 doi:10.20944/preprints201905.0360.v1

Neuropsin in Mental Health

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Key words: Neuropsin, KLK8, Mental disorders, Mental health

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ABSTRACT:

Neuropsin is an extracellular matrix serine protease that governs the proteolytic cleavage of synaptic proteins and consequently synaptic structural plasticity. In the brain, its substrates include the cell adhesion molecules Neuregulin-1 and L1CAM, that have been linked to neurodevelopmental processes and disorders, such as schizophrenia and bipolar disorder. *Neuropsin* mRNA is abundant in the cerebellum and several peripheral tissues from mid-gestation but is mainly expressed in cortical and limbic tissues postnatally. Differential usage of neuropsin splice forms in the fetal and adult brain has only been reported in humans, suggesting that neuropsin may serve a specialized role in human neurodevelopment. Accordingly, both the expression and proteolytic activity of neuropsin are subject to regulation by neural activity as well as by environmental risk factors associated with mental illness, such as psychophysiological stress. Intriguingly, dysregulation of *neuropsin* has been reported in depression and Alzheimer's disease and its implication in mental disorder is supported by genetic and epigenome wide studies. Here we review neuropsin regulation in mental health and provide a summary of clinical and preclinical evidence supporting a role for neuropsin in the pathogenesis of mental disorders.

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NEUROPSIN REGULATION IN THE BRAIN:

The extracellular matrix (ECM) serine protease neuropsin (also known as KLK8, NP, PRSS19, BSP1 or TADG14), was named after its apparent neuronal expression and its sequence homology to trypsin [1]. Although expression is not restricted to neuronal tissues (www.gtexportal.org/home/gene/KLK8), *neuropsin* mRNA is found in numerous brain tissues under non-pathological conditions and its expression is particularly high in the cerebellum throughout life. In cortical and limbic tissues, on the other hand, mRNA is most abundant postnatally and peaking in childhood and adulthood (www.brainspan.org) (**Figure 1**). In the developing mouse cortex and midbrain, the primary source of *neuropsin* mRNA is endothelial cells and microglia [2, 3] whereas its expression has also been reported in pyramidal neurons of the hippocampal CA1–3 subfields and magnocellular neurons of the lateral/basolateral amygdaloid nucleus [1]. In the hippocampal CA1-3 subfields and amygdala, the genes show activity dependent expression evoked by neuronal stimuli [1, 4] and its expression is further induced in oligodendrocytes of the spinal cord following injury [5]. While homologs of neuropsin (type 1) are found in other placental mammals, neuropsin isoforms (type 2-6, **Figure 2A**) expressed due to alternative splicing have only been reported in humans [6-9]. In mice, *neuropsin* mRNA is reportedly expressed in limbic regions, especially in the hippocampus, ventral striatum and the lateral amygdaloid nucleus [1, 10]. In humans, however, type 1 neuropsin is predominantly expressed in the fetal brain [6], whereas type 2, resulting from an in frame splice site in exon 3, is preferentially expressed in human adult brain (particularly in CA1-3 regions of the hippocampus and in the amygdala) [6]. Specifically, a single human-specific T-to-A mutation at position 79 in the coding region (**Figure 2A)** leads to a novel GAA-containing motif in exon 3, which functions as splicing enhancer and creates a novel splicing site 8 bp upstream of the mutation site [11]. Type 2 neuropsin is thus identical with human type 1 neuropsin except from a 135 bp (45 amino acid) insert located in exon 3 (**Figure 2A**) [6]. It has been speculated that human type 2 neuropsin may be important for the adult brain plasticity, whereas both type 1 and type 2 may be necessary for the development of the human nervous system [6].

Neuropsin translation results in a preproprotease containing an activity masking peptide, the serine protease peptide and a signal peptide [12] (**Figure 3A)** that destines the preproprotease for translocation to the endoplasmatic reticulum and eventually for secretion as a proprotease following signal sequence removal. This non-active proneuropsin is stored in the

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ECM, probably mostly in the synaptic cleft [13]. Activation of extracellular proneuropsin is facilitated by removal of the activity masking peptide, amino acids QGSK/QEDK, in mouse and human respectively [14]. The responsible activating endoprotease has not been identified, but activation of proneuropsin follows various stimuli, such as kindling epileptogenesis, LTP and application of drugs that can depolarize synaptic activity [13]. Consequently, no or only a little protease activity has been detected in unstimulated brain tissues [14]. Key amino acid residues for the enzyme activity of neuropsin are conserved between mouse and human [15], and comprise His73/118, Aspl20/156, and Ser212/257 in mouse and human respectively [1, 12]. Neuropsin possesses a typical tryptic S1 subsite and cleaves mainly at P1-Arg residues [16]. Interestingly human type 1 and type 2 neuropsin mRNA transcripts produce the same active protease, as the type 2 specific 45 amino acid insertion is located in the signal sequence of the preproneuropsin [17]. Since type 2 neuropsin was shown to constitute a brain specific variant [6], the difference in signal peptide sequence seems to promote the cell-type dependent release of the proprotease, which is in line with the finding that neuropsin type 1 and type 2 secretion is cell-type dependent [17]. Interestingly an extra endoprotease site has been identified in the type 2-specific 45 amino acid region (pointed out in **Figure 3 B**) causing an intermediate protein form during the activation process [17]. Since protease activation only occurs after cleavage of the activity masking peptide, this intermediate form shows very low amidolytic activity, like the other proneuropsin forms [17].

THE NEUROBIOLOGY OF NEUROPSIN:

Neuropsin possesses the complete triplet (His-Asp-Ser) of the serine protease domain and is shown to exhibit proteolytic activity with trypsin-like substrate specificity [14]. Accumulating evidence support a central role for trypsin and trypsin-like serine proteases in neural development, degeneration and plasticity in the brain [18, 19]. Identified neuropsin substrates are vitronectin [20], fibronectin [14], the cell adhesion molecule L1 (L1CAM) [21], neuregulin-1 (NRG1) [20] and the Eph receptor B2 (EphB2) [22], all of which localize to the synapse [23], thus suggesting that neuropsin plays a role in synaptic plasticity. Accordingly, Komai et al. showed that neuropsin has a regulatory effect on Schaffer-collateral long term potentiation (LTP) [24], which is important for the acquisition of hippocampus-associated memory. LTP consists of two phases – the temporary early LTP (E-LTP) and the long-lasting late LTP (L-LTP). Whereas L-LTP requires protein kinase A (PKA) activation leading to

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altered gene expression and novel protein biosynthesis [25], E-LTP comprises an interaction between the ECM and synaptic membranes and thus mechanically modulates synaptic plasticity. It requires N-methyl-D-aspartate (NMDA) receptor and calcium/calmodulin dependent protein kinase II (CAMKII) activation, which in turn increases the number of AMPA receptors at synapses [26]. Post-synaptic NMDA receptor activation following synaptic stimuli in E-LTP has been shown to induce a rapid activation of the precursor form of neuropsin [21] by removal of an 4-amino acid activity masking peptide (QXXK) [14]. By analysing *neuropsin* mRNA levels in mice, an induction of *neuropsin* expression was also observed in response to focal and generalized amygdaloid kindling, a commonly used model for the development of seizures and epilepsy [1, 27]. In this model epilepsy is provoked by repeated electrical stimulation of the brain leading to the development of a predisposition to seizures.

Active neuropsin cleaves the extracellular domain of L1CAM and produce a neuropsin specific 180 kDa fragment [21, 28] (depicted in **Figure 4**). The function of this neuropsin specific L1CAM fragment has not been investigated yet, but the role of cell adhesion molecules in synaptic plasticity [29-32] suggests that neuropsin-specific L1CAM cleavage decreases synaptic adhesion and subsequent increases the flexibility of synaptic structures. Neuropsin is involved in the synaptogenesis of L1CAM expressing orphan and small synaptic boutons in pre-synaptic membranes of the Schaffer-collateral pathway in the hippocampal CA1 substructure [28]. Collectively, this implies that neuropsin-dependent L1CAM processing might modulate activity-dependent structural changes involved in E-LTP.

In line with these studies, E-LTP and thus memory acquisition, but not memory retention, is significantly impaired in *neuropsin* knock-out (KO) mice [33]. Furthermore, *in vivo* inhibition of neuropsin by a specific inhibitor or neutralizing antibody was shown to impair E-LTP, but not neurotransmitter release from the pre-synapse [33]. The same study shows that recombinant neuropsin, delivered in mouse hippocampus, leads to phosphorylation of the glutamate ionotropic receptor AMPA type subunit 1 (GLUR1) at two specific sites: Ser831, a CAMKII site and Ser845, a PKA site (depicted in **Figure 4**). Phosphorylation of Ser831, an E-LTP specific change, was provoked by a low dose (0.18 mU ml^{-1}, 60 min application) of recombinant neuropsin, whereas a high dose (3.6 mU ml^{-1}, 60 min application) of recombinant neuropsin provoked phosphorylation of Ser845, constituting a long-term depression (LTD) specific change. Neuropsin thus modulates synaptic plasticity in the described pathway as an upstream regulator of AMPA receptor phosphorylation [33]. Ishikawa et al. suggested that

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neuropsin, based on its potential of extracellular modulation, might be important for the acquisition of memory depending on input strength. Their study in *neuropsin* KO mice shows that neuropsin facilitates the association of two synapses in the apical and basal dendrites of CA1 pyramidal neurons, enabling a weak stimulus, normally producing E-LTP resulting in synaptic persistency, when being associated with a separate strongly stimulated pathway [34]. This phenomenon is referred to as synaptic tagging and facilitates the transformation of E-LTP to L-LTP without inducing novel protein biosynthesis. Instead synaptic tagging might capture proteins delivered from transcription and translation sites. Neuropsin signaling is shown to be engaged in local synaptic capture, acting upstream of integrin β1, a receptor involved in cell adhesion and CAMKII signaling [34].

Another pathway through which neuropsin may contribute to the modulation of synaptic plasticity involves NRG1, its receptor ErbB4 and GABAergic transmission in the CA1-3 subfields of the hippocampus [20] (depicted in **Figure 4**). It was shown that neuropsin, activated by neuronal activity as described above, cleaves NRG1, which is localized extracellularly in synaptic elements, at three distinct sites. This results in the removal of the heparin binding domain of NRG1 and releases mature NRG1 from the extracellular matrix. Mature NRG1 binds to its receptor ErbB4, which consequently gets phosphorylated [20]. ErbB4 is expressed in parvalbumin-positive GABAergic interneurons within the CA1 region. These neurons project inhibitory synapses into hippocampal pyramidal neurons. Impairment of GABAergic transmission consequently leads to excessive post-synaptic excitation, which prevents the induction of NMDA receptor dependent LTP [35]. In line with this finding, it has been shown by KO studies in mice that loss of neuropsin in the hippocampus predisposes the animal to global seizure activity [36]. Neuropsin deficiency impairs the activation of ErbB4 positive GABAergic interneurons (**Figure 5**), resulting in hyper-activity of pyramidal neurons in the stimulated state and breakdown of the neuronal excitation–inhibition balance in hippocampal networks [20]. Supporting this theory, a recent study shows that reduced levels of active neuropsin are observed in CA1-CA3 subfields of the hippocampus, dentate gyrus, extrahippocampal temporal lobe and parietal cortex during spontaneous seizures in kainic acid-induced status epilepticus rats [37].

In summary, neuropsin substrate cleavage modulates pre- as well as post-synaptic elements regulating synaptic plasticity. Impaired neuropsin levels, both increased and decreased, may thus negatively affect synaptic plasticity and hereby learning, memory formation and cognition.

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NEUROPSIN IN MENTAL DISORDERS:

Bearing the neurobiology of neuropsin in mind, it is not surprising that unbalanced neuropsin levels have been implicated in several mental disorders (schizophrenia (SZ) and bipolar disorder (BD) [38], depression (D) [39-41], Alzheimer's disease (AD) [42, 43] as well as anxiety (ANX) [22, 44]. **Table 1** contains a descriptive overview of clinical and preclinical studies, in which neuropsin was associated to mental health phenotypes.

A genetic polymorphism screening of the entire *neuropsin* gene in 24 SZ cases identified a total of 28 single nucleotide polymorphisms (SNPs), including nine novel SNPs, of which only one resulted in an amino acid change in exon 6 (G5047>A, Val286Ile) [38] (location depicted in **Figure 2B**). This missense mutation is located at an evolutionarily conserved residue but was found in only one out of the 24 SZ cases. Val286Ile was not detected in any of 178 SZ cases in a follow-up study, leading the authors to the conclusion that this missense mutation constitutes a rare variant [38]. This is in line with the Exome Aggregation Consortium (ExAC) database reporting an allele frequency lower than 0.001% for the Val286Ile variant [45]. In total the ExAC database reports 95 observed missense variants and 6 loss of function (LOF) variants in *neuropsin* and an increased intolerance to variation (z-score=0.57), but no intolerance against LOF variants (pLI=0.00). There is thus a low probability that *neuropsin* falls into the haploinsufficient category. Of the 24 SNPs examined in the SZ case-control study, 5 were additionally assessed in major depressive disorder (MDD) and BD to examine a potential genetic correlation between these disorders [38]. Those SNPs were chosen according to their location in the 5´ untranslated region, close to a human-specific splice site in exon 3, resulting in the brain specific type 2 neuropsin and in the 3´ untranslated region of the gene. A significant difference in genotype distribution, as well as allele frequencies, was found between BD patients and controls for SNP rs1722550 located in the 5´ regulatory region (P=0.019) (location depicted in **Figure 2B**). The influence on the transcriptional activity of this SNP was investigated in a promotor assay in rat cultured cortical neurons and revealed no effect on neuropsin transcription. Another significant differently distributed SNP (P=0.018) was located close to the human-specific splice site in exon 3 (rs1701946) (location depicted in **Figure 2B**). Since type 2 neuropsin is human and brain specific, this SNP might affect memory and cognition in human. The most significant difference in genotype distribution was found for SNP rs1612902, located in the 3´ untranslated region (P=0.0015) (location depicted in **Figure 2B**). Interestingly healthy individuals carrying the bipolar disorder risk allele of SNP rs1612902

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showed a lower score in attention/concentration (assessed with WMS-R, P=0.016) and verbal IQ (assessed with WAIS-R, P<0.001) [38].

Assessment of *neuropsin* mRNA levels in peripheral blood revealed significantly higher neuropsin expression in 186 patients diagnosed with major recurrent depression (MRD) (ICD-10, F32.0-7.32.2, F33.0-F33.8) compared to 105 healthy subjects [40]. Furthermore, it was shown that *neuropsin* mRNA is significantly more abundant in blood samples from patients affected by MRD, compared to patients suffering from first episode depression (severity: 21-item Hamilton Depression Rating Scale (HDRS)). The observed increase in *neuropsin* expression was associated with diminished interpersonal abilities in depressive patients [41]. An association between depression symptomatology score in the general population and blood DNA methylation levels in the promoter region of *neuropsin* was recently identified in a large cohort of monozygotic Danish twins [46], supporting the implication of neuropsin in depression symptomatology. Similarly, a study investigating *neuropsin* expression levels in the neurodegenerative mental disorder AD revealed an 11.5 fold increase in *neuropsin* mRNA levels in the hippocampus of AD patients compared to controls [42].

Psychophysiological stress is known to affect synaptic plasticity, such as memory formation and learning and thus provokes behavioral phenotypes as depression and anxiety. Interestingly, mice exposed to acute or chronic stress show increased *neuropsin* mRNA expression in hippocampal tissue accompanied by depressive-like behavior [39]. This upregulation of *neuropsin* after stress exposure was shown to be dependent on the stress hormone corticosterone in primary cultured hippocampal neurons as well as *in vivo* [39, 47]. Corticosterone exposure causes impairment in spatial memory, neurogenesis, dendritic morphology and demyelination in WT mice, while mice lacking *neuropsin* (KO mice as well as knockdown of neuropsin by viral vectors) exhibit a protective effect on those as well as on the development of depressive-like behavior [39]. In line with this, overexpression of *neuropsin* by viral vectors leads to an increased impairment in spatial memory and depressive-like behavior. A protective effect of *neuropsin* inactivation could be explained by a reduction of reactive oxygen species (ROS) production resulting from prolonged exposure to high levels of plasma corticosterone [39], since KO mice exhibit a reduced response to corticosterone treatment compared to WT mice, in terms of hippocampal neuronal activity. Furthermore, *neuropsin* KO mice exposed to glucocorticoids exhibit higher levels of excitatory amino acid transporter 1 gene expression compared to WT mice, promoting glutamate reuptake into the

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synaptic cleft and thus rescuing an imbalance of glutamate transmission caused by high levels of plasma corticosterone [39]. In further support of a regulatory role of stress on *neuropsin* expression, neuropsin plays a critical role in stress-related plasticity in the amygdala [22]. Stress-induced neuropsin-dependent EphB2 cleavage results in a 70 kDA extracellular EphB2 fragment and leads to the dissociation of EphB2 from the NR1-subunit of the NMDAR (depicted in **Figure 6**). This changes the dynamics of the EphB2–NMDA-receptor interaction in mice and enhances the NMDAR current. An increased NMDAR current in turn leads to *Fkpb5* upregulation and ANX-like behaviour [22]. Neuropsin KO mice, however, are protected against stress-induced EphB2 cleavage resulting in a static EphB2–NMDA-receptor interaction, weakened *Fkbp5* induction and low ANX [22]. Fkbp5 acts as a co-chaperone that modulates glucocorticoid receptor activity by reducing its affinity of glucocorticoids in response to stressors and is known for its role in stress adaption. Fkbp5 has been implicated in the pathogenesis of stress-related psychiatric disorders, like ANX, depression and posttraumatic stress disorder (PTSD) [48-53]. Interestingly, a study investigating neuro-behavioural consequences of acute rapamycin treatment, an antiproliferative and immunosuppressive drug, reported upregulated *neuropsin* and *Fkbp5* expression together with ANX-like behaviour and enhanced neuronal activity in the amygdala of rats, compared to vehicle-treated controls [44]. This underlines the involvement of the neuropsin-EphB2-Fkbp5 pathway in the ANX phenotype.

In summary neuropsin has been implicated in the pathology of various mental disorders (SZ, BD, D, ANX and AD) on the genetic and expression level in clinical and preclinical studies.

 doi:10.20944/preprints201905.0360.v1

CONCLUSION:

Accumulating evidence suggests that the serine protease neuropsin, plays a key regulatory role in human neurodevelopment and mental health. Brain specific splicing of *neuropsin* mRNA is only reported in humans and its expression and activation characteristics links it to neuronal activity and the neuromolecular response to stress. It possesses proteolytic specificity towards synaptic proteins and pre- and postsynaptic neuropsin-dependent substrate cleavage are implicated in synaptic plasticity and maintenance of excitation-inhibition balance in the hippocampus. Neuropsin dysregulation is associated with various mental disorders and plays a role in stress-mediated synaptic plasticity in the hippocampus and amygdala. It is thus conceivable that neuropsin may serve as a biomarker for certain aspects of mental illness.

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 doi:10.20944/preprints201905.0360.v1

COMPETING INTERESTS STATEMENT:

The authors report no biomedical financial interests or potential conflicts of interest.

 doi:10.20944/preprints201905.0360.v1

Figure legends

Figure 1 | *Neuropsin* expression across lifespan in human. Shown is the expression of *neuropsin* in log2 RPKM across lifespan (from postconceptional week (pcw) 8 to year 40 for females and year 37 for males) in human in various brain tissues. In cortical and limbic tissues *neuropsin* mRNA is most abundant postnatally and peaking in childhood and adulthood. Data obtained from www.brainspan.org.

Figure 2 | Genomic structures of human *neuropsin* transcripts and sites associated with mental disorders. A) The kallikrein related peptidase 8 gene (*KLK8*) encoding neuropsin spans 5.4 kb and is located at the long arm of chromosome 19 in human (19q13.4). The *neuropsin* gene comprises 6 exons of which the first is non-coding [15] and *neuropsin* cDNA contains a single open reading frame of 780 bp, resulting in a protein comprising 260 amino acids [1, 15]. Shown are exons (boxes) and their length in base pairs, approximate amino acid locations of the characteristic catalytic triad of serine proteases (H, D, and S) as well as start codon (arrow) and stop codon (star). The base pair sequence of exon 3 is shown in upper-case letters (numbers indicate position in coding sequence), parts of the introns surrounding exon 3 in lower-case letters. The base pair sequence specific for type 2 *neuropsin* is highlighted in grey and contains the 79 T<A point mutation [11] (red box). **B)** Depicted are by Izumi et al. identified single nucleotide polymorphisms associated with MDD and missense mutation identified in schizophrenia [38], as well as the CpG site identified in depression symptomatology [46](yellow arrows).

Figure 3 | A) Human neuropsin preproprotein structure [12]**.** Shown are the signal sequence (S), containing either the type 1 specific amino acid sequence (T1) or type 2 specific amino acid sequence (T2), the activity masking peptide (Q) (QGSK in human and QDEK in mice) and the approximate amino acid locations of the characteristic catalytic triad of serine proteases (H, D, and S) **B) Human neuropsin preprotein amino acid sequence** showing endoprotease splice sites [14] (red line). Type 2 neuropsin shows a novel splice site in the type 2 specific amino acid sequence [17] which is highlighted in grey (red arrow).

Figure 4 | Neuropsin pathways implicated in synaptic plasticity. When an action potential arrives at the pre-synapse, this signal will be transmitted to the post-synapse by activating the NMDA receptor (NMDAR). NMDAR activation leads to the removal of the activity masking peptide of proneuropsin (light red) and results in neuropsin activation [21]. Active neuropsin (dark red) then cleaves (red arrow) its substrate L1CAM (blue) [21], and NRG1 (green) [20]. L1CAM cleavage might lead to an increased flexibility of synaptic structures. After removal of the heparin binding domain (dark green, Hb) mature NRG1 (mNRG1, light green) binds to its receptor ErbB4 (grey), which consequently gets phosphorylated (yellow circle). ErbB4 phosphorylation increases GABAergic inhibitory transmission. Thus neuropsin-dependent NRG1 cleavage modulates E-LTP through the modulation of inhibitory projections into CA1 pyramidal cells in the hippocampus [20]. Recombinant neuropsin furthermore leads to phosphorylation of the AMPA receptor (AMPAR) at two distinct sites, one phosphorylation site specific for E-LTP and one specific for LTD [33]. Illustrations partly taken from https://smart.servier.com/, picture of mice brain taken from [54].

Figure 5 | Impaired neuropsin - NRG1 - ErbB4 pathway in *neuropsin* KO mice. In WT mice active neuropsin (dark red) cleaves (red arrow) its substrate NRG1 (green) [20]. After

 doi:10.20944/preprints201905.0360.v1

removal of the heparin binding domain (dark green, Hb) mature NRG1 (mNRG1, light green) binds to its receptor ErbB4 (grey), which consequently gets phosphorylated (yellow circle). ErbB4 phosphorylation leads to increased GABAergic inhibitory transmission onto CA1 pyramidal cells. In *neuropsin* KO mice however, NRG1 does not get cleaved and consequently mNRG1 cannot bind to its receptor ErbB4 [20]. Thus, GABAergic interneurons do not project inhibitory synapses onto CA1pyramidal cells, leading to hyper-excitability of hippocampal pyramidal neurons and break down of the neuronal excitation–inhibition balance in hippocampal networks. Illustrations partly taken from https://smart.servier.com/.

Figure 6 | Neuropsin - EphB2 - Fkbp5 pathway implicated in stress-induced ANX. In a healthy brain synaptic excitation leads to EphB2 and NMDAR clustering. Hereby interacts EphB2 with the NR1-subunit of the NMDAR. Stress however results in increased neuropsin expression and neuropsin-dependent extracellular cleavage of EphB2 in the amygdala, resulting in the dissociation of EphB2 from the NR1-subunit [22]. This dynamic EphB2/NR1 interaction enhances NMDA receptor current and induces *Fkbp5* gene expression. *Fkbp5* gene expression enhances behavioral signs of ANX. Illustrations partly taken from https://smart.servier.com/.

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Tables and figures

Table 1: Descriptive overview of studies, in which neuropsin was associated to mental health phenotypes.

Phenotype	Organism	Tissue	Parameters assessed	Main finding	Reference
Schizo-phrenia	Human	Peripheral blood	**Singe nucleotide polymorphisms**	Val286Ile missense mutation in exon 6 detected in a patient with schizophrenia	Izumi, Iijima et al., 2008
Bipolar disorder	Human	Peripheral blood	**Singe nucleotide polymorphisms**	Significant allelic association between several SNPs and bipolar disorder	Izumi, Iijima et al., 2008
Depression	*Neuropsin* KO mice	Hippocampus	**Expression** of *neuropsin* following stress, depressive-like **behaviour** of KO mice after stress exposure and after corticosterone injection	KO mice are protected against development of depressive-like behaviours	Chang, Bok et al., 2016
	Human	Peripheral blood	**Expression** levels (mRNA) of *neuropsin*, patients diagnosed with major recurrent depression and first episode depression	Higher *neuropsin* expression in patients with recurrent depression compared to first episode patients	Talarowska, Bobinska et al., 2016
	Human	Peripheral blood	**Expression** levels (mRNA) of *neuropsin*, patients diagnosed with major recurrent depression and healthy subjects who have never been treated psychiatrically	Higher *neuropsin* expression in patients with depression compared to controls	Bobinska, Mossakowska-Wojcik et al., 2017
Alzheimer's disease	Human	Hippocampal and parietal cortex	**Expression** of *neuropsin* in Alzheimer's disease (AD) and control tissue	11.5-fold increase in *neuropsin* mRNA levels in AD hippocampus compared to controls	Shimizu-Okabe, Yousef et al., 2001
Anxiety	*Neuropsin* KO mice	Amygdala	*Neuropsin-EphB2-Fkpb5* signaling in anxiety	Neuropsin is critical for novel neuronal pathway linking stress-induced plasticity in the amygdala to anxiety	Attwood, Bourgognon et al.,2011

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Figure 1

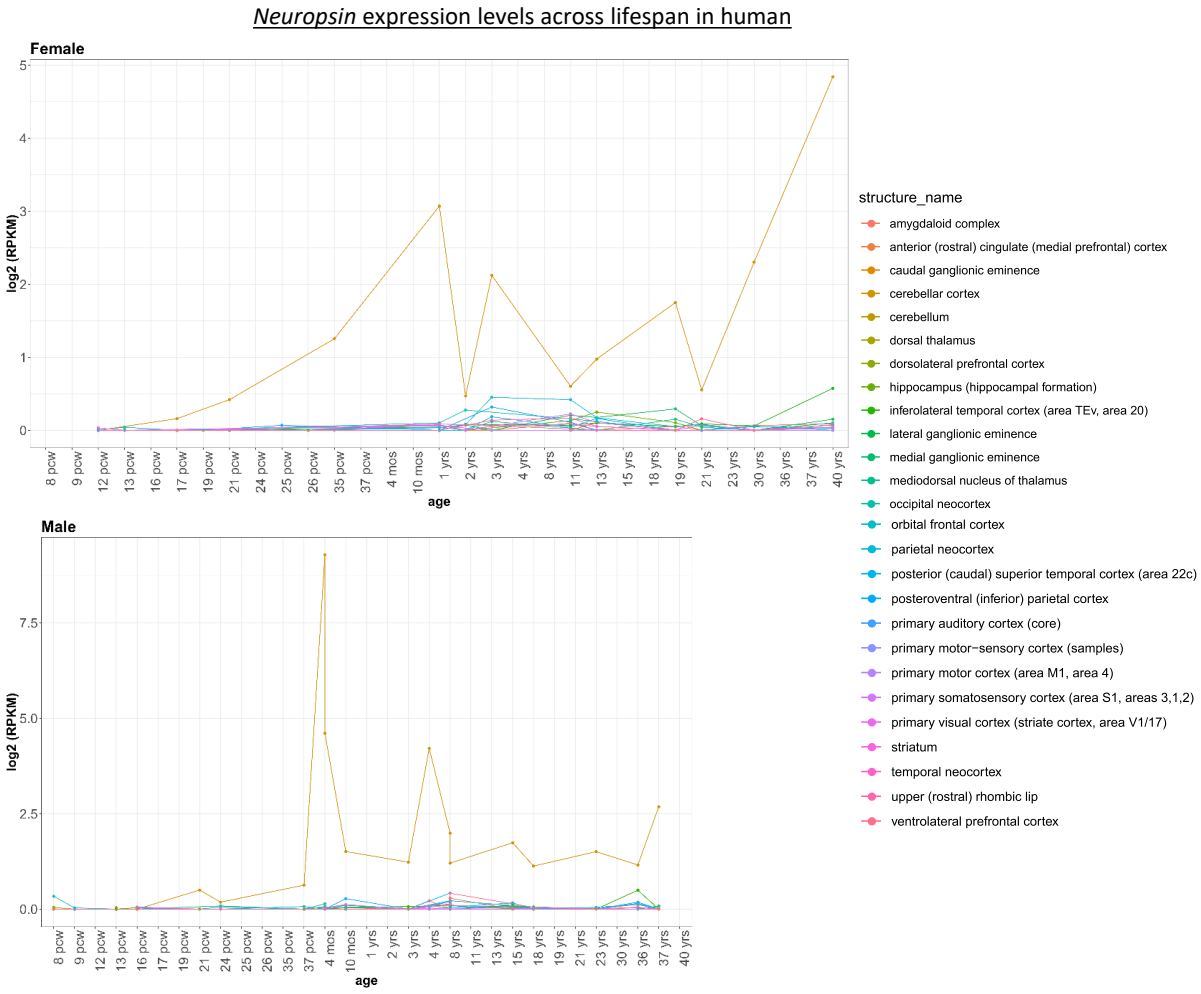

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Figure 2

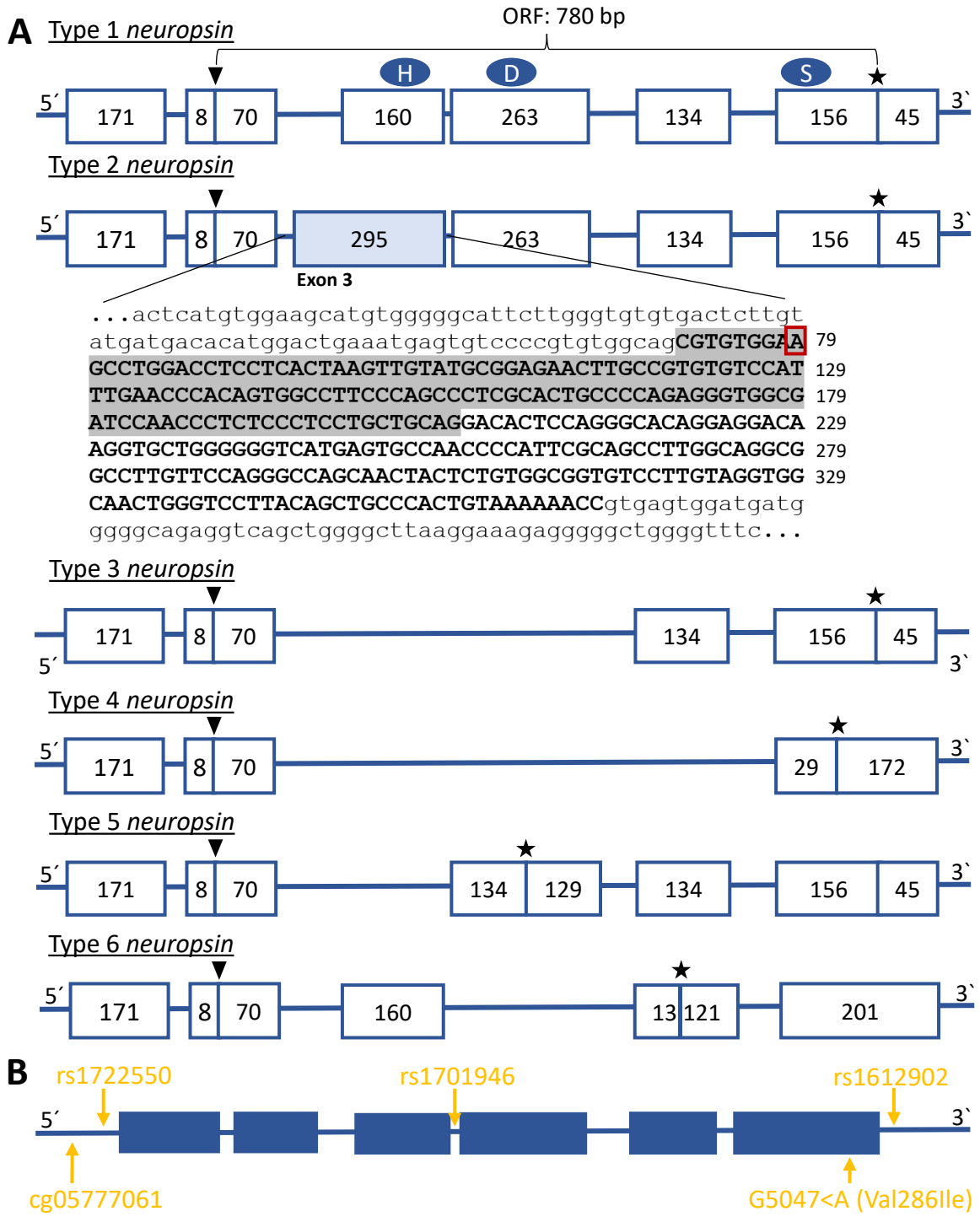

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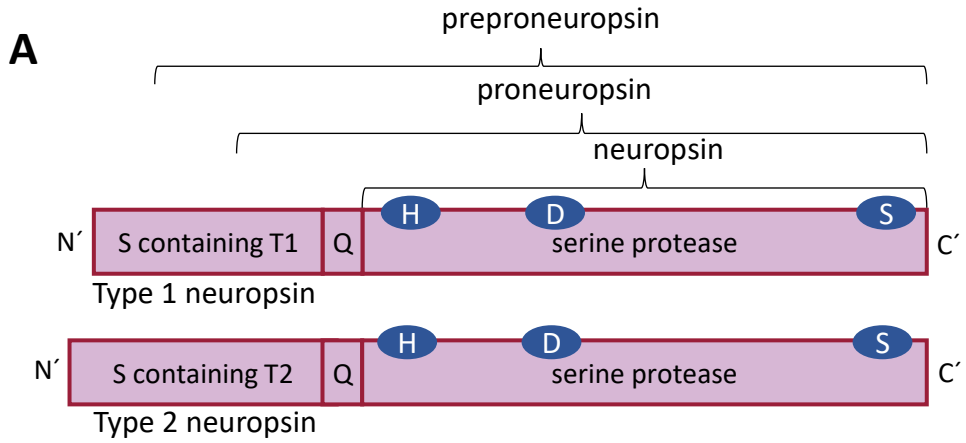
Figure 3



B

Type 1 neuropsin (260 amino acids)

MGRPRPRAAKTWMFLLLLGGAWAGHSRAQEDKVLGGHECQPHSQPWQAALFQGQQLLCGG
VLVGGNWVLTAAHCKKPKYTVRLGDHSLQNKDGPEQEIPVVQSIPHPCYNSSDVEDHNHD
LMLLQLRDQASLGSKVKPISLADHCTQPGQKCTVSGWGTVTSPRENFPDTLNCAEVKIFP
QKKCEDAYPGQITDGMVCAGSSKGADTCQGDSGGPLVCDGALQGITSWGSDPCGRSDKPG
VYTNICRYLDWIKKIIGSKG

Type 2 neuropsin (305 amino acids)

MGRPRPRAAKTWMFLLLLGGAWAACGSLDLLTKLYAENLPCVHLNPQWPSQPSHCPRGWR
SNPLPPAAGHSRAQEDKVLGGHECQPHSQPWQAALFQGQQLLCGGVLVGGNWVLTAAHCK
KPKYTVRLGDHSLQNKDGPEQEIPVVQSIPHPCYNSSDVEDHNHDLMLLQLRDQASLGSK
VKPISLADHCTQPGQKCTVSGWGTVTSPRENFPDTLNCAEVKIFPQKKCEDAYPGQITDG
MVCAGSSKGADTCQGDSGGPLVCDGALQGITSWGSDPCGRSDKPGVYTNICRYLDWIKKI
IGSKG

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Figure 4

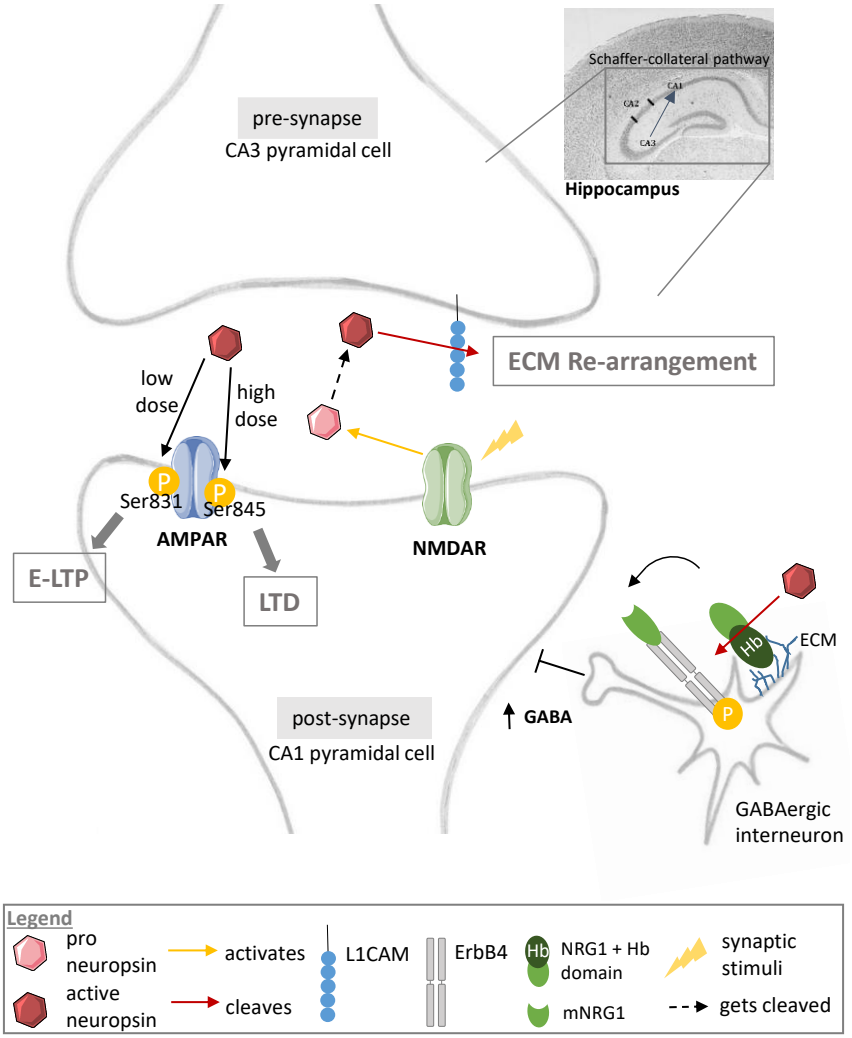

Figure 5

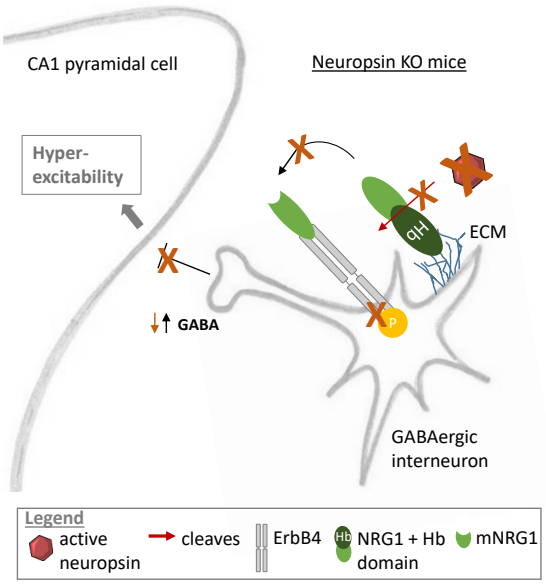

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Figure 6