Chapter 1
General introduction
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For many of us, daily life is full of impulsive actions and decisions: Speaking out of turn in a class or meeting; using money you had set aside for next month’s rent to buy a pair of shoes you really do not need but that look so good on you; needing a new computer and rather than ‘shopping around’ to see what is available, you buy the first Apple MacBook you come across in the store. To summarize impulsive behavior, Barnes and Durana have come up with the following description: “Actions which are poorly conceived, prematurely expressed, unduly risky or inappropriate to the situation and that often result in undesirable consequences” (Durana and Barnes, 1993). A large part of this thesis will concern the neurobiology of impulsivity as we will discuss our recent findings on the role of the endogenous cannabinoid (eCB) and opioid neurotransmitter systems in this multifaceted behavioral construct. In addition, we will address the role of the eCB system in substance dependence, a psychiatric disorder that is in most patients characterized by impulse control-related problems.

1.1. Impulsive behavior

At the start of this introduction, it should be stressed that being impulsive is not a bad thing per se. Otherwise, evolution would have selected against this personality trait. Instead, impulsivity is a relatively conserved trait across most vertebrate species (Williams and Taylor, 2006; Winstanley et al., 2010a). To a certain extent, being impulsive can be reasonable and sometimes even advantageous and adaptive. For instance in rapidly changing or unsecure environments, or when faced with (perceived) competition. In support of this idea, domestic chicks have been found to make more impulsive decisions over time, choosing a small amount of food that can be obtained immediately over a larger amount of food that they will receive after a delay, when repeatedly perceiving (but not really having) competition from other chicks during training (Amita et al., 2010; Amita and Matsushima, 2011). And for men from the Ariaal tribe in Kenya, it has been suggested that carrying certain polymorphisms in the dopamine D4 receptor (DRD4) gene, which are known to be associated with having a more impulsive personality (Nemoda et al., 2011), actually provide health benefits for those individuals that live as nomads, while being disadvantageous for settled Ariaal men (Eisenberg et al., 2008). In our Western society, however, it appears to have long-term benefits to have a less impulsive personality. This has elegantly been demonstrated in a now famous longitudinal study by Walter Mischel and co-workers (Mischel et al., 1972). With their ‘Marshmallow tests’, they found that individuals who at age 4-6 were able to inhibit the impulse to immediately eat one marshmallow in order to get two marshmallows after a delay were more successful in their social and professional life even up to four decades later (Mischel et al., 1989, 2011).

In the introduction and throughout the remainder of this thesis, it is important to
discriminate between trait and state impulsivity. In this thesis, trait impulsivity refers to the baseline, inert level of impulsivity of an individual, which appears to be a stable personality trait in humans and rodents (Dalley et al., 2007; Diergaarde et al., 2008b; Casey et al., 2011). State-dependent impulsivity on the other hand here refers to transient changes in one’s level of impulsivity induced by environmental factors such as acute exposure to drugs of abuse (Cole and Robbins, 1987; De Wit et al., 2002; Fillmore et al., 2003; McDonald et al., 2003; Kieres et al., 2004; van Gaalen et al., 2006a,b). Irrespective of the discrimination between trait and state impulsivity, acting impulsive on a regular basis becomes maladaptive. This endophenotype is frequently observed in a variety of psychiatric and neurological disorders (American Psychiatric Association, 2000). Attention-Deficit/Hyperactivity Disorder (ADHD) likely is the best-known example. However, also patients diagnosed with e.g. Parkinson’s disease, bipolar disorder, or borderline personality disorder often suffer from impulse control-related problems. Moreover, over the last decade impulsivity has also increasingly received attention in relation to substance use-related disorders such as drug addiction (Jentsch and Taylor, 1999; Perry and Carroll, 2008; Verdejo-Garcia et al., 2008; De Wit, 2009; Winstanley et al., 2010a; Dalley et al., 2011). With regard to drug addiction, a high level of impulsivity is thought to be a vulnerability factor for drug abuse and addiction, while repeated exposure to drugs of abuse can, in turn, also (transiently) affect impulsive behavior. Consequently, there is a great need for effective anti-impulsivity treatments.

The first pharmacological agent that was coincidentally found to alleviate impulsivity and other ADHD-related behavioral deficits is the psychostimulant drug amphetamine

### Table 1.1. Overview of frequently prescript anti-impulsivity pharmacotherapies.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Trade names</th>
<th>Pharmacological activity</th>
<th>Clinical efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-amphetamine/ DL-amphetamine¹</td>
<td>Dextedrine®, Adderall®</td>
<td>DA+NE(−5-HT) releasing agent²</td>
<td>~70%</td>
</tr>
<tr>
<td>DL-methylphenidate²</td>
<td>Ritalin®, Concerta®</td>
<td>DA+NE reuptake inhibitor</td>
<td>~70%</td>
</tr>
<tr>
<td>Atomoxetine</td>
<td>Strattera®</td>
<td>NE(−DA) reuptake inhibitor³</td>
<td>50-60%</td>
</tr>
<tr>
<td>Guanfacine</td>
<td>Intuniv®</td>
<td>α₂*-adrenoreceptor agonist (postsynaptic)</td>
<td>50-60%</td>
</tr>
</tbody>
</table>

DA, dopamine; NE, norepinephrine; 5-HT, serotonin.
¹The abbreviations ‘D’ and ‘DL’ indicate the specific stereoisomers of the drugs used in the formulation, solely the dextro-isomer or a mixture of dextro- and levo-isomers.
²Amphetamine has a lower affinity for the 5-HT transporter, hence, at lower, clinically relevant doses, effects on dopamine and norepinephrine most likely prevail.
³Atomoxetine can in the prefrontal cortex also elevate dopamine levels, since dopamine reuptake in that part of the brain is mediated mainly by the norepinephrine transporter.

Table based on (Arnsten and Pliszka, 2011; Heal et al., 2012; Rothman and Baumann, 2003).
(Bradley, 1937). Since then, several other types of anti-impulsivity pharmacotherapies have been developed (Table 1.1), all of which increase monoamine, and particularly dopamine and norepinephrine, neurotransmission in the brain. These medications are reasonably effective, with clinical efficacies (response rates) in the range of 50-70%. However, these drugs are not devoid of side-effects, most notably weight loss, sleep disturbances, and nausea (Heal et al., 2012). Moreover, particularly with the stimulants amphetamine and methylphenidate there is an abuse potential, probably related to the dopamine-releasing properties of these pharmacological agents. This most likely makes these treatments unsuitable for treating e.g. drug addicts (Winstanley, 2011; Heal et al., 2012).

1.1.1. Impulsivity from a scientific perspective: a multimodal behavioral construct

As discussed above, impulsivity in daily life comes in many different flavors. If one is to scientifically study a behavior, however, one preferably tests an isolated, specific behavioral aspect under controlled, standardized conditions. However, doing so is not trivial. Even isolating impulsivity from other executive functions such as attentional functioning, behavioral flexibility, or working memory can be challenging, let alone discriminating between different modalities of impulsivity that are thought to exist. This requires proper definitions of the different behavioral constructs and objective experimental tasks. Impulsive behavior is generally divided into impulsive actions, i.e. an inability to inhibit a response, and impulsive choices, i.e. a distorted judgment with respect to choosing between two differential reward outcomes (e.g. Evenden, 1999; Pattij and Vanderschuren, 2008; Winstanley et al., 2010a). However, splitting the behavioral construct into four or more subcategories would perhaps be more appropriate (Table 1.2), because within impulsive actions there is a clear distinction between action restraint (inhibiting a prepotent, inappropriate response) and action cancellation, i.e. response inhibition or volitional control over responding once the response has been initiated. Moreover, there is a fourth modality of impulsivity, reflection impulsivity, that is, making decisions before adequately sampling and evaluating available information. Although reflection impulsivity has thus far not been studied extensively, it is a behavioral construct that is rather difficult to categorize into either impulsive actions or impulsive choices. Finally, it should be noted that also impulsive choices may be subdivided into delay-, uncertainty-, and effort-based decision making.

In this thesis, the main focus will be on two of the most studied types of impulsive behavior, which from here on will be termed motor impulsivity and impulsive choice. The former refers to action restraint or inhibitory (response) control, the latter to delay aversion, i.e. intolerance to delayed gratification. Although the exact neuronal mechanisms driving these types of impulsive behavior remain to be clarified, there is a wealth of data demonstrating that motor impulsivity and impulsive choice have only partially overlapping underlying neuroanatomy and neurochemistry (see section 1.3; for review, see Evenden,
Moreover, we recently found that these two modalities of impulsivity did not correlate with each other within an individual rat or human (Broos et al., 2012). In the same study, we also failed to find any significant within-subject correlation between the acute effects of amphetamine and atomoxetine on motor impulsivity and impulsive choice in rats. These findings underscore the scientific rationale for distinguishing different aspects of impulsive behavior. More importantly, such a distinction may be clinically relevant, as it argues for a more individually-tailored (pharmacological) treatment of impulse control disorders. To allow for such a personalized treatment approach, however, more mechanistic research is required. To further illustrate the multi-faceted nature of impulsivity, next, several experimental paradigms that can be used to study this behavior will briefly be discussed.

Table 1.2. Overview of different modalities of impulsive behavior and experimental tasks that can be employed to measure those behaviors in humans and rodents.

<table>
<thead>
<tr>
<th>Type of impulsivity</th>
<th>Cognitive domain</th>
<th>Description</th>
<th>Human tasks</th>
<th>Rodent analogue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impulsive action</td>
<td>Action restraint; motor impulsivity</td>
<td>Ability to inhibit prepotent, inappropriate responses</td>
<td>CPT; IMT/DMT</td>
<td>5-CSRTT; Go/No Go task</td>
</tr>
<tr>
<td></td>
<td>Action cancelation; response inhibition</td>
<td>Ability to inhibit actions once initiated</td>
<td>SSRTT</td>
<td>SSRTT</td>
</tr>
<tr>
<td>Impulsive choice</td>
<td>Delay-based decision making</td>
<td>Decision making based on delay-aversion</td>
<td>Experiential or hypothetical DDT</td>
<td>DRT; Adjusting-amount procedure</td>
</tr>
<tr>
<td></td>
<td>Uncertainty-based decision making</td>
<td>Decision making based on risk seeking</td>
<td>iGT</td>
<td>rGT</td>
</tr>
<tr>
<td></td>
<td>Effort-based decision making</td>
<td>Decision making based on effort-aversion</td>
<td>EEfRT; DST</td>
<td>LCLR/HCHR task; rCET</td>
</tr>
<tr>
<td>Reflection impulsivity</td>
<td>Information sampling</td>
<td>Ability to evaluate available information prior to deciding</td>
<td>IST</td>
<td>Uncertain visual discrimination test</td>
</tr>
</tbody>
</table>

CPT, continuous performance task (Rosvold et al., 1956); IMT/DMT, immediate and delayed memory task (Dougherty et al., 2002); 5-CSRTT, 5-choice serial reaction time task (Robbins, 2002); 5 see (Terman and Terman, 1973); SSRTT, stop-signal reaction time task (Logan et al., 1984; Eagle and Robbins, 2003); 6 DDT/DRT, delayed discounting/reward task (Evenden and Ryan, 1999; Pietras et al., 2003; Rachlin et al., 1991); 7 see (Richards et al., 1999); iGT/rGT, Iowa/rat gambling task (Bechara et al., 1994; Zeeb et al., 2009); 9 EEfRT, effort expenditure for rewards task (Treadway et al., 2009); 10DST, demand selection task (Botvinick and Rosen, 2009); 11LCLR/HCHR task, low cost-low reward/high cost-high reward task (Salamone et al., 1994); 12rCET, rat cognitive effort task (Cocker et al., 2012); 13IST, information sampling task (Clark et al., 2006); 14see (Evenden, 1999). See sections 1.2 and 1.3 for more information.
1.1.2. Translational models of impulsive behavior

One way to measure impulsivity in humans is by self-report questionnaires such as the Barratt Impulsivity Scale (BIS-11; Patton et al., 1995), in which subjects are asked to rate their own impulsivity. However, there are objectivity-issues related to such approaches, and perhaps related to this, questionnaire and behavioral measures of impulsivity usually do not correlate well (Dalley and Roiser, 2012). Moreover, these retrospective questionnaires do not allow for studies on the real-time neurobiology of impulsivity. The latter can more elegantly be done with various computerized experimental paradigms that have been developed (Table 1.2). To test motor impulsivity, one can for instance use a continuous performance task (CPT; Rosvold et al., 1956) or a slightly more cognitively challenging variant called the immediate and delayed memory task (IMT/DMT; Dougherty et al., 2002). These tasks primarily measure sustained and divided attention as subjects are required to withhold a response (oftentimes pressing a button) unless a target stimulus is presented. However, failures to refrain from premature responding simultaneously provide a measure of motor impulsivity. Response inhibition is generally measured in a stop-signal reaction time task (SSRT; Logan et al., 1984), a task in which in each trial one has to make a rapid response to a ‘go’ stimulus, except for trials in which the stimulus presentation is followed (with a short, variable delay) by the presentation of a second ‘stop’ stimulus. In such trials, one needs to inhibit the response triggered by the first ‘go’ stimulus. In the SSRT, the estimated reaction time required to successfully abort a ‘planned’ go-response is a measure of response inhibition. Decision making paradigms come in many variations, but subjects are generally asked in a series of trials to choose between obtaining a smaller or a larger reward. For the assessment of delay-based impulsive choice, the catch is that as compared to the smaller reward, the larger reward will come with a longer delay (e.g., delayed discounting task (DDT); Rachlin et al., 1991; Pietras et al., 2003). For uncertainty-based impulsive choice on the other hand, the larger reward will come with a lower probability as compared to the smaller reward (e.g. Iowa gambling task (iGT); Bechara et al., 1994). Finally, in effort-based decision making paradigms more cognitive (e.g. demand selection task (DST); Botvinick and Rosen, 2009) or physical (e.g. effort expenditure for rewards task (EEfRT); Treadway et al., 2009) effort is required to obtain the larger reward option. The level of impulsivity can then be inferred from the subject’s (in)tolerance to delay, risk, or effort, respectively. In addition, although used less frequently, there are several tasks available to measure reflection impulsivity, including the information sampling task (IST; Clark et al., 2006). In this task, subjects are presented with a 5 x 5 matrix of covered boxes that can each be one of two colors. By uncovering boxes one by one, subjects must decide which of the two colors the prevailing one in the matrix is. Impulsivity in the IST is reflected in the number of incorrect, premature decisions.

Our current understanding of impulsive behavior has largely been derived from psychological experiments in humans using one or more of the above-mentioned paradigms. To advance our knowledge on the neuronal mechanisms underlying the different aspects of impulsivity, and the relationships between these behaviors, such
studies in humans remain valuable. However, it clearly is unethical to do experimental pharmacological or genetic manipulations in humans or to use invasive techniques on them. Moreover, performing experiments in humans under controlled conditions and with full knowledge of the subject’s history is often challenging. Thus, to gain in-depth knowledge on the neuronal mechanisms underlying impulsive behavior, or any behavior for that matter, we need proper animal models. Fortunately, many of the human impulsivity tasks have been translated into primate and rodent paradigms (Table 1.2). For instance, there is an operant version of the SSRTT to study response inhibition in rats (Eagle and Robbins, 2003). Operant paradigms to assay reflection impulsivity (uncertain visual discrimination test; Evenden, 1999), probabilistic decision making (e.g. rat gambling task (rGT); Zeeb et al., 2009), and effort-based decision making (e.g. rat cognitive effort task (rCET) or low cost-low reward/high cost-high reward (LCLR/HCHR) task; Salamone et al., 1994; Cocker et al., 2012) in rats have also been developed. These tasks are often strikingly similar to their human equivalents, with the exception that food/liquid rewards rather than monetary rewards are used. However, it should be noted that most rodent impulsivity paradigms have only recently been developed (e.g. rGT) and/or are rather complex to set up and use to for instance study the underlying psychopharmacology (e.g. SSRTT). Consequently, only few studies have been published incorporating these impulsivity tasks. In this thesis, we chose to use two frequently employed operant tasks to assess motor impulsivity and delay-based impulsive choice in rats, the 5-choice serial reaction time task (5-CSRTT) and the delayed reward task (DRT), respectively. Below, these paradigms will be discussed in more detail.

The 5-CSRTT: an attentional task to measure motor impulsivity in rats

Many operant paradigms exist to tax motor impulsivity in rodents, including the Go/No Go task (Terman and Terman, 1973). Go/No Go paradigms use two distinct stimuli, one indicating that a ‘Go response’ (usually making a nosepoke or pressing a lever) is required, and the other that this response should be inhibited in order to obtain a food reward. At the beginning of each trial, one signal is presented, with the ‘Go-signal’ being presented considerably more frequently, thereby priming the motor response. Motor impulsivity can then be assessed by counting the number of responses made during ‘No Go-trials’. However, a more frequently used and better characterized motor impulsivity task in rodents is the 5-CSRTT (Carli et al., 1983; Robbins, 2002; Bari et al., 2008). The 5-CSRTT was intended to be a rodent version of the CPT (Rosvold et al., 1956), and was therefore not designed with the sole purpose of measuring motor impulsivity, but rather to be a test of sustained and visual spatial attention. In the CPT, subjects are asked to press a button whenever they detect a target stimulus within a sequence of stimuli that are being presented on a computer screen. The ‘target’ is usually either a sequence of two symbols, e.g. an ‘A’ followed immediately by an ‘X’ (CPT), or a five-digit number (IMT/DMT). Thus, the subject is required to pay continuous attention to the stimuli presented on the screen. Additionally, motor impulsivity in this task can be inferred from the number of ‘false alarm’ responses made, i.e. responses made when the ‘A’ is not followed by an ‘X’ (CPT) or when the stimulus resembles the target in only four of the five digits (IMT/DMT). Similar to the
Figure 1.1. Schematic overview of the 5-choice serial reaction time task (5-CSRTT). In the 5-CSRTT operant chamber, rats have to respond upon or shortly after (during limited hold) a brief visual stimulus that appears semi-randomly in one of five nose poke apertures (left curved wall) to earn food pellets (delivered in a receptacle in the right wall). In contrast, rats should refrain from responding during the intertrial interval (ITI) preceding each stimulus presentation to prevent a ‘time-out’ penalty. Making a response in an incorrect hole, or making no response at all (omissions), will also result in a ‘time-out’ penalty.
CPT, several variants of the 5-CSRTT have been designed. In the most frequently-used version of the task, which was also used in the experiments described in this thesis, a rat is trained to poke its nose in one of five response apertures whenever a brief stimulus light is presented therein, in order to earn food rewards (Figure 1.1). Responses in any of the other four apertures or omissions of responding will result in a 5 s ‘time-out’ punishment. The ratio between correct and incorrect responses together with the number of omitted trials reflect attentional functioning of the rat. Importantly, at the start of each trial and prior to illumination of a stimulus light, there is a 5 s intertrial interval (ITI) during which the animal must refrain from responding in the apertures. Responses made during this ITI period are recorded as premature, impulsive responses and punished with a 5 s ‘time-out’. Although not identical, these premature responses are thought to bear analogy to ‘false alarm’ errors made in the CPT. Variations on this 5-CSRTT include paradigms in which the length of the ITI is made variable (typically between 3-12 s) rather than fixed in order to increase the cognitive load for the animals and to prevent animals from using a timing strategy to predict stimulus onsets. This manipulation results in a considerable enhancement in premature responding, but its consequences with respect to the underlying neurobiology largely remain to be determined. Furthermore, researchers have recently started to incorporate ‘vigilance trials’ into the 5-CSRTT (Young et al., 2009). During these ‘vigilance’ trials a distinct ‘no response’ stimulus is presented and animals should inhibit responding entirely to obtain the food reward, thereby basically combining the slightly different measures of motor impulsivity provided by the traditional Go/No Go and 5-CSRT tasks.

A clear strength of the 5-CSRTT is its ability to assess multiple aspects of cognitive performance (attention, motor impulsivity, speed of responding, and motivation). When judged properly, this multitude of output parameters may allow for a more accurate interpretation of behavioral effects of a procedural or pharmacological manipulation. On the other hand, the 5-CSRTT is a cognitively demanding task for rodents, requiring extensive (2-3 months) daily training of the animals. Another potential concern regarding the 5-CSRTT (and all other rodent impulsivity paradigms) is the requirement to food restrict animals, albeit only mildly, to get them to work for the food rewards (but see Blasio et al., 2012). Consequently, when a neurotransmitter system under investigation is known to also mediate feeding behavior and/or motivation for food, such as is the case with the eCB and opioid systems (Di Marzo et al., 2009; Kelley et al., 2002), some caution is warranted with the interpretation of the impulsivity data. For any animal model, it is important to have high validity for the clinical phenomenon or disorder it is based on. This is usually judged by three criteria (Mckinney and Bunney, 1969): resemblance in ‘symptomatology’ (face validity), underlying mechanisms (construct validity), and the effects of (pharmacological) manipulations (predictive validity). Of these three criteria, construct validity usually is the hardest to judge due to the simple fact that knowledge on mechanisms underlying a clinical phenomenon or disorder often is incomplete. The 5-CSRTT possesses reasonable face validity, as the behavior rodents are required to display in this task involves an aspect of motor impulsivity, i.e. inhibiting inappropriate responses, and is fairly similar to that asked of humans in, for instance, the CPT or IMT/DMT. Based on current knowledge,
Figure 1.2. Schematic overview of the delayed reward task (DRT). The DRT is performed in a 5-CSRTT operant chamber. Following an initial nose poke in the central aperture in the left wall, rats choose between a small immediate, or a larger delayed food reward by making a nose poke in one of two adjacent apertures. The delay for the larger reward increases over the course of a session. As a result, rats will gradually lose interest in the larger reward. Importantly, due to the adjustable duration of the intertrial interval (ITI), the total length of each trial is the same, irrespective of whether the small or large reward was chosen.
the 5-CSRTT seems to have high construct validity since thus far there appears to be concordance in the neuroanatomy and neurochemistry underlying 5-CSRTT and e.g. CPT behavior (see section 1.3; for review, see Winstanley, 2011). In this regard, however, one concern may be the opposite effects that psychostimulant drugs (e.g. methylphenidate and amphetamine) have in the CPT versus the 5-CSRTT, generally decreasing and increasing motor impulsivity in humans and rats, respectively. This discrepancy may be indicative of a differential role for the dopamine system in mediating CPT versus 5-CSRTT impulsivity (see chapter 6 for further discussion). The latter issue also negatively affects the predictive validity of the 5-CSRTT. Nevertheless, the 5-CSRTT appears to have predictive validity in that all compounds that are known to affect motor impulsivity in the CPT at least also have been found to affect premature responding in the 5-CSRTT (Winstanley, 2011), albeit the direction of the effects may differ in humans versus rodents. An additional problem here is that whereas drugs in humans, particularly when intended as treatment, are usually administered repeatedly, the vast majority of 5-CSRTT experiments to date only involved a single drug administration to otherwise healthy rodents. This, however, is a weakness of most animal models currently employed in neuroscience.

The DRT: a rodent paradigm for assessing impulsive choice driven by delay-aversion

Delay-based decision making in humans is best tested in a DDT (Rachlin et al., 1991; Pietras et al., 2003). In this task, subjects are asked to make choices between a small (monetary) reward now or a larger reward that will be obtained after some delay. Depending on the design, the delays and rewards can either be hypothetical or experienced in real-time. Usually, one of the choice options is kept constant while the other one is adjusted based on the subject’s previous choices in order to determine a subject’s indifference point. This is the situation in which the immediate and delayed options appeal equally to the subject, representing the subjective value of the delayed option. The task is based on the theory that any reward will devaluate according to a hyperbolic function as the delay to its delivery increases (Ainslie, 1975; Mazur, 1987). Importantly, in a DDT, the highest total amount of reward can be obtained by constantly choosing the delayed options. Frequent immediate reward choices, resulting in steeper discounting curves and lower indifference points, are therefore indicative of more impulsive decision making.

Many species, including rodents, will display exactly the same behavior as humans when faced with choices between immediate and delayed rewards. In rodents, impulsive choice can be assessed with an adjusting amount procedure in which choices have to be made between an adjusting immediate and a fixed delayed amount of a liquid reinforcer (Richards et al., 1997). More commonly, however, a DRT is used (Evenden and Ryan, 1996; Cardinal et al., 2000; Cardinal, 2006). As with the 5-CSRTT, many variants of this paradigm exist (e.g. between- versus within-session designs and paradigms incorporating cues to bridge the delay periods or not). These variations may potentially affect the outcome of an experiment. In the DRT used in this thesis (Figure 1.2), rats are given choices between one or four food pellets. Over the course of a session, in blocks of 10 choice trials, the delay for the four pellet-option will then increase from 0-40 s. As in the human DDT, it would be
advantageous for a rat to always choose the larger reward as the total duration of a trial is kept constant, irrespective of a choice for the small or larger reward. Nonetheless, as with humans, large inter-individual differences can be observed in the rate with which rats will discount the larger reward over increasing delays.

Unfortunately, similar to the 5-CSRTT, it requires 2-3 months of daily training for a rat to reach a stable choice preference in a within-session version of the DRT. On the upside, the DRT obviously has high face validity as a test for delay-based decision making, even though a drawback of the rat DRT is that delays within a session are usually presented in fixed (ascending) rather than a random order. The DRT also has reasonable to high construct and predictive validity owing to the seemingly similar neurobiology and pharmacology underlying DDT behavior in humans and DRT behavior in rodents (see section 1.3; for review, see Winstanley, 2011). For instance, in line with their efficacy as anti-impulsivity pharmacotherapies, psychostimulants reduce impulsive choice in humans as well as rats, albeit in the latter species the results may depend on the exact test conditions.

1.1.3. Neurobiology of impulsive behavior

As mentioned above, there already exists a wealth of data on the neurobiology of motor impulsivity and impulsive choice in humans and rodents. A detailed discussion of this literature is beyond the scope of this thesis (for reviews, see Cardinal, 2006; Winstanley et al., 2006a, 2011; Pattij and Vanderschuren, 2008; Dalley et al., 2011; Dalley and Roiser, 2012). Instead, a brief overview of our current understanding of the neuroanatomy and neurochemistry of impulsivity will be provided here.

Neuroanatomy of motor impulsivity and impulsive choice

According to the dual pathway theory on the etiology of ADHD, executive dysfunctioning, including impulse control-related problems, can be caused by failing inhibitory control and/or a disturbed motivational drive (Sonuga-Barke, 2002, 2005). The former is thought to be mediated primarily by cortical (prefrontal cortex) circuitries, whereas the latter heavily depends on subcortical limbic brain regions such as the striatum. Human studies using imaging techniques such as functional magnetic resonance imaging (fMRI) as well as rodent studies seem to support this theory. Specifically, these studies have to date mainly implicated ventral striatal (nucleus accumbens (NAc) shell and core subregions) and prefrontal brain regions (primarily orbital frontal cortex and subregions of the medial prefrontal cortex (mPFC)). Additionally, the insular cortex, the thalamic nucleus reunions, and limbic structures such as the habenula, amygdala, and hippocampus have been implicated in the regulation of motor impulsivity and/or impulsive choice behavior (Abela et al., 2012; Prasad et al., 2012; for reviews, see Cardinal, 2006; Winstanley et al., 2006a; Pattij and Vanderschuren, 2008; Dalley et al., 2011). It is interesting that there appears to be considerable convergence in brain regions involved in motor impulsivity and impulsive
choice (Figure 1.3). However, it should be kept in mind that a multitude of neuronal circuitries exist within each brain region. Therefore, although a specific brain region may be important for both motor impulsivity and impulsive choice, within that brain region, the neurons and/or neural mechanisms regulating both types of impulsivity may be quite dissociable. Sometimes the role of a brain nucleus may even be opposite for two aspects of impulsive behavior. For instance, lesions of the subthalamic nucleus in rats have been reported to induce increased premature responding in the 5-CSRTT but decreased impulsive choice in the DRT (Baunez and Robbins, 1997; Winstanley et al., 2005).

**Neurochemistry of motor impulsivity and impulsive choice**

Traditionally, the vast majority of impulsivity research has been focused around the monoaminergic neurotransmitter systems, and particularly the dopamine system. The interest of researchers in the latter neurotransmitter stems from the clinical efficacy of amphetamine (Dexedrine®; Adderall®) and methylphenidate (Ritalin®; Concerta®) in alleviating impulse control disorders, since these pharmacological agents are thought to exert their effects mainly via modulating dopaminergic signaling (Heal et al., 2012). Indeed, although the exact mechanisms remain to be elucidated, there is overwhelming evidence that dopamine plays a critical role in regulating impulsive behavior in both humans and rodents. High levels of trait impulsivity in humans and rodents are thought to be associated with reduced availability of the dopamine D2 receptor and increased psychostimulant-induced dopamine release, particularly in striatal brain regions (Rosa-Neto et al., 2002, 2005; Dalley et al., 2007; Buckholtz et al., 2010). Moreover, acutely stimulating dopamine release (or inhibiting dopamine reuptake) by administering a psychostimulant drug is known to decrease both impulsive choice and motor impulsivity in humans, as measured in a DDT and CTP, respectively. Intriguingly, the same drugs in rodents generally seem to result in decreased impulsive choice but increased motor impulsivity. It should, however, be noted that methylphenidate at low, clinically more relevant doses has recently been suggested to reduce motor impulsivity in the 5-CSRTT (Pattij et al., 2012). The effects of drugs such as amphetamine and methylphenidate may be baseline-dependent (Loos et al., 2010b) and probably involve a complex interplay between signaling at the two major classes of dopamine receptors, D1 and D2 receptors, as stimulating the activity of these two types of receptors independently with selective agonists seems to decrease motor impulsivity in rats (for reviews, see Pattij and Vanderschuren, 2008; Winstanley, 2011; Dalley and Roiser, 2012). Moreover, for both motor impulsivity and impulsive choice, D2 receptor antagonists do not affect baseline impulsivity but prevent amphetamine-induced impulsivity, while D1 receptor antagonists when administered alone have effects opposite to amphetamine and the dopamine transporter inhibitor GBR 12909. Finally, it is interesting that rodent studies employing either intracranial drug infusions into discrete brain regions or techniques to measure neurotransmitter release in the brain have indicated that partly distinct dopaminergic circuitries may be involved in regulating motor impulsivity and impulsive choice. For the 5-CSRTT, particularly dopamine signaling in the NAc appears to be critical, whereas prefrontal dopamine signaling in e.g. the orbital frontal cortex but not the mPFC may additionally be involved (Cole and Robbins, 1987, 1989; Puumala and Sirvio, 1998;
Figure 1.3. Overview of the neuroanatomy of motor impulsivity and impulsive choice. Schematic overview of coronal sections (adapted from Paxinos and Watson, 1998) at various levels of one hemisphere of the rat brain (in anterior to posterior direction depicted from top to bottom) illustrating the considerable overlap in anatomical regions of the brain that have been shown to be involved in motor impulsivity and delay-based impulsive decision making. Red and blue colors indicate areas that have been implicated specifically in motor impulsivity or impulsive choice, respectively, whereas green indicates areas that have been implicated in both aspects of impulsivity. Dark grey/black areas indicate ventricles in the brain, whereas light grey areas indicate fibre tracts. Not shown are the midbrain nuclei containing the cell bodies of dopamine-, norepinephrine-, and 5-HT-neurons that are thought to be relevant for impulsivity, i.e. the ventral tegmental area, locus coeruleus, and raphé nuclei. Abbreviations: ACC, anterior cingulate cortex; BLA, basolateral amygdala; HAB, habenula; HPC, hippocampus; IL, infralimbic cortex; MS, medial striatum; NAC, nucleus accumbens core; NAS, nucleus accumbens shell; NRe, thalamic nucleus reuniens; OFC, orbitofrontal cortex; PL, prelimbic cortex; STN, subthalamic nucleus. Illustration adapted from (Pattij and Vanderschuren, 2008).
Dalley et al., 2002a,b, 2007; Pattij et al., 2007b; Diergaarde et al., 2008b; Winstanley et al., 2010b). In contrast, prefrontal (particularly orbital frontal cortex) rather than accumbal dopamine transmission has been reported to be involved in regulating impulsive choice in the DRT (Kheramin et al., 2002; Winstanley et al., 2005, 2006b; Diergaarde et al., 2008b; Loos et al., 2010a; Zeeb et al., 2010).

Another monoamine that has recently been gaining increased attention in relation to impulsivity is norepinephrine. Drugs like atomoxetine (Strattera®) and guanfacine (Intuniv®) that target the norepinephrine system appear to work well in ADHD patients (Heal et al., 2012). Unfortunately, systematic analyses of the role of this neurotransmitter system in impulsivity are yet to be performed. Nevertheless, it appears that increasing norepinephrine signaling reduces motor impulsivity (and response inhibition) in humans and rodents. In contrast, impulsive choice behavior at least in rodents may be less dependent on norepinephrine signaling (Robinson et al., 2008; Baarendse and Vanderschuren, 2012; Broos et al., 2012). With respect to the underlying mechanisms, data thus far implicating a variety of adrenergic receptors in motor impulsivity in the 5-CSRTT, mainly the $\alpha_1$, $\alpha_2$, and $\beta_2$-subtypes (Pattij et al., 2012; for review, see Winstanley, 2011). These receptors may be located in the orbital frontal cortex or NAc shell rather than the mPFC (Milstein et al., 2007, 2010; Sun et al., 2010; Economidou et al., 2012).

The third monoaminergic neurotransmitter system, the serotonin (5-HT) system, has since long been hypothesized to mediate behavioral inhibition (Soubrie, 1986). Nonetheless, it has to date not really been exploited as a target for anti-impulsivity pharmacotherapies, perhaps related to the pharmacological complexity of this neurotransmitter system as well as the complex role it may play in impulsive behavior. The 5-HT system contains over 14 different receptor subtypes, with sometimes completely opposite cellular functions (Bockaert et al., 2006). Overall, systemically reducing 5-HT levels increases motor impulsivity in humans and rats, whereas the effects of such a manipulation on impulsive choice are less consistent and may be gender-, genotype-, and task-specific (for reviews, see Winstanley, 2011; Dalley and Roiser, 2012). Conversely, increasing 5-HT signaling has been reported to decrease motor impulsivity in rats, while not affecting impulsive choice in this species (Baarendse and Vanderschuren, 2012). The role of 5-HT in impulsivity is not straightforward, as exemplified by several in vivo microdialysis studies suggesting that premature responding in the 5-CSRTT and impulsive choice in the DRT correlate with increased 5-HT release in the rat mPFC (Dalley et al., 2002a; Winstanley et al., 2005, 2006b). With respect to 5-HT receptor subtypes that have been found to be involved in impulsivity, to date mainly the $5-HT_1A$, $5-HT_2A$, and $5-HT_2C$ have been positively identified. And at least for motor impulsivity, these receptors may be located in the mPFC and/or NAc (for reviews, see Winstanley, 2011; Dalley and Roiser, 2012). Finally, it should be noted that 5-HT signaling has also been reported to modulate amphetamine's effects on motor impulsivity and impulsive choice in rats, probably via an interaction with the dopamine system (Winstanley et al., 2003, 2005; Fletcher et al., 2011).
1.2. Cannabinoids, opioids, and impulsive behavior

Being such a complex and multifaceted behavior, impulsivity is unlikely to be regulated by just three neurotransmitter systems. Indeed, there now is evidence for a role of other neurotransmitter systems, and particularly the glutamatergic system, in regulating this behavior (for reviews, see Pattij and Vanderschuren, 2008; Winstanley, 2011). The research described in this thesis concerned two other neurotransmitter systems, the cannabinoid and opioid systems. Below, both neurotransmitter systems will be introduced, and the rationale for studying them in relation to impulsive behavior will be provided.

1.2.1. The endogenous cannabinoid system

The endogenous cannabinoid (eCB) system is named after the main active ingredient in herbal Cannabis sativa, Δ9-tetrahydrocannabinol (Δ9-THC), that targets its receptors. This evolutionary conserved neuromodulatory system (Elphick and Egertova, 2001) remains relatively unexplored to date. It consists of at least two G<sub>i/o</sub>-protein coupled receptors, denoted CB1 and CB2 receptors, and several endogenous ligands (‘endocannabinoids’) including N-arachidonoyl-ethanolamide (anandamide, AEA) and 2-arachidonyl glycerol (2-AG) (Matsuda et al., 1990; Devane et al., 1992; Munro et al., 1993; Mechoulam et al., 1995; Sugiura et al., 1995; for reviews, see Di Marzo, 2009; Heifets and Castillo, 2009; Kano et al., 2009). In both humans and rodents, the cannabinoid CB1 receptor is densely expressed throughout the brain, particularly in mesocorticolimbic brain areas (Herkenham et al., 1990; Mailleux and Vanderhaeghen, 1992; Burns et al., 2007). In contrast, cannabinoid CB2 receptors are mainly expressed peripherally in the immune system, and only at low levels in the brain (Onaivi et al., 2006; Xi et al., 2011). Although central CB2 receptors might be behaviorally relevant (Onaivi et al., 2006; Xi et al., 2011), we have in this thesis only studied CB1 receptors, because the latter receptors are generally accepted to mediate the vast majority of cannabinoid-related behavioral effects (e.g. Kano et al., 2009). An important notion about CB1 receptors, and many other G-protein coupled receptors, is that they have been hypothesized to exhibit constitutive activity (Kenakin, 2001). That is, activity in the absence of an (endogenous) agonist. However, evidence for constitutive activity of CB1 receptors stems mainly from in vitro experiments (Pertwee, 2005), and the vast majority of in vivo CB1 receptor activity is likely to be eCB-induced (Kano et al., 2009). Nonetheless, CB1 receptor antagonists are usually differentiated into inverse agonists and neutral antagonists, with only the former class of compounds being able to reduce constitutive CB1 receptor activity. As opposed to ‘classical’ neurotransmitters, eCBs are lipid molecules thought to be synthesized “on demand” following a rise in intracellular calcium and/or activation of G<sub>i/o</sub>-protein coupled receptors such as group I metabotropic glutamate receptors (Heifets and Castillo, 2009; Kano et al., 2009; Di Marzo, 2011). However, the existence of intracellular eCB storage pools has recently been suggested as well (Min et al., 2010). Particularly for AEA, multiple synthesis pathways are thought to exist (Di Marzo,
After synthesis and release, eCB signaling is terminated by reuptake into neurons and glia cells and subsequent intracellular hydrolysis. The existence of an active transporter protein that mediates reuptake of AEA and/or 2-AG from the synapse remains subject to vigorous debate (McFarland and Barker, 2004; Glaser et al., 2005; Fu et al., 2012). Hydrolysis of AEA and 2-AG on the other hand is known to be primarily mediated by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively (Blankman et al., 2007; Ahn et al., 2008). Interestingly, AEA and 2-AG have recently been demonstrated to have partly divergent behavioral functions since only dual inhibition of FAAH and MAGL reproduced the behavioral phenotype of an exogenous CB1 receptor agonist (Long et al., 2009). Such a functional differentiation in the eCB system may be related to differences in the distribution of the CB1 receptor and the enzymes responsible for AEA and 2-AG synthesis and hydrolysis in the brain (Kano et al., 2009). Moreover, AEA is a partial agonist for the cannabinoid CB1 receptor, whereas 2-AG acts as a full agonist at this receptor (Hillard, 2000; Pertwee, 2007). Finally, it should be mentioned that eCBs may also activate non-cannabinoid signaling mechanisms (for review, see Pertwee et al., 2010). For instance, AEA potently activates TRPV1 receptors that are known to mediate behavioral effects that are sometimes opposite to those produced by cannabinoid CB1 receptor activation (Kauer and Gibson, 2009; Serrano and Parsons, 2011).

Cannabinoid CB1 receptor activation functions to reduce neuronal activity through a negative feedback mechanism involving, among other processes, inhibition of the intracellular adenylyl cyclase-cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA)-pathway (Wilson and Nicoll, 2002; Heifets and Castillo, 2009; Kano et al., 2009). In addition, CB1 receptor activation can stimulate and inhibit inwardly rectifying potassium and voltage-gated calcium channels, respectively. Via these mechanisms, eCB signaling can (in)directly regulate the release of many other neurotransmitters, including monoamines (Schlicker and Kathmann, 2001; Egerton et al., 2006; Lopez-Moreno et al., 2008). Due to its widespread distribution throughout the brain, the eCB system is an important modulator of a wide range of physiological processes and behaviors such as pain sensation, memory and learning, energy homeostasis, stress/anxiety, and reward and motivational processes (Mariscano et al., 2002; Hohmann et al., 2005; Solinas et al., 2008; Hill and Gorzalka, 2009; Maccarrone et al., 2010). In addition, cannabinoids are thought to play a role in many executive functions, including time perception, attention, behavioral flexibility, working memory, and as will be outlined in section 2.3, impulsivity (Pattij et al., 2008).

1.2.2. The endogenous opioid system

"Opiates" is the term used to describe a class of molecules that are structurally related to morphine, a plant alkaloid derived from the juice of the opium poppy (Papaver somniferum). Other renowned opiates are heroin (diacetylmorphine), methadone, and codeine. Endogenous opiate-like substances, "opioids", also exist, of which the endorphins
(mainly β-endorphin), enkephalins (met- and leu-enkephalin), and dynorphins (dynorphin A and B) are the most studied. Although these peptides have part of their chemical structure in common, they are derived from three different precursor gene products: proopiomelanocortin (POMC), proenkephalin, or prodynorphin (Akil et al., 1984, 1997; Khachaturian et al., 1985). The different classes of endogenous opioids have divergent functions, probably related to their differential affinity profiles for the various opioid receptors (Akil et al., 1997; Goldstein and Naidu, 1989). There are three major classes of opioid receptors, µ-, δ-, and κ-opioid receptors, which are to a large degree conserved throughout vertebrate species and are characterized by a high affinity for the competitive antagonist naloxone (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973; for reviews, see Akil et al., 1984, 1997; Waldhoer et al., 2004). Although none of the endogenous opioids is highly selective for a particular opioid receptor subtype, β-endorphin, enkephalins, and dynorphins have the highest affinity for µ-, δ-, and κ-opioid receptors, respectively (Akil et al., 1997; Goldstein and Naidu, 1989). Further functional differentiation is accomplished by the partly distinct distributions of the precursor molecules as well as the different opioid receptor subtypes throughout the brain (Mansour et al., 1988).

Similar to cannabinoid CB1 receptors, all opioid receptors are G-protein coupled receptors that can (in)directly modulate signaling in many other neurotransmitter systems, including the monoaminergic systems (Khachaturian and Watson, 1982; Mansour et al., 1988), via inhibition of neuronal activity (North, 1986; Eguchi, 2004). Consequently, it should come as no surprise that also opioid systems have been implicated in regulating many physiological and behavioral processes, including pain perception/analgesia, euphoria, sexual-, social-, and drug-related reward, eating and drinking, locomotor activity, learning and memory, and addictive behaviors (van Ree et al., 1999, 2000; Cota et al., 2006; Bodnar, 2011). And as will be discussed in the next paragraph, opioids have also been found to be involved in regulating impulsive behavior.

1.2.3. Cannabinoids and opioids in impulsivity

As discussed in the previous paragraphs, the monoaminergic neurotransmitter systems are thought to critically regulate impulsive behavior, and the eCB and opioid systems are well known to reciprocally interact with the monoaminergic systems, and particularly the dopamine system (Di Chiara and Imperato, 1988; Hurd and Herkenham, 1992; Spanagel et al., 1992; Roth-Deri et al., 2008; Solinas et al., 2008; El Khoury et al., 2012). Moreover, particularly the cannabinoid CB1 and µ-opioid receptor systems are thought to heavily interact with each other; there is considerable overlap in receptor distribution, both receptors share intracellular signaling pathways, there is an extensive literature on (partly) reciprocal pharmacological interactions, and even the formation of receptor heterodimers has been suggested (Schoffelmeer et al., 2006; Robledo et al., 2008; Parolaro et al., 2010). Altogether, this puts the eCB and opioid systems in an excellent position...
to modulate impulsivity.

Indeed, several studies have linked polymorphisms in cannabinoid-related genes to impulsivity or impulse control disorders (Ponce et al., 2003; Ehlers et al., 2007; Lu et al., 2008; Hariri et al., 2009). Moreover, a recent study suggested that ADHD-patients may have reduced AEA hydrolysis (Centonze et al., 2009), and heavy cannabis users have been found to display heightened levels of reflection impulsivity (Clark et al., 2009). In addition, acute challenges with Δ9-THC have in humans been reported to induce deficits in response inhibition but not impulsive choice (McDonald et al., 2003; Ramaekers et al., 2006, 2009). Our laboratory was the first to investigate the role of the cannabinoid CB1 receptor in impulsivity in rats (Pattij et al., 2007a). In line with human studies, the CB1 receptor agonist WIN55,212-2 was found to slightly impair response inhibition in the rat SSRTT. More importantly for this thesis, WIN55,212-2 did neither affect motor impulsivity in the 5-CSRTT nor did it alter impulsive choice in the DRT. In contrast, the cannabinoid CB1 receptor antagonist SR141716A only reduced premature responding in the 5-CSRTT, an effect that was prevented by co-administration of WIN55,212-2 and that was later confirmed by others using the CB1 receptor antagonist SLV330 (de Bruin et al., 2011). Thus, although the inverse agonistic properties that both SR141716A and SLV330 are thought to posses may have been a confounding factor, motor impulsivity, but not impulsive choice, under baseline conditions seems to be modulated by an endogenous tone at the cannabinoid CB1 receptor.

Clinical efficacy of the opioid receptor antagonist naltrexone in the treatment of impulse control disorders has already been suggested in the late 90’s (Kim, 1998). Circumstantial evidence for a role of the opioid system in impulsive behavior has been provided by studies showing that opiate addicts tend to display impulse control deficits, particularly in the decision making domain (Madden et al., 1997; Clark et al., 2006; Verdejo-Garcia et al., 2007). A recent rat study suggested that such deficits may be a consequence rather than a cause of opiate abuse (Schippers et al., 2012). Moreover, it has been reported that the expression and activity of particularly the μ-opioid receptor may be positively correlated with trait impulsivity in humans and mice (Love et al., 2009; Olmstead et al., 2009). Clinical studies examining the effects of acute manipulations of opioid receptor activity on impulsive behavior have yielded inconsistent results, but generally seem to support a role for the μ-opioid receptor in impulsive choice, albeit probably in a genotype and/or personality-dependent way (Mitchell et al., 2007; Boettiger et al., 2009; Zacny and De Wit, 2009). However, it should be noted that the compounds used in those studies, the μ-opioid receptor agonist oxycodon and the preferential μ-opioid receptor antagonist naltrexone, also have affinity for the κ-opioid receptor, which might have obscured data (Mitchell et al., 2007; Zacny and De Wit, 2009). A systematic examination of the role of the different opioid receptors in distinct modalities of impulsivity in rodents is still warranted. Although a null effect has also been reported (Garcia-Lecumberri et al., 2011), there is evidence indicating that the μ-opioid receptor agonist morphine acutely increases impulsive choice behavior in rats, whereas blocking opioid receptors with naltrexone may be without effect (Kieres et al., 2011).
Moreover, κ-opioid receptor activity has consistently been demonstrated not to affect baseline motor impulsivity in the 5-CSRTT (Paine et al., 2007; Shannon et al., 2007; Nemeth et al., 2010), whereas δ-opioid receptors have inconsistently been implicated in inhibitory control (Olmstead et al., 2009; Befort et al., 2011). When we set out to study the role of the opioid system in impulsive behavior, we therefore decided to focus on the μ-opioid receptor. Prior to this thesis, we characterized the effects of acute administration of morphine and the opioid receptor antagonist naloxone in the 5-CSRTT, DRT, and SSRTT (Pattij et al., 2009). The results extended previous findings by showing that morphine made rats more impulsive, particularly in the 5-CSRTT and DRT. Whereas naloxone did not affect impulsivity when tested alone, it completely prevented morphine-induced impulsivity in the 5-CSRTT and DRT. Therefore, although μ-opioid receptors may be silent under baseline conditions, these receptors seem to be in position to alter signaling within impulsivity-related neural circuitries upon activation.

Altogether the above discussed data shows that with respect to baseline (trait) motor impulsivity and impulsive choice, cannabinoid CB1 and μ-opioid receptors play important, but distinct roles (Table 1.3). As mentioned earlier on in this introduction (section 1), one can additionally study the neurobiology underlying (temporary) state-dependent changes in impulsivity. In this type of experiments, an environmental factor (e.g. administration of a drug or stressor) is used to induce a transient increase or decrease in impulsivity. This type of experiments will provide additional valuable information on the neurobiology of impulsivity, and may particularly be relevant in relation to patients diagnosed with e.g. drug dependence or Parkinsonism, people in whom impulse control-related problems, at least in part, seem to be pharmacologically-induced (e.g. Voon et al., 2009; Winstanley et al., 2010a). The data obtained on the role of the eCB and opioid systems in baseline impulsivity raised our interest in the role of these neurotransmitter systems in state-dependent impulsivity. Specifically, a major aim of this thesis was to examine the putative role of cannabinoid CB1 and μ-opioid receptors in psychostimulant-induced impulsivity (see section 4).

Table 1.3. Summary of the reported role of cannabinoid CB1 and μ-opioid receptors in motor impulsivity and impulsive choice.

<table>
<thead>
<tr>
<th>CB1 receptor</th>
<th>μ-opioid receptor</th>
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<tbody>
<tr>
<td>activation</td>
<td>inactivation</td>
</tr>
<tr>
<td>Motor impulsivity</td>
<td>♦</td>
</tr>
<tr>
<td>Impulsive choice</td>
<td>♦</td>
</tr>
</tbody>
</table>

Arrows indicate the direction of the effects of cannabinoid CB1 and μ-opioid receptor (ant)agonists on baseline motor impulsivity (5-CSRTT) and impulsive choice (DRT).
1.3. Drug addiction: a psychiatric disorder characterized by impulse control deficits

At this point, the focus of the introduction will switch from the neuropharmacology of impulsivity to a pathological condition in which maladaptive impulsivity is an endophenotype and in which cannabinoids and opioids are known to play a role: drug addiction. Due to the lack of adequate pharmacotherapies, drug addiction represents a major global health problem with tremendous societal costs (Kalivas and Volkow, 2011; Potenza et al., 2012). Importantly, drug addiction is not a natural progression from repeated drug use, since depending on the drug, only 10-30% of the initial users are estimated to become addicted (O’Brien, 2008). One can become addicted to a variety of substances, including psychostimulants, nicotine, alcohol, and opioids. In the current research, the focus was on the classical opiate heroin. Although opiates are not the most widely abused substances, in 2009, the United Nations Office on Drugs and Crimes (UNODC) estimated the annual prevalence rate of opiate (heroin and prescription opiates) abuse among the world’s population aged 15-64 to be 0.3-0.5% (12-21 million people abusing opiates at least once that year) (www.unodc.org). Nevertheless, heroin is thought to be one of the most addictive substances, with an estimated risk of addiction of about 25% of initial users (O’Brien, 2008). Moreover, heroin was recently found to be the second most harmful drug by independent expert panels in The Netherlands and the United Kingdom, its harm to users and others only being exceeded by that of alcohol (Nutt et al., 2010; RIVM-rapport 34001001/2009, www.RIVM.nl). Heroin addiction, as all other substance use-related disorders, is a chronic psychiatric disorder characterized by a compulsive pattern of drug seeking and taking (American Psychiatric Association, 2000). Drug addicts will spend most of their time seeking and taking drugs, will repeatedly take more drugs than initially intended, and will continue this behavioral pattern even when faced with serious negative consequences such as loss of health, income/financial security, and/or social life. And even when addicts manage to abstain from drug use for longer periods, they remain at high risk of relapse throughout the rest of their lives. Particularly exposure to drug-associated cues, the previously abused drug (priming), or a stressful situation are known triggers for a relapse (De Wit and Stewart, 1981; Shaham et al., 2003; Epstein et al., 2006; Koob and Volkow, 2010). The persistent vulnerability to relapse is a major challenge in treating drug addiction. The development of effective pharmacotherapies against this phenomenon therefore is of utmost importance. To this end, valid rodent models for relapse behavior are required to aid in elucidating the underlying neurobiology.

1.3.1. A rodent model for relapse to drug seeking

Acquisition, extinction, and reinstatement of drug seeking can be studied in rodents with, for instance, conditioned place preference (CPP) models (Tscherntke, 2007). In CPP models, an animal learns to associate a specific environment with a rewarding stimulus,
e.g. a drug injection it just received from the experimenter. Then, the rewarding value of the reinforcer can be assessed by allowing the animal the choice of spending its time in the reward-associated environment versus another familiar environment that lacks emotional value. Following testing, the environment-drug reward association can be extinguished and subsequently reinstated by drug priming or exposure to stress. However, there are considerable differences in the neuronal consequences of passive versus active drug administration (Jacobs et al., 2003; Chen et al., 2010). Therefore, a more standard, and perhaps more valid way to study drug seeking, or relapse to drug seeking, is by using a self-administration approach. The first paper reporting drug (morphine) self-administration in rats dates from the early sixties (Weeks, 1962). Since then, many rodent self-administration paradigms have been developed, some of which are suitable to study relapse behavior, or at least the reinstatement of drug seeking behavior. In so-called reinstatement models of drug relapse (Figure 1.4a; De Wit and Stewart, 1981; Shaham et al., 2003), a rat initially learns to make an operant response (making a nosepoke or pressing a lever) on the ‘active’ operandum to obtain a reward, e.g. an intravenous injection of heroin. Responses on a second available ‘inactive’ operandum will remain without consequence. Drug delivery is always paired with the presentation of discrete audiovisual cues such that a stimulus-reward association is established upon repeated reward presentations. This way, over a series of daily sessions, a rat will learn to stably self-administer a drug. At this point, the response requirements, i.e. the number of active responses a subject needs to make to obtain one drug reward, can be increased to test a rat’s motivation for obtaining the drug. Following a pre-determined self-administration period, operant responding can gradually be extinguished during a series of extinction sessions in which operant responses are no longer rewarded with drug delivery or cue presentations. In this way, the rat will learn that the previously drug-associated context (the operant chamber) no longer predicts drug availability. This new memory will compete with (and over time suppress) the old context-drug association memory, resulting in a gradual reduction in display of drug seeking behavior. Following extinction of the behavior, operant responding on the previously drug-paired operandum, a behavior that is thought to reflect drug seeking, can be reinstated by response-contingent presentations of the drug-associated (audiovisual) cues, a priming injection of the previously self-administered drug, or application of a mild-stressor (e.g. footshocks). Importantly, rats will not receive any drug during a reinstatement/relapse test. In a variation on the traditional reinstatement model that we decided to use in the current research (Figure 1.4b), the extinction period is substituted for a period of forced abstinence during which the rat remains in its homecage. Drug seeking behavior can subsequently be tested by re-introducing the rat to the operant chamber in which it previously self-administered the drug. This abstinence-relapse model may bear more similarity to the human situation in that most drug addicts do not undergo extinction training (but see e.g. Xue et al., 2012). Moreover, subjecting rats to forced abstinence rather than extinction training usually results in more vigorous drug seeking during a relapse test, which facilitates studying the underlying neurobiology.
Figure 1.4. Schematic overview of reinstatement/relapse models for drug seeking. In the reinstatement model (A), rats learn to make an operant response (a lever press or nose poke) to earn drug rewards. Drug delivery is always paired with the presentation of discrete audiovisual cues. Once self-administration has been acquired, operant responding can gradually be extinguished by discontinuing drug delivery and cue presentations upon responding. Following extinction training, drug seeking behavior can be evoked in the absence of drug delivery by response-contingent presentations of the drug-associated audiovisual cues, a priming injection of the previously self-administered drug, or application of a mild-stressor. In abstinence-variants of the model (B), the extinction training is replaced by a forced abstinence period in the homecage. Illustration adapted from (De Vries and Schoffelmeer, 2005).
Reinstatement models are generally accepted to be the most valid model available to study relapse behavior (Katz and Higgins, 2003; Shaham et al., 2003; Epstein et al., 2006). Although this has sometimes been determined retrospectively rather than prospectively, and frequently quite different administration regiments are used in rodent studies versus clinical settings, reinstatement models seem to have predictive validity with respect to putative pharmacotherapies. Examples here are the opioid receptor antagonist naltrexone for alcoholics and heroin addicts and the cysteine pro-drug N-acetylcysteine for cocaine addicts. Moreover, there appears to be striking similarity in the factors that reinstate drug seeking in rodents (drug-associated environments/stimuli, drug priming, and stress) and those that are thought to induce drug craving and relapse in humans. Construct validity of reinstatement models is difficult to assess (Epstein et al., 2006). Nonetheless, there seems to be overlap in the brain areas and neurotransmitters involved in reinstatement of drug seeking in rodents and drug craving and relapse in human addicts. For instance, there is general agreement about involvement of the dopaminergic and glutamatergic systems in relapse behavior, and brain regions thought to be important include the prefrontal and orbital frontal cortex, the amygdala, and the striatum. Although drug seeking behavior is clearly much more simple and repetitive in rodent models as compared to reality, reinstatement models derive face validity from the fact that rodents to some extent control their own drug intake, that drug seeking can be assessed in the absence of drugs, and that the model incorporates some of the cognitive processes (e.g. associative learning) that drug addicts employ to obtain drugs. On the down-side, reinstatement models usually lack availability of alternative reinforcers, hence, animals are basically ‘forced’ to seek and take drugs. Usually there is also no negative consequence of continued drug seeking and taking, particularly not at the level of a subject’s social environment, because rodents are either singly housed or all animals housed together are subjected to drug self-administration. Moreover, most models do not mimic the normal intake pattern of drug addicts (taking several ‘hits’ throughout the day) as animals are only allowed to self-administer during one brief period of the day. However, more recently an increasing number of laboratories have started to incorporate longer self-administration sessions into their models. In this project, we decided to provide rats access to an operant chamber twice a day instead, to more closely resemble the clinical situation. Finally, for studies on the neurobiology underlying drug seeking behavior, there is the problem of a proper control condition for drug self-administration. Rats usually do not self-administer saline, the standard dissolvent of drugs of abuse in rodent studies. Hence, this condition does not suffice as a control. Food self-administration is also not an optimal control condition, in view of data showing e.g. long-lasting neuroadaptations in the mPFC following sucrose self-administration (Van den Oever et al., 2006). Such alterations may be different from those induced by drug self-administration (Grimm et al., 2002; Schmidt et al., 2005; Koya et al., 2006). Moreover, the obesity pandemic that is currently striking the Western World suggests that also food can be consumed in a compulsive, addiction-like, fashion. Nonetheless, we choose to compare heroin seeking with sucrose seeking, as animals that self-administered sucrose will in the relapse paradigm display comparable levels of reward seeking as compared to heroin-trained rats when tested for relapse behavior (Schmidt et al., 2005; Koya et al., 2006).
way, sucrose animals can at least serve as a 'motor activity' control, since any differences found between heroin and sucrose rats are unlikely to be related to motor activity, i.e. lever pressing, per se.

1.3.2. Cannabinoids and relapse to drug seeking

Regarding the neurobiology of relapse behavior, the three neurotransmitter systems that probably have been studied the most are the dopaminergic, glutamatergic, and GABAergic systems, particularly those expressed in mesocortical and mesolimbic brain areas such as the prefrontal cortex, the amygdala, the ventral tegmental area, the striatum, and the ventral pallidum (Jentsch and Taylor, 1999; Everitt and Robbins, 2005; Kalivas and O'Brien, 2008; Koob and Volkow, 2010; Van den Oever et al., 2010; Kalivas and Volkow, 2011).

Interestingly, this neuronal circuitry is remarkably similar to the one thought to mediate impulsive behavior (see section 1.3). This may be a reason why particularly highly impulsive addicts seem to be at risk for relapse (Jentsch and Taylor, 1999; Perry and Carroll, 2008; Verdejo-Garcia et al., 2008; De Wit, 2009; Winstanley et al., 2010; Dalley et al., 2011).

The neuronal circuitry thought to underlie relapse behavior is also similar to the one that mediates natural rewards such as sex, food, and social behavior. In fact, addictive substances have been hypothesized to 'hijack' the neuronal circuitry underlying natural reward and normal learning and memory processes (Hyman and Malenka, 2001; Kelley, 2004). In view of this theory, it is not surprising that also the eCB and opioid systems have been linked to many drug-related behaviors and effects, including relapse to drug seeking (van Ree et al., 1999; Roth-Deri et al., 2008; Justinova et al., 2009; Serrano and Parsons, 2011). These neuromodulatory systems have even already been exploited as a target for anti-addiction pharmacotherapies. The opioid antagonist naltrexone, for instance, has been shown to have efficacy as a treatment for alcoholics and opiate addicts (Epstein et al., 2006; Koob and Volkow, 2010; Potenza et al., 2011), and may additionally be effective against amphetamine dependence (Jayaram-Lindstrom et al., 2008a,b). This thesis, however, will not discuss the role of the opioid system in drug addiction, but instead focus on the role of the eCB system. For the latter neurotransmitter system, many clinical trials have been conducted with cannabinoid CB1 receptor antagonists such as SR144528A (rimonabant or Acomplia®) for the treatment of obesity (food addiction) and drug addiction (mainly nicotine addiction and alcoholism). Unfortunately, despite promising initial results, in 2008, Acomplia® was withdrawn from the European drug market shortly after its release due to unacceptable mood-related side-effects, including feelings of depression and suicide ideation (Cahill and Ussher, 2011).

The clinical setback with Acomplia® should not defer from the importance of the eCB system in drug addiction. It merely indicates that more research on the exact underlying mechanisms is required, allowing for the development of novel cannabinoid-based pharmacological agents with less severe side-effects. Particularly knowledge on the role of
specific eCBs in addictive behaviors is lacking, largely owing to the complex nature of the eCB system. Consequently, although this is lately improving somewhat, the cannabinoid research field has long been hampered by a lack of selective pharmacological tools to manipulate eCB synthesis and hydrolysis (Di Marzo, 2009, 2011; Wiskerke et al., 2012). Measuring levels and/or release of eCBs has also proven to be challenging (Buczynski and Parsons, 2010). Nonetheless, human genome-wide association studies have repeatedly linked polymorphisms in the genes encoding the CB1 receptor and FAAH to the development of drug addiction (Serrano and Parsons, 2011). More compelling evidence comes from a wealth of animal studies, indicating a role for the CB1 receptor in various addictive behaviors including the rewarding and motivational properties of drugs, drug-induced locomotor sensitization (augmented locomotor response upon repeated drug exposure), the initiation, maintenance, and escalation of drug self-administration (drug taking), and relapse behavior (Maldonado et al., 2006; Wiskerke et al., 2008; Justinova et al., 2009; Serrano and Parsons, 2011). Moreover, CB1 receptor activity has been found to modulate drug-induced dopamine release into the NAc (Kleijn et al., 2012; for review, see Serrano and Parsons, 2011), a phenomenon thought to be shared by all addictive substances to subserve their reinforcing properties (Di Chiara and Imperato, 1988; Sulzer, 2011). The effects of cannabinoid drugs on addiction-like behaviors and drug-induced dopamine release are likely related to findings showing involvement of the eCB system in many different short- and long-lasting forms of drug-induced neuroplasticity in the brain (for review, see Sidhpura and Parsons, 2011). It should be mentioned here that the role of the eCB system in drug addiction has been found to be drug-specific, with data generally indicating a more limited role in psychostimulant addiction as compared to alcohol-, nicotine-, and opiate-dependence (Wiskerke et al., 2008; Serrano and Parsons, 2011).

For reinstatement of drug seeking, however, it is generally accepted that CB1 receptor activation bidirectionally controls cue- and drug-induced reinstatement of seeking behavior for virtually all reinforcers, including food (De Vries and Schoffelmeer, 2005; Fattore et al., 2007; Serrano and Parsons, 2011). In contrast, data on stress-induced reinstatement is more inconclusive, with an initial rat self-administration study showing no effect of SR144528A on food shock-induced reinstatement of cocaine seeking (De Vries et al., 2001) and a recent mouse CPP study showing suppression of forced swim-induced reinstatement of cocaine CPP by acute administration of the CB1 receptor antagonist AM251 (Vaughn et al., 2012). Detailed knowledge on the underlying mechanisms remains scarce, but intracranial infusion studies with CB1 receptor (ant)agonists have pointed to the mPFC and NAc, and possibly also the basolateral amygdala and ventral tegmental area, as brain regions of interest for CB1 receptor modulation of cue- and drug-induced reinstatement of drug seeking (Alvarez-Jaimes et al., 2008; Kodas et al., 2007; Hiranita et al., 2008; Oleson et al., 2012). With respect to the underlying neurochemistry, studies have suggested involvement of the dopaminergic, glutamatergic (metabotropic glutamate receptors), and cholinergic (nicotinic αβ receptors) systems in the relapse-suppressing effects of CB1 receptor antagonists (Phillips et al., 2003; Xi et al., 2006; Hiranita et al., 2008; Oleson et al., 2012). Together, these findings indicate that the role of the eCB system in relapse behavior is likely to be neither locally restricted nor simple. The findings that particularly
CB1 receptor activity in the mPFC and NAc is important for drug seeking is interesting in view of the pivotal role prefrontal-accumbal projections are known to play in regulating this behavior (Shaham et al., 2003; Koob and Volkow, 2010; Van den Oever et al., 2010; Kalivas and Volkow, 2011). The mPFC can be divided in a dorsal and ventral subregion, comprising the anterior cingulate and dorsal prelimbic cortex and the ventral prelimbic and infralimbic cortex, respectively (Heidbreder and Groenewegen, 2003). While the dorsal mPFC projects predominantly to the NAc core subregion, the ventral mPFC mainly projects to the NAc shell subregion (Voorn et al., 2004). Importantly, the two prefrontal-accumbal pathways may have distinct roles in relapse behavior, with the dorsal mPFC-NAc core actively driving the behavior while diminished activity in the ventral mPFC-NAc shell pathway impairs inhibitory control over the behavior (Jentsch and Taylor, 1999; Peters et al., 2009; Van den Oever et al., 2010). Unfortunately, it is as yet neither known in which subregions of the mPFC and NAc the drug seeking-mediating CB1 receptors are located, nor which eCB(s) may be involved. These specific issues were addressed in Chapter 5 of this thesis.

Box 1. In vivo microdialysis

With in vivo microdialysis, an animal is implanted with a probe directed at a specific brain region. Via connecting tubing, this probe is subsequently perfused with artificial cerebral spinal fluid (aCSF). The tip of the probe consists of a membrane. Depending on its characteristics, mainly its chemical properties and the pore size, this membrane is permeable for molecules that are released in the brain, including neurotransmitters. Such molecules can passively diffuse across the membrane into the probe, driven by the difference in the molecule concentrations in the perfusate (aCSF) versus the extracellular space (the concentration will always remain lower in the perfusate due to the constant unidirectional flow of perfusate toward the probe outlet). After passage through the probe, the perfusate can be collected and subsequently analyzed for chemical content using techniques such as high pressure liquid chromatography (HPLC) and mass spectrometry to get a quantitative estimate of extracellular neurotransmitter levels in the brain.

1.4. Aim and outline of this thesis

This introductory chapter underlined that hyper-impulsivity and substance dependence are psychiatric conditions posing a serious social and financial burden on society. For many patients, the currently available pharmacotherapies do not effectively alleviate problems. In the search for novel drug targets for pharmacotherapies against maladaptive impulsivity and drug addiction, it is highly interesting that there are ample data indicating that both the eCB and opioid systems are involved in regulating impulsive and addictive behaviors. However, data is lacking about the conditions under which eCBs and opioids may mediate
these behaviors. Nor is there much known about the underlying neural mechanisms. In this thesis, we intended to answer several of the remaining questions. Our first aim was to characterize the role of the eCB and opioid systems in state-dependent impulsivity, specifically impulsive behavior induced by acute exposure to psychostimulant drugs. We hypothesized that under such conditions, the role of the eCB and opioid systems in impulsive behavior would be even more pronounced as compared to under baseline conditions, which would facilitate studies on the underlying psychopharmacology. A second major aim of this thesis was to further elucidate how the eCB system mediates relapse to heroin seeking.

In Chapter 2 we further examined the role of the endogenous opioid system in impulsive behavior. There is ample evidence for a role of the endogenous opioid system, and particularly µ-opioid receptors, in several of the behavioral and neurochemical effects of amphetamine. Moreover, reciprocal interactions between the dopamine and opioid systems are known to exist in the brain. Thus, we aimed to answer the question whether endogenous opioid systems mediate amphetamine-induced impulsivity in the 5-CSRTT and/or DRT. And if so, which type of opioid receptors is involved, where in the brain are these receptors located, and is there an interaction with the dopamine system?

Then, in Chapter 3, we studied the role of the eCB system in impulsivity. To confirm that the previous findings with WIN55,212-2 and SR141716A on impulsive behavior were not compound-specific and not related to inverse agonism at the cannabinoid CB1 receptor, the effects of the CB1 receptor agonist Δ9-THC and the neutral CB1 receptor antagonist O-2050 were first tested in the 5-CSRTT and the DRT. Subsequently, as the eCB system is another neurotransmitter system that has been implicated in several of the behavioral and neurochemical effects of amphetamine, the role of the eCB system in amphetamine-induced impulsivity was investigated.

In Chapter 4, we studied the role of the eCB and opioid systems in drug-induced motor impulsivity in more detail. Specifically, the question was answered whether the findings of chapters 2 and 3 regarding amphetamine-induced impulsivity in the 5-CSRTT would generalize to another psychostimulant drug, nicotine. In addition, putative cannabinoid-dopamine interactions underlying the behavior were studied here, to advance our understanding of the overlap and distinctions between cannabinoid and opioid regulation of motor impulsivity.

Subsequently, in Chapter 5, we switched to studying the role of the eCB system in opiate addiction, and specifically post-abstinence relapse to heroin seeking. To test the hypothesis that relapse to drug seeking is mediated by eCBs, an in vivo microdialysis technique (Box 1) was employed to measure in vivo release of eCBs in the brain immediately prior, during, and following tests for relapse to heroin or sucrose seeking. Based on previous research by our laboratory and others, the mPFC was chosen as the anatomical site for the measurement.

In the final chapter, Chapter 6, we summarized the main findings of this thesis. Moreover,
we there further integrated our findings with those of other studies in order to provide putative models of how the eCB and opioid systems may modulate impulsive behavior and relapse to heroin seek.