Functional, structural, and molecular imaging of the risk for anxiety and depression S.P.A.Wolfensberger

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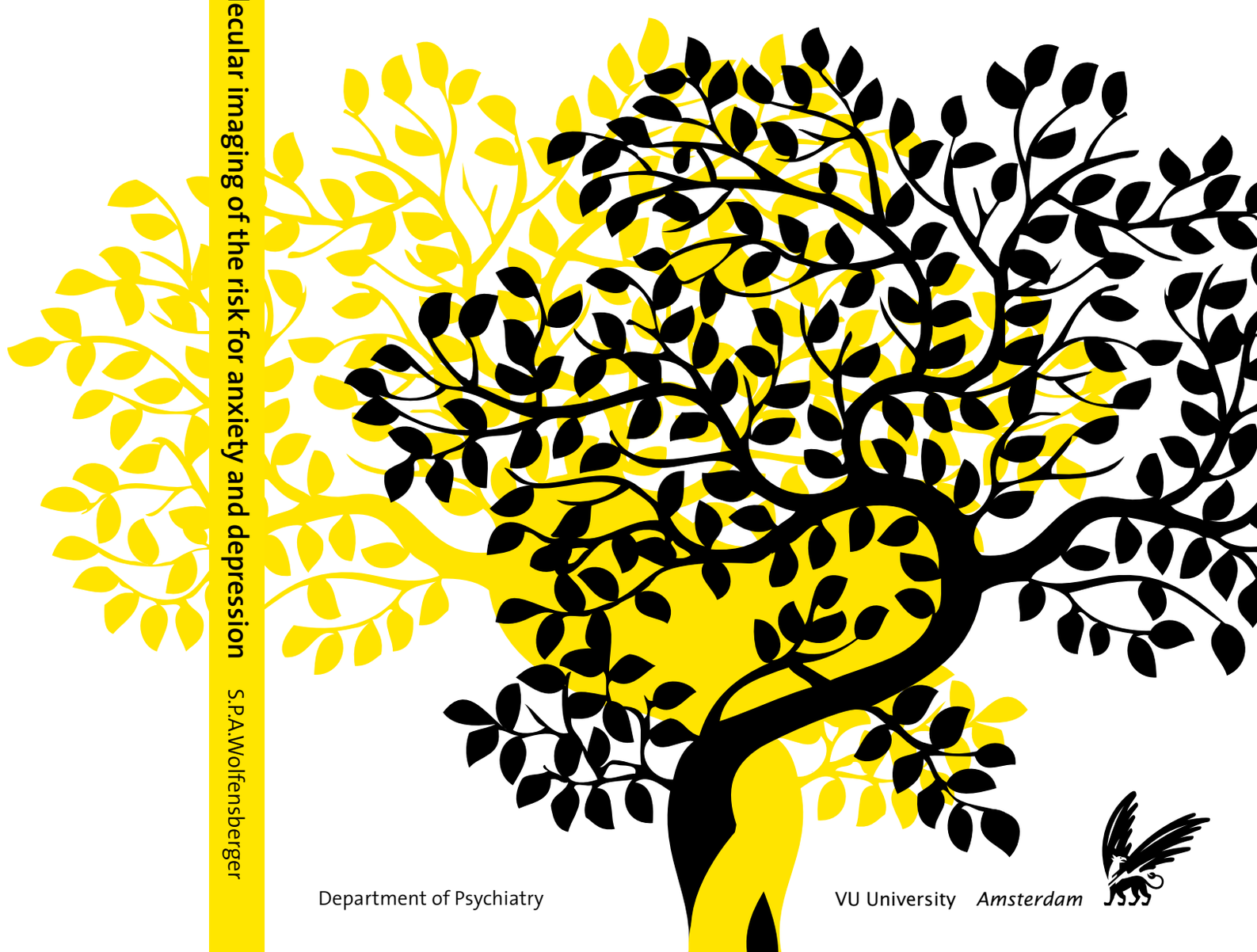

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Summary and discussion

The research described in this thesis examined brain correlates of anxiety and depression using various neuroimaging techniques. Sections A and B report on functional and structural neuroimaging studies in MZ twins at high and low risk for anxiety and depression. In section C, a novel PET ligand for NK1 receptors, which are involved in anxiety and depression, was evaluated *in vivo*. This approach could be the basis of future imaging genetics studies focusing on functional, structural and molecular (receptor ligand) changes, to disentangle the contributions of genetic and environmental risk factors for anxiety and depression.

Summary sections A and B: the MZ discordant/concordant twin study

In sections A and B, the hypothesis was tested that genetic and environmental risk factors for anxiety and depression impact on partly different brain structures or, alternatively, even if they converge on the same brain structures, they do so in entirely different, perhaps opposite, ways.

To this end, dual modal imaging was used in MZ twin pairs strongly concordant or discordant for the risk for anxiety and depression to establish intrapair differences in regional brain structure and function. This risk was computed on the basis of longitudinal survey data on anxiety, depression, neuroticism, and somatic anxiety collected in 1991, 1993, 1997, and 2000 [57], which were shown to have strong predictive value for clinical anxiety and depression, as assessed by the Composite International Diagnostic Interview[58].

Because MZ twins are considered genetically identical, any discordance in their risk for anxiety and depression must arise from differential exposure to environmental influences. Hence, the intrapair comparison of discordant twins highlighted neural activation and brain regions that are particularly susceptible to environmental factors. In MZ twin pairs that are selected to be highly concordant, the risk was either very high in both members of the pair (even though they have been exposed to partly different environments) or very low in both members. Comparing these two groups creates a genetic contrast, which was confirmed by verifying that the parents of these twins indeed had high versus low risk scores as well. Hence, the low / high group comparison of concordant twins highlighted neuronal activation and brain regions that are particularly susceptible to genetic factors.

Section A: Functional Neuroimaging

Previous functional brain imaging studies in subjects suffering from anxiety and depressive disorder have shown deviant amygdala responses to emotional stimuli, but compared to healthy controls both hyperactivity and hypoactivity have been reported. In **chapter 2**, the hypothesis was tested that these discrepant findings are based on different effects of genetic and environmental risk factors on amygdala functioning. To test this hypothesis, amygdala responses to an emotional faces paradigm were assessed during fMRI in monozygotic twin

pairs discordant for the risk of anxiety and depression (n=10 pairs) and in monozygotic twin pairs concordant for high (n=7 pairs) or low (n=15 pairs) risk for anxiety and depression. Main effects (all faces vs. baseline) revealed robust bilateral amygdala activity across groups. In discordant twins, increased amygdala responses were found for negatively valenced stimuli (angry/anxious faces) in high-risk twins compared to their low-risk co-twins. In contrast, concordant high-risk pairs revealed blunted amygdala reactivity to both positive and negative faces compared with concordant low-risk pairs. Post-hoc analyses showed that these findings were independent of serotonin transporter (5-HTTLPR) genotype.

These findings indicate that amygdala hyperactivity was found in subjects who are at high risk for anxiety and depression through environmental factors, but amygdala hypoactivity in those at risk mainly through genetic factors. The hyperactivity of the environmentally at risk twins disappeared after correcting for state anxiety, which indicates that hyperresponsiveness to emotional stimuli went together with increased anxiety. State anxiety was similarly increased in the twins at genetic risk, although here amygdala reactivity to the emotional stimuli was lower compared to neutral stimuli and this hypoactivity did not disappear when state anxiety was taken into account. This pattern of results appears to reflect higher baseline amygdala activation in subjects at high genetic risk for anxiety and depression, which acts to reduce further activation in response to general increases in state anxiety and specific emotion-inducing stimuli (e.g. angry faces).

In **chapter 3**, emotional processing and brain activation were examined during an encoding and recognition paradigm using emotionally salient words in a sample of monozygotic twin pairs at low and high risk for anxiety and depression, using the same study sample as in chapter 2. Psychological theories of major depression have emphasized the role of negative biases in information processing in the etiology and the maintenance of the disorder. Such biases have been reported both for interpretation and storage of emotional information[59-62]. These observations raise the possibility that emotional biases might pre-date the onset of clinical depression and thereby represent a risk factor for subsequent development of illness in predisposed individuals. To investigate emotional bias prior to onset of depression it is necessary to study people who are at risk for anxiety and depression, but who are not clinically depressed. To date, studies combining brain imaging and neuropsychological testing in groups at high risk for depression and anxiety, but not yet affected, have been scarce and have focused primarily on executive functioning.

In the present study, performance data did not support the existence of a negative response bias in high risk subjects. At the neural level, however, increased left inferior frontal gyrus activation by negative words was found in high risk subjects through either genetic or environmental risk factors, most prominently during recognition. In other words, genetic and environmental pathways seemed to converge, both pointing towards increased activation in the left inferior frontal gyrus in response to negatively valenced words in both high risk groups. This may indicate that increased activation of left inferior frontal gyrus in a verbal emotional memory task is a useful vulnerability marker for anxiety and depression.

Section B: Structural Neuroimaging

In **chapter 4**, the same study sample as in chapters 2 and 3 was used. Volume reductions in the temporal lobe were observed in high risk twins, most notably in the left posterior hippocampal region, but exclusively in twins at high risk through environmental factors. A group comparison between pairs concordant for low or high risk, which is more likely to reflect differences in genetic vulnerability, did not show reduced temporal lobe and posterior hippocampal volumes in the pairs at high risk for anxiety and depression. This pattern of results suggests that damage to temporal lobe structures may be specific to an environmentally driven aetiology of anxiety and depression. The following potential explanations were considered for this pattern of findings. The high risk discordant twins reported exposure to more, and more severe, life stressors early in their lives. In line with the glucocorticoid cascade hypothesis, these stressors may well account for part of the environmental effects on temporal grey matter volume, especially the hippocampal region, having a high density of glucocorticoid receptors. An alternative explanation is epigenetic reprogramming. Epigenetic reprogramming can create large phenotypic divergence in genetically identical subjects by selectively repressing the expression of some genes. In particular in older twins, remarkable differences in their gene-expression profile have been found[63].

Epigenetic drift, therefore, is a potential source for discordance in amygdala activation, left temporal volumes and the risk for anxiety and depression. Finally, it might be possible that both explanations converge, and that life stress and hypothalamic-pituitary-adrenocortical (HPA)-axis activation somehow have a direct impact on epigenetic reprogramming. Future (longitudinal) studies should address this important question.

Discussion sections A and B

Several lines of evidence suggest the presence of specific neural circuits within the limbic cortical system that mediate stress responsiveness, mood and emotion regulation[64-66]. A recent comprehensive meta-analysis revealed brain volume reductions in depressed patients in many regions related to emotional processing and stress regulation, such as the frontal lobe and hippocampus, and amygdala[67]. In the studies described in this thesis both volumetric and functional abnormalities were found in those regions.

The hippocampus is well known to be a target for glucocorticoids, which potentially contribute to atrophy in this brain structure[68-79]. Furthermore, amygdala and prefrontal cortex are also involved in HPA-axis regulation. This is important to note, as depression has been associated with disrupted HPA-axis activity and increased cortisol levels[80,81], also mentioned in section B. Several mechanisms have been proposed to explain how prolonged stress can result in limbic and prefrontal (volumetric) abnormalities, such as decreased dendritic branching[82,83], loss of neurons, or decreased expression of brain derived neurotrophic factor[68,82,84]. Evidence for stress induced brain abnormalities in depression is also provided by studies examining genetic variations in the glucocorticoid receptor gene. Especially functional polymorphisms of the NR3C1 (nuclear receptor subfamily 3, group C, member 1) gene are associated with increased susceptibility to depression[68,85,86]. Interestingly, a recent study reported an association of four illness-related polymorphisms of the NR3C1 gene with overall smaller hippocampal volumes in patients with depression[87]. This suggests that risk alleles of the NR3C1 gene influence hippocampal volume, and it would not be surprising when future research will reveal more risk alleles associated with volume changes or differences in activation, potentially confounding study results with un unknown effect size.

A genetic variant that repeatedly has been implicated in amygdala function is the 5-HTTLPR promoter polymorphism. The short allele (S) of the promoter polymorphism is associated with reduced expression of the gene and lower availability of the transporter[88,89], and individuals carrying the s-allele tend to have increased anxiety related temperamental traits, which in turn are related to increased risk of developing depression[68,90]. The few studies that examined the association between 5-HTTLPR polymorphism and brain volumes in depressed patients showed mixed results with respect to amygdala and hippocampus[93-96], but fMRI studies systematically have shown that this polymorphism affects amygdala activity in response to emotional stimuli[38,89,91]. This latter finding was not replicated in the study described in this thesis, but sample size may have been a major issue.

Frontal regions (anterior cingulate, orbitofrontal and prefrontal cortex) are known to exert emotion regulation by inhibiting the activity of limbic regions such as the hippocampus and the amygdala[97,98]. Prominent volume reductions in those frontal regions in major depression were demonstrated[68] in many studies. Although these findings were not replicated, evidence for different functional activation in the left inferior frontal gyrus during emotional word processing was shown.

Limitations of the MRI twin study design

When studying the effects of anxiety and depression on MRI traits, four important effects need to be disentangled: the effects of an ongoing depressive episode, the effects of anti-depressant medication, the effects of vulnerability traits and the effects of increased anxiety at the time of scanning. The main intent was to detect effects of trait vulnerability caused either by detrimental environmental or genetic risk factors, which meant that the other three (confounding) effects needed to be contained as much as possible. It was possible to limit the effects of current depression and antidepressant medication. Apart from one exception in a concordant high-risk pair, no twin was diagnosed with current MDD at the time of scanning, and only one subject used antidepressants (results did not noticeably differ after excluding these subjects). However, there clearly were higher levels of state anxiety and depressive symptoms scores at the time of scanning in the (trait) high risk twins. Increased left inferior frontal gyrus activity during emotional words may therefore reflect state anxiety at the time of scanning rather than genetic or environmental effects on this brain region. Although this first limitation is recognised, it is very hard to envision a design that successfully separates state and trait anxiety.

A second limitation is the absence of baseline activation measurements. A prerequisite of finding a significant BOLD signal is that task related activation in a region of interest is significantly different from the reference baseline activation in that region, as fMRI is based on relative measurements. However, it is likely that the response to stimuli is simply based on ceiling effects, as the response is dependent on the baseline activation[99]. Therefore, interpreting genetic and environmental effects on regional fMRI contrasts should ideally be done in relation to parallel genetic and environmental effects on baseline activation of those regions.

Conclusions sections A and B

In conclusion, the main finding of these studies was that genetic and environmental risk factors for anxiety and depression may impact on partly different brain structures (amygdala, hippocampus), but that they could also converge on the same brain structures (frontal lobe). Therefore, future brain imaging studies on anxiety and depression should aim to avoid an admixture of subjects who are at genetic risk with those at risk for environmental reasons. This can, in principle, be done by selecting patients with known exposure to strong environmental risk factors (e.g. childhood trauma) or with known genetic risk markers. Based on the current understanding of the role of genetics in psychiatric disorders, however, the discordant/concordant MZ twin design remains the most powerful method of separating genetic and environmental risk factors. If fMRI is used as the imaging modality, however, an active attempt should be made to measure baseline activation levels, for example by

using $^{15}OH_2O$ PET for absolute measurements of cerebral perfusion or arterial spin labelling techniques.

Summary section C: Molecular Neuroimaging

NK1 receptors have been implicated in various neuropsychiatric and other disorders, and NK1 receptors are potentially important targets for drug development, as they have been implicated in depression, anxiety and pain perception. R116301 is a selective high affinity NK1 receptor antagonist. In **chapter 5**, a pilot study was performed, evaluating [^{11}C]R116301 as a potential PET ligand for the NK1 receptor. More specifically, the presence of a specific signal was investigated using two dynamic 90 minutes [^{11}C]R116301 scans, separated by 5 hours, in 3 normal volunteers, before and after an oral dose of 125 mg aprepitant. Data were analysed using striatum to cerebellum standardised uptake value (SUV) ratios. Baseline SUV ratios at 60-90 minutes after injection ranged from 1.22 to 1.70. Following aprepitant administration this specific signal was completely blocked. Aprepitant administration did not significantly affect cerebellar uptake, confirming the absence of NK1 receptors in cerebellum. These preliminary results indicated that [^{11}C]R116301 has potential as a radioligand for *in vivo* assessment of NK1 receptors in the human brain.

As a specific signal was found in chapter 5, **chapter 6** describes the evaluation of various quantitative outcome measures of [^{11}C]R116301 binding in healthy subjects using both blocking and test-retest data with the aim to find the optimal model for quantification. Two dynamic 90 minutes [^{11}C]R116301 scans, separated by 5 hours, were performed in 11 healthy volunteers. Data from the blocking study, using the same 3 paired PET studies as in chapter 5, were now used for further evaluation, whilst in the other 8 no intervention was performed (i.e. test-retest). Whole striatum, as defined on a co-registered MRI scan, was used as tissue of interest, as it has the highest density of NK1 receptors. Cerebellum was used as reference tissue. Unfortunately, measured plasma curves were not reliable due to severe stickiness of the tracer. Even after implementing a validated sticking correction, no useful results were obtained when plasma input models were used. In contrast, reference tissue models appeared to be more stable with the simplified reference tissue model (SRTM) performing best. Average (± SD) SRTM derived mean binding potential BP_{ND} of all (first) baseline scans was 0.64 ± 0.31, which reduced to −0.01 ± 0.03 following aprepitant administration. Test-retest results showed good variability (14.0 ± 10.7%) and excellent reliability (intraclass correlation coefficient ICC = 0.93) for SRTM derived BP_{ND}. Striatum to cerebellum SUV ratios minus 1, an approximation of BP_{ND}, showed excellent variability (6.2 ± 3.1%) with excellent reliability (0.98) and correlated well with SRTM (r^2 = 0.96) with acceptable (15%) bias. In conclusion, SRTM was found to be the optimal model for quantification of [^{11}C]R116301 binding, but semi-quantitative SUV methods hold promise for routine clinical applications.

Discussion section C

Subsance P (SP) was first discovered by Ulf von Euler and John Gaddum in 1931[100]. Gaddum and Schild[101] named this new agent SP, P referring to the powder which was obtained after the extraction procedure from equine brain and gut, and which was found to have potent hypotensive and smooth muscle contractile properties.

To date, almost 8 decades later, the neuropeptide SP and its preferred receptor, the tachykinin neurokinin type 1 (NK1) receptor, have been of particular interest because of their potential implication in various neuropsychiatric and other disorders. Depression and anxiety, for example, are related to changes in the release of SP binding to NK1 receptors in the human brain[102-106]. However, despite extensive preclinical and clinical studies on the widely distributed central pathways and NK1 receptors in pain, anxiety, depression, schizophrenia, Parkinson's disease, and Alzheimer's disease and other neuro-psychiatric disorders, to date, NK1 receptor antagonists have only found proven efficacy in the prevention of acute and delayed chemotherapy induced nausea and vomiting.

The NK1 antagonist, aprepitant (EMEND®) is on the market for treatment of radio- and chemotherapy induced emesis. Recently, compounds like casopitant and AV608 have entered phase II clinical trials for various indications such as myalgia, insomnia, irritable bowel syndrome and urinary incontinence. As there is very little known about the functions mediated by SP in humans, PET imaging of NK1 receptors is a useful tool to study this system in health and psychiatric and neurological diseases. Moreover, it could be a useful tool to visualise and quantify specific receptor binding in order to gain more insight in neurotransmitter-receptor function and it could aid in assessing the optimal dose range of new drugs that act by occupancy of central NK1 receptors. For this purpose, quantification of [^{11}C]R116301 binding to the NK1 receptor was evaluated in section C.

Conclusions section C

It can be concluded from these PET studies, that SRTM[107] is the optimal model for quantification of [^{11}C]R116301 binding. This method shows good test-retest variability and good reliability, and it seems feasible to shorten scan durations. Tissue ratio methods hold promise for clinical applications as even shorter scan durations can be used. In future studies, tissue ratio methods need to be further validated over a larger range of BP_{ND}. Furthermore, it will be necessary to demonstrate a difference in BP_{ND} between healthy controls and patients. In addition, it should be evaluated whether the good correlation with SRTM observed in normal controls also applies to various patient populations, for example those who are at risk for anxiety and depression, and those who are diagnosed with major depression. In other words, this should be performed by comparing groups with abnormal NK1 receptor status with healthy controls.

At present, there is no ideal PET tracer for NK1 receptors. Of the available tracers [^{18}F]SPARQ shows a much higher specific signal than [^{11}C]R116301. Therefore, at least in theory, it should provide a more accurate assessment of NK1 receptor status or occupancy, which could be important dealing with small differences between groups. For example, the expected reduction in binding of NK1 receptors in anxiety and depression (12-21% panic disorder) can better be studies using [^{18}F]SPARQ, as far fewer patients would be required. Nevertheless, for clinical applications and also for research purposes,[^{18}F]SPARQ is hampered by its slow kinetics, requiring very long study durations, thereby increasing the risk of movement artefacts. At the cost of a reduced signal,[^{11}C]R116301 has the advantage that studies can be performed within a reasonable time (90 minutes).[^{11}C]R116301 could have a future role in assessing whether a (new) drug results in significant NK1 receptor occupancy.
When ranking the affinity of NK1 ligands, it is clear that the affinity of R116301 is lower than that of SPARQ, which is in agreement with the higher specific signal observed with [^{18}F]SPARQ On the other hand, the lower affinity of R116301 implies that [^{11}C]R116301 could be more sensitive to differences in endogenous substance P concentrations. This would be very interesting in intervention studies, where release of the endogenous ligand could be manipulated by a pharmacological challenge or, for example, a fear stimulus. In fact, [^{11}C]R116301 should be ideally suited for this, as it has a lower affinity than any other (known) available alternative tracer for the NK1 receptor. Clearly, further studies are needed to establish whether this is feasible.

Future perspectives

This thesis has shown the power of a discordant/concordant MZ twin design to detect, using MRI, structural and functional brain differences in subjects at genetically and/or environmentally determined low and high risk for anxiety and depression. Future studies may apply this fMRI and molecular imaging design to other neuropsychiatric illnesses based on a combined genetic and environmental aetiology. First, the issue of differences in baseline activation between groups and within an MZ twin pair could be addressed by making use of either [^{15}O]H_2O PET for absolute measurements or arterial spin labelling (MRI) as an imaging tool for cerebral perfusion. This will allow comparison of baseline activation states within twin pairs to possibly explain differences in neuronal activation measured, using fMRI. One of the drawbacks of arterial spin labelling is its inability to explore the whole brain. It may, nevertheless, be suitable to study one specific region of interest.
Secondly, further use of PET could clarify differences in genetic and environmental risk factors on a molecular (e.g. receptor) level and, therefore, help with interpreting results of functional and structural neuroimaging studies in the same subjects. Various ligands for neuroreceptor mapping can be used. For example, serotonin transporter (SERT) status can be investigated using [^{11}C] DASB, a ligand to the serotonin transporter receptor. This can be used to explore possible differences in receptor status between genetically and environmentally determined risk factors. Therefore, receptor density and affinity of the ligand to the receptor in specific brain regions could shed some light on the functional and structural imaging data, i.e. increased activation or volume reduction in a region of interest could be associated with a change in receptor status in this region.
As mentioned in the discussion of sections A and B, genetic and environmental risk factors could lead to differential underlying neurobiological mechanisms, such as differences in receptor status. These differences (different types of vulnerability profiles for the specific disorder) at a receptor level may have differential responses to pharmacologic intervention (treatment). Unravelling these underlying mechanisms in future research could, therefore, lead to novel or more individualised therapeutic or even preventive strategies.

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