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Exaggerated impulsivity: a cause or a consequence of adolescent repeated ethanol withdrawal?

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DPhil in Psychology

University of Sussex

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I hereby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree.

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The thesis incorporates material already published or submitted in the journals listed below:

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I am grateful to the anonymous reviewers and conference attendees who provided helpful feedback on this work

University of Sussex

Sandra Sanchez-Roige

Doctor of Philosophy in Psychology

Exaggerated impulsivity: a cause or a consequence of adolescent binge drinking?

Binge alcohol drinking is a major public health concern world wide and its occurrence is rising among young adults. Using animal and human subjects, this thesis evaluates the impact of binge drinking during a time of neurodevelopment on aspects of impulse control, and studies the potential of addressing a molecular target, the μ-opioid receptor, to alleviate elevated impulsive-like behaviour.

First, the nature of impulsivity is described in a review paper. We demonstrate the suitability of the Five-Choice Serial Reaction Time Task (5-CSRTT) for measuring one facet of impulsivity, waiting impulsivity, in mice. Bridging the animal and human laboratories, we developed a novel human analogue of the 5-CSRTT (paper 2). Elevated impulsive behaviour was detected in both young human binge drinkers and in an ethanol-preferring strain of mice, suggesting impulsivity to occur as a prelude to heavy alcohol use. In a second approach (paper 3), we studied the long term effects of intermittent alcohol exposure using a mouse model of adolescent binge drinking. We revealed disrupted impulsive behaviour in adulthood in two different inbred strains, which differ in baseline impulsivity and ethanol drinking patterns, indicating that impulsivity is also a consequence of ethanol exposure. In paper 4 we studied the ability of an opioid antagonist to improve top-down control of impulsive behaviour. Consilience between species and paradigms will need to be further addressed in future studies, but antagonising μ-opioid systems may aid in preventing binge drinking by facilitating inhibitory control mechanisms.

Collectively, from animal and human evidence, this thesis will argue that exaggerated impulsivity may result from repeated ethanol withdrawal in adolescence as well as being a pre-existing endophenotype contributing to adolescent binge drinking. Disentangling such a relationship may help delineate new lines of intervention for at-risk individuals.

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This thesis has been ‘co-authored’ by Victor Baro. He has been the first audience for all my talks: he knows about Open Field, and Plus Maze; he knows that DBA are grey; he knows that *impulsivity is not a unitary construct.* Thank you for encouraging me to draw, dance, sing, write. Thank you for following me where science takes me.

I dedicate this to my brothers. I wish that you achieve all your purposes in life. To my mum, and dad, g*ràcies per confiar en mi, obrir-me totes les portes i ajudar-me a conseguir els meus propòsits*. Thanks to all my friends, those who I miss (Yas, Patri) and those whom I have had the opportunity to be with during those years at Sussex (Yolandica!, Miguel, Vane, Nadia). Wish, I am forever grateful to you.

I ‘*cannot wait*’ to see what the future holds for all of us!

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LIST OF ABBREVIATIONS

5-CSRTT	Five-choice serial reaction time task (rodent version)
AAIS	The Adolescent Alcohol Involvement Scale
Acq	acquisition
AD	Alzheimer's disease
AUC	Area Under the Curve
AUD	Alcohol Use Disorder
AUDIT	Alcohol Use Disorder Identification Test
AUQ	Alcohol Use Questionnaire
B6	C57BL/6J
BD	Binge drinking (Chapter 1, 6); Binge drinkers (Chapter 3)
BXD	Recombinant Inbred strains
C.L.	Correct Latency
C57	C57BL/6J
CON	control group
CPT	Continuous Performance Test
D2	DBA/2J
DA	Dopamine
DBA	DBA/2J
DD	Delay Discounting paradigm
DDQ	Delay Discounting Questionnaire
DLPFC	Dorsolateral prefrontal cortex
ERP	event-related potential
EtOH	Ethanol
F	Female
fITI	fixed inter-trial interval
fMRI	functional magnetic resonance imaging
GNG	Go/no-Go task
h	hour

i.p.	intraperitoneal injection
IEE	Intermittent ethanol exposure (mouse model of binge drinking)
IGT	Iowa Gambling Task
ITI	Inter-trial interval
KO	knock-out mice
LH	Limited Hold
LOFC	lateral orbitofrontal cortex
Long.	longitudinal
M	Men
Mag lat	Magazine latency
mIGT	mouse Gambling Task
min	minutes
ml	millilitres
mRNA	Messenger RNA
N/Y	not applied / applied
NBD	Non binge drinkers
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NTX	Naltrexone
p	Probability
PDH	Personal Drinking Habits
perf	Performance
Persev	Perseverative response
PFC	Prefrontal cortex
PND	Post natal day
Prem	Premature response
PWS	Prader-Willi syndrome mouse model
R	Reinforcer
rIFC	right Inferior Frontal Cortex
rIGT	rodent Iowa Gambling Task
s	seconds

s.c.	subcutaneous injection
SAMHSA	National Survey on Drug Use and Health
SD	Stimulus Duration
SE	Standard Error of Mean
sess	session
S-MAST	Short-Michigan Alcoholism Screening Test
snca	alpha-synuclein
SP	electroencephalographic potential
SPC	Superior Parietal Cortex
SSRTi	Stop Signal Reaction Time
SSS	Sensation Seeking Scale
SST	Stop Signal Reaction Time Task
SURPS	Substance Use Risk Profile Scale
Sx-5CSRTT	Sussex Five-choice serial reaction time task (human version)
TCIP	Two Choice Impulsivity paradigm
TLFB	TimeLine Follow-Back
TO	Time Out
TT	Total trials
UPPS	Impulsive Behaviour Scale
vITI	variable inter-trial interval
vmPFC	ventromedial prefrontal cortex
w.	week
WT	wild-type
Y	years of age

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Chapter 1

EXAGGERATED IMPULSIVITY AND ADOLESCENT ALCOHOL BINGEING

GENERAL OVERVIEW AND AIMS OF RESEARCH

1.1 Overview

The adolescent brain undergoes complex and rapid changes, and environmental factors might shape the trajectory of development. Heightened impulsivity is common during this time, and inappropriate levels of inhibitory control may put the adolescent at risk for increased drug experimentation. Indeed, alcohol misuse is highly prevalent among adolescents and the consequences of ethanol experience on brain function are of increasing concern amongst neuroscientists, clinicians and politicians. A large fraction of this thesis will focus on how alcohol consumption, and particularly binge drinking (referred to as 'BD') habits during adolescence might result in impulse control deficits. Given the heterogeneity of the impulsivity construct, this thesis will examine the consequences of BD on different impulsivity aspects, using animal and human subjects. The use of homologous measures across species will allow direct translation of discoveries in mouse to human studies; likewise, hunting for vulnerability markers, such as excessive impulsive behaviour, that can predispose to and/or result from BD, may help design effective interventions relevant for alcohol misuse and impulsive-like disorders.

The goal of this introductory chapter is to review recent behavioural and biological research, aimed at understanding the complex relationship between impulsivity and alcohol use. The chapter is organized into 4 sections, corresponding to: (i) heightened impulsivity during adolescence, and its relationship with BD; (ii) the nature of impulsivity and how it can be measured; (iii) impulsivity deficits and alcohol misuse (and *vice versa*) during adolescence and young adulthood. The closing section will provide an overall view of the aims of research and the battery of experiments conducted to study the interaction between inhibitory control and adolescent BD.

1.2 ADOLESCENT IMPULSIVITY AND ALCOHOL BINGEING

For many teenagers, impulsive actions and decisions are part of their daily life: speaking out of turn in class; attending a party the day prior to an important exam; buying the first pair of expensive trainers rather than shopping around to get a better deal. Thus, definitions of impulsivity include a wide range of seemingly maladaptive behaviours including actions that are premature and mistimed, suggesting inability to wait or withhold responses (i.e. restraining, stopping or postponing a response); and defective decision-making (i.e. acting without thinking of possible negative outcomes).

This is unsurprising: adolescence is a developmental period in humans and other species characterised by high levels of impulsivity, novelty seeking and risk-taking (Spear, 2000; Dahl, 2004). Such behaviours may reflect the incomplete maturation of the prefrontal cortex and executive functions (Huttenlocher, 1984; Luna et al., 2004; Mitchell et al., 2008; Helfinstein and Poldrack, 2012), and the subcortical motivational systems to which the prefrontal cortex is linked (Chambers et al., 2003; Ernst et al., 2006a). Although these developmental processes can be advantageous [i.e. facilitate the transition to adult roles (Andersen, 2003)], they may confer increased vulnerability to the addictive actions of drugs (Chambers et al., 2003), such as alcohol.

Indeed, adolescents abundantly indulge in excessive drinking. Alcohol use is common during adolescence (Windle et al., 2008), with statistics informing us that adolescents (12% 13–14 years old, followed by 22% of 15-16 years old, and 28% 17-18) experience at least one episode of heavy drinking (i.e. > 5 consecutive alcohol drinks) in a period of two weeks (Masten et al., 2008). In western countries, adolescents (13-18 years of age) and young adults [19-24 years of age, (NLM, 2014)] engage in BD habits more often and to a greater extent than older (>24 years old) adults (Plant and Plant, 2006; Bava and Tapert, 2010). It is estimated that excessive alcohol use causes a net harm of 3.7% of all deaths and 4.4% of the global burden of

disease (WHO, 2007). Of particular concern are the damaging effects of alcohol abuse in adolescents (Crews et al., 2000; Spear, 2013). Repeated alcohol bingeing may interrupt crucial neuromaturational processes (c.f. Petit et al., 2014), such as inhibitory control mechanisms (Lopez-Caneda et al., 2014), and constitute a vulnerability factor for subsequent alcohol drinking.

BOX 1.1 WHAT IS BINGE DRINKING AND HOW CAN WE MEASURE IT?

The definition of BD is still under debate but what appears most clear is that this pattern entails repeated heavy episodes of alcohol consumption followed by periods of abstinence. Endorsed by The National Institute on Alcohol Abuse and Alcoholism (NIAAA, 2004), BD was characterized by the quantity of alcohol consumed during a drinking event (i.e. 'binge episodes': consumption of ≥5 drinks, in males, or ≥4 drinks, in females, within ≥ 2 hours, that is likely to raise blood alcohol concentration [BAC] to ≥ 0.08 g %). However, this definition accounts for single BD episodes, and does not capture the consumption pattern associated with BD.

Based on the proposal that withdrawal from alcohol may contribute to the development of addiction ["withdrawal sensitization theory of addiction"; (Stephens, 1995)], the work of Duka and colleagues focused on the relevance of patterns of drinking in identifying BD, in addition to including aspects of alcohol consumption (Townshend and Duka, 2002; Weissenborn and Duka, 2003; Townshend and Duka, 2005). Encompassed in their BD Score (Townshend and Duka, 2005), characterization of BD should capture a behavioural/physiological component (i.e. times drunk within the past 6 months, percentage of time being intoxicated when drinking) beyond the mere act of drinking (i.e. drinks consumed per hour), which may only involve levels of intake and BACs that result in intoxication for individuals without dependence of alcohol.

Given the aforementioned, a definition of BD should integrate three factors: a) quantity of alcohol consumed (taking into account gender), b) time-frame of consumption (i.e. "on more than one occasion"), c) time period of past BD episodes [i.e. past week, 2 weeks, 1 month, 3 months, 6 months, 1 year; see (Courtney et al., 2012) for a review]. The operational definition of BD proposed should facilitate inferences across studies.

1.3 DISSECTING 'IMPULSIVITY'

1.3.1 Inhibitory Control Processes

Some authors maintain that inhibitory control is one of the major top-down process (Dempster, 1991; Barkley, 1997; Duncan et al., 1997; Aron, 2007) involved in self-regulation, and associated with executive control capacity (Bari and Robbins, 2013). Top-down processes constantly self-monitor split-second decisions whereby the individual must inhibit a response (Miyake et al., 2000; Stuphorn and Schall, 2006), such as control over drinking. Failure of the inhibitory processes will reduce the ability to self-regulate and weaken control over alcohol-driven behaviours (Tucker et al., 1995; Hofmann et al., 2008). During adolescence, both elevated impulsivity and experimentation with alcohol-drinking behaviours occur. This parallelism gave rise to the idea that adolescent BD may occur as a consequence of defective impulsivity, that is, when top-down control mechanisms are disrupted.

1.3.2 Fractionation of impulsivity

When studying alcohol and inhibitory control mechanisms, one must bear in mind the multifaceted nature of the impulsivity construct. As extensively reviewed, the term 'impulsivity' (often used interchangeably with 'behavioural inhibition') may represent a family of functions rather than a single, unitary construct (Evenden, 1999; Robbins and Everitt, 1999; Nigg, 2000; Friedman and Miyake, 2004; Winstanley, 2007; De Wit, 2009), with independent underlying neurobiological and neurochemical substrates (Dalley et al., 2008). Addressing this diversity, different paradigms have been developed to measure those constructs, including questionnaires, interviews, electrophysiological assessments, and a wide assortment of behavioural tasks. This section will present a brief overview of the measurements available and their effectiveness to capture deficits resulting from alcohol use.

1.3.3 Impulsivity measured by self-report questionnaires

A commonly used technique to quantify and qualify impulsivity in normal adults and patient populations is the use of **self-report questionnaires**, such as the Barratt Impulsiveness scale BIS-11 (Patton et al., 1995), and in more general assessments of personality (Cloninger, 1987). In a clinical setting, questionnaires have been effective tools to predict development of alcohol use (Kirisci et al., 2007), with higher traits of impulsivity being detected in alcoholic subjects (Vonknorring et al., 1985; Finn, 2002) and binge drinkers (see **Table 1.1**, section i). This information may be useful to dissociate the causality role of impulsivity in relation to alcohol use. In this sense, high impulsivity *traits* may predispose individuals to consume alcohol, which in turn may lead to further (*state*) impulsivity, manifested in a failure to control drinking behaviour (Balodis et al., 2009).

Self-report measures can provide evidence of 'trait' impulsivity (stable personality characteristic), whereas performance-based tests may measure aspects of 'state' impulsivity (influenced by environmental variables). Although self-report measures are useful, this methodology is limited as it may be influenced by subjective bias [e.g. less insight of the inhibitory control deficits in alcoholics (Helmers et al., 1995)] or by the subject's state (Wingrove and Bond, 1997). To circumvent those issues, laboratory tests may more readily facilitate our understanding of the consequences of alcohol use on impulsive behaviour.

1.3.4 Impulsivity measured by behavioural paradigms

Using a variety of laboratory-based paradigms, impulsivity can be fractioned into separate and quantifiable different domains, both in human and non-human subjects. A simplified synthesis of these tests has been illustrated in **Figure 1.1**, which can be broadly divided into two distinct categories (Winstanley, 2007): those measuring **impulsive choice** (decision-making without appropriate deliberation over the alternative options), and those assessing **impulsive action** or motoric response (associated with a lack of behavioural inhibition).

Behavioural paradigms to capture the multifaceted nature of impulsivity

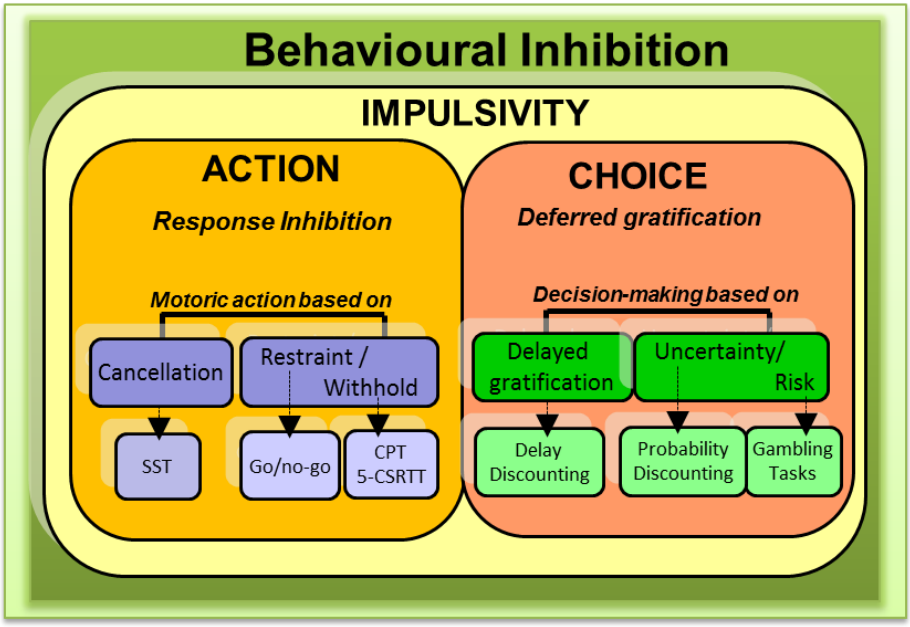

Figure 1.1 Highly schematic depiction of the different facets of impulsivity that can be modelled in laboratory human and animal subjects, using a variety of behavioural paradigms. Broadly, the construct of impulsivity has been divided into impulsive action (response inhibition) and impulsive choice (deferred gratification). Within the former subtype, it has been suggested that tasks that measure action cancellation (i.e. already initiated response), such as the stop-signal reaction time task (SST), are distinct from tasks that measure restraint or withholding a response (i.e. prior initiating a response), such as the go/no-go task, and the continuous performance test (CPT) or five-choice serial reaction time task (5-CSRTT), respectively. Within the second subtype, delay-discounting paradigms have been developed to assess decision-making based on delayed gratification, and tests of probability-discounting and gambling-related decision-making, to measure decisions based on risk or uncertainty. This diagram has been adapted from Winstanley et al. (2010)

1.3.4.1 Alcohol use and 'Action' impulsivity. Impulsivity may occur at the point of inhibiting a response: actions that have to be cancelled when a 'stop' signal is presented after initiating a response, restraint when a 'no-go' signal is presented, or postponed until a 'go' sign appears (waiting). Three well-validated laboratory-based tests that have been developed to measure the level of impulsive action in humans are the stop signal reaction time task (SST), Go/no-Go (GNG) tasks and Continuous Performance Test (CPT); with appropriate animal analogues (Eagle et al., 2008; Winstanley et al., 2010). From those tasks, two main processes can be distinguished (Dalley et al., 2011): action cancellation (SST), and action restraint (CPT, GNG). Thus, although the three paradigms similarly index impulsivity in terms of the ability of a

subject to withhold or inhibit a prepotent response (Band and van Boxtel, 1999), they represent distinct impulsivity facets with distinct behavioural and neural grounds.

The SST is one of the most common measures of behavioural inhibition (Logan et al., 1984; Feola et al., 2000; Eagle et al., 2008). Subjects must learn to respond quickly (i.e. pressing a button) to a 'go' stimulus (e.g. to the direction of an arrow presented on a screen). In some trials, a 'stop' signal is presented after receiving a 'go' signal. On such occasions, the subject must learn to cancel responding. Defective impulsivity will be inferred from the failure to inhibit the response once its execution has been initiated (i.e. ability to 'stop'). Goudriaan and colleagues (2006b) found that alcoholics revealed weaker inhibitory efficiency, reflected by the increased stop signal reaction times. On the other hand, on GNG paradigms, subjects are required to quickly respond in the context of certain stimuli ('go' signal), and restrain in the context of different stimuli ('no-go' signal) (Terman and Terman, 1973). Alcoholic patients have shown more commission errors for pressing the key in the presence of a 'no-go' signal, which indicates less efficient action cancellation [(Bjork et al., 2004; Kamarajan et al., 2006), for a review (Verdejo-Garcia et al., 2008)].

Another facet of impulsivity is acting without thinking, which may be operationalized as poor inhibitory control in tests of stimulus-discrimination. For those purposes, the CPT (Carli et al., 1983) is a test of attentional function that incorporates an aspect of 'action' (or 'motor') impulsivity (Winstanley, 2007). In brief, subjects are required to detect and respond to a brief stimulus. Responding prior to appearance of the stimulus is considered as a failure of inhibitory control, and alcoholic patients have shown difficulties in performing effectively (i.e. inability to 'wait') in a more complicated version of the task (Bjork et al., 2004). A rodent analogue of the CPT, the 5-CSRTT, has been well described in rats (Robbins, 2002) and mice (Sanchez-Roige et al., 2012), as I will review in *Chapter 2*. However, the murine 5-CSRTT and the human CPT are not exact analogues. It is the aim of our research (*Chapter 3*) and that of

others (Voon et al., 2014) to develop a human version of the 5-CSRTT, facilitating comparisons across species.

1.3.4.2 Alcohol use and 'Choice' impulsivity. A final category of procedures used to assess impulsivity relates to decision-making or impulsive choice. In everyday life, individuals are faced with decisions between different outcomes (e.g. rewards) at different *time points* (e.g. sooner or later). Such decisions are captured in delay-discounting paradigms (Ainslie, 1975). Alternatively, impulsive decisions can also occur in the context of *uncertainty*, as measured in the probability discounting task (Mischel and Grusec, 1967), or under *ambiguous*, 'risky', situations, investigated via gambling tasks [humans, c.f. (Brevers et al., 2013); rodents, (Rivalan et al., 2009; Zeeb et al., 2009; van den Bos et al., 2014)].

One of the most successfully utilised measurements of impulsivity is intolerance to delay, using the delay discounting paradigm. Measures of delay discounting are based on the operational definition of a relative preference for a smaller, immediate reward over a larger, but more delayed reward (Rachlin and Green, 1972). Under these circumstances, more impulsive individuals show steeper temporal discounting rates, indicating that delayed rewards have a smaller perceived value amongst impulsive individuals (Ainslie, 1975; Cardinal et al., 2004). On this task, alcohol-dependent subjects were more sensitive to delay than healthy subjects (Petry, 2001; Mitchell et al., 2005; Joos et al., 2013). Intriguingly, early-onset alcoholics were also more impaired in the self-reported questionnaire version of this task (Dom et al., 2006), in comparison to late-onset alcoholics. This finding suggests that drinking onset may have a different developmental effect according to the age of first drink (Cloninger et al., 1981). Such distinction may be clinically relevant, as it argues for a more tailored treatment of impulse and alcohol-related disorders. Studies should incorporate this information when evaluating the effects of BD on impulsive behaviour; failure to do so may explain discrepancies among laboratories or lack of findings in non-differentiated groups (Kirby and Petry, 2004).

Just as delayed delivery can diminish the value of a large reward, so can the probability of receiving such reward. In probability-discounting paradigms, subjects are required to choose either a small but certain reward *versus* a larger but increasingly uncertain reward. On each trial, the subject either gains or fails to gain reward. Risky decision-making would be reflected by the increased preference for large but improbable rewards. On the other hand, the tendency to adopt risky behaviour under ambiguous conditions can be assessed with the Iowa Gambling Task [IGT; (Bechara et al., 1994)], which has long proved its efficacy in detecting decision-making deficits in alcoholic dependent patients (Mazas et al., 2000; Goudriaan et al., 2005; Tomassini et al., 2012; Zorlu et al., 2013). Alcohol-dependent subjects show altered performance on the IGT by making choices that favour large rewards but lead to larger penalties and are thus disadvantageous over the experimental session.

1.3.5 Summary: which facet of impulsivity is most associated with alcohol use?

In the opening paragraphs, I have clarified the nature of impulsivity and discussed the sensitivity of the available measures in detecting deficits resulting from alcohol use. Although alcohol misuse has been associated with impulsivity deficits, studies rarely administered simultaneous multiple testing to evaluate alcohol effects on the different facets of impulsivity (Dougherty et al., 2005). Considering the heterogeneous nature of the impulsivity construct, it is possible that each facet could be involved in separate aspects of initiation and maintenance of alcohol bingeing and their contribution to alcohol use disorders (Lejuez et al., 2010). Moreover, deficits in such control mechanisms may appear either premorbid (Lyvers, 2000; Ersche et al., 2012; Volkow and Baler, 2012), or as a consequence of long-term alcohol use and/or of repeated experiences of alcohol withdrawal (Volkow et al., 2003; Stephens and Duka, 2008; Duka et al., 2011). The following section elaborates this hypothesis further.

1.4 INHIBITORY CONTROL DEFICITS: A CAUSE OR A CONSEQUENCE OF ALCOHOL MISUSE?

1.4.1 Impulsivity deficits: risk factor for alcohol misuse

A very important line of research concerns the source of the inhibitory control deficits. As a cause, deficits in behavioural inhibition may be present prior to alcohol initiation. Multiple studies have documented that **pre-existing levels of high-impulsivity** in childhood are associated with early alcohol use in adolescence [early drinking onset age (Tarter, 2002; Clark et al., 2005; Kirisci et al., 2006; Wong et al., 2006); alcohol dependence (Ernst et al., 2006b)]. For instance, elevated rates of trait impulsivity at age 11 predicted drinking onset by age 14 (McGue et al., 2001); whereas poor response inhibition in early adolescence (12-14Y) prospectively predicted alcohol use in late adolescence [15-17 years (Nigg et al., 2006)]. Similarly, Fernie and colleagues (2013) found that three components of impulsivity, namely response inhibition (SST), risk taking and delay discounting, predicted change in alcohol involvement 6 months later. Hence, from both self-reported and behavioural measures of impulsivity, there is strong evidence to suggest elevated impulsivity to predict early onset of alcohol misuse (Tarter et al., 1999).

There is also a suggestion that the links between impulsivity and alcohol use may be **genetically mediated**. One way to address this issue is to examine adolescents who have no or minimal alcohol exposure, but who have a positive family history for alcoholism. Using this approach, prospective studies have been able to show an association between parental substance use disorders and eventual development of drug misuse in offspring (Tarter et al., 1999; Tarter, 2002; Tarter et al., 2003; Tarter et al., 2004). For instance, Kendler and colleagues (2002; 2003) found that the adolescent offspring of parents with substance use disorders showed elevated alcohol dependency, and lower response inhibition (Nigg et al., 2004; Schweinsburg et al., 2004; Habeych et al., 2006), which is hypothesized to be due to the impulsive endophenotype (Verdejo-Garcia et al., 2008). This hypothesis is further supported by

anatomical and functional structure abnormalities detected in frontal regions involved in inhibitory control in the offspring of alcoholic parents (Schweinsburg et al., 2004; Hill et al., 2009; Heitzeg et al., 2010; DeVito et al., 2013), suggesting that these abnormalities may predispose the children to initiate alcohol use during adolescence (Norman et al., 2011). By this account, adolescents from families with history of alcohol misuse may be predisposed to excessive drinking via disruptions in their inability to inhibit a response (Wetherill et al., 2013) or due to altered delayed gratification, as detected in the steeper delayed gratification in the offspring of alcoholics (Petry et al., 2002), which may lead to heavy drinking. Taken together, the fact that elevated impulsive behaviour is seen in the offspring of parents with a history of alcohol abuse suggests that impulsivity may be a risk factor for alcohol misuse.

Another approach used to investigate the genetic factors contributing to alcohol use and impulsivity is the use of animal lines selectively bred for differences in alcohol consumption. The association between excessive alcohol intake and high impulsivity is not limited to humans, and mouse and rat lines selected for alcohol consumption have also been shown to differ on certain measures of impulsivity [*action* (Wilhelm et al., 2007), *choice* (Wilhelm and Mitchell, 2008; Oberlin and Grahame, 2009)], which again suggests that some genes that contribute to alcohol consumption do play a role in impulsivity [e.g. genetic components for alcoholism (Goodwin et al., 1974), BD (Herman et al., 2003), and impulsivity (Isles et al., 2004)], and shared genetic risk among these attributes (Young et al., 2000; Krueger et al., 2002; Slutske et al., 2002)].

A second strategy is to compare behavioural impulsivity and alcohol use across inbred strains (see **Box 1.2**). As in humans, pre-existing differences in impulsivity have been correlated with differences in alcohol-related behaviour across strains. For instance, Logue and colleagues (1998) showed a genetic correlation between poor inhibitory control and augmented alcohol intake across 13 inbred mouse strains; that is, mice that were able to inhibit a prepotent response during a single-nose poke task (i.e. less impulsive), also consumed less alcohol (and

vice versa). This observation has been replicated for other impulsivity subtypes, where increased sensitivity to delays was associated with higher alcohol intake in inbred rats (Poulos et al., 1995; Poulos et al., 1998; Wilhelm and Mitchell, 2009). Taken together, those studies provide further evidence for a genetic basis of behavioural inhibition (Gubner et al., 2010) and sensitivity to delay (Mitchell et al., 2006; Wilhelm and Mitchell, 2009), in addition to revealing a link between innate levels of impulsivity and response to alcohol [c.f. (Crabbe, 1996; Dick et al., 2010; Mitchell, 2011)]. Although these studies are only correlational (i.e. do not prove that impulsivity gives rise to alcohol abuse), these observations suggest that behavioural undercontrol may be associated with high alcohol consumption.

BOX 1.2 INBRED STRAINS FOR THE STUDY OF IMPULSIVE PHENOTYPES AND GENETIC INFLUENCES CONTRIBUTING TO ALCOHOLISM

Inbred strains are generated by mating male and female siblings over 20 consecutive generations. The offspring crossing procedure results in an inbred strain in which all animals are genetically identical, resembling identical human twins. Thus, any differences between individual members of a particular strain can most likely be attributed to environmental influences.

McClearn and Rodgers (1959) published seminal articles using the inbred strains approach for the study of alcohol-related traits. In those studies, levels of free alcohol consumption differed over tenfold among strains. The mice were raised under identical environmental conditions, thus the behavioural differences detected must have resulted from genetic differences between strains.

A rich diversity of inbred mouse strains is available (Crawley et al., 1997). C57BL/6J (B6) and DBA2/J (D2), pictured in **Figure 1.2**, are perhaps the two most commonly used inbred strains of mice in behavioural and pharmacological genetics, which differ markedly in their sensitivity to the rewarding and locomotor stimulatory actions of ethanol. B6 mice display high levels of ethanol preference as well as relative insensitivity to ethanol withdrawal, while D2 mice display very low ethanol preference and high withdrawal sensitivity (Crabbe et al., 1994). The pair of strains will be used in *Chapters 3* and *4* to disentangle the effects of BD on individuals that present opposed ethanol preference and impulsive phenotypes.

Figure 1.2 *Testing the impulsive phenotype in mice*

1.4.2 Exaggerated impulsivity: consequence of alcohol use

Conversely, alcohol use itself may increase maladaptive impulsive behaviour, either through its direct, acute effects or because of long-term consequences of alcohol use. **Table 1.1** represents a first attempt to synthesize this literature in human binge drinkers.

1.4.2.1 Acute effects of alcohol. A first line of research has investigated the effects of acute alcohol on measures of impulsivity. From overwhelming data from animal and human subjects, it is well known that alcohol can trigger and exacerbate impulsive tendencies (**Table 1.1**, ii).

On the one hand, acute doses of alcohol have been found to alter measures of response inhibition. Under alcohol intoxication, performance on the SST was found to be impaired in young healthy adults (Mulvihill et al., 1997; Caswell et al., 2013), and in young binge drinkers (Fillmore and Vogel-Sprott, 1999; Easdon and Vogel-Sprott, 2000), with three studies indicating that decreased activity in frontal regions may explain the alcohol-induced deficits (Anderson et al., 2011; Schuckit et al., 2012; Nikolaou et al., 2013). Similarly, decreased behavioural control following acute alcohol administration on a GNG paradigm has also been documented in both healthy humans (Marczinski et al., 2005; Rose and Duka, 2007; Fillmore and Weafer, 2012; Miller and Fillmore, 2013) and in rats with high alcohol drinking (Tipps et al., 2014). Further, the acute effects of alcohol may be accentuated in young binge drinkers (Marczinski et al., 2007; Shannon et al., 2011). And this is relevant, as it invokes not only increased impulsivity to elevate alcohol consumption (Weafer and Fillmore, 2008), but suggests that BD during adolescence may alter the response to the disinhibiting effects of alcohol (Norman et al., 2011; Wetherill et al., 2013).

On the other hand, acute doses of alcohol failed to affect aspects of choice impulsivity, using a delay discounting paradigm in healthy young adults (Richards et al., 1999; Ortner et al., 2003), in contrast to findings in laboratory animals (Poulos et al., 1998; Tomie et al., 1998; Olmstead et al., 2006). This raises the question that cross-species paradigms of delay gratification are not

exact analogues; whereas human subjects chose between two *fictional* rewards and delays, animal subjects receive delays and rewards in *real time*, which suggest that rodent delay-discounting paradigms may be more sensitive in capturing the effects of alcohol than questionnaire-based methods (Winstanley, 2011). To improve upon pen-and-paper measures of delay discounting in humans, some researchers have included the Two Choice Impulsivity Paradigm for assessment of choice impulsivity (Dougherty et al., 2005). This task follows a similar approach to delay discounting, but participants are required to make a choice (two shapes presented on a screen) and *experience* the delays (5s vs. 15s) associated with each. Using this measure, McCarthy and colleagues (2012) reported increased choices for immediate options in moderate drinkers following acute doses of alcohol. Thus, the question remains whether the acute effects of alcohol on delay gratification are exacerbated in individuals with BD history, as has been shown in rats (Mejia-Toiber et al., 2014), or if a similar effect is detected for different measures of choice impulsivity. Indeed, when intoxicated, young binge drinkers showed increased risk-taking for probabilistic (Rose et al., 2013), but not delayed, rewards (Bidwell et al., 2013), suggesting that effect of alcohol on the subtypes of impulsivity may be dissociable.

Table 1.1 – Consequences of Human Binge Drinking on Self-reported, Behavioural, Electrophysiological and Neuroanatomical measures of Impulsivity

Authors	Age (Y)	Sample	Impulsivity domain	Task/Measure	Acute EtOH	Key Findings (BD effects)	
Balodis et al. (2009)	19-31	PDH:(4/5 measure),≥1/ w., 6months; M&F	Trait	BIS-11	N	↑ self-report "feeling less intoxicated"	Trait (i)
Balodis et al. (2010)	19-27 (M=19)	See above	Trait	BIS-11	N	↑ ↔polysubstances use	
Carlson et al. (2010)	≥19	NIAAA, M&F	Trait	BIS-11	N	↑ motor impulsivity ↔ # BD events	
Carlson and Johnson (2012)	≥19	See above	Trait	BIS-11	N	↑ ↔ with positive expectancies 'to get drunk'	
Fossati et al. (2001)	M=23	4-point scale alcohol intake; M&F	Trait	BIS-11	N	↑ motor impulsivity	
Wellman et al. (2014)	Long. 22-28	SAMHSA, M&F	Trait	Eysenck	N	↑, ↔ earlier BD onset	
VanderVeen et al. (2013)	18-24	NIAAA, 6 months+ >8AUDIT,M&F	Trait	UPPS	N	↑ in binge drinkers + smokers	
Adan (2012)	18-25	NIAAA, ≥1 past month; M&F	Trait	Dickman's Impulsivity Inventory	N	↑	
Castellanos-Ryan et al. (2011)	Long. 14-16	NIAAA, ≥1 past month; M&F	Trait Action- cancellation	SURPS Stop Task	N	↑Impulsivity ↔ conduct disorder; ↑sensation seeking↔ BD ≠ Stop Task	
Bauer and Ceballos (2014)	18-20	AUDIT: ≥6 drinks, ≥1/w., past month; F	Trait Genetic marker Neural differences	BIS-11, SSS CHRM2,GABRA2, ANKK1 SP	N	↑ planning impulsivity ↔ C*HRM2* genotype Slow SP right parietal cortex ↔ Time Estimation deficits	

Table 1.1 – (continued 2)

Authors	Age (Y)	Sample	Impulsivity domain	Task/Measure	Acute EtOH	Key Findings	
Marczinski et al. (2008)	≥19	S-MAST, PDHQ, M&F	Trait	BIS-11	Y	≠ trait Reduced subjective intoxication in binge drinkers	Acute alcohol (ii)
Marczinski and Fillmore (2009)	≥19	S-MAST, PDHQ, M&F	-	-	Y	BD ↔ tolerance and willingness to drive when intoxicated	
Marczinski et al. (2007)	≥19	S-MAST, PDHQ, M&F	Trait Action – Restraint	BIS-11 GNG	Y	↑ disinhibition in binge drinkers (vs. non binge drinkers) when intoxicated	
Weafer and Fillmore (2008)	21-26	S-MAST, PDHQ, M&F	Trait Action - Restraint	BIS-11 GNG	Y	↑dishinibition ↔ ↑alcohol consumption in BD	
Easdon and Vogel-Sprott (2000)	19-22	PDHQ, M&F	Action - Cancellation	GoStop	Y	↑ when intoxicated	
Fillmore et al. (2012)	21-31	S-MAST Heavy BD, M&F	Action - Cancellation	GoStop	Y	Faster recovery in heavy binge drinkers vs. light-drinkers (alcohol tolerance)	
Shannon et al. (2011)	N/S	AUDIT, NIAAA, M&F	Trait Action – Cancellation	BIS-11 GoStop	Y	≠ BIS-11 ↑ when intoxicated ↔ ↑ alcohol sedation	
McCarthy et al. (2012)	M=21	NIAAA, M&F Drink drivers vs. non drink drivers	Trait Action - Cancellation Choice – Delay	UPPS-P scale SST TCIP	Y	≠ trait ≠ baseline, ↑ TCIP impulsivity in drink drivers while intoxicated	
Rose and Grunsell (2008)	18-25	≥10 drinks/week; M&F	Trait Choice –Delay	BIS-11 TCIP	Y	≠ trait Less sedation to acute alcohol	

Table 1.1 – (continued 3)

Authors	Age (Y)	Sample	Impulsivity domain	Task/Measure	Acute EtOH	Key Findings
Townshend et al. (2014)	18-34	AUQ (BD Score), M&F	Reflexion	Information Sampling Task	N	↑reflection-impulsivity ↔ unplanned sexual encounters
Townshend and Duka (2005)	18-30 (M=20)	See above	Cognitive	Vigilance Task	N	↑disinhibition
Dougherty et al. (2004)	≥21	Drinks/week Age drinking onset; M&F	Trait Action - 'waiting'	BIS-11 Immediate (IMT) and Delayed Memory Tasks (DMT)	N	≠ BIS-11 Early drinking onset (<18 Y) ↔ ↑ commission errors in DMT (but not IMT) vs. Late drinking onset (>21 Y)
Nederkoorn et al. (2009)	M=21	>5 drinks/event, 2w.; M&F	Trait Action - Cancellation	BIS-11 SST	N	↑ response disinhibition in women with heavy BD habits
Scaife and Duka (2009)	18-29	AUQ (BD Score), M&F	Action - Cancellation	Reaction Time Task	N	↑ disinhibition ↔ inferior frontal gyrus function
Henges and Marczinski (2012)	18-21	TLFB,PDHQ (NIAAA,drinks /w.)M&F	Trait Action – Restraint	BIS-11 GNG	N	≠ BIS-11 ↑ GNG ↔ ↑ drinks/event
Moreno et al. (2012)	18-24	CAGE: ≥6drinks/weekend, past month;M&F	Trait Choice – delay, risky Action – Restraint, Cancellation	BIS-11, SSS-V TCIP, IGT GNG, SST	N	↑ BIS-11 (total, motor, non-planning), ↑ SSS dishinibition ≠ GNG, SST, TCIP ↑IGT
Fernie et al. (2013)	Long. 12-13Y	AAIS: 6 months, % 'drunk'; M&F	Choice –delay, risk Action – cancellation	DD, BART SST	N	DD, BART and SST ↔ alcohol involvement 6 months later Alcohol use did not alter impulsivity
Goudriaan et al. (2007)	17.96 Long 2Y	NIAAA, ≥2/w., past month	Choice - risk, ambiguous	IGT	N	↑ early BD-onset
Xiao et al. (2009)	Long. 15-16Y	≥4 drinks, ≥1/w., past month; M&F	Trait Choice – Risk, ambiguous	UPPS IGT	N	↑ urgency and IGT predicted BD 1 year after

Behaviour (iii)

Table 1.1 – (continued 4)

Authors	Age (Y)	Sample	Impulsivity domain	Task/Measure	Acute EtOH	Key Findings	
Petit et al. (2012)	18-25	≥6drinks/3-h event; >3/<4 times/w., past month M&F	Action – Restraint Neural markers	GNG ERP	N	More errors in the alcohol-related context Delayed latency NoGo-P3 in heavy binge drinkers	Electrophysiological (iv)
Smith et al. (2013)	18-21	≥4drinks/ event; ≥1/ month. past year; F only	Action – Restraint Neural markers	GNG ERP	N	↑ dishinibition Amplitude NoGo-P3 alterations in female binge drinkers	
Watson et al. (2014)	18-26	NIAAA(AUQ); ≥2/month, past 6 months M&F	Trait Action – Restraint Neural markers	UPPS, SSS GNG ERP	N	↑ sensation seeking ↑ dishinibition P3 alterations	
Maurage et al. (2012)	≥18	≥5 drinks/3h event; ≥2/w., past year; M&F	Neural markers	ERP	N	4 groups: non-, moderate, heavy and daily drinkers Binge drinkers had a significant ERP amplitude reduction (less intense information processing)	
Lopez-Caneda et al. (2012)	Long. 2Y	≥6drinks/2h event;≥1/month;or≥6drinks/w.; M&F	Action – Restraint Neural markers	GNG fMRI, ERP	N	≠ GNG amplitude NoGo-P3 alterations in binge drinkers hyperactivation in the right IFC	
Wetherill et al. (2013)	Long. 3Y	≥4 drinks/ event, ≥1/ month, past 3 months; M&F	Action – Restraint Neural markers	GNG fMRI	N	Anomalies in inhibitory control circuitry before and after heavy BD	Neuroimaging (v)
Ahmadi et al. (2013)	18-20	NIAAA; ≥2/month, past 6 months M&F	Action – Restraint Neural markers	GNG fMRI	N	Heavy BD (NIAAA+ AUD) > light BD Abnormal function in inhibitory circuitry (left supplementary motor area, bilateral parietal lobule, right hippocampus, bilateral middle frontal gyrus, left superior temporal gyrus, and cingulate gyrus/anterior cingulate cortex)	

Table 1.1 – (continued 5)

Authors	Age (Y)	Sample	Impulsivity domain	Task/Measure	Acute EtOH	Key Findings	
Worbe et al. (2013)	≥18	≥8M≥6F/2h, ≥1/w., past 3months, M&F	Choice – Risk, probability	Risk-choice task fMRI	N	↑ in binge drinkers when anticipating large unlikely losses ↔ DLPFC, SPC, LOFC	Neuroimaging (v)
Xiao et al. (2013)	16-18	≥4 drinks, ≥1/w., past month; M&F	Choice - Risk, ambiguity	IGT fMRI	N	↑ incentive-related behaviours in BD (vs non-drinkers) ↔ hyperactivity cortical regions (amygdala, insula)	

Consequences of human BD on trait (i), behavioural (iii), electrophysiological (iv) and neuroimaging (v) measures of impulsivity, and effects from acute ethanol intoxication (ii). ↑/≠ BD impairment/no effects, ↔ associated, *Long.* longitudinal study, *Y* years of age, *w.* week *h* hour, EtOH acute alcohol exposure, *N/Y* not applied/applied, *N/S* not specified, *ERP event-related potentials, fMRI* functional magnetic resonance imaging, *rIFC* right Inferior Frontal Cortex, *DLPFC* dorsolateral prefrontal cortex*, SPC* superior parietal cortex*, LOFC* lateral orbitofrontal cortex, *SP* electroencephalographic potential. **BD characterization** consisted of: *Personal drinking habits* 4/5 drinks/event, once a week, past 6 months (Dawson and Room, 2000), *NIAAA* (NIAAA, 2004) [consumption of ≥5 drinks, in males, or ≥4 drinks, in females, within ≥ 2 hours, that is likely to raise blood ethanol concentration to ≥ 0.08 g %], *4-point scale* [frequency of intake of alcohol (0 Never; 1 Occasionally; 2 Often; 3 Almost Always)], *SAMHSA* National Survey on Drug Use and Health (2012) (≥5 drinks per episode, ≥ 1 time in the past month)*, AUDIT* adapted from Saunders and Lee (2000) [repeated pattern of drinking that confers the risk of harmful consequences], *AUQ – BD Score* Drinks/hour, times drunk within last 6 months, percentage of time being drunk when drinking, *TLFB* TimeLine Follow-Back (Sobell and Sobell, 1992) [continuous days drinking/abstinence; drinking days; drinks consumed; highest number drinks/event; days drinking ≥5 drinks/ being intoxicated when drinking; past month], *CAGE* BD group ≥one and ≤two points on the CAGE alcohol test (Ewing, 1984) and the presence of BD alcohol use (≥6 drinks/event over the weekend, with ≥1 episode per month), *AAIS* The Adolescent Alcohol Involvement Scale [(Mayer and Filstead, 1979) drinking events past 6 months, percentage 'drunk'] and drinks consumed/past 2 weeks, *S-MAST* Short-Michigan Alcoholism Screening Test (Seltzer et al., 1975), *PDHQ* Personal Drinking Habits Questionnaire [dose consumed and time/occasion; weekly drinking frequency (Vogel-Sprott, 1992)]. **Impulsivity measurements** are abbreviated as follows: *SURPS* Substance Use Risk Profile Scale [measures of impulsivity and sensation seeking], *SSS* Sensation Seeking Scale (Zuckerman et al., 1978) *Eysenck (1977) UPPS* Impulsive Behaviour Scale (Whiteside and Lunam, 2001); *Stop Task v*isual tracking Stop task [from Maudsley Attention and Response Suppression task battery (Rubia et al., 2007)].

***1.4.2.2 Effects of excessive/binge alcohol drinking*.** On the other hand, chronic alcohol use may affect inhibition or decision-making, which could lead to further alcohol intake. It has been postulated that extended drug (alcohol) exposure may disrupt function in frontal cortical regions (Jernigan et al., 1991; Pfefferbaum et al., 1997), involved in inhibitory control (Goldstein and Volkow, 2002; Bechara, 2003), and consequently decrease the subject's ability to inhibit inappropriate responses (Vuchinich and Simpson, 1998; Jentsch and Taylor, 1999; Jacobus and Tapert, 2013). Supporting this claim, poor performance on tasks engaging the frontal networks in young binge drinkers has been extensively documented (Tapert et al., 2004; Squeglia et al., 2012). Subjects with BD habits have shown poor inhibitory control (Dougherty et al., 2004; Scaife and Duka, 2009), revealing deficits in action cancellation (Nederkoorn et al., 2009) and action restraint (Henges and Marczinski, 2012). Moreover, in Ahmadi et al.'s study (2013), heavy drinkers presented more deficits when inhibiting a prepotent motor response in a GNG task than light college drinkers. Although this finding suggests that heavy use may account for behavioural deficits, it is also possible that the patterns of abstinence may be more disrupting than the toxic effects of heavy consumption *per se* (Duka et al., 2004). To address this point, Maurage and colleagues (2012) characterized young non-, moderate-, or intense-binge drinkers and daily drinkers and revealed that electrophysiological measures of inhibitory control were detected in binge, but not daily, drinkers. This indicates that BD patterns are deleterious for brain functioning (Obernier et al., 2002), and that this effect may not be due solely to the effects of alcohol consumption *per se*, but to the drinking patterns (i.e. repeated withdrawal events).

Those behavioural deficits documented in young binge drinkers (**Table 1.1**, iii) parallel alterations in electrophysiological markers of inhibitory control [**Table 1.1**, iv; for a review see (Hermens et al., 2013)], and anomalies in inhibitory control circuitry (**Table 1.1**, v), both before and after BD exposure (Wetherill et al., 2013). From longitudinal fMRI studies on young

individuals with no alcohol history (Squeglia et al., 2009; Norman et al., 2011; Squeglia et al., 2012), BD led to marked cerebral dysfunction in the absence of pre-existing impairment. However, these studies were on working memory, and only one study has explored electrophysiological levels associated with inhibitory control in young binge drinkers. Using a longitudinal approach, Maurage and colleagues (2009) revealed for the first time abnormal cerebral activity (right inferior PFC) following BD, in the absence of pre-existing deficits, suggesting that neural alterations may rapidly arise as the BD pattern commences.

Deficits in decision-making, reflected by intolerance to delayed gratification, have also been documented in young adults with BD habits (Jennison, 2004; Goudriaan et al., 2006a, 2007). Intriguingly, subjects with early BD onset discounted delayed rewards more steeply than subjects with later drinking onset (Kollins, 2003). On a similar note, binge drinkers were also impaired in other measures of choice impulsivity; Xiao and colleagues (2009) demonstrated that binge drinkers took more risks during an IGT session (i.e. hypersensitive to reward) than healthy subjects. In turn, high scores on this task (i.e. higher risky choices) predicted more drinking problems (Johnson et al., 2008; Xiao et al., 2009). Similarly, Worbe and colleagues (2013) found that enhanced risk taking was related to decreased sensitivity to the anticipation of negative outcomes across a range of probabilities. Such behavioural deficits were detected in parallel with abnormal activation of risk-associated regions (i.e. dorsolateral prefrontal cortex, superior parietal cortex and lateral orbitofrontal cortex). Additional fMRI studies also reporting abnormal affective decision-making in young binge drinkers (Worbe et al., 2013; Xiao et al., 2013) suggest that risky choices may be linked to hyperactivity of amygdala and insula (Xiao et al., 2013) and dysfunctional vmPFC as a consequence of BD (Johnson et al., 2008; Blakemore and Robbins, 2012). Taken together, impaired decision-making may underlie risky behaviours in binge drinkers, such as engaging in alcohol binge episodes despite the negative consequences in the long run (De Wit, 2009).

1.4.3 Impulsivity deficits: shortcomings for causality research

Impulsivity has been consistently implicated as both a determinant and a consequence of alcohol abuse. However, research is hampered by the difficulty of studying that interaction in human subjects. *As a cause*, although difficult to execute, longitudinal studies and effective behavioural and neurological measures in early development and throughout the adolescent period are necessary to study further the role of impulsivity on human alcohol intake. This in turn poses an additional limitation, which is to conduct test-retest of some of the measures, which may involve effects of learning. Second, the majority of studies do not employ multiple testing, or do not generally incorporate groups with chronic alcohol consumption, so that it is difficult to know whether the cause of damage is the pattern of drinking (i.e. withdrawal events) or the neurotoxic effects of alcohol itself, or which impulsivity markers are most associated with BD habits. *As a consequence*, long-term effects of BD cannot be readily separated in humans, but only with prospective studies in adolescents; such studies, naturally, take many years. Furthermore, multiple BD definitions make interpretations difficult and groups may not be matched between studies. For those reasons, animal models of BD offer invaluable tools to reveal causality and to study the short and long-term effects of BD. In that context, homologous measures of impulsivity between animals and humans, and similar models of adolescence and BD across species will facilitate cross-species comparisons. By providing common theoretical grounds, we will be able to explore whether different aspects of impulsivity may be associated with different phases of the alcohol cycle (Courtney et al., 2012), and whether BD may exert more deleterious effects in different aspects of the impulsivity construct.

1.5 SPECIFIC AIMS OF THE RESEARCH

From the evidence thus far compiled, it is evident that only comparing behavioural performance on different impulsivity tasks will capture the effects of BD on different varieties of the impulsivity construct. Using such a strategy, an avenue for research is to investigate which, if any, of the aspects of inhibition are most impaired after BD, and to evaluate the long-term consequences of such drinking patterns (only possible using non-human subjects). Third, it has been revealed that early (*vs.* late) exposure to alcohol may differentially impair impulsivity. This differentiation has pointed to possible differences in neurodevelopmental pathways leading to and resulting from alcohol misuse. Animal models of BD will provide invaluable knowledge on this aspect; for instance, by identifying specific individual risk factors (i.e. high impulsive behaviour) as potential biomarkers for BD. Taken together, providing a robust framework of methodologies (i.e. appropriate cross-species analogues) will improve our understanding of psychological underpinnings of alcohol BD.

Several operant tasks have been developed to assess impulsivity in animal and human subjects, and **Chapter 2** is devoted to reviewing the utility of the now well-established five-choice serial reaction time task (5-CSRTT), for measuring 'waiting' impulsivity in mice. Mice are useful tools for dissecting genetic and environmental factors, such as BD, which might influence behaviour in the task.

However, as we will introduce in **Chapter 3**, studies using homologous rodent and human phenotypes for impulsivity remain scarce. Moreover, from the evidence compiled in **Table 1.1**, no studies have yet examined the role of BD on 'waiting' impulsivity in humans. The Five-choice serial reaction time task (5-CSRTT) has proven a useful tool for assessing impulsivity in rodents (Robbins, 2002; *Chapter 2*). In principle, a homologous task would be equally useful for the assessment of 'waiting' impulsivity in humans. To address this question, we developed a novel task, the Sussex Five-Choice Serial Reaction Time Task (Sx-5CSRTT), which is modelled on

the 5-CSRTT in rodents (Robbins, 2002). Given the increased prevalence of BD in youth (Healey et al., 2014), the first aim of our study was to examine the performance of young social binge drinkers on a variety of impulsivity measures. To clarify the origin of those deficits, we tested alcohol-naive B6 (alcohol-preferring) and D2 (alcohol-avoidant) mice on the 5-CSRTT, to examine whether genetic predisposition for alcohol consumption was predictive of elevated 'waiting' impulsivity phenotype.

To investigate the long-term effects of BD, we developed a mouse model of adolescent BD (see **Box 1.3**) in two cohorts of B6 and D2 mice. To cover many facets of impulsivity, we investigated the long-term effects of BD on 'waiting' impulsivity, utilizing the 5-CSRTT, and choice impulsivity/risky decision-making by means of a mouse version of the Iowa Gambling Task (mIGT; **Chapter 4**). The ability of mouse BD to modulate 5-CSRTT and mIGT performance was examined in adulthood.

BOX 1.3 RODENT MODELS OF BINGE DRINKING

One obstacle to studying the long-term effects of BD in human adolescents concerns the difficulty in controlling for drinking patterns among binge drinkers. In this case, animal models may serve to control for such heterogeneity. Environmental manipulations can be controlled in a laboratory setting, the number and time of ethanol exposures can be regulated by the experimenter, and long-term specific effects can be evaluated with well-established tasks. While no complete animal models exist to mimic the complexity of the addiction process [see (Ripley and Stephens, 2011) for a discussion], animal models are useful in studying many elements of the syndrome at multiple levels of analysis – molecular, cellular and behavioural.

Rodent models of adolescent BD

Intriguingly, the propensity for enhanced alcohol consumption during adolescence is not unique to humans. Studies conducted using animal models of adolescence have shown adolescent animals to be more sensitive than adults to rewarding effects of alcohol but less sensitive to alcohol's intoxicating effects (and exacerbated by genetic vulnerabilities), which may serve as a cue to terminate excessive intake (Doremus et al., 2005; Spear, 2013).

To study adolescent BD, most of the current animal models use protocols of self-administration (Finn et al., 2005; Rhodes et al., 2005; Leeman et al., 2010), to replicate the oral route of administration that can lead to alcohol abuse and addiction in humans. However, those procedures may not capture one essential feature of BD, which is drinking to the point where BEC reach levels that have measurable effects on physiology (>≥ 100 mg/ml) and/or behaviour (Finn et al., 2005). Indeed, even mice genetically predisposed to drink alcohol rarely display a drinking pattern that leads to physiological intoxication (i.e. high BEC levels).

Our mouse model of adolescent BD

For those reasons, we describe a model administered by the experimenter that used 4 multiple cycles of intermittent exposure to 2g/kg of ethanol (**Figure 1.3**), with the aim to emulate alcohol BD in two inbred strains of mice. The mouse model of BD we used was timed to occur during the early phase of adolescence, comparable to the age at which the onset of alcohol drinking is associated with the greatest risk for developing alcohol dependence in adult humans (Chou and Pickering, 1992; Grant and Dawson, 1997), and late adolescence (Adriani et al., 2002; Carrasco et al., 2013).

The main advantages of this model over other models of self-administration are that a) it is sufficient to induce significant binge-like blood alcohol levels (Dixon et al., 2012; Tarragon et al., 2012), b) does not require food or water deprivation to promote high intake levels, c) it can control for differences in ethanol intake and patterns of ethanol consumption among inbred strains (Belknap et al., 1993; Rhodes et al., 2007), also including the possibility to d) expose the animals to repeated cycles of withdrawal.

BOX 1.3. RODENT MODELS OF BINGE DRINKING (CONTINUED)

Our mouse model of adolescent BD

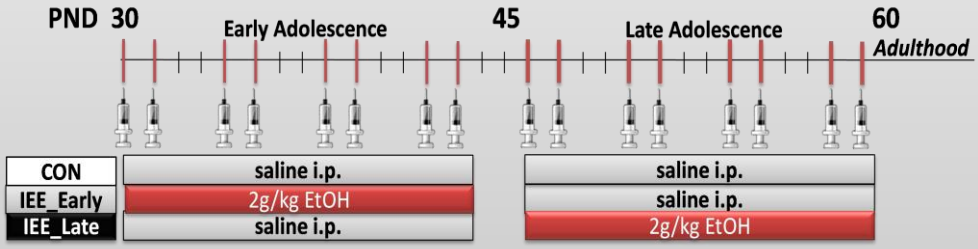

Figure 1.3 Male B6 and D2 mice were exposed to 2.0 g/kg of ethanol (IEE treatment) for 2 consecutive days at 48-h intervals during a 14-day period either during early adolescence (IEE_Early; 8 EtOH injections from PND30 to PND43) or late adolescence (IEE_Late; 8 EtOH injections from PND45 to PND58). CON mice received 16 saline injections throughout the adolescent period

Drawing attention to the relationship between BD and impulsivity, it becomes clear that alleviating impulsive behaviour may constitute an effective strategy for treating BD. Nonetheless, effective interventions to correct for both impulsivity levels and high alcohol intake have been poorly understood. Over the last decade, the opioid antagonist naltrexone has proved effective in the treatment of alcohol dependence (Volpicelli et al., 1992) and impulsivity-related control disorders (Kim, 1998). A major aim of **Chapter 5** was to examine the role of the μ-opioid antagonist NTX on mouse 5-CSRTT and mIGT performance, as a potential aid for treating BD by increasing inhibitory control mechanisms.

In the final chapter, **Chapter 6**, I summarize the main findings of this thesis and integrate our findings in a wider context. Chapter 6 will include a consideration of the limitations of the current programme of research, as well as potential directions for studying the modulatory effects of impulsivity and effects on BD habits.

1.6 REFERENCES

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Chapter 2

Paper 1

MEASURING IMPULSIVITY IN MICE: THE FIVE-CHOICE SERIAL REACTION TIME TASK

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2.1 Abstract

Rationale: Mice are useful tools for dissecting genetic and environmental factors in relation to the study of attention and impulsivity. The 5-choice serial reaction time task (5CSRTT) paradigm has been well established in rats but its transferability to mice is less well documented.

Objectives: To summarise the main results of the 5-choice serial reaction time task (5CSRTT) in mice, with special focus on impulsivity.

Methods: 5CSRTT can be used to explore aspects of both attentional and inhibitory control mechanisms.

Results: Different manipulations of the task parameters can lead to different results; adjusting the protocol as a function of the main variable of interest or the standardization of the protocol to be applied to a large set of strains will be desirable.

Conclusions: The 5CSRTT has proven to be a useful tool to investigate impulsivity in mice.

Key Words: 5-choice serial reaction time task; Impulsivity; Attention; Task parameters; Mice.

2.2 Background

A constellation of neuropsychiatric disorders, such as ADHD, personality disorders, mania, Alzheimer's disease, Parkinson's disease and substance abuse, has been associated with attentional disruptions and impulsive behaviours (Evenden, 1999; Clark and Robbins, 2002). Several operant tasks have been developed to assess both attention and impulsivity in rodents, including the now well established 5-choice serial reaction time task (5CSRTT). This technique has proven to be a useful tool in rat studies and is increasingly used in mouse studies. Mice are particularly useful in the dissection of genetic and environmental factors that might influence behaviour in the task. This review is aimed at presenting the evidence generated from mouse studies, and will discuss the nature of the results found among different studies in relation to the particular procedures implemented. The surveyed data suggest that results obtained may depend on the particular parameters of the test. Hence, information regarding the extent to which the parameters of the task detect (or even produce) differences in impulsivity will be examined, and future directions for research suggested.

2.3 Measuring attention and impulsivity: 5CSRTT

In 1983, Robbins and colleagues initially reported a test for assessing attention in rats. This paradigm was based on another procedure used to monitor attentional function in humans, the continuous performance task (Carli et al., 1983; Robbins, 2002). Briefly, the 5CSRTT assesses attentional performance by the detection of a brief visual stimulus presented pseudo-randomly across several spatial locations, in a 5-hole box, though variations with 9 holes or even 1 hole have also been used. The 5CSRTT also provides information about aspects of inhibitory response control: premature responding (responding before the light stimulus is presented) into the holes is viewed as a failure of response inhibition where the animal has to withhold responding until the stimulus light is illuminated, and provides a measure of

impulsivity (Robbins, 2002); perseverative responding occurs when the animal continues (unnecessary) nose-poking into the holes after a correct detection and may represent a measure of compulsivity (Dalley et al., 2008). As indicated by other excellent reviews, attention and impulsivity are not unitary constructs (Evenden, 1999; Robbins, 2002; Winstanley, 2007), and only one specific form of impulsivity is measured by the 5CSRTT. This impulsivity subtype was initially described as 'motor' impulsivity (Winstanley, 2007), but has been more recently characterised as 'waiting' impulsivity (Robinson et al., 2009).

The flexibility and non-aversive nature of the 5CSRTT makes it suitable for several testing purposes and its use has been well described in rats (Robbins, 2002). However, due to the availability of techniques to manipulate the mouse genome, it is important to be able to perform these studies in mice. A complete analysis of all the variables of the 5CSRTT is beyond the scope of the present review; instead we will focus on premature responses, which provide our main impulsivity measure, and other variables will be introduced as secondary.

The focus of interest when approaching the study of attentional and impulsive phenotypes in the mouse can be subdivided into different domains which we will categorize into 4 main topics. Firstly, and of increasing interest in recent years have been, 1) exploration of the genetic basis of attention and impulsivity; 2) discovery of neurochemical pathways mediating processes of attention; 3) neuropharmacological assessment of drugs and their role in impulsivity and attention; and 4) examination of the role of affective states in attention and impulsivity. Although the early reports using mice focused mainly on attentional function, later studies have emphasised other variables such as premature or perseverative responding in the analysis, expanding the use of the task to study aspects of inhibitory control.

2.4 Evidence from the mouse: test parameters influence the results

The possible trial sequence that a mouse has to follow to obtain a reinforcer is illustrated in **Figure 2.1**, a similar protocol to that described for rats (Robbins, 2002). Considering all variables together, attention is measured mainly by accuracy of performance and omissions, and also by a measure of processing speed - the reaction time or latency to perform a correct response. Accuracy (percentage of responses that are correct) provides a conservative measure of attention, and, if latencies or total trials completed are not impaired, we can assume any disruptions of the task are true attentional deficits. Taking the reaction time for a correct response, increases in correct latency in the absence of changes in another reaction time measure (i.e. latency to retrieve the reward) suggest that the animal's locomotor function and motivation for the reward are unaffected; thus this measure is likely to reflect a true slowing of processing speed. The average latency to make an incorrect response is rarely reported, presumably because it is affected similarly to the latency to perform a correct response (Amitai and Markou, 2010). The second common measure of attention, omissions (failures to respond), can also reflect failures of signal detection and/or motivational/motor deficits (Humby et al., 1999; Davies et al., 2007). On the other hand, inhibitory control variables, premature and perseverative responses, can also affect attentional performance, and can be associated with each other, as has been argued by Dalley et al. (2011). The aforementioned considerations therefore illustrate a crucial point about the 5CSRTT: it is critical to consider the various measures of the task in combination before a final interpretation is made.

5CSRTT parameters are well defined in rat studies (Robbins, 2002). However, the 5CSRTT protocol used in mice varies and the use of different procedures, according to the particular question asked, can make comparison between studies and laboratories sometimes difficult.

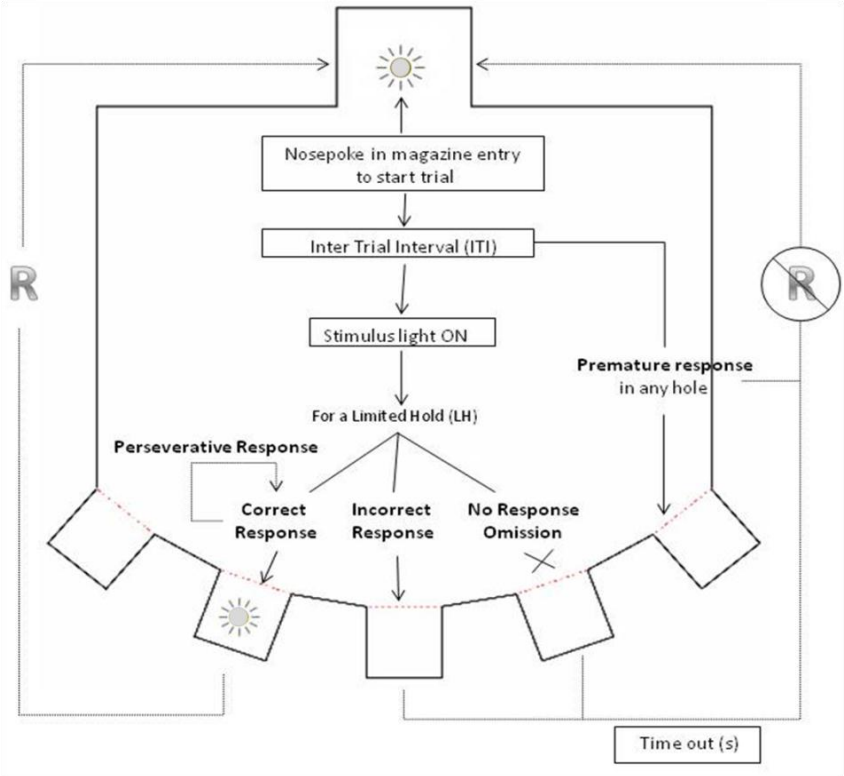

Figure 2.1 Sequence of a session in a 5CSRTT chamber. Different steps may lead to a reward or to a time out, where no reinforcer (R) is available. Correct responses made into the illuminated hole are rewarded by the presentation of a reinforcer in the magazine entry. Omissions (failure to respond to the signalled stimulus within a concrete period of time), incorrect responses (nose-pokes into a non designated hole) and premature responses (responses into the apertures during the inter-trial interval, ITI, prior to the stimulus presentation) are generally followed by a period of 'time out', perseverative responses (responding repeatedly intro the apertures after a correct detection and before collecting the reward) can also be punished by a time out in some protocols. Specifically, mice have to nose-poke into the magazine to start a new trial, and then withhold responding during the inter-trial interval (ITI; s) until the stimulus is presented. If the animal makes a response into one of the holes during this interval, a premature response is recorded. When the ITI period is terminated, the animal is required to nose-poke into the illuminated hole within a limited time (correct response) in order to obtain a reinforcer in the magazine entry. The times to make the correct response and to collect the reinforcer are also recorded (correct latency and magazine latency, respectively).

If the animal nose-pokes in a non-illuminated hole this is recorded as an incorrect response. Both correct and incorrect responses provide a measure of attention (accuracy), usually assessed by the percentage of correct responses (correct / correct + incorrect). However, the animal can also display no response, and this is recorded as an omission. At the same time, some animals tend to nose-poke repeatedly into the holes after a correct response, this measure being considered a form of compulsivity, and recorded as a perseverative response. An additional measure of compulsivity, unfortunately not usually reported, could be responses during timeout (i.e., nose pokes made during the timeout interval) (Amitai and Markou, 2010). To signal appropriateness of behaviour, incorrect, omission, premature and perseverative responses can be followed by a time out, generally signalled by a period of darkness (or by illuminating the chamber, depending on the protocol); during time out, no reinforcer can be obtained. Finally, the total number of trials completed (TT) can be examined, providing a measure of motivation. A camera located inside the box may be useful to detect scanning strategies not easily inferred with the analysis of the variables (Humby et al., 1999)

Pretraining

A number of stages of pre-training in the 5CSRTT, starting with behavioural shaping, gradually introduce the subjects to the different aspects of the 5CSRTT. As reported by Humby et al. (2005), animals perform behavioural shaping to learn how nose-pokes in the magazine lead to a reinforcer, during a small number of sessions, often no more than 2. Studies often limit this pre-training session to 50 reinforcers, but the number of total trials will be increased in subsequent stages of the training. Secondly, during the proper 5CSRTT training, nose-pokes into the signalled holes are required to obtain the reward. Stimuli are initially presented for a longer period of time (usually starting from 30s), and are subsequently reduced according to specific criteria (Humby et al., 1999; de Bruin et al., 2006; Hoyle et al., 2006). Next, parameters are adjusted according to the performance of each animal, also depending on the study. For instance, when using animals modelling Alzheimers disease (AD)-like attentional deficits, the standard task was adjusted to less restrictive conditions: the duration of the session was set to 50 trials or 1 hour (which allows the analysis of possible deficits in sustained attention); the ITI was set at 20 s, and then a larger-than-usual punishment of 10s was used, and a less attentionally demanding stimulus duration of 4s (Romberg et al., 2011). In Relkovic et al. (2010), animals were trained to baseline performance at 0.8s stimulus duration, but only 30 total trials were required to be completed and criteria of 80% of accuracy and <25% of omissions were established. In contrast, when the assessment of attention was the main priority, the number of trials was set at 50, since overtraining could be a confounding factor (Wrenn et al., 2006): attention rather than the ability to learn is being assessed. However, daily sessions of 30 min duration, or limited to 100 trials (whichever comes first) are the most commonly used (Greco et al., 2005; Patel et al., 2006). Testing is routinely carried out daily (5-6 days/week) (Hoyle et al., 2006; Patel et al., 2006; Oliver et al., 2009). Animals can also be tested during the dark phase (Pattij et al., 2007), although is important to note that the time at

which animals are trained, tested and fed should be constant throughout the experiment (Bari et al., 2008), since circadian changes can lead to different results (Yan et al., 2011).

Another aspect of the protocol that needs particular attention is whether punishment is used. In some studies, omissions (failures to respond when a stimulus is presented) and incorrect responses are recorded and punished with a time out, in which no reinforcer is available for a set period of time; in most studies, as originally set up in rats, premature responses are also punished by a time out (Humby et al., 1999). The use of the time out has the consequence of suppressing inappropriate behaviour and thus narrowing the sequence of actions needed to obtain the reward (Bari et al., 2008). Some studies perform the time out punishment less rigorously, using a 2 s period (e.g., Greco and Carli, 2006; Hoyle et al., 2006; Pozzi et al., 2010), whereas others use 4 s (Kerr et al., 2004; Young et al., 2004) or even 10 s (Wrenn et al., 2006; Romberg et al., 2011); 5s is the most common time out interval used (e.g., Humby et al., 1999; Davies et al., 2007; Lambourne et al., 2007; Pattij et al., 2007; Loos et al., 2009; Oliver et al., 2009; Bailey et al., 2010; Relkovic et al., 2010; Yan et al., 2011). The time out might be signalled by a period of darkness (e.g., Hoyle et al., 2006; Oliver et al., 2009; Walker et al., 2011), or might be signalled by the illumination of the house light (Humby et al., 1999; Kerr et al., 2004; Young et al., 2004; Wrenn et al., 2006; Davies et al., 2007; Lambourne et al., 2007). Responses in the holes during the time out period may restart the time out (Greco and Carli, 2006). Some studies add the possibility of avoiding/terminating this time out through a panel push (Davies et al., 2007; Lambourne et al., 2007). To what extent do these variations in the time out procedure lead to the same consequences for behaviour? The importance of this topic is discussed in the study by Hoyle et al. (2006), in which two experiments with different protocols were carried out. In a first experiment, where premature responses were not punished and time available to complete a response (limited hold; LH) was long (LH= 5s), mice showed high levels of premature responding and low levels of accuracy; after modifying task parameters so that time allowed for responding was shortened (LH= 2s) and premature

responses were punished by a time out, mice presented normal anticipatory responding and accuracy levels, but more omissions (Hoyle et al., 2006). Similarly, Bizarro et al. (2003), using rats, found, in the absence of a time out, that acute alcohol decreased premature responding, whereas Oliver et al. (2009), using mice and a time out of 5s after a premature response, found an increase in premature responses after acute ethanol treatment under long ITI conditions. As noted in Amitai and Markou (2011), the absence of a time out decreases the incentive to withhold premature responding. However, punishment for premature responding is not always necessary, and the punishment of perseverative responses has been reported to disrupt training (Bari et al., 2008). In particular, during the training period, or when only attentional assessment is relevant, some studies (Lee et al., 2002; de Bruin et al., 2006) prefer to use a protocol in which premature responses are not punished.

Similarly, the duration of the limited hold (time available to perform the response after the stimulus presentation) has also proven to be important. Both Patel et al. (2006) and Hoyle et al. (2006) argue that a long limited hold offers more time to make an incorrect response, and they propose that some of these supposedly 'incorrect' responses are, actually, impulsive responses - the animal that failed to detect the stimulus presentation 'thinks' that the stimulus has not yet been presented and consequently, makes a response into a random hole. Thus, a long LH diminishes accuracy and could hypothetically increase premature responses (false incorrect), especially when the subjects are not punished (perhaps resembling more compulsive responses). However, when the limited hold is reduced and premature responses are punished, although omission rate is increased, animals make fewer anticipatory responses and display normal accuracy (Hoyle et al., 2006).

Therefore, as discussed, the task may be adjusted depending on the main variable of interest, by modifying the training length, the baseline criteria and/or the task parameters (Bari et al.,

2008). It needs to be borne in mind that such methodological differences in the protocol may account for apparently different findings across laboratories.

2.5 5CSRTT in mice: a summary of main findings

As outlined above, different test parameters can lead to different outcomes. We will divide this section into the different steps and modifications that can be implemented in order to vary task demands. After a period of extensive training in the task, and upon the stabilization of performance under baseline parameters, a variety of behavioural manipulations can be designed to affect specific aspects of attention and impulse control.

In order to investigate stable differences between groups, or the impact of certain drugs, animals are commonly tested under the particular standard conditions used during training. A complementary approach, following training under standard conditions, is to introduce probe sessions from time to time, in which particular parameters are varied. One example is the use of intertrial intervals (the time that the animal has to wait before the stimulus is presented (ITI), that are varied from the ITI used during training. Variations that have been reported in the literature involve lengthening the 'waiting time' – long ITI condition, by shortening it – short ITI condition, or by making the presentation of the stimulus unpredictable – variable ITI condition. Another option might consist of varying the attentional load (e.g., by the manipulation of the characteristics of the stimuli— i.e., short stimulus duration (short SD) condition.

2.5.1 Acquisition of the task / training

Generally mice display no problems in their ability to perform the basic task and to advance across the different stages of training in the 5CSRTT (Hoyle et al., 2006; Davies et al., 2007). Under low attentional demands, mice display high levels of accuracy and short reaction times (Marston et al., 2001; de Bruin et al., 2006) with no particular problems arising from premature responding (Humby et al., 1999). The task has proven suitable for the testing of mice genetically manipulated to mimic Alzheimer disease-related dysfunctions (3xTgAD;

Romberg et al. 2011). Even aged animals (mice of 14, 20 and 27 months old over-expressing human caspase 3, implicated in cell death following neurodegeneration), showed no difficulties in learning the task and did not show differences in performance in comparison to their age-matched wild-type control littermates (Kerr et al., 2004). As previously described for rats (Dalley et al., 2002), mice are able to reach high levels of performance. Indeed, comparing mice and rats, under baseline conditions, the level of premature responding in mice is lower than that seen typically in rats (Humby et al., 1999); on the other hand, omission rate can be higher in mice (Wrenn et al., 2006; Oliver et al., 2009). This impairment may be associated with different motivational processes among different strains of mice and/or a more acute decrement in vigilance within the session in mice, as compared to rats (Dalley et al., 2004); but it may also be related to satiation, a common problem with the procedure in mice (de Bruin et al., 2006). For this reason, the murine version sometimes consists of shorter sessions than are typically used with rats (Wrenn et al., 2006).

Choice of the reinforcer used, liquid reinforcer or food pellets, will help to rule out this confounding variable. When delivering pellets (preferred in order to facilitate comparisons with rats' performance (de Bruin et al., 2006; Patel et al., 2006), sessions will be of shorter duration. On the other hand, liquid reinforcement allows sessions to have a higher number of total trials (100 TT or 30 min; Oliver et al., 2009). In the case of liquid reinforcers, the volume used might be reduced to avoid satiation but also to appropriately restrict body weight; type of reinforcer, on the other hand, can also be used as a motivational factor.

Although performance of mice generally rivals that of rats, some differences in training are seen in mice with specific mutations or depending on the strain. For example, in the study by Greco and Carli (2006), investigating the effects of deletion of neuropeptide Y2 receptors in memory, attention and inhibitory response control, the less anxious Y2-/- mice took twice as many sessions as wildtype mice to nose-poke consistently into an illuminated hole, showing lower accuracy and more premature responding during the training (as well as in long ITI and

variable SD sessions) in comparison to the Y2+/+ mice, suggesting a possible role for anxiety in the learning of the 5CSRTT (Greco and Carli, 2006). Also, a Prader-Willi syndrome (PWS) mouse model, the PWS-IC+/-, with learning impairments thought to arise from attentional deficits, took twice as many sessions and had impaired accuracy, increased omissions and elevated correct reaction times than their wild-type (WT) controls, but no differences were found in premature responses or motivation (latency to collect and consume the reinforcer) (Relkovic et al., 2010).

2.5.2 Baseline performance under standard conditions

Once criteria of stability of performance are reached, data from the last days of training can be used to provide a baseline index of execution. Generally, baseline performance is calculated from the values obtained over the last two (Romberg et al., 2010; Relkovic et al., 2011), three (Humby et al., 1999; Kerr et al., 2004; Greco et al., 2005; Pattij et al., 2007), four (El-Kordi et al., 2009) or even 10 days (Davies et al., 2007) of training in the 5-CSRTT, in which asymptotic performance at the final SD is reached (e.g., 1s; Loos et al., 2010).

Baseline performance has been generally used to study differences between strains (Greco et al., 2005; see Table 2): for example, F1 C57BL/6xDBA/2 vs. C57BL/6x129Sv (Humby et al., 1999), C57BL/6 vs. DBA/2 (Patel et al., 2006; Loos et al., 2010) and C57BL/6JOlaHsd vs. 129S2/SvHsd vs. DBA/2OlaHsd (Pattij et al., 2007) and to compare different genetic manipulations. Some examples include studies of the effects of X-monosomy on visuospatial attention (Davies et al., 2007), a mouse model of Prader-Willi Syndrome (PWS; Relkovic et al., 2010), studies of mice with overproduction of corticoid-releasing hormone (van Gaalen et al., 2003), caspase 3 mutant mice (Kerr et al., 2004), and transgenic mice for the human FTDP-17 tauV337M mutation (Lambourne et al., 2007). Baseline rates have also been used to examine the role of nicotinic α5 (Bailey et al., 2010) and α7 (Young et al., 2004) subunits in attention. Lastly,

pharmacological challenges are also generally implemented under standard conditions, as we will explore later.

5.5.3 Challenge Condition

2.5.3.1 Altering the duration of the ITI: Short, Long and Variable ITI

In order to provoke impulsive responding, a number of experimenters have varied the ITI away from the training conditions. Both shortened (0.5, 1.5, 3.0 or 4.5 s) (Humby et al., 1999; Wrenn et al., 2006; Lambourne et al., 2007) or lengthened (5, 6, 7, 8 or 10 s) ITIs have been used, usually by interleaving occasional long ITI sessions within baseline training sessions; in order to allow sufficient time to complete the same number of trials per session, session duration is usually increased to 45 min. Increasing the ITI from a baseline value of 5s to 7s in the long ITI session has been shown consistently to increase premature responding in both rats (Dalley et al., 2007; Fletcher et al., 2007; Dalley et al., 2008) and mice (Oliver et al., 2009).

Altering the length of the ITI itself has little effect on attentional functioning in the rat and similar findings apply to mice (Robbins, 2002; de Bruin et al., 2006) (see **Figure 2.2**).

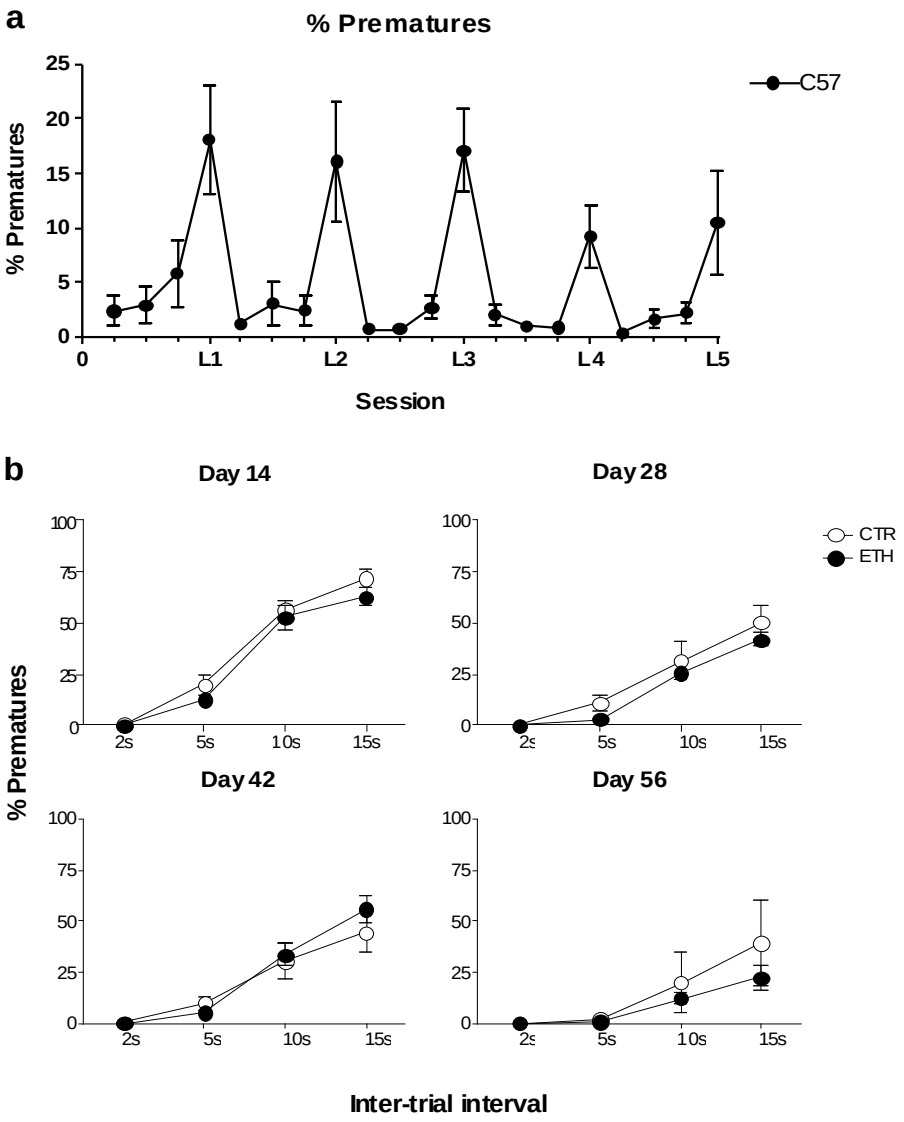

Figure 2.2 a Effects of increasing the inter-trial interval (ITI) on 5CSRTT performance in C57Bl/6JOlaHsd mice. Once stable baseline levels have been achieved, C57Bl/6JOlaHsd mice were tested in 5 long ITI sessions (L1-L5) in which the stimulus predictability was disrupted by increasing the length of the ITI from 5s to 10s. The number of premature responses was accentuated when mice were confronted with a long ITI session but this effect was diminished by repeated sessions (Oliver et al. 2009) **b** Effects of a variable ITI (vITI) on 5CSRTT performance in ethanol-treated and control C57BL/6J mice. Once stable baseline levels had been achieved, the animals were tested in 4 vITI sessions (day 14, day 28, day 42, day 56) in which the stimulus predictability was disrupted by varying the ITI from 2s to 15s. The number of premature responses exacerbated at longer ITIs. Premature responding decreased over sessions (Walker et al., 2011)

Nevertheless, although the main effects of increasing the ITI in mice are seen in impulsivity measures, Marston et al. (2001) additionally described impairments in attention, that were, however, strain-dependent: lengthening the ITI caused greater accuracy deficits in C57BL/6J than in 129P2/OlaHsd mice. In detail, when increasing the ITI, C57BL/6J mice seem to show greater impairments in accuracy in comparison to 129P2/OlaHsd mice, whereas when reducing the SD, 129P2/OlaHsd mice were more affected. At the same time, Yan et al. (2011), using a mouse model with cognitive and inhibitory control deficits that resemble diagnostic features of ADHD (inattentiveness, impulsivity and compulsivity; NK1R-/-), reported that long ITI sessions increased omissions, perseverative responses and latency to collect the reward in NK1R-/- mice in comparison to their wild-type (WT) control group. On the other hand, the same authors report that, using a variable ITI procedure, perseverative responding and premature responding were increased in the NK1-/- mice, and accuracy diminished, indicating that the long ITI and vITI conditions may give rise to rather different outcomes.

In the variable ITI condition (vITI), the stimulus can be presented using different inter-trial delays, for example 4-6-8-10s, in a semi-random fashion, within a single session. By disrupting the temporal predictability of the stimulus onset, the possibility of mice using temporal mediating strategies is minimized. Although mainly implemented in a single session, it may also be interesting to repeat the vITI procedure more than once, to study the evolution of the behaviour and analyze how impulsive responses show adaptation over time and/or repeated testing (Walker et al., 2011). In rats, when introducing a variable ITI condition, at the longest ITI values, levels of premature responding are increased (Fletcher et al., 2007). The same phenomenon is seen in mice (Walker et al., 2011; de Bruin et al., 2006; see **Figure 2.3**), regardless of the strain (Relkovic et al., 2010). Increasing the ITI (from 5 to 6, 7, 8s) produced significant differences in impulsive responding in young mutant tau V337M mice, the deficit being more acute when the animals were older (Lambourne et al., 2007), though changes in accuracy or correct response latency did not occur. In a study comparing 40,XX and 39,XO mice

(Davies et al., 2007), both groups showed a similar increase in the levels of premature responding in a vITI session. In a later study by the same authors, premature responding was especially higher in 40XY in comparison with 39,$X^{Y*}O$ mice (Davies et al., 2009). Consistent with these findings, Greco and Carli (2006) also found premature responses to be increased by vITI, especially in the less anxious Y2-/- mice, whereas no additional effects on accuracy or omissions were reported. Although not consistently reported, impairments in attention can accompany increases in premature responding when using the variable ITI condition (Yan et al. 2011). Humby et al. (1999) report that vITI also resulted in an increase in omissions and a decrease in correct reaction times, in C57BL/6xDBA/2 mice. Although variable ITI is usually implemented as a challenge session, Hoyle et al. (2006) trained animals under a variable inter-trial interval, so that they could be compared for their abilities in coping with temporally unpredictable stimuli. Results under this training procedure showed that fewer reinforcers were obtained and higher correct response latencies were seen, in comparison with the animals trained in a fixed ITI protocol.

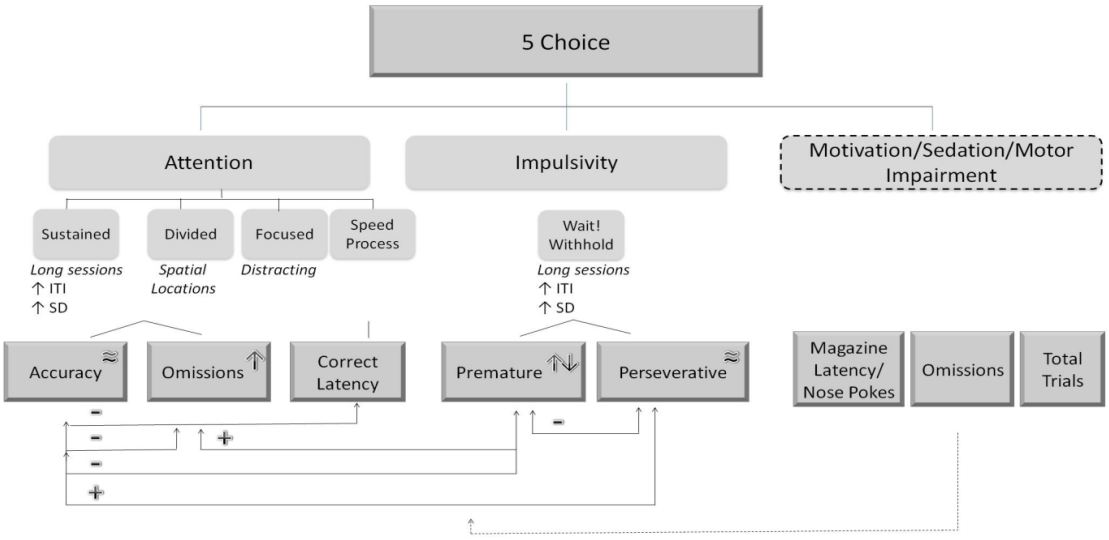

Figure 2.3 The diagram exemplifies the complex relationship between the 5CSRTT variables. Some of the variables covary but, at the same time, can be controlled by independent mechanisms. Symbols indicate positive (+) or negative (-) correlations (Spearman's *r*) from data from 12 BXD recombinant inbred strains and their progenitors (C57BL/6J and DBA2/J), during the baseline and the three long ITI challenge sessions (Pena-Oliver, in preparation). Firstly, from the bottom left to the right, we can see that accuracy shows a negative correlation with omissions; that is, strains that perform more accurately during the challenge sessions, also show fewer omissions (r=-0.790). Furthermore, the BXD study also suggests a negative correlation between accuracy and correct latency, during the long ITI condition. As expected, strains that perform more accurately take less time to make a correct response (r=-0.507). At the same time, accuracy, during baseline but also during the long ITI sessions, correlates with inhibitory control variables in the 5CSRTT: negatively with premature responding, as reported by others (Dalley et al. 2008; Greco et al., 2005), but not in agreement with Loos et al. (2010); and positively with perseverative responses, but not in a model of mice 3xTgAD, where animals with diminished accuracy also increased perseverative responses, similar to rat models of AD and patients (Romberg et al., 2010). Focussing on omissions, values during baseline, second and third long ITI, positively correlate with premature responding, but not with perseverative responses. In the bottom central part of the figure, we can see that the inhibitory control variables have a negative correlation between them: strains with higher number of perseverative responses, show lower premature responding. Those results indicate that perseverative and premature responses might be under different mechanisms, as also suggested in other studies (Greco et al., 2005; Oliver et al., 2009; Loos et al., 2010). Moreover, it is also interesting to assess the stability of those variables over time (symbols inside the boxes), during the standard conditions but also under challenge sessions. Accuracy, and especially, perseverative responses, tend to remain stable over time. Omissions and premature responses, on the other hand, show increments during long ITI sessions as compared with baseline conditions, as also reported in other studies (Walker et al., 2011). However, premature responses tend to decrease over time whereas omissions show a less consistent pattern; indeed, the rate of omissions shown during the baseline does not predict long ITI performance. Above all, as we highlight, special care needs to be taken with motivational, sedation and motor impairment since they can affect overall 5CSRTT performance, as also reported by Bari et al. (2008). If motivation is decreased, fewer trials will be completed by the end of the session, and the latency to collect the reward and the number of head pokes into magazine will increase and decrease, respectively. In the case of sedation, an increase in response latencies and reward collection will be seen. More concrete to this

study, a positive correlation is seen between magazine latency during a long ITI and perseverative responses (+0.608) and correct latency (+0.559); and this last measure correlating negatively with number of total trials (-0.650). In detail, strains that during the long ITI take more time to collect the reward are also more compulsive and completed fewer total trials. Although the high magazine latency may come from lack of motivation, the increase in number of perseverative responses suggests that delay in retrieving the reinforcer is attributable to a longer time spent repeatedly nose-poking: high responding into the stimulus hole may indicate a high motivation or excitatory effects towards the potential reward (or perhaps, insecurity as to whether the nose-poke was effective). In sum, a cautious approach would be required in the analysis of 5CSRTT variables

Under the standard ITI procedure, the stimulus light informs about the correct location for a response. Since the ITI is fixed, timing of the response may be mediated by either the light onset, or by internal timing. Under the vITI procedure, stimulus onset informs both about location of nose-poke, and the appropriate time of responding. If the animal has been trained using a fixed ITI, and thus had the opportunity to employ internal timing to solve the delay aspect of the task, introduction of a variable ITI requires a change in strategy to use only the stimulus onset, and to ignore internal timing. In contrast, under the long ITI condition, as in the standard configuration, the stimulus informs about place, but a possible strategy is for the animal to adjust its internal timing to the new contingencies (wait 7s not 5s), a strategy that is not available for the vITI procedure. The standard, and long ITI procedures may thus have elements in common with differential reinforcement of low rates (DRL) procedures, in which the animal is required to estimate the passage of time to perform efficiently (Stephens and Voet, 1994; Ripley et al., 2001). We may speculate that in the vITI condition, because the animals cannot rely on internal timing they will pay more attention to the stimulus and, for that reason, in the vITI condition, animals may also perform with higher accuracy. On the other hand, under the long ITI condition animals might still use internal timing, appropriately adjusted, and thus rely less on stimulus detection; greater deficits in accuracy might then be expected. To test this notion, we performed correlational analysis using unpublished data from

our laboratory from 46 mice (C57BL/6J and DBA2/J obtained from Jackson laboratories, C57BL/6J from Charles River and C57BL/6OlaHsd from Harlan, UK). Since in these experiments we used a 10-s ITI in the long-ITI version of the task, we compared performance of these mice with mice of the same strains performing the vITI version, selecting the 10-s ITI data from the vITI sessions for comparison. **Figure 2.4** illustrates this comparison. Accuracy and percentage of premature responding in the 10 seconds condition of the vITI session were only weakly negatively correlated (Spearman's rho= -0.325, p=0.03) whereas these two variables in the long ITI condition appear highly negatively correlated (Spearman's rho= -0.673, p<0.0001). Correlations between accuracy and percentage of premature responses were significantly different between the variable and long ITI conditions (z=3.276, p<0.0116), suggesting that the increase in premature responding obtained using the two procedures is achieved by different mechanisms.

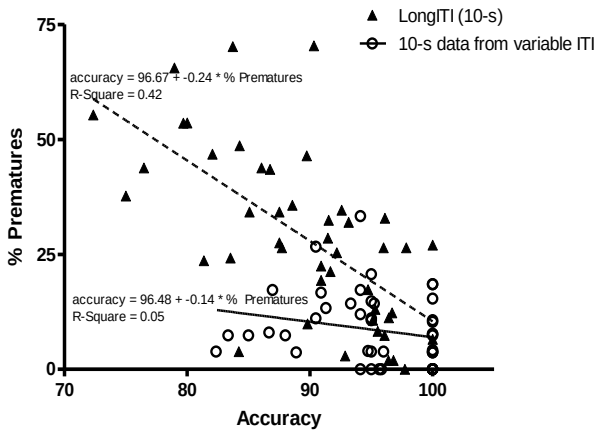

Figure 2.4 Scatter plot of accuracy and percentage of premature responding for the long ITI and variable ITI condition, for C57BL/6 and DBA2/J strains (C57BL/6J and DBA2/J from Jackson laboratories, C57BL/6J from Charles River laboratories and C57BL/6OlaHsd from Harlan; pooled data, n=46) (Pena-Oliver and Stephens, unpublished data). Levels of premature responding are strongly negatively correlated with levels of accuracy when animals are tested under the long ITI condition (Spearman's rho= -0.673, p<0.0001). In contrast, levels of premature responding and accuracy show only a low negative correlation in the vITI condition (Spearman's rho= -0.325, p=0.03). Regression lines for the long ITI (dotted line) and the variable ITI condition (straight line) are significantly different (z=3.276, p<0.0116)

The foregoing discussion raises the possibility that differences in impulsivity between mouse strains, or resulting from pharmacological treatment, or lesions, may arise from differences in internal time estimation or "internal clock" (Wittmann and Paulus, 2008; Coull et al., 2011). We recently examined this possibility in substrains of C57BL/6J mice that differ in expression of alpha-synuclein, a protein involved in regulation of dopamine function (Abeliovich et al., 2000; Anwar et al., 2011). Mice lacking alpha-synuclein (either *snca* KO mice, or the C57BL/6JOlaHsd substrain that exhibits a spontaneous loss of chromosomal material carrying the *snca* gene) showed lower levels of premature responding than wildtype C57BL/6J mice, indicating lower levels of impulsivity, but there were no differences among the groups in their overall timing behaviour, leading us to conclude that at least in this example, differences in impulsivity in the 5-CSRTT were not caused by differences in timing behaviour (Peña-Oliver et al., 2011).

2.5.3.2 Altering the characteristics of the stimulus: Attentional challenge

When the assessment of attentional function is the main goal of the study, due to a ceiling effect as a consequence of extensive training, it is sometimes difficult to discriminate between groups when comparing performance under baseline parameters. The use of testing sessions specifically designed to increase the attentional load are thus sometimes useful in exploring differences between strains or to elucidate pharmacological actions. Typically, attentional challenges are achieved by reducing or varying the stimulus duration (SD; 0.2, 0.4, 0.6 and 0.8s), or reducing stimulus brightness (% reduction: 52, 30, 21 and 12), or imposing a tone distracter. As well as increasing attentional demands, such manipulations may also lead to changes in response patterns leading to premature responding.

2.5.3.2.1 Reducing or varying the stimulus duration

Reduction in stimulus duration has been reported to produce effects on impulsive responding. For example, when stimulus duration was reduced from 1s to 0.3s, premature responses were increased in three inbred strains tested, the C57BL/6JOlaH, DBA/2N and 129SvHsd (Pattij et al., 2007). Reducing the stimulus duration to 0.5s caused an increase in premature responding in DBA/2 mice whereas C57BL/6 (sub strain unspecified) showed higher accuracy (Patel et al., 2006). Another author to report an increase in premature responding as a consequence of reducing the stimulus duration was de Bruin et al. (2006), who described in C57NL/6Jx129sv F2 mice (B6129F2), an increase not only in impulsivity when the SD was reduced from 2s to 1s, but also in perseverative responding when the SD was further reduced to 0.6s. But these manipulations of stimulus duration generally have greater effects on measures of attention. For instance, in de Bruin et al. (2006), an increase in omissions and a decrease in accuracy were also reported, and these were more evident with further reductions of the SD to 0.5s. Marston et al. (2001) described more profound deficits in accuracy in 129P2/OlaHsd mice than in C57BL/6J in sessions employing short SD.

In another study, with 3xTgAD mice, the reduction in SD to 0.6 s caused a decrease in accuracy accompanied by an increase in perseverative responses into the holes, deficits consistent with rat models of AD and AD patients (Lawrence and Sahakian, 1995; Romberg et al., 2011). Moreover, these same 3xTgAD mice, that showed no problems sustaining attention during the less attentionally demanding condition (1.5s), experienced a decrease in performance across the short SD session, possibly due to impairments in vigilance, as shown by an increase in omissions towards the end of the session. Generally, in the short SD session there is a decrease in accuracy accompanied by an increase in number of omissions (Relkovic et al., 2010) but these two variables can help to discriminate between strains, as in Humby et al. (1999), in where C57BL/6x129sv showed an increase in omissions at 0.4s SD while C57BL/6xDBA mice increased the omissions only when the SD was set at 0.2s, suggesting better attentional

abilities in the latter strain. Sometimes the increase in omissions is not accompanied by a decrease in accuracy, as in Wrenn et al. (2006) when the stimulus duration was reduced to 0.8s. Since this reduction (0.8s) is not as low as that used in other studies, and taking into account that they also found an increase in latency to collect the reinforcer, perhaps this attentional disruption was more related to motivational factors.

The short SD challenge has also been used to study the potential cognition enhancer, erythropoietin, in reversing the increment in omissions, with positive results in C57BL/6NCrl mice (El-Kordi et al., 2009; see **Table 2.1**, section h). Surprisingly, mice can learn to perform at very low SDs: Bailey et al. (2010) used one of the lowest SD challenges (0.125s), reporting that nicotinic alpha5 KO mice were less accurate than their WT control group under this protocol. Another study investigating genetics of attention used an extremely short SD of 0.1s, but in a 1-choice procedure, and found that the procedure could discriminate between strains (Davies et al., 2009).

A few studies have employed a variable SD presentation (SDs: 0.25, 0.5, 1.0, 2.0 and 4.0). Under this modification, Y2-/- mice (anxious phenotype), showed less accuracy and more premature responses than the less anxious WT control group (Greco and Carli, 2006). Moreover, a variable SD can also be used during standard training in a two-choice serial reaction time task (Lee et al., 2002). In that investigation, the SD was varied (1, 2 or 5s) according to the days of the week. As expected, and in accordance with other results, when animals were presented the highest SD (5s), the rate of omission was lower, as well as the correct response latency.

2.5.3.2.2 Reducing stimulus brightness (52, 30, 21 and 12% of full)

Reducing stimulus brightness has also been shown to discriminate between levels of attentional ability in mice: Humby et al. (1999) reported an increase in correct latency in C57x129sv and C57BL/6xDBA/2 mice, and described a larger impairment in accuracy and

omissions in the former group. The authors suggested that the differences in disruption of attentional variables could be due to different strategies in the two strains, or to differences in visual acuity. No differences in other variables were reported.

2.5.3.2.3 Imposition of white noise distractor

The main effects seen as a consequence of the white noise distractor challenge are decrements in accuracy (Davies et al., 2007) or in omissions (Humby et al., 1999), but no effects in premature responding have been described (Davies et al., 2007; Humby et al., 1999; Wrenn et al., 2006). Nevertheless, de Bruin et al. (2006) found no disruptive effects of this challenge on attention or premature responding but a reduction in the number of perseverative responses.

Table 2.1 Main effects of pharmacological manipulations in attention and impulsivity in the 5CSRTT in mice

	Compound	Drug	Dose (mg/kg)	Strain	C	Attention			Impulsivity		Learning	
						Accuracy	Omission	C.L	Prem	Persev	TT/criteria	
A	DA	Amphetamine (Loos 2010) [1.1]	1	D2J	b				↑ C57			Impulsivity
		GBR12909	10	C57J					↑C57			
		d-amphetamine (Yan 2011) [1.1]	1	NK1R-/-	c					↓		
					d				↑			
B	Glutamate	PCP (Greco 2005) [1.2]	1.5	D2N	b	↓			↑	=		
		LY379268	3	D2N		=			↓			
			1.0-3.0	C57N		=			↓			
		PCP (Pozzi 2010) [1.1]	1.5	D2	b	↓			↑	↑		
		M100907	10 µg/kg			↗			↘	↘		
C	Ethanol	ketamine (Oliver 2009) [1.1]	10-20.0	C57H CD1	c			↑ C57	↑CD1			
		Ethanol (Oliver 2009) [1.1]	2 g/kg	C57H	b		↑			↓ CD1		
			1 g/kg	CD1	c	=			↑	↓CD1		
		Ethanol (Walker 2011) [1.1]	chronic	C57J	b	=						
					d	=			↑	=		
			enduring			=						
D	GABA	Diazepam (Greco 2006) [1.2, 2]	2	Y2-/-	b	↓ Y2-/-	-	-	↑	=	-	
		FG7142, anxiogenic	10	Y2+/+	c	↓ Y2-/-	-	-	↓Y2-/-	=	-	
		Diazepam (Oliver 2009) [1.1]	1.0-2.0	C57H, CD1	c	-	-	-	↑	-	-	
		Diazepam (van Gaalen 2003) [0]	2.5-15	CRH Tg	b	=	-	-				
E	Ach	Scopolamine (Humby 1999) [1.1]	0.02-0.2-2	C57xD2	b	↓	↑		=	=		
			2	C57x129			↑↑ in C57xD2		=	=		
		methylscopolamine	0.02-0.2		1-CSRTT	=						
			2		b	=						Attention
		Donepencil (Romberg 2011) [1.1]	0.03-0.1-0.3	3xTgAD	e	↑	=		=	=		
		Galanin injection (Wrenn 2006) [1.1,3]	0.5-1.0 nmol	C57J		no eff on sust. att in Gal-tg						

Table 2.1 Continued

	Compound	Drug	Dose (mg/kg)	Strain	C	Attention			Impulsivity		Learning	
						Accuracy	Omission	C.L	Prem	Persev	TT/criteria	
F	Nicotine	Nicotine (de Bruin 2006) [0, 1#]	0.16	C57Jx129	e 1s	↑						Attention
					e 0.5	=						
		Scopolamine	0.16		b	↓	↓	↓				
		Scopolamine (Siegel 2011) [0]	1.4	Apoe$^{-/-}$	b	↓						
		Nicotine (Young 2004) [1.1,1.3]	3-1 µg/kg	α7 $^{-/-}$ C57J	e	↑	↓					
		Nicotine (Hoyle 2006) [0, 1.1]	1	α7$^{-/-}$, WT	b, 0	=						
					b, 1[1]	↑		↑	↓		↓R	
		Nicotine (Bailey 2010) [1.1, 2]	0.03	α5$^{-/-}$, WT	e	↓WT	=	↓	↑	=	=	
		Scopolamine (Pattij 2007) [1.1]	0.1-0.5-1.0	129H	e	↓			↑			
		Nicotine	acute	C57H D2H		↓						
			subchronic			↑						
G		DHEAS (Davies 2009) [1.1-3]	40mg/kg	39,XY*O 40,XY	b	=						
					e		↓					
		Coumate	10mg/kg			↓						
H		Erythropoietin (el-Kordi 2009) [1.1]	chronic	C57N	e		↓ from 4s to 2s				↑	

Number codes underline differences in the protocol implemented (*0* premature responses not punished, *1.1* premature responses punished with a time out (lights off), *1.2* time out can be terminated, *1.3* premature responses punished with a time out (lights on), *2* perseverative responses punished. Letter codes indicate the condition implemented in the task (a, training; b, baseline; c, long ITI; d, variable ITI; e, attentional challenges). Strain identifications are abbreviated as follows: *D2* DBA, *D2N* DBA/2N, *D2J* DBA/2J, *D2H* DBA/2OlaHsd, *C57* C57Bl/6, *C57N* C57Bl/6N, *C57J* C57Bl/6J, *C57H* C57Bl/6JOlaHsd, *α5-/-* α5 nACh KO, *α7-/-* α7 nACh KO, *129H* 129S2/SvHsd. Codes in the index are abbreviated as: *C* task condition, *C.L* correct latency, *Prem* premature responses, *Persev* perseverative responses, *TT* total trials, *number sign* absence of time out during the training, *TO* during challenge. ↘/↗, manipulation reversed effects, *R* reinforcer, *blank spaces* not reported

2.5.4 Pharmacological manipulations

Often, once animals acquire stable performance under baseline parameters, the effects of a series of drugs are tested. Results of some of the key drugs are summarised in **Table 2.1**, based upon the findings from mouse studies.

2.5.4.1 Dopamine

We will limit this review to the impact of different drugs on impulsive behaviour. As can be seen in **Table 2.1**, little is known with respect to the possible role of dopamine in the modulation of inhibitory control and visuospatial attention in mice (**Table 2.1**, section a). Loos et al. (2010) studied the effects of the psychostimulant amphetamine, and the DA uptake inhibitor, GBR12909 in the 5CSRTT (standard condition) and a go/no go task, in C57BL/6J and DBA2/J mice. As in rats (Harrison et al., 1997; Robbins, 2002), amphetamine increased premature responses, but only in C57BL/6J mice (which were showing lower baseline levels of premature responding in comparison with DBA2/J mice). On the other hand, amphetamine had no effects on attentional measures. GBR12909, given at the highest dose (10 mg/kg), decreased accuracy and increased premature responses in C57BL/6J mice, compared with saline. In summary, Loos et al. (2010) found that amphetamine and GBR12909 modulate inhibitory control mechanisms in C57BL/6J but not in DBA2/J mice. Moreover, amphetamine seems to increase impulsivity without affecting accuracy, while a higher dose of GBR12909 increased premature responding and decreased accuracy in C57BL/6J mice. Nevertheless, inconsistent results were found when d-amphetamine was tested in a model of ADHD in mice (NK1R-/-; Yan et al., 2011). As mentioned in the previous section, those animals presented increased perseverative and omission rate during a long ITI challenge, and also increased premature and decreased accuracy during the vITI. When d-amphetamine was administered under the long ITI condition, it decreased the number of perseverative responses and

omissions. In contrast, when the psychostimulant was administered on a variable ITI condition it increased premature responding.

2.5.4.2 Glutamate

In a study performed by Greco et al. (2005), the role of glutamate neurotransmission in impulsivity and attention was investigated using DBA/2N and C57BL/6N mice (see **Table 2.1**, section b). PCP, a non-competitive glutamate receptor antagonist, and LY379268, an mGluR2/3 receptor agonist, were tested. PCP, when given at a 1.5mg/kg dose, on standard conditions, increased the number of premature responses only in the animals that were already more impulsive (DBA/2N mice), and caused disruptions in accuracy, as compared to C57BL/6N. Surprisingly, when administering LY379268, premature responses were diminished but only in the mice that showed low levels during the baseline (C57BL/6N). Three aspects of this study need to be considered: first, PCP increased impulsivity only in baseline high-impulsive mice, whereas LY379168 reduced premature responses only in baseline low-impulsive mice; second, strain contributed to the different effects of PCP and LY379168; and third, perseverative and premature responses seemed to be controlled by different mechanisms, since PCP only affected perseverative responses in DBA/2N but not C57BL/6N mice. Furthermore, accuracy and premature responding might be associated, since PCP increased premature responding and reduced accuracy in DBA/2N mice. However, this apparent relationship should be treated with caution, since the effects of administering LY379168 were limited to premature responding without modifying accuracy (Greco et al., 2005).

A similar result is reported by Pozzi et al. (2010); again, PCP impaired inhibitory response control in DBA/2 mice, but this time increased not only premature but also perseverative responding. This result was accompanied by a decrease in accuracy, in agreement with Greco et al. (2005). However, while Greco et al. (2005) found LY379168 to decrease premature

responding only in C57BL/6N mice, in contrast, in the study by Pozzi et al. (2010), the 5-HT2A antagonist M100907, was able to reverse the effects induced by PCP, by increasing accuracy and preventing perseverative and premature deficits, in both C57BL/6N and DBA2/N mice. Thus, this study adds evidence supporting a role for glutamate and serotoninergic neurotransmission in attention and impulsivity. Similar results were also described in rats, where M100907 reduced and SB242084 increased premature responding in a long ITI session; no treatments in this study significantly impaired accuracy (Fletcher et al., 2007).

In keeping with NMDA receptor blockade increasing impulsive behaviour, Oliver et al. (2009) found that the NMDA receptor antagonist ketamine (10 and 20mg/kg) increased premature responses in CD1 mice, but not in the C57BL/6JOlaHsd strain. No disruptions in perseverative responding or in accuracy were seen.

Ethanol possesses some pharmacological effects as an antagonist of NMDA receptors, but no effects of ethanol were seen during baseline conditions of the task; when the mice were confronted by long ITI sessions, ethanol (1g/kg) increased premature responding in both C57BL/6JOlaHsd and CD1 mice (Oliver et al., 2009), in contrast to a study reported in rats, where ethanol at 1.2 and 1.6g/kg resulted in a reduction of impulsivity (Bizarro et al., 2003). In a later study (Walker et al., 2011) we found that chronic ethanol treatment induced no impairments in impulsivity in baseline conditions of the task. However, when given a vITI challenge, ethanol treated C57BL/6J mice took more sessions to diminish premature responding (after repeated testing) in comparison to control mice. Even though no differences in impulsive responding were seen between groups during the first challenge, the ethanol-treated mice remained impulsive for longer. As in Oliver et al. (2009), the disruption in premature responding was not accompanied by disruptions in attentional ability. Alcohol withdrawal may increase glutamatergic transmission, leading to hyperexcitation, which dissipates over time, perhaps explaining the temporary nature of the learning deficits

(Stephens and Duka, 2008). If the vITI procedure requires a switch in strategy from the use of internal timing to predict stimulus onset under baseline conditions to one in which the timing of the response is externally cued by light onset, these observations might suggest that chronic alcohol impairs the ability to flexibly switch strategies to fit the new requirements, consistent with human data on alcoholic patients (Duka et al., 2011).

Additionally, ethanol may have also resulted in a decrease in sensitivity to TO punishment, taking into account that when animals are not trained under TO periods they acquire the task more slowly (Christakou et al., 2004)

2.5.4.3 GABAergic system

Oliver et al. (2009) reported premature responses to be increased in strains C57BL/6JOlaHsd and CD1, after diazepam administration, with no disruptions in perseverative responding or in accuracy. In this experiment, diazepam mimicked the effects of ethanol in both strains.

GABAergic pharmacological manipulations have also been used to test the hypothesis of impulsivity being associated with anxiety (see **Table 2.1**, section d). The same anxiolytic drug diazepam increased premature responding in an anxious group of mice (Van Galeen et al., 2003). The opposite result was found by Greco and Carli (2006) who reported that diazepam increased premature responses in Y2 -/- and WT mice but this increase was greater in the animals that presented a less anxious phenotype (Y2 -/-). In the same study, the anxiogenic compound FG7142 decreased premature responding again in the non anxious Y2 -/- mice. No effects on perseverative responding were found with any of these compounds. These results seem to indicate the existence of a possible relationship between anxiety and impulsivity, low levels of anxiety being indicative of higher impulsivity in the 5CSRTT, as proposed by Loos et al. (2009).

Interesting results were found by Davies et al. (2009) where the neurosteroid dehydroepiandrosterone sulfate (DHEAS), a compound with activity at both $GABA_A$ and NMDA receptors, and hypothesized to influence ADHD endophenotypes (attention, motor impulsivity, and activity), proved to enhance attentional functioning but showed no effects on inhibitory control (see **Table 2.1**, section g). These findings are not easy to integrate with the above, possibly because DHEAS has a number of additional actions (Yadid et al., 2010).

2.5.4.4 Cholinergic mechanisms

Administration of nicotine increased number of premature responses and decreased correct latency in nicotinic α5 KO and WT mice when tested under a short SD session (Bailey et al., 2010).

But apart from the study of impulsivity, most investigations using the 5CSRTT have been focused in the evaluation of attentional function (see **Table 2.1**, section e). In the very first study using the 5CSRTT in mice, Humby et al. (1999) studied the role of other cholinergic compounds, such as the muscarinic antagonist scopolamine, describing disruptions in accuracy and omissions but reporting no effects in premature or perseverative responding. In line with this report, as replicated in subsequent studies (de Bruin et al., 2006; Siegel et al., 2011), ACh mechanisms proved to be important for attentional functioning in mice, but not for inhibitory control. Specifically, Romberg et al. investigated the impact of donepezil, a cholinesterase inhibitor, in attentional performance of 3xTgAD mice, a mouse model with cholinergic deficits used as a model of human AD (Romberg et al., 2011). Donepezil selectively increased accuracy of responding, reducing decrements in vigilance throughout the session, while no effects in omissions or perseverative responding were reported.

With regard to nicotine, several studies have described an enhancement in attentional performance in mice in the 5CSRTT, in line with effects in humans and rats (Hahn et al., 2002;

Hahn and Stolerman, 2002) (see **Table 2.1**, section f). Specifically, nicotine improved attention in comparison to the vehicle-treated mice (Young et al., 2004; de Bruin et al., 2006), the effect persisting after chronic nicotine treatment (Pattij et al., 2007). Nevertheless, when another protocol (limited LH and punished premature responding; Hoyle et al. 2006) and manipulations (short SD condition; Bailey et al., 2010) were implemented, nicotine failed to show the mentioned attentional benefits. If anything, nicotine caused a general impairment in omissions, exacerbated in nicotinic alpha7 KO mice, suggesting that alpha7 nAChR may be involved in mediating the effects of nicotine in the task (Hoyle et al., 2006). Thus, the beneficial effects of nicotine are restricted to certain conditions (Bailey et al., 2010). Consistent with a role for alpha7 nicotinic receptors in attentional performance, KO mice for α7 nAChR were unable to perform the task equally to the wild-type (Young et al., 2004). Similarly, mice lacking apolipoprotein E (*Apoe*$^{-/-}$) could not acquire the task performance criteria in terms of attention (Siegel et al., 2011), which suggested that apolipoprotein E may alter ACh neurotransmission and, consequently, impair cognition.

So far, pharmacological experiments strongly support the hypothesis that results depend on parameters of the task. **Figure 2.3** exemplifies the complex relationship between the 5CSRTT variables. All those variables build an intrinsic structure where one variable is associated with others but, at the same time, are also controlled by independent mechanisms. Moreover, we emphasise the need to test drugs in other paradigms of attention and impulsivity in order to draw a more complete picture (Dalley et al., 2008; Pattij and Vanderschuren, 2008).

Table 2.2 Strain differences in the main 5CSRTT variables

Strain		Reference	Comparison	Punishment	Condition	Attention			Motivation	Impulsivity		Learning
						Accuracy	Omission	C.L	Mag lat	Prem	Persev	TT/criteria
α7-/-		Hoyle et al. 2006	C57J*	0	a	↓				↑		
				1[a]		=	↑			=		
		Young et al. 2004	C57J**	1[a,d]	a	=	↑					↑ acq
α5-/-		Bailey et al. 2010	C57J	1[a,d]	a	↓						
					e	↓ both	=					
Gal-Tg		Wrenn et al. 2006	WT	1[a,d]	a	=						
					e ~	=	↑	=				
					a 2s		↑					↑days at 2s
					d	=						
					f		↑					
	C57J	Loos et al. 2010	D2J	1[a, 1]	a	=						
					b	=	=	↓		↓		
	C57J	Patel et al. 2006	D2	0	a	=						
					b	↑				↓		
			xD2		b	=						
	C57	Humby et al. 1999	x129	1[a]	d	=	↑ both	↓ C57xDBA			=	
					e	↓ both	↑ C57x129			=	=	
	C57CR	el-Kordi et al. 2009	-	b	e		↓ C57CR					
C57					a	C57xDBA = C57x129sv						
	C57J	Marston et al. 2001	129H	1[a]	e, 0.25s	↑C57 J						
					c	↓ C57J						
	C57H	Pattij et al. 2007	D2N	1[a]	b	↓	↑ and DBA	↓ and DBA	↓	↑C57 than 129		
			129H		e					↑all, ↑↑ D2		
	C57H	Oliver et al. 2009	CD1	1[a]	a		↑			↓		
	C57J	Walker et al. 2011	-	1[a]	d		↑ at 2s ITI	↑ at 7s ITI		↑ at 7s ITI	↓ at 7s ITI	
	C57CR	Greco et al. 2005	D2CR	1[c]	b	↑				↓	=	=
					e					=	↓	
	C57CR	el-Kordi et al. 2009	no EPO treatm	b	see pharmachology							

Table 2.2 (continued)

Strain	Reference	Comparison	Punishment	Condition	Attention			Motivation	Impulsivity		Learning
					Accuracy	Omission	C.L	Mag lat	Prem	Persev	TT/criteria
Caspase 3	Kerr et al. 2004	C57J x CBA		a	no age-deficits						=
39,X^{Y*}O	Davies et al. 2009	40,XY	1^{a-d}	a, b	=						
				e		↑ at 0.1s					
				d					↓		
Apoe $^{-/-}$ M&F	Siegel et al. 2011	C57J Apoe $^{+/+}$	0	a							not acq perf
tau V337M	Lambourne et al. 2007	C57J x CBA/C	1$^{c, 1.4}$	b					↑		
				d					↑ both		
				e	↑both						
NK1R-/-	Yan et al. 2011	129Sv x C57H	1^{a}	c	=	↑	=	↑	=	↑	-
				a	=	↑	=		↓	↑	↑sess
				d	↓	↑	↑	=	↑	↑	-
CRH Tg	van Gaalen et al. 2003	WT	b	e (0.5s)	↓		↑		↓		↓ R
				a		↑	↑		↓		
Y2 $^{-/-}$	Greco et al. 2006	Y2$^{+/+}$	1^{a}	a	↓				↑	=	
				e	↓	↑			=	=	
				d	=	=			↑	=	
LP-BM5	Lee et al. 2002	C57J	0	e, 2CSRTT		↓	↓				
3xTgAD	Romberg et al. 2006	C57J	1^{a}	b, 2s	=						
				e	↓					↑	
Turner, 39,XO	Davies et al. 2007	40,XY^{*Y}		b	=						
			1^{,3}	e	↓ both		↑				
				d	=				=		
DBA/2J	Pozzi et al. 2010	-	1^{b}	see pharmachology							
PWS-IC$^{+/-}$	Relkovic et al. 2010	C57J, M and F	1^{a}	a	↓	↑	↑				↑sess.
				e	↓	↑					
				d					↑		
(C57Jx129)F2	de Bruin et al. 2006	-	0	see pharmachology							

Number codes underline differences in the protocol implemented (*0* premature responses not punished, *1.1* premature responses punished with a time out (lights off), *1.2* time out can be terminated, *1.3* premature responses punished with a time out (lights on), *2* perseverative responses punished. Letter codes indicate the condition implemented in the task (*a* training, *b* baseline, *c* long ITI, *d* variable ITI, *e* attentional challenges). Strain identifications are abbreviated as follows: *D2* DBA, *D2N* DBA/2N, *D2J* DBA/2J, *D2H* DBA/2OlaHsd, *C57* C57BL/6, *C57N* C57BL/6N, *C57J* C57BL/6J, *C57H* C57BL/6JOlaHsd, *α5-/-* α5 nACh KO, *α7-/-* α7 nACh KO, *129H* 129S2/SvHsd. Codes in the index are abbreviated as: *C.L* correct latency, *Mag lat* magazine latency, *Prem* premature responses, *Persev* perseverative responses, *TT* total trials. Abbreviations in the learning column are: *acq* acquisition, *perf* performance, *sess* number of sessions, *R* reinforce,˜ constant illumination condition, * donor 129S7, ** strain backcrossed for a further 6 generations, *blank spaces* not reported

2.6 Looking for candidate genes

As introduced in the first paragraphs of the present review, the use of the 5CSRTT in mice allows the study of the contribution of both genetic and environmental factors, and their interactions, in the study of impulsivity, compulsivity and attention. The publications reviewed here are an example of the increasing number of investigations using different inbred strains with the aim of unravelling the genetics of impulsivity (Humby et al., 1999; Isles et al., 2004; Patel et al., 2006; Pattij et al., 2007; Loos et al., 2009; Loos et al., 2010) (see **Table 2.2**). For instance, Isles et al. (2004) used the strategy of testing 4 inbred strains to investigate the genetic contribution to impulsive behaviour by using a delayed-reinforcement paradigm, which evaluates impulsive choice. Furthermore, Loos et al. (2009), measured locomotor activity and impulsivity in the 5CSRTT in 12 different inbred strains of mice: after repeated testing in a variable ITI condition, the authors concluded that both genetic and environmental factors contribute to the stability of impulsivity over time. The authors reported genetic correlations between impulsivity and the expression of the genes Frzb, Snx5 and BC056474 in dorsal mPFC (Loos et al., 2009). Frzb gene inhibits the Wnt signalling pathway, which has shown an important role in axon path finding (Bovolenta et al., 2006) and synapse structure and function (Ataman et al., 2008); and the Snx5 in intracellular trafficking (Otsuki et al., 1999) and in response to ethanol treatment (Kerns et al., 2005).

Inbred strains of mice represent a powerful tool to study the contribution of genetic factors in behaviour, and taking this approach a step further, the BXD recombinant inbred strains of mice have proven to be an invaluable tool for behavioural genetics (Crabbe et al., 1999; Chesler et al., 2003). BXD mice derive from the cross of C57BL/6J and DBA2/J mice, two strains that differ in a variety of behavioural traits (see present review, also Crawley et al., 1997; Phillips et al., 1998). Because this inbred panel is composed of genetically identical individuals (within each strain) they can be repeatedly tested and data collected from different laboratories can be

compared and added to a large database to allow multi-trait analysis. Using web QTL database (www.webqtl.org, Chesler et al., 2003), data collected from Affymetrix microarrays in the BXD strains can be used to carry out genetic correlation analysis of gene expression with any other trait of interest, such as with behavioural traits: i.e., impulsivity or compulsivity in the 5CSRTT (Chesler et al., 2003).

In our laboratory, we have collected data from 12 BXD recombinant inbred strains and their progenitor C57BL/6J and DBA/2J mice in the 5CSRTT (using long ITI probe sessions; Pena-Oliver, in preparation) with the aim of finding candidate genes responsible of the impulsive phenotype.

2.7 Conclusions

Mice are just as good as rats in the 5CSRTT. The results presented illustrate that mice are capable of learning the complex 5CSRTT, and show many similarities to rats. Findings across laboratories are reproducible, provided that the same procedures are used. However, variations in procedure and differences between strains can give rise to quite marked differences in outcome. Thus, bear in mind to choose the strain and task parameters depending on the question being asked. The 5CSRTT paradigm is based on appetitive learning and, therefore, the confounding effects of stress are less likely to affect the animal performance, especially in stress-reactive strains. This offers the opportunity to test transgenic and knockout mice with similar background as animal models of human psychiatric and neurological diseases. However, understanding the meaning of the different variables and the way they interact is crucial to understanding the mechanisms that lead to different phenotypes. New research approaches, such as the use of inbred strains, will bring us a step closer to the discovery of the genetics of impulsivity and attention.

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2.9 CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Chapter 3

Paper 2

EXAGGERATED WAITING IMPULSIVITY ASSOCIATED WITH HUMAN BINGE DRINKING, AND HIGH ALCOHOL CONSUMPTION IN MICE

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3.1 Abstract

There are well-established links between impulsivity and alcohol use in humans and animal models; however, whether exaggerated impulsivity is a premorbid risk factor or a consequence of alcohol intake remains unclear. In a first approach, human young (18-25 years) social binge and non-binge drinkers were tested for motor impulsivity and attentional abilities in a human version of the Five-Choice Serial Reaction Time Task (Sx-5CSRTT), modelled on the rodent 5CSRTT (Robbins, 2002). Participants completed four variants of the Sx-5CSRT, in addition to being screened for impulsive traits (BIS-11 questionnaire) and impulsive behaviour (by means of the Delay Discounting Questionnaire, Two Choice Impulsivity Paradigm (TCIP), Stop Signal Reaction Time and Time Estimation Task). Using a second approach, we compared one of these impulsivity measures, 5CSRTT performance, in two inbred strains of mice known to differ in alcohol intake. Compared to non-bingers (NBD; n=22), binge drinkers (BD, n=22) showed robust impairments in attention and premature responding when evaluated under increased attentional load, in addition to presenting deficits in decision making using the TCIP. The best predictors for high binge drinking score were premature responding in the Sx-5CSRTT, trait impulsivity in the BIS-11 and decision making in the TCIP. Alcohol-naïve C57BL/6J (B6) mice (alcohol-preferring) were more impulsive in the 5CSRTT than DBA2/J (D2) mice (alcohol-averse); the degree of impulsivity correlated with subsequent alcohol consumption. Homologous measures in animal and human studies indicate increased premature responding in young social BD and in the ethanol-preferring B6 strain of mice.

Key Words: Premature responding, Attention, adolescent, Sx-5CSRTT, Alcohol, Mouse.

3.2 INTRODUCTION

Impulsivity has been consistently implicated as both a determinant and a consequence of alcohol abuse. Binge patterns of alcohol consumption, in particular, have been associated with impaired attentional function and executive function (Townshend and Duka, 2005; Scaife and Duka, 2009) and increased impulsive behaviour in both humans and rodent models (Duka et al., 2003; Stephens and Duka, 2008; Bell et al., 2013). However, it is unclear whether high levels of impulsivity are a cause or consequence of alcohol intake.

Given the prevalence of binge drinking in adolescence (Healey et al., 2014), the first aim of our study was to examine the relationship of binge drinking to measures of waiting impulsivity and attentional abilities in human adolescents (18-25 years old) and in rodents, which consume alcohol. Taking the rodent 5CSRTT (Robbins, 2002) as a model, we developed a novel, iPad-based task (Sussex 5CSRTT; Sx-5CSRTT), to assess both attentional performance and anticipatory behaviour in humans. BD were also characterized in four additional measures of impulsivity, based on different operational definitions of the construct. The Stop Signal Task, used to assess ability to inhibit a prepotent response (Logan, 1994), served as an additional measure of 'motoric impulsivity'. The Delay Discounting Questionnaire measured preference for immediate over delayed rewards (Petry, 2001), and, combined with the Two Choice Impulsivity paradigm (Dougherty et al., 2005) provided an index of 'choice' impulsivity or decision making; the fourth behavioural measure used was the Time Estimation Task. Although each of these five measures has been used in the clinical context as an index of impulsive behaviour, the sensitivity of each task to characterize impulsive phenotype in young social BD, and their relationship to one another has not been explored. We anticipated binge drinking might be differentially associated with different aspects of impulsive behaviour, but generally that high binge drinking scores would be associated with high levels of trait and behavioural motor impulsivity and aversion to delay.

The role of premorbid impulsivity as a predictor of elevated alcohol intake cannot be easily disentangled in human studies, as impulsivity measures are almost inevitably assessed after a period of alcohol use. Animal models are more powerful tools in this respect as they allow the exclusion of alcohol experience as a potential contributor to impulsivity. Therefore, having established that 5-CSRTT waiting impulsivity was associated with human binge drinking, we asked whether waiting impulsivity in alcohol-naïve mice predicted alcohol drinking in two widely-used B6 and D2 inbred strains, that also differ in alcohol consumption (Crabbe et al., 1994). We have previously reported (Walker et al., 2011) that prior exposure to high alcohol concentrations over several weeks in adulthood has only transitory effects in increasing impulsivity in B6 mice. We predicted greater impulsivity in the high-ethanol preferring mice indicating a potential causal relationship between waiting impulsivity and high alcohol consumption.

Using homologous measures of impulsivity in mouse and humans, we provide evidence that waiting impulsivity is associated with binge drinking in young adult humans, and predicts alcohol consumption in mice.

3.3 MATERIALS AND METHODS

3.3.1 Human study

3.3.1.1 Recruitment and Procedure

44 participants (22 male; age 18-25 years, *M*=21.18, *SD*=1.89), recruited from the University of Sussex subject pool, were assigned to the binge (binge score >32; BD) or non-binge (binge score <16; NBD) condition using scores from Alcohol Use Questionnaire (Mehrabian and Russell, 1978). To assess alcohol drinking patterns, a “binge drinking” score (Townshend and Duka, 2002), was calculated based on the speed of drinking (number of drinks per hour), the number of episodes of alcohol intoxication in the past 6 months, and the percentage of alcohol

intoxications out of the total number of times of going out drinking (see Supplemental Material). An overall score for weekly alcohol-unit consumption was also estimated.

Participants were healthy social drinkers (see Supplemental Material for further details of inclusion criteria). Upon arrival at the laboratory, a breathalyser (Lion Alcolmeter SD-400; Lion Laboratories Ltd, Barry, UK) was used to ensure zero breath alcohol levels. Participants completed: a) The Barratt impulsivity scale, version 11 (Patton et al., 1995), a 30-item checklist that gives a total impulsivity score and three sub-scores of attentional, motor and non-planning impulsiveness; b) the Alcohol Use Disorder Identification Test (AUDIT) (Saunders et al., 1993), to evaluate heavy drinking and/or active alcohol abuse or dependence; and c) the National Adult Reading Task (NART) (Nelson and O'Connell, 1978), an estimate of verbal IQ. Following instructions and practice trials, participants were presented with five computerised tasks in random order (see below). At the end of the session (90 minutes), subjects were debriefed, informed of risks associated with binge drinking and entered into a prize draw to win £25. All participants gave informed consent to take part in the study, which was approved by the University of Sussex ethics committee.

3.3.1.2 Behavioural Measures of Impulsivity

The Sx-5CSRTT was administered using an iPad (iOS 6 operating system; Apple Inc), programmed in Mac OS X (Apple Inc). **Figure 3.1** depicts an example trial of the task. In brief, participants were required to detect and respond to the brief (0.5s) highlighting of one of five moving visual stimuli. Responding before stimulus onset was considered a measure of poor inhibitory control, recorded as a premature response and followed by a 5s time-out period. Following practice trials in which the stimulus was presented every 5s (ITI 5-s) participants performed four task variants: a fixed (fITI) and a variable (vITI) session under simple task conditions; and, in order to increase the attentional load, a fITI and vITI session in combination

with a dual task (Hogarth et al., 2008) in which subjects were also required to respond to a 659 Hz tone by performing a key press with the non-dominant hand. Main outcome variables were 'accuracy', 'percentage of omissions' and 'percentage of premature responding'.

The Stop Signal task (SST) (Logan, 1994) to test response inhibition; a delay discounting questionnaire (DDQ) (Petry, 2001), and Two Choice Impulsivity paradigm (TCIP) (Dougherty et al., 2005), to assess preference for a small immediate over a large delayed reward, and the Time Estimation Task (TE) to evaluate the subject's time perception were added. Main outcome variables included the Go RT and calculated SSRTi from SST; the slope and area of the discounting curve (*k and* AUC parameters) from DDQ, and proportion of immediate choices and maximum number of consecutive delayed choices from the TCIP; and the subject's accuracy of performance in TE. See Supplementary material for details of the tasks and analysis of main variables.

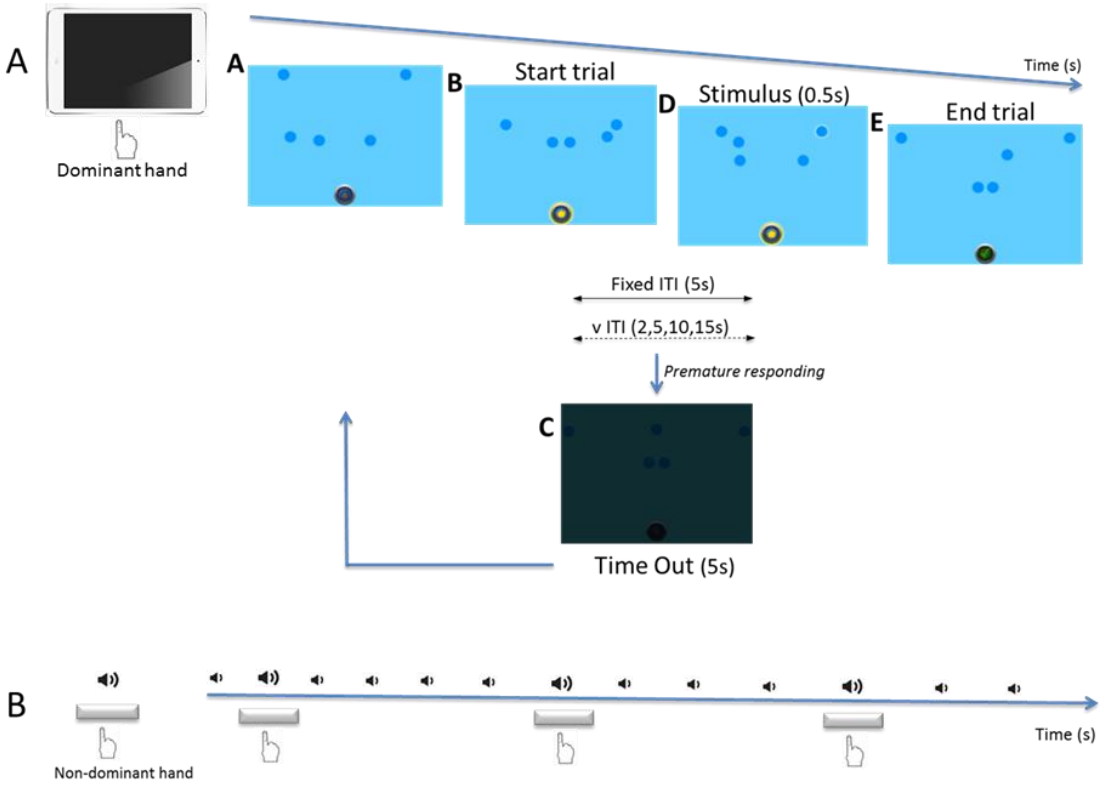

Figure 3.1 (A) Participants were comfortably seated in front of a touch screen. Viewing distance was approximately 30 cm with a vertical visual angle of -30° and a horizontal visual angle of 0°. The task consisted of five independently-moving blue circles (stimulus) represented in a "circular" motion in a tactile screen. We adopted moving targets in an attempt to increase attentional load, which, in the mouse task, comes about because reinforcer retrieval, as well as spontaneous locomotor activity diverts attention from the 5-choice array. Below the stimuli and at the bottom of the screen a home button was located. Trial commenced by the illumination of the house button (B). The participant was required to tap and hold onto the home button, and withhold responding until the stimulus presentation. After a designated inter-trial interval (ITI; s), one of the 5 circular visual stimuli modified its contour (D) and the participant was then required to tap into the highlighted circle and return to the home button. Illumination of the home button signalled the start of a new trial (E). Omissions (failure to respond to the signalled stimulus within a concrete period of time), incorrect responses (tapping into a non-designated circle) and premature responses (responses into the circles during the inter-trial interval prior to the stimulus presentation) were followed by a designated time out period of 5-s (C). Perseverative responses (responding repeatedly to the circles after a correct detection) were also assessed. Total number of trials completed was determined, providing a measure of motivation. Following practice trials (correctly responding in each of the five signalled stimuli, or after 3 minutes, whichever came first), participants performed four task variants: a fITI and vITI session under simple task conditions (n=31-32; panel A); and a fITI and vITI session in combination with a dual task (n=44; panel B). During the dual task, participants were required to discriminate between sequences of low and high pitched tones (13 blocks of 10 trials; 1 high tone/ block, presented in random order) and respond to the latter by pressing a space bar located in an external keyboard whilst performing the Sx-5CSRTT with their dominant index finger

3.3.2 Mouse study

3.3.2.1 Subjects

Two cohorts of mice from B6 (n=21) and D2 (n=22) strains, purchased from The Jackson Laboratory (Bar Harbor, Maine, USA), were used. Mice were housed in groups of two per cage on a 12-h light/dark cycle (lights off at 19.00) at a temperature of 19-21°C and 50% humidity. Before starting 5-CSRTT training, mice were food-restricted to reduce their body weights to 85% of their free-feeding weight. Water was available *ad libitum*. Behavioural testing took place between 8:00 and 14:00 hours, 5-6 days per week. Experiments were approved by the institutional ethics committee and performed under United Kingdom legislation on animal experimentation [Animal (Scientific Procedures) Act, 1986].

3.3.2.2 Five-choice serial reaction time task (5-CSRTT)

Testing of performance followed the protocol previously described (Pena-Oliver et al., 2012; see Supplemental Methods). In brief, following training under fITI conditions, mice were tested under vITI (2, 5, 10, 15s) conditions to increase premature responding (Robbins, 2002; Sanchez-Roige et al., 2012).

3.3.2.3 Alcohol Consumption

Following completion of the 5CSRTT, mice were given free access to food and water for 14 days before being tested for alcohol consumption and preference, using a 2-bottle choice test (Belknap et al., 1993). See Supplemental Material for details.

3.3.3 Statistical analysis

Statistical analysis was performed using the “Statistical Package for Social Sciences” (SPSS, version 20.0). Following 2-way ANOVA with group (2 levels: BD, NBD) and gender (2 levels) as between subject factors, group differences were explored using one-way ANOVA. ‘Binge drinking’ scores were square-root transformed to obtain homogeneity of variance, though untransformed means are shown throughout. Daily cigarette use, Total BIS Score, the

behavioural measures of “accuracy” (fITI and vITI with dual task), “percentage of omissions” (fITI and vITI with dual task), and “percentage of prematures” (vITI with dual task) were analysed by Mann-Whitney *U* tests.

Pearson’s correlation coefficient *r* was used to determine relationships between Binge Scores and behavioural impulsivity measures and trait impulsivity measures from BIS-11. Trait impulsivity from BIS-11 was also correlated with the impulsivity measures. Finally, the variables “SSRT”, “Maximum delayed choices”, “BIS-trait” and “premature responding” (the main variables denoting impulsivity) were entered into multiple regression analyses with binge drinking score as the dependent variable. Outliers (>3 SD above group mean) were removed. Assumptions of normality (Shapiro–Wilk statistic) and homogeneity of variance (Levene’s test) were met, unless otherwise stated.

Repeated measures ANOVA was used to compare performance across ITI conditions (4 levels) as within-subject factors and strain as between factors. Where sphericity assumptions were violated, the Greenhouse–Geisser correction was applied and epsilon (ε) values reported.

3.4 RESULTS

3.4.1 Human Study

22 BD were compared with 22 NBD. Groups were matched on gender and IQ, but BD were younger ($F(1,43)= 14.712$, $p= .001$), reported an earlier drinking onset ($F(1,42)= 4.707$, $p= .036$) and presented higher scores on the AUDIT Test ($F(1,43)= 23.214$, $p= .001$). Compared to NBD, BD subjects presented higher binge drinking scores (see **Table 3.1**; $F(1,43)= 296.443$, $p= .001$). BD subjects displayed significantly higher scores in the motor impulsivity and non-planning subscales of the BIS-11 questionnaire ($F(1,43)= 6.820$, $p= .012$; $F(1,43)= 4.525$, $p= .039$, respectively; **Figure S3.1**), and a marginal tendency to present higher total scores of trait

impulsivity (*U*(42)=164.5, *p*= .068). There were no statistical group differences on scores for the attentional subscale (*F*(1, 43)= 2.344, *p*= .133).

Table 3.1 Group characteristics (age, smoking per day, verbal IQ, alcohol use, onset of drinking) and additional impulsivity measures for NBD and BD

Variable	Non-Binge Drinkers	Binge Drinkers	Statistics
N	22 (11M, 11F)	22 (11M, 11F)	
Age [a]	22.14 (1.83)	20.23 (1.44)	*F*(1, 43)= 14.712, *p*= .001
Cigarette per day (N) β	1.55 (.86)	1.23 (.97)	*U*(42)= 188.5, *p*= .165
Binge Drinking Score¥ [a]	5.96 (4.08)	48.39 (13.10)	*F*(1, 43)= 296.443, *p*= .001
AUQ – weekly units [a 1]	10.10 (8.34)	25.22 (13.23)	*F*(1, 43)= 23.618, *p*= .001
NART - IQ [°]	114.53 (9.91)	112.0 (8.17)	*F*(1, 38)= .173, *p*= .679
Onset [a °]	15.76 (1.73)	14.73 (1.39)	*F*(1, 42)= 4.707, *p*= .036
AUDIT [a]	5.77 (3.85)	12.59 (5.40)	*F*(1, 43)= 23.210, *p*= .001
Barratt Impulsivity Scale			
Total Score β	61.50 (10.34)	68.90 (7.61)	*U*(42)= 164.5, *p*= .068
Time Estimation			
Accuracy	99.48 (19.70)	91.48 (26.19)	*F*(1, 43)= 1.310, *p*= .259
Stop Signal Task			
Go Reaction Time	448.57 (28.81)	423.26 (28.09)	*F*(1, 43)= .270, *p*= .606
SSRTi	169.65 (12.52)	187.67 (12.00)	*F*(1, 43)= .899, *p*= .348
Delay Discounting			
AUC	.27 (.27)	.23 (.26)	*F*(1, 43)= .169, *p*= .683

Values are expressed as mean ± SD. AUQ, Alcohol Use Questionnaire; NART (National Adult Reading Test; verbal IQ); SSRTi = Stop Signal Reaction Times (milliseconds, ms); AUC= Area Under the Curve of Delay Discounting; Delayed choices = maximum number of consecutive delayed choices in a Two-Choice paradigm

β, non-parametric; ¥**,** SQRT transformed

[a] significant group differences

[1] one alcohol unit = 8 grams of alcohol

[°] The onset age of drinking for one male NBD was not recorded. All except 8 spoke English as their first language; the National Adult Reading Test (NART) scores from these 8 were discarded

3.4.1.1 Binge Drinkers' performance on The Sussex-Five Choice Serial Reaction Time Task

Simple Task Conditions

During the fITI session, BD displayed higher levels of premature responding (F(1, 30)= 4.656, p= .039; **Figure 3.2C**) compared to NBD, but a group x gender interaction indicated that male BD showed more premature responding than male NBD (F(1, 27)= 4.655, p= .04; **Figure S3.3A**). No other group differences were found (F< 3, ps>.05).

During the vITI session no group differences on premature responding or attentional (accuracy and omissions) performance were found (F<1.6, ps >.05). A significant group x gender interaction revealed lower levels of accuracy in male BD than male NBD (F(1, 28)= 6.058, p= .02; **Figure S3.3A**).

Dual Task conditions

During the fITI-dual task session, group differences appeared in measures of attention; BD showed lower accuracy (U(44)=157, p= .027; **Figure 3.2D**) and higher levels of omissions (U(44)=138, p= .019; **Figure 3.2E**). No effects of binge drinking were detected for premature responding (p >.05; **Figure 3.2F**).

When participants performed a vITI-dual task session, attentional deficits in BD were again detected; BD subjects showed lower accuracy (U(44)=146.5, p= .009; **Figure 3.2D**) and more omissions (U(44)=161, p= .049; **Figure 3.2E**). Under these task conditions, BD also showed high percentage of premature responses (U(44)=118, p= .003; **Figure 3.2F**).

The groups did not significantly differ on accuracy in detecting tones during the dual task in any of the conditions (F<3.8, ps> .05).

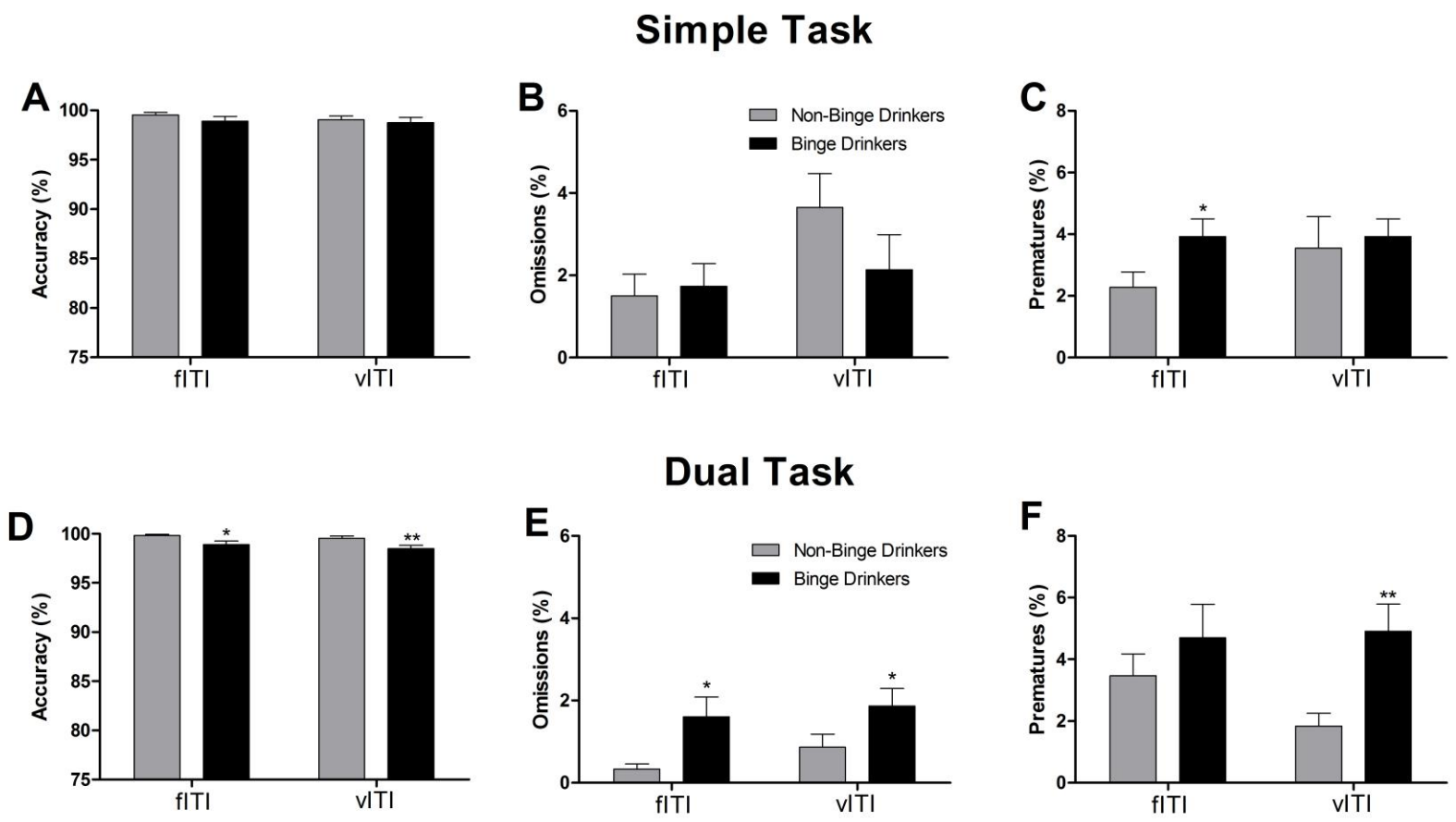

Figure 3.2 Sx-5CSRTT performance during a Fixed ITI (fITI) and vITI sessions under Simple Task (top panels) and Dual Task (bottom panels) conditions for NBD and BD. Mean (±SEM) of the percentage of A-D) Accuracy of responding, B-E) Percentage of omissions, and C-F) premature responses. **p*< .05, ***p*< .01 compared to NBD (independent sample *t*-test or non-parametric Mann-Whitney *U*-test)

3.4.1.2 Binge drinkers' performance on additional behavioural measures of Impulsivity

There were no group differences in any of the SST measures (F<1.4, ps> .05). Similarly, with regards to DDQ 'choice' impulsivity, both groups showed a similar linear decrease of indifference point as a function of increased delay (R^2 values ranged from 0.92 to 0.94; k values for NBD and BD were 0.008 and 0.01, respectively; see **Table 3.1**). In contrast, BD chose delayed options less frequently than NBD during the TCIP (F(1, 43)= 5.533, p= .023; **Figure 3.3B**) and a tendency to display more immediate choices was detected (F(1, 43)= 3.627, p=.064; **Figure 3.3A**). One-way ANOVA failed to reveal group differences on the accuracy of time estimation (F(1, 43)= 1.31, p= .259).

Gender differences in performance were detected only during the SST; female subjects displayed faster Go RT than males (F(1, 43)= 4.407, p= .042). There were no group x gender interactions in any tasks (F<2.7, ps>.05).

Figure 3.3 Performance during the Two-Choice Impulsivity paradigm. Mean ±SEM of A) proportion of immediate choices and B) maximum number of consecutive delayed choices. *p< .05 compared to NBD (independent sample t-test), (*) p= .064

3.4.1.3 Correlations between binge drinking scores and Impulsivity Measures

Significant positive correlations were found between binge scores and levels of BIS motor impulsivity trait ($r(44)=0.38$, $p= .011$) and behavioural measures of Sx-5CSRTT premature responding during fITI under simple task conditions ($r(30)=0.413$, $p= .021$) and during vITI under dual task conditions ($r(31)=0.370$, $p= .013$).

Significant correlations were also detected between binge drinking scores and attentional measures in the Sx-5CSRTT under dual task conditions (percentage of omissions during vITI ($r(31)= .363$, $p= .015$) and accuracy of responding during both fITI ($r(31)=- .342$, $p= .026$) and vITI ($r(32)=- .362$, $p= .016$); higher binge scores associated with higher omission rates and lower accuracy, respectively.

High binge drinking scores correlated with lower numbers of consecutive delay choices (TCIP; $r(44)=- .392$, $p= .029$). No other planned correlations showed significance ($ps>.05$).

In the multiple regression analysis the main model was significant ($F(3, 30)= 7.314$, $p< .01$), and accounted for approximately 45% of the variance of binge drinking scores ($R^2= .456$, R^2 Adjusted= .396). Of the four factors included (SSRTi, Maximum delayed choices, BIS-trait and premature responding), only trait impulsivity ($\beta = .454$, $p= .004$) and premature responding during the first (fITI – Simple Task; $\beta = .369$, $p= .016$) and last (vITI – dual task; $\beta = .324$, $p= .033$) sessions were significant predictors of high binge drinking score ($R^2 = .46$, $F(3,30)= 7.55$, $p= .001$), with SSRTi and Delayed Choices not significantly contributing to the model ($\beta =- .121$, $p= .421$; $\beta =- .250$, $p= .086$, respectively).

3.4.2 Mouse Study

3.4.2.1 Strain differences in 5-CSRTT impulsivity

As depicted in **Figure 3.4A**, no differences in impulsivity were found between B6 and D2 mice during baseline conditions ($F(1, 42)=.248$, $p= .621$). Upon the introduction of vITI, the number of anticipatory responses in both strains increased ($F(3, 123)= 68.457$, $p= .001$, $\varepsilon= 0.415$); inspection across the different intervals during vITI revealed B6 mice as showing a steeper increase in premature responding with increasing ITI (ITI x strain interaction, $F(3, 42)= 4.930$, $p= .024$; **Figure 3.4B**). Similarities across the increase in premature responding curves between mice and human subjects can be detected in **Figure 3.4B-C**.

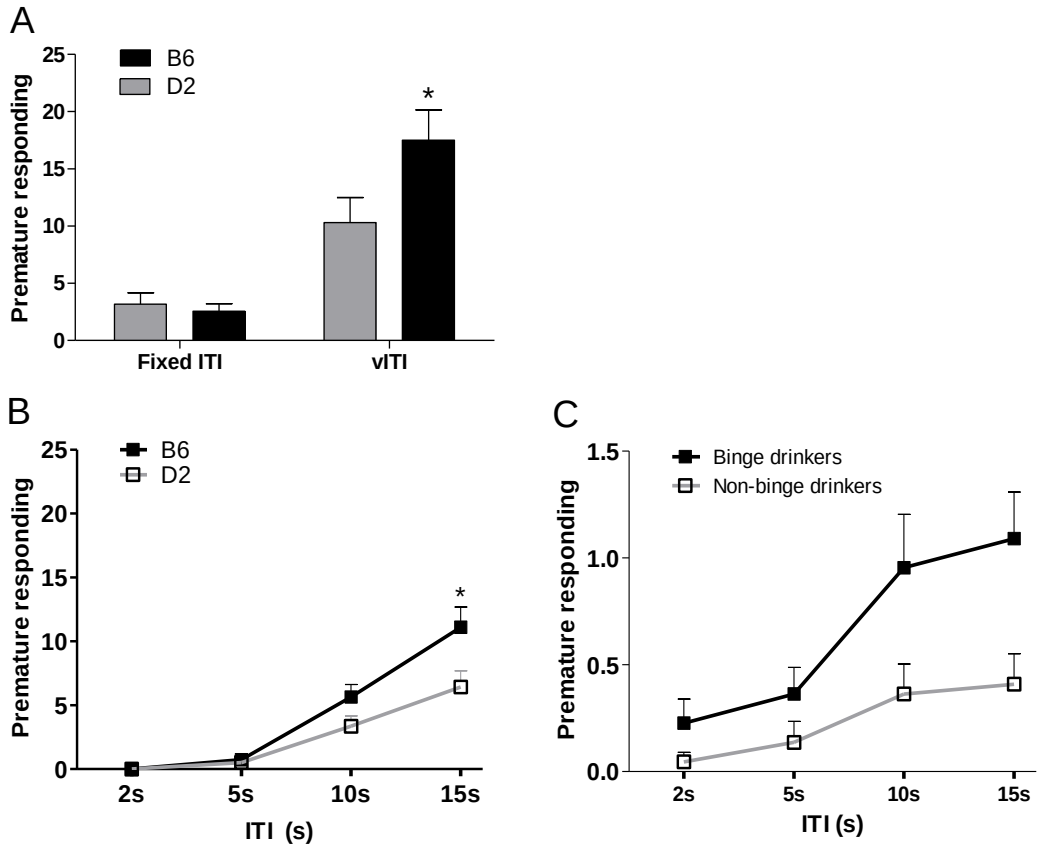

Figure 3.4 Premature responding (mean ± SEM) of C57BL/6J (B6, black bars/lines) and DBA2/J (D2, gray bars/lines) in the 5-CSRTT during a A) fITI (last session in stage 6) and vITI sessions, and across the different ITIs during the vITI session for mice (B) and human participants (C). * $p< .05$ (independent *t*-test)

3.4.2.2 Alcohol Consumption

Alcohol consumption and preference for B6 and D2 mice is illustrated in Supplementary **Figure S3.5**. B6 consumed higher amounts of ethanol in comparison to D2 mice ($F(1, 13)= 37.244$, $p< .001$; **Figure S3.5A**), and showed greater ethanol preference (*vs*. water, $F(1, 13)= 50.501$, $p< .001$; **Figure S3.5B**). Significant positive correlations were found between ethanol preference and number of premature responses during a vITI challenge (Spearman's rho(14)= .585, $p= .028$; **Figure S3.5D**); greater ethanol preference was associated with higher levels of premature responding in the 5-CSRTT. Interestingly, significant correlations appeared at the longest ITI (15s, Spearman's rho(14)= .559, $p= .038$), but only a tendency was revealed at 10s-ITI (Spearman's rho(14)= .518, $p= .058$), with no significant correlations at 5s or 2s-ITI (Spearman's rho(14)< .445, *ps*> .05). Levels of alcohol consumption did not correlate with levels of premature responding (Spearman's rho(14)= .285, $p= .324$; **Figure S3.5C**).

3.5 Discussion

Failure to control alcohol drinking has frequently been ascribed to "impulsivity" (Dick et al., 2010), but this term is used to describe several distinct phenomena (Evenden, 1999). Furthermore, the extent to which "impulsivity" is a cause or consequence of excessive alcohol consumption is not clear. Using a battery of tasks, we reveal impulsivity deficits in human BD, as indexed by the novel Sx-5CSRTT, and increased risky choices, measured in the TCIP. In parallel, we show alcohol-preferring B6 mice to be more impulsive in the mouse version of the 5-CSRTT in comparison to alcohol-avoiding D2, and that waiting impulsivity correlates with alcohol preference. Thus, waiting impulsivity may precede heavy alcohol intake in the mouse, and, by extension, humans.

3.5.1 Binge Drinking and human 5-CSRTT performance

Human BD showed elevated premature responding during the first (novelty component) and last (most challenging condition) session. Increased premature responding in BD subjects was accompanied by attentional impairments, revealed as more omitted trials, and lower accuracy of detection in the dual task condition. Others have recently independently developed a similar human analogue of the 5-CSRTT to measure waiting impulsivity (Voon et al., 2014; Worbe et al., 2014), and have found parallels with rodent performance in substance abusers, and following neurochemical manipulations, suggesting close parallels between measures of waiting impulsivity in humans and rodents.

In our experiments, mouse performance in the 5-CSRTT, differed between B6 and D2 strains. Despite not differing during baseline performance (in keeping with reports from Loos et al., 2010; Patel et al., 2006), the ethanol-preferring B6 strain displayed higher levels of impulsivity when tested under challenging conditions by increasing the ITI, as did the humans (impaired performance was seen in the initial stages of the task and under attentional load).

Comparison of the mouse and human studies reveals that different manipulations of the task parameters lead to parallel behavioural changes. While a fITI session is often unable to detect group differences, increasing the time required to wait before responding, or making stimulus onset less predictable using a vITI, or increasing the task complexity, reveal attentional and impulsivity impairments in both our mouse and human studies. In vITI, the stimulus is presented using different inter-trial delays, in a semi-random fashion, within a single session, thus preventing use of internal timing abilities to predict onset of the stimulus. Since impulsive individuals may overestimate the passage of time (Melges and Fougerousse, 1966), premature responses under fixed interval conditions may reflect errors in time estimation, rather than impulsivity. By disrupting the temporal predictability of the stimulus onset, the participant is required to be more attentive to the stimulus in order to achieve high levels of performance.

From the vITI we can extract two main points; first, although vITI is sufficient to provoke premature responding in mice, vITI under the conditions here described was insufficient to detect differences in impulsivity between human groups, perhaps because in humans this variation is insufficient to challenge cognitive resources necessary for task performance. It was only when the complexity of the task was further extended by compromising attentional resources during dual task performance that reliable group differences emerged. Secondly, from the vITI we can also observe a similarity in outcome between animal and human performance; the longer the ITI, the greater the challenge to the ability of the mouse or human to withhold responding. Interestingly, differences between BD and NBD (with BD showing more premature responses) were also detected during fITI in the simple task, when attention load was not challenged; however, these differences were found only when the task was performed for first time, before performance was optimised.

3.5.2 Binge Drinking and other measures of impulsivity

Although increased 'waiting' impulsivity was detected in BD, no differences in action inhibition or response cancelation appeared when using the SST, again emphasising the multifaceted nature of impulsivity (Evenden, 1999). Furthermore, the two tests that we used to cover other major impulsivity constructs, namely choice impulsivity or decision making, revealed increased impulsive choice in BD only in a behavioural task (TCIP), an effect consistent with previous reports of elevated risky choice in BD (Worbe et al., 2013), but not when using a questionnaire (DDQ), consistent with a number of researchers' findings when using the DDQ as a measure of impulsivity (MacKillop et al., 2007; Fernie et al., 2010). Data from correlational analysis of the human measures revealed positive relationships between percentage of premature responses during fITI and vITI, both behavioural measures of motor impulsivity, but self-reports were poorly correlated with behavioural task measures, consistent with previous studies

highlighting robust differences between self-reported (Patton et al., 1995) and behavioural measures of impulsivity (Dick et al., 2010; Aichert et al., 2012; Vonmoos et al., 2013). Further, that measures of both impulsive choice (TCIP) and waiting impulsivity (Sx-5CSRTT) correlated with binge drinking score, but did not correlate with each other, might suggest that both forms of impulsivity contribute independently to poor control over alcohol consumption. It is therefore of interest that although B6 and D2 mouse strains also differ in measures of "choice" impulsivity in delay-discounting tasks, surprisingly, greater discounting (higher impulsivity) has been reported in D2 than in B6 mice (Helms et al., 2006). Thus, if the TCIP is a true measure of delay discounting, there is a mismatch between alcohol bingeing humans, and alcohol preferring mice.

It should be noted, however, that, in the TCIP, subjects choose between one stimulus that allows an immediate subsequent response for a small reward, and another that requires delaying the subsequent response to obtain a larger reward. Thus, the task not only opposes reward size to delay in obtaining it (as in standard delay-discounting tasks), but also incorporates a choice between responding quickly, or following a delay. The task may thus contain an element of waiting impulsivity in addition to delay discounting.

In contrast to apparent species differences in delay discounting, BD humans, and alcohol-preferring mice both showed high levels of waiting impulsivity in the 5-CSRTT, suggesting analogy between the tasks. Accepting this analogy, the current data become important in interpreting whether changes seen in human BD precede or are the effect of binge drinking. That waiting impulsivity is seen in B6 mice that had not been exposed to alcohol drinking but are prone to alcohol abuse, and also in BD, suggesting that high waiting impulsivity seen in BD subjects may not only be a consequence, but also may precede binge drinking. Although, only prospective studies in adolescents could finally identify behavioural predictors of alcohol binge drinking, studies like this one can guide us to include appropriate tasks in prospective studies.

3.5.3 Limitations

The development of homologous tasks in humans and animals will hopefully facilitate the translation of animal laboratory findings to human studies and in a second step, to clinical populations. In that spirit, it is worth considering limitations of the study. Firstly, although age of first drinking was provided by the participants, we have no independent measure of drinking patterns; such information would only be available from a prospective study. Similarly, illicit substance use was not formally assessed. Although corrections for multiple comparisons were not applied to the correlational data, the size effect of relationships between the important variables can still be used for interpretation of the findings. However, we recognize that our human findings should be considered exploratory and that impulsivity may account for only a small, albeit significant, proportion of differences in patterns of binge drinking. Despite gender-specific effects of binge drinking being reported frequently in the literature (Petry et al., 2002; Scaife and Duka, 2009), both male and female BD subjects were equally impaired when the demands of the task increased. The finding of increased premature responses in BD compared to NBD, in the simple form of the task and in the fITI condition, only in males is difficult to understand. This absence of clear gender differences may derive from our low sample size. Finally, although the present experiments suggest an association between high levels of waiting impulsivity and high alcohol consumption, we have no direct evidence of a causal relationship. It cannot be excluded, for instance, that gene variants that contribute to impulsivity, independently contribute to alcohol drinking (pleiotropy). Similarly, the significant correlations between impulsivity and alcohol consumption in the mouse experiments are only suggestive, and may be influenced by strain effects (see **Figure S3.5**). Tests of a causal relationship need to demonstrate at least that manipulations of impulsivity have predictable consequences for drinking. The introduction of parallel tests in rodents and humans will facilitate such studies.

3.5.4 Conclusions

Altogether, the present findings suggest opportunities for obtaining consilience between animal and human studies of impulsivity. The Sx-5CSRTT was able to detect waiting impulsive elevations and attentional disruptions in a young human population of BD, in line with previous studies describing alcohol-dependent patients with greater deficits in tasks related to prefrontal function (Scaife & Duka, 2009). Findings from our mouse study reveal that strains that differ in alcohol intake also show high levels of premature responding prior to alcohol-exposure, and that the measure of impulsivity correlates with alcohol preference. Thus, trait impulsivity may predispose to binge drinking, as much as being a consequence of alcohol intake.

3.6 Funding and Disclosure

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Supplementary information is available at the *Neuropsychopharmacology* website

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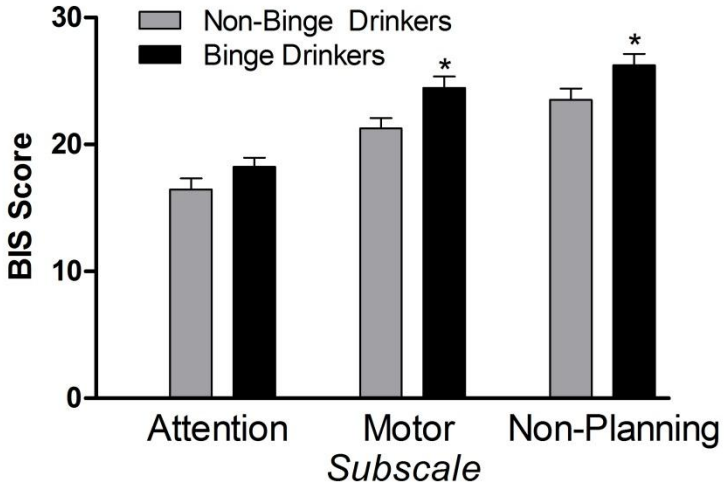
Chapter 3

Appendix 3.1

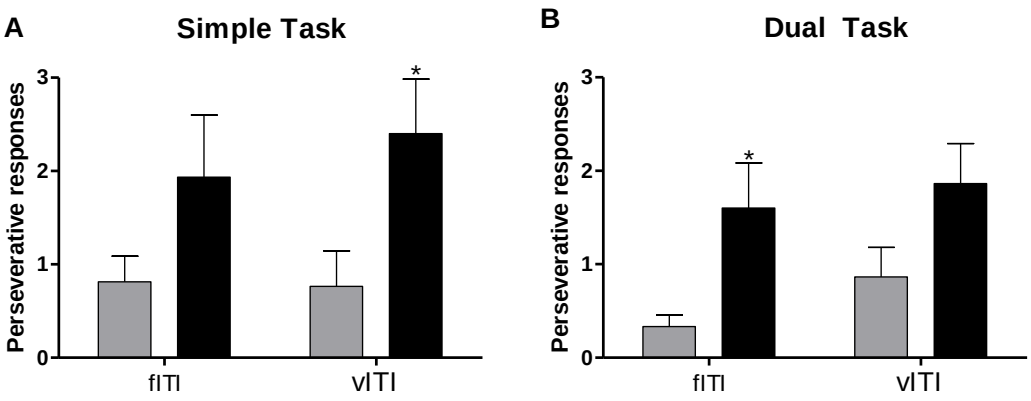
Supplemental Information

Figure S3.1 Trait measurement scores from the Barratt Impulsivity Scale



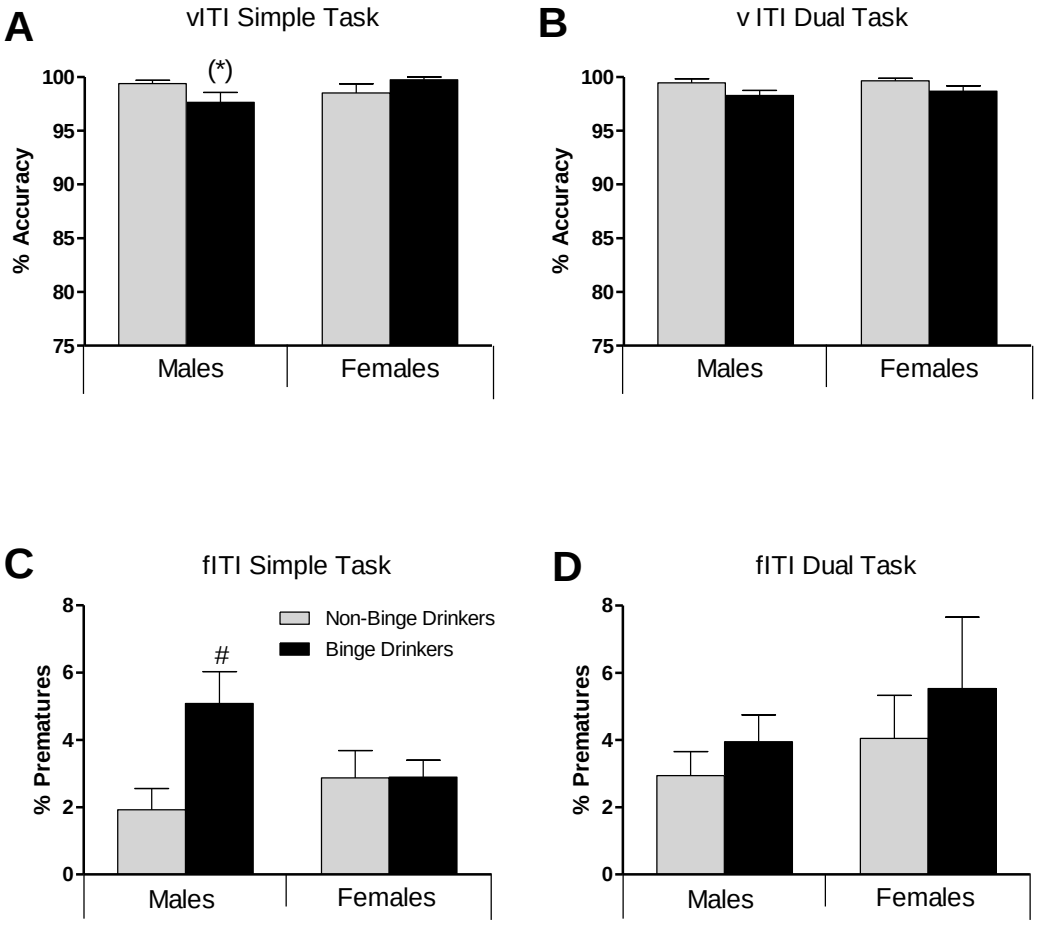
Trait measurement scores for the Attentional, Motor and Non-Planning subscales from the Barratt Impulsivity Scale (Mean ±SEM). *p<0.05 compared to NBD (independent sample t-test)

Figure S3.2 Perseverative responding during the Sx-5CSRT



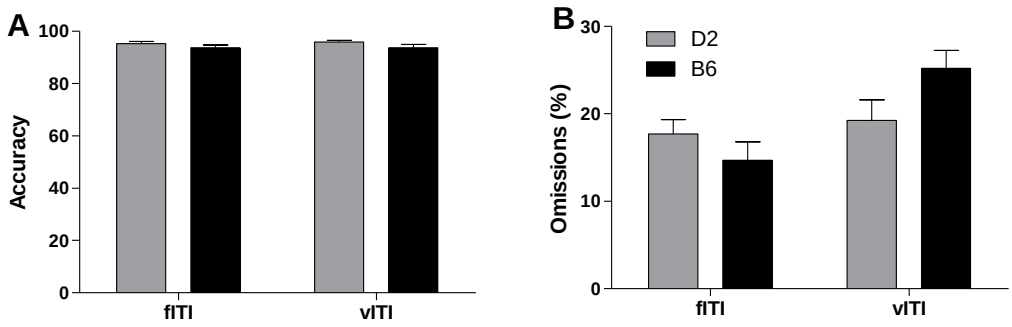
Sx-5CSRTT performance during a fITI and vITI sessions under Simple Task (A) and Dual Task (B) conditions for NBD (grey bars) and BD (dark bars). Mean (±SEM) of perseverative responses. * different from NBD [vITI Simple Task, $F(1, 31)= 5.777$, $p= .023$; fITI Dual Task, $F(1, 31)= 6.714$, $p= .013$])

Figure S3.3 Gender differences on Sx-5CSRTT performance



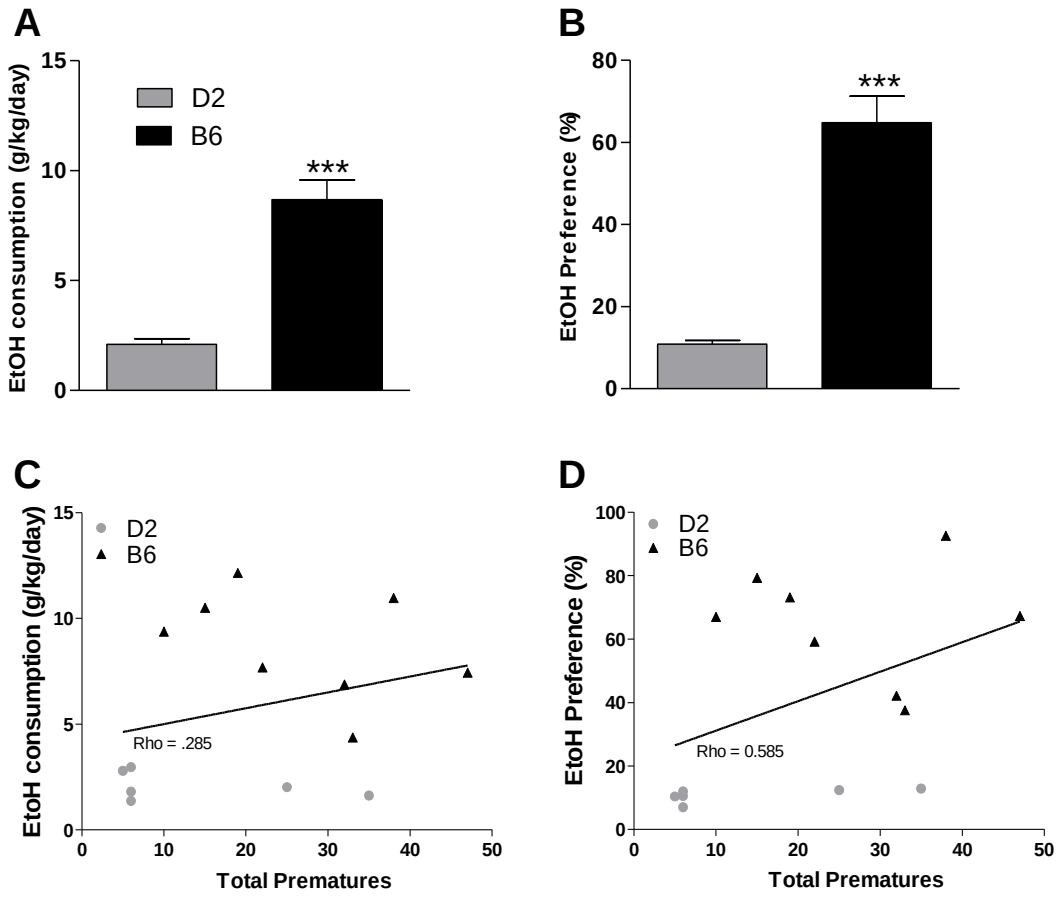
Gender differences on Sx-5CSRTT performance for A) accuracy of responding during a vITI session, B) percentage of premature responding during a fITI session for males and females within NBD and BD groups. # different from NBD group, p= .02, (*) p= .061 (Bonferroni post hoc comparison following significant ANOVA [A, $F(1, 28)$= 6.058, p= .02; C, $F(1, 27)$= 4.655, p= .04])

Figure S3.4 Strain differences on Attentional Performance in the 5-CSRTT



Accuracy of responding (A) and percentage of omitted trials (B; Mean±SEM) of C57BL/6J (B6, black bars) and DBA/2J (D2, grey bars) in the 5-CSRTT during a fITI (last session in stage 6) and vITI sessions. No significant differences were detected in any of the measures

Figure S3.5 Voluntary ethanol consumption and preference in B6 and D2 mice and levels of Premature responding in the 5-CSRTT



Mean ± S.E.M. of ethanol intake (g/kg/day) (A), and ethanol preference (%) (B) of D2 and B6 mice over a three-week period in a 2-bottle choice paradigm. (A) B6 consumed higher amounts of ethanol in comparison to D2 mice ($F(1, 13)= 37.244$, $p= .001$), in addition to showing greater ethanol preference (*vs.* water, $F(1, 13)= 50.501$, $p= .001$; B). (C-D) Scatter plot of total premature responding for the vITI condition for B6 and D2 mice and ethanol consumption and preference in the 2-bottle choice test. Significant positive correlations were found between ethanol preference and number of premature responses during a vITI challenge (Spearman's rho(14)= .585, $p= .028$; D), higher ethanol preference associated with higher levels of premature responding in the 5-CSRTT. Levels of alcohol consumption did not correlate with levels of premature responding (C)

METHODS

Human Study

Participant's inclusion/exclusion criteria - *Restrictions during the study*

Participants were healthy, not currently suffering from a mental or neurological illness or alcohol or substance abuse disorder as documented via a medical questionnaire followed if necessary by a medical interview. Psychiatric diagnoses (including those relating to substance use disorders) were not formally assessed as part of the study; information concerning those was obtained via a medical questionnaire and an additional interview. Participants had to be able to abstain from smoking during testing and not taking medication (excluding the contraceptive pill). In addition the Alcohol Use Disorder Identification Test (AUDIT) was used to evaluate heavy drinking and/or active alcohol abuse or dependence. Prior to the experiment, participants had been instructed to abstain from the use of illicit recreational drugs (≥ 5 days) or alcohol (≥ 12 h), and were asked to eat a low-fat meal the evening before testing and a low-fat breakfast (excluding drinking coffee or tea) on the day of testing. All participants provided written informed consent to take part in the study, which was approved by the University of Sussex ethics committee.

Upon arrival at the laboratory, a standard breathalyser (Lion Alcolmeter SD-400; Lion Laboratories Ltd, Barry, UK), with a detection-limit equivalent to 0.01 g/l of alcohol in the bloodstream, was used to measure breath alcohol concentrations (BRaCs) to ensure zero blood alcohol levels.

Classification of binge drinkers and non binge drinkers and Binge drinking scores

The BD and NBD classification was based on the scores from the Alcohol Use Questionnaire (Mehrabian and Russell, 1978), as previously described in Townshend and Duka (2005). Although previous studies have demonstrated that the number of drinks in a row drunk in an occasion (i.e. 5-drink/episode for men and 4-drink/episode for women) differentiates between

BD and NBD (Wechsler and Austin, 1998), it also implies that BD and NBD consume different quantities of alcohol. The “binge score” [derived from items from the AUQ questionnaire] used here focuses on patterns of drinking rather than quantities and includes drunkenness, which may be a better predictor of alcohol dependency problems. Indeed, we have shown that, unlike the measurement “drinks in a row,” the “binge drinking score” was unrelated to weekly alcohol consumption (Townshend and Duka, 2002).

Thus the binge score used to classify BD and NBD was derived from a database of 245 AUQ questionnaires (Mehrabian and Russell, 1978), completed by volunteers. Total scores from participants close to the upper 33% were grouped as BD and close to the low 33% as NBD. Considering the total scores of binge drinking from our previous population, the cut off for BD in our study was 32 and 16 for NBD.

To calculate the binge drinking score (Townshend and Duka, 2002), we collected the information given in items 10, 11 and 12 of the Alcohol Use Questionnaire (Mehrabian and Russell, 1978), which provides information of: average of drinks consumed per hour (item 10); number of times being drunk in the previous 6 months (item 11); percentage of times getting drunk while drinking (item 12). The binge score is then calculated by using the following equation: [4x(item 10)+Item 11+0.2x(Item 12)]. As the binge drinking score is based on patterns of drinking (Townshend and Duka, 2002), rather than quantity consumed, no differences between the BD scores of males and females volunteers were found. Consequently, in our study we used the same cutoff points for male and female subjects.

The human Sussex Five-Choice Serial Reaction Time Task – Additional Information

Participants were comfortably seated in front of a touch screen. Viewing distance was approximately 30 cm with a vertical visual angle of -30° and a horizontal visual angle of 0°. The task consisted of five moving blue circles (stimulus) represented in a "circular" motion in a tactile screen. Bellow the stimuli and at the bottom of the screen was located a home button.

The session started upon selection (tapping) and holding of the finger on the home button, thus initiating the first trial (see **Figure 3.1**). After a fixed interval (ITI; 5s), the motion of the five visual stimuli was interrupted and one circle briefly changed its contour (referred here as "illuminated") for 0.5s. The participant was then required to tap in the correct circle within a certain period of time and return to the home button. The releasing of the home button before the presentation of the illuminated stimulus was recorded as a premature response and punished with a 5s time out. Correct responses and number of omitted trials provided a measure of attention. Latency to make a correct response (correct latency) and perseveration after correct detection were also recorded. Sessions consisted of a maximum of 50 trials or 10 minutes, whichever came first.

Participants were required to complete two variants of the task: fixed ITI (fITI) and variable ITI (vITI, with pseudorandom presentation of different inter-trial intervals varying from 2, 5, 10 to 15 seconds), referred here as "simple task". Additionally, participants completed the fITI and vITI conditions with the inclusion of an auditory continuous discrimination task (referred here as "dual task"), which we have used previously to increase the attentional load in a study assessing cognitive processing and drug seeking (Hogarth et al., 2008). Due to technical problems with the device, data from one female NBD during the fITI with simple Task and one female BD during fITI with dual task) sessions were lost and therefore not included in the analysis.

The complete list of variables considered in the analysis of the 5-CSRTT were:

- Accuracy (percentage of correct responses): correct responses / (correct responses + incorrect responses) x 100.
- Percentage of omissions (including responses only after stimulus presentation): omissions / (correct responses + incorrect responses + omissions) x 100.
- Percentage of premature responding (including all responses): premature responses / (correct responses + incorrect responses + omissions + premature responses) x 100.
- Correct latency: latency to tap into the correct circle after the onset of the stimulus (s).
- Perseverative responses: total number of responses made into the circles after a correct detection.

Auditory continuous discrimination task (Hogarth et al., 2008). The dual task utilised a PC computer to record detection rates. Auditory stimuli were presented via headphones equipped with an adjusted volume control, set by default to a constant level, and detection rates were recorded using an external keyboard. Participants were required to discriminate between low and high pitched tones and respond to the latter by pressing a space bar located in an external keyboard. This practice trial allowed the high tone task to be practiced in isolation. Otherwise, the auditory dual task was combined with the Sx-5CSRTT Fixed and vITI tests. Accuracy of detection and latency of responding were recorded and considered in the analysis.

Stop Signal Task *(SST; Logan, 1994).* The SST (CANTAB; Cambridge Cognition, Cambridge, UK; http://www.camcog.com) was used as a behavioural measure of response inhibition. The task consisted of five blocks of 64 trials. Subjects were instructed to respond as quickly as possible to the orientation of an arrow (‘go’ stimulus) with a left or right- button on a press pad response box. On 25% of the trials, after the ‘go’ stimulus, an auditory stimulus (‘Stop Signal’)

was presented at a variable delay (Stop Signal Delay, SSD; 100-200-400-500ms), during which participants were instructed to withhold responding. Initial Stop Signal was presented at 200ms, but increased or decreased by 50ms following a staircase procedure according to the subject's performance: increasing times of presentation by 50ms following successful stopping, decreasing presentation times following failure to stop by 50ms. After the subject successfully inhibited his or her responses during Stop trials in 50% of the occasions, the Stop Signal Reaction Time (SSRT; RT Go stimulus – RT successfully Stop Trials) was determined. The Go Accuracy (%), Go Reaction Time (mean; ms) and SSRTi were also calculated. SSRT was calculated using the integration method (SSRTi; see Caswell et al., 2013; Verbruggen et al., 2013 for further details on SSRTi analysis).

Time Estimation Task. Time perception was measured using a Time Estimation task (TE), programmed using E-prime and administered using a screen and an external keyboard. Subjects were instructed to press and hold a spacebar to indicate a 27 seconds interval. Releasing the spacebar indicated the amount of time that the subject considered to have elapsed. The subject's accuracy of performance was calculated.

Delay-Discounting Questionnaire. The Delay-Discounting questionnaire (DDQ) was used to provide an index of the relative value for immediate *vs.* delayed rewards and was programmed using E-Prime (Richards et al., 1999). Subjects were asked to choose between different amounts of money after a period of different delays using a two response buttons on an external keyboard. The questionnaire consisted of 189 questions, such as ' would you rather £x Now, or £1000 in x time?', presented on a computer screen. Whilst 27 monetary rewards could be received immediately (£1, £5, £10, £20, £40, £60, £80, £100, £150, £200, £250, £300, £350, £400, £450, £500, £550, £600, £650, £700, £750, £800, £850, £920, £960, £990, £1000), the second option (£1000) could be received after a certain delay (1 week, 2 weeks, 1 month, 6

months, 1 year, 5 years, 25 years). Each delay was presented in a block, with the monetary amount randomised across trials. The order of the delays was also randomised, as the sequence of delays has been shown to alter the participant's preferences (Stillwell and Tunney, 2012). The point at which each individual was indifferent between the smaller immediate reward and the $1000 delayed reward (e.g. switch from delayed to immediate rewards to delayed rewards) was determined for each of the seven different delays. Within each session, seven indifferent points for seven different delays were determined. The curves that result from the devaluation of reinforcer value (*k*) by delay were also measured using the hyperbolic function of Mazur (Mazur and Coe, 1987):

$$v_d = \frac{V}{(1+kd)}$$

, where Vd is the present subjective value of a reward of amount V (£1000), *d* is the time (delay) until its receipt, and *k* is the parameter that governs the rate at which the subjective value decreases. The subjective value was calculated as the value at which participant switched from immediate, certain rewards to the delayed reward. Thus, seven subjective equivalent points (indifference points), one for each delay, were calculated. Comparison of goodness-of-fit (R^2), nonlinear regression, was used to fit the seven estimated indifference points from each participant to a hyperbolic function, according to the methodology established by Bickel et al. (1999). When the hyperbola is less than 1.0, discounting rate (for the same value of *k*) becomes less steep as the delay increases. A second analysis was conducted by calculating the Area Under the Curve (AUC; Myerson et al., 2001). Delays and indifference points were normalized (e.g. expressed as a proportion of the maximum value, £1000) and the area underneath those points was computed by summing the results of the following equation: (x_2-x_1) x $([(y_1+y_2)/2])$, where x represent successive delays and y correspond to the indifference points associated with those delays. Larger AUCs represent less discounting by delay, thus less impulsivity.

The Two choice impulsivity paradigm (Dougherty et al., 2005). The Two Choice Impulsivity Paradigm (TCIP) is a forced-choice, reward directed procedure, which assesses the participant's tendency to choose between a circle and a square presented on a computer screen. Choosing the circle allows the subject to retrieve a small immediate reward (5 points) after 5 seconds delay by again responding on the chosen symbol; choosing the square allows retrieval of a large reward (15 points) following 15 seconds delay. Subjects were presented with a total of 50 trials. The proportion of immediate choices selected and the maximum number of consecutive long-delayed choices scored were measured and served as an index of 'choice' impulsivity.

Mouse Study

Apparatus: 5-CSRTT

The test apparatus consisted of eight mouse operant chambers (Med Associates Inc., St. Albans, Vermont, USA). Each chamber was housed in a sound-attenuating outer cabinet, with a ventilator fan providing a constant low-level background noise. The left wall of the chamber was curved and contained 5 apertures fitted with infrared detectors to detect nose-poke responses. The apertures were illuminated by a yellow stimulus light located inside each aperture. The right wall of the chamber contained a receptacle hole with a round access opening where the liquid reinforcer was delivered. 30% condensed milk solution was used as a reinforcer (0.01ml) and was delivered into a small cup by means of a dipper. Head entries into the food magazine were recorded by an infrared photo-cell beam crossing the entrance of the receptacle hole, which could be illuminated by a yellow stimulus light inside the aperture. A house-light was located at the top of the wall above the food magazine. The presentation of stimuli and the recording of the responses were controlled by a Smart Control Package 8IN/16out with an additional interface by MED-PC for Windows (Med Associates Inc., St. Albans, Vermont, USA).

Behavioural sequence

Habituation to the reinforcement and to the 5-CSRTT boxes. During the first two-three sessions, animals were placed in the boxes for 30 min and the liquid reward was available into the magazine. The house light, the magazine light and the stimulus lights in the five holes were turned on during the entire session. Magazine head entries and number of reinforcements earned were recorded. When the animal earned 50 or more reinforcements in two consecutive sessions, the animal started the training in the 5-CSRTT.

5-CSRTT Training. The session commenced with the illumination of the house-light. Nose-poking in the aperture started the first trial, and free delivery of the liquid reinforcer (dipper on for 3 s.) was presented and accompanied by the illumination of the food magazine. After a fixed interval (inter-trial interval, ITI), one of the stimulus lights into the holes was turned on for a brief time. The animal was required to nose-poke within a certain period (limited hold, LH) into the correct hole in order to obtain the reinforcer. After a correct detection**,** the animal was able collect the reinforcer in the magazine tray thus initiating the next trial. An incorrect response occurred in the case of the animal making a response in a non-illuminated hole. On the other hand, if the animal failed to respond into any of the holes after the completion of the LH, this was recorded as an error of omission. Any response into the holes during the inter-trial interval, when the stimulus light had not yet been presented, was registered as a premature response. To signal appropriateness of behaviour, incorrect, omission and premature responses were followed by a time out (TO), signalled by a 5-s period of darkness, where no reinforcer could be obtained. After the termination of the TO period, the next trial was restarted by a nose-poke into the magazine. Some animals tended to nose-poke repeatedly into the holes after a correct response, referred to as a perseverative response, but had no programmed consequences. The times to make the correct response and to collect the reinforcer were also recorded (correct latency and magazine latency, respectively). The total number of trials completed was also examined.

At the beginning of training, the stimulus duration (SD) was set to 30 s and the ITI to 2s, but these parameters were adjusted according to the performance of each animal. When the animal was able to perform two consecutive sessions achieving the execution criteria (>50 correct trials, >75% accuracy and < 25% omissions) the stimulus duration was reduced in the following pattern: 30, 20, 10, 5, 2.5, 1.8s (baseline), and the LH and the ITI set at 5s. Testing was carried out daily (5-6 days a week), and the session lasted for 100 trials or 30 min, whichever came first. In the long inter-trial interval (long ITI) sessions, the ITI was set at 10 seconds and the duration of the task increased to 45 min.

Following Oliver et al. (2009), the variables considered in the analysis of the 5-CSRTT were:

- Accuracy (percentage of correct responses): correct responses / (correct responses + incorrect responses) x 100.
- Percentage of omissions: omissions / (correct responses + incorrect responses + omissions) x 100.
- Percentage of premature responding: premature responses / (correct responses + incorrect responses + omissions + premature responses) x 100.
- Correct latency: latency to nose-poke into the correct hole after the onset of the stimulus (s).
- Magazine latency: latency to collect the reward after a correct response (s).
- Perseverative responses: total number of responses made into the holes after a correct response and before the collection of the reward.

Voluntary ethanol consumption in a two-bottle choice paradigm

During the 2-bottle choice testing (Belknap et al., 1993), mice (n=14; B6=8, D2=6) were individually housed and provided with continuous access to two bottles containing water or ethanol. For each ethanol-drinking session (24h/day; 7days/week), one bottle contained 10% v/v EtOH, and the other contained only tap water. The amount of water and EtOH solution

consumed was recorded every 3 days (Monday and Thursday). Solutions were prepared and changed weekly, and provided at room temperature. The positions of the bottles were rotated every three sessions to prevent the development of positional bias. Fluid levels were measured to the nearest 0.1 mL and ethanol and water intake (g/kg or mL/kg, respectively) were calculated as the mean for three sessions based on the animal weights recorded every three days. Ethanol preference [(ethanol consumption/ethanol consumption+water consumption)*100] was also included in the analysis.

Statistical analyses

Strain differences on alcohol consumption and ethanol preference were explored using a one-way ANOVA. Spearman's correlation coefficient rho was used to determine relationships between alcohol consumption and preference and premature responding in the 5-CSRTT.

ADDITIONAL RESULTS

Human Sx-5CSRTT – Additional Measurements

Latency to make a correct detection and perseverative responses were additional variables considered into the analysis and herein characterized. Under simple task conditions (**Figure S3.2A**) group differences appeared in the analysis ($F(1, 30)= 4.816$, $p= .036$); compared with NBD, BD subjects were slower in making a correct response when performing in a fITI session ([NBD, $M= 1.02$, $SD= .24$; BD, $M= 1.17$, $SD= 0.13$] $t(29)=2.195$, $p= .036$). Increasing the task difficulty in a vITI session abolished the differences in latencies of performance ($F(1, 30)= 3.732$, $p= .063$), but group differences on perseverative responding were revealed ($F(1, 31)= 5.777$, $p= .023$), BD showing higher number of perseverative responses in comparison to NBD ($t(30)=2.404$, $p= .023$; **Figure S3.2A**). When a dual task was introduced (**Figure S3.2B**), BD subjects again showed higher levels of perseverative responding in comparison to NBD, both during a fITI ($U(44)=142.5$, $p< .044$), and a tendency during a vITI session ($U(44)= 163.5$,

p= .051). No effects of binge drinking were detected for latency of performance (*F*<0.9, *ps* >.05).

Interestingly, binge drinking scores were positively correlated with perseverative responses under simple task conditions during a vITI (*r*(32)= .363, *p*= .041) and a fITI session under dual task conditions (*r*(41)= .426, *p*= .005). There were no significant correlations between binge drinking scores and latency to correct responses in any of the variants of the task or the later within other variables (*ps*>.05).

Human binge drinking scores and Impulsivity Measures – Additional Measurements

Regression models were generated using each measure (SSRTi, Maximum delayed choices, BIS-trait and premature responding) as individual predictor. When age and gender were included in the same model, then the model still appeared significant (*F*(3, 30)= 7.314, *p*< .01), reached in one step but with a much lower variance explained (R^2= .290, R^2 Adjusted= .263), and only age was a good predictor of binge drinking scores (β = -.538, *p*= .003). As this analysis was only exploratory, age or sex were not included as predictors in the final model.

Mouse 5-CSRTT – Additional Measurements

As illustrated in **Figure S3.4**, no differences in accuracy of responding and percentage of omitted trials were found between B6 and D2 mice (fITI and vITI, *F*<2.4, *p*> .05), suggesting similar levels of attentional performance in the 5-CSRTT across the group.

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Chapter 3

Appendix 3.2

Alcohol Use Questionnaire

Barratt Impulsiveness Scale

National Adult Reading Task

Alcohol Use Questionnaire

The following questions ask you about your habitual use of various types of alcoholic drinks. Please consider your drinking for the **last 6 months** in answering the questions, and take your time to give an accurate answer to each question.

1 On how many days per week do you drink ***wine, or any wine-type product***, e.g. sherry, port, martini? _____

2 On those days you do drink wine (or similar), about how many glasses (pub measure) do you drink?
If unsure, please estimate the number of bottles or parts of a bottle _____

3 How any glasses (pub measure) of wine do you have in a week, in total?____

4 On how many days per week do you drink ***beer or cider*** (at least half a pint)?
Please state usual brand(s) e.g. Carling, Harvey's, Strongbow _____

5 On those days you do drink beer/cider, about how many pints do you typically have?
_____

6 How many pints of beer/cider do you drink in a week, in total? _____

7 On how many days per week do you drink ***spirits*** (e.g. whisky, vodka, gin, rum)?
Please state usual brand(s) e.g. Smirnoff, Bells, Gordon's:__

8 On those days you do drink spirits, about how many shorts (pub measure) do you typically have? If unsure, please estimate number of bottles or parts of a bottle ___

9 How many drinks of spirits do you have in a week, in total? ___

10 On how many days per week do you drink ***alcopops***? ____
Please state usual brand(s) e.g. Hooch, Bacardi Breezer, WKD: ____

11 On those days you drink alcopops, about how many bottles do you typically have?____

12 How many bottles of alcopops do you have each week, in total? _____

13 When you drink, ***how fast do you drink***? (Here, a drink is a glass of wine, a pint of beer, a shot of spirits, straight or mixed). Please circle the correct response:

Drinks per hour: 7+ 6 5 4 3 2 1

or 1 drink in 2 hours

or 1 drink in 3 or more hours

14 How many times have you been drunk in the last 6 months? By 'drunk' we mean loss of co-ordination, nausea, and/or inability to speak clearly _____

15 What percentage of times that you drink do you get drunk? ___

16 At what age did you start drinking?___

17 Do you have anyone in your close family who was alcoholic? Please circle correct response: Yes / No
If yes, what relation are they to you? _____

Barratt Impulsiveness Scale

Please circle the number which corresponds to the choice that best describes you. Try to describe the way you USUALLY act and feel, not just how you are feeling right now.

1 = rarely/never 2 = occasionally 3 = often 4 = almost always/always

1	I plan tasks carefully.	1	2	3	4
2	I do things without thinking.	1	2	3	4
3	I make up my mind quickly.	1	2	3	4
4	I am happy-go-lucky.	1	2	3	4
5	I don't "pay attention".	1	2	3	4
6	I have "racing" thoughts.	1	2	3	4
7	I plan trips well ahead of time.	1	2	3	4
8	I am self-controlled.	1	2	3	4
9	I concentrate easily.	1	2	3	4
10	I save regularly.	1	2	3	4
11	I "squirm" at plays or lectures.	1	2	3	4
12	I am a careful thinker.	1	2	3	4
13	I plan for job security.	1	2	3	4
14	I say things without thinking.	1	2	3	4
15	I like to think about complex problems.	1	2	3	4
16	I change jobs.	1	2	3	4
17	I act "on impulse".	1	2	3	4
18	I get easily bored when solving thought problems.	1	2	3	4
19	I act on the spur of the moment.	1	2	3	4
20	I am a steady thinker.	1	2	3	4
21	I change residences.	1	2	3	4
22	I buy things on impulse.	1	2	3	4
23	I can only think about one problem at a time.	1	2	3	4
24	I change hobbies.	1	2	3	4
25	I spend or charge more than I earn.	1	2	3	4
26	I often have extraneous thoughts when thinking.	1	2	3	4
27	I am more interested in the present than the future.	1	2	3	4
28	I am restless at the theatre or lectures.	1	2	3	4
29	I like puzzles.	1	2	3	4
30	I am future orientated.	1	2	3	4

National Adult Reading Task

CHORD
ACHE
DEPOT
AISLE
BOUQUET
PSALM
CAPON
DENY
NAUSEA
DEBT
COURTEOUS
RAREFY
EQUIVOCAL
SUPERFLUOUS
SIMILE
BANAL
QUADRUPED
CELLIST
FACADE
ZEALOT
DRACHM
AEON
PLACEBO
ABSTEMIOUS
DETENTE
IDYLL

NAIVE
CATACOMB
GAOLED
THYME
HEIR
RADIX
ASSIGNATE
HIATUS
SUBTLE
PROCREATE
GIST
GOUGE
PUERPERAL
AVER
GAUCHE
TOPIARY
LEVIATHAN
BEATIFY
PRELATE
SIDEREAL
DEMESNE
SYNCOPE
LABILE
CAMPANILE

Chapter 4

Paper 3

REPEATED ETHANOL EXPOSURE DURING EARLY AND LATE ADOLESCENCE: DOUBLE DISSOCIATION OF EFFECTS ON WAITING AND CHOICE IMPULSIVITY

IMPULSIVITY FOLLOWING EARLY LIFE ETHANOL EXPOSURE

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4.1 Abstract

Background: A strong association exists between impulsivity and binge drinking, and between adolescent alcohol exposure and alcohol abuse in humans. To understand the extent to which early-life alcohol exposure contributes to increased impulsivity, we developed an animal model of binge drinking using two strains of mice, C57BL/6J (B6) and DBA2/J (D2), that differ in both motor impulsivity and alcohol drinking.

Methods: Mice were treated with 2g/kg ethanol during their early (IEE_Early; PND30-45) or late (IEE_Late; PND45-60) adolescence or with saline (CON) throughout the adolescence period. To determine the consequences of intermittent ethanol exposure (IEE) on waiting impulsivity and attentional function, the number of premature responses and omissions, respectively, were evaluated in adulthood using the 5-choice serial reaction time task (5-CSRTT). To examine the effects of IEE on choice impulsivity, risky decision-making was assessed in adulthood using a mouse version of the Iowa Gambling Task (mIGT). Additionally, the acute effects of ethanol in adulthood on waiting impulsivity and choice preference were investigated.

Results: We provide experimental evidence that IEE during late, but not early, adolescence disrupts waiting impulsivity and attentional abilities in the 5-CSRTT. In contrast, IEE during early, but not late, adolescence altered risky decision making in the mIGT. D2 mice consistently showed lower premature responding than B6 mice in both the mIGT and the 5-CSRTT, but greater risky decision making on the mIGT. IEE and CON mice showed similar responsiveness to the acute ethanol effects on premature responding, but increased risky choices only in B6_IEE_Early mice.

Conclusions: Our observations suggest a direct effect of IEE during adolescence on waiting and choice impulsivity and attention later in life.

Key Words: impulsivity, ethanol, adolescence, inbred mice

4.2 INTRODUCTION

Recent studies have suggested that exposure to ethanol, accompanied by intermittent withdrawals, a pattern that occurs during binge drinking, can have deleterious effects on impulsive behaviour (Henges and Marczinski, 2012; Moreno et al., 2012). Despite the increase in prevalence of binge drinking behaviours in young populations (White et al., 2006), the most vulnerable periods of ethanol exposure and its long-term effects on impulsive behaviour are not known.

One obstacle to studying the long-term effects of binge drinking in human adolescents concerns the difficulty in controlling drinking patterns among binge drinkers, and isolating the ethanol experience to adolescence. Animal models may serve to control for such heterogeneity. However, even mice genetically predisposed to drink alcohol rarely display a pattern that leads to high blood ethanol levels and physiological intoxication (Finn et al., 2005), an essential feature of human binge drinking. Consequently, we used 4 multiple cycles of intermittent ethanol exposure (IEE; 2g/kg) using an injection protocol. IEE was timed to occur during early adolescence (Spear, 2000), comparable to the age of drinking onset with the greatest risk for developing alcohol dependence in adult humans (Grant and Dawson, 1997), and late adolescence, as a period of high alcohol bingeing in young adults (Bava and Tapert, 2010).

As extensively reviewed, impulsivity is a multifaceted construct. We studied two main subtypes, namely waiting and choice impulsivities. In a laboratory setting, 'motor' (Winstanley, 2007) or 'waiting' impulsivity (Robinson et al., 2009) can be assessed with the well-established Five-choice serial reaction time task (5-CSRTT) (Robbins, 2002), in which premature responding before a "go" signal is presented in one of five locations, is assessed. Correct identification of the location of the stimulus, or failure to respond when the stimulus is presented (omission error), serve as measures of attention (Robbins, 2002; Sanchez-Roige et al., 2012). Choice

impulsivity, or decision-making ability, and the tendency to adopt risky behaviour under ambiguous conditions, can be assessed with the Iowa Gambling Task (IGT; Bechara et al., 1994), which has long proved effective in detecting decision-making deficits in alcoholic patients and detoxified subjects (e.g. Goudriaan et al., 2005; Tomassini et al., 2012). Alcohol-dependent subjects show altered performance on the IGT by making choices that favour large rewards but lead to larger penalties and are thus disadvantageous over the course of the experimental session. The task has been recently adapted for its use in rodents (Zeeb et al., 2009), and has proven effective for the measurement of risky choice behaviour in mice (Young et al., 2011).

The distinct nature of motor and choice impulsivities led us to hypothesise that different genetic backgrounds may also display different impulsive phenotypes. We therefore carried out our study of 5CSRTT and mIGT performance in two inbred strains, C57BL/6J (B6) and DBA/2J (D2), selected for presenting different impulsive phenotypes (Helms et al., 2006) and different sensitivities to the actions of ethanol. Compared to D2 mice, B6 mice display high levels of ethanol preference and exaggerated impulsivity in the 5-CSRTT (Sanchez-Roige et al., 2014); in addition, B6 mice show a relative insensitivity to ethanol withdrawal (Crabbe et al., 1994) and to the acute effects of alcohol (e.g. Roberts et al., 1992). Consequently, after establishing baseline performance in the 5-CSRTT and mIGT, the ability of IEE to modulate the two impulsivity types and to alter the response to acute ethanol was assessed in the two strains in adulthood. We anticipated increased impulsive behaviour in B6 mice during baseline and following acute doses of alcohol, and strain-dependent responsiveness to the effects of IEE on adult impulsive-like behaviour. On the other hand, as D2 mice have shown greater delay discounting than B6 mice in other tasks assessing choice impulsivity (Helms et al., 2006), increased risky behaviour in the mIGT was predicted for this strain. Collectively, these manipulations might provide further insights into the relationships between binge drinking during a critical neurodevelopmental period and impulsivity changes in later life.

4.3 MATERIAL AND METHODS

4.3.1 Subjects

Two separate cohorts of B6 (n=24/group) and D2 (n=24/group) male mice obtained from Charles River Laboratories (Arbresle, France) were randomly assigned to different groups (n=8/group): control group (CON), IEE during early adolescence (IEE_Early), IEE during late adolescence (IEE_Late). The mice were housed in groups of two per cage on a 12-h light/dark cycle (lights off at 7:00pm) at a temperature of 19-21°C and 50% humidity. After the IEE treatment, the mice were food restricted to reduce their body weights to 85% of their free-feeding weight and kept under food restriction until the end of the experiments. Water was available *ad libitum* throughout the study. Behavioural testing took place between 8:00 and 2:00pm, 5 to 6 days per week. All experiments were approved by the institutional ethics committee and were performed under United Kingdom legislation on animal experimentation [Animal (Scientific Procedures) Act, 1986].

4.3.2 Intermittent Ethanol Exposure

Mice were exposed to ethanol (2g/kg) in a pattern of intermittent exposure (4 cycles of 2 days ethanol injection, 2 days injection free) during early (PND30-45) and late adolescence (PND 46-59). Ethanol (95%, diluted to 20% [v/v] in saline solution) was administered i.p. at a volume of 10mL/kg to avoid tissue irritation. Controls received saline injections throughout PND 30-60, on the same schedule as IEE mice (**Figure 4.1**).

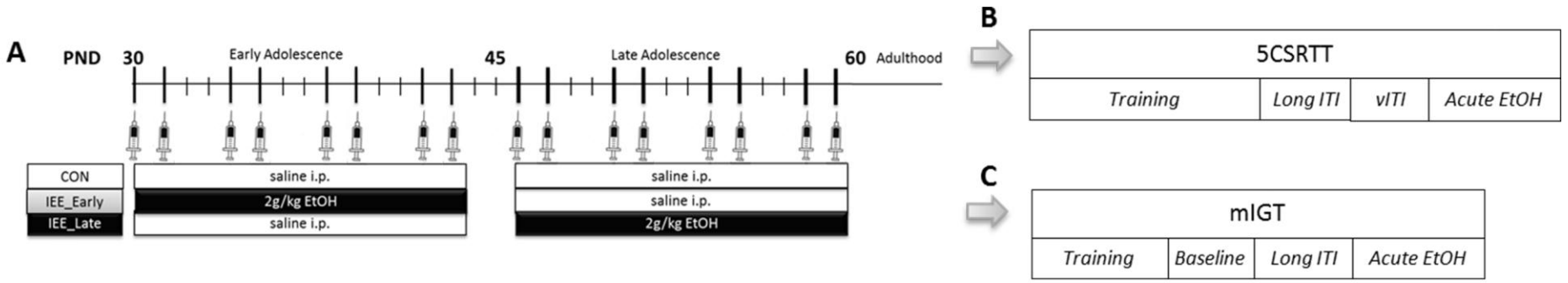

Figure 4.1 IEE protocol details and experimental timeline. A) Adolescent B6 and D2 mice were treated with either 2.0 g/kg of saline (CON group, 16 saline injections from day 30 to 60) or ethanol (IEE treatment) for 2 consecutive days at 48-h intervals during a 14-day period either during early adolescence (IEE_Early, from PND30 to PND43; 8 ethanol injections from day 30 to 45, with a protocol of 2 days on and 2 days off; and 8 saline injections from day 45 to 60) or during late adolescence (IEE_Late, PND45 to PND58; 8 saline injections from day 30 to 45; and 8 ethanol injections from day 45 to 60). The effects of the IEE treatment on waiting and choice impulsivity were assessed in adulthood across different task conditions and acute ethanol administration using two different paradigms, the 5-CSRTT (n= 48; B) and mIGT (n= 48; C), respectively

4.3.3 The Five-Choice Serial Reaction Time Task

The test apparatus consisted of eight mouse operant chambers (Med Associates Inc., St. Albans, Vermont, USA; see Supplementary Material for further description). The training phases of the experiments were based on procedures described elsewhere (Oliver et al., 2009) and in the Supplementary Material. When the mice achieved the performance criteria in the last stage of training [>75% accuracy, <25% omissions for two consecutive days, using the parameters: Stimulus duration = 1.8-s; Limited hold (maximum duration to make a response after stimulus presentation)= 5-s; ITI= 5-s; TO= 5-s], mice were presented with a long ITI session (ITI=10-s vs. 5-s during baseline), which promotes the emergence of premature responses in both rats (e.g. Dalley et al., 2007) and mice (Oliver et al., 2009). After a week of retraining to baseline conditions, animals faced a variable ITI (vITI) session, where different ITIs (2-5-10-15-s) were randomly presented in a single 45-min session. After completion of the 5-CSRTT challenges, one B6 mouse from the CON group failed to reach criterion for performance and was removed from the experiment (n=47). To examine the effects of ethanol in the 5-CSRTT, 3 weeks after the last challenge session, animals were injected (i.p.) with single doses of 0, 0.5, 1 and 2 g/kg ethanol in a Latin square design, 15 minutes prior to the long ITI session. A minimum of 1 week occurred between administrations, during which mice performed under baseline parameters to ensure high performance levels (>75% accuracy, <25% omissions) in case it had been disrupted by the drug testing sessions.

Several performance measures were recorded (see Supplementary Materials). However, main outcomes included in the analysis were percentages of premature responding (premature responses/ [correct responses + incorrect responses + omissions + premature responses] x 100) and omission (total omissions/[correct responses + incorrect responses + omissions] x 100).

4.3.4 The mouse Gambling Task

The test apparatus consisted of eight mouse five-hole operant chambers (Med Associates Inc., St. Albans, Vermont, USA; see 5-CSRTT). Extensive details of the protocol can be found elsewhere (Peña-Oliver et al., 2014) and in Supplementary Material. Briefly, a second group of 24 B6 and 24 D2 mice were habituated to the chamber and reinforcer (30% condensed milk solution). **Figure S4.1** illustrates the procedure: animals were first given experience of all four contingencies. Under Contingency 1, a nose-poke into a defined hole provided a single drop of milk with a probability of 0.9, or a TO of 5-s (probability 0.1). The corresponding values for Contingency 2 were [2 drops, p= 0.8; TO 10-s, p= 0.2], for Contingency 3 [3 drops, p= 0.5; TO 30-s, p= 0.5], and Contingency 4 [4 drops, p= 0.4; TO 40-s, p= 0.6]. Decision-making was assessed for 15 30-min sessions (session 15 referred to as 'baseline' responding). During those sessions, mice had to choose between advantageous options (Contingencies 1 and 2), characterized by a reinforcer of low magnitude, but high probability of reinforcement and short punishment timeouts, over the more disadvantageous or risky options (Contingencies 3 and 4) associated with larger reward size, but lower net gains over the session. In order to examine the effects of lengthening the ITI in premature (any response into the holes during the ITI, prior to the stimulus lights presentation) and choice impulsivity, mice were challenged under a long ITI session. To examine the effects of ethanol on mIGT performance, following a week of retraining under standard conditions, animals were injected (i.p.), 15 minutes prior to testing sessions (baseline conditions), with single doses of 0, 0.5 and 1g/kg of ethanol in a Latin square design. One mouse from the B6_IEE_Early was excluded from analysis (response choice exceeded the group mean responses + 2.5 x S.D.), leaving group sizes of n = 7-8. After the long ITI session, one mouse from the same group (B6_IEE_Early) died, leaving a group size of n = 6.

Several performance measures were recorded (see Supplementary Material), but primary outcomes included in the analysis were percentage of risky choices ([choice 3 + choice 4] / [choice 1+2+3+4] x 100) and percentage of premature responding [Premature responses / (premature responses + total choices) × 100].

4.3.5 Statistical analysis

The statistical analysis was performed using the 'Statistical Package for Social Sciences' (SPSS, version 20.0). Three-way repeated measures ANOVA was used for the analysis of each variable of the 5-CSRTT during vITI condition and of the mIGT during long ITI condition, with treatment (IEE_Early, IEE_Late, CON) and strain (B6, D2) as the between-subjects factor and ITI (4 levels, vITI) or session (2 levels, long ITI) as the within-subjects factor. For baseline (mIGT) and long ITI (5-CSRTT) sessions, a three-way ANOVA was applied, with treatment and strain as the between-subjects factor. The variables "percentage of premature responses" and "omissions" in the long ITI challenge were log10 transformed in order to attain homogeneity of variance and permit valid parametric analysis, though untransformed means are shown throughout. Within-session performance during acute ethanol challenge was analysed using a repeated-measures ANOVA with strain (B6, D2) and treatment (CON, IEE_Early, IEE_Late) as the between-subjects factor and drug dose (four levels, 5-CSRTT; three levels, mIGT) as the within-subjects factor. When significant interactions were found, one-way ANOVAs and Bonferroni comparisons were used for post hoc analysis. Where sphericity assumptions were violated, the Greenhouse-Geisser correction was applied and the epsilon (ε) values are reported. A $p<0.05$ was required for results to be considered statistically significant.

4.4 RESULTS

4.4.1 Effects of IEE in early or late adolescence on 5-CSRTT performance

Mice acquired the 5-CSRTT performance criteria under the final parameters of 1.8s stimulus duration in 27.3±0.5 sessions, all groups learning the contingencies of the task in a similar manner (see **Figure S4.2** for training performance).

During baseline conditions, strain differences appeared on measures of waiting impulsivity, B6 mice showing higher levels of premature responding ($F(1, 42)= 5.882$, $p<0.05$; **Figure 4.2*A***), but IEE effects were not observed ($F(2, 41)<0.3$, $ps>0.05$). Introducing a long ITI session also revealed strain differences in percentages of premature responses, which were higher in B6 mice ($F(1,42)= 11.055$, $p<0.01$; **Figure 4.2*B***), but no main effects of IEE or interactions were observed ($F<1.97$, $p>0.05$). Further, under a vITI, the percentage of premature responses was significantly increased in all groups at longer ITIs ($F(3, 42)= 95.775$, $p<0.001$, $\varepsilon=0.489$; **Figure 4.2*C***), with B6 mice performing more premature responses in comparison to D2 mice (ITI x strain, $F(3, 42)= 4.456$, $p<0.01$). Under this challenge, impulsivity differences emerged among groups (ITI x treatment interaction; $F(6, 42)= 3.526$, $p<0.01$), with the IEE_Late mice displaying a tendency to show more premature responses in comparison to CON mice at longer ITIs ($p=0.062$). Overall, under this challenge, B6 mice were more impulsive than D2 ($F(1, 42)= 4.621$, $p<0.05$) and an overall effect of IEE increasing premature responding was detected ($F(2, 42)=3.214$, $p<0.05$).

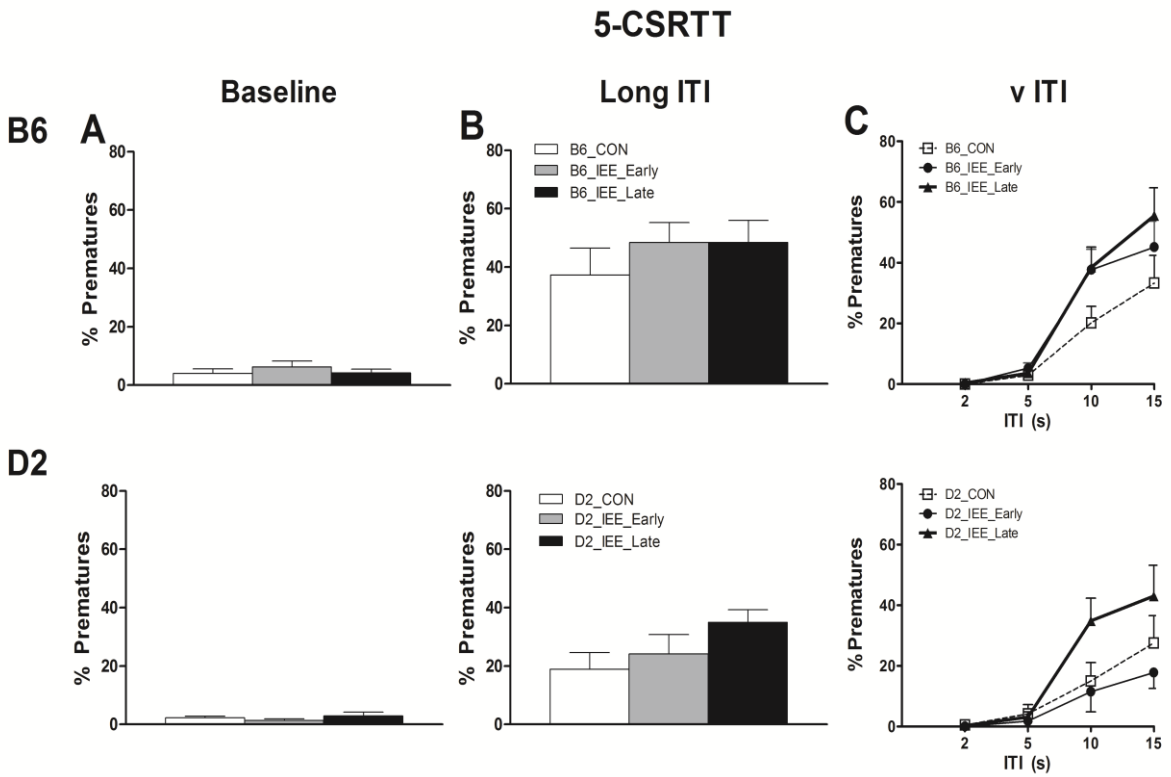

Figure 4.2 Premature responding in the 5-CSRTT during baseline, long ITI, vITI and acute ethanol conditions for B6 (top panels) and D2 strains (bottom panels) and for CON (white bars), IEE_Early (grey bars) and IEE_Late (black bars) mice (n=7-8/group). Across (A, B) conditions, B6 mice displayed higher percentage of premature responses (p<0.05, p<0.001, respectively). IEE altered premature responding during the vITI, where IEE_Late groups presented the highest levels of premature responding (p<0.05, C)

With regards to attention, strain differences emerged during baseline conditions, where D2 mice omitted responding in more trials than B6 mice (F(1, 42)= 8.065, p<0.001; **Figure 4.3*A***). Introducing a long ITI session increased the percentage of omissions (F(1, 42)= 5.821, p<0.01; **Figure 4.3*B***), IEE_Late mice being more affected than both CON (p<0.01) and IEE_Early mice (p<0.05). Similarly, varying the ITI led to increased numbers of omitted trials (F(3, 42)= 13.753, p<0.001 ε=0.808; **Figure 4.3*C***), more markedly at 15-s ITI for both strains (p<0.001), and in B6 mice when the ITI was reduced to 2-s (ITI x strain, F(2, 42)= 6.580, p<0.001; 2-s vs. 5-s, p<0.001). No effects of strain (F(1, 42)= 0.744, p=0.393) or treatment (F(2, 42)= 0.119, p=0.888) were observed across this measure.

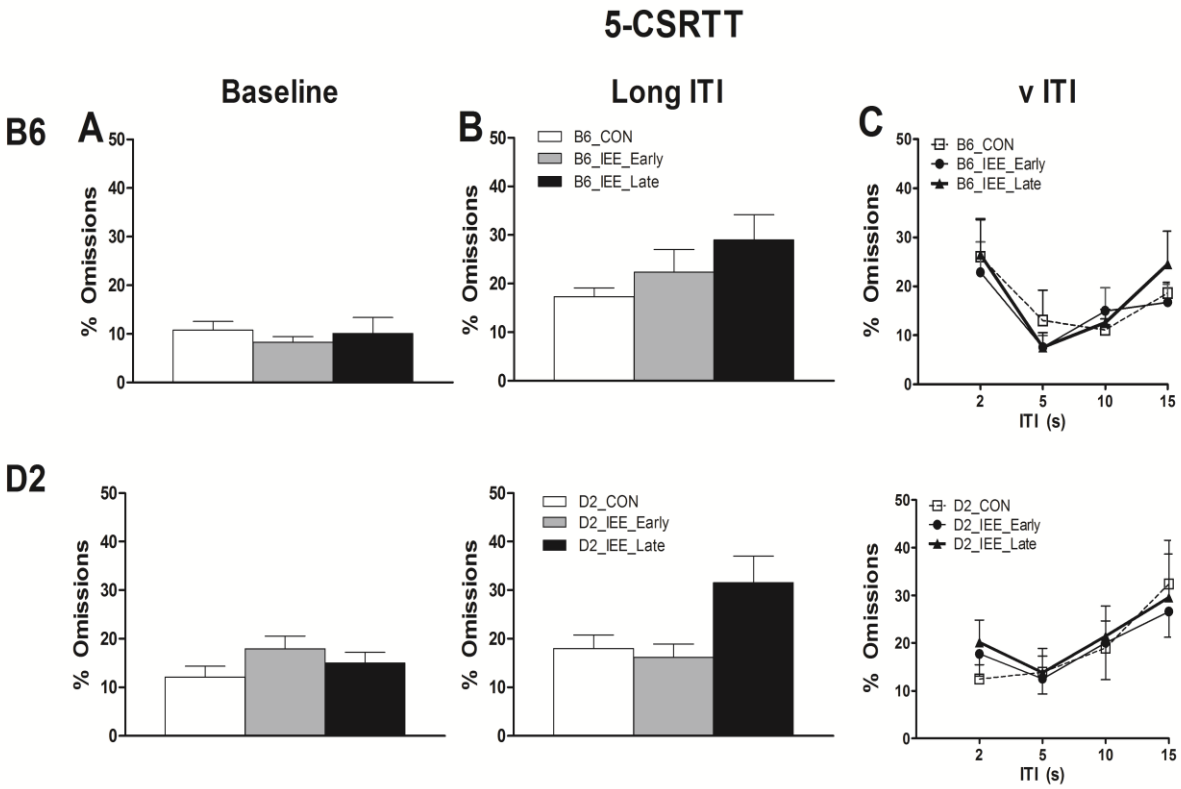

Figure 4.3 Attentional performance (% omissions) in the 5-CSRTT during baseline, long ITI, vITI and acute ethanol conditions for B6 (top panels) and D2 strains (bottom panels) and for CON (white bars), IEE_Early (grey bars) and IEE_Late (black bars) mice (n=7-8/group). During baseline, D2 mice had higher percentage of omissions in comparison to B6 (p<0.05, A). A long ITI increased percentage of omissions (p<0.001, B), markedly in B6 (p<0.001) and in IEE_Late mice than both CON (p<0.01) and IEE_Early mice (p<0.05). Longer ITIs (15-s) during a vITI session also led to increases in % omissions (p<0.001, C)

4.4.2 Effects of acute ethanol under long ITI sessions

Figure 4.4 shows the effects of acute doses of ethanol on 5-CSRTT performance. The two strains showed different responsiveness to ethanol for premature responding. There was a significant dose x strain interaction (F(3, 40)= 5.663, p<0.01, ε=0.775; **Figure 4.4*A***), and a main effect of strain was also found (F(1, 40)= 7.873, p<0.01), attributable to the higher percentage of premature responses in the B6 mice when they received 0.5g/kg (F(1, 40)= 17.112, p<0.001) and 1g/kg (F(1, 40)= 12.452, p<0.001), but those strain differences were abolished under vehicle and 2g/kg conditions. With regards to attentional function, a 2g/kg of ethanol dose

increased percentages of omissions (F(3, 40)= 27.550, p<0.001, ε=0.682; **Figure 4.4*B***), this increase being larger in B6 mice in comparison to D2 mice (dose x strain, F(3, 40)= 9.575, p<0.001). No main effect of strain was found in percentage of omissions (F(1, 40)= 0.005, p=0.945). Surprisingly, no differences among IEE groups were detected in any of the measures reported (F(6, 40)<1.1, *ps*>.05), acute ethanol having the same effect on drug-naïve mice as IEE mice.

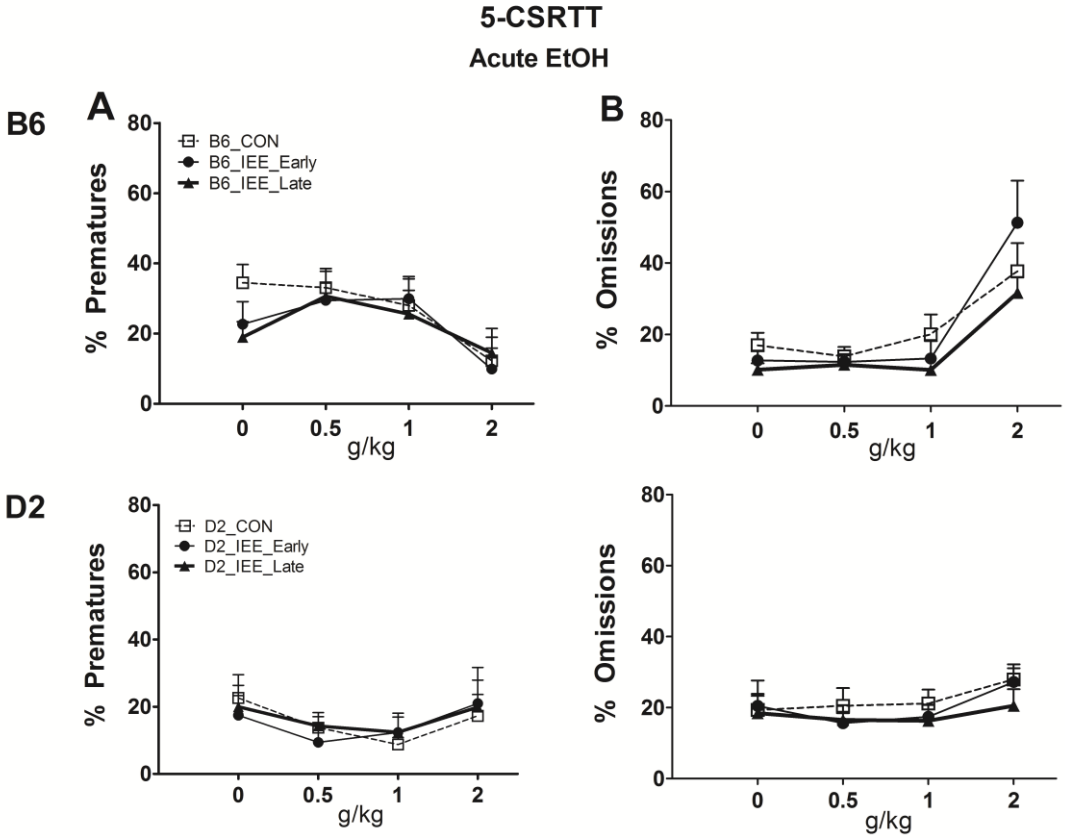

Figure 4.4 5-CSRTT performance under acute ethanol administration for B6 (top panels) and D2 strains (bottom panels) and for CON (white bars), IEE_Early (grey bars) and IEE_Late (black bars) mice (n=7-8/group). The strains showed different responsiveness to the acute effects of alcohol on premature responding (A). B6 mice showed higher premature responding at a dose of 0.5 g/kg compared to the higher dose of 2g/kg (p<0.05), and a tendency for the higher dose (2g/kg) to decrease premature responding compared to the vehicle (p=0.088), but there were no effects of IEE under this challenge. 2g/kg increased the percentage of omitted trials (p<0.001, B), more significantly in B6 mice than in D2 (p<0.001)

4.4.3 Effects of IEE in early or late adolescence on mIGT performance

Under baseline conditions, strain differences emerged for risky decision-making, D2 choosing more risky options than B6 mice ($F(1,41)= 9.622$, $p<0.01$; **Figure 4.5*A***; see **Figure S4.4** for training performance). Introducing a long ITI session did not alter risky behaviour ($F(1, 41)= 1.324$, $p=0.257$, **Figure 4.5*B***), and strain differences were abolished during this session.

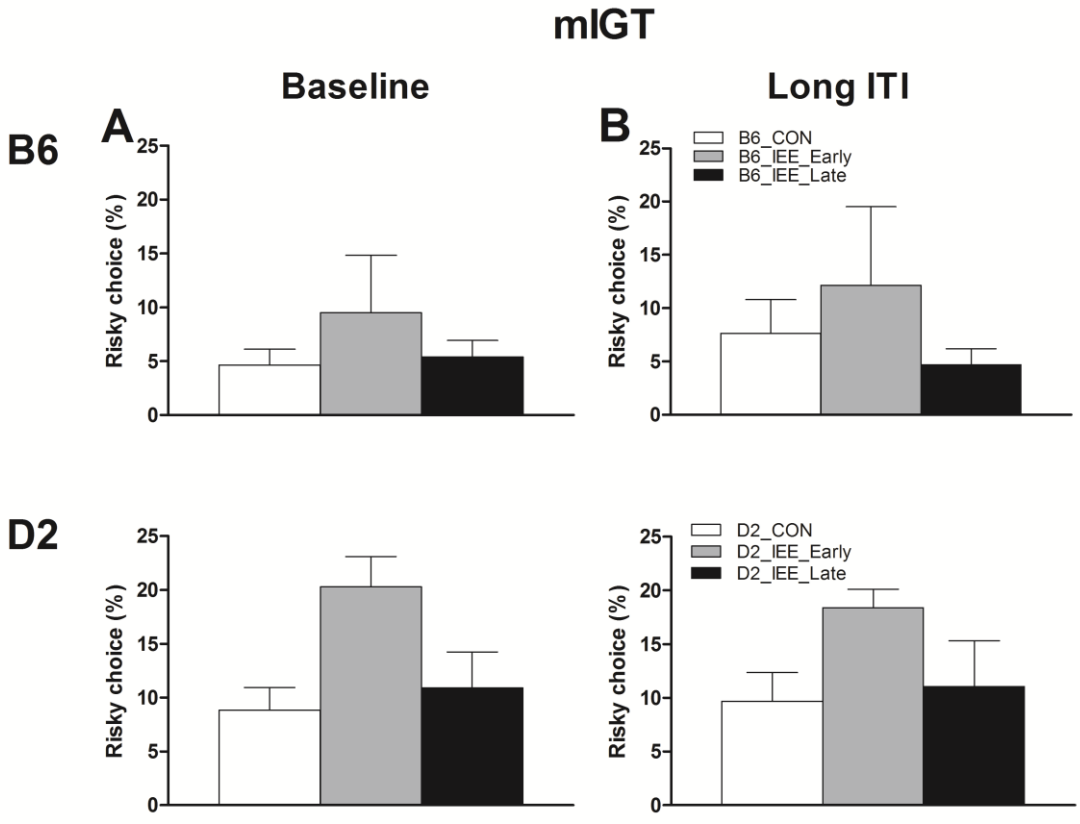

Figure 4.5 Risky decision-making across different task conditions during mIGT for B6 (top panels) and D2 (bottom panels) for CON (white bars), IEE_Early (gray bars) and IEE_Late (black bars) mice (n=6-8/group). Strain differences in choice impulsivity were detected during baseline, D2 showing higher risky choices in comparison to B6 ($p<0.01$, A) but those effects were abolished during a long ITI session (B)

Strain differences were also observed for premature responses during baseline conditions, B6 mice showing a higher percentage of premature responses ($F(1, 41)= 13.676$, $p<0.001$; **Figure 4.6*A***). By lengthening the ITI, premature responses were increased ($F(1, 41)= 53.659$, $p<0.001$;

Figure 4.6*B*), and this effect was greater in B6 mice (session x strain, $F(1, 41)= 13.203$, $p<0.001$). Again, overall, B6 mice displayed more premature responses ($F(1, 41)= 12.789$, $p=0.001$; **Figure 4.6*B***).

With regards to omissions, the D2 strain showed higher rates of omitted trials during baseline choice performance ($F(1, 41)= 6.070$, $p<0.05$; D2=7.9±1.29 vs. B6= 3.35±1.32 [Mean±SE]) and under a long ITI challenge ($F(1, 41)= 13.392$, $p<0.001$; D2=7.11±1.35 vs. B6= 1.48±1.38 [Mean±SE]).

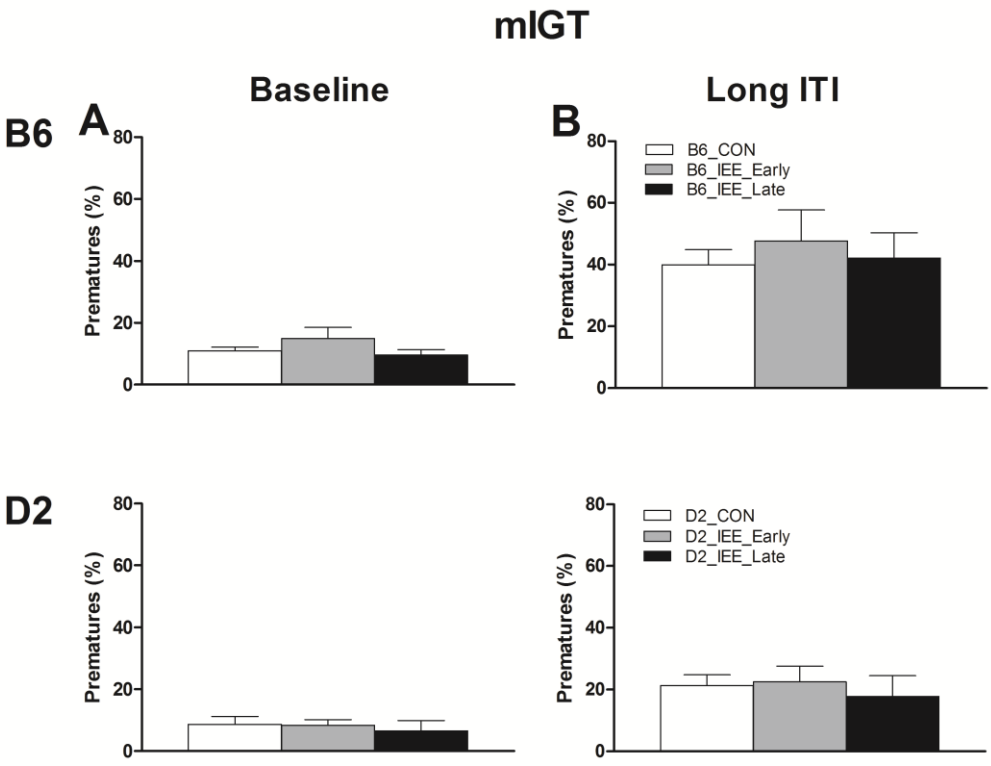

Figure 4.6 Strain differences appeared for waiting impulsivity across different task conditions during mIGT for B6 (top panels) and D2 (bottom panels) for CON (white bars), IEE_Early (grey bars) and IEE_Late (black bars) mice (n=6-8/group). During baseline and long ITI challenge, B6 mice displayed higher percentage of premature responses (A, B; *ps*<0.001)

4.4.4 Effects of acute ethanol on mIGT performance

Figure 4.7 shows the results from the acute doses of ethanol on mIGT performance. Administration of 0.5g/kg increased the percentage of risky choices in B6 mice from the IEE_Early group, as revealed in a dose x strain x treatment interaction ($F(4, 40)= 3.037$, $p<0.05$; **Figure 4.7*A***). Interestingly, a main effect of treatment was found ($F(1, 40)= 3.611$, $p<0.05$), but no reliable effect of IEE_Late treatment ($p=0.067$). As for premature responding, a dose of 1g/kg (vs. vehicle) increased the percentage of premature responses in IEE_Late mice (dose x treatment, $F(4, 40)= 3.019$, $p<0.05$, **Figure 4.7*B***), and a tendency for an ethanol dose x strain x treatment interaction suggested that this increase was more pronounced in B6 mice than in D2 ($F(1, 40)= 2.612$, $p=0.086$). A main strain effect was also found ($F(1, 40)= 7.960$, $p<0.01$), B6 displaying higher percentage of premature responses. There were no differences in the percentage of omitted trials between the groups or as a consequence of ethanol injection.

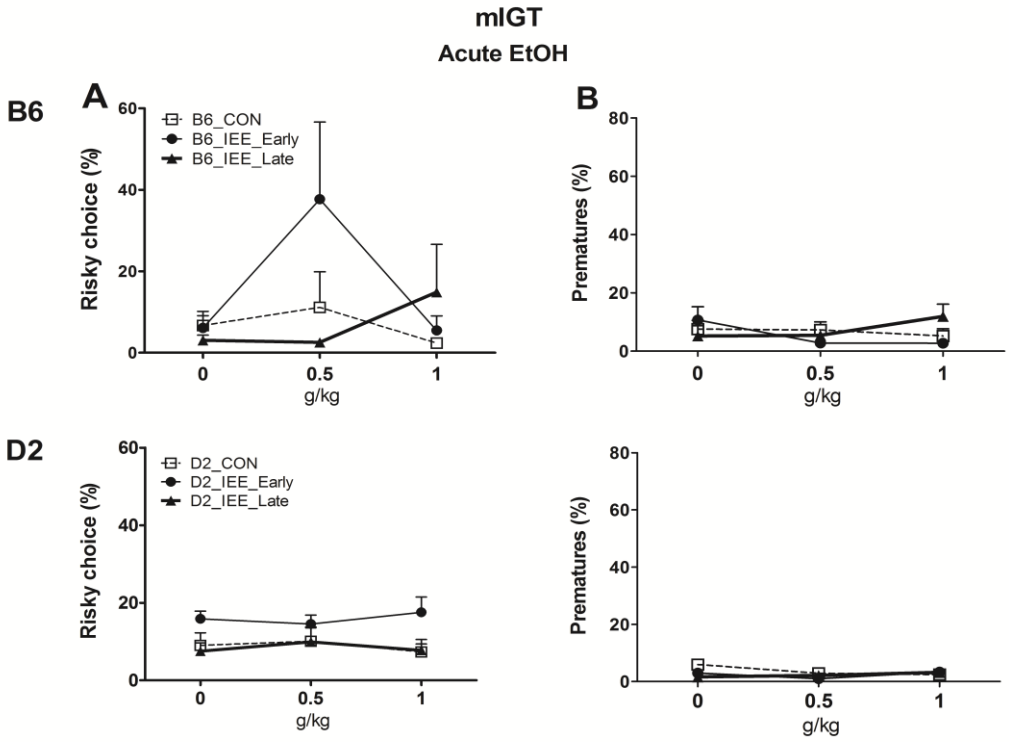

Figure 4.7 mIGT performance under acute ethanol administration for B6 (top panels) and D2 (bottom panels) for CON (white bars), IEE_Early (grey bars) and IEE_Late (black bars) mice (n=6-8/group). An acute 0.5g/kg dose of ethanol increased risky choices in B6 IEE_Early groups (strain x dose x treatment, $p<0.05$, A). A 1g/kg dose increased premature responses in IEE_Late mice (dose x strain, $p<0.05$). B6 mice showed higher percentage of premature responses than D2 mice ($p<0.001$; B)

4.5 DISCUSSION

Intermittent ethanol exposure during adolescence had detrimental effects in adulthood on attention, waiting impulsivity and decision-making, which were dependent on the timing of the alcohol exposure. In the 5-CSRTT, there was a tendency for ethanol exposure during late (but not early) adolescence to increase the number of premature responses, a measure of waiting impulsivity, under the unpredictable stimulus onset (vITI) condition. Inspection of **Figure 4.2*C*** suggests this was particularly true for the D2 strain. Conversely, in the mIGT, ethanol exposure during early (but not late) adolescence gave rise to increased risky choice behaviour in adulthood (**Figure S4.4**). Under particular testing conditions (long ITI, 5-CSRTT), attentional function (errors of omission) was compromised in mice exposed to ethanol during late, but not early, adolescence. Compared to CON mice, IEE mice showed no difference in responsiveness to acute ethanol effects on waiting impulsivity in the 5-CSRTT. In contrast, acute doses of ethanol prior to mIGT testing showed a tendency to increase premature responses in B6_IEE_Late mice (1g/kg; **Figure 4.7*B***), and increased risky decision-making in B6_IEE_Early mice (0.5g/kg; **Figure 4.7*A***). Both studies revealed differences in performance between the strains, D2 mice displaying lower levels of waiting impulsivity than B6 mice but more risky behaviour. Thus, the two types of impulsivity studied here showed double dissociations in their susceptibility both to timing of adolescent ethanol exposure, and to mouse strain differences (see **Table S4.1** for a summary).

4.5.1 IEE disrupts 'waiting' impulsivity and attentional function under challenging situations

During baseline conditions (**Figure 4.2*A***), IEE mice showed similar levels of 5-CSRTT premature responses to CON mice. When the cognitive demands were extended in a vITI session (**Figure 4.2*C***), IEE_Late mice showed a tendency to display higher levels of premature responding, suggesting that interactions between IEE and impulsivity may be dependent on the complexity

or novelty of the situation**.** In contrast, IEE during late adolescence did not impair premature responding in the mIGT. It is possible that during the mIGT, which demands minimal attentional control, impulsivity deficits in IEE_Late groups disappear (or are not detected).

In parallel with waiting impulsivity deficits, IEE also resulted in attentional impairments in the 5-CSRTT. When the demands of the task were increased in a long ITI session, animals treated during late, but not early adolescence, showed greater attentional dysfunction, expressed as an increase in omissions (**Figure 4.3*B***), in agreement with preclinical studies using a two-choice reaction time task in ethanol exposed rats (Slawecki, 2006) and in errors of commission in the human-task equivalent of the 5-CSRTT in abstinent inpatients (Bjork et al., 2004).

Results from Semenova (2012) support a lack of effects of IEE during early adolescence (PND33-36) on the 5-CSRTT performance assessed using a rat model of binge drinking. It is not clear, however, if this failure to find effects in IEE_Early mice is due to a) a lack of effects of IEE at this time, consistent with the late developmental trajectories of the human PFC cortex (Giedd et al., 1999), b) to plasticity leading to recovery during later stages of development (Crews and Nixon, 2009; Toga et al., 2006), or c) different lengths of alcohol withdrawal (14 days) between the groups, although these were small. Additional investigations are needed to resolve whether withdrawal from ethanol during different time points in the adolescence disrupts maturational processes in brain regions that mediate optimal 5-CSRTT and mIGT performance. Further, it is also possible that exposure to multiple withdrawal from alcohol, rather than mere alcohol exposure, may explain the behavioural alterations herein revealed, as previous animal and human findings have suggested (Duka et al., 2011; Stephens et al., 2001).

4.5.2 IEE disrupts decision-making in the mIGT

Compared to CON and IEE_Late mice, IEE_Early mice were more likely to select high-risk choices during the final stage of training (sessions 11-15; **Figure S4.4**). Although a three-way ANOVA failed to reveal effects of IEE on choice impulsivity during baseline conditions, when we analysed each strain separately, D2 mice from the IEE_Early groups displayed significantly more risky choices than CON mice, suggesting that different genetic backgrounds show different responsiveness to IEE.

In addition to providing a measure of risk taking, the mIGT offers the opportunity to test waiting impulsivity (premature responding) and decision-making processes concurrently. In clinical studies, an impulsivity trait has been frequently associated with aberrant decision-making on the IGT (Franken et al., 2008; Zermatten et al., 2005). Although these findings suggest an overlap between the neurobehavioral underpinnings of impulsivity and decision-making, we observed no correlation between risk levels of premature responding and risky choice during baseline or challenging conditions (baseline, Spearman's rho= -0.06, p=0.688; long ITI, Spearman's rho= -0.075, p=0.618), suggesting that impaired decision-making and exaggerated waiting impulsivity can manifest independently (Kreek et al., 2005; van der Plas et al., 2009).

4.5.3 Double dissociation of genetic backgrounds on motor and choice impulsivity

Inbred strains represent a powerful tool for studying the contribution of genetic factors in behaviour. Across several challenges, B6 showed greater 'waiting' impulsivity than D2 mice (see **Table S4.1**). Conversely, D2 displayed more risky choices in the mIGT, in agreement with previous studies assessing different aspects of choice impulsivity (Helms et al., 2006; van den Bos et al., 2006). However, 'poor' decision-making in D2 may be accounted for by factors other than impulsivity. Thus, D2 mice have been reported to show: a) decreased sensitivity to

rewards (e.g. Forgie et al., 1988), consistent with the slow speed of collecting the reinforcer in both the 5-CSRTT and mIGT (D2=1.65±0.49 vs. B6= 1.38±0.33 and D2=1.17±0.23 vs. B6= 0.99±0.24 [Mean±SE], during long ITI and baseline conditions for 5-CSRTT and mIGT, respectively), a measure that has been linked to motivational factors for food reward (Robbins, 2002); and/or, b) greater sensitivity to punishment in B6 mice, which may increase the preference for choices 1-2 in order to obtain the reinforcer more frequently and/or to avoid the large punishment associated with options 3-4.

4.5.4 Acute ethanol effects on motor and choice impulsivity

In the 5-CSRTT, no significant main effects of acute ethanol administration on premature responding were detected (**Figure 4.4*A***), as others have previously reported in rats at small ethanol doses (Bizarro et al., 2003). However, the strains showed different responsiveness to the acute effects of ethanol. Whereas D2 mice were unaffected by the acute effects ethanol on premature responding, the high-impulsive B6 mice showed higher premature responding when they received a small dose of 0.5g/kg, in comparison to the highest dose of 2g/kg, and a tendency for the higher dose (2g/kg) to decrease premature responding compared to the vehicle. In line with our results, ethanol increased premature responses under a long ITI session in B6 mice (Oliver et al., 2009), despite a higher dose of 1g/kg being needed; others have reported that even higher ethanol doses [1.2, 1.6 g/kg (Bizarro et al., 2003); 3g/kg (Semenova, 2012)] decreased 5-CSRTT premature behaviour in control rats.

Furthermore, and in agreement with others (Semenova, 2012), 5-CSRTT premature responses in IEE mice did not change following acute ethanol administration (**Figure 4.4*A***). Conversely, in the mIGT a higher dose (1g/kg) showed a tendency to increase premature responses in B6_IEE_Late mice (**Figure 4.7*B***). Such an observation is in keeping with human findings

describing how both dispositional risk factors and history of alcohol consumption modulate alcohol-induced inhibitory deficits (Marinkovic et al., 2012).

With regard to decision-making, the effects of acute ethanol on measures of choice impulsivity in the mIGT were dependent upon both strain and IEE timing (see **Table S1**). A low dose of ethanol (0.5g/kg) increased risky choice in B6_IEE_Early mice (**Figure 4.7*A***), consistent with human studies where elevated risky decision-making was found in alcohol abusers (Petry, 2001), but not among healthy adults. The lack of ethanol-induced impairments in choice impulsivity in CON mice is in agreement with a previous study conducted in this lab using the same behavioural task (Peña-Oliver et al., 2014) and in different types of choice impulsivity (Wilhelm and Mitchell, 2012). However, ethanol has frequently been reported to impair delay discounting in rodents (e.g. Evenden and Ryan, 1999; Olmstead et al., 2006) and risky choice in human subjects (George et al., 2005; Kyngdon and Dickerson, 1999), suggesting that the effects of alcohol may vary as a function of the behavioural task demands or the impulsivity type assessed.

Additionally, and in agreement with previous studies assessing the effects of acute ethanol on the 5-CSRTT (Bizarro et al., 2003; Oliver et al., 2009), no differences in accuracy were found under this challenge (**Figure S4.3**), suggesting that attentional mechanisms are preserved after acute ethanol and that increases in impulsive behaviour might be independent of attentional performance (Dalley et al., 2011; Oliver et al., 2009; Sanchez-Roige et al., 2012) but see (Patel et al., 2006; Puumala and Sirvio, 1998). Although a higher ethanol dose of 2-g/kg increased the percentage of omitted trials (**Figure 4.4*B***), those effects may result from a general reduction in the vigour of responding, sedation or reduced mobility under ethanol, consistent with the increased time to make a response and the decreases in the total number of trials and speed to collect the reinforcer (**Figure S4.3**).

To our knowledge, no studies have compared the effects of acute alcohol on behavioural inhibition in B6 and D2 strains. At the highest dose of 2-g/kg of ethanol, the high-responding B6 strain appeared to show a reduction in general activity, possibly reflecting sedation at this high dose. However, at low doses, B6 mice (but not D2 mice) displayed higher premature responses in the 5-CSRTT (**Figure 4.4*A***) in comparison to higher doses, where premature responding appeared to be diminished. These findings support the notion that individuals with greater baseline levels of waiting impulsivity may be more sensitive to the acute effects of alcohol in inhibitory control, which may increase further alcohol-seeking behaviour, as has been shown in humans for other measures of impulsivity (Weafer and Fillmore, 2008).

4.5.5 Human implications and concluding remarks

The animal model of binge drinking here described impaired 'waiting' impulsivity in adulthood in two inbred strains of mice and altered decision-making in the D2 strain. Further research is needed to clarify the neurobiological bases underlying the long-term disruption on motoric and choice impulsive behaviours. Unravelling such mechanisms is of major relevance for human health, as greater trait impulsivity and lower executive function have been shown to predict alcohol abuse and dependence in at-risk subjects (Gierski et al., 2013).

4.6 ACKNOWLEDGEMENTS

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Chapter 4

Appendix 4.1

Supplemental Information

Table S4.1. Summary of main strain and IEE effects for 5-CSRTT and mIGT performance

	Strain	IEE
5- CSRTT		
% Prematures		
Baseline	B6 > D2	=
Long ITI	B6 > D2	=
vITI	B6 > D2	IEE > CON (IEE_Late > CON*)
Acute EtOH	B6 > D2 at 0.5-1 g/kg	=
Attention- 5CSRTT		
% Omissions		
Baseline	B6 < D2	=
Long ITI	=	IEE_Late > IEE_Early = CON
vITI	=	=
Acute EtOH	B6 > D2 at 2g/kg	=
mIGT		
% Risky choice		
Baseline	B6 < D2	= (D2_IEE_Early > CON#)
Long ITI	=	=
Acute EtOH	=	B6_IEE_Early > B6_IEE_Late = B6_CON at 0.5 g/kg
% Prematures		
Baseline	B6 > D2	=
Long ITI	B6 > D2	=
Acute EtOH	B6 > D2	IEE_Late > IEE_Early = CON at 1g/kg
% Omissions		
Baseline	B6 < D2	= (IEE_Late > CON#)
Long ITI	B6 < D2	=
Acute EtOH	=	=

* tendency for significance, # final stages of training (session 11-15), >/< higher/lower values, respectively, = no differences

Figure S4.1

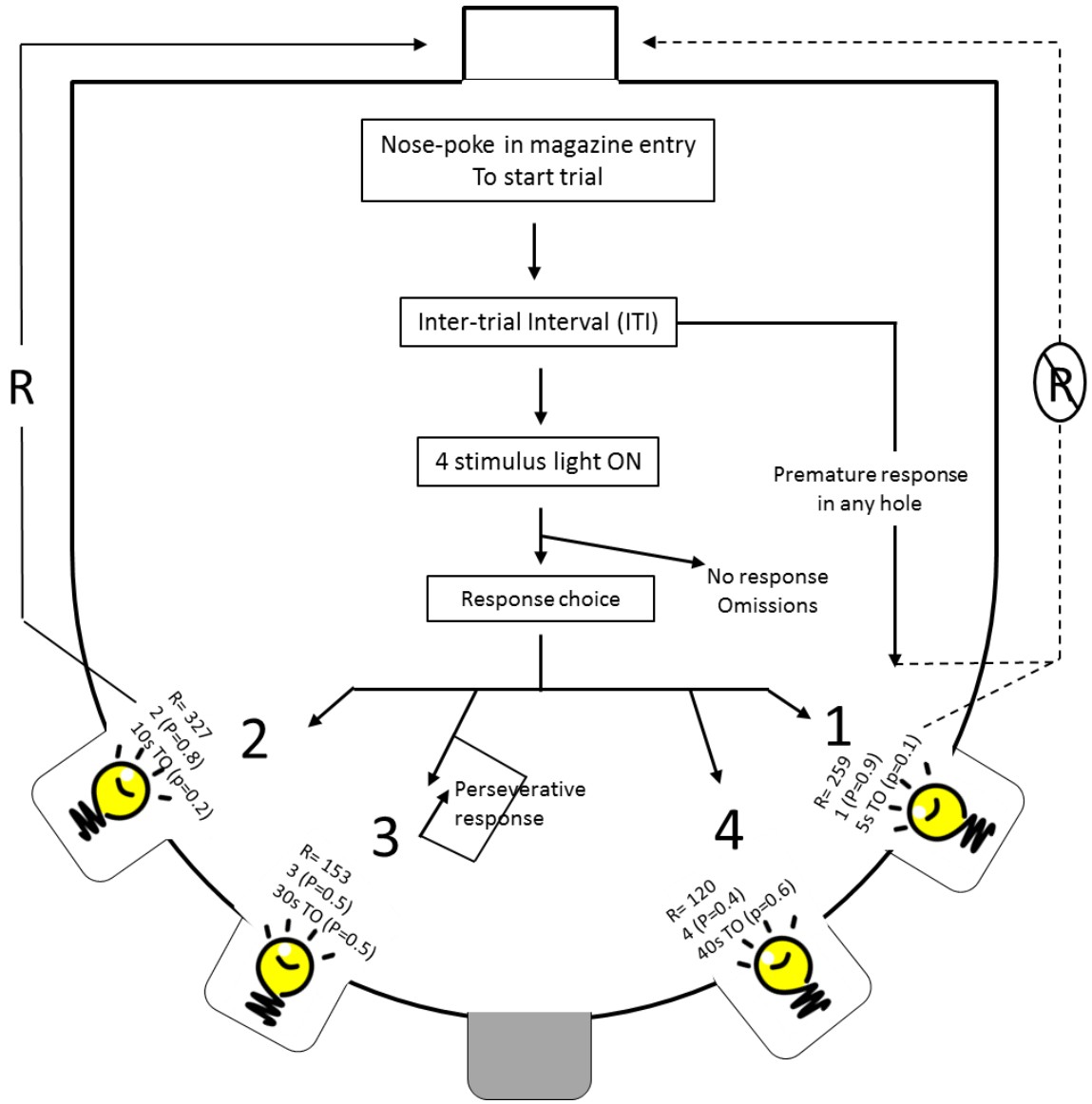

Figure S4.1 Task sequence for a trial example on the mIGT. The task commenced with illumination of the magazine light. A nose-poke response magazine entry initiated a new trial. After an inter-trial-interval (ITI) of 5-s, four stimulus lights were turned on in holes 1, 2, 4, and 5, and the animal was required to respond in one of these holes within 10-s (response choice). A response made during the ITI was classified as a premature response and punished by a 5-s timeout, signalled by a period of darkness. This response was then rewarded or punished depending on the reinforcement schedule for that option (R, total number of reinforcers; # maximum number of reinforcer available and probability of occurrence; # duration of timeout and probability of occurrence). If the animal received a reward, the stimulus lights were extinguished and the animal received the corresponding number of drops of condensed-milk in the magazine. If the animal was punished, the cue light of the selected hole flashed at a 0.5Hz frequency and the remaining cues were extinguished. A nose-poke in the magazine started a new trial. Failure to respond in any of the four illuminated holes resulted in an omission, but led to no programmed consequences. The locations of the positions delivering the different contingencies were counterbalanced across mice

Figure S4.2

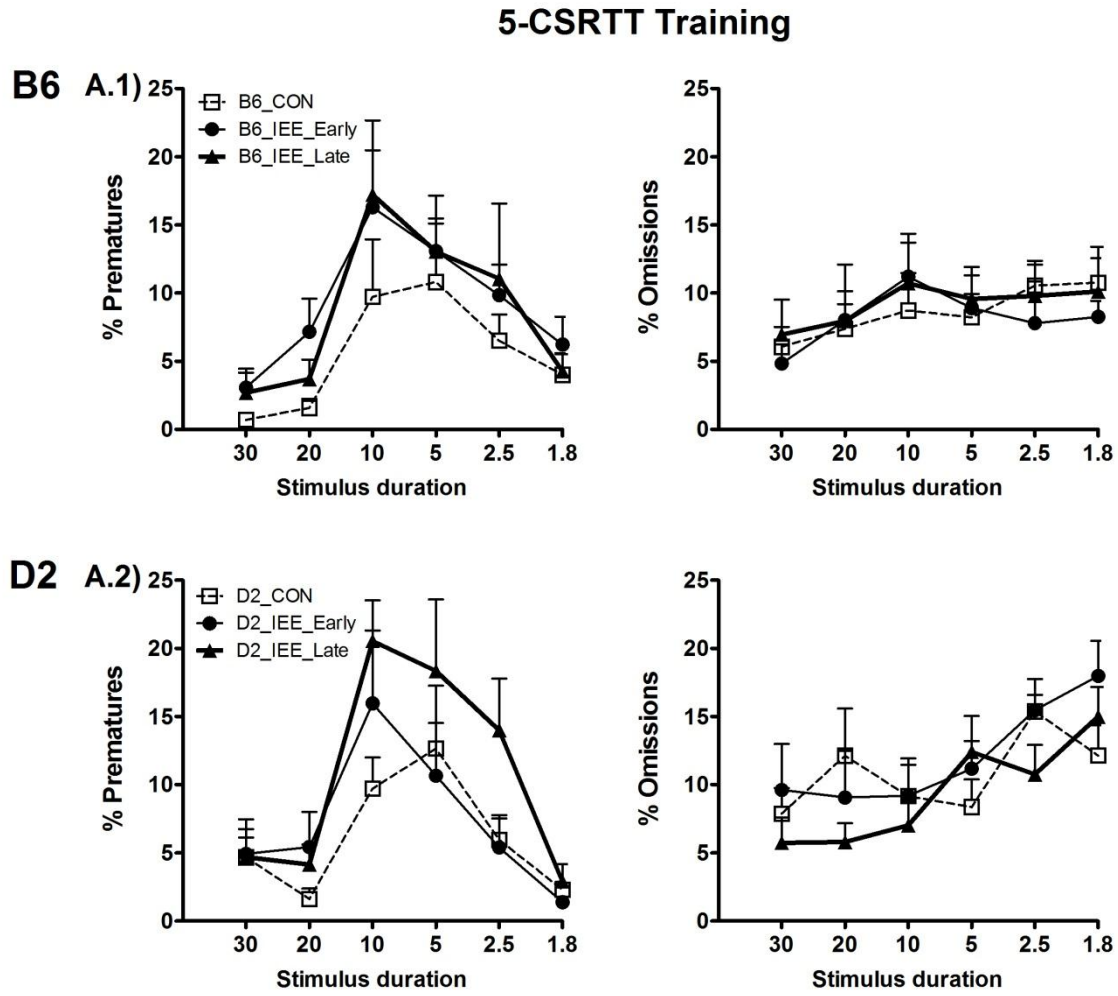

Figure S4.2 Performance at different stages of training in the 5-CSRTT for B6 (top panels) and D2 (bottom panels) mice. Percentage of premature responses (A) reached a peak when the ITI was extended from 2-s to 5-s (stimulus duration=10-s) but all mice learned not to be impulsive in subsequent stages ($F(5, 47)= 20.146$, $p<0.001$, $\varepsilon=0.671$). A main effect of IEE was found in this impulsivity measure ($F(2, 47)=3.227$, $p<0.05$), IEE_Late mice showing overall higher premature levels in comparison to CON mice ($p<0.05$). Levels of omissions (B), required to be below 25% to meet criterion for adequate performance during training, did not differ among groups ($F(2, 47)=.193$, $p=0.825$). The percentage omissions increased as the training progressed, with only a tendency of D2 omitting more trials than B6 mice ($p=0.051$). Data shown are for the last day of each successive training stage (mean ± SE)

Figure S4.3

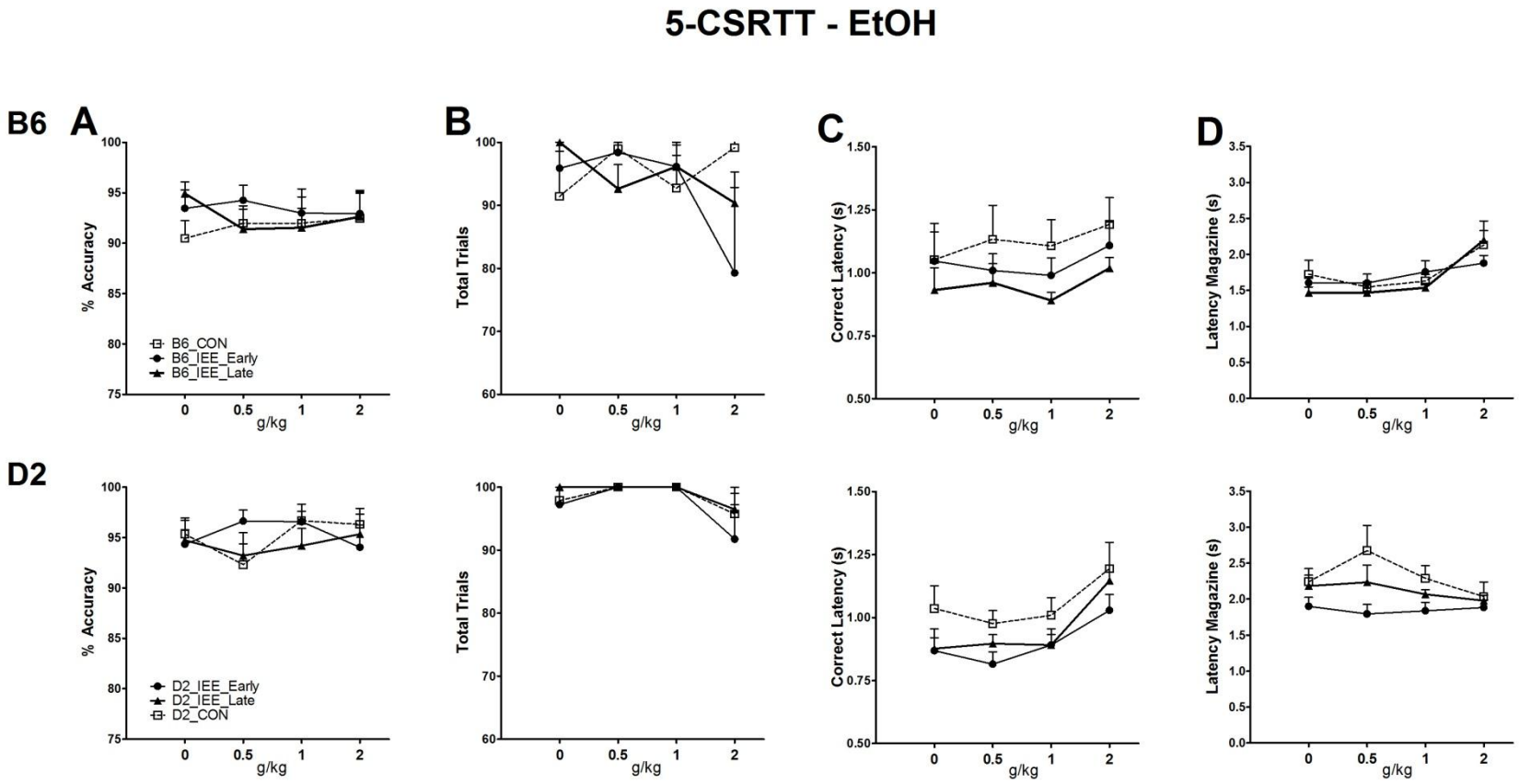

Figure S4.3 Effects of ethanol at different doses on the 5-CSRTT under long ITI condition in B6 (top panels) and D2 (bottom panels) mice. A) The mean ± SE of the accuracy of responding, B) total number of trials, C) latency to make a correct response and D) latency to collect the reinforcer. Ethanol did not impair the attentional performance of the mice (A). A dose of 2g/kg increased the latency of responding in both strains (p<0.001; C) and increased the latency to collect the reinforcer (p<0.05; D), more reliably in B6 mice (p<0.001). No IEE effects were found in any of those measures

Figure S4.4

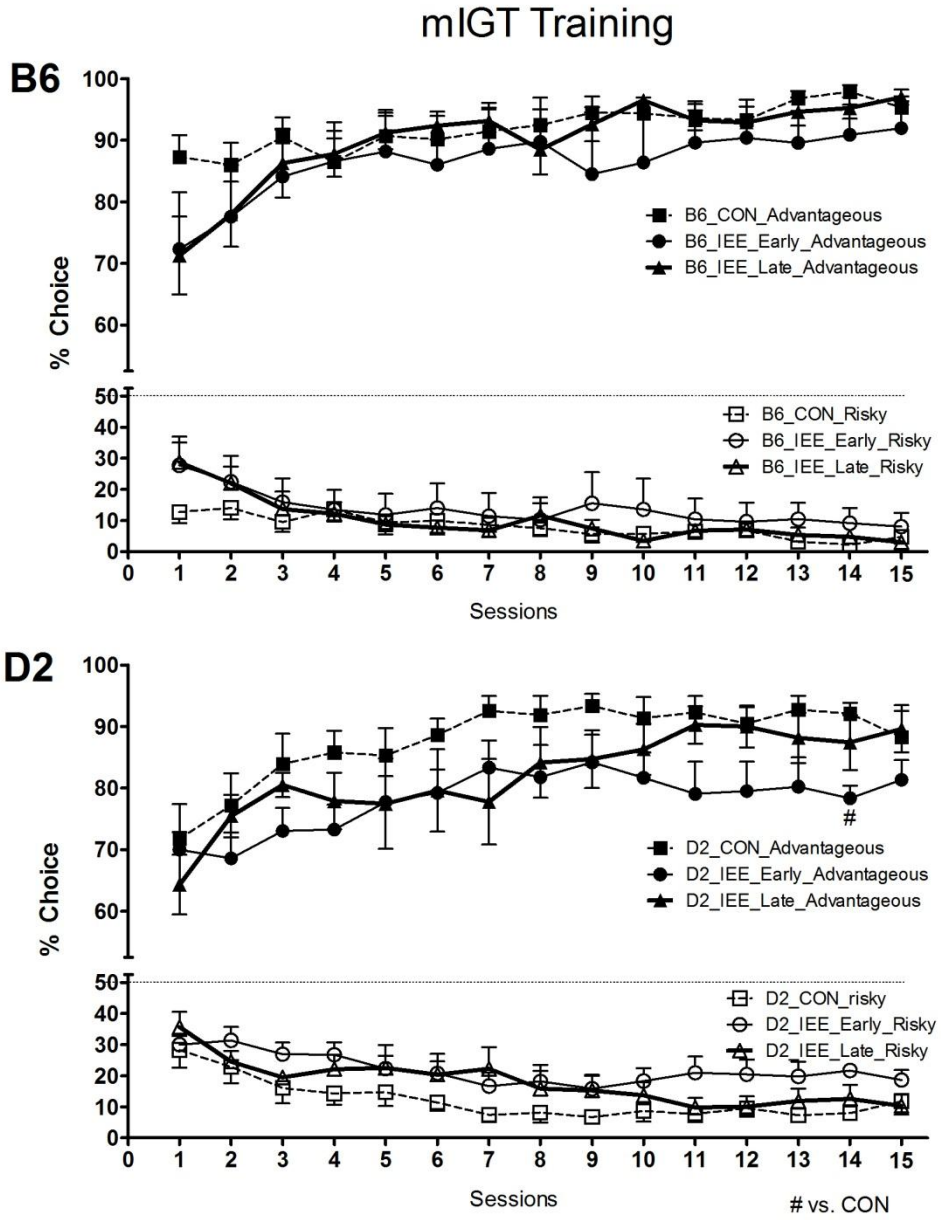

Figure S4.4 Risky (empty symbols) and advantageous (filled symbols) choice performance during mIGT training (3 blocks: initial training, sessions 1-5; middle training, sessions 6-10; final, sessions 11-15) for B6 (top panel) and D2 (bottom panel). D2 mice learned the contingencies of the mIGT at a slower rate than B6 mice (B6=5.22±0.61 vs. D2=13.67±0.59, mean±SEM; $F(1, 41)= 86.271$, $p<0.001$) but no effects of IEE were detected in the analysis, animals from both IEE groups needing as many sessions as mice from CON groups to reach criterion. Over time, all mice developed a preference for the advantageous choices ($F(2, 41)=16.251$, $p<0.001$), performing fewer advantageous choices during the initial training ($p<0.01$, $p<0.001$, vs. middle and final training, respectively), but reaching stable responses over middle and final stages of training. There were no strain or group differences in the learning curve but a main effect of strain appeared in the analysis ($F(1, 41)= 5.361$, $p<0.05$), revealing more risky choices in D2 mice. In order to examine those strain differences further, we employed a two-way ANOVA analysis for the final stage of training. Whereas no effect of IEE was detected in B6 mice, significant effects of IEE were found in D2 mice ($F(2, 23)= 4.738$, $p<0.05$), IEE_Early mice displaying a higher percentages of risky choices in comparison to CON ($p<0.05$)

SUPPLEMENTARY MATERIALS AND METHODS

The Five-Choice Serial Reaction Time Task

Apparatus: 5-CSRTT Boxes

The test apparatus consisted of eight mouse operant chambers (Med Associates Inc., St. Albans, Vermont, USA). Each chamber was housed in a sound-attenuating outer cabinet, with a ventilator fan providing a constant low-level background noise. The left wall of the chamber was curved and contained 5 apertures fitted with infrared detectors to detect nose-poke responses. The apertures were illuminated by a yellow stimulus light located inside each aperture. The right wall of the chamber contained a receptacle hole with a round access opening where the liquid reinforcer was delivered. 30% condensed milk solution was used as a reinforcer (0.01ml) and was delivered into a small cup by means of a dipper. Head entries into the food magazine were recorded by an infrared photo-cell beam crossing the entrance of the receptacle hole, which could be illuminated by a yellow stimulus light inside the aperture. A house-light was located at the top of the wall above the food magazine. The presentation of stimuli and the recording of the responses were controlled by a Smart Control Package 8IN/16out with an additional interface by MED-PC for Windows (Med Associates Inc., St. Albans, Vermont, USA).

Behavioural sequence

Habituation to the reinforcement and to the 5-CSRTT boxes

During the first two-three sessions, animals were placed in the boxes for 30 min and the liquid reward (30% condensed milk solution) was available into the magazine. The house light, the magazine light and the stimulus lights in the five holes were turned on during the entire session. Magazine head entries and number of reinforcers earned were recorded. When the animal earned 50 or more reinforcers in two consecutive sessions, the animal started the training in the 5-CSRTT.

5-CSRTT Training

In brief, a total of 48 adult mice were habituated to the chamber and reinforcer, and were progressively trained to detect a stimulus light of 10-s, which gradually decreased to 1.8-s. The session commenced with the illumination of the house-light. Nose-poking in the aperture started the first trial, and free delivery of the liquid reinforcer (dipper on for 3-s) was presented and accompanied by the illumination of the food magazine. After a fixed interval (inter-trial interval, ITI), one of the stimulus lights was turned on for a brief time. The animal was required to nose-poke within a certain period (limited hold, LH) into the correct hole in order to obtain the reinforcer. After a correct detection, the animal was able collect the reinforcer in the magazine tray thus initiating the next trial. An incorrect response occurred in the case of the animal making a response in a non-illuminated hole. On the other hand, if the animal failed to respond into any of the holes after the completion of the LH, this was recorded as an error of omission. Any response into the holes during the inter-trial interval, when the stimulus light had not yet been presented, was registered as a premature response. To signal appropriateness of behaviour, incorrect, omission and premature responses were followed by a time out (TO), signalled by a 5-s period of darkness, where no reinforcer could be obtained. After the termination of the TO period, the next trial was started by a nose-poke into the magazine. Some animals tended to nose-poke repeatedly into the holes after a correct response, referred to as a perseverative response and a form of compulsivity (Dalley et al., 2011), but had no programmed consequences. The times to make the correct response and to collect the reinforcer were also recorded (correct latency and magazine latency, respectively), and the total number of trials completed (Dalley et al., 2007) were also examined, altogether providing a measure of motivation.

At the beginning of training, the stimulus duration (SD) was set to 30-s and the ITI to 2-s, but these parameters were adjusted according to the performance of each animal. When the animal was able to perform two consecutive sessions achieving the execution criteria (>50

correct trials, >75% accuracy and < 25% omissions) the stimulus duration was reduced in the following pattern: 30, 20, 10, 5, 2.5, 1.8-s (baseline), and the LH and the ITI set at 5-s. Testing was carried out daily (5-6 days a week), and the session lasted for 100 trials or 30 min, whichever came first. When mice achieved the performance criteria at the stimulus duration of 1.8-s (>75% accuracy, <25% omissions, for two consecutive days), several task parameters were modified in order to promote premature responding (Sanchez-Roige et al., 2012). Testing began at approximately PND180. Firstly, mice confronted a long inter-trial interval (long ITI) session, where the ITI was set at 10-s and the duration of the task increased to 45 min, known to provoke impulsive responding in both rats (e.g. Dalley et al., 2007) and mice (Oliver et al., 2009); followed by a second challenge vITI, which is also know to increase premature responding in mice (Walker et al., 2011). Pharmacological challenges (acute ethanol) were introduced lastly (Sanchez-Roige et al., 2012), to avoid possible behavioural disruptions following acute exposure to the compound.

The variables considered in the analysis of the 5-CSRTT were:

- Accuracy (percentage of correct responses): correct responses / (correct responses + total incorrect responses) x 100.
- Percentage of omissions: total omissions / (correct responses + incorrect responses + omissions) x 100.
- Percentage of premature responding: premature responses / (correct responses + incorrect responses + omissions + premature responses) x 100.
- Correct latency: latency to nose-poke into the correct hole after the onset of the stimulus (s).
- Magazine latency: latency to collect the reward after a correct response (s).
- Perseverative responses: total number of responses made into the holes after a correct response and before the collection of the reward.

The mouse Gambling Task

Habituation to the mIGT

During the first two sessions of training, animals were placed in the boxes for 30 min and the liquid reinforcer was available into the magazine entry in an identical manner as the habituation protocol used for the 5-CSRTT. The house light and the magazine light were turned on and the stimulus cues remained off for the entire session. Magazine head entries and number of reinforcers earned were recorded.

Forced-choice program: learning the contingencies

Programs used during training and the mIGT were adapted from both the Rat Gambling Task protocol, developed by Winstanley and colleagues (Zeeb et al., 2009), and the Mouse Gambling Task (Young et al., 2011). Specific details for the protocol here used can be retrieved from (Peña-Oliver et al., 2014). Briefly, the training in the mIGT was divided into 3 stages. During the first stage, mice were trained to nose-poke into any of four illuminated holes (central light turned off throughout the entire experiment) in order to obtain the reinforcer(s). Magazine head entries, number of reinforcers earned and nose-pokes into each hole were recorded. Mice achieved criteria after two consecutive sessions of earning more than 40 reinforcers. In a second stage, mice were trained under the same conditions but required to nose-poke into the magazine entry in order to start a new trial. After two consecutive sessions of earning more than 40 reinforcers in the previous stage, the animals were moved to a forced-choice program, with two counterbalanced options (A and B; see **Figure S4.1**) to control for possible hole preferences. During 5 sessions, only one stimulus light was illuminated and the animals were required to nose-poke into the illuminated hole. As illustrated in **Figure S4.1**, each cue was associated with a fixed probability of reinforcement and punishment, and the aim of this stage was to train the mice on the contingencies associated with each stimulus location. Daily sessions (5-6 days a week) lasted for 100 trials or 30 min, whichever came first.

The mIGT

The session commenced with the illumination of the house-light accompanied by the illumination of the magazine entry. A nose-poke into the magazine initiated the first trial and the mice had to withhold any responses during a fixed interval of 5-s (inter-trial interval, ITI). A response made into the holes during the ITI, when the stimulus lights had not yet been presented, was registered as a 'premature response' and was followed by a time out period (TO), during which the lights were turned off for 5 seconds. Responses made into the holes during this period restarted the time out. If the animal succeeded in withholding responding into the holes, four stimulus lights were turned on for 10 seconds and the animal had to nose-poke into one hole in order to receive a reward (or punishment). If the animal nose-poked in one of the illuminated holes, that was recorded as a 'response choice', the stimulus lights were extinguished and the mouse was rewarded or punished depending on the contingencies associated to the cue selected. In a rewarded trial the magazine light was illuminated and the animal received 1, 2, 3 or 4 delivery presentations of the condensed milk solution. As required in the forced-choice, a nose-poke in the magazine entry started the next trial. However, if the choice led to a punishment, a time-out of certain duration was delivered (5, 10, 30 or 40 seconds, depending on the choice probability). A TO resulting from a response choice was signalled by a stimulus light flashing at a frequency of 0.5 Hz in the selected hole, for the duration of the TO period for that particular cue. Further responses into the selected hole after a response choice and before the collection of the reward, were registered as 'perseverative responses' but had no programmed consequences. Failure to respond in any of the illuminated holes was recorded as an 'omission', but did not lead to a time-out period. The latency to nose-poke into a stimulus hole after the onset of the stimulus (choice latency), and the latency to collect the reinforcer after the response choice (magazine latency), were also registered. Baseline and long ITI sessions occurred at approximately PND100.

The variables considered in the analysis of the mIGT were:

- Percentage of advantageous choices: (choice 1 + choice 2) / (choice 1+2+3+4) x 100.
- Percentage of risky choices: (choice 3 + choice 4) / (choice 1+2+3+4) x 100.
- Percentage of omissions: total omissions / (total choices + omissions) x 100.
- Percentage of premature responding: premature responses / (premature responses + total choices) x 100.
- Correct latency: latency to make a response choice after the onset of the stimulus (s).
- Magazine latency: latency to collect the reward after a response choice (s).
- Perseverative responses: total number of responses made into the selected holes after a response choice and before the collection of the reward.

Statistical analysis

Three-way repeated measures ANOVA was used for the analysis of each variable of the 5-CSRTT during training, with treatment (IEE_Early, IEE_Late, CON) and strain (B6, D2) as the between-subjects factor and stage of training (6 levels) as the within-subjects factor. Within-session performance in the mIGT during training was analysed using a repeated-measures ANOVA with strain (B6, D2) and treatment (CON, IEE_Early, IEE_Late) as the between-subjects factor and drug dose (three levels) as the within-subjects factor.

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Chapter 5

Paper 4

ALLEVIATING WAITING IMPULSIVITY AND PERSEVERATIVE RESPONDING BY μ-OPIOID RECEPTOR ANTAGONISM IN TWO INBRED MOUSE STRAINS

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5.1 Abstract

Rationale Recent evidence has implicated the opioid system in exaggerated ethanol consumption and impulsivity deficits. The opioid receptor antagonist naltrexone (NTX) has proven efficient in reducing alcohol consumption; however, its role on impulsive behaviour is not fully characterized.

Objective The aim of this study was to investigate the effects of NTX on two measures of impulsive behaviour in two inbred mouse strains that differ in ethanol preference and impulsive phenotype.

Methods Two separate groups of C57BL/6J (B6; n=24) and DBA2/J (D2; n=24) male mice were exposed to intermittent ethanol (2g/kg; IEE) during early (PND30-45; IEE_Early) or late (PND45-60; IEE_Late) adolescence, or the respective saline control. The ability of NTX (10mg/kg) alone, or co-administered with ethanol (0.5g/kg), to diminish waiting impulsivity in the Five-choice Serial Reaction Time Task (5-CSRTT), or improve decision-making in a mouse version of the Iowa Gambling Task (mIGT), were examined in adulthood.

Results In the 5-CSRTT, NTX diminished impulsivity in both strains of mice, irrespective of previous ethanol experience. In the mIGT, NTX failed to alter risky decision-making but decreased perseverative responding.

Conclusions Blocking the actions of endogenous opioids may attenuate waiting impulsivity, in addition to alleviating perseverative responding. In a broader context, μ-opiate antagonism may be of potential interest for impulse-control disorders.

Key Words Alcohol, Naltrexone, C57BL/6J, DBA2/J, 5-CSRTT, mIGT

5.2 INTRODUCTION

Following the discovery that increased activity of the opioid system is associated with high alcohol consumption (Reid et al., 1991), naltrexone (NTX), a compound that acts through antagonism of μ-opioid receptors, has proven useful for the treatment of alcohol dependence (Kamdar et al., 2007). Initial research revealed the efficacy of NTX in reducing alcohol consumption, both in human subjects with a history of alcohol abuse (Anton et al. 1999; Davidson et al., 1999; Volpicelli et al., 1992) and in animal models (Boyle et al., 1998; Stromberg et al., 1998). In rodents, NTX dose-dependently decreased ethanol consumption, this effect being specific for ethanol without it affecting consumption of water or sucrose solutions (Oberlin et al., 2010).

The neural mechanisms underlying NTX's efficacy remain unclear. Since μ-opioid receptors have been hypothesized to mediate ethanol reward, it has been suggested that antagonising μ-opioid receptors may reduce drinking by decreasing the rewarding properties of ethanol (Dayas et al., 2007; Sinclair, 2001). Another possibility is that NTX reduces drinking by generating aversive side effects, such as nausea or general anhedonia, when ethanol is consumed (Davidson et al., 1999; de Wit et al., 1999; Mitchell et al., 2009). An alternative explanation accounts for NTX reducing ethanol intake by positing its ability to increase inhibitory control. NTX significantly reduced alcohol craving in alcoholics during abstinence (Monti et al., 1999; Rohsenow et al., 2000), and increased resistance to thoughts, urges, and behaviours associated with drinking (Anton et al., 1999). Thus, it is plausible that NTX reduces drinking and relapse rates by facilitating cognitive control processes.

In humans, this compound has been found not only to reduce alcohol consumption, but also craving and relapse (O'Brien et al., 1996). Thus, the capacity of NTX to affect top-down processes, such as impulsive behaviour, has found increased interest with the hypothesis that such mechanisms may in turn increase control over high drinking. In humans, a 50 mg dose of

NTX increased activity in the orbital frontal cortex of human alcoholics, one of the frontal-cortical areas of the brain that modulates behavioural control through executive functions during a task measuring choice impulsivity (Boettiger et al., 2009; Crews and Boettiger, 2009). In rodents, although a 10mg/kg dose of NTX significantly decreased ethanol intake in C57BL/6NCRL and DBA2/J mice (Tomie et al., 2013) and in high alcohol-preferring mice (Oberlin et al., 2010), it failed to decrease choice impulsivity as measured in a delay discounting task (Kieres et al., 2004; Oberlin et al., 2010), suggesting that the effectiveness of naltrexone might be dependent on the facet of impulsivity measured (Evenden, 1999). Here we aimed to investigate the ability of the same dose of NTX to reduce two aspects of impulsivity, motor (or waiting) impulsivity, by decreasing premature responses in the 5-CSRTT (Robbins, 2002; Sanchez-Roige et al., 2012), and choice impulsivity, using a rodent version of the Iowa Gambling Task (mIGT; Young et al., 2011; Zeeb et al., 2009; Peña-Oliver et al., 2014) in two inbred strains of mice. Since early-life exposure to alcohol may impair behavioural control in adulthood (Lopez-Caneda et al., 2012), we also tested NTX effects in these tasks in animals with previous ethanol experience, using a mouse model of adolescent binge drinking. Mice were exposed to repeated withdrawals from ethanol during early and late adolescence (Sanchez-Roige et al., 2014a), in order to emulate intermittent binge drinking, a characteristic pattern in human adolescents (White et al., 2006). Two cohorts of C57BL/6J (B6) and DBA2/J (D2) mice, known to differ in levels of alcohol consumption, and which additionally present distinct impulsive phenotypes (Helms et al., 2006; Sanchez-Roige et al., 2014b), were used in the study. Following extensive training, the effects of acute NTX, given alone, and in co-administration with ethanol, to inhibit impulsive behaviour was tested in adulthood in the two different impulsivity paradigms.

5.3 MATERIAL AND METHODS

5.3.1 Subjects

Two separate cohorts of B6 (n=24/group) and D2 (n=24/group) male mice obtained from Charles River Laboratories (Arbresle, France) were randomly assigned to different groups (n=8/group): control group (CON), intermittent ethanol exposure (IEE) during early adolescence (IEE_Early), and late adolescence (IEE_Late). The mice were housed in groups of two per cage on a 12-h light/dark cycle (lights off at 7:00pm) at a temperature of 19-21°C and 50% humidity. After the IEE treatment, the mice were food restricted to reduce their body weights to 85% of their free-feeding weight and kept under food restriction until the end of the experiments. Water was available *ad libitum* throughout the study. Behavioural testing took place between 8:00 and 2:00pm, 5 to 6 days per week. Experimental protocols were approved by the institutional ethics committee and were performed under United Kingdom legislation on animal experimentation [Animal (Scientific Procedures) Act, 1986].

5.3.2 Intermittent ethanol exposure

Mice were exposed to ethanol (2g/kg, i.p.) in a pattern of intermittent exposure (4 cycles of 2 days ethanol injection, 2 days injection free) during early (PND 30-45) and late adolescence (PND 46-59). Controls received saline injections throughout PND 30-60, on the same schedule as IEE mice [see (Sanchez-Roige et al., 2014a) for details].

5.3.3 Drugs

Ethanol (95%) was diluted to 20% (v/v) in saline solution and administered intraperitoneally (i.p) at 2g/kg for the IEE treatment and at 0.5g/kg (i.p.) for the NTX challenge. Ethanol was administered at a volume of 10mL/kg to avoid tissue irritation. Naltrexone hydrochloride (Sigma-Aldrich; NTX) was dissolved in saline to produce a 1mg/ml solution and administered

subcutaneously (s.c.; 10ml/kg dose volume) in two separate Latin square designs [phase 1, Vehicle-Vehicle, NTX-Vehicle; phase 2, Vehicle-ethanol, NTX-ethanol]. The dose of NTX (10mg/kg) was chosen on the basis of previous work (Oberlin et al., 2010); we used an acute (0.5g/kg)-ethanol dose, as it previously increased premature responding in the highly impulsive B6 strain (Sanchez-Roige et al., 2014a).

Experimental procedure

5.3.4 The Five-Choice Serial Reaction Time Task

The test apparatus consisted of eight mouse operant chambers [(Med Associates Inc., St. Albans, Vermont, USA); see (Oliver et al., 2009) for further description]. The training phases of the experiments were based on procedures described elsewhere (Oliver et al., 2009). In brief, a group of 24 B6 and 24 D2 mice were habituated to the chamber and reinforcer (30% condensed milk solution), and were progressively trained to detect a stimulus light of 10-s duration, which gradually decreased to 1.8-s. The animal was required to nose-poke within a certain period (limited hold, LH) into the correct illuminated hole in order to obtain the reinforcer in the magazine entry. Time to make the correct response and to collect the reinforcer (correct and magazine latency, respectively), and the total number of trials completed, were recorded, altogether providing a measure of motivation (Dalley et al., 2007). Correct identification of the location of the stimulus, or failure to respond when the stimulus was presented (omission error), served as measures of attention (Robbins, 2002; Sanchez-Roige et al., 2012). Failure to respond, responses into non-illuminated holes (incorrect) and premature responses (response into the holes during the 5-s inter-trial interval ['waiting time', ITI], when the stimulus light had not yet been presented) were punished by a time out (TO), signalled by a 5-s period of darkness, where no reinforcer could be obtained. Perseverative responses (further responses into the holes after a correct response and before the collection of the reward) were recorded but had no programmed consequences. After successful learning

of the task [baseline parameters: Stimulus duration= 1.8-s; LH= 5-s; ITI= 5-s; TO= 5-s; performance criteria: >75% accuracy, <25% omissions for two consecutive days] and completion of previous 5-CSRTT testing reported elsewhere (Sanchez-Roige et al., 2014a), one mouse from each of the D2_IEE_Late, B6_IEE_Early, B6_IEE_Late groups, and two mice from D2_IEE_Early group died, for unknown reasons. Following 5-CSRTT retraining, one mouse from D2_IEE_Late, two mice from the D2_CON and one mouse from the B6_CON group failed to reach criterion for performance and were excluded from the experiment (n=39, n=6-7/group; CON, n= 13; IEE_Early, n= 13; IEE_Late, n= 13). To test the effects of NTX on 5-CSRTT performance, animals were injected vehicle or NTX (10mg/kg) 25-min prior to the long-ITI session, known to provoke impulsive responding (Robbins, 2002; Sanchez-Roige et al., 2012). Following a 10-min interval, animals were injected with vehicle or ethanol (0.5g/kg; 15-min prior to testing). Between each of the drug testing sessions, mice performed a minimum of two days of drug free testing under baseline parameters.

Several performance measures were recorded: a) total trials [total correct responses + total incorrect + total omissions]; b) accuracy [(percentage of correct responses): correct responses/(correct responses + total incorrect responses) x 100], c) percentage of omissions [total omissions/(correct responses + incorrect responses + omissions) x 100], d) percentage of premature responding [premature responses/(correct responses + incorrect responses + omissions + premature responses) x 100], e) correct latency (latency to nose-poke into the correct hole after the onset of the stimulus), f) perseverative responses (total number of responses made into the holes after a correct response).

5.3.5 The mouse Gambling Task

The test apparatus consisted of eight mouse five-hole operant chambers (Med Associates Inc., St. Albans, Vermont, USA). Extensive details of the protocol can be found elsewhere [(Peña-

Oliver et al., 2014; Sanchez-Roige et al., 2014a)]. Briefly, a second group of 24 B6 and 24 D2 mice were habituated to the chamber and reinforcer (30% condensed milk solution). The training in the mIGT was aimed to train the mice on the contingencies associated with each of the four stimulus locations, during which the animals were required to nose-poke into one illuminated hole at a time. Under Contingency 1, a nose-poke into a defined hole provided a single drop of milk with a probability of 0.9, or a TO of 5-s (probability 0.1). The corresponding values for Contingency 2 were [2 drops, p= 0.8; TO 10-s, p= 0.2], for Contingency 3 [3 drops, p= 0.5; TO 30-s, p= 0.5], and Contingency 4 [4 drops, p= 0.4; TO 40-s, p=0.6]. Decision-making was assessed for 15 30-min sessions. During those sessions, a nose-poke into the magazine entry started a new trial. If the animal succeeded in withholding any responding into the holes (ITI= 5-s), registered as 'premature responses', four stimulus lights were turned on for 10 seconds and the animal had to nose-poke into one hole in order to receive a reward (or punishment). Mice had to choose between advantageous options (Contingencies 1 and 2), characterized by a reinforcer of low magnitude, but high probability of reinforcement and short punishment TO, over the more disadvantageous options (Contingencies 3 and 4) associated with larger reward size, but lower net gains over the session. If the animal nose-poked in one of the illuminated holes ('response choice'), the stimulus lights were extinguished and the mouse was rewarded or punished depending on the contingencies associated with the hole selected. In a rewarded trial the magazine light was illuminated and the animal received 1, 2, 3 or 4 delivery presentations of the condensed milk solution. However, if the choice lead to punishment, a TO of certain duration was accompanied by a stimulus light flashing at a frequency of 0.5 Hz in the selected hole, for the duration of the TO period for that particular hole (5, 10, 30 or 40 seconds, depending on the choice probability). Failure to respond in any of the illuminated holes ('omission') and further responses into the selected hole after a response choice and before the collection of the reward ('perseverative responses'), were recorded but had no programmed consequences. Time to nose-poke into a stimulus hole and

to collect the reinforcer (choice and magazine latency, respectively), were also registered. One mouse from the B6_IEE_Early group was excluded from the analysis because its response choice exceeded the group mean responses + 3 x S.D. and was considered to be an outlier (Tabachnick and Fidell 2000); one mouse from the same group died for unknown reasons prior to the experiment, leaving a group size of n = 6 (final n= 46; CON, n= 16; IEE_Early, n= 14; IEE_Late, n= 16). After stabilization of performance and completion of previous mIGT testing reported elsewhere (Sanchez-Roige et al., 2014a), the effects of NTX and ethanol co-administration in risky decision-making were tested under baseline conditions of the task. Due to technical difficulties, data from the Vehicle-Vehicle session was lost for one mouse from the D2_IEE_Late group, and thus this mouse was excluded from phase 1. To allow informal comparisons on the NTX effects on 5-CSRTT and mIGT performance, the same Latin square design as for the 5-CSRTT experiment was used.

The variables considered in the analysis of the mGIT were: a) Percentage of advantageous choices [(choice 1 + choice 2)/(choice 1+2+3+4) x 100], percentage of omissions [total omissions/(total choices + omissions) x 100], percentage of premature responding [premature responses/(premature responses + total choices) x 100), choice latency (s), magazine latency (s), perseverative responses (total number of responses made into the selected stimulus hole after a response choice and before the collection of the reward).

5.3.6 Statistical analyses

The statistical analysis was performed using the 'Statistical Package for Social Sciences' (SPSS, version 20.0). 5-CSRTT and mIGT performance were analysed using repeated measures analysis of variance (ANOVA) with treatment (CON, IEE_Early, IEE_Late) and strain (B6, D2) as the between-subjects factor and drug dose (two levels: Vehicle-Vehicle vs. NTX-Vehicle [phase 1]; Vehicle-Ethanol vs. NTX-Ethanol [phase 2]) as the within-subjects factor. When significant interactions were found, one-way ANOVAs and Bonferroni comparisons were used for post

hoc analysis. To examine IEE effects on 5-CSRTT premature responding during each challenge (four levels), an additional ANOVA analysis was conducted, with treatment and strain as the between-subjects factor. A $p<0.05$ was required for results to be considered statistically significant.

5.4 RESULTS

5.4.1 Effects of NTX and acute ethanol co-administration on 5-CSRTT performance

Figure 5.1 illustrates the effects of NTX given alone (phase 1), and with ethanol co-administration (phase 2), under four long-ITI sessions in the 5-CSRTT. Administration of NTX did not alter the number of total trials completed (dose: $F< 2.8$, $ps>0.05$, phase 1 and 2), but a dose x strain interaction was found during phase 1 ($F(1, 33)= 8.470$, $p<0.01$), attributable to the increased number of total trials in B6 mice (81.7±5.4 vs. 96.5±2.2 [Vehicle-Vehicle vs. NTX-Vehicle, Mean±SE, B6]; 96.3±1.5 vs. 92.4±3.7 [Vehicle-Vehicle vs. NTX-Vehicle, D2]). However, when NTX was co-administered with ethanol, those strain-dependent increases in total number of trials disappeared (dose x strain: $F(1, 33)= 0.2$, $p>0.05$; 99.4±0.7 vs. 98.8±0.9 [Vehicle-Ethanol vs. NTX-Ethanol, B6]; 99.3±0.7 vs. 97.7±1.8 [Vehicle-Ethanol vs. NTX-Ethanol, D2]). Moreover, exposure to IEE did not alter the responsiveness to NTX across this measure (dose x treatment: $F< 1.4$, $ps>0.05$, phase 1 and 2), and no main effects of strain, treatment or interactions were observed ($F< 1.5$, $ps>0.05$).

With regards to attention, NTX alone or co-administered with ethanol did not modify the accuracy of responding (dose: $F< 2.8$, $ps>0.05$, phase 1 and 2; **Figure 5.1a**), but a dose x strain interaction was found, revealing increased accuracy of responding in B6 mice in comparison to D2 mice when NTX alone was administered (dose x strain: $F(1, 33)= 4.913$, $p<0.05$, phase 1). No differences among IEE groups were detected in this variable as a consequence of a dose of NTX

(dose x treatment: $F< 0.93$, $ps>.05$), NTX alone or co-administered with ethanol having the same effect on CON mice as IEE mice. Overall, no strain or treatment differences were observed in this measure ($F< 2.7$, $ps>0.05$).

On the other hand, NTX alone and in co-administration with ethanol increased the number of omissions (dose: $F(1, 33)=11.207$, $p<0.01$, phase 1; $F(1, 33)= 34.347$, $p<0.001$, phase 2; **Figure 5.1b**). A dose x strain interaction indicated that this effect was greater in D2 mice during phase 1 (dose x strain: $F(1, 33)= 6.377$, $p<0.05$), the strain with overall higher omission levels in comparison to B6 (strain: $F(1, 33)= 42.388$, $p<0.001$, phase 1; $F(1, 33)= 29.272$, $p<0.001$, phase 2). The increase in omissions induced by NTX occurred irrespective of early ethanol treatment (dose x treatment: $F< 0.58$, $ps>0.05$), and IEE mice showed similar rates of omitted trials than CON mice (treatment: $F< 1.8$, $ps>0.05$).

Figure 5.1c shows that a dramatic decrease in premature responding occurred in all mice given NTX alone (dose: $F(1, 33)= 12.666$, $p<0.001$) and under ethanol co-administration (dose: $F(1, 33)= 21.973$, $p<0.001$), with a similar decrease in both strains (dose x strain: $F< 0.34$, $ps>0.05$, phase 1 and 2) and treatment groups (dose x treatment: $F< 1.86$, $ps>0.05$). Overall, B6 were more impulsive than D2 mice during phase 1 (strain: $F(1, 33)= 25.003$, $p<0.001$). Under phase 2, those strain differences were not noted (strain: $F(1, 33)= 1.936$, $p>0.05$), but a tendency of IEE to increase impulsivity was detected (treatment: $F(2, 33)= 3.014$, $p=0.063$), with IEE_Late mice showing a tendency to display higher levels of premature responses than CON mice ($p=0.089$). NTX alone did not affect the latency to make a correct response (dose: $F(1, 33)= 0.99$, $p>0.05$; **Figure 5.1d**). However, when NTX was co-administered with ethanol, an increase in correct latencies was detected (dose: $F(1, 33)= 21.489$, $p<0.001$), this effect being more marked in D2 mice (dose x strain: $F(1, 33)= 4.878$, $p<0.05$), but irrespective of IEE treatment (dose x treatment: $F(2, 33)= 0.11$, $p>0.05$). Overall, B6 performed faster detections than D2 mice

during both phases (strain: $F(1, 33)= 5.182$, $p<0.05$, phase 1; $F(1, 33)= 19.475$, $p<0.001$, phase 2), while IEE and CON mice showed similar latencies (treatment: $F< 3.07$, $ps>0.05$).

Similarly, NTX alone increased magazine latencies (dose: $F(1, 33)= 7.642$, $p<0.01$; **Figure 5.1e**), in a similar manner for both strains (dose x strain: $F(1, 33)= 2.86$, $p>0.05$), despite D2 mice displaying higher overall values (strain: $F(1, 33)= 4.935$, $p<0.05$). Equally, NTX co-administered with ethanol increased the latency to collect the reinforcer (dose: $F(1, 33)= 12.400$, $p<0.001$), this effect being more accentuated in D2 mice (dose x strain: $F(1, 33)= 9.611$, $p<0.01$), strain displaying higher overall magazine latency values (strain: $F(1, 33)= 15.778$, $p<0.001$). Exposure to early alcohol did not alter the NTX responsiveness on this measure (dose x treatment: $F< 0.99$, $ps>0.05$), and IEE mice showed similar times to collect the reinforcer than CON mice during the two phases (treatment: $F> 0.73$, $ps>0.05$).

NTX alone decreased perseverative responses (dose: $F(1, 33)= 8.121$, $p<0.01$; **Figure 5.1f**), this effect being accentuated in D2 mice (dose x strain: $F(1, 33)= 14.751$, $p<0.001$), but irrespective of IEE (dose x treatment: $F(2, 33)= 2.6$, $p>0.05$). When ethanol was co-administered, NTX failed to decrease perseverative responding (dose: $F(1, 33)= 2.763$, $p>0.05$) but main effects of strain appeared in the analysis, D2 mice showing higher levels of perseverative responses in comparison to B6 mice (strain: $F(1, 33)= 8.626$, $p<0.01$). IEE mice showed similar levels of perseverative responses to CON mice (treatment: $F< 0.57$, $ps>0.05$).

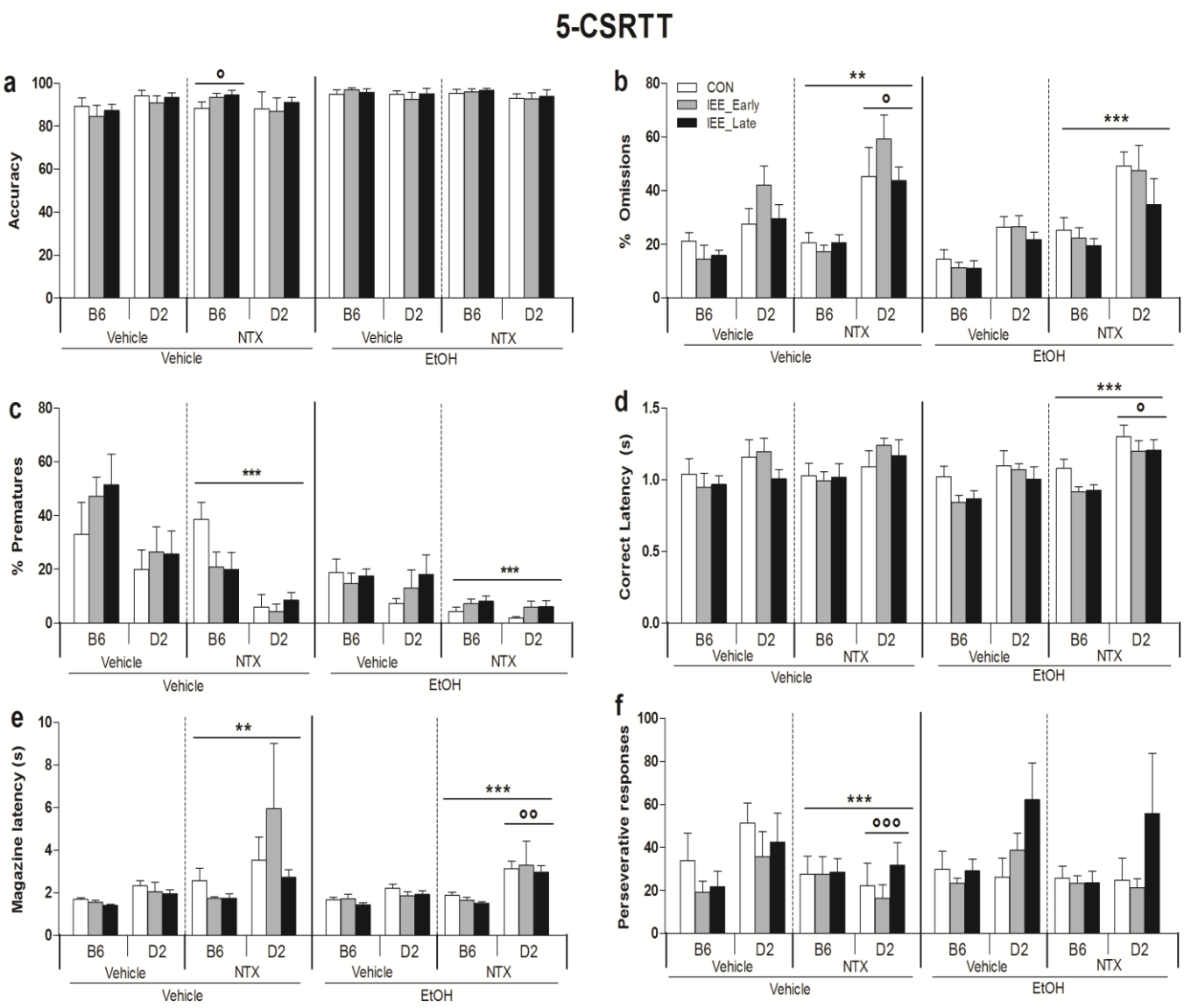

Figure 5.1 Effects of NTX (10mg/kg; Phase 1 [Vehicle-Vehicle vs. Naltrexone-Vehicle]) and ethanol (0.5g/kg; Phase 2 [Vehicle-Ethanol vs. Naltrexone-Ethanol]) co-administration on 5-CSRTT performance of B6 and D2 mice. NTX increased accuracy of responding in B6 mice (phase 1; a) but also increased the percentage of omitted trials in both strains (phase 1 and 2; b). NTX significantly decreased premature responding in both phase 1 and 2 (c) and decreased perseverative responding more substantially in D2 mice (f). NTX also increased correct (d, phase 2) and magazine latencies (e; phase 1 and 2). Data expressed as Mean ± SE (n=39, 6-7/group).

** p<0.01, ***p<0.001 vs. vehicle; ° p<0.05, °° p<0.01, °°° p<0.001 vs. vehicle (D2 mice only)

5.4.2 Effects of NTX and acute ethanol co-administration on mIGT performance

Figure 5.2 depicts the effects of NTX alone, and with ethanol co-administration, under four baseline sessions in the mIGT. The total number of choices that mice performed was reduced under NTX alone (dose: $F(1, 39)= 29.978$, $p<0.001$; Vehicle-Vehicle=96.81±1.09 vs. NTX-Vehicle=80.15±2.87 [Mean±SE]) and co-administered with ethanol (dose: $F(1, 40)= 21.833$, $p<0.001$; Vehicle-ethanol=95.72±1.13 vs. NTX-ethanol=79.87±3.17 [Mean±SE]). This effect was irrespective of strain (dose x strain: $F< 0.22$, $ps>0.05$) or treatment (dose x treatment: $F< 0.76$, $ps>0.05$). No statistical differences between strains, treatment groups or interactions were found in this variable ($F< 1.69$, $ps>0.05$).

NTX alone or co-administered with ethanol had no effect on decision-making (dose: $F>2.0$, $ps>0.05$), as the percentage of advantageous choices remained unchanged across challenges (**Figure 5.2a**). Main effects of strain revealed poorer decision-making in D2 mice, which consistently displayed fewer advantageous choices than B6 mice (strain: $F(1, 39)= 21.123$, $p<0.001$, phase 1; $F(1, 40)= 6.639$, $p<0.05$, phase 2), but no effects of treatment were found ($F< 1.1$, $ps>0.05$).

Additionally, NTX alone and under ethanol co-administration increased the percentage of omitted trials (dose: $F(1, 39)= 39.312$, $p<0.001$, $F(1, 40)= 19.796$, $p<0.001$, respectively; **Figure 5.2b**), this effect being exacerbated in phase 1 for D2 mice (dose x strain: $F(1, 39)= 15.879$, $p<0.001$) and IEE mice (dose x treatment: $F(2, 39)= 3.499$, $p<0.05$). Main effects of strain revealed higher omission rates in D2 mice (strain: $F(1, 39)= 30.642$, $p<0.001$, phase 1; $F(1, 40)= 8.559$, $p<0.01$, phase 2), but no main effects of treatment ($F< 1.1$, $ps>0.05$) were observed across this measure.

Conversely to the effects observed in the 5-CSRTT, NTX failed to decrease the percentage of premature responses in the mIGT (dose: $F(1, 39)= 0.021$, $p>0.05$; **Figure 5.2c**). However, a tendency of NTX to decrease waiting impulsivity was detected under ethanol co-administration

(dose: $F(1, 40)= 2.923$, $p=0.095$), that was irrespective of strain (dose x strain: $F(1, 40)= 0.02$, $p>0.05$) or treatment (dose x treatment: $F(2, 40)= 0.04$, $p>0.05$). During phase 1, a tendency for B6 mice to display a higher percentage of premature trials was observed (strain: $F(1, 39)= 3.771$, $p=0.059$), but impulsivity differences did not emerge among groups (treatment: $F< 0.91$, $ps>0.05$).

NTX alone increased the latency to make a response choice (dose: $F(1, 39)= 28.517$, $p<0.001$; **Figure 5.2d**) and to collect the reward (dose: $F(1, 39)= 14.956$, $p<0.001$; **Figure 5.2e**), and a similar effect was detected when co-administered with ethanol (dose: $F(1, 40)= 38.319$, $p<0.001$, $F(1, 40)= 14.265$, $p<0.001$, respectively), which was independent of strain (dose x strain: $F< 2.4$, $ps>0.05$) or treatment (dose x treatment: $F< 2.48$, $ps>0.05$). Compared to B6, D2 mice showed higher overall choice and magazine latencies during the two phases (strain: $F(1, 39)= 28.157$, $p<0.001$, $F(1, 39)= 9.159$, $p<0.01$, phase 1; $F(1, 40)= 33.561$, $p<0.001$, $F(1, 40)= 11.698$, $p<0.001$, phase 2, respectively for choice and magazine latencies). No main effects of treatment were observed in these measures ($F< 0.82$, $ps>0.05$).

With regards to perseveration, NTX alone, and under ethanol co-administration, decreased perseverative responding in all mice (dose: $F(1, 39)= 33.668$, $p<0.001$, phase 1; $F(1, 40)= 17.024$, $p<0.001$, phase 2; **Figure 5.2f**), irrespective of strain (dose x strain: $F< 3.3$, $ps>0.05$) or treatment (dose x treatment: $F< 2.9$, $ps>0.05$). No statistical differences in perseverative responding were found between strains ($F< 3.3$, $ps>0.05$) or as a consequence of early ethanol treatment ($F< 2.9$, $ps>0.05$).

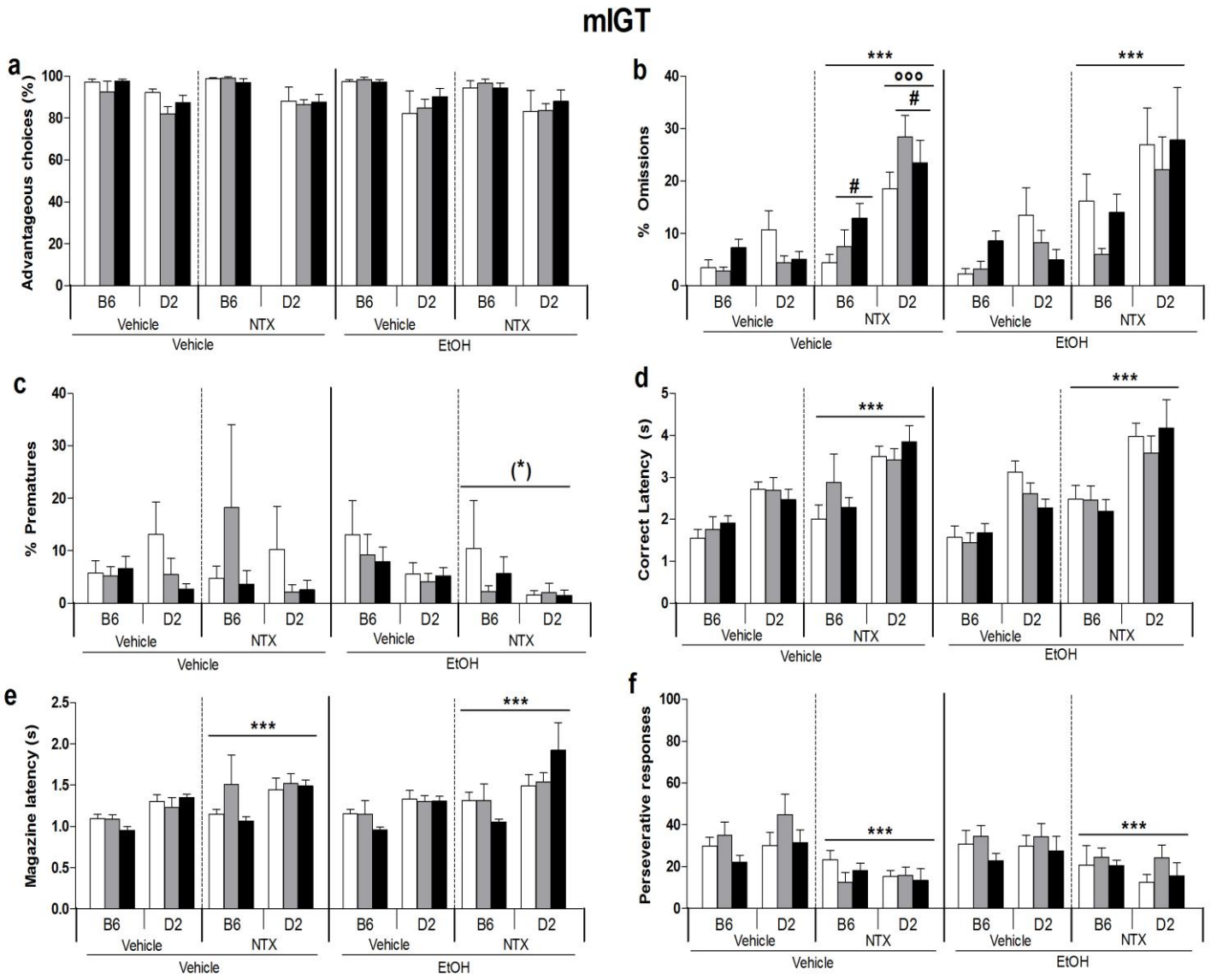

Figure 5.2 Effects of NTX (10 mg/kg; Phase 1 [Vehicle-Vehicle vs. Naltrexone-Vehicle]) and ethanol (0.5 g/kg; Phase 2 [Vehicle-Ethanol vs. Naltrexone-Ethanol]) co-administration on mIGT performance of B6 and D2 mice. A 10mg/kg dose of NTX did not affect decision-making or impulsive behaviour (a) but did decreased perseverative responding in both phases (f). NTX increased the percentage of omitted trials (b), correct latencies (d) and magazine latencies (e) in both strains. Data expressed as Mean ± SE (n=46, 7-8/group). (*) p=0.095, ***p<0.001 vs. vehicle; °°° p<0.001 vs. vehicle (D2 mice only); # p<0.05 vs. vehicle (IEE mice only)

5.5 DISCUSSION

The present study shows that antagonising μ-opioid receptors by NTX decreased two measures of top-down control behaviour [reduced incidence of premature responding (5-CSRTT) and perseverative responding (5-CSRTT, mIGT)], suggesting that NTX can be a potential candidate for treating certain inhibitory control deficits. Despite NTX decreasing waiting impulsivity, it failed to improve decision-making in the mIGT. In addition, NTX increased the number of omitted trials and latencies both to perform a response and to collect the reward (5-CSRTT, mIGT), and decreased the total number of trials completed (mIGT), suggesting the ability of this compound to generate aversive side effects, such as decrease motivation or simply cause sedation. As we have previously reported (Sanchez-Roige et al., 2014a), we found a tendency of IEE during late adolescence to increase 5-CSRTT premature responding. Additionally, the two strains differed in their impulsive phenotype: the ethanol-preferring B6 mice were more impaired on measures of waiting impulsivity (**Figure 5.1c**), while the ethanol-avoiding D2 mice showed more risky behaviour in the mIGT task (**Figure 5.2a**). In addition, D2 mice showed higher levels of perseverative responding. Although strain-dependent effects of NTX on impulsive behaviour were not detected, NTX enhanced accuracy of responding in B6 mice and was more effective in reducing perseverative responding in D2 mice. The NTX-induced motivational decreases were more acute in the D2 strain, altogether suggesting that NTX responsiveness may be genetically mediated or depend on baseline differences in behaviour between the strains.

In keeping with previous findings where the μ-opioid receptor antagonist naloxone increased inhibitory behaviour in a conflict test in 5-HT depleted rats (Soderpalm and Svensson, 1999), or attenuated the amphetamine-induced inhibitory control deficits in the 5-CSRTT in healthy rats (Wiskerke et al., 2011), NTX proved effective in diminishing 5-CSRTT impulsivity (**Figure 5.1c**). In line with our findings, work by Olmstead and colleagues (2009) has revealed that mice

lacking μ-, but not δ-opioid, receptors exhibit lower premature responding in a simpler version of the 5-CSRTT task. Taken together, these results suggest that endogenous opioids may promote impulsivity, and that NTX improves self-control by blocking endogenous μ-opioid activity. However, the mechanisms by which μ-opioid receptors regulate waiting impulsivity remain to be elucidated, as NTX may also act indirectly through its effects on other neurotransmitter systems (Wiskerke et al., 2011) or, at the high dose used, may lose μ selectivity in favour of increased antagonist potency at δ receptors [(Stromberg et al., 1998) but see (Olmstead et al., 2009)]. Using a more selective μ-opioid antagonist (e.g. GSK1521498) might help resolve this possibility (Ignar et al., 2011); on the other hand, dose-response experiments might help elucidate whether lower, receptor selective doses of NTX can still attenuate premature responding.

People with a personal or familial history of alcoholism are reported to have relatively low levels of endogenous μ-opioid agonists (Dai et al., 2005; del Arbol et al., 1995), and thus responsiveness to the opioid antagonist NTX has been hypothesised to be genetically determined (Anton, 2008; Thorsell, 2013). In ethanol-preferring and –avoiding mouse lines, differences in some functions of the endorphin system (De Waele and Gianoulakis, 1994; Jamensky and Gianoulakis, 1997) and behavioural effects of opiates and opiate antagonists also exist (Castellano and Puglisi-Allegra, 1982; Kiianmaa et al., 1983). Although these observations suggest that opioid system differences may be predictive of NTX responsiveness, in our study NTX similarly reduced 5-CSRTT premature responding in the two strains of mice, suggesting that the NTX effect on this measure may be independent of circulating levels of opioid agonists (Gianoulakis and De Waele, 1994).

On the other hand, NTX exerted greater efficacy in reducing alcohol consumption in patients with a family history of alcoholism (Krishnan-Sarin et al., 2007), or in heavy (vs. none, moderate) drinking subjects (McCaul et al., 2000). Here, NTX's ability to reduce impulsive

(premature) behaviour was similar across groups, independently of their previous alcohol history. Nevertheless, experiments conducted only a few weeks after the IEE treatment may help determine if the presumed premature protective effects by NTX in IEE mice also occur at a shorter time into (alcohol) withdrawal. Moreover, deficits in 5-CSRTT premature responses following early ethanol exposure, which we reported elsewhere (Sanchez-Roige et al., 2014a), showed only a tendency for significance during phase 2. It is possible that extensive training or previous exposure to acute alcohol in an earlier experiment may have had residual effects, thereby abolishing (or not detecting) possible differences between groups.

Replicating previous findings (Sanchez-Roige et al., 2014a), strain differences in waiting impulsivity emerged; the ethanol-preferring B6 strain showed greater impulsivity in the 5-CSRTT than the ethanol-avoiding D2 strain during the first phase of testing, but not subsequently, suggesting that B6 mice may present initial deficits in inhibiting pre-potent motor responses, but are able to diminish those responses following repeated testing (Walker et al., 2011). NTX had a similar effect in reducing waiting impulsivity in the two strains, and the reduction of premature responses in all mice was apparent both when NTX was given alone, and in co-administration with ethanol. In contrast, in the mIGT, NTX alone failed to diminish waiting impulsivity and only a tendency to reduce premature responding was detected when co-administered with ethanol (0.5g/kg). That NTX reduced premature responding on the 5-CSRTT, but not on the mIGT suggests that the NTX-induced effects on premature responding may depend on the attentional demands of the task (compared to mIGT, the 5-CSRTT has a short stimulus duration, and a single location). For instance, it is possible that NTX decreases waiting impulsivity when the demands of the task are increased, either by augmenting the attentional complexity (e.g. long-ITI in the 5-CSRTT vs. baseline conditions in the mIGT) or by challenging the inhibitory control further [e.g. an ethanol dose of 1g/kg was needed to disrupt waiting impulsivity in the mIGT (Sanchez-Roige et al., 2014a)]. Also, it is conceivable that the

low baseline levels of mIGT premature responses prevented the NTX reductions on this measure.

Alongside NTX effects on premature responding, this compound attenuated perseverative responding (5-CSRTT, mIGT), more significantly in the D2 mice (5-CSRTT). The common effect of NTX to reduce both premature and perseverative responses may reflect actions on "top-down" cortico-striatal mechanisms responsible for the impulsive-compulsive acts (Dalley et al., 2011; Fineberg et al., 2010), in agreement with human studies where NTX has proved useful for the treatment of other impulse control disorders [e.g. self-injury (Odlaug and Grant, 2010; Rapp and Vollmer, 2005), compulsive sexual behaviour (Raymond et al., 2002); for a review (Kim, 1998)].

In addition to reducing premature and perseverative responding, NTX also decreased the total number of trials completed (mIGT), and increased omitted trials and times of responding (5-CSRTT, mIGT), suggesting that this drug slowed performance. The decrease in premature (5-CSRTT) and perseverative responses in both tasks is consistent with the hypothesis of a general reduction in the vigour of responding that could have been caused by sedation or reduced mobility under NTX; future studies evaluating lower doses of this compound may help clarify the interpretations herein drawn. Furthermore, the strain-dependent increased latencies in D2 mice during 5-CSRTT performance under NTX (**Figure 5.1d-e**) is consistent with earlier studies showing greater NTX-induced depressant effects on locomotor activity in the D2 than B6 strain (Castellano and Puglisi-Allegra, 1982). It is also plausible that baseline differences in behaviour (e.g. D2 showing more perseverative responses and omissions than B6 mice) may explain the different NTX responsiveness between the strains. Increases in omission were not accompanied by accuracy deficits (indeed, an acute dose of NTX increased accuracy of responding in B6 mice; **Figure 5.1a**). Such decreases in total trials and increases in magazine latency, may reflect decreased motivation under NTX (Davidson et al., 1999; de Wit et al., 1999;

Mitchell et al. 2009), as others have suggested for food (Giuliano et al., 2012; Nathan and Bullmore, 2009; Pecina and Berridge, 2005), and drugs of abuse (Contet et al., 2004; Giuliano et al., 2013).

The dual effect of NTX in decreasing both motivation for food rewards and top-down control behaviours (premature and perseverative responding) may have therapeutic implications for a range of disorders characterised by impulsive and compulsive behaviours. These are two closely associated aspects of alcohol misuse, as excessive drinking can be evoked by increased salience of the alcohol reward or associated discrete cues [i.e. motivation, (Marinelli et al., 2009)], and result in loss of control over drinking (Perry and Carroll, 2008). Our findings bring attention to the fact that blocking μ-opioid receptors may play a role in promoting abstinence both by enhancing inhibitory control (Crews and Boettiger, 2009; Dayas et al., 2007), and by decreasing incentive motivation (Myrick et al., 2008).

NTX did not reliably reduce impulsive choice in the mIGT, similarly to previous studies where NTX failed to alleviate other facets of choice impulsivity, such as intolerance to delay gratification using delay discounting tasks in alcohol preferring mice (3 or 10 mg/kg; Oberlin et al., 2010), in rats (10 mg/kg; Kieres et al., 2004), or human alcoholics [(Mitchell et al., 2007), but see (Boettiger et al., 2009)], altogether suggesting that the effects of NTX may depend on the impulsivity type assessed (e.g. waiting vs. choice impulsivity). Intriguingly, some authors revealed an effect of acute NTX dose on impulsive choice in humans to be predicted by personality traits (Mitchell et al., 2007), and by personality traits with family history of alcoholism (Altamirano et al., 2011); however, we did not detect a differential NTX responsiveness in the mIGT between the two strains, or in mice with previous ethanol history. The possibility remains that NTX may alter choice impulsivity, but that we simply used an ineffective dose or that our assay was not sensitive to detect major changes on decision-making, as the mice were showing high baseline levels of advantageous choices and therefore

low risky behaviour (Sanchez-Roige et al., 2014a). Using different doses and impulsivity paradigms will hopefully elucidate those relationships, as it is evident that NTX attenuates a variety of impulse disorders in humans where deficits in decision-making are important (Grant et al., 2009; Kim and Grant, 2001; Marrazzi et al., 1995; Raymond et al., 2002).

5.5.1 Conclusion

Our results provide more evidence to support the notion that the μ-opioid antagonist NTX may not only prove efficacious in attenuating ethanol consumption, but may also interact with top-down processes, relevant for other addiction and impulsive/compulsive-like disorders.

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Chapter 6

GENERAL DISCUSSION

6.1 Review of general aims and summary of main findings

The aim of this thesis was three-fold. At a first stage, using a battery of trait and behavioural impulsivity measures, we intended to gain more insight into the impact of **adolescent BD on impulsive behaviour**. Previous investigations suggested impulsivity to be both a cause and a consequence of elevated drinking. The research described in *Chapter 3* extended those findings by demonstrating elevated impulsivity was observed prior to alcohol consumption in ethanol-preferring B6 mice, and as a consequence, revealing a potential causative role. That young social binge drinkers also showed high impulsivity might be consistent with those findings. More intriguingly, in *Chapter 4* we showed that mouse BD-like alcohol exposure has **long-term consequences**, and divergent roles in affecting distinct impulsivity types (early-BD onset, associated with increased risky behaviour; late-BD onset, related to impaired waiting impulsivity). The last research line of the thesis included addressing a molecular target to **alleviate impulsivity-deficits**. Previous studies in humans and rodents have suggested a modulatory role of the opioid systems in regulating impulsive control. In C*hapter 5* we confirm this association and revealed that μ-opioid system antagonism alleviated one form of impulsivity (waiting impulsivity). Targeting opioid neurotransmitter systems may present a new avenue for the treatment of high-impulsive behaviours. Below, I integrate our findings with the previous literature and suggest future directions to continue expanding our knowledge on the role of impulsivity in alcohol misuse.

BOX 6.1 SUMMARY OF KEY FINDINGS

1. *Pre-existing versus BD-induced impulsive behaviour.* As a cause, reduced inhibition might precede the appearance of BD habits (B6 mice). As a consequence, BD has a direct effect on two forms of impulsivity: augmenting waiting impulsivity in the 5-CSRTT (B6, D2 mice) and Sx-5CSRTT (young binge drinkers), and risky decision-making, as indexed in the mIGT (D2 mice) and TCIP (young binge drinkers).

2. *A critical period in brain development*. The BD-induced changes in impulsivity persist later in life (mouse model of BD): early BD-exposure impairing decision-making, and late BD-exposure affecting waiting impulsivity. Longitudinal studies are needed to study whether those deficits are also seen in human binge drinkers; fMRI studies can help expand correlational parallels between behavioural differences and brain abnormalities that may result from BD.

3. *Vulnerability markers.* We detected premature responses to be a predictor of ethanol preference in mice, and of BD habits in young humans. More studies are needed to investigate whether such habits could result in AUDs in adulthood.

4. *Early prevention and treatment.* Inhibitory control deficits could serve as potential biomarkers to characterize individuals at risk and prevent BD habits. Naltrexone could prove efficient in preventing excessive drinking, by enhancing top-down control mechanisms.

6.2 Pre-existing versus BD-induced impulsive behaviour

6.2.1 An update on BD and impulsive behaviour

Although impulsive behaviour is not necessarily disadvantageous (Paaver et al., 2006; Williams and Taylor, 2006), excessive levels can be maladaptive (Evenden, 1999). In this thesis, I studied two of the widely recognized aspects of the impulsive phenotype: *action* impulsivity, or actions that are premature or inappropriate to prepotent stimuli; and *choice* impulsivity, or actions that may result from a distorted evaluation of delayed consequences of behaviour and are drawn to immediate pleasure.

Deficient levels of impulsivity have been previously observed in young social drinkers (see **Table 1-iii**, for a summary). Multiple paradigms have been used to characterize the impulsive phenotype in young social binge drinkers, yet very few have used those tests simultaneously. To that end, we used a battery of tasks in a cross-species (mice and humans) design. The effects of BD were measured on *action* impulsivity. To capture aspects of 'waiting impulsivity', we used the 5-CSRTT in two cohorts of mice, ethanol naive (to study effects of causality) and a model of BD (to investigate the long-term impact of BD), and the novel human analogue Sx-5CRTT in young binge drinkers; the human SST for 'action cancelation'; along with self-reported measures of 'trait impulsivity', indexed using the BIS-11 questionnaire. To further delineate the interrelationship between action and choice impulsivity, the mouse mIGT and human DDQ and TCIP were used for assessing impulsive choice.

6.2.1.1 **High-impulsivity and alcohol bingeing.** In a first approach, we tried to disentangle the **origin of the impulsivity deficits**. In *Chapter 3* we found that mice from an alcohol-preferring strain (B6) show high impulsivity in the 5-CSRTT, even if they have not been previously exposed to alcohol, and individual level of 'waiting' impulsivity correlated with ethanol preference. This is in agreement with other studies showing elevated ethanol consumption in high impulsive strains [c.f. (Lejuez et al., 2010)], suggesting a biological marker for alcohol use initiation. However, our studies are only correlational and do not provide evidence for causality. It cannot be excluded, for instance, that gene variants that contribute to impulsivity, independently contribute to alcohol drinking (pleiotropy). Tests of a causal relationship need to demonstrate at least that manipulations of impulsivity have predictable consequences for drinking. The introduction of parallel tests in rodents and humans (e.g. 5-CSRTT, Sx-5CSRTT) will facilitate such studies. Moreover, whilst there is a vast amount of literature describing the effects of human BD on decision-making and

aspects of action impulsivity (**Table 1.1-iii**), 'waiting' impulsivity until now has remained relatively unexplored. To that end, in a second line of research, we investigated the impact of **BD on different impulsivity paradigms** in a systematic effort to relate BD to the multidimensional nature of the impulsivity construct.

6.2.1.2 **Human alcohol bingeing and impulsivity changes**. Similarly to other reports demonstrating high trait impulsivity in binge drinkers (**Table 1.1**, i), our population of young binge drinkers also presented deficits in BIS-11 trait (motoric, non-planning) impulsivity, in addition to showing ***impaired performance in the Sx-5CSRT task***. Hence, we show that trait disinhibition is associated with BD patterns, and we are the first group to demonstrate that alcohol bingeing is associated with a state of 'cannot wait' disinhibition. Further, although 'motor' impulsivity subscale from the BIS-11 is supposed to map motor (or 'waiting') impulsivity measured in the Sx-5CSRTT, the two forms did not correlate, supporting the suggestion that self-report and behavioural impulsivity should be grouped into separate domains (Malle and Neubauer, 1991; Reynolds et al., 2008). On the other hand, separable aspects of trait impulsivity have previously shown different patterns of association with alcohol use outcomes in adolescence (Leeman et al., 2009; Stautz and Cooper, 2013). From the sample reviewed, high trait of motor BIS-11 impulsivity was predictive of high BD scores; however, others found that measures of reward-related disinhibition (e.g. response bias for reward as measured by a GNG task) and trait sensation seeking (but not motor trait impulsivity) mediated BD (Castellanos-Ryan et al., 2011). Clearly, further investigation into these traits as potential risk factors for problematic alcohol use in adolescence is warranted. Despite BD-induced deficits in 'waiting' impulsivity, the ability to 'cancel' a response in binge drinkers remained intact, as measured in the SST [in agreement with (Moreno et al., 2012; Fernie et al., 2013) but against others who found increased response disinhibition in women with BD habits (Nederkoorn et al., 2009), revealing possible gender-specific effects].

Such findings support previous observations indicating that measures of behavioural impulsivity assessed between different laboratory tasks are not homogeneous (De Wit, 2009; Dick et al., 2010), suggesting that these impulsivity ('waiting' vs. 'cancelling') subtypes differ from one another (Meda et al., 2009; Broos et al., 2012). For example, the binge drinker's ability to withhold prepotent responses in the Sx-5CSRTT did not correlate with their ability to cancel a response in a SST task, both within the domains of (action) 'response' inhibition, but tapping into different underlying processes (withholding, cancellation, respectively). Along similar lines, the ability of the participants to withhold or cancel a response was not associated with their tolerance to delayed gratification. More evident in the mouse study (*Chapter 4*), and in previous studies (Reynolds et al., 2008; Broos et al., 2012), measures of action and choice impulsivity were not associated.

Our data revealed that ***young binge drinkers were more prone to choose immediate rewards*** as indexed in the TCIP, but did not differ from healthy subjects in tasks of delayed gratification when using monetary incentives in a questionnaire-based test (DDQ), similarly to a previous report (Fernie et al., 2013), and in agreement with previous studies observing pen-and-paper and experiential measures of choice impulsivity to be at a point of divergence [e.g. (Reynolds et al., 2006; Moallem and Ray, 2012; Melanko and Larkin, 2013)]. Instead, deficits in choice impulsivity were detected in tasks requiring a more naturalistic experience of the delays [i.e. experiencing delays in *real time* in the TCIP paradigm vs. '*imagining*' the hypothetical delay in the DD questionnaire (Odum, 2011)]. Although we did not explore other aspects of choice impulsivity in young binge drinkers, such as risky decision-making, other studies using the Iowa Gambling task have been able to show robust impairments following adolescent BD (Goudriaan et al., 2007; Xiao et al., 2009; Moreno et al., 2012; Worbe et al., 2013). Collectively, those findings suggest that young binge drinkers may be more prone to make risky choices on the basis of long-term outcome. And this is relevant, because it is possible that the pleasure for immediate gratification and the

mismatch with increased disinhibition may decrease the willpower to resist alcohol drinking (Bechara, 2005; Redish et al., 2008).

6.2.1.3 Limitations. From an intervention perspective, it is important to know which of these impulsivity facets are predictive of BD. Altogether, our findings revealed that premature responding in the Sx-5CSRTT and risky decision-making in the TCIP effectively distinguished between binge versus non-binge drinkers. However, it is difficult to extrapolate our findings to a wider context. First, although others have recently independently developed a similar human analogue of the 5-CSRTT to measure waiting impulsivity (Voon et al., 2014; Worbe et al., 2014), no other groups have investigated the role of waiting impulsivity and BD. Moreover, only few studies distinguish between different periods of adolescence [see (Witt, 1994; Spear, 2000; Andersen, 2003; Witt, 2010) for further discussion]. In those studies, the mean age of the sample is variable and different time-points in adolescence may be more problematic than others [e.g. early alcohol initiation has been shown to be predictive of later alcohol use problems (Grant and Dawson, 1997)]. Our population of binge drinkers, and that of others (**Table 1.1**), comprises late and young adulthood. Future studies should aim to include wider age ranges. Additionally, the methodology used to characterize the population of binge drinkers (i.e. impulsivity tests; alcohol drinking patterns) is not consistent across published studies, which likely introduced a degree of heterogeneity among the samples studied. More effort should be put towards a universally accepted and appropriately categorized definition of BD, as many studies rely simply on amounts consumed in a defined limited period to characterize BD, without taking into account factors such as body size, or degree of tolerance. Moreover, although we have shown that some impulsivity variables (waiting impulsivity, trait impulsivity), but not others (SSRT, DDQ) were associated with high BD scores, the interpretation of these data needs to be cautious, as correlations may have been skewed by group pre-selection (i.e. binge drinkers, non-binge drinkers). Nevertheless,

even though the correlations might appear influenced by group differences in BD scores, these were only explorative and should be valued against the primary objectives of research, that is, to compare binge drinkers versus non-binge drinkers in a variety of impulsivity measures. Consequently, the correlation between BD scores with different impulsivity measures was set as a secondary objective, for tentative exploration regarding which of the impulsivity variables was most associated with high BD scores. Additionally, the analysis failed to include partial correlations, which makes it difficult to interpret the association between the variables studied. As a result, we cannot conclude that factors acted independently of each other to account for high BD scores, but, instead, those factors may have affected one another. We also recognize that our studies employed a low sample size, and there was little control over potential confounds in outcome (e.g. age difference between the groups, possible other group differences [e.g. illicit substance use]). On the other hand, that BD failed to affect some measures of choice impulsivity (DDQ) may underlie methodological constraints (i.e. the task used being insufficiently sensitive to detect behavioural changes). Considering that other research groups have shown choice impulsivity deficits in binge drinkers as measured in the IGT (see **Table 1.1**), subsequent studies may wish to include this task in order to provide a more accurate characterization on the relative contribution of distinct forms of impulsivity in BD psychopathology. Lastly, all of the studies were conducted in an experimental laboratory setting, so the results reported may not be indicative of drinking behaviour in the natural environment. Future studies may wish to incorporate more naturalistic settings, in addition to examining the effects of acute alcohol during testing.

It is also important to recognize that impairment of inhibitory control accounts for only a small, albeit significant, proportion of the total variance in individual differences in alcohol consumption. Clearly, many other factors (see **Box 6.2** for a review of one of the possible

additional factors) contribute to the individual differences in quantity of alcohol consumed during a drinking episode (Stephens and Duka, 2008).

BOX 6.2 ATTENTIONAL DEFICITS

Impulsivity mechanisms affected by alcohol are likely to be paralleled by impairments in cognitive processes [e.g. (Hermens et al., 2013; Jacobus and Tapert, 2013)]. From our data:

BD impairs attentional capacities *in young social drinkers (Chapter 3) and in late-exposed mice (Chapter 4).*

Attentional deficits ***do not seem to be the cause*** *for elevated alcohol bingeing* (**Figure S3.4**). It is intriguing that, despite showing differences in waiting impulsivity, the two strains did not show different attentional phenotype, suggesting that whereas impulsivity deficits may predict alcohol preference, initial attentional deficits may not explain alcohol intake but appear as a result of BD (*Chapter 4*).

Moreover, whilst not investigated comprehensively here (low sample size), there may be gender difference in BD-effects on impulsivity (see Weafer and Wit (2014) for a review). Consistent with BD-induced gender-dependent differences in a number of clinical human and animal studies (Andersen, 2003; Caldwell et al., 2005; Scaife and Duka, 2009; Squeglia et al., 2011; Squeglia et al., 2012), future studies should assess both genders to have a better grasp of the deleterious effects of BD during development.

6.3 A critical period in brain development: Mouse BD-onset mediated long-term deficits on *waiting* and *choice* impulsivity

As discussed in *Chapter 1*, disentangling the long-term effects of BD in human populations is a difficult task, due to the heterogeneous nature of BD populations and the enormous costs of conducting longitudinal studies in human research. The work presented in *Chapter*

4 described an extensive profile of the long-term behavioural consequences of adolescent alcohol bingeing. On the one hand, our findings suggest that mouse adolescent-BD affects *action* and *choice* impulsivity in adulthood, utilizing the well-established 5-CSRTT and the newly developed mIGT. More importantly, we have described that the type of effect is dependent on the time of BD-exposure. Early-exposed BD mice showed increased risky behaviour in the mIGT. In contrast, late BD-exposure led to significant long-term alterations in 'waiting' impulsivity. These findings demonstrate that repeated alcohol exposure during adolescence results in persistent alterations in behaviour that last into adulthood, which may be relevant to deficits in exerting control over limiting alcohol intake. Although history of BD did not alter inhibitory-induced effects of acute alcohol on 'waiting' impulsivity during adulthood, a small acute dose of alcohol increased risky choices in mice exposed to BD during early adolescence. Collectively, our findings reveal a double dissociation of BD-effect on neurodevelopment implicated in inhibitory control and risky behaviour, manifested in specific behavioural impairments observed in these particular tasks.

6.3.1. Limitations. The interpretation of the findings reported here is not straightforward, for at least three reasons. **First,** although our method of inducing repeated episodes of withdrawal from a 2-g/kg i.p dose of ethanol in the mouse led to long-term increases in two impulsivity measures, similarly to human studies showing impulsivity deficits in young social BD (Bjork et al., 2004; Scaife and Duka, 2009), one could question whether the deficits identified are due to the particular BD model used (i.e. intense alcohol consumption episodes followed by abstinence) or to the global effects of heavy alcohol intake.

Previous studies suggest it is the number of withdrawal events, and not simply ethanol exposure, that is the key determinant for the magnitude of the deficit in top-down control mechanisms (Duka et al., 2002, 2003; Volkow et al., 2003; Duka et al., 2004; Stephens and Duka, 2008; Duka et al., 2011), suggesting that experience of successive withdrawal events

('detoxifications') may be more (or differently) damaging than the experience of the drug itself (Borlikova et al., 2006). Hence daily and intermittent ethanol treatments can have different effects on brain function (Breese et al., 2005). For instance, from previous studies conducted in this laboratory, chronic exposure of mice to high alcohol concentrations had only transient effects on 5-CSRTT impulsivity (Walker et al., 2011), which suggests that it is possible that the pattern of exposure was the cause of the long-term effects of BD on 5-CSRTT impulsivity herein reported. This observation is supported by human studies, where young binge drinkers presented greater performance deficits in tasks of motor (GNG) and choice (DDQ) impulsivity than daily drinkers (Maurage et al., 2012). Future studies might include groups with chronic exposure to ethanol (without intermittent withdrawal) to provide more support to the theory of increased brain damage after multiple withdrawals from alcohol (Veatch and Gonzalez, 1999; Crews et al., 2001).

Second, considering that testing occurred several months following withdrawal from ethanol, we can assume with confidence that our findings reveal prolonged BD-consequences on multiple mouse behaviour (action and choice impulsivity), relative to ethanol-naive mice. However, evidence for increased vulnerability to the acute effects of alcohol in adulthood remains less clear. Similarly to other reports indicating that low doses of ethanol increased behavioural disinhibition in rodents (Oliver et al., 2009) and humans [(Weafer and Fillmore, 2012), see **Table 1.1**-ii], we found that alcohol intake during adulthood primed further premature responding in the 5-CSRTT (B6 mice). Although this effect was independent of adolescent BD experience, others found increased sensitivity to the disinhibitory effects of acute alcohol in human binge drinkers in comparison to non-binge drinkers (Marczinski et al., 2007; Weafer and Fillmore, 2008; McCarthy et al., 2012), whilst other authors reported increased tolerance to the effects of acute alcohol in BD rats (Semenova, 2012) and young binge drinkers (Fillmore and Weafer, 2012). From our sample, what appears evident is that high baseline levels of impulsivity (B6 mice) are associated

with greater acute alcohol-induced deficits on behavioural control in the 5-CSRTT and risky decision-making in the mIGT. This finding supports the idea that alcohol drinking may acutely impair the individual's ability to stop the drinking event by decreasing the 'brakes' on inhibitory control mechanisms or disrupted decision-making in individuals with initial deficit (Moschak and Mitchell, 2013), thus prolonging further the drinking event (Weafer and Fillmore, 2008).

Third, the observed impulsivity differences between early and late BD-onset suggest that neural systems affected by BD during the adolescent period may vary with age. Broadly characterized, adolescent behaviour has been described as *impulsive* and *risky* (Le Moal and Simon, 1991), yet these behaviours rely on distinct neurobiological and developmental trajectories [for a review see (Casey et al., 2008)]. On the one hand, risk taking is exaggerated during adolescence [(Matthews et al., 2004; Galvan et al., 2006); for a review see (Casey et al., 2008)]. Studies measuring reward sensitivity using the IGT (Cauffman et al., 2010) reveal a U-shaped function in performance, increasing between early adolescence (14-16) and then declining (Overman et al., 2004; Steinberg et al., 2008). In sharp contrast, inhibitory control shows a linear increase from preadolescence into the decade of the 20s (Steinberg et al., 2008; Steinberg et al., 2009; Luna et al., 2010; Blakemore and Robbins, 2012; Taylor et al., 2013), mirroring the slow maturation of the PFC (Rubia et al., 2000; Tamm et al., 2002; Rubia et al., 2006; Blakemore and Robbins, 2012). A number of behavioural paradigms, together with fMRI, show that children recruit larger and more diffuse frontal regions; with age, patterns of brain activity become more fine-tuned (Brown et al., 2005; Casey et al., 2005; Durston et al., 2006; Galvan et al., 2007), which allows the transition from impulsive to more controlled behaviour. Taken together, these changes may explain why early adolescence is characterized by an increase in appetitive drive that remains without brakes (Spear, 2000; Bava et al., 2010; Best and Miller, 2010) until the self-regulatory systems mature (Ernst and Korelitz, 2009).

Perturbations occurring early in life (e.g. heavy alcohol drinking) can potentially disrupt brain development (Andersen, 2003; Carpenter-Hyland and Chandler, 2007; Crews et al., 2007; Gogtay and Thompson, 2010). Indeed, compared to adults, adolescents are more sensitive to the neurotoxic actions induced by alcohol (Monti et al., 2005; Crews et al., 2007; Ernst and Korelitz, 2009). By interfering with the ongoing brain development, alcohol could lead to damage of frontal brain regions (Crews et al., 2000; Moselhy et al., 2001; Bava and Tapert, 2010; Pascual et al., 2014), altering higher executive functions in a manner that promotes continued impulsive behaviour in adulthood (Crews and Boettiger, 2009).

In keeping with the aforementioned trajectory of development, we showed that early alcohol bingeing disrupted **choice impulsivity**. The increase in mIGT risky behaviour in IEE_Early mice may be related, at least in part, to BD alteration of neural substrates relevant for optimal decision-making in the mIGT. Although developmental studies examining neuroanatomical mechanisms in adolescent rodents are limited, frontal areas, such as ventromedial and orbitofrontal cortex [vmPFC, OFC; (Zeeb and Winstanley, 2011)], and emotional- and context-dependent structures, such as the amygdala and hippocampus (Labudda et al., 2009), respectively, play important roles in effective mIGT performance. These brain regions undergo extensive remodelling during adolescence. Based on rodent models (Laviola et al., 2003) and human imaging studies (Ernst et al., 2006), young adolescents show exaggerated responses to reward, as indexed by increased activity in limbic areas [e.g. amygdala, accumbens (Spear, 2000; Kelley et al., 2004)] paired with the 'immature' recruitment of top-down control mechanisms (Casey et al., 1997; Tamm et al., 2002; Gogtay et al., 2004; Toga et al., 2006; Galvan et al., 2007; Luna et al., 2010; Ordaz et al., 2013). Considering previous findings reporting the neurotoxic effects of alcohol in those areas (De Bellis et al., 2005; Nagel et al., 2005; Medina et al., 2007; McQueeny et al., 2009; Welch et al., 2013), it is plausible that early, but not late, adolescent BD may have disrupted neurodevelopment of those vulnerable regions. Supporting this hypothesis, recent

anatomical MRI studies found anomalies in amygdala (Xiao et al., 2013) and frontal regions (Worbe et al., 2013) in young binge drinkers, which in turn correlated with deficient IGT performance (Xiao et al., 2013) and risk-taking (Worbe et al., 2013). Additionally, and similarly to our findings, early BD-onset disrupted the development of the rat frontal cortex (OFC), which in turn correlated with decreased executive function performance (Coleman et al., 2014). Consequently, in a broader context, adolescents with substantially less frontal lobe maturation may be more susceptible to immediate environmental risks compared to those with more complete neuroanatomical maturation.

Alternatively, other regions and systems may have also been targeted. For instance, the ventral striatum [a key structure implicated in motivational and reward processes, which undergoes extensive remodelling between adolescence and young adulthood (Sowell et al., 1999)] has been detected to be enlarged in young binge drinkers (Howell et al., 2013) and in early-adolescent B6 mice (Coleman et al., 2014). In parallel, it is also possible to explain the increased risk-taking behaviour in IEE_Early mice in terms of greater neurogenesis inhibitions in hippocampal regions. Based on the implication of this structure in the mIGT (Labudda et al., 2009), and its vulnerability to BD-patterns (Medina et al., 2007; Howell et al., 2013), it is interesting to speculate that early BD-onset may have more deleterious effects on hippocampal neurogenesis, as neuron formation is greater during that period (Crews et al., 2000; Tarter et al., 2004). Additional prospective longitudinal studies should bridge the period between early and late adolescent groups to differentiate some of these possibilities, and explore other BD-induced deficits, such as impairments in brain neurotransmission (Smith and Weiss, 1999; Maldonado-Devincci et al., 2010).

On the other hand, exaggerated **5-CSRTT 'waiting' impulsivity** was a consequence of late, but not early, adolescent alcohol "binge" exposure. In humans, the frontal lobes are key regions for inhibitory control [e.g. (Jentsch and Taylor, 1999)] and optimal 5-CSRTT

performance (Christakou et al., 2001; Robbins, 2002; Chudasama et al., 2003; Christakou et al., 2004; Dalley et al., 2004). However, those structures do not reach maturity until the person is in their 20s [(Giedd et al., 1999) but see (Li et al., 2006; Rubia et al., 2013; White et al., 2014) for how gender and genetic interactions may also influence fronto-striatal mediated top-down control]. Hence, it is not surprising if frontal regions are among those at risk to disruption by BD during late adolescence, suggesting that some plasticity can occur during later stages of development (Semenova 2012) to compensate the effects of ethanol during early but not late adolescence phase (Toga, Thompson, & Sowell, 2006). Supporting this idea, alcohol exposure resulted in frontal neurodegeneration in rodents (Crews et al., 2000) and in human binge drinkers (De Bellis et al., 2005; Medina et al., 2008). In turn, dysfunction of frontal areas have been associated with the number of drinks per bingeing event, and with the loss of self-control and goal setting in tasks of executive functioning in humans (Duka et al., 2003; Weissenborn and Duka, 2003), which may also underlie the inhibitory control deficits herein observed. Collectively, these results highlight that even mild forms of alcohol misuse (i.e. BD) may alter neurodevelopmental trajectories for effective impulse control (Chambers et al., 2003), and should reinforce the importance of early intervention (i.e. identification of biological markers, such as risky choice or waiting impulsivity, of subgroups at greater risk, that is, younger/older adolescents, respectively).

Nevertheless, the aforementioned hypotheses do not come without reservations, primarily due to the limited information available regarding: **a)** mouse brain development; **b)** effects of BD on brain mechanisms, as the majority of studies have used patterns of chronic alcohol exposure (without intermittent withdrawal) or used different BD patterns from our IEE model, making any comparisons between our study and others difficult; **c)** although the neural network for optimal 5-CSRTT performance has been described in rats (Dalley et al., 2011), the mouse mIGT is a novel paradigm, for which we possess limited evidence of the neural network that may regulate its performance. Moreover, **d)** the findings from our

mouse studies (*Chapter 4*) suggest that a BD pattern of alcohol intake continued over a long period of time during adolescence may lead to chronic changes in impulsive behaviour. Although it is possible that the same effects could be observed in human subjects, longitudinal studies are needed to directly test this hypothesis. These issues represent a major shortcoming for the tentative hypothesis herein drawn (i.e. explain the possible behavioural differences detected according to the time of IEE exposure considering the distinct neurodevelopmental trajectories of choice and waiting impulsivity), but those open new venues for research, which I consider in the following paragraphs.

As I have previously argued, research on anatomical differences associated with human BD is comparatively limited, and developmental studies with fMRI data on human Sx-5CSRTT performance are non-existent. As such, even though the neural network that regulates 5-CSRTT performance has been described in rodents (Dalley et al., 2011), there are no studies assessing neuroimaging correlates in humans. With the development of appropriate task analogues (e.g. 5-CSRTT and Sx-5CSRTT), it will be important for future human studies to: (i) examine neurobiological correlates of task performance that have been detected in animals to more directly compare findings across species; (ii) study the nature of age-dependent neurodevelopmental trajectories on the human Sx-5CSTT (i.e. whether performance improves during late vs. early adolescence); and (iii) elucidate the influence of BD during different time-points of the adolescent period on these networks (i.e. whether late BD-onset also causes greater Sx-5CSRTT performance disruption; whether early BD-onset impairs IGT performance).

Even more challenging is the study of mouse neurodevelopment, and the difficulty of identifying homologues of human brain structures in the mouse brain. For instance, neuroimaging studies in humans pointed to the ventromedial prefrontal cortex (and related structures) as having a key role in decision-making using the IGT. However, it is notoriously

difficult to find the rodent homologue of this structure (Preuss, 1995; Ongur and Price, 2000). Furthermore, although essential brain structures that regulate IGT performance in humans have been described, the mouse IGT is a novel paradigm, for which only few studies have explored the neural substrates for its performance (Zeeb and Winstanley, 2011, 2013). Additionally, it is also possible that the mouse and human IGT may not be exact task analogues, as mice are extensively trained at the point of assessment, hence reducing the ambiguity aspect that is a part of the human IGT.

Further, the age range to be determined as "adolescence" is not consistent, not only in humans but also in animal studies (Spear, 2000), with disagreement over how best to define the period of adolescence (Dahl, 2004; Roenneberg et al., 2004). Even more challenging when comparing animal and human research, it may be impossible to measure the current aspects of impulsivity in juvenile animals, considering that behavioural tests take several weeks to train and adolescence of rodents lasts only from PND30-PND60. This constraint emphasises the necessity to include larger age ranges and wider battery of tasks to address the complex mechanisms of development (Best and Miller, 2010).

Although previous studies have shown that 2g/kg of ethanol results in high BEC levels in adult B6 mice (Dixon et al., 2012), assessment of BEC levels were not examined in the current studies. Hence, we cannot exclude that age-dependent increases in impulsivity are not a consequence of differences between early and late adolescent mice in BEC levels attained. Future studies should further examine potential group differences that may result from repeated doses of 2-g/kg of alcohol (e.g. differences in ethanol pharmacokinetics, by measuring BEC levels at different time intervals during the IEE protocol) in order to examine the level of intoxication attained, in addition to examining additional effects that may result from our IEE model (e.g. ataxic and sedative effects of ethanol, using a Rotarod apparatus or wire hang tests, and the duration of loss of righting reflex (Dixon et al., 2012).

Additionally, future studies covering the whole adolescent period (PND35-PND60) to allow potential interactions between early- and late-adolescent exposure, may provide additional insights of the effects of IEE on impulsive behaviour.

In sum, we are only beginning to understand the neurobiological mechanisms underlying impulsivity and the impact of BD on those structures. Future assessments of the neural mechanisms underlying impulsive behaviour and BD across the species will likely provide exciting new information and further our understanding of the double dissociation of BD-effects on impulsive behaviour depending the time of exposure herein reported. A next step will be to test this approach in humans: without longitudinal follow-up, it is unclear **a)** how human adolescent BD habits affect impulsivity in adulthood, **b)** how those behavioural deficits induced by BD map onto the trajectory of the human and mouse brain development and, **c)** more importantly, what portion of at risk groups (e.g. high impulsivity) leads to the development of alcohol misuse in adulthood (but see Whelan et al., 2014, for initial longitudinal data from the IMAGEN project). Future investigations using both experimental animals and humans will be required to fully understand the role of inhibitory control impairments in BD and subsequent alcohol addiction (Morein-Zamir and Robbins, 2014).

6.4 Implications for human adolescent alcohol use and emergence of alcohol-use disorders

The work presented here, in addition to the existing literature (**Table 1.1**-iii), suggests that adolescent BD may have important long-term consequences on behaviour. Our ability to reduce or prevent underage alcohol drinking is limited and offering new venues of treatment is an important priority.

From the animal data we have shown that genetic predispositions that led to increased waiting impulsivity in the 5-CSRTT, also showed increased ethanol preference in adulthood. Alarmingly, heightened waiting impulsivity was also exacerbated by mouse adolescent alcohol bingeing. Thus, identifying useful biomarkers such as impulsivity (i.e. high premature responding) in at-risk individuals could be important to minimize, delay or **prevent** onset of alcohol use in adolescence by promoting enhanced inhibitory control over drinking behaviour (Conrod et al., 2008; Conrod et al., 2011), and reduce the adverse impact of BD on the developing brain (Castellanos-Ryan et al., 2013).

6.4.1. Limitations. From a clinical perspective, therapies aiming to restore the inhibitory control could also be effective for the **treatment** of disorders where impulsivity is a core symptom (Stevens et al., 2014). Findings from *Chapter 5* revealed promising benefits of NTX, a compound that acts through μ-opioid antagonism, as a pharmacological therapy to alleviate waiting impulsivity. However, additional work is still needed. Although the experiments discussed in Chapter 5 suggest that blocking μ-opioid receptors by NTX may reduce motor impulsivity in the 5-CSRTT, and this is consistent with previous observations (e.g. Olmstead et al., 2009), the mechanisms by which μ-opioid receptors regulate waiting impulsivity remain to be elucidated, as NTX, at the high dose used, may lose μ selectivity in favour of increased antagonist potency at δ receptors [(Stromberg et al. 1998) but see (Olmstead et al., 2009)]. Indeed, although NTX is more potent and selective for μ-opioid receptors, it also shows affinity for different opioid receptors [(Raynor et al., 1994); e.g. 25-fold selective at rat μ- vs. δ-, κ-opioid receptors (Ignar et al., 2011)]. Using a more selective μ-opioid antagonist (e.g. GSK1521498), or using lower doses of NTX and/or comparing the time course and dose response of receptor occupancy with levels of inhibitory control, might help resolve this possibility (Ignar et al., 2011). Additionally, NTX may also act indirectly through its effects on other neurotransmitter systems (Wiskerke et al., 2011),

such as inhibiting NAc dopamine signalling (Yoshida et al., 1999), either via local GABAergic mechanisms (van Dongen et al., 2005; Aono et al., 2008), or indirect GABAergic mechanisms (e.g. via blockade of μ-opioid receptors on VTA GABAergic interneurons, thereby increasing GABAergic and decreasing mesolimbic dopaminergic transmission), or via effects on GABAeric output neurons (Svingos et al., 1997). Furthermore, it should also be noted that NTX reduced responding for food in rodents (Giuliano et al., 2012), consistent with previous observations in humans [(for review see Yeomans and Gray, 1997; Berner et al., 2011) but see (Kamdar et al., 2007)] which could be a confounding factor of the apparent increases in inhibitory control detected in this task. Although these results may be interesting in providing evidence that opioid mechanisms generally underlie incentive motivation, which may have therapeutic potential in the treatment of a wide range of addictive behaviours, it may also reveal non-specific effects (e.g. sedation, nausea) resulting from NTX (de Wit et al., 1999). Using smaller doses of NTX may help disentangle the possible relationship between inhibitory control improvements by NTX and additional non-specific effects, such as nausea or sedation. Moreover, future studies should examine the effects of NTX in other aspects of impulsivity, such as GNG inhibition, which is an important aspect of action impulsivity that we have not studied. Further, it will be important to know whether the NTX-induced effects on impulsivity measures are also seen in human binge drinkers.

6.5 Future directions

The collection of studies tell us a suggestive story, but there is still more effort that needs to be addressed in future investigations. Clearly, progress has been made in linking the behavioural effects of adolescent-BD to underlying neural mechanisms (Witt, 2010), but we **need to create a broader interdisciplinary approach**. As such, we must approach this problem at multiple levels of analysis, including genetic, cellular and molecular systems, neuroimaging and behavioural, with an emphasis on integrating the various levels of analysis. Those should include more developmental studies of inhibitory control in adolescents (Schumann el al., 2010); clinical research should focus on the areas which may be affected by BD. Advances using animal models will further our understanding on the neural aspects of the behavioural disruption herein reported, by controlling for dose and pattern of exposure. For that, we need stronger translational links between animal and human research, as it is crucial to understand when and which effects result from BD. This knowledge could be used to define better intervention in high-risk youth at a time when some of these systems are more plastic to change.

A better understanding of the **pre-existing impulsivity differences** in youth prior to initiation of alcohol drinking is needed. This issue has only been partially addressed and still needs to be confirmed and extended. We have shown a correlation between heavy drinking and impulsivity across two mouse strains (not proof of causality), and that human binge drinkers show heightened impulsivity (direction of causality not known). Longitudinal studies should explore how different aspects of impulsivity (i.e. early detection of elevated premature responding) may predict aspects of alcohol use [e.g. age of onset of drinking, frequency of binges (Wiers et al., 2010)]. As such, prevention programs could be developed to help enhance impulsive control and decrease the likelihood of alcohol use.

Altogether, this thesis has opened new venues for research (see **Box 6.3**), which hopefully will expand our understanding on exaggerated impulsivity and alcohol misuse.

BOX 6.3 MANY CRITICAL QUESTIONS REMAIN

i. Comorbidity with other psychiatric disorders and the potential of other substances to contribute to the observed impairments (Hermens et al., 2013).
ii. To what extent different forms of impulsive behaviour may be important during different stages of the alcohol-addiction cycle, i.e. whether exaggerated impulsivity may shift towards a more compulsive pattern of behaviour (Belin et al., 2008; Everitt et al., 2008; Dalley et al., 2011).
iii. Extending the role of attention in alcohol use disorders, and its relation with impulsive behaviour.
iv. While a wealth of literature demonstrates the deleterious effects of alcohol on the adult brain [for review, see (Kril and Halliday, 1999)], the adolescent brain is still actively developing, making it difficult to draw conclusions about adolescents based on findings from adults. It is essential to understand the neuromaturational deficits that may underlie the double dissociation of early/late BD-effects on choice and action impulsivity, which may provide additional information about their contributions to maladaptive alcohol use.

6.6 Concluding remarks

Collectively, the results presented in this thesis highlight that even mild forms of alcohol misuse (i.e. BD) may alter neurodevelopmental trajectories for effective impulse control, and should reinforce the importance of early intervention (i.e. identification of biological markers, such as risky decision-making or 'waiting' impulsivity, in subgroups at greater risk, that is, younger/older adolescents, respectively). Antagonising the opioid system can enhance the inhibitory control, which may prove a good addition to the existing interventions for BD and other disorders where impulsivity is a core deficit. Delineating homologous measures of the impulsive phenotype between species, and BD definitions across laboratories, will contribute to a more accurate applicability of the findings, facilitating a common theoretical background relevant for alcohol misuse and impulsivity related disorders (Stephens et al., 2013).

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