Chapter 6

Summary and concluding remarks
The original aim of the research founding this thesis was to characterize the neurobiological substrate of panic disorder (PD), and to characterize the effects of treatment on the recovery of the affected circuitry. Along the way, the focus partially shifted to the larger concept of the neurobiology of anxiety. When studying the patterns of brain activation before, during and after a pentagastrin challenge in PD patients and healthy volunteers we discovered that different states of anxiety showed a similar pattern of brain activation, wherein the same brain areas were involved. LeDoux (1996) described a fear network in the brain, based on clinical and preclinical research, by which conditioned as well as unconditioned stimuli produce anxiety. A few years later, Gorman et al (2000) revised their neuroanatomical hypothesis of PD (Gorman et al 1989), and described the anxiety in PD in the same terms. The amygdala plays an important central role in the fear network in the brain. In the amygdala, signals from sensory cortical regions and from the thalamus, hippocampus, insula and prefrontal cortex come together and are recognized as anxiety-related. The amygdala projects to brain areas as the locus coeruleus, the hypothalamus and the periaqueductal gray matter (PAG) to generate fear behavior and endocrine and autonomic responses. Furthermore, projections from the amygdala to the orbitofrontal cortex and the anterior cingulate cortex were shown (see also the introduction of this thesis). Damasio et al (2000) discuss different emotions (anger, fear, sadness and happiness), based on a theoretical framework which proposes that all emotions are part of a multitiered and evolutionary set neural mechanism aimed at maintaining organism homeostasis. They argue that different emotions engage (activate or deactivate) cortical and subcortical regions concerned with representing and/or regulating this organism homeostasis, namely the insular cortex, the secondary somatosensory cortex, the anterior and posterior cingulate cortex, the hypothalamus and nuclei in the brainstem tegmentum (upper pons and midbrain). Most of these regions have been implicated in the fear network in the brain, as described by LeDoux (1996). In our study, all brain regions affected by pentagastrin were also part of this fear network. However, it is difficult to interpret increases and decreases in blood flow in functional imaging studies (1999). Malizia (1999) reviews a number of caveats that apply to the definition of the direction of change in the final calculation of brain activity. This leads some investigators to believe that in studies of affect or mood the direction of the change could be of less heuristic value than the location of the changes. Therefore, in the following part, changes in blood flow are not discussed specifically as increases or decreases but only in terms of activation differences, to facilitate reading.

In chapter three we reported anticipatory anxiety in both PD patients and healthy volunteers, and we analyzed the differences in brain activation between PD patients and healthy volunteers at rest. The PD patients in our study experienced a higher level of anticipatory anxiety than the normal control subjects, as witnessed by the higher number of experienced panic-related symptoms in the prechallenge condition. The same rCBF pattern was observed in rest, when comparing PD patients with healthy control subjects, a situation in which PD patients are subject to their normal baseline level of anxiety, while the control subjects are at rest, feeling no anxiety at all (Boshuisen et al 2001). We discovered that during anticipatory anxiety the following regions showed altered brain activity: the precentral gyrus, the inferior frontal...
gyrus, the insula, the amygdala, the parahippocampal gyrus, the hippocampus, the
temporal lobe, the orbitofrontal cortex, the anterior cingulate gyrus, the hypothala-
mus, the thalamus and the midbrain. At rest, we discovered the following differen-
tces in rCBF between untreated PD patients and healthy volunteers, reflecting a pro-
bable trait difference related to the presence of the anxiety disorder, and thus related
to the feeling of anxiety: the precentral gyrus, the insula, the parahippocampal gyrus,
the hippocampus, the temporal lobe, the orbitofrontal cortex, the thalamus and the
midbrain.

In chapter four we described panic induced by pentagastrin in PD patients, and
compared these data with the anxious arousal experienced by healthy volunteers
during a pentagastrin challenge. A similar pattern of anxiety related brain activity
was seen during pentagastrin-induced panic, noticed by the large increase in symp-
tom experience and anxiety in the PD patients, and during pentagastrin-induced
anxious arousal, as shown by the increase in symptom experience and by the small
increase in anxiety in the healthy control subjects (Boshuisen et al submitted). In
the PD patients we found changes in the rCBF in the inferior and superior frontal
gyrus, the insula, the parahippocampal gyrus, the hippocampus, the medial and
inferior temporal lobe, the orbitofrontal cortex, the thalamus, the basal ganglia and
the cerebellum whereas in healthy volunteers we noticed changes in the rCBF in the
precentral gyrus, the superior frontal gyrus, the insula, the amygdala, the parahippo-
campal gyrus, the inferior and superior temporal lobe, the orbitofrontal cortex, the
anterior and the posterior cingulate cortex, the hypothalamus, the basal ganglia, the
cerebellum. In the fifth chapter of this thesis we reported partial normalization of
the rCBF pattern in PD patients after successful pharmacological treatment with the
SSRI sertraline. The findings show that the rCBF differences observed between PD
patients and healthy control subjects are at least partially state dependent, and
change when anxiety diminishes. The fact that the normalization of the rCBF pattern
is only partial could reflect trait differences between PD patients and control sub-
jects. It is conceivable that a follow up period of 12 weeks is too short to achieve
complete normalization of the activation pattern in the brain. Longer follow up pe-
riods can control for this and show if complete normalization is possible. Up until
now, no neuroimaging studies with long follow up intervals have been published.

Finally in chapter six, we described the rCBF pattern during a spontaneous panic
attack and compared this to the panic induced by pentagastrin. No significant diffe-
rences were shown in the rCBF pattern related to the experience of a spontaneous
panic attack as compared to the pentagastrin induced panic attack. Both in sponta-
neous panic and in pentagastrin induced panic we observed a similar pattern of
brain activation. Activation differences during pentagastrin induced panic were seen
in the precentral gyrus, the inferior frontal gyrus, the amygdala, the parahippocamp-
al gyrus, the hippocampus, the medial and superior temporal lobe, the orbitofron-
tal/prefrontal cortex, the anterior and posterior cingulate gyrus, the thalamus and
the cerebellum after pentagastrin challenge. During spontaneous panic activation
differences were seen in the precentral gyrus, the inferior and medial frontal gyrus,
the hippocampus, the anterior and posterior cingulate gyrus, the medial and supe-
rior temporal lobe, the prefrontal/orbitofrontal gyrus, the thalamus and the cerebel-
rum.
We observed an unexpected decrease of the rCBF in the amygdala in PD patients during anticipatory anxiety, and furthermore we observed an increase in the amygdala of healthy controls subjects during anxious arousal, while this could not be observed in PD patients. The amygdala is considered to be crucial in the experiencing of anxiety and fear, and plays a central role in the fear network in the brain (Davis 1997; LeDoux 1996; LeDoux 1998; Windmann 1998) (see also the introduction of this thesis). One may expect increased levels of activity in the amygdala in PD patients, because of this key role of the amygdala in the acquisition and expression of fear (LeDoux 2002). Increased blood flow in the amygdala region is frequently reported in some but certainly not in all anxiety-related studies (Liberzon et al 1999; Pissiota et al 2002; Tillfors et al 2001). Our finding that activation of the amygdala is not present during pentagastrin induced panic attacks in PD patients might be explained by the fact that the amygdala in PD patients is already habituated to anxious arousal in connection with panic. It is hypothesized that the amygdala is specifically involved in the initial acquisition of emotional learning, a phase PD patients have passed, while the situation may be new to the healthy control subjects. In an experiment by Cheng et al (2003) subjects receiving paired presentations of a conditioned stimulus and a shock showed greater amygdala activation than subjects receiving unpaired presentations, thereby exhibiting that the amygdala is important for the expression of learned behavioral responses during Pavlovian fear conditioning. Inhibition of the amygdala could be a consequence of the increased rCBF of the prefrontal cortex, due to a cognitive representation of anticipatory anxiety, present in PD patients but not in healthy control subjects. This inhibition of the amygdala by the prefrontal cortex has been observed previously in rats, monkeys and also in humans (Hariri et al 2003; Kalin et al 2001; LeDoux 1998; Ochsner et al 2002). In our studies activity in the prefrontal cortex is observed and could thus be an explanation for the absence of activity in the amygdala. The role of the amygdala and the prefrontal cortex in anxiety is discussed in a review by Davidson, in which these issues are also addressed (2002).

The remarkable similarities in the rCBF pattern in different forms of anxiety as observed in the studies presented here, were also observed by Reiman (1997). Reiman (1997) reviewed six of his PET studies that investigated brain regions participating in emotion, anxiety and anxiety disorders. He concludes his paper by outlining a fear network in which the different areas and their potential functions are described as being involved in anticipatory anxiety, during panic and in panic disorder patients compared to control subjects. However, the conclusions of Reiman (1997) are based on studies with different designs, and the involvement of only a few fear network related brain regions could be demonstrated. Conclusions in this thesis are based upon studies with a similar design, wherein rCBF differences were consistently shown in most of the fear network related brain regions in different anxiety states.

As explained in the introduction of this thesis, the disorder of PD as it is nowadays categorized in the DSM-IV only exists for several decades. From the DSM-III (1980) onwards the diagnosis is delimited from the anxiety neurosis. The DSM-III came forth from the Research Diagnostic Criteria (RDC) (Spitzer et al 1978), and both classification systems were a result of the development of a taxonomy that could be
accepted by the psychiatric community at the time. Furthermore, a process of scientification of the psychiatric profession started. Research in psychiatry became mainly focused on the search for biological parameters explaining psychiatric disorders, supported by the developments in psychopharmacology. We presently tend to think in this categorical differentiation of anxiety disorders, but several groups have argued for the so-called neurotic disorders to be dimensionally organized (Goldberg 1996; van Praag et al 1987; van Praag 1989; 1990). The categorical or nosological model has great advantages: it allows us to identify relatively homogenous patient groups for research and it is a quick short-hand for clinicians (Goldberg 1996). However, the long-term usefulness of such a model is debated, especially for illnesses such as anxiety and mood disorders (Goldberg 1996; Kendell 1975; van Praag et al 1990; van Praag 2000).

Klein and Klein (1989), favoring the nosological approach, discuss the utility of the panic disorder (PD) concept by stating that spontaneous panic is not simply a severe form of ordinary anxiety or generalized anxiety disorder (GAD), but is distinguishable by the pattern of development, the psychopharmacological responsivity and familial aggregation. According to them it is often mistakenly thought that panic is simply the quantitative extreme of generalized anxiety. They state that the central distinction between panic and anxiety is the sudden crescendo and the spontaneity of the panic attack. This concept of PD as a stable clinical entity is supported by the data of O’Rourke et al (1996). This group states that it is difficult to reconcile their data, derived of the present state examination, with the view that PD is but one facet of a general neurotic syndrome.

Goldberg (1996), favoring the dimensional approach to anxiety disorders, argues that dimensional models are a way of trying to account for variations between the myriad different sets of symptoms offered by patients in the most economical way. According to him, these dimensional models offer considerable advantages over categorical models when relating continuously distributed social variables to clinical variables. He declares: “comorbidity for example, has some meaning if we are referring to combinations of diabetes and schizophrenia, or even of depression and alcohol dependence, but it is surely stretching the concept to absurdity to allow one or two symptoms from correlated domains to produce the phenomenon. The size of comorbidity between anxiety and depression is not a measure of the frequency with which two independent morbid conditions coexist; it merely indicates the position of an arbitrary dividing line on a dimension, which could equally easily, and plausibly, be drawn elsewhere.” In different papers concerning the topic of the denosologization of psychiatry, van Praag (1987; 1989; 2000) argues for a reaction model instead of the nosological approach. The reaction model conceives abnormal psychic states as reaction patterns to noxious stimuli. Noxious stimuli can disarrange various neuronal circuits and various psychological systems. The degree of the neuronal disruption varies as a function of ego strength, strength of the stimuli and neuronal adaptability. Therefore, according to van Praag (2000), psychiatric conditions lack symptomatological consistency and predictability. He argues in favor of verticalization of syndromes, the dissection of syndromes into their component parts, namely the psychological dysfunctions (i.e. functionalization of the diagnosis), and use these psychological dysfunctions as subjects for research into psychiatric disorders.
The outcome of the different studies of this thesis, concerning the activation of fear network related brain regions during anxiety, led us to believe that panic and anxiety are not different entities, but neuronal fear network threshold related differences. After crossing a certain threshold, anxiety may become panic. It is also conceivable that the threshold of anxiety in PD patients is lower than in healthy control subjects. Based upon this one may argue that antipanic drugs exert their clinical effects by virtue of increasing this threshold, for example by improving cerebral plasticity (Saarelainen et al 2003). Therefore, this thesis favors the notion of a more dimensional approach to the diagnosis of anxiety and the concept of anxiety disorders in psychiatry. This notion is corroborated by the outcome of a recent study by Mataix-Cols et al (Mataix-Cols et al 2003), finding support for a dimensional model of obsessive-compulsive disorder. They observed the same brain regions implicated in the mediation of anxiety in response to symptom-related material in healthy volunteers as were previously discovered in OCD patients.

Suggestions for further research

More research in this field is needed to support the idea of a more dimensional concept of anxiety, instead of the nosological definitions of anxiety disorders as can be found in the DSM-IV (1994). This can be done by repeating the experiments in different anxiety disorders as categorized in the DSM-IV, especially the distinction between panic disorder and generalized anxiety is in this respect interesting, because these two disorders are seen as two fundamentally different concepts of anxiety. Furthermore, several parts of the experiments founding this thesis can be changed, to elucidate subjects that are still unclear, or improve the impact of the data gathered.

It would be interesting to assess autonomic functions more precisely in the different experiments, by measuring skin conductance response, continuous heart rate and blood pressure, contingent with heart rate variability. The somatic component of anxiety (e.g. the increased autonomic nervous system reaction) over time almost disappeared in the categorical model of anxiety disorders, but for patients this is still a key component of anxiety.

According to neuroimaging methods, it is preferable to use fMRI in future experiments, because this technique provides better spatial resolution than PET, and experiments can be repeated more often because of the absence of radiation. Difficulties in this respect are the practical aspects of the injection of pentagastrin and the monitoring of the different functions. Furthermore, many patients with anxiety disorders are afraid of small spaces, and therefore do not want to participate when the experiment is performed in the MRI scanner. Partially, this problem can be solved by making a structural MRI scan of every subject in addition to the PET scan, and correlating the PET and MRI data. Then the brain regions with increased or decreased rCBF can be localized more accurately.

We chose a double-blind procedure to administer pentagastrin to the subjects. During the experiments we discovered that pentagastrin is such a powerful panic inducing agent, that after injection it is immediately clear if a subject received penta-
gastrin or placebo. A single-blind procedure is easier performed and has the advantage that the researcher can plan the panic induction. Some subjects were so anxious while anticipating a possible panic attack during pentagastrin, that they experienced a spontaneous panic attack during placebo injection in the second gift. The number of subjects with spontaneous panic attacks might have been much higher when a single-blind procedure was used and all subjects had received placebo during the second gift and pentagastrin during the third gift.

In addition to the placebo gift, it might be interesting to compare the effect of pentagastrin with the effect of thyrotropin releasing hormone (TRH). Coupland et al (1996) discovered that in healthy volunteers TRH induces similar objective and subjective symptoms as pentagastrin. The main differences between TRH and pentagastrin were that TRH induced more urinary urgency and pentagastrin induced dyspnoea and anxiety, and more tingling in the extremities. The mechanism of action of CCK₄ and pentagastrin in anxiety induction is still unclear. Radioactive labeling of pentagastrin or CCK₄ would be a nice way to observe if these substances act directly in the central nervous system, or if cognitive mediation is a more likely mode of action. Labeling of CCK₄ or pentagastrin has not been conducted yet. CCK receptor antagonists have been labeled for the use as CCK receptor ligands in PET studies, but the results for the CCK₄ receptor are not very hopeful. Biodistribution studies showed very low brain uptake after intravenous injection in the rat (Haradahira et al 1998).

PD has a higher incidence in women than in men. Gender differences should be addressed in research of this kind, even more because gender differences also exist in cerebral blood flow (George et al 1996). Unfortunately this subject is beyond the scope of this thesis, but we are planning to address this subject in future research.

Conclusion

The outcome of the different studies of this thesis led us to believe that panic and anxiety are not different entities, but neuronal fear network threshold related differences. After crossing a certain threshold, anxiety may become panic. It is conceivable that the threshold of anxiety in PD patients is lower than in healthy control subjects. More research is needed to further elucidate this subject, nevertheless this thesis provides a conceptual framework suitable as a starting point.