universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

"Interactions between the prefrontal cortex and ventral striatum in the mediation of risk-based decision making in rodents"

Verfasserin

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angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2012

Studienkennzahl lt. Studienblatt:	A 490
Studienrichtung lt. Studienblatt:	Diplomstudium Molekulare Biologie
Betreuer:	Prof. Thomas Klausberger

Table of Contents

1. Introduction 3
   1.1. The role of the Prefrontal Cortex in decision making 5
   1.2. The role of the Nucleus Accumbens in decision making 8
   1.3. The role of the Amygdala in decision making 9
   1.4. The role of the Dopamine System in decision making 11
   1.5. The role of interactions between the PFC, the NAc and the BLA in decision making 12
2. Methods 19
   2.1. Animals 19
   2.2. Apparatus 19
   2.3. Training of the animals 20
   2.4. Surgery and microinfusion protocol 23
   2.5. Histology 27
   2.6. Data Analysis 28
3. Results 30
4. Discussion 33
5. References 45
6. Appendix 50
   6.1. Acknowledgement 50
   6.2. Summary 51
   6.3. German Summary (Zusammenfassung) 52
   6.4. Curriculum Vitae 53

1. Introduction

In our everyday life, we routinely have to decide between options associated with different costs and benefits. Greater outcomes are often related to higher costs, meaning that we have to work harder to achieve our goal, wait longer to be rewarded, or that we have to take the risk not to be rewarded at all or even to suffer losses.

Decisions under risk involve choices between smaller but more certain rewards and larger, probabilistic rewards. Real-world situations requiring assessments of the relative risks and rewards associated with different options include not only playing the stock market or gambling at a casino. These decisions have to be made all the time, for example when we decide whether to cross the street in order to catch the bus, even if we take the risk to be knocked down by an approaching car, or to wait for the next bus. Still, gambling is the most obvious example, what makes it a popular basis for studies on the neuropsychological background of risk-based decision making.

Different types of gambling tasks, that are designed to simulate real-life decisions, have been developed to assess this form of executive functioning. The original version of the task was developed by Bechara, Damasio, and colleagues and is now referred to as the "Iowa Gambling Task". In this task, subjects choose between decks of cards that yield either high immediate monetary gain but larger future loss, or smaller gains and smaller future loss. Healthy subjects eventually develop the optimal strategy, shifting their choice preference away from the high-risk decks, selecting the low-risk decks to obtain long-term gains. Patients with damage to specific brain regions displayed impaired decision making, making more high risk choices that led to long-term loss (Bechara *et al.*, 1994). These findings led to an increase in research attempting to reveal neural circuits that mediate cost-benefit decision making. The assessment of different patient populations, development of new tasks, and functional brain imaging techniques helped identifying some of the distributed neural circuits mediating different aspects of this type of decision making. The Iowa Gambling Task and similar tasks revealed distributed neural networks that mediate decision making. These networks incorporate different regions of the prefrontal cortex (PFC), the

ventral striatum, the amygdala, and the dopamine system, as well as the hippocampus that will not be discussed in detail in this thesis.

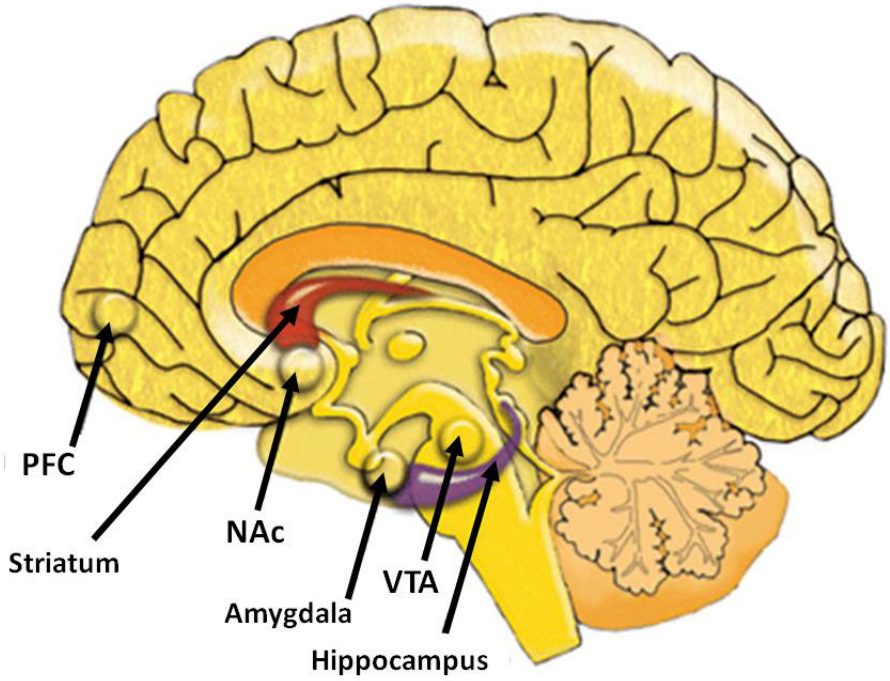

Figure 1: The prefrontal cortex (PFC), the nucleus accumbens (NAc) region of the ventral striatum, the amygdala, the hippocampus, and dopaminergic input from the ventral tegmental area (VTA) have been shown to play a role in different types of decision making.

To help clarify how these brain regions contribute to different aspects of decision making, rodent models, that allow much greater experimental control, have been established. These tasks are very similar to human tasks, except for the fact that humans typically experience "punishment", meaning the loss of previously obtained monetary rewards. Rats, in the contrary, receive food reinforcement as rewarding stimuli, that are usually consumed immediately and can't be taken away later. Nevertheless, animal models can help to assess the evaluation of costs and rewards associated with certain actions (Floresco *et al.*, 2008a). In these studies, rats usually have to choose between a small reward associated with low costs, and a larger reward associated with higher costs. Costs can take multiple forms, including delaying reward delivery, requiring more effort to obtain it or, again, making the reward probabilistic.

1.1. The role of the Prefrontal Cortex in decision making

Patients with damage to the ventromedial regions of the prefrontal cortex (PFC) – encompassing the orbitofrontal cortex (OFC) and ventral aspects of the anterior cingulate cortex – displayed impaired decision making in the Iowa Gambling Task, making more high risk choices that led to long-term loss (Bechara *et al.*, 1994; Bechara *et al.*, 1999). Other areas of the PFC that have been associated with impaired performance in risk-based decision making tasks are the medial and lateral regions of the OFC (Ernst *et al.*, 2004; Clark *et al.*, 2003; Rogers *et al.*, 1999a; Rogers *et al.*, 1999b; Manes *et al.*, 2002; Clark *et al.*, 2008; Fukui *et al.*, 2005), the dorsolateral PFC (Labudda *et al.*, 2008; Brand *et al.*, 2004; Ernst *et al.*, 2002; Manes *et al.*, 2002; Fellows and Farah, 2005), the dorsal (Brodmann's Area 24) and ventral (Area 32) regions of the anterior cingulate (Lawrence *et al.*, 2009; Ernst *et al.*, 2002; Labudda *et al.*, 2008), and the insular cortex (Clark *et al.*, 2008; Ernst *et al.*, 2002; Bar-On *et al.*, 2003; Smith *et al.*, 2009).

It is important to note, that different studies show considerable discrepancies in terms of which regions of the PFC play a critical role in decision-making processes requiring cost-benefit evaluations about risks and rewards. For example, damage to the OFC or dorsolateral PFC has been associated with impaired decision making in some studies (Bechara *et al.*, 1994; Bechara *et al.*, 1999; Rogers *et al.*, 1999b; Brand *et al.*, 2004; Fellows and Farah, 2005), but not in others (Manes *et al.*, 2002; Clark *et al.*, 2003). Reasons for these discrepancies may be due to differences in specific loci of damage, that vary considerably between patients. Often, damage may also encompass multiple regions of the PFC, what makes conclusions even more difficult. Another fact that makes this type of decision-making studies so difficult to interpret is that impairments in the task are not necessarily due to impairments in the evaluation of relative risks and rewards. They may reflect a simple failure to learn the reward magnitudes or win-lose contingencies associated with different choice options, a general deficit in strategy acquisition and maintenance, or a preference for high-risk options regardless of losses. For example, impaired decision making displayed by patients with ventromedial PFC damage may have various reasons (Clark *et al.*, 2004). Regions of the anterior cingulate have been implicated to contribute to multiple aspects of decision making, including monitoring of changes in reward contingencies, loss or negative

feedback, increasing potential or received gain in order to adapt behavior (Bush *et al.*, 2002; Cohen *et al.*, 2008; Rogers *et al.*, 2004). Although it is apparent that multiple frontal lobe regions are facilitating risk/reward decisions, the specific contributions of different PFC regions to risk-based decision making are still not clear.

To assess how different regions of the PFC contribute to aspects of cost-benefit decision making, several rodent studies have been conducted. Different forms of these judgments seem to be mediated by dissociable regions of the rat PFC. The OFC, for example appears to be a critically involved in delay discounting (impulsivity), where animals have to chose between small immediate, and large delayed rewards. The dorsal anterior cingulate cortex has been shown to mediate decisions related to effort costs associated with larger rewards. (Floresco and Ghods-Sharifi, 2007; Winstanley *et al.*, 2004; Rudebeck *et al.*, 2006; Zeeb *et al.*, 2010)

Studies on the contribution of PFC regions to risk-based decision making showed controversial results. Lesions of the OFC (encompassing portions of the medial PFC) increased the preference for small certain food rewards relative to larger probabilistic rewards in one study (Mobini *et al.*, 2002), whereas another study, using a procedure modeled after the Iowa Gambling task, showed the opposite effect (Pais-Vieira *et al.*, 2007). Bearing in mind that, in both studies, lesions to the OFC were induced prior to training on the task, impairments on the task indicate a possible role of the OFC in learning the risk/reward contingencies, but not necessarily a role in the final decision making process. St. Onge and Floresco (2009) investigated the effects of reversible inactivation of different subregions of the rat PFC on risk-based decision making, using a probabilistic discounting task which was originally modified from that described by Cardinal and Howes (2005).This task was conducted in operant chambers, where rats chose between responses that yielded a Small/Certain food reward (one sugar pellet), or a Large/Risky food reward that was delivered in a probabilistic manner (four sugar pellets), where the odds of receiving the large reward decreased over a session from 100% in the first trial block, to 50%, 25% and 12.5% in the latter trial blocks. Rats were well trained on the task prior to receiving reversible inactivation of one of four different subregions of the PFC. Subregions analyzed in this study

were the prelimbic medial PFC (mPFC), the OFC, anterior cingulate cortex and insular cortex. Only inactivation of the prelimbic region of the medial PFC significantly altered risk discounting. Normal controls biased their choice towards the Small/Certain reward when the session progressed and the probability of obtaining the Large/Risky reward decreased. Rats with inactivation of the medial PFC showed a slight shift into the same direction, but much less than normal controls. The result was a significant increase in the proportion of choices directed towards the Large/Risky option. To clarify the specific nature of the disruption in risk-based decision making induced by medial PFC inactivation, subsequent experiments were conducted. Rats were tested on the same task, but this time the odds of obtaining the Large/Risky reward increased over the session. Controls now shifted their choice away from the Small/Certain reward, choosing the risky option more often as the session progressed. Again, rats with inactivated medial PFC failed to shift their choice as much as control groups, what led to a decrease in the over-all choice of the Large/Risky option. These findings indicate that the animals were slower to adjust their choice behavior as the probability of obtaining the large reward changed, what suggests that the medial PFC may facilitate adjustments in choice behavior under changing conditions. A subsequently conducted within-session reversal task showed that this alteration in choice behavior was not due to general perturbations in response flexibility. Also the possibility that medial PFC inactivations led to a fundamental disruption in calculating the relative value of the Large/Risky option could be eliminated. (St. Onge and Floresco, 2009)

This region is anatomically homologous to Area 32 of the human anterior cingulate cortex and also shares functional homology to the dorsolateral PFC (Uylings *et al.*, 2003; Uylings and van Eden, 1991). Both regions have been shown to play an important role in mediating risk-based decision making in humans. These findings suggest that the medial PFC of the rat may share functions that are similar to those mediated by the ventral anterior cingulate cortex and the dorsolateral PFC in humans, which include monitoring choice information and adjusting behavior in response to decision outcomes. (St. Onge and Floresco, 2009)

1.2. The role of the Nucleus Accumbens in decision making

Another brain area heavily implicated in contributing to this type of decision making is the nucleus accumbens (NAc) region of the ventral striatum. In a financial risk task, conducted by Kuhnen and Knutson (2005), humans were asked to choose between a safe bond that always yielded a small gain, or two risky stocks, which yielded either a large gain or loss. In this study, NAc activation preceded risky choices and risk-seeking mistakes. Other studies employing different risk-based decision making tasks, showed similar results, indicating that NAc activation may bias choice toward riskier options associated with greater rewards (Rao *et al.*, 2008; Knutson *et al.*, 2008; Kuhnen and Knutson, 2005; Samanez-Larkin *et al.*, 2010; Matthews *et al.*, 2004).

The findings that the NAc plays a role in cost-benefit decision making are further supported by rodent studies, whereas most of these studies focused on the contribution of the NAc to effort-based decision making or impulsivity. Inactivation or lesion to this nucleus reduced the preference to work harder or to wait longer for the larger reward (Cardinal *et al.*, 2001; Hauber and Sommer, 2009; Ghods-Sharifi and Floresco, 2010).

In a probabilistic discounting task, conducted by Cardinal and Howes (2005), rats had to chose between a Small/Certain option that always led to delivery of one reward pellet, and a Large/Risky option that led to delivery of four pellets with a probability that decreased as the session progressed (the original version of the task utilized by St. Onge and Floresco in 2009, described above). Excitotoxic lesions of the NAc, induced after initial training, caused rats to be relatively indifferent to the two alternatives, choosing the Large/Risky option on about 50% of all trials, regardless of the probability of delivery. With extended training, lesioned rats eventually biased their choice toward the Small/Certain option earlier in the session, when the Large/Risky option was more advantageous. In a control experiment, the probability of receiving the large reward was fixed to 100%. In this task, lesions to the NAc did not significantly reduce choice of the large reward option. These results indicate, that the NAc is involved specifically in the processing of differently valued rewards under conditions of uncertainty and that lesions of the NAc lead to risk aversion. (Cardinal and Howes, 2005)

The NAc can be subdivided into two regions that differ in a variety of neurochemical and anatomical characteristics: NAc core and shell (Brog *et al.*, 1993; Ikemoto and Panksepp, 1999). Lesions of the NAc core and shell have been found to cause differential impairments on behavioral tasks. Cost-benefit decision making tasks using subregion-selective manipulations of the NAc also identified dissociations between the subregions, indicating that the core plays a more critical role than the shell in both delay- and effort-based decision making (Ghods-Sharifi and Floresco, 2010; Hauber and Sommer, 2009).

Stopper and Floresco (2010) utilized a probabilistic discounting task (modified from the one described by Cardinal and Howes (2005), see above) to investigate the contributions of both NAc core and shell to risk-based decision making. Animals were trained prior to reversible inactivations to the entire nucleus, and then to either the core or shell. NAc inactivation caused a significant decrease in the proportion of choices directed toward the Large/Risky option. Further analysis showed that inactivation of the NAc decreased the probability of choosing the Large/Risky option following receipt of the large reward. The same effect was induced by inactivation of the shell, whereas inactivation of the NAc core had no effects on choice preference but increased choice latencies. Controls experiments using fixed probabilities demonstrated that inactivation of the NAc biased choice away from the Large/Risky option, especially when that option had greater long-term value than the Small/Certain option (Stopper and Floresco, 2011).

1.3. The role of the Amygdala in decision making

The amygdala plays a major role in different forms of cost-benefit decision making. Functional imaging studies have reported an increase in amygdala activation when subjects chose options associated with larger reward magnitudes (Smith *et al.*, 2009), or risky options in the context of loss (De Martino *et al.*, 2006). Also, patients with amygdala damage make more disadvantageous, risky choices on decision making tasks simulating real-life decisions associated with risk, reward and punishment (Bechara *et al.*, 1999). These findings are not surprising, considering that the amygdala has a well established role in reward-related associative learning (Seymour and Dolan, 2008). It is assumed, that the contribution of the basolateral amygdala (BLA) to different forms of decision making is related to its proposed

role in integrating information about potential outcome values with action-outcome associations to guide behavior (Ghods-Sharifi *et al.*, 2009).

Various animal studies focused on the role of the BLA in cost-benefit decision making. Inactivations or lesions of the rat BLA reduced preference for larger, delayed rewards (Winstanley *et al.*, 2004) or larger rewards that require rats to climb a barrier to obtain them (Floresco and Ghods-Sharifi, 2007).

Ghods-Sharifi and colleagues (2009) investigated the role of the BLA in risk-based decision making, using the discounting paradigm described above (Cardinal and Howes, 2005). Reversible inactivation of the BLA induced a risk-averse pattern of choice, decreasing the proportion of times, the Large/Risky option was chosen. A targeted analysis of these data showed, that this reduction of Large/Risky choice occurred mainly in the middle part of the session, when the probabilities of being rewarded were either 50% or 25%. These two middle trial blocks provided the most uncertainty in terms of the probability of receiving the larger reward (50%) or the overall amount that could be obtained after selection of either option (25% probability of receiving the large four pellet reward versus 100% probability of receiving the small one pellet reward – both options were yielding the same long term outcome). Furthermore, BLA inactivation led to a significant increase in response latencies and trial omissions in the middle and later trial blocks, where decision-making was harder. Thus, inactivation of the BLA reduces preference for larger, probabilistic rewards in rodent studies, especially under conditions of great uncertainty about the most profitable course of action, under which the decision making process also slows down. These findings are in great conflict with the results of human studies, described above, where subjects with amygdala damage biased their choice towards riskier options associated with larger rewards. One possible explanation for these discrepancies is the fact that human studies involve the loss of already obtained reward, experienced as punishment, whereas this study did not employ any explicit punishments, the risk was just a lost opportunity to gain reward. Both situations, being rewarded and getting punished, are accompanied by strong emotions. Considering the BLA's central role in emotional processing, one argumentation could be, that in healthy patients, the fear of punishment is stronger than the excitement for being rewarded, causing

subjects to choose safer strategies. In amygdala patients, this factor drops out and subjects play riskier. In the rodent task described above, the strongest experienced emotion probably was winning four sugar pellets. After inactivation of the BLA, the strong emotions associated with win could not be experienced anymore, causing rats to chose a safer strategy.

Amygdala lesions in animals have shown to interfere with the ability of punishers to affect instrumental behavior in other studies (Killcross *et al.*, 1997). Another consideration is, that in the human task described above, choosing the high-risk option is usually disadvantageous, yielding smaller gains or long-term loss. In the rodent task, rats also made more disadvantageous choices after BLA inactivation, selecting the large-risky option less often, when it was beneficial to do so. These findings indicate that the BLA might be important to bias choice towards maximal long-term gains. Disruption in amygdala function may increase or decrease risky choice, dependent on which pattern is less profitable in the long term (Ghods-Sharifi *et al.*, 2009).

1.4. The role of the Dopamine System in decision making

In both human and non-human species medial regions of the PFC are thought to monitor choice information in response to decision outcomes, whereas the amygdala and ventral striatum play a role in value representation. All these regions receive a dense dopaminergic input from the ventral tegmental area, what suggests that alterations in dopamine transmission may affect decision-making. Several studies on neuropsychiatric patients support this assumption: Patients with diseases of the dopamine system, like schizophrenia (Hutton *et al.*, 2002), Parkinson's disease (Pagonabarraga *et al.*, 2007), major depression (Must *et al.*, 2006) and stimulant abusers (Rogers *et al.*, 1999a) all show altered choice behavior in risk-based decision making tasks. Both schizophrenics and stimulant abusers also show impulsive choice on delay-based decision making tasks (Heerey *et al.*, 2007). As many of these studies also used medicated patients, the alterations in decision making can in some extend be attributed to treatments, increasing or decreasing mesocorticolimbic dopamine activity (Floresco *et al.*, 2008a). St. Onge and colleagues (2010) investigated the effects of dopaminergic manipulations on risky choice on rats, using the task described above (St. Onge and Floresco, 2009). The dopamine agonist amphetamine increased the preference for

Large/Risky choice when the probabilities of receiving the large reward decreased over a session, but reduced preference when probabilities increased. Flupenthixol, a dopamine antagonist, consistently decreased preference for Large/Risky options. These findings led to the conclusion, that reductions in normal dopamine release bias choice away from larger, probabilistic rewards, whereas increased dopamine activity may disrupt behavioral adjustments in response to changes in the relative value of certain versus uncertain rewards (St. Onge *et al.*, 2010).

1.5. The role of interactions between the PFC, the NAc and the BLA in decision making

All of the above-mentioned regions are intimately interconnected: PFC and amygdala are reciprocally connected with each other, and both send unidirectional projections to the ventral striatum. The reciprocal connection of PFC and amygdala allows top-down processing of information that is sent from the PFC to the amygdala, as well as bottom-up processing of information sent from the amygdala to the PFC (Sesack *et al.*, 1989; Brog *et al.*, 1993; McDonald, 1987; McDonald, 1991b; McDonald, 1991a; McDonald *et al.*, 1996).

Functional imaging studies showed that these regions interact with each other when subjects make decisions about probabilistic rewards. The human anterior cingulate cortex was shown to be functionally connected with the amygdala, when subjects anticipated reward outcomes (Marsh *et al.*, 2007), and to the NAc, when they chose high-risk, compared to low-risk gambles (Cohen *et al.*, 2005). Based on accumulating evidence, it is suggested that a cortico-limbic-striatal circuit, linking NAc, BLA and prelimbic medial PFC, mediates risk-based decision making. Functional imaging techniques can reveal which regions share functional connectivity, but to directly test whether connectivity between these regions is essential for task performance, rodent models that enable the direct disconnection of specific pathways provide much better opportunities (St. Onge and Floresco, 2010).

St. Onge and Floresco (2010) assessed this question, employing an asymmetrical disconnection approach, based on the assumption that information is transferred serially from one brain structure to another, on both brain hemispheres in parallel and that functional regions in one hemispheres can, at least to a certain extent, compensate for dysfunctions of the same structure in the other hemisphere. Inactivation of the origin of the

pathway on one side of the brain, and of the terminal region on the contralateral side blocks the pathway.

To visualize the principle underlying this disconnection design, imagine the disconnection of two structures, A and B. A gets inactivated in the left hemisphere, B in the right hemisphere. In this case, the left A cannot send any information to the left B. The right A can still send information to the right B, which is however inactivated itself and therefore cannot process incoming information. The same happens to information normally transferred from B to A: either the origin or the terminal is inactivated, preventing both structures from interacting with each other. To be maximally effective, possible contralateral connections between the relevant structures have to be minimized by transection of the corpus callosum in the appropriate region. All other A and B functions are presumably not affected, as the right A and the left B can at least partially compensate for their contralateral equivalents. Therefore, potential behavioral disruptions can be attributed mainly to a disruption in communication between those two brain regions. In addition to saline control groups, control groups receiving ipsilateral inactivations of both regions (for example right A and right B) are used to discriminate between behavioral effects that are due to either a contralateral disconnection, or rather to an inactivation of the structures themselves or disconnection of both structures on only one side of the brain.

The animals were first trained on a probabilistic discounting task, similar to the one described above. Again, rats had to chose between a Small/Certain and a Large/Risky option, whereas the probability of obtaining the small reward was always 100%, for the large reward it was 100% in the first trial block, and decreased to 50%, 25% and 12.5% in the latter blocks. Once stable choice patterns were established, different groups of animals received different disconnection manipulations:

- Disconnection of BLA and NAc, by asymmetrical inactivation of both structures.
- Disconnection of mPFC and BLA, by asymmetrical inactivation of both structures.
- Disconnection of mPFC-to-BLA top-down processing, by asymmetrical inactivation of the BLA and the ventromedial internal capsule that is part of the descending neuronal pathway connecting both structures.

- Disconnection of BLA-to-mPFC bottom-up processing, by asymmetrical inactivation of the mPFC and the ventrolateral amygdalofugal pathway, the ascending pathway connecting both structures.

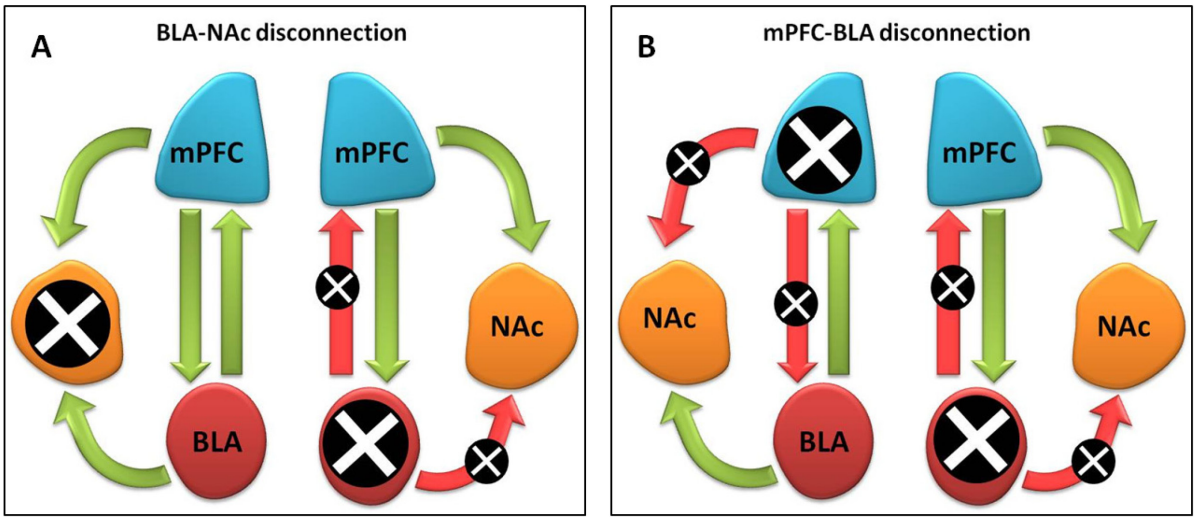

Figure 2: Asymmetrical disconnection design: A) BLA and NAc disconnection: Inactivation of the BLA in one hemisphere and of the NAc in the contralateral hemisphere selectively disrupts communication between these structures. B) medial PFC and BLA disconnection: Inactivation of the mPFC in one hemisphere and of the BLA in the contralateral hemisphere selectively disrupts communication between these structures. Arrows indicate functional (green) and dysfunctional (red) signaling between structures.

Projections from the BLA to the NAc and PFC are primarily ipsilateral. The PFC, however, sends both ipsi- and contralateral projections to the BLA. In the experiment where descending PFC inputs to the BLA were selectively disconnected, the procedures included a transection of the corpus callosum in a region caudal to the PFC, where axons crossed over to the contralateral BLA.

Asymmetrical, as well as ipsilateral disconnection of the BLA-NAc pathway significantly reduced choice of the Large/Risky option. A subset of rats showed no decrease in risky choice after ipsilateral inactivation (n=7), but still showed reduced risky choice after functional disconnection of the BLA-NAc pathway. This effect was, as subsequent experiments showed, not attributable to a general disruption in discriminating between

rewards of different magnitudes. These findings indicate that neural activity within this subcortical amygdalar-ventral striatal pathway plays a role in biasing choice behavior towards options yielding larger rewards that may be risky, but may also be more profitable when probabilities of being rewarded are high.

Choice biases after BLA-NAc disconnection

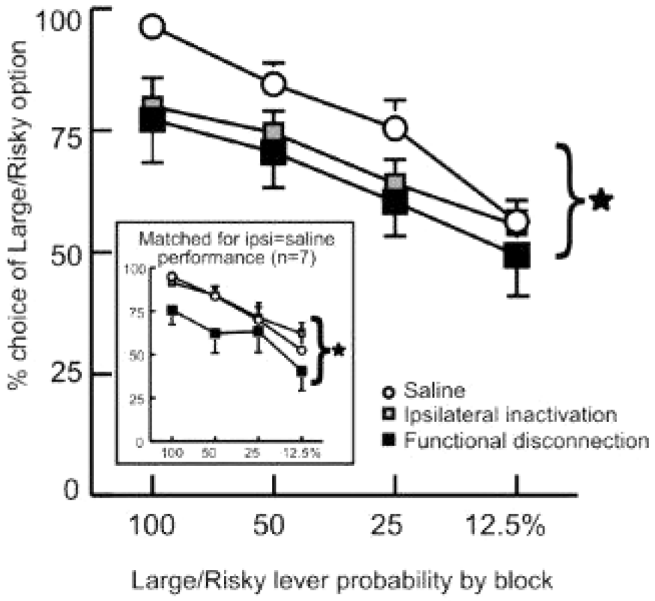

Figure 3: Percentage of choice directed towards the Large/Risky lever following functional BLA-NAc disconnection and control treatments across all four trial blocks. Symbols represent mean +SEM. * $p<0.05$ (ipsilateral/functional disconnection vs. saline). The inset shows data from a subset of rats that showed no decrease in choice of the Large/Risky option after ipsilateral inactivation, yet showed a decrease in risky choice after functional disconnection. (n=7) * $p<0.05$ (functional disconnection vs. saline); (St. Onge and Floresco, 2010)

Asymmetrical disconnection of the medial PFC-BLA pathway significantly increased choice of the Large/Risky option in the last two trial blocks, when the Small/Certain reward would have been more advantageous. These treatments also caused an increase in response latencies and trial omissions (when the animal made no choice within ten seconds). The medial PFC-BLA disconnection especially reduced lose-shift tendencies. Thus, the medial PFC-BLA circuit seems to enable adjustments in decision making when reward probabilities change, by mitigating choice behavior in response to negative feedback (reward omission). A similar result was observed after disruption of descending inputs from the medial PFC to the

BLA. Disruption of the ascending pathway didn't cause any effects on task performance, indicating that regulation of decision making by prefrontal-amygdalar circuitry is achieved primarily by top-down control by the medial PFC. Thus, when larger, riskier options seem to be profitable, information sent from the medial PFC to the BLA may bias choice towards larger rewards, driven by the BLA-NAc circuitry. (St. Onge and Floresco, 2010)

Choice biases after mPFC-BLA disconnection

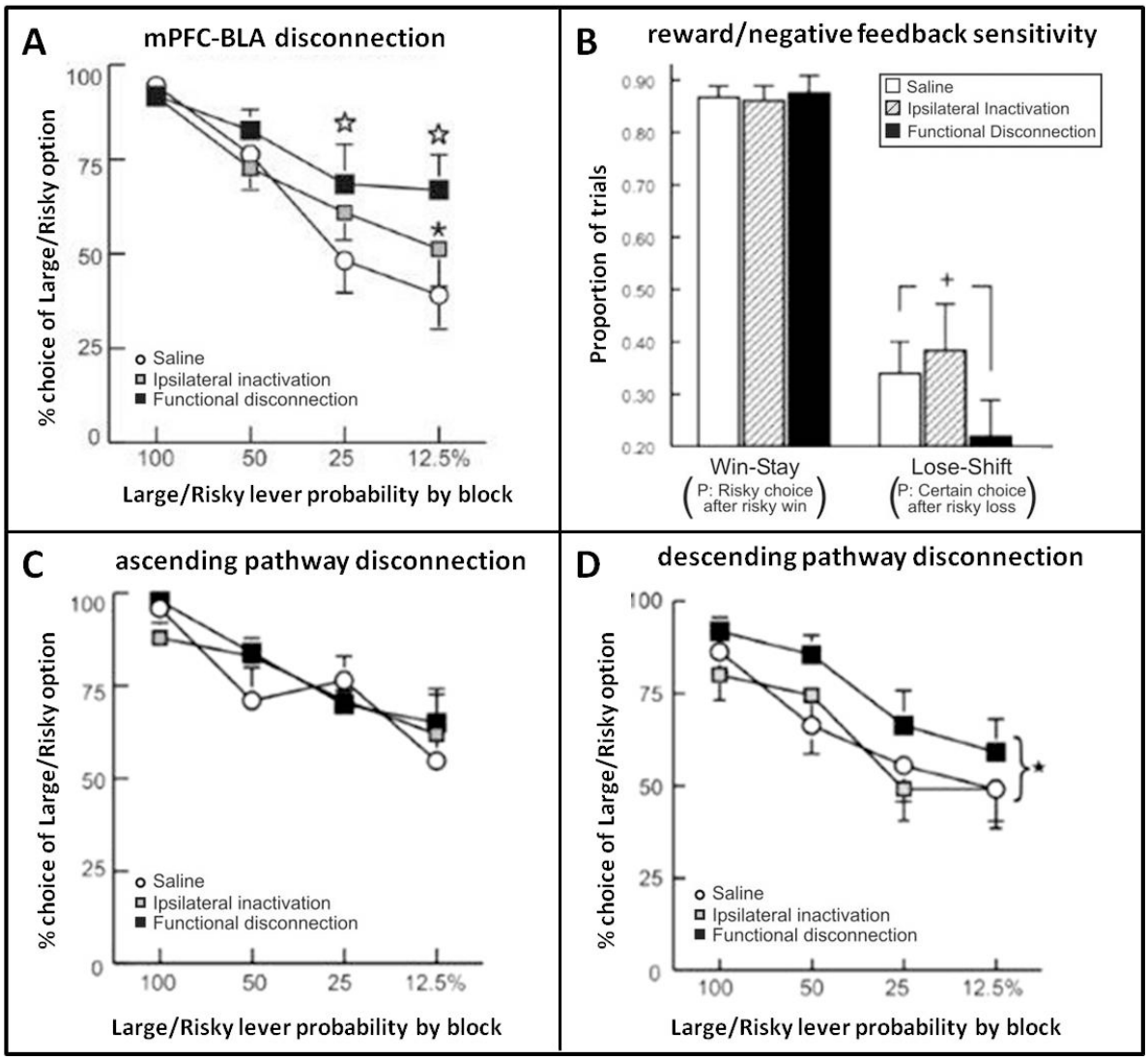

Figure 4: Disconnection of the medial PFC-BLA pathway: Symbols represent mean +SEM. A) Percentage of choice directed towards the Large/Risky lever following functional medial PFC-BLA disconnections, and control treatments. Disconnection of this pathway increased choice of the Large/Risky option. Open star: $p<0.05$ (saline vs. functional disconnection), * $p<0.05$ (ipsilateral inactivations vs. functional disconnection) B) Win-stay and lose-shift data for functional medial PFC-BLA disconnections. An increase in risky choice after disconnection of this pathway was attributable to a reduced negative feedback sensitivity (decreased lose-shift performance) + $p=0.059$ C) Percentage of choice directed towards the Large/Risky lever following disconnection of the ascending BLA-medial PFC pathway, and control treatments. Disconnection of this pathway had no significant effect on risky choice. D) Percentage of choice directed towards the Large/Risky lever following functional disconnection of the descending medial PFC-BLA pathway, and control treatments. Disconnection of this pathway increased choice of the Large/Risky option. * $p<0.05$ (functional disconnection vs. saline); (St. Onge and Floresco, 2010)

These experiments clarified the role of interactions between the medial PFC and the BLA, as well as interactions between the BLA and the NAc. What remained unclear is, whether the medial PFC directly interacts with the NAc when rats make choices associated with risk, and how this interaction contributes to performance in a risk-based decision making task. The purpose of my diploma thesis was to assess this topic.

2. Methods

Similar methods were described before (St. Onge and Floresco, 2009; Stopper and Floresco, 2011).

2.1. Animals

For this experiment, male Long Evans rats from Charles River Laboratories (Montreal, Canada) were used. On arrival, the rats were group housed and free fed for one week to acclimatize to the colony. They were handled for a minimum of five minutes each day to get used to human handling and had ad libitum access to water for the duration of the experiment. One week before behavioral training the animals were single caged and food restricted to 85%-90% of their free-feeding weight (13-18 g food per rat per day). At the beginning of behavioral training they weighted 250-300 g. Body weights were monitored at the end of the experimental day before feeding, which occurred in the rats' home cages. For the duration of the experiment, the animals were kept on food restriction that allowed them to gain weight constantly, as they were still growing (20-22 g food per rat per day). The lights in the animal house went on at 7:00 am and off at 7:00 pm. All testing was in accordance with the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia.

2.2. Apparatus

Behavioral training and testing was conducted in twelve operant chambers (30.5 x 24 x 21 cm; Med-Associates, St Albans, VT). Each chamber was fitted with two retractable levers, one located on each side of the central food receptacle where food reinforcement (45 mg sugar pellets; Bioserv, Frenchtown, NJ) was delivered via a pellet dispenser. The chambers were enclosed in sound-attenuating boxes equipped with a fan to mask extraneous noise and to provide ventilation. A single 100-mA houselight, located in the top center of the wall opposite the levers, enabled illumination of the chambers. To monitor locomotor activity, four photobeams were mounted on the sides of each chamber. The number of photobeam breaks during a session was used as an index of locomotion. All experimental data were recorded by an IBM personal computer connected to the chambers via an interface.

2.3. Training of the animals

One day before the rats were first exposed to the operant chambers, they were given approximately 25 reward pellets in their home cages to get familiar with them.

Phase 1: Lever press training (2 days)

On the first day, rats were trained to acquire the lever press response. Half of the rats started with the left lever manually inserted into the chamber, the other half with the right one inserted. Before placing the rats in the chambers, three to four pellets were delivered into the central food receptacles, and some crushed pellets were placed on the extended levers. Rats were trained to a criterion of 50 presses in 30 min. All rats reached the criterion on the first day of training and could be trained with the opposite lever extended on the next day. As they reached the criterion again on the following day, they could move to the next training phase.

Phase 2: Retractable lever press training (3 days)

Each session started with the houselight off. Every 40 sec, one of the two levers was extended and the houselight came on. In every pair of trials, the left or right lever was presented once, and the order within the pair of trials was random. The rats had to press the lever within ten seconds of its insertion to receive a food pellet, otherwise, the lever retracted and the light extinguished until the next trial started (referred to as an “omission”). On every press, the rats received one food pellet with a probability of 50%. This schedule familiarized the rats with the probabilistic nature of the full task. Each 60 min session consisted of 90 trials. Rats were trained on the task for three days. At this point, they made fewer than five omissions over one session and could move to the next phase immediately.

Phase 3: Side preference test (1 day, immediately after last Phase 2 training)

For different reasons, rats often prefer one lever over the other, what can retard the training period. Therefore, a side preference test was started right after the rats had finished Phase 2. For the first time, rats got both levers inserted into the chamber. On the first trial, a press on either lever gave them one pellet. 20 sec later, both levers came out again. If the rat chose the same lever as before, it got no pellet. This continued until it chose the other lever,

got a pellet and could move to the next trial. The program continued until seven trials, and kept track of the first choice (left or right lever) made in each trial. From these data, the side preference of the rat was determined. In the final training phase, each rat was trained the program where the risky lever was opposite the one it preferred during the side preference test. Seven rats preferred the left lever, nine the right one.

Phase 4: The final Probabilistic discounting task (48 min)

This task was modified from the original procedure described by Cardinal and Howes (2005) and described before (St. Onge and Floresco, 2009; Stopper and Floresco, 2011). On this decision making task, the rats were trained for the duration of the experiment on five to six days a week. Each 48 min session consisted of 72 discrete choice trials, separated into four blocks of 18 trials. A session began with both levers retracted and the houselights off (the intertrial state). A trial started every 40 sec with the illumination of the houselight and, three seconds later, insertion of either one or both levers into the chamber. The lever preferred during the side preference test was designated the Small/Certain lever, the other the Large/Risky lever, which remained consistent throughout training . If the rat did not respond within ten seconds of lever presentation, the chamber was reset to the intertrial state until the next trial started (omission). A response on either lever caused both levers to be retracted. Choice of the Small/Certain lever always delivered one pellet. Choice of the Large/Risky lever delivered four pellets in a probabilistic manner that was varied across the session (see below). Multiple pellets were always delivered 0.5 sec apart.

The four blocks each started with eight forced choice trials where only one lever was presented (four trials for each lever, randomized in pairs), permitting animals to learn the amount of food associated with each lever press and the respective probability of receiving reinforcement during each block. This was followed by ten free-choice trials, where both levers were inserted. After food was delivered, the houselight remained on for another four seconds before the chamber was reset to the intertrial state until the next trial started. The probability of obtaining four pellets after pressing the Large/Risky lever was 100% in the first block, then 50%, 25% and 12.5%, respectively, for the next three blocks. For each session and trial block, the probability of receiving the large reward was drawn randomly from a set

probability distribution. Therefore, on any given day, the probabilities in each block may vary, but on average across many training days, the actual probability experienced by the rat would approximate the set value. Using these probabilities, selection of the Large/Risky lever would be advantageous in the first two blocks, and disadvantageous in the last block, whereas rats could obtain an equivalent number of food pellets after pressing either lever in the 25% block. Therefore, in the last three trial blocks, selection of the large reward option carried with it the risk, of not obtaining any reward on a given trial. Response latencies and locomotor activity (photobeam breaks) were also recorded.

Rats were trained on the task until as a group they chose the Large/Risky lever on at least 80% of successful trials during the first block and demonstrated stable baseline levels of discounting for three consecutive days. Stability was assessed using statistical procedures similar to that described by Stopper and Floresco (2011) and Ghods-Sharifi and colleagues (2009). In this procedure, data from three consecutive sessions were analyzed with a repeated-measures ANOVA with two within-subjects factors: Day and Trial Block. If the effect of Trial Block was significant (at $p < 0.05$ level), but there was no main effect of Day or Day x Trial Block interaction (at $p > 0.1$ level), animals were judged to have achieved stable baseline levels of choice behavior. After this criterion was reached, rats were provided food *ad libitum* and were subjected to surgery two to five days later.

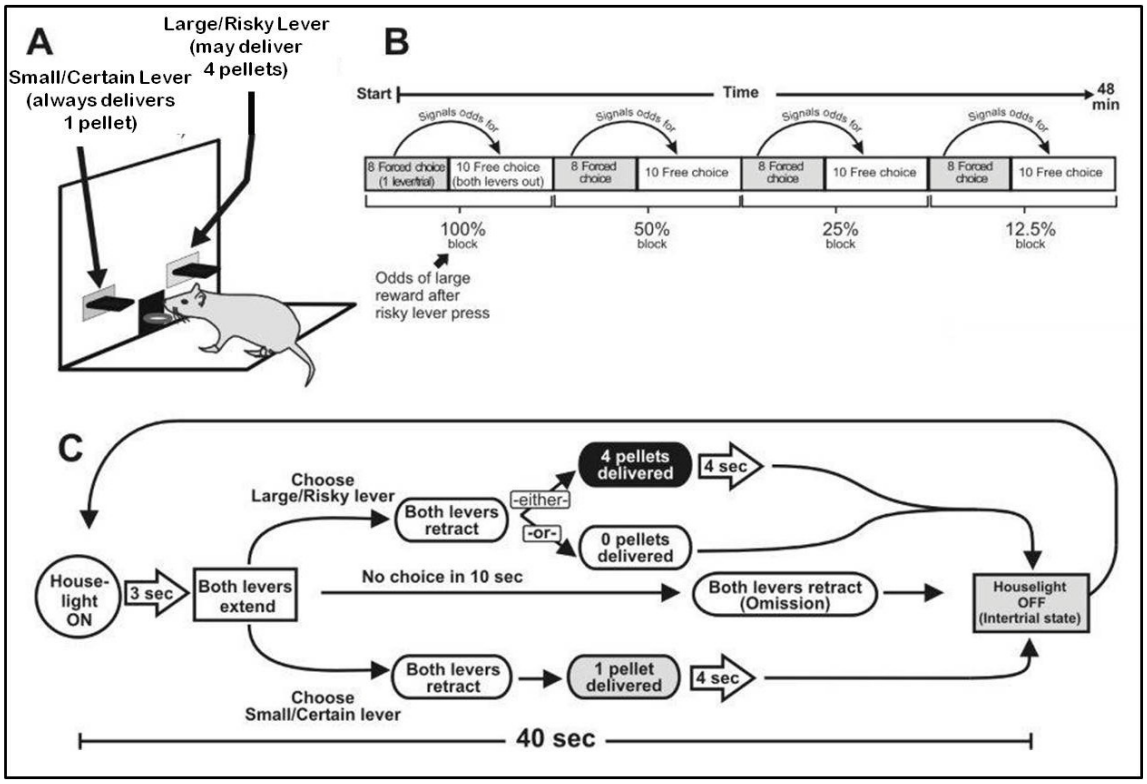

Figure 5: Design of the risk-discounting task: A) Assembly of operant chambers: Each chamber is fitted with two levers, one located on each side of the central food receptacle. The Small/Certain lever delivers one pellet on every press. The Large/Risky lever delivers four pellets with a certain probability that decreases across the four blocks of a session (100%, 50%, 25%, 12.5%). B) Format of the sequence of forced and free choice trials within each block of a training session C) Format of a single free-choice trial.

2.4. Surgery and microinfusion protocol

Rats were anesthetized with 100 mg/kg ketamine hydrochloride and 7 mg/kg xylazine and firmly fastened in a stereotaxic apparatus via ear and mouth bars. The mouth bar was set to -3.3 mm (flat skull). Afterwards rats were implanted with two sets of bilateral 23 gauge stainless steel guide cannulae to facilitate targeted infusions. Each rat was implanted in both NAc regions and both mPFC regions to enable left-right counterbalanced infusions on different test days, contralateral as well as ipsilateral. The guide cannula targeting the NAc transected the corpus callosum, what prevented contralateral interactions. Callosotomy alone usually has little to no effect on behavior (Dunnett *et al.*, 2005; Floresco *et al.*, 1999). For the placement of implants, standard stereotaxic techniques were used.

Stereotaxic coordinates of NAc and medial PFC implants:

Axis	NAc implants	medial PFC implants
anteroposterior [AP]	+ 1.5 mm	+ 3.4 mm
medial-lateral [ML]	± 1.4 mm from bregma	± 0.7 mm from bregma
dorsoventral [DV]	- 5.9 mm from dura	- 2.8 mm from dura

The guide cannulae were held in place with stainless steel screws and dental acrylic. Thirty-gauge obdurators, flush with the end of guide cannulae, remained in place until the infusions were made and were reinserted after each infusion to keep the cannulae clean and free of blood clots. Before testing, rats were given at least seven days to recover from surgery. During this period, they were food restricted to 85% of their free-feeding weight and handled at least five minutes each day. Their body weights were monitored daily to ensure a steady weight loss. After this recovery period, the rats were retrained on the probability discounting task for twelve days, until as a group they displayed stable patterns of choice for three consecutive days. Before the animals could be infused and tested, they had to get used to infusion procedures to avoid high stress levels on the following microinfusion test days. Therefore, the obdurators were removed and a mock infusion procedure was conducted. Stainless steel injectors were placed in either two ipsi- or contralateral guide cannulae for two minutes and 15 sec, but no infusion was administered. After the mock infusions, rats were left in their home cages for ten minutes, and were then placed in their operant chambers to perform the task. This was the same procedure conducted on following test days. As the animals' task performance differed from normal patterns on the first mock infusion day, this procedure was repeated two times in the following days, which included also normal training days without mock infusions.

After the rats had reached stable levels of choice for three consecutive days again, they were ready to be tested. Inactivation of both, the medial PFC and the NAc, was achieved by infusion of a drug solution containing the $GABA_A$ agonist muscimol (Sigma-Aldrich Canada, Oakville, Ontario, Canada) and the $GABA_B$ agonist baclofen (Sigma-Aldrich Canada). GABA

(gamma-Aminobutyric acid) acts at inhibitory synapses by binding to transmembrane receptors, what causes the opening of ion channels and subsequently, either the influx of negative ions or the efflux of positive ions, leading to a hyperpolarization of the cell. Two general types of GABA receptors are known: $GABA_A$ receptors that are part of a ligand gated ion channel complex, and metabotropic $GABA_B$ receptors. Both receptor types are bound by either muscimol ($GABA_A$) or baclofen ($GABA_B$), what causes a temporary inactivation of the infused brain region. Both drugs were dissolved separately at a concentration of 500 ng/µl in physiological saline and combined in equal volumes. The final concentration of each compound in solution was 250 ng/µl. For each microinfusion, a volume of 0.5 µl was used, so that the final dose of both baclofen and muscimol was 125 ng per inactivated brain region. These doses have been found to effectively alter risk-discounting when infused in different brain regions (Ghods-Sharifi *et al.*, 2009) and infusions of comparable doses of these drugs into the OFC have been shown to disrupt cognition (Takahashi *et al.*, 2009). Note that even much lower doses of these drugs have been reported to affect behavior in previous studies (Corrigall *et al.*, 2001). Van Duuren and colleagues (2007) reported that administration of muscimol into the brain induced a near complete suppression of neural activity that lasted for two hours. Thus, inactivation induced by a combination of muscimol and baclofen would be expected to persist over the duration of the test session used in the present study (48 min). Furthermore, an infusion volume of 0.5 µl should still be in an acceptable range, regarding a functional spread of the drugs into surrounding areas. Previous studies used an infusion volume of 0,3 µl of baclofen/muscimol solutions with similar concentrations, and observed dissociable behavioral effects when the adjacent brain regions were separated by only 1 mm (Floresco *et al.*, 2006; Floresco *et al.*, 2008b), suggesting that the functional spread of these treatments was likely less than 1 mm. The increased volume used in the present study is expected to diffuse slightly farther but not much more than 1 mm in radius, and therefore not outside the infused regions. The infusions were administered by a micro syringe pump at a rate of 0.5 µl/75 sec via a 30-gauge injection cannulae that protruded 0.8 mm past the end of the guide cannulae.

Rats received three different counterbalanced infusions, on separate test days:

1) functional disconnection: drug infusions in one NAc region and one medial PFC region in opposite hemispheres
2) ipsilateral inactivation (control): drug infusions in NAc and medial PFC regions in the same hemisphere
3) saline (control): saline infusion in one NAc region and one medial PFC region in opposite hemispheres

Ipsilateral inactivations were used as a within-subjects control to determine whether potential effects observed after contralateral inactivations were due to an actual disruption in communication between NAc and mPFC or rather a "mass action" effect that may occur when brain regions are simultaneously inactivated or the effect of disconnection in only one hemisphere. Minor deviations from normal patterns (i.e. saline control) are usually expected, as we can't assume a complete compensation of the loss of inactivated brain region by their contralateral equivalents. The order of treatments was counterbalanced across animals, to eliminate possible influences of factors that may vary between test days (e.g. different stress levels due to noise). Therefore, the animals were divided into three groups, depending on their choice behavior in the last three days of training. Rats in one group had a similar preference for the Large/Risky lever. Also the hemispheres that received contra- and ipsilateral infusions were counterbalanced across animals. This should eliminate the influence of possible functional differences between both hemispheres, and also enable a differential analysis of both sides of the brain. The rats' choice behavior in the last three days of training was averaged between rats belonging to one group to define a pre-infusion baseline for each group. After each microinfusion test day, rats received one or two days of baseline training (without infusions) until an individual rat's choice of the Large/Risky lever deviated by less than 15% from its pre-infusion baseline. When this criterion was reached, a rat received a second counterbalanced microinfusion on the next day. This was followed by one or two days of baseline training and the final infusion.

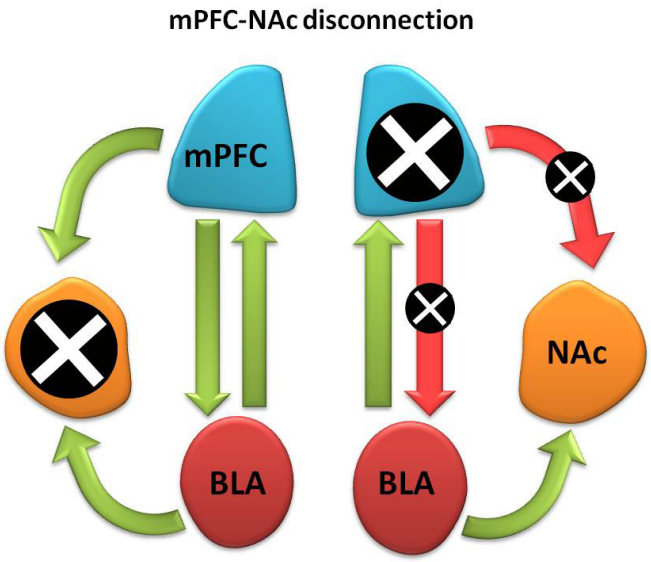

Figure 6: Asymmetrical disconnection design: Inactivation of the medial PFC in one hemisphere and of the NAc in the contralateral hemisphere selectively disrupts communication between these structures. Arrows indicate functional (green) and dysfunctional (red) signaling between structures.

2.5. Histology

After all behavioral tests were completed, the animals were euthanized in a carbon dioxide chamber. Their brains were removed and fixed in a 4 % formalin solution, frozen and sliced in 50 µm sections, mounted, and finally stained with Cresyl Violet. All cannulae placements were verified with reference to a neuroanatomical atlas (Paxinos and Watson, 1998). The locations of acceptable infusions are presented in Figure 7. Data from rats whose placements were outside the borders of the medial PFC or NAc were removed from analysis.

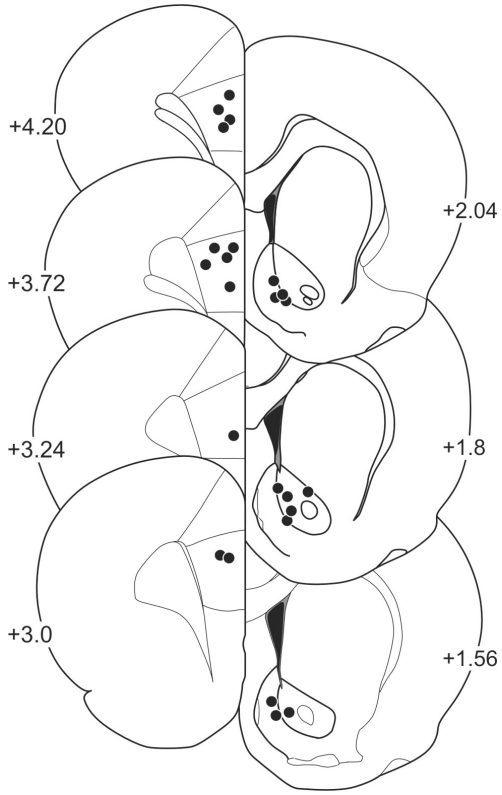

Figure 7: Histology: Schematic of coronal sections of the rat brain, showing acceptable locations of infusions in the medial PFC (left) and the NAc (right). The numbers beside each plate correspond to millimeters from bregma.

2.6. Data Analysis

For the analysis of choice behavior in the probabilistic discounting task, the primary dependent measure of interest was the percentage of times the Large/Risky lever was chosen in each block of free choice trials, factoring in trial omissions. This was calculated by dividing the number of Large/Risky choices by the total number of successful trials. Choice data were analyzed using one or two-way within subjects ANOVAs, with Treatment and Trial Block as within-subjects factors. A significant main effect of Trial Block indicates that rats bias their choice towards the Small/Certain lever, as the probability of obtaining the large reward decreases over a session. A significant main effect of Treatment would indicate that one or more infusion treatments affected choice patterns, relative to other treatments. A significant main effect of Treatment x Trial Block would indicate that one or more infusion treatments affected choice patterns, relative to other treatments, in one or more Trial Blocks, relative to other Trial Blocks. The number of trial omissions, response latencies and

locomotor activity (i.e. photobeam breaks) were analyzed with one-way repeated-measures ANOVAs. The n-value represents the number of rats that had acceptable cannulae placements.

Win-Stay and Lose-Shift Analyses

A supplementary analysis of win-stay and lose-shift performances was conducted to obtain further insight into how these treatments may affect choice patterns. This choice-to-choice analysis should show possible alterations in negative feedback sensitivity (lose-shift performance), or alterations in the likelihood of choosing the risky lever after obtaining the larger reward (win-stay performance) (Stopper and Floresco, 2011). The rats' choices were analyzed according to the outcome of each previous free-choice trial (reward or non-reward) and expressed as a ratio. The proportion of lose-shift trials was calculated from the number of times animals shifted their choice to the Small/Certain option after choosing the Large/Risky lever on the previous trial and were not rewarded (loss), divided by the total number of free-choice trials resulting in a loss. Conversely, win-stay performance was calculated from the number of times rats chose the Large/Risky option after choosing the risky option on the preceding trial and obtaining the large reward (win), divided by the total number of free-choice trials resulting in a win. Changes in lose-shift performance served as an index of negative feedback sensitivity, whereas changes in the win-stay performance indicated what impact a win of the Large/Risky reward had on subsequent choices. This analysis was conducted for all trials of a session. A block-by-block analysis was not conducted, as there were many instances where rats didn't obtain the large reward at all during the latter blocks.

3. Results

The rats required 27 days of training on the probabilistic risk discounting task, before they were subjected to surgery. After surgery, they were retrained for twelve days prior to receiving infusions of either saline or muscimol/baclofen. The data from four rats had to be eliminated due to inaccurate cannulae placements.

Analysis of choice behavior of the twelve remaining rats showed that disconnection of the medial PFC and the NAc did not affect the rats' choice patterns in the probabilistic discounting task [n=12; effect of Treatment ($F(2,22)=2.40$, $p=0.11$); effect of Treatment*Trial Block ($F(6,66)=0.60$, $p=0.73$; Figure 8)]. The analysis showed no significant effects of the side of injection (left or right hemisphere) and the side of the Large/Risky lever (left or right lever) on choice behavior ($p>0.05$). The main effect of Trial Block was significant ($p<0.05$), indicating that rats biased their choice towards the Small/Certain lever, as the probability of obtaining the large reward decreased over a session.

Choice biases after mPFC-NAc disconnection

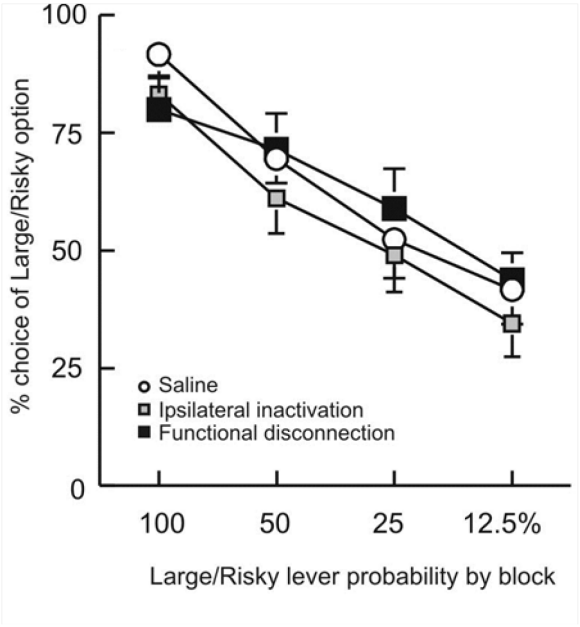

Figure 8: Neither the functional disconnection of medial PFC and NAc, nor ipsilateral inactivations of these regions had a significant effect on risk discounting. The percentage of Large/Risky choices in free choice trials (y-axis) is plotted as a function of the Large/Risky lever probability by block (x-axis). The symbols represent means + standard error of the mean (SEM).

An analysis of win-stay and lose-shift performances showed no effect of treatment on win-stay performances [n=12; effect of Treatment ($F(2,22)=0.75$, $p=0.48$)]. Lose-shift performance, however, was significantly affected after rats received asymmetrical disconnection of the NAc and the medial PFC, compared to rats infused with saline [n=12; effect of Treatment ($F(1,11)=13.18$, $p=0.004$)]. The proportion of trials animals shifted their choice to the Small/Certain lever after they chose the Large/Risky lever on the preceding trial without being rewarded (loss) decreased after these regions were disconnected. This finding indicates, that interactions between NAc and medial PFC play a role in negative feedback sensitivity, even if the effect of contralateral disconnections between these regions was not sufficient to generally alter choice biases.

Win-stay and Lose-shift performance

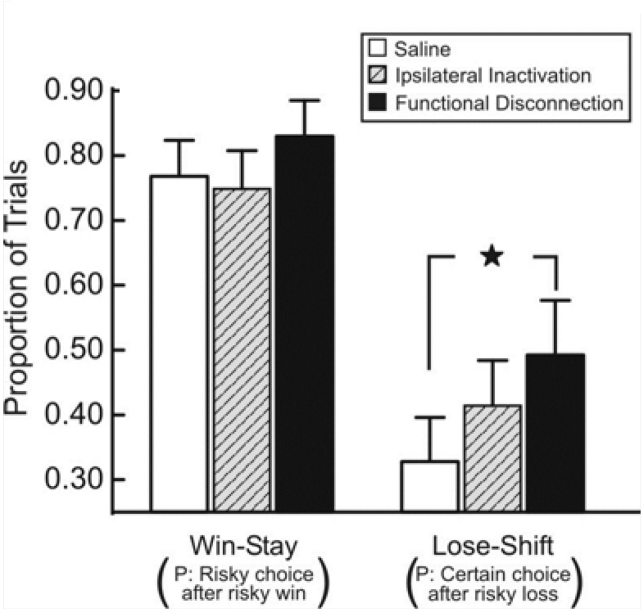

Figure 9: Win-stay and lose-shift performance: Win-stay data are displayed as the proportion of Large/Risky choice after selecting this lever on the previous trial and obtaining the large reward (win). Lose-shift data are displayed as the proportion of Small/Certain choice following selection of the Large/Risky lever on the previous trial without being rewarded (loss). Disconnection of the medial PFC and the NAc selectively reduced lose-shift tendencies compared to saline infusions. Stars denote a significant main effect of treatment at $p<0.05$

Furthermore, disconnection of these regions increased over-all response latencies compared to saline treatment [n=12; effect of Treatment ($F(1,11)=7.57$, $p=0.02$); effect of

Treatment*Trial Block (F(3,33)=0.21, p=0.89)]. Also trial omissions increased after disconnection treatments, compared to both ipsilateral drug infusions and saline infusions [n=12; effect of Treatment (F(2,22)=3.92, p=0.04)]. The locomotion of rats was significantly reduced after disconnection treatments, compared to ipsilateral drug infusions and saline infusions [n=12; effect of Treatment (F(2,22)=5.30, p=0.01)]. Whereas in saline controls, a mean of 1142 (SEM: 92) photobeam breaks was recorded and in ipsilateral controls a mean of 1007 (SEM: 108), the light beams of rats that had received disconnection injections were broken only 778 (SEM: 80) times, on average. These latter results suggest that disrupting communication in this pathway impairs attention and vigilance aspects of task performance and increases deliberation times.

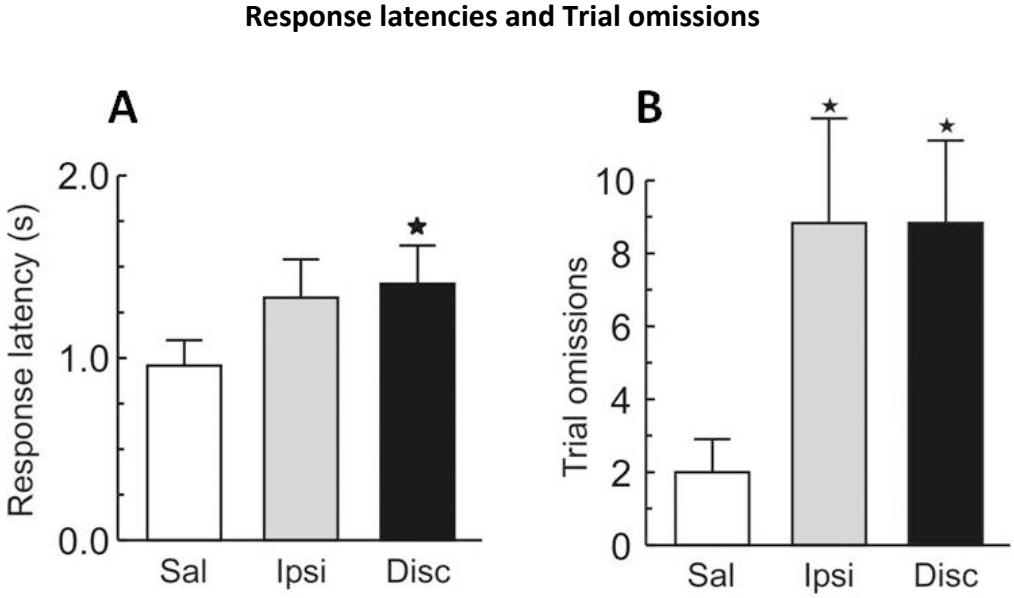

Figure 10: Response latencies and trial omissions: a) Disconnection of the medial PFC and the NAc significantly increased response latencies. Star denotes a significant ($p<0.05$) main effect of treatment. b) Disconnection of the medial PFC and NAc, as well as ipsilateral inactivation of medial PFC and NAc significantly increased trial omissions. Stars denote a significant ($p<0.05$) main effect of treatment.

4. Discussion

If we can freely choose between options associated with different rewards, we intuitively go for larger payoffs, provided that all other factors associated with these options are equal. Other factors that help biasing our choice can be multifarious. For example, we may have to work harder, invest more money or sometimes just have to wait longer to achieve more valuable or larger payoffs. Another important factor that has a great impact on our everyday choices is uncertainty. Almost every decision we make is associated with a certain degree of uncertainty, even if we are sometimes not aware of it. Strictly speaking, nothing is certain and for every decision we make, we have to account for this probabilistic factor. The challenge is to integrate information about magnitude, effort, delay and probability and other factors to chose options that are most valuable in the long term. Another factor that makes this process even more complicated is variability. We are living in a complex dynamic environment that requires permanent monitoring and adaptation to changes. Decisions that were profitable once, may become disadvantageous when the probability of achieving reward decreases. In this case, it may become advantageous to forgo this option and to decide in favor for smaller but more certain returns. Monitoring and integration of these factors requires a complex interplay of various brain regions. Dysfunction in one of these regions or disruption of communication between these regions may lead to imbalance and disabilities in decision making. The present data, in addition to findings from previous studies, suggest that parallel prefrontal cortical, amygdalar and ventral striatal circuits contribute to bias choices and help regulate whether one retains established strategies or shifts to different options that may be smaller but more reliable and therefore advantageous in the long term. The present study examined the contribution of interactions between the NAc and medial PFC of the rat to these decision making processes, whereas the rat medial PFC is anatomically homologous to Area 32 of the human anterior cingulate cortex and shares functional homology to the dorsolateral PFC.

PFC projections to the NAc have been shown to play a critical role in working memory, including a number of executive functions like attention, cognitive flexibility and also effort-based decision making.

Working memory is defined as the ability to retain and manipulate mnemonic information to guide ongoing behavior (Baddeley, 1986). One important component of working memory is the short-time storage of trial-unique information, which is discarded after a response was executed. Another component attributed to working memory involves a multitude of cognitive processes that are characterized as "executive functions". Executive functions include supervisory processes for the temporal organization of behavior and utilization of the short-term memory to plan a sequence of responses (Floresco *et al.*, 1999). Several studies showed that interactions between the PFC and its cortical and subcortical connections are involved in behaviors that require working memory. Disconnection of PFC projections to the NAc disrupted performance on a delayed spatial win-shift version of the radial arm maze task, conducted by Floresco and colleagues (1999). In this task, rats were given information about the location of food in the maze 30 min before each test session, what enabled them to plan an efficient foraging strategy. These data are consistent with the general theory that executive control over motor functions is dependent on interactions between the PFC and striatal systems, and confirm that inputs from the PFC to the NAc facilitate the transformation of a plan of action into appropriate behavioral output. It is suggested that information is routed from the hippocampus to the PFC, where it is integrated in prospective foraging strategies, and that interactions between the PFC and the NAc transform this memory, processed by hippocampal-cortical circuits, into a sequence of motor responses. (Floresco *et al.*, 1997; Floresco *et al.*, 1999). An involvement of the PFC and NAc in working memory suggests that this circuit is of general importance for cognitive processing.

Parkinson and colleagues (2000) studied the effects of bilateral excitotoxic lesions of either the anterior cingulate cortex or the NAc as well as disconnection of anterior cingulate cortex and NAc core on the acquisition of appetitive Pavlovian conditioning in an autoshaping procedure. Lesions to both regions as well as disconnection of both regions impaired the rats' task performance. These findings suggest that the NAc and anterior cingulate are "nodes" of a corticostriatal circuit that is involved in stimulus-reward learning. Considering that the anterior cingulate cortex is critical if two or more similar stimuli predicting different outcomes have to be discriminated, it is suggested that its contribution to stimulus-reward

learning is highly specific (Cardinal *et al.*, 2002). Furthermore, Christakou and colleagues demonstrated the involvement of serial interactions in parallel prefrontostriatal circuits that connect the prelimbic/infralimbic cortex with the dorsal and ventral striatum, in attention (Christakou *et al.*, 2001) and affective modulation of action (Christakou *et al.*, 2004). In a strategy set shifting task, conducted by Block and colleagues (2007), disconnection between the PFC and the NAc impaired the rats' ability to shift from one discrimination strategy to another. The PFC was earlier proposed to play an important role in early set shifting, suppressing previously correct strategies (Ragozzino *et al.*, 1999), whereas corticostriatal circuits including the NAc were believed to mediate the maintenance of a novel strategy (Floresco *et al.*, 2006). The findings from Block's study, together with findings from Floresco and colleagues (2006) suggest that this corticostriatal circuit facilitates both, the suppression of previously established response rules as well as the maintenance of a novel strategy.

The impact of interactions between the PFC and the NAc on effort-based decision making was examined by Hauber and Sommer (2009). They reported that disconnection of the anterior cingulate cortex and the NAc core, using an asymmetrical excitotoxic lesion procedure, impaired performance of rats in an effort-based decision making task. Rats that received disconnection of these regions chose the large reward option, accompanied by higher effort, less often. This finding provides strong support to the assumption that anterior cingulate cortex and NAc core operate functionally and serially in effort-based decision making.

Given that the PFC and the NAc play a critical role in both effort-based and risk-based decision making, the assumption that interactions between both regions may play a role in risk-based decision making as well was quite obvious. In some studies, lesions of the PFC and the NAc were shown to have similar effects on both types of decision making (Floresco *et al.*, 2008a). Therefore, it was surprising that in the present probabilistic discounting study, disconnection of medial PFC and NAc showed no significant effect on choice patterns suggesting that these two regions work relatively independently in guiding risk-based decision biases. Considering that bilateral inactivations of the NAc and medial PFC had qualitatively opposite effects on probabilistic discounting in former studies, one explanation

could be that inactivation of one region may have negated the effect of the other. Inactivation of the medial PFC increased choice of the Large/Risky lever in a probabilistic discounting task with descending probabilities of obtaining the large reward (St. Onge and Floresco, 2009). NAc inactivations however, decreased choice of the Large/Risky lever in the same task, making rats risk-averse (Stopper and Floresco, 2011).

Nevertheless, we could find an effect on lose-shift performances. After disconnection of the medial PFC and the NAc, rats showed less sensitivity to negative feedback, meaning that the percentage of times they shifted their choice to the Small/Certain lever after choosing the Large/Risky lever on the previous trial without being rewarded decreased. Previous studies on inactivations of involved regions had very different outcomes concerning positive and negative feedback sensitivities. Bilateral inactivations of the NAc for example reduced win-stay performances, but had no effect on lose-shift performances in the same task, conducted by Stopper and Floresco (2011).

Findings from previous studies are mixed regarding the effects of reward omissions on ventral striatal activation: Increased NAc activation has primarily been associated with increased firing of dopaminergic cells in the midbrain in response to reward (Hollerman and Schultz, 1998). In some fMRI studies, the absence of expected reward was associated with decreased activation of the ventral striatum (Knutson *et al.*, 2001), whereas in other studies, this effect could not be found (Pagnoni *et al.*, 2002). Various studies examined the mesencephalic dopaminergic system, that may participate in learning by providing a prediction error signal to its targets that include the ventral striatum, the OFC and medial PFC regions, as well as by showing sensitivity to the degree of uncertainty associated with individual stimuli (Aron *et al.*, 2004).

Rodriguez and colleagues (2006) found an increased NAc activity after unexpected negative feedback, but not after positive feedback, in a fMRI task examining prediction error sensitivity during probabilistic classification learning with purely cognitive feedback (presentation of the right answer on the screen). In this task, subjects were scanned while they learned which combination of features (eyeglasses, hat,…) on a “Mr. Potato Head” figure predicted if it would chose chocolate or vanilla ice cream. When the subject’s

prediction matched the outcome, the feedback was positive, when it did not match, it was counted as negative feedback. Increasing activation of the NAc was correlated with increasing degree of prediction error on negative feedback trials. In a previous study, they found higher activation of the midbrain for negative versus positive feedback (Aron *et al.*, 2004). A possible explanation is that positive feedback should be of little informational value compared to negative feedback that may generate surprise and suggests that the subject should change expectancy. Another explanation would be, that higher fMRI signals cannot necessarily be interpreted as higher dopaminergic activation of a specific region. fMRI signals in the midbrain for example could as well indicate activation of GABAergic signals that arise from the ventral striatum in response to reward omission and result in decreased neural firing but increased fMRI signal (Aron *et al.*, 2004).

With regard to the present study, these findings could indicate increased activity of the NAc and PFC due to an active dopaminergic "reward system" either in response to obtained reward or in response to unexpected non-reward on lost trials, reporting prediction errors. Disconnection of the NAc and the PFC affected negative-feedback sensitivity, suggesting that these regions do not only work separately from each other but also communicate with each other when negative feedback is processed. This effect, however, was not strong enough to generally affect decision making on this task.

Nevertheless, this disconnection manipulation disrupted other aspects of task performance, increasing response latencies, reducing the number of choices made (increasing trial omissions) and reducing locomotor activity. An increase in choice latencies and trial omissions could be attributed to many reasons. The animal could simply show less motivation to gamble, what would explain that it takes more time until decisions are made and levers are pressed. This goes hand in hand with an increase in trial omissions, as the levers were retracted when no choice was made after ten seconds. What speaks against this explanation, is that the rat's choice patterns were not affected, and a lack of motivation may also have negative effects on choice biases. As mentioned above, interactions between the PFC and the NAc also play a critical role in attention (Christakou *et al.*, 2001), suggesting that extended response periods may be attributed to attentional deficits. The fact that rats also

showed reduced locomotor activity, could theoretically indicate a general disability in motor function. This effect was, however, only significant in free-choice trials and not in forced choice trials, what makes this assertion rather unlikely. Also other studies examining effects of disconnection of the medial PFC and the NAc reported, to my knowledge, no such effects on motor functioning. Furthermore, bilateral inactivation of the NAc in a previous study significantly increased response latencies, primarily in the second and last trial blocks, when decision-making was more difficult than in the first trial block, where the probability of obtaining the large reward was 100% (Stopper and Floresco, 2011). A similar effect was observed for bilateral inactivation of the medial PFC and OFC, where response latencies were increased primarily in the latter two trial blocks (St. Onge and Floresco, 2009). These findings indicate, that dysfunctions of the NAc and PFC more specifically delayed the decision-making process on this task rather than inducing a general aversion or disability to move towards or press the levers, even if Treatment*Trial Block interactions were not significant for response latencies in the present study. This suggest that disrupting interactions in this pathway may impair attention and vigilance aspects of task performance and therefore increase deliberation times.

To highlight the importance of these findings, the present data, together with information gathered by St. Onge and Floresco (2010) have to be combined and integrated to create an overall picture. As mentioned above, this experiment was the last missing part of their study, examining interactions between the medial PFC, the BLA and the NAc in risk-based decision making. The present data, together with data from St. Onge and Floresco indicate that parallel amygdalar, ventral striatal and prefrontal circuits make differential contributions to this type of decision making.

St. Onge and colleagues suggested a subcortical amygdala-striatal circuitry that drives choice towards larger but uncertain rewards and is under top-down control of the medial PFC. Several studies indicate that projections from the BLA modulate NAc activity and influence the direction of behavior towards reward-related stimuli (Floresco *et al.*, 2001; Ambroggi *et al.*, 2008; Everitt *et al.*, 1999). St. Onge and colleagues could show that disconnections of the BLA and the NAc shifted the rats' choice biases away from larger but uncertain rewards. This

effect was particularly striking in the first three trial blocks, when choosing the Large/Risky option would have been advantageous, yielding an equal or greater reward than the Small/Certain option. This fact indicates, that disconnection of these regions does not induce a general risk aversion, but seems to reduce the bias toward larger-magnitude rewards when the odds of obtaining them are good. One would expect, that a general risk aversion would hardly affect choice patterns in the first trial blocks, when there was little to no risk, but decrease risky choice especially in the last trial blocks, when the probability of being rewarded was low. The choice patterns observed in this study, however, suggested a different contribution of the BLA-NAc circuitry to decision making. Note that not only asymmetrical disconnection of this circuitry, but also ipsilateral disconnections evoked a similar effect. As ipsilateral inactivations either had a reduced effect or no effect on cognitive performance in several other tasks (Dunnett *et al.*, 2005; Block *et al.*, 2007), this result was somewhat surprising. According to a recent report however, ipsilateral disconnection of the BLA and the OFC was shown to attenuate drug context-induced cocaine seeking behavior (Lasseter *et al.*, 2011). Unilateral inactivations of the BLA or OFC alone did not affect behavior in this task, suggesting that this effect could not be attributed to either reduced BLA or OFC activity, but probably to blocked communication between these regions in one hemisphere. This finding confirms, that ipsilateral disconnections can have an effect on behavior and that the contralateral circuitry cannot always compensate for the loss. Even if, to my knowledge, effects of unilateral inactivations of the BLA or NAc on risk-based decision making have not been examined, it is suggested that the observed effect of ipsilateral inactivations is attributable to disrupted communication between both structures in one hemisphere. Risk-based decision making requires a great level of cognitive processing and seems to be much more complex than, for example, effort-discounting. This complexity, amongst others, may explain why rats displayed impairments in task performance after disconnection of the BLA and the NAc, even if it was only in one hemisphere. Note that ipsilateral disconnection did only disrupt choice behavior in a subset of rats whereas asymmetrical disconnection significantly reduced risky choice of all rats. Collectively, these data confirm that communication between these two brain regions plays a crucial role in biasing choice towards larger rewards, even if they are risky. In a subsequent experiment St.

Onge and Floresco examined, if this effect was possibly due to a disability in discriminating between smaller and larger rewards. As reward magnitude discrimination was shown not to be affected by the described disconnection manipulation, it is safe to assume that this amygdala-striatal circuitry specifically contributes to decision-making processes when the relative value of a large reward is diminished by its uncertainty, rather than inducing a general preference for larger rewards. Several previous studies showed, that the BLA as well as the NAc help overcoming various cost in order to gain objectively larger rewards instead of choosing a smaller reward without investing much time and effort or taking risk (Floresco *et al.*, 2008a). Bilateral inactivation of the NAc had a similar effect on the same risk-discounting task, significantly reducing risky choice in the first trial blocks (Stopper and Floresco, 2011). The same effect was caused by bilateral inactivation of the BLA in a similar study that also reported reduced choice of the large reward in an effort-discounting task (Ghods-Sharifi *et al.*, 2009). These findings are in keeping with neurophysiological studies that report increased activation of the amygdala and NAc when subjects chose larger or more valuable rewards, even when their receipt was uncertain, indicating that activity within this amygdala-striatal circuitry encodes the relative reward value associated with different options (Kuhnen and Knutson, 2005; Marsh *et al.*, 2007; Smith *et al.*, 2009). Thus, activity within the BLA-NAc circuitry seems to be causally-linked to the direction of choice. With respect to the study conducted by St. Onge and colleagues, this presumption can be expanded, suggesting that increased NAc activity is driven by excitatory inputs from the BLA, associated with choice of larger but uncertain rewards. Findings from other studies led to the presumption that activity within the NAc circuitry may influence behavior through descending projections to motor sites (Zahm and Heimer, 1990).

Activity of the amygdala-NAc circuit correlates with choice of larger, uncertain rewards. Importantly, preference for larger, uncertain rewards decreases when the odds of being rewarded are getting worse, meaning that the activity within this circuit drops as well. That suggests that there has to be a mechanism that controls the neuronal activity within this circuit, depending on the expected long term outcome that depends on both, the reward value as well as on the probability of being rewarded. This mechanism has to assure that the circuitry is active, when choice of larger but risky rewards seems to be advantageous. When

the odds are bad, however, it has to be inhibited to ensure that choice is biased towards safer options, even if these are associated with objectively smaller rewards. On the basis of information gathered by St. Onge and colleagues in their medial PFC–amygdala disconnection experiment, it is suggested that this regulatory mechanism is mediated by the medial PFC.

Whereas disconnection between the BLA and the NAc decreased risky choice in the probabilistic discounting task with descending probabilities, disconnection between the BLA and the medial PFC had the opposite effect, biasing the rats' choice towards the Large/Risky lever. This effect of asymmetrical disconnections was observed in the last two trial blocks relative to the saline control group and in the last trial block (12.5%) relative to the ipsilateral disconnection control group. In contrast to disconnection of the BLA-NAc circuit, ipsilateral inactivations of the medial PFC and the BLA did not affect choice relative to saline infusions, confirming that the observed effect was indeed due to a disruption in communication. The same effect on choice behavior was found after bilateral inactivation of the medial PFC in a former study (St. Onge and Floresco, 2009). These findings indicate that inactivation of the medial PFC, as well as the blockade of interactions between the medial PFC and the BLA, decrease the rats' sensitivity to changes in reward outcomes, rather than generally increasing the rats' preference for risky options. Bilateral inactivation of the BLA decreased risky choice in a previous study (Ghods-Sharifi *et al.*, 2009). Considering that selective inactivation of either structure, the medial PFC and the BLA, had opposite effects on choice behavior, one expected outcome of a disconnection between these structures would be that one effect effectively cancels out the other, causing no net change in choice. The fact that this disconnection induced a prefrontal-like profile suggests however, that the medial PFC has a supervisory role in regulating down-stream processes mediated by the BLA, obviously in cooperation with the NAc. This communication between frontal and temporal lobes may help tracking decision outcomes to detect changes in reward probabilities over time and facilitate an adjustment of choice biases. If inputs from the medial PFC fail to reach the BLA, or are not even produced, the BLA-NAc circuit has to work independently and biases choice towards larger, uncertain rewards, irrespective of reward outcomes. This assumption was affirmed by experiments examining the direction of this pathway. A neuroanatomical study

showed that the medial PFC and the BLA are reciprocally connected with each other, meaning that the medial PFC projects to the BLA, facilitating top-down processing of information, but also receives projections from the BLA, enabling bottom-up processing (St. Onge and Floresco, 2010). Asymmetrical disconnection of both structures could only reveal that they communicate with each other, but gave no evidence about the direction of information transfer. To examine in which direction information has to be sent to cause the observed effect, another series of experiments was performed, selectively disrupting bottom-up (BLA-to-PFC) and top-down (PFC-to-BLA) signaling. Therefore, specific structures in ascending and descending pathways were selectively inactivated. Disruption of the ascending (BLA-to-PFC) axonal pathway had no reliable effect on decision-making. Thus, even though information about reward value may be sent from the BLA to the PFC, disruption of this pathway was not sufficient to alter over all choice patterns in this probabilistic discounting paradigm. Disruption of communication in the descending (PFC-to-BLA) axonal pathway had the same effect as an overall blockade of interactions between these structures, suggesting that top-down processing of information transferred from the medial PFC to the amygdala is the crucial process in risk-based decision making. This effect was reflected by a reduced tendency to shift choice towards the certain option following a non-rewarded risky choice, indicating a reduced sensitivity to negative feedback. This finding indicates that information transferred from the PFC to the BLA provides information about reward omissions that help biasing the direction of subsequent choices.

These results are in keeping with the above mentioned assumption, that the PFC has a supervisory role in regulating BLA activity. Even if this study was the first using a combined neuroanatomical/disconnection approach to directly demonstrate top-down control of the amygdala by the PFC, this idea was not new, although it came primarily from indirect evidence provided by lesion and imaging studies. Support comes from a study conducted by Hayden and colleagues (Hayden *et al.*, 2011) that showed that disengagement from options providing decreasing reward outcomes in favor of exploring novel strategies was associated with increased activity within the anterior cingulate cortex. Several previous studies suggested a role of medial regions of the human PFC, including the anterior cingulate cortex, in cognitive control and in monitoring of changes in reward contingencies and other

parameters of decision-making. Shima and Tanji (Shima and Tanji, 1998) showed that unit-firing in the anterior cingulate codes changes in reward magnitude associated with current response output. This finding was replicated by Bush and colleagues, in an event-based fMRI study (Bush *et al.*, 2002). Different parts of the anterior cingulate and medial PFC were shown to play distinct roles in monitoring changes. Increased activation of the rostral part of the anterior cingulate cortex, for example, was associated with increasing levels of expected reward, whereas activation of the dorsal anterior cingulate was strongly associated with increases in the number of decision options in a decision-making task incorporating variations in the level of expected reward, as well as the number of available decision options (Marsh *et al.*, 2007).

In a reward-guided choice task, conducted by Kennerley and colleagues (Kennerley *et al.*, 2006), anterior cingulate lesions did not impair macaque monkeys' performance immediately after errors, but impaired their ability to sustain rewarded responses. This study, along with others, suggests that the anterior cingulate may form and maintain representations of actions associated with different reward values and update them over time through repeated experiences of gain and loss in order to determine which options are more profitable in the long term, rather than monitoring whether single actions achieved their expected outcomes or signaling the need for adaptive behavior. This assumption makes sense, especially considering situations that provide only a probabilistic chance of being rewarded. Reward omission on a particular occasion may not necessarily mean that we have to switch to alternative strategies. This updating function may influence whether decision policies promote the exploitation of a currently beneficial situation or lean towards exploration of new strategies when reward omissions increase, for example by inhibiting the subcortical BLA-NAc circuit (St. Onge and Floresco, 2010). Further support for the notion, that the medial PFC modulates BLA-NAc function via feed-forward inhibition comes from transcranial magnetic stimulation studies in humans that showed that the PFC can override decision-making biases mediated by other brain regions (Figner *et al.*, 2010; Knoch *et al.*, 2006). Disruption of PFC-to-BLA input impairs the use of this information to adapt behavior, and thereby permits the subcortical amygdala-striatal circuit to persist in biasing choice towards the larger but riskier option, even if this option may not be profitable anymore

because the odds of being rewarded are decreasing. Collectively, findings from the present study and the study conducted by St. Onge and colleagues showed that projections from the medial PFC to the BLA aid in adjusting choice biases, whereas projections to the NAc enable staying "on task" and ensure that decisions are made in a timely fashion. In addition, these data highlight the impact of circuit analysis approaches in studies examining the neural basis of complex cognitive processes like decision-making, rather than analyzing different brain regions in isolation.

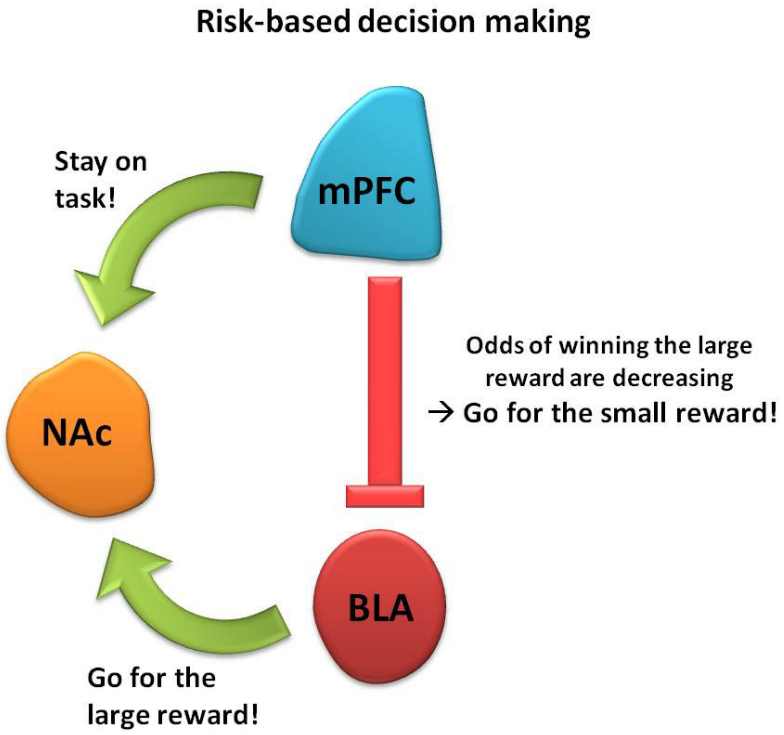

Figure 11: Interactions between the medial PFC, the BLA and the NAc in the mediation of risk-based decision making: The subcortical BLA-NAC circuitry biases choices towards larger, probabilistic rewards. Activity within this circuitry is regulated by the medial PFC that exerts top-down inhibition on the BLA. Medial PFC projections to the NAc enable staying on task.

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6. Appendix

6.1. Acknowledgement

I would like to express my appreciation to Stan Floresco (University of British Columbia, Department of Psychology) for giving me the opportunity to be part of this project and for his encouraged guidance within my stay at University of British Columbia. Special thanks to my colleagues Maric Tse and Colin Stopper for their technical support, Jen St. Onge for providing background information and Naghmeh Shafiei for her hospitality and friendship.

I also want to gratefully acknowledge Thomas Klausberger (Medical University of Vienna, Center for Brain Research) for his supervision, and the University of Vienna for providing a "KWA" research stipend that facilitated my stay abroad.

Finally, I want to thank my family and friends, for everything they did for me.

6.2. Summary

The ability to make advantageous decisions that yield outcomes of greater value implies the evaluation of relative costs and benefits associated with different choices. Choosing between smaller, certain rewards or larger, uncertain rewards is one type of decision making that requires a high level of cognitive processing, including the integration of information about probabilities and reward magnitudes and permanent monitoring of our environment to adapt to changes. Research on the neuropsychological basis of decision making in both human and non-human species has revealed distributed neural networks that mediate this process. These networks incorporate different regions of the prefrontal cortex (PFC), the nucleus accumbens (NAc) part of the ventral striatum, the basolateral amygdala (BLA), and the dopamine system. Disrupting communication between the BLA and the NAc has revealed that this subcortical circuit plays a role in biasing choice towards larger probabilistic rewards on a risk-based decision making task, employing rats. In contrast, disconnection between the medial PFC and the BLA increased risky choice on the same task, suggesting a supervisory role of the medial PFC. The present study was the last part of this experimental series, examining the interactions between the medial PFC and the NAc. Asymmetrical disconnection between these structures had no effect on overall decision biases, but did affect other behavioral measures like locomotion, response latencies and response omissions. Furthermore, we observed a decreased sensitivity to negative feedback. These findings provide insight into the dynamic interactions between cortical and subcortical circuits and help clarify the neuropsychological mechanisms underlying risk-based decision making.

6.3. German Summary (Zusammenfassung)

Die Fähigkeit vorteilhafte Entscheidungen zu treffen, die höhere Gewinne erzielen, erfordert die Auswertung damit verbundener relativer Kosten und Nutzen. Die Entscheidung zwischen kleineren, sicheren Gewinnen und größeren, unsicheren Gewinnen erfordert einen besonders hohen Level an kognitiver Verarbeitung, der die Integration von Informationen über Wahrscheinlichkeiten und Ausmaße des Gewinnes beinhaltet, sowie die permanente Beobachtung unserer Umwelt und Anpassung an Veränderungen. Die Erforschung neuropsychologischer Hintergründe der Entscheidungsfindung an Menschen, sowie anhand von Tiermodellen, offenbarte neuronale Netzwerke, die diese Prozesse vermitteln. Diese Netzwerke beinhalten verschiedene Regionen des prefrontalen Kortex (PFC), den Nukleus Accumbens (NAc) des ventralen Striatums, die basolaterale Amygdala (BLA) und das Dopaminsystem. Im Zuge eines Experiments, das die Entscheidungsfindung in Verbindung mit Risiken an Ratten untersuchte, wurde die Interaktion zwischen BLA und NAc unterbunden. Das Ergebnis zeigte, dass dieser subkortikale Kreislauf Entscheidungen in Richtung größerer Gewinne verschiebt, deren Erhalt mit höheren Risiken verbunden ist. Die Unterbindung der Interaktion zwischen medialem PFC und BLA hingegen, zeigte einen gegenteiligen Effekt und erhöhte die Anzahl an riskanten Entscheidungen, was auf eine regulative Funktion des medialen PFC hinweist. Die aktuelle Studie war Teil dieser experimentellen Versuchsreihe und untersuchte die Interaktion zwischen medialem PFC und dem NAc. Die Interaktionsunterbindung durch asymmetrische Inaktivierung dieser beiden Regionen hatte keine Auswirkungen auf die allgemeinen Entscheidungstendenzen, veränderte jedoch andere Verhaltensparameter wie Bewegung, Reaktionszeiten und das Ausbleiben von Reaktionen. Des weiteren wurde eine verringerte Empfindlichkeit gegenüber negativen Rückmeldungen festgestellt. Diese Ergebnisse geben Einblick in die dynamischen Interaktionen zwischen kortikalen und subkortikalen Kreisläufen und helfen, die neuropsychologischen Mechanismen der mit Risiko verbundenen Entscheidungsfindung aufzuklären.

6.4. Curriculum Vitae

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Education

since 10/2005	**Diploma Program in Molecular Biology** University of Vienna **Specializations**: Microbiology and Immunology, Neurobiology and Cognitive Science
06/2005	**High school graduation with distinction** GRG 12, Erlgasse 34, Vienna

Work Experience

02/2011 – 09/2011	**Diploma thesis, Neurobiology** University of British Columbia, Vancouver Investigation of the neuropsychological mechanisms of risk-based decision making: "Interactions between the prefrontal cortex and ventral striatum in the mediation of risk-based decision making in rodents"
12/2007 – 02/2009	**Technical Assistant, Vaccine Development** (10 hours per week) Intercell AG, Vienna Maintenance of strain collection and database, PCR-optimization (primer design, temperature, cycles, buffer, polymerase, DMSO,…), supervision of interns

08/2007-09/2007 **Internship, Vaccine Development**

Intercell AG, Vienna

Isolation of pathogenic bacteria and purification of genomic DNA, PCR amplification of bacterial antigens for vaccine development

since 2004 **Climbing instructor**

Sportunion Wien, Vienna

Practical Experience

06/2010 – 07/2010 **Research Internship, Ethology**

Konrad Lorenz Research Station, Grünau im Almtal

Feeding behaviour of the Northern Bald Ibis (*Geronticus eremita*)

11/2009 – 12/2009 **Internship, Neurobiology**

Center for Brain Research, Medical University of Vienna

Quantitative PCR, electrophysiology (patch clamp), radioactive binding assays (ligand gated ion channels), synaptogenesis, neuroimmunology (ELISA, ELISPOT,…), immunohistochemistry, magnetic resonance and optical imaging,…

2005 - 2010 **Laboratory Courses**

University of Vienna

Organic and Anorganic Chemistry, Analytical Chemistry, Genetics, Biochemistry, Cell Biology, Microbiology, Immunology, Ethology, Structural Biology and Bioinformatics, Neuroscience

Other

Languages: German (mother tongue)

English (fluent)

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