DISSERTATION

Investigation of neuronal structures and networks on the modulation of decision-making and impulse control by temporary inactivation via local microinfusion of the $GABA_A$ receptor agonist muscimol in rats

Malte Feja

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1. Gutachter: Prof. Dr. Michael Koch

2. Gutachter: Prof. Dr. Ursula Dicke

Erklärung

Die Untersuchungen, auf denen die hier vorliegende Dissertation beruht, habe ich selbständig durchgeführt und ausgewertet. Lediglich einige praktische Teile der Studien wurden von Master-Studenten unter meiner Supervision im Rahmen meines Lehrauftrags in der Arbeitsgruppe ausgeführt. Die enthaltenen Manuskripte habe ich eigenständig verfasst und lediglich die endgültige Fassung mit meinem Betreuer und Mitautor, Herrn Prof. Dr. Michael Koch, überarbeitet. Es wurden keine anderen als die angegebenen Quellen und Hilfsmittel benutzt. Wörtlich und inhaltlich entnommene Stellen aus den angegebenen Quellen sind gekennzeichnet.

(Malte Feja)

Bremen, den 05. August 2014

List of publications

(■) indicates publications included in this thesis. Articles have been published or submitted to international neuroscientific journals.

Articles

- ■ Hadamitzky M, **Feja M**, Becker T, Koch M (2009) Effects of acute systemic administration of serotonin$_{2A/C}$ receptor ligands in a delay-based decision-making task in rats. Behav Pharmacol 20: 415-423.
- ■ **Feja M**, Koch M (2014) Ventral medial prefrontal cortex inactivation impairs impulse control but does not affect delay-discounting in rats. Behav Brain Res 264: 230-239.
- ■ **Feja M**, Hayn L, Koch M (2014) Nucleus accumbens core and shell inactivation differentially affects impulsive behaviours in rats. Prog Neuropsychopharmacol Biol Psychiatry 54C: 31-42.
- ■ **Feja M**, Koch M (2014) Frontostriatal systems comprising connections between ventral medial prefrontal cortex and nucleus accumbens subregions differentially regulate impulse control in rats. Psychopharmacology (Berl) (*under revision*).

Poster presentations

- ■ 8th Federation of European Neuroscience Societies Forum, Barcelona, Spain: **Feja M**, Hayn L, Koch M (2012) Nucleus accumbens core versus shell: decision-making and impulse control.
- ■ 15th European Behavioural Pharmacology Society Biennial Meeting, La Rochelle, France: **Feja M**, Koch M (2013) The NAc-mPFC connection and its relevance to impulse control.

Abbreviations

5-CSRTT	5-choice serial reaction time task
5-HAT	serotonin
AC	anterior cingulate cortex
ADHD	attention-deficit/hyperactivity disorder
ANOVA	analysis of variance
AP-5	D-(-)-2-Amino-5-phosphonopentanoic acid
BES	binge eating disorder
BLA	basolateral amygdala
BODIPY TMR-X	(6-((4,4-Difluoro-1,3-Dimethyl-5-(4-Methoxyphenyl)-4-Bora-3a,4a-Diaza-s-Indacene-2-Propionyl)amino)hexanoic acid
CPT	continuous performance test of attention
CREB	calcium and cAMP response element-binding protein
CRF	continuous reinforcement
DA	dopamine
DLPFC	dorsolateral prefrontal cortex
DOI	(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropan hydrochloride
DRL	differential reinforcement of low rates of responding
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
FC	forced choice
FCM	fluorophore-conjugated muscimol
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
HR	high reward
ICD-10	International Classification of Diseases, 10th Revision
IL	infralimbic cortex
ITI	intertrial interval
LC	locus coeruleus
LED	light-emitting diode
LH	limited hold period
LR	low reward
mPFC	medial prefrontal cortex
mRNA	messenger ribonucleic acid
MSN	medium-sized, spiny neurons
NA	noradrenaline
NAc	nucleus accumbens
NMDA	N-methyl-D-aspartic acid
OFC	orbitofrontal cortex
PBS	phosphate-buffered saline
PFC	prefrontal cortex
PIT	Pavlovian-instrumental transfer
PL	prelimbic cortex

SD	stimulus duration
SEM	standard error of the mean
SSRT	stop-signal reaction time
vmPFC	ventral medial prefrontal cortex
VTA	ventral tegmental area
WHO	World Health Organization

Contents

1 Zusammenfassung

Kognitiv-exekutive Funktionen, wie Entscheidungsfindung und Impulskontrolle, stellen essentielle Aspekte des täglichen Lebens bei Menschen und Ratten dar. Ein Ungleichgewicht zwischen Verhaltensaktivierung und dessen Inhibition ruft Impulsivität hervor, die durch rasche Entscheidungsfindung und defizitäre Impulskontrolle charakterisiert ist. Hochgradige Impulsivität ist in zahlreichen neuropsychiatrischen Erkrankungen mit frontostriatalen Dysfunktionen, einschließlich Aufmerksamkeitsdefizit-/Hyperaktivitätsstörung (ADHS), antisozialer Persönlichkeitsstörung, Borderline-Persönlichkeitsstörung, Schizophrenie, Drogenmissbrauch und anderer Suchtformen, prävalent.

Impulsives Verhalten gilt als ein multifaktorielles Konstrukt, das in Abhängigkeit von den partizipierenden neuroanatomischen Strukturen und der je nach speziellem Verhaltenstest abverlangten spezifischen Art von Impulsivität moduliert wird. Die Verhaltensparadigmen zur Bewertung von Impulsivität lassen sich weitgehend in zwei Kategorien einteilen: 1. die Messung impulsiver Wahl oder impulsiver Entscheidungsfindung, 2. die Messung impulsiver Aktion oder von Impulskontrolldefiziten. Es wird behauptet, dass jede Form von Impulsivität eine impulsive Aktion in der Art beinhaltet, die notwendig ist, um eine Reaktionsalternative auszuwählen. Der konzeptionelle Unterschied liegt darin, dass es, im Gegensatz zur impulsiven Handlung, bei impulsiver Wahl keine allgemein vorherrschende Reaktion gibt, die dann gewaltsam inhibiert wird.

Eine der meistgenutzten Methoden zur Messung von Impulskontrolldefiziten ist die *5-choice serial reaction time task* (5-CSRTT), in der Ratten impulsive Reaktionen in Erwartung eines visuellen, belohnungsankündigenden Stimulus, der randomisiert in einem von fünf Zielorten präsentiert wird, zurückhalten müssen. Die Anzahl der Reaktionen vor Onset des Lichtreizes wird generell als Maß für die Impulskontrolle angesehen, da hohe Level verfrühter Antworten Verhaltensdisinhibition reflektieren. Die meisten Entscheidungsfindungsprozeduren verwenden Diskontierungsprozesse, wobei die Individuen mit zwei Optionen konfrontiert werden, die in Kosten und Nutzen differieren. Da impulsive Probanden eine Intoleranz gegenüber Belohnungsverzögerung aufweisen, reflektiert die zeitliche Diskontierung, die durch die Präferenz für eine kleine sofortige über eine größere verzögerte Belohnung indiziert wird, am ehesten impulsives Verhalten. Bei Tieren wird die Verzögerungsdiskontierung üblicherweise in operanten Boxen mit zurückziehbaren Hebeln oder in verzögerungsbasierten Entscheidungsfindungsaufgaben in T-Labyrinthen evaluiert.

Es wird angenommen, dass frontostriatale Systeme, die den präfrontalen Kortex (PFC) und das Striatum umfassen, welches weiter in dorsale und ventrale Abschnitte mit dem Nucleus accumbens (NAc) als Bestandteil des ventralen Striatums untergliedert werden kann, eine Schlüsselrolle bei der Impulskontrolle und impulsiven Entscheidungsfindung spielen. Allerdings herrschen kontroverse Theorien hinsichtlich der Beteiligung dieser Strukturen an distinkten Formen von Impulsivität. Unter den PFC-Regionen ist möglicherweise der ventrale mediale PFC (vmPFC) am meisten in impulsives Verhalten involviert. Bezüglich des NAc suggerieren die unterschiedlichen Konnektivitätsprofile dessen Kern- („core“) und Schalenregion („shell“), besonders im Hinblick auf die topographischen Projektionen vom mPFC, einen differentiellen Einfluss der NAc-Subregionen auf impulsive Verhaltensweisen.

Die vorliegende Arbeit zielte darauf ab, die Beteiligung des vmPFC auf frontaler und des NAc auf striataler Ebene an der Modulation von Entscheidungsfindung und Impulskontrolle bei Ratten anhand der reversiblen Inaktivierungstechnik mittels Mikroinfusion des $GABA_A$-Rezeptoragonisten Muscimol zu erläutern. Angesichts der Tatsache, dass die NAc-Subregionen funktionelle Dichotomie hinsichtlich zahlreicher Verhaltensweisen zeigen, intendierte die Arbeit, eine potentiell heterogene Rolle von NAc core und shell bei impulsiver Wahl und impulsiver Aktion aufzuklären. Zudem diente die simultane temporäre Inaktivierung von vmPFC und NAc core oder shell dazu, die Verwicklung der verschiedenen frontostriatalen Verbindungen in inhibitorische Kontrolle zu analysieren.

1.1 Studie 1 (in *Behavioural Brain Research*, 2014)

„*Ventral medial prefrontal cortex inactivation impairs impulse control but does not affect delay-discounting in rats*” betrachtet die Relevanz des ventralen medialen präfrontalen Kortex (vmPFC) für verschiedene Formen von Impulsivität. Als Teil des PFC scheint der vmPFC entscheidend in die Top-down-Kontrolle von impulsiver Entscheidungsfindung und motorischer Impulsivität involviert zu sein. Anhand von bilateraler Mikroinfusion des γ-Aminobuttersäure $(GABA)_A$-Rezeptoragonisten Muscimol (0,05, 0,5 µg/0,3 µl) wurden die Effekte der reversiblen Inaktivierung des vmPFC in der *5-choice serial reaction time task* (5-CSRTT) und in einem Verzögerungsdiskontierungsparadigma in einer Skinner-Box untersucht.

Die intra-vmPFC-Applikation von niedrig dosiertem Muscimol erzeugte erhöhte Level motorischer Impulsivität, was sich durch vermehrtes verfrühtes Antworten in der 5-CSRTT äußerte. Infolge der hoch dosierten Muscimol-Injektion war die 5-CSRTT-Performanz sowohl durch Defizite der Impuls- als auch der Aufmerksamkeitskontrolle gekennzeichnet. Im Gegensatz dazu induzierte die temporäre Inaktivierung des vmPFC keine impulsiv-ähnlichen Verhaltensweisen in der verzögerungsbasierten Entscheidungsfindungsaufgabe. Trotz einer Erhöhung der Auslassungsrate beeinflusste hoch dosiertes Muscimol nicht die Verzögerungsdiskontierung, während niedrig dosiertes Muscimol eine Abflachung der verzögerungsabhängigen Verschiebung in der Präferenz für die große gegenüber der kleinen sofortigen Belohnung verursachte.

Schlussfolgernd stützen diese Ergebnisse die Verhaltensdissoziation von impulsiver Wahl und impulsiver Aktion auf der Ebene des vmPFC bei Ratten. Demzufolge ist ein intakter vmPFC offensichtlich essentiell für die Aufrechterhaltung der Impulskontrolle in der 5-CSRTT, wogegen Verzögerungsdiskontierungsprozesse scheinbar durch andere neuronale Pfade reguliert werden, wobei der vmPFC, wenn überhaupt, eine untergeordnete Rolle spielt.

1.2 Studie 2 (in *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 2014)

In „*Nucleus accumbens core and shell inactivation differentially affects impulsive behaviours in rats*" wurde die Beteiligung der Nucleus accumbens (NAc)-Subregionen core und shell an Aspekten der Impulskontrolle in der *5-choice serial reaction time task* (5-CSRTT) und verzögerungsbasierter Entscheidungsfindung im T-Labyrinth bei Ratten analysiert. Zu diesem Zweck erfolgte die vorübergehende Inaktivierung von NAc core und shell via bilateraler Mikroinfusion des γ-Aminobuttersäure $(GABA)_A$-Rezeptoragonisten Muscimol (0,05 µg/0,3 µl). Zusätzlich wurde Fluorophor-konjugiertes Muscimol (FCM) in äquimolarer Konzentration (0,27 µg/0,3 µl) injiziert, um die räumliche Verteilung von Muscimol in beiden Arealen zu evaluieren.

Die Inaktivierung des NAc shell führte zu einer signifikanten Verringerung der Impulskontrolle in der 5-CSRTT, wogegen die Muscimol-Injektion in den NAc core starke Beeinträchtigungen der generellen Leistung in dem Paradigma nach sich zog. Die temporäre Deaktivierung sowohl von NAc shell als auch von NAc core löste impulsives Wahlverhalten in der verzögerungsbasierten Entscheidungsfindungsaufgabe im T-Labyrinth aus, wobei die

intra-NAc core-Injektion von Muscimol größere Defizite in der Wartekapazität der Ratten verursachte als die Mikroinfusion in den NAc shell. Das Ausmaß der FCM-Diffusion war auf die jeweilige Subregion beschränkt. Die FCM-Behandlung zeigte jedoch keinen Effekt auf die Verhaltensparameter.

Somit scheinen beide Regionen des NAc an dem neuronalen Netzwerk zu partizipieren, welches für die Vermittlung von Impulsivität verantwortlich ist, allerdings mit variierenden Einflüssen hinsichtlich unterschiedlicher Formen von impulsivem Verhalten. Diese Studie deutet darauf hin, dass die shell-Region durch die Regulation sowohl von impulsiver Aktion als auch impulsiver Wahl eine besondere Rolle bei der Verhaltenskontrolle zu spielen vermag. Demgegenüber scheint der NAc core einen zusätzlichen Einfluss auf die lokomotorische Aktivität und motivationale Aspekte auszuüben und offenbart bezüglich der Impulskontrolle eine funktionale Dichotomie im Vergleich zu dem shell-Areal.

1.3 Studie 3 (eingereicht bei *Psychopharmacology*)

„*Frontostriatal systems comprising connections between ventral medial prefrontal cortex and nucleus accumbens subregions differentially regulate impulse control in rats*" beabsichtigt, das neuronale Netzwerk, welches der inhibitorischen Reaktionskontrolle unterliegt, präziser zu erläutern. Beteiligte Hirnstrukturen bilden parallele, funktionell getrennte, jedoch partiell überlappende frontostriatale Schaltkreise, einschließlich des ventralen medialen präfrontalen Kortex (vmPFC) und des Nucleus accumbens (NAc).

In dieser Studie diente ein Diskonnektionsansatz in Form von reversibler Inaktivierung des vmPFC und NAc core oder shell bei Ratten durch simultane kontralaterale Mikroinfusionen des γ-Aminobuttersäure $(GABA)_A$-Rezeptoragonisten Muscimol (0,05 µg/0,3 µl) zur Untersuchung der funktionalen Beziehung und potentiellen Abgrenzung der unterschiedlichen Verbindungen des vmPFC mit den NAc-Subregionen hinsichtlich der Impulskontrolle in der *5-choice serial reaction time task* (5-CSRTT).

Die Diskonnektion von vmPFC und NAc shell führte zu spezifischen Beeinträchtigungen der inhibitorischen Kontrolle, die sich durch vermehrte antizipatorische Reaktionen und Antworten innerhalb der Time-out-Phase äußerten. Dagegen bewirkte die simultane kontralaterale Inaktivierung von vmPFC und NAc core keine Veränderung der Impulskontrolle, sondern resultierte lediglich in einer geringfügigen Erhöhung der

Auslassungsrate und der Futteraufnahmelatenz, was auf Aufmerksamkeits- und Motivationsdefizite schließen lässt.

Zusammengefasst weisen die Resultate auf eine funktionale Spezialisierung frontostriataler Systeme mit einer prädominanten Rolle der Verbindung von vmPFC und NAc shell bei der Vermittlung von Impulskontrolle in der 5-CSRTT hin, während der vmPFC-NAc core-Pfad einen größeren Einfluss auf Aufmerksamkeitsprozesse und motivationale Aspekte zu haben scheint.

1.4 Fazit

Insgesamt betrachtet bekräftigen die gegenwärtigen Befunde die Hypothese, dass impulsives Verhalten nicht nur von der Top-down-Kontrolle kortikaler Strukturen abhängt, sondern auch auf subkortikaler Ebene reguliert wird. Die erzielten Ergebnisse deuten auf distinkte Impulsivitätsprozesse im vmPFC und NAc hin, wobei die Impulskontrolle in der 5-CSRTT durch beide Strukturen reguliert wird, während impulsive Entscheidungsfindung vorwiegend einer Modulation seitens des NAc, und nicht des vmPFC, unterliegt. Des Weiteren suggerieren die aktuellen Untersuchungen in Bezug auf impulsive Aktion sowohl funktionelle Dissoziationen als auch enge Interaktionen zwischen vmPFC und NAc in Abhängigkeit von der involvierten accumbalen Subregion. Eine wichtige Erkenntnis der durchgeführten Studien besteht darin, dass der NAc shell die entscheidende Struktur bei der Vermittlung beider Impulsivitätsformen darstellt, wogegen der NAc core über impulsives Wahlverhalten hinaus an unspezifischen Verhaltensbeeinträchtigungen beteiligt zu sein scheint. Folglich deutet die vorliegende Arbeit auf mehrere frontostriatale Systeme hin, die auf differentielle Weise an verzögerungsbasierter Entscheidungsfindung und insbesondere an der Impulskontrolle mitwirken.

2 Abstract

Cognitive-executive functions, such as decision-making and impulse control, are essential aspects of daily life in humans and rats. An imbalance of behavioural activation and its inhibition induces impulsivity, characterised by rash decision-making and deficient impulse control. High levels of impulsivity are prevalent in numerous neuropsychiatric disorders underlain by frontostriatal dysfunctions, involving attention-deficit/hyperactivity disorder (ADHD), antisocial personality disorder, borderline personality disorder, schizophrenia, drug abuse and other forms of addiction. Impulsive behaviour is considered as a multifactorial construct that is modulated in dependence on the participating neuroanatomical structures and on the specific type of impulsivity which is demanded in a particular behavioural task. Behavioural paradigms for assessing impulsivity can be broadly divided into two categories: 1. measuring impulsive choice or impulsive decision-making, 2. measuring impulsive action or deficits of impulse control. It is assumed that each type of impulsivity involves a kind of impulsive action, which is necessary to choose a response alternative. The main difference is that impulsive choice requires no forcible inhibition of a prepotent response compared to impulse control.

One of the most commonly used methods for measuring impulse control deficits is the 5-choice serial reaction time task (5-CSRTT), where rats are required to withhold from impulsive responding to a visual, reward-predicting cue, which is randomly presented in one of five apertures. The number of responses before the onset of the light stimulus is generally regarded as an index of impulse control, as high levels of premature responses reflect behavioural disinhibition. Most decision-making procedures utilise discounting processes and confront the individuals with two options differing in cost and benefit. Since impulsive subjects are intolerant to delay of gratification, temporal discounting indexed by the preference for a small immediate over a larger delayed reward is deemed to mostly reflect impulsive behaviour. In animals, delay discounting is typically evaluated in lever-equipped operant chamber versions or in delay-based decision-making T-maze tasks.

Frontostriatal systems comprising the prefrontal cortex (PFC) and the striatum, which can be further divided into dorsal and ventral parts with the ventral striatum encompassing the nucleus accumbens (NAc), are considered to play a key role in impulse control and impulsive decision-making. However, controversial assumptions exist regarding the contribution of these structures to distinct forms of impulsivity. Among PFC regions, the ventral medial PFC

(vmPFC) might be most critically involved in impulsive behaviour. Regarding the NAc, the distinct connectivity profiles of its subregions core and shell, particularly concerning the topographical projections from the mPFC, suggests a differential influence of the NAc subregions on impulsive behaviours.

Using the reversible inactivation technique via microinfusion of the $GABA_A$ receptor agonist muscimol the thesis aimed to elucidate the participation of the vmPFC on frontal and of the NAc on striatal level in the modulation of decision-making and impulse control in rats. Given that the NAc subregions show functional dichotomy in several behaviours, the thesis intended to clarify a potentially heterogeneous role of NAc core and shell in impulsive choice and impulsive action. Moreover, simultaneous temporary inactivation of the vmPFC and NAc core or shell was applied to analyse the involvement of different frontostriatal connections in inhibitory control.

2.1 Study 1 (*Behavioural Brain Research*, 2014)

"*Ventral medial prefrontal cortex inactivation impairs impulse control but does not affect delay-discounting in rats*" considers the relevance of the ventral medial prefrontal cortex (vmPFC) for different types of impulsivity. As part of the PFC, the vmPFC seems to be critically involved in the top-down control of impulsive decision-making and motor impulsivity. By use of bilateral microinfusion of the γ-aminobutyric acid $(GABA)_A$ receptor agonist muscimol (0.05, 0.5 μg/0.3 μl), the effects of reversibly inactivating the vmPFC were examined in the 5-choice serial reaction time task (5-CSRTT) and in a delay-discounting paradigm in a Skinner box.

Intra-vmPFC administration of low-dose muscimol generated enhanced levels of motor impulsivity indicated by increased premature responding in the 5-CSRTT. Following injection of high-dose muscimol, 5-CSRTT performance was characterised by both impulse and attentional control deficits. On the contrary, temporary inactivation of the vmPFC did not induce impulsive-related behaviours in the delay-based decision-making task as measured by the preference for small immediate over large delayed rewards. High-dose muscimol did not affect delay-discounting though raising the rate of omissions, while low-dose muscimol caused a flattening of the delay-dependent shift in the preference of the large reward in the task.

In conclusion, these data support the behavioural dissociation of impulsive choice and impulsive action on the level of the vmPFC in rats. Hence, an intact vmPFC is obviously essential for the maintenance of impulse control in the 5-CSRTT, whereas delay-discounting processes seem to be regulated by other neuronal pathways, with the vmPFC playing, if at all, a minor role.

2.2 Study 2 (*Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 2014)

In "*Nucleus accumbens core and shell inactivation differentially affects impulsive behaviours in rats*" the contribution of the nucleus accumbens (NAc) subregions core and shell to aspects of impulse control in the 5-choice serial reaction time task (5-CSRTT) and delay-based decision-making in the T-maze task was analysed in rats. For this purpose, NAc core and shell were transiently inactivated via bilateral microinfusion of the γ-aminobutyric acid $(GABA)_A$ receptor agonist muscimol (0.05 µg/0.3 µl). Additionally, fluorophore-conjugated muscimol (FCM) was injected in an equimolar concentration (0.27 µg/0.3 µl) to evaluate the spatial distribution of muscimol in both areas.

Inactivation of the NAc shell significantly reduced impulse control in the 5-CSRTT, whereas muscimol injection in the NAc core produced severe impairments in the general performance of the task. Transient deactivation of the NAc shell as well as the NAc core induced impulsive choice in the delay-based decision-making T-maze task, with higher deficits of the rats' waiting capacity following intra-NAc core injection of muscimol compared to shell. FCM showed diffusion extent restricted to the respective subregion, albeit having no effect on any behavioural parameters.

Thus, both regions of the NAc seem to be part of the neural network mediating impulsivity, with varying influences concerning distinct types of impulsive behaviour. This study indicates that the shell region might play a specific role in behavioural control by regulating both impulsive action as well as impulsive choice. In contrast, the NAc core seems to have an additional impact on locomotor activity and motivational aspects and shows functional dichotomy regarding impulse control in comparison with the shell.

2.3 Study 3 (*Psychopharmacology*, under review)

"*Frontostriatal systems comprising connections between ventral medial prefrontal cortex and nucleus accumbens subregions differentially regulate impulse control in rats*" aims to more precisely elucidate the neuronal network underlying inhibitory response control. Participating brain structures form parallel, functionally segregated, yet partly overlapping frontostriatal circuits, including the ventral medial prefrontal cortex (vmPFC) and the nucleus accumbens (NAc).

In this study, a disconnection approach by reversible inactivation of the rats' vmPFC and NAc core or shell, respectively, via simultaneous contralateral microinfusions of the γ-aminobutyric acid $(GABA)_A$ receptor agonist muscimol (0.05 µg/0.3 µl) was used to investigate the functional relationship and a potential distinction between the connections of the vmPFC and the NAc subregions concerning impulse control in the 5-choice serial reaction time task (5-CSRTT).

Disconnection of the vmPFC and the NAc shell induced specific deficits in inhibitory control, as indicated by increased premature and time-out responding. In contrast, simultaneous contralateral inactivation of the vmPFC and the NAc core had no effect on impulse control, but slightly increased the rate of omissions and latency of reward collection suggesting attentional and motivational deficits.

Taken together, the results point out a functional specialisation of frontostriatal systems with a predominant role for the connection of the vmPFC and the NAc shell in mediating impulse control in the 5-CSRTT, while the vmPFC-NAc core pathway seems to have a greater impact on attentional processes and motivational aspects.

2.4 Conclusion

Summarising, the present results corroborate the hypothesis that impulsive behaviour is not only dependent on top-down control by cortical structures, but also regulated at subcortical level. The results achieved indicate distinct impulsivity processes in the vmPFC and NAc, with impulse control in the 5-CSRTT being regulated by both structures, while impulsive decision-making in delay-discounting tasks is principally modulated by the NAc, and not the vmPFC. Further, the current investigation suggests both functional dissociations and close interactions between the vmPFC and NAc in terms of impulsive action, depending on the involved accumbal subregion. A fundamental finding of the current studies was that the NAc

shell constitutes the critical region mediating both types of impulsivity, whereas the NAc core seems to be implicated in non-specific impairments beyond impulsive choice. Consequently, this work points towards various specific frontostriatal systems differentially contributing to delay-based decision-making and particularly impulse control.

3 General introduction

3.1 Behavioural control and impulsivity

Cognitive-executive functions, such as behavioural control, adaptation, planning, organisation and decision-making, are essential aspects of daily life of both humans and rats. They require the right balance of behavioural inhibition and activation to enable the promotion of positive outcomes (Chudasama 2011;Ghazizadeh et al. 2012;Paulus 2005;West and Gardner 2013). Behavioural inhibition is highly influenced by motivational states ('impulses') and is associated with resisting temptation, deferred gratification, motor inhibition, and impulse control (Aron 2007;Jentsch and Taylor 1999). Many psychological theories focus on dualistic processes underlying and competing for control of behaviour, often termed impulsive versus reflective (Gladwin et al. 2011;Strack and Deutsch 2004). Impulsivity is a behavioural characteristic that may beneficially affect living conditions and can function as a dimension of normal personality (Eysenck and Eysenck 1977). In case of dysfunctional behavioural control, the term 'impulsivity' refers to premature, unduly risky, poorly conceived actions. Impulsivity is characterised by deficits in attention, lack of reflection, inability to wait, insensitivity to unfavourable or delayed consequences, difficulty withholding responses and impaired decision-making (de Wit 2009;Evenden 1999b;Reynolds et al. 2006).

High levels of impulsivity are associated with several psychiatric disorders, like attention-deficit/hyperactivity disorder (ADHD), obsessive-compulsive disorder, antisocial personality disorder, borderline personality disorder, schizophrenia, pathological gambling and substance dependence (de Wit 2009;Evenden 1999a;Herpertz and Sass 1997). Moreover, the classification of the *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition* (DSM-5) includes a discrete diagnostic category of 'disruptive, impulse-control, and conduct disorders' (American Psychiatric Association 2013;Berlin and Hollander 2014). The *International Classification of Diseases, 10th Revision* (ICD-10) by the World Health Organization (WHO) lists 'Habit and impulse disorders' in its code set (World Health Organization 2010). According to the WHO World Mental Health survey on the global burden of mental disorders, the lifetime prevalence of impulse control disorders, such as ADHD, ranges from 4.1 % in the European Union up to 25.0 % in the United States population (Kessler et al. 2009).

3.2 Subdivision of impulsivity

A few decades ago, the majority of human and animal research studies assumed that impulsivity is a unitary construct. Over the last 40 years, the complexity of impulsivity became more and more apparent. Increasing evidence from human and animal studies indicated multiple varieties of this behavioural phenotype (Evenden 1999b). Two key terms have emerged among published definitions of impulsivity: decision-making and impulse control (Bari and Robbins 2013;Evenden 1999b). Buss and Plomin (1975) already defined impulse control as the core feature and decision-time as another important aspect of impulsivity. Other groups differentiated between motor (acting without thinking) and cognitive impulsiveness (quick cognitive decision-making) (Patton et al. 1995). Motor impulsivity is closely related to impulse control and reflects reduced response inhibition, whereas cognitive impulsivity affects the evaluation of alternative outcomes associated with a diminished waiting capacity resulting in a loss of long-term rewards (Brunner and Hen 1997). In line with this, other studies found that impulsive subjects show resistance to delay of reinforcement (Logue 1988) and prefer a rapid, but less valuable outcome to a later but more valuable one (Evenden 1999b). Evenden (1999b) distinguished the decisional aspect of impulsivity from premature responding, indicating impaired response withholding more related to execution processes. Accordingly, the multifaceted construct of impulsivity is generally determined by intolerance to delay-of-gratification (impulsive choice) and deficits in impulse control (impulsive action) (Bari and Robbins 2013;Winstanley et al. 2006).

3.3 Assessment of impulsivity

Regarding impulsive behaviour in operant reinforcement tasks, distinct measures of impulsive action or motor impulsivity are distinguishable from impulsive choice or impulsive decision-making (Pattij and Vanderschuren 2008). It has been argued that each type of impulsivity involves a kind of impulsive action, which is necessary to choose a response alternative. The main difference is that impulsive choice requires no forcible inhibition of a prepotent response compared to impulse control (Winstanley et al. 2006).

3.3.1 Impulsive action and the 5-choice serial reaction time task

Impulsive actions (e.g., responding prematurely without foresight) are regarded as an endophenotype of impulsivity that results from a failure in impulse control. It can be defined as the inability to resist making a response. A loss of impulse control – the active inhibitory control mechanism that regulates internally or externally driven urges for reinforcers like food, drugs or money – leads to a disinhibition of rapid conditioned responses. These are transiently suppressed under normal conditions (Bari and Robbins 2013;Robbins 2002;Winstanley et al. 2006). Dysfunctional response inhibition is prevalent in traditional impulse control disorders, such as pathological gambling, trichotillomania, kleptomania, pyromania and intermittent explosive disorder, in substance dependence and in Parkinson's disease and characterises one of the fundamental deficits of ADHD (Bechara 2005;Dell'Osso et al. 2006;Nombela et al. 2014;Winstanley et al. 2006).

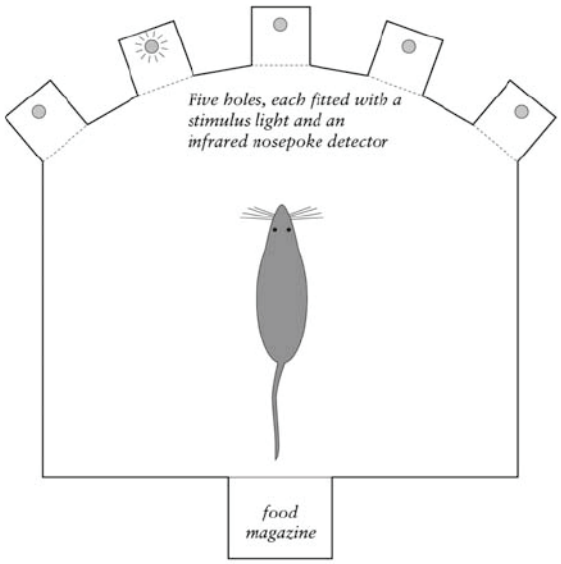

Fig. 3.1 Schematic representation of an operant testing chamber for the 5-choice serial reaction time task (Dalley et al. 2004).

One of the most commonly used methods for measuring response inhibition deficits is the 5-choice serial reaction time task (5-CSRTT). The 5-CSRTT was originally developed to assess visuospatial attention in rodents. Growing interest in impulse control disorders raised the number of studies using the 5-CSRTT for the investigation of motor impulsivity (Eagle and Baunez 2010;Robbins 2002). In this operant-based test paradigm, rats are required to

withhold from impulsive responding to a visual, reward-predicting cue, which is randomly presented in one of five apertures (Fig. 3.1). The number of responses before the onset of the light stimulus is generally regarded as an index of impulse control. Low levels of premature responses presuppose the ability to inhibit actions, while high levels of anticipatory responding reflect behavioural disinhibition (Carli et al. 1983;Muir et al. 1996;Pattij and Vanderschuren 2008;Robbins 2002).

Besides the assessment of impulsive action, the 5-CSRTT allows the registration of perseverative responses, another aspect of inhibitory response control that is more attributable to compulsive rather than impulsive behaviour. Moreover, the flexibility of the 5-CSRTT provides dissociable measurements of reaction time, motivation and particularly sustained, spatially divided and selective attention (Robbins 2002).

3.3.2 Impulsive choice

Impulsive choice is more related to decision-making than to impulse control as required in the 5-CSRTT (Winstanley et al. 2006). Many neurological patients show impairments in decision-making, especially subjects with damage to the prefrontal cortex (PFC) and patients suffering from ADHD, substance-dependence, schizophrenia and anxiety disorders (Damasio 1996;Denk et al. 2005;Ernst and Paulus 2005;Marco et al. 2009). Decision-making is generally considered as the emergence of preferences between alternative conducts based on a rational evaluation of their outcome (Sanfey and Chang 2008). It is composed of at least three distinct processes: 1) the judgment of different alternatives, 2) the selection and execution of an action, and 3) the assessment of the corresponding consequences (Ernst and Paulus 2005). Most decision-making procedures utilise discounting processes and confront the individuals with two options differing in cost and benefit. The increment of costs for the usually more-preferred larger reward leads to a discounting in the value of this option. Discounting models assess the choice behaviour in relation to delay, effort or probability of reward (Floresco et al. 2008b). Since impulsive subjects are intolerant to delay of gratification, delay-discounting is deemed to mostly reflect impulsive behaviour (Bizot et al. 2007). Despite probable negative consequences in the future, drug addicts, obese people and pathological gamblers display higher rates of delay-discounting due to the highly rewarding potential of drugs, food and the chance of gaining money in the immediate situation (Bari and Robbins 2013;Bickel et al. 2012). Consequently, the selection of the smaller immediate reward represents an operational measure for choice impulsivity, while the preference for the deferred, but more profitable

reinforcer indicates self-control (Bizot et al. 1999). Delay-discounting in animals is typically evaluated in lever-equipped operant chamber versions or in delay-based decision-making T-maze tasks.

3.3.2.1 Delay-based decision-making T-maze task

The delayed reinforcement task was originally developed by Thiébot and colleagues (1985). In this procedure, based on prospective timing, animals are placed in a T-shaped maze that initially demands the decision for one of two side arms both of which giving access to determinate food rewards differing in size and delay (Fig. 3.2). The preference choice of the arm immediately offering a low reward over the opposite arm, in which the animal is detained for a short period of time before achieving a high reward, is proposed as an index of delay aversion and thus impulsive-related behaviour (Bizot et al. 1988;Bizot et al. 1999;Thiébot et al. 1985).

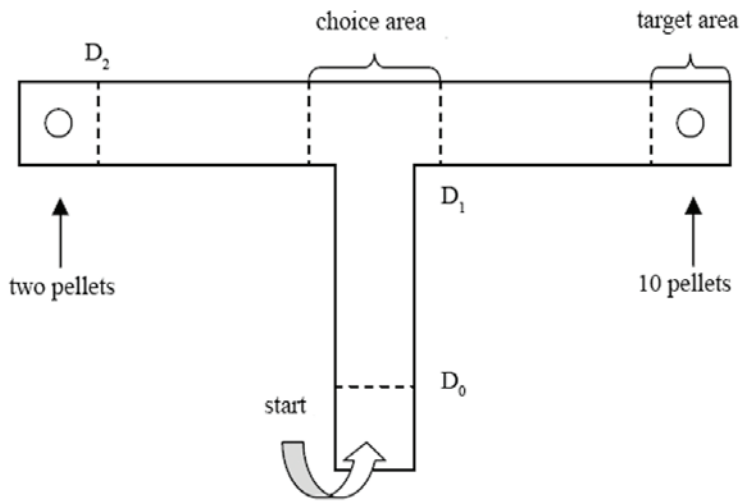

Fig. 3.2 Schematic representation of the delay-based decision-making test apparatus (T-maze). Removable guillotine doors are marked as D_0 in the starting area, D_1 at the choice area and D_2 at the target area. Once a rat is introduced into the starting area, D_1 are elevated to allow choice between the two target arms. In case of a decision for the high reward option (10 pellets), the rat is retained between the lowered D_1 and D_2 for the period of a 10 s-delay. The choice of the alternative side leads to unrestricted access to the low reward of two pellets.

Previous studies have shown that untreated rats usually prefer the larger but delayed reward under a 10-15 s delay condition, whereas a delay of ≥25 s induces a shift towards the smaller immediate option (Bizot et al. 1988;Hadamitzky et al. 2009;Thiébot et al. 1985;Wischhof et al. 2011). Thus, under shorter delay conditions the decision-making T-maze

task is well-suited for the detection of drug-induced impairments in waiting capacity. Longer waiting periods provide a better evaluation of improved tolerance of delayed gratification following drug treatment (Bizot et al. 2007).

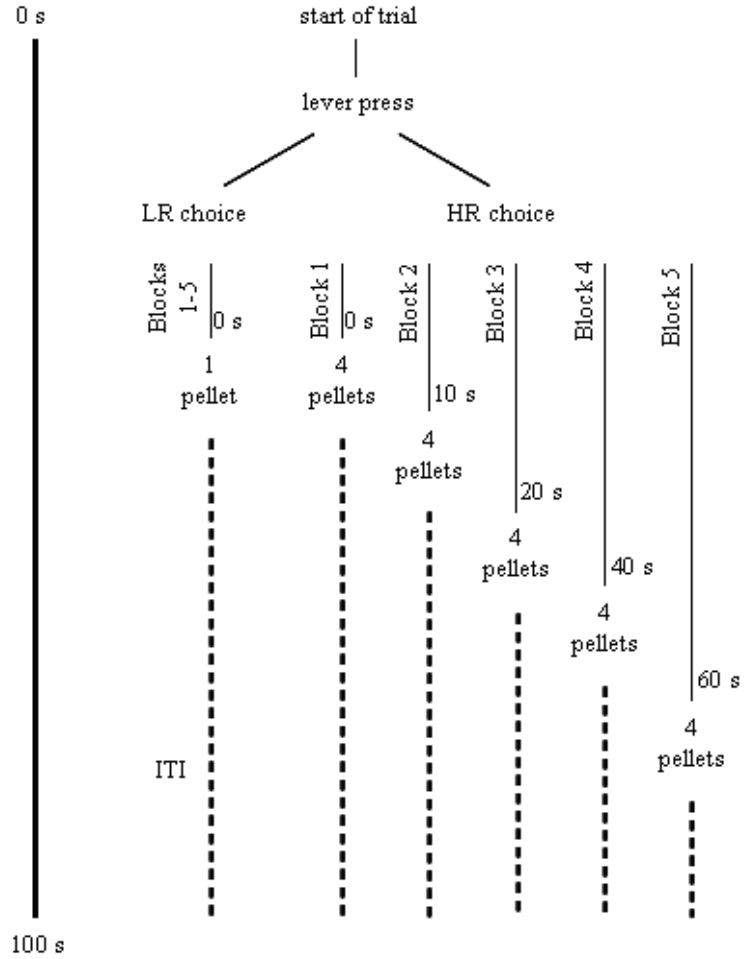

Fig. 3.3 Schematic representation of the operant testing schedule of one trial in the delay-discounting task (Winstanley et al. 2004).

3.3.2.2 Delay-discounting task in operant chambers

Charrier and Thiébot (1996) transferred the T-maze model of Thiébot et al. (1985) into an operant chamber version. In this task, rats were subjected to a choice between two levers associated with food reinforcers varying in both magnitude and delay, closely resembling the paradigm of the T-maze test. Evenden and Ryan (1996) and Cardinal and colleagues (2001) refined the version of Charrier and Thiébot (1996). They established a progressive delay-discounting procedure involving lever-pressing in an operant chamber (Cardinal et al.

2001;Charrier and Thiebot 1996;Evenden and Ryan 1996). Within this automatised paradigm the animals are faced with the choice between one lever leading to a small immediate reward or another leading to a large reinforcer. This is preceded by a delay being gradually increased as the test session progresses (Fig. 3.3). This operant schedule enables the evaluation of drug-induced shifts in delay-discounting both towards preference for the low immediate reward (increased choice impulsivity) and towards the high but delayed reinforcer (decreased choice impulsivity) (Evenden and Ryan 1996).

3.3.3 Translatability of animal models of impulsivity

Particularly due to the division into distinct behavioural aspects, impulsivity is measurable in both human and non-human subjects. In clinical psychology, impulsivity is commonly identified by self-report questionnaires, such as the Barratt Impulsiveness Scale, the Karolinska Scales of Personality or the Tridimensional Personality Questionnaire (Winstanley et al. 2006). However, with the exception of delay-discounting rates, which have shown long-term stability in a monetary choice questionnaire (Kirby 2009), impulsive traits as evaluated by self-reports rarely correlate with impulsivity in behavioural tests. A recent report directly comparing different aspects of impulsivity revealed that, in healthy volunteers, self-reported impulsivity is not associated with behavioural measures. A principal component analysis yielded three independent factors: self-evaluated impulsivity as assessed by the Barratt Impulsiveness Scale, impulsive choice in the delay-discounting task, and impulsive action as measured by the immediate and delayed memory task as well as the stop signal task (Broos et al. 2012).

Behavioural tests of impulsivity have some advantage compared to self-report studies. In laboratory tasks, the individuals' behaviour is valued by the experimenter on the basis of observable data instead of predefined lexical categories in a set of questions potentially entailing various meanings in different subjects and in diverse sociocultural contexts. Further, behavioural models are less biased by the participants' self-perception and social desirability, and thus imply greater objectivity. Moreover, the opportunity of using analogue behavioural measures of impulsivity in humans and animals leads to a broader range of pharmacological and neurological manipulations (Bari and Robbins 2013).

By use of a cross-species translational behavioural approach, decisional and motor impulsivity were successfully proven as distinct endophenotypes of impulsivity that emerge but do not correlate in both humans and rats supporting the concept of the non-unitary nature

of impulsivity (Broos et al. 2012). In rat studies, premature responding in the 5-CSRTT represents the most widely used behavioural index of impulsive action and deficient impulse control as a consequence of its high reliability and reproducibility (Dalley et al. 2008;Eagle and Baunez 2010;Pattij and Vanderschuren 2008;Robbins 2002;Winstanley et al. 2006). The 5-CSRTT is modelled after its human analogues, the continuous performance test of attention (CPT) and Leonard's five choice serial reaction time task, both used to monitor attentional processes in clinical settings. The 5-CSRTT was originally developed for the preclinical investigation of ADHD-related deficits in children and has meanwhile been validated by its sensitivity to drugs used for human ADHD treatment (Carli et al. 1983;Navarra et al. 2008;Robbins 2002;Robinson et al. 2008b). Similar versions of the 5-CSRTT exist for mice (Fletcher et al. 2007;Humby et al. 2005), monkeys (Spinelli et al. 2004;Weed et al. 1999) and humans (Jones et al. 1995;Sahakian et al. 1993).

The human analogue of the 5-CSRTT, the CPT, is principally used as a model of human attention, but is also capable of measuring premature responding (Day et al. 2008). Besides, many other tests of impulsive action exist, such as the differential reinforcement of low rates of responding (DRL) task, the go/no-go task and the stop-signal reaction time (SSRT) task. While impulsive action expressed in the DRL task closely resembles premature responding in the 5-CSRTT, the go/no-go task is frequently used to investigate inhibitory deficits in patients, albeit having important differences compared to the 5-CSRTT (Eagle and Baunez 2010). Thus, very recently a novel analogue of the rodent 5-CSRTT was developed for clinical subjects, which provided evidence of translatability by provoking impairments in impulse control in alcohol- and methamphetamine-dependent subjects and current smokers compared with healthy volunteers (Voon et al. 2014). Exposure to these drugs has previously induced impulsive action in the 5-CSRTT in rat studies (Dalley et al. 2007b;Irimia et al. 2013;Semenova et al. 2007).

Similar to human substance-dependent individuals, impulsive choice behaviour has been found in animals following chronic drug administration in delay-discounting tasks (Bari and Robbins 2013;Setlow et al. 2009). As mentioned above, the majority of human delay-discounting studies is based on questionnaires that involve imaginary delays and rewards. Real-time operant delay-discounting tasks including real rewards carry some difficulties due to the temporally limited duration of laboratory measures leading to a tendentially insufficient aversion to delayed rewards in human subjects (Winstanley et al. 2006). Nevertheless, in the more recent past there is growing evidence for the reliability of real-time discounting tasks. Exemplarily, methylphenidate, approved for the treatment of ADHD, reduced impulsive

choice in a human real-time discounting task (Pietras et al. 2003). Another report of undergraduate students varying in self-reported ADHD symptoms revealed that higher self-reported levels of impulsivity were associated with greater discounting in a real-time discounting task but not in a hypothetical discounting task (Scheres et al. 2008). In accordance with this, methylphenidate treatment reduced the discounting of delayed experiential, but not hypothetical rewards among children with ADHD (Shiels et al. 2009).

3.4 Neural substrates of impulsivity

The neuroanatomical network associated with impulsivity in rodents and humans involves cortical, striatal and limbic brain regions (Pattij and Vanderschuren 2008). It is generally assumed that impulsive behaviour is modulated depending on which neuroanatomical system participates and which type of impulsivity is investigated in a particular method (Evenden and Ryan 1999). On the one hand, lesion studies have shown considerable overlap in neuronal pathways mediating impulsive choice and impulsive action. On the other hand, some brain areas are differentially involved in distinct forms of impulsivity (Fig. 3.4). For example, parts of the medial prefrontal cortex (mPFC), such as the anterior cingulate cortex (AC) and the infralimbic cortex (IL) seem to be primarily implicated in impulsive action, while limbic regions like the basolateral amygdala (BLA) and the hippocampus appear to primarily modulate delay aversion. Frontostriatal systems comprising the PFC and the striatum, which can be further divided into dorsal and ventral parts with the ventral striatum encompassing the nucleus accumbens (NAc), are considered to play a key role in impulse control and impulsive decision-making (Pattij and Vanderschuren 2008).

3.4.1 Medial prefrontal cortex

The mammalian PFC is a heterogeneous structure that has been classically defined by anatomical criteria, such as cytoarchitectonic features (presence or absence of granular characteristics), reciprocal connectivity with the mediodorsal nucleus of the thalamus or input of dopaminergic fibers from the ventral mesencephalon. Translational research of the PFC is complicated by the enormous variation across species in relation to these criteria (Dalley et al. 2004;Heidbreder and Groenewegen 2003). The human PFC comprises the dorsolateral prefrontal cortex (DLPFC), the orbitofrontal cortex (OFC) and the AC (Krawczyk 2002). In

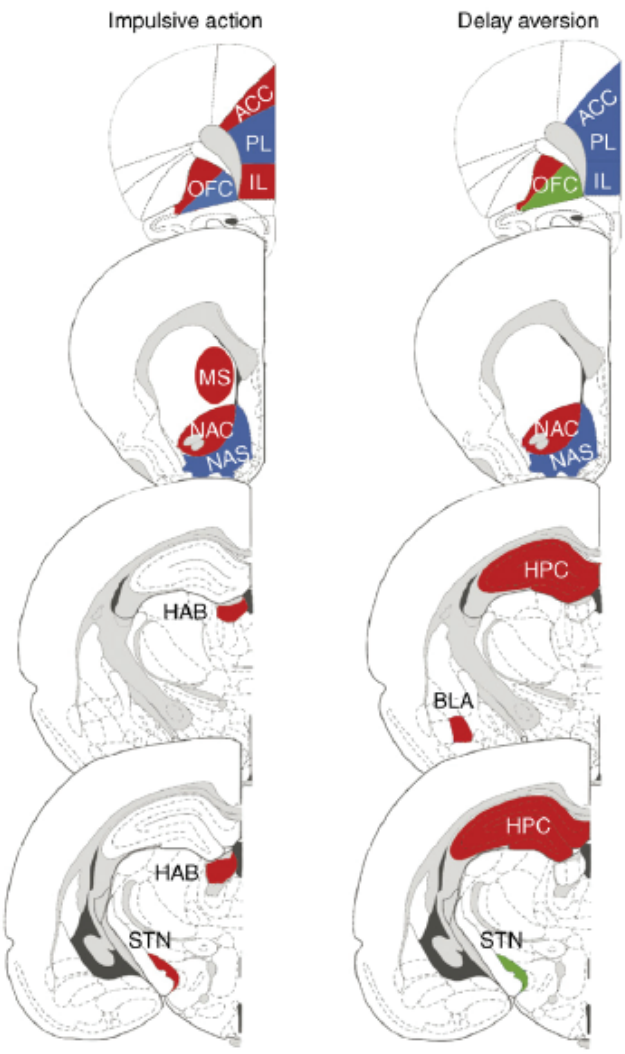

Fig. 3.4 Schematic overview of the neural substrates in the brain involved in impulsive action and delay aversion. Red: lesions of these regions increase impulsive action or delay aversion; green: lesions induce beneficial effects on impulsivity; blue: lesions of these regions neither affect impulsive action nor delay aversion. ACC, anterior cingulate cortex; BLA, basolateral amygdala; HAB, habenula; HPC, hippocampus; IL, infralimbic cortex; MS, medial striatum; NAC, nucleus accumbens core; NAS, nucleus accumbens shell; OFC, orbitofrontal cortex; PL, prelimbic cortex; STN, subthalamic nucleus (Pattij and Vanderschuren 2008).

rats, at least three different regions can be identified: firstly, the mPFC, which forms the major part of the medial wall of the hemisphere anterior and dorsal to the genu of the corpus callosum, secondly, the ventrally located OFC and thirdly a laterally situated division including the agranular insular and lateral orbital cortices. The mPFC of rats can be further divided into dorsal (anterior cingulate and medial precentral cortices) and ventral subdivisions (infralimbic and prelimbic cortices) (Heidbreder and Groenewegen 2003;Ongur and Price 2000). Although lacking certain areas that arised during primate evolution, rats feature many

earlier evolved prefrontal regions that have a homologue in primates, such as the AC, IL and prelimbic cortex (PL) (Fig. 3.5) (Wise 2008). The rat mPFC seems to combine elements of primate AC and DLPFC (Preuss 1995;Seamans et al. 2008;Uylings et al. 2003;Vertes 2004). While rat dorsal mPFC is supposed to anatomically and functionally resemble primate DLPFC (Seamans et al. 2008;Uylings et al. 2003), the ventral medial prefrontal cortex (vmPFC) of rats appears to be equivalent to the primate AC (Preuss 1995). Concomitantly, the literature provides growing indications for a functional-behavioural differentiation of the mPFC into dorsal and ventral components (Heidbreder and Groenewegen 2003).

The mPFC as a whole is involved in a variety of cognitive and executive processes, including decision-making, inhibitory response control, working memory, behavioural flexibility, temporal processing and attentional selection (Dalley et al. 2004;Heidbreder and Groenewegen 2003). In humans, inconsistent conceptions of the relevance of the DLPFC to aspects of impulsivity exist. Dysfunctions of the DLPFC in psychopathic offenders generate impairments in impulse control (Yang and Raine 2009). However, the DLPFC is not linked to motor impulsivity evaluated with the Barratt Impulsivity Scale (Cho et al. 2013). In terms of impulsive choice, human OFC is heavily implicated, while the DLPFC seems to play a minor role (Bechara et al. 2000;Krawczyk 2002). Yet, recent investigations using repetitive transcranial magnetic stimulation associate the DLPFC with delay-discounting (Cho et al. 2010;Figner et al. 2010). By contrast, human AC function is related to both impulse control (Bechara 2004;Brown et al. 2006;Fineberg et al. 2010;Liu et al. 2012) and delay-discounting (Hinvest et al. 2011;Hoffman et al. 2008;Li et al. 2013).

In animal studies findings of electrophysiological recordings (Hayton et al. 2011), lesions (Chudasama et al. 2003;Muir et al. 1996;Pezze et al. 2009) and transient inactivation (Izaki et al. 2007;Murphy et al. 2012;Narayanan et al. 2006;Paine et al. 2011) associate the rodent mPFC with impulse control. During 5-CSRTT performance, attentional control appears to depend selectively on dorsal parts of the mPFC, while ventral regions seem to be critical for inhibitory response control (Dalley et al. 2004). Accordingly, the vmPFC is suggested to be more critically involved in impulsive behaviour (Chudasama et al. 2003;Kesner and Churchwell 2011). In terms of behavioural choice, the AC of rats is primarily associated with effort-discounting, whereas the vmPFC is more implicated in delay-based decision-making (Kesner and Churchwell 2011). However, controversial results exist regarding the contribution of the mPFC to impulsive choice. Increased delay-discounting was observed in vmPFC-lesioned (Gill et al. 2010) or -inactivated (Churchwell et al. 2009) rats.

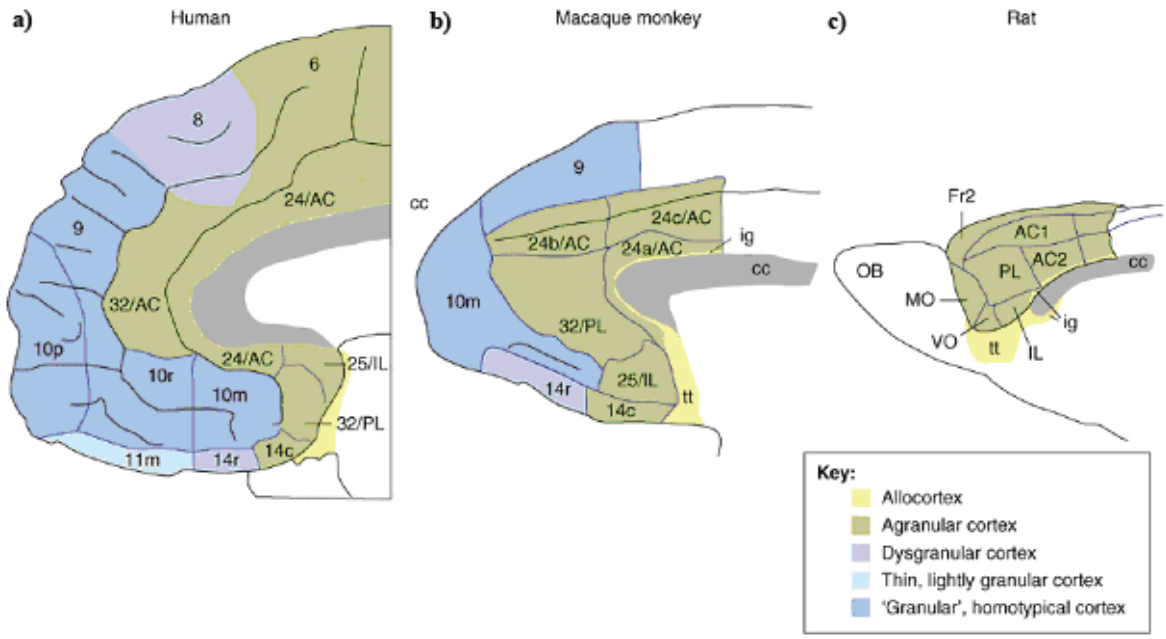

Fig. 3.5 Architectonic maps of the medial frontal cortex of humans (a), macaque monkeys (b) and rats (c). a, agranular; AC, anterior cingulate cortex; c, caudal; cc, corpus callosum; Fr2, second frontal area; ig, indusium griseum; IL, infralimbic cortex; m, medial; MO, medial orbital area; OB, olfactory bulb; p, posterior; PL, prelimbic cortex; r, rostral; tt, tenia tectum; VO, ventral orbital area. Numbers indicate cortical fields, except after certain regions, such as Fr2 and AC1, where they indicate subdivisions of cortical fields. The letters 'a, b or c' after a number also indicate regional subdivisions (Wise 2008).

Another delay-discounting study showed that neither the AC nor the vmPFC is the primary site of this action (Cardinal et al. 2001).

3.4.2 Nucleus accumbens

In both rats and primates, all major regions of the cerebral cortex project to the striatum (Ferry et al. 2000;McGeorge and Faull 1989). This subcortical input structure of the basal ganglia includes the caudate-putamen complex, generally termed as dorsal striatum, and the NAc as part of the ventral striatum (Voorn et al. 2004). The mPFC innervates predominantly the respective medial parts of the NAc and the caudate-putamen complex (Berendse et al. 1992). On the basis of anatomical and neurochemical criteria, the rat NAc is further divided into distinct subterritories which are also present in the primate brain: a dorsolateral core region surrounding the anterior commissure, and a shell region that is located ventromedially to the core (Fig. 3.6) (Heimer et al. 1991;Jongen-Relo et al. 1994;Meredith et al. 1996;Sokolowski and Salamone 1998;Voorn et al. 1989;Voorn et al. 1996;Zaborszky et al. 1985;Zahm and

Brog 1992). The core region sends fibers to the conventional basal ganglia circuitry involving subcommissural ventral pallidum, globus pallidus and substantia nigra. In contrast, shell projections extensively reach subcortical limbic structures, such as lateral hypothalamus, ventral tegmental area (VTA), the ventromedial part of the ventral pallidum and the extended amygdala (Heimer et al. 1991;Zahm and Brog 1992).

As a critical element of the mesocorticolimbic system, the NAc is generally implicated in reward and motivation, integrating input signals of emotion (BLA), context (hippocampus), arousal (midline thalamus) and executive-cognitive information (PFC). Accumbal efferents reach brain sites involved in feeding and drinking (lateral hypothalamus), motivational (VTA, substantia nigra) and locomotor behaviour (caudal mesencephalon) as well as higher cognitive-executive functions, such as behavioural control and decision-making (via medial thalamic nuclei to the PFC) (Carlezon, Jr. and Thomas 2009;Groenewegen and Trimble 2007).

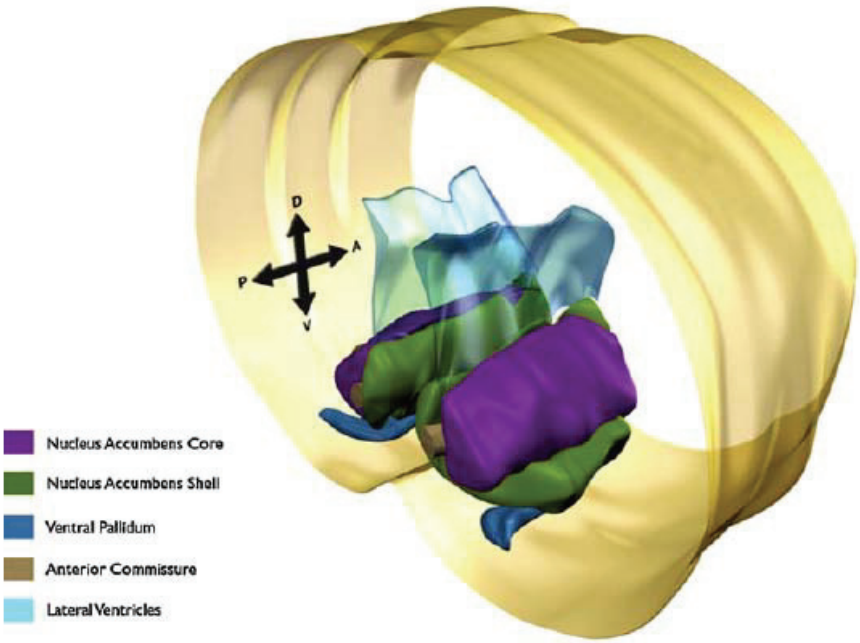

Fig. 3.6 Three-dimensional schematic representation of the rat nucleus accumbens and adjacent structures. A, anterior; D, dorsal; P, posterior; V, ventral (Basar et al. 2010).

The original concept of the NAc as a functional interface between the limbic and motor systems is still valid, but the distinct connectivity profiles of core and shell suggest that the NAc should be viewed much more differentiated (Groenewegen and Trimble 2007;Heimer 2003;Mogenson et al. 1980). Previous studies have already demonstrated a

differential influence of the NAc subregions on a variety of behaviours. These include goal-directed instrumental action (Corbit et al. 2001), Pavlovian-instrumental transfer (Corbit and Balleine 2011;Saddoris et al. 2011), behavioural flexibility (Floresco et al. 2006), stress-, cue- or cocaine priming-induced reinstatement of drug- or food-seeking behaviour (Floresco et al. 2008a;McFarland et al. 2004;Vassoler et al. 2013), working memory (Jongen-Relo et al. 2003), locomotor activity (Jongen-Relo et al. 2002;Pothuizen et al. 2005a;Robbins and Everitt 1996), motivational behaviour (Bassareo et al. 2002;Stratford and Kelley 1997) and attentional processes, like prepulse and latent inhibition (Jongen-Relo et al. 2002;Pothuizen et al. 2005a).

Taken as a whole, the NAc is implicated in decision-making (Assadi et al. 2009;Day et al. 2011;de Visser et al. 2011a) and anticipation of reward in humans, other primates and rats (Cromwell and Schultz 2003;Knutson et al. 2001;Martin and Ono 2000;Rademacher et al. 2014). Human functional magnetic resonance imaging studies found activation of the NAc during performance in delay-discounting tasks (Ballard and Knutson 2009;Hariri et al. 2006;McClure et al. 2004;Wittmann et al. 2007). Evidence from rat studies indicates that the NAc is also heterogeneously involved in impulsive behaviours. Lesions of the core induce impulsive choice (Bezzina et al. 2007;Bezzina et al. 2008a;Cardinal et al. 2001;da Costa et al. 2009;Pothuizen et al. 2005b) and deficits in impulse control in the 5-CSRTT and DRL task (Christakou et al. 2004;Pothuizen et al. 2005b). By contrast, shell lesions do neither affect delay-discounting nor premature responding in response inhibition tasks (Cole and Robbins 1989;Murphy et al. 2008;Pothuizen et al. 2005b). However, recent pharmacological manipulations highlighted a potential involvement of the NAc shell in aspects of impulsivity. More precisely, motor impulsivity in the 5-CSRTT was found to correlate with enhanced dopamine (DA) release due to decreased DA D2/3 receptor availability and higher D1 receptor messenger ribonucleic acid (mRNA) expression in the shell, but reduced DA release caused by lower D1 receptor binding in the core (Diergaarde et al. 2008;Jupp et al. 2013;Simon et al. 2013).

3.4.3 Frontostriatal systems

In primates, five parallel circuits connect discrete regions of the frontal lobes with specific subregions of the striatum. Three of these circuits comprise prefrontal regions at the cortical level and mediate cognitive, emotional, and motivational processes. Firstly, the projection from the DLPFC that terminates within the dorsolateral portion of the caudate nucleus.

Secondly, the lateral OFC that projects to the ventromedial sector of the caudate nucleus, and thirdly the AC circuit, which is connected to the ventral striatum, including the NAc. The dorsolateral circuit is involved in the mediation of executive functions, such as response inhibition. The orbitofrontal circuit is implicated in the selection and control of appropriate behaviour, and the AC circuitry is responsible for regulating motivation and maintaining activity (Alexander et al. 1986;Cummings 1995;Krawczyk 2002).

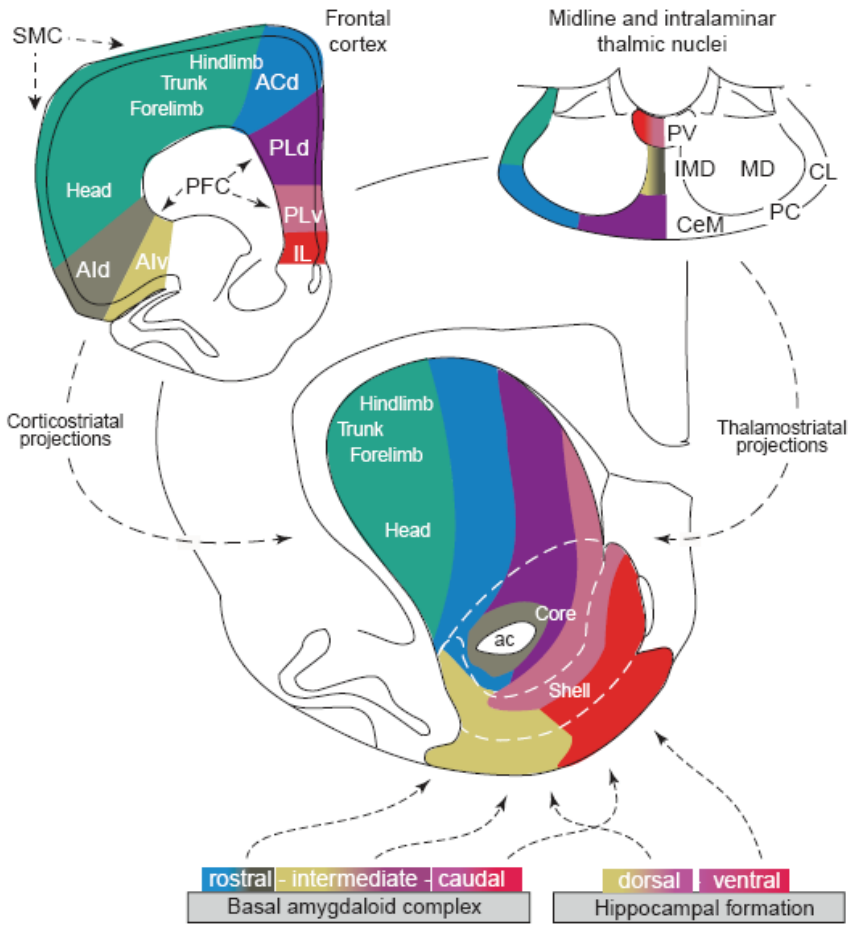

Fig. 3.7 Cortical, thalamic, basal amygdaloid and hippocampal inputs to the rat striatum. Corticostriatal projections are topographically arranged and distribute to zones with a dorsomedial-to-ventrolateral orientation. Areas of the frontal cortex and their corresponding striatal projection zones are depicted in the same colours. ac, anterior commissure; ACd, dorsal anterior cingulate cortex; AId, dorsal agranular insular cortex; AIv, ventral agranular insular cortex; CeM, central medial thalamic nucleus; CL, central lateral thalamic nucleus; IL, infralimbic cortex; IMD, intermediodorsal thalamic nucleus; MD, mediodorsal thalamic nucleus; PC, paracentral thalamic nucleus; PFC, prefrontal cortex; PLd, dorsal prelimbic cortex; PLv, ventral prelimbic cortex; PV, paraventricular thalamic nucleus; SMC, sensorimotor cortex (Voorn et al. 2004).

The organisation of frontostriatal systems of rats is similar to primates and supports the assumption that the frontal agranular areas in rodents, particularly the mPFC, are homologous to those in primates (see Fig. 3.5). The connection between the mPFC and the striatum in rats is highly topographically arranged, characterised by a shift along the dorsal-ventral axis (Fig. 3.7). Consequently, dorsal regions of the mPFC primarily project to the dorsal striatum, whereas the ventral striatum is predominatly innervated by the vmPFC. More precisely, the medial precentral cortex terminates in the central part of the caudate-putamen complex, while the AC targets more medially and also projects to the NAc core. Efferent connections of the dorsal PL include the ventromedial sector of the caudate-putamen and the NAc core. The ventral PL and the IL primarily project to the shell region of the NAc (Heidbreder and Groenewegen 2003;Wise 2008).

Impulse control is thought to be a principal function of frontostriatal systems. Dysfunction of this network generates impulsivity in a number of pathological states, like drug abuse, pathological gambling, ADHD and Parkinson's disease (Cho et al. 2013;Jentsch and Taylor 1999). Besides impulse control (Aron et al. 2007;Christakou et al. 2004;Diekhof and Gruber 2010;Morgane et al. 2005), a functional relationship between the PFC and NAc has been found to be implicated in impulsive choice (Costa Dias et al. 2013;Diergaarde et al. 2008), behavioural flexibility (Coppens et al. 2010;Goto and Grace 2005) and drug seeking in both humans and rats (Bossert et al. 2012;Peters et al. 2008). Disconnecting the mPFC from the NAc core by lesions induces motor impulsivity in the 5-CSRTT (Christakou et al. 2004), whereas an implication of the mPFC-NAc shell connection was not examined as yet. However, there is evidence to suggest that the anatomically heterogeneous connectivity between the mPFC and the NAc subregions is paralleled by functional subregional specifity (Heidbreder and Groenewegen 2003).

3.5 Permanent and reversible inactivation techniques

Numerous findings concerning impulsivity are derived from patients with damage to distinct cerebral regions due to traumatic brain injury, particularly lesions of the frontal lobe (Aron 2007;Crews and Boettiger 2009;Sellitto et al. 2010). Accordingly, the lesion technique was most frequently used to study brain function in animals. The permanent removal or destruction of nervous tissue is commonly produced by physical (surgical excision or aspiration) or chemical ablation (injections of excitotoxins, like ibotenic or kainic acid)

methods. Nevertheless, the lesion technique entails some serious drawbacks. The major obstacle is a phenomenon known as 'recovery of function', characterised by significant behavioural deficits in the days immediately after the ablative manipulation, but followed by a partially or complete decline of these deficits in the subsequent time. This indicates that the animals' performance in a behavioural test, which is typically carried out after a necessary recovery period from the manipulation lasting several days, might be compensatorily influenced by other brain areas. This would mean that the approach would not examine the true function of the lesioned tissue, but rather the functional adaptability of remaining intact structures, or restorative processes that occur during recovery time (Lomber 1999;Majchrzak and Di Scala 2000).

In contrast to these permanent deactivations, temporary inactivation techniques do not irreversibly destroy brain tissue. This allows behavioural testing of the animals and functional investigation of the involved region at the time of inactivation. Transient inactivation methods have additional advantages compared to lesion procedures. The reliability of results is increased by within-subject designs as the same site can be repeatedly inactivated in the same animal during multiple behavioural sessions (Martin and Ghez 1999). Since each animal serves as its own control, fewer animals are necessary in the studies. Moreover, two or more sites can be separately or simultaneously inactivated to investigate the specific contribution or the combined function of these areas in a neural network with regard to a particular behaviour (Lomber 1999).

The two principal methods for reversible inactivation are chemical and cryogenic. Depending on the drug or on the extent of temperature reduction, respectively, both techniques can either selectively block cell bodies or, non-selectively, inhibit synaptic transmission together with axonal action potential conduction. However, it should be considered that the cooling method generates a zone of neuronal hyperexcitability surrounding the inactivation site. On the contrary, the pharmacological technique only depresses neuronal activity. Another feature of chemical inactivation being more beneficial compared to cooling consists in the readily usability on both cortical and deep brain structures (Lomber 1999;Martin and Ghez 1999).

Pharmacological agents producing reversible inactivation are dividable into two groups: sodium channel blocker and inhibitory neurotransmitters, in particular γ-aminobutyric acid (GABA) and its agonists (Lomber 1999). GABA is the main inhibitory neurotransmitter in the adult brain and, beside glutamate, the principal fast-acting transmitter of frontal-subcortical circuits (Cummings 1995). There are two main types of GABA receptors: the

$GABA_A$ receptor, a ligand-gated chloride ion channel, and the metabotropic G-protein coupled $GABA_B$ receptor. GABA acts primarily through activation of the $GABA_A$ receptor, whereas the distribution of the $GABA_B$ receptor is more limited (Wu and Sun 2014). The most commonly used $GABA_A$ receptor agonist is muscimol (3-hydroxy-5-aminomethyllisoxazole), the psychoactive ingredient of the mushroom *Amanita muscaria*. Muscimol has a striking structural similarity to GABA and even a more potent pharmacological profile as the inhibitory neurotransmitter itself (Baraldi et al. 1979;Krogsgaard-Larsen and Johnston 1978). The substance selectively induces a rapid hyperpolarisation lasting up to several hours on postsynaptic neurons via activation of $GABA_A$ receptors on the surface of local cell bodies without affecting fibers of passage, thereby allowing behavioural testing almost immediately after injection (Heiss et al. 2010;Martin and Ghez 1999). Intracerebral microinjections of muscimol have already been used to induce highly site-specific reversible inactivation in diverse species in a variety of behavioural tasks and indeed revealed contradictory contributions of distinct brain regions in terms of impulsivity compared to lesion studies (Cardinal et al. 2001;Churchwell et al. 2009;Majchrzak and Di Scala 2000;Murphy et al. 2012;Paine et al. 2011;Passetti et al. 2002).

Imaging the spatial distribution of muscimol via fluorescence may help to evaluate the spatial extent of drug-infused brain tissue and to localise drug effects more precisely. For this purpose, fluorophore-conjugated muscimol (FCM) molecules (Fig. 3.8) have been demonstrated to be useful for producing local and reversible brain inactivations resulting in behavioural effects similar to muscimol in order to assess the function of these regions (Allen et al. 2008).

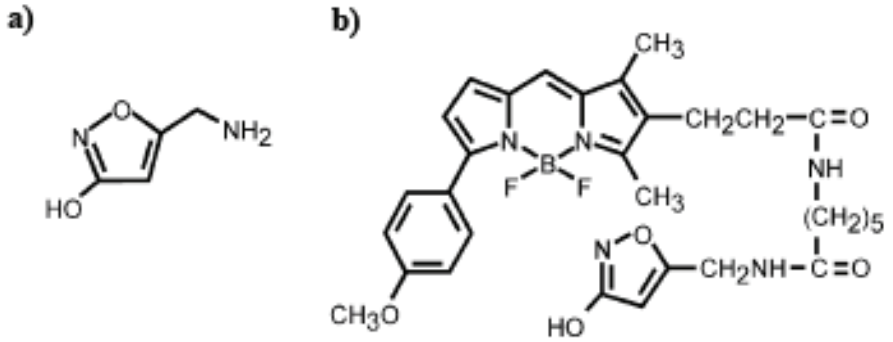

Fig. 3.8 Chemical structures of a) muscimol (M = 114.10 g/mol) and b) fluorophore-conjugated muscimol (FCM, M = 607.46 g/mol) (Allen et al. 2008).

3.6 Aim of the thesis

This thesis further investigated the contribution of specific structures of the frontostriatal network to distinct aspects of behavioural inhibition. Dysfunctions of frontostriatal systems have been heavily implicated in the aetiology of impulsivity and associated psychological disorders. In the intact brain, frontostriatal circuits reveal highly specialised and heterogeneous functions depending on the involved cerebral structures and the situationally demanded behavioural component. Using the reversible inactivation technique via microinfusion of the $GABA_A$ receptor agonist muscimol, the thesis aimed to elucidate the participation of the vmPFC on frontal and of the NAc on striatal level in the modulation of decision-making and impulse control in rats. Given that the NAc subregions show functional dichotomy in several behaviours, the thesis attempted to clarify a potentially differential role of NAc core and shell in impulsive choice and impulsive action. Moreover, simultaneous transient inactivation of the vmPFC and NAc core or shell was applied to analyse the involvement of different frontostriatal connections in inhibitory control.

4 Ventral medial prefrontal cortex inactivation impairs impulse control but does not affect delay-discounting in rats

Malte Feja · Michael Koch

4.1 HIGHLIGHTS

- The $GABA_A$ agonist muscimol was used to reversibly inactivate vmPFC in rats
- Deactivating vmPFC with low-dose muscimol induced impulsive action in the 5-CSRTT
- High-dose muscimol infusion impaired impulse and attentional control in the 5-CSRTT
- Muscimol into vmPFC did not affect delay-discounting in a Skinner box
- The control function of vmPFC is impulsivity type-specific

4.2 ABSTRACT

Maladaptive levels of impulsivity are found in several neuropsychiatric disorders, such as ADHD, addiction, aggression and schizophrenia. Intolerance to delay-of-gratification, or delay-discounting, and deficits in impulse control are dissociable forms of impulsivity top-down controlled by the prefrontal cortex, with the ventral medial prefrontal cortex (vmPFC) suggested to be critically involved. The present study used transient inactivation of the rats' vmPFC via bilateral microinfusion of the $GABA_A$ receptor agonist muscimol (0.05, 0.5 μg/0.3 μl) to analyse its relevance for impulse control in a 5-choice serial reaction time task (5-CSRTT) and delay-discounting in a Skinner box. Intra-vmPFC injection of low-dose muscimol impaired impulse control indicated by enhanced premature responding in the 5-CSRTT, while flattening the delay-dependent shift in the preference of the large reward in the delay-discounting task. Likewise, high-dose muscimol did not affect delay-discounting, though raising the rate of omissions. On the contrary, 5-CSRTT performance was characterised by deficits in impulse and attentional control. These data support the behavioural distinction of delay-discounting and impulse control on the level of the vmPFC in rats. Reversible inactivation with muscimol revealed an obvious implication of the vmPFC in the modulation of impulse control in the 5-CSRTT. By contrast, delay-discounting processes seem to be regulated by other neuronal pathways, with the vmPFC playing, if at all, a minor role.

4.3 Introduction

Cognitive-executive functions, such as behavioural control and decision-making, are essential aspects of daily life in both humans and rats (Chudasama 2011;Paulus 2005). These processes are closely related to impulsive behaviour. Impulsivity is a behavioural characteristic that both adversely and beneficially affects living conditions and can function as a dimension of normal personality (Eysenck and Eysenck 1977). In case of an imbalance of behavioural activation and its inhibition, the term 'impulsivity' refers to maladaptive behaviours including inability to wait, difficulty withholding responses and insensitivity to unfavourable or delayed consequences (de Wit 2009;Reynolds et al. 2006). High levels of impulsivity are found in psychiatric disorders, involving attention-deficit/hyperactivity disorder (ADHD), antisocial personality disorder, borderline personality disorder, schizophrenia, drug abuse and other forms of addiction (de Wit 2009;Evenden 1999a;Herpertz and Sass 1997). Moreover, the classification of the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5) lists a discrete diagnostic category of 'disruptive, impulse-control, and conduct disorders' (American Psychiatric Association 2013).

However, impulsivity is not a unitary construct, but rather a multifactorial phenome-

non, largely determined by intolerance to delay-of-gratification (impulsive choice), or delay-discounting, and deficits in impulse control (impulsive action) (Evenden 1999b;Winstanley et al. 2006). Hence, the dominant behavioural measures of impulsivity are delay-discounting and response inhibition tasks. The delay-discounting model is used in both humans and animals to assess impulsive decision-making, reflected in the preference for a small immediate over a larger-but-delayed reward (Broos et al. 2012;de Wit 2009;Moeller et al. 2001;Swann et al. 2002). By contrast, response inhibition paradigms, e. g. the 5-choice serial reaction time task (5-CSRTT), require to withhold from premature responding which is regarded as an index of deficient impulse control (Carli et al. 1983;Robbins 2002). The 5-CSRTT also has translational properties and is modelled after its human analogues, the continuous performance test of attention and Leonard's five choice serial reaction time task (Muir et al. 1996).

Neuropsychological evidence suggests that executive processing relies on the intact function of the frontal cortices, with the prefrontal cortex (PFC) playing a major role (Elliott 2003;Fuster 2000;Robbins et al. 1996). Patients with damage to the PFC show impaired decision-making and behavioural disinhibition (Elliott 2003). The human PFC is a heterogeneous region of the brain, comprising the dorsolateral prefrontal cortex (DLPFC), the orbitofrontal cortex (OFC) and the anterior cingulate cortex (AC). The PFC subregions appear to be engaged in separable multi-component neural systems mediating distinct cognitive processes (Fuster 2001;Krawczyk 2002). Concerning the DLPFC, inconsistent conceptions of its relevance to aspects of impulsivity exist. Decreased functioning of the DLPFC in psychopathic offenders generates impairments in impulse control (Yang and Raine 2009). However, the DLPFC is not associated with motor impulsivity measured with the Barratt Impulsivity Scale (Cho et al. 2013). In terms of impulsive decision-making, the OFC is heavily implicated, while the DLPFC seems to play a minor role (Bechara et al. 2000;Krawczyk 2002). Yet, recent studies using repetitive transcranial magnetic stimulation link the DLPFC with delay-discounting (Cho et al. 2010;Figner et al. 2010). AC function is related to both impulse control (Bechara 2004;Brown et al. 2006;Fineberg et al. 2010;Liu et al. 2012) and delay-discounting (Hinvest et al. 2011;Hoffman et al. 2008;Li et al. 2013).

The medial prefrontal cortex (mPFC) of rats seems to combine elements of primate AC and DLPFC (Preuss 1995;Seamans et al. 2008;Uylings et al. 2003;Vertes 2004) and shares many features with the human medial frontal cortex (Narayanan et al. 2013). Based on cellular structure and lamination of the cortex, the mPFC of rats can be further divided into dorsal (anterior cingulate and medial precentral cortices) and ventral subdivisions (infralimbic and prelimbic cortices) (Heidbreder and Groenewegen 2003;Ongur and Price 2000). While rat dorsal mPFC is supposed to anatomically and functionally resemble primate DLPFC (Seamans et al. 2008;Uylings et al. 2003), the ventral medial prefrontal cortex (vmPFC) of rats appears to be equivalent to the primate AC (Preuss 1995). Animal studies further strengthen the assumption of distinct aspects underlying impulsive behaviour. Findings of electrophysiological recordings (Hayton et al. 2011), lesions (Chudasama et al. 2003;Muir et al. 1996;Pezze et al. 2009) and transient inactivation (Izaki et al. 2007;Murphy et al. 2012;Narayanan et al. 2006;Paine et al. 2011) associate the rodent mPFC with impulse control. On the other hand, controversial results exist regarding the contribution of the mPFC to impulsive choice. For example, increased delay-discounting appears in mPFC-lesioned (Gill et al. 2010) or -inactivated (Churchwell et al. 2009) rats, whereas another delay-discounting study shows that the mPFC is not the primary site of this action (Cardinal et al. 2001). There is evidence to suggest that the anatomical dichotomy of the mPFC is paralleled by functional subregional specifity, with the ventral medial prefrontal cortex (vmPFC) appearing to be more critically involved in impulsive behaviour (Chudasama et al. 2003;Kesner and Churchwell 2011). However, lesion studies revealed discrepancies in the role

of the vmPFC in impulsivity, ranging from direct participation (Chudasama et al. 2003), a mere tendency of involvement (Chudasama and Muir 2001) to no important role (Passetti et al. 2002).

The lesion technique was the most widely used method to investigate brain function, nevertheless carrying some drawbacks in comparison to inactivation tools. Following lesions, brain tissue is destroyed irreversibly and a compensation of function by other brain areas might occur. In contrast, chemical agents like the $GABA_A$ receptor agonist muscimol allow acute, reversible inactivations of distinct brain regions, and hence, within-subject designs concomitant with increased reliability (Lomber 1999). Muscimol is the psychoactive ingredient of the mushroom *Amanita muscaria* and has even a more potent pharmacological profile as the inhibitory neurotransmitter GABA itself (Krogsgaard-Larsen and Johnston 1978). After injection, muscimol selectively induces a rapid hyperpolarization lasting up to several hours on postsynaptic neurons via activation of $GABA_A$ receptors on the surface of local cell bodies without affecting fibers of passage (Heiss et al. 2010;Martin and Ghez 1999).

In the present study, temporary inactivation of the rats' vmPFC via bilateral microinfusion of the $GABA_A$ receptor agonist muscimol was used to further clarify its contribution to different aspects of impulse control in the 5-CSRTT and delay-discounting in a Skinner box.

4.4 Material and methods

4.4.1 Subjects

A total of 23 adult male Lister Hooded rats (280 – 340 g) obtained from Harlan (Borchen, Germany) were housed in groups of four to six in standard Macrolon cages (type IV) under controlled ambient conditions (21 – 22 °C, 45 – 55 % humidity, 12 h light/dark cycle, lights on at 7:00 a.m.). The animals were maintained on their experimental body weight by controlled feeding of 12 g laboratory rodent chow (Nohrlin GmbH, Bad Salzuflen, Germany) per rat per day and received tap water ad libitum. Behavioural testing took place between 8:00 a.m. and 6:00 p.m. The experiments were performed in accordance with the National Institutes of Health ethical guidelines for the care and use of laboratory animals for experiments and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

4.4.2 Experiment 1: 5-CSRTT

4.4.2.1 Apparatus

The 5-CSRTT was conducted in two operant aluminium chambers (26 x 26 x 26 cm; Campden Instruments Ltd., Loughborough, UK), wherein five apertures (2.5 x 2.5 cm, 4 cm deep) were inserted 2 cm above floor level in the concavely curved rear wall. This assembly provided five response options located equidistant to the food magazine on the opposite. Inside each hole, a light-emitting diode (LED) generated visual stimuli of variable duration. Nose-poke responses of the animals were detected by infra-red photo cell beams at the entrance of the apertures. The rats could be placed in the box through a Plexiglas® door on the upper part of the front wall. Underneath the door, a small Plexiglas® panel provided access to the food magazine which was lighted via two LEDs and automatically supplied with casein pellets (45 mg Dustless Precision Pellets, Bio-Serv®, UK) by an electromechanical feeder. Food collection was detected by a microswitch monitoring the movement of the hinged panel. Each chamber was illuminated by a 3 W house light mounted on the ceiling. A noise-damped fan served as ventilation and background noise of about 60 dB. The extendable grid floor facilitated the removal of excrements. For the purpose of sound attenuation, the wooden cabinet was reinforced with an insulating plate at the interior of the door. The apparatus was controlled by specific software written in Turandot (Cambridge Cognition Ltd., version 1.23) which was run on a personal computer connected to the BNC Mark 2 System (Behavioral Net Controller, Campden Instruments Ltd., Loughborough, UK).

4.4.2.2 Training

The animals (n = 12) were trained to detect the occurrence of brief light stimuli in one of the five rear wall apertures. The general procedure was based on the protocol of Campden Instruments and was divided into a habituation, pretraining and baseline training phase (Campden Instruments Limited 2005). Before training and tests the rats were acclimatised to the laboratory for at least 30 min in their home-cages.

The first experimental phase comprised two daily half-hour habituation sessions. The boxes were prepared as follows: before the first training session, the tray panel was opened to facilitate access to 15 freely available pellets in order to reinforce the meaning of the magazine as location of food reward. During the second session, no panel manipulation was carried out. Besides the reward in the tray, two pellets were placed in each aperture to promote exploration of these areas. The chambers were permanently illuminated by the house light during both sessions.

The daily training session lasted 30 min or was finished after completion of 100 trials. Each session started with the simultaneous illumination of the box and the food magazine and the delivery of a single pellet into the tray. Once the rat opened the panel for food retrieval, the first trial was initiated. The magazine light faded and a fixed intertrial interval (ITI) of 5 s started. At the end of the ITI, a light stimulus of determinate duration (stimulus duration, SD) was randomly presented in one of the five holes. The rats had to respond with a nose-poke into the appropriate aperture during the stimulus presentation or within a subsequent limited hold period (LH). A correct response was followed by the supply of a pellet into the lighted food magazine. The next trial was triggered by the panel movement. Inappropriate responses led to a punishment in terms of a predefined 5 s period of darkness (time-out) without reward delivery. The task procedure offered various opportunities for such reactions:

- *incorrect responses* in a hole where no stimulus appeared,
- *omissions* in the form of absent reaction to the occurrence of the stimulus within the LH,
- *premature responses* before the onset of the stimulus during the ITI in one of the apertures
- and *perseverative responses*, meaning additional responses after a correct response and before reward intake.

Every response during the time-out phase reinitialised the period of darkness. Following the time-out, the box and the tray were illuminated again and the next trial was started by a nose-poke into the food magazine. Within a session, the visual stimuli were randomly presented in equal number in each hole. The progressive decrement of the variables SD (60 → 1 s) and LH (60 → 5 s) over eight training levels enabled the acquisition of the 5-CSRTT.

The baseline training session was determined by the conditions of the eighth training level (SD = 1 s, LH = 5 s). After showing a stable baseline performance (>80 % accuracy and <20 % omissions with <10 % variation over five consecutive training sessions), rats underwent surgery.

4.4.3 Experiment 2: delay-discounting

4.4.3.1 Apparatus

The delay-discounting procedure was carried out in two standard, automated Skinner boxes (29.8 cm long x 24.1 cm wide x 28.2 cm high; Modular Test Cage System, Patent No. 3830201; Coulbourn Instruments, Whitehall, PA, USA). Each operant chamber consisted of two transparent polycarbonate side walls, one hinged to enable insertion of the animal, an aluminium rear wall, an aluminium roof and a grid floor composed of parallel, 6.4 mm metallic rods mounted over a plastic excrement pan. The aluminium front panel comprised three modular columns including a 2 W house light on top and a tray at the bottom of the middle column. The food magazine (5.3 cm wide x 6 cm high) was equipped with a 1 W miniature bulb and a hinged Plexiglas® panel connected with a microswitch to detect nose-poke entries into the receptacle. A motor-driven (50-L series; Ledex Inc., Datton, OH, USA) food dispenser delivered 45 mg casein pellets into the tray. Each lateral column included a retract-

able lever (Bilaney Consultants GmbH, Düsseldorf, Germany) and a stimulus light above the lever. The chambers were integrated in sound-attenuating boxes and controlled via a self-modified interface (Med Associates, Inc., St. Albans, VT, USA) by self-programmed software based on InstaCal (Measurement Computing Corporation, Norton, MA, USA).

4.4.3.2 Training

The delay-discounting task is a modified version of the procedure developed by Evenden and colleagues (Evenden and Ryan 1996). The training schedule was divided into a habituation, pretraining and baseline training stage. For the purpose of habituation, the rats (n = 11) were first introduced in the operant chambers for a period of 30 min with a few pellets placed on the extended levers and into the food magazine.

The pretraining was divided into three different phases: the two-lever continuous reinforcement (CRF) mode followed by two nose-poke training levels. During the CRF mode, the animals learned to lever press for food. Both levers were presented at the same time with only one lever determined as the rewarding over the entire daily session of 30 min. After reaching the criterion of 200 presses in one session, the reinforcing lever shifted, counterbalanced between the subjects. Pressing the rewarding lever delivered one pellet into the simultaneous illuminated magazine. The house light as well as the stimulus lights was permanently active over the session.

The following pretraining steps served to associate nose-poking with trial initiation. Each daily session consisted of 90 trials. Each trial started with both levers retracted. The rats had the chance to nose-poke into the lighted tray to trigger the presentation of a single lever. In that case, the magazine light was shut down and the house light together with the lever-corresponding stimulus light was powered on. If the animal failed to respond inside 30 s, the program proceeded to a 10 s period of darkness (ITI). A lever press within 30 s was rewarded with one pellet and accompanied by lever retraction and shutdown of the house light and stimulus light. By contrast, the food tray was illuminated either until the rat nose-poked again to collect the food or for 30 s from lever extension on. Thereafter, the next trial followed. In every pair of trials, each lever was presented once in a random order. When the animals performed at least 80 successful trials within a session over three consecutive days, they achieved the last pretraining stage. Herein, the time to initiate a trial and the duration for lever press and food collection were shortened to 10 s. The criterion to reach the subsequent baseline training was consistent with the previous.

During the baseline training session, the pretraining sequence was expanded by a progressive delay-discounting procedure. A daily training session comprised 60 trials divided into five blocks with 12 trials. Each trial lasted 100 s. Each block started with two forced-choice trials with only one lever presented randomly in every pair of forced-choice trials. In the following ten trials, the animals had the free choice between both levers, whereby one lever was always defined as the high reward (HR) lever while the other one was determined as the low reward (LR) lever, equally distributed among the rats. The selection of the LR lever delivered one pellet per press immediately, whereas the choice of the HR lever was associated with four pellets per press, but only provided after a delay increasing with each block (0, 10, 20, 40 and 60 s). The levers were retracted immediately after a press. Following food collection, the chambers returned to the ITI state. Omitted responses at the food magazine or on the levers within 10 s, respectively, were scored as omissions and punished with the return into the dark ITI. The switching of the lights remained just as in the preceding pretraining phase. The rats executed the baseline training until they achieved a stable delay-discounting performance.

4.4.4 Surgery

The rats were anaesthetised with chloral hydrate (360 mg/kg; Sigma-Aldrich, Steinheim, Germany) and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Stainless steel 21 gauge guide cannulae were implanted bilaterally 1 mm above the target

injection site into the vmPFC (anteroposterior +2.7 mm, mediolateral ±0.8 mm, dorsoventral -4.0 mm from Bregma). Jeweller screws were anchored in the skull serving to fix the cannulae which were embedded in dental cement and closed by removable 26 gauge stylets of the same length. After surgery, the rats were kept individually for three days with free access to food and water. Following a total recovery period of five days, the animals were reintroduced to the baseline training until they re-established the presurgical performance.

4.4.5 Microinfusion procedure

The test design comprised three 4-day sessions for the animals. Each session started with an injection day, followed by a day without training. The second and third post-testing day were used to achieve the baseline performance and to ensure the washout process of the drug. Before infusion, the stylets were exchanged for 27 gauge injection cannulae connected with microlitre syringes (SGE Scientific Glass Engineering, Darmstadt, Germany) via polyethylene tubes. The rats received bilateral intra-vmPFC microinjections of the $GABA_A$ agonist muscimol (0.05, 0.5 µg/0.3 µl) and 0.9 % saline as vehicle (0.3 µl) according to a pseudorandom Latin square design. The injection rate was 0.1 µl/30 s. The injectors were left in place for 1 min to guarantee diffusion and to avoid reflux of the solution. Ten minutes after the microinjection, the rats underwent behavioural testing. The sequence of the test sessions matched with the baseline training.

4.4.6 Drugs

The $GABA_A$ agonist muscimol was purchased from Tocris Bioscience (Ellisville, MO, USA) and dissolved in 0.9 % saline. Aliquots of stock solutions (0.5 µg/0.3 µl) were prepared and stored at -20 °C until use. If necessary, aliquots were further diluted to a dose of 0.05 µg/0.3 µl on the treatment day. Doses were based on previous studies (Diederich and Koch 2005).

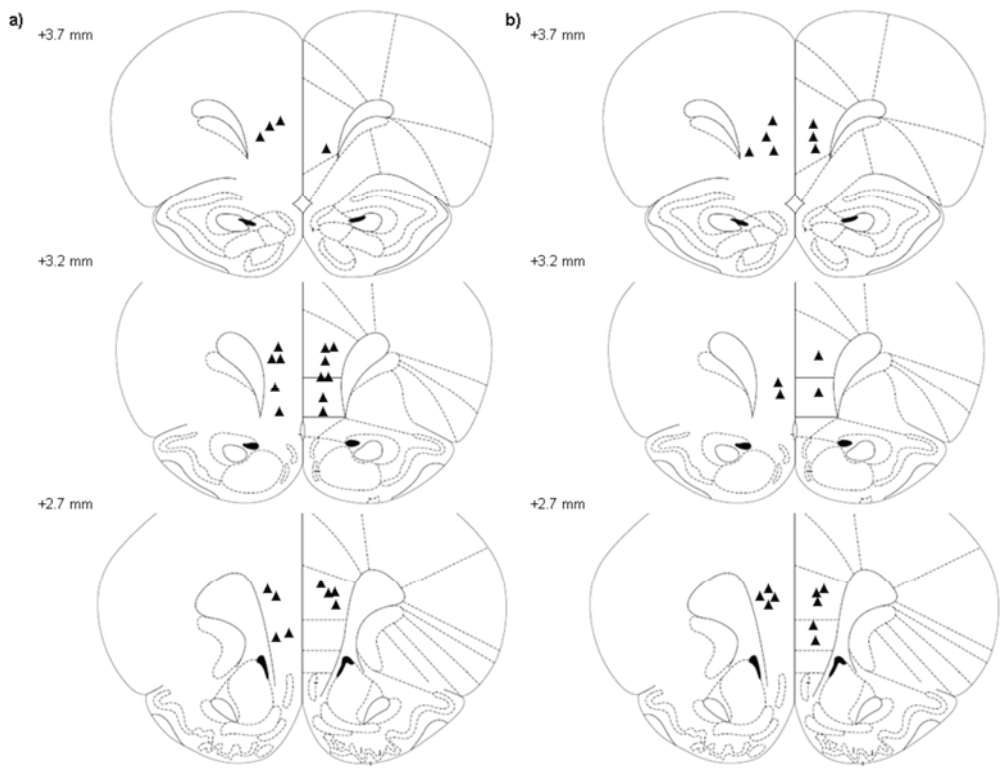

Fig. 4.1 Location of the bilateral injection sites (triangles) in the ventral medial prefrontal cortex in **a)** the 5-choice serial reaction time task (n = 12) and **b)** the delay-discounting task (n = 10) depicted on schematic drawings from the rat brain atlas of Paxinos and Watson (1998). Rostral distance (in mm) to bregma is indicated by numbers.

4.4.7 Histology

Upon termination of the experiment, the rats were euthanised with a lethal dose of chloral hydrate. The brains were removed from the skull and immersion-fixed in a 4 % formalin/30 % sucrose solution for 48 h. Coronal 50 µm sections of the vmPFC were cut on a cryostat (Jung CM 3000; Leica Instrument GmbH, Nussloch, Germany), mounted on gelatine-coated glass slides and Nissl-stained with thionin. Then, the sections were analysed using a light microscope and injection sites plotted on standardised coronal sections of a rat brain stereotaxic atlas (Paxinos and Watson 1998).

4.4.8 Data analysis

The descriptive statistics is based on means and variance and is indicated by the standard error of the mean (±SEM). The statistical analyses were conducted by the software SigmaStat (version 2.0 for Windows).

In experiment 1, the drug effects within the testing group on the following behavioural parameters were investigated using two-way repeated measures analysis of variance (ANOVA; factors: drug treatment and behavioural parameter): percentage of correct responses (accuracy; 100 x number of correct responses/number of correct and incorrect responses), percentage of omitted responses (100 x number of omitted responses/total number of correct, incorrect and omitted responses), number of premature responses, number of perseverative responses, number of trials completed, number of time-out responses, latency of correct responses [s] and latency of reward collection [s]. In the case of significant main effects ($P < 0.05$), one-way repeated measures ANOVA and post hoc Tukey`s *t*-tests for pairwise comparisons were conducted.

In experiment 2, the training performance of the rats had to be examined statistically at first to verify that they achieved the baseline criteria. Therefore, the forced-choice trials were excluded from the data and the percentage choice of HR (HR lever responses/total responses) was calculated per day and delay for each animal. The data from five consecutive sessions were analysed by two-way repeated measures ANOVA with the within-subject factors day and delay. If the effect of delay was significant ($P < 0.05$) and there was no main effect of day or delay x day interaction, the rats reached a stable baseline performance. The data of the behavioural tests were analysed by two-way repeated measures ANOVA with the within-subject factors drug treatment and delay. Additionally, the omission rate [%] and the latency of lever responses [s] were calculated.

4.5 Results

4.5.1 Histology

A depiction of the injector tips located within the vmPFC is shown in Fig. 4.1.

The histological analysis revealed that all except one rat, which had to be excluded from the results, had acceptable injection sites accurately positioned to the target structure with minimal tissue damage as indicated in the representative section in Fig. 4.2.

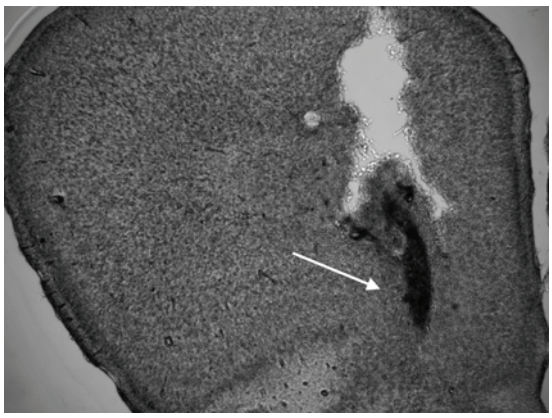

Fig. 4.2 Representative photomicrograph of the injection site in the ventral medial prefrontal cortex of rats indicated by the arrow.

4.5.2 Experiment 1: effects of inactivation of the vmPFC on rats` performance in the 5-CSRTT

The rats performed a stable baseline throughout the entire term of the experiment with high levels of accuracy (90.52±0.99 %), fast

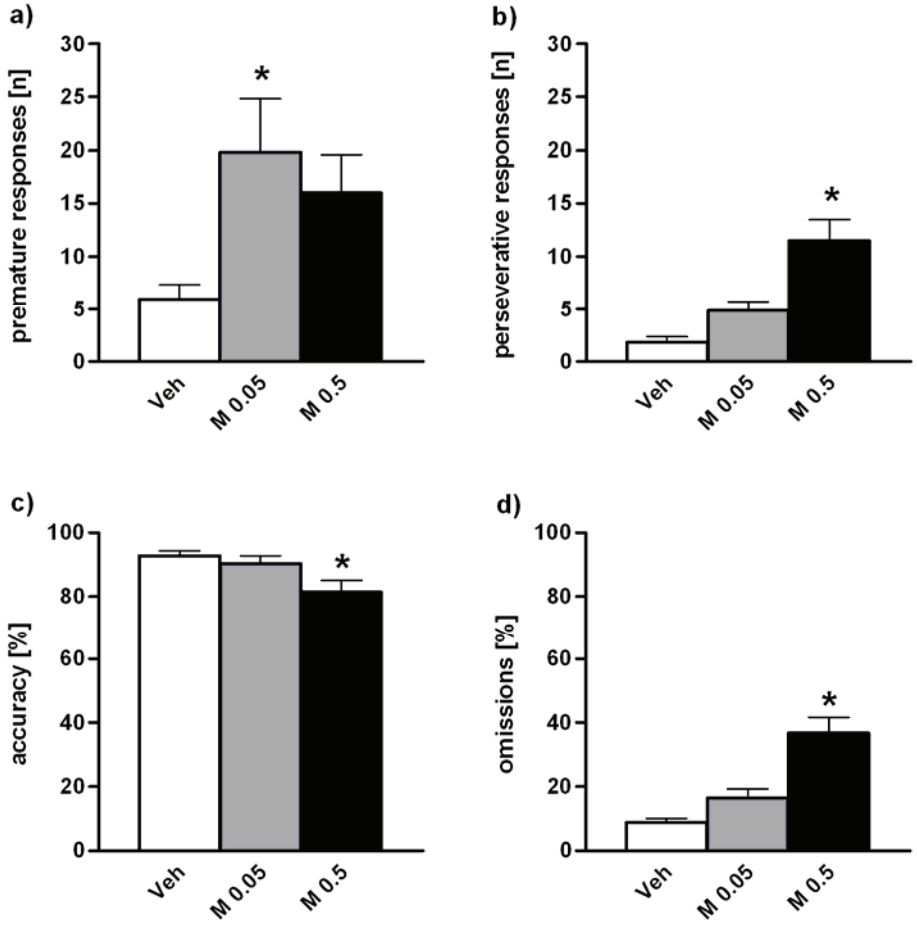

Fig. 4.3 Effects of local bilateral infusions of the $GABA_A$ agonist muscimol (M; 0.05, 0.5 µg/0.3 µl) into the ventral medial prefrontal cortex on the rats` (n = 12) performance in the 5-choice serial reaction time task. Data of **a)** premature responses, **b)** perseverative responses, **c)** accuracy and **d)** omissions are means ±SEM. Statistically significant differences between drug treatment compared to vehicle (Veh) are indicated by asterisks (one-way repeated measures ANOVA, post-hoc Tukey`s *t*-test, $P < 0.05$).

latencies (correct responding: 0.69± 0.01 s; reward collection: 1.17±0.04 s) and low percentages of omissions (11.16±0.71 %) as well as low numbers of premature (7.42±1.15) and perseverative responses (2.64±0.62) before testing. Analysis of the training data demonstrated no significant differences in the pre- and postoperative sessions and the 'drug-free days' between testing excluding any carry-over effects of drug treatment or surgery (data not shown). Two-way repeated measures ANOVA on the 5-CSRTT performance showed main effects of drug treatment [$F_{(2,154)} = 10.829$; $P < 0.001$] as well as a statistically significant interaction between both factors [$F_{(14,154)} = 4.816$; $P < 0.001$]. Further one-way repeated measures ANOVAs and post hoc Tukey`s *t*-tests revealed that administration of the lower dose of muscimol (0.05 µg/0.3 µl) specifically enhanced impulsive behaviour reflected by a significant increase in premature responding compared to vehicle ($P = 0.033$), while no other measured parameter was affected (Fig. 4.3 and Table 4.1). Intra-vmPFC injection of the higher dose of muscimol (0.5 µg/0.3 µl) also appeared to augment premature responses, but this effect did not reach statistical significance (Fig. 4.3a). By contrast, this dose induced significant differences in perseverations ($P < 0.001$), accuracy ($P = 0.016$), omissions ($P < 0.001$), time-out responses ($P = 0.035$) and the latency of reward collection ($P < 0.001$) in comparison to vehicle (Fig. 4.3 and Table 4.1).

Table 4.1 Effects of local bilateral infusions of the $GABA_A$ agonist muscimol (0.05, 0.5 µg/0.3 µl) into the ventral medial prefrontal cortex on the rats` (n = 12) performance in the 5-choice serial reaction time task. Data are expressed as means ±SEM. *M* muscimol, *Veh* vehicle. *$P < 0.05$ vs. Veh (one-way repeated measures ANOVA, post-hoc Tukey`s *t*-test).

Treatment	Trials completed [n]	Time-out responses [n]	Latency of correct responding [s]	Latency of reward collection [s]
Veh	100.00±0.00	12.75±2.43	0.70±0.02	1.11±0.03
M 0.05	96.33±2.45	51.33±16.56	0.67±0.02	1.26±0.06
M 0.5	96.42±1.92	56.67±11.76*	0.78±0.04	1.85±0.18*

4.5.3 Experiment 2: effects of deactivation of the vmPFC on delay-discounting

Vehicle microinfusions affected delay sensitivity of the rats in a similar way as observed in the baseline training phase and in the sessions between the testing days (data not shown). The two-way repeated measures ANOVA of the choice of HR resulted in a main effect of delay [$F_{(4,72)} = 26.770$; $P < 0.001$], but there was no significant effect of treatment [$F_{(2,72)} = 1.527$; $P = 0.244$] indicating that none of the doses of muscimol affected the choice of the large reinforcer (Fig. 4.4a). Post-hoc comparisons confirmed the typical delay-dependent within-session shift in the preference of the HR with statistically significant differences between no delay and the longer delays of 40 s (vehicle: $P < 0.001$; 0.05 µg/0.3 µl muscimol: $P = 0.035$; 0.5 µg/0.3 µl muscimol: $P = 0.013$) and 60 s ($P < 0.001$ in each case) for any drug treatment. The analysis of the omission rate yielded main effects of delay [$F_{(4,72)} = 3.201$; $P = 0.024$] and treatment [$F_{(2,72)} = 6.305$; $P = 0.008$], but the effects of different treatments did not depend on what level of delay was present [interaction: $F_{(8,72)} = 1.634$; $P = 0.130$]. The omission rate increased with the delay, but the sole significant effect compared to vehicle was obtained in the 40 s delay period by the higher dose of muscimol ($P = 0.007$) (Fig. 4.4b). After administration of saline (5.20 ±3.41 %) and 0.05 µg/0.3 µl muscimol (7.00 ±3.89 %), the omission rate remained very low throughout the session and the latencies to respond on the lever were similar in all delays. Highly-dosed muscimol slightly prolonged the lever response

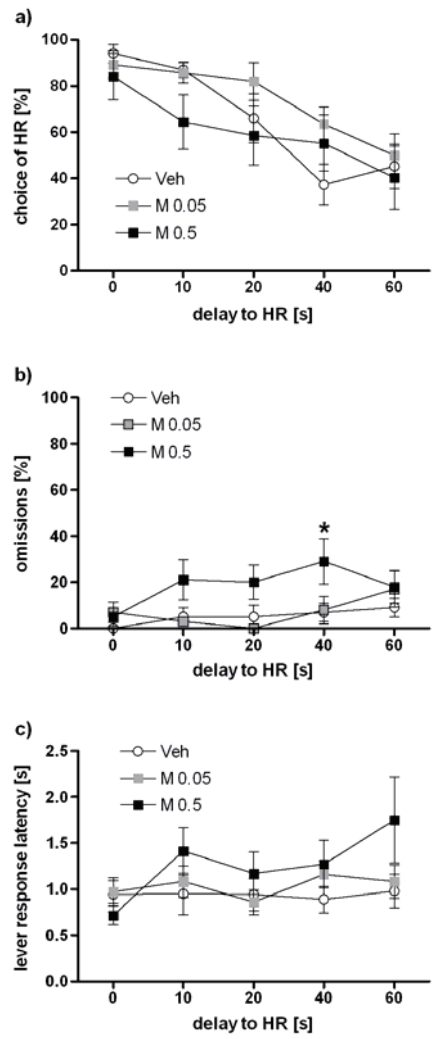

Fig. 4.4 Effects of local bilateral infusions of the $GABA_A$ agonist muscimol (M; 0.05, 0.5 µg/0.3 µl) into the ventral medial prefrontal cortex on the rats` (n = 10) performance in the delay-discounting task. Data of a) choice of high reward (HR), b) omissions and c) lever response latency are means ±SEM. Statistically significant differences between drug treatment compared to vehicle (Veh) are indicated by asterisks (two-way repeated measures ANOVA, post-hoc Tukey`s *t*-test, $P < 0.05$).

latency (Fig. 4.4c) without reaching significance as shown by two-way repeated measures ANOVA [treatment: $F_{(2,72)} = 1.276$; $P = 0.303$, delay: $F_{(4,72)} = 2.333$; $P = 0.074$, interaction: $F_{(8,72)} = 1.476$; $P = 0.181$].

4.6 Discussion

The main findings of this study are that reversible inactivation of the rats` vmPFC by the $GABA_A$ agonist muscimol induced deficits in impulse control but did not affect delay-discounting. Our results support the relevance of the vmPFC for the control of impulsive action in the 5-CSRTT as indicated by increased premature responding after administration of muscimol. Both doses of muscimol produced a marked increase of impulsive action, whereby the lower dose (0.05 µg/0.3 µl) was sufficient to significantly enhance anticipatory responses with lacking effects on any other behavioural parameters. The current behavioural effects of muscimol support previous inactivation studies and corroborate the theory that the ventral part of the mPFC, including the prelimbic (PL) and infralimbic (IL) cortices, is critically involved in controlling premature responding in the 5-CSRTT in rats (Chudasama et al. 2003;Murphy et al. 2012;Paine et al. 2011). Besides premature responses, the high dose of muscimol also significantly increased perseverative behaviour and time-out responses, representing other aspects of inhibitory control, more related to compulsivity and behavioural flexibility (Robbins 2002). Perseverative responding reflects the inability to stop a compulsive repetition of reaction without purpose after a correct response has already been made (Carli et al. 2006;Robbins 2002). In the 5-CSRTT, such inappropriate responses are punished by a period of darkness, called time-out (Bari et al. 2008). Hence, it is not surprising that ongoing responding during the time-out comes along with increased perseverative reactions. In this regard, vmPFC lesions or inactivation result in behavioural inflexibility and increased perseverative errors in reversal learning tasks in rats (Kosaki and Watanabe 2012;Ragozzino 2007;Ragozzino et al. 1999). Moreover, abnormalities in the AC, the putative human equivalent of the rodent vmPFC, are correlated with obsessive-compulsive disorder, a syndrome hallmarked by perseverative behaviours (Kuhn et al. 2013;Remijnse et al. 2013). Cognitive constructs such as impulsivity, compulsivity and flexibility are closely interrelated executive processes in the context of inhibitory control, hierarchically top-down mediated by the PFC (Bari and Robbins 2013;Wise 2008). Latest findings support the role of the mPFC in action monitoring and motor impulsivity in that low-frequency oscillations within rat and human medial frontal cortex synchronise local and motor cortex neurons facilitating the representation and exertion of adaptive control (Narayanan et al. 2013).

The methodological advantage of the 5-CSRTT is based on the dissociation of several behavioural aspects like impulse control, motivation, reaction time and attention (Robbins 2002). High-dose muscimol (0.5 µg/0.3 µl) infusions caused a significant decrease in accuracy and an increase in the omission rate, a combined effect most likely reflecting an attentional deficit. High numbers of omissions might also indicate sensory, motor or motivational factors (Robbins 2002). Indeed, $GABA_A$ receptor activation in the vmPFC slightly increased the latency of reward collection following the high-dose injection of muscimol, suggesting incentive motivational influences (Rogers et al. 2001). However, the latency of correct responding and the number of completed trials were not affected by muscimol excluding locomotor or sedative drug effects (Robbins 2002). Besides, $GABA_A$-mediated inhibition of the vmPFC enhances feeding behaviour in rats (Kelley et al. 2005). The attentional impact of the mPFC is confirmed by results of mPFC lesions causing impairments in accuracy in the 5-CSRTT and maze tasks (Passetti et al. 2002;Pezze et al. 2009;Ragozzino et al. 1998). Many authors postulate that high scores of impulsivity in the 5-CSRTT inversely correlate with attentional accuracy (Blondeau and Dellu-Hagedorn 2007;Dalley et al. 2008;Puumala and Sirvio 1998). Considering the central role of prefrontal dysfunction to the pathophysiology of ADHD, incorporating attentional and impulsive dysfunctions (Castellanos and Tannock 2002), it

seems obvious that this relationship could also be valid for the mPFC. But there is evidence to suggest, as unveiled by the different pattern of results following both muscimol treatments in our study, that the mPFC of rats should not be viewed uniformly. Lesion studies suggest dissociable roles of the dorsal and ventral subregions of the mPFC on the 5-CSRTT performance. IL and PL seem to be more implicated in impulsive and compulsive behaviours, whereas attentional and motivational parameters like accuracy, omissions and the latency of reward collection appear to be rather modulated by the dorsally located anterior cingulate cortex (AC) (Chudasama et al. 2003;Chudasama and Muir 2001;Passetti et al. 2002). In our study, the effects on the latter aspects could be attributed to the involvement of adjacent brain areas due to diffusion of muscimol. According to Fick's law of diffusion, the spatial extent of a drug correlates with its initial concentration (Edeline et al. 2002). Autoradiographic estimation of intracortical spread and glucose metabolism after administration of a slightly lower dose of muscimol (1 µg/µl) revealed a mean radial spread of 1.66 mm and reduced neuronal activity in a radius of several millimeters (Martin 1991;Martin and Ghez 1999). In conjunction with the suggestion that drug diffusion goes up along the cannulae by capillarity forming an ellipsoidal area of inactivation (Hupe et al. 1999), the AC might additionally be affected by higher doses of muscimol. This hypothesis is supported by large lesions encompassing dorsal and ventral subregions of the mPFC, which cause deficits in both cognitive domains, namely attention and impulsivity, in contrast to those restricted to the respective subareas (Pezze et al. 2009).

To our knowledge, this is the first study using the inactivation technique with muscimol at the level of the vmPFC with respect to impulsive behaviour specifically comparing impulse control with delay-discounting in rats. The present study demonstrates that in contrast to impulsive action, impulsive choice in the delay-discounting task is not significantly controlled by the vmPFC. We observed a flattening in the typical delay-dependent within-session shift in the preference of the HR for both muscimol doses, similar to the findings of Cardinal et al. (2001) following mPFC lesions, primarily PL and IL. For clarification, in case of no delay rats with inactivated vmPFC chose the HR less than controls and after treatment with low-dosed muscimol the preference of the large reward was even higher at the maximum delay compared to control. One possible explanation for this observation might be that reversible inactivation of the vmPFC caused insensitivity to the task contingencies by a disruption of temporal discrimination (Cardinal et al. 2004). Delay-discounting describes the function by which a reward is subjectively devalued by a delay to its delivery. The two reinforcers vary in both size and delay, so the the choice of the LR could reflect impulsivity or changes in motivational behaviour, as motivation in goal-directed behaviour depends on the expected value of the anticipated reward and impulsive subjects are characterised by a greater delay aversion compared to normal individuals (Spreckelmeyer et al. 2009;Winstanley et al. 2006). Consequently, an impulsivity-promoting or demotivating impact of the transiently inactivated vmPFC would have been expected to result in a greater reduction of the delayed reward choice expressed as a steeper discounting curve. Instead, the animals appeared to lack temporal stimulus control and average their choice of the high reward over the session, independent of delay. Aspiration lesions of the mPFC have shown to generate a general deficit in timing ability in the peak interval procedure in rats (Dietrich and Allen 1998). More recently, intra-mPFC administration of muscimol in rats impaired time interval discrimination in the range of a few seconds indicating that the mPFC might be part of an internal clock in the brain (Kim et al. 2009;Kim et al. 2013). More precisely, Narayanan and colleagues lately found that prefrontal D1 dopamine signalling in rats is necessary for temporal control in fixed-interval timing and temporal expectation in simple reaction time tasks (Narayanan et al. 2012;Parker et al. 2013). Such deficits in temporal control following vmPFC inactivation might explain our results of the 5-CSRTT and delay-discounting task, which both require rats'

waiting capacity. However, in contrast to the delay-discounting paradigm, the 5-CSRTT primarily demands the rats to suppress their drive to respond and to a lesser extent internally timing their behaviour. Since the mPFC, particularly the PL, plays a distinctive role in the detection of instrumental contingencies (Balleine and Dickinson 1998), another possibility could be that the present inactivations abolished the learned association between response and reward delivery. Yet, recent findings suggest that the prelimbic sector is not critical for the formation of action-outcome associations (Tran-Tu-Yen et al. 2009).

High-dose muscimol further led to a delay-dependent increase in the omission rate, an effect less attributable to a general impairment of performance as mPFC disruptions do not impair primary motivation in decision-making tasks (Gill et al. 2010;Walton et al. 2002;Walton et al. 2003). Another explanation might be motor side-effects of treatment. Indeed, the enhanced rate of omissions was accompanied by slowed lever response latency and intra-mPFC injection of muscimol (0.05 µg/0.5 µl) reduces locomotor activity in an open field (Paine et al. 2011). Since vmPFC-inactivated rats did not show impairments of response latency in the 5-CSRTT and even respond faster in a simple reaction time task (Narayanan et al. 2006), the impact on omissions and latencies might also be more linked to deficits in temporal task contingencies.

Impulsive choice is more related to decision-making than to motor inhibition as required in the 5-CSRTT (Winstanley et al. 2006). Decision-making is considered as the emergence of preferences between alternative conducts based on a mental evaluation of their outcome (Sanfey and Chang 2008). It is composed of three distinct processes: 1) the judgment of different options, 2) the selection and execution of an action, and 3) the experience or evaluation of the corresponding consequences (Ernst and Paulus 2005). Most decision-making procedures confront the individuals with two alternatives differing in cost and benefit. The increment of costs for the usually more-preferred larger reward leads to a discounting in the value of this option. Discounting models assess the choice behaviour in relation to delay, effort or probability of reward (Floresco et al. 2008b).The execution of a decision necessitates the interaction of multiple underlying systems. However, earlier investigations found remarkable discrepancies in the role of different subregions of the PFC in these decision-making processes (Floresco et al. 2008b). In humans, the DLPFC may participate by monitoring and timing of actions (Ernst and Paulus 2005). Accordingly, enhanced lateral prefrontal activation is observed during the selection of later rewards suggesting that an intact DLPFC is required for the choice of delayed gratification (Ballard and Knutson 2009;McClure et al. 2004). Altered activity in human AC is associated with impulsive decision-making in delay-discounting tasks (Hinvest et al. 2011;Hoffman et al. 2008;Li et al. 2013;Xu et al. 2009). In rats, lesion and reversible inactivation studies have already linked the mPFC with effort-, risk- and delay-discounting, though revealing inconsistencies regarding dorsal and ventral parts of this area (Churchwell et al. 2009;de Visser et al. 2011b;Paine et al. 2013;Rivalan et al. 2011;Rudebeck et al. 2006;St Onge and Floresco 2010;van Enkhuizen et al. 2013;Walton et al. 2003;Walton et al. 2002). Among those three types, temporal discounting mostly reflects impulsive behaviour (Bari and Robbins 2013). Unfortunately, the precise relevance of the mPFC for impulsive choice is still unclear. Delay-discounting procedures produce contradictory results depending on the inactivation technique, the mPFC subregion and the behavioural task that is used. Permanent deactivation via excitotoxic lesion of the AC does not affect rats` performance, irrespective of which delay-discounting type is applied (Cardinal et al. 2001;Rudebeck et al. 2006). In contrast, effects of lesioning the PL and IL seem to be task-specific. While the typical paradigm measuring choice between large-but-delayed and small immediate rewards in an operant chamber, as in our case, also detects no impulsivity-related behaviour, PL/IL lesions induce impulsive choice in a sustained response task with a single response operandum (Cardinal et al. 2001;Gill et al. 2010). Unlike our study, transient inacti-

vation of the ventral subareas of the mPFC by muscimol increases delay-discounting in a T-maze task (Churchwell et al. 2009). However, the findings of Churchwell et al. (2009) and Gill et al. (2010) have to be compared carefully with those of our present study. The study of Gill and colleagues (2010) probably reflects more a mixture of impulsive choice and motor impulsivity because subjects must make a decision to initiate responding but the task also requires them to maintain their response and to inhibit their natural tendency to withdraw. Generally speaking, the results of Churchwell et al. (2009) appear discrepant with the present, particularly as both tasks used different delay ranges. However, closer considering the discrete session blocks in our study reveals a trend towards delay aversion for the shortest delay duration (10 s) following treatment with high-dosed muscimol. Interestingly, the duration of the delay is similar to that of Churchwell et al. (2009) suggesting that the vmPFC, at least partially, might be implicated in impulsive decision-making with rewards delayed in the range of a few seconds. Previous studies have shown that prolonged delay periods of ≥25 s are suitable to assess drug-induced improvements of waiting capacity, but to a lesser extent a decrease in the tolerance of delay (Bizot et al. 2007). Other groups already proved the feasibility of discounting paradigms using shorter delays to detect impulsive choice (Floresco et al. 2008c). Further experiments on temporal discounting with short delays could help to elucidate a potential involvement of the vmPFC in impulsive decision-making.

Increasing evidence suggests that impulsive behaviour is controlled depending on which neuroanatomical system is involved and which aspects of impulsivity are investigated in a special task (Evenden and Ryan 1999). Within the rat literature, impulse control in the 5-CSRTT has been most widely used as an index of impulsive action in comparison with impulsive choice in delay-discounting tasks (Eagle and Baunez 2010). It is claimed that each form of impulsivity includes a kind of impulsive action, which is necessary to choose a response option. The conceptional difference consists in that there is no need to forcibly inhibit a prevalent reaction in impulsive choice compared to impulse control (Winstanley et al. 2006). Participating neuronal structures form cortico-limbic-striatal circuits with considerable overlap in relation to impulsive action and inter-temporal choice (Pattij and Vanderschuren 2008). Rats showing high levels of impulsivity in the 5-CSRTT also exhibit a greater propensity for impulsive decision-making in a delayed reward task (Robinson et al. 2009), suggesting that the vmPFC may be involved in both types of impulsivity. However, in a recent study both rats and humans display no correlation in measures of impulsive choice and impulsive action (Broos et al. 2012). Motor impulsivity is assumed to be principally top-down controlled by the mPFC, while impulsive decision-making depends more on the functional integrity of the OFC, which in turn is less involved in impulse control (Dalley et al. 2008;Hadamitzky and Koch 2009;Wischhof et al. 2011). Together with the present findings, this supports the assumption that the various forms of impulsivity rely on separate, yet partly overlapping neural pathways (Dalley et al. 2011;Pattij and Vanderschuren 2008).

4.7 Conclusions

This is the first study directly comparing the role of vmPFC in two main types of impulsivity. Taken together, the vmPFC comprising PL and IL is critically implicated in impulse control in the 5-CSRTT, but does not mediate impulsive decision-making in the delay-discounting task in rats. Our data confirm the hypothesis of various dimensions underlying impulsivity with a clear-cut distinction of impulsive choice and impulsive action on the level of the vmPFC.

Acknowledgement

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5 Nucleus accumbens core and shell inactivation differentially affects impulsive behaviours in rats

Malte Feja · Linda Hayn · Michael Koch

5.1 HIGHLIGHTS

- The $GABA_A$ agonist muscimol was used to inactivate NAc core and shell in rats
- Deactivation of NAc core as well as shell induced impulsive choice in the T-maze
- Muscimol into NAc shell, but not core, reduced impulse control in the 5-CSRTT
- Muscimol-BODIPY was not suitable to assess the spatial extent of inactivation
- NAc subregions differentially contribute to distinct types of impulsivity

5.2 ABSTRACT

Impulsivity is a multifactorial phenomenon, determined by deficits in decision-making (impulsive choice) and impulse control (impulsive action). Recent findings indicate that impulsive behaviour is not only top-down controlled by cortical areas, but also modulated at subcortical level. The nucleus accumbens (NAc) might be a key substrate in cortico-limbic-striatal circuits involved in impulsive behaviour. Dissociable effects of the NAc subregions in various behavioural paradigms point to a potential functional distinction between NAc core and shell concerning different types of impulsivity. The present study used reversible inactivation of the rats' NAc core and shell via bilateral microinfusion of the $GABA_A$ receptor agonist muscimol (0.05 µg/0.3 µl) and fluorophore-conjugated muscimol (FCM, 0.27 µg/0.3 µl) in order to study their contribution to different aspects of impulse control in a 5-choice serial reaction time task (5-CSRTT) and impulsive choice in a delay-based decision-making T-maze task. Acute inactivation of NAc core as well as shell by muscimol increased impulsive choice, with higher impairments of the rats' waiting capacity in the T-maze following core injections compared to shell. Intra-NAc shell infusion of muscimol also induced specific impulse control deficits in the 5-CSRTT, while deactivation of the core caused severe general impairments in task performance. FCM did not affect animal behaviour. Our findings reveal clear involvement of NAc shell in both forms of impulsivity. Both subareas play a key role in the regulation of impulsive decision-making, but show functional dichotomy regarding impulse control with the core being more implicated in motivational and motor aspects.

5.3 Introduction

Impulsivity is a behavioural phenomenon that both adversely and beneficially affects living conditions (Eysenck and Eysenck 1977). From a theoretical point of view, impulsive behaviour results from the relation between an incentive (impulsive drive) and an inhibitory dimension (impulse control) (Herpertz and Sass 1997). Impulse control is described as an active inhibitory mechanism, which modulates an internally or externally driven prepotent desire for a primary (food) or secondary (money) reinforcer. Rapid, conditioned reactions are transiently suppressed so that slower cognitive patterns can guide behaviour (Eagle and Baunez 2010;Winstanley et al. 2006). Dysfunctional impulse control (e.g., acting prematurely without foresight) is referred to as impulsive action or motor impulsivity (Brunner and Hen 1997;Dalley et al. 2011) and is often measured in the 5-choice serial reaction time task (5-CSRTT) in rats, which was modelled after its human analogues, the continuous performance test of attention and Leonard's five choice serial reaction time task (Carli et al. 1983;Muir et al. 1996;Robbins 2002). As a multifactorial phenomenon, impulsivity is generally distinguished into impulsive action and impulsive choice (Evenden 1999b;Pattij and Vanderschuren

2008;Winstanley et al. 2006). The dominant behavioural model to assess impulsive decision-making in both humans and rodents is the delay-discounting task, where impulsive tendencies are reflected in the preference for a small immediate over a larger-but-delayed reward due to delay aversion and reduced waiting capacity (Broos et al. 2012;de Wit 2009;Moeller et al. 2001;Swann et al. 2002). High levels of impulsivity are expressed in many psychiatric disorders, involving attention-deficit/hyperactivity disorder (ADHD), antisocial personality disorder, borderline personality disorder, schizophrenia, drug abuse and other forms of addiction (de Wit 2009;Evenden 1999a;Herpertz and Sass 1997). Functional magnetic resonance imaging (fMRI) studies in ADHD individuals suggest a contribution of corticostriatal circuitry to impulse control disorders, including the nucleus accumbens (NAc) as part of the ventral striatum (Costa Dias et al. 2013;Jupp et al. 2013). Moreover, previous studies associated the NAc with impulsive cocaine-, alcohol- and food-seeking (Kalivas and Volkow 2005;Koob 1992;LaLumiere et al. 2012).

The NAc is implicated in decision-making (Assadi et al. 2009;Day et al. 2011;de Visser et al. 2011a) and anticipation of reward in humans, other primates and rats (Cromwell and Schultz 2003;Knutson et al. 2001;Martin and Ono 2000;Rademacher et al. 2014). Human studies found activation of the NAc during performance in delay-discounting tasks (Ballard and Knutson 2009;Hariri et al. 2006;McClure et al. 2004;Wittmann et al. 2007) and a negative correlation between striatal dopamine D2/3 receptors and impulsive choice or action (Ghahremani et al. 2012;Lee et al. 2009). Such a reduced D2/3 receptor availability in the NAc was also observed in a 5-CSRTT study of impulsive rats (Dalley et al. 2007a).

As a critical element of the mesocorticolimbic system, the NAc is generally implicated in reward and motivation. The original concept of the NAc as a functional limbic-motor interface is still valid, but findings of the past two decades revealed much more differentiated insights indicating that the NAc should not longer be viewed in the sense of an anatomical entity (Groenewegen and Trimble 2007;Heimer 2003;Mogenson et al. 1980). On the basis of anatomical, neurochemical and electrophysiological criteria, the NAc in the rat brain is divided into distinct subterritories which are also present in the human brain: a dorsolateral core region surrounding the anterior commissure, and a shell region that is situated ventromedially to the core (Meredith et al. 1996;Sokolowski and Salamone 1998;Zaborszky et al. 1985). In rats, considerable differences exist in the input-output features of core and shell. In particular, the medial prefrontal cortex (mPFC) projects topographically to the NAc. Dorsal regions of the mPFC (anterior cingulate and dorsal prelimbic cortices) primarily innervate the core while the shell receives afferents from ventral parts of the mPFC, including ventral prelimbic and infralimbic cortices (Berendse et al. 1992;Brog et al. 1993;Heidbreder and Groenewegen 2003). The efferents also contribute to the core-shell dichotomy. The core region sends fibers to the conventional basal ganglia circuitry, whereas shell projections extensively reach subcortical limbic structures (Heimer et al. 1991;Zahm and Brog 1992).

These differences in connectivity suggest that the NAc subregions might also differ functionally (Corbit et al. 2001). Lesion studies and intracerebral pharmacological manipulations previously demonstrated that NAc core and shell are differentially involved in goal-directed instrumental action (Corbit et al. 2001), Pavlovian-instrumental transfer (Corbit and Balleine 2011;Saddoris et al. 2011), behavioural flexibility (Floresco et al. 2006), stress-, cue- or cocaine priming-induced reinstatement of drug- or food-seeking behaviour (Floresco et al. 2008a;McFarland et al. 2004;Vassoler et al. 2013), working memory (Jongen-Relo et al. 2003), locomotor activity (Jongen-Relo et al. 2002;Pothuizen et al. 2005a;Robbins and Everitt 1996), motivational behaviour (Bassareo et al. 2002;Stratford and Kelley 1997) and attentional processes, like prepulse and latent inhibition (Jongen-Relo et al. 2002;Pothuizen et al. 2005a).

The functional dichotomy at the level of the NAc also holds true for impulsive behav-

iours. While there is strong evidence that core lesions promote impulsive choice (Bezzina et al. 2007;Bezzina et al. 2008a;Cardinal et al. 2001;da Costa et al. 2009;Pothuizen et al. 2005b), shell lesions do not (Pothuizen et al. 2005b). Additionally, rats' exposure to an adjusting-delay schedule in inter-temporal choice is associated with enhanced neuronal activity in the NAc core (da Costa et al. 2010). However, the effect of core lesions remains unclear due to discrepancy with other studies yielding no choice impulsivity (Acheson et al. 2006;Cardinal et al. 2001;Gill et al. 2010).

Regarding impulse control, accumbal DA depletions as well as excitotoxic lesions of NAc shell lack an effect on anticipatory responding in response inhibition tasks (Cole and Robbins 1989;Murphy et al. 2008;Pothuizen et al. 2005b), whereas accumbal 5-HT depletions and lesions of the core show impairments in 5-CSRTT and differential reinforcement for low rates of responding (DRL) tasks (Christakou et al. 2004;Fletcher et al. 2009;Pothuizen et al. 2005b). More insights are provided by recent pharmacological manipulations, highlighting a potential involvement of the shell. In both NAc core and shell, dopamine D_1-like and D_2-like receptors are involved in inhibitory response control (Pattij et al. 2007). Other findings support divergent roles of core and shell in regulating impulse control (Besson et al. 2010;Economidou et al. 2012;Sesia et al. 2008). DA function in the NAc varies between the subregions and further underlines the heterogeneity of core and shell. Impulsive action in the 5-CSRTT correlates with increased DA release due to reduced dopamine D2/3 receptor availability and higher D1 receptor mRNA expression in the shell, but decreased DA release caused by lower D1 receptor binding in the core (Diergaarde et al. 2008;Jupp et al. 2013;Simon et al. 2013).

The lesion technique was the most widely used method to investigate brain function, although carrying some drawbacks due to permanent destruction of brain tissue and a potential functional compensation by other brain areas. These shortcomings can be avoided using reversible acute inactivation procedures (Lomber 1999). Up to now only a few studies investigated the role of NAc subregions in impulsivity using lesions or transient inactivation methods. Local microinfusion of the $GABA_A$ receptor agonist muscimol allow repeated reversible inactivation of distinct brain regions, and hence, within-subject designs with increased reliability (Lomber 1999). Muscimol represents an appropriate inactivation tool, as $GABA_A$ receptors are widely distributed throughout the NAc located on medium-sized, spiny neurons (MSN) (Schwarzer et al. 2001). Muscimol selectively induces a rapid hyperpolarization lasting up to several hours on postsynaptic neurons via activation of $GABA_A$ receptors on the surface of local cell bodies without affecting fibers of passage, thereby allowing behavioural testing almost immediately after injection (Edeline et al. 2002;Heiss et al. 2010;Krupa et al. 1999;Martin and Ghez 1999). In contrast, the lesion technique requires several days for the animals to recover, enabling the development of adaptive functions of remaining structures (Martin and Ghez 1999). Additionally, fluorescent conjugates, like fluorophore-conjugated muscimol (FCM), may help to evaluate the spatial extent of drug-infused tissue.

In the present study, reversible inactivation of the rats' NAc core and shell via bilateral microinfusion of the $GABA_A$ receptor agonist muscimol and FCM was used for the first time to analyse their contribution to impulse control in the 5-CSRTT and impulsive choice in a delay-based decision-making T-maze task.

5.4 Methods

5.4.1 Subjects

The study was conducted using a total of 32 adult male Lister Hooded rats (210 - 310 g) obtained from Harlan (Borchen, Germany) which were assigned to two testing cohorts (n = 16). Each cohort was further subdivided into a NAc core and shell group (n = 8 each). The first cohort was trained in a delay-based decision-making task (T-maze), the second performed the 5-CSRTT. The animals were

housed in groups of four to six in standard Macrolon cages (type IV) under controlled ambient conditions (21 – 22 °C, 45 – 55 % humidity, 12 h light/dark cycle, lights on at 7:00 a.m.). The rats were kept on their experimental body weight by controlled feeding of 12 g laboratory rodent chow (Nohrlin GmbH, Bad Salzuflen, Germany) per rat per day and received tap water ad libitum. Behavioural testing took place between 8:00 a.m. and 6:00 p.m. The experiments were performed in accordance with the National Institutes of Health ethical guidelines for the care and use of laboratory animals for experiments and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

5.4.2 Experiment 1: 5-CSRTT

5.4.2.1 Apparatus

The 5-CSRTT was conducted in two operant aluminium chambers (26 x 26 x 26 cm; Campden Instruments Ltd., Loughborough, UK), wherein five apertures (2.5 x 2.5 cm, 4 cm deep) were embedded 2 cm above floor level in the concavely curved rear wall. This assembly provided five response options located equidistant to the food magazine on the opposite. Inside each hole, a light-emitting diode (LED) generated visual stimuli of variable duration. Nose-poke responses of the animals were detected by infra-red photo cell beams at the entrance of the apertures. The rats could be placed in the box through a Plexiglas® door which filled the upper part of the front wall. Underneath the door, a small Plexiglas® panel provided access to the food magazine which was lighted via two LEDs and automatically supplied with casein pellets (45 mg Dustless Precision Pellets, Bio-Serv®, UK) by an electromechanical feeder. Food collection was detected by a microswitch monitoring the movement of the hinged panel. Each chamber was illuminated by a 3 W house light mounted on the ceiling. A noise-damped fan served as ventilation and background noise. The extendable grid floor facilitated the removal of excrements. For the purpose of sound attenuation, the wooden cabinet was reinforced with an insulating plate at the interior of the door. The apparatus was controlled by specific software written in Turandot (Cambridge Cognition Ltd., version 1.23) which was run on a personal computer connected to the BNC Mark 2 System (Behavioral Net Controller, Campden Instruments Ltd., Loughborough, UK).

5.4.2.2 Training

The animals (n = 16) were trained to detect the occurrence of brief light stimuli in one of the five rear wall apertures. The general procedure was based on the protocol of Campden Instruments and was divided into a habituation, pretraining and baseline training phase (Campden Instruments Limited 2005). Over the entire course of the test procedure, the rats were initially positioned in the laboratory for an acclimatisation period of 30 min.

The first experimental phase comprised two daily half-hour habituation sessions. The boxes were prepared as follows: before the primary session, the tray panel was pasted back to facilitate access to 15 available pellets to reinforce the meaning of the magazine as location of reward. During the second session, no panel manipulation was carried out. Besides the reward in the tray, two pellets were placed in each aperture to promote exploration in these areas. The chambers were permanently illuminated by the house light within both sessions.

The daily training session lasted 30 min or was finished after completion of 100 trials. Each session started with the simultaneous illumination of the box and the food magazine and the delivery of a single pellet into the tray. Once the rat opened the panel for food intake, the first trial was initiated. The magazine light faded and a fixed intertrial interval (ITI) of 5 s started. At the end of the ITI, a light stimulus of determinate duration (stimulus duration, SD) was randomly presented in one of the five holes. The rats had to respond with a nose-poke into the appropriate aperture during the stimulus presentation or within a subsequent limited hold period (LH). A correct response was followed by the supply of a pellet into the lighted food magazine. The next trial was triggered by the detection of the panel movement. Inappropriate reactions led to a punishment in terms of a predefined 5 s period of darkness (time-out)

without reward delivery. The task procedure offered various opportunities for such reactions:

- *incorrect responses* in a hole where no stimulus appeared,
- *omissions* in the form of absent reaction to the occurrence of the stimulus within the LH,
- *premature responses* before the onset of the stimulus during the ITI in one of the apertures
- and *perseverative responses*, meaning additional responses after a correct response and before reward collection.

Every response during the time-out phase reinitialised the period of darkness. Following the time-out, the box and the tray were illuminated again and the next trial was started by a nose-poke into the food magazine. Within a session, the visual stimuli were randomly presented in equal number in each hole. The progressive decrement of the variables SD (60 → 1 s) and LH (60 → 5 s) over eight training levels enabled the acquisition of the 5-CSRTT.

The baseline training session was determined by the conditions of the eighth training level (SD = 1 s, LH = 5 s). After showing a stable baseline performance (>80 % accuracy and <20 % omissions with <10 % variation over five consecutive training sessions), rats underwent surgery.

5.4.3 Experiment 2: delay-based decision-making task

5.4.3.1 Apparatus

The delay-based decision-making task was carried out in a T-shaped maze (Fig. 5.1) constructed of plastic. The starting runway (60 cm long) was vertically arranged to two side arms (each 58 cm long). The height of the apparatus was 30 cm with an inner diameter of 15 cm elevated 80 cm above floor level. At the end of the side arms (target area) a metallic feeding dish each was inset in the maze bottom and refilled with casein pellets (45 mg Dustless Precision Pellets, Bio-Serv®, UK) before each run. One dish always contained a high reward (10 pellets), while the other contained a small one (two pellets). For closing the discrete areas, removable grey plastic guillotine doors early in the starting runway (D_0), at the margins of the choice area (D_1) and in front of the target areas (D_2) were insertable into notches of the maze wall.

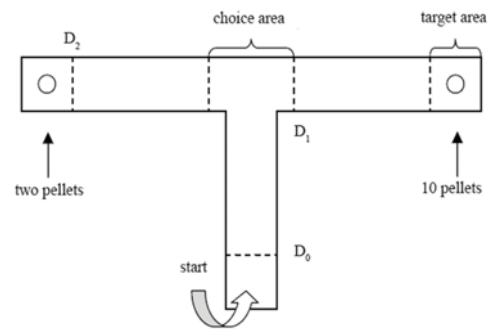

Fig. 5.1 Schematic representation of the delayed reward apparatus (T-maze). Guillotine doors are marked as D_0 in the starting area, D_1 at the choice area and D_2 at the target area.

5.4.3.2 Training

The general schedule of the experiment was based on previous studies (Bizot et al. 1999;Hadamitzky et al. 2009;Wischhof et al. 2011) and divided in a habituation, pretraining and baseline training stage. For the purpose of habituation, the rats (n = 16) were first introduced in the maze for five minutes on two consecutive days, where they were able to move freely. Pellets were dispersed on the bottom and in equal quantity in the food wells to enhance exploration of the new environment.

During the pretraining, the guillotine doors were inserted and one arm was defined as the high reward (HR; 10 pellets) arm while the other one was used as the low reward (LR; two pellets) arm, randomly distributed among the rats. The side determination of HR and LR remained constant throughout all subsequent training and testing sessions for each animal. The rats performed daily sessions consisting of nine trials. Each session started with two forced-choice trials, in which one side offered free entrance, whereas the respective opposite direction was blocked by D_1, with changeover in the second trial. In the following seven trials, the animals had the free choice between both targets. Once a rat was introduced into the starting runway, guillotine doors were immedi-

ately elevated to allow access to the selected reward. After consuming the pellets, animals were returned to their home cage for an inter-trial interval of 1 min. When the rats chose the HR in >70 % of the trials (five of seven) within a session over three consecutive days, they achieved the baseline training stage.

In the baseline training section, the pretraining sequence was expanded by an introduction of a delay in the HR arm. Once a rat chose the HR option, it was retained in the side arm between the lowered doors D_1 and D_2 and hindered of achieving the gratification for the period of delay. The decision for the alternative side with the LR led to unrestricted access to food. Initially, the delay of the HR was set to 5 s. After choosing the HR in at least 70 % of trials over five consecutive days, the delay was increased to 10 s. Animals reached stable baseline performance following manifestation of the 10 s-delayed HR choice in more than 70 % of trials over five successive daily sessions and subsequently underwent surgery.

5.4.4 Surgery

The rats were anaesthetised with chloral hydrate (360 mg/kg; Sigma-Aldrich, Steinheim, Germany) and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Stainless steel 21 gauge guide cannulae were implanted bilaterally 2 mm above the target injection site into the NAc core (anteroposterior +1.2 mm, mediolateral ±1.8 mm, dorsoventral -6.8 mm from Bregma) or shell (anteroposterior +1.2 mm, mediolateral ±0.5 mm, dorsoventral -7.3 mm from Bregma). Jeweller screws were anchored in the skull served to fix the cannulae which were embedded in dental cement and closed by removable 26 gauge stylets of the same length. After surgery, the rats were kept individually for three days with free access to food and water. Following a total recovery period of five days, the animals were reintroduced to the baseline training until they re-established the presurgical baseline performance.

5.4.5 Microinfusion procedure

The test design comprised three 4-day sessions for the animals. Each session started with a testing day, followed by a day without training. The second and third post-testing days were used to achieve the baseline performance and to ensure the washout process of the drug. Before infusion, the stylets were exchanged for 26 gauge injection cannulae connected with microlitre syringes (SGE Scientific Glass Engineering, Darmstadt, Germany) via polyethylene tubes. The rats received bilateral intra-NAc core or shell microinjections of the $GABA_A$ agonist muscimol (0.05 µg/0.3 µl) and phosphate-buffered saline (PBS) as vehicle (0.3 µl) according to a pseudorandom Latin square design. For each subject, FCM (0.27 µg/0.3 µl) was administered as third and last injection to exclude differences in the FCM spread between the rats before perfusion. The injection rate was 0.1 µl/30 s. The injectors were left in place for 1 min to guarantee diffusion and to avoid reflux of the solution. Ten minutes after the microinjection, the rats underwent behavioural testing. The sequence of the test sessions matched with the baseline training.

5.4.6 Drugs

The $GABA_A$ agonist muscimol (M = 114.10 g/mol) was purchased from Tocris Bioscience (Ellisville, MO, USA) and dissolved in PBS. Aliquots of stock solutions (0.5 µg/0.3 µl) were stored at -20 °C until use. On the treatment day, aliquots were further diluted to a dose of 0.05 µg/0.3 µl. FCM (M = 607.46 g/mol), a stable highly lipophilic conjugate of muscimol and the BODIPY® TMR-X fluorophore, was acquired from invitrogen (Life Technologies GmbH, Darmstadt, Germany) and dissolved in PBS in an equimolar concentration to muscimol (0.27 µg/0.3 µl). Doses were based on previous studies (Allen et al. 2008;Diederich and Koch 2005).

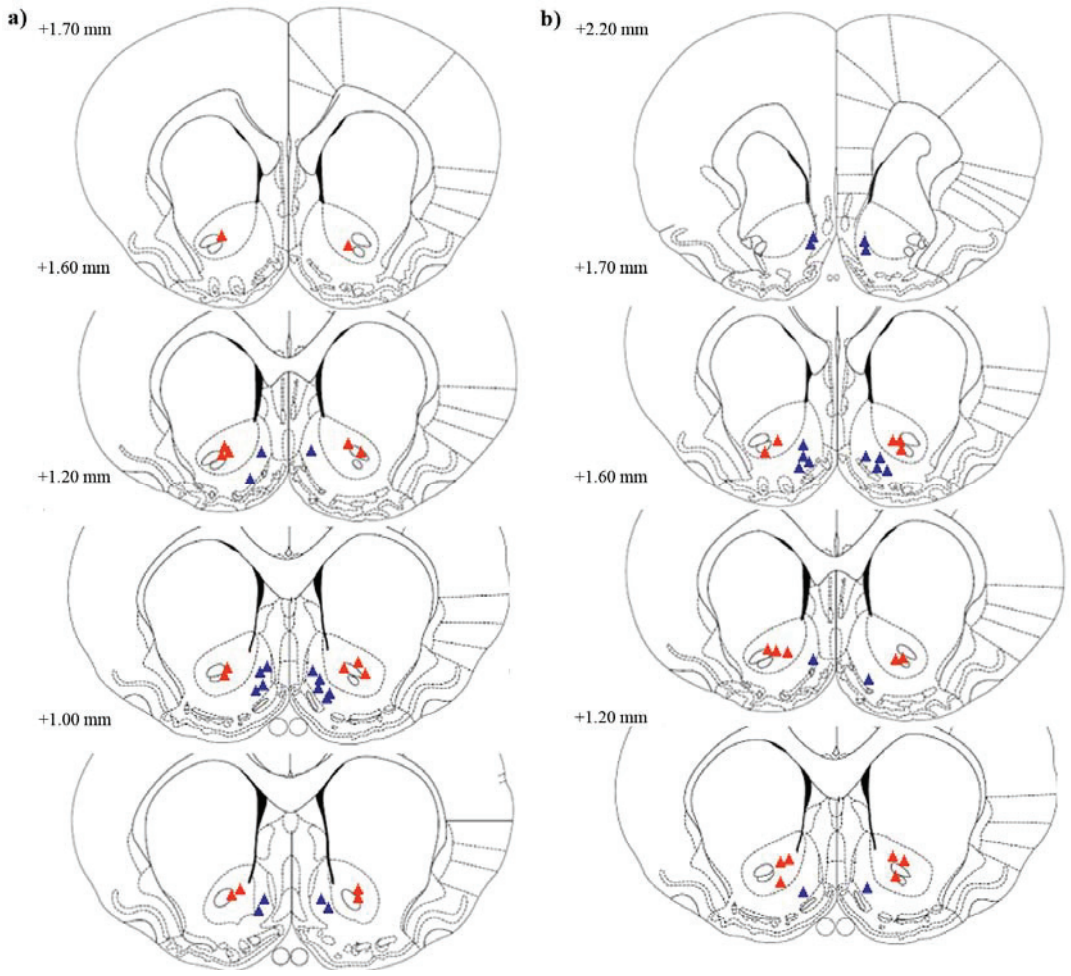

Fig. 5.2 Location of the bilateral injection sites in the nucleus accumbens core (red triangles) and shell (blue triangles) in **a)** the 5-choice serial reaction time task (n = 16) and **b)** the delay-based decision-making task (n = 16) depicted on schematic drawings from the rat brain atlas of Paxinos and Watson (1998). Rostral distance (in mm) to bregma is indicated by numbers.

5.4.7 Histology

After termination of the experiment, the rats were euthanised with a lethal dose of chloral hydrate and transcardially perfused with 200 ml 0.01 M PBS followed by 200 ml 4 % paraformaldehyde in 0.1 M PB. The brains were removed from the cranium and cryoprotected in 30 % sucrose solution for 48 h. Two series of coronal 50 µm sections of the NAc were cut on a cryostat (Jung CM 3000; Leica Instrument GmbH, Nussloch, Germany). The first series was mounted onto gelatinised glass slides and Nissl-stained with thionin to identify location of injection. These sections were analysed using a light microscope and injection sites plotted on standardised coronal sections of a rat brain stereotaxic atlas (Paxinos and Watson 1998). The second series was mounted onto sodium azide-gelatinised glass sides and coverslipped with fluorescence mounting medium (DAKO, Glostrup, Denmark) for imaging. For visualising the spread of FCM, photomicrographs were taken using adequate band pass filter sets (FCM: excitation and emission peaks at 543 and 572 nm). Images were captured by a Zeiss Axiophot microscope (Göttingen, Germany) and the image analysis software Metamorph 4.6 (Visitron Systems GmbH, Puchheim, Germany).

5.4.8 Data analysis

The descriptive statistics is based on means and variance and is indicated by the standard error of the mean (±SEM). The statistical analyses were conducted by the software IBM SPSS Statistics (version 20 for Windows).

In experiment 1, the drug effects within the testing group on the following behavioural parameters were investigated using separate two-way split-plot-factorial analysis of variance (ANOVA; within-subject factor: drug treatment, between-subject factor: region): percentage of correct responses (accuracy; 100 x number of correct responses/number of correct and incorrect responses), percentage of omitted responses (100 x number of omitted responses/total number of correct, incorrect and omitted responses), number of premature responses , number of perseverative responses, number of trials completed, number of time-out responses, latency of correct responses [s] and latency of reward collection [s].

In experiment 2, the forced-choice trials were excluded from the data and the percentage choice of HR (100 x number of HR choice/total number of trials) was calculated. The data were analysed by two-way split-plot-factorial ANOVA with the within-subject factor drug treatment and the between-factor region.

In the case of significant main effects ($P < 0.05$), one-way repeated measures ANOVA and post-hoc Bonferroni tests for the factor drug treatment as well as independent *t*-tests between the NAc subregions were conducted separately for each behavioural parameter.

5.5 Results

5.5.1 Histology

The location of the injector tips within the NAc core and shell is shown in Fig. 5.2.

FCM showed diffusion extent restricted to the respective subregion, with a larger spread in the dorsoventral than in the mediolateral axis. In both regions, fluorescence was consistently observed within an average 0.5-mm radius of the injector tip (Fig. 5.3). The asymmetrical spread of FCM along the dorsoventral axis had a mean radius of 0.8 mm and 0.7 mm in NAc core and shell injected animals, respectively.

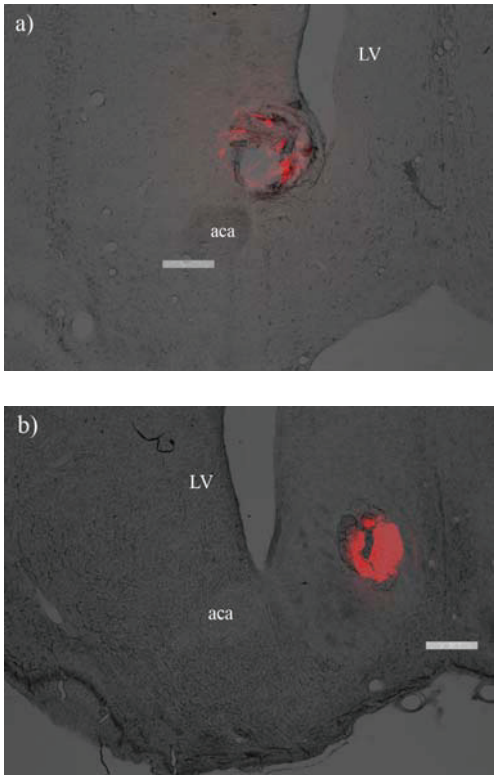

Fig. 5.3 Representative photomicrographs of the injection site in the **a)** nucleus accumbens core and **b)** shell. The red fluorescence of fluorophore-conjugated muscimol (FCM) is overlaid with a bright field image of the corresponding Nissl-stained coronal section. *aca* anterior commissure, anterior part; *LV* lateral ventricle. *Scale bar* = 500 µm.

5.5.2 Experiment 1: effects of inactivation of the NAc core and shell on rats` performance in the 5-CSRTT

The rats performed at a stable baseline throughout the entire experiment with high levels of accuracy (core: 85.72±2.32 %; shell: 86.32±1.44 %), fast correct response (core: 0.69±0.02 s; shell: 0.71±0.02 s) and reward collection latencies (core: 1.14±0.04 s; shell: 1.14±0.06 s), low percentages of omissions (core: 9.97±1.20 %; shell: 7.33±1.00 %) as

well as low numbers of premature (core: 9.45±2.07; shell: 9.53±1.09) and perseverative responses (core: 2.48±0.50; shell: 1.96±0.20) before testing. Analysis of the training data demonstrated no significant differences in the pre- and postoperative sessions and the 'drug-free days' between testing excluding any carry-over effects of drug treatment or surgery (data not shown).

Two-way split-plot-factorial ANOVAs on the 5-CSRTT performance showed main effects of drug treatment [$F_{(2,30)} = 4.299$; $P = 0.024$] and region [$F_{(1,30)} = 6.64$; $P = 0.022$] as well as a statistically significant treatment x region interaction [$F_{(2,30)} = 7.331$; $P = 0.003$] for premature responses, main effects of drug treatment [$F_{(2,30)} = 4.115$; $P = 0.027$] and region [$F_{(1,30)} = 4.651$; $P = 0.049$] for perseverative responses, main effects of drug treatment [$F_{(2,30)} = 32.907$; $P < 0.001$] and region [$F_{(1,30)} = 10.531$; $P = 0.006$] as well as a significant treatment x region interaction [$F_{(2,30)} = 24.594$; $P < 0.001$] for accuracy, main effects of drug treatment [$F_{(2,30)} = 51.35$; $P < 0.001$] and region [$F_{(1,30)} = 39.684$; $P < 0.001$] as well as a significant treatment x region interaction [$F_{(2,30)} = 31.46$; $P < 0.001$] for omissions, main effects of drug treatment [$F_{(2,30)} = 92.535$; $P < 0.001$] and region [$F_{(1,30)} = 24.545$; $P < 0.001$] as well as a significant treatment x region interaction [$F_{(2,30)} = 33.102$; $P < 0.001$] for completed trials, main effects of drug treatment [$F_{(2,30)} = 24.43$; $P < 0.001$] and region [$F_{(1,30)} = 22.915$; $P < 0.001$] as well as a significant treatment x region interaction [$F_{(2,30)} = 21.871$; $P < 0.001$] for latency of correct responses and main effects of drug treatment [$F_{(2,30)} = 21.031$; $P < 0.001$] and region [$F_{(1,30)} = 21.031$; $P < 0.001$] as well as a significant treatment x region interaction [$F_{(2,30)} = 21.026$; $P < 0.001$] for latency of reward collection.

Further one-way repeated measures ANOVAs and post-hoc Bonferroni tests revealed that administration of muscimol into NAc shell specifically impaired inhibitory control reflected by a significant increase in premature ($P = 0.022$) and a trend in time-out responding ($P = 0.093$) compared to vehicle, while no other measured parameter was affected (Fig. 5.4a, b and Table 5.1). Intra-NAc core injection of muscimol led to gross impairments in the general task performance, with the rats showing no anticipatory responses and marginally completed trials in comparison to control ($P < 0.001$; Fig. 5.4a and Table 5.1). Additionally, inactivation of NAc core by muscimol resulted in significantly decreased accuracy ($P = 0.002$; Fig. 5.4c), increased omission rate ($P < 0.001$; Fig. 5.4d) as well as prolonged latencies of correct responding ($P = 0.006$; Table 5.1) and reward collection ($P = 0.008$; Table 5.1). Maximum correct response latency was set to six seconds, consisting of SD (1 s) and LH (5 s). Maximum reward collection latency was set to 1800s, which corresponded to the duration of a complete session, in case an animal did not push the magazine panel by nose-poke and no further trial was initiated. FCM also slightly increased premature responding, but this effect did not reach the level of significance. Except for decreasing the latency of reward collection when injected into the shell (Table 5.1), FCM had no significant effect on 5-CSRTT performance. Independent *t*-tests between the NAc subregions showed significant differences between core and shell following muscimol injection regarding premature ($P = 0.002$) and time-out responses ($P = 0.039$), accuracy ($P = 0.002$), omissions ($P < 0.001$), trials completed ($P < 0.001$) and both latency measures (correct response: $P = 0.002$; reward collection: $P = 0.003$). Microinjection of FCM into NAc shell significantly reduced the omission rate ($P = 0.003$) compared to NAc core. However, vehicle injection into the core region significantly enhanced perseverative responding in comparison to NAc shell ($P = 0.019$), without exceeding baseline performance level.

5.5.3 Experiment 2: effects of deactivation of the NAc core and shell on delay-based decision-making in the T-maze

Data analysis revealed no significant differences in rats` choice behaviour between baseline training, the sessions between the testing days

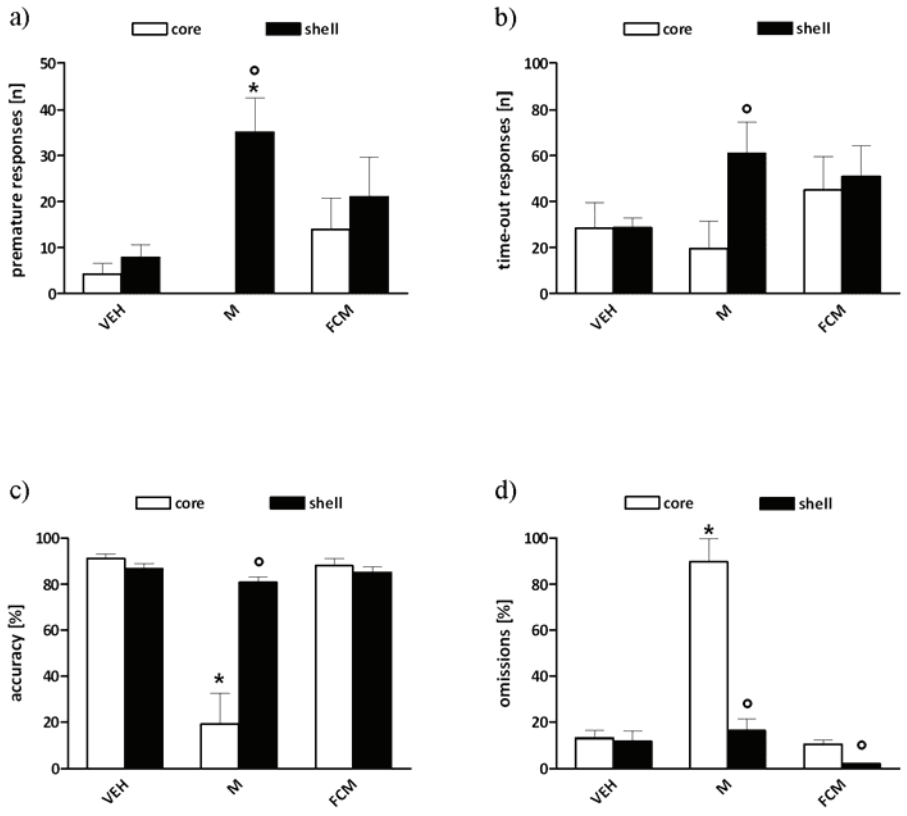

Fig. 5.4 Effects of local bilateral infusions of the $GABA_A$ agonist muscimol (M; 0.05 µg/0.3 µl) and fluorophore-conjugated muscimol (FCM; 0.27 µg/0.3 µl) into the nucleus accumbens (NAc) core (n = 8) or shell (n = 8) on the rats` performance in the 5-choice serial reaction time task. Data of **a)** premature responses, **b)** time-out responses, **c)** accuracy and **d)** omissions are means ±SEM. Statistically significant differences between drug treatment compared to vehicle (Veh) are indicated by asterisks (one-way repeated measures ANOVA, post-hoc Bonferroni test, $P < 0.05$) and between NAc core compared to shell by circles (independent *t*-test, $P < 0.05$).

Table 5.1 Effects of local bilateral infusions of the $GABA_A$ agonist muscimol (M; 0.05 µg/0.3 µl) and fluorophore-conjugated muscimol (FCM; 0.27 µg/0.3 µl) into the nucleus accumbens core (n = 8) or shell (n = 8) on the rats` performance in the 5-choice serial reaction time task. Data are expressed as means ±SEM. *FCM* fluorophore-conjugated muscimol, *M* muscimol, *NAc* nucleus accumbens, *VEH* vehicle. *$P < 0.05$ vs. VEH (one-way repeated measures ANOVA, post-hoc Bonferroni test), °$P < 0.05$ vs. NAc core (independent *t*-test).

	Treatment	Trials completed [n]	Perseverative responses [n]	Latency of correct responding [s]	Latency of reward collection [s]
NAc core	VEH	98.88±1.13	2.50±0.65	0.73±0.04	1.23±0.04
	M	17.88±4.10*	0.25±0.25	4.73±0.83*	1350.51±294.26*
	FCM	91.88±5.74	1.50±0.57	0.73±0.04	1.13±0.05
	Treatment	Trials completed [n]	Perseverative responses [n]	Latency of correct responding [s]	Latency of reward collection [s]
NAc shell	VEH	100.00±0.00	0.63±0.26°	0.70±0.07	1.12±0.05
	M	77.75±7.48°	0.50±0.27	0.77±0.05°	1.16±0.06°
	FCM	94.00±3.95	1.00±0.33	0.62±0.03	1.05±0.05*

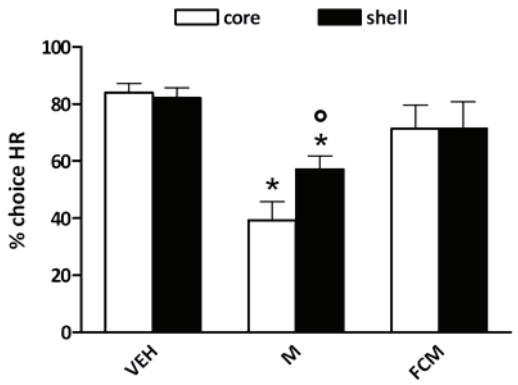

Fig. 5.5 Effects of local bilateral infusions of the $GABA_A$ agonist muscimol (M; 0.05 µg/0.3 µl) and fluorophore-conjugated muscimol (FCM; 0.27 µg/0.3 µl) into the nucleus accumbens (NAc) core (n = 8) or shell (n = 8) on the rats` performance in the delay-based decision-making task. Data of the percentage choice of high reward (HR) are means ±SEM. Statistically significant differences between drug treatment compared to vehicle (Veh) are indicated by asterisks (one-way repeated measures ANOVA, post-hoc Bonferroni test, $P < 0.05$) and between NAc core compared to shell by circles (independent *t*-test, $P < 0.05$).

and control injections for both NAc subregion groups (data not shown). The two-way split-plot factorial ANOVA of the percentage choice of HR resulted in a main effect of treatment [$F_{(2,30)} = 19.857$; $P < 0.001$]. Post-hoc comparisons revealed that reversible inactivation of both NAc core ($P = 0.002$) and shell ($P = 0.001$) by muscimol significantly induced impulsive decision-making as indicated by decreased preference for the large 10 s-delayed reward compared to control (Fig. 5.5). Administration of FCM did neither significantly affect choice behaviour in the NAc core nor in the shell group. Inactivation of the NAc core via muscimol caused a statistically significant higher impairment of the rats' waiting capacity in the T-maze in comparison with the shell region as demonstrated by an additional independent *t*-test ($P = 0.042$) between both subregions. Choice- or reward consumption-latencies were not quantified, as we did not observe obvious changes in these temporal parameters following muscimol or FCM treatment compared to control. Moreover, all rats consumed the food pellets entirely in each trial, indicating that motor or motivational functions were not impaired by the treatment.

5.6 Discussion

In general, the NAc core region is considered to be primarily implicated in impulsivity since NAc shell lesions show no effect on inhibitory control (Murphy et al. 2008) and delay-discounting (Pothuizen et al. 2005b), while core lesions induce impulsive choice behaviour (Cardinal et al. 2001) and tend to increase motor impulsivity in the 5-CSRTT (Christakou et al. 2004). By contrast, the present data indicate that both regions of the NAc are part of the neural network mediating impulsivity, with varying influences on distinct types of impulsive behaviour. Interestingly, the shell region might play a specific role in behavioural control by regulating both impulsive action as well as impulsive choice. The NAc core appears to have an additional impact on locomotor activity and motivational aspects. FCM did not qualify as an adequate tool for evaluating the role of the NAc subregions in behavioural control.

The major findings of the first experiment are that acute reversible inactivation of the rats' NAc shell by the $GABA_A$ agonist muscimol induced deficits in impulse control as indicated by increased premature responding in the 5-CSRTT, while deactivation of the core region resulted in general impairments of task performance. Both muscimol and FCM injections into the shell enhanced impulsive action, but only muscimol was sufficient to significantly increase anticipatory responding. Besides premature responses, intra-shell infusions of muscimol also tended to raise time-out responses, representing another aspect of inhibitory control, more related to cognitive flexibility (Robbins 2002). Other measured parameters indexing attentional (accuracy, omissions), compulsive (perseverative responses), motor (correct response latency) or motivational behaviour (trials completed, reward collection latency) remained unaffected. Intra-NAc core administration of muscimol produced fundamental disturbances in overall

behavioural responding as indicated by a very low number of completed trials concomitant with a strikingly increased omission rate, absent premature responses, very few perseverative reactions and lengthened latencies of correct responses and reward collection.

Damage to the core region has already been shown to impair response inhibition in the 5-CSRTT and DRL task (Christakou et al. 2004;Pothuizen et al. 2005b), even though core-lesioned rats do not show deficits in inhibitory control during the forced choice (FC) and stop-signal reaction time (SSRT) task (Murphy et al. 2008;Eagle and Robbins 2003). While 5-CSRTT and SSRT task measure distinct subtypes of impulsive action, inappropriate premature responding in the FC and DRL task indeed reflects the same aspect of response inhibition, namely a failure of action restraint, as in the 5-CSRTT (Bari and Robbins 2013;Basar et al. 2010;Robinson et al. 2009). The present behavioural effects evoked by muscimol into the NAc shell are in contrast to previous studies, where permanent shell lesions have no effect on premature responding in the FC task, which is modelled after the 5-CSRTT (Murphy et al. 2008), and do not affect inhibitory control of goal-directed behaviour in the DRL task (Pothuizen et al. 2005b). However, the findings of lesion studies have to be compared carefully with those of our present investigation due to different tissue manipulations. Hence, the lack of effect of shell lesions on impulse control could be explained by masking effects owing to functional compensation by adjacent structures.

The present study supports the notion that the core region in contrast to the shell plays an important role in the regulation of locomotion and general responsiveness during 5-CSRTT performance. Generally, application of GABA or muscimol in the NAc reduces motor activity of rats (Anden et al. 1979;Wachtel and Anden 1978). High-dose muscimol (1 µg/µl) even induces cataleptic effects after injection into the NAc (Scheel-Kruger et al. 1977). Considering this, we used a lower dose of muscimol (0.05 µg/0.3 µl), which is known to sufficiently elicit impulsivity-specific effects during 5-CSRTT performance following intraventral mPFC injection in rats (Feja and Koch 2014). The differential impact of the NAc subregions on locomotor activity in the 5-CSRTT experiment is in accordance with more recent discoveries revealing that lesions, higher doses of muscimol and infusions of the NMDA-receptor antagonist AP-5 targeting the core generate decrements in motility or even induce akinesia, whereas shell-treated animals appear normal (Maldonado-Irizarry and Kelley 1994;Maldonado-Irizarry and Kelley 1995;Pothuizen et al. 2005a). In line with this, stimulation of the core region speeds reward collection and response latencies in a reaction time task (Sesia et al. 2010). Specifically, the tremendous decrease in the number of completed trials after deactivation of NAc core but not shell may represent a consequence of motivational dysfunction and may refer to a differential role of both subregions in motivated behaviour in the 5-CSRTT. This is supported by evidence that muscimol injections into the core reduce breakpoint in a progressive ratio schedule in rats (Moscarello et al. 2010), while shell inactivation enhances motivational behaviour in that task (Stratford and Wirtshafter 2012;Wirtshafter and Stratford 2010).

To our knowledge, this is the first study using the inactivation technique with muscimol of the NAc on impulsive behaviour specifically comparing impulse control with choice impulsivity in rats. Impulsive choice is more related to decision-making processes than to motor inhibition as required in the 5-CSRTT. It is widely accepted that these two forms of impulsivity rely on separate neuronal pathways, with potential overlap on the subcortical level of the NAc (Dalley et al. 2011;Pattij and Vanderschuren 2008;Winstanley et al. 2006). Our results support these assumptions and, more importantly, confute the leading opinion that only the core, but not the shell region of the NAc is involved in delay-based decision-making (Basar et al. 2010). In the second experiment we show that inactivation of NAc core as well as shell by microinjection of muscimol significantly decreased the preference for the large delayed reward in the T-maze, with a higher impact of core deactivation on the rats' waiting capacity compared to shell. In rats, lesion and

reversible inactivation studies have already linked the NAc core with various forms of cost/benefit decision-making, including probability- (Cardinal and Howes 2005), effort- (Ghods-Sharifi and Floresco 2010;Hauber and Sommer 2009) and delay-discounting (Bezzina et al. 2007;Cardinal et al. 2001;Pothuizen et al. 2005b). Among those three types, dysfunctions of the shell region to date only contribute to risk-based choice behaviour (Ghods-Sharifi and Floresco 2010;Pothuizen et al. 2005b;Stopper and Floresco 2011). However, it has to be considered that the task design of Pothuizen and colleagues (2005b) lacking effects of NAc shell lesions on choice impulsivity comprised both delayed and probabilistic rewards and thereby differed from conventional delay-discounting tasks. In terms of decision-making, the specific measurement of temporal discounting, as in our study, mostly reflects impulsive behaviour (Bari and Robbins 2013). Thus, our results strengthen the role of NAc core and provide evidence that NAc shell might also be implicated in impulsive choice.

The effects on the choice of the 10 s-delayed high reward in our study are compatible with those of other findings (Bezzina et al. 2007;Cardinal et al. 2001;Pothuizen et al. 2005b), in which similar delays are likewise discounted following NAc core damage. Previous studies using a 15-s delay condition during the T-maze task have shown that rats usually perform a HR choice in 65-70 % of trials, while this rate declines to less than 40 % in case of a 25-s delay (Bizot et al. 1999;Bizot et al. 2007). This implies that prolonged delay periods of ≥25 s are suitable to assess drug-induced improvements of waiting capacity, but to a lesser extent a decrease in the tolerance of delay. Consequently, we applied a shorter delay condition of 10 s, which already established sufficiency in impulsive decision-making in the T-maze (Hadamitzky et al. 2009;Wischhof et al. 2011).

The NAc is ideally positioned to integrate information about the costs and benefits of different response options to regulate decision-making (Floresco 2007;Mogenson et al. 1980). During the T-maze paradigm, animals choose between two reinforcers whose values vary in both size and delay. Human delay-discounting studies have shown that activity of the ventral striatum correlates with the subjective value of a reward and decreases with an increase of the delay preceding the reward, assuming that the NAc helps to value immediate and delayed outcome (Kable and Glimcher 2007;Prevost et al. 2010). A Pavlovian conditioning task using lithium-devalued food reward revealed that both core and shell are necessary for the evaluation of expected outcomes, as devalued rats with lesions of either core or shell showed similar response levels to lesioned, non-devalued rats (Singh et al. 2010), suggesting that NAc deactivation might impair the ability of delays to discount the reward. However, in our case inactivation of NAc subregions reduced the choice of the delayed reinforcer compared to control, pointing towards an effect of delay-discounting.

The effects of NAc core and shell inactivations could not alone be explained by increased delay sensitivity, but also by a reduced sensitivity to the difference in reward magnitude. In a previous study, deactivation of the entire NAc or the shell region slightly reduced preference of larger rewards in a magnitude discrimination task (Stopper and Floresco 2011), but apparently only after a considerable larger number of trials than in our test. Since the rats were faced with the reward magnitudes before each test session due to forced choices and several former investigations indicated that the perception of the relative incentive value and the magnitude discrimination of the rewards remain unaffected after NAc lesions (Balleine and Killcross 1994;Bezzina et al. 2007;Bezzina et al. 2008b;Cardinal and Cheung 2005), the enhanced rate of delay-discounting in our study is more likely based on increased impulsivity than on primary motivational aspects. The NAc is a main target of mesolimbic DA neurons arising in the VTA (Flores 2011). The mesolimbic DA system is more involved in the mediation of the preparatory phase of behaviour and less critical in the consummation of primary rewards (Balleine and Killcross 1994;Blackburn et al. 1992;Salamone 1996). Likewise, lesions of the core do not reduce food motivation in a delayed reinforce-

ment task (Cardinal and Cheung 2005) and muscimol does not affect food intake when injected into the NAc core (Stratford and Kelley 1997) and even increases eating behaviour following infusion into the shell (Basso and Kelley 1999;Lopes et al. 2007;Reynolds and Berridge 2002;Soderpalm and Berridge 2000;Stratford and Kelley 1997;Stratford and Wirtshafter 2011). Rather, NAc function is necessary to bridge action-outcome delays and to maintain a representation of the anticipated reward (Cardinal and Cheung 2005;Roesch et al. 2009).

As the T-maze task used here puts higher spatial demands on the rats than operant chamber versions of delay-discounting, one might argue that deficits in spatial discrimination could have influenced task performance. However, previous studies found that damage to NAc core or shell did not affect retention of a previously acquired instrumental spatial discrimination (Castane et al. 2010) and had little effect on spatial behaviour in a eight-arm radial maze once the rats had learned the task (Klein et al. 2004). Further, core- and shell-lesioned rats showed no deficits in reference memory, which is crucial for the retention of the reward locations (Jongen-Relo et al. 2003). Thus, inactivating NAc subregions entails a delay-dependent process of reward devaluation impairing waiting capacity and favouring a smaller immediate over a larger delayed reward.

In contrast to our findings, a very recent study found that inactivation of accumbens core did not induce but rather decrease delay discounting in rats and that the effect of inactivation depends on baseline levels of discounting (Moschak and Mitchell 2014). However, there are considerable methodical differences making both studies difficult to compare. Firstly and most importantly, the other group used a 140-fold lower dose of muscimol than we did probably only resulting in a partial inactivation of the NAc core. Secondly, their baseline training schedule seems to be inappropriate for investigating delay discounting effects of brain manipulations with delays longer than 5 s, as the discounting curve declines too steep. Thirdly, previous and our delay discounting studies showed far higher levels of high reward choice associated with similar delays following control treatment (Cardinal et al. 2001;Feja and Koch 2014).

Compared to the T-maze paradigm, the 5-CSRTT probably puts higher cognitive demands on the rats, as inactivation of NAc core impaired overall performance while exclusively inducing impulsivity in the decision-making task. As already mentioned, NAc dysfunction is associated with decrements in motor activity (Anden et al. 1979;Scheel-Kruger et al. 1977). However, we suggest that the effects of core treatment were not driven by changes in general inactivity, as we observed no effect on the number of time-out responses and on the number of panel pushes during the ITI in the 5-CSRTT. Hence, we hypothesize that core inactivation does not lead to akinesia but rather to a syndrome with principal deficits in general motivation followed by diminished motor activity. Excitotoxic lesions and DA depletions of the NAc core reduce response initiation and general responsiveness to stimuli (Bezzina et al. 2008b;Gill et al. 2010;Salamone et al. 1995). Furthermore, DA lesions in the NAc have previously shown to increase latencies in 5-CSRTT-trained rats (Cole and Robbins 1989) and to increase the tendency for taking breaks in operant responding (Mingote et al. 2005;Sokolowski and Salamone 1998). During 5-CSRTT performance, the rats are not just required to bridge a time gap by withholding a response (compared to waiting as in the T-maze) but must also react quickly within the LH. Accordingly, inactivation of the core region might slow the behaviour of the rats generating an enhanced omission rate of the expected reward and amplifying the already decreased motivational level. The T-maze paradigm contains no reaction time requirements but offers extended pauses *per se* in terms of longer ITIs compared to the 5-CSRTT, so that the animals are less susceptible to demotivating aspects of the task. Besides, the rats were encouraged to perform the task by being introduced in the T-maze and physically touched before each trial. Further evidence for different cognitive effort required for the tasks derives from two other studies. Following NAc core lesions, a clear cost-based choice deficit was identified in

the T-maze using effort instead of delay (Hauber and Sommer 2009). In contrast, lesions to this site produce a general impairment in response output in a more complex operant task, where decision-making is expanded by aspects of impulse control (Gill et al. 2010).

Another explanation for the task-specific differences following NAc core inactivations could be deduced from Pavlovian-instrumental transfer (PIT) studies. Pavlovian conditioned stimuli (in this case, the stimulus light in the 5-CSRTT) can invigorate instrumental behaviour by altering levels of arousal or behavioural activation. As core lesions reduce responding in instrumental performance and abolish general PIT (general arousal) in contrast to lesions of the shell, it is suggested that NAc core mediates the general excitatory effects of reward-related cues (Corbit et al. 2001;Corbit and Balleine 2011). Deactivation of the core might have reduced the ability of these cues, which are absent in the T-maze task, to elicit appetitive arousal for instrumental performance in the 5-CSRTT. Summarised, the present results indicate that performance in the 5-CSRTT is more influenced by factors like motivation and motor activity than in the T-maze.

Nevertheless, the findings from both experiments might reflect the differential behavioural influence of NAc subregions. In case of the 5-CSRTT, this becomes evident. Other groups have suggested that the core facilitates approach towards rewarding stimuli, whereas the shell mediates the suppression of irrelevant or non-rewarding behaviours (Blaiss and Janak 2009;Floresco et al. 2008a). Thus, it is also possible that the similar effects on delay-discounting following core or shell inactivation were underlain by different types of deficits. Deactivation of NAc core may have reduced general bias to larger, costlier options (Ghods-Sharifi and Floresco 2010), whereas NAc shell is more implicated in behavioural disinhibition than the core (Ambroggi et al. 2011) and may have caused rats to prefer choice of the low-rewarding option. These site-specific functional influences might also contribute to the greater impact of NAc core inactivation on impulsive choice behaviour compared to shell. Accordingly, impulsivity in delay-based cost-/benefit-paradigms, like the T-maze task, could be more susceptible to alterations in appetitive approach behaviour caused by NAc core dysfunction than to deficits in behavioural inhibition following shell inactivation, which is more crucial to motor impulsivity in the 5-CSRTT.

FCM was injected to evaluate its properties as an assessment tool for the spatial spread of the $GABA_A$ agonist in both NAc areas as it is suggested to be useful for exploring the function of small brain regions (Allen et al. 2008). Previous studies using delayed-response tasks showed that performance induced by FCM injection into the dorsomedial PFC in rats is similar to that after application of standard muscimol (Allen et al. 2008;Narayanan et al. 2006). However, one of these reports revealed significant differences between FCM (1.6 mM) and muscimol (8.8 mM) regarding premature responding and ascribed this discrepancy to concentration differences (Allen et al. 2008). Unexpectedly, in our hands FCM did not reproduce any behavioural effect of muscimol although injected in an equimolar concentration, with a ratio being behaviourally effective in a dose-response study in mice (Misane et al. 2013). Owing to its more than five times larger molecular weight, the diffusion of FCM is presumably more limited compared to muscimol. Others already noticed brain region-dependent diffusion gradients in case of FCM (Allen et al. 2008). Due to the highly lipophilic properties of the fluorophore portion, FCM may dissolve in lipid-rich myelinated fibers and cell membranes, e. g., cell clusters in the shell and the anterior commissure integrated in the core (Allen et al. 2008;Zahm and Brog 1992). FCM was not useful for the assessment of the spatial extent of the action of muscimol, since it did not affect behaviour. In general, determining the extent of tissue affected by injected drugs is not easy to determine *in vitro*, since the histological procedures (e.g. rinsing brain sections and washing out BODIPY molecules) could also have changed the parenchymal distribution of FCM. Also, according to Fick's law of diffusion, the spatial distribution of a drug correlates with its initial concentration (Edeline et al. 2002) and steeply declines with distance from the infusion site. Hence, the presence of fluorescent

molecules does not necessarily indicate a concentration of the drug that is sufficient for a functional inactivation and reduction of brain activity in this area.

Although the spread of FCM can not be considered to be equal to the spatial extent of muscimol inactivation, preceding autoradiography studies already estimated the spread of the $GABA_A$ agonist in rats and demonstrated diffusion of radioactive muscimol restricted to NAc core or shell following injection of similar volumes and even higher concentrations (Martin 1991;Martin and Ghez 1999;Pothuizen et al. 2005a). These pieces of evidence suggest that muscimol diffusion in our experiments was restricted to either core or shell. Our behavioural data further indicated region-specificity of injections, as they revealed clear and distinct differences between the NAc core and shell group during 5-CSRTT performance. Moreover, guide cannulae were implanted in the medial, and not ventral, part of the shell region to prevent mechanical tissue damage of the core and possible drug diffusion dorsally into this area. However, it can not be excluded that the injections may have involved adjacent non-accumbal areas, such as parts of the ventral pallidum or the dorsal striatum, especially since FCM infusions showed an asymmetrical diffusion along the dorsoventral axis up the cannula shaft.

5.7 Conclusion

Taken together, the present study corroborates the theory that impulsive behaviour is not only top-down controlled by cortical areas, but also modulated at subcortical level (Dalley et al. 2011). This is the first study directly comparing the role of NAc core and shell in two main types of impulsivity, revealing an involvement of NAc shell in both impulsive choice and impulsive action. We identified a key role for both subregions in the regulation of delay-based decision-making, whereas impulse control was differentially influenced. Muscimol inactivation of the shell specifically induced motor impulsivity in the 5-CSRTT, but core deactivation produced a more complex behavioural change, including motivational and motor aspects, and supporting the functional dichotomy of NAc core and shell.

6 Frontostriatal systems comprising connections between ventral medial prefrontal cortex and nucleus accumbens subregions differentially regulate impulse control in rats

Malte Feja · Michael Koch

6.1 HIGHLIGHTS

- Simultaneous contralateral application of the $GABA_A$ agonist muscimol was used to disconnect the vmPFC from NAc core or shell in rats
- Disconnection of vmPFC and NAc shell reduced impulse control in the 5-CSRTT
- Disconnection of vmPFC and NAc core did not significantly alter 5-CSRTT performance
- Frontostriatal systems differentially contribute to behavioural control depending on the involved NAc subregion
- The regulation of impulse control requires an intact connection between vmPFC and NAc shell

6.2 ABSTRACT

Deficits in impulse control are prevalent in several neuropsychiatric disorders based on impaired frontostriatal communication. The ventral medial prefrontal cortex (vmPFC) and the nucleus accumbens (NAc) are key substrates of impulse control in rats. The NAc core and shell are considered to be differentially involved suggesting a functional distinction between the connections of the vmPFC and particular NAc subregions concerning impulse control. In the present study, simultaneous inactivation of the rats' vmPFC and NAc core or shell via contralateral microinfusion of the $GABA_A$ receptor agonist muscimol was used to analyse their relevance for impulse control in the 5-choice serial reaction time task (5-CSRTT). Disconnection of the vmPFC and NAc shell produced specific impairments in inhibitory control, as indexed by significantly increased premature responding and an enhanced number of time-out responses, closely resembling the effects of bilateral inactivation of either the vmPFC or NAc shell previously reported using the same task. In contrast, disconnection of the vmPFC and NAc core only slightly increased the rate of omissions and latency of reward collection indicating attentional and motivational deficits. Our results extend previous findings pointing out the functional specialisation of frontostriatal networks and show a differential contribution of specific vmPFC-NAc connections to behavioural control depending on the NAc subregion. We conclude that the regulation of impulse control in rats requires an intact connection between the vmPFC and the NAc shell, while the vmPFC-NAc core projection seems to be of minor importance.

6.3 Introduction

Optimal adaptation to the environment is critical for animals' inclusive fitness and requires the right balance of behavioural inhibition and activation (Ghazizadeh et al. 2012;West and Gardner 2013). Behavioral control is highly influenced by motivational states ('impulses'). The active inhibitory mechanism, which modulates such internally or externally driven prepotent desires for reinforcement is referred to as impulse control (Jentsch and Taylor 1999;Winstanley et al. 2006). Deficient impulse control leads to maladaptive impulsive behaviours including inability to wait and difficulty withholding responses, generally defined as impulsive action or motor impulsivity (Brunner and Hen 1997;Dalley et al. 2011;de Wit 2009). The dominant behavioural measures of impulse control are response inhibition paradigms, such as the 5-choice serial reaction time task (5-CSRTT). The 5-CSRTT was modelled after its human analogues, the continuous performance test of attention and Leonard's five choice serial reaction time task and provides dissociable measurements of behavioural control, attention and motivation. Rats are required to withhold from premature responding to a visual, reward-predicting stimulus, generally regarded as an index of impulse con-

trol (Carli et al. 1983;Muir et al. 1996;Robbins 2002). Impulse control is based on cortico-limbic-striatal circuits and dysfunctions of these systems are associated with several psychiatric disorders characterised by high levels of impulsivity, like ADHD (Nigg and Casey 2005), obsessive-compulsive disorder (Anticevic et al. 2014), pathological gambling (Fineberg et al. 2010), schizophrenia (Meyer-Lindenberg et al. 2002;Pantelis et al. 1997;Robbins 1990), drug abuse and other forms of addiction (Kalivas and Volkow 2005;Russo and Nestler 2013). There is evidence that frontostriatal connections are part of parallel, functionally segregated re-entrant striatothalamocortical loops. In both primates and rats, frontostriatal projections are topographically organised so that functionally different subregions of the prefrontal cortex (PFC) have separate targets in the striatum (Alexander et al. 1986;Berendse et al. 1992;McGeorge and Faull 1989). The most pronounced territorial partition of the rodent PFC occurs in the medial PFC, which can be divided into dorsal (anterior cingulate and medial precentral cortices) and ventral subdivisions (prelimbic and infralimbic cortices) (Gabbott et al. 2005;Heidbreder and Groenewegen 2003;Ongur and Price 2000). The anatomical heterogeneity of the mPFC is paralleled by functional subregional differentiation, with the ventral medial prefrontal cortex (vmPFC) being more critically involved in impulsive behaviour (Chudasama et al. 2003;Kesner and Churchwell 2011). On striatal level, the nucleus accumbens (NAc) as part of the ventral striatum and as core element of the mesoaccumbal dopamine (DA) system is generally implicated in reward and motivation and ideally positioned to integrate input signals of executive-cognitive information, such as impulse control, arising from the mPFC (Carlezon, Jr. and Thomas 2009;Groenewegen and Trimble 2007;Mogenson et al. 1980). The vmPFC of rats and its putative primate equivalent, the anterior cingulate cortex (AC), are anatomically and functionally interconnected with the NAc, whereas the rodent dorsal mPFC preferentially innervates the dorsomedial striatum (Alexander et al. 1986;Berendse et al. 1992;Brog et al. 1993;Ding et al. 2001;Ferry et al. 2000;Gorelova and Yang 1997;McGeorge and Faull 1989;Preuss 1995;Sesack et al. 1989;Vertes 2004). Converging lines of evidence further indicate a functional relationship between mPFC and NAc in terms of behavioural inhibition, as both regions have found to be involved in impulse control (Aron et al. 2007;Christakou et al. 2004;Diekhof and Gruber 2010;Morgane et al. 2005), impulsive decision-making (Costa Dias et al. 2013;Diergaarde et al. 2008), behavioural flexibility (Coppens et al. 2010;Goto and Grace 2005) and drug seeking (Bossert et al. 2012;Peters et al. 2008;Vassoler et al. 2013). It is widely accepted, that glutamatergic projections from the mPFC regulate NAc function, in particular the release of DA and its subsequent output structures (Del Arco and Mora 2008;Morgane et al. 2005;Tzschentke and Schmidt 2000). Activation of serotonin $(5\text{-HT})_{2A}$ receptors, implicated in impulsivity (Hadamitzky and Koch 2009;Hadamitzky et al. 2009;Wischhof et al. 2011;Wischhof and Koch 2012), in the mPFC was found to increase excitatory transmission in the NAc (Mocci et al. 2013). Recent findings indicate that impulsive behaviour is not only top-down controlled by cortical areas, but also modulated at subcortical level (Dalley et al. 2011). For instance, intra-NAc injections of DA receptor antagonists reverse behavioural disinhibition induced by vmPFC inactivation (Ghazizadeh et al. 2012) and block premature responding following mPFC lesions in the 5-CSRTT (Pezze et al. 2009). Findings of electrophysiological recording (Hayton et al. 2011), lesion (Chudasama et al. 2003;Muir et al. 1996;Pezze et al. 2009) and reversible inactivation (Izaki et al. 2007;Murphy et al. 2012;Narayanan et al. 2006;Paine et al. 2011) studies already implicated the rodent mPFC in impulse control, but regarding the specific role of the vmPFC in motor impulsivity in the 5-CSRTT, lesion studies revealed discrepancies ranging from direct participation (Chudasama et al. 2003), a mere tendency of involvement (Chudasama and Muir 2001) to no important role (Passetti et al. 2002). The contribution of the NAc to impulsive behaviours turned out to be even more complex, as the NAc can not be regarded as an

anatomical and functional entity (Groenewegen and Trimble 2007;Heimer 2003). Based on anatomical, neurochemical and electrophysiological criteria, the NAc in the rat brain is divided into distinct subterritories, which are also present in the human brain and show considerable different input-output features: a dorsolateral core region surrounding the anterior commissure, and a shell compartiment that is located ventromedially to the core (Berendse et al. 1992;Brog et al. 1993;Heidbreder and Groenewegen 2003;Meredith et al. 1996;Sokolowski and Salamone 1998;Zaborszky et al. 1985). The functional dichotomy of the NAc, as evidenced by a differential involvement of core and shell in goal-directed instrumental action (Corbit et al. 2001), behavioural flexibility (Floresco et al. 2006), drug- or food-seeking behaviour (Floresco et al. 2008a;McFarland et al. 2004;Vassoler et al. 2013), locomotor activity (Jongen-Relo et al. 2002;Pothuizen et al. 2005a;Robbins and Everitt 1996), motivational behaviour (Bassareo et al. 2002;Stratford and Kelley 1997) and attentional processes (Jongen-Relo et al. 2002;Pothuizen et al. 2005a), appears also to hold true for impulse control. While core lesions induce deficits in 5-CSRTT and differential reinforcement for low rates of responding tasks (DRL) (Christakou et al. 2004;Pothuizen et al. 2005b), lesions of the NAc shell lack a significant influence on anticipatory responding in response inhibition tasks (Murphy et al. 2008;Pothuizen et al. 2005b). In line with this, disconnection of the mPFC from the NAc core by lesions caused impulse control deficits in the 5-CSRTT (Christakou et al. 2004), whereas an implication of the mPFC-NAc shell connection was not examined as yet. However, DA D_1-like receptors in NAc shell are involved in inhibitory response control in the 5-CSRTT (Pattij et al. 2007). Interestingly, previous results from our laboratory revealed that transient bilateral inactivation of the vmPFC (Feja and Koch 2014) as well as the NAc shell, but not the core (Feja et al. 2014), via the $GABA_A$ agonist muscimol induced impulsive over-responding in the 5-CSRTT. The lesion technique carries some drawbacks in comparison to inactivation tools due to irreversibly destroyed brain tissue and a potential functional compensation by other brain areas. In contrast, chemical agents like muscimol allow repeated acute and reversible inactivations of distinct brain regions, and hence, within-subject designs accompanied by increased test-retest reliability (Lomber 1999).

The present study extended our above-mentioned findings using an asymmetrical disconnection approach to investigate the relevance of the vmPFC-NAc connectivity to impulse control in the 5-CSRTT. Asymmetrical disconnection designs have successfully been used to show a functional interaction between mPFC and NAc in a variety of behavioural paradigms, including effort-based decision-making (Hauber and Sommer 2009), Pavlovian conditioning (Parkinson et al. 1999), behavioural flexibility (Block et al. 2007), working memory (Floresco et al. 1999) as well as inhibitory and attentional control (Christakou et al. 2004). As bilateral projections from the vmPFC to the NAc subregions are predominantly ipsilateral (Berendse et al. 1992;Brog et al. 1993;Gabbott et al. 2005;McGeorge and Faull 1989;Montaron et al. 1996;Sesack et al. 1989), this procedure results in a disruption of the respective vmPFC-NAc circuitry in both hemispheres. For that purpose, we combined unilateral temporary inactivations by muscimol of the vmPFC and the contralateral NAc core or shell in rats.

6.4 Methods

6.4.1 Subjects

A total of 22 adult male Lister Hooded rats (260 – 340 g) obtained from Harlan (Venray, Netherlands) were used which were assigned to two testing cohorts, defined as vmPFC-NAc core (n = 12) and vmPFC-NAc shell (n = 10) group. The animals were housed in groups of four to six in standard Macrolon cages (type IV) under controlled ambient conditions (21 - 22 °C, 45 – 55 % humidity, 12 h light/dark cycle, lights on at 7:00 a.m.). The animals were kept on their experimental body weight by controlled feeding of 12 g laboratory rodent chow (Nohrlin GmbH, Bad Salzuflen,

Germany) per rat per day and received tap water ad libitum. Behavioural testing took place between 8:00 a.m. and 6:00 p.m. The experiments were performed in accordance with the National Institutes of Health ethical guidelines for the care and use of laboratory animals for experiments and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

6.4.2 Apparatus

The 5-CSRTT was conducted in two operant aluminium chambers (26 x 26 x 26 cm; Campden Instruments Ltd., Loughborough, UK), wherein five apertures (2.5 x 2.5 cm, 4 cm deep) were inserted 2 cm above floor level in the concavely curved rear wall. This assembly provided five response options located equidistant to the food magazine on the opposite. Inside each hole, a light-emitting diode (LED) generated visual stimuli of variable duration. Nose-poke responses of the animals were detected by infra-red photo cell beams at the entrance of the apertures. The rats could be placed in the box through a Plexiglas® door on the upper part of the front wall. Underneath the door, a small Plexiglas® panel provided access to the food magazine which was lighted via two LEDs and automatically supplied with casein pellets (45 mg Dustless Precision Pellets, Bio-Serv®, UK) by an electromechanical feeder. Food collection was detected by a microswitch monitoring the movement of the hinged panel. Each chamber was illuminated by a 3 W house light mounted on the ceiling. A noise-damped fan served as ventilation and background noise of about 60 dB. The grid floor facilitated the removal of excrements. For the purpose of sound attenuation, the wooden cabinet was reinforced with an insulating plate at the interior of the door. The apparatus was controlled by specific software written in Turandot (Cambridge Cognition Ltd., version 1.23) which was run on a personal computer connected to the BNC Mark 2 System (Behavioral Net Controller, Campden Instruments Ltd., Loughborough, UK).

6.4.3 General procedure

6.4.3.1 Training

The animals (n = 22) were trained to detect the occurrence of brief light stimuli in one of the five rear wall apertures. The general procedure was based on the protocol of Campden Instruments and was divided into a habituation, pretraining and baseline training phase (Campden Instruments Limited 2005). Before training and tests the rats were acclimatised to the laboratory for at least 30 min in their homecages.

The first experimental phase comprised two daily half-hour habituation sessions. The boxes were prepared as follows: before the first training session, the tray panel was opened to facilitate access to 15 freely available pellets in order to reinforce the magazine as location of food reward. During the second session, no panel manipulation was carried out. Besides the reward in the tray, two pellets were placed in each aperture to promote exploration of these areas. The chambers were permanently illuminated by the house light during both sessions.

The daily training session lasted 30 min or was finished after completion of 100 trials. Each session started with the simultaneous illumination of the box and the food magazine and the delivery of a single pellet into the tray. Once the rat opened the panel for food retrieval, the first trial was initiated. The magazine light faded and a fixed intertrial interval (ITI) of 5 s started. At the end of the ITI, a light stimulus of determinate duration (stimulus duration, SD) was randomly presented in one of the five holes. The rats had to respond with a nose-poke into the appropriate aperture during the stimulus presentation or within a subsequent limited hold period (LH). A correct response was followed by the supply of a pellet into the lighted food magazine. The next trial was triggered by the panel movement. Inappropriate responses led to a punishment in terms of a predefined 5 s period of darkness (time-out) without reward delivery. The task procedure offered various opportunities for such reactions:

- *incorrect responses* in a hole where no stimulus appeared,

- *omissions* in the form of absent reaction to the occurrence of the stimulus within the LH,
- *premature responses* before the onset of the stimulus during the ITI in one of the apertures
- and *perseverative responses*, meaning additional responses after a correct response and before reward collection.

Every response during the time-out phase reinitialised the period of darkness. Following the time-out, the box and the tray were illuminated again and the next trial was started by a nosepoke into the food magazine. Within a session, the visual stimuli were randomly presented in equal number in each hole. The progressive decrement of the variables SD (60 → 1 s) and LH (60 → 5 s) over eight training levels enabled the acquisition of the 5-CSRTT.

The baseline training session was determined by the conditions of the eighth training level (SD = 1 s, LH = 5 s). After showing a stable baseline performance (>80 % accuracy and <20 % omissions with <10 % variation over five consecutive training sessions), rats underwent surgery.

6.4.3.2 Surgery

The rats were anaesthetised with chloral hydrate (360 mg/kg; Sigma-Aldrich, Steinheim, Germany) and fixed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Stainless steel 21 gauge guide cannulae were implanted unilaterally 1 mm above the target injection site into the vmPFC (anteroposterior +2.7 mm, mediolateral ±0.8 mm, dorsoventral -4.0 mm from Bregma) and 2 mm above the intended injection sites into the contralateral NAc core (anteroposterior +1.2 mm, mediolateral ±1.8 mm, dorsoventral -6.8 mm from Bregma) or shell (anteroposterior +1.2 mm, mediolateral ±0.5 mm, dorsoventral -7.3 mm from Bregma). The sides of the implantations were counterbalanced, resulting in approximately equal numbers of rats with microinfusions in the left or right hemispheres at the level of vmPFC and NAc. Jeweller screws were anchored in the skull serving to fix the cannulae which were embedded in dental cement and closed by removable 26 gauge stylets of the same length. After surgery, the rats were kept individually for three days with free access to food and water. Following a total recovery period of five days, the animals were reintroduced to the baseline training until they re-established the presurgical baseline performance.

6.4.3.3 Microinfusion

The test design comprised four 4-day sessions for the animals. Each session started with an injection day, followed by a day without training. The second and third post-testing day were used to achieve the baseline performance and to ensure the washout process of the drug. Before infusion, the stylets were exchanged for injection cannulae (vmPFC: 27 gauge; NAc: 26 gauge) connected with microlitre syringes (SGE Scientific Glass Engineering, Darmstadt, Germany) via polyethylene tubes. The rats received four sets of combined unilateral microinjections of the $GABA_A$ agonist muscimol (0.05 µg/0.3 µl) and 0.9 % saline as vehicle (0.3 µl) into the vmPFC and the contralateral NAc core or shell according to a pseudorandom Latin square design. The subject groups were divided as follows:

Disconnection group I (vmPFC + NAc core; n = 12): vehicle + vehicle; vehicle + muscimol; muscimol + vehicle; muscimol + muscimol.

Disconnection group II (vmPFC + NAc shell; n = 10): vehicle + vehicle; vehicle + muscimol; muscimol + vehicle; muscimol + muscimol.

The injection rate was 0.1 µl/30 s. The injectors were left in place for 1 min to guarantee diffusion and to avoid reflux of the solution. Ten minutes after the microinjection, the rats underwent behavioural testing. The sequence of the test sessions matched with the baseline training.

6.4.4 Drugs

The $GABA_A$ agonist muscimol was purchased from Tocris Bioscience (Ellisville, MO, USA) and dissolved in 0.9 % saline. Aliquots of stock solutions (0.5 µg/0.3 µl) were prepared and stored at -20 °C until use. On the treatment

day, aliquots were further diluted to a dose of 0.05 µg/0.3 µl. Doses were based on previous studies (Diederich and Koch 2005).

6.4.5 Histology

Upon termination of the experiment, the rats were euthanised with a lethal dose of chloral hydrate. The brains were removed from the skull and immersion-fixed in a 4 % formalin/30 % sucrose solution for 48 h. Coronal 50 µm sections of the mPFC were cut on a cryostat (Jung CM 3000; Leica Instrument GmbH, Nussloch, Germany), mounted on gelatine-coated glass slides and Nissl-stained with thionin. Then, the sections were analysed using a light microscope and injection sites plotted on standardised coronal sections of a rat brain stereotaxic atlas (Paxinos and Watson 1998).

6.4.6 Data analysis

The descriptive statistics is based on means and variance and is indicated by the standard error of the mean (±SEM). The statistical analyses were conducted by the software IBM SPSS Statistics (version 20 for Windows).

The drug effects within the testing group on the following behavioural parameters were investigated using separate two-way split-plot-factorial analysis of variance (ANOVA; within-subject factor: drug treatment, between-subject factor: disconnection group): percentage of correct responses (accuracy; 100 x number of correct responses/number of correct and incorrect responses), percentage of omitted responses (100 x number of omitted responses/total number of correct, incorrect and omitted responses), number of premature responses, number of perseverative responses, number of trials completed, number of time-out responses, latency of correct responses [s] and latency of reward collection [s]. In the case of significant main effects ($P < 0.05$), one-way repeated measures ANOVA and post hoc Bonferroni tests for the factor drug treatment as well as independent *t*-tests between the disconnection groups were conducted separately for each behavioural parameter.

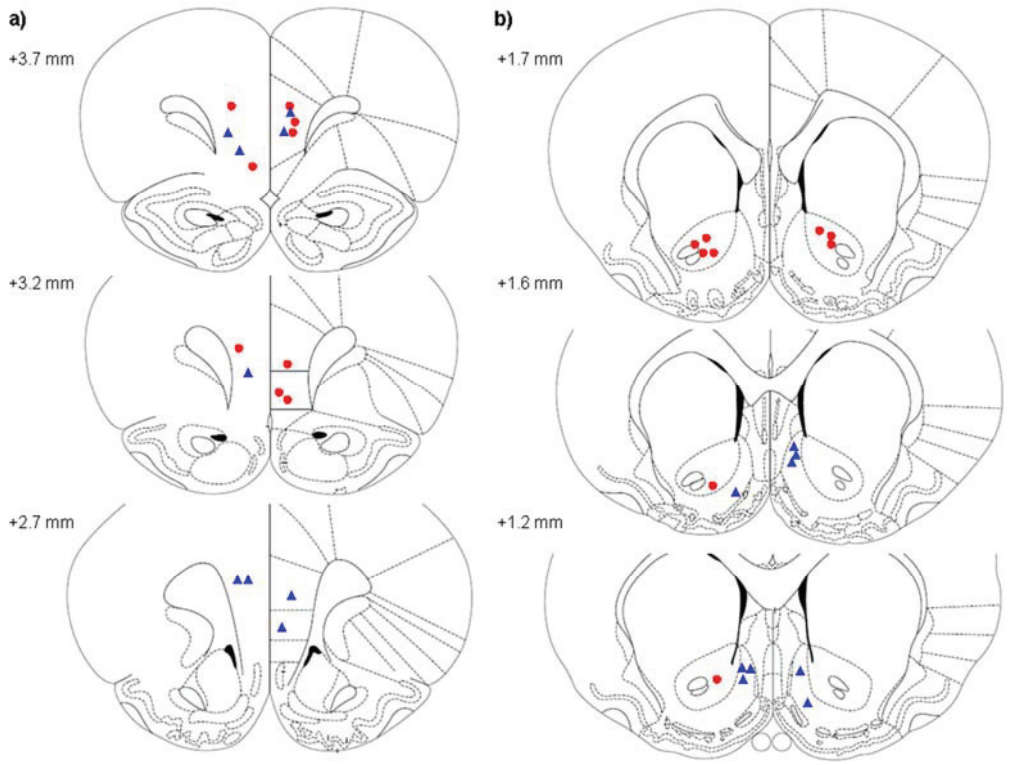

Fig. 6.1 Location of the unilateral injection sites in **a)** the ventral medial prefrontal cortex (circles corresponding to nucleus accumbens core, n = 9; triangles corresponding to nucleus accumbens shell, n = 9) and in **b)** the contralateral nucleus accumbens core (circles, n = 9) or shell (triangles, n = 9) in the 5-choice serial reaction time task depicted on schematic drawings from the rat brain atlas of Paxinos and Watson (1998). Rostral distance (in mm) to bregma is indicated by numbers.

6.5 Results

6.5.1 Histology

In total, 22 rats received unilateral microinjections into the vmPFC combined with contralateral microinfusions into NAc core (n = 12) or shell (n = 10). The histological analysis revealed, as indicated in Fig. 6.1, that 18 rats (n = 9 in each group) had acceptable injection sites accurately located in the target structures with minimal tissue damage.

6.5.2 Effects of inactivation of vmPFC-NAc core and vmPFC-NAc shell connections by muscimol on rats' performance in the 5-CSRTT

Before testing, the rats performed at a stable baseline with high levels of accuracy (disconnection group I: 91.64±1.06 %; disconnection group II: 92.19±0.92 %), fast correct response (disconnection group I: 0.69±0.01 s; disconnection group II: 0.68±0.02 s) and reward collection latencies (disconnection group I: 1.10±0.05 s; disconnection group II: 1.05±0.03 s), low percentages of omissions (disconnection group I: 12.68±1.02 %; disconnection group II: 9.72±1.25 %) as well as low numbers of premature (disconnection group I: 8.10±0.90; disconnection group II: 8.28±1.05) and perseverative responses (disconnection group I: 1.98±0.35; disconnection group II: 2.00±0.54). Analysis of the training data demonstrated no significant differences in the pre- and postoperative sessions and the 'drug-free days' between testing excluding any carry-over effects of drug treatment or surgery (data not shown). Two-way split-plot-factorial ANOVAs on the 5-CSRTT performance showed main

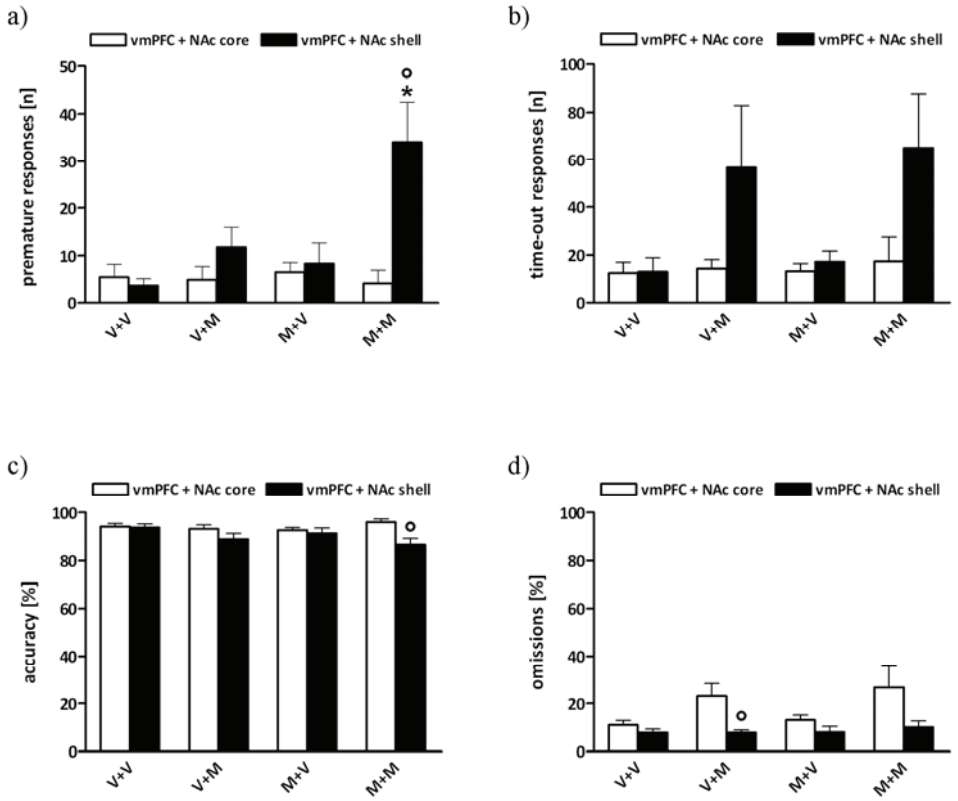

Fig. 6.2 Effects of combined local unilateral infusions of the $GABA_A$ agonist muscimol (M; 0.05 µg/0.3 µl) and 0.9 % saline as vehicle (V) into the ventral medial prefrontal cortex (vmPFC) and the contralateral nucleus accumbens (NAc) core (n = 9) or shell (n = 9) on the rats' performance in the 5-choice serial reaction time task. Data of **a)** premature responses, **b)** time-out responses, **c)** accuracy and **d)** omissions are means ±SEM. Statistically significant differences between drug treatment compared to vehicle are indicated by asterisks (one-way repeated measures ANOVA, post-hoc Bonferroni test, $P < 0.05$) and between subject groups (vmPFC + NAc core compared to vmPFC + NAc shell) by circles (independent *t*-test, $P < 0.05$).

Table 6.1 Effects of combined local unilateral infusions of the $GABA_A$ agonist muscimol (0.05 µg/0.3 µl) and 0.9 % saline as vehicle (V) into the ventral medial prefrontal cortex and the contralateral nucleus accumbens (NAc) core (n = 9) or shell (n = 9) on the rats` performance in the 5-choice serial reaction time task. Data are expressed as means ±SEM.

	Treatment	Trials completed [n]	Perseverative responses [n]	Latency of correct responding [s]	Latency of reward collection [s]
vmPFC + NAc core	V+V	99.22±0.78	1.67±0.37	0.69±0.03	1.21±0.09
	V+M	93.22±4.48	1.89±0.56	0.75±0.04	1.25±0.09
	M+V	100.00±0.00	2.78±0.52	0.71±0.03	1.18±0.06
	M+M	92.22±6.22	1.89±0.61	0.86±0.11	1.51±0.24
	Treatment	Trials completed [n]	Perseverative responses [n]	Latency of correct responding [s]	Latency of reward collection [s]
vmPFC + NAc shell	V+V	100.00±0.00	2.44±0.50	0.67±0.02	1.15±0.04
	V+M	92.44±6.37	2.44±0.58	0.66±0.02	1.11±0.03
	M+V	100.00±0.00	2.56±0.84	0.62±0.03	1.08±0.03
	M+M	91.78±4.50	2.67±0.80	0.71±0.05	1.16±0.06

effects of drug treatment [$F_{(3,51)}$ = 6.119; P = 0.001] and disconnection group [$F_{(1,51)}$ = 5.71; P = 0.03] as well as a statistically significant treatment x disconnection group interaction [$F_{(3,51)}$ = 7.704; P < 0.001] for premature responses, a main effect of disconnection group [$F_{(1,51)}$ = 5.259; P = 0.036] and a statistically significant treatment x disconnection group interaction [$F_{(3,51)}$ = 3.223; P = 0.031] for accuracy, a main effect of disconnection group [$F_{(1,51)}$ = 8.228; P = 0.011] for omissions and a main effect of disconnection group [$F_{(1,51)}$ = 4.754; P = 0.045] for completed trials.

Further one-way repeated measures ANOVAs and post-hoc Bonferroni tests revealed that simultaneous unilateral inactivation of vmPFC and the contralateral NAc shell specifically enhanced impulsive behaviour reflected by a significant increase in premature responding compared to vehicle (P = 0.042), while no other measured parameter was affected (Fig. 6.2 and Table 6.1). Unilateral intra-NAc shell injection of muscimol as well as combined deactivation of vmPFC and NAc shell appeared to augment time-out responses, but this effect did not reach statistical significance (Fig. 6.2b). By contrast, neither unilateral NAc core nor combined vmPFC and NAc core inactivation had any effect on 5-CSRTT performance. Independent *t*-tests between subject groups showed significant differences between the vmPFC-NAc core and vmPFC-NAc shell connection following combined muscimol injection regarding premature responses (P = 0.008) and accuracy (P = 0.006) (Fig. 6.2a, c). Further, unilateral inactivation of NAc core significantly increased the omission rate compared to NAc shell (P = 0.021) (Fig. 6.2d).

6.6 Discussion

In terms of impulsivity, the vmPFC is considered to be primarily implicated in impulse control while there is only limited evidence for an involvement of the NAc, which is more associated with impulsive decision-making. Previous studies revealed that NAc shell lesions showed no effect on premature responding and core lesions merely tended to increase motor impulsivity in the 5-CSRTT. However, the present data show that both the vmPFC and the NAc are involved in the neural network mediating impulse control in the 5-CSRTT in rats,

with a predominant role for the connection of vmPFC and NAc shell. By contrast, the vmPFC-NAc core connection appears to be more crucially involved in attentional behaviour and motivational aspects.

The main findings of this study are that acute disconnection of the vmPFC and NAc shell by simultaneous contralateral inactivation via muscimol considerably enhanced premature responding indicating deficits in impulse control. In contrast, transient disruption of the serial communication between vmPFC and NAc core did not affect impulsive action. Lesion studies have already documented the involvement of the rodent mPFC and NAc in inhibitory response control, but revealed discrepancies regarding different aspects of inhibitory control and specific subregions of the mPFC and NAc (Christakou et al. 2004;Chudasama and Muir 2001;Chudasama et al. 2003;Muir et al. 1996;Murphy et al. 2008;Pezze et al. 2009;Pothuizen et al. 2005b). By use of the $GABA_A$ agonist muscimol, we and other groups have recently shown that the vmPFC, including the prelimbic (PL) and infralimbic (IL) cortices, is critically involved in controlling premature responding in the 5-CSRTT in rats (Feja and Koch 2014;Murphy et al. 2012;Paine et al. 2011). On the subcortical level of the NAc, lesions of the core but not the shell region increased anticipatory responding in response inhibition tasks (Christakou et al. 2004;Murphy et al. 2008;Pothuizen et al. 2005b). Coherently, disconnection lesions of the vmPFC and the NAc core enhanced premature and perseverative responding in the 5-CSRTT, whereas the vmPFC-NAc shell connection was not investigated (Christakou et al. 2004). However, latest work from our laboratory highlighted the role of the NAc shell in terms of motor impulsivity and revealed for the first time that transient deactivation of the shell, but not the core, reduced impulse control in the 5-CSRTT in rats (Feja et al. 2014). The present study verifies our previous findings and confirms that in particular the connection of vmPFC and NAc shell is implicated in the maintenance of impulse control during 5-CSRTT performance.

In comparison to previous lesion studies primarily associating the mPFC-NAc core axis with impulsive action, our results may appear contradictory. However, since the lesion technique carries some drawbacks due to permanent destruction of brain tissue and animals' recovery for several days enabling a potential functional compensation by remaining structures, acute reversible inactivation procedures, as in our case, provide more conclusive evidence of brain area functions (Lomber 1999;Martin and Ghez 1999). Due to different tissue manipulations, the findings of lesion studies have to be compared carefully with those of our present investigation and the lacking effects of shell lesions on impulse control could be attributable to masking effects owing to adaptive functions of adjacent structures.

Asymmetric inactivation of vmPFC and NAc shell also increased the number of time-out responses, although not reaching statistical significance. Time-out responses represent another aspect of inhibitory control, more related to cognitive flexibility (Robbins 2002). The increase of time-out responses substantiates the role of the vmPFC-NAc shell connection in behavioural inhibition. This is further supported by previous findings from our laboratory showing that bilateral injection of muscimol into the vmPFC or the NAc shell increased time-out responses in the 5-CSRTT (Feja and Koch 2014;Feja et al. 2014). Cognitive constructs such as impulsivity and behavioural flexibility are closely interrelated executive processes in the context of inhibitory control, hierarchically top-down mediated by the PFC (Bari and Robbins 2013;Wise 2008). In this regard, vmPFC lesions or inactivations result in behavioural inflexibility in reversal learning tasks in rats (Kosaki and Watanabe 2012;Ragozzino et al. 1999;Ragozzino 2007). The neural network contributing to behavioural flexibility involves both the mPFC and NAc (Coppens et al. 2010). Set-shifting tasks indicated that the mPFC projection to the NAc is important for suppressing inappropriate responses and asymmetrical inactivation of these structures impaired the ability to switch from one discrimination strategy to another (Block et al. 2007). Interestingly, the shell region apparently had a greater impact on the number of time-out responses than the vmPFC, as revealed by unilateral deactivations

of the respective structure. Admittedly, inactivation of NAc shell, in contrast to core, does not impair performance in a set-shifting task in rats, but it was pointed out that the shell mediates the suppression of irrelevant or no-reward behaviours (Blaiss and Janak 2009;Floresco et al. 2006;Floresco et al. 2008a). Thus, unilateral inactivation of NAc shell might have contributed to behavioural disinhibition during 5-CSRTT performance.

Other parameters indexing attentional (omissions), compulsive (perseverative responses), motor (correct response latency) or motivational behaviour (trials completed, reward collection latency) remained unaffected following unilateral intra-vmPFC and intra-shell or combined vmPFC and NAc shell infusions of muscimol.

Taken together, the present behavioural effects on 5-CSRTT performance induced by vmPFC-NAc shell disconnection closely resemble the deficits observed following bilateral vmPFC (Feja and Koch 2014) or NAc shell (Feja et al. 2014) inactivation in the same task, while unilateral control deactivations of the respective regions alone did not produce significant deficits. The asymmetrical manipulation method used in this study is particularly suited to investigate the interaction between components of cortico-subcortical networks (Gaffan and Wilson 2008;Peters et al. 2008). Since neuronal projections, such as frontostriatal connections from the mPFC to the NAc, are predominantly ipsilateral (Berendse et al. 1992), learned behaviours can be preserved by an intact single hemisphere and unilateral manipulations, as in our study, often lead to minor or no cognitive impairments. Via crossed unilateral inactivation of the vmPFC and NAc core or shell, the serial communication between these structures can be bilaterally impeded (Gaffan et al. 1993;Gaffan and Wilson 2008;Setlow et al. 2002). For example, a previous study showed that disconnection of the IL and NAc shell reinstates cocaine seeking in rats after extinction learning, whereas unilateral inactivation of either IL or NAc shell does not alter seeking behaviour (Peters et al. 2008). Consequently, as the effects of the vmPFC-NAc shell disconnection on premature responding in the 5-CSRTT are more pronounced than the additive effect of the single unilateral inactivations, our findings provide evidence that impulse control requires serial information transfer between this specific frontostriatal system.

Unexpectedly, the transient disconnection of vmPFC and NAc core as well as unilateral manipulations of vmPFC or the core region did not produce any significant behavioural effect in the 5-CSRTT compared to control treatment. Contralateral inactivation of vmPFC and NAc core tended to increase the omission rate as well as the reward collection and correct response latencies indicating marginal attentional and locomotor deficits and a slightly reduced motivation for food. Moreover, unilateral deactivation of the core significantly augmented the omission rate compared to the respective manipulation of the shell, implying the impact of NAc core on attention. Previously, we have shown that the core region in contrast to the shell plays an important role in the regulation of locomotion and general responsiveness with a bilaterally inactivated core severely impaired 5-CSRTT performance (Feja et al. 2014). Particularly the strong decrease in the number of completed trials after deactivation of NAc core but not shell represents a consequence of motivational dysfunction and points towards a differential role of both subregions in motivated behaviour in the 5-CSRTT. This is supported by evidence that muscimol injections into the core reduce breakpoint in a progressive ratio schedule in rats (Moscarello et al. 2010), while shell inactivation enhances motivational behaviour in that task (Stratford and Wirtshafter 2012;Wirtshafter and Stratford 2010). However, lesions of the core do not reduce food motivation in a delayed reinforcement task (Cardinal and Cheung 2005) and muscimol does not affect food intake when injected into the NAc core (Stratford and Kelley 1997) and even increases eating behaviour following infusion into the shell (Basso and Kelley 1999;Lopes et al. 2007;Reynolds and Berridge 2002;Soderpalm and Berridge 2000;Stratford and Kelley 1997;Stratford and Wirtshafter 2011).

High scores of impulsivity in the 5-CSRTT inversely correlate with attentional accuracy (Blondeau and Dellu-Hagedorn 2007;Dalley et al. 2008;Puumala and Sirvio 1998). Considering the central role of frontostriatal impairments to the pathophysiology of ADHD, incorporating attentional and impulsive dysfunctions (Nigg and Casey 2005), it seems obvious that this relationship could also be valid for the vmPFC-NAc shell connection, as simultaneous inactivation of vmPFC and NAc shell produced a significant decrease of response accuracy compared to vmPFC-NAc core disconnection. But since the effect of the vmPFC-NAc shell disconnection on accuracy did not differ from control treatment, we suppose the reducing impact on accuracy should be rather seen in consequence of rash-spontaneous impulsive behaviour of the rats leading to some kind of 'careless mistake'.

Meanwhile there is a scientific consensus that impulsive behaviour is not only cortically top-down controlled but also regulated by subcortical areas (Dalley et al. 2011). Most interestingly, impulse control seems to be more depending on an intact NAc shell than on the vmPFC, as bilateral inactivation of the shell enhances premature responding at almost the same rate as the vmPFC-NAc shell disconnection, while bilateral deactivation of the vmPFC only produces approximately half the number of anticipatory responses (Feja and Koch 2014;Feja et al. 2014). Accordingly, we hypothesize that the NAc, particularly the shell region, might function as kind of a bottleneck for impulse control, receiving serial parallel information input from the vmPFC, integrating these input signals of impulse control with emotional (basolateral amygdala), contextual (hippocampus) and arousal content (midline thalamus) and conveying the multiplexed information to downstream brain sites involved in feeding and drinking (lateral hypothalamus), motivation (ventral tegmental area, substantia nigra) and locomotion (caudal mesencephalon). Thus, the original concept of the NAc as a functional interface coordinating limbic, cognitive and motor processes is still valid, expanded by differential contributions of the NAc subregions so that the NAc should not longer be viewed in the sense of an anatomical entity (Carlezon, Jr. and Thomas 2009;Groenewegen and Trimble 2007;Mogenson et al. 1980).

6.7 Conclusion

In conclusion, our results extend previous findings pointing out the functional heterogeneity of frontostriatal systems and show a differential contribution of the vmPFC-NAc connection to behavioural control depending on the involved accumbal subregion. We hypothesize that the maintenance and regulation of impulse control particularly requires an intact connection between the vmPFC and the NAc shell, while the vmPFC-NAc core projection seems to be of minor importance.

7 General discussion

In clinical research, the involvement of specific brain regions in the control of behaviour can be investigated by observing the behavioural alterations in people with injury to these structures. In general, patients with frontostriatal damage display impaired behavioural inhibition, particularly expressed by impulsive decision-making and deficient impulse control compared to healthy control subjects (Costa Dias et al. 2013;Eagle and Baunez 2010;Jentsch and Taylor 1999). Since the human and rat genome encode similar numbers of genes and due to the fact that 90 % of rat genes have orthologs in the human genome, the rat has been the animal model of choice for research in human neurobiology and experimental medicine (Gibbs et al. 2004;Mullins and Mullins 2004). Moreover, both rats and macaque monkeys show homologous organisation of frontostriatal projections from the PL and IL to the NAc (Wise 2008). Thus, lesioning discrete areas of the frontostriatal network in rats has been widely used for comparison with human brain damage and for complementation of clinical research (Eagle and Baunez 2010). Nevertheless, permanent inactivation via the lesion technique requires consideration of concomitant factors. The resulting behavioural effects might not completely be attributable to the destroyed region but could also be influenced by adaptive processes of adjacent remaining structures. Indeed, lesion studies yielded contradictory results regarding impulsive behaviour in rats.

Particularly lesions of the mPFC produced controversial effects on motor impulsivity in the 5-CSRTT, ranging from direct participation (Chudasama et al. 2003), a mere tendency of involvement (Chudasama and Muir 2001) to no important role (Passetti et al. 2002). Inconsistent findings also exist concerning the contribution of the mPFC to choice impulsivity. While one study found increased delay-discounting in mPFC-lesioned rats (Gill et al. 2010), another group declared that the mPFC is not the primary site of this action (Cardinal et al. 2001). In case of the NAc, the core region seems to be clearly involved in impulsivity since excitotoxic lesions induce impulsive choice behaviour as well as impulse control deficits in the 5-CSRTT and DRL task (Bezzina et al. 2007;Bezzina et al. 2008a;Cardinal et al. 2001;Christakou et al. 2004;da Costa et al. 2009;Pothuizen et al. 2005b). By contrast, lesions of the NAc shell do neither influence premature responding nor delay-discounting in rats (Murphy et al. 2008;Pothuizen et al. 2005b). However, the effect of core lesions on impulsive decision-making remains unclear due to discrepancy with other studies lacking any implication in choice impulsivity (Acheson et al. 2006;Gill et al. 2010).

In animals, the lesion method can be extended to study the connectivity between different brain regions by combined unilateral lesioning of each structure in opposite hemispheres (Gaffan and Wilson 2008). Using this approach, disconnection of the mPFC and the NAc core enhances impulsive responding in the 5-CSRTT (Christakou et al. 2004). Unfortunately, a contribution of the mPFC-NAc shell connection was not examined as yet.

By application of transient inactivation tools, many drawbacks accompanying the lesion technique are avoidable, particularly the recovery of function by originally non-involved structures, allowing acute functional investigation of the inactivated regions (Martin and Ghez 1999). In the present thesis, we were able to extend the current knowledge of integral constituents of the frontostriatal network and could further clarify its role in the modulation of decision-making and impulse control. This was achieved by systematically and reversibly inactivating the vmPFC, the NAc subregions core and shell and also by disconnecting the linkage between these structures via local administration of the $GABA_A$ agonist muscimol in rats.

Summarising, the present work revealed that the frontostriatal network differentially contributes to impulsive behaviour depending on the involved NAc subregion and distinct types of impulsivity. The vmPFC and the NAc shell as well as an intact connection between both structures were crucially implicated in the maintenance of impulse control, whereas the core region seems to be more involved into motivational and motor aspects. In comparison to the vmPFC, the NAc appears to have a greater regulative impact on delay-based decision-making as both subareas of the NAc turned out to play a key role, while inactivation of the vmPFC did not affect delay-discounting. Most interestingly, our studies figured out that the priorly often unregarded shell region of the NAc and also its connection with the vmPFC might critically participate in the modulation of impulsivity, at least specifically in terms of impulse control.

7.1 Decision-making

The findings of study 1 and 2 strongly emphasise the NAc as a subcortical key structure in the regulation of impulsive choice and underline the heterogeneity of the PFC in terms of delay-discounting as its ventral medial part displayed no relevance for the top-down control of this specific type of decision-making despite strong connections with the NAc. Thus, choice impulsivity seems to be primarily modulated by other frontocortical regions.

In line with this, previous studies have shown that particularly selective lesions and inactivation of the OFC impair choice behaviour in delay-discounting paradigms (Mobini et al. 2002;Rudebeck et al. 2006;Zeeb et al. 2010). The mPFC is more sensitive to effort-related and probabilistic reinforcement, more associated with motivational and risk-taking behaviour, respectively (St Onge and Floresco 2010;Walton et al. 2002;Walton et al. 2003). The effects of lesioning the mPFC in rats on delay-discounting, mostly reflecting impulsivity-related behaviour, have been rather inconsistent.

The results of study 1 confirm the majority of preceding work pointing out that the mPFC plays a rather minor role in impulsive decision-making (Cardinal et al. 2001;Rudebeck et al. 2006). In our case, reversible bilateral inactivation of the rats' vmPFC by the $GABA_A$ agonist muscimol did not increase the preference for smaller, immediate over larger, delayed rewards. The observed flattening in the typical delay-dependent within-session shift in the preference of the high reward, in accordance with a former report (Cardinal et al. 2001), might be explainable by an insensitivity to the task contingencies due to a disruption of temporal discrimination ability (Cardinal et al. 2004). Another reason could be an abolishment of the learned action-outcome association between response and reward delivery following deactivation of the vmPFC (Balleine and Dickinson 1998). By contrast, transient inactivation of both NAc subregions, independently of one another, markedly decreased selection of the high reward indicating enhanced delay aversion, and thus impulsive choice.

However, comparing study 1 and 2 it has to be considered that delay-based decision-making was assessed using different test designs. In study 1, vmPFC-inactivated rats were tested via a typical delay-discounting paradigm in operant conditioning chambers, while study 2 was conducted in a T-maze using a delayed gratification procedure to investigate the effects of NAc deactivation. Both tasks differed in the range and sequence of their delays, with the discounting model progressively increasing the delay with each session block (0, 10, 20, 40, 60 s) and the T-maze task maintaining the delay constant at 10 s throughout testing. Previous studies demonstrated the impact of the delay duration in that prolonged delay periods of ≥25 s are suitable to assess improvements of waiting capacity, but to a lesser extent an increase of delay aversion and the emergence of impulsive choice (Bizot et al. 2007). Findings from OFC lesion studies revealed that rats even increase the selection of the large reward when confronted with the same delay-discounting protocol as in study 1. However, under shorter delay conditions of 15 s this manipulation induces choice impulsivity in both the T-maze and operant chamber decision-making tasks (Mobini et al. 2002;Rudebeck et al. 2006;Winstanley et al. 2004). This suggests that the vmPFC, at least partially, could also

contribute to impulsive decision-making with rewards delayed in the range of a few seconds.

In keeping with this, local administration of the DA D_1 receptor antagonist SCH23390 and the DA D_2 receptor antagonist raclopride into the mPFC significantly increase impulsive choice in case of delay durations ranging from 0 – 8 s before reinforcer delivery (Pardey et al. 2013). Closer considering the discrete session blocks in study 1 indeed unveiled a trend towards delay aversion for the shortest delay duration (10 s) following intra-vmPFC injection of high-dosed muscimol. Unlike our study, another group found that reversible inactivation of the vmPFC by muscimol causes impulsive choice in the T-maze task under a 15 s delay condition (Churchwell et al. 2009). However, the findings of Churchwell et al. (2009) have to be compared carefully with those of study 1. In contrast to our experiment, that study probably reflects more a mixture of impulsive choice and impulsive action since the rats were able to choose the small immediate reward at any time during the delay, thus requiring them to maintain their response and to increase behavioural inhibition. As a consequence, those results might also point to the fact that the vmPFC is more implicated in impulse control processes than in delay-based decision-making. Further investigations on temporal discounting with short delays could help to elucidate a potential involvement of the vmPFC in choice impulsivity.

In respect of the NAc, the core region is already associated with the principal forms of cost/benefit decision-making, including probability- (Cardinal and Howes 2005), effort- (Ghods-Sharifi and Floresco 2010;Hauber and Sommer 2009) and delay-discounting (Bezzina et al. 2007;Cardinal et al. 2001;Pothuizen et al. 2005b). Dysfunctions of the shell region to date only lead to risk-based choice behaviour (Stopper and Floresco 2011). The current transient deactivation of NAc core as well as shell induced choice impulsivity in the T-maze, with a higher impact of core inactivation on the rats' waiting capacity compared to shell. Thus, the results of study 2 contradict the leading opinion that the NAc shell does not contribute to impulsive choice behaviour and strengthen the hypothesis that the NAc core is preferentially involved in the control of decision-making under delayed reinforcement conditions (Cardinal et al. 2001;Pothuizen et al. 2005b).

The fact that intra-NAc shell injection of muscimol elicited impulsive choice underlines the greater significance of reversible inactivation studies compared to lesion experiments, as selective excitotoxic NAc shell lesions have no effect on temporal discounting (Pothuizen et al. 2005b). The lacking impact of the permanent inactivation method could be attributable to the formation of adaptation processes of adjacent brain structures during animals' postoperative recovery period resulting in the takeover of

regulating rash decision-making. By contrast, transient inactivation by muscimol allows acute investigation of the actual function of the manipulated region providing higher data reliability. Impaired waiting capacity following reversible inactivation of NAc subregions was caused by delay-based reward devaluation, substantiated with evidence that functional NAc is necessary to bridge action-outcome delays and to maintain a representation of the anticipated gratification (Cardinal and Cheung 2005;Roesch et al. 2009).

One might suggest that the disparate impact of vmPFC versus NAc inactivation on choice impulsivity is to be ascribed to the difference between reward ratios (4:1 in study 1, 10:2 in study 2), as it has been proposed that increasing the proportion of the high to the low reward may ameliorate impulsive behaviour (Cardinal 2006). However, this would have implied a greater potential of the T-maze task to generate impulsive decision-making than the delay-discounting paradigm of study 2. Apart from this, lesions of the NAc, as well as bilateral inactivation of the vmPFC, do not disrupt the perception of the relative incentive value and the sensitivity for the magnitude discrimination of the rewards (Balleine and Killcross 1994;Bezzina et al. 2007;Cardinal and Cheung 2005;Churchwell et al. 2009). A further argument against an influence of primary motivational aspects on the delay-discounting rate is that muscimol does not affect eating behaviour when injected into the core and even increases food intake following administration into the shell (Basso and Kelley 1999;Lopes et al. 2007;Reynolds and Berridge 2002;Soderpalm and Berridge 2000;Stratford and Kelley 1997;Stratford and Wirtshafter 2011).

7.2 Impulse control

The results of study 1, 2 and 3 provide evidence of a frontostriatal network (comprising the vmPFC and the NAc) being crucially involved in the regulation of impulse control in rats. In study 1, reversible inactivation of the vmPFC via muscimol induced impulsive action in the 5-CSRTT and efficiently confirmed the assumption that this specific part of the frontal cortex is heavily implicated in impulse control. The findings of study 2 demonstrate that the NAc does not only participate in the modulation of delay-based decision-making, but also contributes to the maintenance of inhibitory response control.

As in several other behaviours, the NAc subregions core and shell showed functional dichotomy concerning motor impulsivity in the 5-CSRTT. Interestingly, transient inactivation of the less explored Nac shell, but not the core, by bilateral microinjection of muscimol

produced impulse control deficits in the 5-CSRTT. The indication of a crucial role of the connection between the vmPFC and the NAc shell gained from study 1 and 2 is further corroborated by study 3. This work elucidates that the regulation of impulse control in the 5-CSRTT primarily requires an intact vmPFC-NAc shell connection compared to a frontostriatal circuit composed of vmPFC and NAc core whose disconnection did not significantly alter 5-CSRTT performance. The present behavioural effects following muscimol injection into the NAc and resulting from disconnection of the vmPFC and the NAc contrast with findings of previous lesion studies. Hence, they illustrate, as above-mentioned, the difficulties in the comparability of both methods, with acute reversible inactivation procedures revealing a more realistic status of brain structure functions than the lesion technique (Lomber 1999;Martin and Ghez 1999).

The current work clearly demonstrates that impulsive action in the 5-CSRTT is not only contingent on top-down control by cortical areas, but is also regulated on the subcortical level of the NAc. Surprisingly, an intact NAc shell even seems to have a greater importance in the vmPFC-NAc shell circuit than the cortical structure, since bilateral inactivation of the shell produced approximately twice as many premature responses compared to muscimol application into the vmPFC.

The present results strengthen the theory that the anatomically heterogeneous connectivity between the mPFC and the NAc is paralleled by functional subregional specifity. The mPFC projects topographically to the NAc, in that dorsal regions primarily innervate the core while the shell receives afferents from ventral parts of the mPFC (Berendse et al. 1992;Brog et al. 1993;Heidbreder and Groenewegen 2003). As the vmPFC is critically involved in impulse control in the 5-CSRTT (Chudasama et al. 2003;Feja and Koch 2014), it is obvious that the NAc shell may act as the accumbal output structure of impulsive action from the vmPFC. Further support for the contribution of the vmPFC-NAc connection to impulse control comes from patch-clamp recordings investigating the mechanisms underlying response inhibition in the rat vmPFC. This work revealed that impulse control is encoded by a selective strengthening of prelimbic projections to the ventral striatum (Hayton et al. 2010).

The effects of the current studies support the postulated dissociation of different components of 5-CSRTT performance, such as attentional ability and impulse control, which are mediated to some extent by distinct frontostriatal circuits (Robbins 2002). Accordingly, we observed effects on the inhibitory control of premature responding in the absence of affected response accuracy and vice versa. Our investigations reveal that low-dose muscimol injections bilaterally into the vmPFC and NAc shell as well as combined contralateral

inactivation of both structures significantly increase the number of anticipatory responses. Other measured parameters indexing attentional (accuracy, omissions), compulsive (perseverative responses), motor (correct response latency) or motivational (trials completed, reward collection latency) behaviour remained unaffected. On the contrary, the core region seems to play an important role in the regulation of motivational and motor aspects during the 5-CSRTT performance. However, the number of completed trials as well as correct response and reward collection latencies reflecting motivation and locomotion were not significantly affected following vmPFC-NAc core disconnection.

An explanatory approach for the divergent effects of bilateral NAc core deactivation compared to the vmPFC-NAc core disconnection could be that the transient disruption of this specific frontostriatal system induced distinct but overlapping mechanisms, via additional circuits, which are offset against one another. Optogenetic stimulation of mPFC DA D1 neurons activates glutamatergic neurons in the BLA and excitatory transmission from the BLA to the NAc promotes motivated behavioural responding for sucrose intake in mice (Land et al. 2014;Stuber et al. 2011), suggesting that inactivation of the vmPFC might lead to a top-down inhibition of feeding. On the other hand, muscimol inactivation of the mPFC in rats reduces overall response latencies and increases premature errors in a time-estimation task due to deficient adaptive control of the downstream motor cortex (Narayanan et al. 2013). Hence, in case of the present vmPFC-NAc core disconnection, a decreased appetitive drive mediated on both cortical and subcortical level might have been opposed to an enhanced locomotor drive elicited by the vmPFC, resulting in only a slight lengthening of speed latencies.

High numbers of omissions might also indicate motor or motivational impairments (Robbins 2002). We suggest that the mildly enhanced omission rate after vmPFC-NAc core disconnection reflects an attentional deficit. It supports the contention that frontostriatal systems comprising the core region of the NAc might be more involved in attentional control rather than in response inhibition. However, our results also imply that the vmPFC plays a minor role in attentional performance, as unilateral manipulations of this structure changed the omission rate less than core inactivation. Moreover, core-lesioned rats significantly increase the omission rate in a ‘forced choice’ task (Murphy et al. 2008). In line with this, lesion studies suggest dissociable roles of the dorsal and ventral subregions of the mPFC on the 5-CSRTT performance. IL and PL seem to be more implicated in impulsive and compulsive behaviours, whereas attentional and motivational parameters like accuracy, omissions and the latency of reward collection appear to be rather modulated by the dorsally

located AC (Chudasama et al. 2003;Chudasama and Muir 2001;Passetti et al. 2002). As the NAc core is more innervated by dorsal parts of the mPFC, a frontostriatal circuit composed of the dorsal mPFC and NAc core might make a more substantial contribution to aspects of the 5-CSRTT performance than the vmPFC-NAc core connection.

The vmPFC of rats and its putative primate equivalent, the AC, are anatomically and functionally strongly interconnected with the NAc, whereas the rodent dorsal mPFC preferentially innervates the dorsomedial striatum (Alexander et al. 1986;Berendse et al. 1992;Brog et al. 1993;Ding et al. 2001;Ferry et al. 2000;Gorelova and Yang 1997;McGeorge and Faull 1989;Preuss 1995;Sesack et al. 1989;Vertes 2004). Previous studies already dissociated the mPFC-NAc core projection from the connection between mPFC and dorsomedial striatum regarding 5-CSRTT performance. The first system markedly affects aspects of response control, while the latter is principally involved in aspects of visual attention (Christakou et al. 2001;Christakou et al. 2004). Our results further substantiate the concept of functionally segregated frontostriatal connections (Alexander et al. 1986) and give evidence that impulse control is also differentially regulated depending on which vmPFC-NAc subsystem is involved. Increasing evidence points to two independent limbic cortico-basal ganglia-thalamocortical circuits, with the first network involving dorsal parts of the PFC and the NAc core and the second circuit comprising connections from the vmPFC to the NAc shell (Dalley et al. 2008;Groenewegen et al. 1999).

7.3 Impulsive action versus impulsive choice

On the basis of present knowledge it is accepted among experts that impulsivity is not a unitary construct and can be broadly subdivided into impulsive choice and impulsive action in both humans and rats, showing several dissociations particularly following neural and neurochemical manipulations (Broos et al. 2012;Evenden 1999b;Winstanley et al. 2006). However, these two types of impulsivity are similarly modulated by drugs such as the selective noradrenaline reuptake inhibitor atomoxetine and the psychostimulants methylphenidate and D-amphetamine, which are used to treat ADHD (Caballero and Nahata 2003;de Wit et al. 2002;Robinson et al. 2008b;Robinson et al. 2009;Solanto 1998;Winstanley et al. 2006). Impulsive behaviour is regulated in dependence of the involved neuroanatomical system. As participating cortico-limbic-striatal circuits show considerable overlap regarding impulsive choice and impulsive action (Pattij and Vanderschuren 2008), one might

hypothesise that the vmPFC and the NAc contribute to both forms of impulsivity. Thus, in study 1 and 2, we, for the first time, directly compared the role of the vmPFC and NAc subregions, respectively, in these two main types of impulsive behaviour. The present investigations reveal that the control function of the vmPFC is impulsivity-type specific, with a critical contribution to impulse control in the 5-CSRTT, but without an implication in impulsive decision-making in the delay-discounting task. The clear-cut distinction of motor and choice impulsivity on the level of the vmPFC could be explained by the fact that the type of behavioural inhibition required in delay-based decision-making paradigms is probably quite different to response inhibition procedures, like the 5-CSRTT, involving the withholding of a motor response. In delay-discounting tasks, the organisation of motor performance is of minor importance and decision-making processes demanding the ability to discriminate between future outcomes have priority (Bari and Robbins 2013;Evenden 1999b), which are apparently top-down controlled by other cortical regions than the vmPFC.

A recent report claimed that impulsive rats in the 5-CSRTT also exhibit high levels of impulsive decision-making in a delay-discounting task and proposed a combined impulsive phenotype featuring a specific deficit in 'waiting impulsivity'. This group further associated changes in a neural network including the NAc with the impulsive phenotype (Robinson et al. 2009). The findings of study 2 indeed demonstrate the involvement of the NAc in both types of impulsivity, but solely with regard to the NAc shell. Hence, the present results are further evidence for the disparate nature of impulsive choice and impulsive action and the functional heterogeneity of the NAc subregions.

Impulsivity is modulated by multiple neurotransmitter systems. Dysfunctions of the 5-HT and DA systems have long been implicated in impulsivity. In dependence on the involved neuroanatomical system and participating receptor subtypes, bidirectional effects on distinct forms of impulsivity, namely impulsive choice versus impulsive action, are observable (Evenden and Ryan 1999;Pattij and Vanderschuren 2008;Robinson et al. 2008a). Elevated 5-HT levels in the mPFC correlate with impaired impulse control and intra-mPFC administration of the 5-$HT_{2A/C}$ receptor agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropan hydrochloride (DOI) induces impulsive over-responding in the 5-CSRTT. On the other hand, 5-HT-related delay aversion is primarily regulated in the OFC among frontocortical structures (Dalley et al. 2002;Wischhof et al. 2011). At the level of the NAc, 5-HT seems to play a rather minor role (Fletcher et al. 2009;Koskinen and Sirvio 2001;Robinson et al. 2008a;Winstanley et al. 2005), whereas abnormalities in DA

transmission in the corticostriatal circuitry are associated with impulse control disorders (Genro et al. 2010;Zimmer 2009).

The mPFC and NAc are critical elements of the mesocorticolimbic system, comprising dopaminergic projections from the VTA to the mPFC (mesocortical way) and VTA DA neurons innervating the NAc (mesolimbic way). Hence, except for the direct glutamatergic excitatory projections, the mPFC can influence NAc function through cortico-limbic-striatal loops or connections to the VTA (Carr and Sesack 2000;Sesack et al. 2003). In turn, prefrontal cortical inputs are tonically and phasically modulated through D_1- and D_2-like receptors in the NAc (Goto and Grace 2005). Previous studies give reason to assume that PFC efferents exert an inhibitory action on DA release in the NAc and differentially modulate DA function in the NAc subcompartiments, underlining the heterogeneity of core and shell. The blockade of NMDA receptors in the PFC increases the DA release in the NAc (Del Arco and Mora 2008). Following systemic amphetamine administration, nuclear levels of the phosphorylated transcription factor CREB (calcium and cAMP response element-binding protein) reflecting neuronal activation are upregulated in the NAc shell, but not core, in PFC-lesioned rats, indicating that the lesion had upregulated accumbal DA (Dalley et al. 1999;Pezze et al. 2009). In keeping with this, impulsive action in the 5-CSRTT correlates with increased DA release due to reduced dopamine D2/3 receptor availability and higher D1 receptor mRNA expression in the shell, but decreased DA release caused by lower D1 receptor binding in the core (Diergaarde et al. 2008;Jupp et al. 2013;Simon et al. 2013).

Bilateral inactivation of either vmPFC or NAc shell as well as the vmPFC-NAc shell disconnection might have induced an increase of DA levels in the accumbens shell resulting in deficient impulse control. This is supported by a previous study showing that vmPFC inactivation results in the disinhibition of phasic excitations at the level of the NAc shell that can thereby be driven by dopaminergic input from the VTA promoting behavioural cue responding. Besides, deactivation of the vmPFC reduces the basal firing of NAc shell neurons that tonically suppress inappropriate actions (Ghazizadeh et al. 2012). Further evidence for the dependence of behavioural disinhibition on DA signalling in the NAc shell comes from another group demonstrating decreased reinstatement of heroine seeking following combined injections of muscimol and the $GABA_B$ receptor agonist baclofen into the vmPFC and the D1 receptor antagonist SCH 23390 into the contralateral accumbens shell (Bossert et al. 2012). An increase in extracellular DA levels in the shell might occur in consequence of inactivation of this region due to its feedback loop involving the VTA. In normal conditions, terminal DA release in the NAc is tonically inhibited via $GABA_A$ receptors in the VTA (Ikemoto et al.

1997;Rahman and McBride 2002). By implication, activating $GABA_A$ receptors in the shell with muscimol may hyperpolarise the MSN projecting to the VTA leading to disinhibition of DA neurons targeting the NAc shell (see Fig. 7.1). Consistently, blockade of $GABA_A$ receptors within the VTA increases the discharge rate of DA neurons innervating the NAc (Ikemoto et al. 1997).

Diergaarde *et al.* (2008) further revealed that impulsive choice is associated with reduced DA reactivity in both NAc regions, confirming our findings that core as well as shell is involved in impulsive decision-making. The difference in the DA hypothesis in relation to distinct types of impulsivity suggests that impulsive behaviours are modulated by interactions of multiple neurotransmitters in the NAc (Winstanley et al. 2005). This might explain why the present inactivation of NAc shell impaired both impulse control and decision-making, whereas specific 5-HT lesions or DA depletions of the NAc do not change delay-discounting and premature responding (Cole and Robbins 1989;Fletcher et al. 2009;Winstanley et al. 2005). Interestingly, the shell receives, as the only striatal area, significant noradrenergic input from regions of the caudal brainstem, like the locus coeruleus (LC) (Delfs et al. 1998;Groenewegen and Trimble 2007). Human studies provide evidence for phasic activity of the neuromodulatory LC-noradrenaline system in response to the outcome of stimulus evaluation and internal decision-making processes (Nieuwenhuis et al. 2005). Furthermore, the activity of the noradrenaline (NA) transporter is known to be important in regulating impulsive behaviour and systemic treatment with the selective NA reuptake inhibitor atomoxetine decreases choice impulsivity in rats (Robinson et al. 2008b;Sun et al. 2012). The substantial noradrenergic innervation by the LC let suggest that NA might regulate NAc shell function (Berridge et al. 1997). In line with this, neurotoxic denervation of the LC projections reduces the DA release potential in the NAc shell (Haidkind et al. 2002). Besides, the LC sends noradrenergic projections to the VTA resulting in a cross-talk between dopaminergic and noradrenergic systems in the rat VTA with a regulative inhibitory effect of NA on VTA dopaminergic activity, and reciprocally (Guiard et al. 2008). Consequently, NA dysfunctions or DA/NA interactions in a network comprising the mesolimbic system and caudal brainstem might contribute to impulsive choice behaviour (see Fig. 7.1). Moreover, intra-shell but not -core administration of atomoxetine decreases premature responding in the 5-CSRTT (Economidou et al. 2012), substantiating the shell region as a subcortical key element in the regulation of impulsivity.

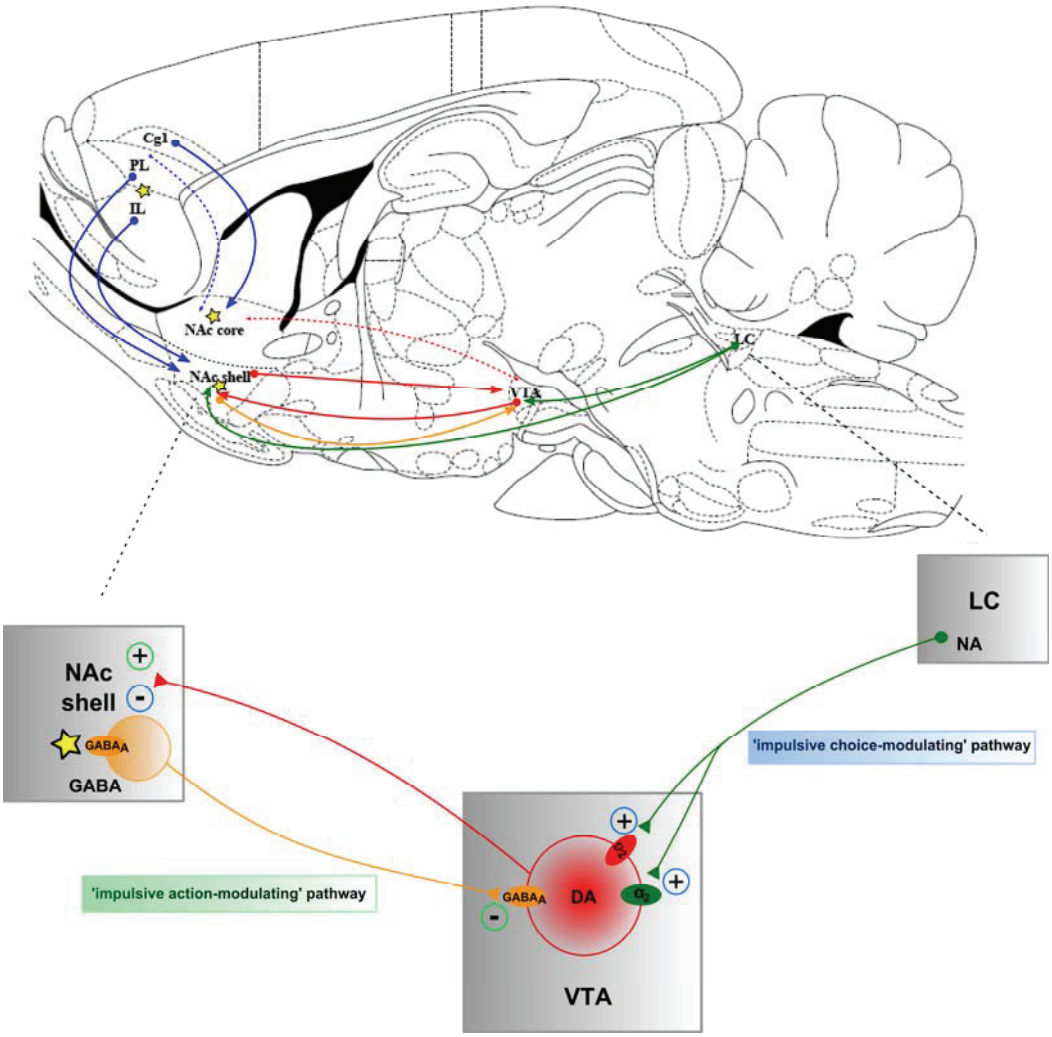

Fig. 7.1 Schematic representation of anatomical connections within frontostriatal circuits (blue arrows) - comprising the dorsal medial prefrontal cortex (Cg1 and dorsal PL), the ventral medial prefrontal cortex (ventral PL and IL) and the nucleus accumbens (NAc) subregions core and shell -, the mesolimbic system from the ventral tegmental area (VTA) to the NAc and its noradrenergic innervation (green arrows) by the locus coeruleus (LC), as well as potential functional relationships between the NAc shell, the VTA and the LC regarding impulsive action and impulsive choice. Dopaminergic projections between the NAc and the VTA are indicated by red arrows, γ-aminobutyric acid (GABA) projections from the NAc shell to the VTA are indicated by orange arrows, and microinfusion sites of the $GABA_A$ receptor agonist muscimol are indicated by yellow asterisks. *Impulsive action-modulating pathway*: intra-NAc shell injection of muscimol hyperpolarises the GABAergic medium spiny neurons projecting to the VTA leading to a reduced tonic inhibition of dopamine (DA) neurons targeting the NAc shell and resulting in increased DA levels in the shell promoting impulsive action. *Impulsive choice-modulating pathway*: noradrenergic projections from the LC to the VTA induce a regulative inhibitory effect of noradrenaline (NA) on the firing activity of VTA DA neurons via activation of DA D_2 and adrenergic α_2-receptors leading to a reduced DA release in the NAc shell associated with impulsive choice. The effects on neuronal activity are indicated by (+) and (-) illustrating increased and decreased neuronal firing, respectively, as well as green and blue circles representing the impulsive action and impulsive choice-modulating pathway, respectively. Cg1, dorsal cingulate cortex area 1; IL, infralimbic cortex; PL, prelimbic cortex.

7.4 Conclusion and future directions

Taken together, the present results corroborate the hypothesis that impulsive behaviour is not only subjected to top-down control by cortical structures, but also regulated at subcortical level. Our data indicate separable impulsivity processes in the vmPFC and NAc when rats make choices involving delay costs or have to control their impulses. Motor impulsivity is regulated by both structures, while choice impulsivity is principally modulated by the NAc, and not the vmPFC. Further, the current investigation suggests both functional dissociations and close interactions between the vmPFC and NAc in terms of impulse control, depending on the involved accumbal subregion. A fundamental finding of our studies is that the NAc shell constitutes the critical region mediating both types of impulsivity, whereas the NAc core caused non-specific impairments beyond impulsive choice. Consequently, our work points towards various specific frontostriatal systems differentially contributing to delay-based decision-making and particularly impulse control.

Although it may be difficult to directly compare the gained knowledge with deficits following human cortical damage or with findings from animal lesion studies, the use of reversible inactivation techniques is an effective analytical tool in the area of basic biological and pharmacological research, especially for dissecting the implications of distinct neuroanatomical structures or systems in specific brain functions. Future animal studies could be conducted in a combination of functional imaging techniques (positron emission tomography or fMRI) with reversible inactivation procedures, like muscimol microinfusion, on the same experimental subjects. This would permit the verification of activated brain regions during performance of a specific task with the subsequent opportunity to temporarily deactivate the respective area and to prove its true involvement in task performance in case of an observed behavioural impairment. Additionally, the use of electrophysiological or metabolic approaches, such as measuring the change in cerebral glucose metabolism during inactivation, might function as an alternative to fluorescent conjugates to estimate the spatial extent of inactivation (Lomber 1999).

Impulse control disorders represent one of the main comorbidities of binge eating disorder (BES) (Hudson et al. 2007). Both individuals with impulse control deficits and BES patients display dysfunctions in the frontostriatal network (Avena and Bocarsly 2012;Balodis et al. 2013;Jentsch and Taylor 1999;Lock et al. 2011;Nigg and Casey 2005). The NAc is suggested to play a key role since reversible inactivation of the shell produces impairments of impulse control as well as intense hyperphagia (Feja et al. 2014;Stratford and Kelley 1997).

Further studies demonstrate the contribution of orexin neuropeptides to the control of binge eating episodes and show extensive projections of orexin neurons to the frontostriatal circuitry (Fadel and Deutch 2002;Piccoli et al. 2012). Thus, an interesting continuation of the current project could deal with the question whether both behavioural phenotypes correlate in animal models and, if so, which impact the orexinergic system exerts in this context within the frontostriatal network.

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