Chapter 8

Summary and general discussion
The aim of this thesis was to investigate the neural substrate of negative symptoms. To this end, the association between negative symptoms and brain activation was assessed during various emotional and cognitive processes, including reward processing (chapter 2), affective forecasting (chapter 3), set-shifting (chapter 6), and self-initiation of behavior (chapter 7), as well as functional connectivity during rest (chapter 4 and 5; Figure 1). Results showed that negative symptoms were associated with ventral striatal activation during reward processing (chapter 2) and with vividness of patients’ imagination of future events and the extent to which the imagination of these events elicited a positive feeling (chapter 3). Although regional brain activation during the imagination of positive future events was not related to negative symptom severity, we did observe an association between severity of negative symptoms and lower functional connectivity between a seed in the precuneus/posterior cingulate cortex (PCC) and the precuneus/paracentral lobule and cerebellum (lobule VI/Crus I) (chapter 3).

Previous studies have shown that negative symptoms may be better described as two separate symptom factors: amotivation and expressive deficits (Liemburg et al., 2013; Stiekema et al., 2016; Strauss et al., 2013). Therefore, in chapter 4 we shifted our focus from negative symptoms to the neural correlates of amotivation and expressive deficits. We found that neither expressive deficits nor amotivation were associated with altered functional brain topology (chapter 4). In chapter 5 we focused on amotivation in particular and found that this factor was associated with lower connectivity between the VTA and an extensive mesocorticolimbic network. Finally, when we narrowed the focus to apathy, a central symptom within the amotivation factor, we found an association between apathy and activation in the superior frontal gyrus and the cerebellum (Crus I/II) during cognitive set-shifting (chapter 6), but not with brain activation during self-initiation (chapter 7).

Figure 1. A. Cognitive processes that may be related to negative symptoms; B: Summary of findings in this thesis. Numbers refer to the respective chapter. Regarding the brain image, colors depict the cognitive process or, in the case of resting-state studies, the symptom factor under study.
Integration of findings

Amotivation

Central to the amotivation factor is a deficit in goal-directed behavior. Patients with amotivation may engage in fewer enjoyable and day-to-day activities, and this may affect professional and social functioning. Multiple models have been proposed to explain this deficit in goal-directed behavior. Levy and Dubois (Levy & Dubois, 2006) proposed a model of goal-directed behavior which emphasizes the role of affective or reward-related processes, executive functioning, and auto-activation. More recently, Kring and Barch (2014) constructed a similar model specifically aimed at explaining the motivational dimension of negative symptoms in schizophrenia. Combining these two models results in a comprehensive framework that may be useful in the integration of the findings in chapter 2, 3, 5, 6, and 7 (Figure 2). The combined framework encompasses several stages related to reward processing (ranging from hedonic responses to approach motivation), as well as construction of action plans (relying on intact executive functioning), and auto-activation or initiation of behavior and thoughts.

According to this framework a pleasurable event will elicit an in-the-moment hedonic response (a sense of liking: “This movie is great!”). In order to translate this positive feeling into future goal-directed behavior, it needs to be maintained and remembered. Then, when the opportunity arises to repeat the behavior (e.g., a new movie from the same director is released), this will trigger retrieval of the memory, which contributes to the subjective value that is ascribed to the behavior. This will result in anticipatory pleasure (looking forward to the action: “This new movie will be great to watch!”). This anticipatory pleasure and the calculated effort it takes to obtain the reward (e.g., buying tickets, going to the movie theater) will influence the subjective value of the action. If the anticipated reward outweighs the anticipated effort, this will result in approach motivation (i.e., the intentions to come into action), after which an action plan to obtain the reward is constructed, a process that relies on executive functioning (e.g., allocation of attention, rule finding, cognitive flexibility, and (sub)goal maintenance). This information feeds back to the effort computation, and if the approach motivation is retained the action is initiated and executed, leading to goal-directed behavior (going to see the movie). In turn, disruptions in any of these subprocesses may lead to reduced goal-directed behavior, resulting in apathy and the superordinate amotivation factor. In this thesis, brain activation and functional connectivity related to reward-related processing (chapter 2, 3, and 5), executive functioning (chapter 6), and self-initiation (chapter 7) have been examined in association with negative symptoms, amotivation, and its central component apathy.

Reward-related processing

In recent years, the neural correlates of reward processing in patients with schizophrenia have received considerable attention. Especially activation in the ventral striatum (VS), an area consistently implicated in the processing of rewarding and salient stimuli (Liu et al., 2011; Menon, 2015), has often shown reduced activation in patients with schizophrenia (Radua et al., 2015). Moreover, several studies have found associations between severity of negative symptoms and activation in this region, although mixed findings have obscured a clear-cut view of this relationship. Therefore, in chapter 2, a meta-analysis was performed on previously published studies that assessed the
association between reward-related ventral striatal activation and negative symptoms. Results showed that reduced VS activation during reward processing is related to stronger severity of negative symptoms. This relationship between negative symptoms and reward-related processing is often thought to reflect a decrease in reward anticipation and learning, which reduces the chance of engaging in goal-directed behavior (Kring & Barch, 2014). More specifically, Maia and Frank (2017) have proposed a model that links altered reward processing to altered dopamine function, explaining both positive and negative symptoms. They have suggested that a combination of increased spontaneous and decreased adaptive (i.e., in response to relevant stimuli) phasic dopamine responses may be involved in schizophrenia: whereas increased spontaneous phasic dopamine release may lead to the occurrence of positive symptoms, decreased adaptive phasic dopamine responses may lead to negative symptoms. Specifically, decreased phasic dopamine response to relevant stimuli may reduce positive prediction errors (i.e., dopamine response to unexpected rewards), leading to reduced value-guided learning. This may in turn reduce VS activation during reward anticipation, resulting in the motivational deficits that are central to negative symptoms. This may account for the association between negative symptoms and lower VS activation during reward processing (chapter 2).

Besides altered VS activation, disrupted value-guided learning may reduce the valuation of positive stimuli, which may explain the less positive valence ratings in relation to negative symptoms during the imagination of positive future events in chapter 3. On the neural level, no striatal involvement was found during the imagination of positive events, nor was an association between striatal activation or connectivity and negative symptoms, making it difficult to explain the neural mechanisms that underlie these behavioral changes in terms of this model.

Figure 2. Model of goal-directed behavior, adapted from Kring & Barch (2014) and Levy & Dubois (2006). Thin backwards arrows represent feedback of information.
The finding of reduced resting-state connectivity between the ventral tegmental area (VTA) and the ventral striatum in relation to amotivation (chapter 5) may also be explained by this model: in case of a relationship between amotivation and decreased adaptive dopamine transients, a reduction of functional connectivity between the VS and the VTA, an area known for its dopaminergic projections to the limbic system, is to be expected. Indeed, we showed that in patients with schizophrenia, negative symptoms were associated with reduced functional connectivity between the VTA and a mesolimbic network, including the VS. However, because no data on dopamine release in response to reward-related stimuli was included these conclusions are speculative.

Taken together, the findings in chapter 2, 3, and 5 contribute to the idea that disruptions of reward-related processing, and especially anticipation of pleasurable events may underlie amotivation and therefore negative symptoms in patients with schizophrenia. However, as Levy and Dubois (Levy & Dubois, 2006) stated, besides intact intention to act (i.e., approach motivation), which may be related to reward-related processing, subsequent executive and auto-activation processes are also imperative for goal-directed behavior. Because disruptions in these processes may lead to the occurrence of apathy and amotivation as well, we investigated these cognitive (chapter 6) and auto-activation (chapter 7) processes and their neural correlates in later chapters.

Executive functioning

In chapter 6, the neural correlates of apathy during set-shifting were examined in a healthy sample. Results showed an association between apathy and activation in the (medial) superior frontal gyrus and Crus I/II of the cerebellum (although this was only at trend level in the nonparametric analysis), during cognitive set-shifting, the switching of a response rule. This suggests a possible involvement of these areas and cognitive set-shifting in the occurrence of apathy. Of course, results in healthy individuals may not translate one-to-one to psychiatric or neurological populations, but these results may be a starting point for further research. In this light, a recent study measuring electroencephalography (EEG) during a set-shifting task has shown an association between apathy and the strength of an event-related potential that has been associated with shifting of attention to novel stimuli (the P3a), both in healthy controls and patients with Parkinson’s disease (Kopp et al., 2006). Future studies on the association between (neural correlates of) executive functioning and apathy in patients with schizophrenia may shed more light on this topic.

Auto-activation

Chapter 7 targeted the third apathy-related process, namely auto-activation. This process reflects the final stage toward execution of goal-directed behavior: the initiation of behavior (Figure 2). After reward-related processing has led to approach motivation and therefore intention to act and a plan has been made on how to obtain the goal, the intention and plan have to be translated into a motor action. In chapter 7 we examined auto-activation with a self-initiative task, in which participants were asked to press one of two buttons, with varying levels of freedom (both in terms of choice of the button to press and their timing of the press). Although fronto-parieto-striatal brain activation that is generally found in relation to self-initiation was replicated in this study, neither behavior nor neural activation during the task was related to apathy. This suggests that
an auto-activation deficit may not play a role in apathy, at least in healthy individuals.

Taken together, the results in chapters 2, 3, 5, 6, and 7 underline the involvement of disrupted reward-related processing and executive functioning in the occurrence of amotivation. First, the results from chapters 2, 3, and 5 suggest an important role of reward processing in the occurrence of disrupted goal-directed behavior in people with negative symptoms and specifically amotivation. Specifically, reduced ventral striatal activation during reward processing (chapter 2) and functional connectivity of a mesocorticolimbic network during rest (chapter 5) suggest that dopamine-driven reward alterations may lie at the base of the occurrence of amotivation. The negative symptom-related reduced vividness, less positive valence ratings, and lower functional connectivity of the precuneus/PCC seed that were found in chapter 3 may in turn suggest less effective anticipatory pleasure in patients with more negative symptoms.

Because in chapter 2 anticipatory pleasure, on-the-moment hedonic responses, and reward learning were combined, the exact subprocess(es) that contribute to the occurrence of negative symptoms could not be distinguished. Similarly, in chapter 5 we focused on VTA connectivity during rest, leaving no room for separation of these subprocesses. Chapter 3 on the other hand was aimed specifically on the anticipation of pleasurable events. Although in this chapter we did not compare anticipatory pleasure to the other subprocesses, the reduced vividness and less positive valence ratings that were reported by patients with more negative symptoms underlie the importance of anticipatory pleasure deficits for the occurrence of negative symptoms. However, whether these deficits are due to specific difficulties in anticipatory pleasure or whether they may also be related to disruption in preceding subprocesses like hedonic response or memory for previous pleasurable experiences (Figure 2) remains to be investigated.

Furthermore, the reduced activation in the mSFG and cerebellum Crus I/II in chapter 6 underlines the importance of a possible disruption of action plan construction due to deficits in executive functioning (especially cognitive set-shifting). Finally, in chapter 7 auto-activation or initiation deficits was not found to be related to apathy in healthy individuals. Of course this does not exclude the possibility of initiation difficulties playing a role in patients with more severe (clinical) levels of apathy. Taken together, these findings emphasize the importance of reward and cognitive processing for the understanding of the amotivation factor.

Expressive deficits
Although amotivation is generally considered to be stronger related to functional outcome (Fervaha et al., 2014b; Strauss et al., 2013), this does not exclude the effect that expressive deficits have on functional outcome, and indeed expressive deficits have been associated with functional outcome (Gur et al., 2006; Walther et al., 2016). In fact, a recent study has shown that people are less likely to desire future interactions with individuals with more pronounced expressive deficits and that expressive deficits are related to the individuals’ satisfaction with social support networks (although this holds for amotivation as well; Riehle and Lincoln, 2017). However, to date not much is known about the neural correlates of expressive deficits. A few task-based fMRI studies have suggested involvement of the amygdala and ACC (Gur et al., 2007; Hager et al., 2015),
but no studies on resting-state connectivity changes underlying this subfactor have been performed. Therefore, in chapter 4, we assessed whether expressive deficits and amotivation are differentially related to changes in the functional connectome, using common functional network measures (i.e., global and local efficiency, and eigenvector centrality and participation coefficient). In this chapter we found no statistically robust relationship between brain topology and negative symptoms, neither for expressive deficits or amotivation, suggesting that the functional connectome, at least when characterized by these network measures, may not be related to expressive deficits nor to amotivation.

Cognition and negative symptoms

Negative symptoms and cognitive deficits are commonly thought to be separable or even independent domains (Harvey et al., 2006; Kirkpatrick et al., 2006). Associations have been found between the two domains, although not consistently and of moderate strength at most (de Gracia Dominguez et al., 2009). These associations may reflect overlap in measurement instruments (see the section Methodological considerations), but it may also reflect common underlying deficits (Foussias et al., 2014a). For example, Fervaha et al. (2016) assessed the relationship between alogia and verbal fluency, a negative symptom and cognitive deficit that are conceptually very similar. Not surprisingly, they found a strong association between alogia and verbal fluency (while there was no association with other negative symptoms), which was robust to controlling for sociodemographic and clinical variables. This may suggest that alogia and verbal fluency may tap into the same deficit and that therefore the difference between the two may be more semantical than etiological.

Moreover, the deficits that characterize negative symptoms and cognitive deficits may be interrelated. As proposed by Levy & Dubois (Levy & Dubois, 2006), disruptions in cognitive processes that are needed to successfully construct an action plan may impair goal-directed behavior, leading to negative symptoms. The association between reduced neural activation during cognitive set-shifting and apathy in chapter 6 may support this idea, although directionality of this relationship could not be definitively established. Conversely, motivational processes underlying negative symptoms may influence cognitive functioning as well: it has been shown that amotivation and effort exertion during cognitive testing may be directly related to cognitive performance (Fervaha et al., 2014c; Foussias et al., 2014b). In addition, effort exertion may partially mediate the association between amotivation and cognitive performance (Foussias et al., 2014b). On the neural level, it has been found that reward processing and the resulting motivation may guide allocation of cognitive resources implemented in the ACC (Hager et al., 2015) and improve executive functioning mediated by frontoparietal regions (Etzel et al., 2016), possibly leading to increased cognitive performance (Foussias et al., 2014b). Taken together, although distinct domains, cognition and negative symptoms may be strongly interrelated.

Negative symptoms and depression

Although negative symptoms (especially the amotivation factor with its symptoms anhedonia and apathy) and depressive symptoms have been found to be associated (Stiekema et al., 2016), associations are generally weak to moderate and have not been
found consistently (e.g., Kirkpatrick et al., 2011; Kring et al., 2013; Rabany et al., 2011; Strauss et al., 2013). Moreover, factor analyses have repeatedly shown that depressive symptoms load on a different factor than negative symptoms (Foussias et al., 2014a). In terms of reward or pleasure processing, it has generally been found that whereas depression is related to in-the-moment experience of pleasure, negative symptoms are characterized by reduced anticipation of pleasure (Millan et al., 2014), although not all studies support this view (Da Silva et al., 2017). Likewise, on the neural level, Simon et al. (Simon et al., 2010) have found that while reduced ventral striatal activation during reward anticipation is related to negative symptoms, reduced orbitofrontal (OFC) activation during the receipt of reward is associated with depressive symptoms. Similarly, it has been found that during effort-based reinforcement learning amotivation is related to different striato-orbitofrontal dysfunction in patients with schizophrenia compared to patients with depression (Park et al., 2017). Therefore, although overlap and comorbidity exist, accumulating evidence supports the idea of negative and depressive symptoms as separate constructs.

Clinical implications
Findings from neuroimaging research are sometimes difficult to relate directly to clinical practice. Although machine learning techniques have the potential of generating clinically relevant information (e.g., diagnostic information or predicting treatment response), currently this approach does not surpass existing clinical assessment instruments (Dazzan, 2014; Fu & Costafreda, 2013). Unfortunately the lack of individual specificity of the group-wise studies in the current thesis may result in modest direct translation into diagnostic information on the individual level. Still, the findings in this thesis may have several clinical implications.

First, knowledge about the neural underpinnings of negative symptoms increases understanding of negative symptoms. Whereas amotivation in patients might occasionally be interpreted by family and friends as laziness, the findings in this thesis support the notion of underlying neural abnormalities that may underline the need for treatment to improve these symptoms.

In addition, the neuroimaging results from this thesis may contribute in guiding the development of treatments. This is of utmost importance, because negative symptoms are generally considered difficult to treat (for a review, see Aleman et al., 2017). One possible reason for this is the heterogeneity of the negative symptoms cluster: targeting interventions directly at amotivation or expressive deficits may yield more promising results, because the underlying cognitive and neural mechanisms of these factors are likely to be different. Insight into the neural mechanisms underlying amotivation and expressive deficits may therefore provide direction for the development and improvement of effective interventions for negative symptoms.

In this light, the findings from chapter 2, 3, and 5 indicate that reward processing and specifically the anticipation of rewards or pleasure may be an important mechanism to improve in people with predominant amotivation. An example of an intervention aimed at improving the anticipation of reward is the Positive Emotions Program for Schizophrenia developed by Ngyen et al. (2016). This intervention focuses on
improving the experience and anticipation of pleasure, as well as reducing defeatist beliefs. A pilot study examining this intervention showed specific improvements of apathy and anhedonia (Favrod et al., 2015). Although replication of these findings in a double-blind randomized control trial is warranted, these results suggest the potential for interventions specifically aimed at the needs of the patient.

Furthermore, the current neuroimaging results may guide neurostimulative treatments, which are used to change brain activation and functional connectivity in order to decrease symptom severity. Neurostimulation of the dorsolateral prefrontal cortex has shown promising effects on negative symptoms in general (Dlabac-de Lange et al., 2015), but further tailoring of the treatment protocol may increase efficacy. Based on the findings in chapter 6, people with amotivation (or more specifically apathy) who experience difficulties in executive functioning may benefit from neurostimulation of the left superior frontal gyrus. However, because the participants in the study of chapter 6 were healthy volunteers, replication of this result is warranted before translation to neurostimulation is desirable.

Methodological considerations and future perspectives
Measuring negative symptoms
In this thesis negative symptoms have been measured using several clinical assessment instruments, all with their advantages and disadvantages. Specifically, the PANSS (used in many studies included in chapter 2, and used in chapter 4 and 5) and the SANS (chapter 3) are two commonly used measures for the assessment of negative symptoms and may therefore enable comparison with many previous studies. Moreover, the SANS was constructed specifically for the characterization of negative symptoms and encompasses a broad range of negative symptoms. However, both the PANSS and SANS have been criticized, among other things for containing measures of cognitive functioning, which is generally considered conceptually separate from negative symptoms. Especially the inclusion of reduced abstract thinking and stereotyped thinking in the negative subscale of the PANSS and the attention deficit subscale of the SANS may be related to cognitive rather than negative symptoms and may therefore hamper the assessment of negative symptoms (Kirkpatrick et al., 2006). In the current thesis, this criticism was met by the use of the PANSS amotivation and expressive deficits factors which do not include abstract thinking or stereotyped thinking (chapter 4 and 5) and the use of a version of the SANS from which the attention subscale was excluded (chapter 3).

Other criticisms of the PANSS and SANS include the focus on observable symptoms rather than internal experience of the participant (Lincoln, Dollfus, & Lyne, 2017) and lack of distinction between anticipatory and consummatory anhedonia (Kirkpatrick et al., 2006). In this respect the use of newer measures like the Brief Negative Symptoms Scale (BNSS) (Kirkpatrick et al., 2011) and the Clinical Assessment Interview For Negative Symptoms (CAINS) (Horan et al., 2011) may provide a more reliable measure of negative symptoms: they provide comprehensive characterization of all negative symptom domains (i.e., blunted affect, alogia, apathy, anhedonia, and asociality), with a focus on both observable symptoms and internal experience (Lincoln, Dollfus, & Lyne, 2017).
The focus on apathy (in chapter 6 and 7) warranted the use of a more specific measure for this negative symptom. To this end, the Apathy Evaluation Scale (AES) was used. This measure is widely used to assess apathy severity in several healthy and clinical populations (Clarke et al., 2011). Because we aimed to assess the neural correlates of different domains that may be disrupted in people with apathy (i.e., affective or reward-related processing, executive functioning, and auto-activation) (Levy & Dubois, 2006), comparison of groups with difficulties predominantly in one of these domains may have given more insight in the different types of disruptions that may underlie apathy. Unfortunately, based on the AES a distinction between these types of apathy cannot be made. However, since the design of this study, a scale has been developed to characterize the subtypes of apathy proposed by Levy and Dubois (Levy & Dubois, 2006): the Dimensional Apathy Scale (DAS) (Radakovic & Abrahams, 2014). The use of this measure may benefit future studies on the neural correlates of the separate domains of apathy.

Although measuring negative symptoms is often done using observer-based interviews or self-rated questionnaires, other more objective measures constructs may give crucial additional information. For example, whereas the AES assesses daily activity level by asking participants what they do on a regular day, this measure may be influenced by lack of insight or socially desirability of certain answers. Therefore, objective measures of motor activity like actigraphy may add crucial information to the assessment of apathy. Indeed, actigraphy has been associated with apathy in patients with schizophrenia (Docx et al., 2013; Farrow et al., 2005) and has been related to cerebral blood flow (Walther et al., 2011), brain volume (Farrow et al., 2005), and structural connectivity (Docx et al., 2017) in patients with schizophrenia. Expressive deficits may be characterized more objective by measuring facial expression using electromyography EMG of the facial muscles (Riehle & Lincoln, 2017) and assessment of gestures and speech production by means of automated analysis of video or audio recordings of social interactions (Alpert et al., 2002; Foussias et al., 2015; Kupper et al., 2010). Including these behavioral measures in neuroimaging studies may aid in the characterization of negative symptoms and may therefore promote understanding of the components of negative symptoms.

Primary versus secondary negative symptoms
With regards to negative symptoms, a distinction can made between primary and secondary negative symptoms. Whereas primary negative symptoms of schizophrenia are thought to be intrinsic to the disorder, secondary negative symptoms may be caused by other characteristics of the disorder (e.g., depressive and positive symptoms, antipsychotic side-effects, environmental effects, and substance abuse) (Carpenter, Heinrichs, & Alphs, 1985; Lincoln, Dollfus, & Lyne, 2017). Although clinically similar, the neural mechanisms leading to primary and secondary negative symptoms have not been compared directly (Kirschner, Aleman, & Kaiser, 2017) and including both in the same study may therefore introduce confounding effects. Therefore, we attempted to account for potential sources of secondary negative symptoms by taking potential causes for secondary negative symptoms into account (i.e., controlling any significant findings for positive symptomatology in chapter 5, 6, and 7, depressive symptomatology in chapter 3 - 7, and antipsychotic medication in chapter 5). Substance abuse was taken
into account by excluding any patients with a substance dependence disorder (chapter 3). Furthermore, in the healthy participants in chapter 6 and 7 substance use was only minimal. Moreover, by studying subclinical apathy in healthy individuals potential confounding factors of medication use and (strongly) impoverished environments have been circumvented, because these participants did not take any psychotropic medication and had the opportunity to undertake physical and social activities (chapter 6 and 7).

Neuroimaging paradigms for the study of negative symptoms

Neural correlates of cognitive and emotional processes are commonly studied using standardized laboratory tasks, yielding control over stimuli and behavior. This may provide good models for cognitive and emotional processing in healthy individuals and may allow characterization of deficits in people with various disorders. However, recently a shift has emerged towards studying the same functions in more naturalistic paradigms, more closely reflecting the complexity of the external world (Hasson & Honey, 2012). Along those lines, in chapter 3 we used an affective forecasting task in which the stimuli were self-generated events. This resulted in a task with complex stimuli that were more closely related to the participants’ lives than a standardized monetary reward task. Although it is not to say that this approach is superior to a standardized task, it may provide additional information, complementary to the traditional approach.

Other approaches to study negative symptoms using more naturalistic paradigms may include virtual reality tasks. For example, instead of a set-shifting task (chapter 6), executive functioning has recently been measured using a virtual reality task (Han, Young Kim, & Kim, 2012). In this study participants were asked to take the quickest bus route to a specific location. Prior to the task participants viewed a conversation between their avatar and a mother figure, telling their avatar that a certain bus route was the fastest. During the task participants were asked to review a schematic representation of the available bus routes and subsequently determine the optimal bus to take. The need for flexibly altering their behavior was introduced by the fact that this schematic showed that the optimal bus route was different from the previously suggested route. Results showed that patients with schizophrenia were less flexible in adapting their behavior. Moreover, in patients that showed inflexible behavior, negative symptoms were associated with longer decision times. Although these results should be interpreted with caution because of a small sample size, a single-trial task design, and possible confounding effects of differences in level of education and general cognitive functioning, this virtual reality approach may have potential as a more naturalistic task for the characterization of executive functioning.

In this thesis, the neural correlates of expressive deficits were studied using a resting-state paradigm. Previous studies have adopted task-related fMRI paradigms in which participants were asked to identify or mimic facial expressions and found that expressive deficits were associated with increased activation of the thalamus, amygdala and hippocampus during the processing of fearful faces (Gur et al. 2007; Lee et al. 2014). An interesting question could be whether these results persist in a more natural setting, for example in a social interaction. An example of this approach is a
study on facial expressions during social interactions in healthy individuals (Riehle & Lincoln, 2017). In this study, facial expressions of healthy participants were measured using EMG of the zygomaticus major (a facial muscle which facilitates smiling). Total amount of smiling during social interactions and synchrony of zygomaticus activations between interaction partners (smiling mimicry) were measured, as well as (subclinical) severity of expressive deficits and the willingness to engage in future interactions. Results showed that expressive deficits were related to the amount of smiling on trend level, but also explained by amotivation. However, because expressive deficit scores in this study were generally low with a narrow range, this may account for the absence of a convincing association. Therefore, repetition of this paradigm in a sample of patients with varying expressive deficit severity may be highly interesting. Unfortunately, given the restrictions that accompany MRI scanning, fMRI may not be of much use for the assessment of the neural mechanisms underlying dysfunctional social interaction using a similar paradigm. Previous studies have examined the neural correlates of various forms of social cognition using near-infrared spectroscopy (NIRS) (Liu et al., 2016; Suda et al., 2011; Takeuchi et al., 2017), leading to the idea that the use of simultaneous EMG-NIRS recordings during social interactions may give an opportunity to assess (cortical) neural correlates of social interaction, smiling behavior and disruptions thereof.

Finally, the functional connectivity networks that were studied using resting-state paradigms in chapter 4 and 5 may also be studied using naturalistic stimuli (e.g., watching a movie or listening to music). This approach may imitate more closely the complex dynamic input of the outside world, while retaining the advantages of resting-state scanning (e.g., low cognitive load and fewer behavioral confounds). Although naturalistic viewing and resting-state clearly reflect distinct mental states, a recent study using dynamic naturalistic stimuli showed similar (but not identical) connectivity patterns compared to traditional resting-state connectivity. Moreover, test-retest reliability of connectivity patterns and graph measures was higher in the naturalistic condition (Wang et al., 2017). Another advantage to this approach is that it may increase experimental control, because the use of naturalistic stimuli may prevent mind wandering. Because it has been shown that during resting-state scanning participants may engage in mind wandering, and that differences in the content of this mind wandering may influence resting-state connectivity (Chou et al., 2017). On the other hand, the nature of the stimuli in a naturalistic paradigm may influence neural activation and connectivity (Vanderwal et al., 2015), leading to the conclusion that although this approach may have its benefits, it may be best used complementary to resting-state paradigms rather than substituting them.

**Concluding remarks**
To conclude, in this thesis the neural correlates of negative symptoms were studied in patients with schizophrenia and healthy individuals. Functional neuroimaging was used during tasks tapping into reward processing, executive processing and auto-activation, as well as during resting-state. Results emphasized the involvement of altered neural activation and functional connectivity during reward processing and executive functioning in the occurrence of negative symptoms. These findings may contribute to enhanced understanding and treatment of negative symptoms, ultimately improving functional outcome of those confronted with these symptoms.