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8 DISCUSSION
1 Introduction

The aim of this work is twofold: first, it aims to advance the idea that there are now grounds for addiction research, and in particular craving, to be viewed in a scheme of the neural substrates of emotion. In this sense, craving will be studied in the context of emotion. Another element in studying cue induced craving is to examine whether the regional changes are static or dynamic. State-trait studies of actually ill and remittent patient groups is one approach. The alternative approach, which is the approach taken in this investigation, examines how the activation in brain regions changes with treatment and how these changes reflect overall symptom specific improvement. The second goal of this paper is a pioneering pilot study designed to explore the possible self-regulation of mechanisms involved in emotion by making unobservable brain processes open to direct conscious experience. This research direction involves the use of real-time fMRI (functional magnetic resonance imaging) applying biofeedback technique.

Studies of neuroadaptations in response to the ingestion of ethanol are, in many cases, directed at generating information on why individuals consume ethanol that is injurious to themselves. In order to approach the problem of sorting out cause and effect relationship between inherent factors that influence excessive consumption of ethanol, investigators have made use of concepts such as alcohol dependence syndrome (Edwards, 1968).

One prominent feature of alcohol dependence is physical dependence which appears when the administration of ethanol is terminated. Jellinek and colleagues (1955) recognized craving as a central component of alcohol dependence syndrome. It was also found that merely the exposure to environmental stimuli can also elicit craving. Stimuli in the environment thus acquire some of the negative affective properties of drug withdrawal and subsequent exposure to these stimuli alone can then elicit withdrawal response that is often associated with compulsive drug-seeking and drug-taking. It was proposed that adaptations in the brain regions during the development of drug dependence contribute to the negative affective component of withdrawal that motivates continued compulsive drug use (Koob and Moal, 1997; Schulteis and Koob, 1996; Koob et al., 1989). It was less clear how stimuli in the environment become associated with both positive and negative effects of abused drugs and thereby impact in the persistence of drug seeking behavior.

Under a number of circumstances dependence to ethanol has been shown to involve learning. Through the use of either classical conditioning approach (Stewart and Eikelboom, 1987) or an
operant conditioning approach, the demonstration of craving has been shown to be dependent upon environmental variables. Laboratory techniques developed by experimental psychologists have proven extremely useful in experimental analyses of drug-seeking behavior. Beginning with the pioneering work of Nichols, Wikler, and Weeks, the conception and techniques of classical and operant conditioning have been applied to the analysis of the behavioral aspects of opiate dependence. A variety of responses to drugs can be classically conditioned and a full constellation of the phenomena of drug abuse must take this into account. Such classical conditioning is relevant to the present context in that it may influence the reinforcement action of a stimuli associated with the drug.

In the opening statement, the idea is advanced that craving ought to be viewed in the context of emotion. This proposition proceeds largely in parallel with the evolution of the concept that the amygdala is implicated in the processes by which environmental stimuli are invested with affective value. Weiskranz (1956), in reviewing the body of data existing at the time, suggested that many of the behavioral changes following amygdala lesions may indicate an important and pervasive role in the association of environmental stimuli with a variety of biologically important aspects of events, thus mediating the impact of their reinforcing value. These findings are extremely important in understanding factors which maintain continued drug-taking behavior in humans. Under this framework, the importance of the amygdala in the phenomena of craving will be investigated using cue-induced craving with ethanol odor.

Studies of drug addiction have been extensively studied in animal models. Animal models are however constrained in that they do without the psychological subjective experience of the human being (Edwards, 1990). All formulations of the dependence syndrome seem to involve ‘loss of control’ of some motivational construct (Stockwell, 1990). Motivation is understood to be a state that can be described as inducing those behaviors that are related to satisfying an organism’s needs. In place or taste conditioning a motivational state is inferred which precedes the act of drug taking. A rat cannot report verbally, so its behavior, by reference to other models, allows one to infer what may lead the animal to drug-taking behavior (Kalant, 1990). In humans, studies have shown that verbal behavior is predictive of drug use (Schuster, 1990).

The method of choice in this thesis for studying the neurobiological basis of craving is the technique of fMRI. When considering brain mechanisms involved in emotion in humans, brain
imaging approaches have been successfully described. FMRI measures changes in blood oxygenation level locally (using signal from deoxyhemoglobin) to provide an index of local brain activity. Experiments using PET (positron emission topography) with cocaine cues to monitor local synaptic activity (reflected in a regional cerebral blood flow rCBF) support the involvement of the amygdala in the psychopathology of craving seen in cocaine addicts (Volkow et al., 1999; Childress et al., 1999; and Grant et al., 1996). So far, the equivalent of approximately eight studies have been conducted among cocaine abusers to study craving, and perhaps only one successful study was conducted in alcoholics that had identified a number of other regions involved in craving (Modell and Mountz, 1995).

Drug addiction and dependence belongs to the most complex realms of human pathology, where psychological, social, cultural factors are closely interwoven. In the case of alcohol, Jellinek (1960) recognized this, and the idea of the multidimensionality of drug dependence has been embodied in the concept of alcohol dependence syndrome (Edwards and Gross, 1976). For example, possible variables would include family environmental influences on the integration transmission of alcohol problems. Also drug taking and activity are taken for euphoria stimulation as well as for euphoria relaxation. Other factors include developmental variables (e.g., attitudes towards deviance, non-functional families). More complexity is added as a result of secondary processes, which may be considered reactivity related and which can serve to augment the drug-rated problem e.g., guilt, depression, ambivalence, vacillation. Some authors have suggested that drug dependence is a multifaceted concept. Unlike the traditional approaches to drug abuse in which the source of difficulty is placed within the individual, today, the importance of the control of an individual’s behavior by environmental contingencies have to be stressed when analyzing drug-seeking and drug taking behavior. This has tremendous implications for the treatment programs which, to be maximally, effective, must consider all the environmental factors maintaining an individuals drug-seeking and drug taking behavior (Schuster, 1990).

A promising new treatment application which does not depend on any of the environmental processes, is proposed by controlling dysfunctional brain mechanisms through operant conditioning using real-time fMRI. In light of the surmounting evidence that craving may be controlled by complex affective processing systems, and the possible role the amygdala plays in response to substance relevant stimuli, the goal of the second research direction was the usage of on-line
biofeedback for manipulating BOLD response in the amygdala as a potentially important method to influence human experience and behavior.

This thesis begins with a description of the amygdala as an important nodal point in the adaptive neural responses to an environment with constantly changing affective contingencies. For this reason, the amygdala plays an important role in a wide range of behaviors. While the notion that the amygdala is influential in affective behaviors has been established for some time now, the challenge next is to establish the role between how the function of the amygdala enables it to participate beyond the normal appreciation of emotion to include stimulus-reinforcement associations, and further to determine whether dysfunction of the amygdala is an important contributor to the etiology of alcohol craving. The relationship between neuroadapations and cues can reveal fundamental evidence crucial for understanding craving. Such evidence, in terms of building theories and models of craving, can complement imaging approaches very effectively. The approach to brain function in terms of neuroadaptation in the brain areas associated with craving will also be made reference to in this study. The thesis will focus on evidence from the alcohol literature, but shall also draw selectively on other drugs to exemplify points where alcohol data is shortcoming.
AMYGDALA AND EMOTION

2 AMYGDALA AND EMOTION

2.1 HISTORICAL PERSPECTIVE

Although the amygdala is now believed to be a key structure in the brain’s emotional system, it has not always been viewed this way. Only after the Klüver-Bucy syndrome was attributed to a damaged amygdala (1939), has the amygdala become an area of special interest in the study of emotion. The significance of the amygdala was either ignored or diminished relative to other structures by early influential theories of brain and emotion. In the Canon-Bard hypothesis, for example, the hypothalamus and cerebral cortex played a key integrative role in emotional processing (Bard, 1959; Cannon, 1931). Canon and Bard based their theory on the discovery that the cat’s strong emotional responses to stimuli depended on an intact diencephalon, which includes the thalamus and hypothalamus. After disconnection of the diencephalon from the midbrain, such responses seized. Stimulation of both the hypothalamus and thalamus elicited different types of affective behaviors and autonomic responses such as hissing and changes in heart rate. Accordingly, Canon posited that critical for emotional experiences are the diencephalic-hypothalamic circuitry, in which afferent stimuli entering the brain get transmitted to the thalamus, which activates the hypothalamus; via the hypothalamus the endocrine system and the autonomic nervous system, and it is these systems that induce the physiological visceral changes which primarily act as adaptive changes and aid in the survival of the organism. The feedback from the hypothalamus to the cortex is what causes emotional experiences according to Canon-Bard. Putting their theory into practice would take this form: at the same time that the diencephalon discharges downward to the basal ganglia, producing motor impulses of emotional behavior such as crying or laughing, it also discharges upward to the cortex, allowing conscious appreciation of emotional states. Along similar lines, Papez (1937) proposed that the mechanism of emotion is part of a unified concept with three divisions through which man’s volitional energy “flow”. First, in Papez’s reasoning, the “stream of movement” is conducted through the dorsal thalamus and the internal capsule to the corpus striatum and then out the central nervous system to the somatic motor neurons. Second, the “stream of thought” arises from the thalamus and ascends the internal capsule to find expression in the lateral cerebral cortex involved in executive function. Third, there are impulses flowing through the medial structures in the “stream of feeling”. Papez proposed that the
circuit of emotion involved the hypothalamus, anterior thalamus, cingulate cortex, and hippocampus.

Not until the late 40’s, was reference ever made to the amygdala in theories of brain and emotion. Yakolev (1948) independent of earlier theories, suggested that three lateral cortical regions - the orbital frontal cortex, temporal lobe, and the insula – and two cortical structures the amygdala and thalamus played a role in motivation and emotion. MacLean (1952) referred to these structures as forming the limbic system. While the amygdala was one of the subcortical nuclei of MacLean’s limbic system (1949, 1952), it was clearly a lesser companion to the hippocampus, which was the centerpiece of the limbic system theory of emotion. Presently, the amygdala is viewed as the brain region most often implicated in emotional processing, with the hippocampus and other limbic systems to which it is connected commonly portrayed as more involved in cognitive than emotional processes.

A strong advocate of this position is LeDoux et al. (1990), who modified Canon’s thalamic-hypothalamic emotion circuit to include the amygdala. According to LeDoux, stimuli entering the brain project to the thalamus which acts as a relay station before they reach the amygdala. He noted that the amygdala receives afferent nerve impulses not only from the cortex and the thalamus but also from visceral pathways. He also made the observation that monkeys with a destroyed amygdala on one side demonstrated normal behavior when they viewed the world with the intact amygdala. In contrast, the monkeys were tame and fearless to threatening stimuli when they viewed the world with the hemisphere of the destroyed amygdala. LeDoux proposed that emotions are consciously perceived at the thalamo-amygdala path. He based his argument on the observation that classical conditioning of fear in rats was possible without cortical participation.

2.2 ANATOMICAL ORGANIZATION OF THE AMYGDALA

As research into this area ensued, data continued to accumulate in support of the amygdala and the strongly interconnected limbic and subcortical and frontal brain regions as the neuroanatomical substrates of emotion. Yet, the precise role of the amygdaloid complex in behavior remains unclear even today, with still many questions begging an answer. For example, studies have revealed rich connections between the amygdala and the ventral striatum, an area once thought to be heavily involved in addiction. Do the prominent projections to the striatum have a role in initiating an
appropriate motor response to a motivating stimulus? The amygdala has reciprocal connects with the orbitofrontal cortex. The orbitofrontal cortex is involved in motivational states, and forms part of the striato-thalamo-orbito-frontal circuits which are believed to be important in the inhibition of common responses in contexts in which they are not adequate. Dysfunction to this circuit results in compulsive behavior and exaggerated motivation to rewarding stimuli. Could the integration of information from the amygdala to the orbitofrontal cortex mediate compulsive behavior, and loss of control to conditioned motivating stimuli. While clearly, there remain unanswered questions concerning the anatomy of the amygdaloid complex, it is quite evident that the design of behavioral analyses directed at unraveling the functional correlates of the amygdala must take the richness of its anatomical organization into consideration. The cortical anatomy description presented below are extrapolated from non-human primates to a human brain since the refinement of the brain’s connectional anatomy is overwhelmingly derived from non-human primate tracer studies.

2.2.1 THE AMYGDALA

The amygdala, so called because it resembles the shape of an almond, is a deep layered structure that forms part of the limbic system. The fundamental description of the amygdala structure that is in widest use today was introduced in 1923 by Johnson. The amygdaloid complex lies in the anterior medial portion of each temporal pole. It has neural connections with many other parts of the brain including the neocortex, the hypothalamus, the septal area, the thalamus, the hippocampus, the reticular formation. Cortical and subcortical areas share phylogenic and cytoarchitectural features which are united by two belts one of which is the orbitofrontal paralimbic division. The amygdala is the subcortical focus of the orbitofrontal paralimbic division having rich interconnections with various neocortical and subcortical regions.

The amygdala contains four deep nuclei from lateral to medial: the lateral nucleus, the basal nucleus (with magnocellular, intermediate, and parvocellular divisions from dorsal to ventral), the accessory basal nucleus (with magnocellular, parvocellular, and ventromedial divisions from lateral to medial) and the paralaminar nucleus on the ventral surface of the amygdala. Six superficial nuclei (the anterior and posterior cortical, medial and central nuclei, the nucleus of lateral olfactory tract, and the periamygdaloid cortex) and three other nuclei (the anterior amygdaloid area, amygdalohippocampal area and collections of cell bodies in the fiber bundles within the amygdala forming the intercalated nuclei) form the remaining part of amygdala.
2.2.2 CONNECTIONS OF THE AMYGDALA

A simplification of the amygdalar complexity is the functional and anatomical separation of a cortical association division (basal/lateral nuclei) from a visceral-subcortical division (central/medial nuclei. The former has stronger connections with association cortex, whereas the latter focuses projections to the brainstem, hypothalamus, and basal ganglia (Heimer et al., 1991).

2.2.2.1 Basal/lateral nuclei

The relatively new basolateral division of the amygdala is involved in all aspects of higher order emotional activity. Hence its rich interconnections with the neocortex (Kirkpatrick, 1996). Rich interconnections with the inferior, middle, and superior temporal lobes are maintained with the lateral amygdala, thereby giving emotional significance to auditory, somato-esthetic and visual information (Steklis and King, 1985). In addition to it’s sharing reciprocal connections with a number of cortical areas the basolateral area projects to the dorsal and ventral striatum, contributing to the enervation of the ventral striatum (Kelley et al., 1982). The basolateral area has been implicated in reinforcement paradigms (Burns et al., 1993) and in certain cognitive tasks (Ingles et al., 1993). Moreover, the extensive connection between the basolateral amygdala and the frontal cortex suggest that it may be involved in the experience and perception of anxiety.

2.2.2.2 Central/medial nuclei

The highly processed sensory information from various cortical (all modalities and polymodal; figure 1) areas reaching the amygdala through its lateral and basolateral nuclei (Amaral, 1987; Burwell et al., 1995) project to the central nucleus of the amygdala (Amaral, 1987; Aggleton, 1985; Savandar et al., 1995). The amygdaloid complex is generally considered to have two major output pathways: the ventral amygdalofugal pathway and the stria terminals. Projections from the central nucleus to subcortical structures: the brainstem or hypothalamus occur via these two extrinsic systems. The finding that the sensory cortical areas project to the amygdala before passing on the brainstem and hypothalamus (Mesulam and Mufson, 1984; Turner et al., 1980) indicates that the amygdala provides a crucial relay between these cortical sensory systems and the deep subcortical structures involved in affective processes. The projections from the medial/central nucleus to a variety of brainstem regions influences or initiates autonomic and somatic components. Portions of the medial amygdala merge with basal ganglia complex and the caudate tail of the caudate nucleus,
making it heavily involved in motivation and coordinating whole body activity. Other efferents project to the modulla, and pons (Price and Amaral, 1981). These projections continue to the substantia nigra (pars compacta), peripeduncular nucleus, the central gray (freezing responses), parabrachial nucleus (stress response; corticosteroid release), locus coeruleus (arousal and vigilance), and dorsal motor nucleus of the vagus (ulcers, urination, bradycardia). These connections between the central nucleus of the amygdala and the variety of targets are suggestive of a heavy involvement in fear.

2.2.2.3 Periamygdaloid cortex

Although the periamygdaloid nucleus forms one of the superficial nuclei, it is important to note for the purpose of this thesis that the periamygdaloid cortex receives projections from the olfactory bulb. The olfactory input is organized in a different fashion than the other sensory inputs, since it originates from the very early stage of olfactory processing (olfactory bulbs) and the projections terminate at the periamygdaloid cortex rather than the deep nuclei (Turner et al., 1978). In addition to the direct connection between the amygdala and the (main or accessory) olfactory bulbs, there are substantial associational connections between all parts of the primary olfactory cortex.

2.2.2.4 Intrinsic connections

The term amygdala has never been much more than a geographical ‘catch all’ for anatomically but neighboring structures (Baars, 1998). Studies on macaque monkeys in which horseradish peroxide has been injected, provided much of what we know about the intrinsic connections of the amygdala. It was found that the intrinsic connections possess highly organized patterns, with the vast majority coursing medially or dorsally within the amygdala. The heavy network of internal connections suggests an enormous amount of additional sensory convergence within the amygdala, especially as it is those regions that receive the greatest concentration of cortical sensory afferents (lateral/basal nuclei) that also provide the major intrinsic efferents. It is also significant that the amygdaloid nuclei receiving the bulk of the intrinsic connections (i.e., the accessory basal, centromedial and central nuclei) are the ones that provide the major amygdaloid output to the hypothalamus and midbrain. From observation based on anatomical and electrophysiological studies the basic intrinsic amygdaloid circuitry is said to be involved in conditioning processes associating neutral and aversive stimuli.
The structural complexity of the amygdala should give a clue to the functional importance of this area. The selective overview of the anatomical connections of this structure indicate that it is strategically placed to receive highly processed information from the cortex and to influence motor systems, autonomic systems, some cortical areas from which it receives inputs and other limbic areas. A logical starting place in the discussion of the amygdaloid function is to consider whether the operations of the amygdala are to influence or modulate many of the brain regions to which it is connected. Behavioral dysfunction produced by amygdala lesion provides one opportunity to investigate the role of the amygdala in emotion.
2.3 LESIONS OF THE AMYGDALA

Psychic blindness is among the most striking features elicited by lesions to the amygdala and surrounding areas. Klüver and Bucy (1937) were the first to demonstrate that destruction of the amygdala in monkeys would produce a complex set of several affective disorders that include tameness coupled with a willingness to approach normally fear-inducing stimuli such as humans, gloves, or animal models (loss of fear); increased and inappropriate sexual behavior (hypersexuality); and a tendency to investigate the environment orally and put inedible objects into their mouth (oral tendency).

Weiskrantz (1956) later suggested that the “effect of amygdalectomy is to make difficult for reinforcing stimuli whether positive or negative, to become established”. Indeed it is proposed that at least part of the importance of the amygdala in emotion is, that it is involved in this type of emotional learning. In an attempt to directly test the nature of underlying emotional responses, a number of experimentally induced amygdala lesions showed that monkeys failed to learn to make responses when a light was signaling that shock would soon follow unless a response was made. Jones and Mishkin (1972) showed that monkeys with bilateral lesions preformed poorly in tasks involving rapid switching of the reward value of the stimuli. Thus, these evidence suggest that selective amygdala lesion impair some type of behavior to learn stimulus-reward associations.

In the first series of amygdalectomies to reduce epilepsy in humans ever to be reported, Narabayashi et al. (1963) found that aggressive or hyperactive patients who received relatively small unilateral or bilateral stereotaxic lesions showed a marked reduction in emotional excitability. Subsequent cases served to confirm their initial findings (Balasubramanian and Ramamurthi, 1970; Vaernet and Madsen, 1970). The full blown Klüver-Bucy syndrome in humans, however, has rarely been described. In those rare cases where dramatic, global losses of emotion were reported, large bilateral, temporal removals extending beyond the amygdala were included (Obraor, 1947; Green et al., 1951). Other areas have been suggested to include both cortical and subcortical temporal lobe damage (Terzian and Ore, 1955).

Consistent with this view is the finding of Klüver-Bucy-like symptoms in more diffuse diseases that include the amygdala. For example, in Alzheimer’s disease, amygdala pathology is common and appears to be correlated with the Klüver-Bucy syndrome (Herzog and Kemper, 1980). These
symptoms are also characteristic with Pick’s disease, that preferentially attacks the temporal lobes (Cummings and Duchen, 1981). Similarly, there is evidence that the amygdala is involved in schizophrenia (Bogerts et al., 1985; Cramer et al., 1992; Gaffan and Murray, 1990; Schneider et al., 1995, 1998) which strongly implicates the hippocampus and frontal lobe (Poletti, 1986).

The findings outlined above point to a central role for the amygdala in the expression and experience of emotion in man and animals. How might, one asks, do tameness and hypoemotionality, the increase in orality and altered food response arise from damage to an area by which stimuli become associated with reward or punishment. As noted earlier, the amygdala receives highly integrated sensory information from all modalities in a late stage of cortical processing (Gloor, 1986). In turn, the amygdala projects to the hypothalamus, and brain stem area (Hopkin et al., 1978) which is involved in behavioral responses made on the basis of learned associations between visual and primary stimuli (unlearned) (Oomura et al., 1970). It was suggested that the Klüver-Bucy syndrome may be produced by lesions that interrupt this pathway (Horal et al., 1975), such as lesions of the entire temporal neocortex or infero-temporal (visual) cortex (Gaffan et al., 1988), lesions of the amygdala (Horal et al., 1975), or lesions of the pathway from the amygdala to the hypothalamus or brain stem (Hopkins and Holstege, 1963). Based on neuron recordings in awake monkeys with a visual discrimination task, Rolls et al. (1977) discovered physical related (shape, size, orientation, color, texture) responses in the infero-temporal lobe. Neurons in the amygdala responded primarily to food and to objects associated with food as well as to one or more aversive and neutral stimuli. However, once the rewarding stimuli were reversed, the population of neurons showed difficulty in reversing their responses. For example, Nishijo (1988) made the observation that neurons may respond to the visual presentation of a slice of watermelon, but not to watermelon that has been salted and thereby made aversive. Neurons with responses more closely related to reinforcement were found in areas to which the amygdala projects, such as the lateral hypothalamus and ventral striatum, areas with orbitofrontal input. It was suggested that the orbitofrontal cortex is more involved than the amygdala in the rapid readjustments of behavioral responses made to stimuli when their reinforcing value is repeatedly changing (Thorpe et al., 1983), somewhat like the manager of the brain. These findings suggest that the amygdala is involved in processes by which visual stimuli are associated with reinforcement. Once formed, these associations may enable learned affective stimuli to influence behavior by producing autonomic behavior via the lateral hypothalamus (LeDoux, 1988), by influencing the striatum (see below) and
orbitofrontal cortex (see below), and by producing emotional arousal which can influence learning (Rolls 1990b; Rolls and Johnston, 1991).

2.4 SECONDARY REINFORCEMENT AND THE AMYGDALA

It is apparent from the foreground evidence that the symptoms of Kluver-Bucy syndrome, including the emotional changes (Jones and Mishkin, 1972) could be a result of deficit in learning stimulus-reinforcement associations. There is also evidence that the amygdala is involved in behavior to stimuli learned as being associated with reward as well as with punishment, referred to as secondary reinforcers. Stimuli that are learned reinforcers through their association with primary reinforcers become secondary reinforcers. This type of learning may thus be called “stimulus-reinforcement association” and most likely occurs via a process of classical conditioning. Most of the studies performed involving secondary reinforcers have been done using abuse drugs. In studies investigating the role of the amygdala in reward-related learning in the rats, Cador et al. (1989) obtained evidence consistent with this hypotheses that the learned incentive (conditioned reinforcers) effect of previously neutral stimuli paired with rewards are mediated by the amygdala. Whitelaw et al. (1996) showed that excitotoxic lesions of the basolateral amygdala in rats impaired behavioral responses to a light associated with delivery for the drug, while self-administration of the drug in a continuous reinforcement (i.e. they were rewarded on every acquisition trial) was not impaired, thus suggesting that the amygdala is not necessary for the primary reinforcement effects of cocaine. In another experiment, Schulties et al. (2000) demonstrated that lesions to the basolateral amygdala disrupted the conditioned suppression of appetitive behavioral response following presentation of withdrawal-associated conditioned stimulus, but not to the primary aversive effect of the opiate withdrawal. The results in this study indicated that the basolateral amygdala is not required for the association of the negative affect of withdrawal, but that it is critical for the association of the negative affect of withdrawal with previously neutral environment stimuli. In another animal model, where rats were exposed to ethanol associated odor compared to water, lever pressing in rats was reinstated after extinction. Interestingly, conditioned auditory cues failed to evoke such effects, suggesting that the involved processes may be modality-specific (Katner and Weiss, 1999). Although the study did not directly investigate the amygdala, considering the evidence so far, amygdala involvement can be inferred.
2.5 **AMYGDALA STRIATUM CONNECTION**

Moreover, there is growing evidence to suggest that the conditioned reinforcing effects which are mediated by the amygdala act through the ventral striatum. Everette and Robbins (1992) have proposed that the ventral striatum is the site at which affective processing occurring in the limbic forebrain may gain access to subcortical elements of the motor system thereby affecting action. Support for this theory was found in a number of experiments of neural manipulation of the ventral/amygdala areas. For example, animal studies demonstrated that pharmacological stimulation of the amygdala induced locomotor activity, similar to stimulation effects of the ventral striatum (Yim and Mogenson, 1982, 1983). Complementing these discoveries was the finding that neurotoxic lesions of the ventral striatum in primates produced some changes in emotionality, including increased activity and aggressive behavior upon the elimination of reward (Stern and Passingham, 1996). Thus, a body of data exist that strongly indicate that simple locomotor responses generated by stimulation of the forebrain limbic structures depend upon direct interaction between these structures and the ventral striatum. It was reasoned that if the basolateral amygdala and the ventral striatum are indeed functionally and serially interrelated than the asymmetric lesions should have a behavioral affect that resemble closely those followed by bilateral lesions of either structure alone (Everitt et al., 1991). This supposition was indeed confirmed by elegantly crafted experiments one of which involved the place preference paradigm. Conditioned place preference involves two phases. Phase one: repeated presentation of primary reinforcers, or nothing in one of two distinctive environments. Phase two: the conditioned approach to the environment previously paired with the reward is measured in a preference test in extinction. In an experimental study of the role of the amygdala in responses to learned positive reinforcers in rats, Everett et al. (1991) showed that a conditioned place preference to a place where rats were given 10% sucrose was abolished by bilateral excitotoxic lesions of the basolateral amygdala. Moreover, the output of the amygdala for this learned reinforcement effect on behavior appears to be via the ventral striatum, for a unilateral lesion of the amygdala and a contralateral lesion of the ventral striatum also impaired the conditioned place preference for the place where sucrose was made available. These data point to the importance of interactions between the amygdala and ventral striatum in the processes by which environmental stimuli gain affective value through their predictive association of an integrated emotional response.
2.6 **AMYGDALA AND ELECTRICAL/CHEMICAL STIMULATION**

A substantial hindrance in our understanding of the brain mechanisms of emotion is the relationship between man and animal emotional systems. The majority of instruments used in animal research have centered on issues relating to how the brain processes the emotional significance of stimuli and produces emotional responses. These issues are not trivial to say the least, but they relate indirectly to how the brain generates the variety of subjective emotional states. Electrical stimulation offers one excellent technique for studying emotional states in humans. One of the strongest arguments for amygdala involvement in emotion comes from observations of patients who have strong emotions associated with medial temporal lobe or amygdala seizures (Halgren, 1981) which are normally connected with abnormal electrical activity. Electrical stimulation of epileptic patients aimed at relieving the epileptic conditions can result in a broad variety of phenomena. For example, electrical stimulation of the amygdala in conscious man produce hallucinations (dreamlike, memorylike, or thoughtlike). Such experiential or hallucinations strike patients as being very similar to vivid life experiences, because they share a quality of experiential immediacy that frequently encompasses perceptual, mnemonic and affective features. Illusion of familiarity are in some situations experienced as a *déjà-vu* sensation. Interestingly, these memory flashbacks are devoid of content, yet contain an ‘affective’ quality. Among perceptual phenomena, visual or auditory hallucinations (e.g., seeing a scene or a person, hearing a piece of music played) are most common. Less frequently observed are olfactory hallucinations and gustatory which are most likely experienced when discharge spreads to the insular cortex, in the latter experience (Hauser-Hauw and Bancaud, 1987). A variety of viscerosensory phenomena have also been reported, referred to as head, heart, chest or stomach. Sexual feelings (Bancaud, 1981) have also been reported. For unexplained reasons, erotic feelings have almost exclusively been reported by women.

The affective experience elicited by electrical stimulation almost always involves fear. This includes altered heart rate and blood pressure (Kaada, 1951), altered respiration (Anand and Dua, 1956), a prominent symptom of fear, especially panic symptoms. Activation of facial motor neurons is also found (Fanardjian and Manvelyan, 1987) which probably mediate some facial expressions seen during fear reaction. Also cessation of ongoing behavior has been noted. This is, in fact, a critical measure of fear or anxiety (Applegate et al., 1983). Additionally, electrical stimulation of the amygdala has shown an increase in plasma levels of corticosterone, indicating an excitatory
effect of the amygdala on the hypothalamo-pituitary axis (HPA) (Dunn and Whitener, 1986). In animals autonomic and defensive reactions have been evoked during chemical stimulation of the hypothalamus (Abrahams et al., 1960). Similar phenomena have been evoked in humans by amygdala stimulation (Iwata et al., 1987). However, on closer examination, it was found that amygdala stimulation mediate autonomic changes via amygdalo-hypothalamic projections (Sun and Guyenet, 1986), and mediate fear responses via amygdala and central gray connections (Bandler, 1982).

Interestingly, the experiences across subjects that have had electric stimulation were dissimilar. Even within a given patient, the category of mental phenomenon evoked tends to be constant across multiple locations in the medial temporal lobe within a given session, but may be different on another day (Halgren et al., 1978a). Extensive reviews of amygdala stimulation have been published in which it was reported that phenomena elicited by electrical stimulation of the amygdala is specific to the person stimulated. Moreover, the content of the sensory hallucinations may be related to the patients personality and ongoing concerns (Mahl et al., 1964; Rayport and Ferguson, 1974). No less significant, is the finding that stimulation of distant sites where the amygdala is known to project, have provoked similar phenomena as those seen during amygdala stimulation. For example, hallucinations were reported after stimulation of the lateral temporal sites (Panfield and Parot, 1963) and fear can be evoked in various limbic sites (Halgren, 1982). However, the full constellation of phenomena observed after medial temporal lobe stimulation has not been observed elsewhere. It would appear therefore, that these phenomena reflect the activity of quite widespread and integrated neural networks, and this is why their content is so dependent on personal and contextual variables (Chauval et al., 1989)

2.7 THE AMYGDALA AND ANXIETY

As was just pointed out, electrical stimulation of the amygdala was shown to increase plasma levels of corticosterone. The mechanism involved in symptoms of anxiety are suggested to result from dysregulation of CRF (corticotropin releasing factor) transmission in the amygdala. Animal studies using microdialyses technique have indicated that CRF in the amygdala increased within 20 minutes of application of a stressor, and began returning to normal levels soon thereafter (Pich, 1993).
A model that has been successfully used to study human anxiety is the fear potentiated startle paradigm since it can be loosely connected with human anxiety on the basis that people startle more when they are afraid. Fear-potentiated startle is defined by an increase in startle in the presence of a cue previously paired with shock. Importantly, the startle paradigm is a sensitive method for determining behavioral changes in excitability resulting from negative emotional arousal (Grillon et al., 1991) and drug withdrawal. A study of the effects of chronic ethanol administration and withdrawal in acoustic startle response revealed increases in startle during ethanol withdrawal in humans (Rassnick et al., 1992; Cooper et al., 1979).

A possible mechanism involved in the aspects of ethanol withdrawal is the overshoot of the brain stress hormone corticotropin releasing factor (CRF). This suggestion is supported by the finding that many of the behavioral effects of stress can be blocked by intraventricular infusion of CRF antagonist helical CRF. Swerdlow et al. (1989) reported that intravenous infusion of CRF causes dose dependent increase in the amplitude of startle reflex which can be blocked by intraventricular infusion of helical CRF. No less significant was the finding that the magnitude of startle is directly related to the number of intervening shocks prior to presenting any startle stimuli suggesting a sensitization effect. Thus, increased anxiety could also result from an overshoot of CRF due to shock sensitization (Groves and Thompson, 1970).

Taken together, it can be assumed, based on the above data, that CRF is functionally involved in the regulation of stressful stimuli, possibly via the amygdala structure. Moreover, that dysregulation of CRF is an important component in the manifestation of anxiety.

2.8 AMYGDALA CONNECTIONS AND REGIONS INVOLVED IN REINFORCEMENT/ANXIETY

The stage for understanding the role of the amygdala in the subject matter of this thesis may be set by considering its anatomical relations to other brain structures. It is however, difficult to separate neuroanatomical from functional criteria, which will become evident in the following:

2.8.1 HIPPOCAMPUS AMYGDALA CONNECTION

The hippocampus consists of a large group of nuclei lying along the floor of the third ventricle deep within the temporal lobe. Besides the amygdaloid complex, the hippocampus formation is the most prominent component of the primate temporal lobe. The hippocampus formation, which is believed to be involved in higher cognitive functions (Squire, 1987) sends projections to the lateral nucleus of the amygdala. A model of anxiety has been built around the functions of the hippocampus and
septum. Gray’s (1982) model is based on a couple of observations; (i) the fact that the effects of antianxiety drugs (alcohol, barbiturates) on behavioral tasks mirror the effects of lesions of the septo-hippocampal system, and (ii) no less important, the traditional distinction between fear and anxiety. Clinically fear is regarded to be more stimulus-specific (associated with sensory stimuli from sensory processing pathways) than anxiety, despite the similar symptoms. According to Gray, thoughts and memory processed in the hippocampus might receive emotional coloring by way of information transmission to the amygdala. Thus, the hippocampus would contribute to anxiety as a cognitive rather than as an affective processing structure.

2.8.2 ORBITOFRONTAL CORTEX AND THE AMYGDALA

The orbitofrontal cortex (OFC) is one of the three parts comprising the prefrontal cortex, receiving projections from the mediodorsal nucleus of the thalamus and the primary olfactory cortex (Price et al., 1999). It is situated in front of the motor and premotor cortices and there exists reciprocal anatomical projections between the orbitofrontal cortex and the amygdala (Amaral et al., 1986).

The orbitofrontal cortex is one of the major brain areas, apart from the amygdala, that is involved in emotion and motivation in humans. It’s role is suggested to involve the correction of behavioral responses made to stimuli associated with primary and secondary reinforcement. In this respect, it is proposed that the OFC is the neural substrate where information about stimuli and incentives are located, and which can be stored for long periods, and recalled whenever each stimulus is seen in the future (Rolls and Treves, 1998). Hence, while it is suggested that the amygdala is involved in coding of new information, the site of permanent representation of affect-laden information is proposed (Kesner and Dimattia, 1987) to be in the orbitofrontal cortex. In particular, it is suggested that the orbitofrontal cortex mediates affect information as it relates to the expectation of rewards and punishment and social behavior. Even though there is very little data on the role of the orbitofrontal cortex involvement in permanent representation of affect-laden information, a case can be made when one combines human and animal data in orbitofrontal cortex function.

For example, humans with orbitofrontal cortex damage show a variety of personality changes, characterized by euphoria, irritability, facetiousness, which may be related to a dysfunction in altering behavior appropriately in response to a change on reinforcement contingencies. Moreover, these patients seem to have a lack of appreciation of social rules and altered affect. Miller (1993)
showed that in the Wisconsin Card Sorting Task, frontal patients either had difficulty in
determining the first sorting principle, or in shifting to a second principle when required to do so.

Damage to the orbitofrontal cortex in the monkey produces emotional changes such as reduced
aggression to humans as well as to stimuli such as a snake (Butter et al., 1970). Orbital cortex-
damaged animals had difficulty changing their behavior when the value of reward was not
consistent with expectations based on prior experiences. Thus, animals with orbitofrontal cortex
lesions display prolonged extinction of a previously rewarding response (Butter, 1969). For
example, monkeys with OFC damage may be impaired on go/nogo tasks in that they respond to the
object which was formerly rewarded with food, and in extinction in that they continue to respond to
an object that was no longer rewarded. These data suggest that the orbitofrontal cortex is involved
in encoding and processing affect information for reward strategies and rules.

Human, animal and lesion studies thus suggest that the amygdala is involved in learning to form
new stimuli reward association, whereas, the orbitofrontal cortex mediates affect information as it
relates to expectation of reward and punishment. Accordingly, it can be predicted that orbitofrontal
damaged patients will display deficits in situations where the expectations of reward or affect are
well established prior to injury, while amygdala damage will display deficits in situations where
expectations and reward are established after injury. Bradly et al. (1954) showed that after
amygdala lesions, cats failed to acquire an active avoidance response, but showed no retention
deficits when amygdala lesions were made after training. In contrast, cats with orbitofrontal cortex
lesions made after training displayed a marked retention deficit. The responses of the animals with
amygdala lesion fits well with the notion that the amygdala is involved in coding of new
information.

2.8.3 CINGULATE CORTEX AND THE AMYGDALA

The cingulate is one of the largest part of the limbic system (MacLean, 1990). This area is divided
into two parts, the anterior and posterior regions, which are characterized by very different
cytoarchitechure, connections and function. The cingulate cortex receives widespread projections
from the amygdala. In general, projections from the cingulate cortices appear to reciprocate the
amygdalocortical projections (Aggleton et al., 1980). Although the anterior part of the cingulate
plays a role in motor function, the anterior cingulate cortex also mediates autonomic activity
associated with affective behavior, suggesting its involvement in emotional behavior.
The suggestion that the cingulate participates in emotion is strengthened by observations of cingulum stimulation. For example, electrically induced seizures to the anterior cingulate in humans have evoked euphoria (Laitinen and Vilkke, 1973). In epileptic patients, electrical stimulation of that area induced fear, pleasure and agitation (Meyer et al., 1973). Also emotional changes are common among patients with cingulate seizure foci (i.e, laughing, irritability and sexual deviancy). However, not all investigators were able to replicate these findings (Lewin and Whitty, 1960). Cingulate tumor and other lesions have caused a variety of affective changes, including apathy, depression and aggression (Levin and Duchowny, 1991).

Theoretically, the cingulate area is postulated to play a role in aspects of attentional processing (Posner, 1985). Interestingly, Lane et al. (1997) found a significant involvement of the cingulate in tasks that required attention to subjective emotional responses compared with conditions requiring attention to context of stimuli. Also, studies of mood induction, have revealed cingulum involvement during sad mood (Mayberg, 1997). Given the inputs to the anterior cingulate from the amygdala, orbitofrontal, and insular cortex, and its output connections to the brainstem areas and ventral striatum, and autonomic brainstem nuclei (van Hoesen et al., 1993), the anterior cingulate cortex may be part of an executive out-put response selection system for some emotional state.

2.9 SUMMARY

One can surmise from the disturbances associated with Klüver-Bucy syndrome, that the fundamental task for the amygdala is to interpret incoming sensory information. A concomitant function might be the evocation or potentiation of visceral and behavioral responses appropriate to this interpretation. While the amygdala may thus play a key role in relating biologically motivated emotions to sensory input, its role may be complemented with the development of alternative routes involving the prefrontal cortex (PFC). Until recently, emotional behavior was understood in terms of autonomic, endocrine, and unconditioned reflexive behavior (Richardson, 1973; Davis et al., 1987). However most emotionally driven behavior is voluntary, representing behavior instrumental in obtaining access to particular goals (Robbins et al., 1989) where incentive is said to play a major role. Evidence has been presented to suggest that the amygdala and ventral striatum are part of the neural system that is impartially involved in mediation of voluntary elements of an integrated emotional system.
In sum, lesion studies have indicated that the amygdala is a focal point of causes contributing to the Klüver-Bucy syndrome (Gloor, 1960 Goddard, 1964), while electrical stimulation has helped demonstrate the crucial role of this structure in emotional behavior. The pathway from the amygdala to the ventral striatum appear to be especially involved in linking conditioned incentives (secondary reinforcers) to behavioral output. We have also seen that the amygdala and orbitofrontal cortex form a unified zone. These are especially important for reward and motivation, not only because they are the parts of the brain where the primary (unlearned) reinforcing value of stimuli is presented, but also because as shown in primate studies, they are the regions that learn pattern associations between potential secondary reinforcers and primary reinforcers.
3  **Brain Imaging – Approaches in the Study of Emotion**

Just drinking steadily and consistently over time can cause a sense of dependence and withdrawal symptoms during periods of abstinence. The craving for alcohol during abstinence, (the pain of withdrawal) is due to the brain’s adaptation to the changes in its own chemistry caused by long term use of alcohol. Unlike the many studies conducted to describe alcohol-specific structural changes and neurochemical mechanisms, research of disease specific functional changes leaves much to be desired for. Drug abuse researchers have known that when addicts are exposed to drug-related cues as diverse as a piece of music, the sight of a Liquor bottle somewhere else, the smell of alcohol or even a particular neighborhood, they can spark powerful drug craving. Using brain-imaging techniques, it is now possible to witness first hand the changes that these environmental cues trigger in the emotional centers of brain as they are happening. It is inevitable that we touch on the technique of fMRI before proceeding with the studies of emotion.

3.1  **fMRI**

Recent advances in the understanding of the human brain have come about in large part as a result of a combination of the availability of new functional imaging techniques, and no less importantly, creative and careful experimentation. The implementation of fMRI has contributed immensely to a more detailed understanding of cortical and subcortical brain function and neural networks. Since its discovery by Kwong et al. (1991) the use of fMRI imaging has grown explosively. Some reasons for this remarkable growth include the noninvasiveness of fMRI imaging, the wide availability of MR scanners capable of functional imaging, and the relative robustness and reproducibility of fMRI results. An important additional feature of fMRI is its capability to follow signal changes in real time, even though the temporal (Menon et al., 1995) as well as spatial resolution (Menon et al., 1993) of fMRI is dictated by characteristics of hemodynamic response. While the time constants of electrical activity of neuronal systems are shorter than some hundreds of msec, the hemodynamic response time is characterized by several seconds. FMRI can take advantage of real-time data acquisition and analyses and follow the time course of the signal change associated with mental activity that can evolve over seconds for particular cognitive, and as presented in the current thesis, for specially designed emotional paradigms.
3.1.1 Principles of FMRI

To understand the potential application of fMRI, requires an understanding not only of the instrumentation, physical, and techniques of fMRI, but also an appreciation for underlying cerebral physiology. This section covers the fundamentals of fMRI. The basic subsystems are the MR imager, the magnet with gradient coils, field correction coils (shims), radio frequency (rf) cabinet and acquisition controlling hardware. The computer system in an MR imaging device is needed to control the events during pulse sequence execution, to store the resulting raw data and to reconstruct the images from the raw data. The computer also controls a number of self-tests and adjusting routines. Shims adjust the magnetic field until it is homogeneous through special coils present within the magnet. At times, when the imager is ready for operation, there are minor variations remaining in the magnetic fields within the magnet. The human body contains an enormous number of hydrogen atoms. It is known that the protons at the center of each of these hydrogen atoms possess a magnetic spin having a magnetic moment vector, causing it to behave like a tiny magnet with a north and south pole.

3.1.1.1 Gradient coils

The spins in the subject can be manipulated by applying magnetic fields and signals produced by motion (precession) of the spins can be detected outside the body. Magnetic resonance imaging requires the application of strong and carefully crafted magnetic fields that vary as precisely defined functions of space and time. Such are provided by gradient coils. Gradients are one of the most stressed components of the MR imager, since they produce the gradients in the magnetic field. The gradient system consists of three coils: two transverse $x$, $y$ and one longitudinal $z$, parallel to the direction of the main magnetic field $B_0$. Each gradient has a separate power supply which is used to permit either slice selection or code position (frequency encoding and/or phase encoding) of the NMR signal (Turner, 1993). The majority of fMRI relevant sequences are 2D slice selective. Slice selection is performed by rf excitation in the presence of slice selection gradient resulting in the selection of (spins) in a slice or plane through the brain transverse to the gradient direction. Frequency encoding allows the capture of different frequency spins in a slice that is being imaged (proportional to a proton spin). The phase encoding gradient is used to impart a specific phase angle to a transverse magnetization vector. Phase encoding performs the same function as frequency encoding but instead of measuring spins it measures phase angles of photon spins (location of
spins). Various methods have been derived for mapping tissue with NMR. Present day MRI is based on the principle of Fourier Transformation (FT) NMR (Kumar et al., 1975; Edelstein et al., 1980). In FT MRI, the spatial distribution of protons within a sample (x,y), is mapped by rendering their Larmor precession frequency (the frequency with which protons oscillate and which is determined by nuclei and field strength). Thus, the signals are first Fourier transformed in the x direction to extract the frequency domain information and then in the phase encoding y direction to extract information about the locations in the phase encoding gradient direction.

3.1.1.2 Magnet system
To generate a magnetic field of sufficient strength and spatial uniformity a specific magnet system is necessary. A high field strength of 1 T (tesla) or more, is required for high-quality imaging, and can be achieved with superconductive magnets. The key property of superconductors is that they have absolutely no electrical resistance when cooled below transition temperature. The way this is done is by surrounding the superconductive magnets with liquid helium. The advantage in superconductive magnets is that it reduces power requirements and the magnet maintains a magnetic field for a very long time. The magnetic field normally used in clinical tomography is 1.5 Tesla - 2 Tesla.

3.1.1.3 RF Coils
Radiofrequency (rf) coils are required to perform two functions – transmitting and receiving signals at the near Larmour frequency of the precessing spins (Schenk, 1986). MRI is almost always done using the Fourier transform technique where a brief, powerful burst of rf energy (lasting at most a few milliseconds) from an rf transmitter is used to excite the spins and is followed by the detection of a signal (the free-induction decay or FID) that lasts roughly ten to several hundreds milliseconds. The ideal rf field \( B_1 \) is transverse to \( B_0 \), and completely uniform over the subject's brain.

3.1.2 SIGNAL AND TYPES OF CONTRAST
A full appreciation of image contrast requires an understanding of the mechanisms underlying proton relaxation. Spin (proton) density is the basic contrast in MRI. Since it is flat (protons are distributed evenly over the human body) it is not used separately. In general when trying to create contrast, one utilizes differences in the spin’s ability to respond to a perturbation (e.g., radiofrequency pulse) or differences in signal evolution following the excitation. The return of the
macroscopic spin magnetization is described by two independent processes, called longitudinal (T1) and transverse relaxation (T2). Conventional images are gradually constructed from a series of repeated excitations and relaxations.

Radiofrequency emitted from the rf coils causes the oscillating nuclei to absorb energy, causing their axis of spin to be tilted away from the axis of the static magnet. The nuclei in their exited state, after having absorbed radio energy, return to align with the field by dissipating their excess energy to the environment, the lattice. This process is termed spin-lattice relaxation, and it is characterized by the decay time T1. The T1 is dependent on the molecular environment of protons and varies from tissue to tissue.

Transverse relaxation time or spin-spin relaxation time (T2) is the second tissue specific time constant. In the T2 relaxation process, no energy is transferred from the nuclei to the lattice. Rather, the spins exchange energy with each other. The random fields generated by an ensemble of nuclei cause the decay of transverse magnetization. It is the rate of the transverse magnetization that determine the T2 relaxation time. T2 is characteristic for the specimen being investigated.

When these protons absorb radiofrequency energy they form a usually synchronous source and their usually random spins are temporarily brought into phase. Whilst they remain in phase their oscillation can be detected as a radio frequency signal; this signal forms the basis of magnetic resonance imaging. Due to slight non-informities of the field, however, spins tend to get out of phase with one another faster, thus loosing macroscopic radiofrequency signal. The time taken for this signal to decay is termed T2* and is multifactorial, being dependent on the homogeneity of both the chemical and magnetic environment of the tissue concerned.

3.1.2.1 Sensitivity to the Blood oxygenation

The emergence of fMRI methodology is fundamentally based on the fortuitous presence of an endogenous contrast agent, paramagnetic deoxyhemoglobin circulation in the brain and the tight combined effect of neuronal activation and hemodynamic/metabolic responses. In 1990, pioneering work of Ogawa at al. (1990ab) and Turner et al. (1991) demonstrated that MR signal in the brain tissue decreased with a decrease in blood oxygenation. This type of physiological contrast was coined “blood oxygenation level dependent” (BOLD).
The first report using this model described human brain activation in the primary visual cortex and motor cortex (Kwong et al., 1992). The working model constructed to explain these observations was that an increase in neuronal activity causes local vasodilatation which in turn, causes an increase in blood flow. This results in an excess of oxygenated hemoglobin beyond the metabolic need, thus reducing the proportion of paramagnetic deoxyhemoglobin in the vasculature. A reduction in deoxyhemoglobin in the vasculature causes a reduction in magnetic susceptibility differences in the vicinity of venules, veins and red blood cells within veins, thereby causing an increase in spin coherence (increase in T2 and T2*), and therefore an increase in signal in T2* - and T2 - weighted images. Ultimately regions of the brain that have enhanced activity appear brighter regions on the MR image.

**Figure 2. A schematic diagram on the principles of functional magnetic resonance imaging (Schneider et al., 1996a)**
3.2 Physical Limitations of fMRI

The signal change during cerebral activation varies with field strength from 3% to 7% at 1.5 T up to 15% at higher fields such as 4T (Turner et al., 1991). The spatial resolution of fMRI is substantially better than other functional imaging modalities down to 1mm (Bucher et al., 1995). Nevertheless, there are some major limitations of fMRI temporal and spatial resolution relating to hemodynamics.

3.2.1 Temporal Resolution

Before proceeding with a description of temporal resolution limitation, it is important to keep in mind that two separate time scales are presented and separately measured during imaging: these are the time from the signal transition from one state to another and the accuracy to which the location of the transition can be measured. These factors are determined by hemodynamic response which can be described as: latency in space and time of hemodynamic response variations, and functional signal to noise ratio (vessel size, tasks, or regional differences in vascular transit rate).

The major limitation of the temporal resolution of fMRI is the physiological time course of cerebral blood flow following functional activation. Evidence from activation studies suggests that the cerebral blood flow changes take several seconds to reach a maximum following the onset of functional activation (BOLD response) (2 seconds after (with a small dip before) the activation onset, and plateaus at 3-4 seconds after activation) (Badittini et al., 1995). The characteristic response delay differs across brain regions and stimulus regimes (Bandettini et al., 1995; DeYoe et al., 1992). This is substantially slower than the tens to hundreds of milliseconds that characterize neural or psychophysiological responses. Savoy et al. (1994) was nevertheless able to show that by synchronizing acquisition of MR scans to the presentation of brief visual flash stimuli, using ultra-fast imaging, it is possible to detect signal changes from stimulus within 300 msec. Another limitation of fMRI is that signal intensity during activation periods generally vary, even with constant stimulus intensities. For example, signal response not only takes time to appear in some cases, but begins to decrease prior to cessation of the stimuli and at times fluctuates during the stimulus presentation. Potential sources for the variations include physiological (i.e., vasculature pulsation’s), experimental, and instrumental elements (Jezzard et al., 1993).
3.2.2 **Spatial Resolution**

The spatial resolution is the measure of the smallest detectable features in the image and reflects the sharpness of the image, and can be expressed as the maximum spatial frequency. A variety of factors limit the spatial resolution in fMRI methods. Evidence suggests that neuronal control of blood oxygenation occurs on a spatial scale of less than 0.5 mm (Frostig et al., 1990; Grinvald et al., 1991). MRI evidence suggests that blood oxygenation increases that occur on brain activation are more extensive than actual activation regions (Frahm, 1994; Haacke et al., 1994). In other words, it is possible that although the local oxygenation may be regulated on a submilimeter scale, the subsequent changes in oxygenation may occur on a layer scale due to a “spill-over” effect. Thus signal would be potentially displaced from the activated neuronal tissue.

To achieve the goal of high spatial resolution fMRI imaging, greater hemodynamic specificity accomplished by proper pulse sequence choice may allow for greater spatial specificity, discussed next.

3.3 **Imaging Methods**

Each MR imaging technique is defined by its time sequence of gradients. As pointed out earlier, the Fourier transformation (FT) is used for mapping the tissue by rendering the Larmor precessing frequency of protons spatially dependent on the magnetic gradient. In conventional methods, since one phase encoding step occurs each TR (repetition time) seconds the time required to produce an image is determined by the product of TR and the number of phase encoding steps. Each time the sequence is repeated the magnitude of the phase encoding gradient is changed. These would require long examination time, between 1-2 hours.

In order to shorten scanning time a number of techniques were developed. One archetype of such scanning technique is gradient-echo imaging, which has come to represent an entire family of different sequences. Two major features distinguish gradient-echo scanning from more traditional counterpart of spin-echo imaging. 1) The radiofrequency (rf) excitation pulse is operator selected and in practice is less than 90° pulse in contrast to spin echo sequence, (which allows shortening of repetition time) and 2) signal is acquired by virtue of reversal of the readout (frequency-encoding) gradient without applying 180° refocusing rf pulse for spin-echo scanning. The advantage with this technique is that it brought acquisition time down to a range of seconds per slice (Atkinson and Edelman, 1990) reducing motion artifacts.
One family member of gradient echo is echo-planar imaging (EPI). EPI image of a single slice can be acquired in a time as short as 30 milliseconds. A timing echo planar imaging sequence holds a 90° slice selective RF pulse which is applied in the presence of a slice selection gradient. There is an initial phase and frequency encoding gradient pulse followed by fast reversal read-out gradient. When reversal occurs at the same time phase encode blips are applied to every phase encoding. 15 to 30 images can be obtained a second. Typically EPI images are $T2^*$-weighted.

EPI has several drawbacks. For example, low spatial resolution (2mm) due to the limited available time to measure the magnetization in the single-shot acquisition owing to the $T2^*$ signal decay. Also, the potential for peripheral nerve stimulation due to the strong and rapidly switching gradient is present. Another disadvantage of EPI is spatial distortions introduced by main field inhomogeneity and poor shimming susceptibility effects. This can be corrected during post processing in most cases. Additionally, a practical, but significant factor to be considered when performing fMRI with EPI is the rapidity with which large amounts of data are collected. The data may then go through several additional transformations (adding to the total required data storage capacity) before a functional image can be created. If 10 slices having 64x64 resolution can be acquired every 2 seconds, then the data acquisition rate is extremely large (approximately 2MB per minute). Nevertheless, the high temporal resolution (improved to about 100ms), rate of information capture (signal to noise), and low sensitivity to motion among a relatively long list of other factors, still greatly outweigh the disadvantages for most purposes related to fMR imaging.

3.3.1 SENSITIVITY

In order to optimize fMRI imaging sensitivity, a number of salient variables are important to consider for example: **Averaging** – averaging of sequentially obtained images increases the signal to noise by the square root of the number of images collected. **Field strength** – Advantage of increasing field strength are signal-to-noise and functional contrast increase. Drawbacks are increased shimming problems, increased physiological fluctuations, and limitations on possible RF coils. **Filtering** – during imaging, noise over time is dominated by physiological fluctuations (heart rate and respiration rates). Filtering out such fluctuation would increase functional-signal to noise ratio. **Gating** – noise collection of images at different phases of cardiac cycle (brain moves with every heart beat) can be eliminated when triggering the scanner to heart beat. The disadvantage lies in changes in signal intensity due to heart rate changes during collection of images ultimately
causing large fluctuations in the data. Paradigm timing – As a rule of thumb in fMRI, the goal is to maximize the number of on/off cycles.

3.4 Principles of Data Analyses

In fMRI, a large number of images – tens to several hundreds – are measured consecutively in a single experiment. The collection of data are a time series of signal intensity from small volume elements or “voxels” covering regions of interest or the whole brain. During data acquisition period inputs for brain activation are presented to the subject in the magnet at appropriate periods. These inputs can be sensory stimulation, sensory input-guided cognitive tasks, subject-initiated mental activity, or even spontaneous brain activity the subject may not be aware of. Images taken during the absence of these inputs are used as controls. Image signals responding to the input are then compared with the control image signal (Bandettini et al., 1993).

The first step following data collection is to attempt to fit them to a linear combination of experimentally controllable variables, or simple terms of seen. In hypothesis led experimental design and analysis, one or more variables are controlled or at least monitored, and the effects on intensity at any voxel in the image are recorded. Both magnitude of intensity variation and spatial extend of co-activated pixels, can be taken to account straightforwardly in making inferences when the data be regarded as a Guassian Random Field (Adler, 1981). Gaussian field makes inferences about the magnitude of intensity variation and spatial extent of co-activated pixels. Inferences are based on the probability of obtaining clusters of voxels above a threshold.

Thus the fundamental question relating to fMRI data analysis is how “well behaved” in a statistical sense the raw data of MRI images are. This means that the data are stationary (due to the fact that neuroimaging samples uniformly and that the point spread function is stationary). However, since the raw data consist of narrow point spread function, and of sharp boundaries represented by abrupt image intensity changes (thus image is not smoothed), and head motion and physiological pulsations result in highly spatially correlated time-varying changes in image intensity, the raw data are not of much use. The strategy of statistical parametric mapping (SPM) (Statistical Parametric Mapping, University College, Department of Cognitive Neurology, London ) when applied to fMRI data is to use a series of image transformations, which have known statistical implications, and to allow robust statistical inferences.
3.4.1 **Real Time fMRI**

In the mean time, new imaging methods are being developed that allow for ‘real-time’ (Cox et al., 1995; Gembris et al., 2000) tracking of brain activation. These advances stimulated the development of “real-time fMRI” which is characterized by steady state data acquisition, image reconstruction, motion correction and statistical image analysis during the ongoing scan, preferably with a time delay of less than a single TR cycle. With real-time fMRI the delay between task initiation and clear display of involved cortical areas is no longer determined by computation time. Instead, it is determined by physiologically delayed signal response (~ 3-6 s) and necessary accumulation time of data due to limited signal-to-noise ratio (SNR). A number of processing methods exist which enable continuous monitoring of fMRI signal changes during the ongoing scan with constant sensitivity. One such method is processing methods for Functional Imaging in REal time (FIRE). In contrast to most previous approaches for real-time fMRI, these methods include real-time image transfer and display (Schor et al., 1998), “sliding-window” correlation analysis (Gembris et al., 2000), reference vector optimization (Gembris et al., 2000) and real-time motion correction (Mathiak and Posse 2000).

This method is well suited for single-run-single trial experiments (Richter et al., 1997). It appears an ideal technique as a diagnostic tool or more so for therapeutic application. For example, central issues in psychiatry and clinical psychology such as etiology of functional disorders, affective disorders, or other may be assisted by using real-time fMRI. Clinically, psychiatry and psychology often deal with emotions, which are difficult subjects for experimental study. Real-time fMRI in combination with smart and innovative techniques could offer a great opportunity to investigate emotional processing in real-time. Within this framework, and using biofeedback technique, the evolutionary idea for the second experiment in this thesis was developed.

3.5 **Positron Emission Tomography (PET)**

PET offers a powerful biological tool which enables regional brain function to be assayed. PET utilizes radioactivity radiolabelled biological probes to perform radioessays with good sensitivity. In principle, PET imaging begins with the injection of a metabolically active tracer, a biological molecule that carries with it a positron-emitting isotope (for example, $^{15}$O, or $^{18}$F). After injecting (or via inhalation), the distribution of radioactivity derived from the administered tracer is measured. The regional distribution of tracer in the brain can be followed during minutes to hours,
depending on the type of tracer and radionuclide attached to it. Mathematical models can then be applied to convert the measured radioactivity into specific tissue function e.g., cerebral blood flow, oxygen or glucose utilization, receptor densities, etc. (Phelps et al., 1986).

PET and SPECT have proven themselves to be well suited to probe the pharmacokinetics and pharmacodynamics of drugs of abuse (Gatley and Volkow, 1989). Volkow et al. (1997) has successfully applied PET to study the amount of cocaine that gets into the brain, its regional distribution and residence time, and its site of binding. On the whole PET/SPECT have offered a window into the human brain, especially when they can be used with study designs that involve pharmacological challenge as well as contributing to basic knowledge of the effects of abused drugs on the brain. However, PET has several limitations one of which is poor spatial resolution. FMRI compared with PET has the highest resolution of all of the imaging technologies that are used for functional mapping of the human brain (Bandettini et al., 1992; Kwong et al., 1992; Ogawa et al., 1992). Limitations posed by signal to noise ratio of PET in certain areas of the brain can be accommodated for by intersubject image averaging and the development of volumetric acquisition for mapping areas in single subjects. (Pardo and Fox, 1993; Watson et al., 1993). Single-subject functional maps, in turn, can be co-registered with a subject’s anatomical image, creating an integrated map of structure and function (Grafton et al., 1993; Watson et al., 1993).
CRAVING

4 CRAVING

Scientific analyses of the problem of alcohol craving is a comparatively recent endeavor. Until the 1990’s researchers and clinicians have not rigorously investigated this phenomenon. Only during the past 5 to 10 years has interest in craving increased spurred by numerous developments which are highlighted next. Animal studies have repeatedly lead to the suggestion that the rewards induced by abuse drugs act on the nucleus accumbens (N. Acc) along with the ventral tegmental area (VTA) (Hert, 1990; Spanagel et al., 1992). Recently, however, the view on the functions of the reward centers as part of the central nervous control of drug seeking/or drug taking behaviors has been challenged (Hodge et al., 1992; Kiyatkin et al., 1993). The newly adopted position based on the recent evidence proposes long-lasting neuroadaptive changes in the brain which occur in the form of sensitization or homeostatic adaptive mechanisms. These neuroadaptive processes can then persist long after the drug has cleared from the brain. Craving and drug taking are suggested to be directly linked to these neuroadaptive mechanisms.

Another challenge is directed at the earlier conception that mainly actions of drug on the reward system are relevant for drug taking (Shippenberg et al., 1988; Wise and Bozarth, 1987). An alternative proposition that has been put forth is that the primary actions of drugs of abuse are influencing emotional centers of the brain which control emotions such as euphoria and anxiety (McGregor and Roberts, 1993; Miller and Gold, 1993). Moreover, there are growing indications that drug seeking and drug taking behavior are based on different neural mechanisms: on the one hand the evaluation and ratings of emotional responses to a preceding drug administration (primary reinforcers) and on the other hand a cue based guidance of subsequent drug seeking and drug taking behavior (Hodge et al., 1992; Kiyatkin et al., 1993; Koob, 1992; Samson et al., 1992).

Although physiological and behavioral correlates of craving have been difficult to illustrate, alcoholic patients repeatedly describe a cognitive and subjective state analogous to craving. Many abstinent participants when asked to define craving indicated there were physical attributes to it (craving) also, “where you can actually taste and smell it” or “craving was wanting something to the excess that you’d do anything to get it” or “you want something more than anything in the world” (Merikle, 1999). These states, may in turn, motivate voluntary drug-seeking (operant) behaviors. If the drug-seeker succeeds in finding the drug, the chain of behaviors is again reinforced by the pleasant drug-induced feeling (brain effect). If on the other hand, the drug is not found, drug seeking and drug taking are motivated by negative-reinforcing effects. In general, substance users...
consistently label the motivational state preceding substance use as “craving” and often attribute both ongoing use and relapse to giving into intense craving.

In humans, both the effects of drug intake on emotional variables and motivation for drug seeking usually depend on environmental variables. Social conditions, distress and many other factors tend to enhance or attenuate the desire to take the drug (Alterman et al., 1990; DeCastro, 1990). The reward system of the brain appears to integrate these variables and to adjust drug seeking and drug taking behavior according to them. The resulting behavior can be defined as ‘controlled consumption of a drug’ (Coper et al., 1990; Wolfframm, 1991). An example of controlled drinking of alcohol is ‘social drinking’ (Kalant, 1977). In the course of drug addiction the consumer loses his/her control of drug seeking and drug taking. Craving for the drug becomes eminent and superimposes ‘normal’ behavior (Coper et al., 1990; Miller and Gold, 1993). According to the new versions of diagnostic manuals like DSM-IV, “loss of control” is regarded as a major criterion of drug addiction.

Environmental cues that may lead to “loss of control” not only are in the form of the drug itself, but also people, sights, sounds, odors and situations that are associated with the drug use can become predictors of the onset of drug effects. The user need not consciously be aware of these cues in order for an association to occur. With repetition, the cues may begin to provoke physiological changes (autonomic responses) that the experienced user interprets as drug craving or withdrawal symptoms. Withdrawal symptoms are made up of two elements. There are the physical signs of withdrawal, which are characteristic for each drug, such as the well known tremor and autonomic hyperactivity of alcohol withdrawal. However, it was quickly realized that addicts continue to crave for drugs even after long prolonged drug-free periods when withdrawal signs and symptoms had dissipated. To account for this craving, it was hypothesized that the drug satisfied some psychological need of the addict, which may be considered more motivational; these signs consist of varying components of a negative emotional state including dysphoria, depression, anxiety, and malaise (American Psychiatric Association, 1994).

Based on the suppositions above, a plethora of studies have been directed at understanding the neuronal changes that occur concurrently with, and are possible determinants of drug-seeking and drug-taking behaviors. The following reviews will describe the current ideas on ways in which the central nervous system (CNS) adapts, or maladapts, to repeated alcohol exposure and stimuli associated with alcohol.
4.1 The Role of Withdrawal in Craving

Physical dependence has been defined primarily by the presence of a characteristic state of stereotypic symptoms that appear when the administration of relatively high doses of ethanol abruptly terminates. The constant impingement of ethanol on neuronal function is postulated to promote neuronal adaptation.

Since its first definition by Himmelsbach (1941) the term neuroadaptation is often linked to the concept of a neural mechanism that oppose to some drug effect, that is, this term is used not only for describing a change but also for defining the direction of the change. According to this view, the initial acute effects of the drug are opposed or counteracted by homeostatic changes in the system that mediate the (primary) drug effect making it progressively less effective and leading to drug tolerance. Rapid removal of the drug unmasks the state of adaptation causing changes in the opposite direction to those produced acutely by the drug (the withdrawal syndrome). This theory was conceptualized by Solomon and Corbit (1974) as the ‘opponent process theory’

More recently, the ‘opponent process theory’ has been reformulated as a homeostatic neuroadaptation model as applied to the affective and motivational component of drug dependence. In this view, chronic self-administration of habit forming drugs triggers the development of tolerance and euphorogenic (positive) drug effects, causing the positive reinforcing effects to diminish while the negative reinforcing effects (relief of withdrawal induced anhedonia) increase in the same brain reward circuits. Withdrawal symptoms will intensify and become longer lasting. The positive hedonic processes are hypothesized to be simple, stable and follow administration of the drug, closely in time. In contrast, negative hedonic processes are of longer latency, slow to build in strength, and slow to decay. Within this framework, the intense euphoria is presumed to reflect the positive hedonic process, and the aversive withdrawal symptoms of anxiety are presumed to reflect the opponent negative hedonic process (Koob and Bloom, 1988; Solomon, 1977). Under this theory, the term craving and withdrawal are used interchangeably, with the assumption that craving might be a form of mild withdrawal. Critics of the opponent process theory of craving argue that patients do not always subscribe to this position; reports of craving usually showed no correlations with withdrawal (Childress, 1993).
4.1.1 ANIMAL MODELS OF ALCOHOL WITHDRAWAL AND MOTIVATION

Ethanol withdrawal, a sedative-hypnotic drug, is characterized by a state of central nervous system (CNS) hyperirritability that can result, in varying degrees of severity in profound physiological disturbance such as tremor, hypothermia, seizures, rigidity, hyperreflexia, and hallucination and delirium (Goodwin, 1992; Jaffe, 1990). The affective or emotional symptomatology include irritability, restlessness, anxiety and mood disturbance (Emmett-Oglesby et al., 1990). Many of the overt physical signs associated with withdrawal from alcohol can be easily quantified, while motivational measures require more than simple observation in many cases. Animal models sensitive to the motivational effects of ethanol withdrawal have included several different approaches, all of which measure some aspects of negative affective state. Intracranial self-stimulation (ICSS) (e.g., self-stimulation sites an animal will repeatedly perform an operant response to obtain one train of stimulation from electrons installed within the brain) behavior has been used to assess changes in reward systems during the course of alcohol dependence. It was shown that acute administration of ethanol can lower ICSS reward threshold (Bain and Kornetsky, 1989; Moolten and Kornetsky, 1990), generally considered to be an index of reward properties of this drug. By contrast, withdrawal from ethanol resulted in an elevation of ICSS thresholds, which is considered as an index of a negative affective motivational state and is opposite to the effect produced by acute administration (Leith and Barrett, 1976). The elevated pulse maze provides a measure of anxiogenic-like responses associated with withdrawal. Animals are placed on an elevated cross-shaped platform that consists of closed and open arms. Rat withdrawal from ethanol increase sensitivity to the elevated pulse maze (Baldwin et al., 1991). Withdrawal from sedative hypnotics (ethanol) is characterized by increased time spent in the enclosed arms.

4.1.2 NEUROBIOLOGICAL SUBSTRATE OF WITHDRAWAL AND MOTIVATION

Animal models of motivational effects of opiate withdrawal have implicated a number of brain sites including the N. Acc and the amygdala. Animal signs of ethanol withdrawal include whole body tremor, hyperirritability, rigidity, augmented susceptibility to audiogenic seizures, and onset of a ventromedial distal (VMDF) limb flexion response (Cooper et al., 1979; Baldwin et al., 1991; Majchrowicz, 1981). These signs may reflect hyperexcitability of the central nervous system (Baldwin et al., 1991; Majchrowicz, 1981)
Investigations have suggested that the N. Acc as part of the CNS, not only is concerned with the rating of reward elicited by drugs of abuse but also the control of appetitive behaviors (e.g., drug seeking) (Amalric and Koob, 1993; Holloway et al., 1993). Opiate antagonists (methylnaltroxone) injected into the N. Acc and amygdala produced place aversion, suggesting that these two sites are involved in the neural substrates of withdrawal for opiate (Stinus et al., 1990). However, overt classical signs of withdrawal were observed in the N. Acc only. N. Acc neurons receive convergent excitatory commands from the ventral hippocampus (subiculum), basolateral amygdala (chapter 2), and prefrontal cortex, which together supply information regarding environmental conditions, behavioral contingencies, emotional states, and motivational needs.

4.1.3 Motivational Effects of Alcohol Withdrawal - Neurobiological Mechanisms

Ethanol (EtOH) withdrawal not only involve the physical signs but also change in motivational state as described above. The amygdala has been identified as an important site mediating anxiety and more specifically EtOH withdrawal-induced increases in anxiety (Rassnick et al., 1993). Neuroadaptation to ethanol may involve endogenous brain corticotrophin releasing factor. It was pointed out in the second chapter that CRF itself has anxiogenic actions. Correspondingly, CRF antagonists, α helical CRF, reverses some behavioral responses to stress. For example, a CRF antagonist microinjection into the amygdala was able to block the place aversion produced by precipitated opiate withdrawal (Rassnick et al., 1993).

It was shown that a variety of drugs that reduce fear or anxiety in humans decrease potentiated startle in rats. Drugs like clonidine, diazepam, and alcohol, too, were found to have depressant effects on startle. In humans, Rassnick et al. (1992), showed that startle reactivity was suppressed during EtOH access and heightened during withdrawals. In another study, recently detoxified male early onset alcoholic patients had increased acoustic startle magnitude during a subacute phase of alcohol withdrawal. On the other hand, drugs like yohimbine induce anxiety in normal people (Charney et al., 1984). Yohimbine in alcoholic patients enhanced startle magnitude and reduced startle latency relative to placebo. The same study also highlighted the importance of the number of episodes of ethanol withdrawal as a factor influencing subsequent neuronal excitability (Ballenger and Post, 1978). For example, enhanced startle magnitude was linked to a number of previous ethanol detoxification, potentially supporting a sensitization effect (Krystal et al., 1997).
Using the place preference paradigm, examination of the sequelae of c-fos staining (which permits quantitative analysis of neural activity) in rats that were in a state of craving, showed increased immunoreactivity in regions of the amygdala, and the dorsolateral prefrontal cortex (Grant et al., 1996; Maas et al., 1998). These regions did not overlap with regions activated during non-beer craving connotations nor during a neutral condition. Elevated c-fos immunoreactivity have also been found consistently activated in the amygdala by stresses of conditioned fear and foot shock (Topple et al., 1998). This finding adds impetus to the proposal that the amygdala is involved in craving, e.g., in stress induced craving.

4.1.4 CONDITIONED REINFORCEMENT AND CONDITIONED WITHDRAWAL

The habitual behavior of many alcoholics means that the aversive symptoms of drug withdrawal are frequently experienced in specific environments (Wikler, 1973). The experience of conditioned withdrawal is believed to promote ‘craving’ for the drug (Wikler and Pescor, 1967). Both the positive and negative affective states can become associated with stimuli in the drug-taking environment through classical conditioning processes, motivating continued drug use and relapse after abstinence upon re-exposure.

Conditioned withdrawal has been repeatedly observed in animals and humans. In the early classical studies by Wikler et al. (1973), rats made dependent by gradually increased daily doses of morphine were exposed to a novel environment each night while experiencing morphine abstinence. After 56 weeks of such pairing, rats exposed to the same distinct environment showed physical withdrawal signs up to 155 days after the last morphine ejection.

Significant clinical evidence of conditioned withdrawal can be made. In the case of opiates, the withdrawal syndrome following large repeated doses of heroin or morphine has been shown to be associated with the environment within which it occurs (O'brien, 1986). Re-exposure of abstinent individuals to the environment previously associated with opiate withdrawal has been hypothesized to result in conditioned responses with opiate that are similar to withdrawal symptoms. Conditioned withdrawal can be elicited experimentally. Opiate addicts maintained on methodane (a synthetic addictive drug used for the relief of pain and as a substitute narcotic in the treatment of heroin addiction) (O'Brien et al., 1977) were given naloxone injections which were repeatedly paired with a tone and peppermint smell. Subsequent presentation of only the tone or odor elicited both subjective reports of discomfort and objective physical signs of withdrawal.
Compelling evidence for motivational properties of stimuli paired with withdrawal is the observation that such stimuli actually come to influence drug-taking behavior. For example, a detoxified alcoholic patient, who is in a state of conditioned withdrawal could be induced very severely by entering a familiar bar and taking a non-alcoholic drink. The setting would trigger negative affect, anxiety, tremor, sweating and so on, all early signs of alcohol withdrawal. Since by experience the alcoholic patient knows that all these unpleasant symptoms can be relieved by taking alcohol, this behavior is likely the consequence. It is argued that because the adaptive changes operate far below the level of consciousness it is proposed that all the individual is aware of is that something has made them crave alcohol and this is what they report (Littleton 1995). One strand of work has shown that responses to alcohol cues are more pronounced in severely dependent drinkers. For example, the work of Pomerleau et al. (1983) showed that if a severely dependent drinker sniffs alcohol after being dried out, the physical responses and subjective craving responses distinguish him clearly from social drinkers. The motivational significance for heavy drinkers of alcohol withdrawal symptoms is also underlined by evidence linking the extent of previous withdrawal history with propensity to experience craving for alcohol and to respond to a priming dose of alcohol with greatly increased withdrawal (Hodgson et al., 1979).

4.2 CRAVING AND THE INCENTIVE SENSITIZATION MODEL

The positive incentive account of drug-seeking behavior is in contrast to the drive-reduction model which has supposed that drug seeking must be maintained by the avoidance or reduction of unpleasant withdrawal symptoms or other states of distress. It is suggested that conditioned stimuli associated with drugs, activate neural systems of the brain associated with positive status. This corresponds with the brain system, posited by Gray (1987), as signaling reward or non-punishment and altering the organism for approach (as opposed to flight or inhibition).

4.2.1 THEORY OF INCENTIVE SENSITIZATION EFFECT OF CRAVING

Craving according to the incentive sensitization theory is hypothesized to manifest in the following manner: with repeated drug use the act of taking the drug and drug associated stimuli become more and more attractive. Conditioned stimuli associated with drug taking come to arouse neural states that mimic those produced by the drugs themselves, and exposure to such stimuli then gives rise to the incentive to take drugs. Drug associated stimuli become more and more able to control
behavior, because the neural system that mediates wanting becomes progressively sensitized. According to Berridge and Robinson (1993), wanting evolves into obsessive craving and this is manifest behaviorally as compulsive drug seeking and drug taking behavior.

4.2.2 Motivational Measures Of Sensitization

Studies have shown that long-lasting neuroadaptation changes within the brain follows repeated administration of some psychotropic drugs leading to a sensitization of rewarding and locomotor effects (Hoffmann and Wise, 1992; Robinson and Berridge, 1993). Also sensitization of drug effect can be linked to stimuli or events that occur when the original effect was elected, often termed context-dependent sensitization.

The effect of sensitization to the behavioral activation effects (motor) was tested in a study using rats. It was found that motor activity was stimulated with rising doses of ethanol intake in alcohol dependent rats compared to alcohol naive rats. In dose effect studies of the chronically administered drug, the amount of drug-induced hyperactivity was found to be directly related to dose of proceeding chronic treatment (Bartoletti et al., 1987). It seems that the association of the previous action of the drug taking with the subsequent experienced drug effects is decisive for the motor response.

According to incentive sensitization the apparent environment control over sensitization, is directly due to drug induced changes in the neural system (or special reinforcement circuit in the brain) that are undergoing sensitization-related neuroadaptations. Animals exposed repeatedly to the reinforcing effects of a drug in the presence of a given environment, when given the test of sensitization, show greater preference for the environment where the drug effects were experienced than animals exposed to the a different environment (Stewart et al., 1993). In another study, using the place preference test, rats treated with lisuride a D2-agonist promoting the rewarding effect of drugs during ethanol administration, revealed enhanced preference for the alcohol reinforcer compartment compared to the water compartment when tested without actual alcohol (Robinson and Berridge, 1993).

4.2.3 The Neural Substrates Of Sensitization

The mechanisms for the context-dependent sensitization is suggested to include the medial forebrain bundle. The concept of a ‘pleasure center’ in the brain was discussed by Olds (1976), a system that has also been called the motivational or reward circuit of the brain. It was assumed that
the effects of the drug in the mesolimbic reward system mediate certain behavioral responses like
sensitization of stimulating actions (Miller and Gold, 1993; Wise and Bozarth 1987). A number of
studies using in vivo microdialysis method have shown that cocaine increases extracellular
dopamine levels in the N. Acc (Hurd et al., 1989), and that the magnitude of this response is greater
in animals given repeated cocaine (Akimoto et al., 1990; Kalivas and Duffy, 1990).

It may be premature, though, to think that factors of drug action, evaluation of reward effect,
motivational control of drug taking, craving and loss of control can be primarily attributed to the
mesolimbic reward system. All the evidence thus far (this section and chapter 2) suggest that overall
actions of abused drugs take place in emotional regions of the brain, which should imply that the
effects may be alternatively mediated by a different mechanism. That is not to say that the
mesolimbic system is not involved. Strong support in favor of the suggestion for the involvement of
emotional centers of the brain stems from the observation that lesions to both the N. Acc and
amygdala blocked drug-induced behavioral sensitization (Mogenson, 1987).

One possible interpretation of the latter observation is that lesions to the nucleus accumbens were
able to block cocaine-induced behavioral sensitization by interfering with limbic motor integration
(Mogenson, 1987). In contrast, the amygdala lesions may have blocked the conditioned induced
behavioral sensitization by interfering with associative learning or environmental context
components. The connection between the amygdala and ventral striatum (chapter 2) make it
possible for processes by which environmental stimuli gain affective value through their predictive
association with primary goals to control instrumental components of an integrated emotional
response. There also appears to be an important role for a stress connection in sensitization (Koob
and Cador, 1993). Activation of the CRF (corticotropin releasing factor) brain system has been
shown to be permissive for psychomotor stimulant sensitization. Stressors can cause sensitization to
stimulant drugs, and there appears to be an important role for extrahypothalamic CRF in stress-
induced sensitization (Cole et al., 1990).

4.2.4 CONDITIONED REINFORCEMENT AND CONDITIONED POSITIVE ‘AFFECTIVE’ STATES

The incentive motivational model of craving emphasizes the positive reinforcing effects of
substance use in the generation of craving. It has been assumed that the stimuli associated with the
drugs trigger off a positive affective state, causing a renewed search for the drug (conditioned
analogous positive ‘affective’ states). These drug-related stimuli (e.g., the sight and smell of
alcohol, positive affect-cues previously paired with substance use, information that the drug is available or a small dose of the substance itself) activate positive affect states that produce craving and drug-seeking behaviors.

Davis and Smith (1987) have tested several drugs in the conditioned reinforcement paradigm such as pairing of previously neutral stimuli with acute positive reinforcing effects of drugs and could show that the presentation of a stimulus, previously paired with the drug administration, maintains lever responding in rats in the absence of the drug. However, proponents of the conditioned negative withdrawal model of craving would argue that the rat’s may have perceived anxiety prior to lever pressing.

As with animals there is evidence in humans that the positive reinforcing effects of drugs can become conditioned to environmental stimuli. In the laboratory, Meyer and Mirin (1979) found that the most powerful stimulus for eliciting craving in their subjects was the signal that drug was available. Subjects maintained reasonably low interest in drugs and low levels of reported cravings as long as the drug was unavailable. As soon as the drug became available the ratings of craving were high. It was found that patients being treated for heroin addiction and allowed to self-administer either saline or heroin report that both saline and heroin injections were pleasurable, particularly after the actual injection (Obrien et al., 1979). However, it is argued that prior to any good feeling associated with drug intake, these subjects reported feelings of discomfort and dysphoria initially, suggesting that conditioned withdrawal may also have had a motivational effect.

4.3 **MODELS OF CRAVING: CRAVING INDUCTION PROCEDURES**

Addicts, even those who have abstained from drug use for many years are confronted with a major dilemma of temptation by small cues, be it visual, or other, i.e., seeing the drug of addiction or stimuli associated with it. These cues can stimulate a very strong craving for the drug through the process of classical conditioning. Conditioned responses are thus elicited when cues acquire conditioned stimulus properties. Classical conditioning theory is considered a prominent explanatory model for craving (Franken et al., 1998). Accordingly, laboratory paradigms can provide important and potentially potent methods for studying craving. One problem with the concept of classical conditioning is that there is no evidence rejecting the possibility of craving as an unconditioned response. Individuals low on conditionability or without a reinforcement history
sufficient enough to show classical conditioned responses might well be motivated strongly to drink alcohol for any of its reinforcing properties. Such an explanation of craving would be consistent with an operant conditioning model of craving (Franklin et al., 1998). Be that as it may, the assumption that classically conditioned responses represent one type of mechanism that leads to craving provides an excellent framework for the investigation of this phenomenon.

According to the conditioning model cue reactivity and conditioning history should be directly related. In alcoholics a relationship between severity of alcohol dependence and cue reactivity was shown (Drummond and Glautier, 1994) as well as enhanced craving effect (Maas et al., 1998). However, there is great individual variability in the response to these cues, with some subjects showing only weak craving responses. Also factors such as motivation appears to play a not too little part (Verheul et al., 1999). The following list some of the methods presently used to evoke conditioned craving.

4.3.1 CUE EXPOSURE

Exposure to alcohol or other drug-related cues, has been used successfully to evoke craving. These include, for example, sight of drug, (Childress et al., 1993; Rankin et al., 1979), or viewing a videotape of actual drug use (Maas et al., 1998).

4.3.2 ACTUAL INGESTION

Alcohol ingestion should prime or stimulate craving according to the incentive sensitization model. (Steward et al., 1984). This hypothesis was successfully demonstrated in abstinent alcohol dependent rats that showed significant increase in craving following alcohol ingestion (Drummond and Glautier, 1994). In humans, alcohol dosage is usually too small to produce a direct pharmacological effect, but it is assumed to induce a desire to consume the drug. Another type of cue assumed to mimic real-life situations is odor. Odor is a powerful means to induce craving in abstinent individuals. As already pointed out in chapter 2, the amygdala is privy to olfactory information through direct olfactory input from the olfactory bulb (Price, 1990). Odor cues may have an advantage in that investigators would not have to worry about the salience of the cue within the stimulus context that patients are presented. Most odor induced-craving have involved asking subjects to hold and smell.
4.3.3 Recall of Autobiographical Memories

In alcohol dependence, there is limited research on the effectiveness of autobiographical memories induced-craving (recalling of images of situations and particular circumstances of drug taking). Nevertheless, there is ample evidence to lend support to the success of imagery induced craving. Opiate-dependent individuals recalling images of situations and particular circumstances reported that imagination was vivid and sufficient enough to provoke craving for heroin (Bradley and Moorey 1988). Also imagery of in vivo smoking were equally effective in eliciting high levels of self-report urges (Tiffany and Drobes 1990). The results reported by Elash et al. (1995) of cigarette smokers have shown augmented self-report of negative affect in response to craving imagery induction. Subjects reported significant increases in subjective craving following the imagery of situations that elicit drink urges (negative affect) and after recalling of autobiographical memories of craving for alcohol. These cues produced an emotional activation of negative affect (anxiety and sadness) and reduction of happiness (Weinstein et al., 1998). However, it was also found that in-vivo exposure to alcohol produced increased craving for alcohol in dependent alcoholic patients than did imagery exposure (Weinstein et al., 1998). Not much different from autobiographical induced craving, is the stress-induced craving which involves brief imagery of a recent personal stressful experience. This method of induced craving has also shown significant increase in craving response. However, it was less certain whether the major component of the psychological stress is activation of negative affect (Sinha and O’Malley, 1998).

4.3.4 Negative Affect-Induced Craving

Negative mood manipulation typically involves imagination of an unpleasant situation or the presentation of stressors that will invoke such a mood. Enhanced urge reactivity for alcohol beverage was demonstrated following exposure to alcohol beverage after mood induction. A previous study by Childress et al. (1994) has indicated that induced negative mood states, in particular depression and anxiety, produced significant increases in subjective rating of craving. Payne et al. (1992) had alcoholics listen to a description of a high risk or low risk situation while they were exposed to alcoholic beverage cues. Their craving increased when exposed to alcoholic beverage cues to the high-risk situation, but not to the low-risk situation. Most recently, negative affect-induced craving via guided imagery when combined with presentation of alcoholic beverage cues predicted time of relapse after inpatient discharge (Cooney et al., 1997). Alternatively, the
hypothesis of positive mood induction and increased craving effect is put forth. In smokers and social drinkers, high levels of pleasure were associated with the desire to consume cigarettes or alcohol respectively (Mucha et al., 1999). Generally, it is argued that induction of negative or positive mood presentation of relevant cues should produce strong craving than would be elicited by either of these presented in isolation.

4.4 Craving Evaluation Methods

The measurement of craving has been difficult because much of craving occurs in primitive areas of the brain where emotions emerge and where language lacks form (Anton, 1997). Nevertheless, a variety of distinct tools were developed to measure craving including self-report scales, questionnaires and physiological measures.

4.4.1 Self-Report Instruments

The field of craving research is not without its difficulties. For one, the literature lacks hypotheses about the clinical features of craving. Examination of the published data on craving reveals that the entity was conceptualized in a variety of ways, each with implications for suitable research techniques (Kozlowski and Wilkinson, 1987). For example, the construct was used to subsume phenomena such as recurrent and persistent thoughts, desire to alleviate unpleasant withdrawal, withdrawal symptoms, cue-induced autonomic response, lack of control over use, and behavioral impulses. To this effect, a variety of relatively distinct tools were developed to measure craving, including self-report scales or questionnaires. Self-report methods include single-item Likert type scales (Drobes et al., 1994), single item visual analogue scale (Tiffany and Hakenewerth, 1991) and multi-items multi-dimensional questionnaires such as The Obsessive Compulsive Drinking Scale (OCDR; Anton et al., 1996) which is a revised version of the Yale-Brown Obsessive Compulsive Scale for Heavy Drinkers (Y-BOCS-hd). Both latter instruments conceptualize alcohol craving as obsession and compulsion relating to alcohol consumption. Clearly, the current conceptual status of craving reflects the lack of a comprehensive etiological model that distinguishes between correlates and/or core components (e.g., desire for alleviation of unpleasant withdrawal symptoms; urgent irritable desires) from precipitants and or causes of craving (e.g., withdrawal symptoms; cue induced autonomic responses) on the one hand and consequence of craving on the other hand (lack of control; behavioral impulses) (Verheul et al., 1999).
4.4.2 PSYCHOMETRIC ISSUES
More recent conceptualizations of craving have fostered a broader perspective on the nature of craving, and consequently, on the source of data, e.g., the behaviors that could provide important information on craving. The behavioral measure most closely related to the concept of alcohol craving is alcohol consumption itself. However, since many researchers are reluctant to conduct studies involving alcohol consumption with alcohol dependent subjects, recent cue reactivity studies have incorporated indirect behavior measures in lieu of actual drinking behavior. One such behavioral measure would be cue-viewing time. That is, the length of time a person chooses to look at a stimulus. Within studies on motivation and emotion, choice cue-viewing time has been related to various subjective and psychological indicators of arousal (Lang et al., 1993).

4.4.3 PSYCHOPHYSIOLOGICAL MEASURES
Instrumental methods for measuring autonomic physiological activity in response to alcohol cues have also gained in popularity in studies of craving. Such methods are particularly popular in craving studies that postulate a role for classical conditioning. Physiological responses to cues include a number of behavioral manifestations: for example research has shown that exposure to alcohol without consumption, can stimulate an increased salivary response in alcoholics (Pomerleau, 1983). Similarly, skin conductance levels and self-reported desire for alcohol were correlated for alcoholic subjects in response to alcohol cues (Kaplan et al., 1983). The relationship was strongest for those most severely dependent subjects. In other studies, alcohol abusers reacted to the smell of alcoholic drinks with increased salivation and increased heart rate (Cooney et al., 1984; Cooney et al., 1987). Stormark and colleagues (Stormark et al., 1995) found that high-potency alcohol odor (beer) elicited a defense reaction in alcoholics, reflected in greater heart rate accelerations and skin conductance responses. A low potency alcohol odor (vodka) was ineffective in eliciting a defense reaction. These responses suggest an anxiety component to craving. What these observations point to is that stressors increase alcohol craving, autonomic changes, and subjective anxiety (Sinha et al., 1997). Significantly, some studies show that autonomic reactions to alcohol cues present changes in skin conductance that can predict later relapse to drinking (Drummond and Glauner 1994). Carter and Tiffany (1999) conducted a metanalyses of cue reactivity effects of psychophysiological variables across addictive liability drugs. Consistent with the findings that alcoholics exhibit increased urges and craving for alcohol cues, their analyses revealed that alcoholics across studies demonstrate significant increases in heart rate and skin...
conductance. Nevertheless, despite some claims to the contrary, published correlations between cue elicited physiological arousal and subjective craving are generally far from perfect (Tiffany, 1990). Clearly, caution should be taken when claims are made as to the importance designated to physiological responses to cue-induced craving.

Researchers frequently use psychophysiological and behavioral indices in conjunction with self report measures to elucidate the full range of responses that may relate to craving. A major advantage of measuring additional response domains is that they provide more objective data than self-reports or single indices and thus may be influenced less by various sources of bias. It is important to note that certain drugs may elicit different autonomic reactions, some may be better assessed with self-report and some with alternative measuring techniques. There still appears a long way to go before an optimum solution to the diverse issues that have just been raised may be found.

4.4.4 FACTORS AFFECTING CRAVING EVALUATION

The utility of any craving assessment instrument depends on its psychometric properties, specifically on whether the instrument reliably and validly measures craving. However, these requirements pose some problems in studies of craving. For example, reliability of test instruments refers to consistency of results when a subject is tested several times. However, craving is considered a reactively transient state that is expected to differ from one occasion to the next. The term ‘validity’ refers in general to the degree to which an instrument measures what it purports to measure. Because the empirical study of craving is relatively in its early stages, measuring craving is not easy since researchers still find it difficult to agree on what criteria can be used to define craving.

An important factor to consider when measuring craving is the timeframe of assessment. The specific timeframe of the assessment instrument used should depend on the goal of the research intention. One timeframe method includes the ‘state measurement’ that measures the ‘status quo’ of the patient. These are best employed by researchers interested in the psychophysiological, subjective and emotional mechanisms of craving, since it can assess craving fluctuations over time. Studies however, found a lack of correlation between state of strength of subjective rating at a given time point and concurrent or subsequent alcohol behavior. Another timeframe includes ‘global measures’. These measures describe the general experience of craving over the course of a day to
perhaps a month or more. The point of weakness in these methods is that they do not demonstrate a sensitivity to the relationship between craving and general alcohol behavior (Drobes and Thomas 1999).

4.5 Neural Substrates of Craving

In the study of neural substrates of craving, the tradition of research has focused on the likely role of the ventral striatum (Koob and Goeders, 1989) as the primary area involved in reinforcement and incentive (Weiss, 1981), which has only marginally implicated the amygdala, or indeed other limbic structures. This was originally inspired by the phenomenon of intracranial self-stimulation from medial forebrain sites (Milner, 1989; Shizgal, 1989). There was also the strongly held view that the ventral striatum system represents a final common element in all-reward functions, including those of natural reinforcers such as food and sex (Blackburn et al., 1986; Phillips et al., 1989). With the evidence presented in the preceding chapters, it is appreciated that craving is an integral part of emotional processes. A full understanding of the neural substrates of craving can be accommodated using fMRI.

The majority of imaging studies involving drugs of abuse thus far have been conducted using positron-emission tomography (PET) and single photon emission computed tomography (SPECT). In such studies, chronic alcoholics showed lower rCBR (regional cerebral metabolism) in the left parietal and right frontal cortex during actual alcohol ingestion (Volkow et al., 1992b). Other workers have reported decreased rCBR in the medial frontal cortex (Gilman et al., 1990; Adams et al., 1993). Craving however, was overall not particularly addressed in substance abuse research. Once the relationship between craving and environmental cues was established the interest in craving has since been heading in a new direction.

Of the few studies that have applied functional brain imaging to investigate brain states associated with craving, the majority have examined the effects of craving among cocaine abusers. Perhaps, only one study has successfully induced and assessed craving among alcoholics (Modell and Mounts 1995). Using SPECT, the authors of this study found a significant increase in blood flow in the right caudate nucleus during craving. The failure to find amygdala involvement may have rested on the fact that they used actual alcohol ingestion, which does not signify a cue in the sense of secondary reinforcement. Another attempt to induce craving in alcoholics was by injecting m-
chlorophenylpiperazine (mCPP) (Hommer et al., 1997). By this method, no evidence of amygdala involvement was found. In contrast to the few imaging studies conducted on alcohol craving, studies investigating craving for cocaine have implicated a number of brain regions during craving such as the nucleus accumbens, insular cortex, and orbital cortex. Support for the participation of the amygdala in craving comes from a number of other studies with cue-induced cocaine (Grant et al., 1996; Childress et al., 1999). Hence, these recent experiments point to limbic structures as the site where functional changes may be found in addicts, thus laying the foundation for the relationship between craving and emotion. From the functional imaging literature, a unifying model of craving experience should be derived.

4.5.1 BRAIN REGIONS INVOLVED IN CRAVING

One of the goals of imaging is to visualize brain areas involved in craving. As indicated earlier, most studies involving drugs of abuse, mostly cocaine, were conducted using PET/SPECT. Yet, as these studies were being carried out, the potential to investigate craving using PET was already being recognized by its capability to measure radiotracer responses to exposure to drug-related cues (Modell and Mountz, 1995; Zubieta et al., 1996).

The technique of measuring blood flow using $^{99m}$TcHMPAO (SPECT) allows investigators to visualize blood flow distribution within the brain during the first 1 to 2 minutes. This method has been used to investigate the functional cerebral anatomy of craving in alcoholic patients. An enhanced increase in blood flow was found during low dose of alcohol ingestion in the right caudate nucleus, which correlated with the subjects’ self-report of craving for alcohol (Modell and Mountz 1995). The caudate nucleus is connected to the prefrontal cortex and is related to motivation, which may explain activation in that area. Actual alcohol ingestion is considered to contain more of a priming effect which may explain the absence of activation in emotional processing centers. However, priming has been also associated with arousal (negative affect) which should have indicated amygdala involvement.

The physiological activity of neurons require energy derived from the metabolism of glucose. The mapping and quantification of regional glucose utilization rates by the $^{18}$F-flourodeoxyglucose [FDG] is a method that provides an index of regional functional activity in the central nervous system. FDG PET measures neuronal glucose use during a period of 20 to 30 minutes. A selective enhancement on the rates of glucose utilization was found during cocaine craving with cocaine

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related cues in cocaine abusers. The hypermetabolism was primarily produced in the amygdala, dorsolateral prefrontal (DLPC), and orbital cortex, and with the amygdala and DLPC correlating with self-report of craving. Hypometabolism was found in the caudate nucleus (Grant et al., 1996). The orbitofrontal cortex showed increased brain rates of glucose in a similar study along with two other regions that included the left insular and cerebellum and with metabolic values in the insular showing significant correlating with self-reports of cocaine craving (Wang et al., 1999). The insular is an area involved in visceral function, olfaction, taste and emotion. By dint of its extensive connections with the amygdala, it is postulated to play a role in the motivational and emotional (Hommer et al., 1999) aspects of cue induced craving.

Memories of rewarding aspects of alcohol use and their salience are proposed to be located in the DLPC (Kalivas et al., 1998). Accordingly, situations that are coupled with alcohol use could be remembered with increased salience, because the DLPC is activated by alcohol related cues, and by the amygdala which has been found to impart valence to stimuli. Also input from the amygdala to the frontal cortex is assumed to relay information about the rewarding aspect of cues (figure 3) (Hatfield et al., 1996). Hypermetabolism was also found by Volkow et al. (1999) in cocaine addicts in the anterior cingulate gyrus, right thalamus, and cerebellum, using methlyphenidate. As suggested earlier, there is now ample evidence to relate the cingulum to emotional processes. The cingulum as well as the thalamus form part of the striatal-thalamic circuit which is suggested to be linked to subjective craving. Given the inputs to the anterior cingulate, and its output connections to the ventral striatum (van Hoesen et al., 1993), the anterior cingulate cortex may be part of an executive out-put response selection network for certain emotional condition. Today, the cerebellum has been attributed to participate in memory retrieval and learning (Buckner et al., 1996). In the case of craving and conditioned responses, cerebellum activation may involve memory retrieval of learned unconscious response. For example, animal and clinical findings indicate that several components of non-declarative memory such as motor and cognitive skill acquisition or certain types of classical conditioning are dependent upon the integrity of the cerebellum (Daum and Ackermann, 1997; see pp 86 for more extensive discussion).
Figure 3. One model of brain regions involved in craving. Alcohol activates the nucleus accumbens, the brain’s “reward center.” Nerve cells (i.e., neurons) in the nucleus accumbens send information to the amygdala, which plays a role in the modulation of stress and emotions; the frontal cortex (shaded area), including the dorsal lateral prefrontal cortex (DLPC), where the reward memory is thought to be located; and the basal ganglion, which plays a role in repetitive thought and behavior patterns. Neurons in the amygdala also send information to the DLPC and the basal ganglia. The DLPC sends information back to the basal ganglia (a connection that may play a role in obsessive-compulsive behaviors) and to the nucleus accumbens. Feedback from the DLPC to the nucleus accumbens may sensitize the latter to further alcohol exposure. The DLPC itself is controlled by the orbitofrontal cortex, which induces impulse control (From: Anton RF, 1999. What is craving: Models and implications for treatment. Alcohol Res Health 23, 165-173).
Administration of $^{15}\text{O}$ is a commonly employed PET technique that measures blood flow using $[^{15}\text{O}]\text{H}_2\text{O}$. In contrast to FDG PET’s long measurements, $^{15}\text{O}$ PET requires only 1 to 2 minutes. A presentation of cocaine related tapes showed increased rCBF in the amygdala, dorsolateral frontal cortex and anterior cingulate and reduced rCBF in the caudate nucleus in cocaine addicts compared to controls (Childress et al., 1999). Cue-induced craving has been suggested to cause anxiety and stress associated with withdrawal. According to the stress hypothesis of craving, amygdala activation may be associated with stress induced craving possibly through its connection with the DLPC, since the memories of stress relief may be encoded in the DLPC (figure 3). Interestingly, a number of craving studies have suggested that withdrawal symptoms although present are not always consciously perceived.

Numerous attempts to link structural changes with psychiatric illness were made, with varied success. FMRI has the potential to map abnormal function of what appears to be a structurally normal cortex. It is a promising imaging modality in studies of craving because of the ease of its use and superior spatial and temporal resolutions. So far this advantage has been slow to be made use of in addiction research. Of the few fMRI studies that have so far been conducted on craving, the areas of the anterior cingulate gyrus and left dorsolateral prefrontal cortices, were activated with a cocaine theme interview in cocaine abusers compared to controls, whereby the anterior cingulate activation only reached significance (Maas, 1998). BOLD response increases in the nucleus accumbens, caudate nucleus, thalamus, and anterior cingulate and insular cortices were found with actual cocaine administration in cocaine abusers, in another study. Self-report of craving was found to be positively correlated with signal increases. However, BOLD signal decreases were found in the amygdala. (Breiter et al., 1997). Here again, the actual administration of the drug itself may have had the affect of decreased amygdala activation. In a more recent investigation in cocaine addicts, increased anterior cingulate and low frontal lobe activation was reported during the presentation of video tapes showing cocaine cue material compared to happy, and sad tapes. A similar response was not found in the control group (Wexler et a., 2001).

The nucleus accumbens is a drug-stimulation site in primates and rats (Rolls et al., 1980), and this area is thought to be the brains ‘reward center’ (figure 3). Anatomical studies have found extensions from the nucleus accumbens to the amygdala and the frontal cortex. Neural recordings in the nucleus accumbens in monkeys have shown that this area responded to particular environmental
stimuli which were cues indicating particular motor response (Rolls et al., 1983b). Hence, it appears that the nucleus accumbens is not directly involved in the development and maintenance of reward and punishment associated with stimuli (Caan et al., 1984), but rather, in motor activity response to the motivational value of drugs of abuse. In this respect, the accumbens may be a system through which conditioned incentives (secondary reinforcers) learned about in the amygdala are connected to behavioral outputs.

Event related fMRI (increasingly being used today) in deprived smokers during presentation of images of smoking cues revealed significant signal increases in the left and right fusiform gyrus, middle frontal gyrus, inferior frontal gyrus, anterior cingulate, ventral tegmental area, and the medial thalamus. Significant, right, but not left amygdala activation was also detected. These activations were not observed in controls except for the area of the right ventral tegmental area during neutral stimuli (Due et al., 2000). As noted above, the thalamus is proposed to form part of the basal ganglia-thalamocortical circuit, that includes the anterior cingulate gyrus, caudate nucleus, amygdala and orbital cortices, which in light of the evidence presented so far, are all associated with cue induced craving. Because of their anatomical connections, it is expected that these regions are functionally linked (Alexander et al., 1986). The ventral tegmental area forms part of the mesocorticolimbic area, which is involved in the rewarding effects of alcohol. Its involvement in cue induced craving has so far not been reported and may still need to be investigated. The pronounced right but not left amygdala activation is consistent with other lateralized effects reported in studies of emotion (Costall et al., 1987). Previous imaging studies have implicated fusiform gyrus in self-monitoring, in the perception of biological motion, and in the attribution of mental states using verbal stimuli or visual depictions of the human form. Castelli et al. (2000) using PET demonstrated that selectively evoked mental state attribution or simple action description during movement animation patterns were associated with increased activation in this area. Its association to craving is unclear, and may have to be addressed in future studies of craving.

4.5.2 SOME POTENTIAL PITFALLS

Variables that can confound the result of cross-sectional imaging experiments encompass: age, sex, handedness, IQ, body weight, time from last drug exposure. A few factors are more difficult to have complete control over. These factors would include, for example, polydrug use. Most abusers use tobacco, alcohol and coffee and the amount and patterns of soft drug use, as well as dietary and
other lifestyle choices may affect imaging results. It is also possible that abusers and healthy control subjects may on occasions mislead researchers about prior drug use. A psychostimulant abuser informed that he/she will receive either a drug or placebo may experience disappointment when the anticipated ‘high’ does not materialize, whereas the drug-naive subject may experience anxiety as well as euphoria when undergoing what for he/she is a new experience. Activation pattern may therefore reflect the difference between disappointment and anxiety (Gatley and Volkow, 1998), and possible building expectancies concerning stimulations. Also the functional imaging studies conducted in cocaine, alcohol and tobacco addicts indicated quite a number of brain regions differing from study to study, that are likely involved in craving. The lack of concordance generally found between studies of the neurobiological correlates of craving may be caused by methodological issues, i.e., the various techniques for quantifying brain activation, and the variety of experimental techniques for eliciting craving.

4.6 SUMMARY

Despite the early views minimizing the importance of alcohol withdrawal symptoms with alcohol dependence (Pederson, 1986), there is sufficient indication to the contrary. The impressive amount of evidence for the various studies suggest the involvement of conditioned negative and positive reinforcers in alcohol addiction. The proposed pattern of reactivity to alcohol exposure as well as cue-induced alcohol craving lends support to Solomon’s Opponent Process Theory of affective dynamics (Solomon, 1977). Moreover, the amygdala was identified as a key element within the circuitry that may sustain compulsive drug seeking and drug use and contribute to drug craving mediated by the motivational impact of conditioned withdrawal. No less important, it was also pointed out that chronic administration of drugs produce sensitization. Sensitization is likely to occur to the rewarding effects of the drug which can also be context-dependent. Sensitization has a neurobiological explanation for changes modulation of the N. Acc and amygdala. In considering brain systems involved in sensitization, the component of CRF is suggested to play a role and should thus also be taken into account.

Conditioned cues exert a wide range of peripheral effects. It was assumed that craving for drugs could be assessed for many of its measurable actions. These must depend on the interaction between the stimuli and the mechanisms of induced craving. Thus, exposure to conditioned cues, can trigger physiological and behavioral and psychological effects. Unfortunately, not all would agree on the
close relationships among behavioral, psychophysiological, and subjective measures of craving. It would appear that responses vary considerably as a result of numerous individual differences (Cooney et al., 1997) and situational factors. It is obvious that the field of craving is handicapped due to the concept’s controversial conceptual status and application of a wide range of scales and questionnaires of unknown reliability and/or validity. Finally, functional imaging studies especially fMRI lends itself as an ideal tool in the investigation of craving. An impressive amount of evidence using a variety of different techniques indicates the amygdala plays a crucial role in cue induced craving, with amygdala projection areas also involved in some aspect of craving. Interestingly, the cerebellum was activated during cue induced craving. In general, studies of the cerebellum do not yet provide a clear picture over the prices involvement of the cerebellum in cognitive, motor and affect processes. Future work using experimentally induced craving with neuroimaging modalities should be fruitful in elucidating the role of the cerebellum in this regard. In a review by Daum and Ackermann (1997), it was proposed that studies of the cerebellum should take different methodological issues into account by using adequately matched clinical as well as non-clinical comparison groups, and theory-driven batteries comprising a wide range of tests.
5 HYPOTHESIS AND RESEARCH QUESTION

Recent neuroimaging studies of cocaine users point to limbic involvement during cue-induced cocaine craving. In particular, the cerebral correlates of craving were suggested to involve the amygdala. In animal studies, Schulteis et al. (2000) have recently identified the basolateral amygdala as a key element that may sustain compulsive opiate drug use and contribute to drug craving and relapse by mediating the motivational impact of conditioned withdrawal. The evidence provided in the preceding pages (chapter 2 and 4), highlight the importance of the interaction of the amygdala in processes by which environmental stimuli gain affective value through their predictive association with primary reinforcers. This phenomenon results from adaptive changes in the neuronal function produced by prolonged drug consumption. Two types of neuronal adaptations have been proposed (chapter 4). There is the sensitization model which proposes a positive incentive account to craving (Stewart et al., 1984); in contrast there is the drive reduction model which has supposed that drug seeking must be maintained by the avoidance or reduction of unpleasant withdrawal symptoms or other states of distress (Solomon, 1980). Correspondingly, Backer et al. (1987) proposed the affect model of cue reactivity, positing that reactivity to substance-relevant stimuli is controlled by complex affective processing systems that can be either appetitively based (positive affect craving systems) or withdrawal based (negative affect craving systems).

The craving for alcohol has not been systematically studied with the full range of methods available in human neuroscience. In order to determine the neural substrates of craving in alcoholic patients, a healthy group of subjects was included in this study as controls and both non-patients and patients groups were exposed to ethanol (EtOH) induced craving. Considering that the amygdala is especially important in emotion and motivation, being that it is a region that learns to form associations between secondary reinforcers and primary reinforcers, it was hypothesized to find amygdala activation in response to cue induced craving.

The following research questions are addressed in this study during the first measurement

Using fMRI, amygdala involvement in response to cue induced ethanol craving in alcoholic patients should become evident.
On the other hand, BOLD effect in the amygdala is not expected to be found in response to neutral room air for both patients and healthy controls.

Moreover, in a comparison to healthy controls, any differential functional activation should suggest a craving effect during ethanol odor.

Correlations were demonstrated between behavioral, physiological, and subjective variables of craving (Stockwell, 1985). Questionnaires have some validity in that they yield a quantitative index of severity. Several studies have shown that the avoidance for withdrawal symptoms after chronic ethanol-self-administration causes continued ethanol consumption, though this proposition has not been without its criticism (Mello and Mandelson, 1977). The same consensus was reached about other drugs of abuse (Wise and Bozarth, 1987). It was therefore of interest to this study to evaluate the possible craving effect on the Craving Rating Scale (CR). This scale is a modified version of the German questionnaire for currently experienced alcohol craving (Szegedi et al., 2000) following cue of ethanol. Similarly, many studies have demonstrated that cocaine dependent individuals show altered mood with increased craving. Such reactivity is thought to provide some of the motivation for relapse to cocaine following treatment. Whether altered mood is a result of craving or a by product of craving or an attribute to craving is debated, with implications suggesting the latter. This study expects to see a change of mood on the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) in alcoholic patients to ethanol cue as they currently experience it. It is thus hypothesized to find levels of craving to be associated with withdrawal ratings as well as difference in mood states.

The following research questions are addressed during the first measurement:

It is expected that patients are likely to score well along the continuum of severity for craving in response to ethanol cue compared to neutral air. Healthy subjects are not expected to show any craving effect on the craving scale.

The alcoholic patients are expected to report altered mood that should correlate to cue induced ethanol craving compared to neutral room air. Altered subjective experience should not be found on the scale for mood in healthy controls in response to ethanol stimulation.
Published studies have found that cocaine craving is high at the end of bingeing which is accompanied with an anhedonic dysphoric state. Craving then diminished markedly, only to emerge after 1-5 days when affective symptoms have more or less normalized (Gawin et al., 1986). Such cycles have not been measured using imaging methods. Much less, have behavioral effects as a result of treatment affect been elucidated for alcohol craving, be it pharmacological or psychological. A review of the literature suggests published studies exist for depression, in which it is demonstrated that recovery is associated with normalization of certain brain areas after drug therapy. These suggest that the defects are state markers of the illness (Baxter et al., 1989; Ebert and Ebmeier, 1996). The second goal of this study was to determine to what extent are the cerebral correlates of craving static or dynamic. The hypothesis, based on data obtained from the cocaine craving literature, is that craving is dynamic.

The following research questions are addressed at the second measurement:

- Recovery from craving after intervention should be associated with normalization of regional abnormalities in the amygdala.

- These should resemble BOLD effect of control subjects at the first measurement

- A reduced mood effect and craving effect on the rating scales should emerge for alcoholic patients. No changes are proposed to be found for healthy subjects

In light of normalized amygdala activation, it can be inferred that its role serves as a state marker.
6 Method

The following section will describe the first study. This experiment focuses on the neurobiological basis of craving in recently detoxified alcoholic patients. Using the minimally invasive technique of fMRI the possible significance of the amygdala area in processes of craving will be investigated within the larger picture of emotion. Any recovery from pretreatment indicated by normalization of overactive regions should suggest a state marker in craving processes. This is the second element of investigation in this study. This and the next study will use the technique of functional imaging.

6.1 Subjects

The subjects were 10 male patients seeking help for alcohol addiction at the psychiatry and psychotherapy department of the Rheinische Friedrich-Wilhelms-University, Bonn. Patient recruitment and evaluation were performed by well trained psychologists and psychiatrists. Patients went through a comprehensive screening interview that included drinking history, and prior hospital stays.

Patients were diagnosed for alcohol-dependence using DSM IV (Diagnostic and Statistical Manual of Mental Disorders) based on the semi-structured clinical interview (German translation of the S-SSAGA, Buchholz et al., 1994) for alcoholism. Patients had no history of prior drug abuse. An initial urine drug screening for opioids, methadone, amphetamines, cannabinoids and sedatives was done for all patients on the day of admission to the detoxification unit. Only patients with negative drug screening results were included in the study. Inclusion criteria included willingness to participate in the research project that comprised three weeks of treatment, followed by a second measurement. Also verification of normal sense of smell was required. Patients were right-handed, native German speakers.

CT scans were evaluated for all patients prior to inclusion. CT scans of five patients were judged to be unobtrusive. Three patients showed slight enlargements of internal and/or external ventricles. One patient showed a large sinus frontalis sphenoidales and another demonstrated single falx calcifications.

Before inclusion in the study, patients underwent an inpatient detoxification treatment (up to 2 weeks; detoxification treatment with disulfiram or doxepine and, if necessary, carbamazepine and/or haloperidol). Patients voluntarily entered an open or closed detoxification unit depending on
the severity of withdrawal. After detoxification patients were transferred to the psychiatric day-clinic program for patients with alcohol related problems. Patients were randomly selected to undergo a breathalyzer (Dräger) test once daily in the detoxification unit and in the 3-weeks inpatient treatment period to control alcohol abstinence.

Initial screening for potential safety hazard and medical history (neurological and psychiatric) was done by questionnaires which the patient completes upon arrival for fMRI examinations. Safety risks included metallic or partly metallic items such as eyeglasses, hairpins, hearing aids. The same applies to any removable dental work. Metallic cosmetics should also be removed, since they may result in image artifacts that obscure parts of the orbitofrontal anatomy (Sacco et al., 1987).

Exclusion criteria for alcohol addicts were current alcohol use or any substance other than nicotine and other psychiatric comorbidity according to the DSM IV, except for a history of prior alcohol abuse. Patients were given a complete description of the study, and were required to give written informed consent.

Inclusion criteria for control subjects were the same as for addicted subjects except for alcohol use. Exclusion criteria for control subjects were the same as for addicted subjects except for an absence of a lifetime history of psychoactive substance abuse or dependence other than nicotine. After inspection of 3D-scans during the fMRI measurements, one healthy subject was excluded due to neuronal atrophy with ventricular enlargement. Control subjects were recruited by notices or word of mouth. Volunteers accepted appointments for an initial evaluation session which included screening for eligibility, and then underwent the information consent procedure. Addiction/medical histories, cognitive incapacity, and mental health assessments were performed. Volunteers who were accepted and agreed to participate were oriented to the testing procedures. Volunteers who completed the research project, were paid DM 25/- per hour.

Subjects entered the study sometime during July 1999 to January 2000. Subjects were measured at two time points with three weeks of intervention between measurement. Treatment did not apply to controls.
### Method

6.2 **Variables**

6.2.1 **Independent Variables**

The independent variables were group sample (detoxified alcoholic patients and non-patients), condition (ethanol and neutral room air), time point of measurement (time point 1 and time 2), subject characteristics, psychopathology and laboratory testing.

6.2.2 **Dependent Variables**

6.2.2.1 **BOLD contrast**

The following imaging protocol was used for performance of the fMRI study. The imaging parameters were selected to optimize examination according to the field strength and equipment.

MR imaging was performed on a 1.5T imager (Magnetom Vision, Siemens). A high-resolution sagittal 3D-dataset was acquired (matrix size 256×256, sagittal slices 128, TR 11.4ms, TE 4.4ms, FOV 230mm).

The intercomissural line AC-PC (figure 4) served as a reference for the position of the subsequent transaxial functional images (EPI 70 measurements, slice thickness 3mm, slice-gap 0.3mm, TR 10s, TE 66ms, matrix 64×64, FOV 200mm, 32 axial slices, α 90°, acquisition time 4s), covering the whole brain.

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**Table 1. Subject demographics of the alcoholic patients and the control group. Values are mean ± SD unless otherwise indicated**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n=10)</th>
<th>Controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41.4±7.7 (30-58)</td>
<td>41.1±7.5 (29-56)</td>
</tr>
<tr>
<td>Education</td>
<td>9.5±1.7 (8-10)</td>
<td>12.7±4.0 (8-18)</td>
</tr>
<tr>
<td>Duration of alcohol use</td>
<td>8.9±3.84 (3-14)</td>
<td></td>
</tr>
<tr>
<td>Alcohol daily dose</td>
<td>11.90±4.97 (5-20)</td>
<td></td>
</tr>
<tr>
<td>Cigarettes smoked per day</td>
<td>(n=9) 22.22±9.39 (10-35)</td>
<td>(n=5) 15.8±5.31 (10-20)</td>
</tr>
<tr>
<td>Number of years smoking</td>
<td>20.66±8.03 (4-34)</td>
<td>24.6±9.58 (11-36)</td>
</tr>
</tbody>
</table>
6.2.2.2 Subjective evaluation

Success in advancing the craving syndrome concept hinges upon developing practical methods for the operational measurement of the syndrome. A number of self-report questionnaires were developed for assessing craving. The following well characterized signs of ethanol craving/withdrawal were rated immediately after each condition using the CR. The CR is a 10 point unipolar intensity scale that includes 7 dimensions of craving. These dimensions include urge for alcohol, withdrawal symptoms, obsessional thoughts, anxiety and depression, irritability and anger, positive affect, and goal for maintaining abstinence.

To determine mood state, subjective ratings were also used and included the PANAS: a 5-point unipolar intensity scale, which includes ratings for factor-referenced emotional descriptors for orthogonal positive and negative dimensions. The scale requires a rating of “how did you actually feel in the last minutes?” Ratings were acquired verbally.
6.3 **PROCEDURE**

The Institute for Medicine, Research Center, Jülich was the location of fMRI experiments. Following initial screening by questionnaires upon arrival of subjects and solicitation of further clarification, written informed consent of experiment participation was obtained. Subjects were positioned supine head first on the examination table. To restrict motion and allow for subject comfort, a head stabilizer was used and padding was present. Earplugs were provided to attenuate the repetitive gradient noise.

![Diagram of experimental conditions](image)

**Figure 5. Sequence of stimulations for each of the 2 experimental conditions (ethanol, neutral room air).**
METHOD

The task paradigm consisted of two conditions: ethanol stimulation and neutral room air stimulation. The stimulus interval of 10s started with 4s data acquisition, during which time the initial 2s were of olfactory stimulation (air/ethanol). This was followed by a pause of 6s without stimulation or data acquisition (figure 5). Each condition comprised 70 whole brain acquisitions (70*32=2240 slices).

The olfactory stimuli were presented using a costume based olfactometer, built to accommodate for MRI application. The olfactometer consisted of a compressor to ‘blow air’ through hollow tubes (length approximately 10m, applied with same temperature and pressure (50 ml/s)) to the right nostril of the subjects; two glass reservoir tubes, filled with different liquid odorant (ethanol - water), each with inflow conduits from the compressed air source and outflow conduits to the subjects nose; two airflow switches and relays to direct the compressed room air to the individual odorant tubes; and a laptop computer to control the airflow.

The olfactory system-mediated odorant was corn spirits, containing 38% ethanol by volume, and unmanipulated air for the neutral stimulus condition.

Each subject underwent two separate evaluations inside of the scanner room after each condition using the PANAS, and CR.

6.4 MEASUREMENT (TIME POINT 2)

The subjects were measured again after three weeks. During that time only the alcoholic patients received a standardized cognitive behavioral group therapy during controlled abstinence. The therapy was conducted by licensed and well-trained clinical psychologists. The 5 hour-a-week program involved relapse prevention, stimulus control and educational intervention. Study patients were medication-free during the three weeks study time except for a daily dose of 150 mg Doxepine. Hence, the second time point fMRI measurement followed after the three weeks of therapy with pharmacological intervention. The same magnetic resonance imaging protocol on the 1.5 T scanner was used at the second measurement (time point 2) and the same experimental design was applied.

Overall time in the imager was approximately 95 minutes per subject for each session.
6.5 **DATA ANALYSES**

6.5.1 **FUNCTIONAL MAGNETIC RESONANCE IMAGING**

Image reconstruction was performed off-line on a SPARC Workstation (Sun Microsystems, Surry UK). Analysis was performed using SPM97d (Statistical Parametric Mapping, University College, Department of Cognitive Neurology, London).

FMRI was performed using boxcar design with alternating epoch of rest and single activation task.

In fMRI time series of human brain, a number of noise components are evident which arise from artifacts (head motion, slow global variations in blood oxygenation, or physiological cardiac reparatory pulsations). Bulk head motion was recognized since the earliest days of fMRI (Kwong et al., 1992) as potentially the most severe and misleading confound in fMRI. Motion effects were removed by realignment of images in a time series to the 10th image, using sinc interpolation for greatest precision. This motion correction reduces motion artifacts by a rigid body transformation (translation in x, y, z direction and rotation around these three main axes), minimizing the total square difference between images and the reference image. Images were low-pass filtered with a Gaussian function.

Functional (mean image of the realigned) data were typically superimposed upon subject’s corresponding anatomical MR images obtained with T1-weighted imaging technique. The anatomical slices were superimposed onto the set of each experimental condition and coregistered with MPITOOL (Version 2.59, MPI for Neurological Research, Cologne). For the superposition, AC as a corresponding reference point was determined.

In many fMRI experiments several subjects are studied, and this raises the question of how best to proceed with data analysis. The comparison of results between subjects, or different groups become difficult as results are not reported in a standard anatomical space. The ability to normalize fMRI data into a common stereotactic space, such as that of Talairach and Tournoux (Talairach J, Tournoux P. 1988. *Co-planar stereotaxic atlas of the human brain*. Thieme, Stuttgart) allows the reporting of significant activations in a common reference space. Normalization sets the average whole-brain image value of all the voxels to a set number. Stereotaxic normalization used in this study was 4×4×4 mm³ resolution.

The data were spatially smoothed, by interpolating to a smaller pixel size, and then convoluted using a Guassian kernel in space, with a full width at half maximum of the original pixel
dimensions. Smoothing in space enhances the signal to noise ratio of data and allows intersubject averaging by blurring differences in gyral anatomy between subjects. It ensures that haemodynamic changes from subject to subject are assessed on a spatial scale. Anatomical scale was chosen at (6×6×6 mm³ Gaussian filter).

As noted, fMRI time series contain a number of signals; uncorrelated (thermal) and correlated (physiological). There is also correlated signal that conforms approximately to changes in neural activity convolved with hemodynamic response function (Friston et al., 1994b). Smoothing in time of fMRI time series with hemodynamic response function will enhance signal relative to noise. Temporal smoothing active was used.

6.5.2 STATISTICAL ANALYSIS (SPM)

Following the above mentioned procedures of realignment, normalization, smoothing, and filtering, from 20 subjects and 70 scans while performing one of two tasks (ethanol odor, neutral odor exposure) at two sessions (before and after intervention), the adjusted measures were subjected to statistical analyses (correlation with boxcar, analyses of covariance, subtraction analyses).

First, a group and single subject analyses were performed for both conditions for both time points. For this, contrasts were defined with alternating periods of ‘baseline’ and ‘activation’ and a between groups difference using delayed box-car reference vector.

For this, the general linear model, or analyses of covariance as it is generally known, is employed to perform the appropriate univariate test at each voxel to search for significant activated voxels. This assessment is in terms of a t value for each and every voxel (i.e., a SPM {t}).

The resulting statistical parametric maps of t statistics generated for each voxel was transformed to a map of corresponding SPM {Z} values.

The objective of further analyses (using SPM), was to test the hypothesis about regional condition-specific effects. Considering the a priori hypothesis with regard to amygdala activation and an interest for increasing sensitivity, Bonferroni corrections were not applied. Performing corrections for multiple comparisons would be unnecessarily conservative on Type I errors.

In view of preceding studies (Schneider et al., 1998; 2000) and the observations made by others (Irwin, 1996), showing that amygdala activation is less robust than activations in other regions, a threshold of Z=1.64 (p=.05) was set and a minimum cluster size (extent) of 1 voxel. The significance level was set at α=0.05. Cluster size was not increased to avoid overlooking small
areas of activation in this already small region. The activated voxels surviving this procedure were superimposed on SPM glass brain projection. Three different brain volumes were found to have a spatial offset of more than one voxel in three different subjects, respectively. Each of these brain volumes were under different conditions blocks, at different time points of the scanning session and only single scans during the time course were affected. Therefore the whole time series were included in the analyses.

To confirm results of SPM, a second order analysis was used for the region of interest (amygdala) in an ANOVA. Here signal courses were extracted at the maxima of activation in patients at T0 as well as for the same location for the other groups. The relative signal intensity (SI) change was then calculated from the mean values of baseline and activation periods according to \((SI_{ACT} - SI_{BL})/SI_{BL}\), where \(SI_{ACT}\) and \(SI_{BL}\) refer to mean SI during activation (ACT) and baseline (BL), respectively. These relative signal changes served as dependent measures in an repeated measures analysis of variance (SAS statistical software package) with two factors, time (T0, T1) and group as a between subject factor.

In this study, the effects of activations due to ethanol odor or deactivation due to neutral air were addressed using the subtractive approach. Cognitive subtraction is a powerful paradigm having been the mainstay of functional neuroimaging over the past decade, and with one of its earliest applications in functional anatomy of word processing (Petersen et al., 1989). The principle of subtraction approach is that sensorimotor or cognitive processes should lead to increased activity in and only in the area of interest both at a level of function and neural (with no interaction among areas). This model involves elaborating on two or more tasks that differ in a separable component. Ensuing differences in brain activity are then attributed to this component. In imaging paradigms, this would mean testing a hypothesis pertaining to activation in one task relative to another. Cognitive subtraction however, is a rather unsound conceptual bases for many lines of research since it depends on the assumption that cognitive states differ in components that can be purely inserted or removed with no interaction among them. The possible fallibility of this assumption has lead to complementary, as well as to the exploration of other experimental designs. An extension of the subtraction technique is the conjunction analyses. Conjunction technique involves combining a series of subtraction. This approach tests several hypotheses, asking whether all activations, in a series of task pairs, are jointly significant.
Another approach to task analyses relates to factorial design. This approach involves combining two or more factors within a task or tasks and looks at the interaction between the different factors, or the effect one factor has on the response of the other factor. This design looks at regionally specific interactions. Generally, interactions can be thought of as a difference in activation brought about by another processing demand such as with the dual task interference paradigm (Shallice et al., 1995). This approach looks at regionally specific interactions rather than regionally specific activations of the cognitive subtraction.

The future direction may involve psychophysiological interaction: the concept of psychophysiological interactions try to explain the physiological responses in one part of the brain region in terms of an interaction between the presence of a sensorimotor or cognitive process and activity in another part of the brain. An illustration of this model approach was conducted by Friston et al. (1997) who demonstrated that activity on the medial parietal region in a face and non-face observation task, was most significantly expressed in the right infero-temporal region. This is an interesting analysis for it allows inferences to be made about brain function with respect to functional connectivity.

6.6 **SUBJECTIVE DATA ANALYSES**

Quantifying the condition effect was derived from the ratings of positive and negative scores of the PANAS. Subjective ratings analysis was performed for the 20 item PANAS (Positive affect and Negative affect). Similarly quantifying the craving intensity for the CR was derived from each condition of each time point and for both groups.

6.6.1 **STATISTICAL ANALYSIS**

A two factor analysis of variance using SAS (Statistical Analysis Systems, 1998) (odor 2, time 2) of craving ratings (CR) in patients and controls. Since controls were not required to respond to all the 7 dimensions of craving included in the rating scale, data for controls and patients were analyzed separately.

A four factor analysis of variance for the PANAS (n=20) with group as a between factor (2), and odor (2), time (2), and scale/mood valence (2) as repeated factors was performed. A similar analyses was done for CR.
7 **RESULTS**

7.1 **SUBJECTIVE RATINGS**

7.1.1 **CRAVING AND MOOD RATINGS DURING ETHANOL FOR GROUPS - TIME POINT 1 AND 2**

A two factor analysis of variance (odor 2, time 2) of subjective ratings CR for craving in patients demonstrated a significant interaction between odor and time ($F(1,8)=6.06, p=0.03$; figure 6). Mean craving ratings for ethanol condition was $17.0 \pm 4.4$ ($\pm SD$), ratings for neutral room air was $14.7 \pm 4.2$. At the second time point, ratings for ethanol decreased ($14.5 \pm 2.5$), whereas for neutral odor, the ratings did not show much variance ($15.7 \pm 3.1$). Analysis for controls showed no significant effect.

![Figure 6. Mean craving ratings (CR) in the alcoholic patients pre- and posttreatment (first and second measurement) during condition ethanol odor and neutral air.](image)

The dependent measure for the PANAS was derived from the ratings of 10 items contributing to a positive and a negative score (table 1). The four factor analysis of variance for the PANAS ($n=20$) with group as a between factor (2), and odor (2), time (2), and scale/mood valence (2) as repeated
factors yielded a significant main effect for group (F(1,18)=7.99, p=0.01), odor (F(1,18)=8.61, p=0.009) and for scale (F(1,18)=58.73, p=0.0001). A similar meaningful interaction (group-by-time-by-odor or time-by-odor) to CR ratings, did not emerge.

<table>
<thead>
<tr>
<th></th>
<th>PANAS Positive</th>
<th>PANAS Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>30.7±8.0</td>
<td>13.1±5.2</td>
</tr>
<tr>
<td>Neutral odor</td>
<td>28.7±8.3</td>
<td>11.9±2.9</td>
</tr>
<tr>
<td>T1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>28.5±8.3</td>
<td>12.7±5.5</td>
</tr>
<tr>
<td>Neutral odor</td>
<td>28.6±9.2</td>
<td>12.6±5.5</td>
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<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>23.4±6.9</td>
<td>11.3±2.1</td>
</tr>
<tr>
<td>Neutral odor</td>
<td>21.8±6.1</td>
<td>11.1±1.8</td>
</tr>
<tr>
<td>T1:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>20.5±7.3</td>
<td>10.9±1.7</td>
</tr>
<tr>
<td>Neutral odor</td>
<td>19.3±6.6</td>
<td>10.6±1.6</td>
</tr>
</tbody>
</table>

**Table 1. Subjective ratings for PANAS during stimulations with ethanol odor and neutral air for the alcoholic patients (before and after psychotherapy with a pharmacological treatment) and the healthy controls (mean±SD).**
RESULTS

7.2 MEASUREMENTS

7.2.1 ACTIVATION AREAS IN BOTH GROUPS WITH ETHANOL AND NEUTRAL AIR
In the ethanol experimental condition there were significant increases of BOLD effect in the right amygdala-hippocampal area, the superior temporal gyrus and the cerebellum in the alcoholic patient group (figure 7 and 8 - 9, table 2). A similar activation during neutral room air was not demonstrated, with only small activation clusters seen in the medial frontal gyrus and the paracentral lobe.

![MRI images of brain sections](image)

**Figure 7. MRI section of the brain of 10 early abstinent alcoholic patients during cue induced ethanol craving at (T0) and at (T1). Planes parallel to the intercommisural line are shown at distances below as indicated by the number on top of the images. Superimposed in bright color are the areas showing significant BOLD signal during the ethanol condition compared to neutral air. The analyses was performed using SPM. The SPM[Z] - threshold was 1.64. (p=.05). The significance level was set at α=0.05**
RESULTS

FIGURES 8 AND 9. GLASS BRAIN VIEWS OF SIGNIFICANT VOXELS AT $\alpha = 0.05$. THIS IS A MAXIMUM INTENSITY PROJECTION OF SPM ($t$) FOLLOWING TRANSFORMATION TO THE Z SCORE. SIGNIFICANTLY ACTIVATED VOXELS DURING CUE INDUCED ETHANOL CRAVING OF 10 ALCOHOLIC PATIENTS BEFORE (ABOVE) AND AFTER (BELOW) INTERVENTION (T0 AND T1). IMAGES ARE SHOWN AT INTEGRATED PROJECTIONS THROUGH SAGITTAL, CORONAL, AND TRANSVERSE VIEWS OF THE BRAIN. ARROWS SHOW THE AREA OF THE RIGHT AMYGDALA. NOTE THAT AFTER INTERVENTION AMYGDALA ACTIVATION IS NO LONGER DEMONSTRATED.
The results of the second order analysis of variance for the amygdala activation revealed the expected group×time interaction for both local maxima (table 2) \((F(1,18)=5.37, \ p=0.032; \ F(1,18)=4.54, \ p=0.047)\) and confirmed results of the SPM analysis.

<table>
<thead>
<tr>
<th>Patients Region</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>max. SPM[Z]</th>
<th>N</th>
<th>Comparison subjects Region</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>max. SPM[Z]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol Amygdala/Hippocampus</td>
<td>R</td>
<td>36</td>
<td>0</td>
<td>-12</td>
<td>3.3</td>
<td>3</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>24</td>
<td>-4</td>
<td>-24</td>
<td>2.5</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sup. temporal gyrus</td>
<td>R</td>
<td>64</td>
<td>-12</td>
<td>8</td>
<td>4.7</td>
<td>67</td>
<td>R</td>
<td>64</td>
<td>-12</td>
<td>4</td>
<td>-</td>
<td>4.9</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>L</td>
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<td>0</td>
<td>0</td>
<td>5.1</td>
<td>17</td>
<td>L</td>
<td>-64</td>
<td>-24</td>
<td>16</td>
<td>4.9</td>
<td>5</td>
<td>5</td>
</tr>
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<td></td>
<td>R</td>
<td>52</td>
<td>12</td>
<td>-20</td>
<td>4.2</td>
<td>8</td>
<td>R</td>
<td>44</td>
<td>12</td>
<td>-12</td>
<td>3.9</td>
<td>6</td>
<td>6</td>
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<td></td>
<td>L</td>
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<td>0</td>
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<td>-60</td>
<td>-40</td>
<td>24</td>
<td>4.0</td>
<td>3</td>
<td>3</td>
</tr>
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<td></td>
<td>R</td>
<td>36</td>
<td>12</td>
<td>-16</td>
<td>3.6</td>
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<td>-</td>
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<tr>
<td></td>
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<td>4</td>
<td>-16</td>
<td>3.1</td>
<td>5</td>
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<td>-</td>
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<tr>
<td></td>
<td>R</td>
<td>28</td>
<td>0</td>
<td>-28</td>
<td>3.2</td>
<td>13</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>L</td>
<td>-28</td>
<td>-64</td>
<td>-24</td>
<td>3.7</td>
<td>33</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>L</td>
<td>-12</td>
<td>-80</td>
<td>-20</td>
<td>3.5</td>
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<tr>
<td>Neutral air Medial frontal gyrus</td>
<td>L</td>
<td>-8</td>
<td>16</td>
<td>44</td>
<td>3.2</td>
<td>32</td>
<td></td>
<td>-</td>
<td>-</td>
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<td>Paracentral lobe</td>
<td>L</td>
<td>-12</td>
<td>-28</td>
<td>52</td>
<td>2.6</td>
<td>6</td>
<td></td>
<td>-</td>
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<td>Sup. temporal gyrus</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>L</td>
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<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 2.** The table shows brain region activation in patients and controls at T0 before therapy (patients only) that correlated with ethanol odor stimulation and neutral room air stimulation. SPM[Z] - threshold = 1.64. x, y and z are Talairach co-ordinates and refer to the center of gravity of the cluster. N is the number of voxels above threshold in the cluster, p < 0.01 for all regions at the voxel level.
7.2.2 Stimulation Using Ethanol in Normal Controls

The analysis of the condition ethanol revealed activation bilaterally in the superior temporal gyrus in the healthy group at T0 (figure 10 and 11) and T1 (figure 12 and 13). Unlike the patient group, the non-patient sample showed no activations in the subcortical-limbic region. A smaller, right temporal gyrus activation was also found during stimulation with neutral room air.

**Figure 10.** MRI images are shown of 10 non-alcoholic subjects. In each, activations are indicated as colored areas on transaxial MRI anatomical scans of the normal subjects. Planes parallel to the intercommisural line are shown at distances above as indicated by the number on top of the images.

**Figure 11.** Significantly activated voxels during cue induced ethanol of 10 non-patients before interventions (T0). Thresholds for significance: p<.05. Notice the non-patient sample showed no activations in the subcortical-limbic region.
RESULTS

**Figure 12.** One row of MRI images are shown of 10 non-alcoholics. In each, superimposed in bright color are the areas showing significant BOLD effect during cue induced ethanol at (T1). Notice activations are more strongly illustrated post-treatment.

**Figure 13.** Significantly activated voxels during cue induced ethanol of 10 non-patients after three weeks (T1). Thresholds for significance: $p=.05$. Images are shown at integrated projections through sagittal, coronal, and transverse views of the brain. Arrow shows the area of the right amygdala where once again no activation is found.
7.3 **GROUP COMPARISON**

7.3.1 **BOLD EFFECT IN GROUPS DURING BOTH CONDITIONS (TIME POINT 1)**

As can be seen in this table a group comparison of healthy controls and alcoholic patients showed small activation in the region of the amygdala and the cerebellum along with small activation areas in the superior and medial temporal lobe during cue-induced ethanol craving. A similar group comparison showed only medial frontal gyrus and the paracentral lobule activation during neutral odor.

<table>
<thead>
<tr>
<th>Region</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>max. SPM[Z]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol Amydala</td>
<td>R</td>
<td>28</td>
<td>0</td>
<td>-24</td>
<td>1.94</td>
<td>1</td>
</tr>
<tr>
<td>Sup. temporal gyrus</td>
<td>L</td>
<td>-56</td>
<td>12</td>
<td>-20</td>
<td>3.2</td>
<td>4</td>
</tr>
<tr>
<td>Med. temporal gyrus</td>
<td>L</td>
<td>-40</td>
<td>-4</td>
<td>-20</td>
<td>2.5</td>
<td>7</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>L</td>
<td>-40</td>
<td>-56</td>
<td>-24</td>
<td>2.8</td>
<td>14</td>
</tr>
<tr>
<td>Med. temporal gyrus</td>
<td>L</td>
<td>-56</td>
<td>12</td>
<td>-20</td>
<td>3.2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>12</td>
<td>-72</td>
<td>-16</td>
<td>3.5</td>
<td>22</td>
</tr>
<tr>
<td>Neutral Paracentral lobe</td>
<td>L</td>
<td>-16</td>
<td>-28</td>
<td>44</td>
<td>3.0</td>
<td>10</td>
</tr>
<tr>
<td>Medial frontal gyrus</td>
<td>L</td>
<td>-8</td>
<td>16</td>
<td>48</td>
<td>2.8</td>
<td>32</td>
</tr>
</tbody>
</table>

**Table 3.** Tabular data are presented on “significant” regions for group comparisons of patients and controls (activation maps of the differences: patients minus comparison subjects) at T0 that correlated with ethanol odor stimulation and neutral room air stimulation. (p < 0.01 corrected for the volume of the SPM [Z]). The location on the maximal voxel in each region is given with the size of the regions N and the peak Z score.
7.3.2 Activation Areas in Both Groups during Ethanol and Neutral Air (Time Point 2)

Ethanol induced craving at posttreatment no longer produced BOLD effect in the amygdala or cerebellum in the alcoholic patients. A BOLD effect was still shown in the superior temporal gyrus similar to pretreatment. Other activation regions were detected in the occipital cortex and the insula. Regions that were activated during the neutral room air were the superior temporal gyrus located more anteriorly, the occipital cortex, the frontal gyrus, the precentral gyrus and cingulate gyrus.

Ethanol induced craving in the non-patient sample showed significant increase in the extent and intensity of activation in the superior temporal lobe (activations showed increased extent and intensity) (figure 12 and 13). Other activation areas were found in the posterior cingulate. Regional activations during neutral air were demonstrated only in the postcentral gyrus.

<table>
<thead>
<tr>
<th>Region</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>max. SPM[Z]</th>
<th>N</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>max. SPM[Z]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol Sup. temporal gyrus</td>
<td>L</td>
<td>-48</td>
<td>-4</td>
<td>4</td>
<td>4.5</td>
<td>25</td>
<td>L</td>
<td>-68</td>
<td>-24</td>
<td>8</td>
<td>5.9</td>
<td>86</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>R</td>
<td>60</td>
<td>-24</td>
<td>8</td>
<td>5.6</td>
<td>69</td>
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<td></td>
<td></td>
<td></td>
<td>R</td>
<td>60</td>
<td>4</td>
<td>0</td>
<td>5.6</td>
<td>58</td>
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<td>17</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L</td>
<td>-48</td>
<td>12</td>
<td>-16</td>
<td>2.7</td>
<td>5</td>
</tr>
<tr>
<td>Posterior Cingulate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L</td>
<td>4</td>
<td>-40</td>
<td>4</td>
<td>4.9</td>
<td>38</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>R</td>
<td>20</td>
<td>-80</td>
<td>4</td>
<td>3.3</td>
<td>40</td>
<td>L</td>
<td>4</td>
<td>-40</td>
<td>4</td>
<td>3.3</td>
<td>40</td>
</tr>
<tr>
<td>Insula</td>
<td>R</td>
<td>40</td>
<td>-4</td>
<td>4</td>
<td>3.3</td>
<td>40</td>
<td>L</td>
<td>4</td>
<td>-40</td>
<td>4</td>
<td>3.3</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 4. Tabular data are presented on “significant” regions for group comparisons of patients and non-patients at T1 following three weeks of behavior therapy with pharmacological intervention (patients only) that correlated with cue induced ethanol odor and neutral air stimulation. (p < 0.01 corrected for the volume of the SPM [Z]). The location on the maximal voxel in each region is given with the size of the regions N and the peak Z score.
7.3.3 **BOLD Effect in Group Comparison During Ethanol/Neutral Air (Time Point 2)**

An analysis of activation maps of the differences between groups (patients minus comparison subjects), demonstrated a left-lateralized activation in the parietal and occipital cortex and a greater left-sided temporal activation in the patient group compared to the non-patient group. Furthermore, activation in right insula, left cerebellum and left lenticular nucleus were found primarily in the alcoholic patients compared to the healthy controls. Activation during neutral room air was shown in the right medial frontal gyrus and the right superior temporal gyrus in alcoholic patients compared to normal controls.

<table>
<thead>
<tr>
<th>Region</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>max. SPM[Z]</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial temporal gyrus</td>
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<td>36</td>
<td>-48</td>
<td>8</td>
<td>3.3</td>
<td>56</td>
</tr>
<tr>
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<td>-48</td>
<td>4</td>
<td>8</td>
<td>3.0</td>
<td>14</td>
</tr>
<tr>
<td>Temporal gyrus</td>
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<td>28</td>
<td>-28</td>
<td>12</td>
<td>2.5</td>
<td>3</td>
</tr>
<tr>
<td>Insula</td>
<td>R</td>
<td>40</td>
<td>-4</td>
<td>4</td>
<td>3.5</td>
<td>7</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>L</td>
<td>-36</td>
<td>-40</td>
<td>44</td>
<td>2.5</td>
<td>4</td>
</tr>
<tr>
<td>N. lenticularis</td>
<td>L</td>
<td>-28</td>
<td>-8</td>
<td>16</td>
<td>2.7</td>
<td>14</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>L</td>
<td>-32</td>
<td>-76</td>
<td>8</td>
<td>2.1</td>
<td>4</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>L</td>
<td>-4</td>
<td>-68</td>
<td>-12</td>
<td>2.6</td>
<td>3</td>
</tr>
<tr>
<td>Neutral</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Medial frontal gyrus</td>
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<td>20</td>
<td>28</td>
<td>40</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>Sup. temporal gyrus</td>
<td>R</td>
<td>48</td>
<td>4</td>
<td>-8</td>
<td>2.4</td>
<td>6</td>
</tr>
</tbody>
</table>

**Table 5. Mean Activated Regions for Group Comparisons (Patients minus Non-Patients) During Ethanol and Neutral Room Air at T1). (p < 0.01 Corrected for the Volume of the SPM [Z]). The Location on the Maximal Voxel in Each Region is Given with the Size of the Regions N and the Peak Z Score**
8 DISCUSSION

8.1 MEASUREMENT (TIME POINT 1)

8.1.1 AMYGDALA

The first research direction of this thesis, was aimed at exploring the neurobiological correlates of craving. Investigating the neural substrates of craving in the more wider context of emotion may help us understand abnormal emotional states. The study confirmed the hypothesis that cue-induced ethanol craving arises from amygdala dysfunction (increased activation) in detoxified alcoholic patients. The increased BOLD effect in the amygdala was associated with increased self-report of craving (CR). Studies with other drugs have supported our hypothesis. Their results have concluded amygdala activation to cues of cocaine (Childress et al., 1999; Volkow et al., 1999) and nicotine (Due at al., 2000). This finding suggests that: first, the subjective experience of craving is mediated by the amygdala and is one of the driving forces motivating continued drug seeking and drug use; and second, that the experience of emotion that motivates continued drug use is also dependent on the interaction between environmental stimuli with affective value and internal variables. This finding is consistent with the hypothesis that the amygdala is especially important in emotion and motivation, being that it is a region that learns associations between secondary reinforcers and primary reinforcers (Everitt and Robbins, 1992).

To account for craving, a number of models were proposed: one proposition is that positive incentives account for craving effects (Stewart et al., 1994). Conditioned stimuli should activate (prime) brain areas associated with positive appetitive states. Thus, patients should be primed to associate alcohol cues with the pleasurable experience of alcohol ingestion (e.g., euphoria, disinhibition, anxiety reduction, sedation and hypnosis). A number of factors have been suggested to contribute to the priming effect. For example, drug seeking may be directly linked to the rewarding properties of a drug. Another factor that may contribute to priming effect is arousal (negative affect), which was measured by increased locomotor activity. Studies have found that the amygdala contributes to an increased level of arousal and attention which provides optimal conditions for sensory processing by lowering detection thresholds for environmental stimuli and enhance neural responsiveness of the amygdala to such stimuli (Kapp et al., 1979). Furthermore, recent studies have suggested that emotional arousal, mediated by the amygdala, can facilitate explicit memory (Cahill et al., 1995) thereby linking environmental cues with drug craving.
On the other hand, Solomon (1980) hypothesized that craving results from opponent processes in the brain. Briefly, anticipation of a drug about to be administered results in conditioned positive association of the drug effect, followed by a slower build-up of a negative conditioned process. This has the opposite qualities of the drug and is usually aversive, which results in craving characterized by anxiety and dysphoria when the drug is not directly taken. Anxyolitic compounds acting as 5-HT receptor subtype agonist were shown to produce anxyolitic effects after local infusion into the amygdala (Costall et al., 1989). Such infusions also can block some signs of withdrawal following administration of ethanol (Costall et al., 1990). Antidepressants were shown to reduce depressive symptoms and to decrease alcohol consumption among alcoholic patients (Naranjo et al., 1995). Serotonergic dysfunction in alcoholism and symptoms of depression during withdrawal were supported using PET (Heinz et al., 1998).

Alternatively, 5-HT has been suggested to be involved in the process of craving more along the lines of obsessive compulsive disorder (OCD). Some aspects of craving were linked to OCD (Modell et al., 1992a,b; Anton et al., 1996). For example, alcoholics may experience recurrent and irresistible thoughts about alcohol during recovery and in various stages during later recovery. These ideation are a hallmark of OCD. Indeed, it is believed that the loss of control over drug-taking is an important factor in the maintenance of addictive syndrome (Koob et al., 1998).

8.1.2 SELF-RATINGS OF MOOD

Variations in general mood states may serve to modulate craving. For example, Baker et al. (1987) proposed that craving may result from negative affect (withdrawal-based urges) or from positive affect (incentive-based urges). Many studies employing correlation designs have now documented that fluctuations in mood states often co-vary with fluctuations in craving (Sherman et al., 1986). However, Robbins et al. (2000) using the Profile of Mood States (POMS) questionnaire found that mood states at the start of a cue induction craving session were associated with higher levels of self-report craving, before and after cue exposure. This would suggest that negative mood states may contribute to craving apart from cues. Cooney et al. (1997) proposed that negative mood and drug cues summate to determine an individual’s overall level of drug desire. However, the PANAS ratings, although showing higher positive and negative affect for ethanol compared to neutral room air in the patient group before intervention, the differences were not significant, which does not
support the hypothesis of a relationship between mood and craving. Therefore, a mood effect on ratings for craving cannot be confirmed by the findings of this investigation.

8.2 OTHER AREAS INVOLVED IN CRAVING

While the contribution of the amygdala is undeniable in cue induced craving, it does not imply that craving can be related to only this brain structure. The amygdala and its connection almost certainly constitute the involvement of other systems in craving processes. This proposition is underlined by brain activity similarly evoked in the amygdala-hippocampus and cerebellum area which were not demonstrated during neutral room air. Additionally, we have activation in superior temporal gyrus in both groups for both types of stimulation.

8.2.1 HIPPOCAMPUS

The amygdala has been implicated in emotional learning by encoding new information. There are a variety of data that pertain directly to this issue. On the other hand, the reciprocal connections between the amygdala and the hippocampal formation may serve to link affective response patterns with encoding of perceptions in memory, thus providing rapid access to appropriate motivational states when situations arise or particular individuals are re-encountered (Kling and Brothers, 1992). Possibly, the amygdala assigns internal relevance of the cue motivating drug seeking behavior, and the hippocampus encodes these cues and selects intentions to these stimuli.

8.2.2 CEREBELLUM

Luciani (1891) and Ferrier and Turner (1893) following investigations in monkeys, along with influential figures such as Babinski (1899) who used clinical reports firmly established the cerebellum as an area of primarily motor control. However, this conclusion seems to have been rather premature. In the past few years, clinical and experimental data point to the role of the cerebellum in the modulation of affect and emotional expression, along with cognitive dimensions of affect. For example, the cerebellum has been associated with arousal (Manzoni et al., 1967) and autonomic function (Xu and Frazier 1997).

Along similar lines, functional imaging studies revealed cerebellar involvement in autonomic behaviors such as hunger and satiation (Tataranni et al., 1999) and thirst (Parsons et al., 2000) and satiation. Reiman et al. (1989) demonstrated cerebellum activation using PET in patients prone to panic disorders by lactate induced panic. In a later experiment using fMRI cerebellum (Reiman et
al., 1997) activation was found during the viewing of disgust generating film clips. Lane (1997) and Beauregard et al. (1998) demonstrated cerebellar activation during induced sadness. Of more significant in the context of craving is the connection between the cingulate gyrus and cerebellum. The cingulate has been suggested to be involved in motivation and goal directed behavior (Devinsky et al., 1995). This may provide one possible linkage between the cerebellum and craving. Alternatively, the neural substrates of reward have long been hypothesized to involve the locus coeruleus (norepinephrine), and the ventral tegmental area (dopamine), which contain efferents to the cerebellum through cerebral cortical input. The question remains to what extent, if any, do these efferents influence the cerebellum in compulsive drug use. Perhaps the cerebellum’s newly defined role in affect, suggests that it is involved in alcohol induced craving in this context. Support for this assumption is provided by having found no activation in this area posttreatment.

While cerebellar functions are being increasingly described in affect, no role has yet been suggested for the cerebellum in olfaction per se. Recently, Sobel et al. (1998), using fMRI, demonstrated that the cerebellum is involved in olfaction in humans. The authors hypothesized that the cerebellar role in olfaction is to monitor sensory input (odor concentration) and modulate the motor output (sniff volume). However, as noted, the fact that no cerebellar activation was found posttreatment, suggests that it was not involved in motor output here.

8.2.3 SUPERIOR TEMPORAL CORTEX

Another area that has more recently been identified in human olfaction is the temporal lobe (Eichenbaum et al., 1983). Lesions to these areas impaired performance in various olfactory tasks. Hence, the bilateral superior temporal cortex activity that was observed during ethanol stimulation in both healthy controls and alcohol-dependent persons, and the slightly smaller activation in the superior temporal gyrus during stimulation with neutral room air in the control group may be related to olfactory stimulation.

Correspondingly, magnetoencephalographic recordings have shown bilateral superior temporal cortex activation following olfactory stimulation (Kettenmann et al., 1996). Observations in macaque monkeys demonstrated a connectivity between the superior temporal cortex and the inferior orbitofrontal cortex, and with the latter linked to the primary olfactory cortex (Carmichael and Price, 1996). Orbitofrontal cortex participation that is generally observed in studies of olfaction was not found in this experiment (Heinz et al., 1998). Susceptibility related signal loss in the inferior frontal area or a habituation effect due to the relatively long stimulation periods (100 s) are
two possible factors that may account for the failure to find any activation in this region. Odorant induced activation may vary with olfactory stimulation parameters. For example rapid habituation was demonstrated after 30-40s in the primary olfactory cortex during continued olfactory stimulation (Sobel et al., 2000), while the orbitofrontal gyrus was continuously activated. However, our stimulation parameters were not designed with the intent of eliciting orbitofrontal activation.

8.3 Measurement (Time Point 2)

The final element in studying the neurobiological substrates of craving was to investigate whether regional abnormalities are static or dynamic. Here, we examined whether a standardized behavioral therapy with antidepressants resulted in BOLD effect changes. The findings disclosed a pattern of brain activity during ethanol stimulation, when patients were no longer in the phase of early abstinence more similar to the non-patient sample. Posttreatment measurements disclosed similar regional activations in the left superior temporal sulcus, an increased right insula, and to a lesser extent, right occipital cortex activation. Although visual stimulation was not included in this experiment, a state of increased attention may have produced the increased activation in the patient sample. A clinical response was reflected in the amygdala and cerebellum normalization.

8.3.1 Superior Temporal Cortex

It was explained above that brain activation in the superior temporal lobe reveal that this neocortical area is involved in olfactory processing. Posttreatment superior temporal cortex activation in the control group corresponded to the first measurements, albeit with increased intensity and extent. This response may reflect sensitization or recognition effect. Earlier evidence from fMRI studies showed an overall heightened activation in the superomedial temporal gyrus (Yousem et al., 1997) with repeated testing within one week of odorant stimulation of the trigeminal nerve. Odorants that stimulate the trigeminal nerve may thus subject an individual to enhanced irritation, danger, or discomfort compared to odorants that do not stimulate the trigeminal nerve. One might theorize that this evolutionary adaptation may trigger and incorporate additional cortical resources. The alcohol concentration used in this odorant stimulus was seemingly high. Although the threshold for nasal pungency for ethanol is high compared to other, longer chained alcohols (log of nasal pungency threshold=3.9; Cometto-Muniz and Cain, 1990), nasal irritation was nevertheless experienced. The nasal pungency threshold was determined in anosmic patients, rendering it free from an olfactory component. Hence, it appears that the larger activation at the second time point was brought on by
the irritating quality of the stimulation used in this investigation. Alternatively, pre-exposing subjects to the stimulus at time point 1 may have lead to recognition of the ethanol odor. Previous stimulus exposure may have thus evoked activation in neighboring areas involved in memory as well as olfactory processing. On the other hand a similar heightened activation was not shown in patients. One explanation may be that ethanol stimuli produce different cognitive associations with different modes of processing in patients. Also, the stimuli may have led to different attention processes with differential activation patterns in patients and non-patients. Some variations in activation between subject groups and measurement time points were noticed during neutral air stimulation. This is probably due to the expectations that arise from an odorant stimulus which is perceived, but which is difficult to categorize.

8.3.2 INSULAR CORTEX
Activation in the insula, an area which is strongly associated to the limbic system and is functionally relevant in visceral function, olfaction, taste, and emotion, may represent a more cortical influence in the processing of alcohol related cues. It may also represent a lasting correlate of craving, as was reported earlier. The fact that only the right insular activation was found during craving in patients, may reflect greater participation of the right hemisphere which is more involved in emotional processes, compared to the left hemisphere which is more associated in non-affect processes.

8.3.3 POSTTREATMENT ACTIVATION IN THE AMYGDALA AND CEREBELLUM
The results obtained from the alcoholic patients indicate that complete abolished ‘alcoholic depravation effect’ requires normalization of the amygdala and cerebellum sites. This theory is supported by the correlation of craving improvement with both area normalization. The finding supports the hypothesis that craving is a dynamic state since there is change with treatment.

In reviewing the literature over trait/state characteristics in craving, it became pretty clear early on that there are only very few studies published to this effect, and non cover functional imaging techniques. Published studies report that acamprosate completely abolished the ‘alcoholic depravation effect’ in a long term alcohol drinking model in rats (Sinclair and Li, 1989). It also suppressed craving in humans. It is proposed that the acamprosate may reduce craving that is associated with conditioned withdrawal (Littleton, 1995). Similar successful effects were seen using naltrexone which is suggested to mediate the endogenous opioid system (locus coeruleus) (Herz,1997). These findings suggest possible recovery from craving.
Just as studies using imaging techniques to examine pharmacotherapy effects on craving are lacking in the literature, investigation of the effects of behavioral therapy in alcohol craving using imaging techniques are in essence, also, nonexistent. A number of procedures employed to treat craving include extinction and coping stress. Other procedures include cue exposure for reducing craving for alcohol, with very promising results. Blakey and Baker (1980) used a repeated series of *in vivo* exposure trials. Hodgson and Rankin et al. (1983) used a priming dose of alcohol, which according to some authors is better suited for controlling the amount of alcohol patients learn to consume rather than as a procedure of learned abstinence. Some success was achieved in a number of cases in those with patients reporting no desire to drink in follow up periods.

Whether the change in activation pattern in our patient group is due to the effect of the pharmacological or psychological intervention or of abstinence itself (time factor) cannot be clearly defined. It may very well be a combination of these factors.
9 BIOFEEDBACK

The association between behavioral variables and illness has been known for many years now. Biofeedback acknowledges the relationship between behavioral variables and illness, emphasizing that behavior can be used to alleviate illness.

9.1 DEFINITION

‘Biofeedback’ is a recently coined term that refers to a group of experimental procedures in which an external sensor is used to provide the organism with an indication of the state of bodily processes, usually in an attempt to effect a change in the measured quantity (Schwartz and Beatty, 1977). The philosophy of biofeedback training is based on the psychophysiological principles of Elmer Green (1977) and his associates, the early biofeedback investigators at Minninger Foundation in Kansas. It proposes that every change that occurs in a physiological state is accompanied by a change in the mental and emotional state, whether conscious or unconscious, and vice versa.

9.1.1 OPERANT CONDITIONING

Biofeedback is a term that is employed to describe procedures of training subjects to control responses governed by the autonomic nervous system (AS responses) and responses of the central nervous system (CNS). The general idea behind biofeedback has not changed much since its conception. Basically, that ordinarily uncontrollable or autonomic bodily processes may be susceptible to modification to the individual regarding their occurrence. This information may be delivered in the form of reinforcement as a form of learning. Or this information may be delivered to a person along with the explicit instructions both regarding what it signifies and what the person should try to achieve in the way of modification or control, in which case it is self-regulation. In this respect, it is a form of learning, differing from other forms mainly in the type of responses that are controlled. Historically biofeedback grew out of the behavioristic traditions of operant or instrumental learning paradigms (Kimmel 1967; Miller, 1969). Operant learning theory focuses on three main elements: 1. Discriminative stimuli (SD’s) 2. Responses and 3. Reinforcers. The basic principle of operant conditioning can be stated quite simply. The probability of an operant response is increased when a reinforcing stimulus follows that response.

Perhaps biofeedback is best understood on hand of a couple of examples: in the first example, subjects were trained to control their palmer skin response. Subjects were given a reward each time
the response occurred. The participants were told that the purpose of the experiment was to study the effectiveness of various devices for measuring thought processes and their task was to think about an emotional experience. The subjects were instructed that each time the apparatus detected an emotional thought they would hear a tone and earn a sum of money as bonus. As a result of the reinforced contingency, subjects showed increased response rate (Shapiro et al., 1964).

In another example, human subjects were trained to change their occipital alpha activity electroencephalograph (EEG) (baseline 20% of the time alpha activity). Subjects were instructed to sit quietly during white noise presentations, and to produce alpha activity in the absence of white noise. Also, they were informed that when white noise is absent, tone is presented when the required pattern of brain electrical activity is successfully produced. Following training, alpha activity was presented only 20% of the time in the presence of white noise, and 80% in the absence of white noise (Black et al., 1977).

In the first example, the reinforcer was the tone of a monetary bonus and the reinforced response was palmar skin potential. In example 2, the reinforcer was the tone and the response was a high alpha EEG activity. In both cases the tone was a reinforcer as a consequence of the particular instructions that were given. The response is reinforced in the presence of the SD. In the first example, the response was reinforced once the apparatus detected an emotional thought. In the second example, the response was reinforced in the absence of white noise.

The power of the biofeedback methods lies in its preciseness, the focus being on specific responses and its potential to bring these under specific control. Still, little is known about the mechanisms underlying the principles of operant conditioning. However, the emphasis appears to lie on external forces, reinforcers and environment that shape behavior and physiology, paying attention to reinforcement and motivation.

9.1.2 INSTRUMENTATION

The instrumentation used in biofeedback continuously monitors the psychophysiological changes within the individual. A neutral physiological signal from the body is amplified by biofeedback instruments and ‘fed back’ to the subject through a sensory modality. In order for training to be successful, the physiological function to be controlled must be continuously monitored, the signal immediately fed back. As a training procedure, biofeedback can be used in the medical setting. On
the other hand, as a scientific tool, it can be used to test hypotheses and investigate self-regulation. When used in this fashion, biofeedback can be in the fore- and backfront of many other disciplines, used by itself, or immersed in other approaches. While at times it is used literally to reeducate a physiological system (e.g., neuromasculator reeducation), at other times it is used to illustrate a principle (e.g., different emotional states correspond to different brain activations). There are however some problems encountered in practice. For example, there are technical difficulties in monitoring many of the psychophysiological processes that one would like to control. Somewhat more complicated is the problem that not all physiological systems are equally responsive to biofeedback intervention.

9.1.3 BIOFEEDBACK APPLICABILITY
Interest in biofeedback is stimulated in part by its potential contributions to clinical research and therapy. For example, it can be used for investigation of the etiology and mechanisms underlying specific disorders. This function should be distinguished from the application of biofeedback as a treatment modality for producing clinical correction on physiological dysfunction.

As a treatment modality, the medical illness to which biofeedback has been applied are extensive (Fotopoulus and Sunderlan, 1978). They include some that are commonly associated with stress—tension and migraine headaches, ulcers, and asthma. They also include some that arise from obvious organic injury, such as paralysis following cerebral strokes or damage to motor nerves. Additionally, biofeedback has been applied to other illnesses such as depression, and to more serious mental disorders such as schizophrenia (Schneider et al., 1992a,b respectively). It has also been applied in alcoholism (Schneider et al., 1993). Although biofeedback’s success in the clinical place is now well documented, the field is by no means closed. Indeed, success pose many questions as they answer and challenge some fundamental ideas about the nature of an ‘illness’ and the definition of a ‘cure’ (Olton and Noonberg, 1980).

9.1.4 LIMITATION OF BIOFEEDBACK
One of the practical concern in the evaluation of biofeedback as a clinical tool in treatment is patients motivation. Several articles have commented on the importance of patient motivation in biofeedback programs. Motivation has a significant effect on performance: it is well established that with increases in levels of motivation, performance increases. An obvious case can be made for a lack in performance with little or no motivation. One good example was well documented by Surwit
(1973) in which he reported that a patient who had made a long trip to receive biofeedback training made no progress as a result of boredom perceived during the training sessions.

An issue closely related to motivation and equally as important is transfer of training. The aim of biofeedback is to train individuals to control their mental and bodily functions in the absence of instrument assistance. In other words, biofeedback training is the instructed transfer of control of mental and bodily functions to visual or auditory surveillance tasks in the absence of concurrent instrumental feedback. Such transfer would depend on unspecified internal processes. Successful transfer would indicate that the observer had acquired a means of regulating ‘mental and bodily functions’ and that the instrumental contingency feedback were no longer necessary (Beatty and O’Hanlon 1979). It is often all too easy to overlook the fact that learning techniques cannot be administered the way most medical treatment can.

9.2 **Biofeedback and Emotion**

Can biofeedback be embedded in a larger context—namely that of emotion? According to the American Medical Association, reference was made at one point that the brain is divided into a conscious and unconscious domain. The conscious domain contains both the cerebral cortex and the craniospinal nervous system, roughly the voluntary muscular system. The unconscious side includes both the subcortical brain, the ‘old’ lower brain structures that man shares with most animals, and the autonomic nervous system, the involuntary nervous system, which lies outside of the brain and brain stem, and which controls among other things, the skin, the internal organs and glands, and the vascular system of the body. The paleocortex, the old brain, includes the limbic system, which has been given a name “visceral brain” coined by MacLean (1970), due to its particular great significance for understanding psychosomatic self-regulation. It is quite clear from research that electrical stimulation of the visceral brain and related neuronal structures through implanted electrodes causes emotional changes in humans. Conversely, it is well known that perceptual and emotional changes are followed by neural changes, or responses, in the limbic system of the brain. If one wants to manipulate emotions one would have to extend conscious control to the unconscious domain.

However, this has been a difficult feat so far in biofeedback studies. One important component of biofeedback is the monitoring and feeding back of biological information from the body in real-time. The whole area of emotion, therefore could be explored through on-line feedback of neural
substrates of emotion. The major problem with present biofeedback technology is not that they cannot be used to study emotion, but rather that they cannot monitor these substrates and they are invasive. A non-invasive technique of real-time fMRI was developed in the current study that allows for on-line monitoring of emotional areas of the brain.

9.3 Emotion and Imaging

When considering brain mechanisms involved in emotion, human brain imaging approaches using mood induction have become pretty common place. A combination of cortical and limbic increases and decreases in regional cerebral blood flow are found following induction of transient sadness. Among them, the amygdala is thought to play a special role in modulating and generating emotional reactions due to its functional relevance in assigning significance to external stimuli and events. Amygdala activation was shown with the processing of especially negative emotional material, i.e. with the presentation of fearful faces with PET (Morris et al., 1996) and fMRI (Irwin et al., 1996), but also sad faces using PET (Blair et al., 1999) and in the context of emotional learning (Breiter et al., 1996; Büchel et al., 1998; LaBar et al., 1998). Its participation was also demonstrated during the subjective experience of emotion, i.e. during states of self-induced (Schneider et al., 1995, 1997, 1998) or externally induced negative mood (Ketter et al., 1996). However, a number of other mood induction studies applying various mood induction techniques could not find any amygdala activation. Evidence for primary participation of the inferior and orbitofrontal cortex (Pardo et al., 1993), the anterior cingulate, medial prefrontal, and mesial temporal cortex, as well as for the brainstem, thalamus, caudatum, putamen (George et al., 1995) was yielded in former PET studies. Recent experiments consistently found an anterior cingulate cortex and prefrontal cortex involvement (Mayberg et al., 1999; Teasdale et al., 1999).

The role of the amygdala is also elucidated in disorders with affective dysfunctions, for example schizophrenia or depression. In depressed patients, greater blood flow was shown in the left amygdala (Drevets and Raichle, 1992). In such patients a correlation emerged between resting regional cerebral metabolic rate in the right amygdala and the dispositional negative affect (Abercrombie et al., 1998). Schneider et al. (1998) described a lack of amygdala activation during sad mood induction in male schizophrenic patients compared to matched healthy controls although a similar subjective experience of sadness was reported. Phobic patients subjected to conditioned
aversive stimuli associated with negative odor showed increased activation in the amygdala and hippocampus which was opposite to that of healthy subjects (Schneider et al., 1999).

In general, the exact pattern of response appears to be highly dependent on the provocation strategies used to elicit the mood states. Lane et al. (1997) found, for example, different regional rCBF changes due to film (amygdala) or recall induced (anterior insula) mood. Provocation strategies would include 1. experience of certain emotional state with the help of emotional material (e.g., text, music, films), 2. presentation of emotional material without the explicit instruction that the experience of a special emotional state is required of the subjects, 3. free recall of personal events, 4. feedback of success and failure leading to satisfaction and frustration, respectively 5, experimental physiological manipulation (e.g., manipulation of facial expressions) (Schneider and Weiss, 1998). However, despite technical differences among studies, limbic and frontal regions have consistently been identified in the published literature, with much emphasis placed on the amygdala.

9.3.1 LATERALITY

There is ongoing discussion pertaining to hemispheric asymmetry in the processing of emotion, favoring a right hemisphere dominance (Adolphs et al., 1996; Davidson 1993). The hypothesis on emotional valence proposes a greater right hemisphere involvement for negative emotional stimuli, whereas a greater left involvement is associated with material of positive affect (Heller, 1990). Along similar lines, Tucker (1981) suggested that the right hemisphere is associated with negative moods, and left hemisphere is associated with positive moods. However, Schneider et al. (1995, 1997) reported left amygdala activation in sad mood experience. Similar results were obtained in a series of other investigations designed to study emotion. (Breiter et al., 1996; Morris et al., 1996). A right hemispheric dominance for emotional expression has been deduced, for example, from the asymmetry found in emotional facial expressions, especially for negative emotions, expressed more intensively on the left side (Asthana and Mandal, 1998; Borod et al., 1988). Furthermore, sad mood induction was associated with declarative left visual field processing (right hemisphere) (Lavadas et al., 1984). Hemispheric asymmetries have been shown in the processing of sad vs. happy facial expressions, with a right hemisphere advantage in the processing speed for sad expressions (Moretti et al., 1996). In general, studies of facial and verbal emotion perception have provided evidence for the right hemisphere dominance, as well as for valence related asymmetries. However, some results contradict predictions of the valence hypothesis. For example, it was shown that the selective
presentation of emotional film clips to the right hemisphere (with contact lenses) did not produce greater negative ratings (Otto and Yeo, 1993). Experiments of dichotic listening also did not support the assumption of the right hemisphere dominance for emotional material, and they fail to do so for the valence hypothesis (Bulman-Fleming and Bryden, 1994; Erhan et al., 1998). In such studies, as noted earlier variables of the emotional task requirement (perception of emotion, facial expression, emotional experience) as well as the method for investigating cerebral asymmetries (dichotic listening, contact lenses, tachistoscopic procedures) exert influence on outcome, and may thus lend some explanation to the different results (Schneider et al., 2000).
10 HYPOTHESIS AND RESEARCH QUESTION

The previously described investigation (study 1) focused on neurobiological correlates of craving. The hypothesis holds that the amygdala activation to cue induced craving may be a response characteristic of negative affect and anxiety. This response may of course come about as a result of dysfunctional neurobiological processes in the dynamic commerce with the external environment. Such commerce was proposed by Schwartz (1975). A major reason for the failure to recognize and appreciate the self-regulation of brain process is that the very brain processes involved in functional disorders are normally unobservable and not available to direct conscious experience. The development of fMRI using real-time *in-vivo* imaging for online observation of brain activation, serves the important function of bringing these unconscious neural processes directly into human awareness. This makes it possible to now manipulate brain processes differentially as a method for exploring the functional relationship of human experience and behavior, with possible treatment. Self-regulation of brain activation using BF (biofeedback) in real-time provides the basis for the next hypothesis: using this procedure subjects may be trained to learn to voluntary control their brain activity. The goal of the study was to control the neural substrate for sadness. Mood induction experiments in healthy subjects have shown successful involvement of the amygdala during sadness (Schneider et al., 1995, 1997). Thus, it was hypothesized to find change in amygdala activation following BF training using real time fMR imaging with the mood induction of sadness.

The following research questions are addressed in this experiment

- Success of BF training should be reflected in increased amygdala activation.

- Successful BF training should be reflected in increased and continuous amygdala activation with each advancing training session.

- Subjective ratings of sad mood state should correlate with amygdala activation.

Postexperimental interviews with the subjects that are expected to perform well are proposed to report feelings of sadness.
11 METHOD

11.1 SUBJECTS

The subjects were 6 healthy controls (one subject performed only two sessions due to illness), male, German speakers, and right handed. Mean age ± S.D. was = 27.58 ± 4.56 years (range 18-45). Inclusion criteria was similar to the inclusion criteria for controls of the preceding study but with willingness to participate in the research project that comprised three measurements. Control subjects were recruited by notices or word of mouth. Volunteers accepted appointments for an initial evaluation session which included screening for eligibility, and then underwent the information consent procedure. Volunteers who were accepted and agreed to participate were oriented to the testing procedures. Participants who completed the research project, received DM 25/- per hour.

Initial screening for potential safety hazard and medical, neurological, and psychiatric history was done by questionnaires which subjects had to complete upon arrival for fMRI examinations. Safety risks were the same as for study one.

Also verification of normal sense of sight was required.

Subjects entered the study sometime during May 1999 to October 2000. Subjects were measured at three time points with a one week interval between measurements.

11.2 VARIABLES

11.2.1 INDEPENDENT VARIABLES

The independent variable was fMRI measurement. Three imaging sessions took place: each measurement was separated by one week.

11.2.2 DEPENDENT VARIABLES

11.2.2.1 BOLD contrast

FMRI imaging was performed on a 1.5T imager (Magnetom Vision, Siemens). High resolution MRI was performed to localize the amygdala. Signal responses in the amygdala were measured using EPI with ECG-gating (TR 3 s, 1 slice, voxel size 6x6x6 mm$^3$, flip angle 30°). Data analysis was performed with "sliding-window" correlation analysis in real-time (Gembris et al., 2000), using Functional Imaging in REal time (FIRE) processing package. Correlation was performed cumulatively over 63 scans. Real time 2D-motion correction was applied.
11.2.2.2 SELF-Rating
Subjects were assessed for subjective experiences of sadness on a 5-point unipolar intensity scale using a two way intercom system. Subjects were subsequently given a short interview over the strategy that they had used to experience sadness.

11.3 PROCEDURE
Following initial screening, written informed consent of experiment participation was obtained. Subjects were positioned supine head first on the examination table. To restrict motion and allow for subject comfort, a head stabilizer was used and padding was present. Earplugs were provided to attenuate the repetitive gradient noise.

The mood induction trials were performed in each subject using 2 repetitions of 30 s rest and 45 s mood induction. The experiment consisted of three sessions with 10 trials per session (figure 2).
Before beginning with the session subjects were given instructions to recall personal situations or imagine events that would make them experience sadness (Schneider et al., 1994). A mirror mounted above the subject allowed uninterrupted viewing of one slice of subject’s corresponding anatomical brain projected to a screen close to the subject. The slice showed - among others - the best possible regional depiction of the amygdala (figure 1). Real time correlation maps allowed monitoring of amygdala activation. The region of the amygdala was identified to the subject before training began. Success and failure was demonstrated by the extent of amygdala activation or lack of it during each trial.

Following each trial, subjective experience of sadness was recorded for each of the subjects on a five point rating scale. In addition to biofeedback, after every session and following each trial, feedback of success or failure of amygdala activation was also given verbally by the experimenter using a two way communication system. This was possible since the activation maps were also monitored by the experimenter on a laptop outside of the scanner room.

**Figure 2.** Experimental design of on-line biofeedback training of amygdala activation during induced sadness depicting the biofeedback training session consisting of 10 trials.
11.4 Measurements (Time Point 2 and 3)

The subjects were measured again during two consecutive weeks using the same magnetic resonance imaging protocol, and the same experimental design. Overall time in the imager was approximately 60 minutes per subject for each session.

11.5 Data Analyses

11.5.1 Real-Time Data Analysis

The scanner was linked to an external SUN Ultra Sparc 100 Workstation operating at 300 MHz for real-time data analysis. From the host computer images were sent to the external workstation where images were uncompressed, rescaled and displayed.

Real time correction and “sliding-window” correlation analysis were performed within a single TR period.

A paradigm control window displayed the time course of the paradigm and the reference vector which was modeled by convolution of the paradigm with a time shifted Poisson function. The paradigm was created by graphic editing using a mouse controlled cursor. The current position within the sliding window was indicated by a moving time marker. The correlator button allowed the selection of the processing strategy which was either correlation with a fixed reference vector and first order detrending of the mean or correlation with a fixed reference vector and higher order detrending or reference vector optimization (RVO) using the graphically defined reference vector as a starting point (RVO parameters control the extent of the RVO search space (starting point, step size, number of steps))

The first 3 image data sets were excluded from correlation to avoid $T_1$ related saturation effects.

During the ongoing scan the following interactive features were available: adjustment of the correlation threshold, a snapshot tool to save correlation maps to disk and simultaneous display of spatially averaged time course data from up to 3 graphically defined regions of interest (ROI).

Within these ROI windows the minimum, the maximum and the mean signal intensities, as well as the maximum percent signal changes with respect to the mean were displayed. As a further option, averaging within these rectangular ROIs was restricted to voxels with an absolute correlation coefficient above the selected threshold.

Since this may cause the number of averaged voxels to fluctuate, image intensities were normalized to the first nondiscarded data set.
A 3D display of correlation images mapped onto interactively volume rendered high-resolution MR images was implemented using AVS visualization toolkit. Coregistration of the two data sets was performed manually.

The graphical user interface could be projected into the scanner using a custom made video display system to enable real-time self-monitoring of brain activation by the subject.

Reference vector was modeled as a linear-exponential function.

All signal changes were detected within a single activation cycle using sliding window widths with 21 time points.

11.5.2 Offline Data Analysis
Image reconstruction was performed off-line on a SunSolaris station, confirming activation patterns that were detected in real-time. Analysis was performed using SPM99 (Statistical Parametric Mapping, University College, Department of Cognitive Neurology, London).
Realignment over ten conditions of one session using the 5th image as reference was performed.
Spatial smoothing using the same voxel dimensions (6x6x6 mm³ Gaussian filter) over the 10 conditions for each subject and each session was applied.

11.5.3 Statistical Analysis (SPM99)
Following the above mentioned procedures of realignment and smoothing from 6 subjects and 63 scans while performing one task comprising 10 trials (induced sadness) at three sessions (biofeedback training), the adjusted measures were subjected to statistical analysis (descriptive analysis).

Scans were viewed for activation using the same reference vector that was applied for data collection. This was done by viewing the data by running it in FIRE and taking snapshots. Snapshots were analyzed for amygdala activation.

11.6 Subjective Data Analysis
Quantifying the session effect was derived from the ratings of the 5-point unipolar scale that was used to rate self perceived sadness of subjects. The subjective ratings of mood were analyzed for subjects for three sessions, separately. For every subject, self-ratings were averaged over the 10 trials for each measurement (sessions 1, 2 and 3).
12 RESULTS

12.1 BOLD EFFECT FOLLOWING BIOFEEDBACK TRAINING USING REAL-TIME FMRI

A descriptive analysis of the data revealed increased amygdala activation in one subject over the last sessions and trials for all the scans (figure 3), and in another subject over the second session in the last 9 trials.

**Figure 3.** Shows an example of successful biofeedback on-line training during induced sadness of one subject. The skewed lines illustrate amygdala activation before and after training.
In another subject marginal activation was observed during the second session and only for one trial. One of the participants showed increased activation in session 1 for all trials and decreased activation as the sessions progressed (session 2 and 3). No change in activation was found throughout the experiment for the remaining subjects.

12.1.1 Subjective Evaluation
Subjective ratings demonstrated an increase in mean sadness ratings of subjects over training sessions (session 1: 3.16±1.13, session 2: 3.70 ±1.32, session 3: 3.43 ±1.00)
The involvement of the amygdala in emotion has been uncovered in the tremendous acceleration in research over the last decade, and more recently with the advent in imaging technique. In the light of such evidence we have advanced the hypothesis that, by manipulation subjective affect (induction of sadness), it may be possible to gain control over the neural substrates of emotion. A considerable amount of evidence congruent with the application of this hypothesis to human subjects has been discussed in the introduction.

The results of this experiment suggest that while learning occurred in two cases, high interindividual variability between subjects makes it difficult to confirm our hypothesis. Moreover, there was no overall correlation between subjective rating and amygdala activation. There are a number of factors that may have lead to these results. One element may have been the lack of attentiveness of subjects to the trial, especially since intense practice appears necessary in this experiment. Another element may have been the length of training trials per period. Stress may have also had an affect. Studies have shown that performance becomes affected in biofeedback learning because one is actively striving. Alternatively, subjects may not have been motivated enough, since the subjects were not rewarded with a bonus. Given the interindividual variability, it may have been more appropriate to allow the participants to perform at their own rate, especially given that subjects were required to perceive sadness. The fact that subjects' reporting of increased sadness did not correlate with amygdala activation is difficult to explain. Overall, many of the problems noted here are the result of subtle qualities that may have affected the goal of the study which was to acquire the control over the neural substrates of emotion.

A number of therapeutic applications of biofeedback have produced preliminary results that are promising enough to merit attempts at replication with more rigorous control (Miller, 1974a). Considering that this is a pilot study, and looking at the preliminary results, there is a great need for careful evaluation of this most promising new application since the evidence is strong enough to justify but weak enough to require the performance of more rigorously controlled studies. Such replication is essential given the various factors noted above and the small number of subjects included in the study. The attractiveness of our hypothesis is that it is possible to subject it to rigorous experimental tests in order to improve it to the level of clinical research.
One of the future tasks for research is to try to improve our training technique of online biofeedback training by discovering more about the laws that govern the learning responses under this laboratory setting. These would include the various parameters of the training procedure. For example, we need to investigate the variables such as spacing of trials, length of training sessions, implementation of reward and effects of instructions.
14 **GENERAL DISCUSSION**

While additional experimental studies are clearly needed, the first study provides strong support for the proposed scheme of studying craving in the context of emotion. The question of whether alcohol addiction and affective disorders share a common overlapping biological basis appears to have come a step closer to being resolved. Evidence is provided to suggest that drug addiction centers on the neurobiological substrates of secondary reinforces, substrates which have been implicated in emotional processing. Much was known about the substrates for acute positive reinforcing effect of drug of abuse. Now there is convincing evidence to suggest amygdala involvement in the secondary reinforcing effect of drugs of abuse. To date, most of the knowledge over drug addiction, and craving have been extracted from animal research. The dissection of craving experience into components that are presented in different brain regions has been one of the constant preoccupations of these studies, a concern that has been adopted by modern neuroscience. Localization of function were investigated by the modern technique of brain activation and imaging. The latter technique often uses linear methods to compare one experimental condition with another in the hope of isolating individual components of behavior and correlating them with observed changes in the measured variable. Craving responses to cue stimulation represents a unique way to isolate components of experiences and correlate them with local change in BOLD signal. According to the evidence presented here craving appears to involve the amygdala, but not only, for alterations in brain activity were also found in the cerebellum. The finding that amygdala was activated in response to ethanol cue is consistent with the classical conditioning model of craving (Franken et al., 1998). In this respect it is proposed that the craving systems are structured within networks that encode information on eliciting stimuli and the meaning of stimuli. This fits well with the notion that the amygdala integrates external stimuli with internal drives and is part of a distributed neural network that marks stimuli and events with positive or negative value. Moreover the amygdala has been identified as belonging to the motive circuit (Pierce and Kalivas, 1997). The neural mechanisms within this circuit are believed to mediate the translation of perception of reward into behavioral activation. This assumption is based on the close temporal link between perception of a reward and the investigation of an appropriate behavioral response to obtain the rewarding stimulus. The role of the amygdala within this circuit serves to cue behavior to conditioned reward (Kalivas and Nakamura, 1999).
Although many unanswered questions remain, it is postulated that recovery from craving, if one can call it that, facilitated by psychotherapy or pharmacotherapy or a combination of both, requires the inhibition of an overactive amygdala. How this adaptation occurred was less clear. One major hypothesis was generated to explain craving: namely homeostatic adaptation. According to the neuroadaptive model of craving, chronic alcohol exposure leads to changes in brain cell function (i.e., neuroadaptation). They are expressed as changes in the activity of various brain chemicals. Neuroadaptation can contribute to certain characteristics of alcohol dependence such as withdrawal consisting of physical signs of tremor and autonomic hyperactivity, or a psychological aspect of withdrawal consisting of anxiety and dysphoria, which usually supersedes the overt responses. During initial abstinence when alcohol withdrawal may occur, neuroadaptation leads to an imbalance in brain function, which results in a subjective feeling of discomfort, and subsequent craving. It appears therefore the case that the amygdala undergoes neuroadaptations during the development of dependence. The hypothesis that a dysregulation of opposing processes of CRF transmission in the amygdala contributes to symptoms of anxiety which is suggested to evoke in patients the perception of craving is certainly attractive (Heilig et al., 1994). As stated in the preceding sections the amygdala plays an important role in modulating stress and mood. The constant impingement of alcohol on brain function (the amygdala is also suggested to be involved with initial euphorogenic effects of alcohol) would cause brain areas to adapt. The abrupt secession of ethanol would unmask the artificial homeostatic state denoted by hyperactivity (Macey et al., 1996) triggering the physiological, behavioral and psychological states. Interestingly, increased activation of the amygdala was also demonstrated in anxious individuals exposed to aversive conditioned cues which was not present in normal healthy subjects (Schneider et al, 1999). This observation is in line with the experimental studies that have shown increased anxiety in alcoholic patients (Krystal et al., 1997; Rasnick et al., 1992), attributed to amygdala dysfunction (Rasnick et al., 1993). ‘Recovery’ from craving can be surmised to be a return to a normal homeostatic milieu of the amygdala, and possibly other regions.

An interesting hypothesis that is gaining support from recent functional observations is that the neuronal substrates for the drug effects may involve a common neural circuitry that forms part of a single entity within the basal forebrain, termed the extended amygdala (Alheid and Heimer, 1988). The term represents a macrostructure, that is composed of several basal forebrain structures: the bed nucleus of the stria terminalis, the central medial amygdala, and the medial part
of the nucleus accumbens (e.g., labeled the shell) (Heimer and Alheid, 1991). Recent studies suggest that cortical inputs into this area may be important for affective aspects of emotional and motivational behavior. Interestingly, these areas are suggested to be involved in fear and anxiety. It remains to be seen what role this area may have in addiction research.

The localization of regional changes in patients with good response to intervention is identical to that demonstrated in the induced sadness experiment. Recovery from craving is associated with decreased activation in the amygdala. Induction of transient sadness showed identical pattern, but in reverse: increases in the amygdala regions. The relationship between the amygdala and overall mood state provide strong evidence for a reciprocal interaction among this area in health and disease. This model maintains that recovery requires normalization form the dysfunctional amygdala, an effect that may be facilitated by using real-time fMRI together with biofeedback training.

On a final note, overall the clinical implication of our findings are numerous. Understanding the biological basis of disease provides important insight into therapeutic intervention. Dysfunctional brain areas that underlie craving provide conceptual anchor for psychotherapeutic as well as for pharmaco-therapeutic intervention. Although imaging studies like the one carried out in this study began to shed light on brain regions involved in craving, future investigation should address several issues. For example, to date, functional imaging studies have treated craving as a state characterized by only one pattern of behaviors or emotion (i.e, a unitary state). The resulting questions, however indicate that the desire to drink elicited by a cue in which a subject knows that alcohol consumption is impossible is likely to differ from the desire to drink in which a subject knows that alcohol is accessible. Also, imaging studies in healthy individuals without alcohol addiction should attempt to identify patterns of brain activity generally associated with appetite and aversive motivation in general. The results of such studies could be compared with the patterns of activation found during craving for alcohol, thereby enabling researchers to understand craving in the context of how the brain deals with motivation normally (Hommer, 1999).

As for biofeedback in realtime, while additional experiments are clearly needed, it is postulated that dysfunctional brain area involved in disease can be facilitated by BF procedures using real-time fMRI, which requires inhibition of overactive areas or for that matter innovation of underactive areas. This technique can also compliment other forms of intervention methods. The idea that
Operant procedures may have an effect on the control of emotional brain areas such as the amygdala using real-time fMRI, making a convincing argument for the value of exploring unconscious regulation with operant conditioning methods on-line. This study provides a useful framework for facilitating the continued study of this novel procedure in emotion research. It is hoped that real-time fMRI using operant conditioning will contribute to the development of new treatments and elucidate the pathogenesis of affective disorders.


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### Abbreviations

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<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>AB</td>
<td>Accessory basal nucleus of the amygdala</td>
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<tr>
<td>ACT</td>
<td>Activation</td>
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<td>AS</td>
<td>Autonomic nervous system</td>
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<td>BF</td>
<td>Biofeedback</td>
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<td>BL</td>
<td>Baseline</td>
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<td>BOLD</td>
<td>Blood oxygenation level dependent</td>
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<td>Ce</td>
<td>Central nucleus of the amygdala</td>
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<td>Co</td>
<td>Anterior cortical nucleus of the amygdala</td>
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<td>CRF</td>
<td>Corticotropin-releasing factor</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>DLPC</td>
<td>Dorsolateral prefrontal cortex</td>
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<td>DSM IV</td>
<td>Diagnostic and statistical manual of mental disorders</td>
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<td>EEG</td>
<td>Electroencephalogram</td>
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<td>EPI</td>
<td>Echo-planer imaging gradient</td>
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<td>EtOH</td>
<td>Ethanol</td>
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<tr>
<td>FID</td>
<td>Free-induction decay</td>
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<td>FIRE</td>
<td>Functional imaging in REAL time</td>
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<td>FMRI</td>
<td>Functional magnetic imaging</td>
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<td>FT</td>
<td>Fourier transformation</td>
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<td>HPA</td>
<td>Hypothalamo-pituitary axis</td>
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<td>ICSS</td>
<td>Intracranial self-stimulation</td>
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<td>L</td>
<td>Lateral nucleus of the amygdala</td>
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<td>M</td>
<td>Medial nucleus of the amygdala</td>
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<td>N. Acc</td>
<td>Nucleus accumbens</td>
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<td>OCD</td>
<td>Obsessive compulsive disorder.</td>
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<td>OFC</td>
<td>Orbitofrontal cortex</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<td>Abbreviation</td>
<td>Term</td>
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<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
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<td>POMS</td>
<td>Profile of mood states</td>
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<td>RF</td>
<td>Radiofrequencies</td>
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<td>SAS</td>
<td>Statistical analysis systems</td>
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<td>SD</td>
<td>Discriminative stimuli</td>
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<td>SPECT</td>
<td>Single photon emission computed tomography</td>
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<td>SPM</td>
<td>Statistical parametric mapping</td>
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<td>SNR</td>
<td>Signal-to-noise ratio.</td>
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<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
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Curriculum Vitae

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Studium

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Diplom Psychologie
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Berufliche Tätigkeiten

1984-1990 General Trading & Services Ltd. (Familien Unternehmen)

1995-1996 Studentische Hilfskraft am Institut für Personalberatung und Betriebspsychologie in Bochum

1997-1998 Studentische Hilfskraft der Psychiatrischen Klinik der Heinrich-Heine-Universität Düsseldorf

Mai 1998–2001 Wissenschaftliche Mitarbeiterin der Psychiatrischen Klinik der Heinrich-Heine-Universität Düsseldorf

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18  PUBLIKATIONVERZEICHNIS

19  I. ORIGINALARBEITEN


Progress in Neuro-Psychopharmacology & Biological Psychiatry (Accepted for publication)


8. Weiss, U., Kühn, E., Salloum, J.B., Devos, H. & Schneider, F. Emotional processing in psychopathy. Aggressive Behavior (Accepted for publication)


20 II PUBLIZIERTE ABSTRACTS


21 III POSTERS


22 IV VORTRÄGE
