- and long-term synaptic plasticity, has generated the succeeding categories of interneurons;

Fast spiking interneurons (FS) , which fire single APs with fast, steady rate and undergo little or no adaptation during prolonged current stimulation, sustaining a firing rate in 500 - 600 Hz for hundreds of milliseconds (González-Burgos et al. 2005);

Late spiking interneurons (LS), which discharge with a considerable delay after a depolarizing step, mainly found in layers II/III and V.

Low threshold spiking cells (LTS), also called, “burst - spiking non pyramidal cells” (BSNP), due to their burst-like response to low intensity-hyperpolarizing pulses of low intensity. They are primarily, located in layer V.

Regular spiking non-pyramidal cells (RSNP), are located in layers II/III & V and are characterized by long lasting action potentials and plasticity of their spike trains’ rate.

Irregular spiking cells (IR) , are also found in layers II/III & V , fire an initial burst of APs followed by irregularly spaced action potentials (Markram et al. 2004)

iii) a widely accepted classification is the one based on interneurons’ specific pattern of expression, especially in terms of calcium binding proteins (CaBPs) ; parvalbumin, somatostatin and calretinin. , which play a role in intracellular signaling by affecting the cytosplasmic calcium level.

Parvalbumin interneurons (PV) exhibit an FS firing pattern, perisomatic-specific targeting and comprise the major cortical inhibitory input.

Calbindin interneurons (CB) have an RS firing pattern, similar to that of the majority of pyramidal neurons and project to distal dendrites of pyramidal neurons.

Calretinin interneurons (CR) fire irregularly spaced, action potentials and they project in distal pyramidal dendrites or even on other interneurons, mediating disinhibition.

An equally important biochemical marker is the protein somatostatin (SST), an inhibitory hormone-peptide regulating endocrine pathways and affecting neural transmission and cell proliferation. Somatostatin, as well as, three other neuropeptides (vasoactive intestinal peptide - VIP, cholecystokinin - CCK and neuropeptide Y - NPY) may also be used for generation of another branching classification scheme (Kawaguchi & Kubota 1997;Cauli et al. 1997).

Despite the extended effort to classify and group subtypes of interneurons according to characteristics of different quality, the quest remains; whether this outstanding diversity, as a whole, represents a need for extreme functional specialization, or there is a distinction between features defining the main functional role of interneurons and others, evolved as an adaptive property, just enhancing the cell's efficacy in different conditions.

1.3.4 Working memory

Working memory is a system of short-term storage, which provide an active retention of information, in order for computational mechanisms to monitor and manipulate it. It constitutes the interface between perception, long-term memory and goal-directed behavior, as it allows processing of external and internal representations and renders possible, the integration of spatially and temporaly, remote information (Baddeley 1998) .

In its simplest form, working memory is the ability to maintain information in mind without the presence of an external stimulus and because of its temporary nature, it has been described as “the blackboard of the brain” or its “RAM memory”. In this way, information, not needed for long-term use, is replaced quickly, as it is not permanently imprinted in neural networks (Goldman-Rakic 1995). It is also, referred to as "representation knowledge" and is considered one of the most flexible innate mechanisms of the brain , constituting a remarkable evolutionary achievement. As mentionned before, the contribution of working memory in a wide range of cognitive mechanisms, such as learning, reasoning, language processing ,perception, decision-making, behavioral inhibition, planning of actions and creativity, is essential for their proper function.

Long-standing evidence, posit that prefrontal cortex (PFC) constitutes the main neural substrate of working memory (Fuster 2008; Goldman-Rakic 1987). The first supporting evidence came from both, lesion studies in non-human primates, showing WM deficits, in grounds of unilateral dlPFC lesions (Butters & Pandya 1969) and functional neuroimaging studies in humans, demonstrating dlPFC activity deuring WM tests.

In animals, working memory function is tested using the delayed-response task, which involves the presentation of a stimulus, followed by a brief delay, after which a task-engaged response to the stimulus is made. In order for a proper, delayed response, the animals submitted to the task, have to hold ‘on-line’ the sensory information presented to them (fig.) .

Studies of non-human primates, performing delayed- response tasks, strengthened the hypothesis of the crucial role of dlPFC in working memory. Delay-dependent impairments, whereby forgetting increases with the length of the delay, were observed after, experimental lesions of the principal sulcus in the dlPFC (Miller & Orbach 1972) . In addition, electrophysiological recordings from dlPFC, often demonstrate persistent, sustained levels of neuronal firing, during the delay period of the task (Funahashi et al. 1989; Kubota & Niki 1971). The presented data have established a strong argument of dlPFC being the main anatomic substrate of working memory (Barbey et al. 2013) and without

opposing to this statement, other functional and anatomical studies delineate a more extended basic neural circuitry (Goldman-Rakic 1987), mediating delayed-response function in adult nonhuman primates.

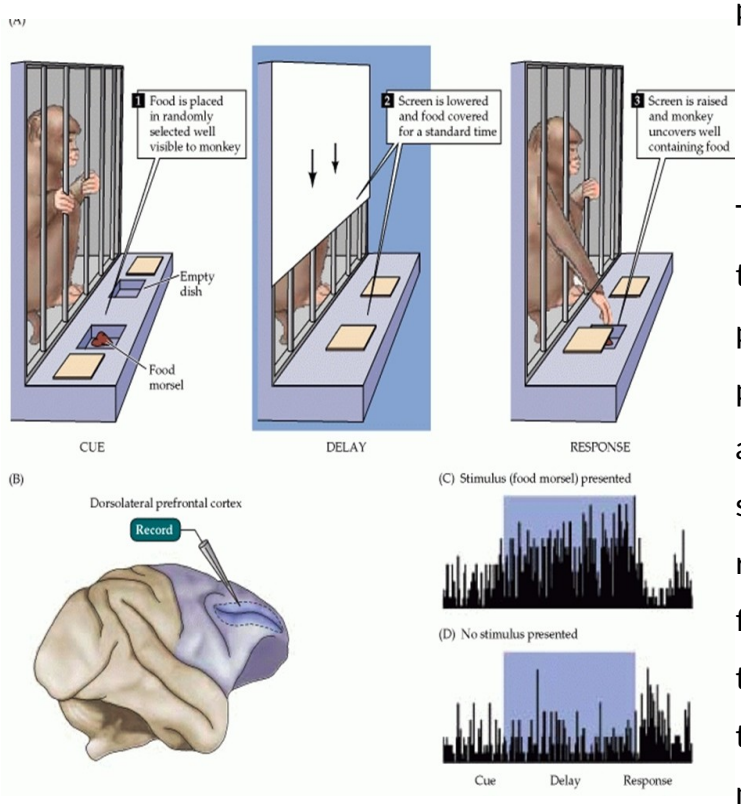


Figure 8 . Schematic representation of delayed response task (adapted from

Persistent Activity

The sustained firing activity, recorded in these tasks, is termed “persistent activity”, because it persists seconds beyond the end of the stimulus presentation and is thought to mediate the active retention of information (e.g location of a stimulus cue), which is necessary for a proper response. Persistent activity, the delay-related firing of pyramidal PFC neurons, is considered the cellular correlate of working memory, but the biophysical mechanisms of its induction and maintenance, have not yet been fully clarified.

The work of many researchers is devoted in identifying these mechanisms and two main directions, have come on the scene; investigation of the contribution of network connectivity and intrinsic cellular excitability. In the first direction, researchers assume that persistent activity is drived by reverberating synaptic excitation in the PFC cortical circuit, based on recurrent interconnections of pyramidal neurons. A self-sustained network activity, emerges via positive feedback loop between the spike firing of a neuronal population and the recurrent synaptic drive. In this context, the induction and stabilization of a network persistent state is influenced by the efficacy of recurrent synapses, balance between excitatory and inhibitory recurrent network synapses (Konstantoudaki et al. 2014) and biophysical properties of cortical synaptic transmission, such as, the synaptic decay time constant of NMDA current, determined by the kinetic of NMDA receptors (Wang 1999). Both, imbalance of excitation/inhibition ratio, that may be caused by altered GABA neurotransmission (Lewis et al. 1999) and NMDA hypofunction have been implicated in prefrontal cortical dysfunction in schizophrenia.

The second approach, does not doubt the recurrent nature of the mechanism inolved in generation and maintance of persistent activity(PA), but is looking for it, at a single-neuron level. Computational studies explore the probability of induction of PA , as an emergent consequence of a single neuron dynamics , examining intrinsic cellular excitability, a product of its individual channels' conductances. A loop of positive feedback between firing activity and cation influx through the membrane , may be dependent of Ca^{2+}-activated non-selective cation current (CAN) and results in a delayed afterdepolarization (dADP). It is suggested that a burst-evoked intrinsic depolarization may constitute a form of short-term cellular memory(Sidiropoulou et al. 2009;Sidiropoulou & Poirazi 2012).

Furthermore, persistent activity seems to be stimulus selective. For example, in spatial working memory tasks, specific pyramidal neuronal populations, exhibit persistent activity in response to as stimulus presented in specific locations of the visual field, configuring its "memory field". Cellular mechanisms enabling stimulus selectivity in PFC have not been determined. In correspondance with, the formation of orientation columns in visual cortex, the mechanisms assumed to play a role, are interactions between pyramidal cells and interneurons and proper inhibitory transmission, along with predictive features of firing activity during the presentation of the preffered stimulus, such as latency to the first action potential or the sequence of inter-spike intervals, which downstream neurons/neuronal circuits, can decode(Sidiropoulou & Poirazi 2012).

Finally, an other issue of debate, concerning persistent activity is whether or not, its emergence is incident of only large scale networks. Structure–function studies of neuronal networks, exploiting the advances in molecular genetics, in vivo imaging and recordings, argue that brain function is encoded in the firing of neuronal assemblies with distinct temporal dynamics (Feldt et al. 2011). Experimental morphological and functional evidence argue that small, tightly interconnected clusters of neurons in the cortex may support similar functionalities. Indicatively, in PFC, there is an old notion of microclumnar functional dissection, that seems to mediate persistent activity by isodirectional tuning of adjacent interneurons and pyramidal neurons (Rao et al. 1999). In addition, a structural description of layers I and II in several cortical areas, including PFC, sets out the existence of small modules interconnected with excitatoty recurrent projections (Arteaga et al. 2015). Observed subcircuits of distinct, regarding their projection area, subtypes of neurons exhibit a distinct response to neuromodulators (Dembrow et al. 2010), an evidence that infers specific regulatory effects of neuromodulatory systems, such as the dopamine system, to a specific functionality served by a distinct PFC subcircuit. Computational studies, investigating network properties, have incorporated simulations of physiological intrinsic and synaptic currents and

connectivity properties of neurons, in a more elaborate way through the years, making it more plausible, to understand microcircuits' functional potential. A biologically realistic layer V-specific PFC microcircuit with morphologically simplified neurons, embodyied with highly reciprocal connections and facilitating synapses, responds to stimulation, with beyond its end, firing activity, indicating that PA can emerge in small-sized clusters of cells (Papoutsi et al. 2013).

2. AIM

This study, has two main components. First, there was a review of evidence, suggesting that distinct, regarding their projection targets, morphology and firing pattern, PFC pyramidal subpopulations, exhibit distinct interconnectivity, integrative properties and responses to neuromodulation (Morishima & Kawaguchi 2006;Dembrow et al. 2010;Wang et al. 2006). These evidence, give rise to the hypothesis of these subpopulations, forming subcircuits with distinct functionality, and contributing in different ways to the emergence of a fundamental PFC function; working memory. The first step towards testing this hypothesis and the first goal of this study, is to investigate whether subcircuits, consisted of distinct pyramidal neuron subtypes, exhibit different features in their stimulus-induced persistent activity, which constitutes the cellular correlate of working memory. Therefore, the first instance of this study, explores mechanisms or attributes of a physiological PFC function.

In contrast, the second goal of this study, was introduced, with a perspective on the pathophysiology of schizophrenia. Consistent evidence of deranged PFC-mediated functions, such as working memory in schizophrenic patients (Callicott et al. 2003;Driesen et al. 2008; Perlstein et al. 2003;Giraldo-Chica et al. 2018;Barch et al. 2003; Park & Holzman 1992), in addition with evidence that associate SCZ-related SNPs in CACNA1C gene, with deficits in working memory performance(Bigos et al., 2010;Zhang et al., 2012;Cosgrove et al. 2017), leads us to investigate the functional effects of SCZ-related SNPs in CACNA1C gene, regarding the pesrsistent activity induced in firing pattern-specific neuronal circuits of the prefrontal cortex. Inferences about a differential impact of the effects of SCZ-associated SNPs, to the firing-specific neuronal subcircuits, are made in the dicussion's section.

3. MATERIALS and METHODS

Four different biophysically detailed multicompartmental model cells were used as foundation. These model neurons; a layer V PFC (non Adapting) pyramidal neuron and three different interneurons, an FS model, an RS model and an IS model, are adapted from (Konstantoudaki et al. 2014) and based on known electrophysiological data. A network, which consists of 16 pyramidal models and 4 interneuron models (2 FS models, 1RS and 1 IS model)(Konstantoudaki et al. 2014), with connectivity properties between neuron models, extracted from experimental anatomical and electrophysiological data, constitutes a layer V PFC microcircuit.

In this work, modification of the layer V PFC pyramidal neuron model's parameters, generated a different, adaptive firing pattern and this alternative PFC (Adapting) neuron model was embedded, seperately in a microcircuit with the same connectivity characteristics, as described above. Based on our testing hypothesis, the same alterations (described below) in one model parameter, in each of these two firing-specific PFC neuron models gave rise in two different versions of each model. Each of this version, was embedded seperately in a microcircuit. Conclusively, we used six PFC neuron models, embedded in distinct microcircuits. All models are implemented in the Neuron simulation environment (Hines and Carnevale, 2001) and simulations were executed on a xeon cluster (8 core xeon processors).

3.1 Description of the different neuronal models

Layer V PFC (nAd) pyramidal neuron model

The layer V PFC pyramidal neuron model used, was adapted from (Konstantoudaki et al. 2014) and consists of five compartments; a soma, an axon, a basal, a proximal and a distal dendritic compartment. It includes modeling equations for 14 types of ionic mechanisms, with compartment-specific distribution , as well as modeling equations for the regulation of intracellular calcium (same equations as in Konstantoudaki et al. 2014). The dimensions of the somatic, axonic, and dendritic compartments of the pyramidal model cell, are presented in comparison with those of the Adapting pyramidal neuron in **Table 1**. The passive and active parameters and properties of the pyramidal neuron model were validated according to the experimental results of (Nasif et al. 2004) (**Table 2** and **Figure 9**). This pyramidal neuron model exhibits a non adaptive firing pattern.

Adapting (Ad) PFC pyramidal neuron model

It is known that regular spiking (RS) pyramidal neurons, can be further categorized as **Adapting (Ad)**, and **non-Adapting (nAd),** regarding their firing pattern (Chang & Luebke 2007). In grounds of limited data about the mechanisms involved in the emergence of this firing pattern, we chose to implement a strictly phenomenogical model of an adapting PFC pyramidal neuron.

Based on evidence, reported in introduction, that pyramidal neurons of layer II, exhibit a principally adaptive firing pattern, we made the assumption that distinct morphological features of the pyramidal neurons in this layer may play some role in their emergent firing pattern. In addition, spike frequency adaptation is a phenomenon, mainly attributed to activity- dependent mechanisms that activate slow hyperpolarizing or shunting currents (Jan Benda and Herz 2003). In order to simulate an adapting, PFC pyramidal neuron of layer II, we had to implement both the adaptive firing frequency pattern and to depict the basic characteristic of layer II pyramidal cells, the shortened length of their apical dendrites (Spruston 2008), which is considered to contribute to their emergent firing activity. First, we reduced to the half, the above mentioned structural parameter.

The adaptive firing pattern emerged through a combination of variations in parameter values of the non adapting pyramidal neuron; in the passive conductance value, in the delayed rectifier K+ , sAHP, both kinds of L-type calcium currents and in all the active ionic mechanisms of the apical dendrites .The passive conductance (G_pas (S/cm2)) value is increased to 120% of its original value in (Konstantoudaki et al. 2014) and is 1.85 fold greater than its value in the non-Adapting cell in layer V , in all compartments of the layer V pyramidal neuron.The aforementioned currents were changed by modulating the conductance value of their channels; for Kdr (delayed rectifier K+) the conductance value is reduced by 10%, for sAHP is doubled , for Cav 1.3-L type, is 3 fold greater than the value of the non adapting cell and for Cav 1.2-L type is increased to 330% of its original value. These modulations took place in all compartments. Finally, a reduction by 90% in the conductance value of all channels in both apical dendritic compartments accompanies their length's reduction . These variations generated an adaptive firing pattern and thus, gave rise to a strictly phenomenogical model of a layer II Adapting PFC pyramidal neuron. The structural parameters and active and passive ionic properties of this neuron model are presented in comparison with those of layer V PFC pyramidal neuron model in **Table 1, 3,4** and **Figure 9**.

Table 1. Comparison of morphological parameters of the non-Adapting (nAd) and Adapting (Ad) pyramidal model neurons.

	Length (μm)	Diameter (μm)	Length (μm)	Diameter (μm)
Compartments	**Non Adapting Cell**		**Adapting Cell**	
Soma	86.3	10.14	86.3	10.14
Basal Dendrite	150	1	150	1
Proximal Apical Dendrite	400	2.6	**200**	2.6
Distal Apical Dendrite	400	2.6	**200**	2.6
Axon	113.22	1.1	113.22	1.1

Table 2. Active and passive ionic properties of non-Adapting (nAd) pyramidal neuron model.

Pyramidal Neuron, mechanisms	Soma	Axon	Basal Dendrites	Proximal Apical Dendrite	Distal Apical Dendrite
Active Properties (Ionic Mechanisms)					
Conductances (S/cm^2)					
Sodium conductance	0.108	0.18	0.0108	0.0072	0.0036
Delayed rectifier K^+	5.4e-3	5.4e-3	4.86e-4	2.16e-5	5.4e-6
Persistent Sodium	18e-7	-	18e-6	54e-6	18e-5
sAHP	0.025	-	2.5e-5	0.0025	–
A-type K^+	7e-4	-	7e-4	7e-4	**7e-5**
N-type calcium	2e-5	-	6e-5	6e-5	0.001
T-type calcium	6e-6	-	6e-5	6e-5	6e-6
R-type calcium	3e-8	-	9e-8	9e-8	15e-7
L-type calcium (a1D)	3e-5	-	3e-5	-	–
L-type calcium (a1C)	1e-5	-	1e-5	1e-5	3e-6
D-type K^+	6e-4	-	0.0012	0.0012	0.0012
fAHP	**2.2e-3**	-	2.2e-6	2.2e-5	2.2e-6
H-current	**9e-6**	-	9e-6	9e-6	9e-5
Passive Properties					
Calcium diffusion model	Yes	No	Yes	Yes	Yes
C_M ($\mu F/cm^2$)	1.2	1.2	2.4	2.4	2.4
G_pas (S/cm^2)	**5.85e-5**	**5.53e-5**	**1.11e-4**	**1.11e-4**	**1.11e-4**
R_A (ohm/cm)	150	150	150	150	150
R_M ($\mu F/cm^2$)	11	11	6	6	6

***The passive conductance (G_pas (S/cm^2)) is the only parameter value changed; reduced to 65% of its original value in (Konstantoudaki et al. 2014), in all compartments of the layer V pyramidal neuron.** This variation is done in order for the model to be more biophysically relevant with an adult layer V pyramidal cell.

Table 3. Active and passive ionic properties of Adapting (Ad) pyramidal neuron model.

	Pyramidal Neuron, mechanisms	Soma	Axon	Basal Dendrites	Proximal Apical Dendrite	Distal Apica Dendrite
	Active Properties (Ionic Mechanisms)					
	Conductances (S/cm^2)					
	Sodium conductance	0.108	0.18	0.0108	0.0072	0.0036
	Delayed rectifier K^+	**4.9e-3**	**4.9e-3**	**4.37e-4**	**1.94e-6**	**1.94e-6**
a	Persistent Sodium	18e-7	-	18e-6	54e-6	18e-5
b	sAHP	**0.05**	-	**5e-5**	**5e-4**	–
	A-type K^+	7e-4	-	7e-4	7e-4	7e-5
	N-type calcium	2e-5	-	6e-5	6e-5	0.001
	T-type calcium	6e-6	-	6e-5	6e-5	6e-6
	R-type calcium	3e-8	-	9e-8	9e-8	15e-7
c	L-type calcium (a1D)	**0.0001**	-	**0.0001**	-	–
d	L-type calcium (a1C)	**3e-5**	-	**3e-5**	**3e-6**	**9e-7**
	D-type K^+	6e-4	-	0.0012	0.0012	0.0012
	fAHP	2.2e-3	-	2.2e-6	2.2e-5	2.2e-6
	H-current	9e-6	-	9e-6	9e-6	9e-5
	Passive Properties					
	Calcium diffusion model	Yes	No	Yes	Yes	Yes
	C_M ($\mu F/cm^2$)	1.2	1.2	2.4	2.4	2.4
	G_pas (S/cm^2)	**1.1e-4**	**1e-4**	**2e-4**	**2e-4**	**2e-4**
	R_A (ohm/cm)	150	150	150	150	150
	R_M ($\mu F/cm^2$)	11	11	6	6	6

Variations The adaptive firing pattern emerged through a combination of variations in parameter values of the non adapting pyramidal neuron; in the passive conductance value, in the delayed rectifier K+ , sAHP, both kinds of L-type calcium currents and in all the active ionic mechanisms of the apical dendrites .1. The passive conductance (G_pas (S/cm2)) value is increased to 120% of its original value in (Konstantoudaki et al. 2014) and is 1.85 fold greater than its value in the Non-Adapting cell in layer V the in all compartments of the layer V pyramidal neuron. 2.The forementioned currents were changed by modulating the conductance value of their channels.(a) Kdr (delayed rectifier K+) is reduced by 10%, (b) is doubled , (c) is 3 fold greater than the value of the non adapting cell and (d) is increased to 330% of its original value. These modulations took place in allcompartments. 3. a reduction by 90% in the conductance value of all channels in both apical dendritic compartments accompanies their length's reduction .

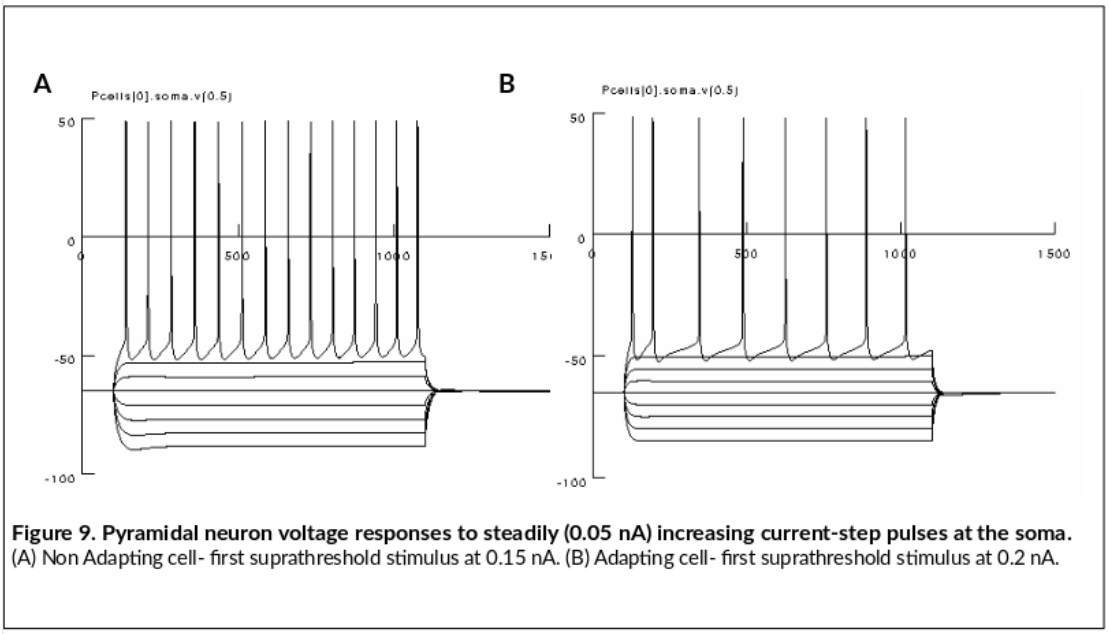


Figure 9. Pyramidal neuron voltage responses to steadily (0.05 nA) increasing current-step pulses at the soma. (A) Non Adapting cell- first suprathreshold stimulus at 0.15 nA. (B) Adapting cell- first suprathreshold stimulus at 0.2 nA.

Table 4. Input resistance values of the model neurons.

	Non Adapting	Adapting
Input Resistance **(MΩ)**	117.300	97.49

CACNA1C variants

In order to study the effects of SCZ-associated polymorphisms in CACNA1C gene, on two microcircuits ,comprised of distinct neuronal subtypes; on a layer V PFC microcircuit and on a layer II PFC microcircuit (a microcircuit, whose pyramidal neurons exhibit an adaptive firing pattern), we first had to link the effect of a genetic variant in the Cav 1.2 channel to a change in the corresponding neuron model parameter.

As mentioned before in introduction, there is contradictory evidence, regarding the effect of intronic SNP variations, associated with SCZ; both, an increase and a decrease in the mRNA expression of Cav 1.2 has been reported. The altered level of expression of an ionic channel can be simulated with a modification of the conductance value of this channel in the neuronal models of our circuits.

In order to study the opposite effects reported, there was both an increase of the Cav 1.2 channel's conductance (the value of the variant was 1.5 fold greater than the control value) and a decrease (the conductance value of the channel was reduced by half). Since, the Cav 1.2 channel, is not one of the included ionic mechanisms in interneurons, these neuron models were not modificated at all, whereas both subtypes of pyramidal neuron models were submitted to a change in their conductance value of Cav 1.2 channel, in both directions. This procedure, gave rise to 4 variant models, named after their variation and pyramidal subtype; CACNA1C_0.5 nAd, CACNA1C_1.5 nAd and CACNA1C_0.5 Ad, CACNA1C_1.5 Ad.

Validation of synaptic mechanisms

The conductances of excitatory and inhibitory synaptic mechanisms were adjusted according to electrophysiological recordings. The AMPA and NMDA currents were validated with a simulated voltage clamp protocol to replicate the results of Wang et al. (2008) for all the instances of the pyramidal neuron model.

Interneuron models

All three interneuron models; fast spiking (FS), regular spriking (RS) and irregular spiking (IS) subtype, were adapted from (Konstantoudaki et al. 2014), without modification in the included, compartment-specific, ionic mechanisms and modeling equations for the regulation of intracellular calcium buffering mechanism. Each different interneuron model subtype included ionic mechanisms

known to be present in each type (Toledo-Rodriguez et al., 2005). Morphological parameters of the interneuron model subtypes, as well as, their active and passive ionic parameter and properties are presented in **Table A1-4** and **Figure –** in the **Appendix**.

3.2 Description of the microcircuit model

The PFC microcircuit (graphically ilustrated in **Figure 10**) consists of 16 pyramidal neuron models and 4 interneuron models; 2 fast-spiking interneuron models (FS), 1 regular spiking interneuron model (RS) and 1 irregular-spiking interneuron model (IS). Connectivity properties, including the location and number of synapses, which are listed in **Table 5**, the latencies between pairs of neurons, as well as the synaptic conductances, were based on anatomical and electrophysiological data.

Specifically, all neurons are fully connected through recurrent connections, incorporating the high reciprocity of connections reported in (Wang et al., 2006). The axon of each model neuron type, forms synapses in specific compartments of its target model neuron. Pyramidal neuron model projects to the basal dendrite of the other pyramidal neuron models. FS interneuron model projects to the soma of all pyramidal neurons models. The axon of the RS model project to the distal apical dendrite of all pyramidal neuron models. IS interneuron model projects to the distal apical dendrite of all pyramidal neuron models and to the dendrite of the RS nterneuron model, mediating a disinhibition input to the microcircuit. The pyramidal neuron and the FS interneuron models also form autaptic synapses.

A network property of recurrent networks, due to reverberating excitation is the induction of persistent activity (PA); neuronal firing activity that persists after the end of stimulation . Persistent activity in this microcircuit is induced by providing external synaptic simulation to all 16 pyramidal neurons in their proximal apical dendrites.

After embedding each version of the pyramidal neuron model in a microcircuit, given that we have implemented two distinct, control pyramidal neuron subtypes; non Adapting (layer V), Adapting (layer II) and two genetic variants for each of them; namely CACNA1C_0.5 nAd, CACNA1C_1.5 nAd, CACNA1C_0.5 Ad, CACNA1C_1.5 Ad, six microcircuits were generated.

Table 5. Synaptic connections in the microcircuit

Type of connection	Location	No. of synapses	References
Thalamocortical (incoming)	Proximal dendrite	150	Kuroda et al., 1998
Pyramidal (recurrent)	Basal dendrite	24	Thomson and Lamy, 2007; Peters et al., 2008
Autapses in Pyr	Basal dendrite	8	Lubke et al., 1996
Pyr-to-FS	Dendrite	12	Markram et al., 2004; Thomson and Lamy, 2007
Pyr-to-RS	Dendrite	14	Markram et al., 2004
Pyr-to-IS	Dendrite	7	Cauli et al., 1997; Markram et al., 2004
Autapses in FS	Soma	1	Bacci et al., 2003
FS-to-Pyr	Soma	15	Tamás et al., 1997a,b; Markram et al., 2004
RS-to-Pyr	Distal dendrite	12	Tamás et al., 1997a,b; Markram et al., 2004
IS-to-Pyr	Distal Dendrite	10	Tamás et al., 1997a,b
IS-to-RS	Dendrite	2	Murayama et al., 2009

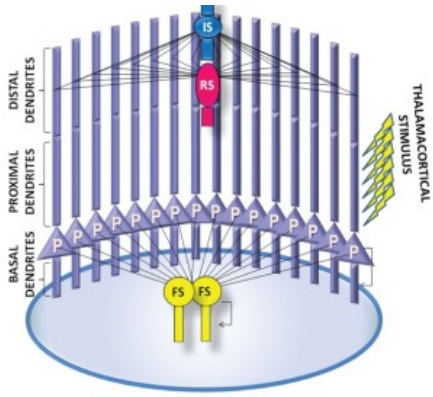


Figure 10 . Schematic representation of PFC microcircuit (adapted from Konstantoudaki et al., 2014)

3.3 Stimulation Protocol

The microcircuit was stimulated by activating 150 excitatory synapses, 10 times at 20 HZ, (AMPA and NMDA receptors included in each one), located at the proximal apical dendrites of all pyramidal neurons (Kuroda et al., 1998). Based on the idea that neurons within a microcircuit share similar stimulus properties (Yoshimura and Callaway, 2005; Petreanu et al., 2009), the same initial stimulus was delivered to all pyramidal neurons.

3.4 Backround noise

In vitro observations of membrane potential fluctuations attributed to stochastic ion channel noise (Linaro et al., 2011), rend necessary the simulation of backround noise, using an artificial current with Poisson characteristics, injected in the soma of all neuron models.

3.5 Implementation and data analysis

The microcircuit model is implemented in the NEURON simuation environment (Hines and Carnevale, 2001) and simulations were executed on a parallel cluster (8 core xeon processors). Data analysis was performed in MATLAB.

4. RESULTS

According to the two testing hypotheses of this study, features of persistent activity, induced in each of the six implemented microcircuits were estimated and presented with the following way in the subsequent sections; in the first section, we used detailed compartmental models of Ad and nAd pyramidal neuron sub-types, embodied in distinct microcircuits and features of PA in the non Adapting (layer V) PFC microcircuit (control nAd) are presented in comparison with those of the Adapting (layer II) microcircuit (control nAd), in order to investigate whether these two PFC microcircuits, consisted of two firing pattern-specific pyramidal neuronal subtypes, exhibit distinct functionality, in matters of their stimulus-induced property;PA.
In the second section, features of PA in the non Adapting (layer V) PFC microcircuit are presented in comparison with those of its two implemented genetic variants; namely CACNA1C_1.5 nAd, and CACNA1C_0.5 nAd, the functional effects of SCZ-related SNPs in CACNA1C gene, in this layer and firing pattern-specific subcircuit.
The third section includes, the respective comparison between the properties of PA in the control Adapting (layer II) microcircuit (control Ad) and in its variants; CACNA1C_1.5 Ad, CACNA1C_0.5 Ad.

Each of the six microcircuits was stimulated by activating 10 times at 20 HZ, 150 excitatory synapses, randomly distributed, in the proximal apical dendrites of all pyramidal neurons (Kuroda et al., 1998). This stimulation protocol was repeated 100 times and the location set of dendritic branches, containing the activated synapses, varied between trials; this variability in the location of incoming contact, is assumed to represent different incoming stimuli. In this way, persistent activity was induced in a probabilistic manner, in a percentage of these trials.
We define persistent activity, as the neuronal activity, recorded in the pyramidal neuron models, which continues past the end of the initiating stimulus and lasts at least 2 seconds. In this way, trials

exhibiting self-terminated persistent activity (<2000 ms), were classified as not having persistent activity ('no PA' trials).

Specifically, we estimated the following properties of persistent activity; the probability of its emergence across different NMDA/AMPA (1, 1.25, 1.5, 1.75) and GABAb/GABAa ratios (0.2-0.5), average firing rate (in comparison with average firing frequency of the stimulation period) in a specific GABAb/GABAa ratio (0.2 or 0.3) and across the different NMDA/AMPA ratios, the mean ISI (interspike interval) in 500 ms bins, both during stimulation (500-1000 ms) and during PA, in a specific combination of AMPA/NMDA and GABAb/GABAa ratio, coefficient of variation of interspike Interval, in 500 ms bins, during stimulation (500-1000 ms) and PA. Finally, differences in AP (action potential) latencies between 'PA' and 'no PA' trials were computed. The latency of the first, stimulation-induced, action potential, is assumed to be a feature that may contain predictive information, regarding the emergence of persistent activity, providing a mechanism for encoding preferred stimuli, decoded by downstream neurons (Sidiropoulou & Poirazi 2012) .

Results are listed in the following sections, in a way, that conceptually corresponds to the questions imposed by our hypotheses. Indicatively, representative traces of each distinct pyramidal neuron model, during both the stimulation period and PA, are shown in **Figure 11,** giving a first glance, of the different firing pattern, observed during persistent activity, especially between adapting and non-adapting pyramidal neuron models.

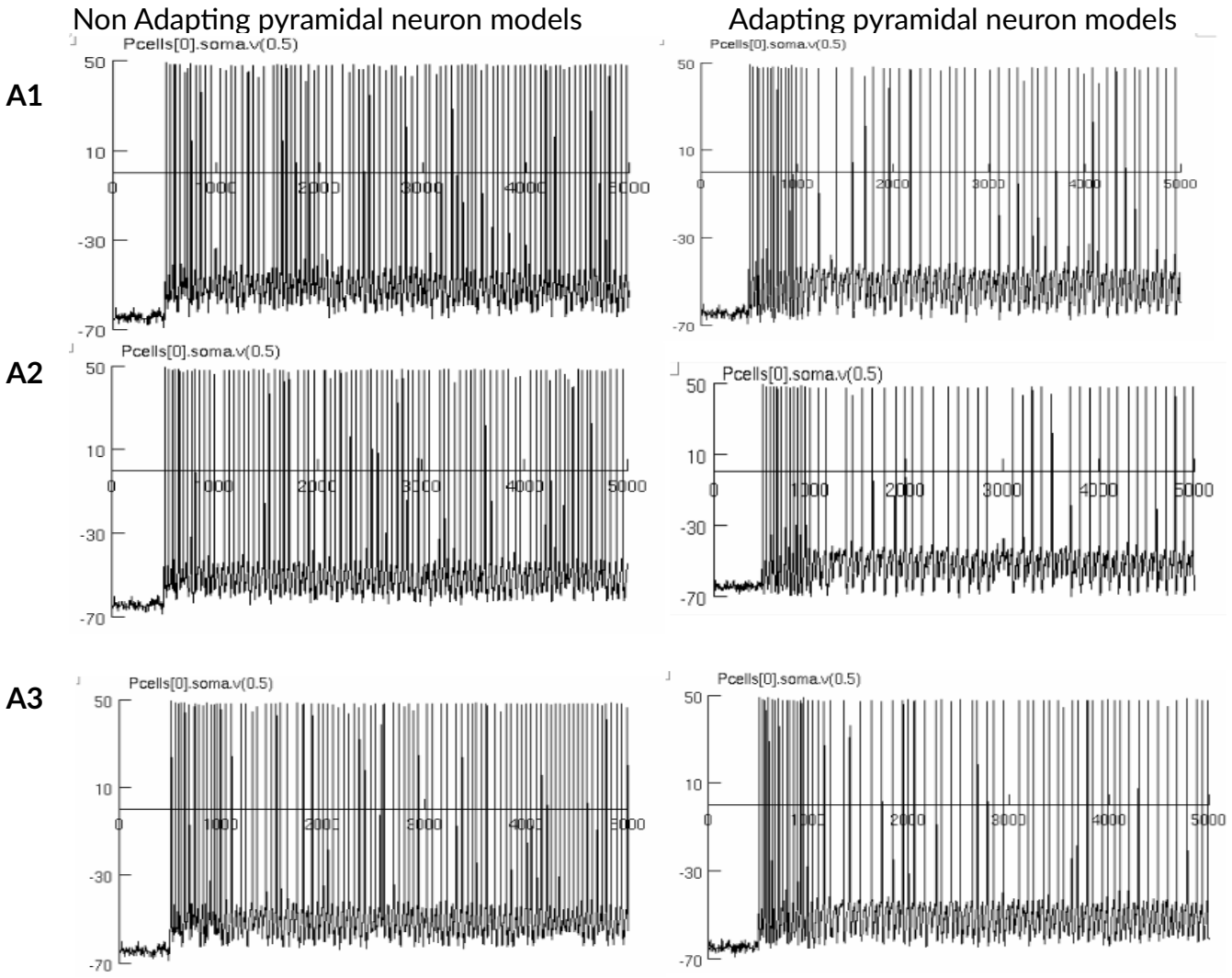


Figure 11 . Representative traces of control and CACNA1C variants of non Adapting and Adapting pyramidal neuron models during the stimulus(500-1000ms) and PA (1000-5000 ms) in PA trials in the same AMPA/NMDA and GABAb/GABAa ratio (1.25 and 0.2, respectively) in the 6 different model microcircuits.
(A1) non adapting control,(A2) non adapting CACNA1C_1.5 variant, (A3) non adapting CACNA1C_0.5 variant, (B1) adapting control, (B2) adapting CACNA1C_1.5 variant,(B3) adapting CACNA1C_0.5 variant. Each neuron model had a different firing pattern during persistent activity, depending on its own electrophysiological characteristics

3.1 Non-adapting (nAd) vs Adapting (Ad) network

We assessed the properties of emergent persistent activity of firing-pattern specific pyramidal neuron models, embedded in distinct networks. Each pyramidal neuron model subtype, exhibited a different firing pattern during persistent activity, depending on its own electrophysiological characteristics (**Figure 11 (A1),(B1)**).

First, the probability of persistent activity induction (as measured in 100 trials) was dependent on the GABAb -to-GABAa and NMDA-to-AMPA ratio on both pyramidal neuron model subtypes, as

previously seen (Papoutsi et al. 2013; Konstantoudaki et al. 2014). This is in accordance, with the notion that regulation of NMDA receptors in pyramidal neurons modulates PFC function (Wang et al. 2008). The AMPA and NMDA components of excitatory postsynaptic currents (EPSCs) are regulated in a way that maintains a constant ratio (1.25 and 1.5, when PFC neurall activity is modulated by dopamine). In **Figure 12. A1, A2** , we notice that the adapting pyramidal neuron model exhibits a smaller range of NMDA-to-AMPA ratios in which persistent activity is induced, a smaller range of GABAb-to-GABAa ratios, in which persistent activity is induced, for each NMDA-to-AMPA ratio(1.25,1.5) and across almost, all the combinations of ratios NMDA-to-AMPA ratio GABAb-to-GABAa , persistent activity is induced with a reduced probability.

Average firing frequency during stimulation(500-1000 ms) is similar and with a slight incease, across the different NMDA/AMPA ratio, in both models, while the average firing frequency during persistent activity is decreased, compared to the stimulation period, for each NMDA/AMPA ratio, with the Adapting neuron model showing a greater decrease than the non Adapting (**Figure 12. B1, B2)**.

The mean interspike interval (ISIs) of the two pyramidal neuron models during stimulation is similar, but we notice a statistically significant difference in the increase, they both exhibited during persistent activity and especially, in its initial phase (**Figure 12 C)**.

The coefficient of variation (CV) of the ISIs (**Figure 12. D)**, was similar during persistent activity compared to the CV during the stimulus, for both models, although a greater CV was expected for the adapting neuron model, in grounds of the increased ISI observed during its persistent activity. The first bin of 500ms of PA(100-1500 ms) constitutes an exception, altough, not statistically significant, as it exhibits an increased CV compared with that of the non-adapting model and the CV of the stimulation.

Finally, the difference in the AP latency (**Figure 12. E)**, between PA and 'no PA' trials, a feature that may gives information to downstream neurons for the preferred stimuli (those who will finally, induce persistent activity), in the non Adapting pyramidal neuron is small and does not appear to be modulated across different NMDA-to-AMPA ratios, indicative of limited discrimination capability, unaffected by neuromodulation, whereas the Adapting model exhibits a NMDA-to-AMPA ratio(1.5-0.3)-dependent increase of its discriminative ability, concerning PA and 'no PA' trials.

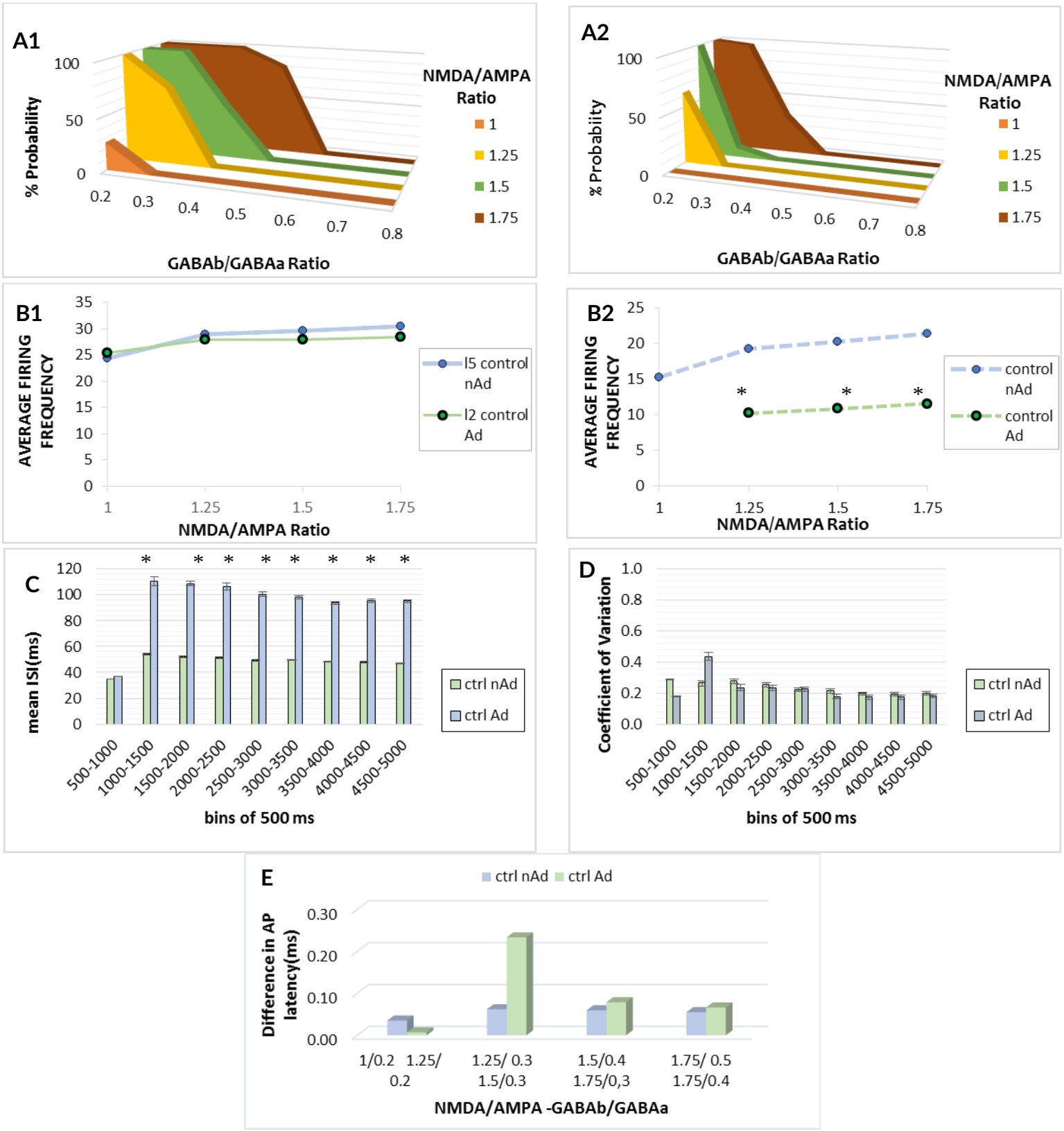


Figure 12. (A) Probability of emergence of PA across different NMDA/AMPA and GABAb ratios for (A1) a nAd and (A2) and Ad PFC microcircuit (B) Firing Frequency(FF) in GABAb/GABAa ratio 0.2 and across different NMDA/AMPA ratios, (B1) average FF during stimulation(two way ANOVA, $F(1,98)=1.1$, $p=0.2$) and (B2) during PA (two way ANOVA, $F(2,557)=620$ $p<0.001$, (C) mean ISI (interspike interval) in 500 ms bins during stimulation (500-1000 ms) and PA in AMPA/NMDA ratio 1.25 and GABAb/GABAa ratio 0.2 (repeated measures ANOVA, $F(1,98)=3.5$, $p=0.001$), (D) Coefficient of Variation of Interspike Interval in 500 ms bins during stimulation (500-1000 ms) and PA (repeated measures ANOVA, $F(1,98)=1.2$, $p=0.2$, (E) Differences in AP Latencies values between PA and no PA trials

3.2 CACNA1C variants vs. control non-adapting network

The 'CACNA1C variants' of the non-adapting pyramidal neuron model, constitute an implementation of the two reported, opposite effects, of the SCZ-associated SNPs in the CACNA1C gene, concerning the Cav 1.2 L-type channel mRNA expression (Bigos et al., 2010; Roussos et al., 2014; Gershon et al., 2014). In order to link this effect of a genetic variant in the Cav 1.2 channel, we had to modulate the value of Cav 1.2 channel's conductance, which corresponds to an altered level of an ionic channel's density in the cell's membrane . In order to validate, the impact of the modified channel's coductance to the current amplitude, we recorded the trace of the current passing through the Cav 1.2 channel, using current clamp recording. Comparative results, concerning the recorded Cav 1.2 channel-mediated, current traces in the control , CACNA1C_1.5 variant nAd pyramidal cell (increased Cav 1.2 channel's conductance) and CACNA1C_0.5 variant (decreased Cav 1.2 channel's conductance) are shown in **Figure 13**, while the values of maximum current amplitude, for each pyramidal cell are listed in **Table A5** in **Appendix,** depicting a proportional relationship between the L-type current's maximum amplitude and the Cav 1.2 channel's conductance.

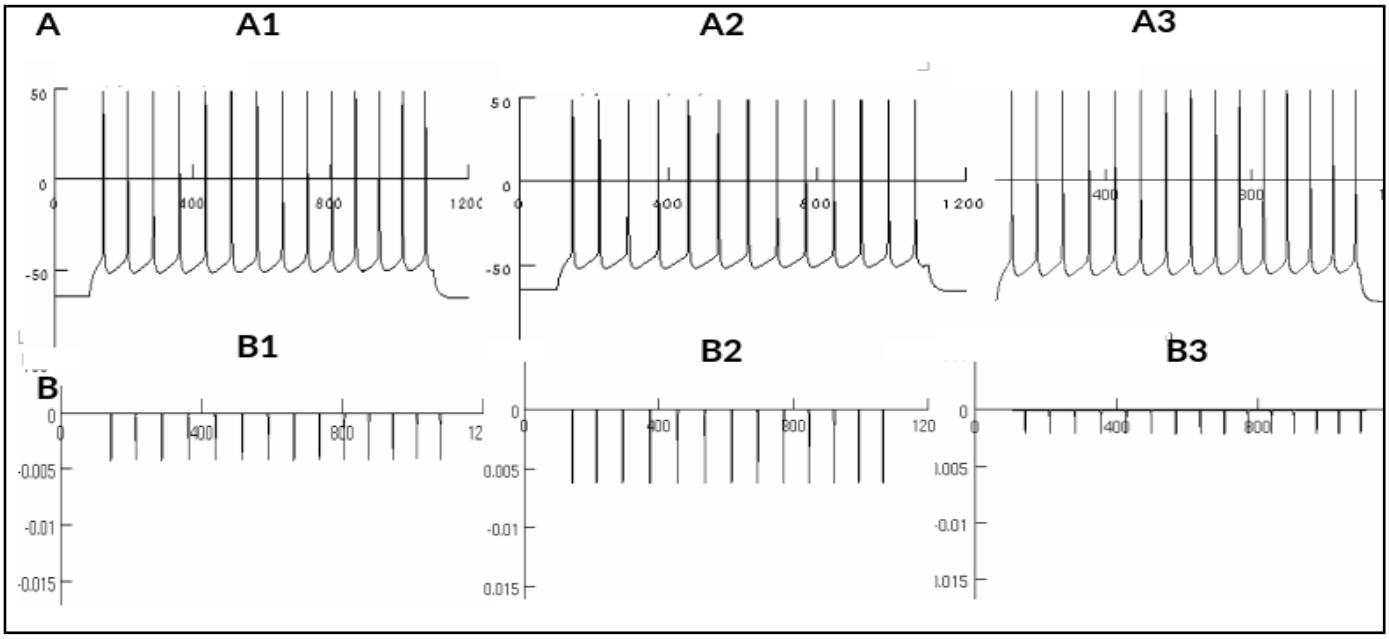


Figure 13. Comparison of the Cav 1.2 channel current trace in control and CACNA1C variant non Adapting cells.
A. Voltage response to a single current step pulse (0.15 nA) in **(A1)** the control nAd pyramidal cell, **(A2)** in the CACNA1C_1.5 variant nAd pyramidal cell **(A3)** the CACNA1C_0.5 nAd variant. We notice that number of spikes is reduced in (A2) and is elevated in (A3). **B.** Cav 1.2 channel current traces during the stimulus in **(B1)**. control nAd (max amplitude 0.11 nA) **(B2)** in the CACNA1C_1.5 variant nAd pyramidal cell, in **(B3)** the CACNA1C_0.5 variant. Current amplitude appears to be in a proportional linear relationship with the variation of CaV 1.2 channel's conductace.

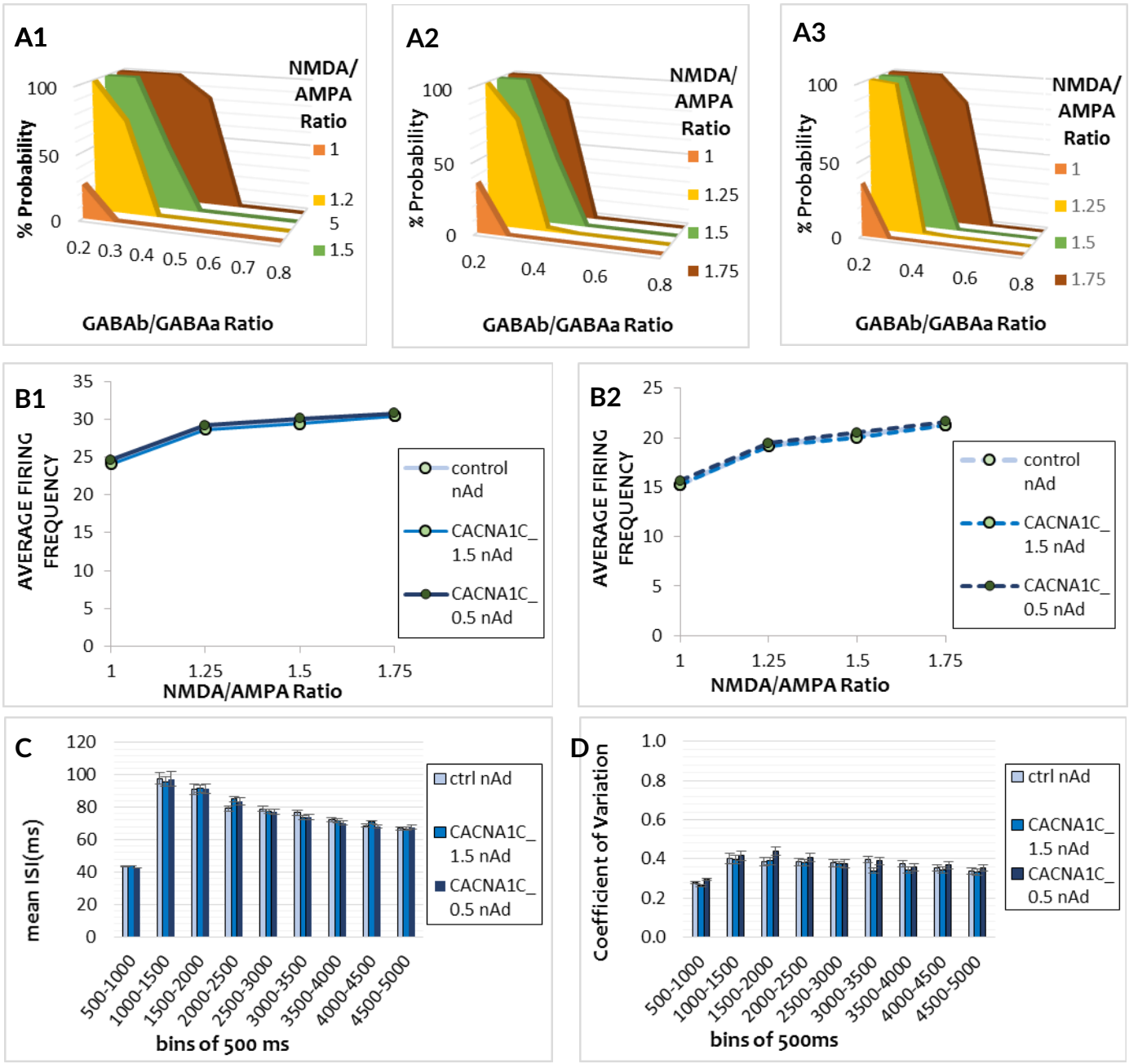
The probability of persistent activity induction, as shown in **Figure 14. A1, A2, A3**, is almost the same, in control nAd pyramidal cell and its CACNA1C variants.

Average firing frequency during stimulation(500-1000 ms) and during PA, is slightly inceased following an increase of the NMDA/AMPA ratio. This occurs in all the three models, with no difference exhibited between them, both during dtimulus response and PA. Average firing frequency during PA is decreased, compared with that of the stimulus-induced response (**Figure 14. B1, B2)**.

The mean interspike interval (ISIs) of all neuron models, is, initially increased, compared to the stimulus mean ISI, and gradually decreased during persistent activity (**Figure 14. C)**.

Coefficient of variation (CV) of the ISIs, does not present any difference between the models and is slightly greater during persistent activity, compared to the CV during the stimulus(**Figure 14. D)**.

Although, all the properties of persistent activity appear the same, the difference in the AP latency, between PA and 'no PA' trials, exhibits a differential modulation by the NMDA-to-AMPA ratios, in each of the three models. The effect of an increased NMDA-to-AMPA ratio (from 1 to 1.5) and GABAb-to-GABAa (from 0.2 to 0.4) to the discriminative ability of each neuron model, in matters of an increase or decrease of the difference in AP latecy between PA and 'no PA' trials, is different for each model. For the non Adapting pyramidal neuron, there is almost no effect, for the CACNA1C_1.5 variant, increasing the NMDA-to-AMPA ratio from 1 to 1.5 coincides with an increase in the observed difference, indicative of an enhancing effect of increased NMDA-to-AMPA ratio to the discriminative abilities of this model. On te other hand, for the CACNA1C_0.5 variant, an increase of the NMDA-to-AMPA (from 1 to 1.25) and GABAb-to-GABAa ratio (from 0.2 to 0.3), decreases the difference in AP latecy between PA and 'no PA' trials, whereas for the third combination of ratios (1.5/0.4), shown in **Figure 14. E,** the difference in AP latecy between PA and 'no PA' trials, is actually reversed. If we assume that the parameter we assess is actually a predictive feature that discriminates between PA and no PA trials, 'no PA trials' exhibiting a diminished latency, compared with PA trials, in this NMDA-to-AMPA ratio, in contrast with what occurs in the other NMDA-to-AMPA ratios, equals with with a reversal of value of the predictive feature.


A1
100
50
0
% Probability
0.2 0.3 0.4 0.5 0.6 0.7 0.8
GABAb/GABAa Ratio
NMDA/ AMPA Ratio
1
1.2 5
1.5
A2
100
50
0
% Probability
0.2 0.4 0.6 0.8
GABAb/GABAa Ratio
NMDA/ AMPA Ratio
1
1.25
1.5
1.75
A3
100
50
0
% Probability
0.2 0.4 0.6 0.8
GABAb/GABAa Ratio
NMDA/ AMPA Ratio
1
1.25
1.5
1.75
B1
AVERAGE FIRING FREQUENCY
35 30 25 20 15 10 5 0
1 1.25 1.5 1.75
NMDA/AMPA Ratio
control nAd
CACNA1C_ 1.5 nAd
CACNA1C_ 0.5 nAd
B2
AVERAGE FIRING FREQUENCY
25 20 15 10 5 0
1 1.25 1.5 1.75
NMDA/AMPA Ratio
control nAd
CACNA1C_ 1.5 nAd
CACNA1C_ 0.5 nAd
C
mean ISI(ms)
120 100 80 60 40 20 0
500-1000 1000-1500 1500-2000 2000-2500 2500-3000 3000-3500 3500-4000 4000-4500 4500-5000
bins of 500 ms
ctrl nAd
CACNA1C_ 1.5 nAd
CACNA1C_ 0.5 nAd
D
Coefficient of Variation
1.0 0.8 0.6 0.4 0.2 0.0
500-1000 1000-1500 1500-2000 2000-2500 2500-3000 3000-3500 3500-4000 4000-4500 4500-5000
bins of 500ms
ctrl nAd
CACNA1C_ 1.5 nAd
CACNA1C_ 0.5 nAd

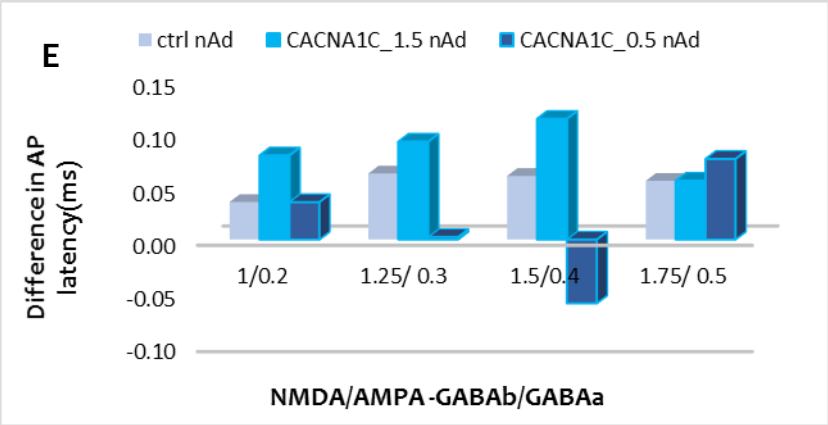


Figure 14 . (A) Probability of emergence of PA across different NMDA/AMPA and GABAb ratios for **(A1)** a control nAd and **(A2)** CACNA1C_1.5 nAd PFC microcircuit and **(A3)** CACNA1C_0.5 nAd PFC microcircuit **(B)**Firing Frequency(FF) in GABAb/GABAa ratio 0.2 and across different NMDA/AMPA ratios, **(B1)** FF during stimulation and **(B2**) during PA, in AMPA/NMDA ratio 1.5 and GABAb/GABAa ratio 0.3:, **(C)** mean ISI (interspike interval) in 500 ms bins during stimulation (500-1000 ms) and PA and **(D)** Coefficient of Variation of Interspike Interval in 500 ms bins during stimulation (500-1000 ms) and PA, **(E)** Differences in AP Latencies values between PA and no PA trials

3.3 CACNA1C variants vs control adapting network

The 'CACNA1C variants' of the adapting pyramidal neuron, have emerged through the same alterations in Cav 1.2 channel's conductance and the proportional relationship between the L-type current's maximum amplitude and the Cav 1.2 channel's conductance is presented through the depiction of the recorded Cav 1.2 channel-mediated, current traces in the control , CACNA1C_1.5 variant Ad pyramidal cell and CACNA1C_0.5 variant in **Figure 15** while the values of maximum current amplitude, for each pyramidal cell are once again, listed in **Table A5** in **Appendix.**

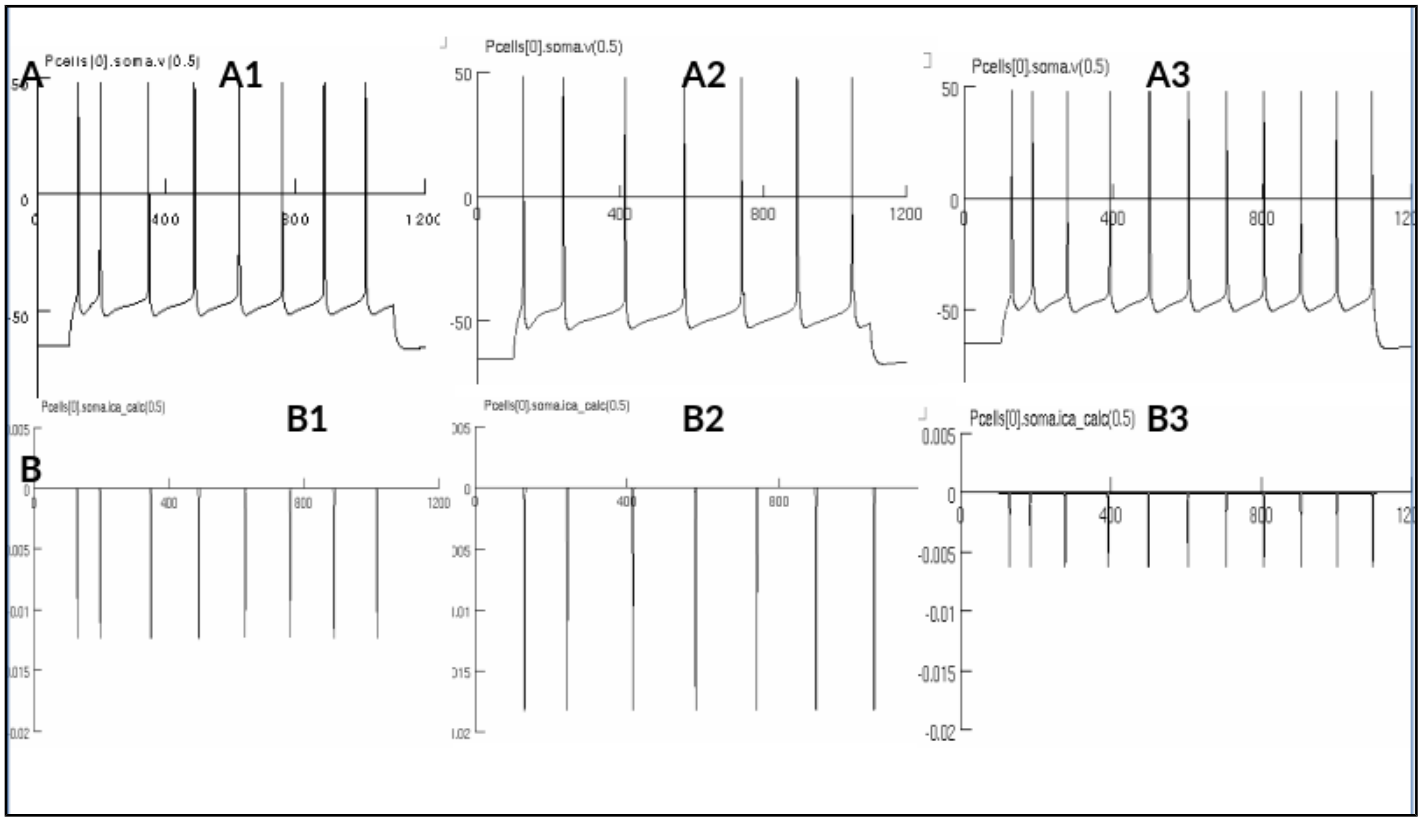


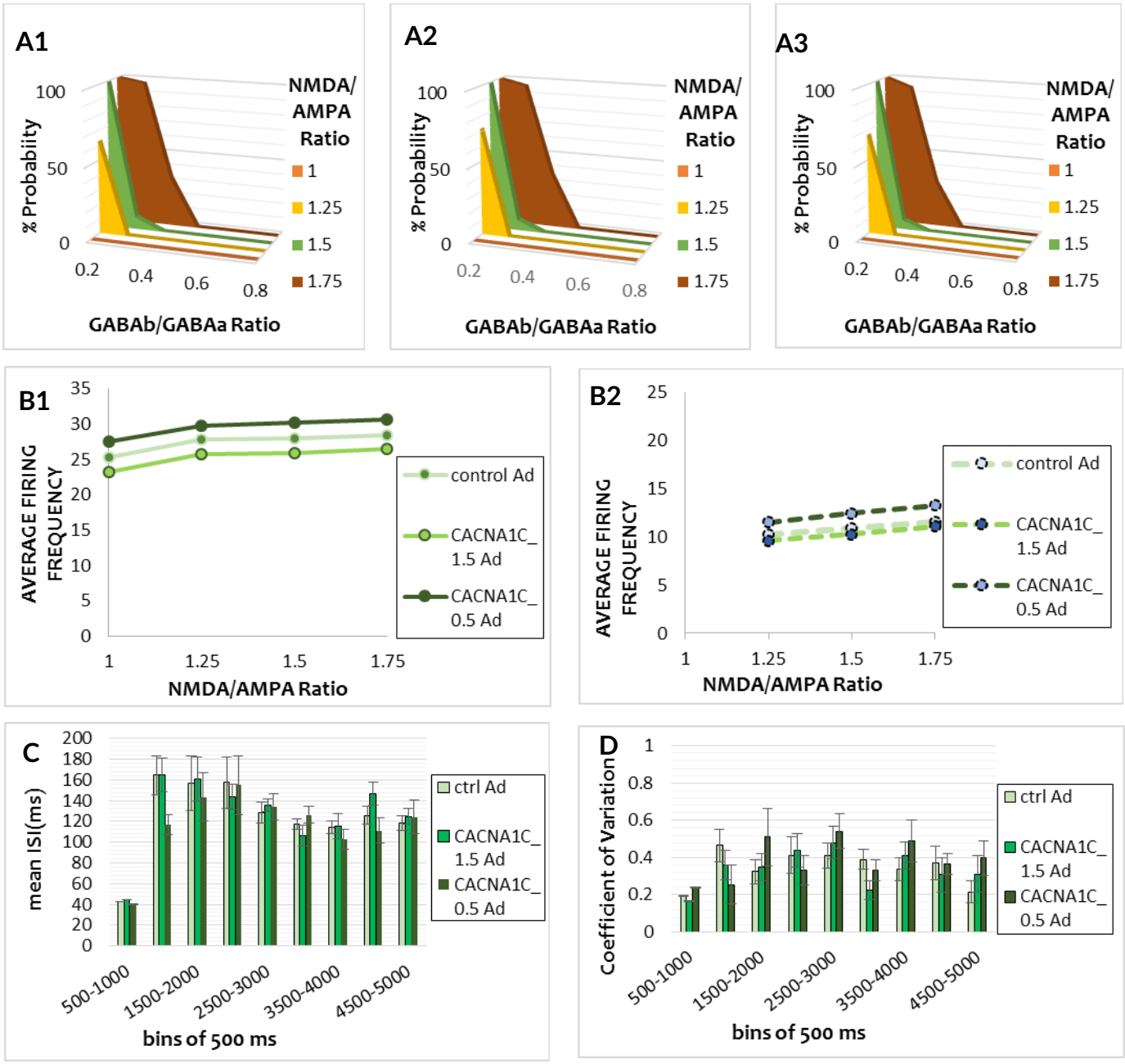
Figure 15. Comparison of the Cav 1.2 channel current trace in control and CACNA1C variant Adapting cells.
A. Voltage response to a single current step pulse (0.2 nA) in **(A1)** the control Adapting pyramidal cell, **(A2)** in the CACNA1C x 1.5 variant and in **(A3)** the CACNA1C x 0.5 variant.We notice that the firing rate is decreased in (A2) and increased in the case of the variant with the reduced calcium conductance (A3).
B. Cav 1.2 channel current traces during the stimulus in **(B1)** control Adapting (max amplitude 0.11 nA) **(B2)** in the CACNA1C x 1.5 variant, in **(B3)** the CACNA1C x 0.5 variant.

The probability of persistent activity induction, as shown in **Figure 16. A1, A2, A3**, presents almost no difference, between the control Ad pyramidal cell and its CACNA1C variants.

Average firing frequency during stimulation(500-1000 ms) (**Figure 16. B1)** shows some, not statistically significant, differences between the three models, with CACNA1C_0.5 Ad having increased and CACNA1C_1.5 Ad decreased average firing frequency values, across all the NMDA-to-AMPA ratios. During PA (**Figure 16. B2)**, average firing frequency is decreased for all the models and across all the NMDA-to-AMPA ratios, compared to that of the stimulus-induced response. The differences of the average frequency during PA are slighter, with that observed in CACNA1C_1.5 Ad , being almost similar with control Ad.

During PA, the mean interspike interval (ISIs) of all neuron models (**Figure 16. C)**, and coefficient of variation (CV) of the ISIs (**Figure 16. D)**, both need further investigation, as there is not an obvious relationship between the three models, across the different bins of PA.

In **Figure 16. E,** which depicts the difference in the AP latency, between PA and 'no PA' trials, the greatest difference for the control Ad model is exhibited in the NMDA-to-AMPA / GABAb-to-GABAaratio, 1.5/0.3, as seen also in **Figure 12. E.** The CACNA1C variants models, both present, a reversal of the difference in the AP latency, between PA and 'no PA' trials, in the second and forth combination of NMDA-to-AMPA / GABAb-to-GABAa ratios.


A1
A2
A3
% Probability
100
50
0
0.2
0.4
0.6
0.8
NMDA/ AMPA Ratio
1
1.25
1.5
1.75
GABAb/GABAa Ratio
B1
AVERAGE FIRING FREQUENCY
35
30
25
20
15
10
5
0
1
1.25
1.5
1.75
NMDA/AMPA Ratio
control Ad
CACNA1C_ 1.5 Ad
CACNA1C_ 0.5 Ad
B2
AVERAGE FIRING FREQUENCY
25
20
15
10
5
0
1
1.25
1.5
1.75
NMDA/AMPA Ratio
control Ad
CACNA1C_ 1.5 Ad
CACNA1C_ 0.5 Ad
C
mean ISI(ms)
200
180
160
140
120
100
80
60
40
20
0
500-1000
1500-2000
2500-3000
3500-4000
4500-5000
bins of 500 ms
ctrl Ad
CACNA1C_ 1.5 Ad
CACNA1C_ 0.5 Ad
D
Coefficient of Variation
1
0.8
0.6
0.4
0.2
0
500-1000
1500-2000
2500-3000
3500-4000
4500-5000
bins of 500 ms
ctrl Ad
CACNA1C_ 1.5 Ad
CACNA1C_ 0.5 Ad

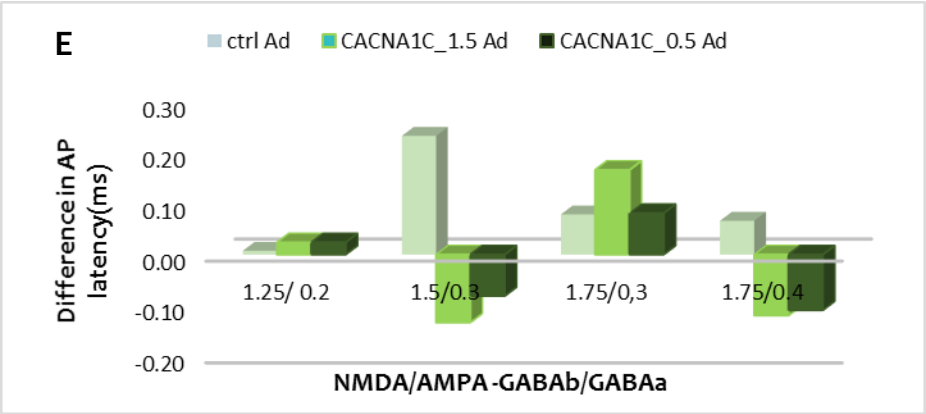


Figure 16 . (A) Probability of emergence of PA across different NMDA/AMPA and GABAb ratios for **(A1)** a control Ad, **(A2)** CACNA1C_1.5 Ad PFC microcircuit and **(A2)** CACNA1C_0.5 Ad PFC microcircuit **(B)**Firing Frequency(FF) in GABAb/GABAa ratio 0.2 and across different NMDA/AMPA ratios, **(B1)** FF during stimulation (two-way ANOVA, F(2,(arithoms persistent trials total-2)=2.6 p=0.01) and **(B2**) during PA, in AMPA/NMDA ratio 1.5 and GABAb/GABAa ratio 0.3: (two-way ANOVA, F(2,(arithoms persistent trials total-2)=2.4 p=0.02) **(C)** mean ISI (interspike interval) in 500 ms bins during stimulation (500-1000 ms) and PA, (repeated measure ANOVA, F(2,(arithoms persistent trials total-2)=1.5 p=0.2) **(D)** Coefficient of Variation of Interspike Interval in 500 ms bins during stimulation (500-1000 ms) and PA (repeated ANOVA, F(2,(arithoms persistent trials total-2)=1.8 p=0.15) , **(E)** Differences in AP Latencies values between PA and no PA trials

5. DISCUSSION

The essential role of PFC, in the mediation of cognitive functions is underlined by its position at the summit of multiple, distinct processing streams, given the hierarchical way that brain processes information. Its extended structural and functional connectivity with other cortical and subcortical brain areas, the existence of distinct, concerning the morphology, firing pattern and projection targets, pyramidal subpopulations, the delicate regulation of the excitability of these distinct neuronal populations, mediated by synaptic gain control mechanisms; such as NMDA receptors, neuromodulatory receptors, like D1 dopamine receptors and excitation-inhibition balance; all support the leading place of PFC in the functional integration, which is necessary for a normal cognitive and behavioral output and strengthen the suspicion of its impairement being in the center of the pathophysiology of psychiatric diseases.

In this study, we chose to concentrate on a salient functional feature of PFC microcircuits, namely self-sustained persistent activity, considered the cellular correlate of working memory.

Features of the firing pattern of PA (average firing frequency, ISIs, CV of ISIs) and the latency of the first AP during the stimulus-induced response, one of the features assumed to mediate PA's stimulus selectivity, can differentiate either in the context of functional specialization or in the context of a pathologically altered state of function. We decided to investigate differences in the properties of persistent activity displayed by PFC pyramidal neurons, which make part of PFC microcircuits, in both contexts.

Implementation of firing-pattern specific PFC microciruits.

For the first instance of differentiation and based on evidence of distinct, regarding their morphology and firing pattern subpopulations, forming subcircuits, we chose to assess the aforementionned PA characteristics in comparison between two firing-pattern specific PFC microciruits. In order to do that, we used a PFC microcircuit consisted of pyramidal neurons which exhibit a regular spiking, non adaptive firing pattern (Konstantoudaki et al. 2014) and via variations made in the ionic conductances and morphology of this pyramidal neuron model, we generated a PFC pyramidal neuron model subtype, which exhibit an adaptive firing behavior and this, was also embedded in a microcircuit.

Without knowing the exact morphological characteristics or ionic properties, that generate this subtype of pyramidal neuron, we based our implementation on associations made between structural or intrinsic excitability mechanisms and the occurrence of the adaptive firing pattern. Litterature review about the spike frequency adaptation phenomenon,drived our choise to modulate the conductance values of channels that either, directly mediate or indirectly influence spike-dependent after-hyperpolarization currents and evidence of layer specific occurrence of this adaptive firing pattern, reasons the modification of a structural parameter value.

Implementation of CACNA1C variants of PFC microcircuits

For the second instance of differentiation of features of PA, in the context of pathophysiological alterations, we chose to investigate the functional effects of SCZ-associated SNPs in CACNA1C gene at a microcircuit level. Limitations are imposed to computational approaches, trying to translate genomic data in fuctional concequences in a cellular or circuit level, by the lack of data for the exact effect of single nucleotide polymorphism (SNP) variants, identified through GWASs, and by the fact, that effects to dynamics of protein translation and phosphorylation can not be simulated by the the biophysical parameters of computational models of moderate complexity. We chose to implement an SNP's effect on the Cav 1.2 channel's conductance value, based on the intronic location of SNPs and the documented specific interaction of an enhancer region, with the proximal CACNA1C gene promoter (Roussos et al. 2014) associated with evidence of both increased and decreased mRNA expression of CACNA1C. An other reason for our choise is that, even without genetic manipulations, study of variants that affect the expression level of the ionic channel and thus, the current density, is experimentally plausible, as it can be imitated by the use of partial pharmacologic blocks and pharmacological agonists.

Our results, arose from strictly phenomenological implentations of both the firing-pattern specific pyramidal neuron subtype and the CACNA1C variants and thus, can only provide insights of how differences in morphology and intrinsic excitability at a single neuron level, can be translated in differences in an emergent property of networks underlying the context of normal, functional segregation or pathophysiological function. First, the firing pattern-specific microcircuits exhibited differences in the NMDA-to-AMPA ratio dependent, probability of

induction of persistent activity and in the same NMDA-to-AMPA ratio, differences in the firing pattern (mean ISIs) of persistent activity are statistically significant, a fact that is consistent with evidence of neuromodulators, that are hypothesized to enhance mnemonic persistent activity by influencing NMDAR-mediated changes in synaptic efficacy, such as dopamine, affecting distinct pyramidal subpopulations, in a discrete way; (Dembrow et al. 2010;Dembrow & Johnston 2014).

In the case of the variants effect, it is interesting that we observed a differential impact of the implemented CACNA1C variant's effect, to the firing-pattern specific subcircuits, concerning their firing pattern during both the stimulus response period and during persistent activity, without the probability of induction of pesistent activity being affected. This is an observation, conceptually consistent with an altered, rather than diminished, functionality of distinct subpopulations, which may compromise their regulation and communication with other areas. It is also noticable, in the case of the variants of the non adapting subcircuit, that their impact did not concern any of the features of PA (avg firing frequency, mean ISIs, probability of induction) but, both variants differentiated the impact of neuromodulation, in terms of NMDA-to-AMPA ratio, in the AP latency of PA and no PA trials, what is considered one of their discriminative features. Although, in order to test whether differences in the AP latency can actually discriminate between 'persistent' and 'no persistent' trials in a more systematic manner, we should assess the ability of the AP latency values to predict the emergence of persistent activity using Linear Discriminant Analysis (LDA), the paradigm of an anaffected firing activity, which still can, theoretically, give altered information to downstream neurons is intriguing.

Conclusively, persistent activity being both a confirmed cellular substrate of a cognitive fuction, and a property emerging through network activity, provides, functional genomics, with a substrate for investigating the effects of expanding genetic findings to network functionality Finally, from the systems biology perspective, differentiated features of a network activiry, either as a product of functional specialization or as a pathological state, may be informative of how specific or abnormal synaptic connectivity can be translated into computational specializations or impairments at the level of neuronal circuits.

6. REFERENCES

van Aerde, K.I. & Feldmeyer, D., 2015. Morphological and physiological characterization of pyramidal neuron subtypes in rat medial prefrontal cortex. *Cerebral cortex* , 25(3), pp.788–805.

American Psychiatric Association, 2013. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*, American Psychiatric Pub.

Arteaga, G. et al., 2015. Human prefrontal layer II interneurons in areas 46, 10 and 24. *Colombia medica* , 46(1), pp.19–25.

Baddeley, A., 1998. Working memory. *Comptes rendus de l'Academie des sciences. Serie III, Sciences de la vie*, 321(2-3), pp.167–173.

Barbas, H., 2000. Connections underlying the synthesis of cognition, memory, and emotion in primate prefrontal cortices. *Brain research bulletin*, 52(5), pp.319–330.

Barbey, A.K., Koenigs, M. & Grafman, J., 2013. Dorsolateral prefrontal contributions to human working memory. *Cortex; a journal devoted to the study of the nervous system and behavior*, 49(5), pp.1195–1205.

Barch, D.M. et al., 2003. Working memory and prefrontal cortex dysfunction: specificity to schizophrenia compared with major depression. *Biological psychiatry*, 53(5), pp.376–384.

Barch, D.M. & Dowd, E.C., 2010. Goal representations and motivational drive in schizophrenia: the role of prefrontal-striatal interactions. *Schizophrenia bulletin*, 36(5), pp.919–934.

Bayer, T.A., Falkai, P. & Maier, W., 1999. Genetic and non-genetic vulnerability factors in schizophrenia: the basis of the "two hit hypothesis." *Journal of psychiatric research*, 33(6), pp.543–548.

Benda, J. & Herz, A.V.M., 2003. A universal model for spike-frequency adaptation. *Neural computation*, 15(11), pp.2523–2564.

Berridge, K.C. & Robinson, T.E., 1998. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain research reviews*, 28(3), pp.309–369.

Bhat, S. et al., 2012. CACNA1C (Cav1.2) in the pathophysiology of psychiatric disease. *Progress in neurobiology*, 99(1), pp.1–14.

Bigos, K.L. et al., 2010. Genetic variation in CACNA1C affects brain circuitries related to mental illness. *Archives of general psychiatry*, 67(9), pp.939–945.

Brown, A.S. & Derkits, E.J., 2010. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. *The American journal of psychiatry*, 167(3), pp.261–280.

Buda, M. et al., 2011. A specific brain structural basis for individual differences in reality monitoring. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 31(40), pp.14308–14313.

Butters, N. & Pandya, D., 1969. Retention of delayed-alternation: effect of selective lesions of sulcus principalis. *Science*, 165(3899), pp.1271–1273.

Cachia, A. et al., 2014. Deviations in cortex sulcation associated with visual hallucinations in schizophrenia. *Molecular psychiatry*, 20(9), pp.1101–1107.

Callicott, J.H. et al., 2003. Abnormal fMRI response of the dorsolateral prefrontal cortex in cognitively intact siblings of patients with schizophrenia. *The American journal of psychiatry*, 160(4), pp.709–719.

Cardno, A.G. & Gottesman, I.I., 2000. Twin studies of schizophrenia: from bow-and-arrow concordances to star wars Mx and functional genomics. *American journal of medical genetics*, 97(1), pp.12–17.

Carlén, M., 2017. What constitutes the prefrontal cortex? *Science*, 358(6362), pp.478–482.

Cauli, B. et al., 1997. Molecular and physiological diversity of cortical nonpyramidal cells. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 17(10), pp.3894–3906.

Cavelti, M. et al., 2018. Formal thought disorder is related to aberrations in language-related white matter tracts in patients with schizophrenia. *Psychiatry research. Neuroimaging*, 279, pp.40–50.

Chalkiadaki, K. et al., 2018. Development of the MAM model of schizophrenia in mice: Sex similarities and differences of prefrontal cortical and hippocampal function. Available at: http://dx.doi.org/10.1101/295741.

Chang, Y.-M. & Luebke, J.I., 2007. Electrophysiological diversity of layer 5 pyramidal cells in the prefrontal cortex of the rhesus monkey: in vitro slice studies. *Journal of neurophysiology*, 98(5), pp.2622–2632.

Collins, A.G.E. et al., 2014. Working memory contributions to reinforcement learning impairments in schizophrenia. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 34(41), pp.13747–13756.

Connors, B.W. & Gutnick, M.J., 1990. Intrinsic firing patterns of diverse neocortical neurons. *Trends in neurosciences*, 13(3), pp.99–104.

Corlett, P.R. et al., 2007. Disrupted prediction-error signal in psychosis: evidence for an associative account of delusions. *Brain: a journal of neurology*, 130(Pt 9), pp.2387–2400.

Cosgrove, D. et al., 2017. Cognitive Characterization of Schizophrenia Risk Variants Involved in Synaptic Transmission: Evidence of CACNA1C's Role in Working Memory. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, 42(13), pp.2612–2622.

Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *The Lancet*, 381(9875), pp.1371–1379.

Ćurčić-Blake, B. et al., 2017. Interaction of language, auditory and memory brain networks in auditory verbal hallucinations. *Progress in neurobiology*, 148, pp.1–20.

Davis, K.L. et al., 2003. White Matter Changes in Schizophrenia. *Archives of general psychiatry*, 60(5), p.443.

Dembrow, N.C., Chitwood, R.A. & Johnston, D., 2010. Projection-specific neuromodulation of medial prefrontal cortex neurons. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 30(50), pp.16922–16937.

Dembrow, N. & Johnston, D., 2014. Subcircuit-specific neuromodulation in the prefrontal cortex. *Frontiers in neural circuits*, 8, p.54.

Deserno, L. et al., 2013. Reinforcement Learning and Dopamine in Schizophrenia: Dimensions of Symptoms or Specific Features of a Disease Group? *Frontiers in psychiatry / Frontiers Research Foundation*, 4. Available at: http://dx.doi.org/10.3389/fpsyt.2013.00172.

Driesen, N.R. et al., 2008. Impairment of working memory maintenance and response in schizophrenia: functional magnetic resonance imaging evidence. *Biological psychiatry*, 64(12), pp.1026–1034.

Elston, G.N., Oga, T. & Fujita, I., 2009. Spinogenesis and Pruning Scales across Functional Hierarchies. *Journal of Neuroscience*, 29(10), pp.3271–3275.

Erk, S. et al., 2010. Brain function in carriers of a genome-wide supported bipolar disorder variant. *Archives of general psychiatry*, 67(8), pp.803–811.

Feldmeyer, D. et al., 2002. Synaptic connections between layer 4 spiny neurone-layer 2/3 pyramidal cell pairs in juvenile rat barrel cortex: physiology and anatomy of interlaminar signalling within a cortical column. *The Journal of physiology*, 538(Pt 3), pp.803–822.

Feldt, S., Bonifazi, P. & Cossart, R., 2011. Dissecting functional connectivity of neuronal microcircuits: experimental and theoretical insights. *Trends in neurosciences*, 34(5), pp.225–236.

Flynn, S.W. et al., 2003. Abnormalities of myelination in schizophrenia detected in vivo with MRI, and post-mortem with analysis of oligodendrocyte proteins. *Molecular psychiatry*, 8(9), pp.811–820.

Fries, P., 2015. Rhythms for Cognition: Communication through Coherence. *Neuron*, 88(1), pp.220–235.

Friston, K. et al., 2016. The dysconnection hypothesis (2016). *Schizophrenia research*, 176(2-3), pp.83–94.

Friston, K.J. & Frith, C.D., 1995. Schizophrenia: a disconnection syndrome? *Clinical neuroscience* , 3(2), pp.89–97.

Funahashi, S., Bruce, C.J. & Goldman-Rakic, P.S., 1989. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *Journal of neurophysiology*, 61(2), pp.331–349.

Fuster, J.M., 2008. Anatomy of the Prefrontal Cortex. In *The Prefrontal Cortex*. pp. 7–58.

Gao, W.-J. et al., 2012. The Unique Properties of the Prefrontal Cortex and Mental Illness. In *When Things Go Wrong - Diseases and Disorders of the Human Brain*.

Garrison, J.R. et al., 2015. Paracingulate sulcus morphology is associated with hallucinations in the human brain. *Nature communications*, 6, p.8956.

Gershon, E.S. & Grennan, K.S., 2015. Genetic and genomic analyses as a basis for new diagnostic nosologies. *Dialogues in clinical neuroscience*, 17(1), pp.69–78.

Giraldo-Chica, M. et al., 2018. Prefrontal-Thalamic Anatomical Connectivity and Executive Cognitive Function in Schizophrenia. *Biological psychiatry*, 83(6), pp.509–517.

Gold, J.M. et al., 2008. Reward Processing in Schizophrenia: A Deficit in the Representation of Value. *Schizophrenia bulletin*, 34(5), pp.835–847.

Goldman-Rakic, P.S., 1995. Cellular basis of working memory. *Neuron*, 14(3), pp.477–485.

Goldman-Rakic, P.S., 1987. Development of Cortical Circuitry and Cognitive Function. *Child development*, 58(3), p.601.

González-Burgos, G. et al., 2005. Functional Properties of Fast Spiking Interneurons and Their Synaptic Connections With Pyramidal Cells in Primate Dorsolateral Prefrontal Cortex. *Journal of neurophysiology*, 93(2), pp.942–953.

Grace, A.A., 1991. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*, 41(1), pp.1–24.

Gradin, V.B. et al., 2011. Expected value and prediction error abnormalities in depression and schizophrenia. *Brain: a journal of neurology*, 134(Pt 6), pp.1751–1764.

Hamshere, M.L. et al., 2013. Genome-wide significant associations in schizophrenia to ITIH3/4, CACNA1C and SDCCAG8, and extensive replication of associations reported by the Schizophrenia PGC. *Molecular psychiatry*, 18(6), pp.708–712.

Hattox, A.M. & Nelson, S.B., 2007. Layer V Neurons in Mouse Cortex Projecting to Different Targets Have Distinct Physiological Properties. *Journal of neurophysiology*, 98(6), pp.3330–3340.

Heyes, S. et al., 2015. Genetic disruption of voltage-gated calcium channels in psychiatric and neurological disorders. *Progress in neurobiology*, 134, pp.36–54.

Hirtz, J.J. et al., 2012. Synaptic refinement of an inhibitory topographic map in the auditory brainstem requires functional Cav1.3 calcium channels. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 32(42), pp.14602–14616.

Holloway, T. et al., 2013. Prenatal stress induces schizophrenia-like alterations of serotonin 2A and metabotropic glutamate 2 receptors in the adult offspring: role of maternal immune system. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 33(3), pp.1088–1098.

Jackson, M.E., Homayoun, H. & Moghaddam, B., 2004. NMDA receptor hypofunction produces concomitant firing rate potentiation and burst activity reduction in the prefrontal cortex.

Proceedings of the National Academy of Sciences of the United States of America, 101(22), pp.8467–8472.

Jacobson, S. & Trojanowski, J.Q., 1977. Prefrontal granular cortex of the rhesus monkey. II. Interhemispheric cortical afferents. *Brain research*, 132(2), pp.235–246.

Kawaguchi, Y. & Kubota, Y., 1997. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cerebral cortex* , 7(6), pp.476–486.

Kolb, B. et al., 2012. Experience and the developing prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, 109 Suppl 2, pp.17186–17193.

Konstantoudaki, X. et al., 2014. Modulatory effects of inhibition on persistent activity in a cortical microcircuit model. *Frontiers in neural circuits*, 8, p.7.

Kubota, K. & Niki, H., 1971. Prefrontal cortical unit activity and delayed alternation performance in monkeys. *Journal of neurophysiology*, 34(3), pp.337–347.

Kuroda, M., Yokofujita, J. & Murakami, K., 1998. An ultrastructural study of the neural circuit between the prefrontal cortex and the mediodorsal nucleus of the thalamus. *Progress in neurobiology*, 54(4), pp.417–458.

Lancaster, T.M. et al., 2014. CACNA1C risk variant affects reward responsiveness in healthy individuals. *Translational psychiatry*, 4, p.e461.

Letinic, K., Zoncu, R. & Rakic, P., 2002. Origin of GABAergic neurons in the human neocortex. *Nature*, 417(6889), pp.645–649.

Lewis, D.A. et al., 1999. Altered GABA neurotransmission and prefrontal cortical dysfunction in schizophrenia. *Biological psychiatry*, 46(5), pp.616–626.

Lewis, D.A., Hashimoto, T. & Volk, D.W., 2005. Cortical inhibitory neurons and schizophrenia. *Nature reviews. Neuroscience*, 6(4), pp.312–324.

Linaro, D., Storace, M. & Giugliano, M., 2011. Accurate and Fast Simulation of Channel Noise in Conductance-Based Model Neurons by Diffusion Approximation. *PLoS computational biology*, 7(3), p.e1001102.

Li, Z. et al., 2017. Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. *Nature genetics*, 49(11), pp.1576–1583.

Liu, Y. et al., 2014. Cav1.2 and Cav1.3 L-type calcium channels regulate dopaminergic firing activity in the mouse ventral tegmental area. *Journal of neurophysiology*, 112(5), pp.1119–1130.

Lodge, D.J., 2013. The MAM Rodent Model of Schizophrenia. In *Current Protocols in Neuroscience*.

Maia, T.V. & Cleeremans, A., 2005. Consciousness: converging insights from connectionist modeling and neuroscience. *Trends in cognitive sciences*, 9(8), pp.397–404.

Markram, H. et al., 2004. Interneurons of the neocortical inhibitory system. *Nature reviews. Neuroscience*, 5(10), pp.793–807.

Maynard, T.M. et al., 2001. Neural Development, Cell-Cell Signaling, and the "Two-Hit" Hypothesis of Schizophrenia. *Schizophrenia bulletin*, 27(3), pp.457–476.

McClelland, J.L. & Rumelhart, D.E., 1981. An interactive activation model of context effects in letter perception: I. An account of basic findings. *Psychological review*, 88(5), pp.375–407.

Millar, J.K. et al., 2000. Identification and Analysis of Genes Disrupted by a Translocation Segregating with Schizophrenia. *Biochemical Society transactions*, 28(5), pp.A302.1–A302.

Miller, M.H. & Orbach, J., 1972. Retention of spatial alternation following frontal lobe resections in stump-tailed macaques. *Neuropsychologia*, 10(3), pp.291–298.

Miller, M.L. et al., 2018. Adolescent exposure to Δ-tetrahydrocannabinol alters the transcriptional trajectory and dendritic architecture of prefrontal pyramidal neurons. *Molecular psychiatry*. Available at: http://dx.doi.org/10.1038/s41380-018-0243-x.

Mitchell, K.J., 2011. The genetics of neurodevelopmental disease. *Current opinion in neurobiology*, 21(1), pp.197–203.

Morishima, M. & Kawaguchi, Y., 2006. Recurrent connection patterns of corticostriatal pyramidal cells in frontal cortex. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 26(16), pp.4394–4405.

Morris, R.W. et al., 2012. Disambiguating ventral striatum fMRI-related BOLD signal during reward prediction in schizophrenia. *Molecular psychiatry*, 17(3), pp.235, 280–9.

Mortensen, P.B. et al., 1999. Effects of family history and place and season of birth on the risk of schizophrenia. *The New England journal of medicine*, 340(8), pp.603–608.

Mrzljak, L. et al., 1990. Neuronal development in human prefrontal cortex in prenatal and postnatal stages. *Progress in brain research*, 85, pp.185–222.

Murray, E., Wise, S. & Graham, K., 2016. *The Evolution of Memory Systems*, Oxford University Press.

Murray, G.K. et al., 2008. Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Molecular psychiatry*, 13(3), pp.239, 267–76.

Nestor, P.G. et al., 1998. A neuropsychological analysis of schizophrenic thought disorder. *Schizophrenia research*, 29(3), pp.217–225.

Orellana, G. & Slachevsky, A., 2013. Executive Functioning in Schizophrenia. *Frontiers in psychiatry / Frontiers Research Foundation*, 4. Available at: http://dx.doi.org/10.3389/fpsyt.2013.00035.

Otsuka, T. & Kawaguchi, Y., 2008. Firing-pattern-dependent specificity of cortical excitatory feed-forward subnetworks. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 28(44), pp.11186–11195.

Papoutsi, A. et al., 2013. Induction and modulation of persistent activity in a layer V PFC microcircuit model. *Frontiers in neural circuits*, 7, p.161.

Parajuli, L.K. et al., 2012. Quantitative regional and ultrastructural localization of the Ca(v)2.3 subunit of R-type calcium channel in mouse brain. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 32(39), pp.13555–13567.

Park, S. & Holzman, P.S., 1992. Schizophrenics show spatial working memory deficits. *Archives of general psychiatry*, 49(12), pp.975–982.

Paulus, F.M. et al., 2014. Association of rs1006737 in CACNA1C with alterations in prefrontal activation and fronto-hippocampal connectivity. *Human brain mapping*, 35(4), pp.1190–1200.

Perez-Reyes, E., 2003. Molecular Physiology of Low-Voltage-Activated T-type Calcium Channels. *Physiological reviews*, 83(1), pp.117–161.

Perlstein, W.M. et al., 2003. Prefrontal cortex dysfunction mediates deficits in working memory and prepotent responding in schizophrenia. *Biological psychiatry*, 53(1), pp.25–38.

Petanjek, Z. et al., 2011. Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proceedings of the National Academy of Sciences*, 108(32), pp.13281–13286.

Peters, B.D. & Karlsgodt, K.H., 2015. White matter development in the early stages of psychosis. *Schizophrenia research*, 161(1), pp.61–69.

Petreanu, L. et al., 2009. The subcellular organization of neocortical excitatory connections. *Nature*, 457(7233), pp.1142–1145.

Preuss, T.M. & Goldman-Rakic, P.S., 1987. Crossed corticothalamic and thalamocortical connections of macaque prefrontal cortex. *The Journal of comparative neurology*, 257(2), pp.269–281.

Rao, S.G., Williams, G.V. & Goldman-Rakic, P.S., 1999. Isodirectional tuning of adjacent interneurons and pyramidal cells during working memory: evidence for microcolumnar organization in PFC. *Journal of neurophysiology*, 81(4), pp.1903–1916.

Ratajczak, P., Wozniak, A. & Nowakowska, E., 2013. Animal models of schizophrenia: developmental preparation in rats. *Acta neurobiologiae experimentalis*, 73(4), pp.472–484.

Renard, J. et al., 2017. Adolescent THC Exposure Causes Enduring Prefrontal Cortical Disruption of GABAergic Inhibition and Dysregulation of Sub-Cortical Dopamine Function. *Scientific reports*, 7(1). Available at: http://dx.doi.org/10.1038/s41598-017-11645-8.

Renard, J., Rushlow, W.J. & Laviolette, S.R., 2018. Effects of Adolescent THC Exposure on the Prefrontal GABAergic System: Implications for Schizophrenia-Related Psychopathology. *Frontiers in psychiatry / Frontiers Research Foundation*, *9*, p.281.

Ripke, S. et al., 2013. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nature genetics*, 45(10), pp.1150–1159.

Rosene, D.L. & Van Hoesen, G.W., 1977. Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey. *Science*, 198(4314), pp.315–317.

Roussos, P. et al., 2014. A role for noncoding variation in schizophrenia. *Cell reports*, 9(4), pp.1417–1429.

Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*, 511(7510), pp.421–427.

Sidiropoulou, K. et al., 2009. Dopamine modulates an mGluR5-mediated depolarization underlying prefrontal persistent activity. *Nature neuroscience*, 12(2), pp.190–199.

Sidiropoulou, K. & Poirazi, P., 2012. Predictive Features of Persistent Activity Emergence in Regular Spiking and Intrinsic Bursting Model Neurons. *PLoS computational biology*, 8(4), p.e1002489.

Simms, B.A. & Zamponi, G.W., 2014. Neuronal voltage-gated calcium channels: structure, function, and dysfunction. *Neuron*, 82(1), pp.24–45.

Sohal, V.S. et al., 2009. Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature*, 459(7247), pp.698–702.

Spalletta, G. et al., 2013. Fronto-thalamic volumetry markers of somatic delusions and hallucinations in schizophrenia. *Psychiatry research*, 212(1), pp.54–64.

Spruston, N., 2008. Pyramidal neurons: dendritic structure and synaptic integration. *Nature reviews. Neuroscience*, 9(3), pp.206–221.

St Clair, D. et al., 2005. Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. *JAMA: the journal of the American Medical Association*, 294(5), pp.557–562.

Stuss, D.T. & Alexander, M.P., 2000. Executive functions and the frontal lobes: a conceptual view. *Psychological research*, 63(3-4), pp.289–298.

Stuss, D.T. & Levine, B., 2002. Adult clinical neuropsychology: lessons from studies of the frontal lobes. *Annual review of psychology*, 53, pp.401–433.

Susser, E. et al., 1996. Schizophrenia after prenatal famine. Further evidence. *Archives of general psychiatry*, 53(1), pp.25–31.

Vinckier, F. et al., 2016. Confidence and psychosis: a neuro-computational account of contingency learning disruption by NMDA blockade. *Molecular psychiatry*, 21(7), pp.946–955.

Wang, H. et al., 2008. A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, 105(43), pp.16791–16796.

Wang, X.J., 1999. Synaptic basis of cortical persistent activity: the importance of NMDA receptors to working memory. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 19(21), pp.9587–9603.

Wang, Y. et al., 2006. Heterogeneity in the pyramidal network of the medial prefrontal cortex. *Nature neuroscience*, 9(4), pp.534–542.

Wheeler, D.G. et al., 2012. Ca(V)1 and Ca(V)2 channels engage distinct modes of Ca(2+) signaling to control CREB-dependent gene expression. *Cell*, 149(5), pp.1112–1124.

Whitford, T.J. et al., 2009. Delusions and dorso-medial frontal cortex volume in first-episode schizophrenia: A voxel-based morphometry study. *Psychiatry Research: Neuroimaging*, 172(3), pp.175–179.

Woodward, N.D. & Heckers, S., 2016. Mapping Thalamocortical Functional Connectivity in Chronic and Early Stages of Psychotic Disorders. *Biological psychiatry*, 79(12), pp.1016–1025.

Yohn, N.L. & Blendy, J.A., 2017. Adolescent Chronic Unpredictable Stress Exposure Is a Sensitive Window for Long-Term Changes in Adult Behavior in Mice. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, 42(8), pp.1670–1678.

Yoshimizu, T. et al., 2015. Functional implications of a psychiatric risk variant within CACNA1C in induced human neurons. *Molecular psychiatry*, 20(2), p.284.

Yoshimura, Y., Dantzker, J.L.M. & Callaway, E.M., 2005. Excitatory cortical neurons form fine-scale functional networks. *Nature*, 433(7028), pp.868–873.

Zamberletti, E. et al., 2014. Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats. *Neurobiology of disease*, 63, pp.35–47.

Zhang, Q. et al., 2011. The Effects of CACNA1C Gene Polymorphism on Spatial Working Memory in Both Healthy Controls and Patients with Schizophrenia or Bipolar Disorder. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, 37(3), pp.677–684.

Zhu, J. et al., 2016. Neural substrates underlying delusions in schizophrenia. *Scientific reports*, 6, p.33857.

Appendix

Table A1. Morphological parameters of Interneuron model neurons.

FS Interneuron	Length (μm)	Diameter (μm)
Soma	27	29
Dendrite	22	7
Axon	115	1.5
RS Interneuron		
Soma	42	42
Dendrite	22	7
Axon	113.22	1.1
IS Interneuron		
Soma	27	27
Dendrite	22	7
Dendrite	22	7
Axon	113.22	1.1

Table A2. Active and passive ionic properties of FS interneuron model

FS interneuron mechanisms	Soma	Axon	Dendrite
Sodium conductance, S/cm^2	0.135	1.35	0.09
Delayed rectifier K^+, S/cm^2	0.036	0.018	0.0075
N-type calcium, S/cm^2	0.0003	–	–
D-type K^+, S/cm^2	0.0000725	–	–
H-current, S/cm^2	0.00001	–	–
A-type K^+, S/cm^2	0.0032	–	0.032
fAHP, S/cm^2	0.0001	–	–
Calcium diffusion model	Yes	No	No
C_M ($\mu F/cm^2$)	1.2	1.2	1.2
G_pas (S/cm^2)	1.4e-4	1.4e-4	1.4e-4
R_A (ohm/cm)	150	150	150
R_M ($\mu F/cm^2$)	10	10	10

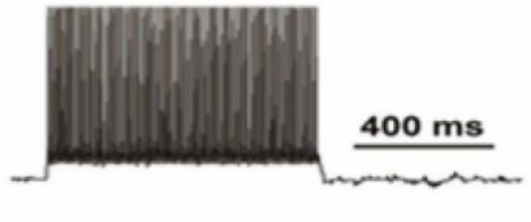


Figure 17 . FS neuron model response to 0.3 nA depolarizing current injection.
(adapted from Konstantoudaki et al., 2014)

Table A3. Active and passive ionic properties of RS interneuron model

RS interneuron mechanisms	Soma	Axon	Dendrite
Sodium conductance, S/cm^2	0.075	0.75	0.018
Delayed rectifier K^+, S/cm^2	0.018	0.009	0.009
T-type calcium, S/cm^2	0.003	–	–
H-current, S/cm^2	0.000002	–	–
A-type K+, S/cm^2	0.035	–	0.00875
fAHP, S/cm^2	0	–	–
Calcium diffusion model	Yes	No	No
C_M ($\mu F/cm^2$)	1.2	1.2	1.2
G_pas (S/cm^2)	2.5e-4	2.5e-4	2.5e-4
R_A (ohm/cm)	150	150	150
R_M ($\mu F/cm^2$)	40	40	40

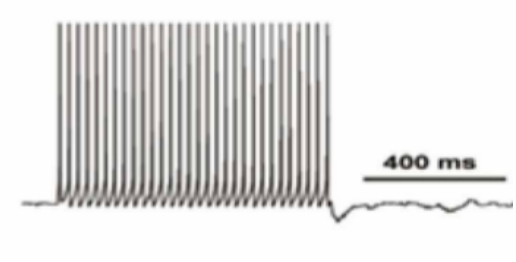


Figure 18 .RS neuron model response to 0.2 nA depolarizing current injection.
(adapted from konstantoudaki et al., 2014)

Table A4. Active and passive ionic properties of IS interneuron model

IS interneuron mechanisms	Soma	Axon	Dendrite
Sodium conductance, S/cm^2	0.015	0.75	0.075
Delayed rectifier K^+, S/cm2	0.018	0.009	0.009
D-type K+, S/cm^2	0.000725	–	–
N-type calcium, S/cm^2	0.001	–	–
fAHP, S/cm^2	0.00003	–	–
Calcium diffusion model	Yes	No	No
C_M ($\mu F/cm^2$)	1.2	1.2	1.2
G_pas (S/cm^2)	1.1e-4	1.1e-4	1.1e-4
R_A (ohm/cm)	150	150	150
R_M ($\mu F/cm^2$)	20	20	20

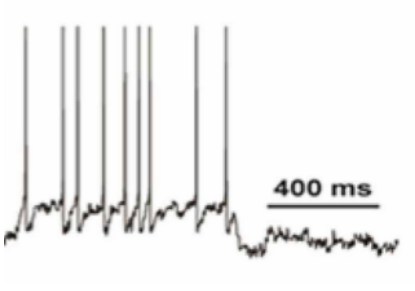


Figure 19. IS neuron model response to 0.2 nA depolarizing current injection.
(adapted from konstantoudaki et al., 2014)

Table A5. a. Analytic description of compartmental conductances of Cav 1.2 channel of the non-adapting and adapting cell.

b. Current recordings from the Cav 1.2 channel using current clamp at the soma. Effects of the CACNA1C variants in current amplitude are shown.

Compartments	CONDUCTANCES Cav 1.2 (S/cm^2) Non Adapting	Adapting
Soma	10^{-5}	$3*10^{-5}$
Basal Dendrite	10^{-5}	$3*10^{-5}$
Proximal Apical Dendrite	10^{-5}	$3*10^{-6}$
Distal Apical Dendrite	$3*10^{-6}$	$9*10^{-7}$
CONDUCTANCES of Cav 1.2	**ICLAMP**	
g_Cav 1.2	Spikes:14 / 1000ms icalc=116.4 pA(~0.1nA)	Spikes: 8 icalc= 340.92 pA
g_Cav 1.2 (variant x1.5)	Spikes: 13 icalc=179.8 pA	Spikes: 7 icalc=523.8 pA
g_Cav 1.2 (variant x0.5)	Spikes: 15 icalc=58.2 pA	Spikes: 11 icalc=174.6 pA