Prefrontal gene expression changes in mood disorders and suicide

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CHAPTER 8

SUMMARY AND GENERAL DISCUSSION

SUMMARY OF MAIN FINDINGS

The research described in this thesis was centered on two questions. Firstly, we investigated whether suicide, as specific entity and as part of the symptomatology of depressive disorder, is characterized by specific neurotransmitter changes in the prefrontal cortex (PFC). To this end, we studied two independent patient cohorts: (i) the Stanley Medical Research Institute (SMRI) cohort, comprising patients who were diagnosed with major depressive disorder (MDD) and who had accomplished suicide, and (ii) a Netherlands Brain Bank (NBB) cohort, consisting of elderly MDD and bipolar disorder (BD) patients who had died of non-suicidal causes. Secondly, we investigated possible differences in molecular changes in the PFC, between Alzheimer's disease (AD) cases with major depression as comorbidity, and patients suffering from MDD per se. We also used the APP/PS1 double-transgenic mouse model of AD, which overexpresses mutated forms of the human amyloid precursor protein (APP), and presenilin 1 (PS1), to test the hypothesis that molecular changes in the frontal cortex may induce hypothalamic-pituitary-adrenal (HPA) axis hyperactivity in AD.

In **Chapter 1**, we first provide a general introduction on the stress system and mood disorders, including the anatomy and general physiology and the interaction of the HPA axis and PFC, and the interaction between HPA and PFC changes in relation to mood disorders, such as MDD and BD, suicide and depression in AD. In addition, neurotransmitters involved in these mood disorders are discussed and the main aims and outline of this thesis are presented.

In **Chapter 2**, we briefly discuss possible reasons for the discrepancies between patients with depression and those that have committed suicide based on the available literature.

In **Chapter 3**, we set out to answer the first question mentioned above and used real-time quantitative PCR (Q-PCR) to determine the expression of genes related to two main neurotransmitter systems, i.e. gamma-aminobutyric acid (GABA) and L-glutamatic acid (glutamate). In the NBB cohort, we studied the anterior cingulate cortex (ACC) and dorsolateral PFC (DLPFC) of elderly patients with MDD and of patients with BD who did not commit suicide. In this cohort, we found that only expression for GABA-A receptor beta-2 (GABRB2) and for post-synaptic density-95 (PSD-95) were significantly decreased in the ACC, but not the DLPFC. In follow-up studies in the SMRI cohort, we found that depressed patients who had committed suicide (MDD-S), had a different expression pattern

with respect to GABA and glutamate than those who died of causes other than suicide (MDD-NS) (**Chapter 4**). Similar comparisons were made for monoamines and nitric oxide (NO) (**Chapter 5**) and glial markers (**Chapter 6**).

In **Chapter 4**, we describe an enhanced expression of GABA/glutamate related genes in the ACC in the MDD-S group, while transcript levels in the MDD-NS group from the SMRI cohort were decreased. Moreover, in the DLPFC, there was a decrease in gene expression in the MDD-S compared to MDD-NS patients, while both groups showed increases in gene expression compared with control subjects.

In **Chapter 5**, we report that the expression levels of corticotropin-releasing hormone (CRH) and neuronal nitric oxide synthase (NOS)-interacting DHHC domain-containing protein with dendritic mRNA (NIDD) were increased in the ACC of the MDD-S group. Other changes, i.e. decreased NIDD and increased 5-hydroxytryptamine receptor 1A (5-HT1A) expression levels, were only present in the NBB depression group. These changes were more pronounced in the ACC than in the DLPFC.

In **Chapter 6**, we report that in MDD-S patients, a significant increase was found in the ACC for EAAT3, EAAT4, ASCT1, SNAT1, SNAT2 levels, which are neuronal components of the glutamate-glutamine cycle. The components located in the astroglial compartment (i.e. EAAT1, EAAT2 and GLUL) on the other hand, showed a significant decrease. In contrast, within the DLPFC, nearly all these components were significantly increased.

Concerning the second question of this thesis, we showed that the GABRA5 mRNA level was significantly decreased in the DLPFC of depressed AD patients. Moreover, vGluT1 mRNA levels were positively correlated to the Cornell score for depression severity. However, these alterations were different from those observed in patients suffering from MDD alone. In the APP/PS1 mice, there were no GABA or glutamate-related gene expression changes, and no comparison could be made to those observed in depressed AD patients. Furthermore, the HPA axis was not activated in APP/PS1 mice under basal conditions. No difference in sucrose preference, an indication for depressive-like behaviour, was found either (**Chapter 7**).

In conclusion, based on the results described in this thesis, we speculate that mood disorders and suicide are two different entities, while mood disorders *per se*, and depression in AD are also different when it comes to specific molecular alterations. Later on in the Discussion we will give some suggestions for further research.

Severe mood disorders and suicide: two different disorders?

Suicide is a cause of death persistently associated with mood disorders (Joiner et al., 2005; Knorr et al., 2016), and there is a strong relationship of suicide ideation with mood disorders, which in fact is one of the DSM criteria for depression (Vannoy et al., 2007). However, we found that some neurobiological factors seem to contribute to suicide *per se* that are independent of the psychiatric disorders (see before in Summary of main findings).

The availability of appropriate brain collections has provided a unique opportunity to investigate morphological and chemical alterations in diseased brain tissue for molecular details that neither *in vivo* neuroimaging nor animal models could offer. Post-mortem studies therefore represent an essential complementary source for the understanding of specific molecular aspects of the neurobiology of psychiatric disorders. So far, post-mortem studies on mood disorders and suicide have often been inconsistent. One critical issue for interpreting this inconsistencies is the different case-control matching strategies. For instance, studies that have claimed to determine molecular alterations in relation to *depression* had often selected mood disorder patients who had committed suicide, who were then compared with controls who did not suffer from a psychiatric disease, and had not committed suicide. This may be due, at least partly, to the fact that suicidality is generally perceived as a medical complication and an integral aspect of depression, rather than a disorder in its own right. Indeed, suicide was only listed as a symptom of psychiatric disorders in the DSM-4, while the DSM-5 still does not code suicidal behaviour, although it is the most prominent emergency in psychiatry (Aleman and Denys, 2014).

On the other hand, the studies that claimed to show changes in relation to suicide per se appeared to compare suicide cases to matched controls without any psychiatric disorder, thus disregarding the fact that the great majority of suicides suffered from a psychiatric disorder (Hawton and van Heeringen, 2009; Knorr et al., 2016). Most suicides occur in mood disorder patients. However, suicide attempts and accomplished suicide only counts for a small percentage in these patients (Beck and Steer, 1989; Cullberg et al., 1988). While the presence of mental illness is a major risk factor for suicide, other factors, such as psychosocial crises and notable life events, also play a role (Cavanagh et al., 1999; Hawton and van Heeringen, 2009; McClatchey et al., 2017; Pandey, 2013).

Our studies showed that GABA and glutamate-related genes, the neuronally-located components of the glutamate-glutamine cycle, were increased in the ACC

of MDD-S patients as compared to MDD-NS patients and non-psychiatric control subjects (Zhao et al., 2016, Chapter 6). In contrast, both GABA and glutamate-related genes were lower in the depressed patients who did not commit suicide, confirming our earlier study (Zhao et al., 2012, Chapter 3). In another brain area, DLPFC, GABA and glutamate-related genes, astroglia located components were found to be decreased in the MDD-S patients as compared to MDD-NS subjects (Zhao et al., 2016, Chapter 6). Consistent with this, in another study on CRH and serotonin related genes in the same patient cohort. We have also shown that especially CRH and NIDD were associated with suicide, whereas an increase in 5-HT1A and rather a decrease in NIDD was associated with the presence of depression (Zhao et al., 2015, Chapter 5). These data suggest that depression and suicide have different gene expression patterns in various neurotransmitter systems, which suggests that suicide may be an independent disorder.

Ketamine and other NMDA antagonists:

In **Chapter 4**, we reported increased AMPA-receptor subunit and NMDA-receptor subunit expression in the ACC of MDD-S patients. Further studies are needed to establish whether selectively suppressing ACC activity by targeting glutamatergic synapses locally, could be used as a therapeutic approach towards the prevention of suicide. Ketamine is a NMDA receptor antagonist that was used as anesthetic compound, but was abandoned because of its serious side effects, such as hallucinations (Strayer and Nelson, 2008). The emergence of intravenous ketamine therapy has been celebrated as perhaps the "most important breaking through in antidepressant treatment in a decade" (Insel, 2014). Studies have shown that antidepressant responses to ketamine started at 3 to 4 hours post-infusion across all open-label investigations and controlled trials N = 101. The antidepressant response observed to ketamine was very similar to the effects achieved by giving classic antidepressants for six weeks (aan het Rot et al., 2010; aan Het Rot et al., 2012). More important, this rapid antidepressant effect is also true for patients with treatment-resistant depression (Fekadu et al., 2009; Nemeroff, 2007). Furthermore, ketamine decreased suicidal ideation with treatment-resistant depression within 40 minutes, and this decrease remained significant throughout the first 4 hours post-infusion. This indicates that a decrease in suicidal ideations is one of the earliest and most prominent effects of this drug (DiazGranados et al., 2010). Recent pilot studies demonstrated that suicidal thoughts in BD were less (trend significant) after ketamine than after midazolam infusion (Grunebaum et al., 2017).

The mechanism as to how exactly ketamine infusion can produce glutamatergic activation of AMPA receptors remains obscure. Recent animal studies have shown that, by blocking the NMDAR effect in the lateral habenula (LHb), ketamine exerts an antidepressant effect. This suggests that ketamine, by modulating NMDARs, may quickly elevate mood due to a disinhibition of reward centres, such as the LHb (Yang et al., 2018). Studies suggest that NMDA receptor antagonists, such as ketamine, trigger glutamate release from presynaptic terminals, which causes more glutamate to bind to AMPA receptors (Adams and Moghaddam, 2001; Moghaddam et al., 1997; Moghaddam and Adams, 1998). It might also be a metabolite of ketamine called hydroxynorketamine (HNK) whose antidepressant action is to increase the levels of AMPA-receptors at synapses, thereby enhance neural activity (Zanos et al., 2016). The necessity of AMPA activation implies that ketamine induces synaptogenesis by increasing glutamate signalling rather than by protecting neurons from glutamate excitotoxicity (Newport et al., 2015). The linkage between NMDA antagonism and AMPA receptor activation is a crucial point here, as this might be helpful for clarifying the neuroprotective of ketamine in some contexts (Brunson et al., 2001; Yan and Jiang, 2014) but potentially neurotoxic in others (Yan and Jiang, 2014; Yan et al., 2014; Zuo et al., 2014). This dual potential of ketamine must be considered when contemplating its therapy in the clinical setting.

TECHNICAL CONSIDERATIONS

Human postmortem brain material: practical limitations

As Dr. Webster, director of the Stanley Foundation, mentioned in a review, that are inherent limitations to the use of postmortem human tissue that could confound the measurement of the biological effect of interest (Webster, 2006). Here, we discuss the potential issues that have to be taken into account when using human brain samples in general, with a special focus on human gene expression profiling studies. Firstly, age is a major factor that affects gene expression (Erraji-Benchekroun et al., 2005; McKinney et al., 2015). In addition, the pH of the tissue affects RNA integrity, yield and individual gene expression (Altar et al., 2005; Mexal et al., 2006). A long agonal state is associated with reduced brain pH levels (Hardy et al., 1985). For this reason most brain banks determine the pH of cerebrospinal fluid (CSF) at autopsy.

Gender also influences gene expression in certain brain areas (Cantuti-Castelvetri et al., 2007; Preece and Cairns, 2003), as does lateralization and PMD (Bamford et al., 2000; Tomita et al., 2004). For instance, it is well known that women are more vulnerable to depression than men. Also differences in race may confound gene expression (Drentea and Goldner, 2006). When designing studies to measure gene expression, equal numbers of cases and controls must be matched as a group for the individual variables. Alternatively, a matched-pairs design can be employed, where each case has a gender-matched control that also matches age, sex, race, PMD, pH, and hemisphere as closely as possible. In reality, the limited availability of postmortem material tends to be the reason for a group match. In our studies we have matched all these factors. Although some of the group sizes were rather small, they were well matched for possible confounders. The confounding variables can also be corrected for in the statistical analysis by including the variables as covariates in an ANCOVA analysis if the dependent variable is reasonably normally distributed. However, if one uses 6 extra covariates (age, sex, etc.), loss of resolution is a serious risk. This could be repaired by stepwise elimination of non-significant covariates.

Another major concern when using postmortem tissue is medication, particularly for studies on depression. This is not only because only very few depressed patients do not receive medication, but also because treatment of depression typically includes long-term administration of medication that may have strong effects on brain neurochemistry. Many antidepressant and other psychotropic drugs have been shown to alter gene expression (Gasso et al., 2017; Gonzalez et al., 2014; Nugent et al., 2013; Sibille et al., 2011). In general, there are several different ways to assess such medication effects, based on the review of McCullumsmith (McCullumsmith et al., 2015). For example, animal models are widely used, especially rodent models up to 4 weeks with medication administration (Duric et al., 2013; Gumuslu et al., 2013; Qiu et al., 2014). However, such a relatively short treatment in rodents does not resemble the human situation where drug treatments often last six months or longer (Preti, 2011; Tamminga et al., 1994). However, long-term treatment are limited due to the high cost and long experiment schedule. Similar to nonhuman primates, which are more suitable treatment models, this is also limited by the access to primate colonies as well as the huge cost (Eggan, 2008; Konopaske et al., 2008). Another approach might be to study patients who have stopped antidepressant medication before death. This might, however, not reverse long-term changes induced by these

types of medication that are often given for many years. A third way to probe for medication effects is to perform statistical analyses to see whether the data were affected by the medication. Even so, patient related factors, such as non-compliance, should be taken into account (Basil et al., 2006). Matching is also a possibility provided people without a psychiatric disorder in the control group get the same medication, which rarely occurs. In our NBB patient cohort, we have tried to select patients who did not take steroid medication for at least the last three months. The influence of medication on gene expression in depressed patients is a very important issue but complicated at the same time due to combinations of drugs, some of which may not even have been recorded. An appropriate statistical analysis would require a number of observations that by far exceeds the number of available patients in our studies.

RNA integrity

Several studies have suggested that RNA isolated from post-mortem human brain is stable (Cummings et al., 2001; Leonard et al., 1993; Yasojima et al., 2001). However, RNA integrity in fact depends on numerous factors such as the post-mortem interval, i.e. the duration of time elapsed between death of the patient and processing/fixation of the brain tissue, but also hypoxia and agonal state (Harrison et al., 1995; Li et al., 2004; Preece and Cairns, 2003; Ross et al., 1992; Walker et al., 2016; Yates et al., 1990). The introduction of the RNA Integrity Number (RIN) as a quantitative measure of RNA integrity allows standardization and a more systematic approach in quality control and inclusion/exclusion procedures for gene expression studies. Studies using Q-PCR based analyses usually make use of a cut-off RIN value of 5 to exclude suboptimal samples from analysis (Fleige et al., 2006). Others prefer a RIN value of 3.95 or higher (Weis et al., 2007). Our RNA had good RIN values (most of them are higher than 7), which surely helps to minimize the impact of integrity.

Difficulties in establishing protein changes

Quantitative, or real-time, PCR is frequently used to study gene expression changes in various cell types or specific tissues in relation to diseases. In the present thesis, we explored gene expression changes in the human prefrontal cortex of postmortem tissue of patients who suffered from depression, suicide or AD, and also made an effort to quantify, by means of Western blot, protein levels of those genes that showed clear mRNA expression changes across the different disease groups.

A study based on label-free liquid chromatography mass spectrometry showed that in depression patients, which were mostly composed of suicide victims, a hyperglutamatergic state was present in the anterior PFC (Gottschalk et al., 2015). However, with Western blotting, we could not find a significant difference at the protein level in 7 genes (4 from SMRI and 3 from the NBB AD patient cohort) that showed mRNA changes. Various other studies, too, showed that mRNA levels are often not predictive for protein levels, especially on the individual gene level. Indeed, independent of the cell or tissue type investigated, the most significant reported correlations of mRNA vs. protein concentrations centre around R^2 = 0.4 (De Sousa Abreu et al., 2009). Also users of microarrays report that protein expression changes correlate with mRNA data only in less than 50% of the cases (Chuaqui et al., 2002). Nevertheless, a study reported that when high quality mRNA is used, in particular high expression levels, it would improve the correlation between mRNA and protein (Ostlund and Sonnhammer, 2012). Diametrically opposed to these findings, however, is the report of Guo and colleagues, that high mRNA levels do not predict a better correlation between mRNA and protein levels (Guo et al., 2008).

There may be technical and/or biological reasons for the discrepancies between mRNA and protein levels. Both techniques used Q-PCR and Western blotting, which may introduce a substantial amount of variability to the data, even if the experiments are properly conducted. Small expression changes might therefore not be detectable. Post-transcriptional mechanisms might also be a key factor in explaining why the overlap between mRNA and protein levels is very limited. This may concern alternative splicing, polyadenylation, the interaction with stabilizing or destabilizing RNA-binding proteins and/or microRNAs, which influence mRNA half-lives and translation, and which in turn determine an increase or decrease of the corresponding protein levels (Pascale and Govoni, 2012). Changes in translation efficiency and regulation can lead to non-linear translation rates from mRNA to protein. Moreover, posttranslational modifications and protein turn-over rates can either lead to protein accumulation or to fast degradation. Post-transcriptional mechanisms further affect the relative quantities of mRNA and protein to different degrees (De Sousa Abreu et al., 2009) and the correlation of mRNA and protein might even vary for different individuals (Guo et al., 2008). Finally, mRNA is mainly present in the cytoplasm around the nucleus, while the protein changes may only be clear in the terminals of these neurons, which may even be localized in a different brain area. These factors make it very difficult to

predict protein levels based on mRNA data. Our group is currently performing proteomics in order to get an overview of the protein changes in mood disorders.

FUTURE PERSPECTIVES ON DEPRESSION AND SUICIDE RESEARCH

Changes in another brain area: hippocampus

HPA axis activation is a major and commonly found alteration in both mood disorder and suicide (Bao et al., 2008; Bao et al., 2012; Turecki, 2014; Turecki and Meaney, 2016). Several brain structures are involved in HPA axis regulation; apart from the ACC (MacLullich et al., 2006), the hippocampus also inhibits stress-induced HPA activation (Jacobson and Sapolsky, 1991), whereas the amygdala may enhance glucocorticoid secretion via the HPA axis (Herman et al., 2005).

Systematic review and meta-analyse have shown that depressed patients had significantly smaller hippocampal volume relative to control subjects (Geerlings and Gerritsen, 2017). However, there are only a few studies that have focused on hippocampal GABA and glutamate changes in MDD. Higher neuronal densities of glutamic acid decarboxylase (GAD), the key enzyme of GABA synthesis, were found in MDD patients (Bielau et al., 2007), and decreased glutamate receptor GluA1 expression was reported in the hippocampus of MDD patients (Law and Deakin, 2001). In one study, increased expression was found, particularly in tissue of BD patients, of the hippocampal alpha5-subunits of the GABA-A receptor (Dean et al., 2005). Since then, various microarray-based investigations have been performed on the hippocampus of suicide victims with and without major depression and in psychiatrically normal controls. These yielded a vast amount of expression changes (Sequeira et al., 2007), especially in the glutamatergic and GABAergic pathways (Sequeira et al., 2009). These studies suggest that GABA and glutamate-related changes in the hippocampus are suicide-related, but whether these changes are related to MDD per se or whether suicide also plays a critical role in these alterations in MDD patients remains unknown, and further studies are warranted.

Suicide in other diseases

In the ACC of suicide victims, we found pronounced gene expression changes, especially for GABA and glutamate-related genes (Chapter 4). Evidence from numerous empirical studies, suggests that over 90% of suicide victims have a

diagnosable psychiatric illness (Singhal et al., 2014; Wasserman et al., 2012; Whiteford et al., 2013). Apart from the two most prevalent psychiatric disorders related to suicide, MDD and BD, also schizophrenia and multiple sclerosis (MS) have a high risk for suicide (Kasckow et al., 2011). An important question for future research is thus whether our GABA and glutamate findings are limited to MDD and BD, or whether they are a general phenomenon in suicide per se. The NBB has collected tissue that would make it possible to study suicide in relation to MS in the future. Schizophrenia has a 4-5% life-time risk of suicide. This risk increases strongly after the onset of the first psychotic symptoms (Palmer et al., 2005). Also in multiple sclerosis, the risk for suicide is twice the risk in the general population. Here, young male patients early in the disease appear to have the highest risk (Feinstein and Pavisian, 2017). More specifically, in a cohort of 140 MS patients, 29% had suicidal ideations, although only 6% actually did a suicide attempt (Feinstein, 2002). The main risk for suicide in schizophrenia resides in depressive and affective symptoms, rather than psychosis, and the lifetime prevalence of depression in MS patients is well over 50% (Chwastiak and Ehde, 2007; Feinstein, 2002). Other studies have shown that the estimated prevalence of depression in MS has been 24% and the 1-year incidence in a clinical sample was 4% (Marrie et al., 2015; Patten et al., 2010).

Patients with other neurological diseases, such as epilepsy or Parkinson's disease, also run a higher risk of mood disorders and an increased risk of suicide (Lewis et al., 2014). A different group of patients that might be interesting to study in terms of specificity of the ACC changes, are patients who deem their life is complete and patients who ask for euthanasia or help with suicide.

Proteomics

Extensive gene expression data has been collected from postmortem brains on changes in neurotransmitter and pathways prior to a successful suicide attempt (Flory et al., 2017; Mann et al., 2001; Mann et al., 2009; Sequeira et al., 2009), but proteomic studies are still scarce. Our current gene expression data set suggests that suicide, rather than mood disorder, is related to alterations in GABA and glutamatergic transmission in the ACC, and that these two disorders have different gene expression patterns (Zhao et al., 2012; Zhao et al., 2015). Changes in functional peptidergic networks most likely contribute as at least one of the underlying causes of suicide, along with other molecular mechanisms.

Concerning the possible mechanisms involved, it might be that, in addition

to genetic factors (Padurariu et al., 2016), also epigenetic changes, such as hypermethylation of the ribosomal-RNA promoter and 5' regulatory region, could cause aberrant changes in protein synthesis in the suicide brain (Almeida and Turecki, 2016; McGowan et al., 2008). Also psychoactive drugs can change the risk of suicide, and there are other potential biomarkers for the prediction of suicidal behaviour (Coryell and Schlesser, 2007; Falcone et al., 2010; Hunter et al., 2010; Magno et al., 2010; McGuffin et al., 2010; Neves et al., 2010; Sudol and Mann, 2017).

In the past two decades, advances in proteomics have unravelled the intricate molecular cerebral processes and further advanced our neurobiological insights in the depressed and suicide brain (Robinson et al., 2009). Gottschalk et al. found that MDD and BD (the majority of these patients were suicide victims) were linked to a hyperglutamatergic state and hyperfunction of energy metabolism in the anterior PFC (Gottschalk et al., 2015), which supports our findings in the suicide patients. Our group is currently applying proteomics on the ACC and DLPFC of mood disorder patients to further study this possibility.

Imaging molecules in major depression and suicide

Imaging techniques can be used to visualize, characterize and measure molecular and cellular levels in living systems. Two techniques, namely magnetic resonance spectroscopy (MRS) and positron emission tomography (PET), help to shed light on psychiatric disorders. MRS is a non-invasive technique that allows the assessment of levels of biochemical metabolites in the brain, while PET allows radioligands to be injected into the bloodstream that bind, and can thereby visualize (the location and amount of), specific proteins or receptors in the brain. Studies using MRS have generally shown a reduction of GABA and glutamate/glutamine in the ACC of MDD patients (Bhagwagar et al., 2008; Hasler et al., 2007; Yuksel and Ongur, 2010), and their increased response to antidepressant (Brennan et al., 2017) and repetitive transcranial magnetic stimulation (Dubin et al., 2016), indicating a role for GABA and glutamate/glutamine in MDD. Suicidal ideation might be a promising aspect to further explore with MRS in this respect.

PET studies have shown differences between patients with mood disorder and controls with regard to their neurotransmitter systems (Schur et al., 2016), especially the serotonergic system (Drevets et al., 2007; Meltzer et al., 2004; Parsey et al., 2010; Saijo et al., 2010). However, contradictory results have been reported as well (Miller et al., 2009; Sullivan et al., 2009), indicating that these

results obtained with PET are insufficiently robust to be used for diagnostic purposes. Furthermore, the combination of imaging studies, such as fMRI and PET, could help to provide new insights into the functional changes implicated in the aetiology of mood disorders. A recent study that combined PET and MRS on the MDD patients showed an inverse relationship between mGluR5 availability and glutamate levels in the ACC (Abdallah et al., 2017). Obtaining data from multiple receptors, such as GABA, glutamate, serotonin etc., both in patients and in healthy volunteers, will be a promising future direction. Integrating clinical data with results on altered receptor functions may also identify new goals. Moreover, longitudinal, rather than cross-sectional data allow to better combine a clinical state with specific changes in neuro-receptor functioning and hence help understand the relation between drug use and clinical outcome. On the downside, the costs for such imaging studies remain a challenge.

Genetic and epigenetic factors in relation to suicide

Like Moffit and Caspi, Turecki, too, classified familial and genetic predisposition, as well as early-life adversity (ELA) as distal factors that increase the lifetime risk of suicide (Turecki, 2014; Turecki and Brent, 2016). Their combination may induce a change in stress-related responses, possibly through epigenetic effects and subsequent emotional and behavioural alterations. Suicidal behaviour further has a strong familial link (Baldessarini and Hennen, 2004; Tidemalm et al., 2011; Turecki, 2001; Turecki and Brent, 2016). There is about a 3-10 fold greater risk of suicidal behaviour from relatives of probands who committed suicide comparing to relatives of control individuals (Baldessarini and Hennen, 2004; Tidemalm et al., 2011; Turecki, 2001). It has also been reported that a positive family history of suicide attempts or completion, increases the risk of suicidal ideations by almost fivefold (Blum et al., 2012; Lieb et al., 2005). However, there is no clear link between suicidal ideation itself and a family history of suicidal ideation (Brent, 2010; Brent et al., 1996; Lieb et al., 2005).

Suicidal behaviour is furthermore influenced by experiences like sexual or physical abuse and parental neglect (Afifi et al., 2008; Angst et al., 1992; Brezo et al., 2007; Collishaw et al., 2007; Dutta et al., 2017; Fergusson et al., 2000; Gilbert et al., 2009; Lansford et al., 2002; Nemeroff, 2016). The effects of early-life experiences could affect the differential regulation of biological systems and development (Hertzman, 2012; Nelson, 2017; Turecki et al., 2014). Several epigenetic studies have investigated central nervous system and peripheral samples from

individuals exposed to ELA. A postmortem study showed decreased hippocampal glucocorticoid receptor mRNA levels from individuals with a history of abuse or extreme neglect (McGowan et al., 2009). In addition, a more recent study showed that specific types of early-life traumas (particularly under the age of seven) influence the later response to stress and antidepressants (Williams et al., 2016). This is reflected in women with a history of childhood abuse, by enhanced ACTH levels following exposure to stress, as measured with the dexamethasone/CRH test (Heim et al., 2008). Furthermore, a recent study suggests that the development of the HPA axis can be disrupted by early childhood trauma, which in postpartum women may impair affective expression during mother-infant interactions (Juul et al., 2016). Peripheral samples show epigenetic changes that reflect risk factors for depression and suicide, such as child abuse and neglect. Such samples are easier to access than brain samples, but how well they reflect cerebral functions remains elusive.

Animal models for major depression and suicide

Animal models are a commenly-used tool to investigate the etiology of neuropsychiatric disorders, as well as their course and potential treatments. There are indeed a large number of animal models for depression or, rather, for symptoms of depression (Banasr et al., 2017; Czeh et al., 2016; Krishnan and Nestler, 2011). However, as it is not possible to assess any suicidal behaviour in animals, there is no accepted animal model for suicidal behaviour so far (Preti, 2011).

It is suggested that social support is associated with a decreased likelihood of suicide attempts (Kleiman and Liu, 2013). There are social dynamics that precede the occurrence of suicide (lack of social support and guide) and social dynamics that accompany it (surveillance, prompt rescue). Precipitating factors such as conflicts with friends, colleagues or partners have a social basis, and they develop in close interaction with the patients (Brent et al., 1993). Although it is hard to model these interactions in animals, it is possible to create models for the symptoms related to suicide, such as hopelessness, pessimism and low self-esteem which are major cognitive risk factors for suicide. Similarly, hope and pessimism are impossible to capture in animals. So far, the current models of learned helplessness assume that animals will normally avoid aversive stimuli (Seligman, 1972). This model has many symptoms that are observed in human depression, such as weight loss, motor retardation and/or agitation, sleep disturbances, libido changes, and distorted perception of pleasure (Henn and Vollmayr, 2005). Although this model shows

stress-related changes, it does not reflect human suicidal behaviour as animals in which learned helplessness is introduced, fail to show signs of self-inflicted harm.

Gilbert developed an animal model that attempts to imitate (aspects of) suicidal behaviour. In this model, the defeat (the stressful event) and entrapment (no escape from the situation) are combined, which leads to severe "helplessness" (increased morbidity and mortality) in animals (Gilbert and Allan, 1998). Williams et al., who mention suicidal behaviour as a reaction to stressful situations, especially in those situations were no chance of escape is perceived (Williams et al., 2005), have described the notion of defeat and perception in humans. In this model, there is an important role for stress-related changes as well (Brown et al., 1995). Another model is based on adversities during childhood, including abuse and neglect, growing up in a dysfunctional family or being exposed to loss of a parent. In these cases, there is an increased likelihood of developing psychopathology and suicidal behavior later in life (AFIFI et al., 2009; Agerbo et al., 2002; Lu et al., 2008). Maternal deprivation in rodents causes a reduction in the activity of inhibitory neurotransmitters (Newport et al., 2002). In addition, in similar primate models, the development of depression-like symptoms is related to maternal separation earlier in life and coincides with reductions in cerebrospinal fluid (CSF) levels of noradrenaline (Kraemer et al., 1991). However, this situation is not validated as a model for suicide. Moreover, primate models based on maternal separation are very questionable ethically and are associated with high costs in relation to the amount of time needed.

Animal models for the study of depression in AD

Our study shows that the GABA-A receptor subunit GABRA5 mRNA level was significantly decreased in depressed Alzheimer patients. In addition, VGluT1 mRNA levels were positively correlated to the Cornell score for depression severity. However, in the commonly used APP/PS1 mouse model, we failed to find any GABA or glutamate pathway changes that were in any way comparable to the changes we observed in depressed AD patients. Moreover, the basal activity of the HPA axis was not activated in the double transgenic AD mice either, and no difference in sucrose preference was found (Chapter 6).

Since double transgenic AD mice only showed Aβ accumulation, but no tangle formation, we next invested in the breeding of a triple transgenic AD mouse model. This model contains three mutations associated with familial Alzheimer's disease (PS1-M146V, APPswe and tauP301L) (Oddo et al., 2003). According to

the original publication, these triple transgenic AD mice display both plaque and tangle pathology. Aβ deposition is progressive and initially occurs particularly in the intracellular compartment, and would occur in some brain regions from age of three or four month old. Deposits of Aβ in the frontal cortex become extensive by the age of one year but do not appear before the age of six months; tau accumulation occurs even later, after which it extends into the hippocampus (Billings et al., 2005; Oddo et al., 2003). Interestingly, in most of these AD mouse models, the memory impairments, both spatial and contextual as well as LTP alterations, develop before the actual occurrence of plaques and tangles. At 6.5 months of age, triple transgenic mice e.g. show considerable cognitive deficits, but were more capable of performing fear-conditioned tasks (Stover et al., 2015). These changes did not occur after these mice were treated with Aβ antibodies that cleared the intraneuronal amyloid (Billings et al., 2005). Interestingly, these triple transgenic AD mice do exhibit an activated central HPA axis (altered mRNA levels of the glucocorticoid receptor and CRH in the paraventricular nucleus of the hypothalamus) at a young age (3-4 months), together with mild behavioural changes (elevated plus maze and open field) (Hebda-Bauer et al., 2013), indicating that this may be a more promising model to test our hypothesis that AD-related depression is accompanied by changes in GABAergic and glutamatergic pathways and an activation of the HPA axis. It is possible that the mice had high HPA activity because they were low in social hierarchy.

For the reasons mentioned above, we started to breed this triple transgenic mouse model with the same background as in the original publication. A study is now in progress to determine whether this triple transgenic mouse model would be suitable for mimicking aspects of depression in AD pathology. In a pilot study, Aβ and hyperphosphorylated Tau (P-Tau) were immunocytochemically stained in tissue obtained from 12-month-old male and female mice. Aβ was stained with the 4G8 antibody and P-Tau was stained with AT8 (Bossers et al., 2010). In both sexes, intracellular 4G8-stained Aβ was observed in the neurons of cortex, hippocampus and amygdala, while 4G8-stained extracellular plaques were only seen in the hippocampus of female triple transgenic AD mice. AT8-staining was negative in the brains of all these mice.

These pilot data show that Aβ aggregation is stronger in female than in male triple transgenic AD mice, and that Aβ, rather than P-Tau, might be the major - or even only - AD pathology present in this 12-month-old model, which indicates that this mouse model may be used to explore the mechanism of AD pathogenesis, but only for the alterations in relation to Aβ and sex differences in Aβ (Rodriguez

et al., 2008). The expression of CRH in the hypothalamus and depression-related behaviours of this triple transgenic mouse model have still to be analyzed.

CONCLUSIONS

As discussed before, there are many genetic polymorphisms and individual developmental epigenetic differences that can give rise to variable functional changes in the complex network of neurotransmitters and neuropeptides involved in mood disorders and suicide. It is our hope that, in the future, the current data will ultimately allow the identification of the vulnerable neurobiological systems in a depressed individual, and thereby allow a better prediction of the suicide risk, which may lead to an optimal tailor-made anti-depressive and anti-suicidal therapy.

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